International Cotton Genome Initiative
ICGI–2004 Workshop

Book of Abstracts

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International Cotton Genome Initiative

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International Cotton Genome Initiative

ICGI 2004 Workshop

10 – 13 October, Hyderabad, India

Sessions

Structural Genomics

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Commercial Perspectives On Cotton Genomics

Moderators
Dr. David Stelly
Dr. C. D. Mayee

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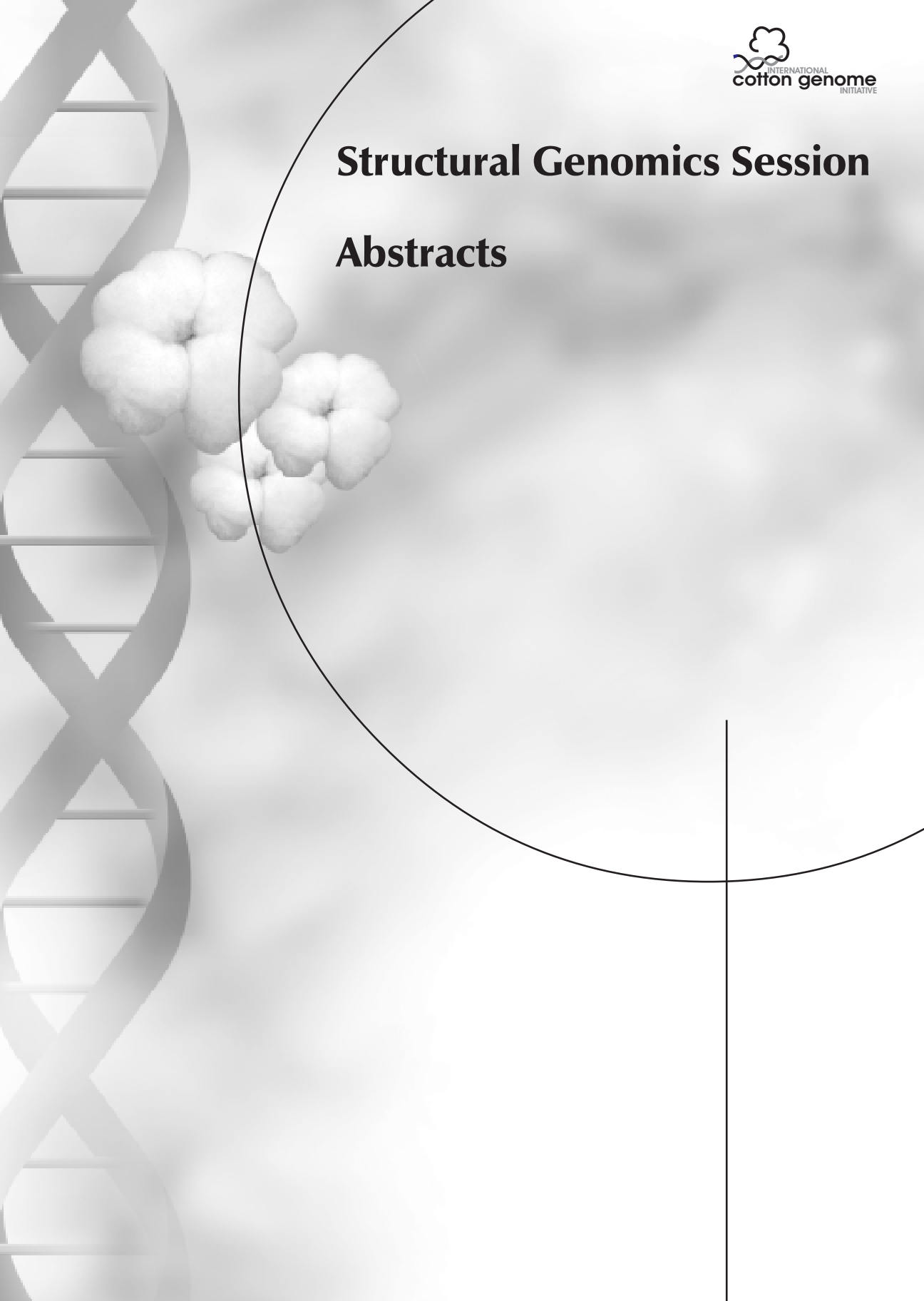

Structural Genomics Session

Abstracts

1. Integrated physical mapping of the cotton genome

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Although cotton (*Gossypium* spp.) has large collections of germplasms, productivity potential of this important crop seems to reach its plateau and continued genetic improvements appear to be difficult. The problem is in part due to the shortage of effective tools to facilitate gene hunting in the cotton germplasm and to transfer useful genes into elite cotton cultivars. Integrated physical maps and other genomic tools will provide new public research resources for continued cotton genetic improvement. We are developing an integrated physical map for the Upland cotton (*G. hirsutum*) by use of its genetic standard (acc. TM-1). The physical map consists of 5,466 contigs assembled from 101,376 TM-1 BAC and BIBAC clones, 675 fiber EST clones anchored to the BAC contigs, and nearly 1,000 SSR markers isolated from the BACs. BAC-derived SSRs will enable integration of cotton physical and genetic maps, and locating mapped genes and QTLs to BACs, BIBACs and/or their contigs for detailed analysis. We are also leveraging cotton genomic resources with the model plant *Arabidopsis* that has the closest relationship among the flowering plants. DNA sequences of BAC ends of TM-1 physical contigs were determined and high levels of synteny between cotton and *Arabidopsis* were observed upon sequence alignment and mapping analyses. Single or low-copy numbers in cotton counterparts indicated potential cross-utilization of genomic information between the two species. An integrated physical map of the cultivated tetraploid cotton and genomic resources derived from other plant species will not only facilitate the continued genetic improvement in cotton, but it will also lay a foundation for basic genomic research on other polyploid crops.

2. Wide-Cross Whole-Genome Radiation Hybrid (WWRH) resources for cotton genomics

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Genome maps are important to genome analysis, genetic manipulation, cloning and genetic engineering. Physical and linkage mapping methods are mutually complementary. Due to significant limitations of linkage maps alone, radiation hybrid (RH) mapping has been used extensively for physical mapping of human and animal genomes. However, RH mapping has been used very little in plants. We have developed a simple alternative approach that uses a wide cross in combination with whole-genome radiation to produce wide-cross whole-genome radiation hybrids (WWRH) that can be used for map construction. We report preliminary test results from cotton, where the genome of one species, *Gossypium barbadense*, was used to rescue radiation-segmented chromosomes of another species, *G. hirsutum*. Mature pollen grains from *G. hirsutum* were gamma-irradiated and used to pollinate emasculated *G. barbadense* flowers to generate nonchimeric WWRHs between those two species. A 5-Krad gamma ray WWRH mapping population was constructed and used to assemble a map. Results were compared to linkage maps, and further tested against cytogenetic stock data. A second generation WWRH panel was constructed and used to create another map, which was tested against the first map. The results indicate that WWRH mapping is robust and complements traditional linkage mapping, and that it will be useful for genomic analysis of cotton and perhaps other plant species. Plans are to provide these materials to the cotton research community to facilitate communication among laboratories and assembly of integrated resources. Toward those goals, collaborators and ideas are solicited from ICGI mapping groups. We gratefully acknowledge support from Texas Agric. Experiment Station, the TAMU Nuclear Science Center, and the Texas Advanced Technology Program.

3. Identification of D genome - specific RAPD fragments in interspecific cross derivatives between *G. arboreum* and *G. hirsutum*

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Gossypium arboreum (2n=2x=26) is still being cultivated in Asia; however, genetic improvement in this species is not in the same magnitude like that of *G. hirsutum*. The present day *G. arboreum* though resistant to sucking pests and abiotic stresses like moisture stress have been replaced by *G. hirsutum* due to their small boll size, short, coarse and weak fibres. *G. hirsutum* genes for fibre, seed, boll and foliage features were transferred to *G. arboreum* through distant hybridization. *G. arboreum* (2n=2x=26 A_2A_2) was colchiploidized to make 2n=4x=52 $A_2A_2A_2A_2$. This genetic stock was hybridized with *G. hirsutum* (2n=4x=52 AADD). The resultant F_1 was backcrossed to 4n *G.arboreum*. In the advanced backcross generation different *G. arboreum* genetic stocks possessing very specific characters but not found in the parent *G. arboreum* were isolated and characterized. Fifteen different genetic stocks with different but specific characters were used for RAPD analysis using decamer primers. Some of the very special characters of genetic stock included 31 mm fibre length, 28 g/tex at 3.2 mm gauge fibre strength (ICC mode), 4.2 fibre fineness, 10.5 g seed index, boll weight of 3.0g and foliage with broad lobes etc. These genetic stocks are unique in nature because the germplasm lines with such values are not present in *G. arboreum* germplasm pool maintained in India. These introgressed derivatives were phenotyped along with *G. arboreum* and *G. hirsutum* parents, G. *raimondii*, five races of *G. arboreum*, *G. herbaceum* and four races of *G. hirsutum* and genotyping was done using RAPD analysis. All the tested decamer primers in RAPD analysis produced polymorphic amplicon products, however, the extent of polymorphism varied with each primer. Few primers, namely OPZ 14, OPP 01 and OPY 02 produced genome specific amplifications, wherein amplicon products ranging between 1000 and 1500 bp were present in 'D' genome (*G. raimondii*), AD (*G. hirsutum*) and in some introgressed *G. arboreum* derivatives but absent in 'A' genome. Presence of such amplified fragments has been considered as resultant of introgression of AD genome into *G. arboreum*. The study hinted at the greater involvement of 'D' genome in traits like long and fine fibers

and bolder seed size than 'A' genome. These genotypes are unique cotton with very exceptional phenotypic values. These valuable genetic stocks can be used for molecular studies both at structural and functional levels especially for seed, boll and fibre specific genes. Further, these genetic stocks being resistant to sucking pests like Jassids are preferred candidates for Bt gene transfer for making cotton cultivation more remunerative as the genotypes emanated from transformation will be resistant to both sucking pests and bollworms.

4. On the hunt for new molecular markers

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Molecular based markers have been developed for a number of plant species. These markers have subsequently been used for a variety of purposes including diagnostic markers for resistance genes, creating fingerprints for identification, and assembling genomic maps. Most of the major crop species have well established maps that combine a number of molecular and physical markers that are freely available for use by the research community. Initially cotton lagged behind in developing an easily accessible map, however, recent coordinated efforts by cotton researchers have accelerated the process. Cotton scientists can now take advantage of the work already done in other crops to increase the speed and efficiency of these efforts. To date, Restriction Fragment Length Polymorphism (RFLP), Random Amplified Polymorphic DNA (RAPD) Amplified Fragment Length Polymorphism (AFLP) and Simple Sequence Repeat (SSR) markers have been used successfully on Cotton. SSR markers can be derived from several sources including random genomic fragments and Expressed Sequence Tags (ESTs). Useful markers can be found both within and outside the genus Gossypium. Preliminary results indicate that soybean (*Glycine max*) and *Arabidopsis thaliana* SSRs can be informative markers for cotton.

5. BAC end sequence – derived SSRs and the CMD

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6. An intraspecific AFLP map of *G. hirsutum*

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A genetic linkage map of Upland cotton (*Gossypium hirsutum* L.) was constructed with Amplified Fragment Length Polymorphism (AFLP) markers using 138 $F_{2:3}$ lines developed from the intraspecific cross of Paymaster 54 and Pee Dee 2165. A new DNA isolation protocol was established, which allows high quality DNA to construct a genetic map with AFLP markers. A total of 32 EcoRI/MseI primer combinations were screened for parental polymorphism. Twenty primer combinations were selected to assay the mapping population and to provide 200 polymorphic loci. Linkage analysis was performed at a LOD score of 4.0 and a maximum recombination fraction of 0.34. The genetic linkage map comprised 143 markers assembled into 25 linkage groups and covered 1773.2 cM, 38% of the estimated genome size, with a mean interlocus spacing of 12.4 cM and no dense clustering of loci. The 143 linked markers were assigned to 13 major and 15 minor linkage groups. The 13 major groups ranged from 50.3 to 205.1 cM in length and each group carried 3 to 19 markers. The 15 minor groups ranged from 7.5 to 49.3 cM in length and each group carried 2 to 6 markers. Significant deviations from the expected Mendelian segregation ratio were observed for 67 loci. This map provides a useful tool for of QTL identification for agronomic and fiber quality traits in Upland cotton which will be the primary target.

7. Construction of molecular linkage map in cultivated tetraploid cotton using SSR

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8. Cotton genomic studies at NIBGE

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Genetic improvement of cotton plant has been a major activity to its continued productivity. Globally, exploration of cotton genome lagged behind due to its complex genome. Our group exploited DNA fingerprinting approaches for estimation of genetic diversity among different cotton cultivars and species, development of preliminary genome maps, identification of DNA markers linked with different traits and launching of marker-assisted selection (MAS). We have a funded project for molecular characterization of *Gossypium* and wheat germplasm with novel DNA markers approaches, which represents another area of our genome projects. Recommendation to further utilization of *Gossypium* and wheat germplasm resources will be made according to the level of uniqueness of the DNA marker profiles; which will also be helpful in understanding complex genetic mechanism against drought. EMS treated knock-out population of *G. arboreum* was screened for fibre quality genes in collaboration with Cotton Functional Genomics Lab, UC Davis, USA, and isolation of novel genes using differential display technology is the dawn of functional genomics studies in Pakistan. Further, molecular approaches to elucidate genetic mechanism against Burewala virus disease (an emerging stress to cotton crop) using NIBGE-115, NIBGE-114, NIBGE-160 and NIBGE-253 cotton stains as a resistant source is being employed. The genomic tools and resources (GMOs detection and DNA fingerprinting services) developed by our group are made available through PIBs facility to public and private sector community.

9. Molecular mapping of QTLs for fiber qualities in upland cotton using SSR markers

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The improvement of cotton fiber quality is extremely important because of changes in spinning technology. The identification of the stable QTLs affecting fiber traits across different generations will be greatly helpful to be used effectively in molecular marker-assisted selection to improve fiber quality of cotton cultivars in the future. Using three elite fiber lines such as 7235, PD 6992 and HS 427-10 in Upland cotton (*Gossypium hirsutum* L.) as parents, three linkage maps were constructed to tag QTLs for fiber qualities using SSR markers. There were 38 QTLs detected by composite interval mapping for fiber traits, in which 11 QTLs were for fiber length, 10 for fiber strength, 9 for Micronaire and 8 for fiber elongation. Most of QTLs detected in (7235 x TM-1) F_2 and $F_{2:3}$ are confirmed in its RIL lines. Among them, 15 stable QTLs (39.47%) could be found in both F_2 and $F_{2:3}$ segregating populations. At least 3 identical QTLs could be identified in two populations. These identical QTLs detected in different populations suggested that there existed elite fiber genes, possibly of the same origin. In addition, we found three pairs of putative homoeologous QTLs, qFL-7-1c and qFL-16-1c, qFS-D03-1a, qFS-A02-1b and qFS-A02-1c, qFECD03-1a and qFE-A02-1c. Our results provided a better understanding of the genetic factors of fiber traits in AD tetraploid cottons. Both stable and identical QTLs have a great potential for molecular-assisted selection in improving fiber quality.

10. Improvement of cotton fiber quality through Marker-Assisted Breeding: limits and prospects of a QTL approach

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The marker-assisted introgression of quantitative traits in crop plants is the topic of an ever-increasing number of reports (recent interrogation of bibliographic database using marker-assisted breeding or QTL as key words produced nearly 500 and 2500 hits respectively). Different papers based both on theoretical and experimental evidence, have also emphasized the limits of QTL-based breeding strategies when applied to quantitative traits. Among these limitations the molecular breeder may face are the precision, power and confidence associated to QTL detection, the important number of progenies to be manipulated when the number of QTL increases. A combination of marker-based and classical phenotype-based selections is often recommended. Our program of interspecific *G. hirsutum* / *G. barbadense* marker-assisted backcross selection, MABS, aims at transferring cotton fibre quality QTLs of *G. barbadense* into *G. hirsutum*. In the preceding seasons, the BC1, BC2 and BC2S1 served for 3 separate QTL analyses (Lacape *et al*., Crop Sc, in press). The pooled QTL data from the 3 populations revealed a total of 50 QTLs meeting permutation-based LOD thresholds (between LOD 3.2 and LOD 4.0 on a per-trait basis) for 11 fibre quality-related traits. When reducing the detection threshold to LOD 2.5, this total reached 80 QTLs. Individual phenotypic effects were essentially in the range of 5-15%, and rarely exceeded 20%. The direction of the effect of 70% of the QTLs was as expected from the parental value (better fibre length, strength and fineness conferred by the *G. barbadense* parent, and better maturity and colour conferred by the *G. hirsutum* parent alleles). A majority of the QTLs were co-localized within QTL-rich chromosome regions: when considering only the donor segments, for which positive contributions to fibre quality derived from the presence of the *G. barbadense* alleles, 19 regions on 15 different chromosomes were defined. Interestingly, 1/3rd (26 out of 80) of the QTLs (LOD 2.5) detected confirmed QTLs reported in other interspecific populations. Using these 15 regions as target candidate regions for introgression, we undertook marker-based selection within 411 and 450 plants in each BC_3 and BC_4 generations respectively: ca 10% of individuals were kept at seedling stage based on the presence of *G. barbadense* alleles at chosen SSR and AFLP loci. The allelic constitution of 37 selected BC_3 plants showed that 5 to 16% (8% on average) of donor *G. barbadense* alleles were still present genome-wide, corresponding only to 3 to 6 of the 15 initial QTL-rich target regions. The marker-trait associations in this BC_4 generation only partly confirmed the associations observed in the previous generations (around cases). Some of the BC_4 plants having

introgressed these particular regions however proved highly interesting as regards to their fibre quality. The field evaluation of these BC_4 as F_2 progenies value using agronomical as well as fibre quality measurements was underway during summer 2004.

11. Isolation and characterization of plant defense genes in the cotton genome

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Proteinase inhibitors (PIs) are antimetabolic proteins that interfere with digestive process of insects. It is one of the defense strategies existing in plants against insect and pathogens. PIs are primary gene products and they are excellent candidates for engineering pest resistant into plants. Inhibitor genes of plant origin are particularly promising to use directly without any modification coding sequence but designing with constitutive promoter. In the present report, proteinase inhibitor genes were isolated by polymerase chain reaction (PCR) from cotton genomic DNA. PCR amplification of the target genes was carried out using forward and reverse primers designed on the basis of published sequences. The amplified product was resolved on 1.5 % agarose gel indicated that the expected fragment of the same size as full-length genes of 650 bp for Kti3 and 250 bp for C-II and PI - IV primers. The fragments were cloned into pDrive vectors (Quiagen) and transformed into host bacteria. The transformed bacterial plasmid was isolated and reamplified the cloned PI genes and the results showed the identical fragment length.

12. Comprehensive gene and gene associated marker discovery in cotton by methylation filtering

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The analysis of methylation filtered (GeneThresher) sequence sets from over a dozen plant species, including both monocots and dicots, demonstrates substantial genome reduction and gene enrichment. This strategy exploits the genome architecture of plants where the vast majority of genes lie within the genome in small hypomethylated islands separated by large oceans of hypermethylated repeats. Methylation filtered libraries are constructed through the selective cloning of hypomethylated gene rich DNA fragments which are subsequently sequenced. This not only results in the enrichment of genes, but also of polymorphic gene-associated genetic markers (SSRs), gene-associated SNPs, promoters, microRNAs and active transposable elements. Unlike EST approaches, low coverage methylation filtered sequences contain near complete and unbiased gene tagging and an even representation of all gene features, including the promoters, UTRs, and introns. Additionally, methylation filtered subclones yield the high fidelity sequence and reproducible genome reduction required for efficient SNP discovery. Methylation filtration can also be used to efficiently finish a genome to the level where all genes are fully sequenced, mapped to the genetic and physical maps, and ordered relative to each other. This is accomplished by the integration of low level sequence coverage from an anchored, whole genome BAC tile, as was done to finish the rat genome (Gibbs *et al*, 2004). In this way, a comprehensive sequence of the cotton genome can be ascertained by leveraging DNA methylation, resulting in rapid completion for as little as one third the cost of whole genome sequencing strategies.

13. Cotton genetic improvement in China

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7878 accessions of cotton germplasm including *Gossypium hirsutum* 6522, *Gossypium barbadense* 565, *Gossypium hirsutum* race 350, *Gossypium arboreum* 378, *Gossypium* species 46 etc., were collected and kept in China. We evaluated more than 6372 cotton germplasm for 66 morphological and chemical traits including 41 morphological and agronomic characters, 13 fiber quality, 4 seed quality, 8 traits with disease – insect - adversity resistance, and also classification of these germplasm. Some of them have been utilized. We found some fiber mutants carry potential high fiber yield and quality genes. When the germplasm was used properly, they can break the negative relationship between fiber yield and quality. In the case of cotton, genetic diversity at the molecular level in *G. hirsutum* is relatively low, therefore, it is very important to develop new germplasm and varieties. Main techniques for germplasm enhancement were multiple crosses, Inter-specific cross, Gene/DNA transformation such as *Agrobacterium* mediated, particle gun bombardment, pollen tube pathway, physical irradiation inducement etc. In China, 26 wild species were used for transformation of the elite characters, and 130 varieties (lines) with high fiber strength, drought resistance, *Verticilium* wilt resistance etc., were gained. 300 new cotton varieties and lines have been bred by using 390 accessions of germplasms in 1984-2003 in China. Moreover, a lot of transgene cotton varieties were bred in recent years. CRI 29 shows fast stand with strong seedlings, growth early with strong growth force, growth stability at square formation period and a high potential productivity. CRI 38 was an insect-resistant cross bred by using double recessive male sterile lines, examined and approved nationally in May 1999. It possesses a strong growth force, high potential yield and better comprehensive characters. The genetic diversity among different cotton varieties and mutants were detected. Genetic analysis on naturally colored lint, fuzz mutants, fuzzless and lintless mutants were carried out and genetic interactions between lint and fuzz traits were discovered. Molecular cluster analysis was carried out on different fiber mutant such as GZnn, GZNn, H-154, Xin and XZ142w, and the Dendrogram of these 6 lines was also built. The SSR fingerprint atlas of 18 colored cotton lines had been obtained based on SSR technology. The genetic analysis of short season cotton earliness and its relative traits by mixed major gene and poly gene model showed that earliness and its relative traits were not only controlled by major gene generally, but also modified by minor effect polygene. There was one major gene at least

expressing at different growth and development stages. The result of the genetic diversity analysis based DNA molecular markers technologies (RAPDs and SSRs) indicated that polymorphism among the short-season upland cotton cultivars was very poor. Fluorescent *in situ* hybridization (FISH) is used to explore the origin and evolution of tetraploid cotton in this study. The contents include the diploid ancestors of tetraploid cotton, polyphyletic or monophyletic origin of tetraploid cotton, and rDNA concerted evolution. Molecular linkage maps based upon DNA markers are widely recognized as essential tools for crop molecular genetics and breeding research in many species. Several ongoing efforts in China are devoted to development molecular breeding and promoting molecular tools and genetic engineering strategies in cotton. The maps derive from either intraspecific *G. hirsutum* (Zuo *et al*. 2000; Zhang *et al*. 2003), or interspecific *G. hirsutum* / *G. barbadense* (Zhang Jun *et al*. 2001; Gao Yu-qian *et al*. 2002; Wu Mao-qin *et al*. 2002; Ling Zhong-xiu *et al*. 2003; and Lu Ying-zhi 2004) populations were constructed and published. Molecular marker analyses have been performed in parallel using a unique collection of fiber developmental mutants, near-isogenic lines, BSA populations for molecular loci of morphological, agronomic characters, fiber quality, disease, insect, adverse resistance trait. The new fiber mutant GZnn was analyzed using traditional genetic method and it was sure that GZnn was qualitative trait controlled by one recessive gene, we named it n4. Using SSR molecular marker technology, we located this gene on chromosome 10, and was closely linked with marker sloc1 and their distance was 10.8 cM.175 F_2 individuals developed by a cross of *G. barbadense* 15-3493 *G. hirsutum* Shi Hezi 875 were employed for a molecular marker population of *Verticillium* wilt resistance. Some SSR loci related to *Verticillium* wilt resistance were found. The 207 F_2 population of CCRI 36, a short season cotton variety, and TM-1 were tagged and mapped with 73 polymorphism markers, including 25 SSR, 35 RAPD and 13 SRAP markers. 43 of the informative loci were used in linkage map construction and were assigned into 5 linkage groups. The linkage map construction for study on short season cotton, QTLs of earliness mapping and clustering have not been reported before. The results are valuable to further research for earliness MAS of short season cotton. Short season upland cotton plays an important role in the cultivation reform in China; there are three ecotypes in cotton growing region. The biochemical genetic mechanism in the earliness-but-not-decrepitude varieties was illustrated. Antioxidant enzymes (such as CAT, POD, and SOD) activity, MDA, IAA and ABA contents etc. biochemical traits had an authoritative normal distribution, so they were typically quantitative character. Endogenous hormone and free radical scavengers, such as GSH and SOD enzyme, might be involved in regulating dark-induced leaf senescence. Evidence was presented to show that the dark-induced leaf senescence of derooted cotton seeding may be attributed to

decreasing cytokinin contents, blocking the entrance of calcium ions into the cytosol, and subsequently reducing SOD activity and increasing the level of lipid peroxidation, consequently causing protein loss and chlorophyll degradation. Gene identification and clone related to fiber development have made some progress. A series of genes and cDNA

for fiber initiation and elongation such as GhPFN1, GhRLK1, GhTub1, GhSH2, GhPRPR40, GhSLSP, GbKTN1, Ghkorriga, GhLIM1, GhCaM1, GhGA20-1, GhGA20-2, GhDET2, GhDWARF1, GhGAI, GhBRI, GhGA20-1, GhDET2, GhDWARF1 etc., were analyzed. A cysteine proteinase gene from cotton (*Gossypium hirsutum*), which designated Ghcysp gene, was isolated by rapid amplification of cDNA ends using polymerase chain reaction (RACE-PCR).

14. Molecular mapping and characterization of EST-SSR markers contributing to fiber initiation and development

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Tagging of the fiber quality loci is one of the main targets of cotton genome research programs worldwide since the outcome of mapping of fiber-associated QTLs with portable flanking DNA-markers promise to be effective in molecular cotton breeding. Collaborative research to map fiber specific EST-SSR markers in recombinant-inbred lines (RILs) of *G. hirsutum*, segregating for lint yield and fiber quality has been carried out. This should tag genes that contributing to fiber initiation and development in cotton. Fourteen of 85 fiber specific EST-SSR markers showed polymorphic PCR-products between parental lines. Three statistically significant QTL regions coincided with the three EST-SSR markers EST- 47 (LOD of 3.30), EST-32 (LOD of 4.50) and EST-56 (LOD of 9.97) in interval mapping of 78 RI individuals. Detailed sequencing analysis of two highly linked EST-SSR markers from RILs revealed that EST-32 marker presents several allelic variations, having an expansion in microsatellite loci (7,9, 10, 11 (CAT)n repeats) and several SNP mu.

15. Molecular markers linked to grey mildew resistance gene locus in cotton genome

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The grey mildew disease commonly known as Dahiya disease of cotton is caused by *Ramularia areola* Atk (*Ramularia gossypii* (Speg) [Ciferri]. The disease was first reported on upland cotton (*Gossypium hirsutum*) in Auburn, Alabama (USA) in 1890 as areolate mildew of cotton. The disease occurrence is known to be restricted only to the Genus *Gossypium*. In India there were several outbreaks of the disease during the crop seasons in 1988-89 and 1993-1994 has resulted in heavy yield losses. The disease causes substantial loss on an average of 10 to 12 %. Seven germplasm lines belonging to *Gossypium arboreum* (desi cotton) namely, G 135-49, 30805, 30814, 30826, 30838, 30856, and EC 174092 were identified as source of resistance and were found immune (no disease) to grey mildew. They were subjected to RAPD and SSR analysis. Polymorphism among the immune lines and susceptible genotype AKA 8401 were identified using 40 decamer primers and 15 SSR primers. The template DNA were amplified in a PCR thermal cycler and resolved on agarose gel electrophoresis. RAPD marker named as OPC 02 (1700 bp) was found to be unique in all the immune lines where as it was absent in the susceptible line which can be used for screening germplasm line by marker assisted selection. For tagging the resistant gene, a mapping population was developed by crossing the immune line G 135-49 and susceptible line AKA 8401. The F_2 mapping population was screened for the disease by spraying artificially with the inoculums and the resistant plants and susceptible plants were scored by grading for the disease. Bulk segregant analysis was carried with ten resistant DNA pool and ten susceptible DNA pool. Three RAPD markers and two SSR markers were identified to be linked with the grey mildew resistant gene.

16. Use of mapped SSR markers to assist the selection of low-gossypol seeds and high-gossypol plant cultivars in upland cotton

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Mapped SSR markers were used to assist the phenotypic selection of genotypes expressing the low-gossypol seed and high-gossypol plant trait in BC1, BC2, BC2S1, BC2S2, BC2S3, BC2S4, BC2S5, BC2S2, BC1-S1, BC3, BC3S1, BC3S2, BC3S3, BC2S2BC1 and BC2S2BC1S1 genotypes obtained from the G. hirsutum L. x G. raimondii Ulb. x G. sturtianum (HRS) trispecific hybrid. Two hundred and six mapped microsatellite markers uniformly distributed on the 26 linkage groups of the *G. hirsutum* genetic map were used to monitor the introgression of *G. sturtianum* Willis and *G. raimondii* Ulb. chromosomal segments in the progenies of the HRS hybrid. Out of 146 polymorphic SSRs amplified on the analysed materials, 188 alleles were introgressed from the wild donor species *G. sturtianum* into the HRS hybrid. A total of 14 *G. sturtianum* alleles mapped on c2-c14, c3-c17, c6-c25, c12-c26 and A03-D02 homeologous chromosome pairs were conserved on the selected BC2S4 and BC2S5 genotypes while the *G. sturtianum* or *G. raimondii* origin of a locus on c12 could not be determined. For the selected BC3S3 materials, three alleles were conserved on c6-c25 chromosomes. The two selected BC2S2, BC1S1 genotypes conserved respectively 13 and 11 alleles of *G. sturtianum* on c2-c14, c3-c17 and c6-c25 homeologous chromosomes pairs. All selected plants in this work presented a normal density of gossypol glands on their aerial parts and produced regularly an important proportion of almost totally glandless seeds. These plants constitute valuable genetic stocks for the introgression of interesting agronomic traits from the wild parental species of HRS into *G. hirsutum*.

17. Permanent recombinant inbred mapping population for cotton genome research

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The lack of a permanent mapping population in cotton (*Gossypium* spp.) hinders progress of many independent genetic studies that otherwise could be coordinated more effectively. We report on the characterization of 191 cotton recombinant inbred lines (RILs) that were derived by single seed decent from an interspecific F_2 hybrid between TM-1 and 3-79, two genetic standards of *G. hirsutum* and *G. barbadense* respectively. At F_7 generation on average, this mapping population retains the phenotypic variability of the original F_2, including fiber properties and other morphological traits. Approximately 200 cotton microsatellite or Simple Sequence Repeat (SSR) markers were used to genotype the 191 RILs. Genetic segregation and allele distribution were assessed to provide a genomic evaluation for expanded uses of this permanent mapping population that is distributed (in DNA stocks) as a reference population to the international cotton research community. Linkage relations of the SSRs and graphical genotypes of the RILs facilitate further genomic characterization of this population that is at the foundation of internationally collaborated efforts to develop a high-density integrated map of the cotton genome and a basis for germplasm characterization.

18. Molecular markers run on a test panel: a data source for the cotton community

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Unlike other major crops such as soybean and corn, cotton has no coordinated public marker database. Cotton Incorporated (CI) has identified the need for more markers and a database as critical action points. The Mid South Area Genomics Laboratory in cooperation with scientists from the Crop Genetics & Production Research Unit at Stoneville, Mississippi responded and initiated a project to screen and evaluate molecular markers. To facilitate comparison of marker data among different groups, a standardized set of DNA from a range of cotton varieties and wild species was selected to use for the screening. The DNA set is being maintained centrally by the ARS Unit at College Station, Texas, so that future screening projects by other groups can all use the same testing set. Simple Sequence Repeat (SSR) markers, from a variety sources, were screened on the standardized CI DNA panel. Protocols were developed to automate the procedures and minimize the amount of primer, DNA and PCR reaction components required. The Mid South Area Genomics Laboratory is currently working with Clemson University Genomics Institute (CUGI) developing a database to make the markers and evaluation data publicly available. The database would allow scientists to search for markers that may be useful for their individual experiments.

19. Approaches for rapid development of SSRs and SSR - based SNP markers in cotton

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Utilization of multiple marker development approaches is crucial for the rapid development of a dense genetic linkage map in cotton. Simple Sequence Repeat (SSR) markers have proven to be extremely useful in many plant systems considering their abundant distribution and high degree of polymorphism. However, the intra-specific polymorphism with respect to SSRs in cotton seems to be 10-15%. Thus, in order to utilize the untapped variation in cotton genome, efforts need to be invested not only in developing large numbers of SSRs but also in exploring and developing potential marker systems such as Single Nucleotide Polymorphisms (SNPs). SNPs are attractive since they are known to be the most abundant forms of variation in genomes studied so far. By developing and employing an improved protocol we have rapidly developed a large number of SSR markers belonging to different repeat classes in a short span of time. In addition, we have also explored the presence of SNPs in SSR flanking regions and observed that many of them contain SNPs/InDels type of variations. Since the PCR primers of the existing SSRs are utilized for this approach, it not only saves resources but also provides a faster way of discovering SNPs. Results obtained from the SSR development and SSR-based SNP discovery projects will be presented.

20. Construction of BAC library toward map-based cloning of Rf1 gene in upland cotton

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Cotton is a very important economic crop, but its genomics research lags behind that of maize, soybean and wheat. Bacterial Artificial Chromosome (BAC) libraries containing large genomic DNA insert are widely adaptable and publicly available for genomics research and important tools for genome physical mapping, map-based cloning and genome sequencing. To positionally isolate genes in cotton and to physically map the cotton genome, high - quality libraries are needed that comprise BAC clones containing large cotton DNA inserts. We have developed a BAC library from 0-613-2R, the restoring line for isolating cotton Rf1 gene and genomic research of cotton. The BAC library contains 97,825 clones stored in 255 384-well microtiter plates. Random samples of BACs digested with Not enzyme indicated an average insert size of 130 Kb with a range of 80 to 275 kb. According to the insert size distribution of BAC clones, 95.7% of BAC clones in the library have an average insert size greater than 100 kb. Based on a cotton genome size of 2250 Mb, library coverage is 5.7x haploid genome equivalents. Recently more and more scientists are focused on the isolation and cloning of Rf1 gene. DNA markers flanking Rf1, the restoring cotton gene, have been identified via linkage analysis of three BC_1 populations derived from 0-613-2R, a common parent. They can be used to screen the BAC library for the isolation of the Rf1 gene.
Key words: BAC library, map-based cloning, Rf1 gene, cotton.

21. Preliminary study of cotton pyramiding breeding by molecular marker assisted selection of fiber strength

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In order to explicit the effect of molecular marker assisted selection to cotton fiber strength and pyramid the high strength and the resistance to budworm into the commercial variety, High strength line 7235, high yield cultivar Tai 121, transgenic bollworm-resistant cotton cultivars SGK321 and SGK9708 were crossed, backcrossed, and self-crossed into two populations: (Tai121 x 7235) x SGK321 BC_1F_2; (Tai121 x 7235) x SGK9708 BC_1F_2, which were planted in Anyang experiment field in 2003. One leaf of plant was rubbed in 0.3% Kanamycin in seedling stage. After 5 days, the plant whose leaf color was changed into yellow was uprooted (no transgenic bollworm-resistant cotton), and the plant whose leaf color was not changed was retained. Each individual's DNA was extracted in seedling stage by CTAB micro-extracting method and agronomic characters of each individual were investigated. 307 plants were harvested. Pesticides were not sprayed ithroughout the growth period. Fiber samples from each plant were tested in the Supervision, Inspection and Test Center of Cotton Quality, Ministry of Agriculture, China. 2 SSR markers (S1521, S2961) tightly linked to high strength fiber QTL were used to screen the 307 plants from the two crosses BC_1F_2 generations. The results showed the normal distribution for fiber strength. The fiber strength mean of plants with and without the S1521 marker were 29.74 cN/tex and 28.03 cN/tex. The difference was significant and prominent. The results of S2961 marker were the same as S1521. The results revealed that the major QTL of fiber strength associated with the 2 markers was inherited steadily in different genetic backgrounds and segregating generations. It was concluded that increasing fiber strength is possible efficiently through the MAS in seedling stage. We had obtained 5 plants with resistance to budworm, high yield and super fiber: 6271-13, 6272-10, 6276-01, 6277-27, 6278-21. In conclusion, pyramiding breeding by MAS and other technologies was a new breeding method, which helped achieving a cotton variety with high yield, super fiber quality

and resistance rapidly, prominently and efficiently. Though there were not many successful examples of cotton breeding by MAS, the primary success exploited broad prospect for MAS in cotton breeding. With the improvement of biotechnology and cotton molecular map, the role of MAS in cotton breeding will become more and more important.

22. Assessment of genetic purity, identified in F_1 hybrids, their parents and F_2 population of cotton

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The maintenance of genetic purity of parents and hybrids of cotton is one of the most important criteria in production of good quality seeds. The genetic identify and purity assessment is a major component in seed production and certification. The present investigation was taken up in order to find out specific morphological
characters (DUS) besides other diagnostic characters so as to identify five cotton hybrids (NHH-44, Savitha, PKVHy-2, H-8 and DCH-32), their parents and also to detect F_2 seed lots by field (GOT) and laboratory methods. Key morphological characters were identified for five cotton hybrids and their parents. These characters were found distinct, uniform and stable, which were least influenced by the environmental situations where it is grown. The electrophoretic banding pattern of each genotype was unique and distinct between hybrids, their parents and F_2 population for total soluble seed proteins and useful for identification. F_2 population of different cotton hybrids segregated into plant types closer to morphological characters of hybrid, female parent, male parent and recombinant plants which were considered as true type, selfed plants and off types respectively for computing genetic purity. Varietal identification and genetic purity assessment could be possible based on the specific morphological characters. Flow chart was developed with key characters useful for field functionaries involved in seed production/certification. All cotton hybrids recorded genetic purity above the minimum seed certification standard (90%) while F_2 population recorded genetic purity ranging from 31.5 to 43.6%.

23. Molecular marking of useful wild genes in interspecific derivatives of haploids x wild *Gossypium*.L hybrids

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Cotton is susceptible to biotic and abiotic stresses and genetic variability in the cultivated species for these characters is limited. Wild *Gossypium species* are a rich source of resistance genes; therefore interspecific hybridization of cultivated *Gossypium species* followed by breeding may result in genotypes with improved characters. The success of a cross i.e., identification of a true hybrid can be established using morphological, cytological, isozyme and molecular markers. Morphological and cytological characters have been used for the identification of cotton hybrids. However, these characters may not be significantly distinct and such assessments require laborious experiments. Although isozyme markers are used to identify the hybrids of cultivars the paucity of isozyme loci restricts their usefulness in breeding. Molecular marker analysis offers an efficient alternative to this approach as genetic relationships are estimated on the basis of genotype not phenotype. Among the variety of molecular marker techniques available Random Amplified Polymorphic DNA (RAPD) can be used for DNA fingerprinting of genotypes because of its simplicity, requirement of a small quantity of DNA and the ability to generate polymorphisms. Interspecific hybridization was carried out between *G arboreum* x *G. thurberi* with view to introgress the genes responsible for pink boll worm tolerance and high fibre strength from wild *G. thurberi* to cultivated *G. arboreum* cotton. The molecular analysis of the interspecific derivatives is done by using RAPD markers. The progenies were confirmed for introgressed characters at molecular level. The detailed gene introgression study and marker identification of importance traits in wild genomes is in progress at Biotechnology Centre and Cotton Improvement Project, MPKV, Rahuri.

24. Initiatives in cloning bacterial blight resistance genes from cotton

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Identification of molecular markers associated with *R* genes can greatly facilitate their cloning. With the broad objective of development of molecular markers for cloning and pyramiding bacterial blight resistance (*BBR*) genes, seven near isogenic lines of Acala 44 cotton having individual *BBR* genes, *B1, B2, B4, B5, B6, b7, Bin* were subjected to RFLP analyses. Four sets of primers were designed based on conserved sequences of plant *R* genes cloned from wide sources, including those from Fusarium resistant cotton lines. The primers were used in PCR to amplify resistance genes analogues (RGAs) from *Gossypium hirsutum IM216* (source of all *BBR* genes). PCR products of 215 to 240 bp were amplified with each of the four sets of primers. High quality genomic DNA was extracted from leaves of seven BBR iso-lines and IM216, by a modified protocol and transferred on Nylon membranes. Southern hybridisation was done with RGAs as probes, following PCR radiolabelling with 32P. RFLP analysis using four RGA probes detected polymorphism in BIn3 gene with one of the four probes used. We are presently designing new sets of primers that could serve as markers for other *BBR* genes. These RGAs can also serve as potential probes for identification and cloning of *R* genes from the library of cotton genome.

25. Significance of grow - out test in assessing genetic purity of cotton hybrids and their parental lines

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The genetic purity of cotton hybrids and their parental seed lots is determined in grow-out-test (GOT) on the basis of distinct, uniform and stable morphological characters expressed during different stages of crop growth by comparing with authentic samples under identical environmental conditions. Fifty eight thousand eight hundred eighty one hybrid cotton seed lots of sixteen hybrids (NHH-44, NHH-390, NHH-302, Savitha, PKVHy-2, DCH-32, JKHy-1, CAH-468, HHB-224, TCHB-213, H-4, H-6, H-8, H-10, Varalakshmi and Surya) and their parental lines (1986 seed lots) were subjected to GOT and evaluated for genetic purity assessment based on specific morphological characters from 1994 to 2000 at Amaravathi GOT Farm, Guntur district, Andhra Pradesh State Seed Certification Agency, AP, India. Among the hybrid seed lots, NHH-44 comprised 73.1% followed by Savitha (11.7%), H-8 (7.1%), DCH-32 (2.4%), PKVHY-2 (2.3%) and JKHY-1 (2.1%). Of the 1986 seed lots of parental lines and varieties, BN-1 (female parent of NHH-44) constituted 19.4% followed by AC-738 (male parent of NHH-44) (15.9%), T-7 (9.5%), M-12 (7.9%) and LRA-5166 (6.4%). Distinguishable key morphological characters of all the hybrids, parents and varieties mentioned above were identified and procedures for conduct of GOT were standardized. Floral trails (petal colour, pollen colour and spots at the base of petal), hairiness on leaf and stem and growth habit were important key characters to establish genetic purity status of the seed lots. Out of 58,881 hybrid seed lots evaluated, 9548 seed lots were found substandard for genetic purity (below 90%). Similarly, of the 1986 seed lots of parental lines, 325 lots were recorded below prescribed standard of genetic purity (99%). The failure seed lots were high in Savitha (31.4%), DCH-32 (24.7%), PKVHy-2 (22.3%) and NHH-44 (13.4%). The failures on account of genetic purity might be due to lack of purity in parental lines, usage of their own source parental line seeds for seed production, inadequate

availability of genetically pure seed, physical admixtures at the time of processing and malpractices of producers/seed growers in selling the F_2 generation seed as F_1 seed.

26. Evaluation of laboratory and field plot techniques (GOT) in identification and genetic purity assessment of cotton genotypes

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In cotton several new varieties / hybrids are being released by the public and private sector in the market in quick succession and it is very difficult to distinguish each and every variety in the absence of specific morphological characters. Most of the hybrids developed were with the narrow genetic base; quite often most of the traits are similar in the hybrids. There is a need to search a technique to distinguish the marked characteristic feature under PVP regime. It is a challenging task to seed technologists, seed certification officials and seed producers to distinguish as well as to assess the genetic purity. Since varietal description / morphological characters gives by the breeder are some times in adequate. There is a need to develop key characters for identification of genotypes. The present investigation was taken up with four hybrids (NHH-44, Savitha, PKVHy-2 and H-8) and their parents along with F_2 generation lots to find out a suitable method for obtaining accurate and reproducible results. The morphological characters in the present study were growth habit, pigmentation, leaf shape, leaf colour, hairiness on the leaf, stem colour, depth of sinus in leaf lobes, leaf texture, inter-nodal distance, flower colour (petal), anther and pollen colour, flower opening, petal spot, bract shape, boll shape, boll texture and gossypol glands. Based on the variation of these morphological characteristics it was possible to group the genotypes through flow chart for identification purpose. F_2 seed lots could be identified based on segregation in morphological characters which are nearer to plant types of female as selfed plants, male as offtype, recombinant type as offtype and hybrid as true type and computed genetic purity assessment, when two fixed characters are taken into consideration besides other diagnostic characters. Grouping of cotton genotypes could be possible based on differential growth response of seedlings to added chemicals, viz., Dithane M-45, Thiram, BHC, Kobiol, GA_3, KNO_3 and PEG. This method may not give accurate reproducible

results for varietal identification for cotton genotypes. None of the genotypes responded positively to the phenol colour reaction of the seeds. The electrophoretic banding pattern of total soluble seed protein and globulins of each genotype was unique and distinct between hybrids and parents. Even though electrophoretic technique is a quick method for varietal identification, it may not be useful for routine testing of genetic purity of large number of samples in a laboratory because this method is more expensive and requires a greater expertise than field GOT. This method can supplement a GOT in case of controversy (disputed/law enforcement sample). The correlation between GOT and electrophoretic technique did not exist. Field Grow Out Test (GOT) is a foolproof method for identification and genetic purity testing rather than electrophoresis because large number of samples could be possible by field plot technique (GOT) only.

27. Drought tolerance and decrepitude resistance of transgenic cotton lines transformed with Rol genes

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The Rol genes cloned from *Agrobacterium rhizogenes* were transferred to the cotton genome via *Agrobacterium*-mediated transformation. The results showed that the expression of Rol genes greatly increased the rooting ability of the transgenic plants, and changed the plant development. Four progeny lines of the plants transformed with 35Sp-rolB displayed strong abilities of drought tolerance and decrepitude resistance. These plants had thicker and smaller leaves with dark green color, shorter but more internodes, and highly developed root system. The mean weights of both fresh and dry roots were increased more than 30 folds. This resulted in the plants setting more bolls and finally increasing the lint and seed cotton yields by 14%, when compared with the control variety. Further analysis of hormonal metabolism for the drought resistant plants showed that the expression of single 35Sp-rol B gene altered the internal concentrations of two phytohormomes. The overexpression of 35Sp-rol B led to three - fold increase of cytokinins in shoot apexes and 23 to 40% reduction in flower stems, and four fold reduction of abscisic acid in the flower stems.

28. Marker – assisted breeding in cotton

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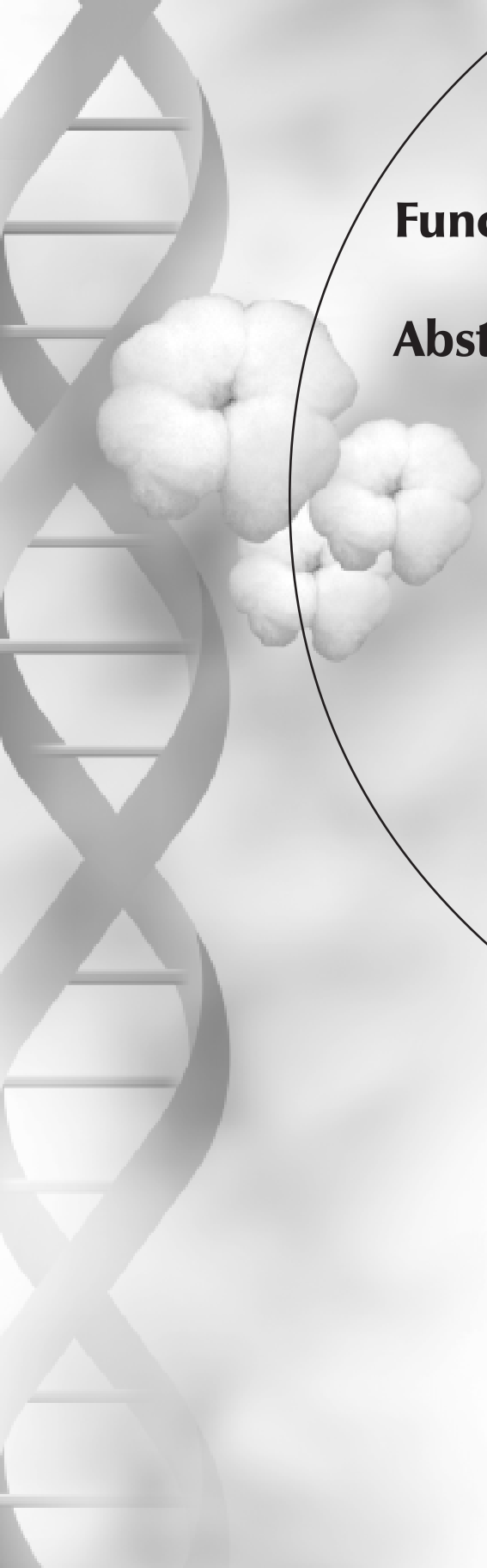
29. Insect resistance in cotton Jassid

KALYAN SUNDARAM, *Punjab Agricultural University, Ludhiana, Punjab, India*



Functional Genomics Session

Abstracts

1. The cotton fiber transcriptome

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2. Expression profile of cotton fiber elongation by cDNA microarrays

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Gene chips, as well named gene microarray, are characterized the media, such as glass or silicon, highly density arranged lots of target nucleotide fragments in proper order. One of the widest applied gene chips is the analysis of large-scale gene expression profiles in pure study. In our study, the gene expression profile chips were made with 1436 ESTs (Expressed Sequence Tags) obtained from the good quality 7235 line (*Gossypium hirsutum* L.) cDNA library onto specially treated glass slides. 1436 ESTs were sequenced from their 5-terminal and classified 13 categories by BLASTX function analysis. 13 categories were cell growth/division, cell structure, disease/defense, energy, intracellular traffic, metabolism, protein destination and storage, protein synthesis, secondary metabolism, signal transduction, transcription, transporters and transporters, respectively. Further, cotton fiber mutant was used for detecting fiber elongation expression profile. Seven different pairs of development stages of cotton fiber from the cultivar Ligon (*Gossypium hirsutum* L.) wild and its Ligon-lintless mutant strain were detected (0, 2, 4, 6 and 8 DPA (days post anthesis) with fiber and ovule both normal and its mutant material and 10, 12 DPA with fiber and ovule in mutant and only fiber in normal material). There were more different expression genes in four stages (0, 2, 10, 12 DPA), whereas less in the other three stages (4, 6, 8 DPA). It was roughly equal between the up-regulation expression genes and down-regulation ones in each detected stages. The different expression genes in 4-8 DPA were a few, with increased progressively in successive stages. It was consistent with the past viewpoint, which believed 3-5 DPA was the principal stage of the source of enormously different morphology between the wild and mutant strain. Reasons for the more different expression genes in 0-2 DPA needed further study. The different expression genes about metabolism were the most in every stage; the second was the genes about secondary metabolism and cell growth/division. This suggested that it need enormous material about cell growth and metabolism and produced enormous product about

secondary metabolism in the developmental progress of cotton fiber. In our study, 6 DPA was focused as an important stage analyzed. Six ESTs with the entire ORF (Open Reading Frame) regulating upward in 6 DPA and after 8 DPA were obtained. These genes would play important roles in the development of cotton fiber. Their functional analysis was underway.

3. Isolation and characterization of fiber specific genes from long staple cotton using suppression subtractive hybridization and microarrays

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4. Proteomic analysis of three fiber developmental mutants in upland cotton (*Gossypium hirsutum* L.)

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Two dimensional gel electrophoresis was used to compare the protein profiles of ovules and attached hairs of three mutants and the wild type at early fiber development stages. The three mutants involve lintless-fuzzless, lintless-fuzzed, and linted-fuzzless, the wild type variety, TM-1, was used as a control. Three days before anthesis (-3 DPA, day post anthesis) represents fiber cell differentiation, 0 DPA fiber initiation, 2~4 DPA fiber primary elongation and fuzz cell initiation, 4~8 DPA fiber, fast primary elongation. Fifty proteins were found that differed in amount between the 4 lines during the early development stages, six of these proteins were identified by tryptic in-gel digestion followed by matrix-assisted laser desorption / ionization (MALDI) / TOF mass spectrometry. The unidentified are being investigated by PSD and LC-electrospray ionization tandem mass spectrometry (LC-ESI MS/MS), the hyphenation of these techniques enable scientists to better understand the mechanism of cotton fiber development.
Keywords: *Gossypium hirsutum*; Cotton; fiber; lint; fuzz; MALDI / TOF; PSD; LC-ESI MS/MS.

5. Function analysis of promoter trapping system after inserting cotton genome

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The technique of promoter trapping was developed to investigate its viability in cotton (*Gossypium hirsutum* L.) functional genomics. A population, 141 independent transformants of cotton, was generated via *Agrobacterium tumefaciens* mediated transformation, of which 97% showed positive response by PCR detection. The reporter, GUS gene, expressed to different extent in different organs, with a frequency of 48% in roots, 9.2% in vascular bundles, 5.2% in leaves, and 51% in flowers. Meanwhile, we discovered that there existed great differences in expressive patterns among different transgenic lines. Their GUS expression patterns were organ or tissue specific or ubiquitous in all of the plants. The promoter trapping system established here was characterized as an effective method for creating mutants with diverse expressive patterns, different in developing stages, which laid a solid foundation for further research of functional genomics in cotton15

6. Genes induced by water-deficit stress in tolerant *Gossypium* genotypes

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Water-deficit stress limits the yield stability and potential of modern cotton cultivars. In an effort to address this problem, we began to profile the drought-responsive transcriptome in Siokra L-23, a water-deficit stress tolerant cotton cultivar. Our early work (1996-1998) utilized differential display of leaf transcripts from stressed *vs* non-stressed plants. Subsequent to this, pathways suggested to be involved in metabolic protection were investigated with emphasis on detecting genes involved in trehalose and proline cycling. Recently we began investigation of genes in the roots of Siokra L-23 that are responsive to osmotic shock. Forty-five Siokra L-23 plants were grown to early reproductive stage using a hydroponics system developed for the experiment in a controlled atmosphere growth chamber. The plants were transferred to a medium containing ten percent polyethylene glycol (PEG, -0.3MPa) plus nutrients or nutrients alone to induce osmotic shock or maintain control conditions, respectively. Photosynthesis, stomatal conductance, and leaf expansion were monitored for one week. Based on these evaluations, the stressed plants progressed through a period of damage (048 h) followed by recovery (~96 h). Gene expression was assayed at 0,1, 4, 24, and 96 hours after PEG application using microarray analysis and cDNA-AFLP to identify genes involved in the response to and recovery from water-deficit stress in cotton roots. In addition to identifying specific genes of interest, trends in expression of various classes of genes over time were noted. We also noted that *G. darwinii* was highly resistant to wilting compared to *G. hirsutum*. A number of transcripts responsive to water-deficit stress were identified in this species by cDNA-AFLP.

7. Identification of salinity responsive transcripts of cotton through differential display of cDNAs

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Cotton is one of the oldest fiber crops of the world and soil salinity is a major abiotic stress that limits plant growth and productivity. Selection for salt tolerance is possible and tolerance has shown to be a heritable character. Further, salinity resistance has been extensively investigated through selection under *in vitro* stress conditions. Considerable varietal differences of salinity tolerance in cotton at different growth stages are reported and few salt tolerant genotypes have been identified. Plants are uniquely suited for coping with abiotic stresses such as salinity during certain stages of their life cycle through triggering a set of physiological and developmental changes. These changes are characterized by a number of biochemical changes that ultimately result from a selective increase or decrease in the biosynthesis of a large number of distinct proteins that alter enzyme activity. Changes in the protein profile are due to changes such as transcription rate, RNA stability, post-transcriptional control, and protein turnover, etc., In the present study, the differential expression of transcripts under different levels of salinity stress in a salinity tolerant Indian cotton cultivar were studied through differential display of cDNAs. The results indicated genetic up regulation and down regulation of few genes during salinity stress. The salinity responsive transcripts identified in the study could be used either to identify the candidate genes influencing salinity tolerance or as markers for selecting salt tolerant cotton lines.

8. Exploring the role of the Brassinosteroid response pathway in cotton fiber development

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Cotton fiber development is known to be regulated by plant growth regulators including auxins, gibberellins and abcissic acid. In addition to these classic phytohormones, our recent work has shown that brassinosteroids (BR) play an important role in fiber development. Exposure of cultured cotton ovules to the brassinosteroid synthesis inhibitor brassinazole (BRZ) strongly inhibits fiber elongation while supplementation with exogenous BR restores fiber development. Therefore, understanding the mechansisms by which phytohormone-mediated responses are regulated in cotton fibers will provide important new tools for the genetic optimization of fiber productivity through both transgenic technologies and molecular breeding. To this end, we are studying cotton orthologs of *Arabidopsis* genes involved in BR perception and signal transduction. We have identified two GhBRI1 genes that encode putative BR receptors and correlated them to the A and D subgenomes. These genes are expressed in elongating fibers and other rapidly expanding tissues. Likewise, cotton orthologs that encode additional BR signal transduction components including the negative regulatory protein kinase BIN2 and positive factor BRZ1 have also been characterized. Modification of the expression of these genes in transgenic cotton is now underway in order to specifically evaluate their functions in cotton fiber development. Recent experiments have also shown that BR signaling involves specific ubiquitination and proteolytic degradation of BZR1. We have identified specific RING finger proteins that interact with BZR1s. These proteins are putative E3 ubiquitin ligases that regulate BZR1 stability and, thereby, mediated BR signal transduction. The role of these factors in cotton fiber development is also being investigated.

9. mRNA transcripts for salt tolerance in Cotton (*Gossypium sp.*)

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10. Novel techniques to improve regeneration and transformation efficiency in cotton: development of different Bt transgenics

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11. Genetic transformation and regeneration of local cotton cultivar

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Transgenic cotton (*Gossypium hirsutum* L.) plants by using of particle bombardment and *Agrobacterium*-mediated transformation have been obtained. Depending on the method of genetic transformation it is necessary to use appropriate recipient system. And on the contrary, depending on the type of plant regeneration it is necessary to choose method of genetic transformation. In experiments on *Agrobacterium*-mediated transformation apical meristems isolated from 5-7-days old seedlings have been used. Using of apical meristems isolated from cotton immature embryos carried out particle bombardment. Hormonal conditions of induction of plant regeneration, root and shoot formation from explants of different origin are optimized. Depending on using of certain system of plant regeneration it is necessary to optimize a number of conditions of plant genetic transformation, its main parameters. By the example of difference between selective agent lethal concentrations the importance of individual approach has been showed. Comparative analysis suggests that amount of transgenic plants obtained using particle bombardment is lower, than using *Agrobacterium*-mediated transformation one. Thus, to obtain genetic modified cotton plants it is effective to conduct *Agrobacterium*-mediated transformation.

12. Functional analysis of a novel promoter system for co-ordinated expression of two insecticidal protein genes from a single locus in transgenic cotton for widening spectrum of target insect pests and insect resistance management

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Expression of two transgenes from a single transgenic integration event in plants is usually achieved by placing each gene downstream of a dedicated promoter element. Co-expression of two genes using a system that employs a bipolar promoter is an emerging concept in plant biotechnology and would be an appropriate technology to adopt since it decreases the length of DNA that needs to be introduced into the plant system. A bipolar expression system has been developed in-house using the Cauliflower mosaic virus 35S promoter. The bipolar element was constructed by fusing a short region of the 3′ end of the promoter in opposite orientations to a fragment representing the central core. This configuration may help in getting co-ordinated, comparable expression of both the genes that are driven by the bipolar element. The system has been validated using a selectable marker-reporter gene combination in tobacco and rice. Following this, we have used this promoter system to develop insect pest-tolerant cotton expressing two different crystal protein genes of *Bacillus thuringiensis*. This strategy addresses two issues viz., expanding the insect target spectrum and management of resistance development in insect populations. The two Cry genes chosen have overlapping target spectra. The targets in the overlapping regions are the major pests of cotton that we intend to control. Molecular data for the expression of two genes from this bipolar expression system will be presented. Bioassay data generated using representative insect pests will also be discussed.

13. Drought tolerance and decrepitude resistance of transgenic cotton lines transformed with Rol genes

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The Rol genes cloned from *Agrobacterium rhizogenes* were transferred to the cotton genome via *Agrobacterium*-mediated transformation. The results showed that the expression of Rol genes greatly increased the rooting ability of the transgenic plants, and changed the plant development. Four progeny lines of the plants transformed with 35Sp-rolB displayed strong abilities of drought tolerance and decrepitude resistance. These plants had thicker and smaller leaves with dark green color, shorter but more internodes, and highly developed root system. The mean weights of both fresh and dry roots were increased more than 30 folds. This resulted in the plants setting more bolls and finally increasing the lint and seed cotton yields by 14%, when compared with the control variety. Further analysis of hormonal metabolism for the drought resistant plants showed that the expression of single 35Sp-rol B gene altered the internal concentrations of two phytohormomes. The overexpression of 35Sp-rol B led to three - fold increase of cytokinins in shoot apexes and 23 to 40% reduction in flower stems, and four fold reduction of abscisic acid in the flower stems.

14. Isolation and characterization of plant defense gene in the cotton genome

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Proteinase inhibitors (PIs) are antimetabolic proteins that interfere with digestive process of insects. It is one of the defense strategies existing in plants against insect and pathogens. PIs are primary gene products and they are excellent candidates for engineering pest resistant into plants. Inhibitor genes of plant origin are particularly promising to use directly without any modification coding sequence but designing with constitutive promoter. In the present report, proteinase inhibitor genes were isolated by polymerase chain reaction (PCR) from cotton genomic DNA. PCR amplification of the target genes was carried out using forward and reverse primers designed on the basis of published sequences. The amplified product was resolved on 1.5 % agarose gel indicated that the expected fragment of the same size as full-length genes of 650 bp for Kti3 and 250 bp for C-II and PI - IV primers. The fragments were cloned into pDrive vectors (Quiagen) and transformed into host bacteria. The transformed bacterial plasmid was isolated and reamplified the cloned PI genes and the results showed the identical fragment length.

15. Construction of Bacterial Artificial Chromosome library of Chinese elite cottons as genomic resources

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In this study, Bacterial Artificial Chromosome (BAC) libraries of two Chinese elite upland cottons have been constructed following the partial digestion of genomic DNA with HindIII. The pIndigoBAC-5 (HindIII- cloning ready) cloning vector was used for the libraries of two varieties. The BAC library of CCRI12, a Chinese elite variety with high yield, good fiber properties and *Fusarium* wilt resistance, included 38800 clones. Analysis of 132 recombinants showed that the insert DNA size ranged from 50 to 150 kb, averaged 120 kb with less than 1% of empty clones. This indicated that the library was two haploid genome equivalents based on an AD genome size of 2240 Mb. As much as 87.7% clones had inserts over 100 kb, and 56% clones over 110 kb. The library of the other variety, Suyuan7235 with high fiber strength and used as a parent of molecular mapping population in China, consisted of 39000 BAC clones. Inserts of checked 140 clones varied between 70 and 150 kb, with a combined average insert size of 120kb. The other parameters were similar to those of CCRI12 library. More clones for two libraries will be developed by using other restriction enzymes. Both of BAC libraries will serve for genomic resources in further research of disease resistance gene cloning, fiber strength gene analysis and SSR marker development.

16. Differentiation of isolates of cotton root rot pathogens *Rhizoctonia solani* and *R bataticola* using pathogenicity and RAPD markers

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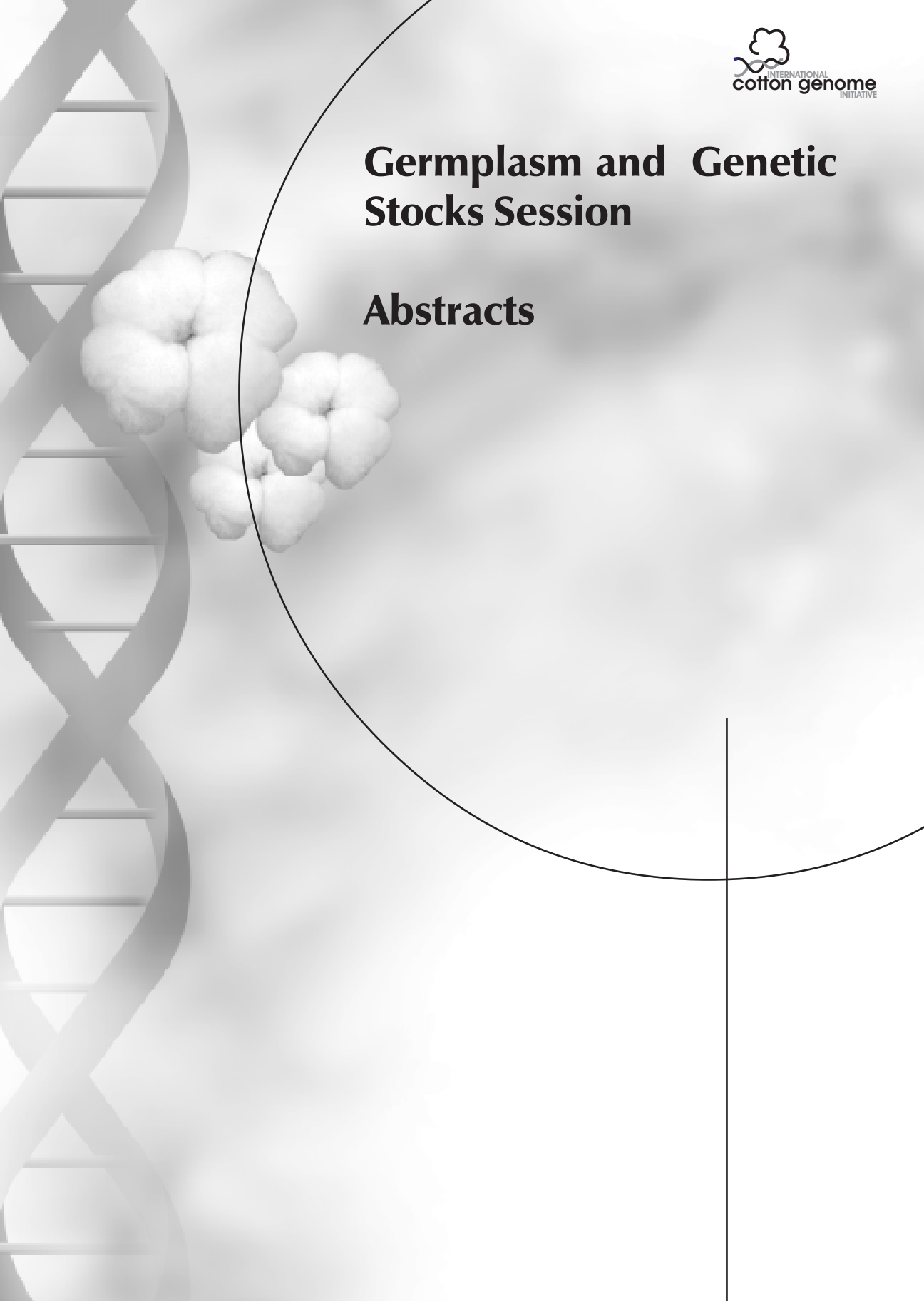
Root rot caused by *Rhizoctonia solani* and *R. bataticola* is a serious disease of cotton in the irrigated northern cotton zone. In the present investigation an attempt has been made to study differentiation of *Rhizoctonia sps*, isolates causing root rot of cotton using pathogenicity and molecular means. Twenty three isolates of *Rhizoctonia solani* and twenty five isolates of *Rhizoctonia bataticola* causing root rot of cotton were obtained after collection of root rot samples from cotton belt of north India. Pathogenicity of these isolates was studied on *Gossypium arboreum* variety RG-8. Five isolates (HR-7, RJ-1, HR-3-10, RJ-3-6 & RS. Coimbatore) cutting across geographical boundaries showed 100% mortality and minimum mortality of 16.6% at par with control was shown by one isolate (HR-4) of *R. solani*. All other isolates showed mortality in between this range. In case of *R. bataticola* the highest mortality of 83.3% was shown by one isolate (RJ-28) followed by HR-3-6, HR-3-10 and IHG-32 and lowest by isolates HR-15, 16 and HR-3-7 (16.6%). Twenty three and 15 RAPD primers were used to fingerprint the individual isolates of *R. solani* and *R. bataticola* respectively. The similarity value of RAPD profiles in *R. solani* isolates ranged from 0.29 to 1.00 and it ranged from 0.12 to 1.00 in case of *R. bataticola*. The isolates were grouped in four in case of *R. solani* and in five groups in *R. bataticola* using unweighted pair group method with arithmetic average (UPGMA). The RAPD method revealed polymorphism within isolates of *R. solani* and *R. bataticola* thereby indicating usefulness of DNA fingerprinting for race/biotype characterization. No clearcut relationship between pathogenicity and differentiation based on molecular groupings could be established, however the maximum pathogenic variation was noted in isolates belonging to Group I followed by Group II & III in both the pathogens. These studies shall help in better understanding the variability in this important cotton pathogen and ultimately planning of strategies for its management.



INTERNATIONAL cotton genome INITIATIVE

Germplasm and Genetic Stocks Session

Abstracts

1. Characterization of new alien monosomic addition lines of *Gossypium australe* on *G. hirsutum*

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The pentaploid *G. hirsutum* x *G. australe* was backcrossed to *G. hirsutum* to produce 253 euploid and aneuploid plants. Among this population, 142 individuals distributed in 39 phenotypic classes presented morphological signs of introgression from *G. australe* into *G. hirsutum*. The screening with mapped SSR markers of the DNA extracted from representatives of the identified phenotypic classes permitted the isolation of three new monosomic alien addition lines (MAAL) of *Gossypium australe* on *G. hirsutum* whose supernumerary chromosomes were homeologs of the c02-c14, c05-D08 and A02-D03 chromosome pairs of *G. hirsutum*. These three new MAAL were characterised morphologically. They complement to the seven MAAL already obtained from the same interspecific hybrid at Gembloux Agricultural University and constitute valuable genetic stocks to carry out fundamental and applied investigations. The contribution of this work regarding the level of genetic material exchanges occurring between diploid species and *G. hirsutum* genomes using the pseudophyletic introgression scheme is discussed.

2. Cytogenetic resources in dissecting complex QTLs and enhancing germplasm in upland cotton

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Stagnant yield, declining fiber quality, threat from biotic and abiotic stresses limit the profitability in world cotton production. Two major limitations to the genetic improvement of cotton are the lack of information about genes that control quantitative traits (QTL) such as fiber yield or fiber quality, and the need for more extensive usage of diverse germplasm. The narrow germplasm base in Upland cotton signifies the need for new genetic resources and novel methods to improve genetic variability in Upland cotton (*G. hirsutum L.*). We have developed 14 backcrossed chromosomal substitution (CS-B) lines, which were used to dissect the genetic effects of quantitative traits associated with specific chromosomes or arms. Fourteen cotton lines with specific chromosomes or chromosome arms from *G. barbadense* L. substituted into *G. hirsutum* (CS-B) were crossed with the recurrent parent and data collected in five diverse environments. Data for chromosome-specific F_2s and their parental lines were analyzed using the additive and dominance (AD) genetic model. Results showed that both additive and dominance effect were significant for most of the traits. CS-B25 had additive effects increasing fiber strength and fiber length and decreasing micronaire and CS-B 16 and CS-B 18 had additive effects related to reduced yields. The heterozygous chromosomal effects between some CS-B lines were different from the homozygous effect for the corresponding CS-B lines. The results provided information on the association of specific chromosomes with genes for agronomic and fiber traits. These new genomic resources will provide an additional route to Upland cotton improvement and will enable development of chromosome-specific recombinant inbred lines for higher resolution mapping.

3. Transfer of fiber length, strength and fineness from Tetraploid to Diploids in *Gossypium spp*.

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Diploid cottons belonging *G.herbaceum* and *G.arboreum* have been under commercial cultivation in sizable area, which can never be replaced by tetraploid upland cottons because that area is considered as very harsh environmentally. Conversion of poor fibre quality diploids into superior quality cottons makes cultivation of such cottons as remunerative. The present research has therefore focused on improvement fibre length and strength of cultivated diploid cottons. Study resorted to inter-specific hybridization using *G.barbadense* (4x) as donor and *G.herbaceum* (2x) and *G.arboreum* (2x) as recipient parents. F_2 and $1BC_2$ populations were subjected to selection and fibre quality analysis. Recipient type plants with elevated fibre properties were selected. Selected Jayadhar type plants possessed fibre length of 24-26mm, fibre strength of 20-23 g/t as against 22 mm and 16g/t of Jayadhar. Similarly selected A-82-1 (*G.arboreum*) plants have fibre length 24 mm and 25g/t against 16 mm and 13 g/t of A-82-1.

4. Advanced backcross generation populations evaluated in Georgia and Texas

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Introgression of genetically diverse germplasm is essential to sustain creation of improved cultivars, but exploiting diversity is not straightforward because diverse germplasm tends to be in a non-elite form and hence is often shunned. Among the USDA-ARS cotton germplasm collection is a large set of day-neutral flowering germplasm lines bred from short-day flowering races of *hirsutum* collected in the wild. Accessing useful genetic diversity among the CRS without relationships established with neutral molecular markers requires sampling strategies to prioritize the CRS to use as parents. One sampling strategy was to select CRS donors found by the USDA-ARS to contribute additive genetic effects for yield, yield components, or fiber quality when mated with a common tester. We selected three CRS as donor parents followed by mating with DES 56 to produce BC_2F_1 populations. DES 56 was chosen as the tester because of its ubiquitous genetic contribution to modern cultivars. Sufficient BC_2F_1 seed was generated to conduct a field trial in 2002 with 61 BC_2F_1 progeny created from MDN63/3*DES56. We did not find significant variation in seedcotton yields among the BC_2F_1 progeny, but five BC_2F_1 lines had greater lint fraction than that of the high parent MDN 63. Segregation for fiber properties was unremarkable, except that most of the BC_2F_1 lines had higher ($P<0.10$) fiber length uniformity than the recurrent parent DES 56. Since upper half mean fiber length of the BC_2F_1 lines was uniformly less ($P<0.10$) than that of DES 56, the enhancement in length uniformity index must reflect greater mean fiber length, and thus possibly less short fiber. Another project led by Texas A & M University involved testing BC_2F_2 populations created by mating 79 CRS with elite line TAM 94-L25. Trials conducted at Tifton, GA in 2002 and 2003 and in 2003 at College Station and Thrall, TX revealed several CRS that improved the yield of 94-L25. The BC_2F_2 population created from TX0072/3*94-L25 yielded more ($P<0.10$) than 94-L25 in both Tifton trials, but not in the TX trials. This finding was not a surprise because the USDA National Cotton Variety Trials have demonstrated the uniqueness of production environments between GA and TX hence the mechanism by which TX 0072 improved the yield of 94-L 25 is likely environment specific.

Nonetheless, findings from both sampling approaches lend support to further efforts to mine the CRS for novel genes. Another effort to expand genetic diversity available for improvement of *hirsutum* is to mine the tetraploid cotton *G. mustelinum*. We have constructed BC_3F_1 populations from a *G. mustelinum* donor and elite germplasm PD94042. To date, interesting segregation was noted in backcross generations in that a number of glabrous plants were recovered, unlike either the donor or recurrent parent. Boll size in the BC_3F_1 generation resembles 94042 compared with the small bolls of *G. mustelinum*. We will next self the BC3F1 in the winter greenhouse followed by selection of BC_3F_2 plants that flower under long days in the summer of 2005 at Tifton, GA. A parallel effort is underway to construct a linkage map in the *mustelinum* / *hirsutum* F_2 population.

5. Intergenomic crosses between A and B genome serve as genetic resources for molecular study

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Among the four cultivated species *G.herbaceum* (2n=26=A_1A_1) is still under cultivation in 1.0 m ha in rainfed tracts of Gujarat and Karnataka of India, but possess short, coarse and weak fibres. *G. anomalum* 2n=26=BB belonging to sub section *Anomala* of sub genus *Gossypium* possess smooth and strong fibres. Distant hybridization was effected to impart smooth and strong fibre characters to *G.herbaceum* Var. Jayadhar. This hybridisation not only appeared to be good source of genetic recombination for fibre properties but also for boll rind and bracts. F_1 of this cross was found to be highly heterotic for number of fruits, plant height, leaf shape, size, flower traits, etc. The notable factor in the F_1 was bundles strength, which was 50.2 g/tex at 3.2 mm gauge with halo length of 22 mm. and very smooth fibre. This value fibre strength has not been observed in diploid and tetraploid cotton germplasm. However, the female parent Jayadhar possessed bundle strength of 17.8 g/tex at 3.2 mm gauge. The cross sectional perimeter (57.8), degree of thickening (0.707), circularity (0.714), reversal density (25.86/cm) convolution angle (5.050) did not give proper explanation for the high strength of the fibre in F_1 hybrid. The fibre strength in BC_1 F_1 generation, ranged from 17.1 to 41.2 g/tex at 3.2 mm gauge. The F_1 plant along with Jayadhar and *G. anomalum* serve as good genetic stocks for fishing out genes involved in fibre strength and fibre fineness. Bolls of *G.anomalum* are ovoid shaped with prominent beak 15 to 20 mm long, three loculed, prominently gland dotted and few glands raised upto 1 mm diameter. On the contrary, Jayadhar possessed round bolls with less prominent beak, 3-4 loculed and obscurely gland dotted or almost absent. F_1 plant possessed gland dotted boll rind with less raised glands compared to *G. anomalum*. In BC_1 F_1 to BC_1 F_3 generation various recombinant types of both parents were isolated. Near isogenic lines, with Jayadhar bolls (round) and prominent gland dots on the boll rind are of interest because these recombinants can be used in identifying genes expressed in boll rind. Hence, the cross between *G.herbaceum* and *G. anomalum* serve as good source for isolation of genetic recombinants useful for biotechnological studies of fibre properties and boll rind.

6. Interspecific germplasm introgression to upland cotton from wild species in China

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There are two cultivated tetraploid *Gossypium* species from the late 80s of the last century in China: Upland cotton (*G. hirsutum*) and Sea-island cotton (*G. barbadense*). The latter is not dominant with less 5% of total cotton production hectares and so the works of genetic improvement has been focusing on Upland cotton. Here we provide the status, in China, about germplasm introgression to Upland cotton from wild *Gossypium* species including races of *G. hirsutum* mainly by interspecific hybrids. There is an over 30 year-history of wild germplasm introgression to Upland cotton via interspecific hybrids in China. The national research projects started from 70s last century and went on in large scale in late 80s. Great contributions to genetic improvements of Upland cotton have been made till today. A key achievement in interspecific introgression from wild species to tetraploid cottons was the overcoming of hybridization incompatibility between cultivated tetraploid and wild diploid species. In early period of the interspecific hybridization, the great efforts were put on preserving interspecific pollinated bolls *in vivo* with some hormones, or culturing the pollinated embryo *in vitro*. Over 20 interspecific hybrids of cultivated cottons (including *G. arboreum*) as female parents with about 13 diploid cottons (also including *G. arboreum*) as anther parents were got by using these methods. In middle 80s last century, as the National Wild Cotton Plantation was established in Hainan Island, we noticed that the hybridization between tetraploid accessions with diploid cottons were relative easily succeeded in winter. With references to other experiments of interspecific hybridization in greenhouse, we concluded that relative lower temperature (20 - 25) during flowing time could be helpful to fertilization after pollination and development of youth embryo for cotton, though it was tropical crop with intendance to higher temperature and sunlight. Just within middle to late 80s in the plantation, we harvested nearly 100 interspecific combinations covering 4 cultivated cottons and 26 wild species, including some between wild diploid species. Another advantage or useful facility for the wild germplasm introgressions of cotton in the place of plantation (Hainan Island) was easily fruiting by backcross with upland cotton to the hybrids. To Upland cotton cultivars or lines, over 25 wild species were used, as male parents, in their interspecific hybrids in China. Among these wild cottons, all three

wild tetraploid species (*G. mustelinum, G. tomentosum* and *G. darwinii*) were included and others were from six wild diploid genomes (B, C, D, E, F and G). Mainly for the consideration of D-subgenome progenitors of tetraploid cottons, D genome species native to central and southern America were emphasized to be used, 11 of total 13 species in the genome were engaged in their hybrids, including *G. davidsonii* and *G. klotzschianum* which bear hybrid lethal genes. Plenty of Upland lines or stocks, which were developed from the interspecific hybrids, exhibited genetic stability. Most of the lines or stocks were provided to breeders and have contributed to developing new cultivars with at least one or two advantage(s) of characteristics, such as high yield potential or better resistances to diseases and/or pest insects, high quality of fiber, and early maturity. An interesting result of interspecific introgression was that, *G. anomalum* from B genome and *G. sturtianum* from C genome, which were distantly related to tetraploid cottons, showed more efficient enhancement to Upland cotton (e.g. more wild germplasm improved accessions were bred from these two species) than ones from D genome. The lines or stocks hybridized by *G. anomalum* exhibited high strength of fiber and those by *G. sturtianum* exhibited better resistances to wilt diseases. There have been at least five commercial varieties, which were directly improved from wild species: Shiyuan 321 (from *G. thurberi*), SGK321 (from *G. thurberi*), Jinmian 21 (also named Yuan 820, from *G. thurberi* and *anomalum*) and Jimian 25 (from *G. thurberi*), Qinyuan 4 (from *G. sturtianum*). Shiyuan 321 is the most outstanding among them with high yield potential, resistance to wilt diseases, and the largest yearly production area of 300 thousand hectares. SGK321 is a variety developed with Bt and CpTI genes into Shiyuan 321. Races or primitive stocks of *G. hirsutum* were also well utilized to improve cultivars. There were some germplasm lines inbred from the races with better traits, mainly in tolerances to diseases (*Fusarium* or *Verticillium* wilts) or drought stress. Another important improvement to Upland cotton was the fiber color. Three commercial cultivars of Upland cotton with brown or green color were developed,- directly introduced with natural color germplasm from races. It is believed that the wild germplasm will play more important role in genetic improvement of cotton varieties in China.

7. Evaluation of fiber quality and other agronomic traits of *G. hirsutum* accessions from Uzbek cotton germplasm

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Fiber yield and qualities are two most important goals of cotton breeding programs worldwide. The modern DNA marker technologies are proven to be useful in tagging candidate genes for trait of interest and therefore, are greatly accelerating the breeding programs to develop well-adapted cotton varieties with superior fiber quality. However, the narrow genetic base of currently available cotton cultivars makes it difficult to map agronomically important traits using molecular markers; therefore, there is a need to explore novel germplasm resources and identify candidate genetic resources for important agronomic traits including fiber quality and yield. As a collaborative research program between USDA-ARS and Uzbekistan, a number of wild *G. hirsutum* races (ssp. *palmeri*, ssp.*richmondi*, ssp. *morilli*, ssp. *mexicanum*, ssp.*latifolium*, ssp. *malum*, ssp.*yucatanense*, ssp. *punctatum*, ssp. *purpurascens* etc.), and *G. hirsutum* accessions belonging to ssp. *euhirsutum* (according to Mauer) that represent a wide range of ecological niche.

8. Evaluating the *Gossypium* C-, G- and K-genome for *Fusarium* wilt resistance

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9. Genetic diversity of Chinese cottons with *Fusarium* and *Verticillium* wilts resistance revealed by AFLPs

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Fusarium and *Verticillium* wilts caused great losses of yield each year in the vast of cotton growing-areas in the world. The most effective and economical methods for controlling the diseases should be obtained through the utilization of highly resistant varieties. An understanding of genetic relationship of existing cottons is critical for further utilization of cotton genetic diversity in the development of elite resistant varieties. Since 1949, several hundreds of cottons with *Fusarium* and other wilt resistance were bred in the country. These varieties have become the basic germplasm resources for breeding resistant cultivars. Up to now, pedigree relationship and genetic variation based on SSR and RAPD markers were investigated by using parts of varieties of this population. AFLP technique has distinct advantages over other molecular markers such as RAPD, RFLP and SSR. Therefore, it was adopted to analyze genetic diversity and phylogenetic relationships among a wide range of crop species. The objective of the present study was designed to estimate genetic diversity existing among 105 China-bred cottons of the disease resistance based on AFLPs. This could provide important information for exploiting the genetic potential of the population and broadening cotton genetic base. The main results were as follows: Twenty polymorphic primer combinations were selected from 100 primer pairs to perform the AFLP fingerprinting. A total of 1498 DNA fragments were scored among all materials, averaging 74.9 for each primer combination, of which, 232 (15.5%) bands were polymorphic. The number of DNA bands per primer combination ranged from 49 to 111, with the average of 11.6 polymorphic markers. Forty six varieties of 105 materials (43.81 %) had specific bands. The primer combination, E41 / M50, made 10 materials produce specific markers. Total mean pairwise genetic distance of 105 varieties, calculated by using SPSS (11.5) software, was 4.353 with the range of 1.732 to 6.708. Mean genetic distance for each variety was among 3.531-5.705. Less than 50% of materials were below the total in

the mean genetic distance. The limited genetic diversity was revealed by these results in the population. Based on dendrogram of AFLPs, 105 varieties were classified into five Upland Cotton Groups (UCGs). The number of cottons was different in each UCG, and their geographic origins and descents were diverse. Genetic diversity among 72 varieties from the Yellow River Valley (YRV) and 29 varieties from the Yangtze River Valley (YTRV) was investigated. Twenty primer combinations revealed 200 polymorphic bands among the YRV cottons and 127 polymorphic bands among the YTRV cottons, respectively. Average genetic distance value was 4.356 for YRV cottons and 4.391 for YTRV ones, respectively. Comparisons of average, maximum, minimum, percentage and cumulative percentage of pairwise genetic distance showed that similar genetic diversity existed between the two kinds of cottons. In addition, several pairs of variety with far genetic relationship were screened out according to pedigree and molecular polymorphism. These materials could be used for the construction of mapping populations, such as F_2 and RIL, in the future efforts of molecular research.

10. Genetic variation for improving the salt tolerance of domesticated diploid cotton

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11. Towards the construction of mutant library induced by T-DNA insertion and EMS in diploid and tetraploid cotton

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Mutation is a very useful and valuable tool for genomic research in plants. The methodology to obtain many mutants has been focused in cotton. Physical treatment and radiation had been employed to induce mutations and most of aberrant chromosomes, for example, deficiency and translocation, have been found. A few morphological mutants also have been identified. Such mutations appeared randomly. In our lab, chemical treatment and T-DNA insertion techniques have been developed in cotton mutation induction for point mutations and direction-oriented mutations. Seed treatment with some chemicals like EMS (Ethyl Methane Sulfonate) causes point mutation of base transition or base transversion. Seeds of diploid cotton (*Gossypium arboreum* L. race *sinense* var Jinghua zhongmian) were treated with EMS at concentrations of 0.33% and 0.25% for 10 hours, respectively. The experimental results indicated that mutagenic effects of EMS-treated cotton were apparently different with the concentrations of treatment. There were more mutants in M_1 population treated by high concentration of EMS, than in that treated by low concentration. It was also found that most of mutants in M_1 are virescent and chimera. A few others like cup, crinkled leaf, small round, mosaic-yellow leaves and double-headed plants also appeared. One of the mutants with male sterile and cup-leaf induced with Co60 was crossed with its parent. F_1 showed fertile and normal leaf, and F_2 appeared in the ratio of 73 to 5 for normal and cupped leaf plants. Two tests demonstrated the inheritance of one pair gene controlling leaf shape. The ratio of F_2 fertility will be assessed this summer. Mutation induction by T-DNA insertion in tetraploid and diploid cottons is being investigated. Calli with T-DNA insertion have been obtained and the plants from them would be regenerated by the end of this year.

12. *In Vitro* Mutagenesis in cotton (*Gossypium hirsutum* L.)

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Plant breeding has a big challenge in the development of varieties with new agronomic, commercial and social characteristics. In order to face this challenge the breeders need to exploit the variability generated not only by traditional crosses but also the variability available by new biotechnology methods such as genetic transformation and induced mutation *in vitro*. The application of mutagenic agents and the recovery of mutant lines have already proven to be very useful for generation of new variability in plant breeding. During the last years the potential of this technique has been increased due to the induction and selection of mutant lines *in vitro* and the use of doubled haploids to shorten the breeding cycle of the mutants to obtain a new variety. Conventional mutagenesis coupled with modern biotechnology tools can help to harness the full potential of induced mutagenesis in crop improvement programs. Exploitation of *in vitro* selection system is a unique opportunity of selecting desirable mutants against various kinds of stresses. The zygotic embryos of cotton (*Gossypium hirsutum* L.) were excised from *in vivo* grown plants and cultured on MS medium fortified with 1.5 mg/l of IAA, 0.5 mg/l of KIN and 250 mg/l of casein hydrolysate. The zygotic embryos were separated and exposed/treated with gamma rays, EMS and SA. Then the both embryos were washed with sterile MS basal medium and were transferred to MS medium supplement with appropriate concentration of hormones for further growth and development. The plant regeneration from treated ovule, the plants was transferred to field conditions after hardening. The plants in the M_1 generation have been studied intensively on their economic characters. The seeds of M_1 plants were collected separately in each dose/concentration of mutagenic treatments. The seeds of M_1 generation to be used for advancing the further generations have to be analyzed thoroughly to select induced variants. The details of work carried out will be discussed.

13. Evaluation of germplasm with microsatellites

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Diversity and relationships based on morphological phylogenetic studies tend to provide poor differentiation among taxa and/or germplasm, making potentially important germplasm accessions from the primary and secondary gene pools almost impossible to recognize useful genetic resources for cotton breeding. Microsatellites are useful for estimating genetic relationships among collections comprising different taxonomic levels, and for analyzing speciation. A set of selected genomic and EST microsatellites were used to examine the origin of allelic diversity in tetraploid cottons, and to assess recently collected diploid and tetraploid cotton germplasm. Amplification of microsatellites via PCR yielded both monomorphic and polymorphic DNA fragments among New World allotetraploid cottons AD_1 and AD_2 (*G. hirsutum* and *G. barbadense*), the two Asian-African progenitor diploid genomes A_1 and A_2 (*G. herbaceum* and *G. arboreum*), and 12 accessions of D-genome species representing all taxonomic subsections of subgenus *Houzingenia*. Estimates of genetic similarities among all taxa showed extensive allelic diversity in the D genome. Phenetic trees (N-J, Jackknife, BootStrap, and UPGMA) based on genetic similarities, as measured by microsatellite diversity, produced clades consistent with current taxonomic species delineations. However, the topology of the consensus tree also revealed clades that suggest additional undocumented relationships within the D genome.

14. Comparative study of the existing cotton cultivars in Bangladesh

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15. Cotton genetic resources at CIRAD

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The cotton genetic resources managed by CIRAD is located in Montpellier (South of France) and represents one of the most important collection of seed resources from *Gossypium* species in the world. It includes 3185 accessions representing a large range of diversity. Major part of the 50 species of *Gossypium* genus (all 5 tetraploid and 35 of the 45 diploid species) are present. These accessions originate from 99 different countries, with United States of America, the French West Indies, French-speaking Africa, and Latin America as most frequent origins. *G. hirsutum* L. accounts for 68 % of the collection, and 87 % of the total are tetraploid accessions. The collection comprises both cultivars and primitive accessions. Close to 1700 accessions are cultivars, with a particular emphasis on the varieties that Cirad developed in partnership in different tropical countries. Two millions hectares are cultivated to day with these varieties in West and Central Africa, South America and South East Asia. Numerous primitive landraces as well as early domesticated forms were collected in the eighties under the umbrella of former IBPGR throughout the diversification areas of *G. hirsutum* and *G. barbadense*. Finally a series of interspecific material derived from hexaploid and tetraploid hybrids and developed for possible introgressive breeding (backcrosses to cultivated *G. hirsutum*) are conserved. Seed renewal is undertaken under tropical conditions once every 12 years for cultivars and 15 years for others. The seeds (300 and 100 grams per accession respectively) are stored in a cold room at 5oC and 40% RH. Storage conditions have recently been completed by a parallel storage in an 18 oC freezer. More than 2700 accessions of the collection have been described for morphological and fiber quality traits. The cultivated germplasm is permanently used for seed exchange and several breeding programs had been launched using samples of this germplasm. The primitive pool and the obsolete varieties are freely available. Most recent cultivars, when patented in partnership with Cirad, can be released upon contractual agreement. A database program allows the management of the collection and the printing of catalogs. Recently, a preliminary study of the genetic diversity of a small collection of 56 accessions belonging to the tetraploid pool (landraces and cultivars) has been undertaken using over 200 microsatellites markers. A subset of highly informative markers has been selected and assembled for constituting a genome-wide genotyping kit of chromosome-specific and multiplexable microsatellites. Two or three 3-plex, ie. 6 to 9 microsatellites, per pair of homoeologous chromosomes,

constitute the kit. In the future, the molecular analysis of the diversity of the collection using this kit of microsatellites combined with the botanical, morphological and technological descriptions will permit to structure the variability and to create core collections. This will then facilitate the screening of the collection for traits of economical interest (adaptation to adverse environment, plant mapping, disease and insect resistances). An internet site will also be developed to promote the collection and to give information on the different accessions (description, origin).

16. Origin and cytogenetics of new cytogenetic stocks of cotton *G. hirsutum L.*

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Cytogenetic stocks are especially helpful to genetic mapping. Our independent studies have been directed to development of cytogenetic cotton stocks. We used 4 types of radiation: combined treatment of seeds with colchicine and gamma rays, fast and thermal neutrons and pollen gamma irradiation. As a results over 200 chromosome translocations as heterozygotes, 90 primary and 22 tertiary monosomics, 14 monotelodisomics, 4 monoisodisomics, 3 haploids and 31 desynaptic plants were recovered. But our observations indicated the different effectiveness of the radiation treatments. Combined treatment of seeds with colchicine and gamma rays gave rise to a lot of plants containing several translocations per PMC and multiple interchanges. After irradiation of seeds by thermal neutrons it was observed certain rare mutation types did not observed before in our experiments. In comparison with the seed treatment the pollen irradiation was the most effective to produce highest number and the widest spectrum of aberrations. Translocations have been confirmed as homozygotes according to the scheme. As a result 29 new homozygous translocation stocks have been isolated. 27 translocation stocks are simple reciprocal interchanges, involving only 2 non-homologous chromosomes, whereas the remaining 2 (Tr 2 and Tr 20) are interchanges involving 3 non-homologous chromosomes. The differences between these stocks were indicated on chromosome configurations and frequencies of multivalents in M_1 of meiosis, pollen fertility and morphological characters. It was established that there are the differences between translocation stocks in a number chromosomes in common involved into the interchanges. It was shown, the chromosomes in Tr 3, Tr 7-8 and Tr 16 were more frequently involved into interchanges, but chromosomes from Tr 1, Tr 10 and Tr 20 were less frequently involved. These translocation stocks were also crossed with hybrid *G. thurberi* x *G. raimondii* to determine genome location of the interchanged chromosomes. At present time we can only determine AA-subgenome location for translocation stocks – Tr 1, Tr 7-8, Tr 16 and AAD – for Tr 2. From 90 primary monosomics only 72 were produced directly from irradiated treatments, 2 monosomics from translocations and 16 from

the progenies of desynaptic plants. Apparently, the origin of these monosomics resulted from irregular segregation. The transmission of the monosomics was studied by us in the selfed or outcrossed progenies. The frequency of the aneuploid progeny was depended on the rate of haplo-deficient gamete transmissions. Different monosomics were distinguished by transmission rates. Four monosomics – Mo 3, Mo 15, Mo 40 and Mo 56 usually occurred in much lower frequencies and required larger populations for their recovery. Various transmission rates indirectly pointed out different monosomes to be specific chromosomes of cotton genome. Identification of the monosomes by means of translocations from our collection revealed that 9 monosomics (Mo 3, Mo 10, Mo19, Mo 27, Mo 39, Mo 48, Mo 53, Mo 56 and Mo 73) are homologous with a chromosome involved in the interchanges of 6 translocation stocks (Tr 11, Tr 3, Tr 16, Tr 8, Tr 5, Tr 12, Tr 12, Tr 5 and Tr 3 respectively), since hybrid monosomics formed 24 bivalents plus one trivalent. So, as genome tests showed that translocation stocks Tr 7-8 and Tr 16 were AA-subgenome location monosomes, Mo 19 and Mo 27 may be considered as specific A-subgenome chromosomes in cotton.

17. Studies on distant hybridisation between *Gossypium herbaceum* x *G. anomalum* and useful genetic stocks for biotechnological studies

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Among the four cultivated species *G.herbaceum* ($2n=26=A_1A_1$) is still under cultivation in 1.0 m ha in rainfed tracts of Gujarat and Karnataka of India, but possess short, coarse and weak fibres. *G. anomalum* 2n=26=BB belonging to sub section *Anomala* of sub genus *Gossypium* possess smooth and strong fibres. Distant hybridization was effected to impart smooth and strong fibre characters to *G.herbaceum* Var. Jayadhar. This hybridisation not only appeared to be good source of genetic recombination for fibre properties but also for boll rind and bracts. F_1 of this cross was found to be highly heterotic for number of fruits, plant height, leaf shape, size, flower traits, etc. The notable factor in the F_1 was bundles strength, which was 50.2 g/tex at 3.2 mm gauge with halo length of 22 mm. and very smooth fibre. This value fibre strength has not been observed in diploid and tetraploid cotton germplasm. However, the female parent Jayadhar possessed bundle strength of 17.8 g/tex at 3.2 mm gauge. The cross sectional perimeter (57.8), degree of thickening (0.707), circularity (0.714), reversal density (25.86/cm) convolution angle (5.050) did not give proper explanation for the high strength of the fibre in F_1 hybrid. The fibre strength in BC_1 F_1 generation, ranged from 17.1 to 41.2 g/tex at 3.2 mm gauge. The F_1 plant along with Jayadhar and *G. anomalum* serve as good genetic stocks for fishing out genes involved in fibre strength and fibre fineness. Bolls of *G.anomalum* are ovoid shaped with prominent beak 15 to 20 mm long, three loculed, prominently gland dotted and few glands raised upto 1 mm diameter. On the contrary, Jayadhar possessed round bolls with less prominent beak, 3-4 loculed and obscurely gland dotted or almost absent. F_1 plant possessed gland dotted boll rind with less raised glands compared to *G. anomalum*. In BC_1 F_1 to BC_1 F_3 generation various recombinant types of both parents were isolated. Near isogenic lines, with Jayadhar bolls (round) and prominent gland dots on the boll rind are of interest because these recombinants can be used in identifying genes expressed in boll rind. Hence, the cross between *G.herbaceum* and *G. anomalum* serve as good source for isolation of genetic recombinants useful for biotechnological studies of fibre properties and boll rind.

18. Introgression derivatives from *Gossypium arboreum* (A_2) and *G. hirsutum* (AD_1) serve as source to identify 'D' Genome Genes- A Study

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Gossypium arboreum (2n=2x=26) is still being cultivated in Asia; however, genetic improvement in this species is not in the same magnitude like that of *G. hirsutum*. The present day *G. arboreum* though resistant to sucking pests and abiotic stresses like moisture stress have been replaced by *G. hirsutum* due to their small boll size, short, coarse and weak fibres. *G. hirsutum* genes for fibre, seed, boll and foliage features were transferred to *G. arboreum* through distant hybridization. *G. arboreum* (2n=2x=26 A_2A_2) was colchiploidized to make 2n=4x=52 $A_2A_2A_2A_2$. This genetic stock was hybridized with *G. hirsutum* (2n=4x=52 AADD). The resultant F_1 was backcrossed to 4n *G.arboreum*. In the advanced backcross generation different *G. arboreum* genetic stocks possessing very specific characters but not found in the parent *G. arboreum* were isolated and characterized. Fifteen different genetic stocks with different but specific characters were used for RAPD analysis using decamer primers. Some of the very special characters of genetic stock included 31 mm fibre length, 28 g/tex at 3.2 mm gauge fibre strength (ICC mode), 4.2 fibre fineness, 10.5 g seed index, boll weight of 3.0g and foliage with broad lobes etc. These genetic stocks are unique in nature because the germplasm lines with such values are not present in *G. arboreum* germplasm pool maintained in India. These introgressed derivatives were phenotyped along with *G. arboreum* and *G. hirsutum* parents, *G. raimondii*, five races of *G. arboreum*, *G. herbaceum* and four races of *G. hirsutum* and genotyping was done using RAPD analysis. All the tested decamer primers in RAPD analysis produced polymorphic amplicon products, however, the extent of polymorphism varied with each primer. Few primers, namely OPZ 14, OPP 01 and OPY 02 produced genome specific amplifications, wherein amplicon products ranging between 1000 and 1500 bp were present in 'D' genome (*G. raimondii*), AD (*G. hirsutum*) and in some introgressed *G. arboreum* derivatives but absent in 'A' genome. Presence of such amplified fragments has been considered as resultant of introgression of AD genome into *G. arboreum*. The study hinted

at the greater involvement of 'D' genome in traits like long and fine fibers and bolder seed size than 'A' genome. These genotypes are unique cotton with very exceptional phenotypic values. These valuable genetic stocks can be used for molecular studies both at structural and functional levels especially for seed, boll and fibre specific genes. Further, these genetic stocks being resistant to sucking pests like Jassids are preferred candidates for Bt gene transfer for making cotton cultivation more remunerative as the genotypes emanated from transformation will be resistant to both sucking pests and bollworms.

19. Effect of mitochondrial genomes on male sterility, yield and other economic characters in cotton

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India is the pioneer country for commercial cultivation of hybrid cotton. Hybrid cotton covers about 45% of the total cotton area and contributes 55% to the national production. Seed production is generally carried out by conventional method, which involves hand emasculation and pollination. As a result the cost of hybrid seed is very high. Use of male sterility system provides scope for the solution of the above problem. It has many advantages over the conventional methods viz. reduction in cost, higher seed setting, lesser immature seed, lesser shedding of crossed bolls, high crossing efficiency, high quantity of seed production, check over use of F_2 seeds and rapid spread of hybrids. So far, 16 genes in tetraploid cotton and two genes in diploid cotton have been identified for genetic male sterility; ms5ms6 (Gregg ms 399) is the most stable and being utilised by hybrid cotton growing countries. The expression of male sterility varies in extent and stability among the loci. In diploid cotton, out of two genes identified for GMS, one is from a cross between *G. anomalum* x *G. arboreum* (ar.ms) and the second source is a spontaneous mutant in arboreum variety DS 5 (ams1). Meyer (1975) first developed the cytoplasmic genic male sterility in cotton from *G. harknessii* species carrying $D_{2\text{-}2}$. Two new sources have been developed recently, one through the use of ***G. aridum*** (D_4 genome) by Dr. L.D. Meshram and the other using *G. trilobum* (D_8) by Dr. Stewart. The two sources of CMS available in India were taken for the present study ($D_{2\text{-}2}$ and D_4 source). It was found that restorer lines developed for restoring fertility against *aridum* cytoplasm was effective in restoring the fertility of *G. harknessii* and vice-versa. The study further showed that there was effect of $D_{2\text{-}2}$ and D_4 cytoplasm on yield and various yield attributes. Same genotypes with harknessii and aridum background were taken and crossed with the same set of R-lines. It was found that hybrids with *aridum* source of male sterility performed better than the same combination in *harknessii* background. Similarly, the performance of *aridum* R-lines were better than the *harknessii* R-lines. The hybrids were also found to possess high fibre strength and length. The aridum cytoplasm has been found to offer greater resistance to diseases and pests. The results are encouraging and needs further indepth investigation.

20. Wild species of *Gossypium* and their utilisation in India

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The genus *Gossypium* that belongs to the family *Malvaceae* includes about 50 species (including seven new Australian species), out of which four species are cultivated for their spinnable fiber. The remaining 46 species are distributed throughout the tropics and subtropics of the world in wild forms. The species of *Gossypium* are grouped into two ploidy level i.e., diploids and tetraploids. There are 43 diploid species with 2n=26 chromosomes which have been classified into 8 genomes A, B, C, D, E, F, G and K and 7 tetraploid species with 2n=52 chromosomes with genome designation (AD). The wild species of *Gossypium* are important sources of useful traits such as special and superior fiber properties, cytoplasms for male sterility, resistance to biotic and environmental stresses etc., which can be introgressed into the cultivated species for improvement. Since variability available in cultivated germplasm can be expanded through suitable breeding programmes, it has become a necessity to develop new germplasm enriched with rare useful genes from wild species through introgression. In India, varieties as well as hybrids have been developed for commercial cultivation through introgressed germplasm. In upland cotton PKV081, Rajat, Gujarat 67, MCU 2, MCU 5, Deviraj, Devitej, Khandwa 2 and Badnawar 1 had been derived from inter-specific hybridization with good fiber yield and quality. At the Central Institute for Cotton Research, Nagpur, a species garden with 25 species is maintained *in situ* from material obtained from Dr. R. J. Kohel in 1981 and already available material in CICR Coimbatore Center. These wild species are being utilized in basic studies and cotton improvement potentials through breeding. Bacterial blight resistance was transferred from *G. anomalum* to *G. hirsutum* in the form of a short duration short staple drought tolerant variety Arogya, which was released for cultivation in the Vidarbha region of Maharashtra State in India. A vast number of inter-specific hybrid derivatives have been developed for important agronomic characters like high yield, altered fiber attributes of value for new requirements in textile industry particularly strength, elongation and congenial seed coat attributes, modified plant types, improved physiological parameters like high biomass associated with high harvest index and

optimum leaf area and resistance to bacterial blight, jassid, aphids, whitefly and American bollworm. These inter-specific derivatives amounting to over 145 variants developed at CICR and its cooperating centers will be added to the National Gene Bank of Cotton at the Institute and also inducted into the breeding

programs for the development of superior cultivars / hybrids for meeting the challenging requirements of cotton farming and textile industry. These are also being evaluated for their seed utilization potential as well as the physiological efficiency in varied ecosystems of the country. In due course, when a full-fledged *Gossypium* Genomic Studies Laboratory is established at the CICR, some of unique germplasm so derived would be used as experimental material for cytogenetic and genome analysis to find out the nature of gene alterations in relation to the original parents to increase the basic knowledge for improving gene introgression and improve the bioinformatics of the functional aspects and proteomics.

21. Need for analysis of genome diversity in relation to racial and geographic diversity in cultivated *Gossypium* Species Linnaeus

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Gossypium is one of the largest genera in the Dicotyledonous plant kingdom having nearly 50 species. Four are cultivated for their lint with bonus output of seed oil and protein. Two of them are diploid species with 2n=26 chromosomes of A_1 and A_2 genomes and another two are allotetraploid with 2n=52 chromosomes of 2 $(AD)_1$ and 2 $(AD)_2$ genomes. All these four cultivated species of *Gossypium* had been subjected to intensive genetic analysis of economically and taxonomically important attributes, cytogenetic characterization and breeding and genetic engineering investigations for over a century now in India. The agronomic potential of the cotton crop and its products have been improved substantially for widely varying environments through breeding and great advancement has been made in terms of fiber yield and its quality for various end uses in all the pure species as well as some of their intra-ploidy hybrids that showed much dramatic changes in the last three decades. Similarly, the seed attributes in relation to fiber processing characters and seed oil and protein improvement potential have also been exploited both quantitatively and qualitatively. Still the zenith of the cotton plant's potential has not been reached in terms of very many economic attributes based on the latent genetic potential available and implied in the varied geographical and land races and also the innumerable wild relatives. Hence cotton offers immense possibilities for improving the boll load per plant in shorter plant frame with optimum boll weight, modifying its plant types and leaf canopy for varying management conditions and mechanical harvesting, improved levels of multiple resistance to biological and environmental constraints and various combinations of fiber quality parameters and seed characters for improving its competition for challenging farming conditions and profitable alternative crops, alternative fibers with admirable handling comfort and durability and enhancing the sustainability in all the four cultivated species. India is the only country today to commercially cultivate all the four cultivable species and some of the inter-specific and intra-specific hybrids. In India, over 18 geographical races with large number of representatives under each and several land

races are available as genetic stocks in the National gene Bank of Cotton at the Central Institute for Cotton Research (CICR), Nagpur. These have been evaluated for their agronomic potentials and technological properties and catalogued by the CICR besides cytogenetic features by a few workers in India. The A genome of the Diploid cottons and the A of AD of the allotetraploid cottons have common lineage or connection as per studies and hypotheses made thereon by several stalwarts on cotton in advanced countries of the cotton world including India, besides postulating the monophyletic origin of the two allotetraploid cultivated species and subsequent divergence. The racial differentiation associated with geographical diversification has been distinct in both the diploids and *hirsutum* cotton. *Hirsutum* and *barbadense* have developed with frequent mutual introgressions. The study of the structural and functional genomics as well as the proteomics of the four cultivated species using these geographical and land races may throw more light on the role of introgression and human selection in the development of the genomes of the four cultivated species. The CICR being the major holder of germplasm of all the four cultivated species can therefore be considered as a collaborative center for the Genomic Research in *Gossypium* under the International Cotton Genome Initiative, which would be deliberating on the establishment of new institutions for the same at the ICGI-2004 Workshop.

22. Markers associated with histo-morphological attributes, following pedigree of crosses involving unique parents of *Gossypium arboreum* L. and *G. herbaceum* L.

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Diploid cultivated cotton species of *G. arboreum* and *G. herbaceum* present novel and some unique genetic material for study of marker attributes and changes following mutation induction. The present investigation aims at characterization and inheritance analysis of several economically important plant attributes and identification of associated molecular marker traits in these two Asian species that are highly cross compatible. A set of unique phenotypic marker parents (including red flower and stem color, brown lint color, naked seed and large-sized boll traits) and their segregating generations from inter and intra-specific combinations have been scored. In the first phase of this study, histo-morphological changes in parents and their offspring have been compared through successive stages of leaf and floral bud development. In the case of mature leaves, differences are observed between several traits of *G. arboreum* and *G. herbaceum* L, showing dominant inheritance pattern in the segregating generations. Histological analysis of floral buds has also revealed significant and possibly key developmental differences between the species and their progeny in crosses, as also in terms of primordial anther and carpel related attributes, which appear to be dominant and recessive in nature.

23. Overview of Cotton Research in Uzbekistan

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According to archeological findings cotton growing in Uzbekistan traces long back in our history. Although Uzbek farmers had grown mostly diploid cottons (*G. herbaceum*) for the past, the growing of long staple fine fibered Egyptian cottons (*G. barbadense*) began after the conquer of the Turkistan (now Central Asia) by Russian imperialism, yet Egyptian cottons failed to give crop yield because of their long vegetation period. In the beginning of XVIII- century, farmers successfully began growing American Upland cottons, which consisted of plant mixture of cottonseeds. The first varieties, Triumph Nevrotskogo and variety 1306, developed in 1930s, replaced cottonseed mixtures in the Republic. These first cotton varieties were then consequently replaced by more productive and early-maturing varieties (C-450, C-460, 18819, 108f and 4727) during Former Soviet Union period. During former Soviet Union (FSU) period, Uzbekistan was one of the main republics to produce cotton fiber for the entire country, and it had accelerated cotton.

24. Molecular screening of induced photoperiod related cotton mutants with microsatellite markers

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Photoperiodic response of plants is one of the important physiological reactions that determines an important aspect of plant development in ontogenes is such as plant flowering. Furthermore, photoperiodic response in plants is species-dependent complex networking process and connected with light signal perception, circadian rhythm and molecular clock pathways. Although photoperiodic pathway genes are well studied in model plant systems, cotton photoperiodic response genes and their significance in cotton photomorphogenesis have not been studied yet. Hence, determinations of molecular basis of cotton photoperiodic response is of particular importance since understanding cotton flowering will be useful in manipulation of wild photoperiodic cotton germplasm in applied breeding. One of the approaches toward this goal is molecular screening of photoperiod released induced mutant germplasm to identify candidate loci for further tagging of useful photoperiod related mutations (cottonseeds of several photoperiodic wild cottons).

25. Heterosis and combining ability for plant type traits in cotton

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The study was made using diallel analysis involving 12 cotton genotypes representing four different plant type classes. The main plant type features viz., plant height, number and length of sympodia, number and length of monopodia, angle of sympodia on main stem, leaf area, leaf area index and plant yield were studied. The heterosis over mid parent ranged from – 62 % for number of monopodia to as high as 123.50 % for sympodial length. Overall, compact x medium compact plant type combination had high heterotic hybrids for yield with low to moderate heterosis for plant type traits, indicating the physiological efficiency of the plant type traits in enhancing the yield *per se*. Combining ability studies showed importance of non - additive component governing plant type traits. In general low x low combining parents were found to be important for breeding compact plant types.

26. Intergenomic hybridization in *Gossypium* for the improvement of cultivated allotetraploid cottons

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Gossypium herbaceum is designated A_1 genome, *G. arboreum* as A_2 genome and *G. anomalum* as B_1. Six geographical races in *G. arboreum* and five in *G. herbaceum* are maintained in the Indian gene banks for cotton. Four out of the five in the former namely *africanum*, *acerifolium*, *persicum* and *wightianum* and all the six of the latter namely, *indicum*, *soudanense*, *bengalense*, *cernuum*, *sinense* and *burmanicum* were used in crosses with *G. anomalum* and the ten inter-specific hybrids each with *G. anomalum* as female as well as male were studied for morphology, fertility and cytogenetic behaviour. Amphiploids induced by colchicine in six combinations and their self-fertilized progenies were also similarly evaluated. The amphiploids and their progeny showed increased fertility of pollen (60 - 87%) and ovule (47 - 55%) with setting of bolls. The lint was extremely fine, silky and strong, but with a brownish tinge in contrast with the comparatively white lint of the Asiatic cottons. Some of the amphiploid progenies that were fertile and produced bolls tended towards *G. anomalum* for certain morphological attributes like bracteoles and pubescence and lint. The Asiatic cotton races of *G. arboreum* namely, *burmanicum*, *sinense* and *soudanense* were found to make differential contributions for ginning percentage, fiber fineness and boll size. Inter-racial diploid hybrids of *G. arboreum* as well as *G. herbaceum* (total 6 races of the former and 4 of the latter) with *G. anomalum* and induced amphiploids of 5 combinations representing the two Asiatic cottons were analyzed for cytological behaviour. The formation of 10 - 13 bivalents at meiosis in the hybrids revealed a high degree of structural similarity between the genomes of the races of *G. herbaceum* and *G. arboreum*. The differentiation between the races within each of the species *G. herbaceum* and *G. arboreum* was inferred to be primarily genic. The hybrids of *G. herbaceum* x *G. anomalum* showed complete bivalent pairing while the hybrids between *G. arboreum* x *G. anomalum* frequently showed one quadrivalent indicative of structural differences between the genomes of these parental species. All the hybrids were highly pollen and seed sterile. The chromosome association in the induced amphiploids ranged from a minimum of 26 bivalents in the 4n (*G. herbaceum* x *G. anomalum*) and one quadrivalent plus 24 bivalents in 4n (*G. arboreum* x *G. anomalum*) to a maximum of 6 quadrivalents and 14 bivalents in both cases. In the amphiploids derived from the 5 different races of the cultivated parents with *G. anomalum*, the maximum association indicated that in the basic chromosome set of the present day diploids (x=n) comprising 13, six are

differentiated from the rest in inter-genomic homology between the Asiatic species and *G. anomalum* leading to the conclusion that the basic number of this genus could be 6 or 7. The existence of cryptic structural differentiation in the genomes of the cultivated diploid parents is indicated by the greater restoration of the pollen and ovule fertility in the amphiploids of the various combinations. In the self-fertilized amphiploid progeny of which two plants were studied cytological, meiosis showed high bivalent formation (26 bivalents) with a reduction in quadrivalent frequency from the induced amphiploid generation. There were distinct morphological differences between the plants of the progenies indicating recombinants resulting from the heterogenetic associations of chromosomes in the quadrivalents formed in the progenitor. The allotetraploid derivatives from crosses of the amphiploids with both *G. barbadense* and *G. hirsutum* exhibited marked vigour for growth, leaf and floral characters besides having a tendency for perennial nature. Intergenomic pairing and multivalent formation between A, B and D genomes characterized the chromosome associations in the allotetraploid derivatives. Though high female sterility was observed in these allotetraploids, further utilization in breeding by crossing with the cultivated tetraploids using the former as pollen parents was found to be a promising proposition in so far as these allotetraploids exhibited a pollen fertility of 6 to 19 per cent. The varying behaviour of the allotetraploids of the different combinations indicated varying extent of differentiation between the various D and A genomes. The study brought out the potential of utilizing *G. anomalum* for the transference of desirable fiber characters as extreme fineness and strength of lint to the cultivated allotetraploid cottons by using the synthesized allotetraploid derivatives through the backcross and intermating techniques. The extensive study of the self-fertilized progenies of the amphiploids themselves can lead to good progress in fiber breeding. The potentialities for improving the two diploid cultivated cottons for their fiber qualities are also indicated. Cotton fiber development is a multistage overlapping developmental process involving fiber initiation, cell elongation, secondary wall deposition and maturation taking in all about 45 to 55 days from the time of fertilization of the ovules. Cotton is a continuous flowering crop extending for a period of 60 to 90 days or more and bears bolls of different ages in a given time during the crop duration of 180 to 200 days. Botanically the commercial lint fibers are called trichomes since the cells do not form part of the vascular tissues and arise rather from the ovular epidermis and this is a unique case in the plant kingdom. These trichomes or lint hairs are highly elongated structures and of exceptional chemical make up that makes the cotton fiber as a unique model for studies on plant cell elongation and cell biogenesis (Hee Jin Kim and Barbara A. Triplett, 2001). The study of fuzz hairs for better exploitation is also being thought of in the context of genetic engineering. Application of this technique to the screening of the synthetic

allopolyploids and their derivatives can give a better clue to the manipulation of fiber development and seed development in inter-genomic or inter-specific breeding by better understanding of functioning of the various fiber genes involved in the process. Since superior strength and Micronaire value coupled with long staple are important for modern spinning, the combined role of breeders, physiologists and molecular biologists would prove more productive in exploiting the potential of transferring useful fiber genes into both the cultivated diploid and tetraploid cottons rather than searching for gene sources outside the *Gossypium* genome. Special efforts to build up new germplasm through the inter-specific breeding is still a time consuming process, but for the sake of posterity, it may be treated as one essential prerequisite for breeding better cottons for the future.

27. Interspecific hybridization in cotton varietal improvement: A Mini-Review

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India is a pioneer in the utilization of inter-genomic hybridization techniques in *Gossypium* species for the transference of useful characters to cultivated cottons in the varietal improvement programme. The first ever inter-specific Asiatic-American species hybrids synthesized at the Cotton Research Station, Surat, Gujarat state involved variety Co 2 (*G. hirsutum*) with Gaorani (*G. arboreum*) cotton resulting in improved cultivars 170-Co. 2; and with 1027 ALF (*G. herbaceum*) leading to variety 134-Co2-M. The *G. hirsutum* x *G. tomentosum* crosses made at Surat named Co-tom, subsequently bred at Indore resulted in several CTI selections, with increased pilosity and resistance to jassid. Further hybridization with *G. hirsutum* genetic stocks resulted in improved varieties B 1007, SRT1 and Khandwa 2, which became choice varieties for extensive cultivation in Maharashtra, Gujarat and Madhya Pradesh. These proved to be popular in cultivation for long time being highly drought-resistant, jassid-tolerant and widely adopted to rain-grown ecosystems. The utilization of *G. barbadense* variety Sea Island cotton in the multiple cross programme involving *G. hirsutum* varieties in Coimbatore, resulted in the first extra-long staple variety MCU 5, which is a significant milestone in the Indian cotton varietal improvement programme. The introgression of *G. anomalum* genes into *G. arboreum* and *G. hirsutum* cultivars at Coimbatore and Nagpur are other instances worthy of record. It is worth mentioning that some of the varieties mentioned above have been used as proven parents in the development of very successful hybrid cotton cultivars in India. Work on male sterility systems, both CMS and GMS have been intensified in recent years to reduce the cost of production of hybrid seed, which is extensively cultivated in India. In this context, the diversification of cyto- sources to *G. anomalum*. *G. arboreum*, *G. raimondii*, *G. thurberi* etc., has been resorted to by workers at Akola, Coimbatore, Hisar and other research centres to develop new MS lines in *G. hirsutum* and *G. arboreum*. Success has been reported in development of practically usable CMS/ GMS in both American upland and Asiatic cottons. The genetic stocks and germplasm resources derived from the inter-genomic hybridization carried out in India have considerably added to the genetic resources pool available for cotton varietal improvement. In recent years further active research has been undertaken in R & D programmes of the Technology Mission on Cotton and the National Agricultural Technology Project at selected research centres and progress reported is encouraging

with high potential for broadening the genetic base of variability in the National Gene Bank of Cotton and also induction in future varietal improvement, to meet the new norms for fibre quality in modern textile processing systems and changing requirements for sustainable cotton farming.

28. Screening *Gossypium hirsutum* and *G. barbadense* germplasm accessions for fiber quality traits

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An investigation has been undertaken with 102 genotypes, 51 each of *G. hirsutum* and *G. barbadense* at Department of Cotton, TNAU, Coimbatore during kharif 2003 to understand the magnitude of genetic divergence for fibre quality traits and their contribution to genetic diversity by using D2 statistics and principal component analysis (PCA). The cluster pattern based on Tocher's method grouped the genotypes into eight clusters. Among the clusters, cluster I had 58 genotypes, cluster II had 36 genotypes and cluster III had 3 genotypes. All other clusters had only one genotype showing uniqueness in the breeding point of view. Based on the inter-cluster distance, clusters VI vs VIII, III vs VIII, II vs III and II vs VI were highly divergent with each other. Hence genotypes of cluster II, III, VI and VIII could be crossed among themselves to produce wider segregation among the progenies and to improve fibre quality of cotton. Among the fibre quality traits, elongation percentage and 2.5% span length showed greater contribution towards total divergence than other characters and hence selection would be effective for these characters. The principal component analysis grouped 102 genotypes into nine clusters. The genotypes from clusters V, VIII and IX recorded more diversity. The biplot analysis based on IPCA I and IPCA II scores revealed that the genotypes viz., T-142 DA, 71/3, ISC 71 and SH 467 were highly divergent and could be utilized as parents in a crossing programme. The clusters V, VI and VII showed low inter-cluster distance and formed a sub group while the clusters I, II, III and IV formed another sub group. Comparing PCA clustering and D2 grouping based on Tocher's method, due importance should be given for PCA as it measures divergence between varieties in terms of spatial distance (Biplot) rather than quantifying divergence as D2 does and it determines effective number of traits (axes) of differentiation. However, the accessions T-142 DA and 71/3 were found to be highly divergent in both D2 and principal component analysis and were also superior in mean expression for fibre quality traits indicating that these accessions can be used in the breeding programme for improving fibre quality in cotton.

29. Narasimha - A versatile *hirsutum* cultivar with diverse germplasm integration and selection for drought resistance and wide adaptability in India

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Narasimha was released from Cotton Research Center, Nandyal of Andhra Pradesh, India in the year 1993. It has proved to be a versatile *hirsutum* cultivar in Southern India and has shown not only wide adaptability in several regions of the south, but also exhibited high tolerance to drought and high yield potential. The parentage of Narasimha is somewhat complex involving the multiple crossing, backcrossing and selection programme using LRA 5166 (A 179 x B1007) x Strain 6-6. LRA 5166 is a three-way cross derivative of (Laxmi x Reba B50) x Khandwa 2 (a selection from derivatives of COTOM (*hirsutum* x *tomentosum*) inter-specific breeding programme). LRA 5166 proved to be so versatile variety of Coimbatore that it covered the largest ever area of large geographical regions by any variety in India for its good adaptability and yielding ability over wide range of environments. However it was susceptible to jassids and blackarm. A 179 was an Indian Punjab variety with high ginning out turn and early maturity suited for north zone states. B 1007 was a highly jassid resistant variety having been derived from earlier inter-specific hybridization programme in
Buri in Madhya Pradesh and a popular rainfed cultivar in Vidarbha region of Maharashtra state. Strain 6-6 was a highly drought tolerant germplasm gene pool
maintained at Nandyal of Andhra Pradesh. Very skillful breeding procedures like intense selection towards high GOT, high drought tolerance, high jassid and bacterial blight resistance, high boll number coupled with high yield potential and medium staple were practiced at all stages and in the final evaluation the Narasimha (NA 1325) stood in top that it was released for the state of Andhra Pradesh, which is having a total area of 0.8 to 1.0 million hectares under cotton. After the release of Narasimha, the whitefly problem also became a matter of the past. Narasimha has recorded 20q/ha seed cotton yield under rainfed conditions and 30-36q/ha under irrigated conditions with best management practices. Even under adverse drought conditions as witnessed in 2002-03, Narasimha provided higher and stable performance. Narasimha has recorded 36 to 37 % ginning out turn, 27 to 28mm staple length (29mm 2.5% span length) with spinning potential of 40s HSC. It has a high boll load bearing potential in normal as well as stress situations. The Variety has spread fast and occupied large areas and could not find a replacement so far in variety areas. Another special feature of

Narasimha is the existence of somewhat higher residual variability for boll weight (3.5 to 5.0 g), highly sympodial nature and higher staple length (29 - 30mm) so that a large number of reselections have been made by private seed companies for use as parent in the hybrid cotton and transgenic hybrid cotton programme in the south and central India. This is because of the high and superior combining ability in the heterosis-breeding programme. The amenability for reselection has been a boon to many seed companies for their hybrid development programme in the south and central zone states of India. Thus in terms of germplasm utilization in India for a successful variety development programme, Narasimha could serve as a desired and highly sought after parent for heterosis breeding making it a unique achievement in skillful and effective choice of basic parents and their manipulation in breeding superior cultivars and as a parent, making it a two – in - one achievement.

30. Genetic diversity studies in introgressed lines of *G.hirsutum* cotton using cluster analysis

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Eighty two lines of *Gossypium hirsutum* along with two local checks developed out of introgression involving different wild species for tolerance to biotic and abiotic stresses and improvement in fibre quality at different Cotton Research Centres across the country were evaluated at Regional Agricultural Research Station, Lam during *kharif* 2003-04 to elucidate genetic divergence using a non-hierarchical Euclidean cluster analysis for yield and its components. The genotypes were grouped into ten clusters irrespective of geographic and genetic diversity. Cluster IX contained the largest number of sixteen genotypes and had the genotypes of heterogeneous origin, which showed that there was no parallelism between genetic and geographic diversity. The maximum genetic distance occurred between cluster III and VI. Cluster VI is monogenotypic and had high mean values for seed cotton yield, number of sympodia, boll weight, seed index, lint index, ginning out turn, 2.5% span length and maturity coefficient. It is further suggested to go for series of diallel analysis with the genotypes grouped in the above clusters for identifying superior heterotic combinations and isolating desirable recombinants in segregating generations. Genotypes were also identified which may serve as potent genetic donors for some metric and quality traits.

31. Genetic variability for cottonseed oil content and quality

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In addition to lint, cotton produces other useful products also. Cottonseed oil is the most important among them. Cottonseed contains hull and kernel. The hull produces fibre and linters. Cottonseed oil is extracted from kernel. Refined cottonseed oil is considered as one of the purest cooking media available. An additional benefit that accrues from cottonseed oil is its high level of antioxidants / tocopherols. Existence of genetic variability is the prerequisite for the genetical improvement of any trait. It serves as a reservoir of genetic wealth for a plant breeder to choose from. The seeds of desi cotton varieties are small in size in comparison to tetraploid species and contain lower percentage of seed oil. A wide array of variability for seed oil content in cottonseed has been observed among the germplasm collection as well as breeding material at Central Institute for Cotton Research, Nagpur. Significant genotypic and year differences were detected for oil content and fatty acid profile. Extent of year effects seemed to be generally higher in comparison to entry effects particularly for oil content and index. Highest seed oil content (29.8 %) was recorded in line Acala 5-1 among *G. hirsutum*, while among *G. barbadense* EC 97635 recorded highest seed oil content (25.8 %). Similarly sizeable variability has been recorded for fatty acid profile, which indicates the possibility of improvement in linoleic and oleic acid content through adoption of apt breeding strategies.

32. Study of genetic variability in introgressed lines of *G. hirsutum* cotton

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Reduction in genetic variation of cultivated cotton makes it vulnerable to biotic as well as abiotic stresses. With the changing needs of textile industry, genetic variability was created through introgression breeding at different Cotton Research Centres across the country involving wild relatives of cotton. So eighty-two introgressed lines along with two checks were evaluated for yield and its component traits at Regional Agricultural Research Station, Lam during *kharif* 2003-04 under Cotton Technology Mission Project to assess the nature and extent of genetic variability in the present material. These genotypes have showed considerable variability for all the traits studied. Wide range of genotypic coefficient of variation and phenotypic coefficient of variation, high heritability accompanied by high genetic advance was observed for number of bolls per plant, seed cotton yield per plant and number of monopodia revealing the role of additive gene action. Genotypic correlation coefficients were higher than the corresponding phenotypic one for most of the character combinations revealing the less influence of environment on these traits. The phenotypic and genotypic correlations revealed strong and positive correlation of number of bolls per plant with seed cotton yield. Path analysis further confirmed this relationship. It clearly indicated that boll number is amenable to selection. However, care should be taken in boll weight and boll number since they are negatively correlated with each other *Vis a Vis* boll weight is positively correlated with important fibre characters.

33. Pre-Breeding in cotton - A New Initiative

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Pre-breeding is defined as an interface between the germplasm evaluation and plant breeding. It is in fact an activity wherein the raw germplasm is refined, upgraded and made potentially useful for a breeding programme. Pre-breeding is governed by two approaches viz., introgression and incorporation. Introgression is the method of including desirable favourable alleles from one species into another. This is achieved by introducing a simple crossing programme between unadapted and elite populations. Further, backcrossing of F_1s with elite cultivars as recurrent parents leads to the inclusion of desirable traits from unadapted germplasm as donor parent. The second approach designated as incorporation is a process of broadening the genetic base of existing elite cultivars by introgressing the desirable traits from unadapted germplasm (indigenous or exotic). With the growing needs of developing superior varieties and the available potential of unused germplasm lines available in *G. hirsutum*, pre-breeding or developmental breeding was initiated at CICR, Nagpur to introduce the favourable untapped genes in unadapted germplasm into elite cultivar background. In order to broaden the base of the elite cultivars, these unused exotic germplasm were used as male parents and combined with a wide array of female parents selected across the three zones viz, North (irrigated), Central (rainfed) and South (irrigated and rainfed). The selection of parents as trait -specific donors was done from working collections of *G. hirsutum*. In the first season, seven parents as unadapted *G. hirsutum* germplasm were involved in a crossing programme involving ten elite cultivars as female parents. Thus, this base broadening approach led to evolution of F_1s. These are being evaluated in subsequent generation. In the second year, these F_1s were evaluated. In order to increase the parental base, more number of female parents from different zones were taken along with few new additional germplasm lines to be used as donor parents. The traits aimed at were mean halo length, lint index, ginning percentage, lint index, seed index, lint yield etc. The use of unadapted germplasm lines has resulted in enhanced level of heterosis for creating diverse heterotic genepools in *G.*

hirsutum. Three cross combinations recorded F_1 mean value of above 30 mm staple length indicating their superiority in staple length. One cross combination had the highest expression for boll weight, seed index and staple length. Thirteen cross combinations recorded the seed index of above 8 g; four cross combinations exhibited the lint index of above 5; nine cross combinations displayed high ginning outturn of more than 38%. Thus, an attempt to define the role or new unadapted germplasm lines has been initiated. This will lead to the development of new diverse genepools that would result in value addition to the breeding programme.

34. DUS Testing in cotton - in IPR perspective

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Among the fibre crops, cotton has a unique position as single largest source of natural fibre and plays an important role in the country's agrarian as well as industrial economy. Among the field crops, cotton is one of the important major crops that enjoy equal participation of public as well as private sector in its genetical R&D. This scenario manifested in terms of plethora of varieties and hybrids coming from public and private sectors makes it all more imperative to come out with a well defined system of varietal protection so as to avoid any possible disputes related to varietal or hybrid legacy. To have an effective and foolproof system of varietal protection under DUS concept, one has to identify a set of characters that remain true over the time and space. In a study conducted in Asiatic cotton (*G. arboreum*) at Central Institute for Cotton Research, Nagpur, on a few morphological traits, it was observed that a few particular traits viz., bract shape and size, leaf lobe, petal blotch, petal claw and orientation of locule in capsule stay unchanged over environments and filial generations and more importantly offer pronounced inter genotypic variability to fit into criterion of distinctiveness. Detailed investigation of a particular character like leaf lobe led to a huge number of sub sets on the basis of variation in leaf lobe shape & size, sinus and notch length, and vein reticulation and geometry. Similarly bract shape and size along with serration pattern observed at a specific stage of plant growth could also be an important criterion in varietal characterization. Petal blotch observed at the reproductive phase of plant in terms of its presence / absence & its geometry along with morphological variation in petal claw could also be used as an efficient differential trait. Further, efforts are on to develop a DUS index based on additional uniform and stable differential traits to serve as ready reckoner in determining distinctiveness of new varieties and hybrids.

35. Host plant resistance to insect pests in cotton - The Indian scenario

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Host plant resistance to insect pests forms the corner stone for effective pest management in cotton. The strength in India lies in its ready access to a vast germplasm pool belonging to the four species of *Gossypium* as well as to the wild races and species. On the other hand a large insect pest complex poses a tremendous challenge to successful cotton cultivation especially by the resource poor farmer. Cotton is attacked in the vegetative and reproductive phases by the sucking pests and / or the bollworm complex the latter which posses several adaptive traits like insecticide resistance and intra-specific variation. Till date, search for bollworm resistance in India has been elusive while breakthroughs have been achieved in identifying sucking pest resistant lines. Several reports do suggest the existence of morphological, biochemical parameters that impact host plant resistance to insects directly. These parameters are however, often influenced by the biotic and abiotic factors of the region wherein the germplasm line is evaluated. There have been very few examples in India wherein breeding for resistance against insect pests has been successful with the use of these parameters. This paper discusses the status of host plant resistance to insect pests in India. It categorizes important germplasm lines into lines tolerant to bollworms and sucking pests (especially jassids) with the help of the work done by the first author and published references already available. Proper identification and characterization of insect resistant germplasm has not been satisfactory thus far mainly due to non - availability of appropriate screening facilities, procedures and efforts. A SWOT analysis of the procedures adopted in evaluating host plant resistance to insect pests in India is presented with special reference to bollworms and jassids. It suggests the identification of location specific promising insect tolerant lines that need to reach the farmers field through intensive breeding procedures carried out by special workgroups identified for the purpose.

36. Scope for development of abiotic stress tolerant cotton (*Gossypium hirsutum* L.) through *Agrobacterium*-Mediated transformation

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Plants experience a wide range of environmental suboptimal conditions during their life cycle. To survive these adverse conditions plants exhibit a number of responses including metabolic adaptation. Low water availability, high salt and low temperature are among the most common environmental stresses for plants. Agricultural and the damaging effects of salt accumulation in agricultural soils have influenced ancient and modern civilizations. The detrimental effects of salt on plants are a consequence of both a water deficit that results from the relatively high solute concentrations in the soil and a Na^+ specific stress resulting from altered K^+ / Na^+ ratios and Na^+ ion concentrations that are inimical to plants. The alteration of ion ratios in the plant is caused by the influx of Na^+ through pathways that function in the acquisition of K^+. Genetic engineering provides powerful tools to enhance the modification of plants to the potential benefit of society and genetic manipulation of crop plants for enhanced abiotic stress tolerance holds great promise for sustainable agriculture. Abiotic stresses have been shown to have a quantitative character, and thus they are controlled by multiple genes. However, there are number of instances where single-gene transfers have led to the development of tolerant plants. The increasing problem associated with the rise in soil and water salinity is a major threat to agricultural productivity worldwide. Recent reports have described production of transgenic plants with improved tolerance to salinity after transfer of a single gene such as Na^+/H^+ antiporter. Recently, over expression of trehalose biosynthetic genes has also contributed towards development of abiotic stress-tolerant genotypes in rice. But considering the complex metabolic reactions operating in the cell in response to abiotic stresses, there is a need to test the possible contribution of other candidate genes towards this multigenic trait. The glyoxalase system has been long known in animal systems and has been proposed to be involved in various functions that include regulation of cell division and proliferation, microtubule assembly, and protection against oxoaldehyde toxicity. Glyoxalase enzymes are important for the glutathione (GSH) based detoxification of methylglyoxal (MG), which is formed primarily as a

byproduct of carbohydrate and lipid metabolism. MG is a potent mutagenic and cytotoxic compound known to arrest growth, react with DNA and protein, and increase sister chromatid exchange. The reaction catalyzed by glyoxalase I (gly I) and glyoxalase II (gly II) is as follows: First Methyl glyoxal + GSH converted into hemithioacetal Secondly Gly I act on hemithioacetal and converted into S-D lactoyl GSH Finally the Gly II act on S-D lactoyl GSH and converted into D-lactic acid + GSH. The physiological significance of the glyoxalase system has not been clearly defined in plants; however, this system has been often regarded as a "marker for cell growth and division". Gly I has been shown to be up-regulated in tomato in response to salt and osmotic stress and to phytohormonal stimuli. Based on the importance of Glyoxalase system in salinity tolerance, we designed the experiments to over express the Glyoxalase I gene (Gly I) for improved salinity tolerance in cotton. Cotton is the world's leading natural fibre and the second largest oilseed crop. Cotton is a mainstay of global economies and it is considered as the number one value-added crop in the country. It provides benefits to the Indian economy, both in terms of domestic use and as a trade commodity in world market. The details of the aim of this work and results will be discussed. In the pioneering work by Veena *et al*. (1999) and Singla-Pareek *et al*. (2003) showed that over expression of Gly I leads to salt, drought and heavy metal stress tolerance in Brassica and tobacco respectively. The work being carried out in our laboratory has great potential to provide salt and drought tolerant transgenic of cotton, which would have long term beneficial effects and help the poor farmers.

37. Genotype specificity of callus induction and somatic embryogenesis in cotton germplasm

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Cotton is most important fiber crop being cultivated all over the world. Regeneration of cotton by somatic embryogenesis is an important aspect for micropropagation, germplasm preservation, synthetic seed technology and transgenic technology. Cotton is recalcitrant to regeneration by somatic embryogenesis. The regeneration of cotton *G. hirsutum* cv. Coker-312 by somatic embryogenesis (Trolinder and Goodin, 1987) led to the development of first transgenic cotton by incorporating Cry IA gene in the world. The other genotypes, which exhibit somatic embryogenesis, include Coker-310 (Davidonis and Hamilton, 1983; Kumria *et al*., 2003 and Leelavathi *et al*., 2004), Coker-315 (Finer, 1988) and MCU-5 an Indian cultivar (Kumar and Pental, 1998). Direct shoot organogenesis by multiple shoot induction (Gupta *et al*., 1997; Agrawal *et al*., 1999; Nandeshwar *et al*., 2002) is another alternative which may prove rewarding to micropropagation but may not be useful at the same time for transgenic cotton development because of frequent chimeric effect. At CICR, Nagpur over 10,000 germplasm lines belonging to *G. hirsutum*, *G.arboreum* and *G.herbaceum* are being maintained. About 50 germplasm lines from both *G.hirsutum* and *G.arboreum* were selected from germplasm pool. These were evaluated for somatic embryogenesis. Seven days old *in vitro* germinated seedlings, grown under dark condition initially constituted the material for tissue culture. Upper, middle and lower hypocotyl sections of all germplasm lines were isolated aseptically and cultured in callus induction medium. The callus induction medium consisted of Murashige and Skoog basal salts with different combinations of growth regulators involving 2,4-D, Kinetin, NAA and IAA. In some of the media combination anti-oxidant like PVP and AA were used to minimize tissue browning. In each combination 30 explants of upper, middle and lower hypocotyl sections replicated twice, were used. Depending on the response of genotypes, three Coker lines namely Coker-W-9, Coker-413 and Coker-wild staple were crossed with adaptive cultivars like Bikaneri narma, Khandwa-3, LRK-516, DHY-286 and Vikram and their F_1 were also evaluated *in vitro* for embryogenic response. Most of the germplasm lines showed callus proliferation within a week. After 20-25 days, the whole explants formed callus. The developmental pattern, texture and coloration of callus were highly depended on the genotype beside type of growth regulators used.

In case of upper hypocotyl sections coupled with either 2,4- D + Kin (0.1 mg/L each) or 2,4- D (0.1 mg/L alone) formed callus which was mostly friable and pale in colour. In media containing IAA or NAA (2 mg/L) + Kin (1 mg/L), callus was compact and hard in nature with brownish - green sectors. In some cases it was brownish-yellowish also. But in both the cases there was frequent root formation from nodular structure embedded within callus mass. Many globular structures appeared on the callus surface. These were confirmed by cytological observation to be early stage somatic embryos. Efforts were made to induce growth and maturity of somatic embryos. Genotypes Khandwa-3, PKV-081, Coker lines and some of the lines like MHR-10 and MHR-14 were observed to be embryogenic in nature.

38. The use of novel fluorescent proteins as visual markers in cotton transformation

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Three novel fluorescent proteins (NFPs) obtained from ClonTech have been assessed for their suitability in cotton transformation optimisation programmes. These include AmCyan from *Anemonia majano*, ZsGreen from *Zoanthus* sp. and AsRed from *Anemonia sulcata*. All three NFPs allow for the non-destructive visualisation of transgenic foci without the requirement for any external substrates or co-factors. Parallel experiments were performed to compare the three proteins with the aim of identifying the FP most suitable for transformation improvement. The constitutive Cestrum Yellow Leaf Curling Virus promoter was used to drive expression of the NFP coding sequences, the promoter: FP fusions were cloned into binary vectors for *Agrobacterium* mediated DNA delivery. Both transient and stable expressions of all three NFPs were visualized in transformed cotton cells using fluorescence microscopy. Fertile transgenic plants containing the NFPs have been obtained. The results suggest that AmCyan is the best visual marker. Detailed observation of early event tracking and expression patterns in the mature transgenic plants and their offspring will be presented.

39. SSR marker location and analysis of genetic similarity of fiber development mutants in cotton

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The new fiber mutant GZnn was analyzed using traditional genetic method and it was sure that GZnn was a qualitative trait controlled by one recessive gene. Using SSR molecular marker technology, we located this gene on chromosome 10. Since its position was different from n1 (on chromosome 12), n2 (on chromosome 26) and n3 that have been found recently in America, we named it n4. The gene was closely linked with marker sloc1 and their distance was 10.8 cM. The gene was also linked with sloc2-1 with their distance of 20.4 cM. Using SSR marker technology, we undertook genetic polymorphism analysis on 3 groups of fiber mutant NILs (Near Isogenic Lines) and their SSR fingerprint atlas had been also built. The similarity coefficients between Xin and Zh12, Xin and Yu4 were 0.471 and 0.587 respectively. They differed from each other significantly. Therefore, Xin may originate from Yu4. XZ142w and XZ142 belonged to the second group of NILs. Although having only differences of fiber and fuzz, they showed large genetic differences, and 181 polymorphism SSR allele loci were found between them. XZ142w possessed similar mutant characters as Xin and their SSR genetic similarity coefficient reached 90 percent. However, their SSR fingerprint atlas showed they had different patterns, which maybe due to their different genetic background. SSR genetic similarity analysis also revealed the difference between 4 other NILs (TM-1, GZnn, GZNn and H-154) was significant and the similarity coefficients ranged from 0 .5 to 0.62. This proved that dominant or recessive mutation could lead to large genetic difference. But the similarity coefficient of 2 fiber mutants H-154 and GZnn reached 0.94, which resulted in their same phenotype of fuzzless-linty character controlled by single recessive gene. With the application of software NTSYS 1.8, cluster analysis was also carried out on TM-1, GZnn, GZNn, H-154, Xin and XZ142w and the Dendrogram of these 6 lines was also built. When the genetic distance was 3, the 6 lines could be divided into 3 groups. GZnn, H-154 and GZNn belonged to one group and all of them were fuzzless and linted. Meanwhile, the mutants Xin and XZ142, which were fuzzless and lintless lines, were clustered in another group.

Key words: cotton fiber mutant; SSR marker; fingerprint atlas.

40. Preliminary study on the biochemistry and molecular biology of colored cotton

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Colored cotton is a kind of cotton with natural color in its fiber. Since the colored cotton needs no artificial dyeing, the garments made by it are prized as "green clothing". Also colored cotton is healthy and profitable for mankind and its prospective market is very wide, which will trigger a revolution in the textile and clothing industry in the future. Recently we have made some progress on the research of colored cotton: 1. The SSR fingerprint atlas of 18 colored cotton lines had been obtained based on SSR technology. After 110 pairs of SSR primers screening, we got 10 pairs that had excellent amplification results. Then 4 of them were used to build a molecular searching pattern of these colored cotton lines. Meanwhile, cluster analysis had been made using the 10 pairs of primers, and we also made a discussion on their phylogenetic relationship. 2. Different methods (methanol dipping, ethanol distilling, HNO_3 / ethanol distilling) of extracting the pigment in fiber were compared and there was obvious color in the ethanol extraction liquid. Chemical tests proved that there were flavonoids in the ethanol extraction liquid of green cotton. We also found the wax content of white cotton TM-1 was lower than that of four green cotton lines. Among these lines, the wax content differed from each other with the range from 6.25 to 18.35 percent and there was no relationship between the fiber color and the wax content. 3. The textile made by brown and green cotton had been treated in different ways (acid, alkali, water, soap fluid, washing powder, different washing times and drying methods, etc.). Analysis on color system revealed: acid could deepen the color of colored cotton, alkali could deepen the color of brown cotton while made the color of green cotton lighter; the washing effect of water and soap was better than that of washing powder; the effect of cool drying was better than that of drying in the sun.
Key words: colored cotton, SSR, Pigment, Wax.

41. Fiber characteristics of major *Gossypium barbadense* germplasm pools contributing to the development of American Pima cotton

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The cotton breeding and molecular genetics communities have devoted considerable attention to the *Gossypium barbadense* L. species in their efforts to improve fiber quality in upland cotton. However, the range in fiber attributes within *Gossypium barbadense* often has been overlooked. An overview of the genetic contributors to the creation of the modern Pima germplasm and their unimproved progenitors serve to illustrate the fiber variability inherent in the species. The modern Pima germplasm is the result of a series of hybridizations among lines originating in the Sea Island, Egyptian, Peruvian Tanguis, and American Egyptian germplasm pools, among others, and all possess distinctive fiber characteristics. Sea Island cottons are characterized as possessing exceptional fiber length and fineness, accompanied by low lint percentages and lower fiber strengths than present in modern extra-long staple cultivars. Egyptian cultivars vary considerably in their fiber traits, but are generally characterized as possessing high fiber strengths. Peruvian Tanguis cottons are distinguished by their fibers with exceptionally high Micronaire values, shorter fiber lengths, and high dye efficiencies. Among unimproved or land race *G. barbadense* accessions, there is little evidence of the fiber strengths or lengths achieved within modern cultivars. These accessions are characterized by fiber length that rarely exceeds 28 mm and strength that rarely exceeds 25 cN. In the investigationandutilizationof *G. barbadense* germplasm, awareness and attention to the broad range of variability available, will benefit the nvestigator.

42. Secondary gene pool contributions in domesticated cotton

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Pedigree analysis indicated that the Upland cotton gene pool, *Gossypium hirsutum*, have substantial exposure to both secondary and tertiary gene pools. However, the extent of the contribution of these donors is largely unknown. We surveyed about 330 cotton lines/cultivars using over 300 evenly spaced RFLP loci to address the amount and genomic locations of introgressed genes in the cotton genome. Preliminary analysis indicated that the cotton genome is largely homogeneous, and that rare alleles, which have likely arisen through introgression, are restricted to only a small group of cultivars. Historical accounts indicate that these cultivars were clearly developed through interspecific hybridization. These results suggest that the use of secondary and tertiary gene pools remains largely uncapitalized in cotton improvement.

43. Differential response of four Haryana genotypes of cotton for callus induction and somatic embryogenesis

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Plant regeneration in cotton is highly genotype specific and major limiting factor in developing cotton transgenics of commercial value. The study was undertaken to develop regeneration system in four cultivars of Haryana viz. H777, H1117, H1098 and H974. The hypocotyls, immature embryos, cotyledons and anthers were used for callus induction. Out for several media tried, MS medium supplemented with 0.1 mg/l 2,4-D and 0.1 mg/l NAA showed best callusing response. H1117 and H777 showed better callus induction over the other cultivars. The induced calli were sub-cultured on MS medium with B5 vitamins, 1.9 g/l KNO_3, 750 mg/l $MgCl_2$ for initiating embryogenesis in callus cultures. The calli were also transferred to liquid medium for developing embryogenic suspension cultures. The cultures turned embyogenic and developed somatic embryos. The liquid cultures showed higher rates of embryogenesis and somatic embryos at different stages of development. The embryo germination after brief dessication treatment was observed, albeit at very low frequency which needs further investigation.

44. Variability for fibre quality characters in Indian *Gossypium barbadense* germplasm accessions

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The super fine extra-long staple cotton varieties capable of spinning more than

100^{s} counts are mainly bred from the *G. barbadense* species of cotton. In India, Suvin (released in 1976) is the only barbadense variety being cultivated commercially, which has spinning potential of upto 120^{s} counts and is spun exclusively for export purpose. The main draw back of this variety is the poor fibre maturity as reflected in low micronaire value (2.2 to 2.5), which causes difficulty while spinning at higher counts. Hence, a search was made in the germplasm accessions of *G. barbadense* being maintained at Central Institute for Cotton Research, Regional Station, Coimbatore (the National Active Germplasm Site for *G. barbadense*). About 318 accessions were evaluated for fibre quality along with Suvin using High Volume Instrument (HVI) to assess the variability for the characters *viz.*, 2.5 % span length (mm), uniformity ratio (%), micronaire, bundle strength at 3.2 mm gauge (g/tex) and elongation percentage.

The data indicated wide variability for elongation percentage (CV=35.8 %) and micronaire (CV=18.8%). Several accessions had one or more superior fibre quality character than the variety Suvin. For micronaire, the highest value recorded was 4.6 as compared to 2.2 noted in Suvin, while the mean value was 3.2. For 2.5 % span length and bundle strength also higher value was recorded in germplasm accession than the Suvin. The highest value observed for 2.5 % span length was 37.8 mm (for Suvin, 36.4 mm) and for bundle strength it was 30.4 g/tex (for Suvin, 26.6 g/tex). Since, for bundle strength also superior accessions are available in germplasm, simultaneous improvement for micronaire and bundle strength may be undertaken. Because, for high speed open end spinning, higher bundle strength is desirable.

When correlation coefficients were calculated between these fibre quality characters, it was found that 2.5 % span length was positively correlated with bundle strength and elongation percentage, whereas, it was the other way with micronaire. Similarly, micronaire was negatively with bundle strength and elongation percentage. Hence, care should be taken while improving the micronaire, because it is likely to affect the 2.5 % span length and bundle strength. The present study clearly indicates that it is possible to improve the micronaire and bundle strength of the variety Suvin utilizing the variability available in the germplasm following suitable breeding

methods including molecular marker based selections. Molecular markers for these specific fibre properties are of immense interest to effect suitable improvement in fibre properties.

Key words: 2.5% Span Length, Micronaire, Bundle Strength, High Volume Instrument

45. Interspecific hybridization between *Gossypium hirsutum* and *Gossypium aridum* – Cytomorphological and RAPD analysis

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The cultivated tetraploid species *Gossypium hirsutum* cv. Anjali (belonging to AD genome) was crossed with a wild diploid species, *G. aridum* (D4 genome) and the hybridity of the cross assessed using Random Amplified Polymorphic DNA (RAPD) analysis apart from morphological and cytological characterization. Morphologically the hybrids were taller than their maternal parent, with more number of nodes/plant, monopodia/ plant, sympodia/ plant, inter node length, number of leaves, stem girth and number of squares. However, the triploids had lesser leaf area and petiole length than their tetraploid female parent. The floral morphology of the hybrid was intermediate between the parents. The triploid flowers are characterized by light purple petal with shallow petal spot and had intermediate bract size.

Meiotic study of the hybrid at metaphase I indicated 39 chromosomes while the parents showed 52 and 26 chromosomes respectively in *G. hirsutum* and *G. aridum*. The mean diameter of the pollen grains was the least in triploid (55.7 μm) as compared to 96.6 μm and 107.7 μm recorded in *G. aridum* and *G. hirsutum* cv. Anjali, respectively.

The hybridity was also confirmed using RAPD molecular markers. When the parents and the hybrid was screened with 60 random primers, 21 primers generated polymorphic marker bands. Based on the presence or absence of DNA bands, seven types of DNA markers (Type I-VII) could be classified. Of these, Type IV markers shared bands in male parent and hybrid, which is more useful in identifying status of the cross.

Presence of about 13.3% Type IV markers clearly confirmed the hybrid status of the cross. Genetic similarities were calculated to determine the genetic relatedness between the parents and offspring, which indicated that the triploid hybrid was more similar to female parent (65%) than to the male parent (55%).

Key words: Cotton, Interspecific hybrid, Molecular marker

46. Temporal expression of metabolic and gene products during fibre elongation in Cotton genotypes

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Cell expansion has been reported to be controlled by genetic, hormonal and environmental factors. Experiments were carried out to study the significance of temporal expression of metabolic and gene products during cell expansion under *in vitro* and *in vivo* conditions. It was observed that short staple cotton cv. *Arogya* had higher reducing sugar and total phenols in seeds during fibre elongation phase compared to medium staple cv. *LRA 5166* and extralong staple cv. *Suvin*. Reducing sugar content in fibre was seen at higher level in *Suvin*, followed by LRA.5166 and *Arogya*, thus indicating better solute accumulation in *Suvin* for efficient positive turgor during fibre development. Higher levels of IAA oxidase (15 units) and Peroxidase (24 units) were seen in cv. *Arogya*, while cv. Suvin had lower levels of these enzymes (9 & 17 units, respectively). The higher activity of these enzymes during fibre cell elongation and secondary wall thickening can have profound effect on cellular levels of IAA and act as a limiting factor for fibre elongation. Higher total phenols coupled with higher IAA oxidase and peroxidase activities besides lesser fibre to seed reducing sugar content act as regulating factors during cotton fibre elongation.

As regards *in vitro* ovule culture, fertilised ovules showed fibre initiation that was independent of hormone source, while fibre elongation was observed only in presence of IAA and GA. The presence of GA was prerequisite all through the culture for fibre initiation when unfertilised ovules was utilised. Comparative aspects of regulation of cotton fibre growth under *in vitro* and *in vivo* conditions will be discussed. Efforts are underway to investigate further the specific gene products associated with fibre quality traits like higher length and strength and enhanced thermal properties through introgression of useful genes from wild and elite cultivars.

47. RAPD analysis in wide hybrids of cotton for bollworm tolerance

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Wide hybridization through introgression of useful genes offers good and steady scope for generation of newer materials. Identification and characterization of biochemical metabolites, metabolic process intermediates and utilization of molecular approaches form the basis for identification of bollworm tolerant materials and newer genotypes of cotton. Eight hybrids developed by involving wild species of cotton viz., *G.armourianum*, *G.aridum* and *G.raimondii*, known for their tolerance to insect pests and four popular cultivars viz., LRA.5166, Sumangala, Anjali and Surabhi (*G.hirsutum*) were subjected to RAPD analysis for hybridity confirmation and elucidation for gene transfer from wild parents. The primers used were found to be polymorphic among parents. Derivatives generated from introgressed materials viz., IRH I-6, IRH II-1, IRH II-15, based on lower incidence of bollworms, were also seen to possess biochemical intermediates and metabolic activity to support bollworm tolerance.

48. Molecular elucidation of gene and gene products for abiotic stress response in cotton

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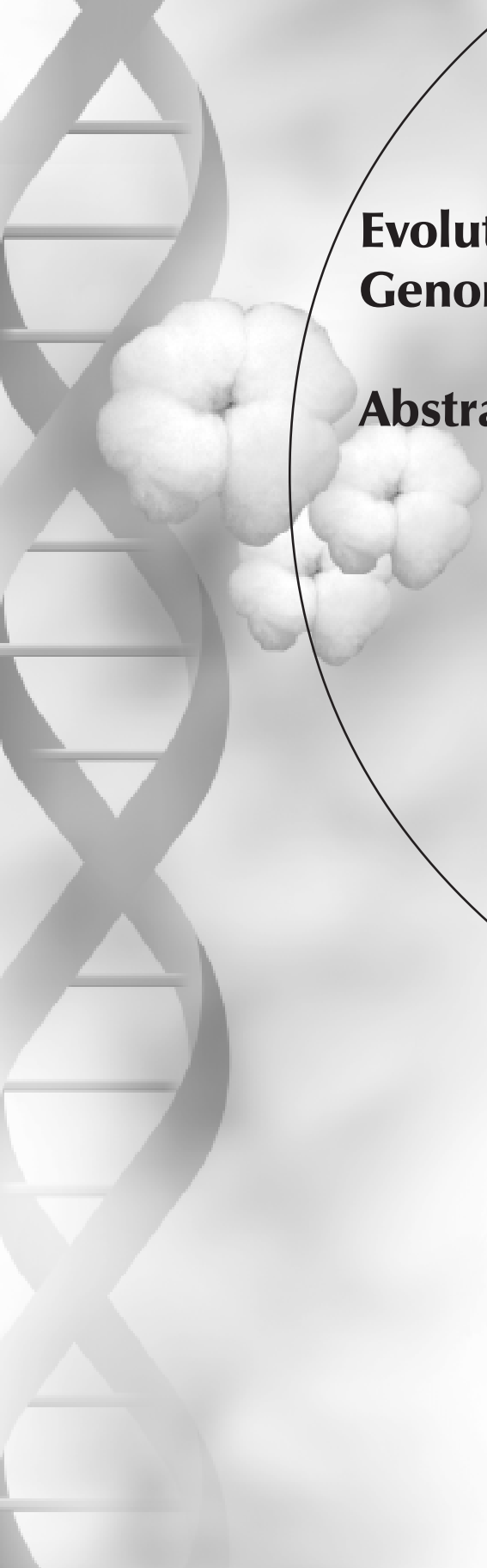
Central Institute for Cotton Research, Regional Station, Coimbatore – 641 003 (India)

Molecular, physiological and biochemical approaches shall unravel the underlying principles of mechanisms of adaptation for achieving profitable yields of cotton even under adverse abiotic stresses. The complex biochemical and physiological processes acting in an integrated way have been studied in cotton genotypes/hybrids with moderate tolerance to waterlogging, salinity and drought stress. Accumulation of osmoprotectants, free radical detoxification, enzyme activation and desirable metabolic changes play key role in stress tolerance. Higher level of activities of enzymes *viz*., nitrate reductase, glutamine synthetase, glutamate dehydrogenase and peroxidase in moderately drought tolerant genotypes point to their possible role in overcoming stress, while susceptible varieties could be characterized by quantum rise in activities of RNase, acid and alkaline phosphatases. The tolerant cotton genotypes exhibited higher photosynthetic rate, chlorophyll content and moderate transpiration rate, while susceptible genotypes revealed drastic reduction in these parameters under waterlogged conditions. Impaired nitrogen assimilation enzyme system was apparent in genotypes susceptible to waterlogging. The temporal distribution of peroxidase was markedly affected in susceptible genotypes, explaining their reduced growth and development due to improper quenching of free radicals. The deleterious effect of chloride and sulphate ions on the growth and metabolism of cotton seedlings could be partly overcome due to application of phytohormones through their beneficial influence on restoring the activities of metabolically important enzymes.

The prospects of inserting desirable foreign genes have opened up new vistas for achieving the goal of development of newer plant types. The breeding programmes, both conventional and modern, including gene isolation, characterization and biochemical markers will help in introduction of favourable tolerance traits into cotton as in the case of other field crops. Wide hybridization techniques can be of help to introgress tolerance genes from wild germplasm of cotton. Intensive efforts are needed to custom-tailor biochemical mechanisms, stress responsive genes, DNA sequencing, gene regulation, protein targeting and suitable selection procedures for generation of transgenic cotton plants with superior quality and high yielding ability even under challenging and adverse situations.



Evolutionary and Comparative Genomics Session

Abstracts

1. Comparative genomics of cotton and *Arabidopsis*

***ANDREW PATERSON**, Distinguished Professor, University of Georgia, Athens, USA*
Junkang Rong, University of Georgia, Athens, USA
John Bowers, University of Georgia, Athens, USA

2. LINEs and gypsy group retrotransposons in *Gossypium* sp.

ESSAM ZAKI, *Department Head, GEBRI, Egypt*

3. A comparative genetic linkage map of tetraploid cotton (*Gossypium hirsutum* X *G. tomentosum*)

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Junkang Rong, Plant Genome Mapping Laboratory, University of Georgia, Athens, Georgia 30602, USA
Carl J. Rogers, Gary J. Pierce, Plant Genome Mapping Laboratory, University of Georgia, Athens, Georgia 30602,
Jonathan F. Wendel, Department of Ecology, Evolution, and Organismal Biology, Iowa State University, Ames, IA, USA
Andrew H. Paterson, Plant Genome Mapping Laboratory, University of Georgia, Athens, Georgia 30602, USA

Comparative mapping is a powerful approach by which to study genome evolution, genetic differentiation and reproductive isolation in diverging species. *Gossypium* comprises 45 diploid and 5 allotetraploid species, the latter involving the rejoining of A and D sub-genomes in a common polyploid nucleus about 1.1-1.9 mya. We report a STS based genetic map (HT) of *G. hirsutum* (GH) and *G. tomentosum* (GB), a Hawaiian endemic tetraploid that offers new information to plant biology and new opportunities to crop improvement. The HT map was compared with a HB map developed from cultivated tetraploid GH and *G. barbadense* (GB). The HT map comprises 589 loci spanning a total recombinational length of 4259.4 cM. The HT and HB maps were aligned by virtue of 165 anchor loci (based on the same size of restriction fragment produced using the same restriction enzyme in both population) and 119 supporting loci (different enzyme used but mapping to the locations that were consistent with those of closely linked loci). The arrangements of loci along the chromosomes of both maps are similar, but not identical. Recombination rates are closely correlated between the HB and HT maps, although there is generally higher recombination in HT. Terminal paracentric inversions on chromosomes 3, 10 and 15 distinguish among the polyploids. Relative densities of the markers along each chromosome were uncorrelated, suggesting that the evolution of genetic polymorphism in GB and GT have been subjected to different forces. At least 63 DNA probes, that were monomorphic in HB, detect a total of 86 polymorphic HT loci. Comparative analysis of the nectariless trait provides a possible example of differential subfunctionalization, in which different polyploid lineages have allocated a common function to different homoeologous genes.

4. Genetic linkage mapping of the *Gossypium* tertiary genepool

CURT BRUBAKER, *Cotton Genetecist, CSIRO Plant Industry, Australia*

5. Microsatellite allelic diversity within tetraploid *Gossypium* germplasm

***JM LACAPE**, Researcher, CIRAD, France*

6. Molecular analyses of induced photoperiod related mutations in cotton

***IBROKHIM ABDURAKHMONOV**, Institute of Genetics and Plant Experimental Biology, Yuqori -Yuz, Kibray region, Tashkent district 702151, Uzbekistan*
F. Kushanov, F. Djaniqulov, Z. Buriev, N. Fayzieva D. Toshmukhamedova and A. Abdukarimov, Institute of Genetics and Plant Experimental Biology, Yuqori -Yuz, Kibray region, Tashkent district 702151, Uzbekistan

Photoperiodic response of plants is one of the important physiological reactions that determines an important aspect of plant development in ontogenes is such as plant flowering. Furthermore, photoperiodic response in plants is species-dependent complex networking process and connected with light signal perception, circadian rhythm and molecular clock pathways. Although photoperiodic pathway genes are well studied in model plant systems, cotton photoperiodic response genes and their significance in cotton photomorphogenesis have not been studied yet. Hence, determinations of molecular basis of cotton photoperiodic response is of particular importance since understanding cotton flowering will be useful in manipulation of wild photoperiodic cotton germplasm in applied breeding. One of the approaches toward this goal is molecular screening of photoperiod released induced mutant germplasm to identify candidate loci for further tagging of useful photoperiod related mutations (cottonseeds of several photoperiodic wild cottons).

7. ISSR analysis of wild species of the genus *Gossypium*

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Twenty-two wild species belonging to seven genomic groups were studied using ISSR-PCR marker to establish phylogenetic relationship within the genus. Among 55 ISSR primers tested, 19 ISSR primers were found to be scorable on agarose gel and 88% markers were scored as polymorphic. Genome specific and unique markers were observed in this study. In support of the evolutionary study, two major clusters were found in the dendrogram, one composed with A and B genome species while other contained D, AD and C genome species. E, F and G genome species were placed outside the major cluster at the end of dendrogram. These results suggested that ISSR-PCR markers are potentially useful to study the genetic relationship among the wild species of genus *Gossypium* and their evolution.
Key words: ISSR, *Gossypium*, Wild Species, Genetic relationship.

8. Molecular marker based genetic purity testing of cotton hybrid

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Vilas Parkhi, Biotechnology Section, Central Institute for Cotton Research, Post Box No. 2, Shankarnagar P.O., Nagpur-440010, India

Hybrid cotton H 6 and its parents G.Cot.10 (male) and G.Cot.100 (female) were studied for identification with three PCR based molecular markers, RAPD, ISSR and microsatellite. Twenty RAPD primers, nineteen ISSR primers and twenty-five JESPR cotton microsatellite loci were used. RAPD primer OPA 11 was found to be successful in differentiating parents and hybrid. Two ISSR primers, IS4 and IS7 showed polymorphism in the parents. IS4 identified a female-specific amplicon of about 500bp and IS7 identified two female-specific amplicons of about 500 and 1200bp in the hybrid H 6. Microsatellite loci JESPR-2 and JESPR-17 were found to be heteroallelic for parents. JESPR-2 identified one male-specific repeat of about 850bp, while JESPR-17 detected two male-specific repeats of about 800bp and 700bp in the hybrid H 6. Results indicated that using all three markers - RAPD, ISSR and SSR - in combination is faster and more reliable than using the three in isolation.
Key words: *Gossypium*, ISSR, RAPD, SSR, hybrid.

9. Marker-associated breeding of cotton sorts

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Ludmila Saranskaya, Institute of Genetics and Plants Experimental Biology, Tashkent Region, 702151, Kibrai, Uzbekistan

On the base of marking of such important traits as tolerance to wilt, salinity and drought, earliness, fiber type and its quality, productivity of heterosis F_1 is shown as a possibility for acceleration of breeding process and improvement of existing cotton varieties. The estimation of breeding materials takes place on the seed level (genotypes) with use of marked enzymes and proteins. This technology opens wide perspectives for use in seed grow, estimation of genetic homogeneity and reveal the varietal purity, and also long maintenance of good variety in production. Using systematic analysis of seeds on all marked traits it is possible to reveal the most adaptable to unfavorable environmental factors genotypes with further their propagation. In the basis of this technology lies analysis of genotypes. A population, developed from such genotypes, has a high morpho-physiological homogeneity. On the base of the elaborated technologies we have obtained lines exceeding the initial sorts by many economic signs. This technology is also applicable to other types of plants.

INTERNATIONAL cotton genome INITIATIVE

Bioinformatics Session

Abstracts

1. Analysis methods for differential gene expression of cDNA microarray data

***JUN ZHU**, Dean and Professor, Zhejiang University, Hangzhou, China*

2. Using statistical methods to evaluate cotton EST libraries

***E TALIERCIO**, Research Geneticist, USDA – ARS, USA*

3. Comparative genomics in fiber traits: from *In silico* prediction to *In vivo* validation

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Fiber yield is a complicated, multigenic trait in cotton, and is affected by various regulatory and biochemical pathways. Shortage in molecular and genomic data in cotton reduces the opportunity for fiber improvement via genetic engineering techniques. To boost the genomic data for gene mining in cotton we have employed a comparative genomics strategy. We compared gene regulation between cotton fiber and leaf trichomes, as it was previously demonstrated that both tissues share a common development trail. Gathering the accumulated data on trichome development in *Arabidopsis* and examining the large genomic data in tomato (over 160,000 ESTs) and tomato trichomes (over 7,000 ESTs) enable us to find homologous genes which possess similar expression pattern in cotton fiber and Arabidopsis/tomato trichomes. ESTs and known cDNA sequences of cotton and tomato, respectively, available at NCBI (http://www.ncbi.nlm.nih.gov/) were clustered using LeadsTM software (Compugen Ltd). The result was 3,404 and 12,800 high quality predicted genes (clusters) of cotton and tomato, respectively. Homology comparison between cotton and tomato clusters revealed 1,781 highly homologous clusters. Out of them 242 of the clusters were built from high percentage of fiber ESTs in cotton and trichome ESTs from tomato. This group of 242 predicted genes is expected to fulfill roles common to fiber and trichome development. This group also includes clusters homologous to *Arabidopsis* genes, already known to affect trichome density (i.e. initiation) and structure (i.e. development). Expression patterns measured using qRT-PCR and RNA extracted from developing fibers and other plant tissues resulted in the association of 23 unknown genes to one of the fiber developmental stages (initiation, elongation, cell wall thickening and maturation). In order to further validate their putative role in cotton fiber morphogenesis, we are about to transform tomato and *Arabidopsis* utilizing constructs that bear the above genes under different promoter combinations. The transgenic plants will be then analyzed for modified trichome appearance.

4. Implementation of a cotton crop module to the TropGENE-DB information system

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***LACAPE JEAN-MARC**, CIRAD, Avenue Agropolis, Montpellier cedex 5, France*

The crop information system, TropGENE-DB developed at Cirad for cocoa, sugar cane and banana (Ruiz *et al*., Nuc Acid Res, 2004, 32: 364-367) has recently been implemented with the marker information and genetic mapping data obtained in our laboratory for cotton. TropGENE-DB is based on the AceDB database management system. The object-oriented system provides usual browsable classes and visualization tools; it also includes a graphical user interface that has been coupled with the Cmap software genetic map viewer (http://www.gmod.org/cmap/index.shtml). The data that is now accessible through the internet at http://tropgenedb.cirad.fr comprise the primer sequence and loci information derived from the series of 392 in-house cotton microsatellites
markers. An always updated version of the interspecific *G. hirsutum* x *G. barbadense* (Guazuncho 2 x VH8) genetic linkage map is also accessible. The nomenclature of markers, loci, and chromosomes / linkage groups, were standardized in order to fit with the most recent international reports on cotton genetic mapping. Data, such as sequence, locus, map data, can be easily submitted for their incorporation in TropGENE-DB using standard Excel submission files. Illustrative of the usefulness of the system, the synteny relationships based on common bridge markers between the 2 densest genetic maps of tetraploid cotton presently publicly available (those of the University of Georgia and of Cirad), can easily be visualized. The future developments of the cotton module of TropGENE-DB include (1) the integration of the phenotypic description and of the microsatellite allelic data of the *Gossypium* collection of Cirad, and (2) the description and localisation of fibre quality QTL data.

5. On-line cotton genomic resources at the University of Georgia

***ANDREW PATERSON**, Distinguished Professor, University of Georgia, USA*
Alan Gingle, Plant Genome Mapping Laboratory, University of Georgia, USA
Hongyu Yang, Plant Genome Mapping Laboratory, University of Georgia, USA
Jamie Estill, Plant Genome Mapping Laboratory, University of Georgia, USA

6. Current status of cotton genomic information on the web and scope for improvement

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P. Vidyasagar, Vibha Agrotech Limited, Hyderabad - 500 082, India
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Cotton is the leading natural fiber and the principal raw material for the textile industry. Of the 50 species of the genus *Gossypium*, four are cultivated namely *G. hirsutum* and *G. barbadense*, which are tetraploid (2n=4x=52) and *G. arboreum* and *G. herbaceum*, which are diploid (2n=2x=26). *Gossypium hirsutum* is by far the most widely grown species worldwide, contributing to nearly 90% of the global production, followed by *Gossypium barbadense* with 8%. The two diploid Asiatic cottons contribute to less than 2% of global production and are primarily confined to India and certain countries of Asia. Molecular marker techniques such as RFLP, RAPD, AFLP and SSR have facilitated the development of extensive genetic and physical maps of the cotton genome. With the advent of the genomics era, a deluge of information has started flowing, in the form of ESTs, GSS, STCs, partial and complete coding sequences. This surge in cotton genomic content has been made possible by BAC library cloning, shotgun sequencing, contig assembly, microarray technology and the application of numerous bioinformatic tools. The flood of cotton genomic information thus generated, has to be stored and classified to facilitate easy retrieval and submission of subsequent information to the database. The databases in the World Wide Web that contain cotton genomic information are GenBank at The National Institutes of Health (NIH); Swiss Prot at the Europian Bioinformatics Institute (EBI); Cotton DB at Texas A&M University (TAMU); Cotton Pilot Project database at the Cotton Genome Center, University of California, Davis; Cotton Genome database at the Plant Genome Mapping Laboratory (PGML), University of Georgia; Cotton Center, Clemson University Genomics Initiative (CUGI); Cotton ESTs and STCs at Arizona Genomics Institute; Cotton Gene Index at The Institute for Genomic Research (TIGR); Plant Genome Database (PlantGDB) at Iowa State University and the AceCot database at Brookhaven National Laboratory. The International Cotton Genome Initiative (ICGI) formed by a select group of cotton researchers in 2000, has been striving towards the establishment of a combined approach in cotton genomic research. While it is obvious that cotton genomics research is forging ahead, the information is scattered on the Internet, making the retrieval and analysis of necessary information difficult. Hence, a unified cotton database that caters to all the requirements of the cotton research community, under the stewardship of ICGI, has been stressed. Also, the research groups have used different genotypes of AD or A or D

genomes for analysis in addition to differences in mapping populations. Hence, the data produced show wide variation and makes it difficult for the researcher to draw biological conclusions from the vast genomic information. The models of the *Arabidopsis* Genome Initiative (AGI) and International Rice Genome Sequencing Project (IRGSP) can be followed for common techniques and resources, accuracy standards, levels of analysis, and a common public release policy for sequence information. The lessons learnt by the researchers and drawbacks in these databases can be scrutinized for the development of an improved, comprehensive cotton database. The unified cotton database can aid in the extension of genomics research towards other minimally explored representatives of A to G and K genomes, which will throw more light on cotton molecular phylogenetics and genetic engineering research. With the genome projects of crops such as Tomato, Soybean, Wheat, Maize making rapid advancements, it will be beneficial to have links to the genomic resources of these crops, which will assist in comparative mapping studies. The established cotton database should lead the direction towards effective manipulation of the cotton genomic information and development of cotton crops with tangible benefits to the farmers and the industry.

INTERNATIONAL cotton genome INITIATIVE

Toward Cotton Genome Sequencing Session

Abstracts

1. Cotton genome sequencing: a reality check

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In this session, I will briefly introduce sequencing strategy, technology capacity and variation, and present and future needs in cotton genome sequencing. Several experts in the field will elaborate a few strategies for sequencing cotton genomes. A discussion session will immediately follow after the last scientific presentation.

2. The roadmap to the cotton genome

***BRIAN SCHEFFLER**, USDA-ARS MSA Genomics Laboratory, 141 Experiment Station Rd., Stoneville, MS 38776, USA*

Cotton is not on the top ten list of species to be sequenced in the United States. With dwindling resources and more groups asking for their organism of interest to be sequenced the Cotton Community must come together and ask itself: What will it take to put cotton on the sequencing priority list? There are several important crop species that have not made the top priority list. For example, soybean is in a similar situation as cotton and is losing its predominant position as the top legume to *Medicago truncatula* a minor crop worldwide. *M. truncatula* is the model organism for the legume community, but some researchers in the soybean community question if the sequence of *M. truncatula* will be applicable to their programs. From their prospective, it makes more sense to sequence soybean before a minor crop like *M. truncatula*. The Cotton Community must ask itself what have other groups done correctly to get their organism on the top priority list and what mistakes have other groups made that are preventing their organism from being put on the list.

3. Methylation "filtering" to enrich for coding sequence regions

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The analysis of methylation filtered (GeneThresher) sequence sets from over a dozen plant species, including both monocots and dicots, demonstrates substantial genome reduction and gene enrichment through the removal of methylated DNA. Unlike EST approaches, low coverage methylation filtered sequences contain near complete and unbiased gene tagging, and an even representation of all gene features, including promoters, UTRs, and introns. This strategy exploits the genome architecture of plants where the vast majority of genes lie within the genome in small hypomethylated islands separated by large oceans of hypermethylated repeats. Methylation filtered libraries are constructed through the selective cloning of hypomethylated gene rich DNA fragments which are subsequently sequenced. A two-stage strategy that leverages DNA methylation to sequence the cotton genome will be presented. The first stage delivers near complete gene tagging and is achieved with as little as a 1.0 x raw sequence coverage of the hypomethylated genome, and can be completed within the first 6 months of the start of the project. To support this, results from the methylation filtered sequence of maize, sorghum and cotton will be presented. The second stage is to generate a high quality deep methyl-filtered sequence of all genes combined with low sequence coverage of all intergenic regions. This allows all gene regions to be ordered, oriented and fully associated with the physical and genetic map. Stage 2 builds on the investment made in stage 1and integrates the skim sequence of a minimum tile of BACs with a deep sequence of the hypomethylated genome. In these two stages, a comprehensive sequence of the cotton genome can be ascertained by leveraging DNA methylation resulting in rapid completion for as little as one third the cost of whole genome sequencing strategies.

4. Efficient sequencing and assembly of the cotton genome, incorporating Cot – based cloning and sequencing

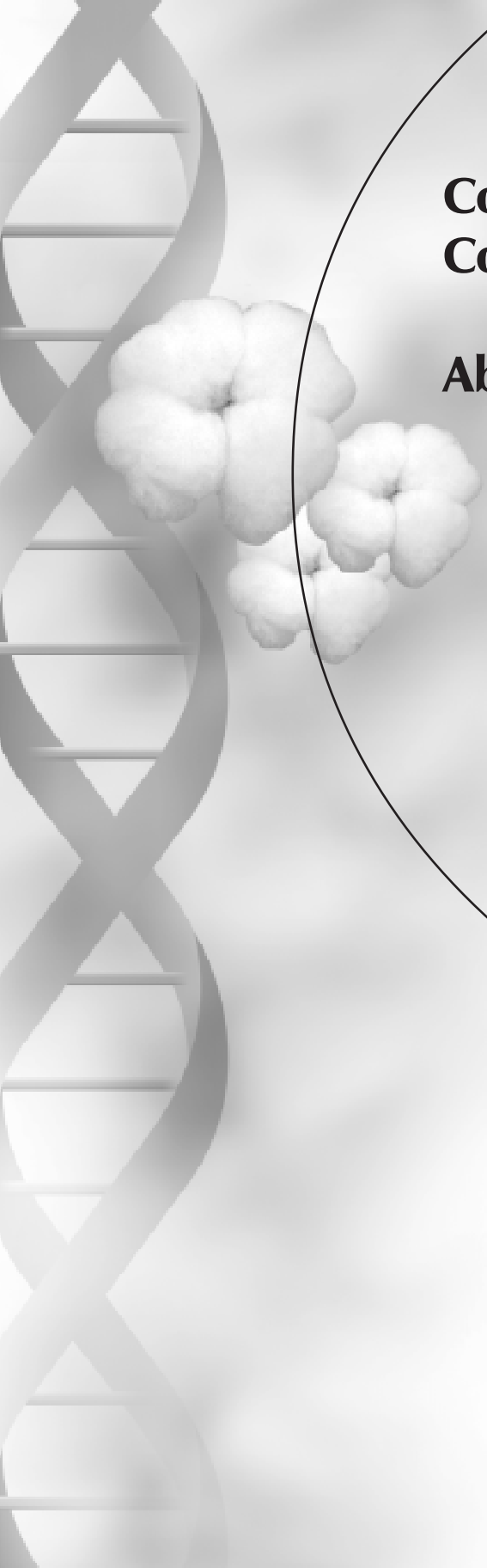
***ANDREW PATERSON**, Distinguished Professor, University of Georgia, USA*
Thomas Wicker, Plant Genome Mapping Laboratory, Univ Georgia, Athens GA Junkang Rong, Plant Genome Mapping Laboratory, Univ Georgia, Athens GA Jamie Estill, Plant Genome Mapping Laboratory, Univ Georgia, Athens GA Daniel Peterson, Mississippi Genome Exploration Laboratory. MS State, Starkville MS, USA

The diverse *Gossypium* (cotton) genus offers a host of exciting and important research opportunities that might be advanced by greater application of high-throughput genomics, and are likely to be ongoing priorities for the next century and beyond. Until the cost of high-throughput genomics drops by several additional orders of magnitude, it will remain necessary to take advantage of both naturally occurring and technological efficiencies to gain maximal new information about the cotton genome at minimal cost. At the taxonomic level, this will involve prioritization of taxa based on a balance between maximizing sequence similarity to cottons of commerce, and minimizing complicating factors such as sequence duplication or repetition. At the technological level, this will involve prudent application of a series of techniques that maximize information yield per unit cost. One model by which to achieve such an integration will be discussed, in particular addressing the roles of Cot-based cloning and sequencing as a component of an integrated genetic-physical-evolutionary approach to provide an initial 'template' for exploring the productivity, quality, ontogeny, and phylogeny of cotton.

5. A minimal tiling path for maximal genome coverage: International collaboration on the global BAC sequencing platform

***JOHN Z. YU**, USDA-ARS, Southern Plains Agricultural Research Center, College Station, TX 77845, USA*

Cotton is an allotetraploid crop with profound gene duplication and sequence complexity. Information on the structural organization of total DNA sequences in cotton is essential not only in identifying many thousands of cotton genes, but also in understanding the functional and evolutionary potential of any gene within its complex genomic neighborhood. A complete complement of genes encoded by the cotton genome will be likely achieved if the vast sequence environment and its unknown role are examined. A reasonably complete genome sequence of an inbred cotton line can serve as a standard reference for comparison with other cotton genotypes and species. By use of a cotton physical map that consists of overlapping BAC contigs, a minimal tiling path (MTP) of cotton BAC clones (whole genome or gene-rich regions) can be determined for sequencing, which reduces sequence redundancy and increases genome coverage. Moreover, such a genomic framework provides a sequencing platform for ready international collaboration where participating countries normally prohibit the transfer of research funds across their borders. An integrated genetic, physical, transcript, and sequence map of the cotton chromosomes will lay the solid foundation for many basic and applied cotton genetic studies to understand and improve the worlds leading fiber crop. This strategy has been discussed for numerous crop species over the past few years and it will be elaborated for cotton in this presentation.



INTERNATIONAL cotton genome INITIATIVE

Commercial Perspectives on Cotton Genomics Session

Abstracts

1. Opening remarks

Moderators
David Stelly
C. D. Mayee

Presenters

2. P. Vidyasagar

VIBHA SEEDS

3. M. Prabhakar Rao

NUZIVEEDU SEEDS

4. Raju Barwale

MAHYCO SEEDS

5. A. R. Sadananda

EG TECHNOLOGIES AND SERVICES

6. Donald Keim

DELTA PINE AND LAND CO.

7. Proposal for international research collaborations on cotton genomics

***JINHUA XIAO**, MONSANTO*

8. Avenues for improving the pace of cotton genomics

***SIVA P. KUMPATLA**, Department of Trait Genetics & Technologies, Dow Agro Sciences LLC, 9330 Zionsville Road, Indianapolis, IN 46268 USA*

Although cotton is the leading natural fiber crop and one of the most important dicotyledonous species, efforts to develop molecular resources and dense genetic maps lagged behind faster paced advances in other commercial crops. Initiatives during the last 2-3 years have paved the way for the development a series of maps. However, in order to rapidly generate saturated maps for marker-assisted selection, gene cloning and other downstream applications, there is a need for the launch and acceleration of collaborative projects involving public institutes and interested industry partners. Such efforts should focus on the large scale development and mapping of multiple types of molecular markers, generation and sharing of genetic stocks and mapping resources, providing support for functional genomics projects and transformation of cotton database to an exhaustive repository of information and a single-stop portal for useful computational tools. By leveraging diverse global talent and resources it is possible to rapidly achieve progress in multiple areas of cotton genomics. Some ideas and proposals for networking and industry-academia collaborative initiatives will be floated for discussion among ICGI participants towards probable action plans.

9. Priorities in cotton genomic research: an industry perspective

***JOHN JACOBS**, BAYER CROPSCIENCE*