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The Third International Cotton Genome Initiative Workshop

第三届国际棉花基因组协会(筹)学术研讨会

June 3-6, 2002
Nanjing, China

Organizers

National Key Laboratory of Crop Genetics & Germplasm Enhancement, Nanjing Agricultural University
National Key Laboratory of Plant Molecular Genetics, Institute of Plant Physiology & Ecology, The Chinese Academy of Sciences
The Society of Cotton Sciences in China; Cotton Research Institute, China Academy of Agricultural Sciences

Preface

Cotton research and production face both unprecedented opportunities and challenges in the 21st century. A desire to take advantage of genomic technologies and resources on cotton improvement led to the birth of the International Cotton Genome Initiative (ICGI) when a small group of cotton scientists held a meeting at CSIRO, Canberra, Australia in February of 2000. The concept of international collaboration and coordination to maximize the benefits of limited cotton genomic resources resulted in the second ICGI workshop held at CIRAD, Montpellier, France in June of 2001. However, much work needs to be done before the ICGI becomes a real coordinator to facilitate global communication, collaboration, and exchanges of genomic information. The third ICGI workshop to be held in Nanjing, the People's Republic of China, in June of 2002 will move it toward a new stage. Chinese colleagues have made significant achievements in cotton genome research and cotton production over the past decade. Vast plain of the lower reaches of the Yangtze River is one of three cotton production centers in China. Nanjing Agricultural University and National Key Laboratory of Crop Genetics and Germplasm Enhancement are among the core researchers in cotton genomics, genetics, and breeding.

The ICGI currently has 221 registered members (http://icgi.tamu.edu/list.html). The third ICGI workshop brings together over 100 participants representing 17 countries of all cotton growing continents: Australia, Belgium, Brazil, China, Egypt, Ethiopia, France, India, Kyrgyzstan, Namibia, Pakistan, Singapore, Thailand, United Kingdom, United States, Uzbekistan, and Zimbabwe. This workshop has also attracted more than 80 research abstracts covering all critical topic areas: genetic mapping & QTL analysis, physical mapping, functional genomics, genetic resources & cytogenetics, and bioinformatics. This workshop provides a forum on recent developments and future plans of the cotton genome research. The workshop program and all accepted abstracts are included in this publication that is invaluable to not only the workshop participants but also all interested cotton researchers.

The workshop organizers would like to thank all individuals and institutions involved in participation in the scientific discussion and financial contributions to this workshop. We look forward to a successful workshop!

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Nanjing Agricultural University, China

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ICGI: Past, Present and Future Direction

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Cotton is viewed as the most important cash crop in the world, and sustains the agricultural economies of many nations by providing a sustainable fiber product for the textile industries. Unfortunately, many challenges face cotton production at present and in the future. Concerns over water use and pesticide inputs abound. In some important growing regions of the world, a production plateau has been reached and stability of production is a serious concern and intense competition from man-made fibers may jeopardize future profitability of cotton production. With the advent of genomic technologies, the scientific community has an unprecedented opportunity to make significant genetic improvements in cotton. The International Cotton Genomics Initiative (ICGI) at the fundamental level is an effort to develop a framework for collaboration and cooperation on cotton genomics research and its application. The last decade has seen unprecedented advances in the use of DNA technology to unravel the genetic secrets of plants and animals. International collaborative efforts are underway to map and characterize the genomes of many important organisms. The recent publication of the sequencing effort for rice illustrates the scope and power of the technology. The study of the complex allopolyploid cotton genome is scientifically very challenging and requires a coordinated multidisciplinary research effort. The ICGI was created at a meeting of cotton genetics/genomics researchers in Canberra, Australia in February 2000. The objectives of ICGI are to: 1) reduce redundancy of research effort and maximize rate of progress in research to understand the cotton genome by providing a forum for international researchers, 2) Foster tool development to begin integrating genetic and physical maps, 3) Accelerate development of consensus cotton linkage map comprised of framework markers that are portable from lab to lab, 4) Foster rapid application of new genomic tools to cotton improvement, 5) develop comprehensive forum for exchange and communication within cotton scientific community and with the *Arabidopsis* model genome community and 6) develop standardized nomenclature for DNA markers, maps and etc. Progress has been made in many of these areas the past two years yet much of the results have not made it out of the individual labs working many times in isolation from each other. Barriers still exist that prevent meaningful collaboration that would enable real gains in cotton genomics that would lead to sustained genetic improvements in cotton germplasm. ICGI is the only forum that can facilitate the necessary multidisciplinary research efforts on a global scale to address some of these issues. The workshop in 2002 in Nanjing will launch a new period for ICGI where individual working groups on critical topic areas can be formed and move forward. The importance of ICGI to the future of the cotton industry is illustrated by the generous financial support Monsanto, Delta and Pineland Co., Stoneville Pedigreed Seed Co., CIRAD (FR), Cotton Research and Development Corporation (AUS), Cotton Incorporate (USA), Cotton Foundation (USA), Syngenta, and Dow AgroSciences. This support has allowed ICGI to grow during these first critical years of development.

Molecular Tagging of Fiber Quality and Yield QTLs and Their MAS Breeding in China

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There are great progresses obtained in cotton genomic research in China.1) DNA marker screening. Many DNA markers such as RFLP, RAPD, SSR, ISSR etc. have been used. A molecular linkage map was constructed with 58 doubled and/or haploid plants from the cross of the two cultivated allotetraploid cotton, *Gossypium hirsutum* L. and *G. barbadense* L., developed by means of Vsg. Among the total of 624 marker loci (510 SSR and 114 RAPD), 489 loci were assembled into 43 linkage groups and covered 3314.5 centi Morgan (cM). Most of linkage groups were associated with the chromosomes of allotetraploid and some of the unassociated groups were connected to corresponding A or D subgenome. DNA markers associated with *Verticillium* and *Fusarium* disease resistance, fertility restorer gene, QTLs for fiber quality, lint percent and other yield components have been identified. A new breeding program has been initiated to pyramid yield, fiber quality and resistance gene to insects and disease combing conventional recurrent selection and marker assisted selection. A basic pool of recurrent selection to develop *Verticillium* wilt was constructed using genic male sterile lines(ms_{14}) in *Gossypium hirsutum* L.in Cotton Research Institute, Nanjing Agricultural University. The male sterile plants segregated out from the recurrent selection population were crossed respectively with high fiber quality germplasm lines, transgenic Bt and antifungus protein strains. Some elite plants pyramided super fiber quality, resistance to insect and *Verticillium*, and high productivity can be selected by intercrossing between genic male sterile and fertile plants and MAS. These plants will be crossed with semigamy line in *Gossypium barbadense* to produce haploid plants and at last to develop homozygous lines or cultivars in *Gossypium hirsutum* L. in a short time.

Comparative Mapping of Cotton and *Arabidopsis*

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A high-density inferred consensus map for 13 homoeologous groups of cotton was constructed through the integration of three genetic maps (At, Dt and D) of homoeologous chromosomes. The consensus map included 2843 markers and spanned about 2242 cM in 13 linkage groups. 1777 mapped probes were sequenced and compared to the *Arabidopsis* using the BLAST algorithm to study possible collinearity between the two species. 1253 cotton sequences mapped on 1500 loci were found to have matches with more than one sequence in *Arabidopsis*. A comparison of the map locations of the matching sequences revealed that each cotton chromosome could be divided into several blocks defined by the collinear relationship between the two species. Overall, about half of the matching sequences show some collinear relationships with segments of *Arabidopsis* chromosomes. This comparison indicates that discernible and potentially useful similarities in gene order have persisted since the divergence of cotton and *Arabidopsis* from a common ancestor.

Overview of Activities and Major Achievements in Molecular Genetics at CIRAD/France

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The Cotton Programme of CIRAD undertakes different research programs aiming at utilizing DNA molecular markers for an applied molecular breeding of cotton. These programs cover areas from marker-assisted selection for fiber quality to functional genomic study of cotton fiber development. The present communication will give an overview of major achievements in these areas.

A major result has been the construction of a saturated and combined RFLP/ SSR/ AFLP genetic map of tetraploid cotton. The ′ GV ′ mapping population comprised 75 BC1 of a ′ Guazuncho II′ *(Gossypium hirsutum*) × ′ VH8′ (*G.barbadense*) cross, backcrossed to the *G. hirsutum* parent. The map spans 4397 cM and contains 888 loci, shared between 465 EcoRI/MseI AFLPs, 229 SSRs, 192 RFLPs and 2 morphological markers. Additionally we realized an integration of the GV map with 2 other independent interspecific F2 mapsanalyzed in USA: TM1/3-79 at Brookhaven Ntl Nab by B Burr; and TM1/NM24016 at New Mexico State Univ by R Cantrell. The integrated and combined map proved congruent with previously published investigation in cotton RFLP mapping. QTL analysis for different fiber quality and morpho-phenological traits has been undertaken on 3 phenotypic data sets, BC1, BC2 and BC2S1. Selected individual BC3 introgressed for favorable *G. barbadense* QTLs/ alleles, are presently grown in Montpellier.

Future progress towards obtain of a more saturated genetic map of cotton will benefit from the development of new microsatellite markers. An enriched poly-CA library developed in 2001 from a *G. hirsutum* cultivar allowed the definition of 303 new SSR primer couples, that are presently being screened for polymorphism between the 2 parents of the mapping population.

Germplasm analysis using molecular fingerprinting techniques at CIRAD have 2 major goals: the analysis of allelic diversity of SSR loci of a working core collection, and the definition of methods and tools (mainly AFLP, and SSR-derived types of markers) for a commercial application to variety identification.

The functional genomics activity is devoted to the study of genes expressed during cotton fiber development. The strategy combines the study of genes (ESTs) expressed during early stages of fiber elongation, as well as ′ candidate genes′ identified from *Arabidopsis thaliana.* In this latter case cellular elongation and related cellulose synthesis pathways serve as model systems for cotton fiber.

Lastly CIRAD plays an interface role and has developed different collaborative activities with various partners in Europe (INRA/France, Univ of Gembloux/Belgium), in USA (BNL, Univ UC Davis), South America (Coodetec/ Brazil), as well as in Asia (Thailand and Vietnam).

Genetic Linkage Mapping of the Diploid *Gossypium* Species

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Primary germplasm pools represent the most readily accessible source of new alleles for crop improvement, but when the most effective alleles are not available in the primary germplasm pool, breeders must confront the difficulties associated with introgression genes from the secondary and tertiary germplasm pools, in cotton, by using synthetic polyploids as introgression bridges. To develop a suite of *G. australe* chromosome specific molecular markers, two parental AFLP genetic linkage maps using a *G. nelsonii* × *G. australe* F_2 family were inferred. To track the fidelity and frequency of *G. australe* chromosome transmission in a *G. hirsutum*× *G. australe* hexaploid bridging family, the *G. australe*-specific markers to screen first and second generation aneuploids, each of which contained the full complement of *G. hirsutum* chromosomes plus several *G. australe* chromosomes. The distribution of markers among the 18 aneuploids identified potential mapping errors within and undetected linkages among the *G. australe* and *G. nelsonii* linkage groups, leading to the resolution of 13 linkage group assemblages corresponding to the individual chromosomes within these species. Conversely, comparison of the two sets of data identified putatively recombined G. australe chromosomes in the aneuploid back cross families. The distribution of G. australe markers among the aneuploids allocated *G. australe* AFLPs that failed to segregate in the F_2 family to chromosomes, doubling the number of *G. australe* AFLPs assigned to linkage group assemblages. These data suggest that when homoeologous recombination is low, first generation aneuploids from parents that differ in chromosome number and homology are useful adjuncts to genetic linkage mapping. Although locus ordering is not possible, this approach requires few progeny and is insensitive to marker density. The *G. australe* and *G. nelsonii* genetic linkage maps pesented here represent the first AFLP linkage maps for the *Gossypium* G genome.

Construction of Molecular Linkage Map of Cultivated Allotetraploid Cotton *Gossypium hirsutum* L. × *G.barbadense* L.) with SSR and RAPD Markers

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A permanent doubled haploid population from the crossing of *G. hirsutum* × *G. barbadense* were developed by means of Vsg, virescently marked semigamy line in sea island cotton, which was characterized by a cytological mechanism for developing haploids with certain convenience, and thus constructed an allotetraploid cotton molecular genetic linkage map with the high level polymorphic SSR and RAPD markers. The linkage groups were then associated with their corresponding chromosomes of allotetraploid cotton with a series of monosomes and telosomes in genetic background of *Gossypium hirsutum* L. and determined their attributive subgenomes by analysis of markers distribution on *G. herbaceum* L.(A-subgenome). and *G. raimondii* L.(D-subgenome), which are the theoretical progenitor diploid cotton species. Among the 624 SSR and RAPD marker loci detected on the mapping population, 489 loci were constructed into 42 linkage groups totally covering 3312.2 cM of the allotetraploid cotton, the biggest of which was linkage group consisted 47 marker loci covering 320.4cM in chromosome 9, and the smallest was a linkage group consisted only two maker loci covering only 9.6 cM.

The primary study on the connection of the constructed linkage groups with chromosomes and subgenomes of the allotetraploid cotton shows that the molecular markers linkage groups of the present map were associated with 18 chromosomes, which were chromosome 1, 3, 4, 5, 7, 9, 10, 11, 12, 14, 16, 17, 18, 20, 22, 23, 25 and 26 of allotetraploid genome. And some of the unassociated groups were connected to corresponding A or D subgenome.

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Identification of DNA Markers for Cotton Leaf Curl Disease (CLCD) in Cotton (*Gossypium hirsutum* L.)

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Epidemic of cotton leaf curl virus disease (CLCD) was the compelling factor to devise new strategies in cotton breeding programs of Pakistan. The evaluation of cotton genotypes against the CLCD resistance is difficult, expensive and time consuming in field and especially in greenhouse due to uneven distribution of the disease. A marker-assisted selection (MAS), a relatively new tool, can be applied to replace screening methods. Thus DNA marker studies were conducted on F2 populations of crosses CP-15/2xS-12 and LRA-5166xS-12. A total of 300 available decamer random primers were surveyed. A DNA marker $OPN12_{1180}$ with recombination frequency of around 14% was linked with the disease resistance in the population derived resistance from CP-15/2. The cotton parents LRA-5166, CP-15/2 and S-12 were also screened with microsatellite loci (SSRs). Two SSR loci were linked with recombination frequency of around 12.5% at′ a′ and 14% at ′ b′ locus with CM-43 and 16% with CM-162. All these markers were found to be loosely linked on the F2 population derived resistance from LRA-5166.

The linked DNA markers were surveyed on available germplasm to detect efficiency of these markers for MAS. The DNA marker $OPN12_{1180}$ and the SSR locus CM-162 detected only non-susceptible genotypes derived resistance from CP-15/2. Thus the markers are limited to the source of resistance. However, the SSR locus CM-43 detected susceptible and non-susceptible genotypes with 79% confidence level irrespective of resistant source. Further research is in progress to find new DNA markers with additional microsatellite (SSR) loci and amplified fragment length polymorphism (AFLP) techniques. The study reported here laid the foundation of cotton genomics in Pakistan.

Molecular Characterization for Leaf Curl Virus Resistance in Cotton

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RAPD analysis was carried out in germplasm lines CNH 123 (RCLCuV), CNH 1012 (RCLCuV), CNH 1020 (SCLCuV) and CNH 120 (SCLCuV) to establish polymorphism among the cotton leaf curl virus (CLCuV) resistant and susceptible genotypes. These lines were characterized using 80 decamer primers by amplification. Eighty primers amplified 392 scorable DNA fragments out of which 20% were polymorphic. The lines CNH 123 and CNH 120 have similarity (85%) as obtained by the Nei and Lies coefficient of similarity. The primer OPC 02 amplified a unique polymorphic fragment in the CLCuV lines CNH 123 and CNH 1012.The hybrid resulted by the cross between CNH 120 × CNH 123 and CNH 1020 × CNH 123 has amplified the unique 1700 bp fragment as introgressed from the resistant parent. Ten resistant and susceptible F_2 lines DNA were pooled and amplified with the same primer, which also produced the 1700 bp fragment. This fragment is sequenced, designated as SCAR marker and the primer is being developed for screening the F_2 mapping population.

Identification of QTLs Affecting Yield and Fiber Properties in Chromosome 16 in Cotton Using Substitution Line

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Gossypium hirsutum L., one of the two cultivated tetraploid species in cotton, is characterized by its high yield and wide adaptation, while *G. barbadense* L.，another cultivated one, by its super fiber properties. Substitution line in which one pair of intact chromosomes of TM-1 (*G. hirsutum* L.) was replaced by one pair of homozygous chromosome of 3 79 (*G.barbadense* L.) is an excellent material for genetic research and molecular tagging. In this study, substitution line 16 （Sub16）was used to evaluate the performance of the 16th chromosome in *G. barbadense* in TM-1 background. The genetic mode using the major gene plus polygene mixed inheritance model in $F_{2:3}$ generation were revealed that there might exist 2 QTLs respectively for boll size, lint percentage, lint index, fiber length and the 1st fruit branch node, 1 QTL for fiber elongation and flowering date, and no QTL for seed index, fiber strength and Micronaire in chromosome 16. However, 9 QTLs（LOD (logarithm of odds)≥3.0）controlling 6 quantitative traits were significantly identified in linkage group of chromosome 16 constructed in (TM1×3-79) F2 by interval mapping. Among them, 1 QTL for boll size, fiber length, flowering date and fiber elongation could explain 15.2%, 19.7%,12.1%, and 11.7% phenotypic variance, respectively, 2 QTLs for lint index could explain 11.6% and 41.9%, and 3 QTLs for lint percentage could explain 8.7%, 9.6% and 29.2% phenotypic variance respectively. One unlinked SSR marker was associated with one QTL respectively for boll size and flowering date and they could explain 1.60% and 4.63% phenotypic variance. Significantly associated with chromosome 16 from Sub 16 were boll weight, lint percentage, lint index, fiber length, fiber elongation and flowering days.

Molecular Tagging and Mapping of QTLs for Super Quality Fiber Properties in Upland Cotton

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A *G. anomalum* introgression line, 7235, characterized as super quality fiber properties, was used to identify molecular markers linked to fiber property QTLs. By use of (7235×TM-1) F_2 in Nanjing and College Station, USA, and (7235×TM-1)F3 in Nanjing and Hainan. Bulked segregation analysis was employed to produce 3 pairs of mixed DNA pools for fiber strength, micronaire and fiber length according to individual value of (7235×TM-1) F_2 and $F_{2:3}$. A total of 221 pairs of SSR primers, 1840 arbitrary 10 mer oligonucleotide primers and 77ISSR primers were used to screen polymorphism between two parents, and 3 pairs of bulked DNA pools. Fifteen markers amplified by thirteen primers were identified to be linked with fiber quality QTLs through DNA polymorphism surveying between the parents, and then paired bulked DNAs, and screening the individual plant of (7235×TM-1) F_2. Linkage test indicated 15 markers could be mapped to three linkage groups. In the first linkage group, eight markers (two SSR and six RAPD markers) associated with fiber strength were tightly linked with 2.2cM interval genetic distance on average, and located on chromosome 10 in cotton. Two major QTLs for fiber quality characters were identified. One for fiber strength could explain 35% of the phenotypic variation in F_2, and 53.8% in $F_{2:3}$ at Hainan, which has the greatest single QTL effect of fiber strength could be identified in all four environments, and tightly linked to 6 RAPD markers and 2 SSR markers with genetic distance no more than 16 cM in chromosome 10,in which FSR_{1933} is the nearest with the distance no more than 0.6 cM. One QTL linked to FMR_{1603} for micronaire could explain 7.8% of the phenotypic variation in F2, and 25.4% in $F_{2:3}$ at Hainan, and expressed in all four environments. One QTL linked to $FLR1_{1550}$ for fiber length could explain 9.5% of the phenotypic variation in $F_{2:3}$ at Hainan, very little in other environments. So they can be used in marker-assisted selection in increasing fiber quality of commercial cultivars.

Fine Mapping of Fertility Restoring Gene for Cytoplasmic Male Sterility in Cotton (*Gossypium* spp.) Using RAPD and SSR

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The heterosis in cotton is much significant, especially in increasing yield andfiber quality. Comparing with hand-emasculation and pollination, and genetic male sterile lines, utilization of CMS lines is much more effective and economical in producing commercial hybrid seeds. Since 1965 in the world, several CMS lines have been developed, such as CMS lines with *G. arboreum*, *G. anomalum*, *G. harknessii* and *G. trilobum* cytoplasms, other type of CMS lines such as 104-7A, Xiangyuan-a, Jing A-1, Jing A-2 and so on. As cotton fibe and seed is major harvesting product to the people, so fertility of CMS restoration is the most important target to the scientists. Genetic basis of fertility restoration to CMS is not very clear. In this study, Genetic of the CMS fertility restoration was presented by the analysis of classic genetics and molecular markers. Based on F_2 segregation of the cross between CMS and restoring lines, and the testcrosses and $F_1 \times F_1$ populations, together with RAPD and SSR mapping, one dominant gene was identified to control the CMS fertility restoration in cotton. The strategy of genotype representation analysis (GRA) was put forward to screening the molecular markers tightly linked with Rf_1 locus using three F_2 populations with total 635 individuals (CMS line Simian 3A, Sumian 12A, Simian 3A, and fertility restoring line Simian 3R). In the representative BSA, Zhongmiansuo 12A, (Xiang A), Zhongmiansuo 12A (104-7A), Zhongmiansuo 12A (ha), and 0-613-2R, 501R, maintainer lines Zhongmiansuo 12, Simian 3 were also used. Totally 1025 random 10 decamer primers from OPERON and University of British-Columbus, Canada (UBC primers) and 282 pairs of SSR primers were screened on the DNA samples of the fertile and sterile representation, though lots of polymorphic DNA were found, but only two RAPD marker --NAU/RAPD/Rf_13_{1480} and NAU/RAPD/Rf15710,a co-dominant marker, tightly linked to this allele (Rf_1) were detected. Additionally, three reliable SSR markers, NAU/SSR/Rf_12_{135},NAU/SSR/Rf_11_{170}, NAU/SSR/Rf_14_{215} amplified by two primers are tightly linked with Rf1 locus. The NAU/RAPD/Rf_13_{1480} is tightly linked Rf_1 allele with a genetic distance of 0.4cM in population1, 0.1cM in population 2,0.6cM in population3; but NAU/RAPD/ Rf_15_{710} is 1.2cM, 0.3cM, and 0.9cM respectively. The co-dominant markers NAU/SSR/ Rf_12_{135} locates 1.2cM, 0.0cM, and 0.9cM to the Rf1 locus in population 1, 2, 3, respectively. The co dominant marker NAU/SSR/ Rf_14_{215} is 1.2cM, 0.3cM, and 0.9cM from the Rf1 allele in population1, 2, 3. The NAU/SSR/ Rf_11_{170}, a dominant marker is positioned 1.2cM, 0.3cM, and 0.6cM from the Rf1 gene. In the different populations used in the study, the genetic distance is similar, indicating the mechanism of fertility restoration probably is almost the same. So from the population with total 635 individuals, the result is that NAU/SSR/ Rf_11_{170} is 0.3cM to the Rf_1, NAU/SSR/Rf_12_{135}, NAU/RAPD/Rf_13_{1480}, NAU/SSR/Rf_14_{215}, NAU/RAPD/Rf_15_{710} is 0.6cM to the Rf_1.

Additionally, basing on the analysis of monosomic and telesomic lines with one SSR maker, the Rf_1 locus is located on the long arm of chromosome 4. Furthermore, from the molecular marker analysis and fertility inspection, the Rf_1 gene ′ dose effect ′ is also found. At present, the tight RAPD marker is being converted into SCAR markers. The results will be very efficient in marker-aided selection of elite fertility restoring lines, and helpful to ultimately cloning the Rf_1 gene with map-based cloning.

Molecular Linkage Map Construction and QTL Mapping for Partial Agronomic Traits in Upland Cotton

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Molecular map construction is very important to gene marking, map-based cloning and marker-assisted breeding. Some advance was made in this area in the US (Shappley et al, 1996; 1998; Reinisch et al, 1994), but little work was done in China. A preliminary molecular map with RFLP, RAPD and SSR was constructed and QTL maping was dong for some agronomic traits by our research group.

Detection of polymorphic markers: Two hundred and eighty four cDNA and EST probes kindly provided by Paterson and Stewart, 520 decamer rindom primers and 100 SSR primers from maize, were used in polymorphism screening between two parents, Si3Bt and Ejing1. Totally 34 probes, 16 random primers and 5 SSRs, which produced reproducible and clear bands were employed to construct map.

Construction of molecular linkage map: One hundred and fifty two plants were analyzed with 34 probe/enzyme combinations, 16 RAPD primers and 5 SSR primers, which resulted in 67 loci. All the 67 loci in the F_2 population were normally segregated, no loci are distorted from expected ratio 3:1 or 1:2:1. A map was constructed using the above polymorphic markers, and the markers were assigned into 9 linkage groups. The linkage map was composed of 40 RFLP, 11 RAPD and 16 SSR loci. It covers 1337.4 cM, about 26.75% of total cotton genome. The marker distance ranged from 7.8 to 46.8 cM. The number of marker associated with particular group was from two to ten makers while 11 markers were not linked to the map.

QTL mapping of agronomic traits: Mapmaker/QTL 2.0 software was used to analyze the data for 20 traits of the population for mapping, and twelve QTLs related to yield components and fiber length were detected. Totally 3, 1 2 3 1 and 2 QTLs were found to have relation with boll number, boll weight, lint percent, lint index, seed index and fiber length, respectively. The cumulative contribution of 3 QTLs to boll number was up to 57.1%, and the 3 QTLs were located in 3 linkage groups separately.

For construction of molecular map, rich markers were required to get a usable map. Cotton is a hard crop in molecular biology, because polymorphism is very poor among genotypes in upland cotton. Only using RFLP and RAPD would be difficult to make a relative saturated map. For this reason, other markers such as AFLP and cotton SSRs should be developed. Exotic SSRs might not be transferred between species, but a lot polymorphic bands could be produced in cotton by using SSRs from maize and rice, which suggested that the bands could be used as markers in mapping if they are reproducible and clear.

Tagging and Mapping of QTLs Controlling Lint Yield and Yield Components in Upland Cotton Using SSR and RAPD Markers

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Three F_2 populations of cotton (*Gossypium hirsutum* L.) from the crosses of Simian 3 × TM-1, Simian 3 × CARMEN and Xiangzamian2 were characterized for RAPD and SSR. 301 pairs of SSR primers and 1040 RAPD primers were used in the Simian 3 ×TM-1 population analysis, which resulted in 49 polymorphic loci. An analysis of these loci with the MAPMARKER program resulted in the establishment of five linkage groups with 11, 4, 3, 7, 3 loci, respectively, as well as 21 unlinked loci. Using interval mapping, ten QTLs of yield and its components in F_2 and $F_{2:3}$, were localized on the chromosome 9, 10 and 16 with 6, 2, 2, respectively. Two QTLs controlling boll size with 18.2% and 21.0% phenotype variance explained in $F_{2:3}$ generation, one QTL controlling lint percent with 24.9% phenotype variance explained in F_2 generation and 5.9% in $F_{2:3}$ generation, and one QTL controlling 100-seed weight with 15.6% phenotype variance explained in $F_{2:3}$ generation were mapped in Chromosome 9. Additionally, another QTL responsible for 100-seed weight was identified and mapped at the same position in Chromosome 9 in $F_{2:3}$ generation. It is worth for further to be studied whether it is one QTL for pleiotrophism or two closely linked QTLs. 301 pairs of SSR primers were used in the Simian 3 × CARMEN population analysis, which resulted in 40 polymorphic loci. An analysis of these loci with the MAPMARKER program resulted in the establishment of six linkage groups with 4, 2, 2, 3, 2 loci, respectively, as well as 24 unlinked loci. Using interval mapping, one QTL controlling 100-seed weight with 6.4% phenotype variance explained in $F_{2:3}$ generation were mapped in Chromosome 9. Using F_2 and $F_{2:3}$ segregating populations of Xiangzamian 2 as mapping population, QTLs of yield traits were tagged by SSR and RAPD, and QTLs genetic effects on the corresponding yield traits were analyzed, and localized on chromosome. 3 very stable RAPD markers screened from 1040 RAPDs, and 15 SSR markers screened from 221 SSRs revealed the polymorphism between parents of Xiangzamian 2. Mapping result showed that on the conditions of linked markers in data set at minimum LOD 3.00, and maximum distance 50 cM, SSR3994、SSR4030、SSR1053、SSR3452、SSR3140、SSR1672 and SSR3031 had been linked on one chromosome with Mapmaker/EXP (3.0b), which had a length of 217.0 cM. By Mapmaker/QTL (Version 1.1b), 13 QTLs of yield traits were mapped on this linkage group, and variance-explained for corresponding trait variation per QTL was from 13% to 34%. Two QTLs for lint percent in $F_{2:3}$ segregating population had 0.2131 and 0.2491 additive effects, and 1.2789 and 1.2871 dominance effects, so the ratio of dominance effect to additive effect was 6.00 and 5.16, which revealed over dominance.

Reducing the Genetic Vulnerability of Cotton

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The US cotton production system exemplifies the complex challenges that must be met in order to reduce the genetic vulnerability of a major crop. Genetic vulnerability results from a combination of a crop's evolutionary history, trends in breeding and biotechnology practices, and grower Decisions based on inadequate information being available, all in response to the inevitable pressures imposed by processor and consumer requirements. We are engaged in the development of (1) a Web-accessible resource that empowers producers to reduce short-term field genetic vulnerability through better-informed decisions about deployment of existing germplasm;(2) ′ user-friendly′ germ- plasm containing new inter-specific gene combinations useful for short-term cotton improvement, plus new genetic stocks (NIILs) useful for long-term research; and (3) genomic tools needed to expedite deployment of these new gene combinations and gain better understanding of the function and control of genes responsible for economically important traits. Efforts in year one concentrated on advancing germplasm, initial field testing of germplasm developed in preliminary studies, fingerprinting diverse genotypes to assess relatedness, and planning for the development of the database (a component which begins in year two).

Isolate a Gene for Velvet Hairiness in Cotton (*Gossypium hirsutum* L.) by Map-based Cloning

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Cotton crop is prone to many insect pests. Finely dense pubescence (pilose/velvet hairy), one of the important elements of defense umbrella confers in built resistance against several insect pests. The present research was conducted to isolate DNA markers for velvet hairiness, which would be useful to launch map-based cloning. An F_2 population developed from a cross between pilose and sparsely hairy (non-hairy) genotypes was used to search DNA marker linked with hairiness. RAPD technique was applied using bulked segregate analysis (BSA). A total of 320 random primers were used to find polymorphic DNA fragments between the bulks constituted on the basis of hair density. The primer OPC-08, OPC-17, OPI-08, OPN-14, OPR-06 and OPZ-09 amplified polymorphic fragment. However, the primer, OPC-08 and OPN-14 amplified polymorphic DNA fragments of approximately 700 and 900 bp in the F2 individual plant DNA samples. These DNA molecules were designated as $OPC08_{700}$ and $OPN14_{900}$ with recombination frequencies of 1.7% and 8% with the velvet hairiness locus. This work is in progress to look for new DNA markers by using additional random primers. Furthermore, amplified fragment length polymorphism (AFLP) technique and microsatellite loci will be applied to saturate the region of the gene for velvet hairiness.

Development of SSR Markers towards Genetic Mapping in Cotton (*Gossypium hirsutum* L.)

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Availability of informative molecular markers is a prerequisite for genetic mapping and marker-assisted selection projects. Micro-satellites or Simple Sequence Repeat (SSR) markers are PCR-based and currently the most widely used marker system in the plant molecular genetics community due to their high degree of polymorphism, random distribution throughout the genome and their suitability for high throughput genotyping formats. Despite its global economic importance, cotton has slower molecular genetic mapping efforts compared to other crop species due to the lack of sufficient number of markers. One of the reasons for the availability of low number of SSR markers is the high price tag associated with their development since pre-existing sequence information is needed to develop this class of markers. We are interested in utilizing SSR markers towards genetic mapping of *Gossypium hirsutum* L. It has been a general experience in the cotton molecular mapping community that only a small portion (about 15%) of the SSR markers are polymorphic in intra-specific situations like *G. hirsutum*. Hence, in orderto identify informative markers for mapping in *G. hirsutum*, a large number of markers have to be developed and screened. To this end, we have initiated an in-house project towards the large-scale development of SSR markers. By optimizing several steps in an existing protocol, we have developed an improved and economical method for SSR capture and construction of enriched libraries. This method relies on magnetic bead capture of physically shared and size-selected genomic DNA fragments using biotinylated tandem repeat probes followed by magnetic bead capture of target molecules. The captured and purified fragments were then cloned and archived in 384-well plates. A second round of screening was performed by hybridizing replica filters of the library with radioactive repeat sequence probes followed by sequencing of the positive clones. As of today, more than 1200 SSR markers have been developed and tested on a panel containing five different *G. hirsutum* genotypes and one *G. barbadense* genotype. Our SSR marker development strategy and the results from marker screening experiments will be presented.

Mapping and Tagging the QTLs of Yield Traits (*Gossypium hirsutum* L.)with SSRs and RAPDs in Upland Cotton

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Molecular markers provide the opportunity to identify marker- quantitative trait locus (QTL) associations in different environments and populations, and can be used to improve the efficiency of conventional plant breeding by carrying out indirect selection through molecular markers linked to the interest traits of QTL at all the stages of plant growth. One Upland cotton (*Gossypium hirsutum* L.) population, ′ I40001 (line selected from CCRI12) × I40005 ′ , was evaluated with RAPD and SSR markers to identify additional QTLs related to yield traits. The individual plants of F2 and 260 F2-derived lines as well as two parents were grown at Jiangpu experimental station of Nanjing Agriculture University, Jiangpu, Nanjing, China in 1999 and 2000, respectively, and evaluated for yield traits. Three reproducible RAPD markers screened from RAPDs, and fifteen SSR markers screened from 221 SSRs revealed the polymorphism between the parents. On the conditions of linked markers in data set at minimum LOD 3.00 and maximum distance 50 cM, mapping result showed that SSR3994、SSR4030、SSR1053、SSR3452、SSR3140、SSR1672 and SSR3031 had been linked on one chromosome with Mapmaker/EXP (3.0b) (Lander, et al.,1987), which had a length of 217.0 cM. By Mapmaker/QTL (Version 1.1b) (Lander and Botstein, 1989). Thirteen QTLs of yield traits were mapped on this linkage group, and their variances explained for corresponding trait variation per QTL was from 13% ～ 34%. Two QTLs for lint percent in $F_{2:3}$ segregating population had 0.2131 and 0.2491 additive effects, and 1.2789 and 1.2871 dominance effects, respectively. The ratio of dominance effect to additive effect was \{6.00\}and\{ 5.16\}, which revealed over dominance. This finding may be consistent with the higher heterosis for yield over mid-parents in the F1 and F2, which were popularly planted in the Yangtze Valley cotton-growing region, China. Based on single-factor analysis of variance (ANOVA) for other 8 SSR markers and 3 RAPD markers, 47 QTLs for yield traits and plant height were identified in F2 and F\{2:3\} segregating population. Their variance explained for corresponding trait variation per QTL was from 2.84% to 21.78%, but 42 QTLs of them only had less 10% variance explained for corresponding trait variation per QTL. It was also found that one marker associated with several QTLs of correlative traits, and QTL have positive effect for one trait, but negative for other traits, possibly caused by genetic correlation among traits. The results of this research can be applied in cotton breeding with marker-assisted selection for yield traits.

Comparison between Marker-assisted Selection and Phenotypical Selection for Fiber Strength and Resistance to *Helicoverpa armigera* in Upland Cotton

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We conducted this study to determine if marker-assisted mass recurrent selection for fiber strength and resistance to *Helicoverpa armigera* within an upland cotton complex population could be more efficient than conventional phenotype selection. Two cycles of marker-assisted selection, phenotypic selection, and marker and phenotype concurrent selection, beginning in 2000, were completed on a single plant basis. The marker-assisted selection was performed on a PCR marker of transgene Bt along with a RAPD marker flanking a putative major fiber strength QTL, which was previously identified by our institute (Zhang et al, 2001). The phenotypic selection was based on the morphological characters of Bt transgenic lines during all living stages, and the feeling of hands pulling a bundle of cotton fiber at the boll opening stage for fiber strength. Similar selections of second cycle were performed on the individuals randomly sampled from the first marker-assisted selection population in the winter 2000 in Hainan. The total six populations, which were named as M1, P1, MP1, M2, P2 and MP2, the initial population C0 and 5 parents were grown in a randomized complete plot design with three replications at Nanjing in 2001.

Mean fiber length was 32.12, 31.84, 31.60, 31.46, 31.28, 31.23 and 30.52 cN • tex^{-1} for the M2, MP1, M1, MP2, C0, P2 and P1 populations, respectively. The M2 population produced significantly greater fiber strength than did all other populations except MP1. The P1 population′s fiber strength was highly significant lower than other populations. The M1 and M2 populations performed significantly higher fiber strength than did P1 and P2 populations, respectively. As fiber strength was increased by marker-assisted selection than phenotypic selection, there were simultaneously significant increases in fiber length, uniformity and elongation, but no significant change in micronaire among all populations.

The percentages of individual plant for resistance to *Helicoverpa armigera* for the seven populations were bioassayed at seedling-bud stage in 2001. The percentages were taken by arcsine transformation and the results were 65.97, 65.95, 63.29, 60.61, 54.88, 44.10 and 32.79% for the P2, MP2, M2, MP1, M1, P1 and C0, respectively. The analyses of variance of the transformed data indicated that C0 was highly significant lower than all other populations, so did P1 population except C0. The populations of the second cycle selection were all significantly higher than those of the first cycle selection, but no significant difference among them. Within the populations of the first cycle selection, MP1 and M1 populations were greatly significant higher than P1, and MP1 significant higher than M1.

Molecular Marker-assisted Selection for *Verticillium* Wilt Resistance in Upland Cotton (*Gossypium hirsutum*)

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Verticillium wilt is a global important disease of cotton, which threatens the development of cotton production seriously. Recent years, because of the change in climate and cropping pattern, *Verticillium* wilt was broke out in cotton production areas in China, which became one of the most important factors restricting the continuant development of cotton production. Selection and utilization of *Verticillium* wilt resistant cultivars were the most economic and effective method to control this disease. However, comparing with the cotton breeding work for *Fusarirum* Wilt resistance, the progress of *Verticillium* wilt breeding for upland cotton was relative slowness, the *Verticillium* wilt resistance of bred upland cotton cultivars was not good enough for the cotton production. How to increase the efficiency of selection was the key in cotton breeding for *Verticillium* wilt resistance, which was also the great problem for cotton breeders to search after. Three near isogenic lines with different resistant levels to *Verticillium* wilt have been derived from a stable upland cotton strain, Z5601, which came from an offspring of interspecific hybridization between *G. hisutium* and *G. barbadense*. AFLP analysis were done for the three near isogenic lines and their offsprings of intraspecific hybrids, and the molecular marker assistant selection was carried out when interlocked markers with *Verticillium* wilt resistance were got.

The experiment was carried out using the Z5629(R) and Z421(S), which are near isogenic lines each other with different level of *Verticillium* wilt resistance, and their F2 plants and F3 family between Z5629 and Z421. DNA were extracted using the modify CTAB method described by Song et al（1998）. All the materials were planted in the field for judging *Verticillium* wilt resistance, and the inoculated *Verticillium* wilt was Anyang strain, which belong to physiological type III, provided by Cotton Research Institute, CAAS. AFLP kits were brought from Life Technology company, and DNA Taq polymerase was brought from Promega. The method of AFLP analysis was follow the steps described in the kit specification.

The polymorphism among resistant and susceptible gene pool were analyzed with the all 64 pairs of primers provided by the AFLP kit, and total 3840 clear bands were amplified among near isogenic lines, and one polymorphism between resistant and susceptive lines was obtained with the primer combination of E-ACG/M-CTA. Because the materials used in this experiment were near isogenic lines, the genetic materials between the resistant and susceptive lines were almost same except for the *Verticillium* wilt resistant gene or genes. So this special band may be related with the cotton resistant gene to *Verticillium* wilt. In order to verify the relationship of the special polymorphism band with the cotton resistant gene to *Verticillium* wilt, the primer combination of E-ACG/M-CTA was used to analyze the F2 plants of Z5629×Z421. The results shown that this AFLP polymorphism band was coseparation with the *Verticillium* wilt resistance of upland cotton, with the linkage value of 8.3% and genetic distance of 9.29 cM, according to the statistical analysis with 110 F2-F3 families for AFLP analysis and *Verticillium* wilt resistance judgment. This marker has been used in cotton breeding program for judging the *Verticillium* wilt resistance in the offsprings of hybridization with Z5629, and about 90% of the resistant plants has this band as the judgment of *Verticillium* wilt resistance in this field. However, for the breeding practices, the utilization of AFLP marker was limited as it is too expensive and complex.

RAPD and SCAR Markers for Dominant Glandless Gene in *Gossypium hirsutum L.*

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Glandless or gossypol free cottons are of great value to develop the utilizations of cottonseeds and cotton scientists have been interested in developing more applicable glandless cultivars. Common glandless cotton are generated by recessive genes, such as gl_2 and gl_3. A dominant glandless mutant has been discovered in Egyptian cotton (*Gossypium barbadense* L.)The gene was transformed into *G. hirsutum* and was proved as dominant single gene Gl2e (Xian-he Zhan, 1987), being attractive for many genetists and breeders to pay more attention to study on it including its genetic markers.

We established the nearly isolated lines (NILs) from the offsprings of CRI 12 and TM-1 (*G.hirsutum* L.) as female parents crossed with a dominant glandless line, which derived from an improved CRI 12 stock with dominant glandless gene introduced from a *G.barbadense* breeding line 'Hai 1'. The Hai 1 was selected directly from an Egyptian variety 'Alexander 4'. For RAPD experiments, 240 random primers were applied. One band, produced by primer OPD 06 showed polymorphism in all template DNA pools and parents from the NILs. The polymorphism was very stable in the same RAPD experiment with three repeats of the PCR amplifications. The PCR products were about 1530bp from dominant glandless parents of two NILs by the primer OPD 06, and did not exist in other parents with glands. The products were well recovered in a F_2 population of 200 plants prepared from CRI 12-NILs with 138 out of 153 glandless plants and 5 out of 47 gland plants showing the band, which named as OD06.1530.

In order to transform the RAPD marker, into SCAR marker we extracted and purified DNA from the band, and got its sequence with 1611 bases. Two pairs of primer were designed based on the sequence by using Primer 3.0: one pair was 5'-ACCTGAACGGAGAGGGAT-3' (forward) and 5'-ACCTGAACGGGCTGATC-TA-3' (reverse); the other was 5'-ACCTGAACGGAGAGGGAT-3' (forward) and 5'-TTGAAACAAGTAAACAGAGT-3' (reverse). The PCR products resulted from SCAR primers were the same with the RAPD amplifications in the template DNAs of parents and of 200 plants of F2 population. The RAPD and SCAR markers were linked to the gene Gl2e, and they located and overlapped in the same side of the gene with their genetic distance of 10.1 cM by using the Mapmaker/Exp 3.0. The markers may be applied in marked-assistant breeding.

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Toward a Genetically-anchored Physical Map of the Cotton Genomes

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We are using a high-density (1-cM) molecular map of the cotton genome based on RFLP, SSR, and EST markers as a foundation for development of a robust BAC-based physical map. The ′ overgo′ approach is providing an efficient means by which to accomplish hybridization-based anchoring of genetically-mapped cotton sequences, and also heterolo -gous sequences from other genomes that are useful for comparative biology. The inference of a′ consensus map′ for a hypothetical-diploid cotton ancestor (Rong et al) has proven useful for taking advantage of sequence-tagged sites that are mapped onto only a subset of the taxa and/or subgenomes under study. A Microsoft Access database has been developed for the management of BAC hybridization data in three species of cotton, including the two cultivated ′ AADD′ tetraploid species *G. barbadense* (Pima S6) and *G. hirsutum*(Acala Maxxa and Tamcot GCNH), and the wild DD genome species *G. raimondii*. The MS Access BAC data management application BACMan allows for the flexible management of hybridization data, and facilitates data tracking by indexing autoradiographs using a barcode system. Our application also provides for the deconvolution of multiplexed BAC hybridization data, and is directly linked to a visual basic application that allows for the automation of film data entry. The physical mapping data set is available online (http://www.plantgenome.agtec.uga.edu/cotton)using Active Server Page technology which directly queries the MS Access database. Genetically mapped probes and BACs are available on request (paterson@uga.edu), on a cost-recovery basis. We thank the National Science Foundation and USDA National Research Initiative for supporting various aspects of our work.

Toward Development of a Whole-genome, BAC/BIBAC-based Integrated Physical/Genetic Map of the Cotton Genome Using the Upland Genetic Standard TM-1: BAC and BIBAC Library Construction, SSR Marker Development, and Physical/Genetic Map Integration

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Integrative physical mapping is the centerpiece of and essential for advanced genomics research. Upland cotton (*Gossypium hirsutum* L.) genetic standard line TM-1 is used as the reference genotype to develop a whole genome,BAC/BIBAC based integrated physical/genetic map of the cotton genome. From the TM-1 line we have constructed two BAC libraries with HindIII and EcoRI, respectively, and one plant-transformation-competent binary BAC (BIBAC) library with *Bam*HI. Three large-insert BAC and BIBAC libraries constructed with different restriction enzymes not only reduce the number of clones needed, but also enhance the coverage of a genome-wide physical map. TM-1 is used for this effort because from this line extensive genetic mutants and cytogenetic stocks were developed. In addition, TM-1 is one of the two parents of our permanent recombinant inbred (RI) mapping population used in the integrated genetic/physical mapping of the cotton genome. Furthermore, seed stock of TM-1 for the libraries was maintained at fifty-forth selfing generation by single-seed decent to ensure the plant homogeneity and homozygosity that are essential to a successful assembly of BAC contig maps by fingerprint analysis that truly reflect the genome structure of the Upland cotton. The three TM-1 BAC/BIBAC libraries contain 158,000 large-insert clones, have an average insert size of 130, 152 and 154 kb, respectively and cover >10x of the haploid $(AD)_1$ genomes. They are now being used to construct the whole-genomeBAC/BIBAC-based integrated genetic/physical map of Upland cotton and other aspects of genomic research. To have a large number of portable SSR markers for the cotton genetic map and facilitate physical/genetic map integration, we have been developing SSR markers from the TM-1 BAC libraries. The BAC-derived SSR markers have many advantages over those developed from small-insert DNA clones. The SSR marker-containing BACs provide direct bridges to physical contig maps with genetic linkage maps. They will not only streamline high-resolution mapping and positional cloning of QTLs and genes of interest, but also lead to the development of many different kinds of DNA markers that are well suited for marker-assisted breeding. An initial set of 1000 such SSR primer pairs have been developed from the TM-1/*Hind*III BAC library. Approximately 60% of these BAC-SSR primer pairs amplify only single fragments, which are very useful for integration of physical/genetic maps and identification of the A- or D-subgenome specific BAC contigs. High-density filters and pools of the TM-1/HindIII BAC clones have been distributed to the international cotton research community for specific genomic studies. TM-1 BAC-derived SSR markers will be distributed to the cotton research community once we determine the value of polymorphism information content (PIC) on each SSR marker with a diverse cotton panel. The TM-1×3-79 genetic map has approximately 1000 DNA markers and itis now being augmented with the 1000 BAC-derived SSR markers and other 1000 SSR markers currently funded by the Cotton Incorporated. Integration of genetic and physical maps with a large number of portable SSR markers on the BAC contigs will certainly benefit the cotton genome research including molecular breeding, germplasm evaluation, and functional analysis of thousands of cotton genes.

Toward Development of a Whole-genome, BAC/BIBAC-based IntegratedPhysical/Genetic Map of the Cotton Genome Using the Upland Cotton Genetic Standard TM-1: BAC Fingerprinting and Physical Map Contig Construction

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We are developing a whole-genome, BAC/BIBAC-based integrated physical/genetic map of the cotton (*Gossypium hirsutum* L.) genome using its genetic standard line TM-1 as the reference genotype. Whole-genome physical maps integrated with genetic maps will provide revolutionized tools and platforms for all kinds of genomics research, including large-scale gene mapping, cloning and target DNA marker development. Development of such an integrated physical map for cotton will accelerate its genomics research many fold. To ensure that the map is widely applicable to the cotton community, three large-insert TM-1 BAC and BIBAC libraries are used. We have developed essential strategies, tools and techniques, and automated the procedure for genome physical mapping with BACs and BIBACs using a robotic workstation (Autogen 960), two capillary sequencers (ABI 3100) and advanced computer programs. Experiments were conducted on the feasibility of development of the cotton physical map from the TM-1 BAC and BIBAC libraries using the strategies, techniques and procedure developed. The result showed that they allowed not only assembly of BAC/BIBAC contigs according to their origin of A or D subgenome, but also sorting of the contigs of A subgenome from those of D subgenome. This result indicates that it is feasible to develop a robust physical map of the AD genome cotton using the techniques and strategies that we developed. Using the automated procedure, approximately 2000 BACs can be fingerprinted and analyzed daily. Thus, the clones covering 10 x cotton AD genomes (about 150000 clones) could be analyzed in 3 - 4 months. Prior studies show that the resulting data would be sufficient to construct a whole-genome physical map of the cotton AD genome. To effectively manipulate, disseminate, access and use integrated physical/genetic maps of agricultural genomes, we have established a core facility of bioinformatics and developed a web-based, integrated Genomic Information System (GIS). The GIS system allows users to access the results from many areas of genome research, not only from integrated map to BAC/BIBAC contigs to BACs/BIBACs to gene/transcript/sequence map to gene expression map to protein sequence, structure and function, but also from genes/ESTs to BAC/BIBACs to BAC/BIBAC contigs to integrated physical/genetic map to association with traits. The system also allows genome macro- and micro-synteny search.

Construction of BAC Library for Egyptian Cotton Varieties

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BAC library for the Egyptian cotton *Gossypium barbadens*e Giza 70, Giza 86, and Giza 75 varieties have been constructed and characterized. The isolation and purification of high molecular weight DNA from nuclei embedded in agarose microbeads was an essential part of this work. Several experimental parameters were investigated including optimization of megabase-size DNA restriction enzyme digest and CHEF gel conditions to achieve the highest resolution and separation of such DNA. Fragments ranging in size from 200Kb to 500Kb were selected and recovered from agarose gel to be used in BAC library construction. The BAC vector, pBeloBAC II derived from the endogenous *E.coli* F-factor plasmid was used in library construction. Different insert: vector ligation ratios examined indicated 15:1 insert:vector to be the optimum ligation ratio. The maximum construct-host transformation efficiency calculated at 1.8KV electroporation voltage. Purification and isolation of BAC clones was followed by restriction enzyme digest using NotI to characterize insert sizes. Characterization of insert sizes and integrity using CHEF gel electrophoresis conditions at 6V/cm, 5 and 15 sec initial and final switch times respectively, and 12℃ for 12 hrs. BAC libraries for Giza 70, Giza 86, and Giza 75 varieties contained 45237, 45742, and 46531 clones,respectively,with an average insert size of 100 Kb. Considering the haploid genome size for cotton to be 2118 Mb, BAC genome equivalent. Egyptian cotton BAC library constructed for Gossypium barbadense Giza 70, Giza 86, and Giza 75 varieties provide 88% probability of isolating a specific genomic region and are optimized for future gene identification, isolation, target gene mapping, and chromosome walking.

FISH Loci of 18-26s rDNA in Four Gossypium Species

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Detection of specific nucleic acid sequences such as RNA or DNA in chromosomes by *in situ* hybridization has important applications in many areas of biology. The genes encoding 18-26s rRNA are located nucleus organizer regions (NORs) in plant chromosomes. Fluorescent *in situ* hybridization (FISH) with 18-26s rDNA as probe to somatic chromosomes may directly provide insight into genetic mapping and then, by comparisons with karyotypes, physical loci of NORs of the genome. There were many studies on karyotypes of *Gossypium* species but differences existed between authors especially about their satellite chromosomes. This paper reports the details in the field by rDNA-FISH.

We carried out the FISH experiments with 18-26s rDNA as probe to somatic chromosomes of *Gossypium hirsutum*, *barbadense*, *arboreum* and *bickii* according to the procedure described by C. Y. Wang (2001). Hybridized signals were clearly observed on the chromosomes, six for both *hirsutum* and *barbadense*, four for *arboreum* and eight for *bickii*, respectively. The karyotypes based on their rDNA-FISHs were as following: 2 n = 4 x = 52 = 32 m + 18 sm(4 sat) + 2 st (2 sat) for *hirsutum*, 2n = 4 x = 52 = 36 m + 16 sm (6 sat) for *barbadense*, 2 n = x = 26 = 24 m + 2 sm (2 sat) + 2 st (2 sat) for *arboreum*, 2 n = 2 x = 26 = 18 m (4 sat) + 8 sm (4 sat) for *bickii*. 18-26s rDNA in pares of homologous chromosomes located in number 12, 19 and 26 of *hirsutum*, 10, 19 and 25 of *barbadense*, 12 and 13 of *arboreum*, and 4, 11, 12 and 13 of *bickii*. Loci of NORs in former three species were fixed in sm chromosomes or st chromosomes. G. *bickii* was distinct with the largest number of Loci of NORs and half in m chromosomes and half in sm chromosomes. If taking number 1 to 13 as A sub-genome and number 14 to 26 as D sub-genome of two allotetraploid species *hirsutum* and *barbadense*, there were two loci of NORs in A sub-genome and four in D sub-genome for both species. Most NORs of these cottons located in end of short arm. But two loci of NORs in both of *arboreum* and bickii were adjacent to the centromere with larger satellites in the short arms, which was in concord with the results of early non FISH karyotypes. It was clear that numbers of NOR loci in these *Gossypium* species did not correlate with their ploidy. The NOR loci in the cottons may be used as molecular markers and further analysis with the rDNA FISH data may be useful in studies on their chromosome construct and plant evolution.

Current Status and Progresses in Chinese Cotton Genomic Research

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Cotton fiber, a single-celled trichome, 30-40mm in length and 15m in cell-wall-thickness, is a differentiated epidermal cell originated from the outer integument of the ovule. There are several groups in China that work on cotton gene cloning or fiber improvement using biotechnological approaches. Dr. Xiaoya Chen's group from the Institute of Plant Physiology and Ecology, *Academia Sinica*, obtained transgenic cottons that express a rabbit keratin gene, a silkworm fibroin gene, IAAM and PAT gene. The last two genes were cloned from plants and were involved in IAA biosynthesis and transport. Transgenic cotton expressing the rabbit keratin gene improved both fiber length and strength significantly. There will be approximately 100 acres of this cotton in Jiangsu and Shanghai this year. We have also produced transgenic cotton with the silkworm fibroin gene incorporated in its genome that was intended to improve the length of fibers. Cottons with an additional IAAM gene or the PAT gene possess more vigorous vegetative growth and resulted not only in greater cotton plants, but also many more cotton bolls on a single plant. Dr. Jinyuan Liu's laboratory at Tsinghua Univeristy obtained 10 cDNAs that were expressed and accumulated preferentially in early phases of cotton fiber development using FDD-PCR technique. They cloned a full-length cDA encoding the putative reversibly glycosylated polypeptide (RGP). This gene contains an open reading frame of 1080 bp encoding a protein of 359 amino acids which has 78～86% identity with other plant RGPs. Northern blot analysis showed that it is preferentially expressed in fiber cells and its transcripts are abundant both at the primary cell wall elongation stage and at the later stage of secondary cell wall thickening, suggesting that GhRGP1 may be involved in non-cellulosic polysaccharide biosynthesis of the plant cell wall. Using the same approach, Dr. Gui-xian Xia's group at the Institute of Microbiology, Academia Sinica, obtained morethan 100 cDNAs that were specifically expressed during the period of secondary cell wall thickening. She found that a beta-tubulin (*GhTub1*) gene was expressed during fiber elongation and wall thickening. Expression of this tubulin gene in yeast cells was able to promote longitudinal growth. She also isolated a 1.9kb upstream sequence that has strong promoter activity and functions better than the Monsanto company isolated E6 promoter during fiber development. Dr. Yongbiao Xue's laboratory in the Institute of Genetics and Developmental Biology, Academia Sinica, has produced a microarray with 23808 cotton cDNAs. They found about 40 fiber specific genes after screening the array with cDNAs prepared from wildtype cotton ovule and from the fiberless Xuzhou 142 mutant. They also constructed a cotton TAC library with average insert size 50~80kb. With cDNAs prepared from upland cotton fiber as testers and cDNAs obtained from a fiberless mutant, Xuzhou 142 as drivers, people in my lab performed PCR-selected cDNA subtraction (RDA) and isolated more than 280 differentially expressed cDNA fragments belong to 13 functional groups. Upon library screening, we obtained many full-length cDNAs that were missing in the fiberless Xuzhou 142 mutant. For example, one cDNA, 1179bp in length, encoded S-adenosyl-L-methionine synthetase (SAMS) and a second, 2010bp in length, encoded F_1-ATPase beta subunit with mitoc hondria targeting sequence. A third clone, 759bp in length, designated as GhFas, is a fasciclin-like protein. Semi-quantitative RT-PCR, *in situ* hybridization studies and yeast cell elongation assays were used to characterize some of the genes.

Creation of a Gene Knockout Population of Cotton

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To date about 145 spontaneous or selected mutant phenotypic markers have been described in allotetraploid upland cotton. Most have been placed on linkage groups covering about one-half of the 26 haploid chromosomes. To increase the number of mutant phenotypic markers, reverse genetic strategies in cotton were implemented by the National Science Foundation funded Cotton Genomics Research Team. Transposon mutagenesis was selected based on the Ds/lox system, which combines the advantages of transposon-tagging using the maize Ac/Ds elements for producing insertion mutants, and Cre/lox site-specific recombination for inducing gross chromosomal rearrangements. The Cre recombinase catalyzes recombination between lox sites, resulting in chromosomal rearrangements (deletions, inversions, translocations) ranging from a few kilobase pairs to cM lengths. The Ds/lox mutagenesis strategy offers an unprecedented opportunity to unveil gene functions important to agronomic traits in a polyploid genome such as cotton with high levels of repetitive DNA. Ds transposon-tagging strategies combined with Cre-lox site-specific recombination will allow the systematic dissection of large chromosomal regions of the cotton genome. The goal is to move towards saturation mutagenesis of the cotton genome by providing to the cotton research community a foundation knockout population, containing in excess of 100 independent transgenic Ds/lox insertion lines, that should result in at least one mutant site on each chromosome. Targeted induced local lesions (TILLING) will further increase the genetic arsenal for genome-wide functional genomics. TILLING couples chemical mutagenesis (EMS) with dHPLC detection of mutations. EMS produces point mutations (mostly C/G and T/A transitions) in high density and dHPLC is a sensitive high-throughput means for detecting mutations in genes of interest.

Cotton Functional Genomics: Lessons Learned from the *Gossypium arboreum* L. Fiber dbEST and Expression Profiling using cDNA Microarrays

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Over the last decade, no more than 50 cotton fiber genes have been described in the literature. However, our view of the molecular basis of the cotton fiber has changed dramatically in just a few short years as a direct result of high-throughput EST sequencing projects. As part of the NSF Cotton Genome Project, a fiber dbEST containing >36000 sequences was generated from expanding cotton fibers isolated from the A-genome diploid species, *Gossypium arboreum*.

The process of gene discovery was greatly accelerated by two critical factors the use of a diploid species, and the deep sampling of the cDNA library. Current estimates indicate that ~10000 genes are differentially expressed in expanding fibers, and thus, as much as 30% of the cotton transcriptome contributes to the growth and development of the cotton fiber. The expression of the 50 or so cotton genes studied to date is induced, for the most part, in expanding fibers. Global expression profiling of cDNA microarrays, however, revealed the novel discovery that the expression of many genes is, in fact, repressed in expanding cotton fibers. Regulation of secondary metabolism is a case in point; while some pathways are transcriptionally activated in expanding fibers, other branch pathways are strongly repressed. The repression of such genes has biological significance, and is directly related to developmental cues regulating fiber elongation, and ones that also impact the structure and composition of the fiber, and hence its fiber traits. Thus, expression profiling is, without a doubt, a powerful technology that is providing novel insight into the underlying molecular mechanisms that govern fiber properties. In the long run, linking such functional data to molecular maps will significantly enhance marker assisted breeding programs.

Functional Genomic Studies of Cotton Fiber Development

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Cotton fibers are single-cell trichomes derived from the epidermis of cotton ovules. Analysis of transcripts in developing fiber cells may provide valuable information to understand mechanisms regulation fiber initiation and growth, as well as to cotton breeding. We have made an array of more than 5000 clones isolated from a cotton ovule (and the fiber) cDNA library. Hybridization of 1536 clones revealed more that 50 showing stronger signals with the cDNA probes prepared from the wild type (fiber-containing) ovules. Analyses of expression patterns of 14 genes identified 10 that were highly transcribed in cotton fiber cells. There are four genes (*GhRDL*, *GhACY*, *GhFDH* and *GhSCP*) that are for the first time found to have a fiber-specific or fiber-preferential expression. *GhRDL1* was found to have a fiber-specific expression pattern. The *GhRDL1* is highly similar to a group of BURP domain-containing proteins, including those from *Bruguiera gymnorrhiza* (e.g., AB062745), and the RD22 protein of *Arabidopsis thaliana.* BURP domain is present in diverse plant proteins with different expressions, and is assumed to interact with structural components of the cell wall. The cotton genome contains a small family of the *RDL*, and different members of the family have different tissue-specific expression patterns. *GhACY* encodes a protein that belongs to a CoA-dependent acyltransferase family. A number of acyltransferases of this type have been found to participate in secondary metabolism, such as anthocyanin and alkaloid biosynthesis; however, fiber-preferential expression of *GhACY* may suggest a distinct function of this enzyme, which is probably related to cellular growth. FDH protein of *Arabidopsis*, a putative beta-ketoacyl-CoA synthase, has been proposed to be involved in biosynthesis of long-chain lipids found in cuticle. Expression of this cotton FDH homolog (*GhFDH*) in elongating fiber cells suggests that it may play a similar role in these highly specialized epidermal cells. The carboxypeptidase-like protein, *GhSCP*, has a relatively higher expression in fiber cells throughout the investigation stage of 1-18 DPA; this class of proteins has been found to carry out a diverse functions in plants, including processing protein(s) involved in brassinolide signaling, and acting as acyltransferases in plant secondary metabolism.

We isolated a cDNA (*GhABC1*) encoding a putative ATP-binding cassette transporter. This ABC transporter was highly expressed in cotton fiber cells during elongation stage, with a low level of the transcript also detected in hypocotyls, cotyledons and young leaves. Based on sequence similarities, *GhABC1* belongs to the WBC family. In *Arabidopsis*, the WCC family is the largest among the ABC transporter super family. Expression of this cotton ABC transporter in A. *thaliana* resulted in reduced root growth. Further investigation is now undertaking. A protein kinase cDNA, SUB8, was found preferentially expressed in fiber cells during the elongation stage. Autophosphorylation assay demonstrated that it is a catalytically active protein kinase *in vitro*. In the *Arabidopsis* genome there are 12 genes that show high homology to SUB8. Analysis of this model plants may help to explore the function of this novel type of protein kinases.

An Introduction to China Rice Functional Genomics Program

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To discover genes essential for agronomic performances of crops we initiated a program on rice functional genomics of important agronomic traits in rice in 1999. The program was funded by the Ministry of Science and Technology of China through the National Basic Research Initiative and is expected to last for five years. Around 20 research groups were organized to participate in the program, which focuses on the identification of genes related to flowering, plant architecture, fertility, reproduction, metabolic controls and stress responses in rice through a combinatorial approach based on genetics, molecular biology and functional genomics. The program′s five-year research targets have been aimed as follows: 1) Generate 3000 Ds transposant lines and 500 EMS- or radiation-induced mutant lines, determine the flanking sequences of 500 Ds insertions, and accomplish genetic analysis of 50 mutants related to rice development and metabolic controls; 2) Obtain >20000 Uin-EST of indica rice and 1000 full-length cDNA, construct microarray containing >10000 cDNA, and establish expressional profiles of genes related to the biological processes of interests and, in combination with gene functional analyses, further identify over 40 key agronomic genes;3)Establish a platform dealing with the large DNA fragment cloning and multi-gene transfer based on TAC (Transformation-competent Artificial Chroome); 4)Complete genome-wide analysis of 1-2 classes of transcriptional factors and further elucidate the biological functions of 5-10 transcriptional factors; 5) Construct a database for rice genes; 6) Accomplish genetic improvements of 1-2 key agronomic traits; 7) Train young scientists highly qualified in the fields of functional genomics and modern crop breeding in addition to publishing international journal and generating discoveries with intellectual property. Recent progress will be presented.

Differential Expression of Genes during Somatic Embryogenesis in Upland Cotton

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Somatic embryogenesis in upland cotton is strongly genotype-dependent, which is a trouble in cotton genetic engineering. Cloning genes related to somatic embryogenesis and then introducing the gene into mainly cultivated varieties would be greatly helpful for cotton improvement by gene transfer. To study the gene expression during somatic embryogenesis will lay a good basis for the cloning of the genes.

In order to investigate the differential expression of the genes related to somatic embryogenesis, the hypocotyl section (explant), explant after ten days induction on the medium of MS with IBA and kinetin, and embryogenic callus were sampled for total RNA isolation. By the protocol established in our lab, highly qualified RNA was obtained, which meet the needs of differential display very well. After reverse transcription, the first strand cDNA was realized with three anchor primers, and DDRT-PCR was performed with various pairs of anchor primers and 10 mer random primers. Through separating these PCR products on a sequencing gel, we obtained about two hundreds of differentially expressed cDNA fragments, of which 42 cDNA fragments only appeared before the induction treatment of cotton hypocotyls by hormones, 44 cDNA fragments expressed only in the hypocotyl sections after 10 day′ s induction by IBA+KT, and 51 cDNA fragments could be seen only in the embryogenetic callus. Beside the band difference among the three kinds of samples, we also observed the down- and up-regulated gene expressions among them. Up to now, we have isolated 16 cDNA fragments that express specially in one of the samples. For screening the library with the tissue specificly expressed genes, a λ phage cDNA library of cotton embryogenic callus was constructed. The titer of the primary library was above 10^6 pfu • ml^{-1}. After amplifying, the titer of the cDNA library was up to 10^{10}pfu •ml^1. The recombination rate was above 95%. Insert fragment sizes ranged from 500bp to 3kb. The identifying of the differentially expressed cDNA fragments and screening of the cDNA library are under way.

Development of Transgenic Cotton Resistant to Fungal Diseases and of Mutants by T-DNA Insertional Mutagenesis

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Verticillium (*V. dahliae* Kleb.) and *Fusarium* (*F. Oxysporum f*. sp. vasinfectum) wilt are two major fungal diseases in cotton production which cause great crop damage and yield loss world wide. Breeding and application of resistant varieties have effectively controlled *Fusarium* wilt. However, the *Verticillium* wilt-resistant varieties have not been developed to date since there is no resistant resources in upland cotton that can be used in conventional breeding. In addition, because this is a vascular-resided pathogen, it is also difficult to control by fungicides. Genetic engineering of crops with enhanced resistance to fungal diseases provides a new path to overcome these problems.

We have constructed a series of plant expression vectors harboring gene(s) of either glucose oxidase or chitinase plus beta-1,3-glucanase that was to be expressed and localized in the vacuole within cells or in the inter space between cells. The plasmids containing foreign gene(s) have been injected into cotton ovary 24h after flowering. Transgenic plants and lines have been assayed by PCR and Southern blot and evaluated in the greenhouse by inoculation as well as in the diseased nurseries for 8~10 generations. Lines with enhanced resistance to *Fusarium* and *Verticillium* wilt have been selected out. At present these lines are at the stage of environmental release approved by the Ministry of Agriculture.

Gossypium barbadense has many good agronomic characteristics such as higher resistance to *Verticillium* wilt and better fiber quality. *Gossypium arboreum* has smaller genome (diploid). For further isolation and cloning of gene(s) responsible for disease resistance and good fiber quality, we have carried out following research projects: (1) Construction of a BAC library using *G.barbadense* 7124 as a starting material. To date 140,000 clones have been selected. The average size of insertion is 60 ~ 128 kb. (2) By using T-DNA insertional mutagenesis, more than 3000 mutants of *G. barbadense* var.7124 and G. arboreum var. Jinhua have been obtained. Some mutant in the T1 plant has been found. The analysis of inserted copy number and the sequencing of flanking regions are now under way. (3) Activation tagging vector harboring tfda gene, non-promoter GUS, and 4x35S enhancer, has been constructed. More than 100000 seeds transformed with new constructed vector have been harvested and screening for transgenic plants on the way.

Preferential Expression of a Beta-tubulin Gene in Developing Cotton Fibers

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The developing cotton fiber is considered as a model system for studying the function of microtubules in plant cell elongation and cell wall biosynthesis. It has been shown that microtubules undergo active dynamic changes during fiber development. At the molecular level, Dixon et al demonstrated that two alpha-tubulin and two beta-tubulin isoforms accumulated preferentially in fibers, and Whittaker and Triplett showed that the accumulation of alpha-tubulin transcripts changed in a gene-specific manner in the developing cotton fibers. These results led to a speculation that tubulin isoforms have distinct function in elongating fiber cells. The focus of this study is to characterize the gene encoding a beta-tubulin isoform and to probe its *in vivo* function.

The cotton variety, yeast strain and plasmid used in this study were *Gossypium hirsutum* Xuzhou 142, *Schizosaccharomyces pombe* strain Q-01 and plasmid pREP1, respectively.

Total RNAs of cotton roots, leaves, hypocotyls, flowers and fibers were prepared by the method of ultracentrifugation. Northern blot hybridization, yeast transformation and phenotype analysis were performed according to standard techniques.

A cDNA library was constructed using poly(A)+ RNA isolated from –1-15 DPA fibers of cotton (*Gossypium hirsutum*). The cDNA encoding a beta-tubulin isoform (designated GhTub1) was identified through EST analysis. Northern blot analysis using 3′ -UTR of the cDNA as a gene-specific probe was performed to investigate the expression levels of *GhTub1* in various organs and in the developing fibers. The results showed that *GhTub1* gene was preferentially expressed in cotton fiber cells. During fiber development, *GhTub1* transcripts accumulated highly at the stage of cell elongation, with the highest expression appearing at the time of transition between primary and secondary cell wall synthesis. To probe the *in vivo* function of *GhTub1*, its cDNA was cloned in the yeast expression vector pREP1 and transformed into the fission yeast *S.pombe*. Overexpression of *GhTub1* cDNA in yeast cells was found to cause severe changes in cell morphology.

The preferntial accumulation of certain alpha-tubulins and beta-tubulins in developing cotton fibers have been demonstrated at either mRNA or protein level . Here we show that the transcript of a beta-tubulin gene *GhTub1* was abundant in fibers, particularly at the stages of cell elongation and transition between primary and secondary cell wall synthesis. Our results suggested a functional significance of *GhTub1* expression in fiber cell development, and meanwhile, support Whittaker and Triplett′ s speculation that tubulin isoforms have distinct function in elongating fiber cells.

Characterization and Functional Analysis of a Cotton Profilin Gene

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In plants, several cellular processes like cell division, differentiation, polar growth, and response to pathogen attack depend on rapid reorganization of the actin cytoskeleton. The reconstruction of actin filaments is controlled by a wide variety of actin-binding proteins, of which, profilin is one of the key modulators. Cotton fiber is a single epidermal cell of the outer integument of the ovule. It has been shown that the organization of cytoskeleton changes actively in fiber cells during the stages of cell elongation and secondary wall deposition. Thus, cotton fiber is considered as an ideal model for studying the function of cytoskeletal components in plant cell elongation and cell wall synthesis.

Here we report on the molecular characterization and a pilot functional analysis of a cotton profilin isoform that preferentially expressed during elongation of cotton fibers.

The cotton variety, yeast strain and plasmid used in this study were *Gossypium hirsutum* TM-1, *Schizosaccharomyces pombe* strain Q-01, plasmids pREP1 and pBin438, respectively.The cDNA of GhPFN1 was isolated from the total RNA of 6DPA cotton fibers by the method of RT-PCR. Semi-quantitative RT-PCR experiment was conducted using total RNAs from roots, leaves, hypocotyls, flowers and fibers of TM-1. The subcloning and transformation of *Gh*PFN1 cDNA into yeast and tobacco suspension culture cells, and the subsequent phenotype analysis were carried out according to standard protocols.

We have isolated a cDNA encoding a profilin isoform (designated *Gh*PFN1) from 6DPA fibers. Protein sequence alignment showed that *Gh*PFN1 shares 71% identity to profilin1 of *Arabidopsis*. Semi-quantitative RT-PCR revealed that *Gh*PFN1 was expressed preferentially in the cotton fibers with highest expression occurring at the stage of cell elongation. As a pilot study, we have investigated the in vivo function of *Gh*PFN1 in yeast and tobacco suspension culture cells. We found that over-expression of *Gh*PFN1 in yeast cells caused dramatic changes inthe cell length and cell shape, and increased expression of *Gh*PFN1 in tobacco suspension cells resulted in the formation of highly elongated cells with long and thick actin cables. These results suggest that GhPFN1 plays a role in cell elon gation and cell shape maintenance.

As one of the key modulators of the actin organization, profilin has been shown to be essential in the polar growth of the plant cells such as pollen tube and root hair cells. Here we report that the *Gh*PFN1 gene of cotton expressed preferentially in the elongating fiber cells, and over-expression of *Gh*PFN1 in yeast and tobacco suspension cells had remarkable effects on the cell length and cell shape maintenance. Based on these results, we speculate that *Gh*PFN1 may play a role in the elongation of cotton fiber cells that display extreme polar growth during development, presumably by its action on the organization of actin filaments.

Development of Gene Expression and Gene Silencing Vectors Based on Cotton Leaf Curl Virus for Functional Genomics in Cotton

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Cotton leaf curl virus is the first example of a cotton-infecting virus where infectious clones are available. Plant viruses are valuable tools in understanding plant biology as they can be engineered for expression of foreign genes or silencing of genes homologous to cloned genes. Development of these tools for cotton will greatly help in understanding gene function in cotton where transformation is time consuming and highly dependant on genotype. Characterization of genomic components of the virus identified several distinct begomoviruses that are systemically infectious on cotton but essentially requires a DNA satellite called DNA β to cause typical disease symptoms. Since DNA β is about half of full length begomovirus, DNA fragments can be cloned for either gene expression or to cause silencing of homologous genes. We have discovered that the virulence determinant encoded by DNA β is expressed from a strong, constitutive promoter. Based on our understanding of DNA components of cotton leaf curl virus, we are developing several gene expression and gene silencing vectors based on these viruses. The green fluorescent protein (GFP) gene has been cloned by either replacing βC1 or by a fusion with βC1 gene. We have also replaced the coat protein gene encoded by the begomovirus with GFP. Small fragments of gene of interest are also cloned in DNA β to cause silencing of homologous genes. Our paper will discuss the possible use of these vectors for functional genomics in cotton.

Expressional Profiling of Genes Related to Cotton Fiber Initiation and Isolation of GhIAA16 Homologus to *Arabidopsis* IAA16

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The Aux/IAA genes are rapidly and specifically induced by the plant hormone auxin and encode short-lived nuclear proteins that are capable of forming homo- and hetero-dimer. Molecular, biochemical, and genetic data suggest that they play a central role in auxin signaling and plant development. In order to investigate genes associated with fiber initiation in cotton (*Gossypium hirsutum*), a cDNA filter-array containing over 20000 clones randomly selected from an ovule cDNA library was developed and screened by differential hybridization with probes reverse-transcribted from ovule RNA of a wild type cultivar Xuzhou 142 and its corresponding fiberless mutant. Analysis of the differentially expressed cDNAs showed that a total of 10 altered their expression during ovule formation. Among them, GhIAA16 encodes a 208 amino acids protein highly homologous to *Arabidopsi*s IAA16 with 59.4% amino acids identity. Expression analysis revealed that *Gh*IAA transcripts peaked at the -3d ovule (3 day before anthesis) when cotton fibers are initiated and decreased rapidly afterwards in the fiberless mutant but maintained a steady expression wild type ovules, suggesting that it likely plays a role in cotton fiber initiation. Further experiments are required to establish its function during cotton fiber initiation.

Isolation, Identification and Characterization of Genes Differentially Expressed between Pima and Upland Cotton Fibers

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The demand for high strength fiber in raw cotton has increased because of the widespread use of high speed spinning technology in the yarn and textile industry. Improvement of cotton fiber quality through conventional breeding is limited due to the complexity of fiber quality genetics. Therefore, the primary goal of this project was to identify and characterize genes related to cotton fiber quality. A cDNA library was constructed from the high fiber-quality Pima cotton, 3-79, and screened differentially with the cDNA probes obtained from mRNAs of 3-79 and high-yielding Upland cotton, TM-1, respectively. Northern hybridization analysis showed that five cDNAs were differentially expressed in the twenty day-post-anthesis (dpa) fiber tissue of 3-79 cotton. Sequence analysis indicated the presence of a cotton lipid transfer protein (LTP), a mitogen-activated protein kinase (MAPK) and a novel gene with no homologous sequences in the Genbank database. The remaining two separate cDNAs showed high identity in nucleotide sequence to 6-day G. *arboreum* and 7-10 dpa *G. hirsutum* fiber cDNAs, respectively, but of unknown functions. Expression of these five fiber-associated genes was developmentally regulated, but not tissue-specific. The expression of MAPK gene was lower in the early fiber developmental stage (10 to 15 dpa), but higher in the late stage (20 to 25 dpa). The expression of the other four genes was at its highest in the 10 to 15 dpa stage, and lowest in the 20 to 25 dpa stage. The cDNA clones differentially expressed in the 3-79 cotton fiber were presumed to be associated with cotton fiber quality, but their specific contribution had not yet been determined.

Construction of Mutant Populations by T-DNA Insertion for Functional Genomic Study in Cotton

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The use of transfer DNA (T-DNA) as a mutagen has been developed for tagging genes in many crops, and results showed that T-DNA insertion is a random event, and that the inserted genes are stable through multiple generations. Through sequencing PCR-amplified fragments adjacent to the inserted elements, we can construct the T-DNA flanking database, which would be useful for cloning the genes tagged by T-DNA. The cultivar cv. JIHE321, which is a highly embryogenic genotype in cotton cell culture, was used as acceptor for introducing Bt gene by *agrobacterium* tumeficien-mediation. Over 1500 mutants of T-DNA insertion were obtained during five years' study. Form the 1500 mutants, seven kinds of mutants with different agronomic traits were selected for genetic analysis. Through crossing the mutant lines with non-transgenic line, we found that there were co-segregation between T-DNA and the mutant traits in F^2 population. For example, the probability of co-segregation is 85.0%, 96.5%in the strength and boll number, respectively. Every kind of trait mutant population has two extrem populations compared with the parent line. For example, we obtained a sterile line which showed co-segregation between sterility and T-DNA. Study was carried out on these populations for 3 years, and we found that all of the mutation traits were stably heritated. The mutant traits involve in plant height, number of bolls per plant, percentage of lint, average leaf area, fiber strength, micronaire, etc. The variance for plant height ranged from 115.4 to 65.2 cm , for the number of balls per plant from 23 to 5, for fiber strength from 28.2 to 16.6 cN • tex^{-1}, for micronaire from 5.8 to 3.1,and for fiber length from 31 to 26 mm. As for the parent line JIHE321, the plant height, number of balls, fiber strength, micronare and fiber length are 90.7 cm, 14.8, 20.8 cN • tex^{-1}, 4.3 and 29.5 cm, respectively.

The cotton genome sequence flanking Bt fragments were amplified by reverse PCR and cloned. Sequencing results showed that the sequence was similar to that of vector. Flanking fragments forward Bt were composed of highly AT repetitive sequence. Because AT was very rich in Bt constructs, it seems to mean that the rich AT structure in insertion site of cotton genome perhaps was important for inserting Bt and maintaining its stability and expression. After searching in GeneBank, we did not find any structural genes in the locus of Bt insertion area. Anyway, this is just a preliminary study, more investigation is underway.

Genome Analysis and Gene Expression Profiling of Fiber Development

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The goals of our cotton research program are to understand fiber developmental biology and improve fiber production using molecular and genomic tools. In the genome mapping project, we have developed a cotton genetic map using a moderate set of AFLP, SSR, and RFLP markers. Polymorphism was detected in two polyploid cotton cultivars, G. *hirsutum* L. var. Acala44 and G. *barbadense* L. var. Pima S 7. A total of 400 markers were mapped in a F2 segregating population derived from the interspecific hybridization between the two lines. The cotton genetic map consists of 40 linkage groups with an accumulative genetic distance of 2933 cM. The linkage map was compared with existing genetic and chromosomal maps by integrating a set of common RFLP and SSR markers. Furthermore, aneuploid stocks for the whole chromosomes and the long or short arm of chromosomes were used to associate linkage groups with chromosomal locations. Using the linkage maps, we analyzed QTLs controlling fiber quality and fiber-related traits. The QTL cartographer software was employed to determine experiment-wide significant thresholds by performing composite interval mapping and permutation tests. A number of fiber-related traits that are mapped with PCR-based markers can be readily applicable to molecular breeding programs. In the functional genomics project, we have studied-transcriptional profiles during cotton fiber development using cDNA-AFLP display technique. By comparing cDNA profiles in leaves, flowers, ovules, and young fiber tissues, we have identified dozens of cDNA fragments present in mature ovules and young fiber cells but absent in leaves and flowers. The expression patterns of the candidate genes were verified in fiber and non-fiber tissues using RT-PCR. To determine developmental changes in fiber initiation, we examined fiber initiation process in the isogenic lines of the wild-type and lintless fiber mutants. Fiber development was initiated relatively late in the mutants and the process aborted shortly after initiation, such that only short fuzz formed on the ovules. Experiments are underway to analyze genome-wide changes in gene expression and determine effects of the candidate genes on fiber initiation in the lintless mutants and cytogenetic lines.

Determining Cotton Fiber Gene Function via Structuring of Mutant Populations for High-Throughput Reverse Genetics

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The NSF Cotton Genome Centers EST project has released >36000 cotton fiber EST sequences from *Gossypium arboreum*, an A-genome diploid species. Of the approximately 10000 genes expressed in rapidly elongating cotton fibers, 50% or more encode unknown gene functions. The next challenge facing cotton researchers is determining the function of the fiber genes, and what role each plays in determining agronomically important fiber traits. Functional analysis using reverse genetic strategies is rapidly gaining in popularity as a result of the vast amount of sequence data that allows for a direct locus-to-phenotype screening. The value and overwhelming success of reverse genetics is especially evident in model plant systems such as *Arabidopsis*. Such methods, when applied to non-traditional crop species such as cotton, can provide novel insight into the molecular basis of agronomic traits, and pave the way for designing and implementing genetic improvement strategies. One of the newest reverse genetic strategies recently introduced is termed TILLING, or targeted induced local lesions in genomes. TILLING couples chemical (EMS) mutagenesis, which induces point mutations in the genome in high density, and a sensitive high-throughput means for detecting knockouts and mutant alleles.

As a first step towards implementing TILLING in cotton, >2000 M2 families were harvested from EMS-mutagenized *G. arboreum* M1 plants in 2001. The EMS-mutagenized *G. arboreum* M1 plants displayed a greater number and diversity of mutant phenotypes than observed in EMS-treated tetraploid (*G.hirsutum*) populations. This observation reinforced our expectation that more phenotypic variants would be recovered in the diploid species due to the reduced ploidy level, and is consistent with the number of fiber genes discovered in diploid vs. tetraploid fiber cells. TILLING for genetic lesions and mutant phenotypes in fiber genes of EMS-treated diploid species therefore promises to yield novel insight into gene function and novel genetic mechanisms that govern the growth and development, morphology and agronomic traits of the highly specialized seed trichomes of cotton.

Analysis of Functional Genes for Cotton Fiber Development

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Working on screening and identification of more important genes will improve understanding of the fiber development mechanism and may also lead to the development of transgenic cotton that could improve cotton fiber quantity and quality. Many efforts have been directed toward the isolation of fiber-specific genes, however, only a few genes have been cloned out of the thousands which are involved in the process so far. Our study was concerned with the isolation and identification of functional genes that are predominantly expressed in the development of cotton fibers.

The cotton (*Gossypium hirsutum* cv. CCRI 12) plants and the fluorescent differential display-polymerase chain reaction (FDD-PCR) and RACE-PCR were used to isolate fiber-specific genes. By the FDD-PCR technique, ten new fiber-specific cDNAs were isolated from developing cotton fiber cells and showed high amino acid identity to previously recorded cDNAs. Five cDNAs encoding bisphosphate nucleotidase, α -tubulin, β -galactosidase, annexin, and reversibly glycosylated polypeptide were identified while the functions of five other cDNAs were undetermined. Dot blot analysis showed that all transcripts of the 10 cDNAs accumulated preferentially in fiber cells and the majority were expressed in the early phase of cotton fiber development, except for F14 which accumulated at a high level during the late phase of developing fiber cells, indicating that all of the genes were involved in the process of fiber development.

Using the RACE-PCR technique, two full length cDNAs were cloned from cotton fiber cells and encode reversibly glycosylated polypeptide and bisphosphate nucleotidase respectively. The putative reversibly glycosylated polypeptide (RGP), designated GhRGP1, is preferentially expressed in fiber cells and its transcripts are abundant both at the primary cell wall elongation stage and at the later stage of secondary cell thickening, suggesting that *Gh*RGP1 may be involved in non-cellulosic polysaccharide biosynthesis of the fiber cell wall. The bisphosphate nucleotidase, designated *Gh*PAP1, belongs to the protein family of magnesium-dependent, lithium-sensitive phosphatases. The yeast tolerance to Na+ and Li+ was improved with the expression of *Gh*PAP1, and further enhanced by supplement with K+. *Gh*PAP1 also complemented the yeast methionine mutant. *Gh*PAP1 expressed mainly in the elongation stage of fiber development, suggesting that *Gh*PAP1 may be a functional gene involved in the sulfate assimilation and salt metabolism in developing cotton fiber cells. The works to investigate functions of these two genes and other candidate genes are now in progress.

Development and Application of a Transformation-competent Artificial chromosome (TAC) Genomic DNA Library in Allotetrapolid Cotton (*Gossypium hirsutum* L.)

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The technology of cloning and transferring of large DNA fragments in plants is important for high-efficient identification of new genes and study of gene functions. Tranformation-competent artificial chromosome (TAC) vector system has been shown to be very useful for efficient gene isolation in *Arobidopsis thaliana*. In order to develop an efficient platform for gene cloning and functional complementation of mutated genes in cotton (*Gossypium hirsutum* L.), a cotton TAC genomic DNA library was constructed in pYLTAC7 vector (Liu et al. PNAS 96:6535 6540,1999). The library contained a total of over 260000 clones with an average insert size of approximately 50 kb and stored in bulked pools (on average 30 clones/pool) in 26 384-well plates. To speed up clone(s) for a target gene, plasmid DNA was prepared in a hierarchical manner and one round of polymerase chain reaction (PCR) with target gene-specific primers allowed the identification of a positive pool (s) from which positive clones could be identified by a further round of PCR screen. To date, TAC clones containing several target genes, for example, GhMYB9 and GhIAA16 (Suo et al and Liang et al in this book), have been identified and are being used to tranform cotton. The TAC library constructed here is expected to have an impact on gene cloning and genetic engineering in cotton.

A Suppressed Gene in Integument Cells of a Fiberless Seed Mutant in Upland Cotton

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A fiberless seed mutant (*fl*) was identified in a commercial cotton (*Gossypium hirsutum* L.) variety Xu-Zhou 142 (FL). This phenotype is associated with lack of fiber cell initiation in the outer integument of the ovule, as was characterized by analysis of genes related to fiber differentiation and development. Two genes, *fl*-E6 and FL-E6, were cloned from *fl*-integument cells and FL-fiber or integument cells, respectively. Compared with FL-E6, *fl-E6* showed a dramatic change in nucleotide sequence: (1) FL-E6 contained a tandem repetitive sequence in which GGCTCA (Gly-Ser) is repeated five times between the 82nd and the 93rd codon from the first ATG codon, while in *fl-E6* the same sequence is repeated four times; (2) The *fl-E6* gene encodes a polypeptide of 241 amino acids but lacks two codons between the 90th and 93rd codon and three between the 171st and 174th relative to FL-E6; (3) There are also 12 nucleotide substitutions which would result in 7 amino acid differences between *fl-E6* and FL-E6. Analysis of RT-PCR and Northern Blot showed that expression of the *fl*-E6 gene is suppressed in the *fl*-integument cells, but highly expressed in FL-fiber cells. The difference between *fl-E6* and FL-E6 may be associated with lower expression of *fl-E6* in the *fl*-integument cells. Searches of protein databases with the FL-E6 gene sequence showed similarity to the protein backbones of two arabinogalactan-proteins (AGPs), one from the filtrate of suspension-cultured cells of *Pyrus communis* (AGPPc2) and the other from *Nicotiana alata* (AGPNa2). Although the function of the FL-E6 protein in differentiation and development of cotton fiber cells is not known, the data indicate that the mutation of *fl-E6* gene from FL-E6 gene may inhibit the fiber cell initiation from epidermal cells of the outer integument of the ovule.

Establishment of Mutated Gene Bank for Cotton Functional Genomic Research

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Upland cotton is one of the important cultivated allotetraploid species. From the point of functional genomic research, it is hard to obtain homozygous recessive mutants by applying of traditional mutagensis methods in this crop as its many traits are controlled by more than two pairs of alleles. Adopting of specific transposon tagging strategy which is widely used to induce and isolate mutated genes in various plant species can avoid isolating recessive mutants and is easy to find and characterize the mutated genes in the tetraploid cotton species.

A construct that derived from Ac/Ds transposable element in maize was exploited to transform 6 varieties of *Gossypium hirsutum* L. via *Agrobacterium tumefaciens* mediated transformation and to generate gene mutation. This construct is composd of an enhancer quadruplet and a bar gene, flanked by inverted repeats of Ds element at the both ends, and located between CaMv35S promoter and the coding sequence of green fluorescent protein gene (GFP). Upstream the 35S promoter, an Ac element that the inverted repeats were deleted supplies function for the Ds transposition, so that the GFP expression and the Ds excision can be monitored by visualizing the tissues of transgenic plants under microscope with excited green light source.

After 3 days co-cultivation of cotton hypocotyl segments with *A.tumefaciens* on MS medium supplemented with 0.1mg • L^{-1} 2,4-D and 0.1mg • L^{-1} kinetin, the explants were transferred to selection medium with 5 units • L^{-1} hygromycin for 8 to 10 weeks. Then the transformants displayed GFP activity and hygromycin resistance were placed on MS medium without hormone for plant regeneration.

The GFP assay demonstrated that the early excision of Ds occurred during the shoot regeneration. All regenerative plants showed variegation for GFP activity in leaves, elucidating that the Ac element lacking of terminal repeats sufficiently conferred function for Ds transposition in cotton genome. Also the GFP activity expressed in whole plant was observed in some progenies of original transgenic plants. It is possible that Ds excision happened in the reproductive cells. Initial analysis of selfed progeny lines by DNA hybridization separately with Ac and Ds probes revealed that the multiple copies of Ds element existed in the genomes of all the progeny lines. The largest ratio of Ac to Ds copies was 1 to 7, indicating the occurrence of multiple transposition and insertion events in the cotton genome.

Taking this specific tagging strategy, we can efficiently incite dominant mutation by the action of enhancer between the inverted repeats on the nearby gene or genes. Any functional gene would therefore be discovered within a suitable-sized population along with the Ds transposition, and be amenable to easy isolation and cloning in the tetraploidy cotton species.

Functional Analysis, Grouping and Expression Pattern Study of the Cotton Fiber Development-related Genes

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Cotton fiber growth consists of four overlapping developmental stages: fiber initiation, cell elongation, secondary wall deposition and maturation. To date, great progresses have been made on cellulose synthesis and deposition. The iniation and elongation requires rapid cell division, differentiation, growth and elongation, which undoubtedly includes expression of large amounts of genes. We adopted the Reprensentional Difference Analysis (RDA) method to clone the cotton fiber development-related genes. With 10DPA (days post anthesis) cotton fiber cDNA and 10DPA *fls* (a fiberless mutant) ovule cDNA as starting materials, we obtained a group of putative cotton fiber-specific gene fragments after hybridization and PCR-select cDNA subtraction. The gene fragments were then cloned into pGEM T Easy vector, resulting in a subtrated library. Sequence analysis and functional prediction showed that there are about 280 independent fiber-related genes, which included about all of the published cotton fiber-specific or cotton fiber development-related genes. They were furtherclassified into 13 cotton fiber development-related functional groups: cellulose synthesis and deposition; cell division, differentiation and growth; cell structure (including cell wall protein, cell wall extension and cytoskeleton); protein synthesis, storage and destination; lipid metabolism; transporter; glycometabolism and energy; secondory metabolism; signal transduction; disease and defense; transposons; E6; other ungrouped enzymes and proteins. We then carried out differential screening to select those expressing only or much higher in cotton fibers than in the *fls* ovules. The blotting-positive clones were reconfirmed by RT-PCR. The RT-PCR results of more than twenty cotton fiber-specific genes displayed colorful expression patterns: they all have no or little expression in the *fls* mutant ovules; they have no or little expression in the leaf, root or stem tissues of the cotton; they have an increasing or a decreasing or a consistant expression level along with the fiber development stages (0-20 DPA). Cotton fibers are in fact the trichomes of the cotton seeds. Indeed, theoretical analysis revealed that some of the cotton fiber genes showed sequence identities with some of the cloned *Arabidopsis* trichome genes whose mutation led to the defection of trichome development in the different life stages, e.g. early development, branching, expansion and maturation. We infer that these genes include all of those crucial to cotton fiber development. Further identification of these genes will undoubtedly help understand the mechanism of fiber development.

Heredity, Reproductive and Adaptive Heterosis and Variability of Intraspecies Hybrids of *G. hirsutum* L.

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On the patterns of geographically remote *G. hirsutum* L. cotton hybrids produced by intraspecies hybridization of local and imported breeds we have investigated particularities in heredity and variability of the most important economical and physiological features, display of reproductive and adaptive heterosis in different water supplement conditions. The result of the research demonstrated hereditary character of these features. The features F1 studied in the experiments allowed to estimate of a heterosis effect and find out hybrid combinations with high rate of reproductive heterosis. The research of individual reactions identified different plasticity level of initial breeds and hybrid combinations to restricted water supplement. Well adaptive to different water conditions forms showed a low level of genotypically-environmental interaction in the main economicallyvaluable parameters. We have also determined most label and steady features in the water lack conditions detected fluctuation of such parameters as direction and dominating intensity of features and calculated correlations indexes depending on genotypical diversity and water conditions. It is demonstrated that the resistance to limited water supplement is inherited as quantitative features with demonstrates of complete and incomplete domination of one of the original and superdomination with the adaptive heterosis effect. The stability to water stress does not interrelate with heterozygote of genotypes but depends on concentration of propitious genes controlling the adaptive processes. The unsteady hybrid form of F_1 increases its stability in F_2 generation; it depends on rising adaptive genes spectrum activity and leads to the different compensatory reactions. It demonstrates the necessity of genetic analysis of F_2 hybrids for adaptive selection.

Mapping Genes for Cotton Fiber Quality: *A. thaliana* as a Source of Candidate Genes

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Cellulose synthesis could play an important role in determining certain aspects of cotton fiber quality, and despite it's abundance in nature, little is known about the biosynthesis of this polymer. Recent advances in understanding the synthesis of cellulose come from the analysis of *Arabidopsis thaliana* cellulose-deficient mutants, and the isolation of a number of genes involved in this process. Among the genes involved in cellulose synthesis, those encoding the catalytic subunit of the cellulose synthase (*CesA*) appear to play an important role. The complete sequencing of the genome of *A. thaliana* has revealed the presence of 10 members in the CesA gene family. The CesA isoforms appear to have specialized and non-redundant functions in primary or secondary wall synthesis. Furthermore, evidence seems to show the requirement for more than one isoform in the same cell. We are interested in developing a genetic map of the tetraploid cotton genome, based on the analysis of a population stemming from an interspecific cross (*Gossypium hirsutum* var. Guazuncho II× *G. barbadense* var. VH8), and locating QTLs for fiber quality. One of the avenues we have chosen is the mapping of candidate genes. Amongst such genes, those encoding different isoforms of the CesA could be of particular interest. Searches in the public databases allowed the identification of a number of cotton fiber ESTs that are related to the CesA gene family. Based on the strong homology between some of these sequences and that of the *A. thaliana*, it could be inferred that, at least in some cases, the cotton sequences represent the orthologs of the *A. thaliana* genes. The alignment of the variable region (HVR2) of the *A. thaliana* CESA sequences with those from cotton allowed the construction of a phylogenetic tree, and the clustering of the different sequences. The cotton sequences clustered with *A. thaliana* sequences rather than together. This allowed the identification, among the cotton fiber ESTs, of orthologs for 5 of the 10 *A. thaliana* CesA isoforms (AtCesA9/ CesA2/CesA5/CesA6; AtCesA4; AtCesA1/ CesA10; AtCesA3; AtCesA8). Furthermore, one cotton EST sequence (AI727450) formed a branch on its own, with no *A. thaliana* related sequence. This could indicate a specialized function for this gene in fiber development. Based on the sequence of the cotton fiber ESTs, PCR primers were developed for the amplification of gene-specific probes. Amplification prducts were obtained for 5 of the 6 cotton isoforms, and sequencing of the PCR products allowed to confirm the specificity of the primers. These probes were then used to detect RFLP polymorphism between the parents of the mapping population. Among the 5 probes assayed, 4 allowed the detection of polymorphism between the 2 parental DNAs. Using this RFLP information, the corresponding genes were positioned on the saturated map of the cotton tetraploid genome.

Cotton *GhACT1* Gene Is Preferentially Expressed in Fiber and Required for Fiber Elongation

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Each fiber of cotton (*Gossypium hirsutum*) is a single epidermal cell that rapidly elongates to 2.5 ～ 3.0 cm from ovule surface within about 16 days after anthesis. A large number of genes are required for fiber differentiation and development, but it is unknown how these genes control and regulate the process of fiber development. To investigate the role of cytoskeleton during fiber development, *GhACT1* cDNA, encoding actin protein, was isolated from a cotton fiber cDNA library. Northern analysis demonstrated that GhACT1 transcripts accumulated at high levels in fiber, and at very low levels in other tissues. Subsequently, the corresponding *GhACT1* gene including its promoter was isolated, and the *GhACT1* promoter was fused with *GUS* reporter gene. Histochemical assays in a large number of transgenic cotton plants showed that the *GhACT1::GUS* fusion gene was preferentially expressed at high levels in fiber, and at low levels in other tissues such as ovule, seedling cotyledon. To study the role of the *GhACT1* gene during fiber development, anti-sense *GhACT1* gene was introduced into cotton. Fiber cell elongation in transgenic plants was dramatically reduced, as a result of the reduction of actin proteins in fibers. The results suggested that the *GhACT1* gene plays a role in fiber elongation but not fiber initiation.

Characterization of Members of the MYB Gene Family Expressed in Cotton Ovules

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The Myb family of proteins is a group of functionally diverse transcriptional activators found in both plants and animals that is characterized by a conserved DNA binding domain of approximately 50 amino acids. In plants, Myb proteins are involved in control of numerous biosynthetic and differentiation pathways including anthocyanin and flavonoid production and trichome differentiation. To investigate likely functions of Myb proteins in fiber initiation in cotton (*Gossypium hirsutum*), we isolated 72 Myb homologs using a pair of primers designed within the conserved R2 and R3 domain of Myb protein. Among them, *GhMyb9* encodes a protein highly homologous to *Arabidopsis* GL1 and MER which are essential in leaf trichome initiation. Expression analysis revealed that *GhMyb9* transcripts peaked at the -3d ovule (3 days before anthesis) and decreased rapidly afterwards both in a wild type cultivar Xuzhou 142 and its corresponding fiberless mutant, suggesting that it likely plays a role in cotton fiber initiation. Further functional tests in *Arabidopsis* and cotton are in progress.

Cloning and Characterization of Fiber-specific Genes Through High Throughput Analysis

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Our current project is to isolate, identify and characterize cotton fiber-specific genes in order to pick up candidates for fiber quality improvement. Firstly, 10DPA(day post anthesis) cotton fiber cDNA library was constructed with 5X106 primary titer and 1.3kb average insertions. Secondly, with cotton fiber and hairless ovule as starting materials, we performed PCR-Selected cDNA Subtraction in different combinations and isolated two groups of differentially expressed clones, which then were inserted into pGEM-T vector to form subtraction libraries. cDNA array showed that there are 100 clones (including nearly all 18 known fiber specific genes) in the fiber subtraction library indeed representing genes specifically or highly expressed in cotton fiber, which were then confirmed by RT-PCR. Using the above-confirmed clones as a probe, we were able to obtain many full-length genes by screening a cDNA library that was constructed using mRNAs prepared from cotton fibers. One of them, designated as *GhFas* (for *Gossypium hirsutum* Fasciclin) was chosen for further analysis. *GhFas* is the cotton homologue of Fasciclin, a member of the immunoglobulin superfamily, which is specifically expressed at the presynaptic terminals in a variety of animal systems and is supposed to function in regulating a number of proneural gene expressions in vertebrate. In situ hybridization demonstrated that *GhFas* expression was detected exclusively in bubble-shaped epidermal cells of cotton ovule, which would grow up to form fibers, and no signal was detected in other epidermal cells. When transferred into *Schizosaccharomyces pombe* SQ-01, GhFas expression caused host cells to become sigificantly longer than vector-transformed control cells. These results demonstrated that *GhFas* might play an important role in selecting specific epidermal cells of cotton ovule to elongate and form fibers. Further study will be carried out using *Arabidopsis* trichome mutant as model to directly demonstrate the role of *GhFas* in trichome development, since cotton fiber is a homologue of *Arabidopsis* trichome. Our results may shed light on the elucidation of fiber-forming mechanism in cotton.

Cloning of Two Genes Related to Plant Defense Response ofSea Island Cotton (*Gossypium barbadense* L.)

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Verticillium wilt, caused by *V. dahaliae*, is a serious fungus disease of cotton in China. Nearly all cultivated upland cotton (*Gossypium hirsutum*) varieties are sensitive to it. Some species of island cotton (*G. barbadense*), however, have a natural resistance to this pathogen. To investigate the mechanism of SAR signal transduction and response to pathogen, two genes, which play important roles in the development of SAR, are cloned by degenerated PCR.

Casein kinase II (CK2) has been suggested to participate in the SA-induced phosphorylation of proteins that probably enhance binding activity of cis-element to Nuclear Factors in tobacco and *Arabidopsis*. For cloning the *G. barbadense* CK2 gene, CK2 genes from other sources and ESTs sequence showing similarity to CK2 are used for design of degenerated oligonucleotide primers. A cDNA library was constructed using RNA extracted from island cotton 7124 after inoculation with elicitors of *V. dahliae*. The designed primers were used to amplify the cDNA library using touch-down Polymerase Chain Reaction (PCR).A ligation and transformation procedure using the PCR products is then performed. The products were inserted into pMD 18-T vector and then sequenced. A 597 bp fragment was obtained. Analysis of its nucleic acid and deduced amino acid sequence showed 69% and 73% similarity to *Arabidopsis* casein kinase II beta subunit(CKB2). WRKY factor is a key regulator of distinct plant defense responses and also involves in certain developmental programs. The WRKY domain, about 60 amid acids, has the ability of binding to the W-box (TTGACC/T)---an element in the promoter of SAR genes. The WRKY of island cotton 7124 was cloned by the same procedure. The fragment is 591 bp and shows high similarity to *Arabidopsis* WRKY2 at 55% of DNA sequence. The deduced amino acids show presence of a WRKY domain. Of these 64 amino acids, 55 amino acids are same as the *Arabidopsis* WRKY2. The characterization of CK2 and WRKY genes and their roles in island cotton defense response are in progress.

In vitro Expression and Characterization of a *Gh*DREB Transcription Factor Containing AP2 Domain in *Gossypium hirsutum*

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Abiotic stress including drought, low temperature, ABA and high salt is major factor affecting the plant growth. Isolation and functional study of abiotic stress-related gene will be helpful to elucidate the signal transduction mechanism of the target gene under abiotic stress during the growth of plant. By using gene-transfer technique, the target gene is incorporated into the plant to improve the adaptation of plant to abiotic stress.

One-week old seedlings of cotton SimianⅢ grown under drought stress was used to isolate total RNA and Genomic DNA. The cDNA of the target gene was isolated by the nested PCR and RT-PCR technique using two pairs of nested primers and a pair of specific primers. One-step RT-PCR was employed to evaluate the amounts of translatable mRNA of target gene under abiotic stress with one pair of specific primers designed based on the both the ends of the target gene, *Gh*Ubiquitin, as an internal control, was amplified in the same tube using One-Step RNA PCR kit (Takara DRR024). One μg total RNA was added into the each tube for all RT-PCR reactions.

A novel *Gh*DREB transcription factor induced mainly by drought, ABA and low temperature, and induced slightly by high salt was isolated using multi-step nested PCR and RT-PCR techniques. For identifying if this foreign gene could be expressed in the *E. coli* strain, the fusion expression vector was constructed by incorporating the complete cDNA fragment into the pGEX4T-1 vector, and cultured in XL-1 *E. coli*. Detection of SDS-PAGE indicated that this foreign gene is successfully expressed in *E. coli*. strain (XL-1) after induction using IPTG for 6 hours at 37℃, and the molecular weight mass of induced proteins 42.83kD including 26kD from expression vector is identical with that -(16.83-kD) of the protein of interest in fusion vector, suggesting that *Gh*DREB is a gene which could be expressed *in vitro*. *Gh*DREB containing AP2 conserved domain in which there are two conserved binding sites Valine and Glutamic acid at the 14th and 19th respectively (counting is usually based on the conserved domain) encodes 153 amino acids. Data suggested that *Gh*DREB gene is a typical member of family containing AP2 domains, which are widely conserved in the DREB transcription factors in plant, and interact specifically with sequences containing core element 5'-TACCGACAT-3' isolated and identified in rd29A gene regulating the tolerance of the plant to drought and low temperature from *Arabidopsis thailana* by Shinozaki (1994). Interestingly, *GhD*REB has significant homology in sequences of deduced amino acids of the AP2 conserved domains in comparison with AP2 domains in the other DREBs from different plants, suggesting this gene belongs to one dependent family member in the cotton, it is very likely that *Gh*DREB probably play an important role in regulating the tolerance of target gene in cotton to adverse abiotic stress. To elucidate the expression

mechanism of *Gh*DREB gene during stress, we analyzed the translatable mRNA level of the *Gh*DREB gene by using One-Step RNA PCR, *Gh*Ubiquitin was used as an internal control. The data indicate that *Gh*DREB gene are strongly induced by drought, ABA and cold, and much expressed under high salt stress compared to the control without stress induction. In addition, *Gh*DREB gene is mainly expressed in the roots and stems of cotton, little mRNA accumulation was detected in the leaves of the cotton. This suggested that *Gh*DREB gene has multi-pathway signaling transduction in regulatory system of gene during the stress response. Although some different pathways are thought to function independently from each other, it is possible that cross talk between these different pathways certainly exists. In recent years, a number of genes mediating the stress response have been identified, the typical one is DREB1 which provides a first attempt toward that DREB1 overexpressing could increase the tolerance of *Arabidopsis* to water and cold stress. Therefore, it is very necessary that we should learn more about DREB proteins and their regulation mechanism in different plants to improve the plant tolerance to abiotic stress.

Morphological Analyses of a Stable Cotton Homeotic Variant *(chv1)*

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Physiological and genetic variations exist richly in the cotton plants regenerated from somatic embryo. A stable homeotic variant (*chv1*) of cotton from regenerated plants was identified. Using light microscope and scanning electron microscope to investigate the morphological changes in chv1, the results revealed that all the floral organs in the chv1 plants converted into leaves, meanwhile the placenta and ovules bearing on the basal part in central leaf-like organs can be discerned. A flower of the variat consists of three to seven bracts, fourteen to thirty one leaf-like organs. Arrangement of the leaf-like organs is intermediate between spiral and whorl.

The floral organ mutant, chv1, may be a useful material in the research of cotton flower development and somaclonal variation. Firstly, The chv1 provide an evidence to support old theory in cotton, that flower organs are modified leaves. This theory is supported by the observation that triple mutations lacking all three of the ABC gene activities and make all flowers become leaf-like. Secondly, although the carpels of the chv1 have converted to leaf-like organs, ovules still emerged at the base of the organs, which normally bear on the axile placeta. This observation supports Angenent's viewpoint that the ovule should be seen as another class of floral organs. Finally, the chv1 is a somatic variant, and the research of molecular mechanism of somatic variant is still in infancy. Further study on the genes involving the mutation of chv1 will help us to shed light on the mechanism of somatic variations.

Introduction of a Cationic Peptide, CEMA, into Cotton Caused Abnormal Phenotype and Chloroplast Degeneration

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As a strategy for phytopathogen control through transgenic way, antimicrobial peptide genes have been employed over the last two decades. CEMA is a cationic antimicrobial chimeric peptide, which is produced by fusing eight amino acid residues from the antimicrobial peptide ceropin A with a modified meltin. Three transgenic cotton plants were generated by bombardment of cotton shoot tips using BioRad particle gun. The gold particles were coated with a cationic peptide, CEMA, DNA. All of the three transgenic plants showed chimeric phenotype. The leaves in the aberrant shoots became mosaic and twisted, and the flowers were sterile and deformed. In contrast, the leaves, flowers and bolls in the healthy shoots were the same as that in the wild type plants. In lesion regions, the chlorophyll contents dropped by 28%, the palisade tissue were showed absent partially and the vascular tissue more or less in degenerating. Using transmission electron microscope the observation revealed that the structures of neucleus, mitochondrion, endoplasmic reticulum, Golgi complex and microbody appeared nearly in normal. However phagocytose action were active in some part and the secondary walls of mesophyll cells became thicker Obvious changes in chloroplast were found in lesions, i.e., the number of stocks in granum abnormally decreased with aggregation of osmiophile globule and a near absence of thylakoid space. All these abnormal phenotype probably caused by the binding of the foreign cationic peptide to thylakoids membranes or other unclear mechanism. To our knowledge, this is the first report of ultrastructural change observation on transgenic plants with cationic peptide gene.

Cloning and Characterization of Polyphosphoinositide Binding Protein (*Gh-sh2*) Gene from Cotton

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Fiber initiation and early elongation are important developmental stages at which the final fiber number per seed is determined and the fibers show dramatically changes in cell shape and gene expression. In order to identify genes function in fiber initiation and elongation, cDNA-AFLP technique was used to compare the gene expressions of the ovules of Xuzhou142 (WT) and its flint-less fuzz-less mutant (fl) at fiber initiation stage. Some 30 differential fragments were recovered and sequenced, among which fragment 01005 was found to be sequence similar to soybean polyphosphoinositide binding protein (GenBank accession No. T05953), which is presumably a component of a complex involved in regulating actin polymerization/ depolymerization (Dewey, 1998). Y-RACE method was employed to amplify the 3′ and 5′ unknown sequences from cDNAs, two amplified fragments (005A and 005S) were found to be overlapped with the 3′ and 5′ ends of fragment 01005. Joining fragment 01005 and its flanking sequences, the full-length cDNA of cotton polyphosphoinositide binding protein gene (*Gh-sh2*) was obtained, and the ORF sequence was futher amplified by pfu polymerase. The cDNA consists of 955bp, and contains a 744-bp ORF with a putative protein of 247 aa. BLAST analysis showed that Gh-sh2p was homologous to soybean polyphosphoinositide binding protein Ssh2, putative poly-phosphoinositide binding protein of *Arabidopsis thialiana* and sec14 like protein of Oryza sativa, with the identity and positive of 66% and 84%, 62%and 79%, 48% and 72%, respectively. By Y-RACE amplification, the expression of *Gh-sh2* was detected in the WT ovules, but not in fl ovules, at fiber initiation stage; and the transcripts accumulated in hypocotyls, fiber, and ovules in the middle and late developmental stages, but not in root and leaf.

It was reveled (Kost，1999；Zheng and Yang，2000) that two polyphosphoinositide speacies, phosphatidylinositol-4-monophosp-ate and phosphatidylinositol (4,5) biphosphate, play a critical role in directing the polar growth of pollen tube. Plant polyphosphoinositide binding protein may be a key factor in the regulating polyphosphoinositide dependent phenomena, as its mammalian and yeast homologues. It appears that *Gh-sh2* gene expresses in tissues growing rapidly and undergoing cell elongation, and the gene may play a important role in fiber initiation and elongation. The over-expression and anti-sense vector have been constructed and transformed, the research on the biological function of *Gh-sh2* gene is in progress.

YADE, a New Method for PCR Walking of Genomic DNA and cDNA in Cotton

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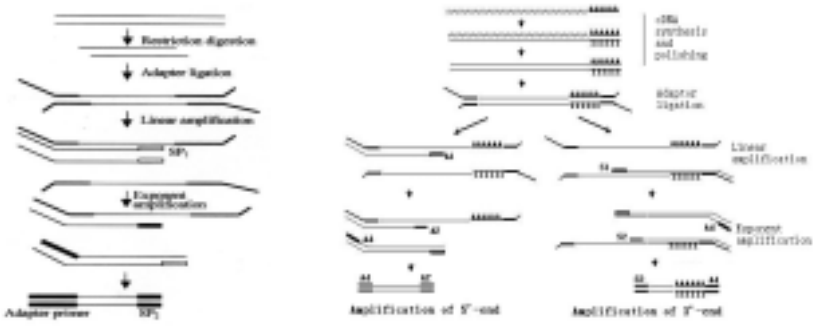
The isolation of flanking sequences is a crucial step in the study of gene cloning and expression. On the basis of Y-shaped adaptor (Prashar and Weissman, 1996), we designed a new method which could be used to amplify the flanking region from both genomic DNA and cDNA. This method was designated as Y-shaped adaptor dependent extesion (YADE). The schematic of this method is showed in fig.1.

Using this method, the 5′ flanking region of gibberellin 20-oxidase (*ga20)* gene and sucrose synthase (*susy*) gene were successfully isolated from cotton (*Gossypium hirsutum*). Firstly, the genomic 5′ fragment of the two genes was amplified by two pairs of primers designed according to the susy cDNA and an EST with sequence similarity to the *Arabidopsis thialiana* GA 20-oxidase, respectively. Then two nested primers were synthesized and used to amplify the corresponding 5′ flanking region. The amplified fragments for *ga20* gene was 1327 bp in length, containing 1249bp upstream to the putative start codon and 50bp overlapped with the EST. The amplified 5′ flanking region of *susy* gene was 1739bp with a 1583bp stretch upstream to the start codon, and the overlapping sequence with the cDNA gene was 89bp. Mutiple light response elememts were detected in the amplified flanking regions. To study the expression character, the two amplified 5′ flanking regions have been fused to a GUS gene, and the further transformation and detections were in progress.

YADE method was also employed in rapid amplification of cDNA ends (RACE). With this method, the 3′ and 5′ terminals of most of cDNA species present in the sample can be amplified from a single reaction of cDNA synthesis. The 3′ and/or 5′ cDNA ends of 4 differential cDNA-AFLP fragments (F027, F010, 01005 and 01068) from cotton ovules and a chitinase-like gene from balsampear, *Momordica charancia*, were successfully amplified. Sequence alignment demonstrated that the two F027 fragments obtained by YADE, although several-base shorter in the ultimate terminal than those amplified by RACE kits (TaKaRa), could be joined to gain the complete coding region. Further amplifying the ORFs with the primers designed according to the YADE products showed that this method was a reliable method to isolate cDNA genes.



A **B**

Fig.1 Schematic of YADE method. A, YADE for genomic walking; B, YADE for RACE.

Isolation and Characterization the ADP-ribosylation Factor(ARF) Gene from Cotton Anther

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ADP-ribosylation factor（ARF）is considered as the family of small GTP binding proteins, with molecular mass around 21KD. It has been known that ARF plays important roles in cells to regulate membrane traffic and structure and intracellular signal transduction system in yeast and mammalian. Although ARFs have been uncovered in several plants in recent years, the function of plant ARFs remains poorly understood. We isolated a fragment from mRNA differential display in developing anther between a upland cotton (*Gossypium hirsutum* L.) male sterile line and its sibling fertile line of "Dong A", then we cloned a gene from cotton anther cDNA library by use of this fragment as probe. The predicated product of this cDNA is related to the ARF.

The positive clone was obtained by GHA27 as probe to screen cDNA library of cotton anther. Sequencing results indicated that this 828bp clone contained a 546bp length of open reading frame (position131-676) encoding a polypeptide of 181 amino acids with a predicted molecular mass of 20.7KD. Sequence comparison of these positive clones with proteins in GeneBank, EMBL, DDBJ and PDB databases revealed a high amino acid sequence identity to ARFs of yeast, mammalians and other plants. This gene isolated from cotton anther putatively encoding ARF was designated as *Gh*ARF (Accession No. AJ421017). Several conservative motifs, P (GLDAAGKT), G (NKQDL), G' (DVGGQ), which are present in the other known ARF proteins as involved in GTP binding motifs, could be also recognized in the *Gh*ARF. On the nucleotide level, *Gh*ARF showed 86%, 84%, 83% identity to the ARFs in Arabidopsis, soybean and wheat respectively. On the protein level, Similarity comparison of *Gh*ARF revealed high identity to *Arabidopsis* and pepper(99%), and to wheat and rice (98%) respectively, indicating that ARF are very conservative in plant kingdom. Probing of a southern blot of genomic DNA digested with *Eco*RⅠ, *EcoR*Ⅴ, *Kpn*Ⅰ(enzymes that do not cut inside the prob) by GHA27 that contained 440bp coding region identified 1 or 2 major and a few minor bands. The hybridization pattern indicated that a few copies of the ARF genes are present in the cotton nuclear genome. To determine the expression pattern of *Gh*ARF gene by RNA dot blotting, the results showed that *Gh*ARF transcripts accumulated in anther, pollen and corolla, but not in leaf, root and ovule. It was weakly detectable in pollen and the amount increased in corolla and anther which are at meiosis and microspore stages. To our knowledge, this is the first report of specificity of ARF in anther, pollen and corolla. The function of *Gh*ARF in cotton floral development is under investigation.

Cloning and Sequence Analysis of a Steroid 5α Reductase Gene from Cotton (*Gossypium hirsuturm* L.) FiberMing

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Brassinosteroids (BRs) are natural growth-promoting products found at low levels in pollen, seeds, and young vegetative tissues throughout the plant kingdom. Recently, the notion that BRs are essential for plant growth and development has been widely accepted by the discovery of BR dwarf mutants of *Arabidopsis*, pea and tomato. The observation that only BRs could rescue the mutant phenotypes to the wild type provided convincing evidence that BRs are indeed essential plant hormones for normal plant growth and development. Cell elongation is developmental process that is regulated by light and phytohormones and is of critical importance for plant growth. The dwarfed phenotype results from a failure in normal cell elongation. The cells of BR dwarf mutants are far shorter than the corresponding wild-type cells. Furthermore, exogenous application of BRs could elongate the cells and rescue the dwarfed phontype. These confirmed the role of BRs in cell elongation. However, the mechanisms of BRs acting on cell elongation, especially in cotton (*Gossypium hirsutum* L.) fiber development are largely unknown. The fibers of cotton are single-cell trichomes that result from elongation of epidermal cells of the ovule and undergo rapid and synchronous elongation. Because the longation occurs at a fast rate over a relatively long period, uninterrupted by cell division, The fibers of cotton are a good experimental system for studying cell elongation. In addition, changes in the cell wall structure of elongating cotton fibers have been well characterized.

To understand the roles and mechanisms of BRs in cotton fiber elongation and to regulate the level of endogenous phytohormones by using the gene of biosynthetic pathway of hormone, we have screened the EST database of cotton fiber using steroid 5α reductase DET2 gene (*Arabidopsis thaliana*), which is the key gene on the biosynthetic pathway of brassinosteroid. Based on a contig sequence that results from three EST fragments, we amplified a 645-bp fragment. A fiber cDNA library of XuZhou 142 (*Gossypium hirsutum*) was screened using this amplified fragment as probe. We obtained a clone (*Gh*DET2), which contains 996 base pairs and a single, long open reading frame that encodes a 248-amino acid protein. BLAST analysi demonstrated that the deduced polypeptide was homologous to *Arabidopsis* DET2p with the sequence identity of 72% and the similarity of 89%, and mammalian steroid 5α reductase with the identity of 36 to 42% respectively. Phylogenetic analysis shows that *Gh*DET2 is very closely related to *Arabidopsis* DET2.

Cloning and Expression Analysis of a LIM-Domain Protein Gene from Cotton (*Gossypium hirsuturm* L.)

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LIM-domain proteins are implicated in multiple cellular and developmental processes in eukaryotes. Their essential roles have been well characterized in animals. It was revealed that LIM-domain protein plays an important role in various cellular processes, including construction of cytoskeleton, transcription control and signal transduction. However, only a few members of this protein family have been identified from plants, and the function of LIM-domain protein in plants is largely unknown. Since LIM-domain protein of plants are structurally related to the animal muscle proteins CRP1, CRP2 and CRP3, which are essential components in the organization of the actin cytoskeleton, particularly at the sites of membrane adhesion. CRP1, CRP2 and CRP3 have been shown to bind the cytoskeletal proteins zyxin and α-actinin, two important regulators of actin cytoskeletal organization. Plant LIM-domain proteins may have similar functions in plant cell. Cytoskeleton plays an important role in the plant developmental processes by regulating the direction of the cell elongation. But the understanding of the mechanism is limited.

The fiber cells of cotton (*Gossypium Hirsuturm* L.) represent a unique cell type that undergoes a period of very rapid elongation. Fiber cells are also single-celled trichomes, which arise in near synchrony from the epidermis of the ovule and may elongate at peak rates in excess of 2mm • d^{-1} during the rapid polar-expansion phase of development. So the cotton fibers are a good experimental system for studying cell elongation.

Based on cotton fiber EST database and contig analysis, the coding region of a cotton LIM-domain protein gene (*Gh*LIM1) was obtained by RT-PCR from cotton 4DPA (day post anthesis) ovule (with fibers). The cloned fragment of 848 bp contains an open reading frame of 570 bp, coding a polypeptide of 189 amino acids. It was demonstrated that the deduced GhLIM1 protein was highly homologous to the LIM-domain protein of Sunflower (*Helianthus annuus*) with the sequence identity of 71%, Tobacco (*Nicotiana tabacum*) with the sequence identity of 89.9% and *Arabidopsis* thaliana with the sequence identity of 76%. Furthermore, same as the other plant LIM-domain protein, GhLIM1 also contains four regions, a short N-terminus (nine residues), two 52-residues LIM domains separated by a 50-residues spacer region, and a short C-terminal region (only 30 residues). Particularly, two intact LIM-domains, with the conserved sequence of double zinc-finger structure (C-X2-C-X17~19-H-X2-C-X2-C-X2-C-X16~24-C-X2-H), were found in the GhLIM1 protein. But the cotton, sunflower, tobacco, and *Arabidopsis* proteins show the same amino acid residues different with the animal CRP proteins. First, the last zinc ligand in each of the LIM domains is a histidine (h), while it is a cystein (c) in the animal proteins. Secondly, the plant proteins have an unusual second LIM domain. RT-PCR analysis showed that *Gh*LIM1 gene expressed in root, hypocotyls, leaf, anther and fiber (4DPA, 12DPA, 18DPA). It was proposed that *Gh*LIM1 gene would be crucial for cotton fiber development.

Cytogenetics and the Utilization of *Gossypium* Germplasm

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Interspecific introgression contributes significantly to genetic improvement of cotton (*Gossypium hirsutum* L.). Cytogenetics has figured prominently in the creation of interspecific hybrids, synthetic polyploids, backcrosses, and other stocks essential to early- and mid-stage manipulation of germplasm at genome-wide and chromosome-specific levels. The spectrum of qualitative and quantitative improvements garnered directly from germplasm introgression includes [1] morphological traits, [2] reproductive traits, [3] biochemical traits, [4] increased tolerance and resistance to specific pests, pathogens and abiotic stresses, [5] improved quality, and [6] increased yield. Although species and interspecific hybrids were used in the early mitotic and meiotic studies that established gross features and differences among 13- and 26-chromosome Gossypium genomes, and related these to geographic distributions, relatively few interspecific hybrids have been used for long-term backcross-mediated introgression in the USA. Future intrgression efforts should embrace complementary whole-genome and chromosome-specific approaches. Hypoaneuploid *G. hirsutum* stocks of the Cotton Cytogenetics Collection are especially helpful to both mapping and introgression viz a viz development of alien chsomosome substitution lines. Hypoaneuploid coverage of the genome is incomplete, but improving. Molecular cytogenetic analyses have raised issue with phylogenetic dogma, suggested major roles for repetitive elements in *Gossypium* genome evolution, and indicated 52-chromosome *Gossypium* species are of at least octaploid origin. Molecular cytogenetics is likely to play important roles in assessing introgression products for alien genome content, and in developing cotton linkage maps, integrated physical maps and genomics. Summarily, opportunities for additional improvement through interspecific introgression abound. For maximal success, introgression efforts must be continuous, sustained and involve cytogenetics. Theoretical and practical findings across crops from the past century clearly manifest the cryptic existence of beneficial alleles among wild relatives. Thus, support for germplasm introgression to improve baseline yield and other multigenic traits should be pursued on that basis, and not relegated to instances where the donor has been demonstrated to carry a desired trait.

Current Status of Cotton Genome Resource in India and Initiative on Utilization of Gene Pool through Molecular Technologies

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The genus *Gossypium* is one of the largest having nearly 50 species of which two are tetraploid cultivars and two are diploid cultivars with 2n = 52 and 26 chromosome respectively and the rest are wild to semi-wild at both these ploidy levels. The tetraploids (AD) are supposed to contain the Asiatic cotton (A) and American diploid (D) species genomes.

The Genome Project on *G. hirsutum* the largest global cotton species under cultivation may be undertaken besides that of *G. arboreum* for better under standing of the molecular level structural and operating mechanism for improving cotton productivity and quality. Later on, the study could be continued in *G. barbadense*, *G. herbaceum* and also the two wild species of the tetraploid cottons.

Cotton offers wonderful possibilities for higher retained burst bolls, modified uniform maturity plant habit, leaf canopy and improved levels of multiple resistance and multiple quality parameters of fibre and seed for serving future needs of our society.

India has the largest area under cotton and has the unique distinction of commercial cultivation of all the four cultivated species of the genus *Gossypium i.e.hirsutum, barbadense, arboreum and herbaceum besides* 50 per cent area under hybrid cottons (both Intra and Interspecific and at both the levels). It has also the second largest collection of cotton genetic resources in the world of all the 4 cultivated species of cotton. The Gene Bank strength includes 5890 *Hirsutum*, 1870 *Arboreum*, 323 *Barbadense*, and 528 *Herbaceum*. We have 25 wild species maintained *ex-situ* at CICR, Nagpur. The available genomes include-A1, A2, A3, B1, B2, B3, C1, D1, D2-1, D3-k, D3-d, D4, D5, D6, D7; E1, E2, F1, G, G1. Besides the above, we also maintain the races of the cultivated species i.e. Arboreum-6, *Herbaceum*- 1, *Hirsutum*-7, *Barbadense*-1.

Utilization of various wild species (Genomes) through introgression in India have already paid rich dividends by the development of new genotypes/varieties/hybrids for various desirable traits such as improvement in quality (MCU 5, DCH 32), disease resistance (Arogya), insect resistance (B 1007, DHY 286), drought resistance (Deviraj, Devitej), male sterility (many), yield (many). The wild species already utilized in the improvement of cultivated cotton include *G. thurberi* (D1), *G. raimondii* (D5), *G. armourianum* (D2-1), *G. anomalum* (B1), *G. tomentosum* (AD-3), *G. harknessii* (D2-2),G. aridum (D4) etc.

The gene pool that exists in the wild species is potentially available and is to be exploited. Some traits from these pools have been transferred to upland cotton. After hybridization with the cultivated tetraploid the high genetic variation and recombination are expected. Due to normal pairing between homeologous chromosomes within the same genomes or sub-genomes, chromosome loss

seldom occurs in subsequent selfing or backcrossing generations. Linkage drag is a problem in the selection of hybrid combinations of interspecific and intraspecific gene transfer in cotton because of polyploidy in nature. The unimproved germplasm carry genes that are linked to the desired traits on the chromosome segment from the exotic germplasm. Molecular technologies had provided numerous methods with which to associate molecular sites on the DNA of plant genome with heritable traits. The works on these areas have already been initiated in India. The wild species of *Gossypium* are important sources of useful traits such as special and superior fibre properties, cytoplasmic male sterility, resistance to biotic and abiotic stresses etc. which can be introgressed into the cultivated species for improvement. A total of more than 65 random sequence decamer primers (Operon Technologies Inc., from groups (A, C, F, and B) were screened using DNA samples. As a result 25 primers were selected for the PCR amplification analysis of all the DNA samples which produced 758 RAPD fragments. From the binary data matrix the genetic distances were estimated which reveals genetic diversity among the species. The dendrogram resulted by UPGMA clustering method of all the species were clustered in to five groups of genomes B, D, F, C, G. Germplasm accessions with economically important characters like good fibre length, jassid and bollworm resistance, leaf curl virus resistance were collected from the gene bank. Genomic DNA was isolated using modified method from our laboratory.

Therefore India can play an important role in the International Cotton Genomics Project and may be a resourceful partner in this endeavor for the benefit of human race in general and cotton in particular.

Gossypium Germplasm Resources

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The genetic variability residing in diploid and tetraploid species of the *Gossypium* genus represents a large, under-utilized resource in current cotton improvement efforts. Genetic diversity in elite germplasm is reported to be narrow. Actual diversity on the land is narrower, due to preferential mass planting of successful cultivars and breeding techniques that tend to promote an over-reliance on a few genotypes. Potential resources available to expand the genetic diversity and improve the agronomic quality of cotton include 49000 accessions of *Gossypium* residing in institutional *ex situ* germplasm collections, worldwide. Of 41 institutions that report having cotton related activities and participate in the World Information and Early Warning System of the FAO, 25 report having *ex situ* collections. The majority of these institutions report holding tetraploid germplasm that is classified as either advanced cultivars, obsolete cultivars, breeder lines, or land races. The Agricultural Research Service of the United States Department of Agriculture has classified the 8986 accessions of its cotton collection into germplasm pools according to relative genetic accessibility and utility. The primary germplasm pool of the collection, defined as tetraploid germplasm that will yield a fertile F_1 hybrid upon hybridization with the AD_1 genome, is currently comprised of 5643 accessions of five species. The secondary germplasm pool is composed of accessions that require significant genetic manipulation to obtain fertile hybrids when crossed to the AD_1 genome, but yield progeny with high chromosome homology to the AD1 genome and high levels of recombination. The secondary germplasm pool is represented in the U. S. collection by 2258 accessions, distributed among the A, D, B, and F genomes of 19 species. The tertiary germplasm pool of the United States Cotton Germplasm Collection exhibits low levels of recombination in hybrid combinations with the AD1 genome. The tertiary germplasm pool is represented by 115 accessions, distributed among the C, G, K, and E genomes of 14 species. Despite their relative under-utilization, germplasm resources have made significant contributions to cultivar improvement. The primary germplasm pool has contributed sources of pest resistance to boll weevil (*Anthonomus grandis* Boh.), bud and boll worms (*Heliothis* spp.), pink bollworm (*Pectinophora gossypiella* Saunders), spider mites (*Tetranychus* spp.), root knot nematodes (*Meloidogyne incognita* Chitwood), and reniform nematodes (*Rotylenchulus reniformis*); resistance to bacterial blight (*Xanthomonas campestris* pv *malvacearum*); and the glandless, nectariless, glabrous, and frego bract morphological traits. The *G. hirsutum* and *G. barbadense* species have been introgressed frequently, with the primary objectives being the transfer of fiber and yield properties. The secondary germplasm pool has contributed fiber strength, resistance to cotton rust (*Puccinia cacabata* Arth. & Holw), blight resistance (*Xanthomonas campestris* pv *malvacearum*), and cytoplasmic male sterility.Problems that continue to retard the expanded use of germplasm resources include photoperiodism, difficulties in identification of desirable traits, ploidy levels, genetic incompatibility between species, and agronomically unacceptable genetic backgrounds.Germplasm collections suffer from deficient characterization and evaluation, unrecognized redundancy, and insufficient support, among many inadequacies. Advances in molecular mapping and genomic analyses offer opportunities for both expanding the use of germplasm and its efficient maintenance in collections.

Cotton Germplasm in China

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Germplasm is the foundation of any crop improvement process. The success of research in establishing PCR-based genetic maps, developing genetic stocks and tool-materials for genomic studies, creating specially-aimed materials, and even training high level educated specialists denpends in large part on the availability of diversity of genetic resources. Cotton germplam also plays very important role in the genomic study.

Gossypium is not originated but there are plentiful kinds of genetic resources in China, totaling over 6800 accessions. Generally three big categories, cultivated, wild species and genetic stocks represent cotton germplam. The cultivated, including (1) obsolete cultivars, (2) improved lines via breeding, induced or natural mutant and biotechnological achievements, (3) current cultivating varieties, constitute a major proportion of the Chinese total. Among the 5900 cultivated accessions, about 5050, 490 and 350 belong to *G. hirsutum, barbadense and arboreum*, respectively, with less than 20 of *herbaceum*. The wild germplasm, involving (1) wild species, (2) semi wild cottons, (3) inter-specific hybrid between cultivated and wild species or between wild ones, and their improved offsprings, (4) perennial stocks of some cultivated cottons, and (5) relatives of *Gossypium*, such as *Thespesia*, assembles to about 700 accessions. There is a collection of about 200 accessions of the genetic stocks or basic research materials in China. Over 60% of the category are morphological variants or morphological markers, and 35% are cytogenetic lines.

Collections of cotton germplasm in large scale in China started from 70s of the last century and over 80% of the total were collected in that time. From 1975 to 1988, Cotton Research Institute of Chinese Academy of Agricultural Sciences (or called as Chinese Cotton Research Institute, or CCRI in brief), which headquarter is located in Anyang City, Henan Province, leaded to surveying and collecting cotton genetic resources in 9 provinces in southern region of China, in which cotton were produced in early cotton production history. And at the same years, by writing letters, calling in telephone or other communications, the institute carried out a series of national projects for cotton collection from 21 provinces, concerning to grown or growing cottons. Introduction from foreign countries has been taking very important role in Chinese cotton collection. More than 2500 accessions were introduced, about 35% of the total collection, mainly from USA (nearly 1500 with 60% of the total introduction), former Soviet Union (about 450 with 18%, especially 140 accessions of *barbadense* with 67% of total exotic sources of the species), Pakistan, Australia and Mexico. There were 56 countries or regions which, directly or indirectly, provided cottons to China. Wild and genetic stocks were mainly imported from USA, Australia and France (former IRCT).

For the purpose of convenience and economy in handling huge collections of crop germplasm in China, the materials are preserved as seed storage in national gene banks and as living plants in national perennial nurseries. For cotton maintenance there are two national gene banks with one located in Beijing, which is used as long term storage (30~50 years) for all crops in China, and another one located in CCRI, which is used as mid term storage (around 10~15 years) only

for cotton. Generally, the materials in long term gene bank are not allowed to exchange and the CCRI gene bank serves to whole country or even oversea as working collection (renewing, exchanging and providing of the materials). Perennial plants of cotton are kept in National Wild Cotton Plantation, which is controlled by CCRI and located in Hainan Island. There are, annually, all wild species already introduced into China (about 40 together with some primitive stocks of 4 cultivated species and nearly 80% of the genus in today′ s world), partly genetic stocks and inter-specific hybrids growing there in relevant nurseries. It should be pointed out that we started to establish a mutant nursery for collecting and assembly keeping natural mutants of cotton nationally wide under support of Chinese Ministry of Agriculture, and a tool nursery in order permanently to maintain the typical plants of aneuploid lines (monosomes and telosomes) while identified by cytology and other genetic stocks which need to be conserved in perennial form.

It is free to use cotton genetic resources in China and many achievements for germplasm utilizations have been made nationally wide. Almost all scientists engaging in studies on cotton genetic improvement in China got some benefits by sharing with the national collection of the germplasm. An other outstanding progress which has been made by Chinese scientists is cotton inter-specific breeding. The work has no longer over 30 years but more than 60 hybrids of 4 cultivated species with wild species (diploids or tetraploids) have been got, and, directly from some of them, a lot of elite breeding lines with high level of fiber quality, high resistance to major diseases (*Fusarium* and *Verticillium* wilt) and to some insects (aphids and bollworms), and good tolerance to drought or cold, have been developed, or even 6 new cultivars were bred directly from the inter-specific hybrids till end of the last century. Now the improved offsprings of the hybrids have constituted a major germplasm and are taking a more and more important role in studies on genetics and breeding in China.

In early stage of this century, emphases of cotton germplasm in China should focused on carrying out projects of core collection; keeping to exchange exotic sources; create new improved breeding lines via common breeding and inter-specific breeding, or even biotechnology with genes out of cotton; propagating the collection for natural and artificial mutants and their evaluation; constitutionally filling the tool nursery with exotic and domestic sources and efficiently utilizing with the germplasm nation wide or even world wide, especially in studies on cotton genetics and even in isolation of functional genes in Gossypium itself. It should be noted that a special nursery should be established in order to perennially keep permanent population (including those for morphology, cytogenetics and molecule) and promote studies on cotton genome.

Genetic Diversity in Upland Cotton(*Gossypium hirsutum* L.) Cultivars Based on RAPDs and SSRs

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Quantification and classification of diversity in germplasm collection is important for both genetic researchers and plant breeders. Some advance was made in this area in the world (Liu et al, 2000) based on SSRs and in China(Xu et al, 2001;2002) based on RAPDs. In this research, 72 cultivars including 14 latest introduced and 30 Bt-transformed ones were evaluated by RAPDs and SSRs. Jaccard's genetic similarity coefficients were calculated and dendrograms were constructed by the unweighted pair group method of arithmetic average(UPGMA) using the NTSYS-pc 2.10 based on the data from RAPDs and SSRs.

Genetic diversity of 72 cultivars. The similarity coefficient matrixes were analyzed. The average similarity coefficient ranged from 0.497 to 0.743,while the maximum similarity coefficient was 0.985 and the minimum one was0.29. DPL 50 and DPL 51 which were introduced latestly from American couldn′t be distinguished from the dendrogram. Another couple of cultivars, Meikang 1999 and SK-2, introduced from American earlier also had very closed genetic relationship with the similarity coefficient of 0.985. Xinluzhong 2, a cultivar from Xinjiang municipality was at the bottom of the dendrogram. As one of the earliest introduced cultivars from American in 1950s′,Deltapine 15, was distinguished from most of the cultivars. That is to say, great genetic improvement in cotton breeding had been obtained in china through the past 50 years.

Genetic diversity in cultivars from china and foreign countries. 17 cultivars selected from different cotton planting areas in China were used to compare with the 14 introduced cultivars. Most of the 14 cultivars were clustered into one group. And interestingly, the cultivar from Iran was also grouped into it. Owing to the cultivars from American came from one certain area, the genetic variation was not as much as it among Chinese cultivars. But the cultivars from foreign countries are still useful for cotton improvement in China.

Genetic diversity in Bt-transformed cultivars. 30 Bt-transformed cultivars were collected from different area in China and American. The dendrogram showed that all the cultivars could be grouped into 2 main clusters, but not based on their origin and the transformed techniques. This partly accorded with that some transformed cultivars were obtained from the local ones which crossed with the transformed materials and were selected from their offsprings.

Comparison of SSR, RAPD and pedigree in genetic diversity. Totally 260 RAPD primers and 72 pairs of SSR primers were used and only 21 polymorphic RAPD primers with 42 polymorphic alleles and 19 pairs of SSR primers with 87 polymorphic alleles were got. Among the 17 cultivars which had detailed pedigree, the data from RAPDs was in line with pedigree better than the data from SSRs though neither of them correlated with the pedigree completely.

Genetic diversity constituted the raw base for plant improvement and it can provide protection against genetic vulnerability to biotic and abiotic stresses. It had been applied successfully in other crops. But cotton is difficult in molecular biology because of poor polymorphism among genotypes in upland cotton. For this reason there must be an efficient method developed to evaluate the cotton germplasm. With the quick development in other biology research, especially in *Arabidopsis*, cooperation studying in cotton in large scale must be applied in the world.

Chromosome Substitution Lines in Cotton Improvement

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Presently cotton breeders are confronting serious challenges due to the extensive use of narrow genetic base in Upland cotton, *Gossypium hirsutum.* Unique germplasm that incorporates new alleles must be developed to provide improved genetic potential for yield, pest and disease resistance. Tetraploid species such as *G. barbadense*, *G. tomentosum*, and *G. mustelinum* are reservoirs of genes for biotic stress resistance and for improved agronomic and fiber traits. Attempts to incorporate genes from wild species into Upland have not generally achieved stable introgression. Poor agronomic qualities of the progenies, distorted segregation, sterility, and limited recombination due to incompatibility between the genomes have been some of the problems associated with these attempts. A complementary alternative approach to introgress alleles from other tetraploid species into an Upland background would be to develop chromosome substitution lines whereby only a chromosome or part of a chromosome from alien species have been introduced. We initiated a plan to develop a set of backcrossed chromosome substitution lines covering about 80% of the genome in Upland cotton using *G. barbadense*, *G. tomentosum*, and *G. mustelinum*, respectively, as donor species.

We used monosomic and monotelodisomic hypoaneuploid *G. hirsutum* stocks of the Cotton Cytogenetics Collection to develop euploid backcrossed chromosome substitution lines from *G. barbadense* for chromosomes 1, 2, 4, 6, 7, 16, 17, 18, 25, 5L, 11L, 12L, 14L, 15L, 22L, 22S and 26S respectively (L= long arm, S=short arm). These lines are genetically similar except that each differs by the replacement of a specific homologous pair of chromosomes or chromosome arms from *G. barbadense* into *G. hirsutum*. These different chromosome substitution lines are being developed in a uniform genetic background. They provided an unique opportunity to map about 140 SSR markers to 21 different chromosomes by deficiency analysis. These chromosome substitution lines were also crossed with *G. hirsutum* (TM-1) to create chromosome-specific F1 hybrids. Self-progeny (F_2) populations were usedto evaluate effects of the substituted chromosome that underwent recombination and segregation therein. F2 families and parental lines were evaluated for agronomic and fiber data from bulk-sample analysis of field trials at three locations in the USA. This provided an opportunity to dissect complex fiber and agronomic traits for individual chromosomes. These lines also initiated the disruption of linkage blocks spanning desirable and undesirable alleles, some of which cotton breeders would likely find difficult to remove using conventional breeding methods. Trait analyses suggested that chromosomes H18, 22L and 22S were associated with an increase of lint percent as these lines produced higher lint percent than both of the parents. We observed that F2 hybrid lines specific to H25 affected micronaire and fiber strength.

We are also developing aneuploid chromosome substitution lines in Upland cotton (TM-1) for chromosome 1, 2, 3, 4, 6, 9,10, 14, 15, 16, 18, 22, 25 of *G. tomentosum*. This species is one of the most heat-resistant species in the genus.

Development and Use of Radiation-induced Chromosomal Translocations and Primary Monosomics of Cotton (*G. hirsutum* L.)

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Over 200 disomie plants with translocations of cotton were recovered as heterozygotes following used by four types of treatments: combined treatment of seeds with colchicines and ɣ-rays, irradiation of seeds by fast and thermal neutrons and ɣ-irradiation of pollen. Numbers of translocations obtained were differed in M1, M2 and M3 generation after irradiation and after treatment by different doses of irradiation.

The number of nonhomological chromosomes of cotton involved in interchanges ranged from two to three. So, 190 translocations from 201 involved two chromosomes and only 11 involved three chromosomes. Chromosome translocations with involving of two chromosomes arose more often than with involving of three chromosomes. Different types and frequencies of multivalent associations were found in heterozygotes of cottons. Only 104 translocations of our collection were characterized by multivalent frequency more than 0.25 per cell.

The translocations have been made homozygous by cytogenetic studying from self-pollination progenies of heterozygotes according to the scheme. As a result, twenty four new reciprocal homopzygous translocation lines have been obtained. Twenty two translocations are simple reciprocal interchanges, involving only two non-homologous chromosomes, whereas the remaining two (Tr2 and Tr20) is associations involving interchanges among three non-homologous chromosomes. The differencesbetween these lines were indicated on chromosome configurations and frequencies f multivalents at MI of meiosis, on pollen fertility and morphological characters. To identify the chromosomes involved in the twenty translocations, more than 240 reciprocal crosses were made between pairs of lines homozygous for different translocations. Translocation tests were shown that, translocation lines Tr2, Tr3, Tr7-Tr8, Tr9, Tr14, Tr16 and Tr19 showed more common chromosomes, but other lines, for example, Tr1, Tr10, Tr13 and Tr20 were rare. On the basis of the rare occurrence common chromosomes in the translocations Tr1 and Tr20 were concluded, these lines has translocated chromosomes which involve only rarely in interchanges.

A total of 86 primary monosomics were obtained by gamma-irradiation of pollen and by seed irradiation with thermal neutrons for period of 1987 and 2001 years in National University of Uzbekistan. The transmission of the monosomics was studied in the selfed or outcrossed progenies in greenhouse and in field.Different monosomics were distinguished by transmission rate. In 32 plants the frequency of monosomics was ranged between 14.29% and\{41.67 %\}. Three monosomics (Mo3, Mo15 and Mo56) usually were occurred in lowest frequencies (\{4.17%\},\{2.78%\} and\{5.00%\}, respectively) and require larger populations to insure their recovery. Various transmission rates indirectly pointed out different monosomes to be specific

chromosomes of cotton genome. The data from 115 crosses between monosomics and translocation lines were obtained. As results, the chromosome associations were observed for the monosomes F1 it was pointed out that seven monosomes (Mo3, Mo10, Mo19, Mo27, Mo39, Mo53 and Mo56) are homologous to one of the chromosomes in translocation lines (Tr11, Tr3, Tr16, Tr8, Tr5, Tr12 and Tr5 respectively). So as genome tests showed that translocation lines Tr8 and Tr16 were AA-subgenome location, monosome Mo27 and Mo19 may be considered as specific A-subgenome chromosomes of the cmplement in cotton.

Among the monosomic plants of our collection new morphological markers were detected, which were not detected in Cytogenetical Collection in USA. Such markers are ribbed flattened bolls (Mo7), spherical bolls with shark beaks (Mo48), dense hairiness (Mo13, Mo34) and reduced stigma (Mo62). The reduction was conveyed in decrease of stigma sizes to 20% from normal size and in absence of stigma protuberance above stamen column. During last five years the monosomics stocks of our collection have been used to determine genetic markers to specific chromosomes.

Resistances level of Indian Diploid and Tetraploid Cotton Cultivarsagainst Plant Selection Marker Kanamycin and Direct Shoot Organogenesis from Shoot Tip Culture

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Selection of transformed tissues in the antibiotic medium is an important step for developing transgenic plants. The toxic level of *G. arboreum* and *G. hirsutum* cotton cultivars of Indian origin were tested against kanamycin using different concentrations. Among these 75~100 ug • ml^{-1} level of kanamycin was found to be toxic to the tetraploid plants, whereas diploid cotton showed high resistance up to 125~150 ug • ml^{-1} kanamycin. Therefore 75~100 ug • ml^{-1} and 150~175 ug • ml^{-1} kanamycin may be used in the medium to select tetraploid and diploid transformants respectively for direct shoot organogenesis. Both the species of cotton plants were regenerated by direct shoot organogenesis via an organogenesis pathway.MS medium supplemented with 1.0 ml B5 vitamin, 2.0 mg BAP, 1.0 mg thidiazuron and 1.5 mg kinetin per litre induced multiple shoots within 2 weeks. Shoot elongation was observed with 0.5 mg of GA3 • L^{-1} within 6~8 days of culture and maximum roots were induced when half MS medium supplemented with 0.4 mg • L^{-1} IBA.

Cotton Germplasm: Resources and Tools for Characterization

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Future improvement of cotton has met new challenges that require integrated tools to characterize existing genetic resources. Fragmentary data and information on germplasm characterization need to be coordinated into an integrated whole. The four domesticated species (*G. hirsutum*, *G. barbadense*, *G. arboreum*, and *G. herbaceum*) of the genus *Gossypium* embody considerable genetic diversity. However, this is dwarfed by the genus whose 49 species have a geographic range that covers most tropical and subtropical regions of the world. The cotton gene pool is subdivided into three sub-pools. The primary pool is limited to the tetraploid species where no genetic barriers are known to exist in any intraspecific hybrids. The secondary pool includes germplasm resources that require manipulation beyond simple crossing to obtain fertile hybrids. The tertiary pool involves species that may or may not hybridize easily with commercial tetraploids and makes trait transfer difficult. There is no reason to believe that this range in diversity does not correspond in physiological and chemical diversity. The domesticated and wild species of cotton, consequently, represent a wide genetic storehouse for potential exploitation by cotton breeders of the world. The US *Gossypium* Collection currently maintains over 8500 seed accessions of *Gossypium* species. The individual seed samples, represented in the collection, were obtained by collectors during planned plant explorations to various parts of the globe, by individuals who independently obtained seeds, and by seed exchanges or donations with other similar collections or ′ seedbanks′ . The rationale behind the US *Gossypium* Collection is to preserve the broadest possible genetic base for cotton and make this available to all legitimate users. Diversity is viewed from the perspective of germplasm utilization in terms of traits that can be utilized for the improvement of the cultivated species. In this sense, more emphasis is placed on genes related to environmental fitness and physiological processes than on the morphological features important in taxonomic classification. The curatorial activities of the collection are focused on acquisition, maintenance, and distribution in order to preserve the broadest possible natural variability of *Gossypium* as a resource for continued efforts to modify and improve cotton cultivars. Current commercial cultivars grown today around the world have a very narrow genetic base. It is for this reason that it is imperative that we take advantage of the available gene pools. As DNA markers/clones, genetic/physical maps, and other genomic tools are being developed from cotton, they needed to characterize the US Gossypium Collection to identify redundancy and duplication from other collections and to provide guidance for evaluation and selection. A subset of the collection that represents the range of diversity of *G. hirsutum* and outliers of other species was subjected to molecular characterization. Pilot experiments included cottons that were genotyped with polymorphic DNA markers. DNA profiles of these cottons were analyzed and results showed strengths and deficiencies of the DNA markers. A set of 104 core reference markers is needed to serve as the standard descriptors to characterize workable sets of *Gossypium* accessions. Every cotton chromosome or linkage group will be covered by at least 4 markers of the core set. Although more molecular markers are needed for detailed characterization, all evaluations of cotton germplasm across different genepools and germplasm sources can be shared and pooled into a common database for gene mining analysis with the core reference markers.

Genetic Engineering of Cotton (*Gossypium hirsutum* L.) for Insect-resistance

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In order to improve insect-resistance of cotton and cultivate new cotton varieties， tissue culture and plant regeneration of cotton (*Gossypium hirsutum* L.) were studied with Xinluzao 4，Xi 550，Jizi 492，Hengwu 89-30，Han 93-2 and Jizi 123 . A system of cotton tissue culture for rapid plant regeneration was developed. The frequency of abnormal embryos was reduced from 80% to 41%, and abnormal plantlets could be recovered to normal ones (the frequency was about 78%) by regulating the kinds and proportions of phytohormones. Root regeneration and transplantation of plantlets were achieved by chemiculture, graft and in combination with cuttage *in vitro*. This system paves the way for Agrobacterium-mediated transformation.

Three plant expression vectors (*pBGb1m*, *pBgbf* and *pBGbfg*) were constructed with gfp gene as a reporter gene, synthetic *Bt* gene *CryIAc*, *CryIAc* fused with *gfp* gene, and the fused *Bt-gfp* gene plus *gna* gene as foreign genes respectively transformed cotton by *Agrobacterium* mediated transformation and Pollen Tube Pathway method; Results from Kan painting on leaves directly, insect bio-assay, PCR, Southern blot and Western blot analysis showed that Bt and *gna* were integrated into cotton genome. In the meantime，the detection method of GFP was proved to be useful in selection of transgenic cotton for its simple，rapid，dependable and economical characteristics.

Now, insect test, Southern blot and Western blot of the progeny of the transgenic plants are underway.

Synthesis of [AG] Complex Genome and the Tri-species Hybrid with [A、D、G]

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Cotton is not only an important source of textile fibre and edible oil, but also a valuable source of high-quality protein and fodder, because 45%~50% of the cotton seed powder is protein. However, the seeds of cultivated cotton usually contain a special polyphenol compound called gossypol, which is poisonous and hinders the utilization of the seeds. Therefore breeding new cotton species with no or low gossypol in seeds with gossypol in plant body is drawing more and more attention from both in China and abroad.

Gossypium bickii, a wild allodiploid cotton originating in Oceania, has the feature of delaying the forming of oil glands in seeds, which means that there is no gland or no gossypol in seeds before germination. By hybridizing wild diploid *bickii* (G_1G_1) with cultivated diploid (*G.arboreum*) [A_2A_2] in 1980, we obtained an allodiploid(A_2G_1), whose chromosomes were then doubled and a fertile allotetraploid ($A_2A_2G_1G_1$)(2n=4x=52) was achieved. The allotetraploid hybrid has steadily passed on the merit of having no gland in seeds and having it in plant body for 8 generations, which makes it possible to breed a cultivated vatiety, with the comprehensive utilization of fibre, protein, oil and resistance to insects.

G.arboreum as the maternal plant was hybridized with the paternal plant *G.bickii* by artificially and dropping GA and NAA inside the bracts to protect the boll. Six hybrid seeds were obtained. Seeds were sowed, only 2 plants grew normally and survived, but were hypersterile. The growing points of the shoot were treated with 0.2% colchicine and 5% DMSO to double the chromosomes of allodiploid, a fertile allotetraploid was obtained. Although there was lethal hamper in the root tip of allotetraploid, 9 normal plants survived and bolls grew through inducement of 0.1mg $\cdot L^{-1}$ IBA and root inducing powder (100 $\times 10^{-6}$ABT). The allotetraploid hasreproduced for 8 generations. Crossing *G. hirsutum* and *G. barbadese* with the allotetraploid produced the triple hybrid.

The allotetraploid hybrid is a kind of small shrub, annual or perennial, with characters between both parents, but more like *bickii*. It is in tower shape, with a slender main-stem, a lot of foliage branches, upward fruit spurs and nodes.

Seed embryo (cotyledon) of the allotetraploid is white and has no gland, which is stably inherited because no gland was observed in hundreds of seeds in the past 5 generations. The gland was observed in hundreds of seeds in 5 consecutive generations. The glands in cotyledon are in light color after germination and become black gradually. All the other organs appear later with glands; the densities of glands are between those in G. arboreum and *G. bickii*, but the glands are darker in color and bigger. The content of gossypol in seed embryo is 0.0158%, lower than the permitted content of 0.02%～0.04% stipulated by FAO and WHO. The behavior of chromosomes in 105 PMC in metaphase I of meiosis revealed that most of the M. (A_2G_1) chromosomes were unpaired; the number of bivalents ranged from 1 to 6,and trivalents were observed in 7 cells. The chromosome configuration was 16.4Ⅰ+4.0Ⅱ+0.4Ⅲ. The average crossing frequency of the rod bivalents was 1.07. Between two poles and in tetrad stage 6～8 or even 10 spores were formed, leading to hypersterile pollen. The PMC meiosis of the allotetraploid was observed, showing that there were 52 chromosomes. Most of them were circular bivalents, and the crossing frequency was 1.83 in average. The chromosome configuration was 2.75Ⅰ+24.5Ⅱ+0.25Ⅲ, and the cells containing 26 bivalents accounted for about 30% of the cells observed. Therefore, in telophase Ⅱ normal tetrads were formed, which led to the development of normal pollen granule. The fertility of ($A_2A_2G_1G_1$) allotetraploid was recovered. Bolls and seeds have been obtained for 8 generations.

There were 12 bands from negative pole to positive pole in the isozymogram. The isozymogram of hybrid displayed an incomplete complementary type of the bands

that emerged in parents. There were 10 bands in *G. arboreum*, 4 bands in *bickii*, 8 bands in allodiploid (F1) among which the 3rd, 4th, 5th and 10th bands were homologous with those of parents. The first,second and ninth bands were homologous only with those of *G. arboreum*. The sixth band was a new one, and the eigthth, 11th and 12th bands did not emerge in the isozymogram of allodiploid. There were 9 bands in allotetraploid, among which the 3rd, 4th, 5th, and 10th bands were homologous only with parents. Just as in the allodiploids the 8th, 9th, 11th and 12th bands were homologous only with *G. arboreum*, and the 7th band was a new band. The 1st and 2nd bands in *G. arboreum* did not present in the isozymogram of allotetraploid.Several wild Australian diploid cotton species, including *G. bickii*, have a very special glandless seeds (low gossypol) and glanded plant trait. After germination, the seedlings became glanded and all the aerial parts became afterwards glanded as well. By transferring this trait into cultivated upland cotton, we could obtain low gossypol cotton seeds and capability of resistance to some insects. By hybridizing the cultivated species of *G. hirsutum* $(AD)_1$ and *G. barbadese* $(AD)_2$ with the allotetraploid the triple hybrid ［*G. arboreum*, *G. bickii* and (AD) complx set］ was produced.

The whole plant in the tri-species hybrid looked roughly like upland cotton. While the character of pubescence was more like *G. bickii*, denser than *G. arboreumand G. hirsutum*, the shape of bract was like *G. hirsutum*, but the size and partly base association were similar to those of *G. arboreum*; the leaf shape and color were similar to those of *G. arboreum*. The boll size and the number of boll cells were intermediate among the three parents; the pink petal, big flower and big petal spot belonged to superdominant characters.

The gossypol contents of the triple hybrids ranged from 0.00125 % to 0.0481 %, much lower than the glanded upland cotton varieties and close to the standard (0.02%～0.04%) set by WHO and FAO, while the contents in other parts (including cotyledon) were equal or higher to the common glanded upland cotton. The analysis of $(A_2 \times G_1) \times (AD)_1$ chromosome karyotype is following as:

The karyotype formula of $(A_2 \times G_1)$: 2n=4x=52=50m(6SAT)+2Sm;

The karyotype formula of $(AD)_1$: 2n=4x=52=50m+2Sm+(2SAT);

The karyotype formula of$(A_2 \times G_1) \times (AD)_1$: 2n=4x=52=2M+44m(SAT)+6Sm(SAT)

Total of 137 RAPD amplified bands of the triple hybrid $(A_2 \times G_1) \times (AD)_1$ F_1 were acquired, among those 73.7% of the DNA bands are similar to the parents. 65.6% of 131 $(A_2 \times G_1) \times (AD)_2$ F_1 bands amplified and 61.4% of 145 $(A_2G_1) \times (AD)_2$ F_1 bands amplified are similar to their parents. The amplification results using primer OPAV-19 shows that the triple hybrid not only has the bands of amphiploid *G. arboreum* × *G. bickii*, but also has its own specific one.

G. bickii and *G.arboreum* have low degrees of homology and show far sibship. Their ecological environment and features are different apparently. Some researchers failed to hybridiz bickii with *G.herbaceum* (A_1) and *G.arboreum*. In our study, the hybridized diploid was treated with colchicines to double the chromosomes and grafted to get seeds. Root-inducing chemical (ABT and IBA) treatment was applied and tissue culture technique was used to overcome the lethal hamper in root tip of allotetraploid. Finally we obtained fertile allotetraploid hybrid with glands in plant and without gland in seed embryo, finding that the root of their offspring can develop normally. All these techniques may be very helpful to the distant hybridization in cotton.

Hybrid F_1 allodiploid and F_3 allotetraploid had the same peroxidase isozymogram and root tip lethal genes. But after hybrids F_4 and F_5 (allotetraploid) were treated with ABT and IBA, their isozyme bands changed apparently, and the number of bands increased by one and there emerged 4 new bands that did not display in allodiploid. This phenomenon suggests that exogenous hormones can inhibit the expression of root tip lethal genes or probably change the lethal genes completely, because once an allotetraploid is induced and developed, its offspring will regularly develop root without lethal hamper. It is worth studying further.

A Genetic Potential of *Gossypium* L. Genus, Its Importance and a Practical Use

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Among crops the most valuable for the mankind, cotton (*Gossypium* L. genus) occupies an important place. On the latest scientific data in the composition of the genus there are 50 species (Fryxell,1992, et al), growing on 5 continents of the world. Biomorphologic diversity of cotton is the richest source of genetic resources. However, its use factor for improvement of existing and development of new cultivars is extremely low. In the practical activity of a man, only 4 cultivated cotton species - *G. hirsutum* L., *G. barbadense* L. (2n=52), *G. herbaceum* L. and *G. arboreum* (2n=26) are mainly used. In the world practice *G. hirsutum* genus cultivars predominate, making up, 90% of annual cotton yield. The rest of representatives of *Gossypium* genus are potential genepool. We have a unique for Middle Asian region collection of cotton biomorphological diversity, being an object of scientific and applied research. It includes more than 40 representatives of wild American, Afro-Asian, and Australian species (living collection seed fund), and 5500 samples of cultivated species from different countries of the world, and diverse hybridous material, obtained on the basis of synthetic breeding. Investigations of many years the existing genepool, carried out by US, with use of methods of botany, genetics, and distant hybridization and experimental polyploidy, allowed by the present time to determine genetic value of many wild species and forms, practical use of which opens broad perspectives before modern breeding. A possibility of drawing into genetic and breeding works of such species as *G. klotzschianum, G. davidsonii, G. bickii, G. australe, G. sturtianum* and others has been determined and demonstrated by our investigations. Transfer of valuable germplasm of wild species to the genome of cultivated ones is of importance in plant protection from affection with wilt, different specific pests, in increasing of drought and salt resistance, productivity, earliness, technological quality of fiber. So, for example, use in breeding of only representative of *G. hirsutum* ssp. Mexicanum gave an opportunity to develop in Uzbekistan a number of wilt resistant cultivars of Tashkent group. On the basis of developed methods for obtaining of hybrids and schemes of phylogenetical relationship in taxons of different ranks (Rizaeva, 1983, 1996; Klyat, 1984; Ernazarova, 1998, et al) has been a success in obtaining of unique hybridous material. Hybrids, both between species of one genome, and belonging to different genomes, as well as compound three-genomic hybrids, with attraction to hybridization of cultivated cultivars. By the present time a three-genomic constant liner material with traits of natural early defoliation (80%~90%) and high indices of fiber technologic quality has been obtained.

With use in genetic and breeding works of *G.klotzschianum* drought-resistant andsalt-tolerant forms have been obtained; when thick-fuzzed Australian species *G.nelsonii*, *G.australe* were used, hybrids resistant to pricking and suctorial insects have been developed.

A new genetic material replenishes the cotton genepool and it can be used as a source of donors of useful traits for improvement of existing cultivars and modeling of new ones.

Agrobacterium-mediated Transformation and Regeneration by Direct Shoot Organogenesis in Cotton (*G. hirsutum*)

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Genotype independent transformation and regeneration of Indian cotton (*Gossypium hirsutum* L.) cultivar was standardized with *Bt-Cry* 1A(b) gene by *Agrobacterium*-mediation. Apical meristem of elite *G. hirsutum* cultivar LRK-516 and LRA 5166 were co-cultivated with *A. tumefaciens* LBA 4404 carrying synthetic *Bt-Cry 1A (b)* +*npt*-II genes. Kanamycin resistant plants were regenerated by direct shoot organogenesis in the kanamycin medium (100g • ml^{-1}). Bacterial concentration, duration of co-cultivtion, stage and size of tissues, selection marker in the medium, media composition and growth hormone all have influence on transformation efficiency and were optimized in our protocol. Integration and expression of the *Bt - cry* gene was confirmed by PCR, Southern blot and ELISA test respectively. Southern analysis indicated the presence of 3~5-copy number. But the CRY protein expression was found to be very low (0.003~0.004 % of leaf protein) and insect bioassay shown less or no effective on *Helicoverpa armegira.* Nevertheless, this protocol may be used to produce genotype independent transformation and regeneration to produce transgenic cotton with other cry genes or any other economically important genes in cotton.

Cytological Analysis Cotton Radicles after Treating the Seeds by Uitra-Violet Irradiation+Analyt+Catalyt

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The cotton seeds were dried in an air, treated by Ultra-Violet Irradiation (UVI) and moistened by analyt and catalyt. Not treated seeds were as control. Seeds were grown in Petri dish in thermostats according to generally accepted methods and their radicles were fixed in 24, 30, 36, 40, 46, 52 and 58h. The mitotic activity was studied on radicles cells. The intensification of cell division in all studied material was higher in radicle meristem of treated seeds by comparison with the control. Varieties Yulduz and L-38 responded to treatment negligibly and the difference between test and control was insignificant. Mitotic activity of cell division of variety Avstraliisky-4 exceeded control during initial period of fixation from 24 to 58 hours. For variety Farkhad there were equal amount of dividing sells in test and in control as well. From 30 to 46 hours the activity of mitotic cycles of cell division was much higher in test than in control. Lines L-49 and L-2708 favorably responded to treatment and their mitotic index exceeded control in all periods of fixation. It is known that the decrease of mitotic activity is caused by the prolonged coming of cells into mitosis phase and by physiologic processes in them. Probably the treating the seeds by UVI + analyt + catalyt stimulates the physiological processes and speeds up the mitotic activity in cells of cotton radicles. Cytological analysis was carried out for clearing up characteristic of UVI + analyt + catalyt effect on M1 cotton plants and for revealing quantity and spectrum of chromosome breakages in meristem cells of cotton radicles. It was established that the electrotechnology did not affect negative influence on chromosomal system. Chromosomal aberrations were on the control level and did not exceed 0.2%~0.5%.

Differentiation of Population Structure and Transformation of Homeostasis on Some Economical Cotton Characters

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As is well known the structure of variety populations is the natural formation that has been shaping by selection and submitting to general regularities of organic world evolution. Our researches are devoted to determining of importance the agro-climatic and agro-technical conditions for forming of population structure. It was established that the change of soil nutrition led to not only divergence of some cotton groups and families between each other but also to differentiation their inner characters and properties. The process of variety differentiation to the families and their forming up to the varieties is going faster on deeply differing agro-technical conditions especially on high organic-mineral background and changed water supply conditions. For several years the biotypes chosen by individual selection have been improved to varieties therefore have relative stability in phenotype and genotype. Some of these varieties are studying on competitive varieties tests, some of ones are cultivating. Varieties and kinds of cotton are facultative self pollinators hence in every generation are creating heterozygous individuals with different phenotypes. Every individual is variable in tens and hundreds genes because of every population consists of individuals with different genotypes, generation, physiology, morphology, biochemistry and so on. That is why every biotype has stable and labile genes influencing on differentiation and forming the structure of cotton varieties. Thus thanks to multi-genomity and heterogeneity of cotton there is the wide polymorphism of the number of characters and properties in cotton populations. During genetic and selective researches of cotton, the genotypes responded to environmental factors and their changeableness.

Fiber Quality Improvement by Inter-specific Hybridization in Upland Cotton

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In order to transfer genes for extra fiber strength to existing cotton cultivars, we introduced 207 interspecific materials obtained by outcrossing where *G. hirsutum*, *G. barbadense*, *G. sturtianum*, *G. thurberi*, *G. harknessii* and *G. somalense* etc. were involved as parents respectively. Under stringent evaluation and acclimatization, only about 20 materials showed approximate homozygosity and adoptable agronomic characters as well as outstanding fiber strength and micronaire value. Using these elite outcrossed germ plasmas lines and another self-bred line from *′ G. hirsutum* × *G. barbadense′* as parents of target character, a lot of cross combinations were made followed by backcross, intercross and pedigree selection. A number of newly bred lines showed improved fiber strength and reduced micronaire value. The yearly interfamily comparisons demonstrated that the negative linkage between yield and fiber strength could be broken in some lines, such as Lu9228, which had a lint yield increase of 20% in contrast with the checker CCRI 12, and fiber strength of 28.2 cN • tex^{-1}. All these interspecific progenies have broadened genetics background of upland cotton and therefore were valuable for practical cotton breeding in view of fiber quality.

Genetic Diversity and Its Correlation with Heterosis of Upland Cotton (*Gossypium hirsutum* L.) Cultivars of Huang-Huai Region in China Evaluated by RAPD

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Sixteen cultivars of Upland cotton (*Gossypium hirsutum* L.) cultivars in Huang Huai Cotton-growing Region were detected by RAPD while the F^1 heterosis of each hybrid involved these cultivars were evaluated. The genetic similarity (GS) of the 16 cultivars through analysis of 115 polymorphic RAPD loci obtained from 70 informative primers were 53% ～ 88%. The pairwise similarity coefficient of Lu 87-340 and Lu 80-9 was the highest (88%) and the lowest pairwise similarity coefficient was the one of Shiyuan 321 and Lu 263 (53%). Shiyuan 321 is an upland cotton cultivar which developed from the germplasm of a triple hybrid of *G. hirsutum* × *G. arboreum* × *G. thurberi*, but no more obvious distinctness were detected in our study. A possible reason may be that the polymorphic loci we detected were not enough to cover the whole genome of the upland cotton (*G. hirsutum*). The 16 upland cultivars could be divided into 3 subgroups based on cluster and similarity analysis and the result showed that these upland cultivars have narrow genetic basis. Correlation analysis between the F1 heterosis of upland cotton and the genetic similarity of the involved cultivars or lines indicated that the genetic similarity detected by RAPD had no marked correlation with the heterosis of corresponding hybridization in a certain extent. It is implicated that alteration of the expression of involved genes or alleles may be the possible reason of the heterosis, and the evaluation based on the genome structure were only revealed the structure or organization of genes or alleles which may have no or less relation with the display of heterosis. The mechanism of the heterosis should be uncovered through the progress of the functional genomics.

Genetic Resources for Cotton Improvement and Application of Genomic Tools

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Progress in cotton improvement slowed in the United States the past 20 years, possibly reflecting genetic stagnation. The pedigrees of popular cultivars contain many of the same parents, evidence of a narrow genetic base. The *Gossypium* genetic base is wide, but only a fraction of the available genetic variation is used in breeding cultivars. Genome incompatibility, photoperiodic flowering, and breeder reluctance to employ other than ′elite′ germplasm in cultivar development hinder exploitation of the Gossypium genetic base. Demonstrating the merits of genetically diverse germplasm for the improvement of upland cotton are necessary.

One effort to convert exotic germplasm into more readily useable forms, while fingerprinting chromatin from the exotic sources shown to improve upland cotton involves creating advanced backcross introgression lines in a common genetic background (cv. DES 56) using three donor parent race stock germplasms. The non-photoperiodic converted race accessions represent an untapped genetic reservoir for use in cultivar development, but do not appear in the pedigrees of US cultivars. Wide segregation for lint fraction and fiber traits was observed among 64 BC_1F_1 plants from the MDN-63/DES56 population. In the spring 2002 greenhouse, we continued the backcrossing effort with ca. 50 BC_1F_1 plants in each of the three populations. The BC2F1 will be phenotyped in the field in short plots summer 2002 and plants from the best rows transplanted to the greenhouse for a final backcross. A companion project is fingerprinting many of the available wild *G. hirsutum* accessions and corresponding non photoperiodic conversion lines to better understand relationships among them for prioritizing and further use.

Another project seeks to transfer desirable alleles from *G. mustelinum* into *G. hirsutum* through an advanced backcross approach. The goal is to create a set of near isogenic introgression lines that can be used in breeding and will also be permanent mapping resources for the improvement of upland cotton. We have created F_1s between *G. mustelinum* accession AD4-8 and the high yielding germplasm line PD94042. The F_1 is being backcrossed to 94042 in a southern hemisphere nursery under short day conditions. *G. mustelinum* accessions have not been characterized for valuable traits that they may carry, but our work reveals that mustelinum has a photoperiodic flowering habit and a juvenile phase prior to anthesis. Accession AD4-8 is densely glanded and the capsule small relative to that of upland cotton, with somewhat sparse, brown tinted lint. We expect that mustelinum will provide genes for the improvement of upland cotton.

A third project seeks to introgress into upland cotton chromatin from *G. barbadense* shown to increase fiber length. Three Tamcot 2111 donor parents introgressed with *G. barbadense* chromatin implicated in controlling fiber length were crossed with germplasm lines GA96211 and PD94042. The F_1 was evaluated for fiber length properties in the field in 2001 and also backcrossed to the recurrent parents to produce the BC_1F_1. Two of the *G. barbadense* introgressed parents enhanced fiber length of the F_1s with 94042 by 4%～8%. We anticipate additional benefits on processing and agronomic qualities as the introgression proceeds.

Genetics and the Genetic Collection of Isogenic Lines of Seed Lint Type and Fiber Output in Cotton

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As a result of long standing investigation on urgent problems of amphidiploid cotton species Gossypium hirsutum genetics, for the first time genetic determination of the most important traits has been established, and the unique genetic collection of homozygous isogenic lines has been developed. A scientifically well-founded theory about combined types of polygene interaction in genetic determination of tracts in cotton, has been developed.

They were, by their special features of functioning divided into two groups; main genes - F_{t1}-f_{t1}. Dominant alleles of these genes, interacting on the polymery type, provide development of lint on the micropylar seed surface.

It has been established, that about 65%~75% of total fiber yield in cotton is controlled by indicated polymeric genes. Seed lint genes have the positive pleiotropic effect on fiber development. Dominant alleles of these genes have the positive pleiotropic effect on development of 30%~35% of total fiber yield.

By the method of experimental mutagenesis in interlinear hybrids and long standing purposeful selection in their progeny original lines have been developed in which a high fiber output (42%~44%) combines with high indices of thousand seed weight (140~145 g).

Genetic collection of isogenic lines is used for carrying out of investigation on molecular, ecologic genetics and genic-cellular engineering, and also as an initial material for practical breeding.

Genetics, Genetic Collection of Characters of Structural Shrub Characteristics of *G.hirsutum* L.

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The structure and shape of a cotton shrub are determined in result of combining their elements: length and amount internodes of a main stem and fruit brunches, amount and shape of leaf blades. Some lines of genetic collection have been crossed between each other for many years and these experimental data showed that phenotypic manifestation of different leaves shape and area control by action and interaction of polygenes.

We can divide their functions into three groups:

1. Genes controlling the leaf blade area (several pares);

2. Genes controlling shape of leaf blade;

3. Genes, controlling a formation of additional segments on the main lobule in a digitate - dissected leaf.

On the base of genetic analysis was supposed that the main stem of a cotton plant (dwarf and law-growing) can be controlled by:

- dominant genes controlling quantity of internodes;
- dominant genes controlling length of internodes;
- the greater concentration of recessive alleles of genes controlling a quantity and length of internodes;
- pleiotropic effect of genes controlling other morphobiological characters;
- interaction of the inter-kind leafness gene with genes of the type of fruit branches;
- lethal genes if they are homozygous.

An analysis of interrelation in inheritance of mutant gene controlling entire-kind shape of leaf blade with fruiting branch genes let to establish that the combining of dominant homozygous state of this gene with recessive homozygous state of fruit branch genes gave development for shrub of determinate type - absolutely non-character shrub architectonic for *G. hirsutum* L.

Some cotton lines of genetic collections on above-mentioned characters were created in the process of studying the next hybrids generations.

Genotypic Reaction of Cotton Plants to Conditions with Lack of Macroelements in Soil

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A group of *G. hirsutum* L. varieties and their hybrids F_1 were studied on two fields with different mineral supply conditions: optimal mineral supply and without fertilizers. The reliable genotypic intraspecies polymorphism of response to the different level of mineral nutrition was identified. An individual reaction of varieties and hybrids was showed by all studied traits. Cotton cultivation in conditions with the deficit of mineral nutrition caused a depression of morpho-physiological and economical features. The processes of growth - bound with a leaf blade area, forming of biomass and the main stem height varied very much as well productive processes bound with fruit quantity and yield. The photosynthesis intensity, quantify of chloroplasts in cells of mesophyllous parenchyma, chlorophyll, weight of bolls and weight of 1000 seeds varied in a smaller degree. The vegetative period duration, index of yield, specific surface area of a leaf and per cent of fiber varied negligibly. For some traits (intensity of photosynthesis, productivity) reciprocal effects in adaptation to macroelements deficit conditions were disclosed, though there were no reciprocal distinctions for these traits in optimal nutrition conditions. The maternal type of reaction inheritance to nutrition stress conditions discovers a role of cytoplasmic genes for regulation of the adaptation mechanisms both in cell and whole organism.

In Vitro Mutagenesis - Alternate Approach to Breeding of *Gossypium hirsutum* L.

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Mutations are possibly the only source of creating heritable variability in all biological system and, many useful mutants in plants have been released for commercial cultivation across the world. To-date throughout the world about 2252 mutants have been officially registered in the FAO/IAEA mutant varieties data base (MVD) of plant breeding and genetics sections. According to Maluszynski et al. (2000) the MVD, the countries in which most of those mutant varieties have been released are China (26.8 %), India (11.5 %), former USSR and Russia (9.3 %), The Netherlands (7.8 %),USA (5.7 %), and Japan (5.3 %). In addition many new genetic stocks have been added in the gene pool evolved through mutagenesis. With the modern techniques of plant biotechnology, it is possible to manipulate and transfer specific gene through on site directed mutagenesis. Conventional mutagenesis coupled with modern biotechnological tools can lead to harness full potential of induced mutagenesis in crop improvement programmes. Exploitation of in vitro selection system is a unique opportunity of selecting desirable mutants against various kinds of stresses (biotic and abiotic).

Cotton is a very important commercial crop of India. It sustains the country cotton textile industry which perhaps the largest segment of organized industries in the country. Cotton provides gainful employment to million of people in the country who are engaged in its cultivation, trading, processing, manufacturing, fabricating and marketing. Almost all parts of the plants are used extensively in several industries and cotton mainly cultivated for its fibre and seed oil. Numbers of Scientists and Plant Breeders have actively engaged in the production of new cotton cultivars in year by year with improved yield and quality. Even though many improvements in this research have been made, quality and yield are not satisfactory to meet the present need of global requirements. Knowing the application of in vitro mutagenesis and the importance of cotton plant, the program envisaged by subjecting two varieties of cotton (var. MCU 5 and MCU 11) *for in vitro* mutagenesis with physical mutagens i.e. (Gamma rays) and chemical mutagens i.e. (Ethyl Methane Sulfonate and Sodium Azide). The desirable mutants/variants where observed in the mutated M1, M2, M3 and M4 generations. The effectiveness and efficiency of the mutagens on inducing qualitative and quantitative mutants were observed in terms of morphological as well as economic characters. Biochemical study such as protein, oil and lipid were estimated on selected mutants. Based on the performance of the plants, 9 mutants were selected through in vitro mutagenesis. All of the selected mutants were bred true to their characters and they showed significant increase in their economic characters and biochemical contents. Further, selected mutants in M4 generations were submitted to Central Institute for Cotton Research (ICAR Regional station), Coimbatore, for testing of the stability of the desirable characters.

Induction of Aneuploids and Their Identification in Upland Cotton

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Aneuploid lines have been playing an important role in crop genetic studies and breeding utilization. Unfortunately, only several genetic materials of aneuploid can be used in Upland cotton. So far, there are 16 monosomics identified in cotton. In order to fill the gap, we carried on the irradiation research of mature pollen grains with γ-rays at different dosages and further produced F_1 generation. The aneuploid plants induced were identified by F1 PMC cytogenetic analyses. Further monosomics identified were selfed to produce new aneuploids. Up to now, 6 different trisomics, 19 primary monosomics and 22 tertiary monosomics were reported. A new trisomic line was confirmed using a set of translocation lines.

The primary and teritary monosomic lines could be effectively induced by γ-ray irradiation at a dosage of 1000 and 1500 R.. Further, primary monosomic lines could be readily separated from the progenies of tertiary monosomics.

Through crossing between induced monosomics and the translocation and gene marker lines, monosomes of the 1st, 2nd and 4th chromosome at the A subgenome and 18th chromosome at the D subgenome were identified. The causes that no more new monosomics were isolated were the similar phenotype between new monosomes and their donors and low transmission rates of n-1 gamates.

The origin, morphology, fertility, transmission rates and cytology of trisomics in Upland cotton were studied and described for the first time. Trisomics could be readily separated from the progenies of monosomics. Further research indicated that Ftr-2 is a secondary trisomic, Ftr-4 may be a tertiary trisomic, and Ftr-1, Ftr-3, Ftr-5 and Ftr-6 are primary trisomics. Their trivalent rates ranged from 0 to 41.67%. Ftr-3 failed to produce any trisomic progeny. Ftr-4, Ftr-5 and Ftr-6 could be transmitted through male gametes.

After crossing separately Ftr-5 and Ftr-6 with a set of translocation lines, PMC chromosome configuration in F1s was analyzed. The result indicated that the extra chromosome of Ftr-5 was neither on the 1,2,3,4,6,7,8,9,10,11,12and 13 Chromosome at A subgenome nor on the 14,15,16, 19,23 and 24 chromosome of D subgenome, While pentavalent was found by observing PMC chromosome configuration in Fs_1 from crossing Ftr-6 with translocation lines-T8-19 and T10-19. The result showed that the extra chromosome of Ftr-6 was related to chromosome 19. This is the first finding of aneuploid related with chromosome 19, which will have an important significance to cotton genetics and molecular biology research.

Influence of Irradiation during Different Development Phases of Male Generative Sphere on Embryo Processes in Cotton Plants Growing in Different Water Supply Conditions

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The influence of irradiation by different doses (32) and (60) on microsporogenesis process and male gametophyte development have been studied on different water supplement conditions. We have studied the pollination, fertilization, micro-sporogenesis and early embryogeny processes in Mo and FoMo. It is established that the processes of microsporogenesis and forming male gametophyte in the highest degree depend on phase of male generative development during treatment and on level of water supplement in the period of plants growing and developing. But the chosen doses 400R, 700R, 20mkCi, 40mkCi and sources of irradiation had less degree of influence on above mentioned processes. Radioresistance of postmeiotic cells is much higher than premeiotic and meiotic ones. Significant depressive effect of irradiation and water deficit in the process of pollen forming were displayed on development phases of archesporium cells and during meiosis in microsporocytes. The less depressive influence was on phases of one- and bi-celled of a pollen grain. Frequency and spectrum of discovered breakages during embryonal processes had almost no difference among irradiated plants by comparison with the control ones. The acceleration of embryonic processes took place both in test and stress water conditions if the pollen grain is irradiated in one- and bi-celled phases. This rate was similar as in case of self-pollination. Water deficit led to sharply decreasing the anthers and seed-bud amount in ovary but the fullness of pollen was high.

Study of Economically-valuable Traits of Cotton Plants Cultivated in Water-deficit Conditions

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The reproductive, adaptive heterosis and economically-valuable traits of hybrids F_1, F2 and F3 obtained as a result intraspecific crossing G. hirsutum L. parents with ordinary type of leaves and imported Okra-leaves parents had been studied. Perspective of use of cotton forms with types okra-leaf was shown for selection of hybrids combining high reproductive heterosis with vegetation growth, high yield index, fiber quality and early maturity. It was found that lengthening of root growth, water retaining ability of tissues, photosynthesis rate and yield index are inherited independently from each other. The fiber yield of intraspecific distant hybrids is inherited completely and incompletely. It was found that adaptive process is not only with genes of nucleus, but and with genes of cytoplasma controlling. The series of genotypes with saved high technological quality of fiber had been obtained. In water-deficit conditions the yield, length and index of fiber of these genotypes were low-variable by comparison with optimal conditions. It was obtained the number hybrid combinations combining high technological parameters of fiber with drought resistance.

Water Stress and Mechanisms of Adaptation for Cotton Plants

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There are many countries in the world including Uzbekistan what have increasing problem of drought in agriculture. In this connection an experiment on different water supplement conditions was organized for study reaction of cotton varieties and hybrids in most sensitive period for plant developing in the period of flower and fruitification. Adaptive heterosis and adaptive reactions of hybrids and parents were studied in water stress conditions. Adaptive heterosis was counted as decreasing or increasing of character value by comparison with the best parent. Coefficient of adaptation that is per cent of harvests waste in stress conditions comparatively with optimal ones showed about adaptation. Resistance to deficit of soil moisture inherited as polygenic character, therefore complete and incomplete dominance of one parent, positive and negative heterosis were manifested. Adaptation to water stress was provided with the different adaptive mechanisms inherited independently from each other: lengthening of root, water-keeping ability of tissues, rate of photosynthesis and index of yield. Combining different mechanisms of stability in some hybrid genotypes resulted in heterosis in drought-resistance. Existing of some reciprocal effects in roots developing and yield in water stress field indicated influence cytoplasmic genes on adaptive processes. In this connection well-adapted maternal parents should be involved in breeding for obtaining drought-tolerant hybrids. Hybrids combining high adaptive a bilities to water stress with complex economical traits were selected for cultivation in droughty areas of agriculture.

Cotton Recombination Inbred Lines and Their Fiber Characters

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Genetic stocks are considered to be most important aspect in plant biological and molecular studies. In the past, a fuzzless-lintless mutant (XZ142w) was introduced into our group from Xuzhou Research Institute of Agriculture Sciences in China. A fuzzless-linted mutant (GZNn) was found in our genetic nursery . Previous studies carried out by our group indicated that the fuzzless of GZNn was controlled by two pairs of genes ($N_1N_1N_2N_2$), Which N_1N_1 present fuzzy, N_2N_2 inhibit fuzzy initiation and development; XZ142W (fuzzless-lintless) was controlled by 4 pairs of genes (n_1 n_1 n_2 n_2 li_3 li_3 li_4 li_4), and the duplicate recessive genes(li_3 li_3 li_4 li_4)which inhibit lint development also stop the further expression of fuzz. In order to further study the fiber quality and quantity, two sets of recombination inbred lines (RILs) population were made by using these two kinds of mutants and the genetic standard line ′ Texas Marker (TM-1)′ from College Station of USDA-ARS in Texas, USA.

The first set of RI population with 180 RILs was produced by single seed descendent method from the cross of GZNn/XZ142w. The RILs of this cross can be divided into three main types: fuzzy-linted,fuzzless-linted, and fuzzless-lintless. There are great variation of fiber Micrnaire Reading value、fiber length and lint percentage among different RILs. For example, even in the population of fuzzy-linted, the micronaire reading value was from 4.1 to 6.8, with a variation coefficient (CV) 13.6 %, and the fiber length was 21.9 mm to 32.6 mm with a CV of 11.9% .

This suggested these specific RI lines are the desirable materials for genetic and molecular study of fiber finesse and fiber length. Meanwhile, the transgressive segregation of fiber length and strength in some RI lines of this cross was found. For instance, the fiber length, and strength of RILNnxu0082 were 32.6 mm and 24 cN • tex^{-1} (ICC standard) respectively,8.3% and 12.6% higher than that of the parent (GZNn) with lint. Moreover, there are other seven RILs with higher fiber strength, 24~25 cN • tex^{-1} (ICC standard), which were separated and selected from the lower generation of offspring with fuzzless-lintless in the cross of GZNn/XZ142w. This indicated that even the parent XZ142w was lintless-fuzzless, it possessed potential genes for higher fiber quality, and this resulted in the transgressive segregation of fiber quality in this cross.

In the second set of RI population with 150 RILs produced from the cross of GZNn/TM-1, there were two kinds of RI population: fuzzless-linted and fuzzy-linted. The great variation of lint percentage was found among the RI lines with fuzzless-linted characters .The lint percentage varied from 8.8% to 27.2% with a CV of 30.8%. However, the variation of fiber length, strength finesse etc was very small. Therefore, the differed RI lines with fuzzless-linted characters in the cross of GZNn/TM-1 can be used as the basic material for molecular and genetic studies of fiber yield (lint percentage).

In this research, molecular marker analysis with the simple sequence repeat (SSR) for some RI lines with the characters of fuzzless lintless, fuzzless-linted fuzzy linted was also carried out. Seven pairs of SSR primers with polymorphism for these RI lines were got. These polymorphic molecular markers maybe have relation to the genes for fuzzless and lintless.

The Effect of Cotton Cultivars on Station and on Farm Condition in Namibia

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In 1998/99 to 2000/01 Cropping season eighteen varieties of cotton were planted and evaluated against fibre quality, yield, pest and diseases resistant. Their performance was measured according to the yield stability, fibre quality in term of micronaire, strength, length and resistant to pest and diseases and their adaptability to soil and rainfall.

The yield performance indicated that cultivar Tetra performed better than others. The average yields in three cropping seasons were 0.73 to 0.98 tons per hectare. All cultivars were planted under dry land condition and land preparation was done mechanically. Planting, Weeding and Harvesting were done by hand. Two hundred and twenty four seed cotton samples were collected and sent to the Tobacco and Cotton Research Institute in Republic of South Africa for fibre analysis in term of micronaire, length and strength. Cotton varieties in term of fibre quality were graded between HX, HA and HB grade hand picked cotton according to the classification system of seed cotton standard of Republic of South Africa.

Genetic Resources for Cotton Breeders, The Current Status and Storms Ahead

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The sources of germplasm for the cotton breeder to use as parents are changing. There are several factors effecting this change. Public collections are available but are operating under some server restrictions. The United States public collection available to breeders has broadened due to accessions from the former Soviet Union. Lines from Russia and Uzbekistan were recently added to the collection but have yet to be characterized. Even prior to these additions several lines in the collection needed characterized. The United States has put into place a Regional Project where the line characterization the being a major objective. Distribution of seed is also regulated due to plant variety protection and patent laws.

Universities no longer serve as major sources for germplasm. Not only are there fewer universities with cotton breeding programs, but those programs are developing fewer lines for release as ′ public ′ germplasm. Universities have moved toward a licensing system in where they license the rights to their best germplasm to a single party. Federal government organizations (United States Department of Agriculture, Agriculture Research Service USDA-ARS) have had reductions in programs. The long standing breeding program the USDA-ARS had at the Pee Dee Station in South Carolina has been closed. Other USDA programs have not been as active in releasing ′ pubic germplasm′ . Recent moves by Cotton Incorporated are geared toward reversing this trend in U.S. public institutions.

Another change is in the germplasm exchange policies between breeders. Large corporations have purchased almost all the US cotton breeding companies and put restrictions on germplasm exchange. In the past, breeders like Drs. Robert Bridge and H.B. Cooper would send germplasm to anybody making a request. Now exchange of germplasm from private breeders is not done and the industry suffers as our germplasm base narrows.

Great strides were made in cotton cultivars due to the free exchange of germplasm. The most threatening storm on the horizon is the use of utility patent on plant cultivars as a protection method. The implications this has to the whole industry must be evaluated. Programs and policies are needed to insure that cotton breeders have the wide and diverse germplasm base to help lead to the improved cotton cultivars of the future. In this paper we will take a detailed look at the resources available, current status, and the issues and policies affecting the germplasm resource.

Cotton Databases and Web Resources

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There are several web sites for which information is available to the cotton research community. Most of these sites relate to resources developed or available to the research community. Few provide bioinformatic tools, which usually relate to the specific data sets and materials presented in the database. Just as the bioinformatics area is evolving, the available resources reflect this evolution. There are many resources available, some are proprietary, and others are in the public domain. Cotton genomics is developing to the point where the ready availability of bioinformatic tools is in an increasing need for the cotton research community. At this time when the need is more obvious, resources seem to be least available. I would like to review the current situation with cotton database resources by focusing on CottonDB. In the US, The National Plant Genomics System (NPGS), which was to provide the coordination and support for plant genomic databases, apparently no longer exists. The central site that moved from the National Agricultural Library to Cornell University has shut down. This action has required that we have moved CottonDB to our hardware site. What is CottonDB? It was established as an AceDB database, which was the standard established by NPGS. It is desirable as a public domain database for which a dedicated team provides continued development and upgrades. This makes it readily available to the research community, and there is on going support. Currently the platforms on which it operates are being expanded, so that its use is less restricted by hardware requirements. Since we had to move the location of CottonDB, we have the opportunity and responsibility to provide greater support for it. The AceDB format has its unique characteristics that can be intimidating to a new or infrequent user. We have attempted to format CottonDB so that its use is more intuitive to the new or infrequent user. In this process there are some restrictions that limit what we are doing. The first is to operate within the limits of the AceDB format, and equally important are to make it accessible to all browsers. What does CottonDB contain? With the initial development of CottonDB, we tried to include a broad spectrum of information that would consolidate in one place information that cotton researchers would need for their reference. At the time of its development, information for the germplasm collection and National Variety Tests were not available as web sites. So, we included that information. As such information became available on the web, we have provided links for data not in CottonDB. As more genomics data became available it was added with specific attribution to its origin. In this capacity CottonDB is a data archival site. We recognize that more information dynamics is needed. This was the support that was to have been provided by NPGS. So, we need to provide more than just links to other sites. There has to be greater accessibility and exchange of cotton data and information. There need to be available tools with which to work with the data, either by linkages or incorporated into the site. This tool incorporation is of a high priority, and will require greater support to implement.

Phylogenetic Analysis of Drought Responsive ATMYB2 Proteins

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Low temperature, drought and salinity are major adverse environmental factors that limit plant productivity. Understanding the mechanisms by which plant perceive and transduce these stress signals to initiate adaptive responses is essential for engineering stress tolerant plants. While studying the stress genes, one of myb related genes, atmyb2, has been identified which is showing response towards water stress in *Arabidopsis thaliana*. ATMYB2 protein was selected and blasted with blast/p at NCBI against non-redundant protein database. The resulting homologous sequences were selected for multiple sequence alignment. We have selected protein sequences from 10 different species and submitted these sequences to Clustal-W at European Institute of Bioinformatics and San Diego Super Computer (SDSC) Biology Workbench for Multiple Sequence Alignment. The output of Clustalw-W and Biology Workbench was taken as input for Phylip. The resulting Phylogenetic tree analysis of both rooted and un-rooted trees showed that there was a close relationship between *Arabidopsis thaliana* and *Gossypium hirsutum* (Cotton). The mechanism of stress tolerant cotton varieties is under study.

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