Theor Appl Genet (2013) 126:109–117
DOI 10.1007/s00122-012-1965-9

ORIGINAL PAPER

# Interspecific chromosomal effects on agronomic traits in *Gossypium hirsutum* by AD analysis using intermated *G. barbadense* chromosome substitution lines

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Received: 30 April 2012 / Accepted: 10 August 2012 / Published online: 4 September 2012

**Abstract** The untapped potential of the beneficial alleles from *Gossypium barbadense* L. has not been well utilized in *G. hirsutum L.* (often referred to as Upland cotton) breeding programs. This is primarily due to genomic incompatibility and technical challenges associated with conventional methods of interspecific introgression. In this study, we used a hypoaneuploid-based chromosome substitution line as a means for systematically introgressing *G. barbadense* doubled-haploid line '3-79' germplasm into a common Upland genetic background, inbred 'Texas marker-1' ('TM-1'). We reported on the chromosomal effects, lint percentage, boll weight, seedcotton yield and lint yield in chromosome substitution CS-B (*G. barbadense* L.) lines. Using an additive-dominance genetic model, we studied the interaction of alleles located on two alien substituted chromosomes versus one alien substituted chromosome using a partial diallel mating design of selected CS-B lines (CS-B05sh, CS-B06, CS-B09, CS-B10, CS-B12, CS-B17 and CS-B18). Among these parents, CS-B09 and CS-B10 were reported for the first time. The donor parent 3-79, had the lowest additive effect for all of the agronomic traits. All of the CS-B lines had significant additive effects with boll weight and lint percentage. CS-B10 had the highest additive effects for lint percentage, and seedcotton and lint yield among all of the lines showing a transgressive genetic mode of inheritance for these traits. CS-B09 had greater additive genetic effects on lint yield, while CS-B06, CS-B10 and CS-B17 had superior additive genetic effects on both lint and seedcotton yield compared to TM-1 parent. The 3-79 line had the highest dominance effects for boll weight (0.513 g) and CS-B10 had the lowest dominance effect for boll weight (−0.702). Some major antagonistic genetic effects for the agronomic traits were present with most of the substituted chromosomes and chromosome arms, a finding suggested their recalcitrance to conventional breeding efforts. The results revealed that the substituted chromosomes and arms of 3-79 carried some cryptic beneficial alleles with potential to improve agronomic traits including yield, whose effects were masked at the whole genome level in 3-79.

## Introduction

Faced with a shrinking supply of land, labor, water and inputs, significant genetic gains will be required from essentially all domesticated plants if civilization is to reliably grow enough to adequately produce sufficient food, feed, fiber, shelter and energy on the path out of poverty. The genetic improvement of crop species will be the major stepping stone to meet this demand. For all crops, in particular cotton, where limited genetic diversity exists among the agriculturally elite types, most commonly as parents in applied breeding, the genetic improvements will significantly depend on innovative exploitation of genetic resources and efficient plant breeding strategies (Bowman

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Communicated by B. Friebe.

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et al. 1996). New genetic resources of key crop must be identified and incorporated into breeding lines and gene pools to meet the global demand.

The narrow genetic base in Upland cotton (Gutierrez et al. 2002; Esbroeck and Bowman 1998; Bowman et al. 1996; Paterson et al. 2004) increases vulnerability to new pathogens, pests and generally constrains opportunities for genetic improvement. Given the immense domestic and global importance of its lint, seed and plants, from which humans derive fiber, feed, food, fuel and hundreds of man-made products, there is an implicit need to exploit multiple avenues of genetic diversification and improvement of cotton including intraspecific and interspecific germplasm introgression.

While some qualitative and quantitative traits have been introgressed, including several disease and pest resistance genes (Endrizzi et al. 1985; Robinson et al. 2007; Campbell et al. 2011), the low levels of diversity present among elite Upland cotton lines suggest that extensive introgression has not been achieved. This indicates that the underlying genetic challenges are complex and demand focused, multi-disciplinary scientific efforts. Such efforts will likely lead to significant improvements in the crop per se, our understanding of the relationships among its complex traits, including fiber properties, the nature of genetic impediments to introgression and recombination, and how complex polyploid genomes evolve in *Gossypium*.

The racestock conversion program of *G. hirsutum* L. was developed to more effectively use intraspecific germplasm by removing photoperiodicity (Wu et al. 2010a; McCarty et al. 2004). The primary gene pool for cotton includes wild types of *G. hirsutum L.*, as well as the other four $2n = 52$ species of allotetraploid *Gossypium*, all of which are readily hybridized and contain $AD_1$ genomes (*G. hirsutum L.*) and thus share a common gross chromosome structure. The primary gene pool of the wild unadapted species is especially accessible reservoirs of important genes for pest and disease resistance, and for improved agronomic and fiber traits (Endrizzi et al. 1985). However, the effects of beneficial genes from wild unadapted germplasm are often obscured by other genes that affect the trait negatively in the wild species. Therefore, breeders very rarely use interspecific crosses in Upland cotton improvement, because the potential value of specific alien genes is usually compromised by co-inheritance of closely linked genes that have deleterious agricultural effects, affecting production and profit (He et al. 2008). To physically separate the beneficial and undesirable alien genes by breeding can thus be extremely difficult requiring several generations of crosses with special breeding strategies. An alternative approach to conventional pedigreed or population-based interspecific introgression is to use alien chromosome substitution lines, increasing the recombination only with the substituted alien chromosomes or chromosome segments (Jenkins et al. 2006, 2011, 2007), thereby greatly reducing the likelihood of undesirable linkages.

Previously, we have reported the development and public release of a number of chromosome substitution lines (CS-B lines) from *G. barbadense* L. (Stelly et al. 2005). Recently, we have extended this program in developing chromosome substitution lines from the primary gene pool species, including *G. tomentosum* L. and *G. mustellinum* L., to improving Upland cotton (Saha et al. 2011a). In conjunction with CS-B line development, previously we developed genetic and statistical approaches for using the CS-B lines to study, identify, quantify and localize several types of quantitative genetic effects of complex traits (Wu et al. 2010b). These approaches are thus now available to help characterize and use existing and forthcoming CS lines. Among these were the experimental definition of genetic effects (Saha et al. 2004), its partitioning into variance components (Saha et al. 2006), the use of topcrosses to query effects related to various cultivars (Jenkins et al. 2006, 2007, 2011) and the use of intermatings among CS lines to detect and quantitate epistasis (Saha et al. 2011b). The chromosome substitution efforts have also led to improved analytical model and statistical approaches that facilitate experimentation and quantitative genetic analyses (Wu et al. 2006a, 2000b, 2008, 2009, 2010b). The resources and advances will facilitate analysis and usage of these cotton germplasm resources.

The objective of this paper was to determine the chromosomal effects on agronomic traits from a partial diallel crossing program among seven selected CS-B lines and to extend our previous studies on intercrossing of CS-B lines (Saha et al. 2010) by including two additional new CS-B lines (CS-B09 and CS-B10). Both of the new CS-B lines harbored some cryptic beneficial alleles for agronomic traits which were not detected in the donor parent (3-79). This is especially important considering the conventional idea which considers that *G. barbadense* is poor in agronomic trait, but carries superior fiber quality characteristics compared to *G. hirsutum* L. The results should add new information to our current CS-B germplasm lines with some beneficial cryptic alleles for improved agronomic traits including yield.

## Materials and methods

A partial diallel mating design was created in the summer of 2006 by intercrossing seven CS-B lines (CS-B05sh, CS-B06, CS-B09, CS-B10, CS-B12, CS-B17 and CS-B18), including five previously released (Stelly et al. 2005) and two new ones, TM-1 (the recurrent parent) and 3-79 (the donor parent) at Mississippi State.

Parent and $F_1$ seeds were grown for selfing in Mexico during the fall/winter of 2006. Fourteen $F_2$ hybrids and the parental lines were planted in a randomized complete block design with four replications in two locations in 2007 and 2008, respectively, and one location in 2009 at Mississippi State. A Leeper silty clay loam (fine, smectitic, nonacid, thermic Vertic Epiaquept, Environment 1, 2007 and Environment 2, 2008) and Marietta loam (Fine-loamy, siliceous, active, Fluvaquentic Eutrudepts, Environment 3, 2007, Environment 4, 2008, and Environment 5, 2009) were the soil types in the two locations. We used standard agronomic practices during the growing season for all environments. All experiments were conducted at the Plant Science Research Center, Mississippi State (33.4°N 88.4°W).

Yield was evaluated by harvesting with a two-row mechanical cotton picker equipped with load cells for individual row. Prior to machine harvest, 25 boll samples were handpicked for all plots for all five environments. These samples were used to determine boll weight (BW, g) and ginned to determine lint percentage (LP, %). Boll samples were ginned on a laboratory 10-saw gin to separate lint and seed. Lint yield (LY, kg/ha) was determined by multiplying seedcotton yield by lint percentage.

### Genetic models and statistical methods

The statistical methods paralleled those reported previously (Saha et al. 2006). These included the use of an additive dominance (AD) with G × E interaction genetic model for data analysis (Zhu 1994; Tang et al. 1996; Saha et al. 2006; Wu et al. 2006c). We used this model based on the following two genetic assumptions: (1) normal disomic segregation and (2) dominance effects (interaction effects between alleles at each locus). The genetic model for parent $i$ at environment $h$ is expressed as follows: $y_{hiik(P)} = \mu + E_h + 2A_i + D_{ii} + 2AE_{hi} + DEE_{hii} + B_{k(h)} + e_{hiik}$.

The genetic model for a $F_2$ between parents $i$ and $j$ at environment $h$ is expressed as follows: $y_{hijk(F_2)} = \mu + E_h + (A_i + A_j) + (0.25D_{ii} + 0.25D_{jj} + 0.5D_{ij}) + (AE_{hi} + AE_{hj}) + (0.25DE_{hii} + 0.25DE_{hjj} + 0.5DE_{hij}) + B_{k(h)} + e_{hijk}$ where $\mu$ = population mean, $E_h$ = environmental effect, $A_i$ (or $A_j$) is the additive effect, $D_{ii}$, $D_{jj}$ or $D_{ij}$ is the dominance effect, $AE_{hi}$ (or $AE_{hj}$) is the additive-by-environment interaction effect, $DE_{hii}$, $DE_{hjj}$ or $DE_{hij}$ is the dominance-by-environment interaction effect, $B_{k(h)}$ is the block effect, and $e_{hijk}$ is the random error. Mathematically if we define $D_{i1}$ as the heterozygous-dominance effects between the female parent $i$ and the male parent TM-1, and $D_{ij}$ as the heterozygous-dominance effect between the female parent $i$ and a CS-B line $j$, then a significant difference of $D_{ij}$ minus $D_{i1}$ can be considered as the interaction effect (heterozygous-dominance effect) due to the specific substituted chromosome or arm from 3-79 and the homologous chromosome or arm in female cultivar parent $i$. This is essentially a probe of the effect of the specific combination between the substituted chromosome in the CS-B line and the homologous chromosome in the cultivar.

The significance of the difference between the genetic effects for a CS-B line and TM-1 was detected using the method of Patterson (1939) based on the standard error of the difference between two effects. Standard errors of variance components and genetic effects were estimated by jackknife procedure (Wu et al. 2008). A one-tailed $t$ test (DF = 19) was used to identify the significance of variance components and two-tailed $t$ test was used to detect the significance of genetic effects. We estimated the predicted genetic effects as deviations from population mean $\mu$, not from TM-1, so the significant difference of specific chromosome effects between 3-79 and TM-1 chromosomes was not directly detected. Since each CSB line is iso-genetic to the recurrent parent TM-1 with only one chromosome or chromosome arm divergent, the difference in genetic effects between a CS-B line and TM-1 can be considered as the substituted chromosome genetic effect deviations from TM-1. A confidence interval test (of 95 %) was utilized to detect the significance of genetic effects among genotypes.

## Results

### Mean comparisons of lines and CS backcross and intercross $F_2$ hybrids

Mean comparisons of parental and CS lines, CS × TM-1 $F_2$ hybrids and CS × CS $F_2$ hybrids are shown in Table 1. The donor line, 3-79, produced less seedcotton (65 %) and lint yield (68 %), smaller bolls (44 %) and lower (4 %) lint percentage compared to TM-1. In contrast to the inferiority of line 3-79 for lint percentage, four CS-B lines (CS-B05sh, CS-B06, CS-B09 and CS-B10) had higher lint percentage than TM-1, and only one had a lower lint percentage (CS-B17). Like the donor, all of the CS-B lines had smaller bolls than TM-1, except CS-B12, for which the difference was nonsignificant. In contrast to the inferiority of the donor for seedcotton yield, CS-B06 produced 15 % higher seedcotton and 17 % higher lint yield than TM-1. CS-B17 had 12 % higher seedcotton yield and almost similar lint yield like TM-1. The conventional $F_2$ hybrid (hetrozygous at the whole genome level) between TM-1 and 3-79 had the lowest boll weight, lint percentage, and seedcotton and lint yield among all the entries including the CS-B lines, the CS-B backcross $F_2$ hybrids, the CS intercross $F_2$ hybrids, and the recurrent and donor parents. Lint percentages were higher among 11 of the 14 CS-B

**Table 1** Mean values of the CS-B lines, TM-1, 3-79 and crosses for four quantitative traits

| Entry | Boll weight (g) | Lint percent (%) | Seedcotton yield (kg/ha) | Lint yield (kg/ha) |
|---|---|---|---|---|
| **TM-1** | **5.51** | **34.29** | **1,938** | **674** |
| CS-B05sh | 4.60 | 36.10 | 1,637 | 599 |
| CS-B06 | 5.21 | 35.07 | 2,226 | 791 |
| CS-B09 | 4.63 | 35.90 | 945 | 340 |
| CS-B10 | 4.38 | 35.51 | 1,684 | 595 |
| CS-B12 | 5.40 | 34.16 | 782 | 268 |
| CS-B17 | 5.00 | 31.00 | 2,169 | 684 |
| CS-B18 | 4.65 | 34.65 | 1,638 | 578 |
| **3-79** | **3.10** | **32.82** | **680** | **218** |
| TM-1 × CS-B05sh | 5.25 | 35.21 | 1,426 | 506 |
| TM-1 × CS-B06 | 5.46 | 34.95 | 1,578 | 549 |
| TM-1 × CS-B09 | 5.26 | 39.20 | 1,849 | 729 |
| TM-1 × CS-B10 | 5.11 | 38.44 | 1,998 | 775 |
| TM-1 × CS-B12 | 5.36 | 35.89 | 1,159 | 416 |
| TM-1 × CS-B17 | 5.31 | 33.85 | 1,929 | 658 |
| TM-1 × CS-B18 | 5.31 | 34.56 | 1,412 | 494 |
| TM-1 × 3-79 | 2.99 | 28.65 | 439 | 127 |
| CS-B05sh × CS-B10 | 4.88 | 39.30 | 1,855 | 737 |
| CS-B05sh × CS-B18 | 4.83 | 36.68 | 1,174 | 435 |
| CS-B06 × CS-B10 | 5.09 | 38.89 | 1,839 | 716 |
| CS-B09 × CS-B10 | 4.68 | 43.74 | 1,478 | 646 |
| CS-B10 × CS-B18 | 5.05 | 39.25 | 1,584 | 628 |
| CS-B12 × CS-B17 | 5.30 | 36.75 | 1,117 | 413 |
| $\text{LSD}_1$ 0.05 | 0.21 | 0.65 | 404 | 150 |
| Parent | 4.72 | 34.39 | 1,522 | 528 |
| $F_2$ | 4.99 | 36.81 | 1,488 | 559 |
| $\text{LSD}_2$ 0.05 | 0.06 | 0.20 | 122 | 45 |

$\text{LSD}_1$ for all entries, $\text{LSD}_2$ for parental lines and $F_2$ two generations over four environments. Mean of the agronomic traits of parent lines of CS-B and the conventional hybrid between TM-1 and 3-79 are shown in boldfaced font

**Table 2** Estimated variance components expressed as proportions to the phenotypic variances for four agronomic traits

| | Boll weight | Lint percent | Seedcotton yield | Lint yield |
|---|---|---|---|---|
| $V_A/V_P$ | 0.330** | 0.024** | 0.091** | 0.083** |
| $V_D/V_P$ | 0.439** | 0.918** | 0.186** | 0.261** |
| $V_{AE}/V_P$ | 0.000 | 0.017+ | 0.000 | 0.019 |
| $V_{DE}/V_P$ | 0.143** | 0.012 | 0.387** | 0.317** |
| $V_E/V_P$ | 0.089** | 0.029** | 0.337** | 0.319** |

+, *, and ** are significant at 0.10, 0.05 and 0.01 probability levels, respectively

$V_A$ additive variance, $V_D$ dominance variance, $V_{AE}$ additive-by-environment variance

$V_{DE}$ dominance-by-environment variance, $V_E$ error variance and $V_P$ phenotypic variance

hybrids (70 %) than the recurrent parent. The average of seedcotton yield of the CS-B hybrids ranged from 1117 kg ha$^{-1}$ (CS-B12 × CS-B17) to 1998 kg ha$^{-1}$ (TM-1 × CS-B10). Lint yield means of the CS-B hybrids ranged from 413 kg $^{-1}$ (CS-B12 × CS-B17) to 775 kg ha$^{-1}$ (TM-1 × CS-B10). The CS-B hybrid (TM-1 × CS-B10) produced 3 % higher seedcotton and 15 % higher lint yield than the recurrent parent TM-1.

## Variance component analysis

Residual variances for boll weight (9 %) and lint percentage (3 %) were low, which suggested that these traits were more stable across all environments (Table 2). Results showed that dominance genetic effects played a major role in boll weight (44 % of phenotypic variance), lint percentage (92 % of phenotypic variance), seedcotton yield (19 % of phenotypic variance) and lint yield (26 % of phenotypic variance). Additive genetic variance contributed 33 % of phenotypic variance in boll weight. Dominance and environment interaction was also a major contributor for seedcotton (39 %) and lint yield (31 %). Additive and environment interaction effects made no or slight contributions to boll weight (0 %), seedcotton yield (0 %) and lint yield (2 %) in this study.

## Additive genetic effects

The additive genetic effects are presented as deviations from the grand mean (Table 3). The donor parent 3-79 had the lowest negative additive effects for all of the agronomic traits. All CS-B lines had significant positive additive genetic effects on boll weight and four CS-B lines had significant positive additive genetic effects on lint percentage. Most of the CS-B lines had significant additive effects for seedcotton and lint yield. CS-B10 had the highest additive effects for lint percentage, seedcotton yield and lint yield among all of the lines, including both donor and recurrent parents. In addition, CS-B06 and CS-B17 had significant higher additive effects in seedcotton and lint yield compared to TM-1.

## Dominance genetic effects

Dominance effects can be considered as equivalent to specific combining ability (Wu et al. 2006b; Jenkins et al. 2007; Saha et al. 2011a, 2011b). We predicted two types of dominance effects (homozygous and heterozygous) in this study (Table 4).

**Table 3** Predicted additive effects for nine parents regarding four agronomic traits

| | Boll weight (g) ±SE | Lint percentage (%) ±SE | Seedcotton yield (kg/ha) ±SE | Lint yield (kg/ha) ±SE |
|---|---|---|---|---|
| TM-1 | 0.205 ± 0.009** | −0.334 ± 0.027** | 49.0 ± 8.7** | 9.5 ± 3.1 |
| CS-B05sh | 0.037 ± 0.008** | 0.077 ± 0.012** | 16.3 ± 13.0 | 6.7 ± 4.5 |
| CS-B06 | 0.234 ± 0.009** | −0.084 ± 0.013** | 179.9 ± 16.8** | 53.1 ± 5.7** |
| CS-B09 | 0.002 ± 0.006** | 0.721 ± 0.068** | 4.9 ± 8.5 | 23.1 ± 2.9** |
| CS-B10 | 0.053 ± 0.009** | 0.983 ± 0.088** | 214.4 ± 17.9** | 106.1 ± 9.4** |
| CS-B12 | 0.261 ± 0.006** | 0.062 ± 0.009** | −247.4 ± 17.0** | −81.7 ± 6.4** |
| CS-B17 | 0.135 ± 0.007** | −0.403 ± 0.043** | 205.6 ± 22.2** | 45.1 ± 6.1** |
| CS-B18 | 0.111 ± 0.008** | −0.063 ± 0.008** | −37.5 ± 15.1 | −17.1 ± 5.3 |
| 3-79 | −1.037 ± 0.018** | −0.961 ± 0.089** | −385.2 ± 24.9** | −144.8 ± 10.9** |

** Significance from TM-1 at 0.01 probability level

**Table 4** Dominance effects for agronomic traits

| | Boll weight (g) ±SE | Lint percentage (%) ±SE | Seedcotton yield (kg/ha) ±SE | Lint yield (kg/ha) ±SE |
|---|---|---|---|---|
| TM-1 × TM-1 | 0.364 ± 0.043** | −0.501 ± 0.087** | 442.4 ± 85.7** | 148.2 ± 29.3** |
| CS-B05sh × CS-B05sh | −0.383 ± 0.033** | −1.431 ± 0.073** | 225.2 ± 75.0 | 73.3 ± 29.6 |
| CS-B06 × CS-B06 | −0.126 ± 0.019** | −1.566 ± 0.099** | 382.0 ± 41.5** | 138.1 ± 16.8** |
| CS-B09 × CS-B09 | −0.238 ± 0.028** | −7.010 ± 0.092** | −367.8 ± 42.8** | −231.3 ± 18.2** |
| CS-B10 × CS-B10 | −0.702 ± 0.050** | −9.893 ± 0.116** | −191.0 ± 28.6** | −210.7 ± 13.3** |
| CS-B12 × CS-B12 | 0.099 ± 0.029 | −3.931 ± 0.082** | 87.1 ± 27.8 | −3.1 ± 9.8 |
| CS-B17 × CS-B17 | −0.125 ± 0.027** | −4.392 ± 0.086** | 277.9 ± 41.2** | 53.3 ± 13.2* |
| CS-B18 × CS-B18 | −0.485 ± 0.026** | −2.304 ± 0.066** | 356.8 ± 40.5** | 120.2 ± 12.3** |
| 3-79 × 3-79 | 0.513 ± 0.025** | 3.040 ± 0.095** | 295.5 ± 33.1** | 117.6 ± 10.1** |
| TM-1 × CS-B05sh | 0.421 ± 0.055** | −0.900 ± 0.145** | −280.1 ± 78.5* | −135.5 ± 30.4** |
| TM-1 × CS-B06 | 0.311 ± 0.042** | −0.263 ± 0.155 | −436.0 ± 69.9** | −200.2 ± 25.6** |
| TM-1 × CS-B09 | 0.510 ± 0.036** | 6.305 ± 0.150** | 734.5 ± 107.9** | 379.3 ± 44.5** |
| TM-1 × CS-B10 | 0.201 ± 0.051* | 3.467 ± 0.100** | 434.7 ± 69.3** | 229.7 ± 30.0** |
| TM-1 × CS-B12 | −0.028 ± 0.041 | 1.939 ± 0.151** | −74.8 ± 42.4 | −31.2 ± 17.1 |
| TM-1 × CS-B17 | 0.236 ± 0.037** | 0.644 ± 0.119** | 140.2 ± 55.0 | 58.1 ± 20.3 |
| TM-1 × CS-B18 | 0.448 ± 0.035** | −1.127 ± 0.098** | −240.1 ± 58.4* | −115.2 ±23.0** |
| TM-1 × 3-79 | −2.531 ± 0.052** | −10.656 ± 0.198** | −1098.3 ± 102.4** | −466.0 ± 30.5** |
| CS-B05sh × CS-B10 | 0.374 ± 0.040** | 2.774 ±0.167** | 342.9 ± 65.6** | 191.9 ± 30.1** |
| CS-B05sh × CS-B18 | 0.025 ± 0.044 | 1.354 ± 0.118** | −492.0 ± 76.0** | −192.3 ± 28.4** |
| CS-B06 × CS-B10 | 0.280 ± 0.051** | 2.998 ± 0.154** | −90.8 ± 92.9 | 8.7 ± 43.1 |
| CS-B09 × CS-B10 | −0.031 ± 0.047 | 11.149 ± 0.159** | 8.3 ± 37.6 | 120.2 ± 15.9** |
| CS-B10 × CS-B18 | 0.657 ± 0.040** | 4.083 ± 0.157** | −30.9 ± 62.7 | 39.9 ± 28.4 |
| CS-B12 × CS-B17 | 0.209 ± 0.053* | 6.221 ± 0.174** | −425.8 ± 64.4** | −93.0 ± 20.8** |

e.g., CS-B05sh × CS-B05sh means the effect of the substituted chromosome 5sh in homozygous condition

CS-B05sh × CS-B10 means the substituted chromosomes 5sh and 10 are in heterozygous condition due to the cross between CS-B14sh and CS-B16

CS-B05sh × TM-1 means the substituted chromosome 5sh was in heterozygous condition due to the cross between CS-B05sh and TM-1, the recurrent parent

* and ** Significance from TM-1 at 0.05 and 0.01 probability levels, respectively

The hybrid of TM-1 × 3-79 had the lowest negative dominance effects for all of the traits when carrying all chromosomes in heterozygous condition in the hybrid. However, results showed that both TM-1 and 3-79, the parental lines, had higher positive homozygous-dominance effects than the hybrid when carrying the chromosome pairs in homozygous condition. Line 3-79 had the highest (0.513 g) homozygous-dominance effects and CS-B10 had

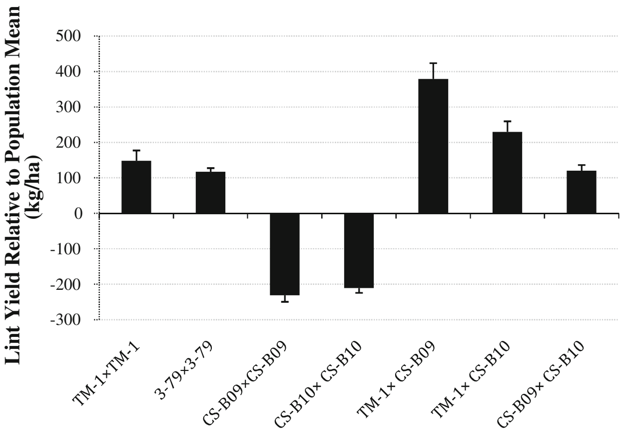

**Fig. 1** Predicted dominance effects on lint yield of the selected CS-B lines, TM-1, 3-79 and the selected hybrids of the CS-B lines. Note that comparative results among the lines including single-chromosome heterozygous versus two-chromosome heterozygous for the respective alien chromosome(s) showing non-additive mode of epistatic gene action

the most negative (−0.702) homozygous-dominance effect for boll weight when carrying the chromosomes in the homozygous condition. All of the substituted chromosomes had negative dominance effects on lint percentage when carrying homozygous substituted chromosome pair, suggesting that these alien chromosomes more likely carried genes that had an effect on lint percentage and could cause inbreeding depression in the cross with any of these lines. CS-B09 × CS-B10 had the highest heterozygous-dominance effect on lint percentage suggesting that the interaction effect of the alleles of the substituted chromosomes caused this effect due to their heterozygous condition. CS-B09 and CS-B10 had opposite dominance effects on seedcotton and lint yield in heterozygous versus homozygous conditions specific to the substituted chromosomes showing non-additive action of epistasis associated with the alleles of the substituted chromosomes for these traits (Table 4; Fig. 1).

### Major and minor chromosomal effects

The AD model provided an opportunity to dissect the quantitative effect into additive and dominance mode of genetic inheritance for the substituted chromosome (Tables 3, 4). Results revealed that some major antagonistic genetic effects for the agronomic traits were associated with most of the substituted chromosomes and chromosome arms suggesting their recalcitrance to conventional breeding efforts. The additive genetic effects were about two times higher on lint percentage in two CS-B lines (CS-B09 and CS-B10), three times more on seedcotton yield in three CS-B lines (CS-B06, CS-B10 and CS-B17) and five times greater on lint yield in three CS-B lines (CS-B06, CS-B10, and CS-B17) compared to TM-1. However, additive genetic effect was about five times lower in seedcotton yield and eight times lower in lint yield of CS-B12 (Table 3). The homozygous-dominance effect on lint percentage of CS-B09 and CS-B10 were about 14 and 20 times lower, respectively, from the homozygous-dominance effect of TM-1 (Table 4). However, the heterozygous-dominance effect in CS-B09 × CS-B10 was about 22 times higher than the homozygous-dominance effect of TM-1. CS-B09 and CS-B10 had homozygous-dominance effect on lint yield about 1.5 times lower than TM-1. Some other CS-B lines had minor genetic effects on the trait compared to the major chromosomal effects. The association of major chromosomal effects in opposite direction for a specific trait in cotton genome is a great impediment in the genetic improvement of Upland cotton using conventional methods of interspecific introgression.

In summary, most of the CS-B lines had significant additive effects for all of the agronomic traits. CS-B06 and CS-B17 contributed significantly higher additive effects in seedcotton and lint yield compared to TM-1. 3-79, and the donor line had the lowest additive effect for all of the agronomic traits among the lines. CS-B09 × CS-B10 had the highest heterozygous-dominance effect on lint percentage.

## Discussion

Our previous reports revealed that CS-B lines open new paradigms in cotton breeding and genetic studies, providing a tool to overcome the problems of interspecific introgression and in the discovery of some novel cryptic genes whose effects could not be readily detected in the donor alien parent (Saha et al. 2006; Jenkins et al. 2007; Saha et al. 2010). These studies demonstrated that the near isogenicity of $BC_5S_n$ CS-B lines with the recurrent parent, TM-1, for 25 chromosome pairs, and between any two CS-B lines for 24 chromosome pairs provided a tool for detection of gross genetic effects by the respective substituted chromosome(s) and/or segment(s) (Saha et al. 2006, 2010). In this report, we extended our previous report on intercrossing of CS-B lines (Saha et al. 2010) by including two new CS-B lines (CS-B09 and CS-B10) and their epistatic interactions involving the seven CS-B lines. Here, for the first time, we are reporting the association of important QTLs with CS-B09 and CS-B10 based on the comparative analysis of CS-B lines and their intercrosses. The overall results on the association of chromosomal effects with fiber traits from the previous studies (Saha et al. 2006, 2008a, b, 2010, 2011b) are concordant with the results of this report.

*G. barbadense* produced superior fiber, but inferior agronomic qualities compared to *G. hirsutum* L. Our results also showed that growing under the same condition, TM-1 had higher boll weight, lint percentage, and seedcotton and lint yield than 3-79. Our previous studies demonstrated that 3-79 had also significantly improved fiber quality traits including fiber strength, length and micronaire (Saha et al. 2006, 2011b). Breeders who targeted at combining the best traits of *G. hirsutum* L. and *G. barbadense* L. using conventional methods of interspecific crosses, however, failed to accomplish their goals. Such unsatisfactory results from previous efforts encourage exploring different approaches to introgress beneficial genes from *G. barbadense* L. into Upland cotton using CS-B lines (Saha et al. 2006, 2010, 2011b; Jenkins et al. 2011, 2007, 2006).

Here, we used a method of partial diallel mating of several CS-B lines to provide new information on the chromosomal effects based on the presence of a single *G. barbadense* L. chromosomes or chromosome arm, or two alien species chromosomes or chromosome arms, in a uniform background of *G. hirsutum* L. Boll weight, lint percentage (LP, %), and seedcotton and lint yield were superior in TM-1 (recurrent parent of the CS lines) than 3-79 (donor parent); these results were concordant with known evidence that the *G. barbadense* L. line is inferior to the recurrent parent TM-1 in these agronomic traits (Saha et al. 2006, 2010). However, we detected for the first time in this study the existence of cryptic novel alleles in the alien chromosomes nine and ten with the potential to improve agronomic traits, including seedcotton and lint yield in Upland cotton (TM-1), contrary to the conventional idea that *G. barbadense* alleles could be used primarily for improving fiber traits but not other agronomic traits.

The use of the AD model provided an opportunity to dissect the genetic effects into additive and dominance effects. However, we could not detect the additive and additive interaction genetic effects using an AD model, because we did not have results from $F_3$ progeny performance (Wu et al. 2010b). The ADAA model in our previous study (Saha et al. 2010) detected that epistatic effects were often involved in important agronomic and fiber quality traits. The comparative analysis from the overall results of the CS-B parents and their hybrids suggested that many of the genetic effects were associated with non-additive mode of genetic actions, highlighting the importance of epistasis in these traits (Fig. 1; Tables 3, 4). The results suggested the occurrence of a transgressive mode of genetic inheritance for some of the agronomic traits in some of the CS-B lines, implying that significant epistatic interaction of the genes associated with the substituted chromosome(s) made a substantial contribution to these genetic effects. A recent study predicted that Upland cotton had about 70,000 genes with 1 gene per 34.5 kb distributed equally between A and D genomes (Lacape et al. 2010). The expression of a gene on different, but specific chromosomes or chromosome arms is not biologically independent. It depends on the interaction of a network of genes located on other chromosomes for the specific trait. This is important considering that we found some chromosomes had different genetic effects in the opposite direction for the same trait in some cases. This result further highlights the challenges associated with the genetic improvement of Upland cotton.

The additive genetic effects are considered as equivalent to the general combining ability and useful for selection of pure lines in a breeding program (Jenkins et al. 2007). Our results suggested that some of the CS-B lines had higher positive additive effects than TM-1 and 3-79 for some of the agronomic traits, suggesting that the respective alien chromosome harbored cryptic alleles from 3-79 conferring significant positive additive effects for these traits in TM-1 genetic background. This genetic effect is transferable to the progenies (McCarty et al. 2004, 2007) and reflects cumulative effects of interactions among different loci, due to the presence of the respective substituted chromosome. Lacape et al. (2010) reported that the average fiber characteristics of RI lines developed from *G. hirsutum* L. × *G. barbadense* L. were intermediate.

All CS-B lines had significant dominance effects for lint percentage in both homozygous and heterozygous condition except TM-1 × CS-B06. A previous study discussed in detail the genetic effects of homozygous and heterozygous dominance (Jenkins et al. 2009). Negative homozygous-dominance effects predict cause of inbreeding depression in the later generation following hybridization with the respective line. All of the CS-B lines had higher dominance effects on lint percentage in heterozygous conditions compared to homozygous conditions, indicating these differences were due to the interactions of different alleles of the substituted chromosome in the same chromosome pair or alleles in other chromosome pair. All crosses between the CS-B lines had an overall higher dominance effects for lint percentage compared to their crosses with the recurrent parent TM-1, suggesting that the interaction of alleles of the two substituted chromosome caused the increase in lint percentage. Our previous study showed that the homozygous-dominance genetic effects on seedcotton and lint yield of CS-B22Lo were nearly four times those of TM-1 and additive effects for lint percentage were higher for CS-B16, CS-B22sh and CS-B22Lo than TM-1 (Saha et al. 2010).

The genetic progress in increasing yield has declined over the past several years based on the historical agronomic and yield data in Upland cotton (Meredith 2006). Our results suggested that the conventional assumption that *G. barbadense* carries useful genes only to improve fiber qualities, but not agronomic traits, may not be correct. For example, TM-1, the recurrent parent, had 65 % higher lint percentage than 3-79 in this study. However, five of the seven CS-B lines had higher average value of lint percentage compared to TM-1. Currently, cotton seed price is very low in the USA market and breeders would consider high lint percentage as a desirable agronomic trait to provide farmers more profits. Results from this report and our previous studies demonstrated that *G. barbadense* carried some cryptic beneficial alleles with potential to improve yield and other agronomic traits, as well as fiber quality traits in Upland cotton (Saha et al. 2006, 2008a, b, 2010, 2011a, b; Jenkins et al. 2006, 2007, 2009, 2011).

Although the alien species in the primary gene pool produce fertile $F_1$ hybrids in crosses with *G. hirsutum*, however, limited alien gene retention and genetic recombination, and $F_2$ hybrid breakdown in subsequent generations, producing nonrandom undesirable genetic products are often observed in conventional methods of interspecific crosses (Endrizzi et al. 1985; Reinisch et al. 1994; Jiang et al. 2000; Saha et al. 2010, 2011a). Conventional methods of interspecific introgression also failed to detect many of these beneficial cryptic agronomic alleles, because the genetic effects of these loci were masked with some other loci carrying negative effects for the trait in the *G. barbadense* genome. By restricting these effects from other chromosomes with the substituted chromosome or chromosome arm, the CS-B lines provide a stepping stone toward understanding the role of epistasis in agronomic traits. Controlled interspecific introgression of *G. hirsutum* L. with *G. barbadense* L. based on individual substituted chromosome provides a way of unveiling the epistasis on a chromosome by chromosome basis and targeted introgression of beneficial alleles from the alien species. Our results suggested additional genetic resolution is essential for scientific and breeding purposes in Upland cotton improvement.

With a prospective content of 500–4,000 alien genes per chromosome, each of the CS-B lines will harbor unique germplasm and thus provide opportunities for science and breeding in the genetic background of Upland cotton (Saha et al. 2011a). Manipulating quantitative traits controlled by many genes located on 26 different chromosomes is difficult in conventional methods of interspecific introgression. That is why conventional methods of interspecific introgression between these two species were not very successful due to incompatibility at the whole genome level. *G. barbadense* L. is well known as a unique source of genetic variation for improving fiber qualities, but it has rarely been used for the genetic improvement of agronomic traits. Our results revealed the discovery of some cryptic novel genes of *G. barbadense* L. with the potential of improving agronomic traits including seedcotton and lint yield. This report demonstrated that chromosome substitution line is one of the most powerful means of targeted interspecific introgression and steps toward novel quantitative trait locus identification in Upland cotton.

**Acknowledgments** We thank Ms. Lillie Hendrix and Dr. Russell Hayes for helping in field and greenhouse research. We acknowledge partial support from the following sources: Texas AgriLife Research, Cotton Inc., Texas State Support Committee and Texas Dept. Agriculture Food & Fiber Research Grant Program. The Joint publication of USDA/ARS, Mississippi Agricultural and Forestry Experiment Station, approved for publication as Journal Article No. 12090 of the Mississippi Agricultural and Forestry Experiment Station.

**Conflict of interest** Mention of trademark or proprietary product does not constitute a guarantee or warranty of the product by the United States Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

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