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# Cotton chromosome substitution lines crossed with cultivars: genetic model evaluation and seed trait analyses

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**Abstract** Seed from upland cotton, *Gossypium hirsutum* L., provides a desirable and important nutrition profile. In this study, several seed traits (protein content, oil content, seed hull fiber content, seed index, seed volume, embryo percentage) for $F_3$ hybrids of 13 cotton chromosome substitution lines crossed with five elite cultivars over four environments were evaluated. Oil and protein were expressed both as percentage of total seed weight and as an index which is the grams of product/100 seeds. An additive and dominance (AD) genetic model with cytoplasmic effects was designed, assessed by simulations, and employed to analyze these seed traits. Simulated results showed that this model was sufficient for analyzing the data structure with $F_3$ and parents in multiple environments without replications. Significant cytoplasmic effects were detected for seed oil content, oil index, seed index, seed volume, and seed embryo percentage. Additive effects were significant for protein content, fiber content, protein index, oil index, fiber index, seed index, seed volume, and embryo percentage. Dominance effects were significant for oil content, oil index, seed index, and seed volume. Cytoplasmic and additive effects for parents and dominance effects in homozygous and heterozygous forms were predicted. Favorable genetic effects were predicted in this study and the results provided evidence that these seed traits can be genetically improved. In addition, chromosome associations with AD effects were detected and discussed in this study.

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## Introduction

Upland cotton *Gossypium hirsutum* L., as an important dual-use crop, provides not only natural fiber to the textile industry but also seed nutrition components for both humans and livestock. Commercial upland cotton cultivars are normally composed of 40–43% lint and 57–60% seed. The importance of fiber quality to the textile industry has been recognized for years due to technology changes in cotton fiber spinning. Breeders have been working to improve fiber quality (Meredith 1993, 2005). However, cottonseed, which has an important nutrition profile, is considered a by-product of lint production. Thus, limited breeding emphasis has been placed on seed nutrition components. It should be a worthy investigation to better understand the genetics and breeding of cottonseed traits.

Among upland cotton lines, oil content ranges from 17 to 27%, protein from 12 to 32%, ash from 4 to 5%, and moisture from 5 to 10% (Turner et al. 1976; Cherry 1983; Cherry et al. 1978a, b; Kohel et al. 1985; Belyea et al. 1989; Blasi and Drouillard 2002; Liu et al. 2002). Cottonseed crude oil, which can be refined to edible oil, can also be considered an important biofuel resource

(Karaosmanoglu et al. 1999; Allen and Watts 2000; Meneghetti et al. 2007). In addition, cottonseed meal, an important food source, is used to feed sheep, dairy and beef cattle (Blasi and Drouillard 2002), and other ruminant livestock. Cottonseed meal was also reported to be better than soybean meal regarding feeding catfish (Barros et al. 2000).

The genetic system controlling plant seed traits is very complicated. For example, genes from the seed nucleus, the maternal plant, and/or cytoplasm may control a seed trait. Previous researches indicated the evidence of cytoplasmic effects in various crops such as cotton (Kohel 1980; Bourland and Bird 1983; Dani and Kohel 1989; Wu et al. 1995a), rice (Shi et al. 1996, 1997), and arabidopsis (Alonso-Blanco et al. 1999, 2003). As one of the most important genetic components, cytoplasmic effects are useful for seed trait improvements; however, only a few studies have been reported for cottonseed nutrition components (Kohel 1980; Bourland and Bird 1983; Dani and Kohel 1989; Wu et al. 1995a; Wang et al. 1996a, b; Ye et al. 2003). One possible major reason is the high cost to measure seed nutritional components while many analytical models required repeated measurements in each environment (i.e. Zhu and Weir 1994a, b). Therefore, it will be helpful to validate the feasibility of various genetic models for seed traits analysis without replications when limited seed supply or financial resources are not available.

A set of cotton chromosome substitution (CS-B) germplasm lines (Stelly et al. 2005), which are near-isogenic to the recurrent parent TM-1 with one chromosome or chromosome arm divergent and introgressed from *G. barbadense* L., 3-79, have been used to associate chromosomes or chromosome arms with agronomic and fiber traits of importance (Saha et al. 2004, 2006; Jenkins et al. 2006, 2007; McCarty et al. 2006). Compared to agronomic traits, the knowledge of the genetics of seed traits regarding these CS-B lines is sparse (Wu et al. 2009).

In this study, the seed traits for five commercial cultivars, 13 CS-B lines, 3-79 (donor parent), TM-1 (recurrent parent), and $F_3$ hybrids were measured in four environments (Jenkins et al. 2006, 2007) without replications. The data set was analyzed by the additive–dominance (AD) model with cytoplasmic effects (ADC model). The first objective of this study was to investigate the appropriateness of this ADC model including G × E interaction effects for our data structure of parents and $F_3$ seed generations by Monte Carlo simulation. The second objective was to detect desirable genetic effects associated with seed traits. The third objective was to determine chromosome associations with seed traits by the comparative method. Thus, this study should not only provide information for genetic models for seed trait analysis in other crops, but also provide valuable genetic information to breeders for cottonseed nutritional improvement.

## Experiment and methods

### Materials and field experiment

Thirteen euploid CS-B lines, their recurrent parent TM-1, donor parent, 3-79, five elite cultivars, 'Deltapine 90' (DP90), 'Sure-Grow 747' (SG747), 'Phytogen 355' (PSC355), 'Stoneville 474' (ST474), and 'FiberMax 966' (FM966) and the $F_3$ seed generations from crosses of the lines with the elite cultivars were used in this study. In each CS-B line, a single chromosome pair or arm of TM-1 has been replaced by the corresponding chromosome from 3-79. The CS-B lines were designated according to the chromosome number specific to the introgressed chromosome or chromosome arm: CS-B02, CS-B04, CS-B06, CS-B07, CS-B16, CS-B17, CS-B18, CS-B25, CS-B05sh (sh, short arm), CS-B14sh, CS-B15sh, CS-B22sh, and CS-B22Lo (Lo, long arm) (Stelly et al. 2005). The 75 top crosses were made at Mississippi State, MS, in the summer of 2002. The $F_1$ seeds were sent to a winter nursery in Tecoman, Mexico, to produce the $F_2$ seeds. The resulting 75 $F_2$ hybrids and their parental lines were planted at two sites in 2003 and 2004 (Environments 1, 2, 3, and 4) at the Plant Science Research Center at Mississippi State, MS (33.4 N 88.8 W). Soil type for Environment 1 was a Marietta loam (fine-loamy, siliceous, active, flulvaquentic, Eutrudepts) and for Environments 2, 3, and 4 was a Leeper silty clay loam (Fine, smectitic, nonacid, thermic Vertic Epiaquept). Planting dates were 28 May for 2003 and 13 May for 2004. Harvest dates were 3 November and 31 October for sites one and two in 2003, respectively, and 2–9 November and 29 October for sites one and two in 2004, respectively (Jenkins et al. 2006). Normal field practices were applied during the growing season. A 25-boll sample was hand harvested from first position bolls near the middle nodes of plants in each plot before machine picking.

### Seed trait measurements

One hundred acid-delinted seed samples were weighed to determine seed index (SI) and also used to determine seed volume (SV) by displacement of 100 seeds in ethyl alcohol. Approximately 10 g of seed sample for each entry was cut by scissors and the embryo was removed by a needle to measure embryo and hull weights and to calculate embryo percentage (EP) (Wu et al. 2009). Twelve grams of acid-delinted seeds for each entry in each of four environments were sent to Mississippi State Chemical Laboratory,

Mississippi State, MS, to determine protein, oil, seed fiber, ash, and moisture content. Determination of seed nutrition components followed the Official Methods of Analysis of AOAC International (crude protein: AOAC 990.03; crude fat: AOAC 920.39; moisture: AOAC 930.15; ash: AOAC 942.05; and crude fiber: AOAC 962.09) (Cunniff 1995). Protein index (PI), oil index (OI), fiber index (FI), ash index, and moisture index were calculated by multiplying SI by their content percentage (Wu et al. 2009). Thus, the index values are the amount of product in grams per 100 seeds.

## Genetic model and data analysis

With the data structure in this study, three genetic models can be used for data analysis. The AD genetic model has been widely used in many studies (Wu et al. 1995b; McCarty et al. 2006; Jenkins et al. 2006, 2007; Saha et al. 2006). The AD model with maternal additive and dominance effects (ADM) and the ADC are two extensions of the AD model. All three genetic models were applied for our data analysis and we found that the ADC model resulted in the smallest residual variance for all seed traits and was chosen to be used in this study. The ADC genetic model for parent and hybrid can be expressed as a linear model as in Eqs. 1 and 2.

For parent,

$$y_{hiik} = \mu + E_h + C_i + 2A_i + D_{ii} + \mathrm{CE}_{hi} + 2\mathrm{AE}_{hi} + \mathrm{DE}_{hii} + e_{hiik} \quad (1)$$

For $F_3$,

$$y_{hijk(F_3)} = \mu + E_h + C_i + A_i + A_j + \frac{1}{8}D_{ii} + \frac{1}{8}D_{jj} + \frac{6}{8}D_{ij} + \mathrm{AE}_{hi} + \mathrm{AE}_{hj} + \mathrm{CE}_{hi} + \frac{1}{8}\mathrm{DE}_{hii} + \frac{1}{8}\mathrm{DE}_{hjj} + \frac{6}{8}\mathrm{DE}_{hij} + e_{hijk(F_3)} \quad (2)$$

where $\mu$ is the population mean, a fixed effect; $E_h$ is the environment effect, either random or fixed (fixed in this study); $A_i$ (or $A_j$) is the additive effect from parent $i$ (or $j$); $D_{ii}$, $D_{jj}$ or $D_{ij}$ is the dominance effect; $C_i$ is the cytoplasmic effect from female parent $i$; $\mathrm{AE}_{hi}$ (or $\mathrm{AE}_{hj}$) is additive × environment interaction effect; $\mathrm{DE}_{hii}$, $\mathrm{DE}_{hjj}$ or $\mathrm{DE}_{hij}$ is the dominance × environment interaction effect; $\mathrm{CE}_{hi}$ is the cytoplasm × environment interaction effect; and $e_{hijk}$ is the random error.

The index for $h$ is from 1 to 4 and the index for $i$ (or $j$) is from 1 to 20 in Eqs. 1 and 2. Due to the complicity of the model and simplification for data analysis by the mixed linear model approaches, all effects were assumed random except population mean and environments. Equations 1 and 2 can be expressed in the forms of vector and matrices as follows.

$$\mathbf{y} = 1\mu + \mathbf{X}_E\mathbf{b}_E + \mathbf{U}_A\mathbf{e}_A + \mathbf{U}_D\mathbf{e}_D + \mathbf{U}_C\mathbf{e}_C + \mathbf{U}_{\mathrm{AE}}\mathbf{e}_{\mathrm{AE}} + \mathbf{U}_{\mathrm{DE}}\mathbf{e}_{\mathrm{DE}} + \mathbf{U}_{\mathrm{CE}}\mathbf{e}_{\mathrm{CE}} + \mathbf{e} = \mathbf{Xb} + \sum_{u=1}^{7}\mathbf{U}_u\mathbf{e}_u. \quad (3)$$

In this model, we assume that the environmental effect is fixed, where $\mu$ is the population mean; $\mathbf{1}$ is the vector with all elements 1; $\mathbf{e}_\mathrm{A}$ is the vector for additive effects, $\mathbf{e}_\mathrm{A} \sim \mathrm{MVN}\ (0, \sigma^2_\mathrm{A}\mathbf{I})$; $\mathbf{U}_\mathrm{A}$ is the incidence matrix for additive effects; $\mathbf{e}_\mathrm{D}$ is the vector for dominance effects, $\mathbf{e}_\mathrm{D} \sim \mathrm{MVN}\ (0, \sigma^2_\mathrm{D}\mathbf{I})$; $\mathbf{U}_\mathrm{D}$ is the incidence matrix for dominance effects; $\mathbf{e}_\mathrm{C}$ is the vector for cytoplasmic effects, $\mathbf{e}_\mathrm{C} \sim \mathrm{MVN}\ (0, \sigma^2_\mathrm{C}\mathbf{I})$; $\mathbf{U}_\mathrm{C}$ is the incidence matrix for cytoplasmic effects; $\mathbf{e}_\mathrm{AE}$ is the vector for additive × E effects, $\mathbf{e}_\mathrm{AE} \sim \mathrm{MVN}\ (0, \sigma^2_\mathrm{AE}\mathbf{I})$; $\mathbf{U}_\mathrm{AE}$ is the incidence matrix for additive × E effects; $\mathbf{e}_\mathrm{DE}$ is the vector for dominance × E effects, $\mathbf{e}_\mathrm{DE} \sim \mathrm{MVN}\ (0, \sigma^2_\mathrm{DE}\mathbf{I})$; $\mathbf{U}_\mathrm{DE}$ is the incidence matrix for dominance × E effects; $\mathbf{e}_\mathrm{CE}$ is the vector for cytoplasm × E effects, $\mathbf{e}_\mathrm{CE} \sim \mathrm{MVN}\ (0, \sigma^2_\mathrm{CE}\mathbf{I})$; $\mathbf{U}_\mathrm{CE}$ is the incidence matrix for cytoplasm × E effects; $\mathbf{e}_\mathrm{e}$ is the vector for random errors, $\mathbf{e}_\mathrm{e} \sim \mathrm{MVN}\ (0, \sigma^2_\mathrm{e}\mathbf{I})$.

The minimum norm quadratic unbiased estimation (MINQUE) approach was proposed by Rao (1971) for estimating variance components. Genetic effects could be predicted by the adjusted unbiased prediction (AUP) approach (Zhu 1993). The variance components in the ADC model can be estimated by solving the following MINQUE normal equations for $u$, $v = 1, 2, \ldots, 7$

$$[\mathrm{tr}(\mathbf{U}_u^T\mathbf{Q}_\alpha\mathbf{U}_v^T\mathbf{U}_v\mathbf{Q}_\alpha\mathbf{U}_u)][\sigma_u^2] = [\mathbf{y}^T\mathbf{Q}_\alpha\mathbf{U}_u\mathbf{U}_u^T\mathbf{Q}_\alpha\mathbf{y}] \quad (4)$$

where the trace tr is the sum of diagonals of a matrix and

$$\mathbf{Q}_\alpha = \mathbf{V}_\alpha^{-1} - \mathbf{V}_\alpha^{-1}\mathbf{X}(\mathbf{X}^T\mathbf{V}_\alpha^{-1}\mathbf{X})^{-1}\mathbf{X}^T\mathbf{V}_\alpha^{-1}, \quad (5)$$

where $\mathbf{V}_\alpha = \sum_{u=1}^{7}\alpha_u\mathbf{U}_u\mathbf{U}_u^T$ and $\mathbf{V}_\alpha^{-1}$ is the inverse matrix of $\mathbf{V}_\alpha$ with prior values $\alpha_u$ in place of $\sigma_u^2$ in $\mathbf{V}$. In this simulation study, we set $\alpha_u = 1$, $u = 1, \ldots, 7$ (Zhu 1989).

The first part of this study was genetic model evaluation for three parameter configurations (Table 1). Bias, Type I error (testing power), and mean square error (MSE) were calculated (Wu et al. 2006, 2008) based on 200 simulations. The second part of this study was the application of this genetic model. The non-pseudo jackknife technique was applied to calculate standard deviation for each parameter by removing 10% of the observations each time (Wu et al. 2008) for 50 times for both simulation and actual data analysis. An approximate $t$ test was used to test the significance of each parameter. All data analyses were conducted by a computer program written by the authors of this study in C++.

**Table 1** Estimated Type I error and testing power for estimating variance components by three configurations regarding the data structure for the AD model with cytoplasmic effects

| | Pre-set value[a] | Bias[b] | Power[c] | MSE[d] |
|---|---|---|---|---|
| Set 1[e] | | | | |
| $\sigma^2_C$ | 0.000 | 0.031 | 0.030 | 0.045 |
| $\sigma^2_A$ | 0.000 | 0.201 | 0.040 | 0.080 |
| $\sigma^2_D$ | 0.000 | 0.000 | 0.040 | 1.291 |
| $\sigma^2_{CE}$ | 0.000 | 0.000 | 0.035 | 0.082 |
| $\sigma^2_{AE}$ | 0.000 | 0.000 | 0.030 | 0.064 |
| $\sigma^2_{DE}$ | 0.000 | 0.209 | 0.060 | 2.043 |
| $\sigma^2_e$ | 20.000 | 0.168 | 1.000 | 0.053 |
| Set 2[e] | | | | |
| $\sigma^2_C$ | 0.000 | 0.000 | 0.025 | 0.063 |
| $\sigma^2_A$ | 20.000 | −0.244 | 0.885 | 0.711 |
| $\sigma^2_D$ | 20.000 | 0.564 | 0.220 | 2.940 |
| $\sigma^2_{CE}$ | 0.000 | 0.000 | 0.025 | 0.141 |
| $\sigma^2_{AE}$ | 20.000 | 0.073 | 0.760 | 0.481 |
| $\sigma^2_{DE}$ | 20.000 | 0.565 | 0.145 | 11.464 |
| $\sigma^2_e$ | 20.000 | −0.049 | 1.000 | 0.061 |
| Set 3[e] | | | | |
| $\sigma^2_C$ | 20.000 | −0.491 | 0.680 | 0.934 |
| $\sigma^2_A$ | 20.000 | 0.700 | 0.885 | 1.164 |
| $\sigma^2_D$ | 20.000 | 0.079 | 0.230 | 2.608 |
| $\sigma^2_{CE}$ | 20.000 | 0.205 | 0.835 | 0.514 |
| $\sigma^2_{AE}$ | 20.000 | 0.008 | 0.780 | 0.447 |
| $\sigma^2_{DE}$ | 20.000 | −0.868 | 0.145 | 12.595 |
| $\sigma^2_e$ | 20.000 | 0.020 | 1.000 | 0.074 |

[a] Pre-set variance component zero is used to determine Type I error and non-zero value is used to determine testing power at 0.05 probability level

[b] Deviation of mean estimate from the pre-set value

[c] It represents Type I error for pre-set variance component zero and testing power for non-zero pre-set value

[d] *MSE* mean square error related to bias and variation (Wu et al. 2006)

[e] The first set was used to determine Type I errors for all variance components except random error; the second one was used to determine Type I errors for cytoplasmic and cytoplasm × environment variance components and testing powers for remaining variance components; and the third one was used to determine testing powers for all variance components

By this MINQUE approach, the predicted genetic effects were deviations from the respective population grand mean $\mu$, rather than from TM-1. However, due to the advantage of chromosome substitution lines, chromosome substitutions with additive and dominance effects can be detected (Jenkins et al. 2006). The significance of the difference between the genetics effects for a CS-B line and TM-1 was detected by the 95% confidence intervals.

## Results

### Genetic model evaluation

The extended AD genetic model with cytoplasmic effects in this study has two unique characteristics. The data sets for seed traits were not repeated within each environment, yet genotype × environment effects need to be estimated. The theoretical appropriateness of using such a genetic model for our data analysis is unclear. Therefore, it was necessary to use simulation data to evaluate the appropriateness under this genetic model for the same data structure used in this study. Even though we conducted various types of computer simulations, we only reported three specific cases (Table 1). The first case was with all variance components set to zero except random error. This case was designed to determine Type I error for all variance components except random error. The second case was with only the variance components for cytoplasmic and cytoplasm × environment interaction set to zero while the remaining components were set to 20. This case was designed to determine Type I error for cytoplasmic effects and its G × E components and to test powers for the remaining components. The third case was all variance components including random error set to 20, which was designed to determine testing powers for different components. Two hundred simulations were used to evaluate Type I error and testing power at the nominal probability level of 0.05. The simulated results are summarized in Table 1.

The results showed that each variance component was estimated in an unbiased or minimally biased manner for both zero and non-zero values (Table 1). Type I error for cytoplasmic variance and cytoplasm × environment variances can be up to 0.05 when more than 9% of the data points were deleted each time. The testing power for both dominance and dominance × environment interaction effects was low compared to other variance components. This is probably due to the data containing no replication. Thus, the test for dominance and dominance × environment interaction effects was conservative.

### Phenotypic results

Since cottonseed ash and moisture contents were not as important as the other seed traits (Wu et al. 2009), only the results for SV, four percentage traits (seed embryo, protein, oil, and fiber), and four index traits (seed, protein, oil, and fiber) are reported in this study. On the average over parental lines and $F_3$ hybrids, percentage for seed protein, oil, and fiber was 20.4, 20.8, and 19.9% (data not shown), respectively. Indexes for seed protein, oil, and fiber were 2.04, 2.07, and 2.00 g, respectively. Mean values for SI,

SV, and EP were 10.0 g, 9.6 ml, and 62.6%, respectively. On the average, cottonseed protein content was lower in 2003 than in 2004 (data not shown). On the other hand, oil content, fiber content, PI, OI, FI, SI, SV in 2003 were higher than in 2004. The results might indicate interaction effects with environmental conditions for these traits.

## Variance components

The variance components expressed as the proportions of the phenotypic variances for seed traits are summarized in Table 2. Cytoplasmic effects, which are contributed by the genes in the female cytoplasm, were significant for oil content, OI, SI, SV, and EP (Table 2). Additive effects were significant for all traits except oil content. Significant dominance effects were detected for oil content, OI, SI, and SV. Cytoplasm × environment interaction effects were significant for protein, oil, fiber percentages, PI, and FI. Dominance × environment interaction effects were significant for all traits except for oil percentage and SV.

## Cytoplasmic effects

Cytoplasmic effects are heritable from the female parents and could be an important component for seed trait improvement. Thus, it is valuable to predict cytoplasmic effects for these seed traits and the results are listed in Table 3. The five elite cultivars and 3-79 had positive cytoplasmic effects for seed oil content while the remaining effects either were not significant or significantly negative (Table 3). Elite cultivars except SG747 had significant positive effects for OI whereas TM-1 and six CS-B lines had significant negative effects. Three elite cultivars (DP90, PSC355, and FM966), CS-B22sh, and 3-79 had significant positive cytoplasmic effects for SI while two cultivars (SG747 and ST474) and three CS-B lines (CS-B25, CS-B15sh, and CS-B22Lo) had significant negative effects. DP90, SG747, PSC355, ST474, and CS-B18 had significant negative cytoplasmic effects for SV while CS-B02, CS-B04, CS-B25, CS-B05sh, CS-B22sh, CS-B22Lo, and 3-79 had significant positive effects. All five cultivars, TM-1, and CS-B22sh had significant negative cytoplasmic effects for EP while CS-B04, CS-B06, CS-B07, CS-B17, CS-B05sh, CS-B14sh, CS-B15sh, and 3-79 had significant positive effects.

**Table 2** Variance components expressed as proportions of the phenotypic variances for seed traits

| Parameter | Protein | Oil | Fiber | PI | OI |
|---|---|---|---|---|---|
| $V_C$ | 0.000 | 0.226** | 0.000 | 0.012 | 0.084** |
| $V_A$ | 0.119** | 0.032 | 0.103** | 0.291** | 0.108** |
| $V_D$ | 0.000 | 0.416** | 0.000 | 0.000 | 0.328** |
| $V_{CE}$ | 0.246** | 0.127** | 0.190** | 0.108** | 0.070 |
| $V_{AE}$ | 0.000 | 0.015 | 0.000 | 0.000 | 0.000 |
| $V_{DE}$ | 0.322** | 0.000 | 0.540** | 0.385** | 0.335** |
| $V_e$ | 0.313** | 0.185** | 0.166** | 0.204** | 0.076** |

| Parameter | FI | SI | SV | EP |
|---|---|---|---|---|
| $V_C$ | 0.010 | 0.024* | 0.156** | 0.197** |
| $V_A$ | 0.265** | 0.316** | 0.199** | 0.212** |
| $V_D$ | 0.019 | 0.279** | 0.322** | 0.000 |
| $V_{CE}$ | 0.126** | 0.024 | 0.000 | 0.000 |
| $V_{AE}$ | 0.000 | 0.000 | 0.005 | 0.000 |
| $V_{DE}$ | 0.384** | 0.257** | 0.000 | 0.397** |
| $V_e$ | 0.196** | 0.100** | 0.319** | 0.194** |

*PI* seed protein index, *OI* seed oil index, *FI* seed fiber index, *SI* seed index, *SV* seed volume, *EP* embryo percentage

*,** Significant at probability levels of 0.05 and 0.01, respectively

**Table 3** Cytoplasmic effects for seed traits

| | Oil (%) | OI (g) | SI (g) | SV (ml) | EP (%) |
|---|---|---|---|---|---|
| DP90 | 1.281** | 0.141** | 0.105* | −0.352** | −0.568** |
| SG747 | 0.525** | −0.029** | −0.241** | −1.283** | −2.108** |
| PSC355 | 0.997** | 0.108** | 0.062** | −0.534** | −1.822** |
| ST474 | 1.122** | 0.063** | −0.094** | −0.374** | −1.505** |
| FM966 | 0.617** | 0.114** | 0.189** | 0.127 | −1.671** |
| TM-1 | −0.414** | −0.027** | 0.025 | 0.076 | −0.352* |
| CS-B02 | 0.040 | 0.006 | 0.006 | 0.412** | 0.107 |
| CS-B04 | 0.035 | −0.016 | −0.070 | 0.264** | 0.444** |
| CS-B06 | −0.515** | −0.066** | −0.081 | 0.053 | 0.665** |
| CS-B07 | −0.185 | −0.014 | −0.013 | 0.104 | 1.208** |
| CS-B16 | −0.964** | −0.096** | −0.031 | −0.008 | −0.128 |
| CS-B17 | 0.039 | 0.008 | 0.011 | −0.012 | 0.858** |
| CS-B18 | −0.015 | −0.045 | −0.148 | −0.296** | 0.522 |
| CS-B25 | −0.411* | −0.080** | −0.134** | 0.354** | 0.270 |
| CS-B05sh | −0.107 | 0.020 | 0.090** | 0.174** | 0.938** |
| CS-B14sh | −0.183 | −0.032** | −0.055 | 0.017 | 1.089** |
| CS-B15sh | −0.630** | −0.079** | −0.093* | −0.013 | 1.079** |
| CS-B22sh | −1.212** | −0.059 | 0.207** | 0.478** | −0.835** |
| CS-B22Lo | −1.123** | −0.110** | −0.094* | 0.252** | −0.228 |
| 3-79 | 1.103** | 0.195** | 0.361** | 0.559** | 2.036** |

*OI* seed oil index, *SI* seed index, *SV* seed volume, *EP* embryo percentage

*,** Significant at probability levels of 0.05 and 0.01, respectively

## Additive effects

Additive effects, which are contributed by the seed nuclear genes, are equivalent to general combining ability effects for inbred line development. DP90, CS-B04, CS-B25, CS-B14sh, and CS-B15sh had negative additive effects for

**Table 4** Additive effects for eight seed traits

| | Protein (%)[a] | Oil (%) | Fiber (%) | PI (g) | OI (g) | FI (g) | SI (g) | SV (ml) |
|---|---|---|---|---|---|---|---|---|
| DP90 | −0.577**·* | −0.128*·* | −0.213 | −0.213**·* | −0.125**·* | −0.162**·* | −0.656**·* | −0.465**·* |
| SG747 | 0.447**·* | −0.343*·* | −0.057 | −0.025 | −0.103**·* | −0.071**·* | −0.297**·* | 0.148** |
| PSC355 | 0.691**·* | −0.030 | −0.871**·* | 0.001 | −0.049**·* | −0.119**·* | −0.278**·* | −0.046 |
| ST474 | 0.593**·* | −0.210*·* | 0.458 | −0.038**·* | −0.093**·* | −0.052**·* | −0.385**·* | −0.331**·* |
| FM966 | 0.577**·* | 0.198 | −1.136**·* | 0.080**·* | 0.044** | −0.080**·* | 0.055 | −0.001* |
| TM-1 | −0.042 | 0.176* | −0.054 | 0.028** | 0.051** | 0.022 | 0.132** | 0.118** |
| CS-B02 | 0.002**·* | −0.073 | −0.178 | 0.017** | −0.001 | 0.004 | 0.084** | 0.087* |
| CS-B04 | −0.565**·* | 0.206* | 0.969**·* | −0.083**·* | 0.018**·* | 0.050**·* | −0.098**·* | −0.132**·* |
| CS-B06 | 0.201 | 0.127 | −0.305* | 0.052**·* | 0.040** | 0.003 | 0.124** | 0.080** |
| CS-B07 | −0.135 | 0.019 | 0.163 | −0.041**·* | −0.015**·* | −0.011 | −0.104**·* | −0.142**·* |
| CS-B16 | −0.128 | 0.020 | −0.147 | 0.026** | 0.030** | 0.024** | 0.164** | 0.066 |
| CS-B17 | 0.034 | 0.128* | −0.525** | 0.079**·* | 0.070**·* | 0.024** | 0.305**·* | 0.314**·* |
| CS-B18 | −0.030 | 0.175* | −0.764**·* | −0.005 | 0.029** | −0.061**·* | 0.001 | −0.120**·* |
| CS-B25 | −0.371**·* | 0.181 | 0.302**·* | 0.035** | 0.082**·* | 0.093**·* | 0.311**·* | 0.116** |
| CS-B05sh | −0.096* | 0.032 | −0.195* | −0.101**·* | −0.051**·* | −0.093**·* | −0.356**·* | −0.425**·* |
| CS-B14sh | −0.320**·* | 0.004 | 0.404**·* | −0.044**·* | −0.006* | 0.024 | −0.042*·* | −0.193**·* |
| CS-B15sh | −0.249**·* | 0.202* | −0.091 | 0.086**·* | 0.106**·* | 0.092**·* | 0.445**·* | 0.315**·* |
| CS-B22sh | 0.118 | −0.027 | 0.021 | 0.018 | 0.001* | 0.007 | 0.031 | 0.054 |
| CS-B22Lo | −0.090 | −0.179*·* | 0.174*·* | −0.122**·* | −0.102**·* | −0.088**·* | −0.453**·* | −0.466**·* |
| 3-79 | −0.060 | −0.477*·* | 2.043**·* | 0.250**·* | 0.073** | 0.393**·* | 1.018**·* | 1.022**·* |

*PI* seed protein index, *OI* seed oil index, *FI* seed fiber index, *SI* seed index, *SV* seed volume

[a] Difference from zero in the first column and difference from TM-1 in the second column after each value

*·** Significant at probability levels of 0.05 and 0.01, respectively

seed protein content whereas SG747, PSC355, ST474, and FM966, and CS-B06 had positive effects (Table 4). DP90, SG747, ST474, CS-B22Lo, and 3-79 had negative additive effects for seed oil percentage while TM-1, CS-B04, CS-B17, CS-B18, and CS-B15sh had positive effects. PSC355, FM966, CS-B17, and CS-B18 had positive additive effects for seed fiber content while CS-B04 and 3-79 had negative effects. DP90, ST474, CS-B04, CS-B07, CS-B05sh, CS-B14sh, and CS-B22Lo had negative additive effects for PI while FM966, TM-1, CS-B06, CS-B17, CS-B15sh, and 3-79 had positive effects. All cultivars except FM966, CS-B05sh, and CS-B22Lo were associated with negative additive effects for OI while FM966, CS-B04, CS-B06, CS-B16, CS-B17, CS-B18, CS-B25, CS-B15sh, and 3-79 were associated with positive effects. DP90, SG747, PSC355, CS-B18, CS-B05sh, and CS-B22Lo were associated with negative additive effects for seed FI while CS-B04, CS-B25, CS-B15sh, and 3-79 were associated with positive effects.

For each CS-B line, there is only one chromosome or chromosome arm divergent compared to the recurrent parent TM-1, the significant deviation of additive effect of a CS-B line from TM-1 could be considered as a chromosome-specific additive effect associated with a seed trait. TM-1 had a significant positive additive effect for oil content, PI, OI, SI and SV and no significant additive effect for protein and fiber content and FI. A comparison of TM-1 and CS-B lines for additive effects showed that several chromosomes from *G. barbadense* had significantly different effects than TM-1 (*G. hirsutum*) chromosomes. Some *G. barbadense* chromosomes had larger effects and some smaller effects for specific traits than the corresponding *G. hirsutum* chromosome (Table 4). With the comparative method and confidence interval test, the chromosome associations with these traits could be detected. For example, chromosome 15sh from *G. barbadense* had significantly greater additive effects than the corresponding chromosome 15sh from *G. hirsutum* for 5 of the 8 seed traits. Also *G. barbadense* chromosome 4 was associated with a reduced additive effect for protein content, while chromosome 6 was associated with an increased additive effect for this trait (Table 4). Other chromosome associations with these seed traits could also be found. Positive chromosome associations could be used to improve seed traits compared to the corresponding *G. hirsutum* chromosome TM-1. This

knowledge can be used by cottonseed breeders in introgressing chromosome and trait-specific genes into *G. hirsutum*.

## Dominance effects

Two types of dominance effects, homozygous and heterozygous dominance effects (Tables 5, 6) were predicted. Large negative homozygous dominance effects are associated with high inbreeding depression following the selfing of hybrids, whereas parents having large heterozygous dominance effects are more likely to produce high heterosis (Jenkins et al. 2006, 2009).

### *Homozygous dominance effects*

Nine out of 20 parents had significant negative homozygous dominance effects for oil content while four parents had positive homozygous dominance effects (Table 5). Nine parents had negative homozygous dominance effects for OI while four parents had positive homozygous dominance effects for OI. Ten parents had negative homozygous dominance effects for SI and eight parents had positive homozygous dominance effects. Six parents had negative homozygous dominance effects for SV and eight parents had positive homozygous dominance effects. These positive homozygous dominance effects can be captured as useful genetic variances in self-pollinated crops such as cotton.

Numerically, there were 9, 10, 9, and 8 CS-B lines with homozygous dominance effects lower than TM-1 for oil content, OI, SI, and SV, respectively (Table 5). On the other hand, there were 4, 3, 4, and 5 CS-B lines greater than TM-1 with respect to homozygous dominance effects for these traits. With the comparative method and confidence interval test, the chromosome associations with these traits could be detected. For example, chromosomes 2 and 16 and chromosome arms 22sh and 22Lo from *G. barbadense* were associated with reduced homozygous dominance effects for oil content, while chromosomes 17 and 18 from *G. hirsutum* were associated with increased homozygous dominance effects for this trait (Table 5). Other chromosome associations with these seed traits are described in Table 5. Positive chromosome associations could be used to improve seed traits compared to TM-1.

### *Heterozygous dominance effects*

Significant heterozygous dominance effects for seed oil content, seed OI, SI, and SV existed among these hybrids (Table 6). The numbers of significant heterozygous dominance effects for oil content, OI, SI, and SV were 39, 45, 50, and 44, respectively. No patterns for these dominance effects were observed by male or female parents. For example, for the same female parent DP90, crosses DP90 × CS-B07 and DP90 × CS-B25 had large positive dominance effects for oil content (2.34 and 1.90%, respectively); however, cross DP90 × CS-B22sh had a

**Table 5** Homozygous dominance effects for four seed traits

| | Oil (%)[a] | OI (g)[a] | SI (g)[a] | SV (ml)[a] |
|---|---|---|---|---|
| DP90 × DP90 | −1.404**·* | −0.436**·* | −1.264**·* | −0.531**·* |
| SG747 × SG747 | −1.771**·* | −0.235**·* | −0.253**·* | 0.926**·* |
| PSC355 × PSC355 | −0.803**·* | −0.215**·* | −0.554**·* | 0.218 |
| ST474 × ST474 | −1.634**·* | −0.287**·* | −0.570**·* | −0.312**·* |
| FM966 × FM966 | 0.394 | 0.016 | −0.112* | −0.071 |
| TM-1 × TM-1 | 0.152** | 0.055** | 0.167** | 0.151** |
| CS-B02 × CS-B02 | −0.153**·* | 0.004 | 0.095** | 0.305** |
| CS-B04 × CS-B04 | 0.532**·* | 0.014 | −0.180**·* | 0.021 |
| CS-B06 × CS-B06 | −0.038 | 0.006 | 0.041 | 0.104** |
| CS-B07 × CS-B07 | −0.079 | −0.035*·* | −0.125**·* | −0.076 |
| CS-B16 × CS-B16 | −0.611**·* | −0.034**·* | 0.138** | 0.057 |
| CS-B17 × CS-B17 | 0.342** | 0.114** | 0.332** | 0.287** |
| CS-B18 × CS-B18 | 0.423** | 0.007 | −0.161**·* | −0.273**·* |
| CS-B25 × CS-B25 | 0.163 | 0.058** | 0.179** | 0.300** |
| CS-B05sh × CS-B05sh | 0.005 | −0.061**·* | −0.275**·* | −0.302**·* |
| CS-B14sh × CS-B14sh | −0.114 | −0.036**·* | −0.105**·* | −0.171**·* |
| CS-B15sh × CS-B15sh | 0.067 | 0.094** | 0.364**·* | 0.287** |
| CS-B22sh × CS-B22sh | −0.896**·* | −0.049 | 0.260** | 0.309** |
| CS-B22Lo × CS-B22Lo | −1.210**·* | −0.248**·* | −0.578**·* | −0.297**·* |
| 3-79 × 3-79 | −0.423**·* | 0.275**·* | 1.464**·* | 1.258**·* |

*OI* seed oil index, *SI* seed index, *SV* seed volume

[a] Difference from zero in the first column and difference from TM-1 in the second column after each value, respectively

*·** Significant at probability levels of 0.05 and 0.01, respectively

**Table 6** Heterozygous dominance effects for four seed traits

| | DP90[a,b] | SG747[b] | PSC355[b] | ST474[b] | FM966[b] |
|---|---|---|---|---|---|
| Oil (%) | | | | | |
| TM-1[c] | −0.250 | −0.376 | 1.355** | 0.397* | −0.269 |
| CS-B02 | 0.443**·* | 0.681**·* | −0.791**·* | 0.506 | −1.014**·* |
| CS-B04 | −0.004 | −0.381 | 0.383 | 0.156 | 0.138 |
| CS-B06 | −0.249 | −0.494 | 1.611** | 1.009**·* | −0.963** |
| CS-B07 | 2.338**·* | −0.169 | −1.062**·* | −1.370**·* | 0.549* |
| CS-B16 | 0.229*·* | −1.078**·* | 0.614** | 1.949**·* | −0.362** |
| CS-B17 | −0.002 | 1.800**·* | −0.063 | −1.486**·* | −0.092 |
| CS-B18 | 1.101 | −0.554 | −0.353* | 0.164 | −0.048 |
| CS-B25 | 1.902**·* | −3.373**·* | 0.775** | −0.190 | 1.743**·* |
| CS-B05sh | 0.522**·* | −0.803** | 0.926** | −0.608**·* | 0.161 |
| CS-B14sh | −0.509* | −0.219 | −0.080* | 0.068 | 0.995**·* |
| CS-B15sh | 0.031 | 1.830**·* | −0.411* | 0.430* | −0.687** |
| CS-B22sh | −2.342**·* | 2.431**·* | 1.370** | −0.235* | 0.386* |
| CS-B22Lo | −0.975**·* | 1.792**·* | −0.371 | 0.630** | 0.165 |
| 3-79 | −0.272 | 0.197 | −2.493**·* | 0.461 | −0.186 |
| OI (g) | | | | | |
| TM-1 | −0.126** | −0.086* | 0.418** | 0.086* | −0.195** |
| CS-B02 | −0.039 | 0.150**·* | 0.026 | −0.156**·* | 0.008* |
| CS-B04 | −0.136** | −0.005 | 0.062 | 0.167** | −0.041*·* |
| CS-B06 | −0.042 | 0.025 | 0.238** | 0.080 | −0.149** |
| CS-B07 | 0.309**·* | 0.088**·* | −0.338**·* | −0.038 | −0.011* |
| CS-B16 | 0.121**·* | −0.228**·* | 0.159**·* | 0.211** | −0.071 |
| CS-B17 | −0.067 | 0.263**·* | 0.142**·* | −0.222**·* | −0.057 |
| CS-B18 | 0.211*·* | 0.031 | −0.101**·* | 0.005 | −0.041* |
| CS-B25 | 0.258**·* | −0.363**·* | −0.006* | 0.004 | 0.323**·* |
| CS-B05sh | 0.055 | −0.088 | −0.193**·* | 0.006 | 0.135**·* |
| CS-B14sh | 0.074 | 0.024 | −0.072* | −0.183**·* | 0.204**·* |
| CS-B15sh | 0.072*·* | 0.194**·* | 0.016* | 0.093** | −0.134** |
| CS-B22sh | −0.248** | 0.037 | 0.116**·* | 0.048 | 0.148**·* |
| CS-B22Lo | −0.318**·* | 0.255**·* | 0.078**·* | 0.103** | −0.036* |
| 3-79 | 0.241**·* | −0.241**·* | −0.315**·* | −0.005 | 0.066* |
| SI (g) | | | | | |
| TM-1 | −0.388** | −0.207 | 1.092** | 0.232 | −0.691** |
| CS-B02 | −0.393** | 0.355**·* | 0.461** | −0.894**·* | 0.516**·* |
| CS-B04 | −0.543** | 0.116 | 0.093* | 0.665** | −0.246**·* |
| CS-B06 | −0.055 | 0.314**·* | 0.272* | −0.073 | −0.193* |
| CS-B07 | 0.286 | 0.440**·* | −0.929**·* | 0.418** | −0.259**·* |
| CS-B16 | 0.397**·* | −0.499** | 0.373**·* | 0.001 | −0.091 |
| CS-B17 | −0.292**·* | 0.370**·* | 0.627** | −0.270**·* | −0.247**·* |
| CS-B18 | 0.459*·* | 0.364**·* | −0.277*·* | −0.043 | −0.179 |
| CS-B25 | 0.284 | 0.060 | −0.415**·* | 0.128 | 0.455**·* |
| CS-B05sh | 0.009 | −0.127 | −1.170**·* | 0.288** | 0.553**·* |
| CS-B14sh | 0.510**·* | 0.227**·* | −0.203**·* | −0.829**·* | 0.387**·* |
| CS-B15sh | 0.290**·* | 0.088 | 0.249* | 0.201 | −0.310 |
| CS-B22sh | −0.127 | −0.937**·* | −0.110 | 0.284** | 0.457**·* |
| CS-B22Lo | −0.964**·* | 0.258*·* | 0.514** | 0.207** | −0.124* |
| 3-79 | 1.217**·* | −1.146**·* | −0.246*·* | −0.255 | 0.349** |

**Table 6** continued

| | DP90[a,b] | SG747[b] | PSC355[b] | ST474[b] | FM966[b] |
|---|---|---|---|---|---|
| SV (ml) | | | | | |
| TM-1 | −0.170 | −0.218 | 1.103** | 0.018 | −0.743** |
| CS-B02 | −1.211**·* | 0.889**·* | 1.140** | −1.019**·* | −0.193 |
| CS-B04 | −1.157**·* | 0.127 | −0.966**·* | 1.206**·* | 0.422**·* |
| CS-B06 | −0.613**·* | 0.423**·* | −0.391*·* | 0.149 | 0.423**·* |
| CS-B07 | −0.545** | −0.067 | −0.343**·* | 0.718**·* | 0.036* |
| CS-B16 | −0.174 | −1.162**·* | 0.739** | 0.724*·* | −0.079 |
| CS-B17 | −0.126 | −0.181 | 0.092 | 0.062 | 0.362** |
| CS-B18 | 0.510 | 0.472*·* | −0.340 | −0.333 | −0.064 |
| CS-B25 | 1.373**·* | −0.270* | −1.086** | −0.065 | −0.262 |
| CS-B05sh | 0.173 | −0.416** | −0.703** | −0.169 | 0.662**·* |
| CS-B14sh | 0.125 | 0.634**·* | −0.203* | −0.778**·* | 0.082 |
| CS-B15sh | 0.403**·* | 0.367*·* | 0.629**·* | −0.473**·* | −0.715** |
| CS-B22sh | 0.469*·* | −0.678**·* | 0.132 | −0.399 | −0.009* |
| CS-B22Lo | −0.837**·* | −0.874**·* | −0.060* | 0.998**·* | 0.208 |
| 3-79 | 1.685**·* | −0.530 | −0.296*·* | −0.840**·* | 0.010* |

*OI* seed oil index, *SI* seed index, *SV* seed volume

[a,c] Female and male parents, respectively

[b] Difference from zero in the first column and difference from TM-1 in the second column after each value, respectively

*·** Significant at probability levels of 0.05 and 0.01, respectively

large negative effect for this trait (−2.34%). On the other hand, comparing different female parents (DP90 and SG474) with the same male parent (CS-B22sh), the cross SG747 × CS-B22sh had a large positive dominance effect for oil content (2.43%). Similar phenomena in Table 6 could be found for other crosses for this trait and the other three seed traits. The results suggested that heterozygous dominance effects were cross-dependent and a large number of crosses are needed to find the useful heterozygous dominance effects which can be utilized for seed trait improvement in a hybrid form.

Comparing the heterozygous dominance effects between a CS-B line and TM-1 with the same female parent allows for the detection of heterozygous dominance effects associated with a specific *G. barbadense* chromosome (chromosome arm) interacting with the homologous chromosome in the female *G. hirsutum* parent. No patterns for specific chromosome associations with heterozygous dominances by female parents or by male parents could be found in this study. For example, chromosomes 2, 7, 18, 25, and chromosome arm 5sh of 3-79 were associated with increased heterozygous dominance effects with DP90 as female parent for seed oil content whereas chromosome arms 22sh and 22Lo were associated with reduced effects with the same DP90 female parent (Table 6). Heterozygous dominance effects for chromosomes 2 and 17, and chromosome arms 15sh, 22sh, and 22Lo with SG747 as a female parent were associated with improved seed oil content while the effects for chromosomes 16 and 25 with the SG474 female parent were associated with reduced oil content. Various chromosome associations with heterozygous dominance effects for the other seed traits can be observed (Table 6).

## Discussion

As previous reports indicated, cottonseed traits may be related to complicated genetic components. With the nature of the data structure ($F_{3}$'s and parents with one replication in each of four environments) in this study, there were several challenging issues to be addressed. Three different genetic models could be applied to this data set, the AD model (Zhu 1992; Wu et al. 1995b), the AD model with maternal genetic effects (ADM model) (Zhu 1992), and the ADC model. Thus, we first determined which genetic model was the most appropriate for our data set. Our simulated results indicated that these models gave unbiased estimations for variance components (simulated results for the AD and ADM models not shown); however, a need existed to determine which model was more appropriate for these seed traits. Therefore, the data analyses for these three genetic models were conducted separately and the residual variances were the smallest for the ADC model. Thus, the ADC model was used for our data analyses.

A second issue involved which resampling method should be used to test for each parameter. When a jackknife method is used, standard deviations for each parameter can be calculated and thus an approximate *t* test can be used to detect the significance for each parameter. However, the pseudo-based jackknife method (Miller 1974) with removal of one observation could result in a large variation for each parameter, thus the statistical power is unacceptably low. On the other hand, the non-pseudo-based jackknife method (Wu et al. 2008) with removal of one observation could result in extremely high Type I error. Type I error and testing power vary with different numbers of individual being removed. According to our simulations, the non-pseudo-based jackknife methods with removal of about 8–10% of total observations each time were recommended to reach favorable power with acceptable Type I error for these data which are without replications. As expected, simulation showed that testing power was much lower for dominance effects for multiple-environment data without replication compared to the data with replications. On one hand, the simulated results indicated that the statistical tests for the parameters obtained from data without replications are conservative. On the other hand, it could be a reasonable option to measure seed traits in multiple environment without replication when limited seed supply or financial resources are the constraint. In this study, each seed sample costs $45 to measure five nutrition components. With this approach, the cost for measuring five seed traits was $17,100 ($4 \times 95 \times 45$) without replication while it could cost $64,400 ($16 \times 95 \times 45$) with four replications. In addition, with the flexibility of the mixed linear model approaches, the applications of the ADC genetic model were not limited to the data structure used in this study. Our additional simulation results suggested that the MINQUE approach can be applied for various seed data structures such as parents with $F_1$, $F_2$, and/or $F_3$ hybrids with or without replications.

A cytoplasmic effect is contributed by the cytoplasmic genes from the female parent. Thus, cytoplasmic genes can be transferred to the next generation during crossing or selfing and are expressed as additive effects due to their haploidy. Therefore, once desirable cytoplasmic effects are detected, they can be used as female parents for a target trait improvement. In this study, significant cytoplasmic effects were detected for seed oil content and OI, SI, SV, and seed EP. All five elite cultivars were detected to have large positive cytoplasmic effects for seed oil content, indicating these cultivars can be used as female parents for oil improvement. However, the use of CS-B06, CS-B16, CS-B15sh, CS-B22sh, and CS-B22Lo as female parents could cause a reduction in seed oil. On the other hand, additive effects are equivalent to general combining ability effects for inbred line development. Our results showed that four cultivars had large positive additive effects for protein content, and negative additive effects for oil content. These same cultivars had no significant cytoplasmic effects on protein content, but significant positive cytoplasmic effects for oil content. The results suggest using these cultivars as female parents, one can capture both the cytoplasmic and the additive effects and improve seed oil and protein contents at the same time.

One of the advantages of using the CS-B lines for cottonseed traits analyses was that various chromosome associations, which are related to specific chromosomes or chromosome arms of 3-79 in the TM-1 background, with additive and both homozygous and heterozygous dominance effects for cottonseed traits can be detected by the comparative method. Using the chromosome model (Wu et al. 2006), it will also be possible to determine chromosome associations with seed traits in these cultivars or other inbred lines that are used to cross with these CS-B lines.

As mentioned, the genetic system for cottonseed traits is very complicated. However, some maternal effects could be confounded with cytoplasmic effects. One way to separate cytoplasmic effects from maternal effects is to obtain reciprocal hybrid seeds at $F_1$ and $F_2$. If a significant difference is observed between reciprocal $F_1$ hybrids but not between reciprocal $F_2$ hybrid seeds, this is strong evidence of maternal effects. On the other hand, if a significant difference is observed between reciprocal $F_1$ hybrids and between reciprocal $F_2$ hybrid seeds, this is an indication of cytoplasmic effects. In addition to cytoplasmic effects being detected in our study, other genetic components may be involved in cottonseed traits (Dani and Kohel 1989; Wu et al. 1995a; Wang et al. 1996a, b). Thus, more complicated genetic models are available and may be utilized for further genetic studies (Zhu and Weir 1994a, b); however, these models require a more complicated genetic data structure.

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