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

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DEVELOPING HYBRID COTTON (*GOSSYPIUM* SPP.) USING HONEY BEES AS POLLINATORS AND ROUNDUP READY® GENE AS SELECTION TRAIT

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ABSTRACT

Cotton (*Gossypium* spp.), the most important textile fiber crop in the United States (US), is cultivated in 17 states across the southern US. and a very important agricultural commodity for several states. The use of hybrids in the US has been limited due to seed cost production. 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 Roundup Ready® gene as selection trait.

This research was conducted during three years (2005-2007) in Louisiana. Crosses between non-transgenic and transgenic varieties were made in 2005 to obtain F_1 cottonseeds using honey bees. In 2006, F_2 cottonseed was obtained. In 2007, F_1, F_2, and parents were field tested using a randomized complete block design with 3 replications in two locations. Data analysis was conducted using the SAS PROC MIXED procedure with estimates of means generated using least square means (LS means).

Results indicate that all crosses exhibited heterosis in the F_1 hybrid populations relative to the best parent. The crosses LA1110023/PHY410R and ARKRM24-12-04/PHY410R exhibited a higher degree of heterosis for yield averaging 33.1% and 20.6%, respectively, across locations. Yield heterosis in the F_2 population was of 20.9% and 19.5%, respectively, and statistically different from the best parent. The ARK9506-40-05/PHY410R cross had yield heterosis averaging 15.6% in the F_1 population and 13.5% in the F_2 population; however, these were not significantly different from the best parent. The lack of significant yield heterosis might be attributed to experimental error and suggests the need for further field testing. Fiber quality descriptors from the six crosses, did not have a significant heterosis in the F_2 population relative to the best parent.

In summary, the use of herbicide resistant varieties as males and Roundup Ready® gene as selection trait, conventional varieties as females and honey bees as pollinators, has proven to be a viable method for developing F_2 hybrid varieties. Further variety testing will be required to determine the best combination of parents. Promotion of this technology among seed companies is required for the development of better and improved cotton varieties as F_2 hybrids.

mean K value. However, the correlated K field may have significant impact on the saltwater intrusion, resulting different from that obtained by the mean K field.

INTRODUCTION

Cotton (*Gossypium* spp.) is the most important textile fiber crop in the United States (U.S.) and in the world as well as the second most important oilseed crop in the world after soybean (Khan, M. A., et al., 2002). Much of the cultivated cotton hectarage throughout the world is in the temperate zone, although cotton is native to tropical and semitropical areas (Smith and Cothren, 1999). Currently, cotton is produced in 17 states across the southern United States. Seven states produced over one million bales each during 2006, and this represented 80% of the cotton in the U.S. Texas devotes more area for cotton production and produces more cotton than any other state, producing 5.8 million bales (bale = 218 kgs), which represented 28% of the U.S. cotton production in 2006 (Louisiana Farm Reporter, 2007).

Despite this importance there has been concern about stagnation in upland cotton yield in the United States (American Cotton Producers, 1999). Recent analyses of cotton yield over time have shown an increase in most of the cotton growing areas in the US. Meredith (2002) concluded that the way to end yield plateaus are new management technologies (e.g., insecticides, equipment, etc) and genetic technology (improved varieties).

The average Mississippi yield for years 2001 to 2005 was 981 kg lint ha^{-1}. This yield was 20% higher than the average yield of 811 kg lint ha^{-1} for the 1986 to 1995 pre-transgenic period. The average yield for the transitional period of 1996-2000, which involved both conventional and transgenic varieties, was 853 kg lint ha^{-1}. The yield increase was attributed to the reduction of insect damage to the crop (10.7% for the 1986-1995 period vs. 7.0% for the 2001-2004 period) (Meredith, 2006).

Current varieties must be changed or new varieties developed either through conventional or genetically enhanced technologies that are better adapted to abiotic and/or biotic stresses to take cotton to new levels of sustainable yield production and fiber quality. Alternatively, the development of cotton hybrids might be one of the solutions to increase cotton yield. 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 has the potential to utilize heterosis to further increase yields. The objective of this research is to determined if Roundup Ready® varieties as male donor crossed with conventional varieties as female receptors, and honey bees as pollinators would increase cotton yield potential to develop F_2 hybrid cotton seed.

REVIEW OF LITERATURE

Plant scientists have discussed the potential advantages of hybrid cotton for the past century. Despite demonstrated hybrid vigor, the commercial use of hybrid cotton has been quite limited in the USA because of 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) high cost of hybrid cotton seed production if by 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 not been successfully accomplished. According to Meredith (1998), in the U.S. Chembred released the first commercial F_2 varieties in 1992, but ceased operations in October 1995. The main factor to the lack of F_2 commercial success was the ineffectiveness of the male gametocide that had to be applied every 14 to 21 days and the varying amount 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 Genetics is commercializing F_1 inter-specific hybrid seeds in California obtained through hand pollination in India. Dong *et al.* (2004) reported that hybrid (F_1) *Bacillus thuringiensis* (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*) using pesticides.

Weaver (1999) reported that an F_2 population produced essentially the same amount of lint ha^{-1} as the F_1 hybrid and that both produced more lint than the parents. Meredith (1990) indicated that F_2 populations can also produce a better combination of yield and fiber quality than their parents grown alone. In that study, F_2 performance was highly correlated (r= 0.86) with F_1 yield performance. Occasionally, F_2 heterosis equaled F_1 heterosis. The highest yielding parent was "DES 119" with an average yield of 1031 kg ha^{-1} and the most widely planted U.S. variety at that time, "Deltapine 50", yielded 959 kg ha^{-1}. The highest yielding F_1 hybrids DES 119/Delcot 344 and DES 119/Coker 81-613, averaged 1145 and 1143 kg ha^{-1}, respectively, or about 15% greater than the average of DES 119 and Deltapine 50. The F_2 hybrids from these two respective crosses averaged 8% higher yields than the average of the parents. No differences in adaptive ability between the parents, F_1's, and F_2's were detected. Schoenhals (1990) reported that the agronomic property of ginned lint percent reflected no differences, and other agronomic properties were generally similar with a few exceptions for the F_1's and their F_2's. Taken together, these studies indicated that hybrids have the potential to increase yield in cotton.

Meredith (1998) cited that due to the genetic variation within an F_2 hybrid, the possibility exists that F_2's might have a broader range of adaptation than conventional varieties. Hybrids tend to have a broader range of adaptation than commercial varieties and they frequently exhibited greatest superiority when grown under stress conditions. Meredith (1998) indicated that using heterosis in cotton will require extensive testing to determine the best (highest yielding) combination of parents. He also reported that the only major trend toward selecting good parents for F_2 performance was that varieties developed in the Mississippi River Delta had the highest general combining ability, because three of the parents (DES 119, Stoneville 453, and Deltapine 50) used were selected in or at Stoneville or Scott, MS – the same location where the evaluation was conducted.

Upland cotton (*Gossypium hirsutum* L.) has large spheroidal and echinate pollen grains with a diameter over 120 µm, which are not wind-disseminated. Insects are the natural agents for pollen transfer. Bees (Hymenoptera: Apoidea) are the most important pollen vectors of cotton (Vaissiere *et al*. 1984). Honey bees (*Apis mellifera* L.) prefer nectar and pollen from plants other than cotton if they are available (Danka, 2005. Personal communication), so to promote cross pollination, cotton should be planted and managed so it blooms as early as possible, and competition from nearby plants should be reduced as much as possible (Moffett *et al*. 1975).

Waller *et al*. (1985) reported that honey bees and wild bees have been used as pollinators for male-sterile hybridization methods and that satisfactory seed yields were obtained in Arizona when the area was saturated with honey bees. Wild bee populations fluctuated too much from year-to-year and between fields during a given year to be dependable pollinators. Vaissiere (1994) cited that honey bees meticulously groom cotton pollen from their body, and the grooming behavior is interpreted as cotton pollen avoidance by honey bees. Vaissiere (1994) observed that in pollination studies for hybrid cotton seed production, honey bee foragers are often found in greater densities in male-sterile flowers than in the male-fertile flowers.

Thomas *et al.* (2001) studied pollen transfer in cotton seed production (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%, concluding that Perkins offered good promise for hybrid production.

Waller *et al*. (1985) demonstrated that in fields where there were few bees, approximately 2 colonies ha^{-1}, that male-fertile varieties averaged 23.6 vs. 18.5 seeds per boll produced by male-sterile varieties, and that the grams of lint per boll averaged 1.5 vs. 0.9 for the male-fertile and male-sterile varieties, respectively. Where the number of bees was higher, approximately 5 colonies per ha, the seed and lint yield between male-sterile and male-fertile varieties were almost identical, as measured by both plot yield and also from harvest weight reported by the seed company. Rhodes (2002) reported that a commercial cotton field managed with bee pollination helped to increase cotton yield up to 15.8% and increase the number of bolls harvested by 11.1%. Currently, the use of insect resistant transgenic cotton varieties (e.g. Bt cotton) and the boll weevil eradication program have dramatically reduced the use of insecticides on cotton.

The introduction of transgenic technology to cotton breeding has provided significant benefits to the industry. The first transgenic traits developed and commercialized in cotton addressed input costs by conferring insect resistance and herbicide tolerance to existing varieties. Though the direct impact of transgenic cotton varieties on yield trends is unclear, the existing transgenic varieties could be used as parents to develop hybrid cotton. When scientists inserted genes for herbicide resistance into cotton, they did not realize that they were also making it practical to produce 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 only one dominant gene is involved in the resistance, the F_2 hybrid will segregate in a 3:1 ratio, which in this case would be 3-resistant to 1-susceptible. The use of herbicide sensitive male-sterile ms5ms6 (1 fertile: 1sterile) varieties would ensure a high percentage of hybrid F_1's but would give some male sterile plants in the F_2 generation (Weaver, 1999).

The objective of this dissertation research is the use of the Roundup Ready® gene as selection trait, varieties with this trait as the male donor, conventional varieties as female receptors, and honey bees as pollinators for the development of F_2 hybrid cotton seed.

MATERIALS AND METHODS

This research was conducted during three years (2005-2007) at different research stations across Louisiana. In year one, crosses between non-transgenic and transgenic varieties were made to obtain F_1 cottonseed[1] using honey bees. In year two, F_2 cottonseed were obtained. In year three, the F_1, F_2, and parents were field tested for yield. Field plots in each research location were maintained by station personnel according to the Louisiana Cooperative Extension Service guidelines.

Year 1

The research was conducted in summer of 2005 in Baton Rouge, Louisiana at the LSU AgCenter Central Research Station. The field dimensions were 13 rows wide and 3 tiers deep (Table 1). Each tier was 15 meters long, rows were spaced 1 meter apart, and the intrarow seed density was 8-10 plants per meter was used.

Eighteen non-transgenic germplasm (Table 2) lines were used as females, selected from the 2005 Regional Breeding Testing Network (RBTN) trial, and a single transgenic commercial variety, Phytogen PHY410R (Dow Agro Sciences, LLC, Indianapolis, IN) was used as the male pollen donor. Female and male were planted in a 1:1 ratio to facilitate pollen transfer from male donor to female receptor. The RBTN varieties were selected for the experiment because of the gene diversity, due to the different objectives and breeding techniques that each breeding program uses. The RBTN facilitates the testing of advanced cotton breeding varieties from public programs over a wide range of environments and also provides a mechanism for the exchange of germplasm among participants (Gerald Myers, 2007. Personal communication).

A week prior to the onset of blooming, honey bees (*Apis mellifera* L.) were placed in the field to effect cross pollination between the transgenic male and non-transgenic female cotton

[1] Cottonseed is seed that has been delinted.

plants. Two honey bee hives were placed in the open field one colony in the first row between tiers one and two and another on the first row between tiers two and three. Additionally, three insect proof mesh cages (3 x 5 x 2.5 meters) were randomly erected over the first ¼ of a tier and three rows, and each cage received one honey bee colony, confining them to transfer pollen and feed from cotton plants within the cage. Cage one was on tier one over rows 1 through 3, cage two on tier one over rows 11 through 13, and cage three on tier 2 over rows 11 through 13 (Table 1 and Figure 1).

Table 1. 2005 Baton Rouge cotton field map

	Tier 1		Tier 2		Tier 3	
Row 1		PHY 410 R	‡	PHY 410 R	‡	PHY 410 R
Row 2	C1 †	ARK 9513-28-01	00 U-82		00 WA-103	
Row 3		PHY 410 R	PHY 410 R		PHY 410 R	
Row 4	ARK 9513-33-04		99 F-87		8824-1-2-25-198-15	
Row 5	PHY 410 R		PHY 410 R		PHY 410 R	
Row 6	ARK RM24-12-04		99 WJ-9		8824-1-2-25-198-7	
Row 7	PHY 410 R		PHY 410 R		PHY 410 R	
Row 8	ARK 9506-40-05		LA 00404065		8824-1-2-25-192-8	
Row 9	PHY 410 R		PHY 410 R		PHY 410 R	
Row 10	LA 1110035		LA 00404204		8824-1-2-25-198-10	
Row 11		PHY 410 R		PHY 410 R	PHY 410 R	
Row 12	C2 †	LA 1110023	C3 †	LA 00405034	8824-1-2-25-30-26	
Row 13		PHY 410 R		PHY 410 R	PHY 410 R	

†C1, C2 and C3 = Insect proof mesh cages, ‡= Open field honey bee hives.

Table 2. Germplasm evaluated as female-receptor for this cotton hybrid study.

Breeding Program	Germplasm	Breeding Program	Germplasm
U. of Arkansas	ARK 9513-28-01	Texas A&M U.	99 WJ-9
U. of Arkansas	ARK 9513-33-04	Texas A&M U.	99 F-87
U. of Arkansas	ARK RM24-12-04	Texas A&M U.	00 U-82
U. of Arkansas	ARK 9506-40-05	Texas A&M U.	00 WA-103
Louisiana State U.	LA 1110035	Mississippi State U.	8824-1-2-25-198-15
Louisiana State U.	LA 1110023	Mississippi State U.	8824-1-2-25-198-7
Louisiana State U.	LA 00405034	Mississippi State U.	8824-1-2-25-192-8
Louisiana State U.	LA 00404204	Mississippi State U.	8824-1-2-25-198-10
Louisiana State U.	LA 00404065	Mississippi State U.	8824-1-2-25-30-26

In fall of 2005 the non-transgenic female germplasm lines were harvested by hand. Among the seedcotton[2] harvested, there was expected to be a mix of self-pollinated non-transgenic seedcotton and F_1 non-transgenic/transgenic hybrid seedcotton. Bolls were ginned at the LSU Cotton Breeding Lab using a 7-saw laboratory gin (Porter-Morrison, Dennis Manufacturing Inc.) and then the fuzzy seed was delinted using 95% sulfuric acid. After delinting, the cottonseed was air cleaned, treated with a mix of Baytan® and Allegiance™ (Bayer CropScience, Durham, NC), packed and stored in a cold room for use in year two. From this harvested cotton, outcrossing percentage using honey bees and the F_2 hybrid cotton was obtained in year two.

Figure 1. 2005 cotton field with the insect proof mesh cages

[2] Seedcotton is the seed that has not been delinted and it is called fuzzy seed.

Year 2

Six random hybrid varieties out of the original eighteen were planted and replicated three times in 2006 in Saint Joseph, LA at the LSU AgCenter Northeast Research Station. Each tier was 12 meters long, with rows spaced 1 meter apart, and planted to an intrarow seed density of 8-10 plants per meter.

Prior to the 4^{th} true leaf stage, glyphosate was sprayed under the canopy by mistake. Soon after, around the $6\text{-}7^{th}$ true leaf stage, Roundup® herbicide (glyphosate at 850 g ai ha^{-1}) was sprayed over the crop to eliminate any self-pollinated non-transgenic plants.

The surviving plants were non-transgenic/transgenic hybrids, and surviving and dead plants were counted to calculate the outcrossing percentage using honey bees. Surviving hybrid plants were allowed to self pollinate and grown to maturity. Their F_2 cottonseed was harvested by hand to be field tested in year three in two locations, Alexandria, LA and Saint Joseph, LA.

Due to a shortage of enough F_1 seed to be planted and field tested for yield, along with the F_2 and parents in year three. The same six random non-transgenic female parents and the one male transgenic parent were grown and cross-pollinated by hand at the Cotton Winter Nursery (Tecoman, Mexico) in winter of 2006-2007 to generate F_1 seeds.

Additionally a single row of non-transgenic female plants were planted and replicated three times in a different block and sprayed with Roundup® herbicide (glyphosate at 850 g ai/ha). Surviving plants were counted to determine the percentage of adventitious Roundup Ready® gene presence among the non-transgenic females. Dr. Ted Wallace, at Mississippi State University (MSU), did the same study in a large scale and his data corroborate the results of the Roundup Ready® gene adventitious frequency observed in this study which are mentioned in the next chapter. The seed used at MSU and in this F_2 hybrid research came from the same lot of seed because every breeding program provided seeds for the 2005 RBTN and part of the seeds that was sent to the LSU Cotton Breeding Program was put aside for this research.

Bolls from F_1 plants, were ginned at the LSU Cotton Breeding Lab using a 7-saw laboratory gin (Porter-Morrison, Dennis Manufacturing Inc.) and then the fuzzy seed was delinted using 95% sulfuric acid. After delinting, cottonseeds were air cleaned, treated with a mix of Baytan® and Allegiance™, packed and stored in a cold room to be yield field tested in year three.

Year 3

The harvested F_2 cottonseeds from Saint Joseph, LA from year two, the F_1 cottonseed from the winter nursery in Mexico, and their parents were planted in a randomized complete block design by generation (Parents, F_1 and F_2 generation), with three replications during the normal growing season of 2007 in two locations for field testing. Varieties were randomized within each generation block and each generation block as randomized within each replication. Varieties were planted by generation to facilitate the application of herbicide of the top.

Due to shortage of seed for the 99WJ-9 and 00U-82 females, they were planted with two replications in the two locations. All data analysis was conducted using the SAS PROC MIXED procedure with estimates of means and standard errors generated using LS MEANS. Combined location data analysis was done where replication was designated as random effects in the model. Location and generation were treated as fixed effects, and varieties were nested in generations. Mean separation was conducted using Fisher's protected least significant difference (LSD) at the 0.05 level of probability.

The research in Alexandria, LA, was conducted on a Norwood silt loam, non-irrigated soil, in 15-meter long plots, and in Saint Joseph was conducted using a minimum tillage system on a Sharkey clay soil, which was given supplemental irrigation, in 12-meter long plots; both locations had rows 1 meter wide. Other management operations were as per Louisiana Cooperative Extension Service guidelines for the respective locations.

Three weeks after planting, at the 3rd-4th true leaf stage, the F_1 and F_2 blocks only were sprayed with Roundup® herbicide (glyphosate at 850 g ai ha^{-1}) over the top in both locations. Ten to fourteen days later the number of live and dead plants was counted on the F_1 and F_2 blocks to determine percent 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 open cotton bolls of each variety were collected by hand prior to machine harvesting in both locations to determine fiber quality descriptors. The twenty five bolls were picked at random from any plant and any portion of the plant in each variety. The twenty five cotton bolls were ginned at the LSU Cotton Breeding Lab using a 7 saw laboratory gin (Porter-Morrison, Dennis Manufacturing Inc.). Lint and cottonseed weights were recorded to determine lint percentage and yield parameters; 100 seeds were counted and weighed before and after delinting (using 95% sulfuric acid) to determine lint index.

Lint collected from the ginning process was analyzed using High Volume Instrumentation (HVI 900™ Zellweger Uster), at the LSU Cotton Fiber Lab. Among the cotton fiber descriptors measured were fiber length (cm), fiber strength (g tex^{-1}), short fiber index (SFI) (%), fiber fineness (micronaire), fiber elongation (%), and fiber uniformity (%). Row length was taken and converted to 15 meters long in both locations. Row gaps bigger than 4 feet were counted and measured for row length and yield adjustment. David Caldwell (2007, Personal communication) reported that, in his experience with adjusting row length for gaps in the rows, plants in rows with a gap lower than 1.2 meters compensate very well. Rows with gaps between

1.2 to 1.8 meters require a 25% length adjustment of the length of the gap added to the cultivated row, and rows with gaps between 1.8 to 2.7 meters require a 50% length adjustment of the length of the gap added to the cultivated row. In this research, there were a small number of row gaps between 1.2 to 2.7 meters, and the others were less than 1.2 m.

Due to the objective to increase lint yield and improve fiber quality for future variety releases and targeting this technology to be used by seed companies, the comparison and discussion of hybrid vigor or heterosis was made with regard to the best or high yielding parent, even though best parent and mid parent heterosis are presented in the cotton lint yield table.

RESULTS AND DISCUSSION

Outcrosses and Roundup Ready® Gene Screening

The cross pollination percentage using honey bees within a cage was not statistically different from the percentage using honey bees in the open field (p =0.48). Cross pollination among plants varied from 21 to 65% within a cage and from 33 to 55% in the open field. The high percent of cross pollination within a cage was expected since the honey bees could only feed at cotton flowers. The high percentage of outcrossing in the open field might have been due to honey bees and other insects such as bumble bees (*Bombus* sp.), that were observed in the plot. The outcrossing variation was high, both within cage and in the open field, but even if we take as reference the lowest cross pollination rate of 21% there probably would be enough hybrid F_1 plants to obtain F_2 hybrid seed for the process to be commercially viable.

The frequency of the adventitious Roundup Ready® genes among the non-transgenic female varieties was zero for LA1110023 and ARKRM24-12-04, below one percent for ARK9506-40-05 (0.5%) and 8824-1-2-25-30-26 (0.7%), and below two percent for 00U-82 (1.1%) and 99WJ-9 (2%). Dr. Ted Wallace in Mississippi State University obtained similar results regarding adventitious Roundup Ready® gene for the first four female varieties mentioned above (Dr. Wallace, 2007. Unpublished data).

There is a threshold of up to 0.5% of seed with adventitious transgenic genes contamination for the seed still to be considered as GMO free seed. Three of the six female varieties used in this experiment were under this threshold. There was no relationship between adventitious presence percentage and cross pollination rates because female varieties above and bellow the 0.5% threshold had similar cross pollination rates. Specifically, pollination means were as follows: ARKRM24-12-04 with 54%, LA1110023 with 43%, and ARK9506-40-05 with

40% for the low or non contaminated varieties and 8824-1-2-25-30-26 with 55%, 00U-82 with 51%, and 99WJ-9 with 33% for the higher contaminated varieties.

Agronomic Traits

There was no location by variety interaction ($p = 0.51$) for plant height (Table 3). The average plant height in Alexandria, LA was, however, significantly higher than the average plant height in Saint Joseph, LA; height was 1.89 m and 1.47 m, respectively ($p < 0.01$). Irrigated plots in Saint Joseph as well as the soil type difference compared to Alexandria might have kept water in the ground available for plants to use during critical moment in their vegetative growth, which might have helped for a normal plant height. In Alexandria, wet cloudy weather during active juvenile growth, high insect pressure and late growth regulator application might have caused greater height. Weed competition in both locations was similar with the tendency of the parents block to have few more weeds among the generation blocks, for this reason weeds were excluded as a factor affecting plant height. There were higher weed populations in alleys and gaps within rows.

There were no significant ($p = 0.\ 40$) differences between varieties for plant height. Over all six crosses, the generation main effect was significant ($p = 0.03$) for plant height indicating the existence of heterosis for height. The height average for the F_1 population was 1.69 meters and 1.71 meters for the F_2 population, which were not statistically different ($p = 0.31$). The average height of the parents was 1.65 m. This shorter than plants of the F_2 population ($p = 0.01$), but similar to plants of the F_1 population ($p = 0.13$).

There was no location by cross interaction ($p = 0.86$) for plant density. 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 row length discrepancies in each location. In Alexandria the seed cone planter was set to plant 140 seeds in 15.2 meters, and in Saint Joseph it was set to plant 140 seeds in 12.2 meters.

Table 3. Plant height and plant density means across locations for six crosses, their F_1, F_2 populations, male and females.

Genotype	G†	Height (meters)	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
LSD (0.05)		0.13	0.8
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

† G= Generation, P= Parents, ♀= female, ♂= male.
‡ Values within a column followed by different letter are statistically different at p-value= 0.05 for comparison within the population cross. LSD for comparison across populations.

There was a generation effect ($p < 0.01$) for plant density. The densities for the F_1 population plots (6.89 plants m^{-1}) and the parent plot (6.82 plants m^{-1}) were similar ($p = 0.37$).

The lower plant density for the F_2 population plots (6.00 plants m^{-1}) was different from both the F_1 population ($p < 0.01$) and the parent plots ($p < 0.01$). The F_1 and the F_2 population plots were sprayed with Roundup® herbicide (glyphosate at 850 g ai ha^{-1}) around the 3^{rd}-4^{th} true leaf stage to eliminate the segregating plants from the F_2 hybrid varieties. Plants in the F_2 generation segregated as expected (3 alive:1 dead) for a single dominant gene. Chi-square analysis was done to test the segregation ratio among the F_2 cotton varieties, and the theoretical segregation ratio was not statistically different from the one obtained in this research (Table 4). Because of this a lower plant density at the F_2 hybrid generation was expected due to segregation for the Roundup Ready® gene. The F_1 plant density was expected not to be affected by herbicide application because they carry the Roundup Ready® gene. The few dead plants in the F_1 populations were presumably reflects pollen contamination from nearby non-transgenic plants or self pollination that proceeded to the application of transgenic pollen via hand cross pollination.

Table 4. Chi-square (χ^2) goodness-of-fit analysis for expected segregation ratio of F_2 population progeny involving Roundup Ready® gene.

Pedigree	Alive	Dead	χ^2 (3:1)	p-value
	--------- %	---------		
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

Cotton Fiber Quality Traits

The variability of the fiber properties in cotton is an unfavorable element in a market that pits this natural fiber against more uniform synthetic fiber. Fiber properties vary as a function of the variety but also as a function of the environment and production practices (Clouvel, *et al.* 1998).

There was no location by variety interaction for fiber length (p =0.28) (Table 5). Fiber length varied by location (p <0.01). Fibers were longer in Alexandria plots (3.03 cm) than in Saint Joseph plots (2.94 cm). There was no generation effect (p =0.09) for fiber length; therefore, there were no differences statistically among the F_1 and F_2 populations and the parents. According to the U. S. Cotton Fiber Chart standards, values of all the parents and their progeny classified them as long fiber.

The main effect of varieties was highly significant (p <0.01) for fiber length. The females 00U-82, LA1110023 and 99WJ-9 had the longest fiber, and they were not statistically different (p 0.05) from each other. The F_1 population for the 00U-82/PHY410R cross had the highest fiber length (3.12 cm), which still was not statistically different than the F_2 population (3.09 cm) (p =0.24) nor was it different from the female parent 00U-82 (3.10 cm) (p =0.07). The F_1 population of the 00U-82/PHY410R cross was highly significant different than the male parent PHY410R (2.90 cm) (p <0.01).

The F_1 population for the LA1110023/PHY410R cross had a length of 3.03 cm, which was higher than the F_2 population (2.97 cm); they both were not statistically different (p =0.09) from each other. The F_1 population for the LA1110023/PHY410R cross was not statistically different from the female parent LA1110023 which was 3.06 cm long (p =0.21). The F_2 population for the LA1110023/PHY410R cross had shorter fiber and was statistically different than the female parent LA1110023 (p <0.01).

The F_1 population for the 99WJ-9/PHY410R cross had a length of 3.03 cm, the F_2 population had a length of 3.07 cm and the female parent 99WJ-9 had a length of 3.04 cm. In this cross, the female parent and progeny were not statistically different from each other ($p = 0.24$); the male parent PHY410R had shorter fiber (2.90 cm) and was statistically different from the F_1, and F_2 populations and the female 99WJ-9 ($p < 0.01$).

The females ARK9506-40-05 and 8824-1-2-25-30-26 had the shortest fiber among all the parents used in this experiment, but they were still considered to have long fiber according to the U. S. Cotton Fiber Chart; the F_1 and F_2 populations exhibited some fiber length hybrid heterosis but population means were not statistically different than their best parents. Parental varieties that had the longest fiber were different statistically from the parental varieties that had the shortest fiber ($p = 0.05$).

The location effect was highly significant ($p < 0.01$) for fiber strength. Alexandria had higher fiber strength than Saint Joseph, 32.88 g tex^{-1} and 31.86 g tex^{-1}, respectively. Across varieties, there was no generation main effect ($p = 0.12$) for fiber strength; therefore, there were no differences statistically among the F_1, and F_2 populations and the parents. According to the U. S. Cotton Fiber Chart all the parents and their progeny had strong fibers.

Varieties main effect was highly significant ($p < 0.01$) for fiber strength (g tex^{-1}). The females LA1110023, 99WJ-9 and 00U-82 had the strongest fiber, and they were not statistically different from each other ($p = 0.05$). These females were, furthermore, not statistically different from their progeny. The females ARKRM24-12-04 and ARK9506-40-05 had the lowest fiber strength among all the parents used in this experiment, and they were not statistically different from their F_1 and F_2 populations progeny ($p = 0.05$). The female LA1110023 and its F_1 and F_2 populations were significantly different from the female ARKRM24-12-04 and its F_1 and F_2 population ($p < 0.01$). High fiber strength varieties are desirable as Artzt (1998) and Suh *et al.*

(1998) found that there is a direct correlation between fiber strength and yarn tenacity or yarn strength.

Table 5. Fiber quality descriptors means for six crosses, their F_1, F_2 populations, male and females*

Genotype	G†	UHM‡ (cm)	GTEX (g tex^{-1})	SFI (%)	Alex	S Joe	E (%)	U (%)
					------- Mic	-------		
LA1110023/PHY410R	F_1	3.03 ab	33.57 a	3.33 b	4.73 a	4.73 b	9.03 bc	85.2 a
LA1110023/PHY410R	F_2	2.97 b	32.98 a	3.58 ab	4.93 a	5.00 a	9.57 ab	84.5 a
LA1110023	♀	3.06 a	33.47 a	3.68 ab	4.46 b	4.66 b	8.70 c	84.9 a
PHY410R	♂	2.90 c	32.60 a	4.17 a	4.83 a	4.96 a	9.85 a	84.5 a
ARKRM24-12-04/PHY410R	F_1	2.94 a	30.70 b	3.70 a	4.93 ab	4.73 b	9.03 b	84.5 a
ARKRM24-12-04/PHY410R	F_2	2.93 a	31.23 ab	3.90 a	5.13 a	5.03 a	9.02 b	84.6 a
ARKRM24-12-04	♀	2.95 a	30.97 b	4.10 a	5.10 a	4.83 ab	8.70 b	84.7 a
PHY410R	♂	2.90 a	32.60 a	4.17 a	4.83 b	4.96 a	9.85 a	84.5 a
ARK9506-40-05/PHY410R	F_1	2.95 a	31.90 ab	3.73 a	4.96 b	4.93 a	9.03 bc	84.6 a
ARK9506-40-05/PHY410R	F_2	2.88 b	30.50 b	3.95 a	5.03 ab	5.03 a	9.22 b	84.5 a
ARK9506-40-05	♀	2.91 ab	31.38 ab	3.67 a	5.20 a	5.10 a	8.50 c	84.5 a
PHY410R	♂	2.90 ab	32.60 a	4.17 a	4.83 b	4.96 a	9.85 a	84.5 a
8824-1-2-25-30-26/PHY410R	F_1	2.97 a	32.80 a	3.52 a	5.00 a	4.86 b	9.18 bc	84.9 a
8824-1-2-25-30-26/PHY410R	F_2	2.93 ab	32.62 a	4.02 a	5.06 a	4.96 ab	9.57 ab	84.4 ab
8824-1-2-25-30-26	♀	2.94 ab	32.28 a	3.97 a	5.06 a	5.16 a	8.88 c	84.1 b
PHY410R	♂	2.90 b	32.60 a	4.17 a	4.83 b	4.96 ab	9.85 a	84.5 ab
99WJ-9/PHY410R	F_1	3.03 a	33.81 a	3.43 bc	4.73 a	5.06 a	8.50 b	85.1 ab
99WJ-9/PHY410R	F_2	3.07 a	32.27 b	3.22 c	4.86 a	4.90 a	8.67 b	85.3 a
99WJ-9	♀	3.04 a	32.54 ab	4.18 ab	4.75 a	4.65 b	8.53 b	84.0 c
PHY410R	♂	2.90 b	32.60 ab	4.17 a	4.83 a	4.96 a	9.85 a	84.5 bc
00U-82/PHY410R	F_1	3.12 a	33.50 a	3.17 b	4.83 a	4.96 a	8.85 b	85.3 a
00U-82/PHY410R	F_2	3.09 a	32.65 a	3.23 b	4.73 a	4.86 a	8.62 b	84.8 ab
00U-82	♀	3.10 a	33.39 a	3.65 ab	4.65 a	4.85 a	8.58 b	84.9 ab
PHY410R	♂	2.90 b	32.60 a	4.17 a	4.83 a	4.96 a	9.85 a	84.5 b
LSD (0.05)		0.07	1.61	0.7	0.23	0.23	0.55	0.7
Total mean generation	F_1	3.01 a	32.71 a	3.48 a	4.90 a	4.88 a	8.94 ab	84.95 a
Total mean generation	F_2	2.98 a	32.04 a	3.65 ab	4.89 a	4.90 a	9.11 a	84.78 ab
Total mean generation	P	2.98 a	32.32 a	3.92 b	4.88 a	4.94 a	8.85 b	84.54 b

* Data presented was combined over locations with the exception of mic

† G= Generation, P= Parents, ♀= Female, ♂= Male, UHM= Length, GTex= Strength, SFI= Short Fiber Index, Mic= Micronaire, Alex=Alexandria, S Joe= Saint Joseph, E= Elongation, U=Uniformity.

‡ Values within a column followed by different letter are statistically different at p-value= 0.05 for comparison within the population cross. LSD for comparison across populations.

There was no location by variety interaction for short fiber index (SFI) ($p = 0.75$); the location main effect was not significant ($p = 0.05$) for SFI. Varieties main effect was not significant ($p = 0.24$) for SFI. As group, generation main effect was significantly different ($p < 0.01$) for SFI; the F_1 population had an average of 3.48% for SFI, the F_2 population had an average of 3.65% for SFI, and the parents had an average of 3.92% for SFI; only the F_1 population was statistically different than the parents ($p < 0.01$).

It is desirable to have cotton varieties with fiber fineness as measured by micronaire (mic) no higher than 4.8. Plant breeders routinely select varieties based in fiber fineness for any given cross. For hybrids, the ideal would be to have negative hybrid vigor for fiber fineness or not higher than the lowest parent. Among all the fiber quality descriptors fineness (mic) was the only one that had location by variety interaction ($p = 0.03$). This means that parents and their F_1 and F_2 population progeny had different fiber fineness in each location. On average Alexandria had lower micronaire than Saint Joseph, 4.89 and 4.91 respectively. Location by variety interaction effect is not altogether unexpected given the large effect that environment has on this fiber characteristic. The female LA1110023 and 00U-82 were the varieties that had the lowest micronaire in Alexandria, where both were not statistically different ($p = 0.17$). The F_1 and F_2 populations from the LA1110023/PHY410R cross were found not to have hybrid heterosis in relation to the best parent (in this case a lower micronaire). They had higher micronaire values and were statistically different from the female parent, but were not statistically different from the male parent which had higher micronaire value.

The F_1 and F_2 generations from the 00U-82/PHY410R cross did not show hybrid heterosis and were not statistically different from either parent ($p = 0.15$). Neither the F_1 nor F_2 populations had lower micronaire nor were they statistically different from the best parent. The females LA1110023, 99WJ-9 and 00U-82 were the varieties that had the lowest micronaire in Saint Joseph, and they were not statistically different ($p = 0.05$) from each other; the 00U-82 and her F_1 and F_2 populations progeny were not statistically different from either parent. Allen (1998) reported that cotton with a micronaire value of 4.5 or greater is more desirable for use in nonwoven roll goods manufacturing since high micronaire cotton contains fewer neps or small bundles of entangled fibers which result in unsightly appearing fabric.

There was no location by variety interaction for fiber elongation ($p = 0.97$); the location main effect was highly significant ($p < 0.01$), where Alexandria had higher fiber elongation than Saint Joseph, 9.24% and 8.68%, respectively. Across varieties, there was a generation main effect ($p = 0.03$) for fiber elongation; there were no differences statistically between the F_1's and the parents ($p = 0.03$), but there was a difference between the F_2's and the parents ($p < 0.01$). Kechagia and Harig, (1998) reported that fiber elongation is correlated with both micronaire and strength.

According to the U. S. Cotton Fiber Chart all the varieties, parents and their progeny, had high elongation. Varieties main effect was highly significant ($p < 0.01$) for fiber elongation. The male parent PHY410R had the highest fiber elongation among all the parents and their progeny; The F_2 population for the LA1110023/PHY410R cross and the 8824-1-2-25-30-26/PHY410R cross had high elongation and were not statistically different from their male parent ($p = 0.30$).

There was no location by variety interaction for fiber uniformity ($p = 0.80$); the location main effect was also not significant ($p = 0.66$). Varieties main effect was not significant ($p = 0.12$) for fiber uniformity. According to the U. S. Cotton Fiber Chart all the varieties, parents and their progeny had high fiber uniformity. The crosses showed hybrid heterosis but none of them was statistically different than the best parent from the same cross. According to Kechagia and Harig (1998) length uniformity is more influenced by ginning rather than by variety or environment.

Within Boll Yield Components

Prior to machine harvest 25 cotton bolls were collected by hand from each variety and the bolls were ginned and the seeds delinted. Yield parameters derived from these 25 boll samples are listed at Table 6, and include: boll weight (g $boll^{-1}$), lint percentage, clean seed or cottonseed wt (g) and lint index (g).

The fraction of the lint separated from a seedcotton sample by ginning is called lint percentage, and is a very important yield determining parameter. After ginning the cotton bolls in the laboratory, 100 seeds of each variety were weighed before and after delinting. The difference in seed weight before and after delinting is called lint index.

There has not been a clear use so far for the lint attached to the seed or lint index; therefore, it is better to have a lower lint index, because this leads to an increase in the lint percentage of the cotton harvested; increasing in this way the lint production.

Lint percentage from a commercial gin could drop a few percentage points in relation to a lab lint percentage, but any field lint percentage above 38-39% would be considered very good (Dr. Jack E. Jones, personal communication).The reason why the lint percentage from cotton bolls harvested by hand and ginned with a laboratory gin are higher than the cotton harvested by machine and ginned in a commercial gin is because the cotton bolls harvested by hand are cleaner and do not undergo any additional stages of cleaning by passage through a lint cleaner. A heavy boll with bigger seeds does not necessarily produce a high lint percentage, but generally a lighter boll with smaller seeds produces a higher lint percentage. Most cotton breeding programs want to have plants that bear heavy bolls with high lint percentage, which could translate into higher lint yields.

There was no location by variety interaction effect (p =0.33) for boll weight. The location main effect was highly significant (p <0.01), the average boll wt in Alexandria was 5.89 g $boll^{-1}$ and in Saint Joseph was 5.54 g $boll^{-1}$. The variety main effect was highly significant (p <0.01), where the male parent PHY410R had the lowest boll wt. and was significant by different from most of the females and their progeny (p =0.05). The female 8824-1-2-25-30-26 variety had the highest boll wt. and was statistically different than its progeny (p =0.05). There was no generation main effect (p =0.43) for boll wt; therefore, there were no differences statistically among the F_1, and F_2 populations and their best parents.

There was no location by variety interaction effect (p =0.94) for lint percentage. The location main effect was highly significant (p <0.01), the average lint percentage in Alexandria was 37.5% and in Saint Joseph was 40.8%. The variety main effect was highly significant (p <0.01), where female parents 99WJ-9 and 00U-82 had the lowest lint percentage, these females and their progeny were not statistically different (p =0.05), the male parent PHY410R had a higher lint percentage and was statistically different (p =0.05) from the female parents.

The female ARKRM24-12-04 variety had the highest lint percentage (40.8); none of her progeny had better lint percentage, neither were they statistically different from each other. There was no generation main effect (p =0.05) for lint percentage; therefore, there were no differences statistically among the F_1 and F_2 populations and their best parents.

Table 6. Cotton yield components across locations for six crosses, their F_1, F_2 populations, male and females.

Genotype	G†	Boll wt (g)	Lint %	100 Seeds (g)	
				Seed wt (g)	Lint index (g)
LA1110023/PHY410R	F_1	5.85 a	39.7 a	10.05 a	1.36 a
LA1110023/PHY410R	F_2	5.78 a	40.2 a	10.20 a	1.23 ab
LA1110023	♀	5.86 a	40.0 a	9.83 a	1.21 b
PHY410R	♂	5.16 b	39.1 a	9.73 a	1.15 b
ARKRM24-12-04/PHY410R	F_1	5.31 ab	40.7 a	9.61 a	1.11 a
ARKRM24-12-04/PHY410R	F_2	5.54 a	40.2 a	9.73 a	1.20 a
ARKRM24-12-04	♀	5.48 ab	40.8 a	9.23 a	1.16 a
PHY410R	♂	5.16 b	39.1 b	9.73 a	1.15 a
ARK9506-40-05/PHY410R	F_1	5.67 a	40.5 a	9.61 a	1.20 b
ARK9506-40-05/PHY410R	F_2	5.70 a	39.9 ab	9.70 a	1.23 ab
ARK9506-40-05	♀	5.72 a	39.3 b	9.71 a	1.36 a
PHY410R	♂	5.16 b	39.1 b	9.73 a	1.15 b
8824-1-2-25-30-26/PHY410R	F_1	5.59 b	39.9 ab	10.03 a	1.28 b
8824-1-2-25-30-26/PHY410R	F_2	5.57 b	39.4 ab	9.75 a	1.21 b
8824-1-2-25-30-26	♀	6.09 a	40.3 a	10.06 a	1.48 a
PHY410R	♂	5.16 c	39.1 b	9.73 a	1.15 b
99WJ-9/PHY410R	F_1	6.01 a	38.1 ab	11.03 a	1.46 a
99WJ-9/PHY410R	F_2	5.82 a	37.9 b	10.71 a	1.38 ab
99WJ-9	♀	5.67 a	37.9 b	10.47 a	1.30 bc
PHY410R	♂	5.16 b	39.1 a	9.73 b	1.15 c
00U-82/PHY410R	F_1	6.03 a	38.2 a	11.18 a	1.33 a
00U-82/PHY410R	F_2	6.08 a	36.9 b	11.25 a	1.31 a
00U-82	♀	5.67 a	36.4 b	11.47 a	1.35 a
PHY410R	♂	5.16 b	39.1 a	9.73 b	1.15 b
LSD (0.05)		0.36	1.2	0.55	0.14
Total mean generation	F_1	5.74 a	0.39 a	10.25 a	1.29 a
Total mean generation	F_2	5.74 a	0.39 a	10.22 a	1.26 a
Total mean generation	P	5.66 a	0.38 a	9.98 a	1.28 a

† G= Generation, P= Parents, ♀= female, ♂= male.
‡ Values within a column followed by different letter are statistically different at p-value= 0.05 for comparison within the population cross. LSD for comparison across populations.

There was no location by variety interaction effect (p =0.90) for 100 seed wt. The location main effect was highly significant (p <0.01). The average 100 seed wt. in Alexandria was higher (10.5 g) than in Saint Joseph (9.85 g). The variety main effect was highly significant (p <0.01), where the parents PHY410R, LA1110023, ARKRM24-12-04 and ARK9506-40-05 had the lowest boll wt. which was significant different from the other parents (p =0.05). The progeny of the parents mentioned above were not significant different from them (p =0.05). The female 99WJ-9 and 00U-82 varieties had the highest 100 seed wt. and were not statistically different than their progeny (p =0.05), but statistically different than PHY410R. There was not generation main effect (p =0.22) for 100 seed wt; therefore, there were no differences statistically among the F_1, and F_2 populations and their best parents.

There was no location by variety interaction effect (p =0.99) for lint index. The location main effect was highly significant (p <0.01). The average lint index in Alexandria was 1.36 gr and in Saint Joseph was 1.19 gr. The variety main effect was highly significant (p <0.01), where parents PHY410R and ARKRM24-12-04 had the lowest lint index, and their progeny were not statistically different (p =0.05). The females 00U-82, ARK9506-40-05 and 8824-1-2-25-30-26 varieties had the highest lint index (above 1.35 gr), and were not statistically different from each other, but were statistically different from PHY410R. The ARK9506-40-05/PHY410R and 8824-1-2-25-30-26/PHY410R progenies had a lower lint index than their female varieties, but were the same as their male parent. There was not generation main effect (p =0.52) for lint index; therefore, there were no differences statistically among the F_1, and F_2 populations and their best parents.

Cottonseed and Lint Yield

Plots were harvested by machine and weights were recorded. Lint yield (kg ha^{-1}) was calculated by multiplying seedcotton yield by lint percentage as determined from the twenty five boll samples and listed at Table 7.

There was no location by variety interaction ($p = 0.97$) for lint yield. There was a location main effect ($p < 0.01$) where the average lint yield in Saint Joseph was 1512 kg lint ha^{-1} compared to 939 kg lint ha^{-1} in Alexandria. The research plots in Saint Joseph were planted in an irrigated Sharkey clay field which could have ensured water for the plants at critical moments in reproductive development reducing plant competition stress. Plant height in Saint Joseph were lower than Alexandria, which might indicate that plants did not expend extra energy in the production of vegetative growth and distributed this energy to lint yield. In Alexandria, wet cloudy weather during active juvenile growth, high insect pressure and late growth regulator application might have induced vegetative growth and limited lint yield production. Weed competition in both locations was similar with the tendency of the parents block to have a few more weeds than the generation blocks, for this reason weeds were excluded as a factor affecting plant height. There were higher weed populations in alleys and gaps within rows.

There was variety main effect ($p = 0.04$) for lint yield. All six crosses showed lint yield increase in the F_1 population, and five of those crosses also displayed lint yield increase in the F_2 population in relation to the best parent. Only LA1110023/PHY410R and the ARKRM24-12-04/PHY410R had significant lint yield increase in both the F_1 and F_2 populations that was statistically different in comparison to the highest lint yielding parent. The LA1110023/PHY410R cross had the highest lint yield (1524 kg ha^{-1}) in the F_1 population and its F_2 population yielded 1384 kg lint ha^{-1}. Both population were not significantly different from each other ($p = 0.26$), but were significantly different from the best parent, PHY410R ($p = 0.05$). The ARKRM24-12-04/PHY410R cross had the second highest lint yield (1428 kg ha^{-1}) in the F_1 population and 1415 kg ha^{-1} in the F_2 population. Both populations were not significantly different from each other ($p = 0.92$), but they were significantly different from the best parent, ARKRM24-12-04 ($p = 0.05$).

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 ($p = 0.05$). According to Dr. Jack E. Jones (2007, personal communication), this was a good yield increase compared to the best parent. The lack of significance might be due to experimental error and suggests the need for further field testing for yields. Across varieties, there were no significant differences among the F_1 population; the LA1110023/PHY410R cross out-yielded the 00U-82/PHY410R cross by 264 kg lint ha^{-1} though they were not statistically different ($p = 0.05$).

There was a generation main effect ($p < 0.01$), therefore, there were differences statistically among the parents and their progeny. The F_1 and F_2 populations showed a yield increase significantly different from the parents which averaged 1077 kg lint ha^{-1} ($p < 0.01$).

Heterosis

The purpose of this research was to evaluate a novel method as a way to develop cotton hybrids. Only one male variety was used as pollen donor and six varieties as females or pollen receptor for the crosses. The lack of at least one additional male variety precluded the ability to do variety by tester or diallel analysis, which would have indicated the best combination of parents for a hybrid and the genetic variability among them. Heterosis and Generation Means Analysis (GMA) were the most appropriate and adaptable analyses for this research.

Heterosis also known as hybrid vigor, is the superior quality found in progeny from crosses of two unrelated parents. It can be taken or measured from either parent or their average (mid-parent) depending on the cross objective. In this research more emphasis was done at the F_2 in relation with its best parent (Table 7).

The only yield parameter that had significant (p =0.05) heterosis in the F_2 generation was lint yield. Most of the fiber quality descriptors, from the six crosses made in this research, did not have a significant F_2 heterosis in relation to the best parent. Only one of the fiber quality parameters, short fiber index (SFI), was found to have heterosis from only one cross (p =0.05). The F_2 population for the cross 99WJ-9/PHY410R had a 3.22 SFI, 23% lower than the best parent.

Analysis of overall means for lint yield, by generation, found that the F_1 population had 27% yield heterosis and the F_2 population had 18% yield heterosis in relation to the mid-parent value. These increases were not statistically different (p =0.09) from each other. In Alexandria, parents yielded an average of 771 kg lint ha^{-1}, and this environment was more discriminative with up to 40% in lint yield heterosis in the F_1 population and up to 27% in lint yield heterosis in the F_2 population in relation to mid-parent heterosis. Lint yield increases in the F_1 and F_2 populations were similar (p =0.08). The Saint Joseph environment was less discriminative among the hybrids and there was less of a drop off from F_1 heterosis to F_2 heterosis. In Saint Joseph, parents yielded an average of 1383 kg lint ha^{-1} and crosses had a 17% lint yield increase in the F_1 generation and up to a 14% increase in the F_2 generation. Both generations were more productive than the parents (p =0.01). All six crosses showed heterosis for lint yield in the F_1 population, and five of those crosses also displayed heterosis in the F_2 population. Only two of six crosses in the F_1 and F_2 populations were, however, statistically different in comparison to the highest lint yielding parent.

Table 7. Cotton lint yield and heterosis in the field across locations for six crosses, their F_1, F_2 populations, male and females.

Genotype	G†	Lint yield (kg ha^{-1} ‡)	HHP (%)	HMP (%)
LA1110023/PHY410R	F_1	1524 a	33.1*	37.3
LA1110023/PHY410R	F_2	1384 a	20.9*	24.6
LA1110023	♀	1076 b		
PHY410R	♂	1145 b		
ARKRM24-12-04/PHY410R	F_1	1428 a	20.6*	22.6
ARKRM24-12-04/PHY410R	F_2	1415 a	19.5*	21.5
ARKRM24-12-04	♀	1184 b		
PHY410R	♂	1145 b		
ARK9506-40-05/PHY410R	F_1	1375 a	15.6 ns	17.8
ARK9506-40-05/PHY410R	F_2	1349 a	13.5 ns	15.6
ARK9506-40-05	♀	1189 a		
PHY410R	♂	1145 a		
8824-1-2-25-30-26/PHY410R	F_1	1304 a	13.9 ns	18.7
8824-1-2-25-30-26/PHY410R	F_2	1230 ab	7.4 ns	12.0
8824-1-2-25-30-26	♀	1052 b		
PHY410R	♂	1145ab		
99WJ-9/PHY410R	F_1	1323 a	15.5 ns	25.4
99WJ-9/PHY410R	F_2	1106 ab	-3.4 ns	4.8
99WJ-9	♀	965 b		
PHY410R	♂	1145 ab		
00U-82/PHY410R	F_1	1260 a	10.0 ns	29.8
00U-82/PHY410R	F_2	1148 a	0.3 ns	18.2
00U-82	♀	797 b		
PHY410R	♂	1145 a		
LSD (0.05)		251		
Total mean generation	F_1	1369 a		27.1
Total mean generation	F_2	1272 a		18.1
Total mean generation	P	1077 b		

† G= Generation, P= Parents, ♂= female, ♂=male, HHP= High-parent heterosis, HMP= Mid-parent heterosis.

‡ Values within a column followed by different letter are statistically different at p-value= 0.05 for comparison within the population cross. LSD for comparison across populations.

* Significantly different from highest yielding parent at p-value =0.05, ns= not significant.

The LA1110023/PHY410R cross had the greatest heterosis for lint yield across locations. Increases over the best parent were 33% in the F_1 and 21% in the F_2. Both populations (F_1 and F_2) were significantly different ($p = 0.05$) than their best yielding parent, which was the

PHY410R variety. 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 their best yielding parent, ARKRM24-12-04.

Among all the parents, the ARK9506-40-05 variety had the highest lint yield and produced F_1 and F_2 populations with 16% and 14% yield increase, respectively. These lint yields in the F_1 and F_2 populations were similar to the best parent (p =0.05).

Generation Means Analysis

Generation Mean Analysis (GMA) provides relative measures of genetic effects. Using mean values of several different generations it is concerned with 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 might be successful (Khan, M. A. 2004). Here, a four generation model consisting of the parent one (P_1), parent two (P_2), hybrid (F_1), and hybrid (F_2) generations was used.

The genetic effects are summarized in Tables 8 and 9. Values of additive or dominance effects for lint yield varied from cross to cross because cotton yield depends on the direct and indirect effect of several 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 other crosses had incomplete dominance. For plant height, there was nearly complete dominance, indicating that the progeny most closely resembled the taller parent. Values of additive and dominance effects for boll weight, lint percentage, seed weight, and lint index varied from cross to cross and presumably reflect the action of different alleles. Across traits and crosses, the relative proportion between the additive and dominance effect estimates the effects for boll weight, lint percentage, seed weight, and lint index are significant.

The genetic effects for fiber quality traits are generally considered to be mostly additive (Gerald Myers, 2007. Personal communication), and the values for the fiber quality traits for this experiment were largely dominant (Table 9). Population sampling differences may explain some of the differences seen here. It is relevant, however, 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 8. Genetic effects for yield, plant height, yield components and seed traits for the six crosses.

Genotype	GMA†	Lint kg ha^{-1} ‡	Plant Height	Yield components		100 Seeds	
				Boll wt	Lint percentage	Seed wt	Lint index
LA1110023/PHY410R	a=	79.29	1.93	0.53	0.02	0.42	0.01
	d=	223.95	14.65	0.88	0.09	1.86	0.05
ARKRM24-12-04/PHY410R	a=	12.74	0.16	0.41	0.02	0.15	0.08
	d=	36.28	14.18	1.24	0.80	2.02	0.40
ARK9506-40-05/PHY410R	a=	27.63	0.34	0.48	0.02	0.47	0.18
	d=	28.14	18.81	0.98	0.08	2.37	0.42
8824-1-2-25-30-26/PHY410R	a=	42.84	1.99	0.74	0.02	0.57	0.22
	d=	15.81	17.44	1.34	0.09	2.12	0.37
99WJ-9/PHY410R	a=	103.30	0.80	0.37	0.01	0.66	0.05
	d=	66.51	13.49	0.56	0.09	1.30	0.00
00U-82/PHY410R	a=	196.27	2.88	0.26	0.01	0.69	0.10
	d=	41.16	14.42	0.68	0.09	1.72	0.23

† d= 0 there is not dominance, d < a for incomplete dominance, d= a complete dominance, d> a overdominance.
‡ Refer to the proportion in the same cross between "a" and "d" and not to the value itself.

Table 9. Genetic effects for fiber quality traits for the six crosses.

Genotype	GMA†	UHM‡	GTEX	SFI	MIC	ELON	UNI
LA1110023/PHY410R	a=	0.09	1.86	0.08	0.08	0.10	4.02
	d=	0.26	7.04	1.67	1.16	2.51	19.11
ARKRM24-12-04/PHY410R	a=	0.06	0.93	0.25	0.32	0.10	4.12
	d=	0.26	8.67	1.49	1.33	2.51	19.86
ARK9506-40-05/PHY410R	a=	0.05	0.91	0.01	0.40	0.23	3.88
	d=	0.25	7.56	1.21	1.23	2.37	19.41
8824-1-2-25-30-26/PHY410R	a=	0.06	1.28	0.23	0.36	0.02	3.58
	d=	0.24	7.14	1.65	1.28	2.42	18.91
99WJ-9/PHY410R	a=	0.08	1.22	0.38	0.10	0.11	3.47
	d=	0.25	6.14	1.91	1.00	2.95	18.00
00U-82/PHY410R	a=	0.06	1.56	0.17	0.16	0.57	3.90
	d=	0.22	7.56	1.84	1.07	2.86	19.00

† d= 0 there is not dominance, d < a for incomplete dominance, d= a complete dominance, d> a overdominance.
‡ Refer to the proportion in the same cross between "a" and "d" and not to the value itself.

SUMMARY AND CONCLUSIONS

Constant evaluation and characterization of the existent germplasm for heterosis is necessary; furthermore hybrids might be the cornerstone for the development of new and better cotton varieties in the United States and the world.

This study found that crosses evaluated had lint yield heterosis at the F_1 hybrid population in relation 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 at the F_1 population, and these two crosses also held a high heterosis into the F_2 hybrid population. The F_2 population for LA1110023/PHY410R and the ARKRM24-12-04/PHY410R crosses had heterosis of 20.9% and 19.5%, respectively. The ARK9506-40-05/PHY410R cross had a yield heterosis of up to 15.6% in the F_1 population and up to 13.5% in the F_2 population, but they were not significantly different from the best parent; the lack of significance might be attributed to high environmental variability, sample size, experimental error or chance. Regardless, this cross had a high yield increase that should be considered for further field testing for yield.

Most of the fiber quality descriptors from the six cross made in this research did not have significant heterosis in the F_2 population in relation to the best parent. Only one parameter, short fiber index (SFI), was found to have heterosis at the F_2 population. For the 99WJ-9/PHY410R cross ($p = 0.05$), SFI was 23% lower in the F_2 than the best parent (PHY410R).

Spraying herbicide (glyphosate) over a F_2 segregating cotton population reduced plant density by up to 25%, which eliminated plants that did not have the Roundup Ready® gene, even though yield was not affected due to plant yield compensation. Furthermore, seed density could be adjusted by increasing the planting rate by 25% to avoid excessive spacing within rows.

In summary, The use of honey bees for cross pollination, the easy transfer of Roundup Ready® gene and its dominant character, the F_2 heterosis that was equaled or was similar to the F_1 heterosis in some cases, and honey bees as pollinators has been proven to be a viable method for development of F_2 hybrid cotton varieties. Further variety testing will be required to determine the best combination of parents. Promotion of this technology among seed companies is required for the development of better and improved F_2 hybrids cotton varieties.

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VITA

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