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BREEDING AND GENETICS

Developing Hybrid Cotton (*Gossypium* spp.) Using Honey Bees as Pollinators and the Roundup Ready® Phenotype as the Selection Trait

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ABSTRACT

Cotton (*Gossypium* spp.) is the most important textile fiber crop in the United States (US). Hybrid cotton is grown in several countries but the use of hybrids in the US has been limited due to seed production costs. The objective of this study was to investigate a novel method for the production of F_2 cotton hybrids using honey bees as pollinators and the Roundup Ready® gene to facilitate identification of hybrid seed. This research was conducted from 2005 to 2007 in Louisiana. Six hybrid populations were developed between non-transgenic and transgenic lines manually or by caging with honey bees (*Apis mellifera* L.) as pollinators. In 2007, F_1, F_2, and parents were field tested in a randomized complete block design at two locations. All F_1 hybrid populations exhibited heterosis compared to the best parent. The crosses LA1110023/PHY410R and ARKRM24-12-04/PHY410R exhibited the highest degree of high-parent heterosis for yield averaging 33.1% and 20.6% increases in the F_1, respectively, and 20.9% and 19.5% increases in the F_2, respectively. Fiber quality measurements did not display significant heterosis in the F_2 population relative to the best parent. Using male contributors containing the Roundup Ready® trait for selection, conventional female lines, and honey bees as pollinators proved to be a viable method for developing F_2 hybrid cotton lines.

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Cotton (*Gossypium* spp.) is the most important textile fiber crop in the United States. Considered primarily a self-pollinated crop, interest in the potential of hybrid cotton has been expressed based upon demonstrated hybrid vigor, notably for yield. The commercial use of hybrid cotton has been, however, quite limited in the U.S. due to the lack of suitable methods to: (1) ensure stable male sterility, (2) adequately restore fertility, (3) provide efficient pollen transfer from male-fertile to male-sterile flowers (Vaissiere et al., 1984) if male-sterile method is used, or (4) reduce the high cost of hybrid cotton seed production if hand emasculation and pollination is used. Alternative techniques such as the male-sterile method have been evaluated using a physical mixing of male and female plants then planting the blend in a single row. Cross pollination is generally much improved with this approach but the male plants harvested in the blend tend to depress the overall hybrid performance (Holland, 1999).

Production of F_1 or F_2 hybrid cotton seed for commercial use by farmers in the U.S. has met limited success. According to Meredith and Brown (1998), Chembred released the first commercial F_2 varieties in the U.S. in 1992, but ceased operations in October 1995. The main factor behind the lack of commercial success was the ineffectiveness of the male gametocide that had to be applied every 14 to 21 days and the varying amounts of both male and female fertility. Incomplete male sterility resulted in non-hybrid seed and female sterility resulted in reduced yields. The competitiveness of some F_2 varieties produced using gametocide seemed to be less than the same F_2's produced by hand crossing. Successful seed production for hybrid cotton is routine in India and China (Holland, 1999) and Hazera Seeds Inc. (Coconut Creek, FL)is commercializing F_1 inter-specific hybrid seeds obtained through hand pollination in India. Dong et al. (2004) reported that hybrid (F_1) *Bacillus thuringiensis* Berliner (Bt) cotton, developed after crossing a Bt variety with a non-Bt variety, resulted in an approximately 20%

yield increase over the Bt cotton parent. Such hybrids are widely used in southern China, because of the difficulties in controlling bollworm (*Helicoverpa armigera* Hübner) using pesticides.

Cotton is an allotetraploid species and primarily considered to be a self-pollinating crop. The pollen of cotton is relatively heavy and pollen movement by wind is minimal (Poehlman, 1987). Thomas et al. (2001) studied pollen transfer in cotton (for isolation standards under California conditions) and reported that it ranged from 6-60% over short distances, dropping to 0.03% at a distance of 48 ft. In another study, pollen transfer as high as 4% was detected at a distance of 60 ft. In a comparable study in a commercial field, Thomas et al. (2001) detected a low level of pollen transfer (0.3%) at distances beyond 100 ft from known transgenic sources, with some transfer being detected as far away as 1 mile. Verhalen et al. (1999) reported that cross pollination at Perkins, OK, fluctuated between 35.0 and 75.4%, and that at Altus, OK, cross pollination was very low, between 0.1 to 3.8%. Rhodes (2002) reported that a commercial cotton field managed with honey bee (*Apis mellifera* L.) pollination helped to increase cotton yield up to 15.8% and increased the number of bolls harvested by 11.1%.

Several studies indicate that the use of F_2 hybrids could potentially increase cotton lint yield. Weaver (1999) reported that an F_2 population produced an equal amount of lint as the F_1 hybrid and that both produced more lint than the parents. Meredith (1990) indicated that F_2 populations can produce a better combination of yield and fiber quality than either of the parents used to create the F_1 generation. In Meredith's study, F_2 performance was highly correlated ($r= 0.86$) with F_1 yield performance. Occasionally, F_2 heterosis equaled F_1 heterosis. No differences in adaptive ability between the parent, F_1 and F_2 generations were detected. Schoenhals (1990) reported that the percentage of lint remaining after the ginning process between the F_1 and F_2 generations did not differ.

It is possible that transgenic lines could be used as parents to develop hybrid cotton (Weaver, 1999). Current evidence is that all of the herbicide tolerance genes used in transgenic cotton are inherited as single, dominant characters. If a single, dominant gene is responsible for the herbicide-resistance, the F_2 hybrid will segregate in a 3:1 ratio (i.e. 3 herbicide resistant to 1 herbicide susceptible) (Weaver, 1999). This mechanism for producing cotton hybrids differs from the use of glyphosate as a chemical gametocide (U.S. Patent No. 4,735,649; WO/1999/046396) (Dhingra, et al., 1988). A Generation Means Analysis (GMA) is an analytical tool that can be used to provide a relative estimate of genetic effects and should be useful for providing important information about potential hybrid development of cotton. Using mean values of several different generations allows for a detailed estimate of genetic effects (additive, dominance, and epistatic effects) rather than genetic variances (diallel analysis). Plant breeders can use information obtained on genetic effects in deciding whether or not a hybrid development program would have a high likelihood of success.

While hybrid cotton production is routine in some countries, notably India, it has enjoyed little success in the U.S. primarily due to the cost of hybrid seed production. The development of a simple, cost-effective method of hybrid cottonseed production could potentially utilize the benefits of heterosis to further increase yields. The objective of this research was to determine if a Roundup Ready® male donor line (Monsanto Co., St. Louis, MO), when crossed with conventional female lines using honey bees as pollinators, is suitable for the development of F_2 hybrid cotton seed.

MATERIALS AND METHODS

Year 1. In the summer of 2005, the original crosses were made at the LSU Agricultural Center Central Research Station near Baton Rouge, LA. The field dimensions were 13 rows wide and 3 tiers deep. Each tier was 15 m long, rows were spaced 1 m apart, and the spacing within a row was 8-10 plants per m.

Eighteen non-transgenic germplasm lines were used as females and a commercially available transgenic variety, Phytogen PHY410R (Dow Agro Sciences, LLC, Indianapolis, IN), was used as the male pollen donor. Female and male rows were planted side by side in a paired-crossing arrangement such that each female was adjacent to the male. Conventional female lines were obtained from public breeding programs throughout the southeastern U.S. because of their diversity in origin and genetics.

One week prior to the onset of blooming, honey bees were placed in the field to facilitate cross pollination between the transgenic male and non-transgenic female cotton plants. Two honey bee colonies (2-storey, 10-frame hives) were placed in

the field; one colony was in the first row between tiers one and two and the other was in the first row between tiers two and three. We estimate that there were about 100 other managed colonies within about 1 km of the field. Additionally, three insect proof mesh cages (3 × 5 × 2.5 m) were randomly erected over the first ¼ of a tier and over three rows, and each cage received one honey bee colony (1-storey, 5-frame hive).

In fall of 2005 the non-transgenic female germplasm lines were harvested by hand. Among the seedcotton harvested, there was expected to be a mix of self-pollinated non-transgenic seedcotton and F_1 non-transgenic/transgenic hybrid seedcotton. Harvested bolls were ginned using a 7-saw laboratory gin (Porter-Morrison, Dennis Manufacturing Inc., Athens, TX) and the fuzzy seed was delinted using a 93% sulfuric acid solution. After delinting, the cottonseed was air-dried, treated with fungicides Baytan® and Allegiance™ (Bayer CropScience, Durham, NC), and stored in a cold room facility.

Year 2. Six hybrid lines out of the original eighteen were randomly selected and planted in 2006 near Saint Joseph, LA at the LSU Agricultural Center Northeast Research Station. Each tier was 12 m long, with rows spaced 1 m apart, and three replications were seeded at a seed density of 8-10 plants per m.

At the 6-7th true leaf stage, Roundup® herbicide (Monsanto Co, St. Louis, MO) (glyphosate at 850 g ai ha^{-1}) was sprayed over the crop to eliminate any non-transgenic plants arising from self-pollinated seed.

The plants surviving the broadcast application of glyphosate were non-transgenic/transgenic hybrids, and the glyphosate-resistant and glyphosate-susceptible plants were counted to calculate the outcrossing percentage produced by honey bee pollination. Surviving hybrid plants were allowed to self-pollinate and F_2 cottonseed was harvested by hand, ginned, cleaned, and stored to be field tested in year three.

Year 3. The F_2 cottonseed from year two, new F_1 cottonseed from crosses made at the winter nursery in Mexico and the parents (for each unique hybrid combination) were planted by generation (Parents, F_1, and F_2 generation) in a randomized complete block design with three replications in two locations (Alexandria and St. Joseph, LA) in 2007. Lines were randomized within each generation and each generation block was randomized within each replication. Lines were planted by generation to facilitate the application of herbicide over the top. Due to a shortage of seed for two of the females (99WJ-9 and 00U-82), they were planted with two replications in both locations.

The trial in Alexandria was conducted on a conventionally tilled Norwood silt loam, non-irrigated, in 15 m long plots, and in Saint Joseph was conducted using a minimum tillage system on an irrigated Sharkey clay soil in 12 m long plots; both locations had rows 1 m wide.

Three weeks after planting, at the 3rd-4th true leaf stage, the F_1 and F_2 blocks were sprayed over-the-top with Roundup® herbicide (glyphosate at 850 g ai ha^{-1} in both locations). Fourteen days after application the ratios of glyphosate-resistant to glyphosate-susceptible plants were determined for the F_1 and F_2 blocks to determine percentage survival and gene segregating ratio in the F_2 generation.

The parameters measured at harvest were plot yield, plant height, and row length and row gaps for yield adjustment. Twenty-five randomly selected cotton bolls of each line were collected by hand prior to machine harvesting in both locations to determine fiber quality. Seedcotton was ginned at the LSU Cotton Breeding Lab using a 7-saw laboratory gin. Lint and cottonseed weights were recorded to determine lint percentage and yield parameters.

Lint collected from the ginning process was analyzed using High Volume Instrumentation (HVI 900™ Zellweger Uster) at the LSU Cotton Fiber Lab. The fiber descriptors measured were fiber length (cm), fiber strength (kN m kg^{-1}), short fiber index (SFI) (%), and fiber fineness (micronaire).

All data were analyzed using the SAS PROC MIXED procedure with estimates of means and standard errors generated using LS MEANS (ver. 9.13; SAS Institute, Cary, NC). Combined-location data analysis was done where replication was designated as a random effect in the model. Location and generation were treated as fixed effects, and lines were nested in generations. Mean separation was conducted using Fisher's protected least significant difference (LSD) at the 0.05 level of probability. Only one male line was used as the pollen donor and six lines as females or pollen receptor for the crosses. Estimates of heterosis were made with regard to the highest yielding parent and to the mid-parent for lint yield. Generation Mean Analysis (GMA) was done using joint scaling test software (Ng, 1990). Tested was a three generation model consisting of the parent one (P_1), parent two (P_2), hybrid (F_1), and hybrid (F_2) generations.

RESULTS AND DISCUSSION

Percentage outcrossing. The percentage of outcrossing using honey bees within a cage was not different from the percentage using honey bees in the open field (P = 0.48, data not presented). Cross pollination among plants varied from 21 to 65% within a cage and from 33 to 55% in the open field. The high percentage of outcrossing in the open field might have been due to honey bees and other insects such as bumble bees (*Bombus* spp.) supplementing the rate of cross pollination. The proximity of numerous honey bee colonies may have also contributed to the high outcrossing rate in the open field.

Plant height, plant density, and segregation of herbicide resistance. There was no location by population interaction (P = 0.51) for plant height so the data are pooled across populations. The average plant height in Alexandria, LA (1.89 m) was, however, significantly higher (P < 0.01) than the average plant height in Saint Joseph, LA (1.47 m). The generation main effect was significant (P = 0.03) for plant height indicating the existence of heterosis for height. The average height 1.69 m for the F_1 population and 1.71 m for the F_2 population, which were not statistically different (P = 0.31) (Table 1). The average height of the parents was 1.65 m. Parent population plants were shorter than plants of the F_2 population (P = 0.01), but not statistically different than the F_1 population (P = 0.13).

There was no location by populations interaction (P = 0.86) for plant density so the data are pooled across populations. The location main effect for plant density was highly significant (P < 0.01). The density in Alexandria was 5.77 plants m^{-1} and the density in Saint Joseph was 7.31 plants m^{-1}. The plant density difference in the locations was likely due to differences in seeding rates. In Alexandria the cone planter was set to plant 140 seeds in 15.2 m, and in Saint Joseph it was set to plant 140 seeds in 12.2 m.

There was a generation effect (P < 0.01) for plant density (Table 1). The densities for the F_1 population plots (6.89 plants m^{-1}) and the parent plot (6.82 plants m^{-1}) were similar (P = 0.37). Plant densities in the F2 populations (6.00 plants m^{-1}) were significantly less than both the F_1 population (P <0.01) and the parent plots (P < 0.01), because F_2 plants segregating for glyphosate resistance died after the Roundup® herbicide application.

Table 1. Plant height and density means of male and female parents and their F_1 and F_2 hybrid progeny across two locations for six cross populations.

Genotype	G[Z]	Height[Y] (m)	Density (Plants m^{-1})
LA1110023/PHY410R	F_1	1.74 a	6.5 ab
LA1110023/PHY410R	F_2	1.75 a	6.0 b
LA1110023	♀	1.70 a	7.2 a
PHY410R	♂	1.75 a	7.1 a
ARKRM24-12-04/PHY410R	F_1	1.69 ab	7.2 a
ARKRM24-12-04/PHY410R	F_2	1.73 a	5.9 b
ARKRM24-12-04	♀	1.60 b	7.1 a
PHY410R	♂	1.75 a	7.1 a
ARK9506-40-05/PHY410R	F_1	1.64 ab	7.1 a
ARK9506-40-05/PHY410R	F_2	1.67 ab	6.0 b
ARK9506-40-05	♀	1.59 b	7.5 a
PHY410R	♂	1.75 a	7.1 a
8824-1-2-25-30-26/PHY410R	F_1	1.67 a	7.2 ab
8824-1-2-25-30-26/PHY410R	F_2	1.72 a	6.2 c
8824-1-2-25-30-26	♀	1.68 a	6.4 bc
PHY410R	♂	1.75 a	7.1 b
99WJ-9/PHY410R	F_1	1.69 ab	6.8 a
99WJ-9/PHY410R	F_2	1.68 ab	6.1 a
99WJ-9	♀	1.59 b	5.7 b
PHY410R	♂	1.75 a	7.1 a
00U-82/PHY410R	F_1	1.67 ab	6.5 ab
00U-82/PHY410R	F_2	1.72 ab	5.7 b
00U-82	♀	1.57 b	6.0 b
PHY410R	♂	1.75 a	7.1 a
Total mean generation	F_1	1.69 ab	6.9 a
Total mean generation	F_2	1.71 a	6.0 b
Total mean generation	P	1.65 b	6.8 a

[Z] G= Generation, ♀= Female parent, ♂= Male parent, P= combined parents.

[Y] Values within a column followed by a different letter are statistically different (P<0.05) for comparison within a population.

Chi-square analysis was done to test the segregation ratio among the F_2 cotton lines, and the theoretical segregation ratio (3 alive: 1 dead) was not different from the observed ratio (Table 2). Plant densities in the F_1 generation were not expected to be affected by glyphosate application because all plants were expected to carry the Roundup Ready® gene for glyphosate resistance. The few dead plants in the F_1 populations presumably reflect pollen contamination from nearby non-transgenic plants, or self-pollination that occurred before hand pollination with transgenic pollen.

Table 2. Chi-square (χ^2) goodness-of-fit analysis for expected segregation ratio of F_2 population progeny possessing the Roundup Ready® gene.

Pedigree	Alive	Dead	χ^2 (3:1)	*P*
	------ % ------			
LA1110023/PHY410R	77.9	22.1	2.49	0.11
ARKRM24-12-04/PHY410R	75.9	24.1	0.23	0.63
ARK9506-40-05/PHY410R	75.5	24.5	0.07	0.80
8824-1-2-25-30-26/PHY410R	78.3	21.7	3.53	0.06
99WJ-9/PHY410R	75.8	24.2	0.22	0.64
00U-82/PHY410R	76.4	23.6	0.62	0.43

Fiber quality heterosis. Heterosis for fiber properties was only noted for short fiber index (Table 3). No heterosis was noted within a population for fiber length (UHM) or strength (kN m kg^{-1}). Short fiber index was found to have significant heterosis in only one cross : 99WJ-9/PHY410R. The F_2 population for the cross 99WJ-9/PHY410R had a 3.22 SFI, 23% lower than the best parent (Table 3). Fineness (micronaire) had a significant location by population interaction which is not unexpected, given the impact of environmental conditions on this trait. Within populations, there were no significant or consistent trends with regards to micronaire in the parent and their F_1 and F_2 progeny, regardless of location. On average Alexandria (4.89) had lower micronaire than Saint Joseph (4.91).

Table 3. Plant height and fiber quality descriptor means of male and female parents and their F_1 and F_2 hybrid progeny across locations for six cross populations.

Genotype	G[Z]	UHM[Y] (cm)	Strength (kN m kg^{-1})	SFI (%)	Alex	S Joe
					------- Mic[X] -------	
LA1110023/PHY410R	F_1	3.03 ab	329.1 a	3.33 b	4.73 a	4.73 b
LA1110023/PHY410R	F_2	2.97 b	323.3 a	3.58 ab	4.93 a	5.00 a
LA1110023	♀	3.06 a	328.1 a	3.68 ab	4.46 b	4.66 b
PHY410R	♂	2.90 c	319.6 a	4.17 a	4.83 a	4.96 a
ARKRM24-12-04/PHY410R	F_1	2.94 a	301.0 b	3.70 a	4.93 ab	4.73 b
ARKRM24-12-04/PHY410R	F_2	2.93 a	306.2 ab	3.90 a	5.13 a	5.03 a
ARKRM24-12-04	♀	2.95 a	303.6 b	4.10 a	5.10 a	4.83 ab
PHY410R	♂	2.90 a	319.6 a	4.17 a	4.83 b	4.96 a
ARK9506-40-05/PHY410R	F_1	2.95 a	312.7 ab	3.73 a	4.96 b	4.93 a
ARK9506-40-05/PHY410R	F_2	2.88 b	299.0 b	3.95 a	5.03 ab	5.03 a
ARK9506-40-05	♀	2.91 ab	307.6 ab	3.67 a	5.20 a	5.10 a
PHY410R	♂	2.90 ab	319.6 a	4.17 a	4.83 b	4.96 a
8824-1-2-25-30-26/PHY410R	F_1	2.97 a	321.3 a	3.52 a	5.00 a	4.86 b
8824-1-2-25-30-26/PHY410R	F_2	2.93 ab	319.8 a	4.02 a	5.06 a	4.96 ab
8824-1-2-25-30-26	♀	2.94 ab	316.5 a	3.97 a	5.06 a	5.16 a
PHY410R	♂	2.90 b	319.6 a	4.17 a	4.83 b	4.96 ab
99WJ-9/PHY410R	F_1	3.03 a	331.5 a	3.43 bc	4.73 a	5.06 a
99WJ-9/PHY410R	F_2	3.07 a	316.4 a	3.22 c	4.86 a	4.90 a
99WJ-9	♀	3.04 a	319.0 ab	4.18 ab	4.75 a	4.65 b
PHY410R	♂	2.90 b	319.6 ab	4.17 a	4.83 a	4.96 a
00U-82/PHY410R	F_1	3.12 a	328.4 a	3.17 b	4.83 a	4.96 a
00U-82/PHY410R	F_2	3.09 a	320.1 a	3.23 b	4.73 a	4.86 a
00U-82	♀	3.10 a	327.4 a	3.65 ab	4.65 a	4.85 a
PHY410R	♂	2.90 b	319.6 a	4.17 a	4.83 a	4.96 a
Total mean generation	F_1	3.01 a	320.7 a	3.48 a	4.90 a	4.88 a
Total mean generation	F_2	2.98 a	314.1 a	3.65 ab	4.89 a	4.90 a
Total mean generation	P	2.98 a	316.9 a	3.92 b	4.88 a	4.94 a

[Z] G= Generation, ♀= Female parent, ♂= Male parent, P= combined parents, UHM= Upper-half mean fiber length, SFI= Short fiber index, Mic= Micronaire, Alex=Alexandria, LA, S Joe= Saint Joseph, LA.

[Y] Values within a column followed by different letter are statistically different at *P*= 0.05 for comparison within a population.

[X] Data presented by location due to interaction.

Yield component and lint yield heterosis. The only parameter that had significant heterosis in the F_1 or F_2 generation when averaged across populations was lint yield (Table 4). No significant heterosis was noted across generations for either boll weight or lint percentage. For yield per se, there was a highly significant (P = 0.01) generation main effect. The F_1 and F_2 generations had a highly significant yield increase over the parents (parents averaged 1094 kg lint ha^{-1}), with 25% heterosis in the F_1 population and 16% heterosis in the F_2 generations relative to the mid-parent value. The difference in the amount of heterosis for lint yield in the F_1 versus the F_2 generation over the mid-parent value (Table 4) was not significant (P =0.09).

The LA1110023/PHY410R cross had the greatest heterosis for lint yield across locations (Table 4). Increases over the best parent were 33% in the F_1 and 21% in the F_2. Both generations (F_1 and F_2) were significantly different (P = 0.05) than the best yielding parent, PHY410R. The second largest heterosis for lint yield was from the ARKRM24-12-04/PHY410R cross. Increases were 21% in the F_1 and 20% in the F_2. Both were significantly different (P =0.05) than the best yielding parent, ARKRM24-12-04. Similar best parent heterosis values have been reported elsewhere (Patil et al., 2012)

Table 4. Heterosis for cotton yield components and lint yield of male and female parents and their F_1 and F_2 hybrid progeny across two locations for six cross populations.

Genotype	G[Z]	Boll wt.[Y] (g)	Lint %	Lint yield (kg ha^{-1})	HHP[X] (%)	HMP (%)
LA1110023/PHY410R	F_1	5.85 a	39.7 a	1524 a	33.1*	37.3
LA1110023/PHY410R	F_2	5.78 a	40.2 a	1384 a	20.9*	24.6
LA1110023	♀	5.86 a	40.0 a	1076 b		
PHY410R	♂	5.16 b	39.1 a	1145 b		
ARKRM24-12-04/PHY410R	F_1	5.31 ab	40.7 a	1428 a	20.6*	22.6
ARKRM24-12-04/PHY410R	F_2	5.54 a	40.2 a	1415 a	19.5*	21.5
ARKRM24-12-04	♀	5.48 ab	40.8 a	1184 b		
PHY410R	♂	5.16 b	39.1 b	1145 b		
ARK9506-40-05/PHY410R	F_1	5.67 a	40.5 a	1375 a	15.6 ns	17.8
ARK9506-40-05/PHY410R	F_2	5.70 a	39.9 ab	1349 a	13.5 ns	15.6
ARK9506-40-05	♀	5.72 a	39.3 b	1189 a		
PHY410R	♂	5.16 b	39.1 b	1145 a		
8824-1-2-25-30-26/PHY410R	F_1	5.59 b	39.9 ab	1304 a	13.9 ns	18.7
8824-1-2-25-30-26/PHY410R	F_2	5.57 b	39.4 ab	1230 ab	7.4 ns	12.0
8824-1-2-25-30-26	♀	6.09 a	40.3 a	1052 b		
PHY410R	♂	5.16 c	39.1 b	1145ab		
99WJ-9/PHY410R	F_1	6.01 a	38.1 ab	1323 a	15.5 ns	25.4
99WJ-9/PHY410R	F_2	5.82 a	37.9 b	1106 ab	-3.4 ns	4.8
99WJ-9	♀	5.67 a	37.9 b	965 b		
PHY410R	♂	5.16 b	39.1 a	1145 ab		
00U-82/PHY410R	F_1	6.03 a	38.2 a	1260 a	10.0 ns	29.8
00U-82/PHY410R	F_2	6.08 a	36.9 b	1148 a	0.3 ns	18.2
00U-82	♀	5.67 a	36.4 b	797 b		
PHY410R	♂	5.16 b	39.1 a	1145 a		
Total mean generation	F_1	5.74 a	0.39 a	1369 a		25.1**
Total mean generation	F_2	5.74 a	0.39 a	1272 a		16.3**
Total mean generation	P	5.66 a	0.38 a	1094 b		

[Z] G= Generation, ♀= Female parent, ♂= Male parent, P= combined parents.

[Y] Values within a column followed by different letter are statistically different at p-value= 0.05 for comparison within the population cross.

[X] HHP= High-parent heterosis, HMP= Mid-parent heterosis.

* Significantly different from highest yielding parent at P=0.05, ns= not significant.

The ARK9506-40-05/PHY410R cross had a lint yield increase of 186 kg ha^{-1} (15.6%) in the F_1 population and a lint yield increase of 160 kg ha^{-1} (13.5%) in the F_2 population in relation to the best parent ARK9506-40-05, even though parents and progeny were not statistically different from each other ($P = 0.05$). In the remaining three populations there was no significant best-parent heterosis despite an average increase in lint yield in the F1 of 10-16%.

Gene effects. The genetic effects calculated from the GMA are summarized in Tables 5 and 6. The genetic effects for fiber quality traits are generally considered to be mostly additive and the genetic effects for the fiber quality traits in this experiment were dominant or over-dominant for all fiber traits and cross populations (Table 5). Significant dominance effects in the F_1 hybrid progeny for most within-boll yield components have recently been reported by Tang and Xiao (2013). Population sampling differences may explain some of the differences seen here. It is relevant to note that F_2 means more closely match mid-parent values than F_1 values. At later generations the approach to mid-parent values would likely become even greater and be in greater accordance with the observation that most fiber traits are under additive control.

Table 5. Genetic effects for fiber quality parameters for six cross populations.

Genotype	GMA[Z]	UHM[Y]	Strength	SFI	MIC
LA1110023/PHY410R	a=	0.09	1.86	0.08	0.08
	d=	0.26	7.04	1.67	1.16
ARKRM24-12-04/PHY410R	a=	0.06	0.93	0.25	0.32
	d=	0.26	8.67	1.49	1.33
ARK9506-40-05/PHY410R	a=	0.05	0.91	0.01	0.40
	d=	0.25	7.56	1.21	1.23
8824-1-2-25-30-26/PHY410R	a=	0.06	1.28	0.23	0.36
	d=	0.24	7.14	1.65	1.28
99WJ-9/PHY410R	a=	0.08	1.22	0.38	0.10
	d=	0.25	6.14	1.91	1.00
00U-82/PHY410R	a=	0.06	1.56	0.17	0.16
	d=	0.22	7.56	1.84	1.07

[Z] d = 0, there is no dominance; d < a, incomplete dominance; d = a, complete dominance; d> a, overdominance.

[Y] Comparisons of additive and dominance should be made within a cross population, not across populations.

Table 6. Genetic effects for lint yield, plant height, and yield components for six cross populations.

Genotype	GMA[Z]	Lint Yield[Y] kg ha^{-1}	Plant Height	Yield components	
				Boll wt	Lint percentage
LA1110023/PHY410R	a=	79.29	1.93	0.53	0.02
	d=	223.95	14.65	0.88	0.09
ARKRM24-12-04/PHY410R	a=	12.74	0.16	0.41	0.02
	d=	36.28	14.18	1.24	0.80
ARK9506-40-05/PHY410R	a=	27.63	0.34	0.48	0.02
	d=	28.14	18.81	0.98	0.08
8824-1-2-25-30-26/PHY410R	a=	42.84	1.99	0.74	0.02
	d=	15.81	17.44	1.34	0.09
99WJ-9/PHY410R	a=	103.30	0.80	0.37	0.01
	d=	66.51	13.49	0.56	0.09
00U-82/PHY410R	a=	196.27	2.88	0.26	0.01
	d=	41.16	14.42	0.68	0.09

[Z] d = 0, no dominance; d < a, incomplete dominance; d = a, complete dominance; d > a, overdominance.

[Y] Comparisons of additive and dominance should be made within a cross population, not across populations.

Values of additive or dominance effects for lint yield varied across cross populations because cotton lint yield depends on the direct and indirect effect of numerous genes and the environment. The relative proportion of the additive and dominance effects for the LA1110023/PHY410R and ARKRM24-12-04/PHY410R crosses are almost 3 times larger for the dominance effect, which indicates overdominance for these specific crosses. The remaining crosses exhibited incomplete dominance for lint yield. For plant height, there was nearly complete over-dominance, indicating that the progeny most closely resembled the taller parent. Values of additive and dominance effects for boll weight and lint percentage varied across cross populations but in general reflect the existence of over-dominance.

SUMMARY AND CONCLUSIONS

Crosses of upland cotton parents had lint yield heterosis in the F_1 hybrid population relative to the best parent. The LA1110023/PHY410R and ARKRM24-12-04/PHY410R crosses had the highest heterosis of up to 33.1% and 20.6%, respectively, in the F_1 generation, and 20.9% and 19.5% in the F_2 generation, respectively. The ARK9506-40-05/PHY410R population had yield heterosis of up to 15.6% in the F_1 population and up to 13.5% in the F_2 population. Not all yield increases were significantly different from the best parent although, in absolute measure, in only one situation was the F_1 or F_2 yield less than that of the best parent regardless of population. In general, fiber quality parameters in these six cross populations did not have significant heterosis in the F_1 or F_2 population relative to the best parent though they were often numerically superior, especially in the F_1. Similar, but slightly larger trends for fiber quality parameter heterosis were noted by Patil et al. (2012). Only one parameter, short fiber index, was found to have heterosis in the F_2 population.

The application of glyphosate over an F_2 population segregating for glyphosate resistance reduced plant density by up to 25%, which was expected since ~25% of the F_2 generation does not contain the Roundup Ready® gene. Due to the extensive compensatory ability of cotton, especially in cotton expressing heterosis, yield was not decreased after a 25% reduction in plant density.

In summary, F_2 hybrid cotton lines having useful heterosis were developed by using honey bees as pollinators to transfer the genetically dominant Roundup Ready® trait, followed by glyphosate application to eliminate self-pollinated F_1 plants. Further line testing will be required to determine the best combination of parents, and the promotion of this technology among seed companies is required for the development of better and improved F_2 hybrid cotton lines.

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