Tri-Species Shuffling of Chromosomes to Study the Effects on Fiber Traits Using Chromosome Substitution Lines

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ABSTRACT

Previous studies revealed difficulties in retention and recombination of alien species germplasm by conventional introgression in upland cotton (*Gossypium hirsutum* L.) due to incompatibility at the whole-genome level. An alternative approach is to use alien species chromosome substitution (CS) lines, whereby retention and recombination can be differentially increased for a specific chromosome or chromosome segment. Here we report for the first time on the development of a set of CS lines from two alien species in a common genetic background of upland cotton. The overall objective of this research is to report on the chromosomal association of important fiber traits using a partial-diallel mating design among CS lines of *G. tomentosum* Nutt. Ex Seem (CS-T), a wild tetraploid species endemic to Hawaii, *G. barbadense* L. (CS-B), a cultivated tetraploid species with improved fiber quality traits, and 'TM-1' (*G. hirsutum*), the recurrent parent with improved agronomic traits and moderate fiber quality traits. The genetic effects associated with CS lines were dissected into additive, homozygous dominance, and heterozygous dominance genetic effects using an additive–dominance statistical model. Five of six CS-B lines and two of six CS-T lines had significant additive genetic effects on lint percentage, indicating that the respective substituted alien chromosome carried alien alleles with potential to improve lint percentage in upland cotton. Fifty-six percent of 16 different significant additive effects associated with the CS-B lines could be useful to improve fiber traits in TM-1. On the contrary, 40% of 15 significant additive genetic effects of the CS-T lines had potential to improve fiber traits. Results suggested that CS lines can unveil many beneficial alleles harbored cryptically in the other AD-genome species and render them more accessible for research and cotton genetic improvement.

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Abbreviations: CS, chromosome substitution; CS-B, chromosome substitution line from *G. barbadense*; CS-T, chromosome substitution line from *G. tomentosum*; GCA, general combining ability; mic, fiber micronaire; QTL, quantitative trait locus; UHM, upper-half mean.

THE primary challenges to genetic improvement of upland cotton (*Gossypium hirsutum* L.) include: (i) low genetic diversity among the elite cultivars, (ii) insufficient information about genes controlling important fiber and yield traits, and (iii) suitable genetic material to study the genes, their interactions, and effects on important complex traits. The current germplasm base of the cotton breeding gene pool has narrowed due to (i) a monophyletic origin, starting with an polyploidization event about 1 to 2 million yr ago (Senchina et al., 2003; Wendel and Cronn, 2003; Udall and Wendel, 2006), (ii) a "genetic bottleneck" occurring during domestication from a common ancestor in cotton (Iqbal et al., 2001), and (iii) the reliance of crosses among closely related elite domesticated genotypes or reselection within existing cultivars for high yield and superior fiber quality (Van Esbroeck et al., 1999). Recent concerns about upland cotton yield stagnation, declining fiber quality, and increasing genetic vulnerability to

biotic and abiotic stresses have stimulated great interest to utilize other species as sources of genetic variation to enhance upland cotton germplasm (Zhang et al., 2011).

The primary genepool of cotton consists of five to seven partially diploidized tetraploid (AD genome, $2n = 52$) species. Two are cultivated, *G. hirsutum* [(AD)$_1$] and *G. barbadense* L. [(AD)$_2$], whereas *G. tomentosum* Nutt. Ex Seem (AD$_3$), *G. mustelinum* Watt (AD$_4$), and *G. darwinii* Watt (AD$_5$) are not. Recent molecular analyses indicate two additional AD tetraploid species (Grover et al., 2015). The 26-chromosome haploid genomes of these species have grossly similar genomic architecture. The primary genepool is typically the first choice for interspecific introgression to improve upland cotton because crosses among tetraploid species normally produce fertile hybrids. However, pedigree analysis of elite cotton cultivars revealed that exotic germplasm has rarely been assimilated anew into an elite cultivar, but once accomplished, such cultivars were used to breed most subsequently successful cultivars. The wild species are also reservoirs of many useful novel genes that remain unknown and underused in various cotton breeding programs because the pedigrees of the elite cultivars reveal imperceptible alien species heritage (Van Esbroeck et al., 1999). Cotton has been domesticated independently four times from four species, two polyploid and two diploid (Wendel and Cronn, 2003). Currently, almost 95% of all cotton produced around the world is accounted for by *G. hirsutum*, the allotetraploid species, because of its improved agronomic characteristics including high yield, whereas *G. barbadense*, the only other allotetraploid species, produces fiber of exceptionally good quality. *Gossypium tomentosum*, a wild species endemic to the Hawaiian archipelago (Fryxell, 1979), is known for its small, rounded silvery gray leaves with dense pubescence and its near absence of foliar and bracteole nectaries, which are present in almost all other members of the cotton genus except *G. gossypioides* Ulbrich (Stephens, 1963, 1964; Fryxell, 1979; Waghmare et al., 2005). *Gossypium tomentosum* has recently been classified as rare by the World Conservation Union and is protected under the Endangered Species Act (https://www.usbg.gov/plants/hawaiian-cotton). The nearly total absence of nectaries in the leaves, bracts, and extrafloral regions in *G. tomentosum* contributes to the reduction of certain insect populations, since little or no leaf or extrafloral nectar is present as a food source for adult insects (Lukefahr and Rhyne, 1960). It was observed that nectariless cottons reduce tarnished plant bug (*Lygus lineolaris* Palisot de Beauvois) numbers (50%), fleahoppers (*Pseudatomoscelis seriatus* Reuter, 50%), boll rot (20%), and bollworm [*Helicoverpa zea* (Boddie)] damage (20%), and that nectariless lines produced lint yield and fiber quality equal to their isogenic commercial parents (Meredith et al., 1973). *Gossypium tomentosum* also produces strong fiber (Meyer and Meredith, 1978)

and is known as the most heat-resistant species in *Gossypium* (Akhtar et al., 1996; Percival et al., 1999). Allozyme analysis revealed no definitive evidence of introgression from other species and no specific pattern of geographic differentiation in *G. tomentosum* from different islands of Hawaii (DeJoode and Wendel, 1992). Another study showed that the genetic diversity within *G. tomentosum* was very low, with only 6.3% of the 351 amplified fragment length polymorphism markers being polymorphic within this species (Hawkins et al., 2005). Several previous reports demonstrated that genotype-by-environment interactions significantly influence cotton lint yield, but such interaction effects were small relative to genotypic effects for fiber quality traits, which suggests the potential of genetic improvement of fiber quality traits in upland cotton using the diverse genetic resources from other tetraploid species (Campbell and Jones 2005; Meredith et al., 2012; Zeng et al., 2014).

Interspecific germplasm introgression can greatly expand opportunities for genetic improvement of upland cotton. However, such efforts are constrained by genetic incompatibilities between the species and our limited knowledge on transmission genetics at the interspecific level (Gardunia, 2006; Saha et al., 2006b, 2013a). *Gossypium barbadense* and the nondomesticated tetraploid species harbor genes of potential value for the improvement of upland cotton. Very little information is available on transmission genetics in interspecific crosses of upland cotton with other species including *G. tomentosum* (Reinisch et al., 1994; Saha et al., 2006b; Rong et al., 2007; Zhang et al., 2011). We have released a set of chromosome substitution (CS) lines using hypoaneuploid-based methods from *G. barbadense* (Stelly et al., 2005; Saha et al., 2011a). We also reported that CS lines from *G. barbadense* (CS–B lines) can be used as an alternative approach to complement conventional pedigreed or population-based interspecific introgression methods (Saha et al., 2006a, 2008, 2010, 2011a, 2011b, 2013b). Results showed that inducing recombination specific only to the targeted substituted alien chromosomes or chromosome segments thereby greatly reduced the presence of undesirable linkages. Here, we report on the use of CS lines from *G. tomentosum* (CS–T lines) and chromosomal effects on agronomic and fiber traits using a partial-diallel mating design among CS–T lines, CS–B lines, and 'TM-1', the recurrent parent, following the overall procedure of our previous studies (Saha et al., 2006a, 2013b). In each CS–B and CS–T line, a pair of chromosomes (or chromosome arms) of TM-1 was replaced by the respective homozygous chromosome (or chromosome arm) pair from *G. barbadense* or *G. tomentosum* (Stelly et al., 2005). Each CS line is near-isogenic to the recurrent parent TM-1 for 25 chromosome pairs; pairs of CS lines that involve different chromosome pairs are near-isogenic to each other for 24 chromosome pairs; pairs of CS lines that involve the same chromosome

pair are near-isogenic to each other for 25 chromosome pairs. The partial diallel provided an opportunity to analyze chromosomal effects on important fiber traits.

Specific anticipated products from this research are: (i) novel genetic materials by infusing beneficial alleles from two different alien species in a common genetic background of upland cotton, (ii) a tool in upland cotton breeding programs for targeted exploitation of useful genes from wild species, and (iii) discovery of unique cryptic alleles from a wild species and an unadapted germplasm for improvement of upland cotton.

MATERIALS AND METHODS

A partial-diallel mating design of six CS–B lines (CS–B01, CS–B04, CS–B07, CS–B08sh [short arm], CS–B15sh, and CS–B18), six CS–T lines (CS–T01, CS–T04, CS–T07, CS–T08sh, CS–T15sh, and CS–T18), and TM-1 was conducted in the summer of 2009 at Mississippi State, MS (Fig. 1). However, we could not use CS–B08sh parent line in the experiment because there were not enough seeds available for this study. The F_1 seeds were sent to Mexico in the fall of 2009 for production of F_2 seed. In 2010, F_2 hybrids and their parents, including TM-1, were grown in two locations at the Plant Science Farm (designated as Environments 1 and 2) at Mississippi State (33.4° N, 88.8° W). In 2011, F_3 hybrids (random bulk sampled from 2010 open bolls) and the parents, including TM-1, were grown in two different locations on the Plant Science Farm (33.4° N, 88.8° W; designated as Environments 3 and 4) and one location at USDA-ARS Florence, SC (34.1° N, 79.4° W; designated as Environment 5). Soil types at Mississippi State were a Leeper silty clay loam (fine, smectitic, nonacid, thermic Vertic Epiaquept; Environments 1 and 3) and a Marietta loam (fine-loamy, siliceous, active, fluvaquentic Eutrudepts; Environments 2 and 4). The soil type at Florence was a Norfolk loamy sand (fine-loamy, kaolinitic, thermic typic Kandiudults). The F_2 populations and their parents were evaluated in a randomized complete block design with four replications within each environment. Standard agronomic practices were followed during the growing season for all environments. Each entry was grown in single-row plots 12 m long with rows spaced 97 cm and plants spaced 10 cm apart (about 110 plants row^{-1}). A 25-boll sample per plot was hand-harvested from the first fruit positions near the middle nodes of plants to determine fiber properties. Samples were ginned on a 10-saw laboratory gin to determine lint percentage, and the lint samples were sent to StarLab (Knoxville, TN) for high-volume instrument fiber measurements. Micronaire (mic), upper-half mean length (UHM), and fiber strength were measured. After the boll samples were harvested, all plots were harvested with a commercial cotton picker modified for plot harvest, and lint weight per plot was calculated.
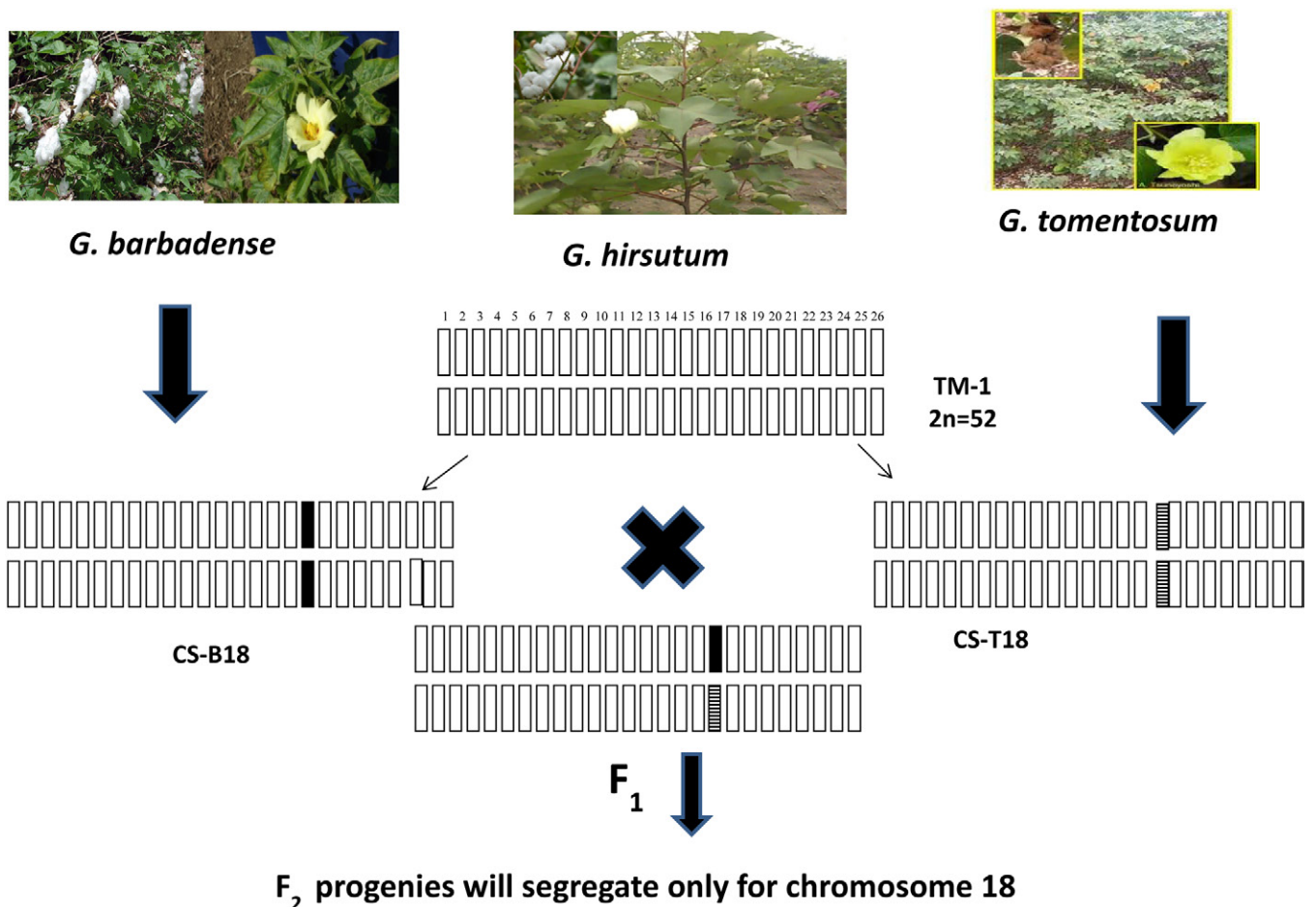

Fig. 1. A representative figure on the use of chromosome substitution lines to show the novel method of targeted incorporation of alien genes by chromosome shuffling creating novel genetic products from the infusion of tri-species germplasm for genetic analysis and germplasm improvement. Notice the differences in plant, leaf, flower, and fiber phenotypes among three different *Gossypium* tetraploid species.

The average value for each trait was calculated for individual CS lines and TM-1.

Genetic Analyses

The data were analyzed following the overall methods using an additive–dominance model of our previous studies (Wu et al., 2006; Saha et al., 2013b) with genotype-by-environment effects included. Values obtained include variance components, proportional variance components, and predicted environmental and genotypic effects. The predicted genetic effects were expressed as deviations from the grand population mean, μ (Zhu, 1994, 1998; Wu et al., 2006; Wu, 2012). Fixed effects (population means) were included at the end of each table, and the predicted genotypic values were calculated by the following equation: $\widehat{GV}_i = \hat{\mu} + 2\hat{A}_i$, where $\widehat{GV}_i$ is the predicted genotypic value for parent i, μ is the estimated population mean, and $\hat{A}_i$ is the predicted additive effect for parent i.

A t-test was used to determine significance of the genetic effects from zero. We provided 95% confidence intervals to compare and detect significant differences between two lines in the tables. A statistically significant difference between two lines indicated that it was unlikely that the alleles for the trait associated with the CS lines came from the same source. The data analyses were conducted by an R package GenMod using the additive–dominance model (Wu, 2012). The additive–dominance model can also handle any missing data in the analysis.

RESULTS

Assuming that all lines are isogenic except for the substituted chromosomes, differences among the lines for all traits were attributed to the substituted chromosomes. However, it should be noted that the identification of a quantitative trait locus (QTL) associated with the substituted chromosome or chromosome segment could also arise due to (i) environmental effects on genes and (ii) the retention of a small amount of residual genes in nonsubstituted chromosomes or chromosome arms from the donor parent during backcrossing and subsequent inbreeding in the development of CS lines. We reported most of our results in the tables as the deviation from the grand population mean. However, we presented some of the results in the text section below, calculating the actual value of fiber traits (given the estimation from the tables) to make readers aware of the actual genetic effects associated with the CS lines.

Average Values of Fiber Traits in Parental Genotypes

Lines CS-B01 and CS-T04 had significantly lower lint percentages than TM-1, whereas CS-T01 and CS-B04 had significantly higher average lint percentages. This could indicate that opposite selection pressure acted on the orthologous substituted chromosomes in these donor species, one domesticated and one wild. Whereas average lint percentage of TM-1 was 35.05%, averages of the CS lines ranged from 37.27% for CS-T01 to 32.51% for CS-T04, which indicates the presence of significant genetic

Table 1. Average parental line values indicating differences from respective overall population means, standard errors, and two-tailed 5% confidence intervals expressed as deviations from the grand population mean. Significance is based on comparison of CI values between the two lines.

Parent line	Lint percentage				Lint yield				Fiber strength				Micronaire				Upper-half mean length			
	Value	SE	2.5% CI	97.5% CI	Value	SE	2.5% CI	97.5% CI	Value	SE	2.5% CI	97.5% CI	Value	SE	2.5% CI	97.5% CI	Value	SE	2.5% CI	97.5% CI
	%				kg ha^{-1}				g tex^{-1}								mm			
CS-B01	-1.076*	0.070	-1.322	-0.830	-29.4	7.1	-54.6	-4.3	-0.704	0.126	-1.149	-0.259	-0.794*	0.020	-0.864	-0.725	0.485	0.050	0.310	0.661
CS-B04	0.679*	0.060	0.468	0.890	-59.8*	9.6	-93.8	-25.9	-3.410*	0.100	-3.763	-3.057	-0.218	0.016	-0.275	-0.161	1.002*	0.073	0.746	1.259
CS-B07	-1.499*	0.113	-1.900	-1.098	82.0	11.7	40.7	123.3	-1.038	0.112	-1.432	-0.644	-0.089*	0.016	-0.147	-0.032	1.261*	0.057	1.062	1.461
CS-B15sh	-0.710*	0.057	-0.912	-0.508	66.9	16.6	8.2	125.5	1.700*	0.121	1.273	2.127	-0.174	0.011	-0.214	-0.134	1.477*	0.046	1.315	1.639
CS-B18	1.073*	0.0491	0.900	1.246	-122.7*	16.9	-182.3	-63.1	0.777*	0.125	0.335	1.219	0.436*	0.039	0.300	0.573	-0.282*	0.050	-0.459	-0.104
CS-T01	2.283*	0.100	1.929	2.636	-128.1*	15.2	-181.7	-74.6	-0.176	0.093	-0.504	0.152	-0.081*	0.020	-0.150	-0.012	-1.510*	0.078	-1.784	-1.245
CS-T04	-2.474*	0.073	-2.732	-2.214	-24.8	20.8	-98.3	48.7	-0.008	0.113	-0.409	0.392	-0.213	0.023	-0.295	-0.130	1.145*	0.045	0.987	1.303
CS-T07	0.0730	0.067	-0.160	0.306	-86.7*	11.2	-126.4	-47.0	1.286*	0.084	0.991	1.581	0.340*	0.018	0.277	0.403	-2.037*	0.074	-2.300	-1.775
CS-T08sh	-0.007	0.072	-0.262	0.248	-37.3	9.9	-72.3	-2.3	0.237*	0.090	-0.081	0.554	0.229*	0.015	0.175	0.284	-0.979*	0.058	-1.185	-0.773
CS-T15sh	-0.279	0.081	-0.566	0.008	-56.1*	11.3	-96.0	-16.2	0.061*	0.102	-0.297	0.420	0.628*	0.020	0.559	0.697	-1.024*	0.077	-1.295	-0.752
CS-T18	-0.660*	0.078	-0.936	-0.383	-82.1*	13.8	-130.9	-33.2	-0.550	0.183	-1.197	0.097	-0.109	0.019	-0.171	-0.046	0.587	0.045	0.427	0.746
TM-1	0.067	0.062	-0.150	0.289	36.9	12.9	-8.5	82.3	-0.640	0.073	-0.896	-0.384	-0.224	0.020	-0.295	-0.152	0.413	0.065	0.185	0.642
Population mean	34.985	0.011	34.946	35.023	824.1	2.0	817.1	831.1	32.014	0.014	31.965	32.064	4.979	0.003	4.97	4.989	27.729	0.01	27.695	27.764

* Significant at the 0.05 probability level.

variation in the *G. tomentosum* genome for lint percentage (Table 1). Lint yields for 9 of the 11 CS lines, including all CS-T lines, were lower than TM-1 (861 kg ha⁻¹). Average fiber strength of only one CS line (CS-B04, 28.60 g tex⁻¹) was significantly lower than TM-1 (31.37 g tex⁻¹). Lines CS-B15sh and CS-T07 produced the highest average fiber strengths (33.71 and 32.01 g tex⁻¹) and significantly exceeded the average mean of TM-1 by 7%. The only CS line with a mic average significantly lower (~12%) than TM-1 (4.76 mic) was CS-B01 (4.20 mic). Most of the CS lines had mic averages higher than TM-1 or non-significantly different from it. The highest average mic value was observed for CS-T15sh (5.61 mic). Average fiber length (UHM) of three CS-B lines and one CS-T line was higher compared with TM-1 (28.14 mm), namely CS-B04, CS-B07, and CS-B 15sh, plus CS-T04. Line CS-B15sh had the highest UHM (29.21 mm). Several CS-B and CS-T lines had significantly shorter fiber, including CS-T07, which had the lowest UHM (25.69 mm). It is likely important that both CS-B04 and CS-T04, the substitutions of TM-1 chromosome 4 by counterparts from *G. barbadense* and *G. tomentosum*, resulted in significantly higher average UHM values.

Variance Components

An examination of the relative contribution to variation (Table 2) indicates that additive effects significantly affected all traits except lint yield and that effects ranged from 22 (fiber strength) to 40% (mic). The relative importance of simple additive vs. dominance effects varied widely among the traits. For example, additive effects highly exceeded dominance effects for mic (3:1) and moderately exceeded them for UHM (about 2:1). Additive and environment interaction effects expressed as proportions of phenotypic variance ranged from 6 (lint percentage) to 7% (mic). Dominance and environment interaction effects as proportions of phenotypic variance for all of the traits except lint yield were negligible and indicated that additive and dominance genetic effects were not very environmentally dependent for most of these traits. Residual effects ranging from 34 (lint percentage) to 48% (fiber strength) of the phenotypic variance measurements suggested that genotypic effects and genotype–by–environment interaction effects potentially influenced many of the fiber traits. Variance components

from five different locations (Table 3) predicted that most of the fiber traits were significantly different among the locations, indicating that the experiments were conducted under diverse environments.

Additive Genetic Effects

The predicted additive effects involving the partial random mating design of a fixed set of homozygous CS and TM-1 lines are comparable with general combining ability (GCA). An additive effect for a CS line is the effect of the alien species chromosome or chromosome segment and the remaining chromosomes of TM-1. However, the deviation of an additive effect of a CS line from the additive effect of TM-1 is considered the additive contribution due to the alien species chromosome or arm segment(s) in the TM-1 background based on our initial assumption that the CS lines are in uniform TM-1 genetic background except the substituted chromosome or arm segment(s) (Fig. 2, Table 4). The same principle is applicable for dominance effects associated with the CS lines (Jenkins et al., 2012). Results showed that all of the substituted chromosomes from *G. tomentosum* and *G. barbadense* harbored several beneficial alleles with additive genetic effects for one or more of the fiber quality traits important to genetic improvement of *G. hirsutum* (Fig. 2).

Five of six CS-B lines and two of six CS-T lines had significant additive genetic effects on lint percentage (Table 4). Substitutions of chromosome 4 of TM-1 from *G. barbadense* and *G. tomentosum* caused an increase (35.30%) and decrease (34.39%), respectively, relative to TM-1 (34.82%). The opposite pattern was observed for chromosome 1, for which substitutions from *G. tomentosum* and *G. barbadense* respectively caused a ~2% increase and a 3% decrease in lint percentage relative to TM-1. We did not detect any additive genetic effect for lint yield with any of the CS lines. Four of six CS-B and three of six CS-T lines exerted significantly higher additive genetic effects on fiber strength than TM-1 (31.76 g tex⁻¹), the two highest being CS-B08sh (33.25 g tex⁻¹) and CS-T07 (32.63 g tex⁻¹). Interestingly, additive effects leading to weaker fiber were significantly different from TM-1 only for CS-B04 (30.96 g tex⁻¹). Additive effects leading to decreased mic relative to TM-1 (4.89 mic) were significant for only one CS line, CS-B01, among all of the CS lines. In contrast, six CS lines exhibited additive

Table 2. Variance components expressed as proportions of phenotype variance for different fiber traits.

Variance	Lint percentage	Lint yield	UHM	Fiber strength	Micronaire
	%	kg ha⁻¹	mm	g tex⁻¹	
Additive	0.28*	0.015	0.32*	0.221*	0.401*
Dominance	0.318*	0.282*	0.186*	0.22*	0.13*
Additive by environment	0.063*	0	0.051	0.068*	0.071*
Dominance by environment	0	0.351*	0.03	0.008	0.006
Residual	0.339*	0.352*	0.413*	0.483*	0.393*
Phenotypic	2.008	67478.73	0.967	3.017	0.16

* Significant at the 0.05 probability level.

Table 3. Environmental effects deviated from population means for different fiber traits on the variance components expressed as proportions of phenotypic variance in five different environments.

Env.	Lint percentage Value	2.5% CI	97.5% CI	Lint yield Value	2.5% CI	97.5% CI	Upper-half mean length Value	2.5% CI	97.5% CI	Fiber strength Value	2.5% CI	97.5% CI	Micronaire Value	2.5% CI	97.5% CI
	%			kg ha^{-1}			mm			g tex^{-1}					
1	1.007**		1.136	−120.391**	−141.637	−99.145	−0.678**	−0.758	−0.598	−0.494**	−0.668	−0.319	−0.18**	−0.217	−0.144
2	1.208**	1.108	1.309	30.951**	3.64	58.261	−0.529**	−0.603	−0.455	0.012	−0.131	0.154	−0.016	−0.061	0.03
3	−1.153**	−1.265	−1.041	220.061**	200.583	239.54	−0.019	−0.099	0.06	−0.693**	−0.835	−0.551	0.068**	0.042	0.094
4	−2.497**	−2.617	−2.376	−1.905	−24.512	20.702	1.162**	1.088	1.235	0.641**	0.492	0.791	−0.362**	−0.406	−0.318
5	1.434**	1.347	1.521	−128.716**	−147.317	−110.114	0.065	−0.032	0.161	0.534**	0.416	0.653	0.49**	0.463	0.518

** Significant at the 0.01 probability level.

effects for significantly higher mic values than TM-1, the two highest being CS-B18 and CS-T15sh from the two donor species. Additive effects on fiber length (UHM) were significantly different than for TM-1 in 10 of the 12 CS lines, four with higher UHM and six with lower UHM. Substitutions from CS-B and CS-T for chromosomes 7 and 15sh had opposite additive genetic effects on UHM relative to TM-1, depending on the donor species; the effect was lower when substituted from *G. tomentosum* and higher when replaced with *G. barbadense*. However, substitution for chromosome arm 8sh from both *G. barbadense* and *G. tomentosum* reduced UHM, indicating that the short arm of chromosome 8 (8sh) from both the donor species harbored alleles causing significant additive genetic effects in a similar negative direction.

Dominance and Nonadditive Interaction Effects

Homozygous dominance effects of individual CS lines (Table 5) on lint percentage ranged over 33.56%. Line CS-T01 exerted the largest positive effect, followed by CS-B08sh, and both differed significantly from TM-1. Only CS-T04 exerted a negative effect on lint percentage significantly different from TM-1. None of the homozygous dominant effects on lint yield was significantly different from TM-1, but the effects for a number of the CS lines were significantly different from each other; for example, CS-T01 (~600 kg ha^{-1}) was significantly lower than effects for five of the six CS-B lines. Collectively, homozygous dominant effects on lint yield by the CS-T lines were more negative than those of CS-B lines. Homozygous dominance effects on fiber strength were significantly larger than TM-1 for two CS lines, CS-B08sh and especially CS-B18; they were significantly lower for only CS-B04. Analogous effects by the CS-T lines were not significantly different from TM-1 for fiber strength, but CS-T18 was significantly different from two other CS-T lines, namely CS-T01 and CS-T04. The effects of substitutions of chromosome 18 by CS-B18 and CS-T18 were positive and negative, respectively, relative to TM-1.
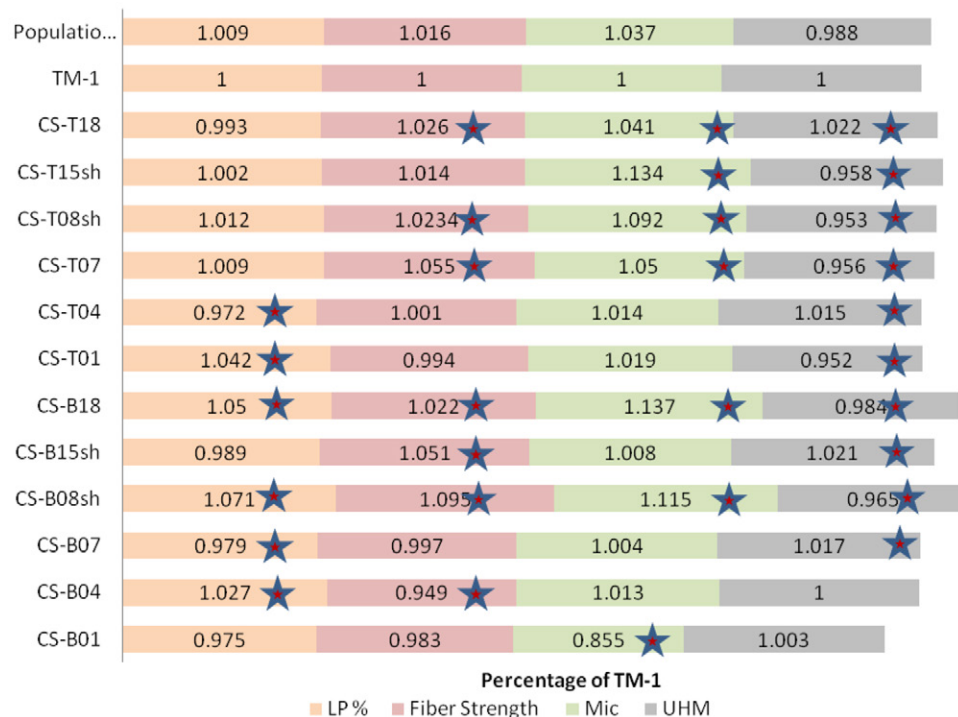

Fig. 2. A comprehensive view of additive genetic effects of chromosome substitution (CS) lines for four fiber traits expressed as percentage of the parental TM-1 line (lint percentage [LP] = 34.66, fiber strength = 31.50 g tex^{-1}, micronaire [mic] = 4.80, and upper-half mean length [UHM] = 28.06 mm). Stars indicate significant differences from TM-1 at the p ≥ 0.05 level. This figure summarizes some of the important CS lines from *G. barbadense* (CS-B) and *G. tomentosum* (CS-T) that are useful to improve specific fiber traits in upland cotton inbred lines.

Table 4. Predicted additive genetic effect of the parental lines expressed as deviations from population grand mean.

Parent line	Lint percentage				Lint yield				Fiber strength				Micronaire				Upper-half mean length			
	Value	SE	2.5% CI	97.5% CI	Value	SE	2.5% CI	97.5% CI	Value	SE	2.5% CI	97.5% CI	Value	SE	2.5% CI	97.5% CI	Value	SE	2.5% CI	97.5% CI
	%				kg ha⁻¹				g tex⁻¹								mm			
CS-B01	-0.604*	0.032	-0.719	-0.490	-4.7	3.8	-18.2	8.8	-0.518	0.039	-0.657	-0.379	-0.438*	0.010	-0.472	-0.404	0.205	0.019	0.137	0.274
CS-B04	0.312*	0.028	0.212	0.412	-15.2	9.6	-49.0	18.7	-1.057*	0.053	-1.245	-0.868	-0.057	0.009	-0.088	-0.025	0.181*	0.023	0.099	0.263
CS-B07	-0.526*	0.029	-0.628	-0.423	8.7	5.9	-12.0	29.4	-0.304	0.044	-0.458	-0.150	-0.079	0.010	-0.115	-0.042	0.400*	0.026	0.308	0.492
CS-B08sh	1.068*	0.058	0.862	1.275	-53.8	34.7	-176.2	68.6	1.238*	0.104	0.872	1.603	0.186*	0.022	0.107	0.265	-0.326*	0.056	-0.525	-0.126
CS-B15sh	-0.354	0.023	-0.435	-0.274	14.9	9.5	-18.6	48.4	0.547*	0.039	0.408	0.686	-0.069	0.009	-0.102	-0.036	0.456*	0.027	0.359	0.553
CS-B18	0.705*	0.029	0.604	0.807	-5.4	4.2	-20.2	9.4	0.084*	0.036	-0.043	0.211	0.241*	0.011	0.204	0.279	-0.060*	0.023	-0.143	0.022
CS-T01	0.562*	0.046	0.400	0.725	9.2	6.3	-13.1	31.5	-0.349	0.040	-0.489	-0.210	-0.044	0.011	-0.082	-0.006	-0.513*	0.033	-0.630	-0.397
CS-T04	-0.647*	0.038	-0.780	-0.513	6.9	5.4	-12.3	26.1	-0.234	0.043	-0.386	-0.082	-0.056	0.010	-0.092	-0.019	0.383*	0.026	0.291	0.475
CS-T07	-0.005	0.027	-0.101	0.092	-4.5	3.2	-15.9	7.0	0.613*	0.035	0.489	0.736	0.030*	0.010	-0.007	0.066	-0.451*	0.030	-0.557	-0.346
CS-T08sh	0.049	0.032	-0.063	0.162	12.3	8.1	-16.3	41.0	0.118*	0.040	-0.024	0.260	0.133*	0.011	0.093	0.173	-0.496*	0.026	-0.589	-0.402
CS-T15sh	-0.123	0.026	-0.216	-0.030	13.3	8.6	-17.0	43.6	-0.033	0.045	-0.192	0.126	0.232*	0.012	0.190	0.273	-0.422*	0.025	-0.508	-0.335
CS-T18	-0.277	0.034	-0.398	-0.156	-2.2	2.4	-10.8	6.4	0.151*	0.037	0.020	0.282	0.010*	0.011	-0.028	0.048	0.477*	0.025	0.389	0.564
TM-1	-0.163	0.038	-0.299	-0.027	20.5	12.8	-24.9	65.8	-0.256	0.038	-0.389	-0.122	-0.089	0.011	-0.129	-0.050	0.167	0.032	0.054	0.279
Population mean	34.985	0.011	34.946	35.023	824.1	2.0	817.1	831.2	32.014	0.014	31.965	32.064	4.979	0.003	4.970	4.789	27.729	0.010	27.695	27.764

* Significant at the 0.05 probability level.

Table 5. Predicted homozygous dominance genetic effect of the parental lines expressed as deviations from population grand mean.

Parent line	Lint percentage				Lint yield				Fiber strength				Micronaire				Upper-half mean length			
	Value	SE	2.5% CI	97.5% CI	Value	SE	2.5% CI	97.5% CI	Value	SE	2.5% CI	97.5% CI	Value	SE	2.5% CI	97.5% CI	Value	SE	2.5% CI	97.5% CI
	%				kg ha⁻¹				g tex⁻¹								mm			
CS-B01	-0.060	0.072	-0.314	0.194	-41.1	13.5	-88.7	6.5	0.173	0.100	-0.179	0.526	0.088	0.020	0.017	0.159	-0.183	0.050	-0.359	-0.007
CS-B04	-0.310	0.077	-0.582	-0.038	11.1	15.0	-41.7	64.0	-1.511*	0.153	-2.052	-0.971	-0.068	0.023	-0.148	0.011	0.337*	0.047	0.169	0.504
CS-B07	-0.681	0.112	-1.075	-0.287	5.8	18.2	-58.4	70.0	-0.664	0.101	-1.022	-0.307	0.075	0.019	0.007	0.143	0.162	0.051	-0.018	0.341
CS-B08sh	0.341*	0.029	0.237	0.444	-27.6	5.0	-45.2	-10.0	0.403*	0.043	0.252	0.554	0.012	0.007	-0.012	0.036	0.034	0.028	-0.064	0.131
CS-B15sh	-0.283	0.067	-0.519	-0.048	-47.6	13.5	-95.4	0.1	0.411	0.098	0.063	0.758	-0.003	0.021	-0.076	0.070	0.231	0.056	0.034	0.428
CS-B18	-0.577	0.079	-0.857	-0.297	-88.8	17.3	-149.7	-27.8	0.716*	0.118	0.298	1.134	0.035	0.036	-0.093	0.162	-0.238	0.062	-0.458	-0.017
CS-T01	0.767*	0.140	0.271	1.263	-223.2	28.9	-325.4	-121.0	0.258	0.124	-0.180	0.697	0.030	0.028	-0.069	0.128	-0.606	0.118	-1.022	-0.191
CS-T04	-1.429*	0.130	-1.889	-0.969	-93.0	34.3	-214.3	28.3	0.271	0.148	-0.252	0.794	-0.064	0.028	-0.164	0.036	0.059	0.070	-0.189	0.307
CS-T07	-0.201	0.108	-0.582	0.179	-93.0	17.5	-154.7	-31.3	-0.185	0.163	-0.759	0.389	0.267*	0.028	0.168	0.365	-1.219*	0.111	-1.610	-0.828
CS-T08sh	-0.494	0.133	-0.962	-0.025	-152.4	18.4	-217.3	-87.5	-0.186	0.162	-0.757	0.386	-0.009	0.024	-0.096	0.077	-0.118	0.096	-0.457	0.222
CS-T15sh	-0.409	0.118	-0.826	0.009	-161.5	21.0	-235.7	-87.2	-0.041	0.168	-0.635	0.554	0.179	0.030	0.074	0.285	-0.362	0.094	-0.695	-0.028
CS-T18	-0.453	0.122	-0.883	-0.024	-103.6	24.9	-191.4	-15.7	-1.170	0.216	-1.934	-0.405	-0.100	0.027	-0.197	-0.004	-0.629	0.074	-0.890	-0.368
TM-1	0.078	0.138	-0.409	0.566	-134.7	34.1	-255.2	-14.1	-0.289	0.147	-0.808	0.231	-0.004	0.032	-0.119	0.110	-0.219	0.103	-0.584	0.146
Population mean	34.985	0.011	34.946	35.023	824.1	2.0	817.1	831.2	32.014	0.014	31.965	32.064	4.979	0.003	4.970	4.989	27.729	0.010	27.695	27.764

* Significant at the 0.05 probability level.

Table 6. Heterozygous dominance effect of the hybrids from all the crosses in the partial diallel mating design of the CS lines expressed as deviations from population grand mean.

Crosses in partial diallel	Lint percentage				Lint yield				Fiber strength				Micronaire				Upper-half mean length			
	Value	SE	2.5% CI	97.5% CI	Value	SE	2.5% CI	97.5% CI	Value	SE	2.5% CI	97.5% CI	Value	SE	2.5% CI	97.5% CI	Value	SE	2.5% CI	97.5% CI
	%				kg ha^{-1}				g tex^{-1}								mm			
CS-B01 × CS-T01	0.630*	0.127	0.181	1.078	63.1	28.2	−36.5	162.7	−1.384*	0.158	−1.943	−0.824	−0.223*	0.031	−0.333	−0.113	0.182	0.072	−0.071	0.435
CS-T01 × CS-T04	−1.441*	0.191	−2.114	−0.768	−294.9*	35.7	−420.9	−168.9	−0.261	0.156	−0.811	0.289	0.177*	0.045	0.019	0.335	−0.191	0.118	−0.608	0.225
CS-T01 × CS-T07	0.014	0.129	−0.443	0.472	19.5	22.2	−58.9	98.0	−0.455	0.158	−1.012	0.102	−0.050	0.027	−0.147	0.046	0.503*	0.105	0.131	0.876
CS-T01 × CS-T08sh	1.224*	0.253	0.330	2.118	323.0*	26.9	228.1	417.8	0.489*	0.122	0.060	0.919	−0.012	0.036	−0.138	0.115	−0.291	0.120	−0.716	0.134
CS-T01 × CS-T15sh	−0.794*	0.130	−1.253	−0.334	284.5*	23.7	200.7	368.2	−0.900*	0.210	−1.644	−0.157	0.195*	0.041	0.050	0.340	−0.359	0.147	−0.877	0.160
CS-T01 × CS-T18	−0.787*	0.119	−1.206	−0.368	40.9	21.5	−35.1	116.9	1.367*	0.162	0.795	1.939	−0.078	0.036	−0.205	0.048	0.828*	0.100	0.474	1.182
CS-B04 × CS-T04	1.007*	0.159	0.444	1.571	−45.1	23.5	−128.2	38.0	2.050*	0.253	1.155	2.944	0.049	0.046	−0.115	0.213	−0.533*	0.089	−0.846	−0.220
CS-T04 × CS-T07	1.063*	0.134	0.590	1.535	114.5*	18.7	48.4	180.5	−0.375	0.211	−1.121	0.370	−0.127*	0.029	−0.230	−0.025	0.198	0.076	−0.071	0.467
CS-T04 × CS-T08sh	0.155	0.146	−0.360	0.670	97.5*	28.0	−1.5	196.4	−0.560	0.180	−1.195	0.075	−0.096	0.033	−0.214	0.022	0.521*	0.064	0.294	0.748
CS-T04 × CST15sh	−0.169	0.137	−0.654	0.316	74.4	26.0	−17.4	166.2	−1.258*	0.145	−1.770	−0.746	0.011	0.068	−0.228	0.250	−0.072	0.082	−0.361	0.217
CS-T04 × CST18	0.788*	0.211	0.042	1.535	143.1*	33.3	25.4	260.9	0.448	0.153	−0.093	0.988	0.034	0.044	−0.122	0.189	0.467*	0.074	0.204	0.729
CS-B07 × CST07	1.064*	0.146	0.548	1.580	−13.2	23.1	−94.8	68.4	0.547*	0.153	0.006	1.089	−0.193*	0.037	−0.323	−0.063	−0.003	0.090	−0.320	0.314
CS-T07 × CST08sh	−2.186*	0.094	−2.519	−1.853	−177.6*	29.8	−282.8	−72.4	−0.222	0.280	−1.211	0.767	0.156*	0.048	−0.013	0.326	0.045	0.104	−0.323	0.413
CS-T07 × CS-T15sh	0.488*	0.127	0.040	0.936	210.6*	23.0	129.3	291.9	1.980*	0.242	1.126	2.835	−0.373*	0.049	−0.546	−0.200	1.094*	0.124	0.657	1.532
CS-T07 × CS-T18	−0.327	0.136	−0.808	0.153	−23.9	17.4	−85.4	37.6	0.372	0.257	−0.537	1.280	−0.079	0.033	−0.196	0.039	0.000	0.102	−0.360	0.359
CS-B08sh × CS-T08sh	0.795*	0.053	0.608	0.983	−123.8*	12.3	−167.2	−80.4	0.697*	0.105	0.327	1.066	0.163*	0.018	0.101	0.225	−0.428*	0.034	−0.547	−0.309
CS-T08sh × CS-T15sh	1.474*	0.152	0.937	2.011	216.6*	26.0	124.8	308.3	0.035	0.227	−0.768	0.838	−0.077	0.039	−0.214	0.059	−0.079	0.117	−0.491	0.334
CS-B15sh × CS-T15sh	−0.031	0.113	−0.431	0.370	−38.7	26.0	−130.5	53.1	−1.095*	0.138	−1.584	−0.606	−0.173*	0.049	−0.345	−0.001	0.143	0.062	−0.076	0.363
CS-B18 × CS-T18	1.150*	0.116	0.740	1.559	89.0*	22.5	9.6	168.3	−0.590	0.206	−1.318	0.138	0.153	0.056	−0.046	0.352	−0.110	0.094	−0.443	0.224
CS-T15sh × CS-T18	−0.024	0.184	−0.675	0.627	−148.5*	27.8	−246.6	−50.3	0.661*	0.175	0.043	1.280	0.188*	0.034	0.067	0.310	0.210	0.074	−0.052	0.472
CS-B01 × TM-1	−1.345*	0.164	−1.925	−0.766	3.7	28.2	−96.1	103.4	0.407	0.132	−0.058	0.872	−0.394*	0.046	−0.557	−0.231	0.411*	0.095	0.077	0.745
CS-B04 × TM-1	0.044	0.114	−0.358	0.446	−28.9	31.0	−138.5	80.6	−0.309	0.166	−0.894	0.276	0.030	0.028	−0.069	0.129	0.060	0.070	−0.187	0.307
CS-B07 × TM-1	−0.429	0.158	−0.985	0.128	30.0	28.2	−69.7	129.7	0.412	0.162	−0.161	0.986	−0.036	0.039	−0.173	0.100	0.123	0.131	−0.338	0.584
CS-B15sh × TM-1	0.108	0.130	−0.352	0.567	184.1*	22.8	103.5	264.7	0.938*	0.194	0.254	1.623	0.110*	0.036	−0.019	0.238	−0.101	0.109	−0.486	0.284
CS-B18 × TM-1	0.979*	0.142	0.479	1.479	70.1	28.2	−29.6	169.9	−0.741*	0.206	−1.469	−0.012	0.020	0.035	−0.103	0.143	0.518*	0.115	0.114	0.923
CS-T01 × TM-1	0.396	0.248	−0.478	1.271	41.7	26.9	−53.1	136.6	0.201	0.163	−0.375	0.777	−0.112	0.035	−0.237	0.012	−0.029	0.131	−0.493	0.434
CS-T04 × TM-1	0.562*	0.182	−0.081	1.205	119.5*	37.9	−14.4	253.4	−0.868*	0.188	−1.531	−0.205	0.025	0.044	−0.129	0.179	−0.084	0.092	−0.408	0.241
CS-T07 × TM-1	0.281	0.119	−0.138	0.700	40.2	21.6	−36.1	116.4	−0.733*	0.214	−1.491	0.025	0.162*	0.033	0.046	0.279	0.100	0.093	−0.230	0.430
CS-T15sh × TM-1	−0.297	0.126	−0.740	0.147	−231.8*	38.4	−367.4	−96.1	0.617	0.205	−0.107	1.341	0.103	0.059	−0.106	0.313	−0.683*	0.134	−1.156	−0.209
CS-T18 × TM-1	−0.275	0.131	−0.738	0.187	98.3*	31.9	−14.4	211.0	0.265	0.196	−0.426	0.957	−0.007	0.043	−0.160	0.147	0.391*	0.085	0.090	0.691
CS-T08sh × TM-1	−0.406	0.167	−0.996	0.183	11.4	16.7	−47.7	70.5	0.077	0.182	−0.568	0.721	0.018	0.039	−0.119	0.155	−0.083	0.107	−0.462	0.296
Population mean	34.985	0.011	34.946	35.023	824.1	2	817.1	831.2	32.014	0.014	31.965	32.064	4.979	0.003	4.97	4.989	27.729	0.01	27.695	27.764

* Significant at the 0.05 probability level.

Heterozygous dominance effects (Table 6) for increased lint percentage were highest in the hybrid of CS-T08sh × CS-T15sh (36.46%). However, it is interesting to note that the CS-T08sh × TM-1 and CS-T015sh × TM-1 hybrids had heterozygous dominance effects for lint percentage of 34.58 and 34.69%, respectively. This suggests that the complementary interaction of the alleles from the two substituted chromosomes caused an increase in nonadditive manner heterozygous dominance effect for lint percentage in the hybrid of CS-T08sh × CS-T15sh. The CS-T01 × CS-T08sh hybrid had the highest heterozygous dominance effect on lint yield (1147 kg ha^{-1}). The chromosome-4 substitutions, CS-B04 × CS-T04, had an increase of 2.05 g tex^{-1} in fiber strength from the population mean (32.014 g tex^{-1}) that was attributable to heterozygous dominance effects. This is in sharp contrast to negative heterozygous dominance effects for fiber strength in the hybrids of CS-B04 × TM-1 and CS-T04 × TM-1, respectively. Nonadditive interaction effects of the heterozygous alleles caused an increase in fiber strength in the CS-B04 × CS-T04 hybrid (Fig. 3, Table 6). Similar nonadditive interaction effects were detected when we compared heterozygous dominance effects in fiber strength among the hybrids of CS-T07 × CS-T15sh, CS-T07 × TM-1, and CS-T15sh × TM-1 (Fig. 2, Table 5). The CS-B01 × TM-1 hybrid reduced mic (4.59 mic) more than all other hybrids. It is interesting to note that the CS-T01 × CS-T18 hybrid had maximum heterozygous dominance effects favoring long fiber (UHM 28.55 mm) (Table 6).

Homologous vs. Homeologous Chromosomes (Chromosomes 1 and 15sh)

Among all of the substituted chromosomes in our analysis, chromosomes 1 and 15 were a homeologous pair of chromosomes in the tetraploid (AD) cotton species. Our results revealed that CS-B01 and CS-B15sh both had a negative average effects of −1.08 and −0.71%, respectively, on lint percentage from the population mean (34.99%), indicating the possibility that similar negative selection pressures for this trait acted in evolution on the substituted paralogous alien chromosome or segment of *G. barbadense* (Table 1). However, CS-T01 had an increase in average effect on lint percentage of 2.28% from the population mean, suggesting that this could be a result of positive selection pressures that favor factor(s) to increase lint percentage for the substituted chromosome 1 in *G. tomentosum*. Relative to the population means, CS-B01 had a negative additive effect of −0.60% on lint percentage and −0.44% on mic, whereas CS-T01 had positive additive effects of 0.56% on lint percentage and 0.23% on mic (Fig. 4, Table 4). Results showed that CS-B15sh had an increase in additive genetic effects on fiber length (UHM 0.46 mm) from the population mean (UHM 27.73 mm). However, CS-T01 and CS-T15sh had negative additive genetic effects of −0.51 and −0.47 mm, respectively, in fiber length from the population mean (UHM 27.73 mm).
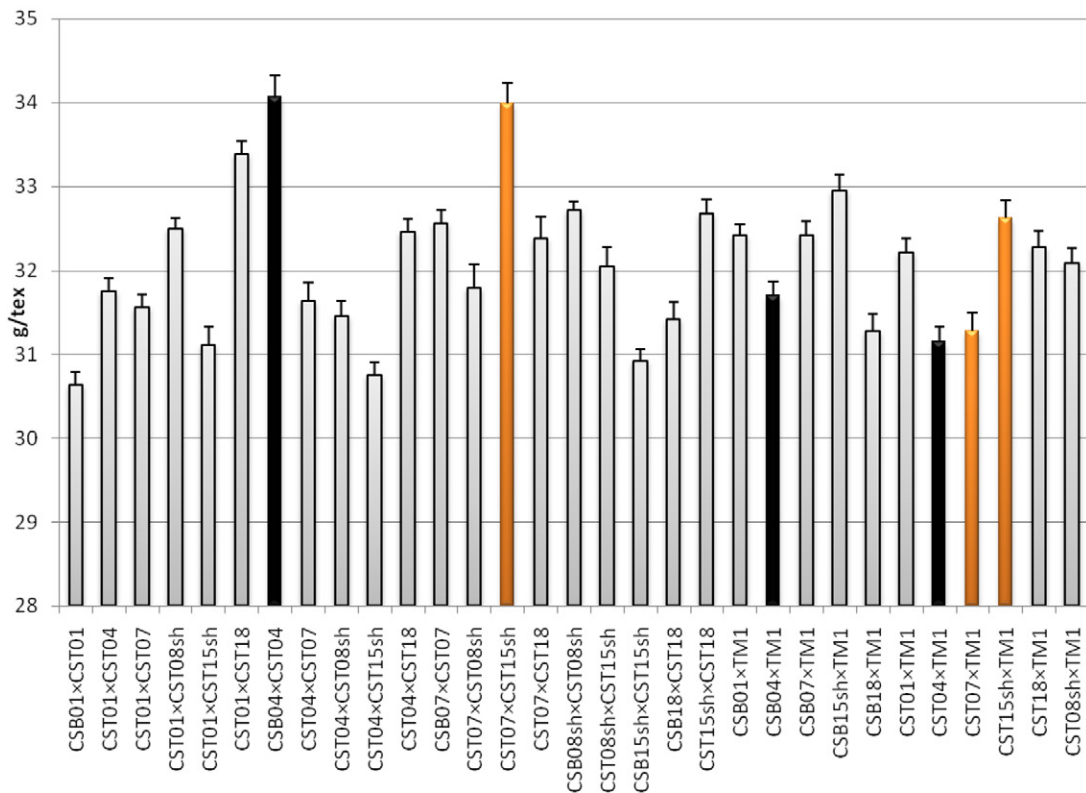

Fig. 3. Heterozygous dominance genetic effects on fiber strength in hybrid population of chromosome substitution (CS) lines. Note the nonlinear epistatic effects for fiber strength on the same heterozygous substituted chromosome pair and two different heterozygous substituted chromosome pairs in the black and orange color among in the hybrids, respectively.
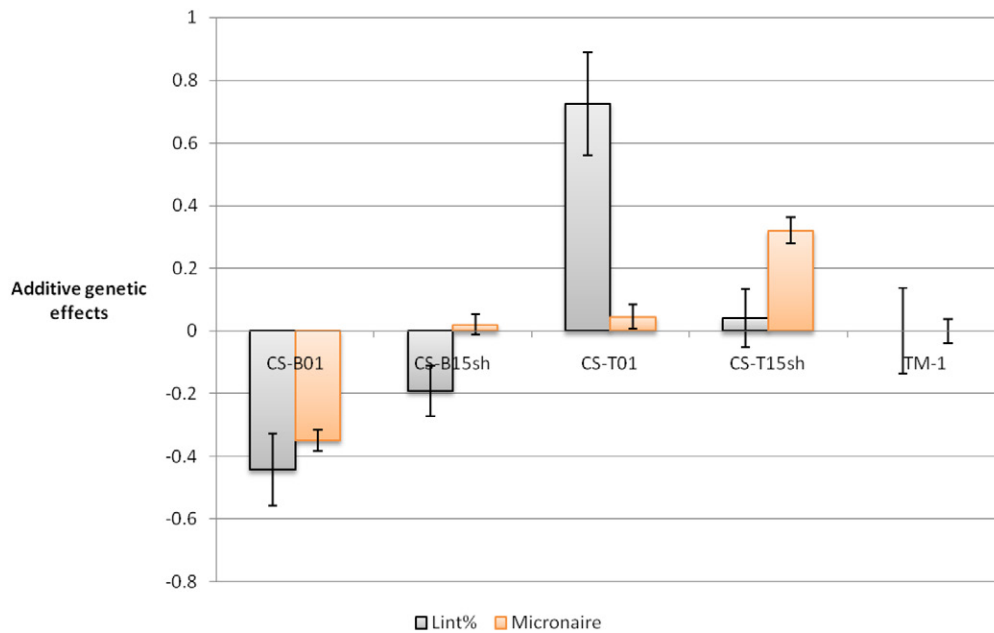
Fig. 4. Comparison of additive genetic effects between homologous versus homeologous chromosomes or segments (chromosome 1 vs, short arm of chromosome 15) of *G. barbadense* L. (CS-B) and *G. tomentosum* (CS-T) lines on two economically important fiber traits. The results presented in the graph as the deviation from TM-1 (lint = 34.82%, micronaire = 4.89) showing 2.5 to 97.5% confidence interval limit.

DISCUSSION

Although development of CS lines is a relatively difficult and time-consuming procedure, it is an exceptionally effective breeding method for germplasm introgression that assures recovery of alien germplasm after extensive backcrossing (Campbell et al., 2003, 2004; Wan et al., 2004; Stelly et al., 2005; Xu et al., 2010). In this report, we have analyzed CSs of *G. hirsutum* involving germplasm from two alien species providing opportunities for breeding use and genetic inference. *Gossypium hirsutum* and *G. barbadense* have been cultivated as tropical and subtropical annual crops since prehistoric times of human civilization. In contrast, *G. tomentosum* has grown as a wild tetraploid perennial shrub in Hawaii. The *G. barbadense* donor line (3–79) produces a considerably reduced amount of lint cotton and has lower lint percentage and mic, but longer and stronger fibers than the genetic background parental line, *G. hirsutum* (TM-1) (Saha et al., 2006a). The wild species *G. tomentosum* produces only very short orange–brown fibers that are unsuitable for spinning or twisting into thread (Saha et al., 2006b). Thus, intercrosses among these lines leads to many new genetic combinations and contributions to fiber traits that are important to cotton breeders. The overall method for genetic analysis and targeted incorporation of useful genes from three tetraploid species is depicted in Fig. 1. A partial-diallel mating design in concert with the appropriate additive–dominance model analysis provided an effective quantitative genetic platform for dissecting genetic effects into additive, homozygous dominance, and heterozygous dominance effects for each of the CS-B and CS-T lines (Wu et al., 2006).

Several studies highlighted the paramount importance of polyploidy with reference to the domestication and evolution of fiber traits in allopolyploid cotton species from the union of the two diverged diploid A and D genome species (Small et al., 1999; Wendel and Cronn, 2003; Adams and Wendel, 2004; Udall and Wendel, 2006; Grover et al., 2015). This genomewide gene duplication in the tetraploid cottons provided an opportunity in evolution to make changes influenced by strong selection that produced remarkable phenotypic divergence between the wild and domesticated forms (Jiang et al., 1998; Udall and Wendel, 2006; Rong et al., 2007). The accumulating evidence from cytogenetic and evolutionary data suggested that the A genome of the allopolyploid upland cotton genome $[(AD)_1]$ is more similar to the A genome of the ancestral diploid domesticated species than the D genome of the allopolyploid is to the D genome of the diploid wild predecessor species (Wendel and Cronn, 2003). Using preliminary evidence, it is tempting to speculate on the evolution of fiber traits in our study by comparing genetic effects of the substituted homologous versus homeologous chromosomes (chromosome 1 and the short arm of chromosome 15) of *G. barbadense* and *G. tomentosum*, respectively (Fig. 4). Our results revealed that CS-B01 and CS-B15sh both had negative average effects on lint percentage from the population mean, indicating that similar negative selection pressure acted in evolution on the substituted chromosomes on paralogous alien chromosomes or segments of *G. barbadense* (Table 1). However, CS-T01 had an increase in average effect on lint percentage from the population mean, indicating the possibility that a different positive selective force might

have caused an increased lint percentage on the orthologous chromosome 1 of *G. tomentosum*. Results showed that substitution of the short arm of chromosome 15 of *G. barbadense* and *G. tomentosum* had an increase effect on average of fiber strength, respectively, suggesting a similar positive selection pressure on the chromosome segment between the orthologous chromosomes for this trait in the two species. Substitution of *G. tomentosum* chromosomes one also produced a positive additive effect on lint percentage and negative additive effects on fiber length (UHM), whereas substitution of the *G. tomentosum* chromosome 15sh induced positive additive effects on mic and negative additive effects on fiber length (UHM) relative to TM-1, indicating different precedents in fiber trait evolution between *G. tomentosum* chromosomes 1 and 15sh.

Results from sequence and gene expression analysis revealed that most of the fiber development genes in the D_t subgenome originated from nonreciprocal DNA exchanges from the A_t subgenome of the tetraploid cottons (A_tD_t) and that most mutations are convergent, with A_t genes converted to the D_t state at more than twice the rate (25%) or the reciprocal (10.6%) (Paterson et al., 2012). Our preliminary results suggested the presence of considerable differences in fiber traits between the A_t (chromosome 1) and D_t (chromosome 15sh) subgenomes of the tetraploid cottons, possibly due to differences in selection pressures and evolutionary rates between the paralogous and orthologous chromosomes or segments of *G. barbadense*, a cultivated species, and *G. tomentosum*, a wild species (Fig. 4). It has been suggested that one possible outcome of gene duplication during allopolyploid species formation was relaxation of selection, allowing divergence between the duplicated genes with an overall acceleration in evolutionary rate in the D_t–genome, especially with fiber traits, relative to the A_t–genome in allopolyploid *Gossypium* (Wendel and Cronn, 2003; Adams and Wendel, 2004; Chee et al., 2005a, 2005b; Rong et al., 2007). A previous study also showed appreciable differences in fiber traits, including fiber length and elongation in the number of QTLs between the homeologous loci of the subgenomes in the tetraploid cotton (Chee et al., 2005a, 2005b). The current study also provided insight into the effects of the evolution and domestication of polyploid cotton. However, it is important to note that our study was based on very preliminary results from a comparative analysis between a complete chromosome with the paralogous segment of another chromosome (chromosome 1 vs. chromosome 15sh) between a wild and domesticated tetraploid species. Further research is needed using more direct evidence from molecular techniques at the whole-genome level to confirm the results on the evolution of homologous vs. homeologous chromosomes.

Cotton breeders are challenged to prevent genetic erosion of breeding materials when they rely most extensively on crosses among closely related elite genotypes that have and beget progenies with improved yield and fiber qualities; the continued reliance on elite germplasm leads to increased genetic uniformity and vulnerability to pests and diseases (Van Esbroeck and Bowman, 1998; Van Esbroeck et al., 1999). For example, between 1986 and 1996, farmers in the United States cultivated nearly 25% of the successful cotton lines (those that occupy 1% or more of the planted area) that were originated simply from reselections and 60% cultivars from two-way crosses (Kuraparthy and Bowman, 2013). Our results show that CS lines provided an opportunity to conserve all of the genes associated with the substituted chromosome or chromosome segment from the wild and unadapted germplasm by restricting recombination in its hypoaneuploid based development stages (Stelly et al., 2005), and they also unlocked the gateway for discovery and targeted introgression of beneficial genes including many cryptic alleles, for some of which the effects cannot be detected directly in the donor alien species. For example, substitution of chromosome 7 (CS-T07) from *G. tomentosum*, a wild species that never produces spinnable lint fibers, caused about a threefold increase in additive genetic effects for fiber strength compared with TM-1 (Table 4). This is important, considering that fiber strength is largely determined by a few major genes and is considered as one of the important quality traits influencing the strength, manufacturing process, and cost of yarn (Meredith, 1977; Bradow et al., 1997; May, 1999; Kumar et al., 2012).

Results also showed that all of the substituted chromosomes from *G. tomentosum* and *G. barbadense* harbor beneficial alleles with additive genetic effects for one or more the fiber quality traits important to genetic improvement of *G. hirsutum* (Fig. 2). To date, *G. tomentosum* has not been extensively used in cotton breeding. As with other noncultivated species, alleles that could be used to enhance crop performance for a quantitative multigenic trait are difficult to discover natively because they are often masked by negative effects also present in the wild genetic background (Tanksley and McCough, 1997). Additionally, they are difficult to recover or use from conventional introgression due to extensive loss of alien germplasm during backcrossing, selection, and genomic incompatibility at the whole-genome level. To be successfully introgressed, alien alleles must be free of all linked, agriculturally deleterious alleles before being deployed in a cultivar and must be free of genomic incompatibility effects. Chromosome substitution lines provide opportunities to overcome some of the genetic drag effects and genomic incompatibilities that can hamper conventional wide-cross breeding approaches.

Most *Gossypium* studies on interspecific transmission genetics are based on crosses between the cultivated species *G. hirsutum* and *G. barbadense*. These studies revealed

that alien species gene retention and genetic combinations are often very difficult due to incompatibility at the whole-genome level, and that they result in nonrandom undesirable products in conventional interspecific introgression methods (Rong et al., 2004; Zhang et al., 2011). It was suggested that the hybrid breakdown in some *Gossypium* interspecific populations was not necessarily due to structural differences at the chromosomal level, but to incompatible allelic interactions (Harland, 1939). However, very little information is available on transmission genetics in interspecific crosses of upland cotton with *G. tomentosum* (Waghmare et al., 2005; Zhang et al., 2011).

Gossypium tomentosum plants were reported to have produced a large number of flowers in a field experiments in Texas without setting any seeds (Gardunia, 2006). Findings suggested that this could be due to: (i) the special characters of the flowers with long style and stigma extended far from the anthers, making self-pollination difficult, or (ii) due to the lack of suitable nocturnal pollinators because *G. tomentosum*, whose flowers remain open well into the night, could be receptive to pollination more in night than day time (Fryxell, 1979). Another study also revealed some impediments in conventional methods of interspecific introgression from *G. tomentosum* to *G. hirsutum* (Zhang et al., 2011). For example, this study showed that only ~10% of the F_2 plants produced flowers, and heterozygotes occurred in BC_3F_2 generation at an average frequency of 28.9% loci, whereas *G. tomentosum* homozygotes occurred at 1.8% loci due to biological constraints. This suggested that *G. tomentosum* introgressions might only be useful in F_1 hybrid cottons, mitigating linkage drag associated with introgression by virtue of the presence of adapted alleles from one parent. Our results showed the presence of almost 100% flowering, with most of the CS-T lines producing 100% fertile F_2 hybrids because the genomic incompatibilities were restricted to only the substituted chromosome or segment, with the potential of transferring all genes from that substitute. This is due to introgressing alien chromosome or segments using hypoaneuploid-based methods in successive backcrosses during development of CS lines (Stelly et al., 2005). Molecular results also revealed almost 100% homozygosity for the alien chromosome or segment in most of the CS lines (Saha et al., 2015). This study suggested that CS lines can overcome some of the impediments of conventional breeding methods—by limiting the scope of introgression to ~5% of the donor genome per line and producing sets of isogenic lines, one gains facility, speed, and efficacy in downstream research and breeding.

Previous studies (Meredith, 1984; May, 1999) on the genetic basis of fiber properties concluded that, even though environmental effects (such as locations, years of testing) affect fiber length, strength, and fineness, the magnitude of genetic variation is generally greater than that of nongenetic factors. Their results revealed that, among the genetic factors, additive effects generally play a more important role rendering moderate to high heritability in many fiber traits. The additive genetic effect provided an estimation of GCA of the specific CS line (Wu et al., 2006; Jenkins et al., 2006, 2007). The additive genetic effects or GCA represent the average performance of CS parents in hybrid combinations with all other parents in the study (Ragsdale and Smith, 2007). Our results from variance component analysis revealed that additive and dominance genetic effects were not very environmentally dependent for most of these traits. Considering at least one QTL or additive genetic effect, a total of 31 significant additive effects were detected for five fiber traits in CS-B and CS-T lines. Sixteen different significant additive effects for fiber traits were associated with the CS-B lines, and 56% of these effects could be useful to improve fiber traits in TM-1, the recurrent parent. On the contrary, based on the similar assumption of at least one QTL or additive genetic effect, we observed that 15 QTLs with additive genetic effects were associated with the CS-T lines, of which 40% had genetic potential to improve fiber traits in TM-1. Three of the six CS-T lines and six CS-B lines had higher additive genetic effects with potential to improve fiber strength in TM-1, indicating that some of these alien alleles from different CS lines could be used in stacking multiple favorable alleles into a single line using specific breeding strategy (Fig. 1, Table 4) Fiber strength is one of the most important traits determining the yarn quality in textile industries because it helps to withstand mechanical impacts of the yarn-spinning process, so that it can be spun at a greater speed compared with weaker fibers (Meredith et al., 1991; Deussen, 1992; Kumar et al., 2012). Therefore, fibers with good strength and higher tenacity are preferable, because they can tolerate more powerful mechanical handling with less costly disruption in textile processing (Chee et al., 2005a, 2005b; Kumar et al., 2012) and generally produce more long-lasting fabrics that maintain cotton's natural qualities after chemical processing (Deussen, 1992; May, 1999).

Previous studies reported ~80 QTLs for fiber strength, the majority of which from the early generation of interspecific hybrid populations, with the favorable alleles originating primarily from *G. barbadense* (Paterson et al., 2003; Kumar et al., 2012). The number of QTLs that were detected in each study ranged from 1 (Zhang et al., 2003) to 21 (Paterson et al., 2003), explaining 2.4 to 53.8% of the total phenotypic variation (Kumar et al., 2012). Our results showed that, in addition to the substituted chromosomes or segments of *G. barbadense*, CS-T-07, CS-T08sh, and CS-T 18 from *G. tomentosum* carried cryptic beneficial alleles with potential to improve additive genetic effects for fiber strength (Fig. 1, Table 4). Previous studies reported a strong inverse relationship between lint yield

and fiber length, and possibly an even stronger inverse relationship between lint yield and fiber strength in interspecific introgressed lines (Culp and Harrell, 1974; Zeng et al., 2010). Our results revealed that several of the CS lines carried alien alleles with potential of additive genetic effects to improve fiber length and strength (Fig. 1 and 4; Table 4). Over the last 5 yr, fiber length of US cotton has increased only 4%, suggesting that CS lines may be a new genetic resource to overcome this problem (Kuraparthy and Bowman, 2013).

None of the lines except CS-B01 had lower additive genetic effects on mic than TM-1, suggesting that the substituted chromosome in CS-B01 carried alien alleles useful to improve mic in upland cotton. It has been reported that spinning larger numbers of finer fibers (lower mic) together produces stronger and more uniform yarns compared with spinning fewer and thicker fibers (Ramey, 1982). Normally any values of mic <3.5 or >4.9 are in the discount range in price for poor fiber quality in the US cotton market. It has been reported that US cotton has an upward trend (7%) in mic from 2000 to 2010 (Kuraparthy and Bowman, 2013). Almost all of the CS lines harbored alleles with antagonistic additive genetic effects on some desirable fiber traits, suggesting that additional breeding strategies would be required to get rid of the alien alleles associated with negative genetic drag effects (Fig. 1).

Dominance effects are grouped into two categories homozygous and heterozygous dominance (Tables 5 and 6). The deviation from TM-1 of a homozygous dominance effect of a CS line estimates the difference of dominance effects between the substituted homozygous pair of chromosomes and the respective homozygous pair of TM-1 chromosomes. The deviation of heterozygous dominance effects of the substituted chromosome(s) (chromosome interaction effects in hybrids) from TM-1 measures the difference in the interaction effects between the alien alleles in the substituted heterozygous chromosome(s) from the interaction effects of the TM-1 alleles in the same chromosome(s) (Jenkins et al., 2006, 2007; Wu et al., 2006). Dominance effects correspond to specific combining ability (Jenkins et al., 2006, 2007; Wu et al., 2006). The isogenic nature of the CS lines and the partial-diallel mating design provided an opportunity to compare dominance effects from a double heterozygous combination vs. a single heterozygous combination for any two specific substituted chromosomes or chromosome arms, revealing genetic effects on a chromosome-by-chromosome basis. Results revealed that some of the double chromosomal heterozygous dominance genetic effects in the hybrid from the cross of two CS-T lines had potential to improve several fiber traits compared with the hybrid from the same single-chromosomal heterozygous dominance genetic effects. Several CS-B and CS-T lines were intercrossed with each other and the recurrent parent to unveil

that chromosomal or interlocus interactions of potentially interacting alleles played a major role in the nonadditive mode of genetic regulation of several fiber traits. For example, comparative analysis of CS-T07 × CS-T15sh, the double heterozygous hybrid, with the single heterozygous hybrid for the same chromosomes, respectively, in CS-T07 × TM-1 and CS-T15sh × TM-1 hybrids revealed a nonadditive mode of complementary interaction at the interlocus of the two alien chromosomes, causing an increase in heterozygous dominance genetic effect for fiber strength (Fig. 4). This result is in concordance with our previous studies, with CS lines showing that interaction effects of genes played a major role in controlling most of the fiber traits (Saha et al., 2006a, 2008, 2010, 2011a, 2011b, 2013b).

Currently, the US cotton industry has shifted from a domestic-based market to an export-oriented market, because nearly two-thirds of US cotton fiber is sold on the world market (Agricultural Marketing Service, 2011). This market shift, along with the improvement in spinning technology in textile industries, demand the production of cotton with greater fiber quality than the standards established for the domestic market. Additionally, fiber quality in the United States has declined after 2000 due to the narrow genetic base in upland cotton (Bowman and Gutiérrez, 2003). Global competition in the textile fiber market has demanded new research and breeding strategies to elevate the genetic potential of upland cotton and US-produced cotton. The global competition demands new thinking and research strategy to unveil the genetic potential of upland cotton. This study reports a new strategy to complement conventional interspecific introgression using a novel method of chromosome shuffling among three species to make US cotton competitive in the world market. It also provides new information on the following: (i) development of a unique set of germplasm by targeted introgression of desirable genes from three species, including wild and unadapted types, into genetic backgrounds readily usable by the breeders with minimum linkage drag, (ii) comparative analysis of fiber traits on a chromosome-by-chromosome basis between a domesticated and wild species (*G. barbadense* vs. *G. tomentosum*), (iii) new information on cryptic alleles associated with the substituted chromosome or segment of *G. barbadense* and *G. tometosum* that can be useful for the genetic improvement of upland cotton, and (iv) preliminary information on the evolution of fiber traits from comparative analysis of the substituted chromosomes of a domesticated vs. wild species. The narrow genetic base and the low utilization of genetic resources from wild and unadapted germplasm are the major factors limiting global productivity in many crop species.

This research is a stepping-stone to provide a breeding tool for chromosome-specific introgression of valuable traits from wild and unadapted species into upland cotton.

These CS lines provide powerful analytical tools for high-resolution, chromosome-specific genetic dissection of complex traits and validation of other genome maps. This research also demonstrated a novel way of exploiting new and diverse sources of variation from wild and unadapted germplasm, especially in crops where a substantial number of CS lines are available.

Acknowledgments

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