# Introgression of *Gossypium* Germplasm to Increase Genetic Diversity for Cotton Improvement

**Agreement No. 04-524TX**
**Texas Agricultural Experiment Station and Cotton Incorporated**
**Annual Report for 2006**

## SUMMARY

Cotton improvement efforts are seriously constrained by the limited amount of genetic diversity available to breeders. This project will lead to better US cottons by [1] diversifying cotton elite cotton germplasm through interspecific introgression, [2] developing superior methods for interspecific introgression, and [3] developing well-trained cotton geneticist-breeders. The targets for introgression include germplasm four species: two 52-chromosome species, *G. tomentosum* (Hawaiian species) and *G. mustelinum* (Brazilian species) and two 26-chromosome species, *G. longicalyx* (African species) and *G. armourianum* (Mexican species).

For introgression of *G. tomentosum* and *G. mustelinum*, field performance evaluations (mean, dispersion) were completed for conventional backcross-inbred (BCI) and backcross-random-mated (BCR) breeding populations at the first backcross, and are forthcoming for second and third backcross levels. SSR analyses revealed patterns of retention and recombination for an entire chromosome through the first backcross. For each species, a doubly complex population containing wild germplasm from these species, plus diverse *G. hirsutum* cultivar germplasm, was advanced one cycle of random-mating, and scored for several traits. For each species, the development of most of the disomic chromosome substitution (DCS) lines was advanced one generation.

For introgression of *G. longicalyx* and *G. armourianum*, we selected advanced backcrosses as homozygous and heterozygous resistant by MAS, and then screened field-grown self-progeny for deleterious traits. Fiber samples were collected from selected plants in [a] progeny-row tests of single-plant selections, and [b] the BC4 doubly complex population (see below). Young leaf tissue and DNA samples were prepared from critical plants in the nematode resistance program as well as the genome-wide introgression program. Two doubly complex trispecies populations, one at BC2 and one at BC4, were advanced and evaluated. Plants in both populations were classified for fertility, and plants of the latter were also sampled for DNA genotyping. Molecular markers tightly linked with the resistance and suitable for MAS of the reniform resistance were identified.

# Introgression of *Gossypium* Germplasm to Increase Genetic Diversity for Cotton Improvement

**Agreement No. 02-259**
**Texas Agricultural Experiment Station and Cotton Incorporated**
**Annual Report for 2006**

## PURPOSE & GOALS

Cotton improvement efforts are seriously constrained by the limited amount of genetic diversity available to breeders. This project will lead to better US cottons by [1] diversifying cotton elite cotton germplasm through interspecific introgression, [2] developing superior methods for interspecific introgression, and [3] developing well-trained cotton geneticist-breeders.

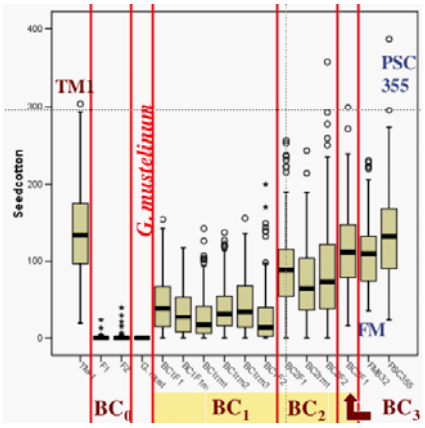
The targets for introgression include germplasm four species: two 52-chromosome species, *G. tomentosum* (Hawaiian species) and *G. mustelinum* (Brazilian species) and two 26-chromosome species, *G. longicalyx* (African species) and *G. armourianum* (Mexican species). Genome-wide introgression is sought for all four species, with the intent of finding and extracting beneficial genes for all traits, e.g., agronomic, yield, fiber quality and other traits. Three breeding methods are being used for *G. tomentosum* and *G. mustelinum* introgression, because each offers advantages and disadvantages for breeding purposes. The methods are to be compared by field performance evaluation, as well as genetically, by molecular analysis of DNA markers. Field data will guide breeding efforts and help define parameters for selection of breeding strategies for future introgression projects. The molecular data will help us understand the genetic basis of performance responses to the various breeding methods.

By way of expansion to CI core Project 02-259, this project also includes efforts aimed at *G. longicalyx* and *G. armourianum*. These efforts are collaborative with the CI-sponsored USDA-ARS effort to specifically transfer and genetically characterize reniform nematode resistance into Upland cotton from *G. longicalyx* (totally immune to the nematode). We are participating in that effort through logistics, cytogenetic analysis, genetic analysis and molecular marker tagging of the immunity trait. The resistance is expected to be of great value to cotton in reniform-affected areas, perhaps $100M annually. However, genome-wide introgression will likely enhance many traits, e.g., agronomic, yield, and fiber quality, and thus will lead to benefits of even greater economic and wider geographic impact. Thus, we used early generation materials from the nematode resistance project to establish a genome-wide introgression effort, too.

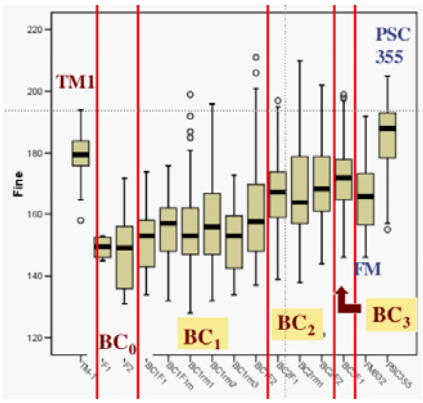
## MAJOR ACTIVITIES & ACCOMPLISHMENTS IN 2006

### *G. tomentosum & G. mustelinum*

**[1] Completed the field performance evaluations (mean, dispersion) conventional backcross-inbred (BCI) and backcross-random-mated (BCR) breeding populations.**




**Yield of various backcross-inbred and -random-mated *G. mustelinum* introgression populations in 2004.** Yields trend upward across backcross generations. BC3 performance was near that of the recurrent parent and on par with the checks PSC 355 and FM832. The broad range of yield performance suggests these materials present great opportunities for genetic gain for yield.



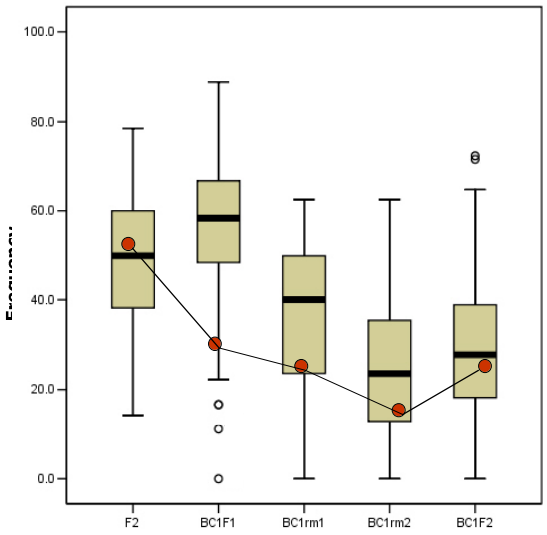

**Fiber fineness of various backcross/inbred and /random-mated *G. mustelinum* introgression populations in 2004.** *G. mustelinum* hybrids have strong thick fiber. Fineness trends upward across backcross generations, approaching the recurrent parent, TM-1. The level and range of fineness in advanced backcrosses (BC2 and BC3) are good and suggest excellent opportunities for selection and genetic advancement for improved fineness.

| | F2 | | | | BC1F2 | | | |
|---|---|---|---|---|---|---|---|---|
| | *mm* | *Mh* | *hh* | | *mm* | *mh* | *hh* | |
| bnl1066 | 12 | 23 | 6 | 2.4 | 2 | 15 | 24 | 4.2 |
| bnl836 | 4 | 14 | 9 | 1.9 | 4 | 12 | 28 | 0.5 |
| bnl36491 | 8 | 14 | 13 | 2.8 | 6 | 9 | 28 | 0.4 |
| bnl3649b | 2 | 24 | 8 | 7.9* | 3 | 13 | 27 | 1.5 |
| bnl3592 | 12 | 14 | 8 | 2.0 | 6 | 13 | 24 | 0.9 |
| bnl1408a | 14 | 15 | 11 | 3.0 | 5 | 12 | 22 | 0.8 |
| bnl1408b | 5 | 23 | 11 | 3.1 | 5 | 8 | 26 | 0.4 |
| bnl2895 | 1 | 21 | 8 | 8.1* | 1 | 10 | 24 | 3.0 |
| bnl2805 | 16 | 13 | 9 | 6.4* | 7 | 11 | 25 | 0.6 |
| bnl2632a | 14 | 19 | 7 | 2.6 | 6 | 11 | 24 | 0.3 |
| bnl2632b | 12 | 19 | 9 | 0.6 | 3 | 18 | 20 | 8.0* |
| bnl1681 | 10 | 18 | 6 | 1.1 | 2 | 10 | 32 | 3.1 |
| bnl3411 | 13 | 23 | 5 | 3.7 | 2 | 8 | 31 | 3.5 |
| bnl1151a | 12 | 20 | 8 | 0.8 | 2 | 10 | 30 | 2.6 |
| bnl1151b | 13 | 22 | 5 | 3.6 | 3 | 17 | 20 | 6.7* |
| bnl1034a | 12 | 18 | 6 | 2.0 | 4 | 14 | 25 | 1.5 |
| bnl1034b | 6 | 20 | 10 | 1.3 | 3 | 21 | 19 | 13.1* |
| bnl2960 | 6 | 15 | 14 | 4.4 | 0 | 12 | 31 | 6.2 |
| expected | 1 | 2 | 1 | | 1 | 2 | 5 | |

Statistical analyses were completed for F1, F2, BC1F1, BC1F2, BC1R1[a] and BC1R2 populations tested in 4-rep RCBD experiments in College Station, Texas during 2003, 2004, and in Mexico during the winter-spring of 2005-2006, for *G. mustelinum* introgression populations. In addition to field and fiber data collection, these generations were tested with microsatellite markers from one chromosome to measure effects of selection and recombination. Distored segregation was obseved in each generation, though not necessarily for the same loci, e.g., see Table, with illustrative data from two populations. To better understand the genetic mechanisms underlying

[a] R = random-mating. Denoted as "rm" in some figures.

breeding responses during introgression, we will attempt to extend this analysis to additional




Boxplots of individual plant heterozygosity by population and scatterplot of allele frequency by *G. mustelinum* population. Red circles mark *G. mustelinum* allele frequency.

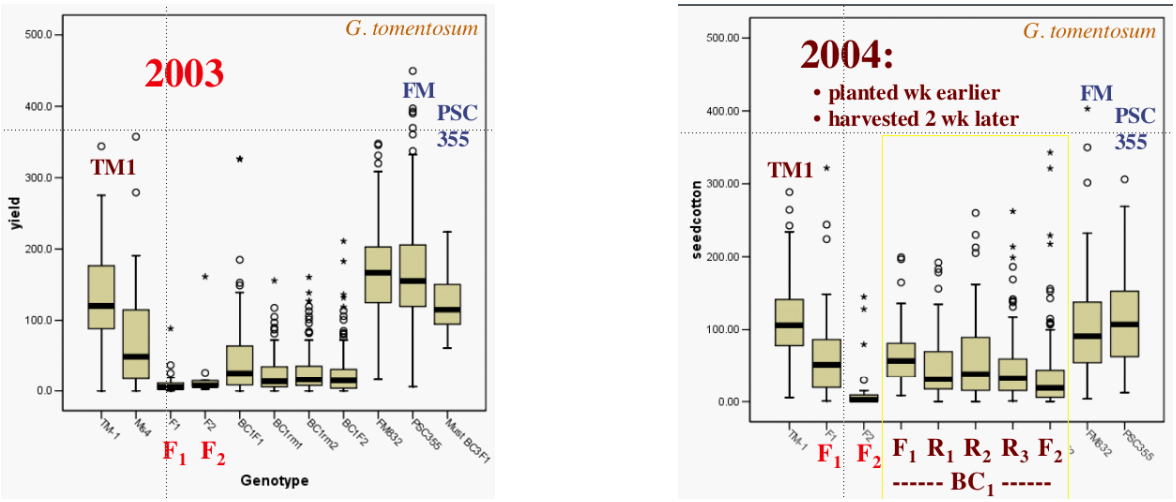
chromosomes and generations.

For *G. mustelinum*, backcross generations improved fiber quality, and introgression barriers were found to include strong day-length sensitivity and, when in short-day conditions, "hybrid breakdown". After just one backcross, means and variances of most traits differed little between random-mating and conventional backcross-inbred populations. Gametophytic selection could be exerting a funnel-like effect on certain potential genotypes available among BC1R1 plants, effectively limiting their variability to similar constaints upon selfing, i.e., among BC1S1, i.e., BC1F2 plants. Microsatellite markers showed decreased frequency of *G. mustelinum* alleles and decreasing heterozygosity, but no increase in map distances in

| | F2 | | | | BC1F2 | | | |
|---|---|---|---|---|---|---|---|---|
| | *mm* | *Mh* | *hh* | | *mm* | *mh* | *hh* | |
| bnl1066 | 12 | 23 | 6 | 2.4 | 2 | 15 | 24 | 4.2 |
| bnl836 | 4 | 14 | 9 | 1.9 | 4 | 12 | 28 | 0.5 |
| bnl36491 | 8 | 14 | 13 | 2.8 | 6 | 9 | 28 | 0.4 |
| bnl3649b | 2 | 24 | 8 | 7.9* | 3 | 13 | 27 | 1.5 |
| bnl3592 | 12 | 14 | 8 | 2.0 | 6 | 13 | 24 | 0.9 |
| bnl1408a | 14 | 15 | 11 | 3.0 | 5 | 12 | 22 | 0.8 |
| bnl1408b | 5 | 23 | 11 | 3.1 | 5 | 8 | 26 | 0.4 |
| bnl2895 | 1 | 21 | 8 | 8.1* | 1 | 10 | 24 | 3.0 |
| bnl2805 | 16 | 13 | 9 | 6.4* | 7 | 11 | 25 | 0.6 |
| bnl2632a | 14 | 19 | 7 | 2.6 | 6 | 11 | 24 | 0.3 |
| bnl2632b | 12 | 19 | 9 | 0.6 | 3 | 18 | 20 | 8.0* |
| bnl1681 | 10 | 18 | 6 | 1.1 | 2 | 10 | 32 | 3.1 |
| bnl3411 | 13 | 23 | 5 | 3.7 | 2 | 8 | 31 | 3.5 |
| bnl1151a | 12 | 20 | 8 | 0.8 | 2 | 10 | 30 | 2.6 |
| bnl1151b | 13 | 22 | 5 | 3.6 | 3 | 17 | 20 | 6.7* |
| bnl1034a | 12 | 18 | 6 | 2.0 | 4 | 14 | 25 | 1.5 |
| bnl1034b | 6 | 20 | 10 | 1.3 | 3 | 21 | 19 | 13.1* |
| bnl2960 | 6 | 15 | 14 | 4.4 | 0 | 12 | 31 | 6.2 |
| expected | 1 | 2 | 1 | | 1 | 2 | 5 | |

random-mating populations. Upper-half mean length (UHML) and upper quartile length by weight (UQLw) were highly heritable, as measured with parent-offspring regression. Most other agronomic traits had moderate heritabilities. The composite generations were found to be favorable for selection and breeding.

For *G. tomentosum* populations, day-length sensitivity was not a problem, but hybrid breakdown was led to especially low yields for F2 and BC1F2 generations. At the first backcross (BC1F1) level, there was little or no increase in variances for random-mating




**Yield of various backcross-inbred and -random-mated *G. tomentosum* introgression populations in 2003 and 2004. Note that yields of most F2 plants were common across years, reflecting genetic sterility. In contrast, yields of F1 and other non-F2 generations were better in 2004 than 2003, particularly relative to the checks. Thus, their low yields in 2003 were due in part to GxE effects.**

populations over conventional populations. *G. tomentosum* populations did not show improvements in fiber length as seen in *G. mustelinum* populations, but did have increased strength in BC1F1 and F1 generations over the recurrent parent. Mapping distances increased in the random-mating populations for *G. tomentosum*, and the average frequency of alien alleles did not decrease in random-mating populations. Generation means approached recurrent parental values for most traits within three backcrosses. Composite generations were found to be the most useful for breeding and selection.

For both species, additional fiber data from 2005 statistical analyses of field plant and fiber data combined from 2004 & 2005 are forthcoming, allowing extension of these analyses to more advanced generations: BC2F1, BC2rm1, BC2F2, BC3F1, BC3rm1, and BC3F2 and composite generations.

## [2] Advanced doubly complex populations for interspecific germplasm introgression into Upland cotton

In 2006, we grew the most advanced generations of the doubly complex populations for *G. tomentosum* and *G. mustelinum*. These populations were originally created in 2004 by separately intermating our *Ms*$_4$-containing BCR complex populations for *G. tomentosum* and *G. mustelinum* with the complex population elite Upland populations (BCR-UP11), developed by Daryl Bowman and Johnie Jenkins (another CI-sponsored project). In 2006,

individual plants in both populations were visually scored for pollen fertility, based on anther size and pollen shed, and also for plant habit and boll set, proximity to main stem, size and storm-proofness. Some difficulty was noted in classification of male sterility, as there seemed to be a gradation of male infertility among the plants, with both genotype and environment exerting influence. Progeny row-testing may be needed to verify *Ms4* genotypes. Development of a marker for MAS of *Ms4* plants might be helpful, too.

Progeny-row tests of single-plant selections.

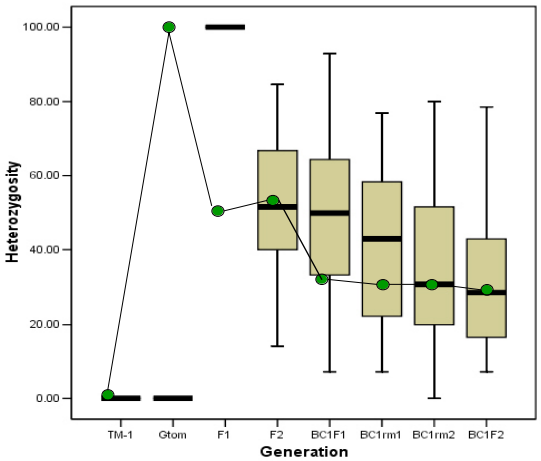
Progeny tests of single-plant selections from 2005, most from the doubly complex populations, suggest that the new germplasm has considerable merit in yield and other traits. This is especially true for the doubly complex germplasm populations. Additional fiber quality data analysis from 2005 and 2006 progeny rows (single-plant selections) are forthcoming.

**[3] Advanced the development of disomic chromosome substitution (DCS) lines**

DCS lines feature lossless gene introgression on a chromosome-by-chromosome basis. We advanced backcross generations in both *G. tomentosum* and *G. mustelinum* chromosome substitution materials. Most lines (one per available chromosome or arm) were moved into the BC3 and BC2 plant generations, so are producing BC4 and BC3 seed. Chromosome-substitution plant and DNA materials are being shared with other groups, e.g., the USDA-ARS group at Mississippi State, Shafter, College Station, and University of Arkansas.

**[4] Molecular analyses of retention and recombination**

Genotypic data were generated for multiple chromosome-11 marker loci during the early generations (through BC1), to study locus retention and recombination. Localized distortions of marker retention were apparent, and could be considered congruent with the expectation of intense selection due to digenic epistatic effects at specific loci. If effects are relegated to a minority of loci and others nearby that affected by linkage drag, the strong local effects could be muted by over-reliance on statistics averaged across all loci. We anticipate that more marker data from these early generation populations and the more advanced generations (BC2




Heterozygosity and allele frequency from *G. tomentosum* F2, BC1F1, BC1rm1, BC1rm2, BC1F2 populations. Boxplots illustrate heterozygosity of each population with center line marking median heterozygosity, box edges the first and third quartile. Green circles represent *G. tomentosum* allele frequency for each population.

and BC3) will shed light on causes that underly the complex field performance behavior of these wide-cross germplasm materials.

## ***G. longicalyx & G. armourianum***

**[1] Examined self-progeny from advanced backcross plants selected as homozygous and heterozygous resistant by MAS, to screen for deleterious traits.**

By random chance or, more often, due to linkage drag and impaired recombination, wide-cross introgression products that carry a desireable trait can be afflicted with one or more undesirable traits. In 2006, we examined self-progeny from reniform-resistant backcross plants that had been genotyped by molecular marker analysis, so we knew their genotypes at the resistance locus with a high degree of accuracy. Among the field-grown progeny, we screened visually for the potentially deleterious recessive traits. Selected rows were harvested for fiber testing. Based on the very positive results, we are moving forward as fast as possible with plans for public release of elite germplasm highly resistant to the reniform nematode, initially with just 1-2 lines, and subsequently with many more that trace to different early generation lineages.

**[2] Fiber samples were collected from plants in the genome-wide introgression project.**

Samples from 2005 were submitted for fiber analysis and seedcotton samples from 2006 were harvested from individual plants and are still being ginned, for subsequent submission for HVI analysis.

**[3] Young leaf tissue and DNA samples were prepared from critical plants in the nematode resistance program as well as the genome-wide introgression program.**

**[4] Doubly complex trispecies populations were advanced and evaluated.**

In previous years, we intermated BC1 and BC3 generations involving these two wild species with the complex population composed from 11 elite Upland populations (BCR-UP11), developed by Daryl Bowman and Johnie Jenkins (another CI-sponsored project). We are integrating the *Ms4* gene to facilitate large-scale population breeding management for cycling and line extraction. In 2006, we grew these two complex populations in nearby plots for evaluationa and comparison. We classified and tagged most of the plants for male fertility on 2-3 occasions and also collected young leaf tissue for DNA extraction from the more advanced one, and self- and topcross seed were produced from most of these same plants. These materials will likely be among the target for marker retention analysis and possibly also marker-trait association, using progeny performance evaluations.

**[5] Molecular markers suitable for MAS of the reniform resistance were identified.**

Concomitant phenotyping for reniform resistance, progeny tests for resistance, and genotyping of molecular marker loci were used to establish a body of data suitable for linkage mapping of molecular markers relative to the resistance-conferring locus or haplotype. At this time, a good codominant marker has been localized to approximately 1-2 cM from the resistance gene or haplotype, and

Root-feeding reniform female. (by KH Wang)

perhaps less. We do not know the physical distance from the resistance locus, but we have localized the gene to chromosome-11 and determined that the locus is recombinationally separate from another locus on chromosome-11 that contributes resistance to root-knot nematodes. We are now poised to deliver to the US producer, elite germplasm with extremely high resistance to the reniform nematode, as well as markers that will enable cost-effective MAS breeding with the trait. Growers could save $100M or more annually once resistant varieties are available.

## 2006 PUBLICATIONS, MEETINGS ATTENDED & PRESENTATIONS :

### Refereed articles

Frelichowski JE Jr, Palmer MB, Main D, Tomkins JP, Cantrell RG, Stelly DM, Yu J, Kohel RJ, Ulloa M. 2006. Cotton genome mapping with new microsatellites from Acala 'Maxxa' BAC-ends. Mol Genet Genomics. 275:479-491.

Gao, W., Z. J. Chen, J. Z. Yu, R. J. Kohel, J. E. Womack, D. M. Stelly. 2006. Wide-cross whole-genome radiation hybrid mapping of the cotton (*Gossypium barbadense* L.) genome, Molecular Genetics and Genomics 275:105-113. DOI: 10.1007/s00438-005-0069-5
http://www.springerlink.com/(unugb445s35my055z4ca1v55)/app/home/contribution.asp?referrer=parent&backto=issue,1,11;journal,5,834;linkingpublicationresults,1:107567,1

Jenkins, J.N., J. Wu, J. C. McCarty, S. Saha, O. Gutiérrez, R. Hayes and D.M. Stelly. 2006. Genetic effects of thirteen *Gossypium barbadense* L. chromosome substitution lines in topcrosses with Upland cotton cultivars: I. Yield and yield components. Crop Sci 46:1169-1178. DOI: 10.2135/cropsci2005.08-0269
http://crop.scijournals.org/cgi/content/abstract/46/3/1169

Lee, J. J.,O. S. S. Hassan, W. Gao, N. E. Wei, R. J. Kohel, X-Y Chen, P. Payton, S-H Sze, D. M. Stelly, Z. J. Chen. 2006. Developmental and gene expression analyses of a cotton naked seed mutant, Planta 223:418-432. DOI: 10.1007/s00425-005-0098-7.

Saha, S., J. N. Jenkins, J. Wu, J. C McCarty, O. A. Gutierrez, R. G. Percy, R.G. Cantrell, and D. M. Stelly. 2006. Effects of chromosome specific introgression in Upland cotton on fiber and agronomic traits. Genetics 172:1927-1938.
http://www.genetics.org/cgi/content/abstract/172/3/1927?etoc

Wu, J., J. N. Jenkins, J. C. McCarty, S. Saha, D. M. Stelly. 2006. An additive-dominance model to determine chromosomal effects in chromosome substitution lines and other germplasms, TAG Theoretical and Applied Genetics 112(3):391-399.
DOI: 10.1007/s00122-005-0042-z
http://www.springerlink.com/(5y5ivb551wyq2l45yvtcw145)/app/home/contribution.asp?referrer=parent&backto=issue,1,21;journal,7,794;linkingpublicationresults,1:100386,1

**Beltwide**:

Gardunia, B. W., D. Stelly, C. Wayne Smith and O. A. Gutierrez, 2006. Usefulness criteria for selection of generations for interspecific introgression from G. tomentosum and G. mustelinum. Proc. Beltwide Cotton Proc. Res. Conf., 2006 San Antonio Jan. 3-6.

Gutierrez,O. A., J. Wu, J. N. Jenkins, J. C. McCarty, D. A. Raska and D. M. Stelly. 2006. An intraspecific SSR linkage map of cotton. Proc. Beltwide Cotton Proc. Res. Conf., 2006 San Antonio Jan. 3-6.

Robinson, A. F., A. A. Bell, D. Stelly, N. Dighe and M. Menz. 2006. Progress report on introgression of reniform nematode resistance from G. longicalyx into Upland cotton. Proc. Beltwide Cotton Proc. Res. Conf., 2006 San Antonio Jan. 3-6.

Yang, S. S., C. Foo, N. E. Wei, J. J. Lee, S-H. Sze, D. M. Stelly, P. Thaxton, B. Triplett, C. D. Town and Z. J. Chen. 2006. Analysis of cotton ovule ESTs reveals enrichment of transcription factors and hormonal regulators implicated in fiber cell development. Proc. Beltwide Cotton Proc. Res. Conf., 2006 San Antonio Jan. 3-6.

Gardunia, B. W., C. A. Braden, C. Wayne Smith and D. Stelly. 2006. Quantile-quantile comparison as a method for testing AFIS distributions. Proc. Beltwide Cotton Proc. Res. Conf., 2006 San Antonio Jan. 3-6.

**Plant & Animal Genome Conference**:

Yang, S. S., C. Foo, N. E. Wei, J. J. Lee, S-H. Sze, D. M. Stelly, P. Thaxton, B. Triplett, C. D. Town, Z. J. Chen. 2006. Analysis of cotton ovule ESTs reveals enrichment of transcription factors and hormonal regulators implicated in fiber cell development. Plant & Animal Genomes XIV Conference, January 14-18, 2006. San Diego, CA. http://www.intl-pag.org/14/abstracts/PAG14_W331.html

Ulloa, M., J. E. Frelichowski, M. Palmer, Y-H. Park, M. S. Alabady, T. A. Wilkins, J. Yu, R. Kohel, R. Cantrell, D. Main, J. P. Tomkins, D. Stelly, A. Van Deynze. 2006. Development of PCR-based markers from fiber ESTs and BAC-end sequences for the construction of a consensus cotton genetic map.Plant & Animal Genomes XIV Conference, January 14-18, 2006. San Diego, CA. http://www.intl-pag.org/14/abstracts/PAG14_W156.html

Gutierrez, O. A., S. Saha, J. N. Jenkins, J. C. McCarty Jr., D. A. Raska, D. M. Stelly. 2006. Linkage group identification and SSR localization in upland cotton (*Gossypium hirsutum* L.) by deficiency testing. Plant & Animal Genomes XIV Conference, January 14-18, 2006. San Diego, CA. http://www.intl-pag.org/14/abstracts/PAG14_W157.html

Yang, S. S., C. Foo, N. E. Wei, J. J. Lee, S-H. Sze, D. M. Stelly, P. Thaxton, B. Triplett, C. D. Town, Z. J. Chen. 2006. Analysis of cotton ovule ESTs reveals enrichment of transcription factors and hormonal regulators implicated in fiber cell development. Plant

& Animal Genomes XIV Conference, January 14-18, 2006. San Diego, CA. http://www.intl-pag.org/14/abstracts/PAG14_W159.html

**Other meetings:**

Dighe, N., F. Robinson, A. Bell, M. Menz, and D. Stelly. 2006. Reniform nematode resistance-marker development and cytogenetics of the *Gossypium longicalyx* introgression products. Crop Sci. Soc. Amer., Nov. 14 2006 Indianapolis, (Abstr.) 246-7.

Robinson, A. F., A. A. Bell, N. Dighe, D. Stelly, and M. Menz. 2006. Transfer of reniform nematode resistance from *Gossypium longicalyx* L. (F genome) to *Gossypium hirsutum* L. (AD genome). Phytopathology 96:S11. http://www.apsnet.org/meetings/2006/abstracts/a06ma68.htm

**TSSC Annual Meeting:** *Interspecific Introgression to Expand Cotton Germplasm. (presented by CW Smith)*