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Combining ability, heritability, and genotypic correlations for lint yield and fiber quality of Upland cotton in delayed planting

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ABSTRACT

Delayed planting is a necessary practice in Upland cotton (*Gossypium hirsutum* L.) production when double cropping cotton with small grain crops. All current cultivars used for delayed planting were selected under environments of normal planting. The information about genetic values of parents in delayed planting will help determine if it is necessary for testing parents under environments of delayed planting before their use in development of cultivars for the delayed planting system. General combining ability (GCA), specific combining ability (SCA), broad sense heritability, and genetic correlation were compared between two treatments of planting date, the normal planting (mid-April to early May) and delayed planting (late May). Ten F_2 hybrid populations derived from a half diallel crossing design and their five parents were planted in 2012 and 2013 with four replicates each year. GCA effects for lint yield and two yield components of boll no. and seeds per boll were different between delayed planting and normal planting. Both direction changes of SCA effects for yield components were observed between treatments of planting date. However, effects of SCA × year were not significant in delayed planting. Reductions in broad sense heritability of yield traits were observed in the delayed planting (0.01–0.25) compared with that of the normal planting (0.10–0.88). Combining ability effects and heritability for fiber properties were similar between treatments of planting date. Genotypic correlation between lint yield and fiber strength became more negative, −0.83, in delayed planting compared with −0.34 in normal planting. The change of genotypic correlations between lint yield and fiber quality in the delayed planting implies an increase of complexity for interrelationships among fiber traits in the double cropped cotton. Overall, the results should inform cotton breeders the need of testing parents for lint yield under environments of delayed planting.

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1. Introduction

Delayed planting might sometime be a necessary practice in the production of Upland cotton (*Gossypium hirsutum* L.) due to adverse weather conditions such as cold temperature and flooding which can prevent equipment from getting into the field or compromise stand establishment to the point that growers might have to make a decision for replanting in order to avoid loss of profit (Wrather et al., 2008). Double cropping cotton with small grain crops such as wheat (*Triticum aestivum* L.) was suggested as an alternative to the double cropping soybean (*Glycine max* L. Merr.) with wheat (Smith and Varvil, 1982; Bauer et al., 1998). In the Mississippi Delta region of U.S., cotton growers plant in mid-April, late April, or early May as a normal practice (Wrather et al., 2008; Boquet and Clawson, 2009). In this region, a typical harvest time for wheat is in late May or early June. A delayed planting of cotton during this time is necessary in order to double cotton with wheat.

Effects of planting date on cotton lint yield and fiber quality have been analyzed in many studies. In the mid-south of U.S., there were reports for effects of planting dates on lint yield and fiber quality (Smith and Varvil, 1982; Cathey and Meredith, 1988; Pettigrew, 2002; Wrather et al., 2008; Boquet and Clawson, 2009; Pettigrew et al., 2009). Four cotton cultivars with different maturity types were monocropped or double cropped after wheat (Smith and Varvil, 1982) during 1976 and 1979 in Arkansas, U.S. The double cropped cotton of a short season cultivar yielded 35 to 50% less than the monocropped while the double cropped cotton of full season cultivars yielded 50 to 65% less than the monocropped. Five cultivars with early, intermediate, and full season maturity types were planted in mid-April, early May, and mid-May during

Abbreviations: Elg, elongation; GCA, general combining ability; MIC, micronair; PD, planting date; SCA, specific combining ability.

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1982 and 1984 (Cathey and Meredith, 1988). Plants of the late planting had greater height, less flowers, and lower lint yield in this study. Another five years experiment was conducted in this region at different planting dates in late April, early May, mid-May, and late May under four planting densities (Wrather et al., 2008). Lint yield and lint percentage of plants planted in late April were greater than those planted in May. Micronaire of the late planted plants was significantly lower than those planted in late April. Six to eight Upland cotton cultivars were planted at eight planting dates between 25 March and 5 June during 2002 and 2005 (Boquet and Clawson, 2009). Optimum planting dates for lint yield was determined to be between 15 April and 5 May and planting dates after 5 May had yield decline. In summary, lint yield of the late planted cotton declined compared with the normal planted cotton with no exception among different studies conducted in this region. Mixed results were observed for effects of planting dates on lint yield and fiber quality in other studies conducted in the southeast and south regions of U.S. Cotton was planted 23 April, 28 April, and 1 May as full season crop and planted after wheat on 1 June, 10 June, and 18 June, in 1982, 1983, and 1984, respectively, in Tifton, GA (Baker, 1987). All double cropped cotton had lower yield than the earlier planted full season cotton. Three cotton cultivars were planted in the late April and late May in the Southeast Coastal Plain (Bauer et al., 1998). Plants planted in late May had higher fiber strength, elongation, and fiber length and lower micronaire and maturity with no yield reduction in two of the three experimental years in this study. In the same region, Buntin et al. (2002) identified that delayed cotton planting did not consistently affect plant stand, but significantly reduced seed cotton yield in a two years experiment of double cotton with Canola (*Brassica napus* L.). In south Texas, planting was delayed due to unusually adverse weather in spring 1997. However, twenty-two fields of the early and medium-early cotton cultivars yielded higher than the historical average (Scott et al., 1998). The yield advantage of the delayed planting in this survey was either due to early maturity of the cultivars planted in these fields or the long season in south Texas.

Although reports on effects of planting dates on lint yield and fiber quality were numerous, the reports on genetic values of yield and fiber quality under delayed planting were rare. Development of cotton cultivars suitable for late planting system might be necessary as suggested by May and Bridges (1995) because current cultivars including both full season and early maturing types were selected for the normal planting system. However, results in a few experiments were inconsistent on supporting this hypothesis. Effects of genotype × planting date for lint yield and lint percentage were significant in a study of six cotton cultivars with different maturing types at early and late planting dates (Porter et al., 1996). Green and Culp (1990) evaluated 33 advanced Pee Dee germplasm lines at normal planting date (May 1) and late planting date (June 1) between 1987 and 1989 and ranked them for lint yield and fiber strength. Two germplasm lines ranked at top for the normal planting, but ranked nearly at bottom for the late planting. These studies indicate the necessity to develop cotton cultivars with high stability for agronomic performance in late planting system. Twenty five Pee Dee germplasm lines and two check cultivars were evaluated at normal planting and late planting systems in Florence, SC (May and Bridges, 1995). No significant genotype × planting system interactions were observed for lint yield and fiber quality in that study. Selection gain for lint yield of late planting was 2.1% increase from the lines selected in the normal planting system. Selection for 2.5% span length and strength in normal planting system also resulted gain in both late and normal planting systems. Because selection of parents for their combining ability is critical in breeding, it is necessary to determine combining ability of parents for lint yield and fiber properties in delayed planting. It is hypothesized that the genetic values of F_2 hybrids are similar between the delayed planting and normal planting.

In this study, F_2 hybrids derived from crosses among five germplasm lines and breeding line of a half diallel design were planted at a late planting date and a normal planting date in 2012 and 2013, respectively. The objective of this experiment was to determine general combining ability, specific combining, and heritability of yield traits and fiber properties in the F_2 hybrids under the treatments of delayed planting and normal planting. Another objective was to analyze genotype correlations among fiber traits in delayed planting. It is expected that this information about genetic values of F_2 hybrids in delayed planting will be useful in determination of necessity to test parents under environments of delayed planting.

2. Materials and methods

2.1. Genetic material

Three elite germplasm lines including MD52ne (PI 634930, Meredith, 2005), Deltapine 90ne (DP90ne, Bowman et al., 2006), and TAM98D-99ne (Thaxton et al., 2005; PI 636491), one exotic germplasm line SP103, and one breeding line, JJ1145ne, were crossed in a half diallel design. These lines were selected for this study based on their reputation on maturity with TAM98D-99ne and JJ1145ne as early type, DP90ne and MD52ne as intermediate early type, and SP103 as full season type. SP103 is a non-released germplasm line derived from species polycross (SP) germplasm population. This germplasm population was developed by U.S. cotton breeders in 1960s and 1970s through multiple crosses among five tetraploid species in *Gossypium* and followed by long terms of random mating and selfing as described by Zeng et al. (2007). JJ1145ne is a non-released breeding line derived from a cross of JAJO 9596/JAJO 9550. JAJO 9596 was selected from a cross of MD 51/LA 887 and JAJO 9550 was selected from a 4-way cross of [(LA 887/LA 850082) × (MD 51/LA 887)] (personal communication, Jack Jones, JAJO Genetics, Baton Rouge, LA). The crosses were made in the summer of 2011 at Delta Research Center, Stoneville, MS. The F_1 hybrids were grown and self-pollinated to produce F_2 seeds into a winter nursery at Colima, Mexico. Same source of F_2 seeds were used for the evaluation tests in 2012 and 2013.

2.2. Experiment design and planting

The ten F_2 populations and their five parents were evaluated at Stoneville, MS in 2012 and 2013. The experimental design was a split plot in a randomized complete block with four replications. In the split plot design, the treatments of planting date were arranged as main plots and genotypes were arranged as subplots. Subplots were fifteen single rows of 12.2 m × 1.0 m. Replications were nested within treatments of planting date to allow a continuous block with a same planting treatment in order to facilitate field management. This type of split plot design has advantages in irrigation management and pest control which can reduce experiment errors. These advantages were believed to offset their shortcomings compared with the typical split plot design in which treatments of planting date were randomized within replications (Popp et al., 2002; Boquet and Clawson, 2009). In 2012, seeds were planted on May 1 (normal planting) and May 25 (delayed planting). In 2013, seeds were planted on April 29 (normal planting) and May 29 (delayed planting). All other field practices followed conventional standard. The soil was a Beulah fine sandy loam (a coarse-loamy, mixed active thermic Typic Dystrochrepts). Due to lack of sufficient F_1 seeds, only F_2 plants and parents were analyzed in this study.

2.3. Plant harvest and fiber traits measurements

Fifty naturally open bolls from each plot were hand harvested randomly before mechanical picking. Boll samples from individual plots were ginned using a 10-saw laboratory gin. Lint percentage was calculated from each plot as percentage of lint weight from the boll sample divided by the lint weight plus seed weight of the boll sample from that plot. The remaining bolls from each plot were mechanically harvested by a spindle picker equipped with a weighing system for lint yield. In 2012, the plants of normal planting were defoliated on October 2 by a full rate of Ginstar with active ingredients of thidiazuron (12%) and diuron (6%) and mechanically picked on October 9. The plants of delayed planting were defoliated on October 9 by a full rate of Ginstar and mechanically picked on October 17. In 2013, the plants of normal planting were defoliated on September 27 by a full rate of Ginstar and mechanically picked on October 17. The plants of delayed planting were defoliated on October 4 by a full rate of Ginstar and mechanically picked on October 23. Total seed cotton weight of each plot was the sum of seed cotton weight of the sampled bolls and the seed cotton mechanically harvested from that plot. Lint yield of each plot was calculated from seed cotton weight of that plot and lint percentage of the same plot. Boll weight was calculated in each plot as seed cotton, i.e., lint weight plus seed weight of the boll sample, divided by 50. Lint index was calculated in each plot as lint weight (g) per 100 seeds, i.e., 100 [(lint wt. per boll sample)/(seed wt. per boll sample/seed wt. per seed)]. The yield component of seeds per boll was calculated as (seed wt. per boll sample/seed wt. per seed)/50. Twenty grams of lint from each boll sample were submitted to Cotton Fiber Laboratory, Louisiana State University AgCenter (Baton Rouge, LA) to determine fiber quality. The fiber properties of fiber length, fiber uniformity, fiber strength, elongation, and micronaire were determined using high volume instrument (HVI). Plant heights were measured before harvest as distances between soil surface and top points on main stems. The parameter of days to 1st open boll was measured in all plots as number of days when 1st open bolls were observed on nearly half of plants in that plot.

2.4. Statistical analysis

The GLIMMIX procedure in SAS (9.4) (SAS Institute, 2013) was used for analysis of variance. The categorical variables, year and planting date, and the interaction term of year × planting date were included in the model as fixed effects while genotype and replication and all interactions with these two terms were included in the model as random effects. To simplify the model, only significant terms were analyzed in the model. All the terms of replication and all interactions with replication including replication × year, replication × planting date, replication × genotype, replication × planting date × genotype were not significant for the analyzing parameters. These terms were removed from the model. Mean comparisons were analyzed in each year between treatments of planting date in output by SLICE statement in PROC GLIMMIX for the estimating parameters.

GCA and SCA effects for lint yield and fiber properties were analyzed using Griffing's diallel analysis (Griffing, 1956) Model 1, Method 2. GSA and SCA effects were estimated using the SAS codes published by Zhang and Kang (1997) and modified to fit the mixed linear model by PROC GLIMMIX. In order to analyze effects of GCA and SCA under each treatment of planting dates, these two variables and the variable of year were included in the model as fixed effects while replicate and all interactions with replicate were included in the model as random effects. Variance components of the additive ($2\sigma^2{}_{\mathrm{gca}}$), the non-additive ($\sigma^2{}_{\mathrm{sca}}$), and the environmental ($\sigma^2{}_{\mathrm{year}}$) were computed by PROC GLIMMIX. The broad sense heritability (H^2) for each planting treatment was calculated as the following equation.

$$H^2 = \frac{\left(2\sigma^2_{\mathrm{gca}} + \sigma^2_{\mathrm{sca}}\right)}{\left(\sigma^2_{\mathrm{year}} + \sigma^2_{\mathrm{year\times gca}} + \sigma^2_{\mathrm{year\times sca}} + \sigma^2_{\mathrm{yea\times gcar\times sca}} + 2\sigma^2_{\mathrm{gca}} + \sigma^2_{\mathrm{sca}}\right)}$$

2.5. Genotypic correlations

Genetic correlations (R_g) among fiber traits were calculated by the equation (Kempthorne, 1957):

$$R_g = \frac{\sigma^2_{\mathrm{gxy}}}{\left(\sigma^2_{\mathrm{gx}} \times \sigma^2_{\mathrm{gy}}\right)^{1/2}}; \sigma^2_{\mathrm{gx}} = \frac{1}{r\left(V_x - V_{\mathrm{ex}}\right)}; \sigma^2_{\mathrm{gy}} = \frac{1}{r\left(V_y - V_{\mathrm{ey}}\right)}; \sigma^2_{\mathrm{gxy}} = \frac{1}{r\left(\mathrm{Cov}_{\mathrm{xy}} - \mathrm{Cov}_{\mathrm{exy}}\right)}$$

where $\sigma^2{}_{\mathrm{gxy}}$ is the genotypic component of covariance between variable x and y; $\sigma^2{}_{\mathrm{gx}}$ and $\sigma^2{}_{\mathrm{gy}}$ are genotypic components of variance for x and y, respectively; V_{ex} and V_{ey} are errors for x and y, respectively; $r$is the number of replicates; Variance and covariance for fiber traits were calculated using MANOVA statement by PROC GLM procedures (SAS Institute, 2013).

3. Results and discussion

3.1. Fixed effects and variance components estimates for random effects

Year was a significant source of variation for all yield and fiber traits (Table 1). Effects of planting dates were significant on yield traits and most fiber properties (Table 1). The interactions of year and planting date were highly significant for most of traits analyzed.

There were ten F_2 populations and five parents analyzed in this study. Although the five parents for the diallel crossing design were selected for their different maturity reputation, the F_2 plants analyzed were considered as a portion of hybrids that will be produced in a continuing breeding program. The independent variable of genotype was included in the linear mixed model as a random effect because our interests will not be limited to the performance of these hybrids per se, but rather to lint yield and fiber quality performance for the late planting system in the current and future cultivars.

The variance component of genotype ($\sigma^2{}_g$) was significant for all traits analyzed except for boll no. (Table 2). The variance components of the first order interactions, i.e., $\sigma^2{}_{g\times \mathrm{Yr}}$and $\sigma^2{}_{g\times \mathrm{PD}}$, and the second order interactions, i.e., $\sigma^2{}_{g\times \mathrm{Yr}\times \mathrm{PD}}$, were significant for less than half of the traits analyzed. The variance component of $\sigma^2{}_{g\times \mathrm{Yr}\times \mathrm{PD}}$ was highly significant for lint yield, lint percentage, boll weight, and fiber length. In order to see if the variance component,

Table 1
Type III tests of fixed effects ($p > F$) for yield traits and fiber properties.

Sources	Lint yield	Lint percentage	Boll no	Boll wt.	Lint index	Seed boll^{-1}	Length	Strength	Elongation	Micronaire
Yr	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.112	0.070	0.016
PD[a]	0.001	0.001	0.003	0.001	0.001	0.001	0.001	0.094	0.094	0.001
Yr × PD	0.001	0.001	0.001	0.073	0.001	0.133	0.084	0.001	0.001	0.001

[a] PD, planting date.

Table 2
Variance component estimates for random effects of yield traits and fiber properties.

Sources	σ^2_g	$\sigma^2_{g\times Yr}$[a]	$\sigma^2_{g\times PD}$[b]	$\sigma^2_{g\times Yr\times PD}$	$\sigma^2_{residue}$
Lint yield	21836***	3611	0.00	9610**	125288***
Lint percentage × 10^{-4}	3.0***	0.03	0.00	0.19***	1.7*
Boll no × 10^8	6.5	4.3	1.2	11.9	274***
Boll wt.	0.18***	0.01	0.00	0.03***	0.18***
Lint index	0.31***	0.00	0.03***	0.00	0.23***
Seeds per boll	0.28*	0.12	0.11	0.04	5.5***
Length	0.15***	0.00	0.01	0.05***	0.20***
Strength	2.1***	0.18***	0.03	0.06	1.2***
Elongation	0.18***	0.01	0.01*	0.00	0.22***
Micronaire	0.01***	0.01***	0.00	0.00	0.04***

* Significant at $p \le 0.05$.
** Significant at $p \le 0.01$.
*** Significant at $p \le 0.001$.
[a] Variance component of genotype by year.
[b] Variance component of genotype by planting date.

$\sigma^2_{g\times Yr\times PD}$, caused significant crossover interactions, genotypes of F_2 hybrids and parents were ranked for lint yield at different planting dates in different years (data not shown). There were some genotypes ranked differently for lint yield with treatment of planting dates in different years. F_2 hybrids of MD52ne × TAM98D-99ne, SP103 × TAM98D-99ne, and SP103 × JJ1145ne ranked at bottom under normal planting while ranked either at top or middle under late planting in 2013. In 2012, these three hybrids ranked either at top or at bottom under both planting treatments. Overall, there was a lack of dramatic changes in rankings of other traits as responses to variance component of $\sigma^2_{g\times Yr\times PD}$ with significant second order interactions (data not shown).

Although variance component, $\sigma^2_{g\times Yr\times PD}$, was significant for half of traits, it was relatively small relative to σ^2_g (Table 2). For all traits except for micronaire, σ^2_g was 2 to 44 times greater than those interaction components associated with it. These results were consistent with lack of dramatic crossover interactions for these traits. Therefore, combining ability among parents could be estimated with combined data of F_2 hybrids across years.

3.2. Mean analysis of lint yield and fiber properties in parents and F_2

Significant difference among parents was observed for lint yield in delayed planting and normal planting (Table 3). JJ1145ne had highest lint yield, 1806 kg ha^{-1} in normal planting and highest lint yield, 1670 kg ha^{-1} in delayed planting. TAM98D-99ne had highest lint yield, 1680 kg ha^{-1}, in normal planting, but had lowest lint yield, 1283 kg ha^{-1}, in delayed planting. Significant difference among parents was also observed for yield components and fiber properties except for boll no. and seeds boll^{-1} in delayed planting (Table 3).

The significant interactions of year × planting date as shown in Table 1 indicated that effects of planting date were different across years. In both F_2 hybrids and parents, lint yield under normal planting was significantly higher than that of the delayed planting in 2012 (Table 4). In contrast, lint yield of F_2 hybrids and parents under delayed planting was either significantly higher or not different from that of the normal planting in 2013. This result can be explained by different responses of lint yield to the treatments of planting date under different weather conditions during the experimental years. In the early season of 2013, there was an unexpected cold weather with 15.7 °C of average minimum temperature in May in contrast to 19.1 °C average minimum temperature in May of 2012 (Table 5). The late growing season of 2013 was warmer than that in 2012, 16.7 vs. 13.7 °C for mean temperature in October of 2013 and 2012, respectively. The cold weather in early growing season and warm weather in the late growing season might have attributed to the high lint yield of the delayed planting in 2013. Same trend was observed for yield components. Reductions of lint percentage, boll weight, and lint index under the late planting ranged from 10 to 20% in 2012 whereas the reductions under the late planting ranged from 3 to 7% in 2013 (Table 4). The increase of lint yield in delayed planting compared with that in normal planting of 2013

Table 3
Means of yield and fiber traits in parents and F_2 hybrids of normal planting and delayed planting.

Genotypes	Lint yield (kg ha^{-1})	Lint percentage (%)	Boll no (10^5 ha^{-1})	Boll wt. g	Lint index (g)	Seeds boll^{-1}	Length (mm)	Strength (g tex)	Elongation (%)	Micronaire
Normal										
SP103	1225c[a]	34.7d	5.14b	6.63a	6.98c	33.0a	30.7ab	33.9d	4.90b	4.84b
MD52ne	1464b	37.2c	8.07a	4.81c	5.98d	30.0bc	30.9a	39.2a	4.90b	5.01ab
JJ1145ne	1806a	41.8a	7.27a	5.85b	7.93a	30.9ab	30.9a	33.8d	6.33a	4.88ab
TAM98D-99ne	1680ab	39.6b	8.43a	5.14c	7.35b	27.9c	30.0c	36.6b	6.00a	5.08a
DP90ne	1545ab	38.4bc	7.60a	5.24c	6.21d	32.3ab	30.2bc	35.3c	5.78a	4.94ab
Av. of parents	1544	38.3	7.30	5.53	6.89	30.8	30.5	35.8	5.58	4.95
F_2	1662	38.1	7.48	5.83	7.03	31.7	30.7	35.2	5.45	4.94
Delayed										
F_2										
SP103	1231bc	32.6c	5.94a	5.63a	6.28b	29.2a	31.7bc	33.7c	5.19b	4.13b
MD52ne	915c	33.1bc	5.65a	4.60b	5.40c	28.3a	32.2ab	38.1a	4.89b	4.34ab
JJ1145ne	1670a	37.5a	7.18a	5.59a	7.14a	29.4a	32.3a	34.5c	6.06a	4.28b
TAM98D-99ne	1283b	36.2a	6.31a	5.29a	6.50b	29.5a	31.3c	37.4a	5.34b	4.55a
DP90ne	1021bc	34.5b	5.91a	4.74b	5.51c	29.7a	31.5c	36.1b	6.00a	4.31b
Av. of parents	1224	34.8	6.40	5.17	6.17	29.2	31.8	36.0	5.50	4.32
F_2	1345	34.6	6.74	5.37	6.16	30.2	31.9	35.6	5.14	4.30

[a] Mean comparisons were made among parents within treatments of planting. The means followed by the different letters were significantly different at $p \le 0.05$.

Table 4
Mean comparisons between treatments of planting date for yield and fiber traits in F_2 hybrids and parents.

Genotypes[a]	Planting dates	Lint yield (kg ha^{-1})	Lint percentage (%)	Boll no (10^5 ha^{-1})	Boll wt. g	Lint index (g)	Seeds boll^{-1}	Length (mm)	Strength (g tex)	Elongation (%)	Micronaire
2012											
F_2	Normal	1494a	37.9a	7.3a	5.39a	6.89a	29.8a	30.1b	35.9a	5.52a	5.10a
	Delayed	570b	32.9b	3.6b	4.83b	5.49b	29.0a	31.5a	35.6a	4.93b	3.97b
Parents	Normal	1152a	37.2a	6.1a	5.16a	6.58a	29.2a	30.1b	36.2a	5.64a	5.07a
	Delayed	475b	33.0b	3.1b	4.60b	5.51b	27.6a	31.3a	35.6a	5.24a	3.98b
2013											
F_2	Normal	1830b	38.3a	7.7b	6.27a	7.16a	33.6a	31.3b	34.6b	5.39a	4.78a
	Delayed	2120a	36.3b	9.9a	5.90b	6.84b	31.3b	32.3a	35.6a	5.36a	4.63b
Parents	Normal	1936a	39.5a	8.5a	5.91a	7.20a	32.5a	31.0b	35.3a	5.53a	4.83a
	Delayed	1973a	36.6b	9.3a	5.74a	6.82b	30.8a	32.3a	36.3a	5.75a	4.66a

[a] Mean comparisons were made between treatments of planting dates within F_2 and parents, respectively. The means followed by the different letters were significantly different at $p \leq 0.05$.

Table 5
Monthly averages of weather parameters during growing seasons of 2012 and 2013 at Stoneville, MS.

Month	Maximum temperature (°C)	Mean temperature (°C)	Minimum temperature (°C)	Precipitation[a] (mm)
2012				
May	30.9	22.7	19.1	51.6
June	31.7	23.0	20.3	162
July	33.9	26.2	23.5	116
August	33.8	24.3	21.1	109
September	30.8	21.7	17.8	82.8
October[b]	20.3	13.7	10.8	133
2013				
May	26.2	18.6	15.7	145
June	30.4	23.9	21.0	92.7
July	31.5	22.6	20.4	48.5
August	33.8	24.7	21.3	50.6
September	32.3	21.4	18.8	130
October	25.6	16.7	13.8	150

[a] Precipitation was defined as the accumulated rainfall during a month in the growing seasons.
[b] Monthly averages were from Oct 1 to Oct 17 in 2012 and from Oct 1 to Oct 23 in 2013.

Table 6
Days to 1st open boll in parents under environments of delayed planting.

Source	Fixed effects
Genotype (G)	0.001
Year (Y)	0.001
G × Y	0.007
2012	
Parents	Days to 1st open boll[a]
SP103	128a
JJ1145ne	126ab
MD52ne	125bc
DP90ne	123 cd
TAM98d-99ne	122d
2013	
Parents	Days to 1st open boll
SP103	109a
TAM98d-99ne	108ab
MD52ne	108ab
DP90ne	106bc
JJ1145ne	104c

[a] Mean comparisons were made among parents within each year. The means followed by the different letters were significantly different at $p \leq 0.05$.

could be attributed to the difference in boll number, 9.9×10^5 ha^{-1} in delayed planting vs. 7.7×10^5 ha^{-1} in normal planting, in that year (Table 4). The low boll number in F_2 plants in normal planting of 2013 might have been caused by poor seedling survival during early growing season in 2013. Although plant establishment was not numerically recorded at early growing seasons during the experiments, plant emergence and establishment were visually estimated by walking through the plots.

3.3. Growth parameters in parents and F_2 plants

Growth parameter of days to 1st open boll was analyzed among parents for their response to the treatment of delayed planting (Table 6). Difference among parents was highly significant for their maturity in delayed planting. However, genotype × year was also highly significant. Therefore, the maturity of parents in delayed planting was compared in each experimental year. The maturity of SP103, MD52ne, and DP90ne was consistent across years, whereas TAM98D-99ne and JJ1145ne performed differently across years. TAM98D-99ne performed like a typical early season line in 2012, but had the latest maturity in delayed planting of 2013. JJ1145ne performed like a typical early line in 2013, but had a relatively late maturity in delayed planting of 2012.

Growth parameters were further analyzed among F_2 plants and parents by comparisons between delayed planting and normal planting. Plant height of the plants in delayed planting was consistently greater than those in normal planting of all genotypes (Table 7). The increase of plant height in delayed planting was also observed in other studies (Cathey and Meredith, 1988; Porter et al., 1996; Boquet and Clawson, 2009). Although larger portion of assimilates were partitioned to vegetative growth, the increase of planting height appeared not to have effects on lint yield which might have been confounded with other yield components such as boll number ha^{-1} in this study.

Days to 1st open boll in the experiment of 2013 were consistently shortened across all genotypes for plants in delayed planting (Table 7). This result implies the ability of these lines to adjust for maturity under delayed planting conditions although SP103 was a full season line. The decrease of days to 1st open boll in these lines under delayed planting conditions might partially contribute to the maintenance of lint yield in delayed planting in this experiment.

3.4. Combining ability and heritability with treatments of normal planting and delayed planting

The genotypic variability among the diallel crosses was partitioned into general combining ability (GCA) and specific combining ability (SCA) effects under each treatment of planting dates (Table 8). GCA effects were significant for all traits except for boll no. ha^{-1} and seeds boll^{-1}. GCA effects for these two yield components were significant in normal planting, but became non-significant in delayed planting. SCA effects for most yield components were different between treatments of planting date especially for yield components of lint index, boll wt., and seeds per boll. The general lack of SCA effects indicates that fiber traits in these F_2 hybrids are mainly controlled by additive genes.

Table 7
Mean comparisons of growth parameters between treatments of plant date in 2013.

No	Genotype	Planting date	Height (cm)	Days to 1st open boll[a] (no. days)
1	SP103 × MD52ne	Normal	70a	129a
		Delayed	97b	104b
2	SP103 × JJ1145ne	Normal	52a	127a
		Delayed	96b	108b
3	SP103 × TAM98D-99ne	Normal	51a	125a
		Delayed	99b	105b
4	SP103 × DP90ne	Normal	62a	127a
		Delayed	102b	104b
5	MD52ne × JJ1145ne	Normal	71a	125a
		Delayed	108b	107b
6	MD52ne × TAM98D-99ne	Normal	73a	128a
		Delayed	106b	104b
7	MD52ne × DP90ne	Normal	62a	126a
		Delayed	109b	108b
8	JJ1145ne × TAM98D-99ne	Normal	77a	126a
		Delayed	111b	106b
9	JJ1145ne × DP90ne	Normal	64a	128a
		Delayed	109b	108b
10	TAM98D-99ne × DP90ne	Normal	68a	124a
		Delayed	113b	106b
11	SP103	Normal	62a	128a
		Delayed	97b	109b
12	MD52ne	Normal	59a	128a
		Delayed	105b	108b
13	JJ1145ne	Normal	77a	129a
		Delayed	115b	104b
14	TAM98D-99ne	Normal	66a	127a
		Delayed	101b	108b
15	DP90ne	Normal	62a	125a
		Delayed	112b	106b

[a] Mean comparisons were made between late planting and normal planting for each genotype.

The broad sense heritability of lint yield and yield components with the treatment of normal planting was higher than that with delayed planting (Table 8). With the treatment of normal planting, broad sense heritability of lint percentage, lint index, and boll weight was as much as 0.88, 0.86, and 0.42, respectively. In delayed planting, broad sense heritability of these three yield components was 0.25, 0.20, and 0.13, respectively. For fiber properties, the broad sense heritability was generally similar between the two treatments. The reduction in the broad sense heritability of yield traits in the delayed planting was due to high year × planting date interactions (Table 1) which indicated differential yearly environmental effects on lint yield between treatments of planting dates. When environmental factor (year) was randomized in the linear model, the effect of year factor for lint yield in delayed planting was 9.5 times of that in normal planting (data not shown).

With the treatment of normal planting, the cultivar JJ1145ne had better general combining ability for increasing lint yield than MD52ne, TAM98D-99ne, DP90ne, and SP103 (Table 9). However, with the treatment of delayed planting, there were no significant differences among parents for their combining ability of lint yield. Although JJ1145ne had 381 kg ha^{-1} positive GCA in delayed planting, it was not significantly different from the average of the five parents due to high standard error under the environment of delayed planting. SP103 had negative GCA for boll no., $-1.8\ 10^5$ ha^{-1}, while MD52ne, JJ1145ne, and TAM98D-99ne had positive GCA for boll no., 0.52, 0.49, and 0.63 10^5 ha^{-1}, respectively, with the treatment of normal planting (Table 9). These GCA effects were not significant with the treatment of delayed planting. SP103 was more likely to increase seeds per boll, 1.5 seeds boll^{-1}, and TAM98D-99ne was more likely to decrease seeds per boll, −1.4 seeds boll^{-1}, with the treatment of normal planting. These GCA effects were not significant in delayed planting. GCA effects of parents for fiber properties were generally similar between treatments of planting date except for fiber length (Table 9).

Both direction changes of SCA effects for yield traits were observed between delayed planting and normal planting (Table 10). Three of four crosses with SP103 as one of parents had negative SCA effects for lint yield, −200 to −658 kg ha^{-1}, in normal planting, but these effects were not observed in delayed planting. The SCA effects for yield components of boll no., boll wt., and seeds per boll were different between treatments of planting date. All crosses with SP103 as one of parents had negative SCA effects for boll no., −1.9 to 2.4 10^5 ha^{-1}, with the treatment of normal planting, but these effects were not observed in delayed planting. SP103 × II1145ne and JJ1145ne × TAM98D-99ne had positive SCA effects for boll wt., 0.71 g and 0.58 g, in normal planting, but the effects became non-significant in delayed planting. SP103 × MD52ne, SP103 × JJ1145ne, and SP103 × TAM98D-99ne had positive SCA effects for seeds per boll, 2.1 to 3.0 seeds boll^{-1}, in normal planting, but these effects were not observed in delayed planting.

3.5. Genotypic correlations between fiber traits between treatments of planting date

Genotypic correlations between lint yield and fiber traits were different between the two treatments of planting date (Table 11). Genotypic correlation coefficients of lint yield vs. lint percentage (0.62) and lint yield vs. elongation (0.44) in delayed planting were less than those of lint yield vs. lint percentage (0.88) and lint yield vs. elongation (0.90) in normal planting. Genotypic correlation of lint yield vs. strength was more negative in delayed planting (−0.83) compared with that in normal planting (−0.34). Genotypic correlation coefficient of lint yield vs. boll wt. in delayed planting (0.95) was greater than that in normal planting (0.09). Genotypic correlations between other fiber traits were similar between treatments of planting date. Generally, the difference between genotypic correlations and phenotypic correlations was

Table 8
Effects ($p > F$) of general combining ability (GCA) and specific combining ability (SCA) and broad sense heritability under treatments of each planting date.

Sources	Lint yield	Lint percentage	Boll no	Lint index	Boll wt.	Seeds boll^{-1}	Length	Strength	Elongation	Micronaire
Normal planting										
GCA	0.001	0.001	0.001	0.001	0.001	0.011	0.001	0.001	0.001	0.001
GCA × year	0.701	0.410	0.816	0.910	0.147	0.448	0.565	0.402	0.096	0.117
SCA	0.089	0.196	0.416	0.011	0.035	0.050	0.001	0.003	0.377	0.221
SCA × year	0.001	0.031	0.002	0.700	0.571	0.604	0.003	0.006	0.631	0.298
H^2	0.20	0.88	0.10	0.86	0.42	0.11	0.22	0.77	0.96	0.11
Delayed planting										
GCA	0.001	0.001	0.391	0.001	0.001	0.601	0.001	0.001	0.001	0.001
GCA × year	0.032	0.923	0.244	0.717	0.001	0.023	0.046	0.051	0.316	0.007
SCA	0.837	0.051	0.851	0.627	0.393	0.586	0.014	0.122	0.008	0.134
SCA × year	0.960	0.274	0.976	0.026	0.143	0.821	0.926	0.337	0.816	0.198
H^2	0.01	0.25	0.00	0.20	0.13	0.00	0.25	0.96	0.59	0.04

Table 9
General combining ability (GCA) for yield traits and fiber properties of five parents.

Parents	Lint yield (kg ha^{-1})	Lint percentage (%)	Boll no (10^5 ha^{-1})	Lint index (g)	Boll wt.	Seeds (boll^{-1})	Length (mm)	Strength gtex	Elg (%)	Mic
Normal planting										
SP103	−259*	−0.038***	−1.79***	0.163	1.07***	1.54*	0.439*	−1.94***	−0.727***	−0.111
MD52ne	−150	−0.013***	0.520*	−1.01***	−0.750***	−0.750	0.255	3.01***	−0.574***	0.106
JJ1145ne	399***	0.040***	0.490*	1.35***	0.351*	−0.577	0.457*	−2.16***	0.894***	−0.083
TAM98D-99ne	70.3	0.007	0.633*	0.167	−0.232	−1.40*	−0.868***	1.46***	0.201**	0.153*
DP90ne	−59.5	0.003	0.153	−0.673***	−0.435*	1.19	−0.314	−0.376	0.205**	−0.065
S.E.$_{GCA}$	251	0.01	0.63	0.23	0.44	1.9	0.58	0.62	0.19	0.16
Delayed planting										
SP103	47.3	−0.024**	−0.143	0.156	0.563*	0.180	−0.031	−2.36***	−0.474***	−0.187
MD52ne	−225	−0.014*	−0.171	−0.729**	−0.530*	−0.661	0.368*	1.97***	−0.539***	0.081
JJ1145ne	381	0.029***	0.823	0.954***	0.315	−0.29	0.595**	−1.52***	0.676***	−0.141
TAM98D-99ne	28.9	0.006	0.055	0.160	0.161	0.653	−0.639**	1.73***	−0.071	0.274*
DP90ne	−232	0.003	−0.564	−0.543*	−0.510*	0.117	−0.294	0.189	0.408***	−0.026
S.E.$_{GCA}$	769	0.02	3.1	0.67	0.59	1.6	0.45	0.46	0.26	0.35

* Significant at $p \leq 0.05$.
** Significant at $p \leq 0.01$.
*** Significant at $p \leq 0.001$.

greater in delayed planting than those in normal planting which indicated greater environmental influence in delayed planting.

3.6. Implications to breeding and management option

Selection of parents for desirable combining ability is the first step in breeding for genetic improvement of lint yield and fiber quality. The objective of this study was to investigate genetic values in F_2 populations under treatments of planting date and answer the question: whether there is a need to test parents for combining ability and heritability under environments of delayed planting? The change of GCA and SCA effects and broad sense heritability for lint yield and yield components between delayed planting and normal planting was observed. Although the change of combining ability could be in positive and negative directions, the increase of environment influence upon the treatment of delayed planting should warrant the need of testing and selection of parents for lint yield under environments of delayed planting. However, considering the current small acreage of the delayed planting cotton in U.S.A., the decision for testing parents under environments of delayed planting would have to weigh between the cost for testing and profitability of double cropping cotton. May and Bridges (1995) suggested that this type of testing was not necessary for small acreage of late planting cotton. In the current study, the changes of specific combining ability between planting treatments were considered moderate. Furthermore, the effects of SCA × year with the treatment of delayed planting were not significant which implies similar SCA effects across experimental years in delayed planting. These results were consistent with the contention that current Upland cotton cultivars can be directly used for small acreages of delayed planting. For large acreages of delayed planting when this management option becomes a common practice, testing parents for their combining ability and heritability under environments of delayed planting will be needed in order to maintain lint yield.

Although it is difficult to determine whether the changes of genotypic correlations between treatments of planting date are

Table 10
Specific combining ability (SCA) for lint yield and yield components between treatments of delayed planting and normal planting.

Hybrids	Lint yield(kg ha^{-1})		Lint percentage (%)		Boll no (10^5 ha^{-1})	
	Normal	Delayed	Normal	Delayed	Normal	Delayed
SP103 × MD52ne	−109	272	−0.025***	−0.010*	−2.32***	0.029
SP103 × JJ1145ne	−658***	−334*	−0.078***	−0.052***	−2.28***	−0.965
SP103 × TAM98D-99ne	−329**	18.4	−0.045***	−0.030***	−2.42***	−0.198
SP103 × DP90ne	−200*	279	−0.041***	−0.027***	−1.95***	0.421
MD52ne × JJ1145ne	−549***	−606***	−0.053***	−0.043***	0.030	−0.994
MD52ne × TAM98D-99ne	−221*	−254	−0.020***	−0.021***	−0.114	−0.226
MD52ne × DP90ne	−91.2	7.30	−0.016**	−0.018***	0.367	0.392
JJ1145ne × TAM98D-99ne	328**	352*	0.033***	0.022***	−0.143	0.768
JJ1145ne × DP90ne	458***	613***	0.037***	0.025***	0.337	1.39*
TAM98D-99ne × DP90ne	130	261	0.004	0.003	0.480	0.619
	Boll wt. g		**Lint index (g)**		**Seeds boll^{-1}**	
Hybrids	Normal	Delayed	Normal	Delayed	Normal	Delayed
SP103 × MD52ne	1.82***	1.09***	1.17***	0.885***	2.29*	0.841
SP103 × JJ1145ne	0.714***	0.248	−1.19***	−0.798***	2.12*	0.470
SP103 × TAM98D-99ne	1.29***	0.402*	−0.005	−0.004	2.95**	−0.473
SP103 × DP90ne	1.50***	1.07***	0.836***	0.700***	0.357	0.063
MD52ne × JJ1145ne	−1.10***	−0.845***	−2.35***	−1.68***	−0.173	−0.371
MD52ne × TAM98D-99ne	−0.517*	−0.691***	−1.17***	−0.889***	0.656	−1.31
MD52ne × DP90ne	−0.315*	−0.020	−0.331	−0.186	−1.94	−0.778
JJ1145ne × TAM98D-99ne	0.584***	0.154	1.18***	0.795***	0.829	−0.943
JJ1145ne × DP90ne	0.786***	0.825***	2.03***	1.50***	−1.75	−0.410
TAM98D-99ne × DP90ne	0.202	0.670***	0.841***	0.703***	−2.59**	0.536

* Significant at $p \leq 0.05$.
** Significant at $p \leq 0.01$.
*** Significant at $p \leq 0.001$.

Table 11
Correlations and genotypic correlations among yield, yield components, and fiber properties in normal planting and delayed planting.

	Lint yield	Lint percentage	Boll no.	Boll wt.	Lint index	Length	Strength	Elongation
Normal planting								
Lint percentage	0.82***,[a]0.88							
Boll no.	0.67**0.61	0.63*0.75						
Boll wt.	0.060.09	−0.25−0.25	−0.65**−0.71					
Lint index	0.72**0.81	0.56*0.54	0.090.07	0.56*0.61				
Length	0.100.20	−0.06−0.03	−0.19−0.24	0.380.44	0.300.35			
Strength	−0.33−0.34	−0.17−0.16	0.380.51	−0.76g−0.80	−0.66**−0.71	−0.43−0.50		
Elongation	0.79***0.90	0.94***1.0	0.55*0.68	−0.14−0.14	0.63*0.73	−0.16−0.14	−0.32−0.31	
Micronaire	−0.07−0.09	−0.06−0.11	0.420.69	−0.52−0.67	−0.40−0.56	−0.65**−0.80	0.77***0.95	−0.080.00
Delayed planting								
Lint percentage	0.490.62							
Boll no.	0.86***0.21	0.360.51						
Boll wt.	0.79***0.95	−0.00−0.01	0.56*−0.74					
Lint index	0.62*1.0	0.62*0.61	0.57*−0.66	0.74**0.81				
Length	0.420.69	0.130.20	0.470.59	0.110.11	0.250.29			
Strength	−0.52*−0.83	−0.05−0.04	−0.27−0.36	−0.62*−0.70	−0.55*−0.58	−0.22−0.27		
Elongation	0.270.44	0.74**0.78	0.130.23	−0.08−0.09	0.420.45	0.090.15	−0.12−0.10	
Micronaire	−0.29−0.33	0.01−0.02	−0.070.21	−0.30−0.48	−0.29−0.46	−0.60*−0.75	0.66**0.83	−0.12−0.21

* Significant at $p \leq 0.05$.
** Significant at $p \leq 0.01$.
*** Significant at $p \leq 0.001$.
[a] Upper values are correlation coefficients and lower values are genetic correlation coefficients.

favorable or unfavorable for most pairs of fiber traits, the fact that the genotypic correlation between lint yield and fiber strength became more negative in delayed planting implies an increase of complexity for interrelationships among fiber traits in the double cropped cotton. This increased complexity is believed to be due to increased environmental covariance component for the pair of fiber traits, Cov_{exy}, in delayed planting. The increase of environmental effects with the treatment of delayed planting suggests a requirement for sufficient number of environments for testing parents in the breeding for delayed planting system. It also informs breeders to test parents under environments of delayed planting in order to simultaneously improve lint yield and fiber quality in the delayed planting system.

4. Conclusion

Changes of combining ability and heritability for yield traits were observed with treatment of delayed planting. Changes of genotypic correlations between yield traits and fiber properties were also observed between treatments of delayed planting and normal planting. These results are the first time to identify differential genetic values for yield traits between delayed planting and normal planting. These results indicated the need to test parents for lint yield under environments of delayed planting.

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References

Baker, S.H., 1987. Effects of tillage practices on cotton double cropped with wheat. Agron. J. 79, 513–516.
Bauer, P.J., May, O.L., Camberato, J.J., 1998. Planting date and potassium fertility effects on cotton yield and fiber properties. J. Prod. Agric. 11, 415–420.
Boquet, D.J., Clawson, E.L., 2009. Cotton planting date: yield, seedling survival, and plant growth. Agron. J. 101, 1123–1130.
Bowman, D.T., Gutiérrez, O.A., Percy, R.G., Calhoun, D.S., May, O.L., 2006. Pedigrees of upland cotton cultivars released between 1970 and 2005. Miss. Agric. Exp. Stn. Bull 1155, Mississippi State University.
Buntin, G.D., Raymer, P.L., Bednarz, C.W., Phillips, D.V., Baird, R.E., 2002. Winter crop, tillage, and planting date effects on double-crop cotton. Agron. J. 94, 273–280.
Cathey, G.W., Meredith Jr., W.R., 1988. Cotton response to planting date and mepiquat chloride. Agron. J. 80, 463–466.
Green, C.C., Culp, T.W., 1990. Selecting for maturity differences and improved yield stability in Pee Dee germplasm lines. In: Proceedings of Beltwide Cotton Conference, 10–13 Jan 1990. Las Vegas, NV, U.S.A, pp. 81–83.
Griffing, B., 1956. Concept of general and specific combining ability in relation to diallel crossing system. Aust. J. Biol. Sci. 9, 464–493.
Kempthorne, O., 1957. An Introduction to Genetic Statistics. John Wiley and Sons, Inc., New York, NY.
May, O.L., Bridges, B.C., 1995. Breeding cottons for conventional and late-planted production systems. Crop Sci. 35, 132–136.
Meredith Jr., W.R., 2005. Registration of MD52ne high fiber quality cotton germplasm and recurrent parent MD 90ne. Crop Sci. 45, 807–808.
Pettigrew, W.T., 2002. Improved yield potential with an early planting cotton production system. Agron. J. 94, 997–1003.
Pettigrew, W.T., Molin, W.T., Stetina, S.R., 2009. Impact of varying planting dates and tillage systems on cotton growth and lint yield production. Agron. J. 101, 1131–1138.
Popp, M.P., Keisling, T.C., McNew, R.W., Oliver, L.R., Dillon, C.R., Wallace, D.M., 2002. Planting date, cultivar, and tillage system effects on dryland soybean production. Agron. J. 94, 81–88.
Porter, P.M., Sullivan, M.J., Harvey, L.H., 1996. Cotton cultivars response to planting date on the southeastern coastal plain. J. Prod. Agric. 9, 223–227.
SAS Institute, 2013. SAS 9.4 TS1M1 (9.4M1). Window version. SAS Institute Inc., Cary, NC, U.S.A.
Scott Jr., A.W., Lukefahr, M.J., Cook, C.C., Spurgeon, D.W., Summy, K.R., Raulston, J.R., Kilgore, T.P., 1998. The strategy of delayed planting with rapid fruiting cotton for the lower Rio Grande Valley of Texas. In: Proceedings of Beltwide Cotton Conference, 5–9 Jan 1998, San Diego, CA, U.S.A, pp. 578–579.
Smith, C.W., Varvil, J.J., 1982. Double cropping cotton and wheat. Agron. J. 74, 862–865.
Thaxton, P.M., Smith, C.W., Cantrell, R., 2005. Registration of TAM98D-102 and TAM98D-99ne upland cotton germplasm lines with high fiber strength. Crop Sci. 45, 1668–1669.
Wrather, J.A., Phipps, B.J., Stevens, W.E., Phillips, A.S., Vories, E.D., 2008. Cotton planting date and plant population effects on yield and fiber quality in the Mississippi Delta. J. Cotton Sci. 12, 1–7.
Zeng, L., Meredith, W.R., Boykin, D.L., Taliercio, E., 2007. Evaluation of an exotic germplasm population derived from multiple crosses among *Gossypium* tetraploid species. J. Cotton Sci. 11, 118–127.
Zhang, Y., Kang, M.S., 1997. Diallel-SAS: a SAS program for Griffing's diallel analyses. Agron. J. 89, 176–182.