GERMPLASM AND GENETIC STOCKS SESSIONS

Oral Presentations

1. Study of the Determinism of the *glanded-plant and glandless-seed* Trait Introgressed in *G. hirsutum* from *G. sturtianum*.

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Two hundred and six mapped microsatellites markers evenly distributed on the 26 chromosomes of *Gossypium hirsutum* L. were used to monitor the introgression of DNA fragments coming from the Australian species *G. sturtianum* Willis and the wild American diploid species *G. ramondii* Ulb. in a population of BC_1, BC_2, BC_2S_1, BC_2S_2, BC_2S_3, BC_2S_4, BC_2S_5, BC_2S_2/BC_1, $BC_2S_2/BC_1/S_1$,BC_3, BC_3S_1, BC_3S_2 and BC_3S_3 derivatives obtained from the *G. hirsutum* x *G. raimondii* x *G. sturtianum* (HRS) trispecific hybrid. In the most advanced backcrossed progenies of the HRS hybrid, the only plants that are still showing a drastic inhibition of gossypol synthesis in a part of their seeds and a normal glanding pattern in their other organs contain fragments of *G. sturtianum* DNA related to c02-c1, c03-c17 and c06-c025 linkage groups of *G. hirsutum*. In these plants, all the SSR markers associated to the *G. sturtianum* c06-c025 DNA fragment introgressed in *G. hirsutum* remain heterozygous after numerous generations of selfing. One can thus suppose that this alien chromosomic fragment may also carry a recessive lethal factor, which expresses itself when it becomes homozygous. If this hypothesis proves to be true, it will be necessary to break the linkage that exist between this lethal factor and the gene(s) responsible for the expression of the trait of interest in order to develop stable homozygous cotton lines with very low gossypol content in the seed and high gossypol content in the aerial parts.

2. New Low Gossypol Cotton Germplasm.

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Cultivated cotton and its wild relatives typically have glands on both the reproductive and vegetative parts of the plant. These glands contain compounds that are toxic to many pests and help protect the plant from tobacco budworms (TBW), bollworms (BW), plant bugs and possibly some diseases. Unfortunately these same compounds are toxic to humans and non-ruminant animals. Cotton seed would be an even more valuable source of high quality protein if these toxic substances, especially gossypol, could be reduced. The focus of our research is to decrease the levels of gossypol in the seed while maintaining a high enough concentration of toxins in vegetative parts of the plant to offer protection from pests. Work done by us, and others, showed that crosses between cotton varieties with different gland densities and distributions produced a range of types. By selecting within the resulting progeny, we have identified and advanced to the F_7 generation genotypes that have total gossypol amounts less than 0.30% total gossypol in the de-hulled seed, while still possessing glands at

critical locations on the vegetative plant parts. Fiber quality analyses indicated that fiber properties have been maintained or improved compared to the parental lines. In 2006, two of these lines will be tested in yield trials and evaluated for insect and disease susceptibility. The resulting elite lines will be a valuable source of germplasm for developing low seed gossypol cultivars.

7. NILs of *G. herbaceum* Introgressed with *G. anomalum*

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Tagging molecular markers with economically important traits in agriculturally important crops enhances plant breeding strategies in crop improvement. Suitable mapping populations, NILs and RILs are required especially in tagging for QTLs. Improvement in fiber strength is the first and the foremost objective of all cotton researching countries in the world. *G. anomalum* is the source of fiber strength. *G. herbaceum* (being agronomically superior in seed cotton yield, drought resistance and sucking pest resistance) is an important choice for fiber strength improvement. Plants with superior fiber strength (23g/tex) have been obtained in a BC_4 population with *G. herbaceum*, as the recurrent parent. Genetic polymorphism for microsatellite markers linked to fiber strength has been identified.

8. Interspecific Chromosome Substitution Lines in Upland Cotton Improvement

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Despite the economic importance of Upland cotton, the genetic base of US cultivars and elite germplasm is very narrow. Two of the primary impediments to genetic improvement of cotton are: 1) insufficient information about genes that control important traits and 2) under-utilization of diverse germplasm. Wide-cross introgression typically requires considerable time and effort to circumvent and overcome biological, genetic and cytogenetic obstacles, and the choice of breeding strategies to introgress germplasm can greatly influence success. Conventional methods of interspecific introgression into cotton typically entail inbreeding immediately after hybridization or after a few backcrosses. A complementary approach is alien chromosome substitution. The overall goal of this project is to develop chromosome substitution lines from the other tetraploid species into *Gossypium hirsutum*. Development of each alien species chromosome substitution line involves three stages: [1] development of the respective TM-1-like hypoaneuploid stock, [2] use of the cytogenetic stock as recurrent seed parent in a recurrent backcrossing program to create a monosomic or monotelodisomic substitution stock, followed by [3] inbreeding to recover a euploid disomic substitution line. TM-1, the highly inbred Upland cotton (*G. hirsutum*) genetic standard, serves as the recurrent parent. The backcrossed chromosome substitution lines are expectedly quasi-isogenic to TM-1 and to each other,

except that each line differs by the replacement of a specific homologous pair of chromosomes or chromosome segments from the donor alien species. When possible, the cytogenetic and genetic constitution of the disomic lines is confirmed by cytological analysis and chromosome-specific SSR markers. We released 17 backcrossed (BC_5) *G. barbadense* chromosome or chromosome arm substitution lines (CS-B). These CS-B lines are providing important information about the association of *G. barbadense* substituted chromosome association with important fiber and agronomic traits. They also have been used to discover their merits in Upland cultivar improvement. For high-resolution analysis of QTLs, we are using many of the CS-B lines in development of chromosome-specific recombinant inbred lines. In addition to the disomic CS-B lines, we also have developed 13 monosomic and 32 monotelodisomic substitution stocks (BC_0F_1) for different chromosomes or chromosome arms of *G. tomentosum* and *G. mustelinum*. Research is also underway to use these for disomic chromosome substitution line development. These new genomic resources will complement the conventional types of interspecific introgression in Upland cotton improvement. All of these materials will provide additional tools in genomic analysis and genetic improvement of Upland cotton.

9. Diversity among Landraces of *Gossypium hirsutum* and D-genome Species Determined by SSR Markers.

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New genetic research tools, such as molecular markers, enable us to better understand the genetic parameters associated with diversity and their relationships with desirable production and quality traits in cotton. Morphological traits and microsatellite (SSR) marker data were collected from a field planting of over 450 landrace accessions of *Gossypium hirsutum* and all known D-genome diploid species collected during recent exploration trips. Thirty morphological traits and 125 selected SSR markers for which chromosomal locations were known were evaluated and assessed on these accessions in Shafter, CA, USA and Tecoman, Colima, Mex. Origins of allelic diversity and genic regions revealed by microsatellites derived from EST and genomic DNA sequences of polymorphic amplicons suggested four scenarios of transferability and rearrangement of amplicons in the allotetraploid AD cottons (*G. hirsutum* and *G. barbadense*) compared to the progenitor A and D diploid genomes. The variation in the morphological and in the SSR data was used separately to cluster the accessions by relatedness. SSR marker data was more helpful than morphological data in characterizing the diversity among the landraces and D species according to their origin of collection. Estimates of genetic similarities among all taxa showed extensive allelic diversity for these genomes. Partial results indicated that tetraploid cottons underwent a higher rate of DNA rearrangement than expected at the gene level following allopolyploidization. This research will be helpful in updating the morphological characterization of the collection, in integrating portable mapped SSRs to understand the genomic basis of genetic diversity of *G. hirsutum*, and in revealing unique sources of genetic variation in the collection.

10. Genetic Structure of and *in situ* Conservation of Natural Populations of *Gossypium mustelinum*

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Gossypium mustelinum is a tetraploid cotton species that occurs only in the semiarid area of Northeast Brazil. Three natural populations are known; two in Bahia State, at Macurur and Jaguarari cities, and one in Rio Grande do Norte State, at Caic. The Jaguarari, Macurur, and Caic populations have not more than 150, 60, and 20 adult individuals, respectively. Expeditions to find both new and previously described populations had failed. However, new subpopulations were found, one at Macurur and another at Jaguarari. The small size of each population, associated with the restricted number of individuals, places the species in a fragile situation. In addition, extensive ranching in the region, mainly of goats, enhances the hazard. Goats feed on leaves, bolls, seeds, young stems, and during the dry season remove the bark of stems to drink the liquid release. To protect these populations, more adequate strategies for *in situ* preservation are needed. However, to meet this aim, the genetic structure of these wild populations should be known. Samples of 51 individuals (20 each from the Jaguarari and Macurur populations and 11 from the Caic population) were evaluated with 24 BNL SSR markers, chosen for being polymorphic when used in moco cotton (*G. hirsutum*) and dooryard plants of *G. barbadense*. Primers amplified 27 loci, 19 polymorphic. Thirty-one private alleles were amplified from 17 loci, with frequency amplitudes ranging from 1.0 to 0.03. The genetic diversity (HE) for all populations was 0.25, and 0.22 in Caic, 0.14 in Jaguarari, and 0.12 in Macurur. Endogamy coefficients were 0.81, 0.95, and 0.57, respectively for Macurur, Jaguarari, and Caic, and 0.78 over all populations. Probably these very high inbreeding coefficients were caused by selfing and related matings. The diversity among populations was very high (FST=0.52), and could be the consequence of geographic isolation and genetic drift due to bottleneck. The great number of private alleles and the high amount of diversity among populations made all populations extremely important. Therefore, these *G. mustelinum* populations must be safeguarded to guarantee the in situ maintenance of diversity in the species. As the populations occur only in restricted areas, *in situ* conservation of *G. mustelinum* is not only achievable, but also quite simple. The Jaguarari population is the best conserved, which is due mainly to a mere fence that keeps the cattle away. Similar measures should be taken to protect the other two populations, with good chances of reaching satisfactory results.

11. Genetic, Biochemical and Molecular study on Colored Cottons

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Naturally colored cottons do not need artificial dying, and the garments made of it are labeled as green clothing. Colored cottons are healthy and profitable for mankind, and its prospective market is very wide. The genetic improvement of colored cottons in China has made some breakthroughs in recent years. The present research was conducted for the purpose of making a thorough analysis of the inheritance of the colored fiber lines, to identify and evaluate the genetic diversity, and the enhancement effect of the colored cotton lines, so as to accelerate the application in the colored cotton breeding. The main results are as follows: 1. Genetic improvement of color cottons has made great advancements in China since 1987. We bred a series of new colored fiber lines, which initiated and promoted the commercialization of colored fiber cottons in China. The yield and fiber quality of most lines reaches over 85% of that in normal white cotton varieties. 2. The inheritance of the colored fiber was analyzed by using F_2 populations of different combinations of new colored cotton lines. The first important result was that there was an interaction between the genes controlling fuzz color and the genes controlling lint color. It was further shown that brown and green colored lint were each controlled by one pair of incompletely dominant major genes on non-homologous chromosomes. Brown and green fuzz were each controlled by one pair of genes on non-homologous chromosomes, and green fiber color genes showed relative dominance to brown fiber color genes. Secondly, different white fiber lines and varieties contained different pairs of dominant genes, the more major genes with white fiber. In other words, colored fiber was not only controlled by the dominant color fiber genes, which derived from the white fiber genes, but also affected by the numbers of dominant white fiber genes. Thirdly, genetic variation was analyzed in 40 brown and 21 green cotton lines. Sixty-six polymorphic SSR loci were detected among those lines, in which 340 and 321 alleles occurred in 40 brown and 21 green colored cottons, respectively. The average number of alleles per locus was 4.9 for green cottons and 5.2 for all the colored cottons. It suggested that the genetic diversity of brown cottons was greater than that of green cottons. Forth, we also used SSR technology to tag the brown lint genes in the F_2 population of brown 128 and FB20. It showed that 1 out of 16 SSR loci maybe linked with the brown lint genes, and the distance was 18.4 cM. Fifth, forty-eight colored cotton lines and F_1 hybrids were studied for wax content, cellulose content, and fiber quality. The content of wax was five to eight times greater in green cotton and two times greater in dark brown cotton than that of white cotton. However, the content of the cellulose of the white cotton was greatest, brown cotton was next, and green fiber was lowest.

12. Biotechnology of Accelerated Breeding and Improvement of Cotton Varieties

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On the base of marking of such important traits as tolerance to wilt, salinity and drought as well earliness, fiber type and its quality, is shown a possibility of acceleration of breeding process and improvement of existing cotton varieties. The estimation of breeding materials takes place on the seeds level (genotypes) with use of marked enzymes and proteins. Using systematic analysis of seeds for all marked traits it is possible to reveal the most adaptable to unfavorable environmental factors genotypes with further their propagation. The basis of this technology is the analysis of genotypes. A

population, developed from such genotypes, possesses a high morpho-physiological homogeneity. This technology is also applicable and to other cultivars. One of the major factors causing a genetic polymorphism of natural populations including varietal is constantly occurring micro evolutionary processes, which are accelerated by the action of both external and artificial environmental factors. It leads to appearance of genetically heterogeneous individuals in the population. This develops in distinctions of members of the population by many diversified traits: morphological, physiological, biochemical and both qualitative and quantitative traits. But physiologically and biochemically adaptive biotypes may not develop morphologically. That is why a phenotype selection especially to pathogens not always answers to desirable or is long because of the factor of accidental. This relates to biotypes with the other signs or to biotypes possessing a complex of signs. Each trait is marked for enzymes or separate proteins of seeds, which have either direct or indirect relation to developing traits. Progeny of genotypes, selected according to these traits, possess high tolerance to pathogens in comparison with source cotton material. The main distinction of our elaboration is that we use only natural genotypes of the plant population, enriched during adaptation. It is possible to prolong preservation of the cotton variety for many years repeating this method periodically. Advantages of this method of increase of tolerance to unfavorable environmental factors are obvious. First of all it is exception of accidental. Besides this is essential economy of time. The screening technique can be also used for marking of other traits such as early ripening, fiber quality, high oil content, natural early leaf fall, tolerance to salinity and drought. Selection within an existing cotton variety can produce progeny that are superior to the original variety. This process can be accelerated if the selection can be carried out on a part of the seed with the remainder of the seed being planted and used to produce the next generation. Using this combination of methods, scientists at the Institute of Genetics and Plants Experimental Biology developed new elite lines that are currently being tested in the Uzbek State Variety Trials. The first line, Shodlik-9, is superior to its source variety, Bayaut-2 for Verticillium wilt and agronomic traits. Fiber analyses at the Uzbek State Testing Lab and StarLab (USA) showed that Shodlik-9 was superior to FM 832, SG 747, and Namangan 77 for fiber length, and it was similar to FM 832 and Acala 1517 for strength, 2.5 %, and 50% span length. Two other lines (L2 and L3) are currently being evaluated for Verticillium wilt resistance and agronomic traits. The lines are significantly earlier than US varieties.

13. Toward to the Construction of Mutant Library Induced by T-DNA Insertion and EMS in Diploid and Tetraploid Cotton.

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Mutations are very useful and valuable tools for genomic research in plants. There has been a focus in cotton on the methodology for maximizing the obtaining of mutants. Physical treatment, radiation for instance, has been employed to induce mutation and many of the chromosome aberrants (for example, deficiencies and translocations) have been found this way. A few morphological mutants also have been identified, but these mutations have been random in occurrence. The more desirable mutations are point-mutations or even direction-oriented mutations resulting in gene inactivation. To obtain these mutants, chemical treatment and the T-DNA insertion technique are being developed in cotton in our lab. Seed treatment with chemicals like EMS (ethylmethanesulfonate) causes point mutation of base transition or base transversion. Seeds of diploid cotton (*Gossypium arboreum* L. race *sinense* var Jinghua Zhongmian) were treated by EMS with the concentration of 0.33% and 0.25% for 10 hours.

The results indicated that the mutagenic effects of the EMS-treatments of cotton were apparently different. More mutants from seeds treated by 0.33% of EMS appeared than that treated by 0.25% in M1 populations. Most of the mutants in M1 are virescent or chimera mutants. Others, like cup-, crinkled- leaf, small-round-, and mosaic-yellow leaves, male sterile, and double-headed plants, also appeared. Genetic research has been done on two of the mutants. One is a male sterile//cup-leaf mutant. Crossed with its parent, 35 Male-fertile//normal leaf F_1 plants were obtained and self-pollinated. Of 88 F_2 plants, 73 were male-fertile//normal leaf and 15 male sterile//cupped leaf. The test results of the two generations demonstrated the inheritance of one pair of recessive genes conferring male fertility and leaf-shape. The other mutant is a dwarf-sterile mutant. Because of both male and female sterility in the mutant, the mutant traits are transmitted through self-pollination of its heterozygote. Among the progenies of the self-pollinated heterozygote, the sterility and dwarf plant characters co-segregated and no recombination between them was found. Based on the segregation ratio of fertility and plant height in the population from the self-pollinated heterozygote, genetic analysis showed that the dwarf and sterile trait were controlled by one pair of recessive genes. The regeneration system and T-DNA insertion to induce mutation in diploid and tetraploid cotton is still on the way.

Germplasm

Poster Presentations

P1. Tracking Gene Flow Between *Gossypium barbadense* and *Gossypium hirsutum* in the Region of the Mato Grosso, using Microsatellite Markers

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Gossypium barbadense is a wild naturalized species, widely distributed in Brazil. The natural occurrence of the wild species *G. mustelinum* and naturalized wild *G. barbadense*, along with the large commercial cultivation of *G. hirsutum* var. marie-galante makes Brazil an important center of diversity of *Gossypium*. *G. hirsutum* and *G. barbadense* coexist in many regions of Brazil, and are reproductively compatibile. In the present investigation, genetic analyses were carried out with the use of SSR, Simple Sequence Repeats, markers to verify the presence of alleles from the commercial cotton marie galante in wild *G. barbadense*. The polymorphism of this molecular marker is based on the length differences of the sequences amplified through PCRs, Polymerase Chain Reaction reactions, according to the number of repetitions in each microsatellite that is highly variable in the genome. The leaf sample of adult individuals and seeds of *G. barbadense* were collected in three regions of the Mato Grosso (Baixada Cuiabana, Pantanal and Cerrado Region). Half-sib families of 44 accessions with 23 descendants for each accession were planted in the green house. The genomic DNA was extracted from leaves of *G. barbadense* and *G. hirsutum*, according to CTAB 2% protocol. The DNA of 1059 individuals was genotyped with eight microsatellite primers. The detection of the amplified fragments was carried out in denaturing gel of polyacrylamide 4%, stained using silver nitrate. *G. barbadense* and *G. hirsutum* are allotetraploid species which was detected in two loci. The results had been compared with the alleles of one Gossypium locus according to Liu and collaborators. Direct analysis of gene flow shows the same alleles of locus BNL3103 occurring in the two species of *Gossypium* coexist in 66 % of the half-sibs families (19% of the Baixada Cuiabana

region, 27% of the Pantanal region and 20% of Cerrado region) evidencing an introgression. Amplifications of seven primers did not show the same alleles between and *G. hirsutum* and *G. barbadense*. The occurrence of introgression suggests a risk in the genetic variability of *G. barbadense*, the wild species.

P2. Determination of Genetic Diversity of *Gossypium hirsutum* Accessions in Argentina with SSR Markers

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Maintaining genetic diversity among cotton (*Gossypium hirsutum*) genotypes offers a measure of protection against potential widespread losses from cotton pests, diseases and facilitates the creation of segregating populations from which plants with superior gene combinations can be selected. The INTA (Instituto Nacional de Tecnologa Agropecuaria, Argentina) cotton germplasm bank contains about 700 accessions for use in breeding. The amount of genetic diversity at the molecular level in this germplasm is unknown, since they have not been surveyed with DNA markers. The main objectives of our study were to (i) investigate the molecular genetic diversity among 71 accessions cotton by simple sequence repeat (SSR) markers and (ii) to compare genetic distance estimated with SSRs. The level of polymorphism detected was low. Forty-nine SSR primer pairs generated 70 loci and 200 alleles. The polymorphism information content (PIC) value for all 70 SSR marker loci was 0.328, with a range from 0 to 0.675. The most informative SSR markers were BNL3257, BNL1317, CIR246 and BNL3545. Genetic distances among cultivars from the same seed companies were generally lower than the mean of all cultivars, an exception was the Acala. The high genetic divergence within Acala cotton was in part attributed to interspecific germplasm introgression into the Acala cotton. On average, cultivars from INTA were most similar to those from Stoneville Pedigreed Seed Company, Delta and Pine Land Company and Bayer Cotton Seed International.

P3. Genetic Diversity Among Feral Populations of Moco Cotton from the Brazilian Semiarid Region

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Moco cotton (*Gossypium hirsutum* var. marie-galante) was largely cultivated in the semiarid of Brazil until the seventies, when the introduction of boll weevil, associated with economic problems, caused a

dramatic reduction in cultivation. Due to the dry climate, it has been difficult to find a crop to substitute for cotton. Thus abandoned fields can be observed, as well as feral populations developed by them. Dooryard plants are grown as a fiber source for wicks or as ornamental plants. The presence of both feral and dooryard plants is continually decreasing, and both can be a valuable source of genetic diversity, since some of them are local varieties no longer cultivated. Furthermore, the ability to form feral populations demonstrates that these varieties have the ability to reproduce despite of drought conditions, and therefore are useful as a genetic resource. Diversity studies can help strategies for both in situ and ex situ conservation. It is particularly important to consider the region called Serid, where local varieties were first developed. Moco cottons from Serid were localized and collected in expeditions in 2004 and 2005, and 25 plants from Rio Grande do Norte and 17 from Paraiba were analyzed with 24 pairs of cotton microsatellite primers developed by CIRAD, which amplified 26 loci, 7 monomorphic and 19 polymorphic. The endogamy was 0.44 in average, slightly greater in the individuals from Rio Grande do Norte than from Paraba. Diversity between individuals from each state population (Neis unbiased estimator) was similar, indicating that comparable variabilities have been maintained. Moreover, the proportion of diversity between populations was extremely low (GST=0,006). These data lead us to conclude that genetic similarities between populations are high, in agreement with information provided by local inhabitants, who affirmed that the plantations were probably sown around 30 to 70 years ago, and genetically similar seeds should have been used, from local varieties shared among farmers. Even though Embrapa has good collections of moco cotton, maintained in germplasm banks, *in situ* conservation can be important. Considering the high similarity among genotypes, as well as difficulties on adopting strategies, which would allow in situ conservation, the most dissimilar ones and those, which are easier to protect, should be prioritized for preservation.

P9. Brasilian Cotton Cultivar Transformation by Pollen Tube Pathway Methodology for Insect Pest Resistance

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Pollen-tube pathway transformation is a method that delivers foreign genes directly into germline cells, combining genetic engineering and conventional breeding to achieve crop improvements. In recent years, in China, strong progress has been made in cotton transformation processes, generating a large number of transgenic cotton cultivars using this technique. Presently, cotton transformation methods in *Gossypium hirsutum* are effective mainly to Coker varieties, hindering their use in other commercial cultivars. An effective and reproducible method to transform plants from cells or tissues is essential for gene transfer involving genetic engineering and plant breeding studies. Transformation using the pollen tube pathway method avoids all the tissue culture steps that are usually very long, demanding, and also limiting to the production of transgenic plants. The main goal of this work is to transform Brazilian cotton varieties, such as BRS Cedro, using pollen tube pathway

technique to obtain transformed plants resistant to insect-pests. Approximately 1,200 cotton flowers were microinjected with a vector containing bcti and tar genes under control of 35Sd promoter, from which 20,000 seed were obtained. Forty plants were screened for resistance to kanamycin and also by PCR amplification. The integration and expression of target genes in potential transgenic plants are currently being analyzed by Southern, Northern, and Western blot molecular techniques. Supported by Embrapa, Facual, Fialgo, CNPq, CAPES, and FAPDF.

P10. Agronomic and Fiber Properties of an Introgressed Recombinant Inbred Population of Cotton (*Gossypium hirsutum* L.)

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Modern spinning techniques and new end uses of cotton have created demand for improved fiber quality in commercial Upland cotton (*Gossypium hirsutum* L.). However, the potential for improvement is limited by the available fiber trait variability within the species. Genetic resources for fiber improvement exist in *G. barbadense*, but past trait introgression efforts between the two species have been a limited success. The objectives of this study were to investigate the genetic variation and heritability of agronomic and fiber traits within a diverse recombinant inbred line (RIL) population created using a stable introgressed parent. The population (n=98 lines) had as its parents NM24016, a stable *G. hirsutum* line with significant introgression from *G. barbadense*, and TM1, the *G. hirsutum* genetic standard. Yield, plant height, boll size, lint percent, and fiber length, strength, micronaire, and elongation were measured in randomized, complete block tests at Las Cruces, NM and Maricopa, AZ, in 2001 and 2002. Significant genotypic variation within the population of RI lines was obtained for all traits measured. The greatest genetic variation, as measured by genotype coefficients of variation, was obtained for the agronomic traits boll size and plant height. Among fiber traits, fiber length (2.5% SL) and micronaire produced the highest genotype CVs. Broadsense heritability estimates of traits were generally high and ranged from 0.69 for lint yield to 0.92 for 2.5% span length. An exception was fiber elongation, which produced a broadsense heritability of 0.39. The distribution of the RIL population deviated from normality for the traits plant height and fiber micronaire. In the case of plant height, twenty five percent of the RI lines were transgressive segregates for taller plants, and in the case of micronaire, fourteen percent of the lines were transgressive segregates for lower micronaire. Transgressive segregant RI lines were identified for most of the traits measured. Phenotypic correlations among fiber traits suggested that simultaneous genetic improvement of multiple traits might be possible. Fiber strength and 2.5% span length were favorably correlated ($r = 0.59$, $P = 0.001$), as were 2.5% span length and micronaire ($r = -0.47$, $P = 0.001$). The lack of often observed negative correlation of lint yield with various fiber quality traits suggested that lines can be identified within the RIL population for simultaneous improvement of yield and fiber quality. The NM24016/TM1 RIL population presents valuable genetic variation for fiber quality improvement efforts in *G. hirsutum*.

PRESENTED AS ORAL

P13. The Evaluation of Cotton Varieties in Northern Communal of Namibia

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The different varieties of American and African Upland cottons (*Gossyppium hirsutum*) were evaluated in Namibia since 1998/9 to 2004/05 under dry land and supplementary irrigation. The four varieties, Tetra, Siokra V15, CA 223, and Delta Opal, were found to be suited for climatic conditions of Namibia. Since 1998/99 they were tested under dry land conditions and supplementary irrigation to determine their agronomic potential to produce high yielding varieties as well as improved production technologies for varying environmental conditions prevailing in the growing region. The varieties were tested and evaluated to introduce and recommend new varieties with high yield potential and high ginning out turn % and resistant to pest and diseases with good fibre quality. The performance compare with other cultivars showed good potential despite harsh environmental condition of Namibia. Their yield performance indicated that cultivar Tetra performed better than the other three with the average yield of 0.89 t/ha of seed cotton under dry land conditions. However, the total average seed cotton yield of the four best varieties namely Tetra, Siokra V15, CA223 and Delta Opal were 0,77 to 0,90 t/ha. The evaluation of different varieties of cotton at various research stations with different agro ecological zones for adaptability indicated that these varieties can be grown in Namibia successfully both under dry land and irrigated conditions. They are drought tolerant, but also perform well under irrigation, since they have very good recovery potential from dry conditions. They can produce good crops if managed well under small scale farming system. All these varieties are early maturity, they can produce high yield if they are established early with good weeding, pest control, and adequate nutrition. They were fair to good pest and diseases tolerance especially to jassids and aphids, which were the major sucking insects. The micronaire values range from 3.3 to 4.3, and their fiber lengths 22 to 36mm. Strength ranges between 23 to 36 (g/tex), length uniformity is less than 80%, elongation is less than 6, and ginning out turn (GOT %) is less than 35%. These varieties also have grown under the dry and irrigated condition in the Institute for Industrial Crops in Rustenburg and Loskop Republic of South Africa, Cotton Research Institute, Kadoma Zimbabwe, and in various locations in Africa. In Kadoma in 2001 to 2002, the recommended varieties were divided into middle veld and low veld dry land and irrigated. The best promise varieties were Albar SZ 9314, middle staple, Albar RQ 902, middle staple, LS 9219, long staple, and CY 889, long staple. These four varieties also have been tested in Namibia for three growing seasons, and they were found to be suitable to abiotic and biotic stress of Namibia. Albar SZ 9314 was considered to be the best variety in Zimbabwe, because of high lint out turns, good fibre quality, and middle staple. While LS 9219 variety was a good option for the growers who have fertile soils in good rainfall and produces a high quality fibre that attracts a premium prices. Its lint out turn for the three growing seasons in Namibia was only 35 to 36%. However, most of them were susceptible to verticillium wilt diseases. This variety can grow in Namibia successfully only in area with good rainfall and good soil or under irrigation areas. The four best varieties, Tetra, Siokra V15, CA223, and Delta Opal, were also graded between HX, HA and HB grades hand-picked cotton according to RSA grading Standard. They have high ginning out turn and perform well under the harsh and erratic rainfall of Namibia. However, varieties in Namibia need to be thoroughly tested over a wide range of seasons and good sites to come up with the best varieties for each site or region, and varieties need resistance to sucking insects such as aphids, leafhoppers (jassids), and for diseases such as alternaria, fusarium wilt, and wet weather blight, which are the common diseases in Namibia.

FUNCTIONNAL GENOMICS

Oral Sessions

1. Accumulation of Genome-specific Transcripts, Transcription Factors, and Phytohormonal Regulators During Early Stages of Fiber Cell Development in Allotetraploid Cotton

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Gene expression during early stages of fiber cell development and in allopolyploid crops is poorly understood. We report computational and expression analyses of 32,789 high-quality ESTs derived from *Gossypium hirsutum* L. Texas Marker-1 (TM1) immature ovules (GH_TMO). The ESTs were assembled into 8,540 unique sequences including 4,036 tentative consensus sequences (TCs) and 4,504 singletons, representing ~15% unique sequences in the cotton EST collection. Compared to ~178,000 existing ESTs derived from elongating fibers and non-fiber tissues, GH_TMO ESTs showed a significant increase in the percentage of the genes encoding putative transcription factors such as MYB and WRKY and the genes encoding predicted proteins involved in auxin, brassinosteroid (BR), gibberellicacid (GA), abscisic acid (ABA), and ethylene signaling pathways. Cotton homologues related to MIXTA, MYB5, GL2, and eight genes in auxin, BR, GA, and ethylene pathways were induced during fiber cell initiation but repressed in the naked seed mutant (N_1N_1).

4. A Proteomic Approach toward Understanding Fiber Development of *Gossypium hirsutum*

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Using proteomic analysis, an investigation aimed at a better understanding of the molecular mechanisms involved in fiber initiation and expansion was carried out in Upland cotton. Proteins were extracted from the ovules of -3 and 0 DPA (days post anthesis) of a fuzzless-lintless mutant (fl) and its wild-type variety, Xuzhou 142 (FL), the lintless mutants (Li_1Li_1) and the wild-type plants (li_1li_1) of Ligon lintless-1 line. An average of 1,600 spots were separated and visualized on each CBB-stained 2-DE gel. Both of the protein profiles of the wild ovules of FL and li_1li_1 at each stage were set as the controls, spots with qualitative (>10 fold) and quantitative difference between the control and one or two mutants were isolated out and digested, followed by tandem MALDI-MS/MS analysis. Thirty-nine protein spots were found to be higher in fl mutant compared to the controls, only 9 proteins were observed to preponderantly express in the wild ovules. Thirty out of the 48 differentially expressed proteins, which are involved in cotton fiber initiation, were identified by MALDI-MS/MS. For the purpose of identification of the proteins related to the disrupted fiber development of Li_1, comparisons of the 2-DE gel profiles have been made between the proteins of -3 and 0 DPA ovules of the lintless mutants and those of the controls, and those between the proteins

extracted from the fiber of 4 and 8 DPA of mutant ovules and those from the wild type plants of the Li_1li_1 selfed population. Sixty-five protein spots displayed positive/negative difference or marked changes in abundance. Forty-four of them were identified with MALDI-MS/MS. The identified proteins included factors for protein biosythesis, RNA processing proteins, molecular chaperons, anti-oxidative enzymes, proteins linked to energy pathway, transcription factors, proteins involved in signal transduction, proteases, as well as enzymes of metabolism and secondary metabolism. This result implies that the mRNA processing, protein isomerization, processing, and decay, altered carbohydrate and energy metabolism are possibly responsible for the aberrant fiber development.

5. Improving Cotton Fiber Quality

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With technological improvements in cotton fiber spinning there is also an increasing demand for high quality cotton fiber. Integrating and building our knowledge of fiber biology and cotton genetics is a rational approach to improve cotton fiber quality parameters such as length and strength. Ultimately a better understanding of the key processes in fiber development will facilitate the design of new strategies to bring specific improvements to cotton fiber. The cotton fiber is a unique biological system pivotal to the study of cell length and strength. Cotton fibers are single cells of the cotton seed coat that elongate reaching up to 5 cm in certain genotypes, are amongst the longest cells in the plant kingdom, and are highly rich in crystalline cellulose. Fiber growth goes through 4 major developmental stages, from initiation, through elongation, secondary cell wall synthesis, and to maturation. Over the last years, we have investigated the cotton fiber elongation stage of development. In the course of these investigations, we have uncovered new knowledge about fiber elongation and the roles of the sucrose synthase enzyme and plasmodesdata gating during fiber elongation.

7. Expression Profiling Identifies Genes Expressed Early During Lint Fiber Initiation in Cotton

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Cotton fibers are a subset of single epidermal cells that elongate from the seed coat to produce the long cellulose strands or lint used for spinning into yarn. To identify genes that might regulate lint fiber initiation, expression profiles of 0 days post-anthesis (dpa) whole ovules from six reduced fiber or fiberless mutants were compared with wild-type linted cotton using cDNA microarrays. Numerous clones were differentially expressed, but when only those genes that are normally expressed in the ovule outer integument (where fibers develop) were considered, just 13 different cDNA clones were down-regulated in some or all of the mutants. These included: a Myb transcription factor (GhHD1) (GhMyb25) similar to the *Antirrhinum* Myb AmMIXTA, a putative homeodomain protein (related to *Arabidopsis* ATML1), a cyclin D gene, some previously identified fiber-expressed structural and metabolic genes, such as lipid transfer protein, α-

expansin and sucrose synthase, as well as some unknown genes. Laser capture microdissection and reverse transcription–PCR were used to show that both the GhMyb25 and the homeodomain gene were predominantly ovule specific and were up-regulated on the day of anthesis in fiber initials relative to adjacent non-fiber ovule epidermal cells. Their spatial and temporal expression pattern therefore coincided with the time and location of fiber initiation.
Reduced expression of GhMyb25 and GhHD1 in cotton using RNAi constructs decreased the amount of lint as well as the number of leaf and stem petiole trichomes. There is also a decreased seed production suggesting an embryo as well as fiber function. Over expression of GhMyb25 in cotton using a 35S:GhMyb25 cDNA construct is in progress.

8. A Transcriptome Model of Gene Response to Osmotic Stress

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Identification of genes and biochemical processes differentially regulated during water-deficit stress is an important step for developing strategies to improve drought tolerance of cultivated cotton (*Gossypium hirsutum* L.). The objective of this study was to develop a model of the responses to osmotic stress in cotton roots. The cultivar Siokra L-23 was used as a model because of its relative tolerance to water-deficit. A polyethylene glycol (PEG)-induced phenotype derived from leaf measurements revealed a decline in photosynthetic rate and an increase in leaf temperature between 0 and 54 h, followed by a return to initial values from 54 to 96 h after stress imposition. Due to the biphasic nature of the measurements, the two phases were designated as response and recovery, respectively. Time-course root gene expression and metabolite profiles were developed utilizing cotton fiber microarrays, HPLC, and atomic absorption spectroscopy. During the response phase, root sucrose content decreased 42% while organic acid content decreased only 16%, producing a 65% increase in the root organic acid:sugar ratio. The regulation of several genes related to glycolysis and the glyoxylate cycle suggested sucrose was converted into organic acids via the glyoxylate cycle to produce this shift. Osmotic adjustment occurred in the response phase since K+ content increased 29% in stressed roots, consistent with up-regulation of two genes involved in K+ transport. Potassium accumulation was coupled to simultaneous reductions in the expression levels of seven aquaporin genes in the response phase. In addition, the yeast two-hybrid system was used to identify interaction of aquaporins with a vesicle-associated protein that aids in immediate response to osmotic shock by removing aquaporins from the plasmalemma. Regulation of membrane permeability to water, decreased cellular solute potential, and efficient production of carbon skeletons are the primary root-localized mechanisms by which Siokra L23 acclimated to PEG-induced osmotic shock.

12. The Biochemical Inheritance and QTLs Of Short-Seasoned Cotton Cultivars That Express Early Maturity Without Premature Senility

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The competition for more planting acreages between cotton and food crops has been intense and will remain so in the near foreseeable future under the situation of a large population needed to feed and clothing in a limited farm land in China. Therefore, the coordinated development between them is the major concern of both policymaking and scientific research community, and double or multiple cropping farm practice provides a valuable possible way in doing so. One of the critical measurements for multiple cropping practices is the exploitation and planting of short season cotton cultivars (SSC). Some varieties show earliness and premature senescence. The earlier the premature senescence occurs, the greater the yield and fiber quality loss. So it is very important to breed Early Maturity Without Premature Senescence cultivars. Six cultivars from two types of short-seasoned cotton (SSC) were selected, three SSC with no premature senescence and 3 SSC with premature senescence. We selected 6 x 6 diallel crossing design, including 6 parent lines and 30F1 and 30F2, all materials were planted in Zhongmiansuo experiment fields in 2004 and 2005. The plot size was 3-row 8m with row spacing of 0.7m, plant population was 5,500 plants per mu. All parents and F_1 and F_2 are planted in a random complete block design with 3 replications. Using 279 F_2 individuals developed by crossing the SSC with no premature senescence cultivar Liao 4086 and the premature senescence cultivar Zhong Miansuo 10 in *Gossypium hirsutum* L., a genetic linkage map was established with 21 linkage groups. A total of 60 of 2,082 SSR primer and 6 of 153 SRAP primer, and 23 of TRAP primer combination analyzed identified polymorphism between Liao 4086 and Zhong Miansuo 10. The 89 polymorphic primer pairs amplified 124 marker loci. A total of 94 marker loci were used to construct genetic linkage groups with 648.4cM. QTL mapping and analyzing of yield, fiber quality traits, early mature traits, and biochemical traits (including the antioxidant enzymes, CAT, POD, SOD, chlorophyll, and MDA content) were performed with composite interval mapping (CIM) and Multiple Interval Mapping (MIM) method using phenotypic data from F_2 progenies.

FUNCTIONNAL GENOMICS

Poster Session

P1. Proteomic Analyses of Susceptible and Resistant Cotton Roots to *Meloidogyne incognita*

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In the last decade the world cotton consumption comes growing considerably. However, the production of this culture in Brazil is affected by great losses caused mainly by pests. Among them

stands out the root-knot nematode, *Meloidogyne incognita*. Currently, there is a global effort to control the progress of this plant pathogen. A quite promising approach to achieve this target relies on the proteomic technology. With the objective to identify proteins related to cotton resistance mechanism, a comparative studies using susceptible and resistant cotton roots from a time course (5 and 15 days after infection) have been accomplished using two-dimensional electrophoresis (2DE). The experiments were conducted in green house using plants with two months age and 30cm height. As cotton is a recalcitrant plant, three methods for the protein sample preparation have been tested. The best result has been obtained extracting the proteins with buffer (40mM Tris-HCl pH 7.0, 250mM sucrose, 1% Triton X-100, 10mM EDTA, PMSF and DTT 1mM) and precipitating them with 10% trichloroacetic acid in acetone at -20C, overnight. The proteins were resolubilized in buffer containing 7M urea, 2M tiourea, 4% CHAPS, 2% IPG Buffer, and 0.3% DTT for 2h at room temperature and submitted to 2DE. Preliminary results have been demonstrated the presence of several proteins with different expression level for the susceptible and resistant plants with and without infection. The comparison between the non-infected control plants allowed the identification of six spots with higher expression in relation to the susceptible genotype and three spots present only in the resistant genotype. Five days after infection with 20,000 second stage juvenile nematodes, two spots with increased expression and two new spots, found exclusively in the resistant genotype, were visualized. Fifteen days after infection were visualized four new spots present only in the resistant genotype and two spots with increased expression in relation to susceptible genotype. These spots were excised from 2D gels, digested with tripsin and are being analyzed using a MALDI-ToF/ToF mass spectrometer. The results demonstrated the potential of proteomics in the search of proteins and/or genes of biotechnological interest for the resistance to nematodes

P3. A Single-Cell-Based Genetic Transformation System in Cotton

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The currently established cotton genetic transformation system, which utilizes an antibiotic selectable marker gene, requires lengthy processes involving tissue culture selection and somatic embryogenesis. In our recent experiments on particle bombardment of embryogenic calli with the green fluorescent protein (GFP) gene, we observed that transgenic somatic embryos could be recovered directly from a single transformed embryogenic cell without an intervening callus stage. Globular embryos emitting green fluorescence were observed 15 days after particle bombardment. Our results show that bombardment transformation of embryogenic callus with GFP visual selection can reduce the time to produce transgenic cotton plants.

P4. Proteomic analysis of growing- and sporulating- *Bacillus thuringiensis* (S811) and activity towards *Anthonumus grandis* (Coleoptera: *Curculionidae*) larvae

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Gram-positive spore-forming entomopathogenic bacteria use a wide range of proteins to invade, infect, and kill their hosts. Many of these proteins are synthesized during sporulation and are commonly founded in parasporal crystals. *Bacillus thuringiensis* (B.t.) is a well characterized group of bacteria known by their specific activity to different insect orders, as Lepidoptera, Diptera, Coleoptera, and Hymenoptera. For years, B.t. entomopathogenic proteins have been used to control insect pests as bioinsecticide formulations, and more recently, their genes have been introduced in crop plants aiming to control insects. Beside of the Cry proteins, other B.t. proteins, such as the VIPs (Vegetative Insecticidal Proteins), which are soluble and synthesized during vegetative growth, have also potential of controlling insects. However, these proteins were not well explored as the Cry proteins for biotechnology purpose. The aim of this study is to characterize the proteome of the *B. thuringiensis* strain S811, from which Cry proteins show a high activity towards Coleoptera, Lepidoptera, and Diptera. Four stages of bacterial growth, including vegetative and sporulation stages, secreted and intracellular proteins, as well as bacterium activity, were analyzed against Coleoptera larvae. The different stages were defined at different time intervals after bacteria inoculation: 8 hours after inoculation (HAI) vegetative stage, where exponential growth begins; 16 HAI beginning of spore formation; 24 HAI when is possible to visualize spore and crystal into the cell; and 32 HAI beginning of sporulation process, period that is possible to observe spores and crystals in the medium. The cell features, in each stage, were evaluated by contrast-phase microscopy. Intracellular and secreted proteins from different stages were used to the bioassay with *Anthonomus grandis* larvae and to establish two-dimensional reference maps. Specific proteins from stages exhibiting high activity towards this insect were excised from the gel and identified by MALDI-TOF MS/MS.

P5. Construction and Analysis of Two ESTs Libraries of Cotton (*Gossypium hirsutum*) Roots from Resistant and Susceptible Varieties to the Root-Knot Nematode (*Meloidogyne incognita*)

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The cotton (*Gossypium hirsutum*) crop is significantly affected by phytonematode infections, mainly by the root-knot nematode *Meloidogyne incognita*. One of the most effective methods to control this pest is the utilization of resistant varieties, which have been developed by conventional plant breeding.

Aiming the study of the nematode resistance mechanisms, we have constructed ESTs libraries of roots from resistant and susceptible varieties of cotton, both under attack of the *M. incognita*. The libraries were developed using the "Superscript Plasmid System with Gateway Technology for cDNAs synthesis and Cloning" (Invitrogen) kit. The cDNAs were sequenced by Automated Sequenciator ABI 3700 and resulting sequences were submitted to control quality by PHRED bigger than 20 for 250-bp long and filtered to rRNA, PoliA+ and vector. Then, approved sequences from both libraries were assembly together, aiming the prospection of differentially expressed genes. The generated data resulted in 2,262 sequences, and the assembly produced 1,827 groups comprising 234 contigs and 1,523 singlets. Gene annotation was done throughout BLAST analysis of both, contigs and singlets, compared with several data banks: nr, MIPS, GO, KOG and PFAM. We find 36 (15%) exclusive ESTs of resistant variety, 76 (32%) of susceptive variety and 122 (53%) of both varieties. The sequences putatively involved in resistance are being analyzed by Real Time PCR experiments. Moreover, selected sequences will be used to transform cotton plants aiming the introduction of resistance to nematode

STRUCTURAL GENOMICS SESSION

Oral Presentations

1. Construction of a PCR-based Genetic Linkage Map with Functional Markers

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A saturated genetic map is necessary to enhance genomics and molecular breeding. A BC_1 mapping population with 140 individuals was produced by crossing between *G. hirsutum* L. cv. TM-1 *G. barbadense* L. cv Hai7124 and then backcrossing with TM-1, and used to construct a PCR-based genetic map in tetraploid cotton. In the construction of the map, microsatellites or simple sequence repeats occurred in expressed sequence tags (EST-SSRs) as functional markers are explored since many ESTs are publicly available now. From 931 ESTs contained SSRs from *G. arboreum*; 544 EST-SSR primer pairs were developed, and a total of 111 loci produced by these 99 EST-SSRs were integrated into our backbone map. We further developed 943 and 1540 EST-SSR primer pairs from *G. hirsutum* and *G. raimondii* ESTs, and together, 932 loci were integrated into our allotetraploid cotton genetic map. Additionally, more than 30 cloned full-length genes and mutated genes such as P_1, R_1, ml, ms_{14}, ms_{15}, n_2 etc were also mapped. So, the present PCR-based genetic map includes more than 2000 loci in which more than 1000 loci are functional markers such as EST-SSR. Using BAC clone screened with linkage group-specific SSR markers as BAC-FISH (fluorescence in situ hybridization) probes hybridized with ten translocation heterozygotes (NT) of *G. hirsutum*. L, we assigned the last six linkage groups LGA01, LGA02, LGA03, LGD02, LGD03 and LGD08 to chromosomes 13, 8, 11, 21, 24, and 19,

respectively. The 13 homoelogous chromosome pairs were therefore established and a new chromosome nomenclature in tetraploid cotton was proposed. A set of chromosome specific-SSR markers was also presented. This map and these data provide a set of powerful tools and also a complete framework for cotton genome research.

2. Physical Mapping of Fiber Genes in Cotton

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Understanding the physical location of fiber genes in cotton chromosomes is important for basic genetic research and improvement of fiber yield and quality. Our pilot study demonstrated an approach to integrating physical and transcript mapping information of these genes in the cotton genome. We collected 39,384 fiber unigenes (18,490 EST contigs and 20,894 EST singletons) from 16 fiber cDNA libraries. We designed an initial set of 600 overgo primers from above non-redundant EST unigenes, and identified 2,640 positive BAC/BIBAC clones from TM-1 cotton BAC and BIBAC libraries. We fingerprinted these positive clones and assembled them into physical contigs. We also used SSR markers from these BAC clones to integrate the contig maps to the genetic maps. The average number of positive BAC clones per unigene was 4.4, which is consistent with the characteristics of the tetraploid cotton genome. There were 87 contigs and 1,471 singletons in the FPC database. Five of the 87 contigs were assembled and anchored to chromosomes 3, 5, 10, 13, and 14. Currently, these contigs and singletons are being edited and merged with the whole cotton BAC contig map. The five integrated contig maps contained 1,381 fiber ESTs, 5 BNL SSR markers, 3 CIR SSR markers, and 31 TMB SSR sequences. We sequenced both ends of BAC clones from these contigs to identify fiber-gene SSR markers and to verify the integrated physical and transcript maps. The information obtained from the study is used to understand genomic distribution, functional implication, and evolutionary events of the fiber genes in cotton.

3. A Strategy for Developing SNP Markers for the MIC-3 Root-specific Gene Family Associated with Nematode Resistance in *Gossypium* spp.

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Candidate gene association mapping using SNPs has become a powerful tool to find genes that are associated with complex traits and diseases. SNP discovery in candidate genes of interest is key among the early steps in association mapping. Disomic polyploids such as cotton usually contain two divergent paralogous copies of each gene, one per subgenome, which complicates the SNP discovery process due to the presence of duplicate loci. Efficient discovery of any SNP markers needs to distinguish differences between subgenomes as well as allelic variants at a locus. MIC-3 is a recently identified gene shown to exhibit increased root-specific expression following nematode infection of plants that are resistant to root-knot nematode (RKN). We cloned and sequenced PCR-amplicons (derived from MIC-3-specific degenerate primers) from individual plant DNAs to [1] identify sequence variation; [2] discover SNPs in MIC-3 family members of selected diploid and tetraploid species in cotton, and [3] discover the chromosomal location(s) of the MIC-3 family members. Phylogenetic trees were constructed based on the analysis of consensus sequences using the most parsimonious algorithm of the PAUP software. SNP markers were discovered from the comparative analysis of the consensus sequences of the tetraploid lines within individual Phylogram clades. Parsimony analysis revealed the presence of several putative haplotype members of MIC-3 gene. BLASTn results suggested that MIC-3 is a novel gene present only in cotton. Five different SNP markers associated with five different MIC-3 family members were delimited to chromosome arm 04sh by hypoaneuploid cytogenetic deficiency tests. Intergenic regions of these clustered genes were localized to the same chromosome arm, further confirming their clustering and physical location within the cotton genome. Chromosomal localization via deletion analysis would be possible only if the associated SNP marker could detect variation at a single locus. In addition, the phylogenetic tree suggested significant homology of the clustered MIC-3 members with the diploid ancestral A-genome. Recombination among the clustered genes associated with disease and pest resistance may confer evolutionary robustness to the organism by creating new variants that might offer opportunities for response to selection pressures from evolving pests and pathogens.

4. QTL Mapping for Resistance to Root-knot Nematodes in the M-120 RNR Upland Cotton Line

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Root-knot nematodes *Meloidogyne incognita* can cause severe yield loss in cotton. Utilizing an interspecific F_2 population developed from crossing M-120 RNR and a susceptible *Gossypium barbadense* cultivar Pima S-6, inheritance analysis indicated that the data fits a single dominant gene model. Association analysis using SSR markers detected a minor and a major dominant QTL on Chromosome 7 and Linkage group A03, respectively. The major QTL on Linkage group A03, Mi1-A03, had a LOD score of 19.21 and accounted for 63.7% of the total phenotypic variation, while the minor QTL locus on Chromosome 7, Mi1-C07, had a LOD score of 3.48 and accounted for 7.7% of the total phenotypic variation. The M-120 RNR allele in the Mi1-A03 locus, derived from the Auburn 623 RNR, is likely to have originated from the Clevewilt 6 cultivar. Results from this study indicated that the SSR marker CIR316 may replace the laborious greenhouse screening in breeding programs to identify genotypes resistant to *M. incognita*.

7. Linkage Disequilibrium Based Association Mapping of Fiber Quality Traits in Cotton Using Diverse Cotton Germplasm from Uzbekistan

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QTL-mapping, a now-classical approach to identify molecular markers linked to complex traits in specific experimental populations, is extremely time-consuming, high-risk and expensive work — prohibitively expensive if dozens, let alone hundreds or thousands, of germplasm accessions are to be examined. However, use of linkage disequilibrium (LD) based association mapping circumvents the need for large F_2 or RI mapping populations by making use of information contained within the genetic recombinations that have occurred in cotton genome during the course of recent evolution. By this method, certain alleles at a marker locus can be "associated" with alleles at a linked locus affecting a trait of interest. LD mapping permits much finer-scale mapping than does QTL-mapping, and in conjunction with new technology for rapid genotyping, LD-mapping will ultimately be more powerful for isolating genes with specific effect. Although novel to cotton research, the association genetics strategy is, in fact, highly applicable to the identification of markers linked to fiber quality and yield through the examination of linkage disequilibrium of DNA-based markers with fiber quality and yield traits in a large diverse germplasm collection. We selected 1,000 *Gossypium hirsutum* wild (ssp. palmeri, richmondii, morrilli, mexicanum, latifolium, malum, yucatanense) and variety (from 37 country of origin) accessions from Uzbek Cotton Germplasm Collection. These selected variety and exotic cotton accessions were grown in Uzbekistan and Mexico growing environment and phenotypically analyzed for at least 14 morphological traits as well as for fiber quality and yield characteristics. Furthermore, 288 exotic *G. hirsutum* accessions were genotyped with a core set of 100 SSR markers and 337 variety accessions (from Uzbekistan, Latin American and Australian ecotypes) were genotyped with 210 chromosome-specific SSR markers, covering 21 chromosomes (an average 10 SSR primer pairs per chromosome) of cotton. The molecular phylogenetic analyses using SSR marker genotypes and analyses of morphological/agronomic trait characteristics revealed a wide range of genetic diversity within studied accessions. The primer pairs for 100 core set SSR markers generated 394 marker loci in the 288 exotic cotton panel, and primer pairs for 210 chromosome specific markers generated 1143 marker loci in the 337 variety accession panel. We estimated genome-wide linkage disequilibrium level between pairs of genotyped marker loci in the exotic and variety cotton accession panel using squared allele-frequency correlations (Rsq) and a rapid permutation test (p-values for significance of pair wise LD among all possible pairs of SSR loci) with TASSEL. The results revealed that the pair wise LD estimates were higher in exotic accessions than variety accessions. We also estimated the population structure and kinship among studied accessions to conduct the mixed linear model (MLM) for association mapping of fiber quality traits in these diverse cotton germplasm. The calculation of LD decay for cotton genome using SSR data is in progress. The details genome-wide LD estimates and association mapping of fiber quality traits of cotton will be discussed.

STRUCTURAL GENOMICS SESSION

Poster Presentations

P1. Molecular Marker Study of Fiber-related Traits in Upland Cotton (*Gossypium hirsutum* L.)

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The modern textile industry depends on the improvement of fiber quality, especially strength, for meeting the needs of higher spinning speed. Advances in the use of DNA markers for marker-assisted selection (MAS) are promising for streamlining plant breeding programs. Molecular marker study of fiber related traits in Upland cotton were conducted in the present paper. Cross 97080 x 153 was made between two parents, 0-153 with high fiber strength, originating from crossing *G. hirsutum* L with *G. arboreum* L and backcrossing with *G. hirsutum* L, and 9708, commercial transgenic variety with resistance to budworm. A total of 940 SSR primers were used to screen polymorphism among two parents 0-153 and 9708, and their F_1, which resulted in 57 polymorphic loci in F_2 population from the cross of 97080 x 153. Linkage test indicated 36 loci could be mapped to 11 linkage groups and covered a total genetic distance of 497.9 cM, approximately 11.07% of total cotton genome. Twenty QTLs for fiber yield related traits were found in F_2 and $F_{2:3}$ populations using IM (Interval Mapping) and CIM (Compose Interval Mapping) methods. Ten were detected by the two methods. QTL numbers for plant seed yield, boll weight, lint percent, lint index, seed index, boll branch numbers, first harvest percent, and resistance disease index were 1, 4, 4, 3, 1, 4, 2, and 1, respectively. Sixteen QTLs for fiber quality characters were found, 9 of them were detected by the two methods, QTL numbers for fiber strength, fiber length, micronaire, fiber elongation, and fiber uniformity were 5, 5, 2, 2, and 2, respectively. No QTLs for fiber yield related traits were detected in both generations, which shows the need of multiple self-crossing lines for QTL analysis of yield traits. Two QTLs for fiber strength, 2 QTLs for fiber length, 1 QTL for micronaire, and 1 QTL for fiber elongation were detected in both F_2 and $F_{2:3}$, which indicated these QTLs show stable genetic effects and could be used for marker assisted selection breeding. One QTL of fiber strength, linked tightly to marker s159L explain 12.37% of phenotypic variance in F_2, and linked tightly to marker s111H could explained 20.36% of variance in $F_{2:3}$. The QTL for fiber strength, linked tightly to marker s5295f170 in both generations, explained 10.68% and 27.77% of variance, respectively. Moreover, the very high additive genetic effects from 0-153, and the highly negative dominant effects for the two QTLs showed that heterozygous plants for these two QTLs tend toward the lower parent in fiber strength, which indicated it is very necessary to select fiber strength through the molecular marker.

P2. Development of Gene-based Markers and their Chromosomal Localization in Cotton (*Gossypium* spp)

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Molecular markers are being used extensively in genetic studies and breeding programs of cotton. A majority of the marker systems were developed based on random genomic sequences, mainly due to the dearth of polymorphism detection systems that can distinguish fine differences in genic regions between different genotypes. Therefore, an objective of our research program is to develop and map gene-based markers for cotton through the use of improved bioinformatics tools and polymorphism detection systems. We report our initial attempts in marker development using candidate genes and expressed sequence tags (ESTs). Candidate genes: using twenty primer pairs for different candidate genes in cotton, we screened for polymorphism by polyacrylamide gel electrophoresis (PAGE) and derived phylogenetic relationships among 32 genotypes that include 12 genotypes that make cotton microsatellite database (CMD) panel and other diploid and tetraploid genotypes. Polymorphism information content (PIC) for these markers ranged from 0.840 - 0.997. Cleaved Amplified Polymorphism (CAP) was observed between *G. hirsutum* (TM-1) and *G. barbadense* (3-79) for 12 of the 20 primer pairs using *Rsa*I, *Msp*I, *Hha*I, and *Hae*III restriction enzymes. We were able to localize five of these markers to chromosomes in the tetraploid genome using 17 chromosome substitution lines of *G. barbadense* in *G. hirsutum* background (CS-B lines, USDA, Mississippi State, MS). Similarly, we mapped these markers to their respective chromosomes based on the absence of the respective allele in 44 *G. tomentosum* and 41 *G. barbadense* aneuploid lines (USDA, Mississippi State, MS) for different chromosomes. We are currently analyzing over 100 fiber quality-related genes for marker development and genome localization. Expressed Sequence Tags: to utilize the EST resources, we collected 38,893 *G. arboreum* ESTs from GenBank and clustered them using StackPackTM software to reduce the redundancy. The non-redundant (NR) sequences were searched for the presence of simple sequence repeat (SSR) motifs. A total of 725 NR sequences had SSR of length 18 or above. A subset of the SSR-containing ESTs was used for designing 200 primer-pairs, which were designated, Mississippi *G. arboreum* EST-SSR (MGAES) markers. One hundred and forty seven (74%) MGAES primer-pairs were successful in amplifying the two reference genotypes, TM-1 and 3-79. A total of sixty-five (44%) EST-SSR markers displayed fragment length polymorphism on polyacrylamide gels. The candidate gene and EST-SSR markers will be genetically mapped using the *G. hirsutum* (TM-1) × *G. barbadense* (3-79) recombinant inbred line (RIL) population.

EVOLUTIONARY AND COMPARATIVIE GENOMICS AND BIOINFORMATICS

Oral Presentations

1. Methylation filtering analysis of Gossypium genomes

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The large size and tetraploid nature of the *Gossypium hirsutum* genome will make it costly and technically challenging to determine its complete DNA sequence, based on today's technology. Therefore, it has been proposed that *G. raimondii* would be a desirable species to sequence because it may be representative of the D genome and its genome is one-third the size of *G. hirsutum*. An alternative strategy would be to use an approach that would concentrate on gene rich regions. In our pilot study, we used GeneThresher technology (methyl filtration) to evaluate the genome composition of the *G. hirsutum* (AD_1), *G. barbadense* (AD_2), *G. raimondii* (D_5), and *G. arboreum* (A_2) genomes. The results from this study demonstrate that there might be an optimal approach for obtaining the maximum gene information for *Gossypium* at the lowest cost.

2. Computational Mining of SSRs in ESTs of Cotton and other Dicotyledonous crops

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Simple sequence repeat (SSR) markers are widely used in molecular genetic studies of many plant and animal genomes. While SSRs, once developed, are very useful markers, their development is expensive since it requires prior knowledge of sequence information. Although repeat enrichment methods reduced SSR development costs over the years, the price tag is still high for crops such as cotton where a large number of SSRs need to be developed and screened. Computational approaches for mining ever-increasing sequence resources in genome databases offers a potential alternative for rapid and economical development of SSR markers. ESTs are excellent candidates for this purpose since they are available in large numbers from many genomes and more over they represent expressed genes. The availability of information on the occurrence and frequency of different types of SSRs in plants is very useful not only for understanding the abundance of SSRs but also for targeted development of specific type of SSRs in a given species. While such information exists for several monocot species it was not available for dicots that comprise many economically important crops. In this study, 1.54 million ESTs from 55 dicotyledonous species were computationally mined and the frequencies of various types of SSRs were surveyed. An overview of the results obtained from this large scale study and an additional study, where mined SSR-ESTs were used for marker development in cotton, will be presented and the potential of this bioinformatics approach for marker development will be discussed.

3. Cotton Microsatellite Database for comparative characterization of SSRs in *Gossypium*

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The Cotton Microsatellite Database (CMD) (http://www.cottonssr.org) is a curated database resource providing centralized access to the largest collection of publicly available cotton SSRs. Microsatellite markers can be used in various applications including gene tagging, genome mapping, selecting progeny for a desired phenotypic trait, localizing qualitatively as well as quantitatively inherited traits, pedigree analysis, variety protection, and introgressing novel genes into breeding germplasm from exotic germplasm. The novelty of the CMD is in its specific orientation toward researchers involved in molecular marker development and application to cotton breeding. It is being actively used by the international cotton community, and can be viewed as an important vehicle toward increased collaboration among academic, government and industry cotton scientists, both nationally and worldwide. The CMD Advisory Board includes 5 scientists from the USDA, 1 researcher from academia, and 6 representatives from international companies. The present collection of 3,610 SSRs in CMD was generated through collaboration with major cotton research groups from the USA, France and China. Currently, the cotton SSRs from 9 projects deposited in the CMD are represented by 2,285 EST-SSRs and 1,325 genomic SSRs, of which 2 are chloroplast-derived and 192 are BAC-derived. CMD provides a suite of online tools for data mining and comparative analysis. Future development of the CMD will focus on the establishment of a standard nomenclature for cotton SSRs, adding new microsatellite data from both public and private sources, enhanced SSR data mining and analysis capabilities, such as full sequence processing facilities, and provision of a quarterly newsletter for the cotton community. Annotation of SSRs will include further classification using gene ontology and KEGG terms.

4. CottonDB.org: New Website for Cotton Genome Database

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CottonDB was initiated in 1995 under a project sponsored by the United States Department of Agriculture (USDA) Agricultural Research Service (ARS) as a resource to contain the developing knowledge base in Cotton Genetics. CottonDB.org is the new home for the expanded and enhanced version of CottonDB. The purpose of CottonDB is to provide a convenient medium for cotton researchers to exchange information regarding germplasm, markers, genetic/physical maps, sequences etc. through a website interface. CottonDB has been updated with several new features that allow

researchers to easily browse and retrieve data from the database. Quick Queries allows the user to search for the most frequently requested information. The FPC viewer is a web viewer for visualizing BAC contig fingerprints. Further enhancements in progress include cMAP viewer, which will provide online map comparisons among two or more genetic or physical maps; Genome Browser for comparisons of the cotton vs. Arabidopsis genomes, and BLAST server, which will allow the user to blast their sequence against the available database of cotton sequences through a web interface. In addition to the individual features available for each of these tools, interactions among the tools and with other commonly used public databases (i.e. GENBANK, EMBL, and DDBJ) are also possible. Users of the database can easily submit their comments, suggestions, and corrections to the curator via an online interface so that CottonDB may be continually improved to best serve the cotton community.

5. New Methods for Analyzing Complex Traits

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We propose a genetic model and statistical methods for analyzing genetic architecture of complex traits including additive, dominance, and epistasis effects of QTLs, as well as their interaction with environment effects. Parameter estimation and statistical tests of the genetic effects in the model are achieved in the mixed linear model framework by MCMC (Markov Chain Monte Carlo) algorithm. A permutation test is employed to calculate the experimental-wise significance level and a model selection procedure is conducted to optimize the model. Monte Carlo simulation studies and a real data set in rice are used to demonstrate the utility of the method. The computer software QTLNetwork was developed using the C++ programming language. QTLNetwork-2.0 is user-friendly computer software for mapping quantitative trait loci (QTL) in DH, RI, BC_1, BC_2, F_2, IF_2 and BC_xF_y populations and for graphical presentation of QTL mapping results.

Evolutionary and Comparative Genomics

Poster Presentations

P1. Engineered Cry8H Toxins Activity to the Cotton Boll Weevil, *Anthonomus grandis*, (Coleoptera: Curculionidae)

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The Cry proteins are delta-endotoxins produced in a crystal form by *Bacillus thuringiensis* strains during the sporulation process. These proteins show high specificity and toxicity to certain classes of insects and invertebrates, which has increased the interest in the search of new genes encoding active molecules to target insect-pests. Among the insect-pests that attack cotton crops, the cotton boll weevil, *Anthonomus grandis*, is one of the most important in Brazil. Due to its endophytic behavior, its control with chemical insecticides is not efficient, is expensive, and associated with many health and environmental problems. In this context, we developed new sequences of *cry8H* that were

specific and more effective against the cotton boll weevil, using techniques such as DNA Shuffling and Phage Display. The *cry8H* sequence was fragmented and randomly recombined, thus obtaining the shuffled genes, generating a combinatory library of mutant cry genes with 105 transformants. The created mutants were screened by Phage Display using *A. grandis* border membrane vesicles (BBMV), in which the potential receptor of the toxin is present. The clones were further selected through bioassays to validate their insecticidal activities. These clones were sequenced to confirm the mutations and 4 clones exhibited 2-6 fold enhanced insecticidal activity compared to the wild type gene. The efficiency of the recombination by DNA Shuffling and Phage Display to generate new active molecules of *cry8H* against *A. grandis* is discussed.

P2. Insecticidal Effects of Cry8H Mutants Generated by DNA Shuffling and Phage Display Techniques Against Fall Armyworm (*Spodoptera frugiperda*)

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Bacillus thuringiensis is a gram-positive bacterium that produces delta-endotoxins active against diverse insect pest species and other invertebrates, which cause extensive agricultural damages in the world. The *cry* genes isolated from *B. thuringiensis* have been widely used to develop transgenic plants with enhanced resistance to specific insect pests. Despite the diversity of the *B. thuringiensis* toxins (Cry proteins) the, technique of rapid molecular evolution (DNA shuffling) has been used to generate more potent and specific Cry proteins against diverse agricultural pests. In this context, the present work aimed to develop new mutants for Bt toxins with enhanced activity towards the main insect pest of maize and cotton, the fall armyworm (*Spodoptera frugiperda*). A library was constructed using the recombination of a new cry8 gene combining DNA shuffling and Phage Display techniques. The gene denominated cry8H was initially amplified by PCR using specific primers containing the *Sfi* I restriction site and submitted to DNase I fragmentation. The products of this reaction (30-50 bp fragments) were submitted to a new PCR reaction without primers in order to obtain new recombinant genes. The recombinant genes were digested by *Sfi* I, subcloned into the pCOMB3X phagemid and expressed in *Escherichia coli* (XL1 Blue) thus generating a combinatory library of cry mutant genes with 105 transformants. The obtained clones were screened for Phage Display using *S. frugiperda* gut ligands and the selected molecules exhibited a higher insecticidal activity ranging from 2 to 6 times in relation to the wild type. This result demonstrated that the combination of DNA shuffling and Phage Display techniques is an efficient tool to generate new *cry* genes encoding insecticidal proteins with potential use in the *S. frugiperda* control.