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Construction of Genetic Linkage Map Showing Chromosomal Regions Associated with Some Agronomic Traits in Cotton

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ABSTRACT

Cotton is the world's leading fiber crop and the second most important oil seed crop. In Egypt, plant breeders have made major contributions to the productivity of this crop; however, this has led to a decrease in the genetic variation among Egyptian cotton varieties. Enhancing the productivity of cotton could be addressed through improving different agronomic traits including early flowering and maturation. In the present investigation, an interspecific cross (*G. barbadense* x *G. hirsutum*) was performed between two genotypes, cvs. 'Giza 83' (late flowering) and 'Deltapine' (early flowering) to develop an F2 segregating population. Analysis of segregation among the 71 F2 individuals was performed using 3 RAPD, 10 SSR, and 6 AFLP primer combinations. Twenty four AFLP primer combinations were used in bulked segregant analysis for flowering time. Linkage analysis and map construction were performed using Map Manager. The map showed 22 linkage groups with 140 markers covered a total length of 1556.7 cM. The average length of linkage groups ranged from 1.4 to 649.5. Single point analysis was used to identify the genomic regions controlling traits for plant height, number of nodes at flowering time, bolling date, days to flowering and number of bolls. In total, 30 significant QTL were identified for the five traits on ten linkage groups, among these 11 QTL for plant height, 8 for number of bolls, 4 QTL for each of days to flowering and bolling date and 3 QTL for number of nodes at flowering time. This work represents the first linkage map for the intercross between 'Giza 83' and 'Deltapine' showing chromosomal regions associated with some agronomic traits.

Keywords: AFLP, molecular markers, QTL, RAPD, SSR

INTRODUCTION

Cotton is an economically important renewable source of natural fiber and secondary products such as oil, livestock feed and cellulose (Anderson 1999; Gregory *et al*. 1999). Research efforts are ongoing at many locations to breed varieties with increased fiber quality, yield, and other agronomic traits. A long term challenge facing cotton breeders is the simultaneous improvement of yield to meet the demands of cotton producers as well as textile industries.

Most agronomic traits of cotton are quantitatively inherited and do not consistently fall into discrete classes because environmental conditions greatly modify their performance. Traits such as plant height, number of nodes at flowering time, bolling date, days to flowering, number of bolls and some other agronomic traits are used to assess our understanding of cotton yield. While yield itself is most typically used as a selection criterion, this may hinder future progress in developing highly productive cultivars, since it is the sum of the contributions of several largely independently inherited yield components. It has been suggested that breeding efforts focused on improving individual yield components may be more efficient in raising yield (Lewis 2001).

Recent developments in molecular genetics offer plant breeders a rapid and precise alternative approach to conventional selection schemes for improving cultivars through the identification and characterization of genes controlling important traits. Molecular markers are efficient tools for generating genetic linkage maps and have been developed to enhance breeding for quantitatively inherited traits (Tanksley and Hewitt 1988; Paterson *et al*. 1991).

The identification of chromosomal regions affecting these agronomic traits would increase our understanding of the genetic control of these traits. The association of molecular markers with desirable quantitative traits should contribute to the discovery of genetic variability and aid in the selection of desirable parents and progeny through marker-assisted breeding. Many studies have been performed to construct genetic linkage maps and to identify quantitative trait loci (QTL) for some agronomic traits in interspecific populations of *G. hirsutum* and *G. barbadense* or in intraspecific populations (Shappley 1994; Reinisch *et al*. 1994; Shappley *et al*. 1996; Altaf *et al*. 1997; Shappley *et al*. 1998b; Brubaker *et al*. 1999; Wright *et al*. 1999; Jiang *et al*. 2000; Ulloa and Meredith 2000; Ulloa *et al*. 2000; Zuo *et al*. 2000; Kohel *et al*. 2001; Ulloa *et al*. 2002; Zhang *et al*. 2002a; Paterson *et al*. 2003; Mei *et al*. 2004; Rong *et al*. 2004; Saranga *et al*. 2004; Ulloa *et al*. 2005; Shen *et al*. 2005; Frelichowski *et al*. 2006; Rong *et al*. 2007). Interspecific hybridization between G. *hirsutum* L. and *G. barbadense* L. has generally been found useful for the generation of genetic maps. Most researchers have turned to interspecific hybrids, which have revealed an increasing number of polymorphic loci.

In an attempt to map QTL for agronomic traits, especially earliness and yield component traits, a major effort to map the cotton genome is ongoing by several research groups. As a part of this ongoing effort, our objectives were to construct a genetic map and to identify QTL for some agronomic traits through a population obtained from the intercross between 'Giza 83' (*G. barbadense*) and 'Deltapine' (*G. hirsutum*). Herein we report a partial linkage map revealing QTL that influence plant height, number of nodes at flowering time, bolling date, days to flowering and number of bolls.

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MATERIALS AND METHODS

Mapping population

After screening some cotton germplasm available in the Cotton Research Institute (CRI), Agriculture Research Center (ARC) using different molecular markers, two highly polymorphic cultivars were selected. These two cultivars were used to develop an F2 mapping population (71 plants) from the interspecific cross between *G. hirsutum* and *G. barbadense*. The female parent was *G. hirsutum* cv. 'Deltapine', which is characterized by early flowering time and the male parent was *G. barbadense* cv. 'Giza 83', which is characterized by late flowering time. The F2 trials were grown in a randomized complete block design with two replications in 2005 at one of the cotton research institute experimental stations.

Measurements of traits

Data for plant height, number of nodes at flowering time, bolling date, days to flowering and number of bolls were measured as des-

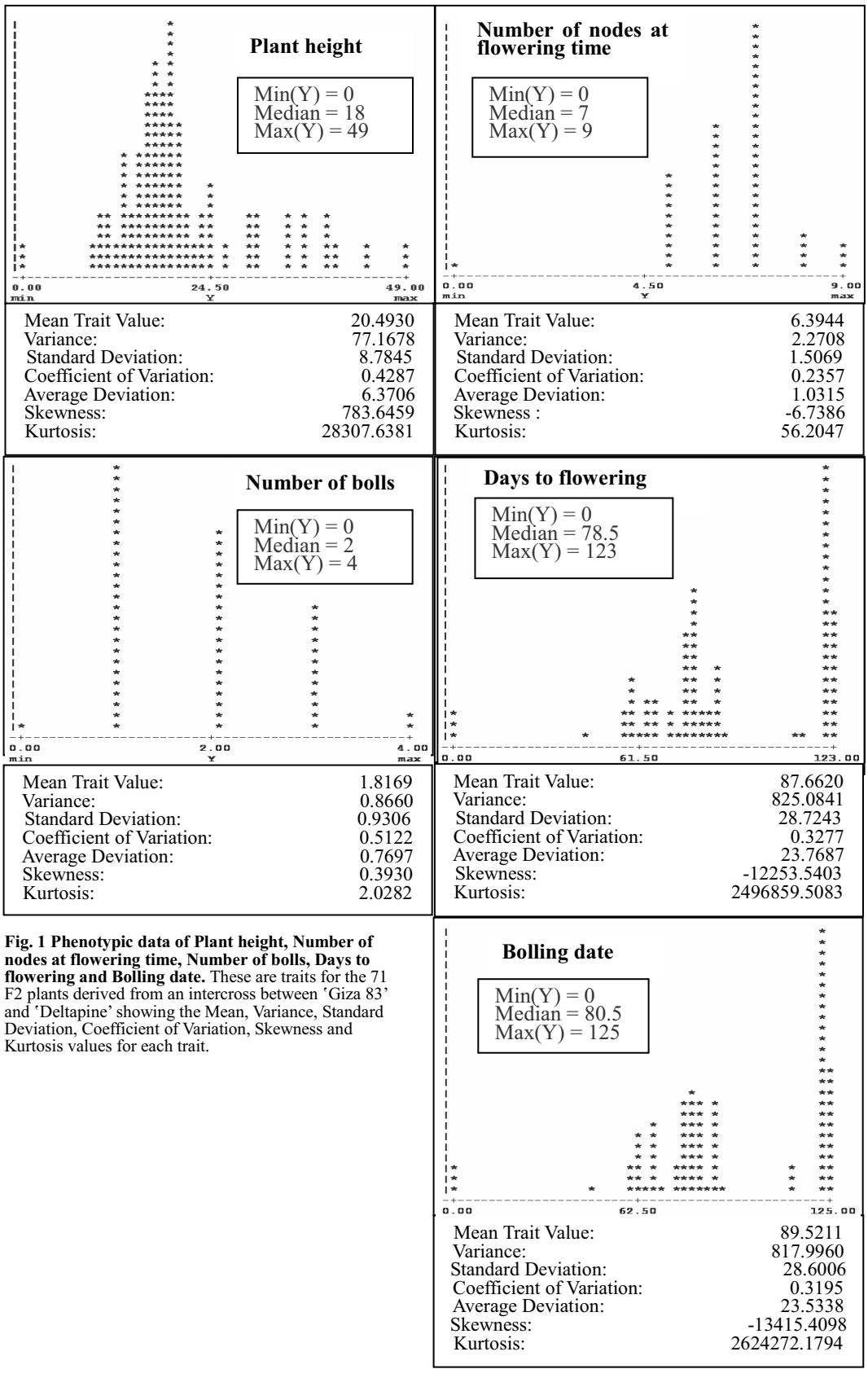

Fig. 1 Phenotypic data of Plant height, Number of nodes at flowering time, Number of bolls, Days to flowering and Bolling date. These are traits for the 71 F2 plants derived from an intercross between 'Giza 83' and 'Deltapine' showing the Mean, Variance, Standard Deviation, Coefficient of Variation, Skewness and Kurtosis values for each trait.

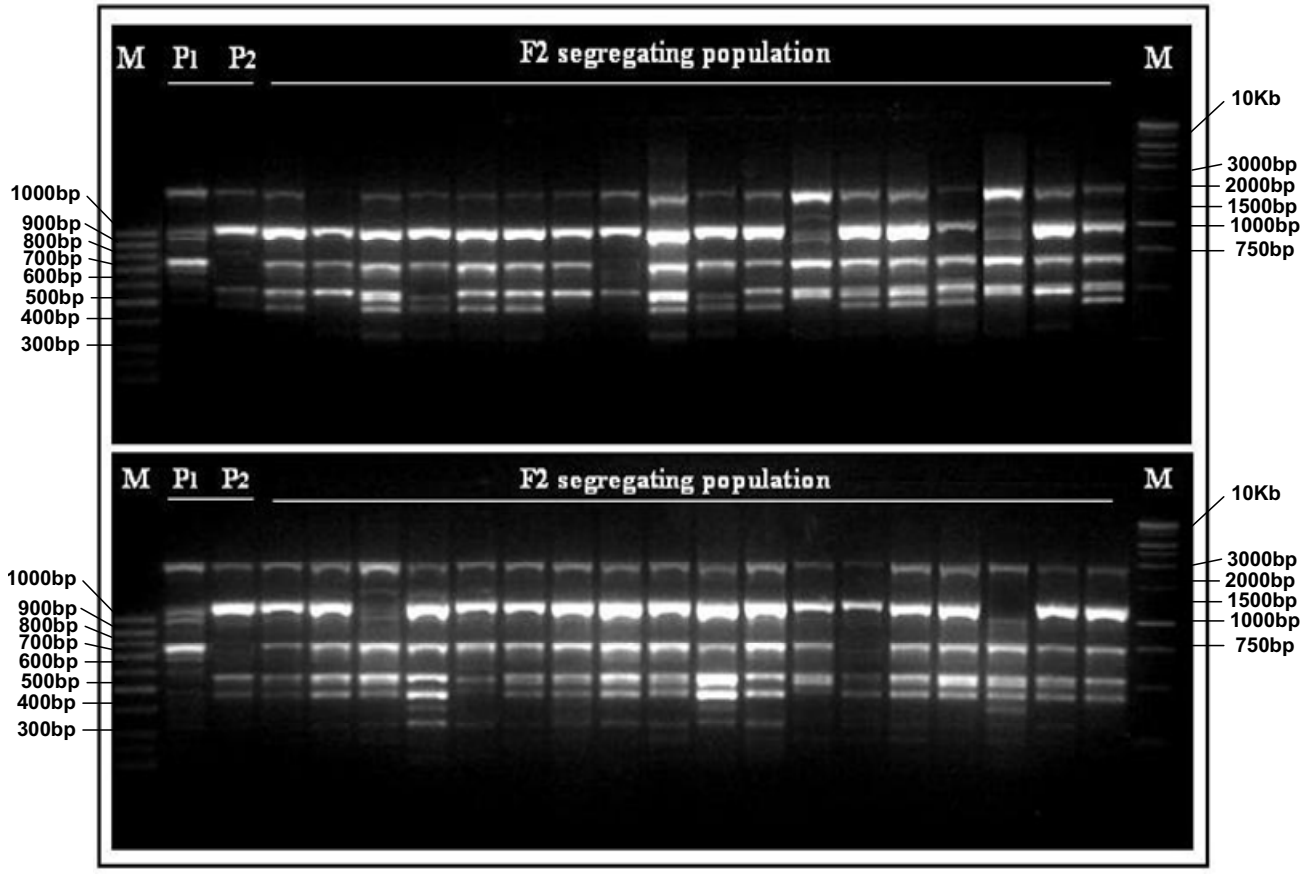

Fig. 2 RAPD agarose gel image showing some polymorphic bands between the 2 parents P1 (*G. hirsutum* cv. 'Deltapine') and P2 (*G. barbadense* cv. 'Giza 83') and segregation among F2 individuals. In the first and last lanes the markers (M) used were 100 bp and 1 kb ladder, respectively.

cribed by Muhanad (2003). Measurements were conducted during flowering and boll development period using the youngest fully expanded leaf per plant.

Statistical analysis

To determine if trait data were normally distributed, the skewness and kurtosis values were calculated for each trait. When seeking to detect a QTL between two markers, the other markers linked with some other QTL are likely to have marker effects, which should be considered in controlling background genetic variation. The program cartographer (Basten *et al*. 2000) was used to analyze the data and to obtain the Mean, Variance, Standard Deviation, Coefficient of Variation, Skewness and Kurtosis values for each trait (**Fig. 1**).

DNA isolation

DNA was isolated from the two parents and the 71 F2 plants using a DNAeasy Plant Mini Kit (Qiagen, Santa Clarita, CA). Fresh young leaves (0.5 mg) were ground in liquid nitrogen and used to extract DNA. The protocol was as described in the manufacturer's instructions. An agarose gel method was used to provide information regarding both DNA quantity and quality. The concentration of genomic DNA was estimated by comparing the size and intensity of each sample band with those of a sizing standard, DNA mass ladder (GIBCO). The DNA samples were diluted to a concentration of 20 ng/µl with TE buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0) to be used as a working solution in marker analysis.

DNA markers

Analysis of segregation among the 71 F2 individuals was performed using 3 RAPD, 10 SSR and 6 AFLP primer combinations. Twenty four AFLP primer combinations were used in bulked segregant analysis for flowering time.

Table 1 Number of total bands, polymorphic bands, primer sequences and codes of RAPD primers.

Code	Primer sequence (5'-3')	Number of bands	
		Total	Polymorphic
MAR-G9	CTGACGTCAC	9	6
MAR-G12	CAGCTCACGA	9	4
MAR-G18	GGCTCATGTG	10	5

RAPD analysis

RAPD amplification was performed as described by Williams *et al*. (1990) with minor modifications. A set of 20 random 10-mer primers were tested in the two parents. Only three RAPD primers revealed polymorphism among the two parents; these primers were used in the screening of segregants in the F2 population. The Number of total bands, polymorphic bands, primer sequences and code of RAPD primers are listed in **Table 1**. The amplification reaction was carried out in 25 µl total volume containing 1x PCR buffer, 1.5 mM $MgCl_2$, 2 mM dNTPs, 10 µM primer, 1 U Taq DNA polymerase and 25 ng template DNA. PCR amplification was performed in a Perkin-Elmer/Gene Amp PCR system 9700 (PE Applied Biosystem). The PCR program was as follows: an initial denaturation step at 94°C for 5 min, followed by 40 cycles at 94°C for 1 min, 36°C for 1 min and 72°C for 2 min and a final extension cycle of 7 min at 72°C. The amplification products were resolved by electrophoresis in 1.5% agarose gels (**Fig. 2**).

SSR analysis

SSR assays were performed as described by Adawy (2007) and Hussein *et al.* (2007). Screening of segregation between the F_2 individual plants was carried out using ten SSR primer pairs derived from Cotton Database and from some published papers (**Table 2**). The PCR reaction was conducted in 25 µl reaction volume containing 1x PCR buffer, 1.5 mM $MgCl_2$, 0.2 µM of each dNTPs, 1

Table 2 Number of total bands, polymorphic bands, primer sequences and codes of SSR primers.

Code	Primer sequence (5'-3')	Number of bands	
		Total	Polymorphic
MAS-L3	F-TTGCTTCAATGGAAAACC	6	4
	R-CGTCGCAAAGTTGAGAATCA		
MAS-L4	F-GCTTGTCATCTCCATTGCT	6	6
	R-TAGCCCGGTTCATGTTCTTC		
MAS-L5	F-TTTGCGGGTAATCCTATTG	4	4
	R-TGTCTATGGGACATTTCGCA		
MAS-L11	F-AAAAACCCCTTTCCATCCAT	2	2
	R-GGTGTCCTTTCCCAAAAATT		
MAS-L12	F-GACAGTCAAACAGAACAGATATGC	4	3
	R-TTACACGACTTGTTCCCACG		
MAS-L13	F-CAGAACCAACATACTTTCTACGG	4	4
	R-ATGTGCAAAAACTTGATGTGG		
MAS-L15	F-TTTTGCTCCATTTTTTTGCC	3	2
	R-TTTATTAATTTCGTTTAGCTTCCG		
MAS-L18	F-AGAGATGCAATGGGATCGAC	3	2
	R-ATGTCGATAATGCGGGGAATG		
MAS-L19	F-CCGAAATATACTTGTCATCTAAACG	2	2
	R-CCCCCGGACTAATTTTTCAA		
MAS-L20	F-GAAAAACCAAAAAGGAAAATCG	4	2
	R-CTCCCTCTCTCTAACCGGCT		

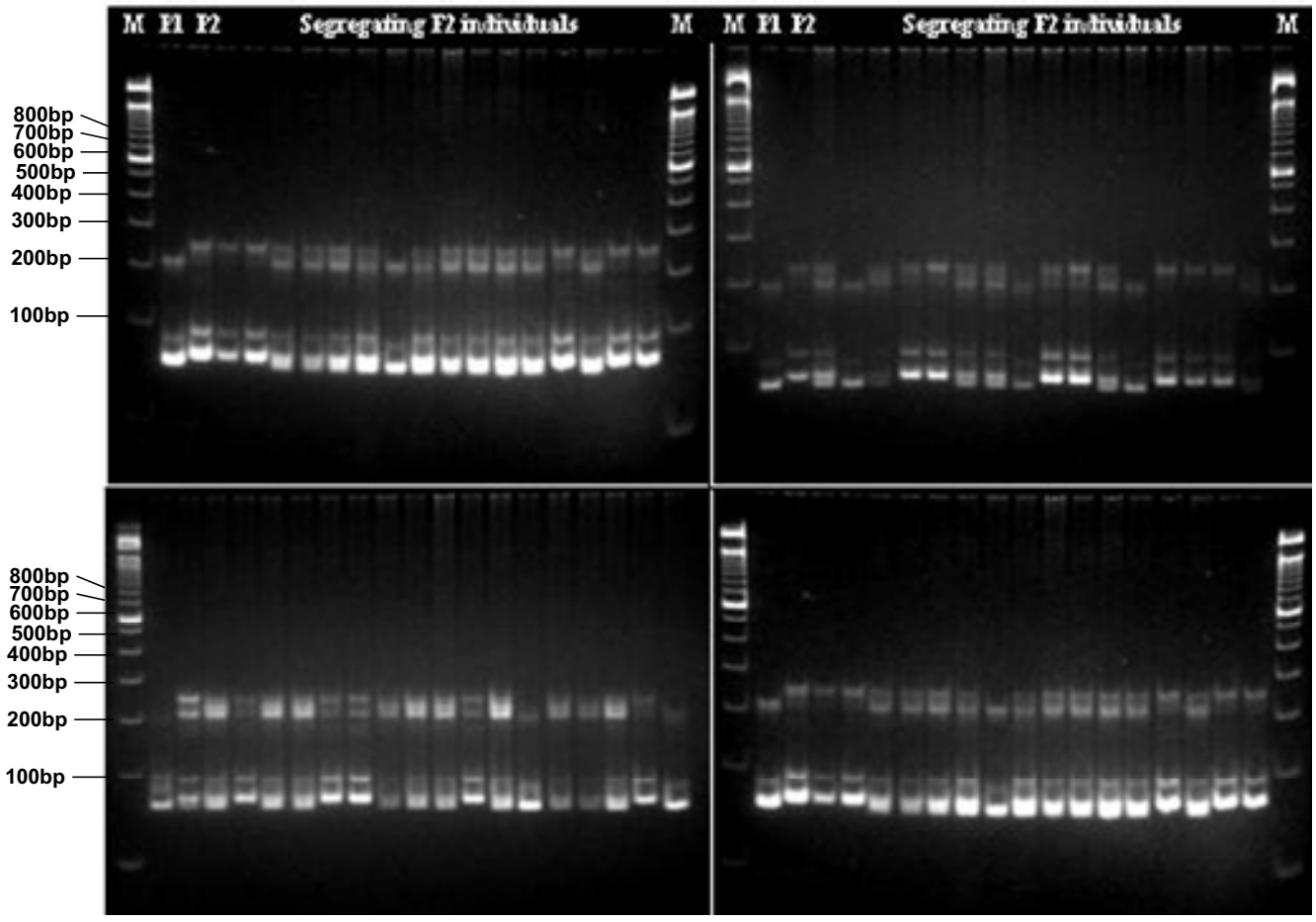

Fig. 3 Segregation of SSR markers among the F2 population derived from the intercross 'Giza 83' and 'Deltapine'. M is the marker 100 bp ladder (*G. hirsutum* cv. 'Deltapine') and P2 (*G. barbadense* cv. 'Giza 83').

μM of forward and reverse primers, 1 U Bioron-Taq polymerase and 25 ng genomic DNA. Touchdown temperature profile was used as follows: an initial denaturation step at 94°C for 5 min, followed by 35 cycles at 94°C for 30 sec, annealing temperature (Ta) for 30 sec and primer elongation at 72°C for 1 min, then a final extension cycle at 72°C for 7 min. The PCR products were detected by electrophoresis on polyacrylamide non-denaturing gels stained with ethidium bromide (**Fig. 3**).

AFLP analysis

Six AFLP primer combinations (**Table 3**) were performed with minor modifications, according to the protocol of Vos *et al*. (1995) using AFLP® Analysis System II (Invitrogen, USA) (Cat. No. 10483-022). Approximately 400 ng DNA of each of the 71 F2 individual plants was digested simultaneously with *Eco*RI and *Mse*I at 37°C for 2 hr. A small aliquot of the digested DNA was run on a 1.5% (w/v) agarose gel to check if the DNA digestion was complete. The digested samples were incubated at 70°C for 15 min to inactivate the restriction endonucleases. *Eco*RI and *Mse*I adaptors

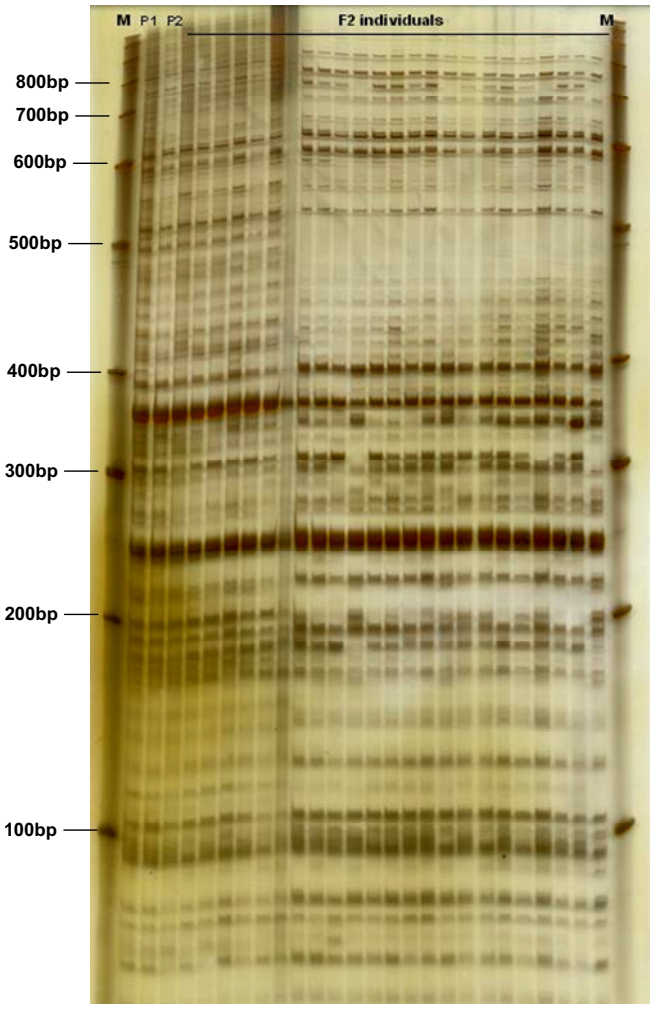

Fig. 4 AFLP segregating pattern in F2 population from the cross of (*G. hirsutum* cv. 'Deltapine') (P1) and (*G. barbadense* cv. 'Giza 83') P2. The amplification was made using different primer combinations. M is the marker 100 bp ladder.

were ligated to the digested DNA samples to generate template DNA for amplification. The ligation products were diluted 10-fold in TE buffer and 5 µl added to preamplification reaction. Preamplification was carried out with 16-primer combinations each carrying one selective nucleotide in a thermocycler for 20 cycles set at 94°C denaturation (30 sec), 56°C annealing (60 sec) and 72°C extension (60 sec). The amplification products were diluted 50-fold in TE buffer and stored at -20°C. Selective AFLP amplification was carried out with *Eco*RI and *Mse*I primers each carrying three selective nucleotides and 5 µL of the diluted PCR products from the preamplification product. The PCR selective amplification temperature profile was as follows: one cycle at 94°C for 30 sec, 65°C for 30 sec, and 72°C for 60 sec; followed by 12 cycles of touchdown PCR in which the annealing temperature was decreased by 0.7°C every cycle until a 'touchdown' annealing temperature of 56°C was reached. Once reached, another 23 cycles were conducted as described above for preamplification. Two µl of the reaction product was mixed with an equal volume of formamide loading buffer (98% [v/v] formamide, 10 mM EDTA, 0.005% [v/v] of each of xylene cyanol and bromophenol blue), denatured by incubating at 92°C for 3 min and quickly cooled on ice. The products were analyzed on 6% (w/v) denaturing polyacrylamide gels. The gel was run at constant power (50-55 W) until the xylene cyanol was about two-thirds down the length of the gel. The gel was silver stained according to the protocol described by the manufacturer (Promega Corp., USA, Silver Sequence DNA Staining Reagents, Lot. No. 171120) (**Fig. 4**).

Bulked segregant analysis

To rapidly find markers closely linked to the traits of interest (earliness), two bulked samples were prepared. For earliness an early bulk consisting of DNA from the earliest 10 F2 individuals and a late bulk consisting of DNA from 10 late flowering F2 individual plants, were prepared. Screening for segregation between the pooled DNA samples for the trait of interest has been performed using twenty four AFLP primer combinations.

Genetic linkage map construction and QTL detection

The markers that showed polymorphism between the parental lines were used to construct the genetic linkage map. For each marker, the F2 individuals were scored as '1' or '3' for presence of the parental band of the female parent (*G. hirsutum* cv. 'Deltapine') or the male parent (*G. barbadense* cv. 'Giza 83'), respectively, or '0' for missing data. Linkage analysis and map construction were performed by using Map Manager QTX14 (Manly and Cudmore 1997) using the Haldane function to convert the recombination frequencies to centiMorgans (cM). The linkage groups were constructed using the "make linkage group" command with a minimum LOD score of 3.0 followed by ripple command for each linkage group to check the final order of markers. The association between phenotype and marker genotype was investigated using single point analysis (SPA), using QTL cartographer (Wang *et al.* 2004). A significance level 5%, 1%, 0.1% and 0.01% were used to declare a QTL.

RESULTS

Statistics and normality test of traits

The mean, variance, standard deviation, coefficient of variation, skewness and kurtosis values for plant height, number of nodes at flowering time, bolling date, days to flowering and number of bolls are presented in **Fig. 1**. A large amount of variation for all traits studied was detected. Plant height and days to flowering were the most variable traits among the 71 plants of the F2 population studied. The least variable trait was bolls per plant. High kurtosis values were observed for bolling date and days to flowering and a large skewness value was obtained for plant height. All traits except bolls per plant showed normal distribution (**Fig. 1**).

Parental polymorphism and genetic linkage map

From a survey of the two parents using 3 RAPD, 10 SSR, 6 AFLP primer combinations a total of 491 major bands were observed for RAPD (28 bands), SSR (38 bands) and AFLP (425 bands). Of these major bands, only 163 (33.2%) were polymorphic between the two parents, 15 for RAPD, 31 for SSR and 117 for AFLP (**Tables 1, 2** and **3**). For RAPD, the number of bands generated ranged from 9 for MAR-G9 and MAR-G12 to 10 for MAR-G18 with a mean of 9 bands. The primer MAR-G9 produced the largest number of polymorphic products (6 in total) (**Table 1**). For SSR, the number of bands obtained by individual primer ranged from 2 for MAS-L11 and MAS-L19 to 6 for MAS-L3 and MAS-L4 with a mean of 3 bands. The primer MAS-L4 produced the largest number of polymorphic bands (6 in total) (**Table 2**). For AFLP, the number of bands generated by individual primer combinations ranged from 51 for MAA-7/3 (*Eco*RI + AGC/*Mse*I + CAG) to 86 for MAA-5/5 (*Eco*RI + ACC/

Table 3 Number of total bands, polymorphic bands and selective nucleotides used of AFLP primer combinations.

Code	Selective nucleotides		Number of bands	
	*Eco*RI	*Mse*I	Total	Polymorphic
MAA-1/3	AAC	CAG	79	23
MAA-4/6	ACT	CTC	71	19
MAA-5/5	ACC	CTA	86	24
MAA-6/6	ACG	CTC	63	16
MAA-7/3	AGC	CAG	51	13
MAA-8/8	AGG	CTT	75	22

Table 4 Distribution of molecular markers, assignment and centiMorgan (cM) coverage across the 22 linkage groups of the genetic map used in QTL mapping.

Linkage Groups	AFLP	RAPD	SSR	Bulk	Markers		cM	cM/Marker
					#	%		
LG 1	0	3	0	0	3	2.14	31.1	10.37
LG 2	15	8	17	0	40	28.57	649.5	16.24
LG 3	7	0	0	12	19	13.57	120.1	6.32
LG 4	2	0	0	0	2	1.43	27.5	13.75
LG 5	4	0	0	19	23	16.43	68	2.96
LG 6	2	0	0	0	2	1.43	1.4	0.70
LG 7	3	0	0	0	3	2.14	27.1	9.03
LG 8	2	0	0	0	2	1.43	2.8	1.40
LG 9	2	0	0	0	2	1.43	2.8	1.40
LG 10	5	1	6	0	12	8.57	202.5	16.88
LG 11	3	0	1	0	4	2.86	57.2	14.30
LG 12	2	0	0	0	2	1.43	10.4	5.20
LG 13	2	0	0	0	2	1.43	14.5	7.25
LG 14	2	0	0	0	2	1.43	17.6	8.80
LG 15	2	0	0	0	2	1.43	19.2	9.60
LG 16	5	0	0	0	5	3.57	93.6	18.72
LG 17	2	0	0	0	2	1.43	21.9	10.95
LG 18	2	0	0	0	2	1.43	21.9	10.95
LG 19	2	0	0	0	2	1.43	27	13.50
LG 20	1	1	0	0	2	1.43	27	13.50
LG 21	3	0	0	0	3	2.14	53.9	17.97
LG 22	4	0	0	0	4	2.86	59.7	14.93
Total	72	13	24	31	140	100	1556.7	11.12

*Mse*I + CTA), with a mean of 21 bands. The primer combination *Eco*RI + AAC/*Mse*I + CAG produced the largest number of polymorphic products (23 in total) (**Table 3**).

Among the 163 polymorphic markers, 23 were unlinked and 140 were constructed into 22 linkage groups covering 1556.7 cM. The linkage groups ranged from 1.4 cM in linkage group 6 to 649.5 cM in linkage group 2 with an average distance of 11.1 cM between loci (**Fig. 5**). The distribution of markers, linkage group assignment and map coverage across the 22 cotton linkage groups are summarized in **Table 4**. The biggest linkage group consisted of 40 marker loci covering 649.5 cM with 16.24 cM average distance in linkage group 2 and the smallest were linkage groups 4, 6, 8, 9, 12, 13, 14, 15, 18, 19 and 20 consisting of two marker loci with average distance of 13.75, 0.70, 1.40, 1.40, 5.20, 7.25, 8.80, 9.60, 10.95, 13.5 and 13.5 cM, respectively.

QTL analysis

A total of 30 QTL with a significance ranging from 0.01% to 5% were identified for the five traits on ten linkage groups (1, 2, 3, 4, 5, 10, 11, 12, 18, and 19). Among these 11 QTL for plant height, 8 QTL for number of bolls, 4 QTL for each of days to flowering and bolling date and 3 QTL for number of nodes at flowering time were identified (**Fig. 5, Table 5**). The most significant QTL of the study was obtained for bolling date on linkage group 19, number of bolls on linkage group 2, 5, 10 and 12, plant height on linkage group 2, 3, 10, 11, 18 and 19, days to flowering on linkage group 19 and for number of bolls on linkage group 1, 2 and 19 (**Table 5**).

Correlation between traits

Since most of the traits studied in this investigation are related, it is of interest to examine the genetic relationships between them. Some genomic regions were found where QTL for different traits overlapped on linkage groups 3, 5, and 19. The linkage groups 3 and 5 showed the most overlapped traits (**Fig. 5**). For example, QTL for plant height, bolling date and days to flowering were mapped to approximately the same chromosomal location. Similarly, QTL for number of bolls per plant, plant height, bolling date and days to flowering were mapped to identical genomic regions and so also were the QTL for days to flowering and bolling date were often mapped to the same regions.

DISCUSSION

The material used, an F2 population derived from the intercross between *G. hirsutum* L. ('Deltapine') and *G. barbadense* L. ('Giza 83') is particularly useful for this kind of study in that *G. hirsutum* dominates the world's cotton fiber production, representing 90% of the production and the second most cultivated species; *G. barbadense*, has superior fiber length, strength, and fineness, giving a higher spinning and manufacturing performance. *G. hirsutum* varieties, however, are usually early-maturing compared with *G. barbadense*, which is a late maturing species. Although bidirectional genome exchanges between the two species are well documented (Brubaker and Wendel 2001) attempts at utilizing deliberate interspecific recombination between *G. hirsutum/G. barbadense* by conventional breeding have had limited impact on cultivar development (Paterson and Smith 1999). In an attempt to overcome the limitations of conventional breeding for the improvement of cotton yield and earliness related traits through interspecific hybridization, we constructed a genetic linkage map showing chromosomal regions associated with some agronomic traits.

Statistics and normality test of traits

The statistical analysis of the five traits presented in **Fig. 1** indicated that these traits segregated continuously and both skewness and kurtosis values suggested that the traits in the present study were normally distributed and the frequency distribution of the traits data showed genetic variation consistent with mutagenic inheritance, thus suitable for QTL analysis. In this study, we chose plant height, number of nodes at flowering time, bolling date, days to flowering and number of bolls as a component of yield and earliness traits in cotton. Each trait is useful and measurements, we show are commonly accepted to the cotton breeders, thus we report data on QTL for each trait.

Genetic linkage map

In cotton, the contribution of new markers to generate a more saturated linkage map will enhance our understanding of its genetics and improve cotton breeding efficiency, especially when quantitative traits are implicated. Reinisch *et al*. (1994) and Shappley (1994) separately reported the first

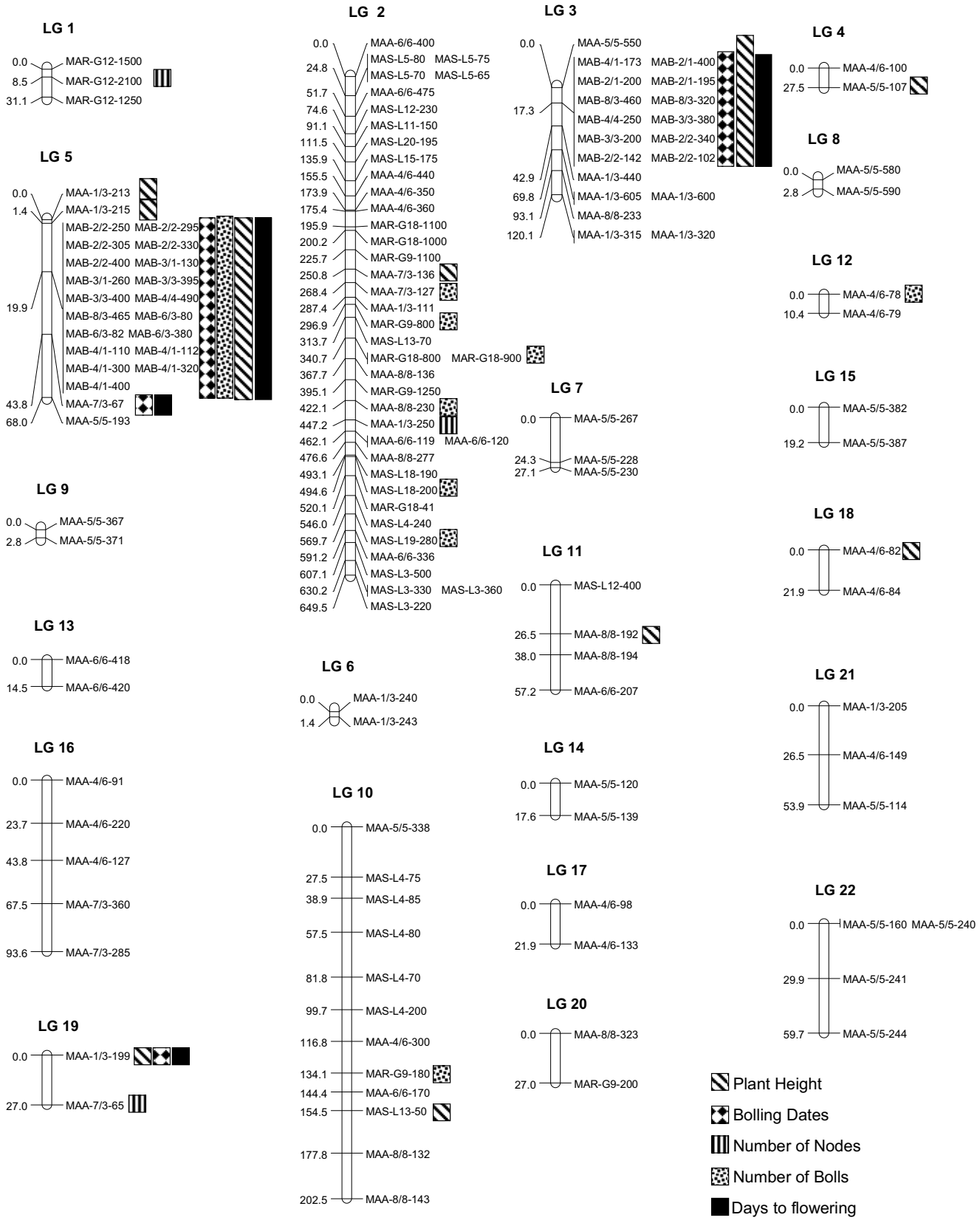

Fig. 5 Molecular linkage groups of cotton (intercross between 'Giza83' and 'Deltapine') showing positions of QTL influencing plant height, number of nodes at flowering time, bolling date, days to flowering and number of bolls. Map distances between adjacent markers are in cM.

constructed linkage map. Shappley (1994) constructed five linkage groups in a cross of Upland cotton, while 41 linkage groups were constructed in a cross between *G. hirsutum* and *G. barbadense*. Jiang *et al*. (2000), Kohel *et al*. (2001), Saranga *et al.* (2001), Shappley *et al*. (1996, 1998a), Ulloa and Meredith (2000), Wright *et al*. (1999) and Yu *et al*. (1998) have developed detailed maps of cotton. Also, different types of markers have been used in linkage map construction, including RAPDs in a study conducted by Kohel *et al.* (2001) and Zhang *et al*. (2002b, 2002c) who both used RAPDs and SSRs to construct 43 linkage groups while Altaf *et al*. (1997) constructed 11 linkage groups using both RAPDs and AFLPs.

In this work, the linkage map constructed in 22 linkage groups consisted of 140 loci covering 1556.7 cM and an average distance between loci of 11.1 cM. Whereas the map

Table 5 The most significant QTL detected by the Single point analysis. This analysis fits the data to the simple linear regression model y = b0 + b1 x + e. The results below give the estimates for b0, b1 and the F statistic for each marker. We are interested in whether the marker is linked to a QTL. We tested this idea by determining if b1 is significantly different from zero. The F statistic compares the hypothesis H0: b1 = 0 to an alternative H1: b1 not 0. The pr(F) is a measure of how much support there is for H0. A smaller pr(F) indicates less support for H0 and thus more support for H1. Significance at the 5%, 1%, 0.1% and 0.01% levels are indicated by *, **, *** and ****, respectively. LG = linkage group.

Trait	LG	Marker	b0	b1	2ln (L0/L1)	F (1,n-2)	Pr (F)	Significance
Bolling date	3	MAB-4/1-173	92.186	-11.664	8.012	8.242	0.005	**
	3	MAB-2/1-400	92.186	-11.664	8.012	8.242	0.005	**
	3	MAB-2/1-200	92.186	-11.664	8.012	8.242	0.005	**
	3	MAB-2/1-195	92.186	-11.664	8.012	8.242	0.005	**
	3	MAB-8/3-460	92.186	-11.664	8.012	8.242	0.005	**
	3	MAB-8/3-320	92.186	-11.664	8.012	8.242	0.005	**
	3	MAB-4/4-250	92.186	-11.664	8.012	8.242	0.005	**
	3	MAB-3/3-380	92.186	-11.664	8.012	8.242	0.005	**
	3	MAB-3/3-200	92.186	-11.664	8.012	8.242	0.005	**
	3	MAB-2/2-340	92.186	-11.664	8.012	8.242	0.005	**
	3	MAB-2/2-142	92.186	-11.664	8.012	8.242	0.005	**
	3	MAB-2/2-102	92.186	-11.664	8.012	8.242	0.005	**
	5	MAB-2/2-250	89.342	18.519	18.727	20.826	0	****
	5	MAB-2/2-295	89.342	18.519	18.727	20.826	0	****
	5	MAB-2/2-305	89.342	18.519	18.727	20.826	0	****
	5	MAB-2/2-330	89.342	18.519	18.727	20.826	0	****
	5	MAB-2/2-400	89.342	18.519	18.727	20.826	0	****
	5	MAB-3/1-130	89.342	18.519	18.727	20.826	0	****
	5	MAB-3/1-260	89.342	18.519	18.727	20.826	0	****
	5	MAB-3/3-395	89.342	18.519	18.727	20.826	0	****
	5	MAB-3/3-400	89.342	18.519	18.727	20.826	0	****
	5	MAB-4/4-490	89.342	18.519	18.727	20.826	0	****
	5	MAB-8/3-465	89.342	18.519	18.727	20.826	0	****
	5	MAB-6/3-80	89.342	18.519	18.727	20.826	0	****
	5	MAB-6/3-82	89.342	18.519	18.727	20.826	0	****
	5	MAB-6/3-380	89.342	18.519	18.727	20.826	0	****
	5	MAB-4/1-110	89.342	18.519	18.727	20.826	0	****
	5	MAB-4/1-112	89.342	18.519	18.727	20.826	0	****
	5	MAB-4/1-300	89.342	18.519	18.727	20.826	0	****
	5	MAB-4/1-320	89.342	18.519	18.727	20.826	0	****
	5	MAB-4/1-400	89.342	18.519	18.727	20.826	0	****
	5	MAA-7/3-67	91.803	9.53	7.969	8.195	0.006	**
	19	MAA-1/3-199	87.004	-8.784	6.411	6.52	0.013	*
Number of bolls	2	MAA-7/3-127	2.023	-0.311	4.661	4.681	0.034	*
	2	MAR-G18-800	2.101	-0.396	6.61	6.733	0.012	*
	2	MAR-G18-900	2.101	-0.396	6.61	6.733	0.012	*
	2	MAA-8/8-230	1.678	0.266	4.432	4.445	0.039	*
	2	MAS-L18-200	1.589	0.325	4.479	4.493	0.038	*
	2	MAS-L19-280	1.977	-0.282	4.299	4.307	0.042	*
	5	MAB-2/2-250	1.82	-0.323	4.885	4.915	0.03	*
	5	MAB-2/2-295	1.82	-0.323	4.885	4.915	0.03	*
	5	MAB-2/2-305	1.82	-0.323	4.885	4.915	0.03	*
	5	MAB-2/2-330	1.82	-0.323	4.885	4.915	0.03	*
	5	MAB-2/2-400	1.82	-0.323	4.885	4.915	0.03	*
	5	MAB-3/1-130	1.82	-0.323	4.885	4.915	0.03	*
	5	MAB-3/1-260	1.82	-0.323	4.885	4.915	0.03	*
	5	MAB-3/3-395	1.82	-0.323	4.885	4.915	0.03	*
	5	MAB-3/3-400	1.82	-0.323	4.885	4.915	0.03	*
	5	MAB-4/4-490	1.82	-0.323	4.885	4.915	0.03	*
	5	MAB-8/3-465	1.82	-0.323	4.885	4.915	0.03	*
	5	MAB-6/3-80	1.82	-0.323	4.885	4.915	0.03	*
	5	MAB-6/3-82	1.82	-0.323	4.885	4.915	0.03	*
	5	MAB-6/3-380	1.82	-0.323	4.885	4.915	0.03	*
	5	MAB-4/1-110	1.82	-0.323	4.885	4.915	0.03	*
	5	MAB-4/1-112	1.82	-0.323	4.885	4.915	0.03	*
	5	MAB-4/1-300	1.82	-0.323	4.885	4.915	0.03	*
	5	MAB-4/1-320	1.82	-0.323	4.885	4.915	0.03	*
	5	MAB-4/1-400	1.82	-0.323	4.885	4.915	0.03	*
	10	MAR-G9-180	2.382	0.617	5.319	5.368	0.023	*
	12	MAA-4/6-78	1.935	-0.28	5.283	5.33	0.024	*
Plant height	2	MAA-7/3-136	19.099	2.537	4.323	4.332	0.041	*
	3	MAA-5/5-550	19.788	2.634	6.282	6.383	0.014	*
	3	MAB-4/1-173	19.608	3.871	9.447	9.819	0.003	**
	3	MAB-2/1-400	19.608	3.871	9.447	9.819	0.003	**
	3	MAB-2/1-200	19.608	3.871	9.447	9.819	0.003	**
	3	MAB-2/1-195	19.608	3.871	9.447	9.819	0.003	**
	3	MAB-8/3-460	19.608	3.871	9.447	9.819	0.003	**
	3	MAB-8/3-320	19.608	3.871	9.447	9.819	0.003	**

Table 5 (Cont.)

Trait	LG	Marker	b0	b1	2ln (L0/L1)	F (1,n-2)	Pr (F)	Significance
Plant height	3	MAB-4/4-250	19.608	3.871	9.447	9.819	0.003	**
	3	MAB-3/3-380	19.608	3.871	9.447	9.819	0.003	**
	3	MAB-3/3-200	19.608	3.871	9.447	9.819	0.003	**
	3	MAB-2/2-340	19.608	3.871	9.447	9.819	0.003	**
	3	MAB-2/2-142	19.608	3.871	9.447	9.819	0.003	**
	3	MAB-2/2-102	19.608	3.871	9.447	9.819	0.003	**
	4	MAA-5/5-107	20.638	-2.059	4.05	4.05	0.048	*
	5	MAA-1/3-213	21.11	-3.311	10.45	10.942	0.001	**
	5	MAA-1/3-215	21.214	-3.364	10.681	11.201	0.001	**
	5	MAB-2/2-250	20.548	-5.699	18.813	20.934	0	****
	5	MAB-2/2-295	20.548	-5.699	18.813	20.934	0	****
	5	MAB-2/2-305	20.548	-5.699	18.813	20.934	0	****
	5	MAB-2/2-330	20.548	-5.699	18.813	20.934	0	****
	5	MAB-2/2-400	20.548	-5.699	18.813	20.934	0	****
	5	MAB-3/1-130	20.548	-5.699	18.813	20.934	0	****
	5	MAB-3/1-260	20.548	-5.699	18.813	20.934	0	****
	5	MAB-3/3-395	20.548	-5.699	18.813	20.934	0	****
	5	MAB-3/3-400	20.548	-5.699	18.813	20.934	0	****
	5	MAB-4/4-490	20.548	-5.699	18.813	20.934	0	****
	5	MAB-8/3-465	20.548	-5.699	18.813	20.934	0	****
	5	MAB-6/3-80	20.548	-5.699	18.813	20.934	0	****
	5	MAB-6/3-82	20.548	-5.699	18.813	20.934	0	****
	5	MAB-6/3-380	20.548	-5.699	18.813	20.934	0	****
	5	MAB-4/1-110	20.548	-5.699	18.813	20.934	0	****
	5	MAB-4/1-112	20.548	-5.699	18.813	20.934	0	****
	5	MAB-4/1-300	20.548	-5.699	18.813	20.934	0	****
	5	MAB-4/1-320	20.548	-5.699	18.813	20.934	0	****
	5	MAB-4/1-400	20.548	-5.699	18.813	20.934	0	****
	10	MAS-L13-50	23.963	4.323	6.487	6.601	0.012	*
	11	MAA-8/8-192	22.894	3.217	4.41	4.422	0.039	*
	18	MAA-4/6-82	20.743	-2.22	4.484	4.499	0.038	*
	19	MAA-1/3-199	21.194	2.446	5.227	5.272	0.025	*
Days to flowering	3	MAB-4/1-173	90.4	-11.984	8.408	8.674	0.004	**
	3	MAB-2/1-400	90.4	-11.984	8.408	8.674	0.004	**
	3	MAB-2/1-200	90.4	-11.984	8.408	8.674	0.004	**
	3	MAB-2/1-195	90.4	-11.984	8.408	8.674	0.004	**
	3	MAB-8/3-460	90.4	-11.984	8.408	8.674	0.004	**
	3	MAB-8/3-320	90.4	-11.984	8.408	8.674	0.004	**
	3	MAB-4/4-250	90.4	-11.984	8.408	8.674	0.004	**
	3	MAB-3/3-380	90.4	-11.984	8.408	8.674	0.004	**
	3	MAB-3/3-200	90.4	-11.984	8.408	8.674	0.004	**
	3	MAB-2/2-340	90.4	-11.984	8.408	8.674	0.004	**
	3	MAB-2/2-142	90.4	-11.984	8.408	8.674	0.004	**
	3	MAB-2/2-102	90.4	-11.984	8.408	8.674	0.004	**
	5	MAB-2/2-250	87.479	18.895	19.418	21.704	0	****
	5	MAB-2/2-295	87.479	18.895	19.418	21.704	0	****
	5	MAB-2/2-305	87.479	18.895	19.418	21.704	0	****
	5	MAB-2/2-330	87.479	18.895	19.418	21.704	0	****
	5	MAB-2/2-400	87.479	18.895	19.418	21.704	0	****
	5	MAB-3/1-130	87.479	18.895	19.418	21.704	0	****
	5	MAB-3/1-260	87.479	18.895	19.418	21.704	0	****
	5	MAB-3/3-395	87.479	18.895	19.418	21.704	0	****
	5	MAB-3/3-400	87.479	18.895	19.418	21.704	0	****
	5	MAB-4/4-490	87.479	18.895	19.418	21.704	0	****
	5	MAB-8/3-465	87.479	18.895	19.418	21.704	0	****
	5	MAB-6/3-80	87.479	18.895	19.418	21.704	0	****
	5	MAB-6/3-82	87.479	18.895	19.418	21.704	0	****
	5	MAB-6/3-380	87.479	18.895	19.418	21.704	0	****
	5	MAB-4/1-110	87.479	18.895	19.418	21.704	0	****
	5	MAB-4/1-112	87.479	18.895	19.418	21.704	0	****
	5	MAB-4/1-300	87.479	18.895	19.418	21.704	0	****
	5	MAB-4/1-320	87.479	18.895	19.418	21.704	0	****
	5	MAB-4/1-400	87.479	18.895	19.418	21.704	0	****
	5	MAA-7/3-67	89.981	9.686	8.171	8.416	0.005	**
	19	MAA-1/3-199	85.131	-8.833	6.428	6.538	0.013	*
Number of nodes	1	MAR-G12-2100	6.475	-0.382	4.566	4.583	0.036	*
	2	MAA-1/3-250	6.863	-0.598	4.209	4.215	0.044	*
	19	MAA-7/3-65	6.481	0.412	5.326	5.375	0.023	*

developed by Frelichowski *et al*. (2006) for the RILs from a cross between *G. hirsutum and G. barbadense* consisted of 433 marker loci and 46 linkage groups with a genetic distance of 2126.3 cM covering approximately 45% of the cotton genome and an average distance between two loci of 4.9 cM. While, Zhang *et al*. (2002b, 2002c) constructed a gene-

tic linkage map of a cotton population derived for the cross between *G. hirsutum* and *G. barbadense* in 42 linkage groups using 489 RAPD and SSR markers. The linkage map covered 3312.1 cM. The longer maps constructed by Frelichowski *et al*. (2006) and Zhang *et al*. (2002b, 2002c) are due mainly to the greater number of mapped markers. On the other hand, Zhang *et al*. (2002b, 2002c) constructed a genetic linkage map for an F2 population consisting of 67 RFLP, SSR and RAPD markers on 9 linkage groups covering 1337.4 cM with average distance between markers ranging from 7.8 to 46.8 cM. The map constructed in the present study is longer and more saturated than the map of Zhang *et al*. (2002b, 2002c) due to a larger number of markers used in this study. Estimated map distance of the cotton genome is around 4660 cM (Reinisch *et al.* 1994), thus the 140 loci in our map covered about 33.4% of the total recombinational length of the cotton genome with an avarage distance between markers of 11.1cM. Hence, more work is needed to obtain a genetic linkage map for the population used in this work with more markers and less distance between loci.

The map constructed in this study was reliable for identifying QLTs for plant height, number of nodes at flowering time, bolling date, days to flowering and number of bolls. On the other hand, the constructed linkage map contains RAPD, SSR and AFLP markers that were not mapped collectively in any other cotton maps for the interspecific cross between *G. hirsutum* and *G. barbadense*.

QTL analysis

Yield, earliness and some agronomic traits are quantitatively inherited traits, controlled by several genetic loci (QTLs). Many quantitative traits can be partitioned between smaller components of a quantitative and/or qualitative nature. In this work QTL analysis for plant height, number of nodes at flowering time, bolling date, days to flowering and number of bolls has been done in relation to mapped genetic markers including RAPD, SSR and AFLP to provide data on genome location and the relative effects of loci and alleles. A total of 30 QTL at significance level of 5%, 1%, 0.1% and 0.01% have been identified for the 5 traits by single-marker analysis (SMA) (**Table 5**). This method investigates the association between trait(s) and one marker at a time. SMA is the method of choice when the number of markers are not enough and when complete genetic map is not avilable (Muhanad 2003), which is the case in this study where only 22 linkage groups were obtained and only 140 markers were mapped. In SMA, the mapping population is partitioned into different genotypic classes that reflect genotypes at the marker locus. This analysis fits the data to the simple linear regression model $y = b0 + b1\ x + e$. The results obtained from this analysis give the estimates for b0, b1 and the F statistic for each marker. We are interested in whether the marker is linked to a QTL. We tested this idea by determining if b1 is significantly different from zero. The F statistic compares the hypothesis H0: $b1 = 0$ to an alternative H1: b1 not 0. The pr(F) is a measure of how much support there is for H0. A smaller pr(F) indicates less support for H0 and thus more support for H1.

QTL for yield, earliness and other agronomic traits have been mapped in cotton (Shappley *et al.* 1998b; Ulloa and Meredith 2000; Saranga *et al.* 2001; Muhanad 2003; Saranga *et al.* 2004; Lacape *et al.* 2005; Ulloa *et al.* 2005; Frelichowski *et al.* 2006; Rong *et al.* 2007).

QTL associated with bolling dates

Bolls are distributed differently over fruiting sites among cultivars of cotton. The fruiting sites that set, mature, and open bolls are under genetic control. Since cotton produces bolls over a period of four to six weeks the opportunity for changing the fruiting pattern in the development of a new variety is a challenge. In the present study, 4 QTL associated with bolling date were identified on linkage groups 3, 5 and 19. The results sugested that these 3 linkage groups contain factors affecting bolling date in cotton population derived from the intercorss *G. barbadense* and *G. hisutum.* On linkage group 5, two QTL were detected; this suggests that this linkage group has more effect on bolling dates than the other linkage groups.

QTL associated with number of bolls per plant

Cotton yield is determined by the number of bolls that mature after fruiting. Boll number is the most important trait in terms of contribution to genetic effects on lint yield, thus plant breeders are interested in combining genes for boll production from early and late season varieties into one variety with higher yield (Guo *et al.* 2007). This work reports the identification of chromosomal region associated with number of bolls per plant as one of the traits related to yield. Saranga *et al*. (2004), Muhanad (2003) and Baogong (2004) identified QTL for number of bolls per plant on linkage groups 2, 5 and 10, respectively. These results are consistent with our findings where QTLs for the same traits were identified on the linkage groups 2, 5, 10 and 12. Of the 9 QTL identified for number of boll per plant, 6 were detected on linkage group 2. This result suggests that this linkage group has a great effect on number of bolls in cotton.

QTL associated with plant height

Plant height is one of the most important traits for cotton plant. It is reported that plant height is positively associated with lint yield and lint percentage and taller plant heights at maturity have been associated with later plant maturity and can present harvest difficulties (Percy *et al.* 2006). In this study 10 QTLs were identified on linkage groups 2, 3, 4, 5, 9, 10, 11, and 18 and three of them were identified on linkage group 5 indicating that this linkage group has a great role in controlling the plant height trait in this population. Shappley *et al.* (1998b) identified 2 QTLs for plant height on linkage groups 6 and 23. This difference in QTL number and position could be due to the effect of different environment and different mapping population used in the two investigations.

QTLs associated with days to flowering

Days to flowering is an important trait for cotton breeders. It has been reported that days to flowering trait is associated with lint percentage, fiber length and fiber elongation (Ren *et al.* 2002). Thus breeders could select for fiber characters and yield through days to flowering. Kohel *et al*. (1977) reported that chromosome 6 is associated with QTL for late flowering in cotton. Ren *et al*. (2002) identified a QTL for days to flowering on chromosome 16. In this work 4 QTLs for days to flowering were identified, two on linkage group 5 and one on each of linkage groups 19 and 3. The inconsistant results for this work and the previous investigations could be due to different environmental effects and different mapping population.

QTLs associated with number of nodes

Node of first fruiting branch is a physiological trait that gives an indication of earliness and is the node at which the plant develops its first nonvegetative branch (Shappley *et al.* 1998b). In this study, 3 markers on three different linkage groups (1, 2 and 19) were found to be associated with QTL for number of nodes; these results suggest that at least three chromosomes contain factors associated with number of nodes in the F2 population derived from the cross between 'Giza 83' and 'Deltapine'. Shappley *et al.* (1998b) identified the same QTL on linkage groups 10, 14, 23 and 31 in an F2 population of upland coton and Guo *et al*. (2008) located QTL for the main-stem node of the first fruiting branch which is related to flowering time in an F2 population consisting of 251 plants from the cross of 'Deltapine 61', a day length neutral flowering cultivar, and 'Texas 701',

a day length sensitive flowering accession. They identified 3 QTL affecting number of nodes on chromosomes 16, 21, and 25 and two additional QTL with minor effects were detected on chromosomes 15 and 16. The difference between the number and position of QTL affecting number of nodes in this study and the previous ones might be due to different types of population, markers and environmental conditions.

Correlation between traits

Multiple QTLs were found for almost all traits at different regions; the number of QTL identified for each trait varied from 3 to 11, indicating that the genome contains multiple genes affecting different traits. We expected several of the traits to be influenced by the same QTL and our data tend to support this (**Fig. 5**). QTL for plant height, bolling date and days to flowering were mapped to approximately the same chromosomal location. Similarly, QTL for number of bolls per plant, plant height, bolling date and days to flowering were mapped to identical genomic region and so also were the QTL for days to flowering and bolling date where often mapped to the same regions. The overlapping of these QTL indicates that either there are closely linked genes or the same gene is affecting different traits. It has been demonstrated that correlated, or components of plant yield traits often have QTL mapping at similar locations. Multiple traits can be correlated due to linkage, pleiotropy, or the correlated traits may be components of a more complex variable. The distinction between linkage and pleiotropy is important for breeding purposes as well as for scientific reasons. However, without fine resolution mapping or molecular cloning of QTLs, such distinction would be difficult and at best one can make inferences based on morphological and/or physiological relationships between traits under consideration.

The genetic linkage map constructed in this work for the F2 population derived from the intercross between *G. hirsutum* and *G. barbadense* promises to provide a better understanding of the cotton crop by possibly providing a core of markers with more practical application than those developed in intraspecific populations. Moreover, the QTL information will help breeders to understand and dissect agronomically important traits and to develop new methods of multi-directional selection. However, efforts to place more markers on the population map and conduct in-depth QTL analyses continue in our lab.

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