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Contents

Technical Highlights

5th Meeting of the Asian Cotton Research and Development Network

Lahore, Pakistan, February 23-25, 2011

The Asian Cotton Research and Development Network was formed in 1999 at a meeting held in Multan, Pakistan. Meetings of the Network have been held every three years since then: Tashkent, Uzbekistan (2002), Nagpur, India (2005) and Anyang (Henan), China (2008). At the invitation of the Government of Pakistan, the 5th Meeting of the Asian Cotton Research and Development Network was held in Lahore, Pakistan on February 23-25, 2011. The Department of Agriculture of the Punjab province hosted the meeting. The Cotton Research Institute of the Ayub Agricultural Research Institute, Punjab made all the necessary arrangements for the meeting. ICAC, CABI-Pakistan and the FAO Sub Regional Office for Central Asia sponsored the meeting. As in previous meetings, the Technical Information Section of ICAC invited international participants and coordinated among international sponsors and the local host. The office of the Director General (Research) of the Ayub Agricultural Research Institute and the Cotton Research Institute, Faisalabad coordinated and invited local participants. Private sector participation and sponsorship for the meeting was overwhelming. The ICAC Secretariat and the Department of Agriculture, Punjab prepared the technical program together (the program is available at http://www.icac.org/tis/regional_networks/asian_network/asian_network.html), along with the list of participants and papers presented at the meeting. Delegates from Azerbaijan, Bangladesh, Canada, China, India, Iran, Kazakhstan, Kyrgyzstan, Myanmar, Tajikistan, Thailand, Turkey, Turkmenistan, USA, Uzbekistan, FAO, CABI, ICAC and a large number of participants from Pakistan attended the meeting. Fifty-nine papers were presented in three days covering all areas of research, from waste (sewage) water to plant nutrition, leaf curl virus disease, biotech cotton, insect pests and hyper sensitive gene response and cell-to-cell movement of proteins to control pathogen attacks. Highlights of the papers presented at the meeting are summarized in this issue of the *ICAC RECORDER*.

Cotton Breeding

During the 1970s, both breeders and physiologists agreed that heat sterility was the main constraint limiting cotton yields in Pakistan. All cotton varieties will start the formation of buds and flowers, but boll formation in Pakistan was delayed by 4-6 weeks due to high temperatures that restricted pollen grain germination and fertilization. The Nuclear Institute for Agriculture and Biology, Faisalabad developed the first heat tolerant variety (NIAB-78) through induced mutation breeding. When NIAB-78 was approved for commercial cultivation in 1983, it revolutionized cotton production in Pakistan. The Institute continues to use irradiation and has released four more commercial varieties since then with superior fiber qualities and higher yields: NIAB-111, NIAB-777, NIAB-846 and NIAB-999. In most cases, the irradiation process is performed either on F_1 seeds or on pollen grains of the male parent. (Sajjad Haidar, Pakistan)

In colored cottons, it is important to understand the mechanism by which pigment deposition takes place in the fiber, the relationship and impacts between fiber pigments and other chemicals, genetic effects and genetic correlation of the color and quality of color fibers. Researchers in China studied cottons of different fiber colors for ultra microstructure, content of cellulose and wax, UV spectrum of extracting solutions, diversity of physical and chemical properties, genetic effects and the genetic correlation of colored cottons

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and QTL locations. Studies showed that the secondary wall of green colored fibers was the thinnest and had poor fiber quality. The secondary wall of brown and white fiber cottons was thicker than that of green cotton. Color pigments were found to be distributed in the primary wall of all kinds of cottons, but there were only one or two layers in the primary wall. The pigment was mainly distributed in the fifth to eighth cellular layer of the secondary wall in green cotton, and there was no pigment detected in the 5th layer outside of the secondary wall. The "non pigment layer" of brown cotton was thicker than that of green cotton, the pigment gathered mainly in the lumen and in the tine layer of the secondary wall in the brown fiber. The wax content was the highest in green cotton reaching 4.65% to 6.81%, while the wax content in brown cotton was 1.09% to 1.21%, and wax content in white cotton was no more than 0.52%. Researchers also found that there were 4 QTLs for lint color and 3 QTLs for fuzz color, with additive effect and dominant effect in the F_2 populations of one cross. The F_2 population of another cross was found to have 5 QTLs for lint color and 4 QTLs for fuzz color, with additive effect and dominant effect. (Xiong-Ming Du, Hong-Jie Feng, Zong-ling Ru, and Jie Sun, China).

Uzbekistan's availability of germplasm and its cotton breeding program are recognized as one of the greatest success stories, not only in the Central Asian countries but also around the world. Uzbekistan has served as one of the primary sources of germplasm resistant to *Verticillium dahliae* Kleb. Researchers used indigenous breeding lines to develop resistance to verticillium race 1 and then later on to race 2. The two famous varieties resistant to race 1 and race 2 are C-4727 and Tashkent-1 respectively. Many commercial varieties resistant to both races were approved for commercial cultivation during the 1980s and in the years since, but improved yields and fiber quality were always the primary breeding objectives.

A third race of *Verticillium* appeared in some parts of Uzbekistan where the varieties available at that time resistant to "A" and "B" proved to be susceptible to the new race. Breeders used interspecific hybridization involving *G. thurberi* and *G. Raimondi* with *G. hirsutum* to develop varieties resistant to the third race. The Sultan variety was developed by crossing the F_1 generation of *G. thurberi* and *G. Raimondi* with *G. hirsutum*. Now even *G. punctatum* and *G. yucatanense* are used to develop resistant germplasm. Researchers found that some non-amphiploid species have horizontal as well as vertical resistance to the races of *V. dahlia*. (V. Avtonomov, O. Kimsanboev and Shadman Namazov, Uzbekistan)

Gossypium herbaceum is one of the four cultivars grown in only a few countries, but as one of the A-genome cottons, it is potentially important as a genetic resource for cotton breeding programs. Collecting native cultivars of this species grown in different countries is an immediate step in broadening the variability of the gene pool. Researchers in Iran undertook an analysis of the intragenomic characteristics of 42 *G. herbaceum* cultivars in the cotton gene bank and their cytological investigations revealed that there were significant variations in chromosome size, chromosome volume and karyotype formulae. Total chromosome length varied from 23.83 µm in one race to 46.46 µm in the other. Average chromosome length ranged from 1.83 to 3.57 µm among 42 genotypes. The longest chromosome measured 5.03 µm and the shortest was 1.2 µm. Chromosome volume ranged from 4.37 μm^3 to 9.32 μm^3. Forty-two genotypes were grouped in six clusters using Unweighted Pair Group Method with Arithmetic Mean (UPGMA). Nine cultivars fell in each of the first and second clusters, seven cultivars in the third, six in the forth, eight in the fifth and three in the sixth cluster. Interestingly, these results were very similar to clustering by morphological characters. The results showed that genomic differences among *G. herbaceum* cultivars could be used as an indicator of variability and heterosis in hybridization. (Omran Alishah, Iran).

Cotton lags behind other major crops in marker-assisted breeding. This shortcoming underscores the need for characterization, tagging, and utilization of existing natural polymorphisms in cotton germplasm collections. In Uzbekistan, researchers conducted molecular genetic analyses in a global set of ~1000 *G. hirsutum* accessions representing 37 cotton producing countries and 8 breeding ecotypes, as well as wild landrace stock accessions. Important fiber quality traits such as fiber length, strength, micronaire, uniformity, reflectance, elongation, etc., were measured in two distinct environments in Uzbekistan and Mexico. This study allowed researchers to design an "association mapping" (mixed linear model-MLM) study to find biologically meaningful marker-trait associations for important fiber quality traits in upland cotton that accounts for population confounding effects. Several SSR (Simple Sequence Repeats) markers associated with the main fiber quality traits, along with donor accessions, were selected for use in marker-assisted selection (MAS) programs. With this study, utilizing previous results of association mapping in Uzbek cotton germplasm resources, and designed with the specific objective of introducing and enriching the currently-applied traditional breeding approaches with more efficient modern MAS tools in Uzbekistan, researchers began marker-assisted selection efforts using molecular markers associated with the more important fiber traits. For this purpose, researchers selected 1) major fiber quality trait–associated SSR markers and 2) donor genotypes that were identified in their previous studies. They selected 23 major (micronaire, fiber strength and length, and elongation) fiber trait-associated DNA markers as a tool to control the transfer of QTLs during genetic hybridization. Researchers also selected 37 (11 wild race stocks and 26 variety accessions from diverse ecotypes) donor cotton genotypes that bear important QTLs for fiber traits.

The donor genotypes were crossed with nine commercial Uzbekistan cultivars (as recipients) in various combinations with the objective of improving one or more of the fiber characteristics of these recipients. These nine parental recipient genomes were preliminarily screened with the DNA-marker panel to compare with 37 donor genotypes. The polymorphic states of marker bands between donor and recipient genotypes were recorded. A subsequent generation of hybrid plants from each cross combination was tested using DNA-markers at the seedling stage and hybrids bearing DNA-marker bands from donor plants were selected for further backcross breeding. Testing a major fiber quality trait using HVI in trait associated marker-band-bearing hybrids revealed that mobilization of the specific marker bands from donors positively improved the trait of interest in recipient genotypes. Researchers developed a second generation of recurrent parent backcrossed hybrids (F_1BC_2), bearing novel marker bands and having superior fiber quality as compared to the original recipient parent (lacking trait-associated SSR bands). The results showed the functionality of the trait-associated SSR markers detected in association mapping efforts in a diverse set of upland cotton germplasm. Using these effective molecular markers as a breeding tool, breeders can plan to pyramid major fiber quality traits into a single genotype. (Ibrokhim Y. Abdurakhmonov, Zabardast T. Buriev, Shukhrat E. Shermatov, Fakhriddin N. Kushanov, Abdusalom Makamov, Umid Shopulatov, Ozod Turaev, Tohir Norov, Chinpulat Akhmedov, Mukhammadjon Mirzaakhmedov, Abdusattor Abdukarimov, Uzbekistan).

The genetic characteristics of *Gossypium arboreum* have not been properly exploited for many reasons, including: non-cultivation of *G. arboretum* in many countries, inability to make cross combinations between diploid and tetraploid species, lower quality fiber, etc. Lately, increased attention has been focused on the *G. arboreum* genome in India and Pakistan. It was found that the presence of a lower palisade layer of leaf lamina in diploid cotton conferred immunity to cotton leaf curl virus (CLCuV) and that the greater sucking distance together with compact alignment of the cortex cells provided resistance to sucking insects. In India, these traits have been transferred to *G. hirsutum* cotton (which usually lacks lower palisade, sucking distance) by introgression with 4n *G. arboreum.* Introgression of AD genome into A2 and A1 genomes (4n *G. arboreum* x *G. hirsutum* and 2n *G. herbaceum* x *G. barbadense)* improved fiber length and fiber fineness substantially in diploid cottons. Further, genetic amelioration of *G. arboreum* was done to improve boll size, fiber length, micronaire and fiber strength by using *G. hirsutum* as a donor source. These introgressed *G. arboreum* genotypes are thought to be the best candidates to substitute *G. hirsutum* genotypes and genetic transformation for insect pest resistance, as they are inherently resistant to sucking pests. Hence, this can be an efficient approach to the "hirsutization" of diploid cultivated species to elevate them to *G. hirsutum* status. These new genetic resources will help in preventing further genetic erosion of diploid cotton.

Apomixis is a phenomenon observed in many crop plants, especially gramineae, which can be used to overcome the hurdles mentioned in the beginning of the paragraph above in connection with hybrid seed production. Apomixis makes it possible to produce seed without meiosis and fertilization and helps to fix hybrid vigor once a desirable cross combination has been obtained. In India work is already going on to utilize apomictic lines in heterosis fixation of biotech cotton hybrids. The facultative apomictic condition in cotton has been observed in inter specific cross *G. hirsutum x G. barbadense x G. arboreum (4n*). Researchers have witnessed improvement in introgressed derivatives with respect to number of locules in bolls. Introgression of *G. arboreum* race *bengalense* with race *cernum* and B genome *G. anomolum* yielded a stable five loculed *G. arboreum* (ABC-5) genotype. (B. M. Khadi, India). Also refer to a paper 'Transference of Virus Resistance from *G. arboreum* and *G. anomalum* into to *G. hirsutum'* presented by Muhammad Arshad of Pakistan at the 4th Meeting of the Asian Cotton R&D Network held in China in 2008. The paper is available at <http://www.icac.org/tis/regional_networks/documents/asian/papers/arshad.pdf>.

Gossypols

There is an abundance of literature proving that many species of insects do not like gossypol. While gossypols are desirable in the plant parts against insects, gossypols in the seed glands prohibit extensive use of cottonseed in livestock feeding. Gossypols occur in two enantiomers referred to as (+) and (-) gossypol and most of the toxicity of gossypol resides in the (-) enantiomer. The ratio of (+) to (-) gossypol in seed has been reported to vary between a high of 98:2 and a low of 31:69. Gossypols offer inbuilt defense against some insects but there is limited information on how the level of (+) and (-) gossypol enantiomers correlates with insect and disease resistance. Chinese scientists have reported on experimentation in which *H. armigera* larvae were raised on artificial diets containing either (+) or (-) gossypol from the 3rd instar through to pupation and the moth stage. The larvae raised on the (+) gossypol diet matured more slowly and percent survival to the adult was also lower. A comparatively recent artificial feeding study in the USA showed that racemic (+) and (-) gossypols were equally effective in reducing days-to-pupation, pupae weight and survival of *H. zea*. The Uzbek researchers undertook a study under field conditions and found that the percent (+) gossypols in seeds does not affect spider mite resistance. In additional studies to verify the impact of gossypols on diseases and other insects, researchers used varieties that exhibited a high percent (>93.0%) of (+) gossypol in seeds and varieties that had low or no gossypols. Researchers concluded that increases in the percent of (+) gossypol correlated with an increase in resistance to black root rot disease caused by a pathogen *Thielaviopsis basicola*. Total gossypol content did not show any correlation with disease intensity. On the other hand *Xanthomonas malvacearum* did not correlate with (+) gossypol percentage. The data on resistance to *Rhizoctonia solani* indicated that increasing the percent of (+) gossypol does not influence plant susceptibility. (Shadman Namazov, Alois A. Bell, Robert D. Stipanovic, Abbos Marupov, Maqsuda Ishanqulova, Rano Yuldosheva, Ikrom Amanturdiev, Tojiddin Rakhimov, Uzbekistan).

The World Health Organization recommended that free gossypol and total gossypol may not constitute more than 0.06% and 1.2% of cotton flour respectively, if cotton flour is to be consumed by humans. The Food and Drug Administration of the USA has set a gossypol limit of 0.045% or 450 ppm in cotton flour for human consumption. Some biotech approaches, including one carried out by Cotton Incorporated in the USA, shows that RNAi-mediated gossypol reduction is restricted to the developing embryo and the kernel in mature seed. Thus, the defensive capabilities offered by the remaining plant organs stemming from gossypol and related terpenoids, were not compromised in RNAi lines. Extensive lab testing and field trials are underway to select one event that has the least amount of gossypol in the seed without any loss of gossypol in other parts of the plant. It is yet to be confirmed that the final event has no negative effect on yield, fiber quality or other aspects of even an agronomic nature.

Cotton Leaf Curl Virus Disease (CLCuV)

The cotton leaf curl virus disease (CLCuV), caused by a single stranded circular Geminivirus consisting of DNA- A and two satellites i.e. DNA-1 and DNA beta and transmitted by whitefly *Bemisia tabaci,* is an important problem in the northern cotton growing region of India. For close to 15 years, the three northern states of Haryana, Punjab and Rajasthan have been planting cotton on an area ranging from 1.2 to 2.0 million hectares. The symptoms that characterize the onset of the disease are characterized by small vein thickening (SVT) on young upper leaves. Other important symptoms of the disease are: upward or downward curling of leaves, formation of a cup-shaped leaf laminar and an out growth of vein tissues on the abaxial side of the leaves. In severe cases there may also be a reduction of internodal length leading to stunting and reduced flowering/fruiting. Effective management of this important disease may be approached through development of resistant varieties, suppression of whitefly and eradication of weed hosts that harbor it. After its appearance in patches around the Sriganganagar district of Rajasthan on *G. hirsutum* in 1993, the disease spread to all of north India in the short span of 4-5 years. It was noted that the most commonly grown cotton varieties in the northern states at that time were highly susceptible to this disease and consequently they were severely affected. Since the appearance of the disease on cotton a number of resistant/tolerant varieties like RS-875, RS-810, RS-2013, F1861, LH-2076, H1117, H-1226, H-1236 and hybrids like LHH144, CSHH198, CSHH238 and CSHH 243 were developed. The disease was brought under control and the damage caused by it was considerably reduced. Biotech cotton hybrids were introduced in north India during 2005/06 as a strategy to combat the menace of the bollworm complex. The greater part of the cotton area (more than 90%) is currently planted to biotech hybrids. In 2009/10, the cotton leaf curl virus disease reappeared in some areas of northern zone in a more virulent form. In certain hot-spot areas even hitherto known resistant varieties also proved susceptible to the disease.

The incidence of CLCuV disease is increasing and has become a threat to cotton cultivation in the region. Research efforts to develop resistant varieties/hybrids through conventional and biotechnological approaches, along with cultural and vector management practices, are in progress for effectively containing this disease. The work done on the original strain showed that minimum temperatures and sunshine hours have a significant negative correlation with incidence and progress of disease, whereas morning and evening relative humidity and rainfall have a positive correlation. This regression equation may be helpful in understanding factors affecting disease development and its prediction. As with the previous strain, the disease is not uniformly spread across areas within a given state, but in 2010/11 the disease was widespread in Haryana state.

Sequencing of DNA A and ß DNA components of the isolates associated with different symptoms revealed the existence of significant variation and recombination with other strains of CLCuV. However, correlation of specific sequences or recombination events with specific symptoms has yet to be established. Sequence identity matrix and RDP (Recombination Detection Program) analysis of the DNA A and ß components of six virus isolates undertaken over a period of four years beginning in 2006 found sequence homology and recombination among several isolates from India and Pakistan. Isolate G6-DC, isolated from cotton cultivar RS 2013, was found to have compromised resistance; severe leaf curl isolate S2, analyzed during 2009/10, showed a close resemblance to several CLCuV isolates from Pakistan. The DNA A component of G6-DC had major recombination events with two Pak strains, beside other Indian strains, while the S2 isolate showed major recombination with three Pak strains. Incidentally, the 'Burewala' strain was the notorious strain that overcame the resistance of popular varieties at Vehari, Pakistan in 2002, including the then highly resistant varieties. Accumulation of recombination events over the years coupled with favorable environmental conditions appeared to have defeated resistance to the disease in India in 2009/10. (Dilip Monga, P. K. Chakrabarty and K. R. Kranthi, India).

The Cotton Research Station at Multan, Pakistan screened germplasm for resistance to CLCuV and found that one tetraploid genotype belonging to *G. barbadense* and seven diploid species viz *G. herbaceum*, *G. arboreum*, *G. anomalum*, *G. capitisviridis*, *G. stocksii*, *G. somalense* and *G. longicalyx* were free of disease symptoms. Researchers are endeavoring to transfer CLCuV resistant genes from *G. arboreum* L. (variety 15-Mollisoni) into *G. hirsutum* L. (variety CRSM-38) through conventional breeding. An artificial autotetraploid of *G. arboreum* L. and an allotetraploid *G. hirsutum* L. were hybridized under field conditions. Exogenous hormones containing 50 mg/l gibberellic acid and 100mg/l naphthalene acetic acid were applied to minimize the chances of boll shedding. Results showed that the F_1 hybrid and BC_1 population are resistant to CLCuV in the field. Grafting also did not transfer the disease. The BC_2 population also showed resistance to CLCuV. Further studies continue. (Saghir Ahmad, Noor-ul-Islam, Abid Mahmood, Khalid Mahmood, Abdul Latif Sheikh, Wajad Nazeer and Mamoona Hanif, Pakistan).

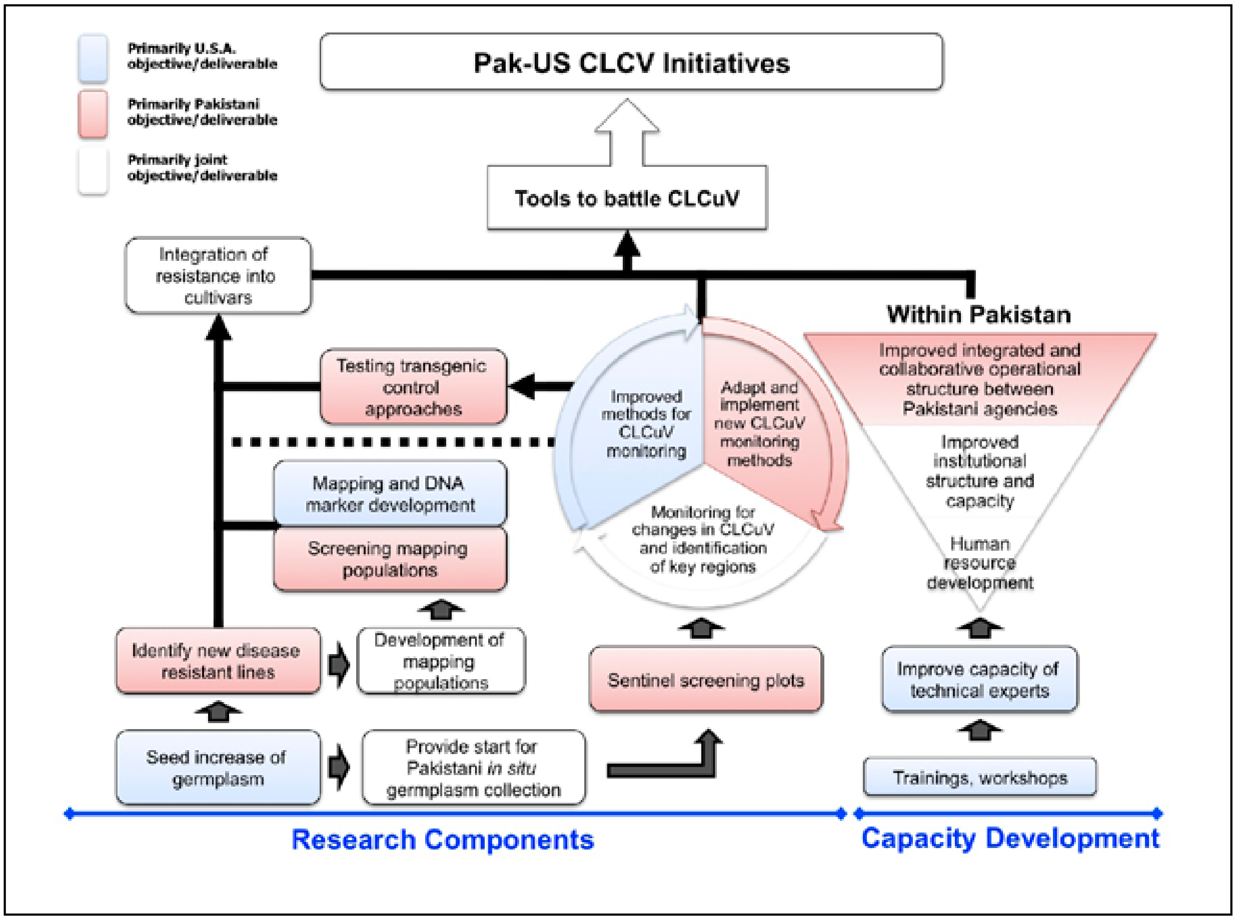
Detail agronomic studies conducted on leaf curl affected plots, planted at two week intervals starting from mid-April to mid-June under Pakistan conditions, revealed that the intensity of CLCuV disease increased with a delay in planting. The same experiment also showed that higher population densities (closer spacing) improved yields in later planted cotton. The findings showed that monopodial branches/plant, sympodial branches/plant, number of bolls/plant and seed cotton yield differed significantly as a result of planting time, plant spacing and genotypes. While the boll weight was significantly different only among genotypes, ginning outturn, fiber length and fiber fineness varied significantly with planting dates and cotton genotypes, but were not significantly affected by plant spacing. Cotton grown with early planting dates had higher seed cotton yield (4,874 and 4,653 kg/ha) at the greatest plant spacing (45cm), while late sown cotton (30th May and 15th June) produced higher seed cotton yields (2,068 and 1,889kg/ha) at the lowest plant spacing (15cm). Ginning outturn, fiber length and fiber fineness improved significantly with late planting, but were not affected by plant spacing. **The present study indicates that when the threat of CLCuV attack is high, seed cotton yield can be improved by planting late and reducing plant spacing (increasing plant population).** (Muhammad Iqbal, M. A. Khan, A. Mahmood and N. I. Khan, Pakistan).

Uzbek researchers carried out surveys of cotton areas in the Tashkent, Fergana and Khorezm regions and observed virus symptoms such as chlorosis or anthocyanosis mosaics and curling of leaves in cotton fields. Virus symptoms were observed in 15 of the 19 districts surveyed. Plants were affected at various stages starting as early as the 6-7 leaf stage. The disease was detected by means of visual symptoms, inoculation of plants or by electron microscopy of purified virus preparations. It was concluded that the virus is not transmitted by mechanical inoculation, but could be transmitted to cotton by grafting. The disease symptoms appeared 7-10 days after inoculation. Electron micrographs of the purified preparations obtained from infected cotton plants showed that the viruses are paired spherical with diameter 15-17 nm (nanometer), typical of geminiviruses and elongated flexuous particles. Researchers studied the effect of viral infection on forming the glucose and restoring sugar, on phospholipids and neutral lipids contents, and also investigated influencing virus diseases to enzyme hydrolysis complex on cotton fiber length in infected and control plants. The study of the phospholipid composition conducted the two-dimensional thin layer chromatography method. Chloroform:methanol:ammonia (65: 30: 4) and chloroform:methanol:acetic acid:water (175: 25: 25: 6) were used as solvents. Phospholipid fractions were found using iodine steam. At determination, hexane:diethyl ether:acetic acid (8: 2: 0:1) were used as neutral lipids. The results of the investigations showed that increased hydrolysis constant of the filaments exists in the course of hydrolysis and increase sugar material besides infecting plants. This is seen in inoculated plants as the length of the fiber develops incomplete maturation of the stringy part of cotton plant. Studying of influencing enzyme of hydrolyses complex on the results of the investigations have shown that increase hydrolysis constant of the filaments exists in the course of hydrolysis and increase sugar material beside virus infection plants. The results of comparative studies of phospholipids in cotton seeds from composition control and from plants with viral diseases have found the phospholipid content to be higher by a factor of 1.5 in infected plants. The study of neutral lipids in seeds from infected plants identified fatty acids, triglycerides, as well as essential oils, which were not found in the seeds of the control plants. (Zarifa N. Kadirova, A. H. Vakhabov, U. M. Juraeva and V. B. Fayziev, Uzbekistan).

Researchers in Pakistan explored a new strategy linked to wax content to tackle the leaf curl disease. The entire waxy coating on leaves is called the "cuticular membrane." The waxy coating consists of (from exterior inwards) epicuticular wax crystalloids or films, the cuticle proper, and a cuticular layer, which interweaves with the epidermal cell wall. The wax regulates non-stomatal water loss, protects plants against ultraviolet radiation, reduces water retention on the surface of the plant thus minimizing deposition of dust, pollen and air pollutants, defends plants against pathogens like bacteria and fungi involved in a variety of plant-insect interactions and have a role in pollen stigma interaction. Researchers tried interspecific crosses, chemical mutagens Ethyl methane sulphonate, Diethyl sulfate and Sodium azoid and physical mutagen Gamma rays to develop wax in wax-deficient mutants. Researchers tested whether whitefly can transmit the cotton leaf curl virus to wax deficient mutant plants or not. Wax deficient plants were chosen as control along with a highly susceptible variety and a diploid species variety having 50% more wax than wax deficient mutants resistant to CLCuV. Studies concluded that wax played an important role as mechanical barrier in hindering the transmission of virus by whitefly. (Tayyab Husnain, Pakistan).

The severity of leaf curl virus disease in the Sindh province of Pakistan is usually lower than the Punjab. Researchers collected samples of whitefly from both provinces to identify species and explore if disease severity is related to different species of whitefly or to environmental conditions. Researchers used a DNA barcode which is a short gene sequence taken from standardized portions of the genome used to identify species. They also used mtDNA because of ease of isolation, high copy number, lack of recombination, conservation of sequence and structure across metazoan and range of mutational rates in different regions of the molecule. COI was also employed because it is relatively well studied at the biochemical level, with size and structure conserved across all aerobic organisms, etc. Studies concluded that COI can be reliably used for species identifications and DNA barcoding data support a single species status for *Bemisia tabaci*. Data on diversity of endosymbionts and GroEL is being generated. (Shahid Mansoor, Pakistan).

Episodic epidemics of cotton leaf curl virus disease were the decisive factor for enriching the conventional system with new genetic sources showing extreme tolerance or resistance to the disease. For the short term, researchers estimated the extent of genetic diversity among the *G. hirsutum* genotypes, but came to the conclusion that narrow genetic base alone is not a decisive factor for the large-scale infection of the disease. Complete elimination of cultivation of locally evolved *G. arboreum* genotypes coupled with intensification in agriculture during the last three decades provided favorable conditions for the occurrence of the epidemic. The genetics of resistance to the disease is not simple. However, at the intraspecific level it may be classified into three groups. The intraspecific population was developed and screened for susceptibility. Parents NIBGE-115 and PGMB-33 were surveyed using 835 SSRs and 520 RAPDs, identified 90 polymorphic primers. These primers are being surveyed on F_2:F_3 populations to develop a genome map. Similarly, we focused our attention on the pyramiding of various sources in order to develop a single super genotype. The sum total of these efforts resulted in the development of genotypes showing fluctuating degrees of tolerance/resistance against the disease. Our study demonstrated the need for bringing in new wild genetic sources for fueling the cultivated cotton species. Bridging through genomic efforts would pave the way for the development of resistant cultivars – a long term strategy to combat the emergence of new strains of virus in future. (Mehboob-ur-Rahman, Muhammad Younus, Tayyaba Shaheen and Yusuf Zafar, Pakistan).



The Government of Pakistan has signed a collaboration agreement with the US Department of Agriculture (USDA) to find a solution to the leaf curl virus problem. Many institutions from Pakistan will be directly involved in a collaborative effort to be undertaken from various angles to find a long term solution of the existing races of geminiviruses causing leaf curl virus disease or other races that evolve over time. Responsibilities assigned, in general, are shown in chart of previous page. (Muhammad Arhsad, Pakistan).

Pakistan's National Institute for Biotechnology and Genetic Engineering is fine tuning RNAi for resistance against the cotton leaf curl disease complex. The RNA interference (RNAi) is a process of selective gene inactivation by destruction of messenger RNA (mRNA). Some applications of RNAi in agriculture include determination of gene function, engineering disease resistance in crops, metabolic engineering, engineering male sterility and insect control. RNAi is almost 1,000 times more effective than antisense RNA. RNAi limits the transcript level by either suppressing transcription (Transcriptional Gene Silencing-TGS) or by activating a sequence-specific RNA degradation process (Post Transcriptional Gene Silencing – PTGS). RNA Interference is a biological system that recognizes dsRNA and inactivates the corresponding mRNAs and/or genes in a sequence specific manner. RNAi has been widely used for resistance against RNA viruses. Reports vary from good success, to partial success or no success at all against geminiviruses. Partial or no success may be due to multiple suppressors, silencing of transgene and sequence targeting. There are multiple viruses responsible for the cotton leaf curl disease complex, thus requiring multiple suppressors and making it difficult to determine which sequence to target for broad spectrum resistance. Success against geminiviruses will depend on targeting of conserved sequences and the two possible targets that are highly conserved are cotton leaf curl BetaC1 gene (only for cotton leaf curl disease) and V2 gene (broad-spectrum for many viruses). RNAi based technologies for virus resistance have identified three highly conserved regions and developed ten different constructs. The work done so far has shown that silencing of V2 provides better protection. A patent for silencing of V2 gene of monopartite begomoviruses for broad-spectrum resistance against cotton leaf curl disease complex and other related viruses has already been filed. (Shahid Mansoor, Pakistan).

Forecasting Insect Pest Populations

The Department of Agriculture, Punjab, Pakistan established the Directorate of Pest Warning and Quality Control of Pesticides in the mid 1980s. While the mandate of the Directorate is apparent from the name, the Directorate earned a reputation in the province by providing information on hotspots for various pests on a weekly basis. The data on hotspots percentages allowed the government and public sector agencies to assess crop damage and accordingly warn growers to take appropriate measures.

Now the Directorate has come up with a model to forecast pest populations in advance of planting. Forecasting techniques rely on fluctuations in biotic factors, simplicity, accuracy of forecasting models and complete knowledge of biology, ecology, quantitative data on previous years' pest damage, field studies on insects and life history studies of insects under scrutiny. **Temperature alone is the single most important factor controlling insect development.** Rainfall, on the other hand, might be a reason for insect outbreaks. The Directorate of Pest Warning and Quality Control of Pesticides used the real pest population data from 2006/7 to 2010/11 from different locations around Multan. The relation of weather conditions to pests' abundance was summarized on the basis of multivariate regression and correlation tactics. The Auto regression integrated moving average method (ARIMA) was used to forecast insect population for the up coming year 2011/12. The meteorological data from the local station at the research institute in Multan was used in the model. The ARIMA sequence using the MINITAB software was employed to forecast insects as indicated in the table below. (Muhammad Sohail Shehzad, Pakistan).

Forecast for Insects on Cotton in 2011/12

Insect	Auto Regression Y=u+φYt-1 + €t	Moving Average Yt= u-Ө1€t-1 +€t	Previous Forecasts Yt=φyt-1 +φ2yt-2 +..........φpyt-p - Ө€t-1-Ө €t-2 + Өq€eq + €t (Lower Limit to Upper Limit))
Jassid	0.2963	−2.2530	14.4940 (11.6281 to 17.3599)
White fly	−1.000	−0.7055	11.6439 (-12.0036 to 35.2913)
Thrips	0.7337	1.4973	3.11425 (2.07439 to 4.15411)
Cotton mealybug	−0.0121	2.0620	61.8928 (34.2260 to 89.5597)
American bollworm	−0.8721	1.0444	-0.20124 (-1.46183 to 1.09535)
Army worm	0.5265	−1.3741	19.3631 (14.0570 to 24.6693)
Pink bollworm	0.3257	2.0138	1.55938 (0.27734 to 2.84142)
Spotted bollworm	0.5811	−0.2471	2.8073 (-9.5787 to 15.1933)
Dusky cotton bug	−0.2031	1.9588	-0.86353(-1.67628 to -0.05078)
Cotton leaf curl virus	0.6911	−1.2783	79.4485 (68.9912 to 89.9057)

Brief History of Cotton Insect pests in Punjab, Pakistan

Year	Insect Pest Situation
1961/62	Out-break of pink bollworm
1973/74	Army worm appeared
1983/84	High pest infestation due to prolonged rainy season
1987/88	Aphids appeared
1988/89	*Helicoverpa armigera* appeared
1990-92	Sever attack of *H. armigera*
1995/96	Severe attack of whitefly
1998/99	*H. armigera* again appeared in abundance
2001/02	Pink bollworm and armyworm became a threat
2003/04	Sever attack of army worm, spotted bollworm and *H. armigera*
2005/06	Mealybug appeared
2006/07	Mealybug increased along with leaf curl virus problem
2007/08	Severe attack of mealybug and leaf curl virus

Mealybug *Phenacoccus solenopsis*

According to Dr. R. Muniappan of Virginia Tech University, USA, the movement of invasive exotic species from one region of the world to another has increased in the recent past. Among the economically important arthropods, whiteflies and mealybugs constitute a major part of this invasion. The spiraling whitefly, *Aleurodicus dispersus*, a native of the Caribbean and Central America, became established in Hawaii in 1978 and since then invaded the Pacific Islands in the early 1980s and then moved to Asia and Africa in the 1990s. It has been effectively controlled by the introduction of the parasitoid *Encarsia haitiensis* and the coccinellid *Nephaspis oculatus*. Other neotropical species that are currently spreading in Asia are the giant whitefly *Aleurodicus dugesii*, that established itself in Hawaii in 2002 and Indonesia in 2007, and the nesting whitefly, *Paraleyrodes minei,* that has become established in the Middle East.

The mealybugs that invaded the old world tropics from the neotropics are the papaya mealybug *Paracoccus marginatus*, solenopsis mealybug *Phenacoccus solenopsis*, cassava mealybug *Phenacoccus manihoti*, Madeira mealybug *Phenacoccus madeirensis* and *Pseudococcus jackbeardsleyi*. The papaya mealybug was first collected in Mexico and then spread to the Caribbean in the 1990s, the Pacific in early 2000 and Asia in late 2000. This mealybug has been effectively controlled by the introduction of the parasitoids *Acerophagus papayae*, *Anagyrus loecki*, and *Pseudleptomastix mexicana*. Solenopsis mealybug was first collected in New Mexico in 1897 and started to spread in the early 1990s. Between 2005 and 2009, it caused severe damage to cotton in Pakistan and India. A fortuitously introduced parasitoid *Aenasius bambawalei*, discovered in the region, successfully suppressed this pest. Recently this mealybug has been reported in Cambodia, Thailand and Indonesia. The cassava mealybug, originally from central South America, caused devastation of cassava in the 1980s when it was accidentally introduced to equatorial Africa. It was reported in Thailand, Cambodia, and Laos in 2009, and Indonesia in 2010. This mealybug has been successfully controlled in Africa by introducing the parasitoid *Anagyrus lopezi.* Madeira mealybug is also of neotropical origin and widespread in tropical South America. It was reported in Pakistan in 1997, Taiwan in 2006 and Thailand in 2010. *Pseudococcus jackbeardsleyi*, another neotropical species was introduced to Singapore in 1958, Hawaii in 1959, the remainder of the Southeast Asia in 1970s and to the Maldives in 1994. It is possible that local parasitoids are keeping Madeira mealybug and *P. jackbeardsleyi* under control in some of these countries. (Rangaswamy Muniappan, USA).

The cotton mealybug *Phenacoccus solenopsis* Tinsley was first reported on cotton in India in 2004 and Pakistan in 2005. It caused huge economic losses to cotton agriculture in 2007 and thereafter in Pakistan. Studies were conducted in Pakistan to maximize biological control of the mealy bug and techniques were developed for utilizing the plant residues (mealybug infested drying shoots and leaves) for onsite production of natural enemies of the mealybug. The predators and parasitoids produced at the site not only naturally dispersed but also could be manually collected and released in farmers' fields. With these interventions, natural enemy populations increased and mealy bug numbers decreased. In pesticides free farms, the biological interventions led to a threefold decrease of the mealybug compared to farms treated with insecticides. The endemic natural enemy complex recorded on the mealybug in bio-control farms comprised of nine coccinellids, a chrysopid, a ceccidomyiid and two unidentified predatory bugs. *Aenasius bambawalei* (Hayat), which may have come with the mealy bug, was also recorded in 2008. It has strong searching ability and attacked mealybug in colonies and scattered individuals. With augmentation and conservation of this parasitoid on pesticide-free farms the mealybug was brought under complete control in cotton and other crops as of 2008. Conversely, in pesticides-treated farms, the mealy bug became a serious threat because the parasitoid was unable to build up its populations. On pesticide-free farms parasitism ranged between 79-93% whereas on pesticide treated farms parasitism did not exceed 8%. In the laboratory, females lived 10-37 days and parasitized 32-170 mealy bugs. The male to female ratio in the progeny was 1:2. The parasitoid seems to be host-specific as of all the mealy bugs tested in the laboratory it completed development on *P. solenopsis* only. *Cryptolaemus montrouzieri* (Muls.) was imported from California, mass bred and released in cotton fields by many government agencies throughout the country. This was recovered repeatedly from some places but not from others. (R. Mahmood, M. N. Aslam, G. S. Solangi and A. Samad, A., CABI-Pakistan, G. S. Solangi and R. Mahmood, CABI-Pakistan).

After the failure to control mealybug effectively with insecticides, the focus of attention in Pakistan shifted to biological control of mealybug. Researchers at the Central Cotton Research Institute, Multan, Pakistan, in collaboration with CABI-Pakistan, collected random field samples of mealybug *Phenacoccus solenopsis* infestations from cotton, brinjal/egg plant, okra, China-rose and *Abutilon* to explore the incidence of parasitoid *Aenasius bambawalei* Hayat and hyperparasite *Promuscidea unfasciativentris* Girault on mealybug. *A. bambawalei,* discovered for the first time in August of 2008 in Tandojam, Pakistan, was released on cotton in Multan and other areas. *A. bambawalei* showed promise by producing an 80%+ infestation of the mealy bug under favorable conditions. Later, in 2010 a tiny Hymenopteran, *P. unfasciativentris* Girault was also found associated with the mealybug however, it was suspected to be a hyperparasite. Both *A. bambawalei* and *P. unfasciativentris* adults emerged in the laboratory from mummified mealy bugs. Incidence of *A. bambawalei* was higher on winter collections of mealybug from cotton (48%) and *Abutilon* (35%) in December and summer collections from China-rose (71% in July). *P. unfasciativentris* did not parasitize mealybug in the laboratory when mealybugs were offered to both the species in one container, however, its adults emerged from mummies of *A. bambawalei* indicating the tendency of *P. unfasciativentris* to act as a hyperparasite. Higher populations of *P. unfasciativentris* in the latter half of the cotton season (26.7% in October) may have reduced the performance of *A. bambawalei*. Studies indicated that *P. unfasciativentris* did not parasitize mealybug for 48 hours as mealybugs continued normal development/reproduction in the laboratory and no mummies were formed. Mealybugs, that were exposed to the parasitoid *A. bambawalei* and then accompanied by *P. unfasciativentris* produced 77.3% mummies from which adults of both the species emerged at rates of 70.7% and 13.8%, respectively, indicating a hyper parasitic tendency in the latter. (M. Iqbal Arif, S. Wazir, Muhammad Rafiq, Abdul Ghaffar and R. Mahmood, Pakistan).

At the meeting, Dr. Keshav Kranthi of the Central Institute for Cotton Research, Nagpur, India presented a paper titled “Package of Practices for Managing Mealybug on Cotton”.. His full paper was published in the March 2011 issue of the *ICAC RECORDER*. The two photos showing how mealybug infects the cotton plant are shown here.

Mealybug Infestation on Young Plants

Mealybug Infestation on Stem and Boll

Irrigation Water

As a result of their differing morphologies and genetic makeup, cotton varieties respond differently to water stress. Researchers at the Central Cotton Research Institute, Multan, Pakistan tested the hypothesis that with efficient gas exchange, tall-stemmed, deep-rooted genotypes may perform better under water stress conditions. Screening of breeding material for physiological parameters might help breeders to select material that requires less water or will tolerate water shortage more effectively. Researchers tested six genotypes of diverse morphology in conditions of no water stress at -1.6±0.2 Mega pascal (MPa) leaf water potential (Ψ_w), as compared to with water stress at -2.0±0.2 MPa Ψ_w. The water stress conditions were imposed 30 days after sowing and was continued until crop maturity. It was concluded that parameters like plant structure development, gas exchange characteristics, water use efficiency, leaf area index and proline content could provide ample guidance to breeders in selecting genotypes tolerant to water stress conditions. (Fiaz Ahmad, Shabab-ud-Din and Rehmat Ali, Pakistan).

Water Use Efficiency For Drip and Furrow Irrigation for Various Plant Spacing

Irrigation Method	Plant Spacing (cm)	Water Use Efficiency (kg seedcotton $ha^{-1}mm^{-1}$)
Drip Irrigation	75x10	7.97
	75x20	7.50
	75x30	6.56
Furrow irrigation		
	75x10	4.95
	75x20	4.75
	75x30	4.34

The conventional approach to combating drought and developing salt tolerance is giving way to molecular mechanisms that are similar in most plants and makes it possible to develop genotypes that are both drought tolerant and salt tolerant. Molecular marker technology has successfully helped to develop desired traits in many crops in less time. Recent improvements in molecular marker technology designed to better exploit the linkage disequilibrium-based association mapping approach have facilitated the use of commercial cultivars for the identification of DNA markers associated with the trait of interest. The association mapping approach can be employed in molecular breeding programs for the development of drought and salt tolerant cotton cultivars. Presently, there is a thrust to develop new marker systems, especially single nucleotide polymorphisms (SNP) in cotton. Determination of locations of quantitative trait loci (QTL) for agriculturally important characters promises increased efficiency for future genetic manipulations in cotton breeding. The use of restricted fragment length polymorphism (RFLP) markers on QTLs for agronomic and fiber traits has been instrumental in identifying QTLs for leaf morphology; QTLs for stomatal conductance were discovered using randomly amplified polymorphic DNA (RAPD) and simple sequence repeat (SSR) markers. Among fiber quality characters, researchers have observed a frequent association of QTLs with multiple traits in connection with maturity and fineness. Because of this association, the use marker-assisted breeding for such traits is more complicated. (Muhammad Saeed, Pakistan).

The plant structure of commercial varieties has changed a lot in Pakistan since the development of heat tolerant varieties almost three decades ago. More recently, the introduction of biotech cotton has accelerated the change in varietal composition. The trend to plant cotton in February as compared to a normal season of May and June has further changed plant's agronomic needs, particularly their water requirement, fertilizer application protocols and pest control. Consequently, ridge sowing has increased in both the major cotton growing provinces of Pakistan. The longer growing season definitely produces higher yields, but the increase should not be achieved to the detriment of water use efficiency. In 2010, researchers at the Central Cotton Research Institute, Multan, tested one of the latest varieties using drip and furrow irrigation methods and planting at various plant intervals. Cotton is normally planted in a configuration of 75x30 cm, but researchers lowered the plant interval to 10 and 20 cm while keeping the row to row separation at 75 cm. The data showed that drip irrigation increased seedcotton yield by an average of 20% by producing bigger bolls and a greater number of bolls. Independently of the irrigation method used, a plant interval of 10 cm produced the greatest number of bolls per unit area. The test for water use efficiency achieved with the different irrigation methods revealed water use efficiency was significantly higher with the drip irrigation system. The data also showed that drip irrigation produced a water-use economy of 53% over furrow irrigation. (Dilbaugh Muhammad, I. Raza, S. Ahmad, M. N. Afzal and M. A. Mian, Pakistan).

Researchers in Iran conducted an experiment on non-agricultural soil to investigate the effect of treated municipal wastewater on the yield and fiber quality of cotton. Apart from treated municipal wastewater other treatments included irrigation with freshwater and different mixtures and intervals of freshwater with treated municipal water. Fertilizer and all other agronomic requirements were provided as for a usual crop in Iran. Plots were irrigated by flood irrigation and the amount of water applied to each plot was the same. During the growing period 819 mm (equal to 32 acre inches) of water was applied. The experimental plots were irrigated with freshwater 20 days after sowing (corresponding to the two-leaf stage of the plants) and irrigation treatments were initiated after this stage. The results showed that cotton yield, number of bolls per unit area, leaf area index (LAI) and plant height benefited significantly with treated municipal water as compared to freshwater irrigation. Seedcotton yield was 2,200 kg/ha and 780 kg/ha under treated municipal water and fresh water respectively. There was no significant difference between interval and mixture treatments with the same percentage of freshwater and wastewater (treated municipal water) application. The treated water showed no significant detrimental effect on the fiber quality characteristics. The use of treated municipal water in irrigation is beneficial for a number of reasons: (a) water shortages can be reduced; (b) large amounts of wastewater can be disposed off during the entire year; (c) high-quality water resources can be devoted to potable uses; (d) economic benefits are possible, attributed primarily to the nutrient content of the wastewater; (e) the availability of additional water near population centers increases the range of crops that may be grown by farmers and, (f) it is an important source of nutrients for less fertile soils. (Ibadollah Baniani, Iran).

Environmental Impact of Cotton Production

Current cotton production practices based on high pesticide application and low water use efficiency take a heavy toll on the environment. The World Widelife Fund-Pakistan and the Better Cotton Initiative (BCI) are working together in Pakistan to minimize damage to the environment and mitigate the social consequences of cotton production. Since 2005, WWF-Pakistan and IKEA collaborated in the "Pakistan Sustainable Cotton Initiatives (PSCI)" for the development and promotion of site-specific Better Management Practices. More recently, collaboration under the BCI, a multi-stakeholder initiative launched in 2005, was further expanded. In Pakistan, 41,000 farmers participated in the program to grow BCI cotton over an area of 150,000 hectares during 2010/11. The project farmers saved 37.5% on water use, 47% in pesticide use and close to 41% in fertilizer use, all of which had a positive impact on farmers' net income. In 2010/11, the farmers involved in the project produced some 62,000 tons of seedcotton, of which 22,000 tons was declared 'Better Cotton' based on BCI third party verification. More than 1,000 tons of Better Cotton lint was produced by designated ginners and most was procured by local suppliers of international brands. (Arif Hameed Makhdoom and Shafiq Ahmad, Pakistan).

Fertilizer Use

Zinc does not affect yield directly. Indirectly, however, it affects yields and quality because of functions such as its activity in biological membrane stability, enzyme activation ability and auxin synthesis. Furthermore, zinc plays and important role in the plant's protein and carbohydrate metabolism. Apart from boron and sulfur, zinc is one of the micronutrients that is commercially applied to cotton. According to the publication *Cotton Production Practices 2008* of the ICAC, zinc is applied to cotton in Australia, Brazil and Pakistan. Dr. Lale Efe from Turkey investigated the effect of different zinc application methods on seedcotton yields, yield components and lint and seed quality. She found that zinc application methods did not affect yield and yield components, but did have a positive effect on plant height. Zinc application methods showed no statistically significant effect on lint quality traits except for the spinning consistency index (SCI), elongation and yellowness (+b). It was observed that zinc application methods did not affect the raw oil and protein ratio of cotton seed, zinc content in cotton seed and leaf. (Lale Efe and Emine Yarpuz, Turkey)

At his private farm in India, Dr. M. S. Kairon tested different fertilizer application protocols with plant spacing intervals of 90 cm, 60 cm, 30 cm and 15 cm while keeping the row to row distance at 90 cm under. He observed that a higher population density produced significantly higher seedcotton yields. In all the three genotypes used in the experiment, the greatest yields were achieved where higher plant population was coupled with higher doses of fertilizer. Fertilizer was applied at rates of 160, 80 and 80 kg NPK respectively. Split application of fertilizer (50% at the time of sowing, 40% in two splits at the squaring and flowering stages and 10% provided through foliar application during boll development) produced significantly higher yields than traditional fertilizers application protocols. (Mian Singh Kairon, India).

Biotechnology

The Government of Iran approved the National Biosafety Law (NBL), which provides for the production, release, import, export and use of living modified organisms (LMOs) in Iran. According to this law, the government "should" facilitate these activities. In 2004, coinciding with the International Year of Rice, the Government officially inaugurated the harvest of an insect resistant biotech rice as the first transgenic crop in Iran. Transgenic cotton with enhanced resistance to Lepidoptera pests and tolerance of fungal diseases is expected to be the next biotech crop in the pipeline and is forecast to be releases in two years. Dr. Behzad Ghareyazie, of the Agricultural Biotechnology Research Institute, presented a paper titled "Iran's Experience in Stacking Insect Resistance and Disease Tolerance in Transgenic Cotton" at the meeting. It is estimated that in Iran more than 30% of the cotton harvest is lost due to the insect pests and wilt disease (*Verticillium dahliae*). In order to enhance cotton resistance to insect pests, hypocotyl explants were transformed using *Agrobacterium tumefaciens* hosting the recombinant binary vector pBI121 containing the *cry1Ab* under the control of a CaMV 35S promoter. In a separate investigation, bean chitinase (*chi*) gene, was expressed in cotton to increase its fungal disease tolerance. The neomycin phosphotransferase (*nptII*) gene was used as selectable marker. Polymerase chain reaction and Southern blot analysis were used to confirm the integration of the *cry1Ab* and *chi* transgenes into the plant genome. Western immunoblot analysis of proteins extracted from leaves of transgenic plants revealed the presence of an immunoreactive band with a molecular weight (MW) of approximately 67kDa for Cry1Ab and 32kDa for the *chi* gene. Homozygous T2 plants carrying the Cry1Ab gene showed significantly higher levels of insect resistance against *Hlicoverpa armigera* larvae than the control plants. As judged by foliar disease symptoms, vascular discoloration and plant height, the homozygous T2 plants with the *chi* gene, showed significantly higher degrees of tolerance to fungi than did the non-transgenic plants. Transgenic plants were also taller than the control plants. Plants with enhanced insect resistance and disease tolerance were crossed to pyramid the two agronomic traits and were backcrossed with Iranian cultivars. Biosafety issues and risk assessments were conducted and the process for field trial and commercialization is under way. (Masoud Tohidfar and Behzad Ghareyazie, Iran).

India has been very aggressive in the adoption of biotech cotton. It is estimated that in 2011/12, the biotech cotton area will be close to 90% of the total cotton area. In the last 10 years or so, since the approval of biotech cotton in India, six events and 619 biotech varieties and hybrids have been approved for

Biotech Cotton Events and Varieties Approved in India

Event Name	Genes Involved	Gene Source/Company	Indian Company Using the Event	No. of Varieties/Hybrids Approved
Mon 531	Cry1Ac	Monsanto	Mahyco-Monsanto	205
Mon 531 + Mon 15985	Cry1Ac + Cry2Ab	Monsanto	Mahyco-Monsanto	309
Event 1	Cry1Ac	Indian Institute of Technology	Indian Institute of Technology and J. K. Agri	33
GFM events	Cry1Ab + Cry1Ac	Chinese Academy of Sciences	Nath Seeds	69
BNLA 106	Cry1Ac	Indian Council of Agri. Res.,Central Institute for Cotton Research, University of Agricultural Sciences (Dharwad) and National Research Center on Plant Biotechnology	Indian Council of Agri. Res.,Central Institute for Cotton Research, University of Agricultural Sciences (Dharwad) and National Research Center on Plant Biotechnology	1
MLS 9124	Cry1c	Metahelix	Metahelix	2

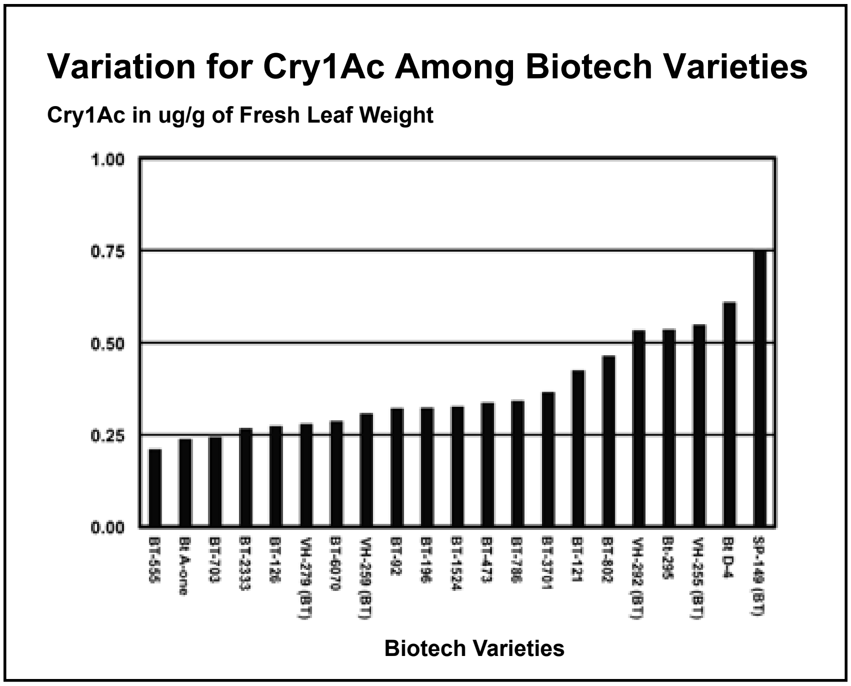
commercial cultivation. The list of events, their sources and varieties are given in the table above.

Of all the countries that have adopted biotech cotton on a commercial scale, India has obtained the highest increases in yields at the national level. There are many factors responsible for such increases. According to Dr. Khadi, biotech cotton seems to have reduced the overall quantity of insecticides substantially. Gujarat State experienced a spectacular reduction in insecticide use on cotton while the rest of the states have shown mixed results despite increases in the area under biotech cotton. One clear impact of Bt-cotton on Indian agriculture appears to be the replacement of large tracts of varietal areas in the North India with biotech hybrids, since the technology is available in India only through hybrids. The Central Institute for Cotton Research, Nagpur has developed diagnostic kits viz., Bt Express, Bt Detect, Bt-Zygosity, Bt Quant for the detection of Bt toxin and these kits have been effectively deployed all over the country to verify the purity of biotech seed and ensure the supply of quality biotech hybrid seeds to the farming community. Due to the large scale cultivation of biotech cotton in India since 2002, changes in the insect pest complex are evident in the country. Mealybugs and mirid bugs are emerging as potential threats. The tobacco caterpillar, *Spodoptera litura*, was also found to be staging a comeback as an economic pest on biotech cotton. There is no certainty that these insects would have become a threat to cotton production in India in the absence of biotech cotton. The concept of refuge (use of non-biotech crop) has been promoted from the very beginning, but it is hardly followed at all. India is lucky that no resistance to biotech cotton toxins has been reported so far. Findings reported in a limited number of papers indicating development of resistance to the toxins would have to be verified. (B. M. Khadi, India).

The National Institute for Biotechnology and Genetic Engineering, Faisalabad, Pakistan is taking the lead in the drive to develop their own Bt and non-Bt genes, get them patented, test these genes in local germplasm under field conditions and ensure availability of biotech cotton to farmers at a reasonable price. The Institute is also working on synthetic genes through different constructs made for enhanced expression using strong plant expressible promoters (CaMV35S, rbcS, etc.), transit peptide and intron. One application for such a patent has already been filed. The newly developed genes are first transferred into tobacco plants for characterization because it is easy to transform tobacco and the transgenic plants can be recovered within 10-12 weeks. It is also easy to obtain the molecular and physiological characterization of transgenes. The insect resistant genes used at the Institute are Cry1Ac, Cry2Ab, Vip3A, Hvt and lectins. Hvt, characterized in tobacco and cotton, is highly effective against *Helicoverpa* spp. and *Spodoptera* spp. but requires confirmation against sap-sucking insects. Lectins were characterized in tobacco and have proved effective against mealybugs. In regard to second generation, Cry1Ac + Hvt and Vip3A + Hvt have been characterized in tobacco and are being transferred into cotton. Transformation of Cry1Ac + Cry2Ab into cotton is in progress and work has not yet started on Hvt + lectin. With regard to the third generation of transgenic plants, preference is given to virus (CLCuV) resistance after insect resistance followed by salinity/drought/heat stress tolerance and herbicide resistance. While developing Bt (Cry1Ac) cotton, 55 independent transgenic events were regenerated, molecular characterization (gene integration, copy number, expression level etc.) was undertaken based on insect bioassays with *Helicoverpa* species and the best performing line was selected and used for further breeding. The Hvt gene has been isolated from the Australian funnel web spider *Hadronyche versuta*. The toxin known as ω-atracotoxin-Hv1a has been biochemically characterized and found to have insecticidal properties. Topically applied purified toxin killed mealybugs in the field within 5 minutes. However, a transgenic cotton having the Hvt gene under the 35S promoter was ineffective for the control of whitefly as well as mealybugs, which may be due to a low level of expression in the cell sap. Further studies continue including risk assessment of Hvt cotton. (Zahid Mukhtar, Sher A. Khan, Shaheen Asad, M. Arshad, Nasir A. Saeed, Allah Dino Shah, M. Nouman Sohail, M. Asif, Afzal Naeem, Yusuf Zafar, Shahid Mansoor and Zafar M. Khalid, Pakistan)

A survey undertaken by the Department of Agriculture, Punjab, Pakistan in 2010/11 showed that 78% of the cotton area in the Punjab province was planted to biotech varieties, as compared to 70% in 2009/10. In addition to the share of biotech cotton, the comprehensive survey also studied types of Bt genes, source (s) of Bt gene used, expression level of Bt gene in different genetic backgrounds and genetic relatedness among Bt varieties planted in 2010. For the sake of estimating the expression level of the Bt gene, Cry1Ac endotoxins was quantified in biotech varieties in 18-day-old leaves of 80-day-old-plants by Enzyme Linked Immunosorbent Assay (ELISA). It was observed that the genetic background had a considerable impact on Cry1Ac expression ranging from 0.21 to 0.75 µg/g of leaf tissue.

The level of expression of Cry1Ac was considerably lower than reported in the donor parent (i.e. 1.56 µg protein/g of leaf tissue). A lateral flow strip test method was used to test target proteins Cry1Ac, Cry2A, CP4 EPSPS and PAT/bar. Polymerase chain reaction (PCR) based assays were used to detect type and source of the Bt gene(s), which showed the most prevalent screens (NPTII, 35S) of the gene Cry1Ac. In total 46 entries were tested and none of them was found positive for Cry2A (Bollgard II). The study cautioned that the considerably low level of expression of Cry1Ac in most of the local biotech varieties may lead to development of resistance by target insects. (Muhammad Zaffar Iqbal and Dr. Ihsan Ullah, Pakistan).



Cotton fibers are differentiated, non-dividing cells that originate from the epidermal layer of developing ovules. Fiber development comprises of four major stages: i) initiation, ii) elongation, iii) secondary cell wall synthesis and iv) maturation. The analysis of fiber development specific genes has revealed that expansins, aquaporins, and sucrose synthase represent the major genes involved in determining fiber characteristics, including primary parameters such as length, strength and micronaire. *Calotropis procera* seed fibers provide an excellent model system in which to study the genes involved in fiber elongation, fineness and strength. The expansin and aquaporin genes were isolated from the cDNA libraries of fibers from cotton and *Calotropis procera*, while the sucrose synthase gene was isolated from developing cotton fibers. Plant transformation vectors were designed for constitutive (35S promoter) and fiber specific expression (LTP3 promoter) of these genes in transgenic cotton plants. The designed cassettes were introduced in *Gossypium hirsutum* var. Coker through *Agrobacterium* mediated transformation. The T_0 transgenics for all the mentioned constructs have been developed at the National Institute for Biotechnology and Genetic Engineering, Pakistan. In addition, the developed vectors are also being used for direct transformation of elite cotton cultivars. The studies provided important information on the use of individual genes to improve fiber traits. These genes may ultimately be used for constructing multiple gene expression vectors to produce transgenic cotton plants generating long and extra-long cotton fibers. (Aftab Bashir, Pakistan).

Proton pumps energize the biological membranes to facilitate the exchange of materials by transporters and exchangers in plants. Transgenic cotton Coker-312 plants containing AVP1-H^+ pump with expression driven by 35S promoter showed quick germination, vigorous seedling growth and higher boll setting than the non-transformed control counterpart. Moreover, hydroponic studies revealed high Na^+/K^+ accumulation in the leaves and efficient root system as compared to control cotton plants. The transgenic AVP1 cotton plant was able to produce more fresh and dry root biomass weight than control cotton plants under hydroponic conditions. Similarly, transgenic AVP1 cotton plants showed improved drought tolerance by showing more negative solute potential and high relative water contents. Transgenic cotton Coker-312 expressing the *AVP1* gene could be used in breeding program to improve the salt and drought tolerance needed to bring marginal lands into production. (Shaheen Asad, Muhammad Arshad, Zahid Mukhtar, Yusuf Zafar, Muhammad Yasin Ashraf and Shahid Mansoor, Pakistan).

Insect Control

Pakistan has one of the most complex patterns of insect pests on cotton in the world. A number of pests appear as key pests at the same time, including whitefly, jassid, mealybug, thrips, aphid, two-spotted mite, spotted bollworms, pink bollworm, cotton bollworm, leafworm or armyworm and beet armyworm. The conditions that favor pest outbreaks include: destruction of beneficial insects by indiscriminate use of pesticides, overwhelming populations brought on by favorable environmental conditions, such as periodic summer showers, for jassids and *Helicoverpa*, and hotter, drier weather with milder winters, for whitefly and mites, along with poor control due to choice of wrong insecticides, their use in insufficient doses or improper application leading to development of insecticide resistance. General improvement in cropping through use of fertilizers, irrigation, etc., is already playing a favorable role in the build-up of insect populations but lately, closer spacing and early sowing have only aggravated the situation in biotech cotton. Whitefly is extremely important in Pakistan not only because of its effects on yield and fiber quality but also as a vector for viruses. Due to continuous use of insecticides, whitefly has developed resistance to various groups of insecticides. The leafworm or armyworm has developed resistance to pyrethroids. Mealybug has developed resistance to a group of organophosphates. The following are some of the more important components of any insecticide resistance management strategy:

- Scout at least once a week, or twice a week when pest pressures are high and environmental conditions favor quick multiplication.
- Spray only at economic threshold levels and follow recommended insecticide use rates, always avoiding under-dosing or over-dosing.
- Delay the first spray on cotton as long as possible to conserve natural enemies and direct insecticide applications at the insect's habitat for improved coverage.
- Never use an insecticide product to which the insect has already developed resistance.
- Apply alternate insecticide classes with different modes of action to avoid development of insecticide resistance
- Do not use any active ingredient more than twice per season
- Never use consecutive sprays of the same pesticide and avoid mixtures if a single product can do the job.
- Use selective insecticides (e.g. insect growth regulators) and avoid using broad-spectrum insecticides, especially early in the season. (Mushtaq Ahmed, Pakistan).

Jassid *Amrasca biguttula* is a serious and historical pest on cotton in Pakistan. Breeders have assigned a high priority to profusely hairy leaves in their breeding programs but insecticides are always required to control the pest. Some renewed efforts are underway to identify the status of natural enemies associated with jassids on cotton in Pakistan. Jassids deposits their eggs inside leaf veins and pesticides can hardly reach them whereas the parasitoids are able to locate their target easily. Some very recent studies indicate that pesticide sprays affected jassid nymphs and adults resulting in lesser number of jassid eggs per leaf on sprayed crop than on unsprayed crop. However, the disadvantage was that parasitoids could hardly survive in the pesticide sprayed environment. The natural control of jassid was severely affected and pesticide applications had to be repeated to save the crop from heavy losses. On the other hand, natural enemies were abundant in the unsprayed crop and pesticides were not needed to control the jassids. The study further indicated that *Arescon enocki* is a dominant parasitoid and that it is effective in regulating the jassid population. In unsprayed cotton not only the jassid was under control, but the mealybug, whose resurgences were seen frequently enough in sprayed cotton, was under good control in the unsprayed crop. Researchers concluded that the agro-ecosystem of the cotton belt in Pakistan is rich in beneficial insects and there is a need to work out strategies that encourage multiplication of jassid predators. (H. A. Sahito, I. Haq, M. A. G. Sulehria, A.A. Nahiyoon and R. Mahmood, Pakistan).

Jassids are also a serious pest in Thailand. Breeders developed a hairy leaf variety, Tak Fa 84-4, from which they selected promising plants and or lines under non-systemic insecticide spraying and artificial leaf roll disease inoculation (jassid serves as a vector for the disease). The number of hairs on the leaf surface per 0.20 cm^2 and number of hairs on leaf vein per 0.20 cm^2 was 62 and 56 respectively on Tak Fa 84-4, as compared to 13 and 16 respectively on the commercial variety Tak Fa 2. Dense hairs on leaves hindered multiplication of the jassid population. (Parinya Seburuang, Thailand).

Dr. S. S. Udikeri of India presented a paper entitled Emerging Pests of Bt Cotton and Dynamics of Insect Pests in Different Events of Bt Cotton. In India, three different species of mirid bugs (Miridae : Hemiptera) viz., *Creontiades biseratense* (Distant), *Compylomma livida* (Reuter) and *Hyalopeplus linefer* (Walker) have been infesting cotton since 2005. *Creontiades biseratense* has the greatest potential and distribution to become a key pest, particularly in Karnataka state. Both adults and nymphs suck the sap from the base of squares and tiny bolls leading to heavy shedding. Almost all cultivated biotech cotton varieties have been found to be susceptible to mirid bugs. The minimum avoidable yield loss with two rounds of protection was calculated to be 20.6 %. Application of Acephate 75 SP @ 700 g.a.i./ha proved to be a good management option. *Helopeltis bradyi* (Waterhouse), which is a common pest on guava/cashew/tea, etc., is emerging as a major pest on interspecific biotech cotton. Affected cotton has suffered total damage and yield loss under heavy infestations. In India, researchers have also found another new insect pest on biotech cotton called the flower bud maggot of cotton *Dasirseura gossypii* Fletcher. (Cecidomyiidae: Diptera). This insect has appeared as a potential pest for the first time in the history of cotton entomology in India and elsewhere. Currently > 90 % fruiting body damage has been recorded in areas cultivating biotech cotton for many years viz Kanaka and Neeraj. (S. S. Udikeri, K. R. Kranthi, S. B. Patil and B. M. Khadi, India).

Country Reports

Iran

Cotton area declined from 325,000 ha in 1996/97 to about 100,000 ha in 2010/11. The main reason for the decline is that cotton producers are shifting to more profitable competing crops. One of the most expensive items in cotton production in Iran is picking labor that usually comprises about 40% of the total cost. The planting seed industry is still in its infancy and unable to assure supplies of certified quality seed for planting. The Government of Iran has designed a five-year plan to encourage growers to return to cotton production. Farmers will be provided with a guaranteed price to shield them from cotton price fluctuation, as well as financial aid to allow them to prepare their land following technical advice for planting on time, use only high quality planting seeds, implement modern irrigation systems to reduce water consumption, etc. All the necessary facilities have been provided to launch a fully mechanized cotton production system designed to lower production costs. The Cotton Research Institute, Gorgan, will play its due role in recovering cotton production in the country. The Institute will continue to produce basic seed to maintain variety purity, develop new varieties and expand the hybrid cotton production program. Interspecific hybrids between *G. hirsutum* and *G. barbadense* will be developed for large scale cultivation. The interspecific hybrid, 'Hysibar," released in 2009 has a staple length of 36.7 mm and matures in about 140 days. Transplanting of cotton allows producers to cut time in the fields and plant early. The Cotton Research Institute has developed pots made of cotton for growing seedlings. The pots decompose in the fields and insure an optimum plant stand and that there will be no seedling mortality during transplanting . Iran is also working on adopting an ultra narrow row system in the north of the country to allow for double cropping with soybeans in the system. (Ali Jafari Mofidabadi, Iran).

Myanmar

Cotton was planted on 359,416 hectares in 2009/10 and almost the same area is forecast for the 2011/12 season. Almost 75% of the area is planted to upland varieties with the remainder planted to *G. arboretum* varieties. In Myanmar, cotton is planted at three different times in largely overlapped cropping seasons and is grown mostly as a rainfed crop. Upland varieties, commonly referred as long staple varieties, are predominantly grown late in the monsoon season that extends from July-August (sowing) to December-January (Picking) and pre monsoon season from February-March (sowing) to June-July (picking). Short staple *G. arboretum* varieties and a certain share of long staple cotton are grown in rainfed conditions during the monsoon season from May-June (sowing) to November-December (picking). The recommended seeding rate is 24 kg of fuzzy seed/ha with a plant configuration of 90x45 cm on fertile soils and 76x45 cm on less fertile soils. Thinning is done and two plants per hill are maintained. Organic manure (usually cow dung) is commonly applied and an application of urea, triple superphosphate and muriate of potash is recommended for upland cotton, depending upon the degree of soil fertility. The nitrogen application rate may range from 62 kg/ha to 125 kg/ha but P and K are applied at the rate of 62 kg/ha for all types of soils. No fertilizer is applied to *G. arboretum.* Three to four hand-weeding passes and three to four inter cultivation passes are practiced during the growing season. Aphids, jassids and whitefly appear at an early stage, but return to former threshold levels after the bollworms, mainly *H. armigera* and *P. gossypiella,* have been controlled. Cotton is sprayed an average of 5-6 times a season, 2 times against early season sucking pests, 1-2 times for mid-

Properties of Three-Layered Particle Boards from Cotton Stalks

Properties	Unit	Flat Pressed Three-layer/Multilayer Particle Board IS 3087-1985		Cotton Stalks Particle Board
		Type I	Type II	
Density	Kg/m^3	500-900	-	750
Average Moisture	%	5-15	-	11
Water absorption	%			
i) 2 hour soaking		10	40	20
ii) 24 hour soaking		20	80	40
Swelling Thickness	%	8	12	9
Swelling due to surface absorption	%	6	9	6
Modulus of rupture	N/mm^2			
i) Up to 20 mm		15	11.0	17.6
ii) Above 20 mm		12.5	11.0	
Internal bond strength	N/mm^2			
i) Up to 20 mm		0.45	0.3	0.51
ii) Above 20 mm		0.40	0.3	
Screw withdrawal strength	N			
Face	1,250	1,250	1,400	
Edge	850	700	860	
Nail withdrawal strength	N	1,250	-	1,300

season pests and 1-2 times for late-season pests (esp. for Red cotton bug control. Insecticides are not used on *G. arboretum.* (Than Than Nu, Myanmar).

Particle Boards from Cotton Stalks

The ICAC implemented a project in India to foster the use cotton stalks for the production of particle boards. The Cotton stalks contain about 69% holocellulose, 27% lignin and 7% ash. In contrast to other agricultural crop residues, cotton stalks are comparable to the most common species of hardwood in respect of fibrous structure and hence it can be used to produce particle boards, pulp and paper, hard boards, corrugated cardboard & boxes, microcrystalline cellulose, cellulose derivatives a substrate for growing edible mushrooms. The data presented in the previous page clearly show that particle boards from cotton stalks possess all the properties needed for internal as well as exterior applications, such as false ceilings, partitioning, paneling, etc.

The paper containing details on the technology and cost analysis of starting up a particle board manufacturing plant using cotton stalks as raw material is available online along with other papers presented at the 5th Meeting of the Asian R&D Network, further detailed information on the project and the final report may be found at <http://www.icac.org/projects/CommonFund/20_ucbvp/english.html>. (A.J. Shaikh, R. M. Gurjar, P. G. Patil, K. M. Paralikar, P. V. Varadarajan & R. H. Balasubramanya, India).

Ginning

Some of the major ongoing concerns afflicting the cotton ginning industry: lowering their per unit power cost, machine operating cost and capital cost of the plant, while improving their machine productivity rates and maximizing their natural fiber parameters (no quality loss in terms of fiber breakage, fiber length, seed breakage, etc.,). A great deal of research and development work has been dedicated to overcoming these concerns and various advances have been achieved in the cotton handling, cotton ginning and cotton processing sectors. The Continental Eagle Corporation, USA developed a 201 saw ginning machine having a throughput capability of up to 25 bales/hour at 280 HP per Machine. This machine compares very favorably with earlier saw gins with 171 saws and producing of 15 bales/hour. The High Speed Rotobar Gins have been modified to increase their capacity from 225 Kg lint/hour to 360 Kg/hour at 25 HP/machine. A number of these machines have been installed in the US and Turkey for trial purposes. They are not available for commercial use yet. The Bajaj Steel Industries Ltd., India has introduced a new double roller gin model named the "Golden Jubilee" model. This new machine can process between 100-130 Kg lint per hour at 5HP/3.7 Kw. and is helping modernize the ginning industry in India and many other countries. The comparative chart shown below for energy/electricity/power cost demonstrates the technology benefits.

Performance of Various Types of New Gins

Type of Ginning Machine	Energy Requirement in HP/machine	Lint Production Capacity Kg/hour	Total No. of Machines Required	Total Electrical Power Required Kwh	Average Production Per Unit of Energy in Kg
201 Saw Continental Gin	280	5,000	1	280	17.9
Balkan HS Rotobar	25	5,000	14	350	14.3
Bajaj Double Roller Gin "Golden Jubilee"	5	5,000	50	250	20.0

Judging from the performance chart above, it is clear that although a double roller gin has a lower throughput and ginners may require a greater number of machines to process the same quantity of lint, double roller ginning is more economical in terms of energy consumed per unit and throughput per unit of energy. (M. K. Sharma and Lav Bajaj, India).

New Chairman

Dr. Noor-ul-Islam of Pakistan was elected Chairman of the Network until the next meeting to be held in three years.

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List of Participants

Countries represnted included Azerbaijan, Bangladesh, Canada, China, India, Iran, Kazakhstan, Kyrgyzstan, Myanmar, Pakistan, Tajikistan, Thailand, Turkey, Turkmenistan, USA and Uzbekistan and CABI and ICAC. Complete list of participants along with contact information is available at http://www.icac.org/tis/regional_networks/asian_network/meeting_5/documents/list_of_participants.pdf.

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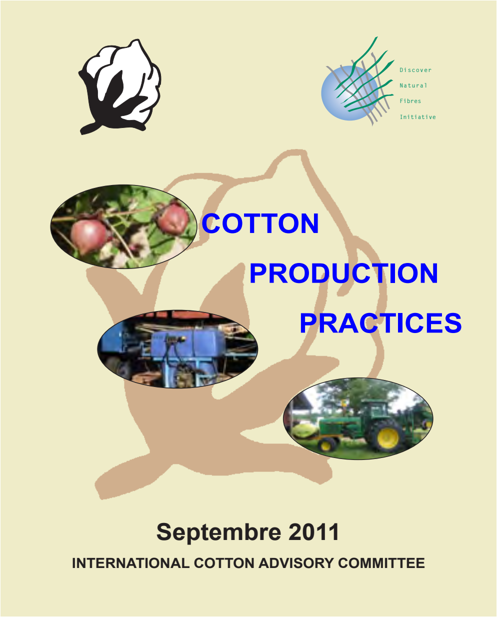

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