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RESEARCH ARTICLE

Linkage map construction and detection of QTLs associated with earliness, fiber quality and yield in an interspecific cross between *Gossypium hirsutum* L. and *Gossypium barbadense* L.

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Abstract

Genomics–based approaches offer unprecedented opportunities for crop improvement. Mapping of the chromosomal regions affecting qualitative and/or quantitative traits using molecular markers is receiving a growing attention in the last few decades. We used 50 EST, 18 EST-SSR, 36 SSR primers and 64 AFLP primer combinations to develop a molecular linkage map of cotton employing F2 segregating population obtained through an interspecific cross between Giza 45 (*G. barbadense*) and Tamcot Luxor (*G. hirsutum*). Analysis of segregation among the resulting 60 F2 individuals was performed using 1 EST, 4 EST-SSR, 14 SSR primers and 10 AFLP primer combinations that revealed consistent polymorphic patterns and were employed for constructing a genetic linkage map that included 210 molecular markers located on 26 linkage groups and spanning a total length of 3503.8 cM. We detected 81 significant QTLs related to earliness, high yield, and fiber quality, which is promising for a better understanding of the cotton crop. Our map is covering ~75.2% of the total recombinational length of the cotton genome with an average marker interval of 16.7cM. SSR markers were used to assign the linkage groups to specific chromosomes. Seven linkage groups were assigned to chromosomes. This map represents the first map for the intercross between Giza 45 and Tamcot Luxor and could be considered as a framework map to overcome the limitations of conventional breeding for the improvement of cotton yield, earliness and fiber quality related traits.

INTRODUCTION

Cotton is a major natural fiber crop that sustains one of the largest industries in Egypt and in many parts of the world. It is the second largest oil-seed crop and a crop for fuel and bio-energy production worldwide. Therefore, it is one of the major mainstays of Egypt and global economies. The genus *Gossypium* includes approximately 45 diploid (2n=2x=26) and 5 allopolyploid (2n=4x=52) species distributed throughout the arid and semi-arid regions of Africa, Australia, Central and South America, the Indian subcontinent, Arabia and Hawaii (Fryxell, 1979 and 1992; Wendel and Cronn, 2003 and Campbell *et al*., 2010). A parallel level of cytogenetic and genomic diversity has arisen during the global radiation of the genus, leading to the evolution of *Gossypium* species into 9 cytological groups or genomes, based on chromosomal similarities, designated: AD, A, B, C, D, E, F, G and K (Endrizzi *et al*., 1985; Percival *et al*., 1999 and Wendel and Cronn, 2003). Allopolyploid cottons appear to have arisen within the last million years, as a consequence of trans-oceanic dispersal of an A-genome taxon to the New World followed by hybridization with an indigenous D-genome diploid. Subsequent to formation, allopolyploids radiated into three modern lineages, including those containing the commercially important species *G. hirsutum* L. and *G. barbadense* L., which represent 90% and 5%, respectively, of the world's cotton production (Wendel and Albert, 1992;

Meredith, 2000; Wendel and Cronn, 2003). *G. hirsutum* (upland cotton) is the most widely cultivated species prized for its higher yield and wider environmental adaptation; while, *G. barbadense* (Pima, Sea Island and Egyptian) boast vastly superior fiber quality (Ulloa *et al*., 2005 and Gore *et al.,* 2014). Guo *et al.* (2008a) estimated the haploid genome size of *G. hirsutum* as ~2.83GB.

Molecular markers are useful tools for characterizing genetic diversity of *Gossypium* germplasm. Molecular markers are differentiated in two types: first non-hybridization-based PCR (RFLP: restriction fragment length polymorphism) and second is PCR based markers (RAPD: random amplified polymorphic DNA, AFLP: amplified fragment length polymorphism, SSR: simple sequence repeats, SNP: single nucleotide polymorphism, etc.). Microsatellite DNA marker has been the most widely used, due to its easy use by simple PCR and to the high degree of information provided by its large number of alleles per locus (Kumar *et al*., 2009). Researchers developed SSRs to identify markers correlated with important quality traits; such as fiber yield and quality (Zhang *et al*., 2005a and Mishra *et al*., 2013) and photoperiodic flowering (Abdurakhmonov *et al.*, 2007b). Chee *et al*. (2004) highlighted the utility of the *G. arboreum* EST sequences in the GenBank database for developing PCR-based markers targeting known-function genes in cultivated tetraploid cottons. Kumar *et al*. (2006) demonstrated that searching cotton ESTs in BLAST against the Arabidopsis database is both feasible and practical for predicting the locations of introns in cotton ESTs. Han *et al*. (2004), Qureshi *et al*. (2004) and Park *et al*. (2005) demonstrated EST-SSR markers as a cost-effective strategy for cotton by exploiting EST databases that would facilitate the development of a high-resolution integrated genetic map of cotton for structural and functional study of fiber genes and fiber quality MAS. Abdurakhmonov *et al.* (2007a) highlighted that SSR and EST-SSR markers associated with fiber development traits have the potential to play a key role in understanding of cotton fiber development and for the development of superior cotton cultivars through marker assisted selection programs. AFLP markers have been used to study diversity and relatedness in cotton (*Gossypium* spp.) plants in Pakistan (Murtaza, 2006), in Syria (Saleh, 2012) and in Australia (Tiwari *et al.*, 2014).

Mapping of chromosomal regions affecting qualitative and/or quantitative traits using molecular markers is receiving a growing attention in the last few decades (Adawy *et al*., 2013; Wang *et al*., 2013 and Gore *et al*., 2014). Genetic linkage maps play fundamental roles in understanding genome structure, explaining genome formation events during evolution and discovering the genetic bases of important traits (Yu *et al.*, 2012b). Additionally, cotton genome mapping offers the possibility to dissecting quantitative traits such as earliness, yield and fiber quality, into their single genetic determinants, the so-called quantitative trait loci (QTLs), thus paving the way to marker-assisted selection (Hoffman *et al,*. 2007; Rakshit *et al*., 2010 and Mishra *et al*., 2013). According to Said *et al.* (2013) results among QTL studies differed due to the use of different genetic populations, markers and marker densities, and testing environments.

According to Fang *et al*. (2014), negative correlation between yield and fiber quality is an obstacle for cotton improvement. Random-mating provides a potential methodology to break this correlation. Identification of stable fiber QTLs in Upland cotton is essential in order to improve cotton cultivars with superior quality using MAS strategy. Abdurakhmonov *et al*. (2008) highlighted that the narrow genetic background of cultivated cotton germplasm is hindering the cotton productivity worldwide. Although potential genetic diversity exists in *Gossypium* genus, it is largely 'underutilized' due to photoperiodism and the lack of innovative tools to overcome such challenge. Abdellatif *et al.* (2012) indicated that the ancestors of all the Egyptian cotton cultivars bred in Egypt are limited to 4 varieties only: Ashmoni, Giza 12, Sakha 3 and Sakha 4, which confirms the narrow genetic background of the Egyptian cotton varieties.

Hence, the objectives of the present investigation were: 1) to develop a molecular linkage map of cotton through the application of different DNA markers (EST, EST-SSR, SSR and AFLP) employing F2 segregating population obtained through an interspecific cross between Giza 45 (*G. barbadense*) and Tamcot Luxor (*G. hirsutum*); 2) to assign QTLs for some agronomic traits related to earliness, high fiber quality, and higher yield (1- Average Boll Weight, 2- Lint Percentage, 3- Fiber Fineness Micronaire, 4- Fiber Strength Presley, 5- Fiber Length per Inch, 6- Fiber Length Uniformity, 7- Date of 1st Flowering, and 8- Node of 1st Fruiting Branch).; to be used as a framework for developing cotton cultivars with superior quality.

Material and Methods

1. Mapping population

Based on preliminary studies (Adawy *et al*., 2006 and Hussein *et al*., 2006) of the genetic polymorphism among 21 cotton genotypes, available in the Cotton Research Institute (CRI), Agriculture Research Center (ARC), Egypt, employing 28 RAPD, 12 ISSR, 24 SSR and 16 AFLP primers/or primer combinations, two polymorphic

accessions were selected to be crossed for generating the mapping population. The two highly polymorphic accessions Giza 45 (*G. barbadense*) and Tamcot Luxor (*G. hirsutum*) were crossed. A mapping population of 60 F2 individuals was developed by intercrossing the two polymorphic cultivars and selfing the resulting F1. The male parent was *G. barbadense* cv. Giza 45, which is characterized by late flowering time and excellent fiber quality (Extra-long Staple). The female parent was *G. hirsutum* cv. Tamcot Luxor, which is characterized by early flowering time and poor fiber quality.

2. Traits measurements

Measurements of the following traits in the 60 F2 individuals were carried out in collaboration with the CRI staff: 1) Average Boll Weight (ABW), 2) Lint Percentage (LP), 3) Fiber Fineness Micronaire (FFM), 4) Fiber Strength Presley (FSP), 5) Fiber Length Per Inch (FLPI), 6) Fiber Length Uniformity (FLU), 7) Date of 1st Flowering (DFF), 8) Node of 1st Fruiting Branch (FFN).

3. Molecular markers

DNA was extracted from the two parents and the 60 F2 plants using CTAB method according to Permingeat *et al*. (1998).

A preliminary screening of the polymorphism between the two parental genotypes was performed using 50 EST, 18 EST-SSR, 36 SSR primers and 64 AFLP primer combinations. Analysis of segregation among the 60 F2 individuals was performed using only 1 EST, 4 EST-SSR, 14 SSR primers and 10 AFLP primer combinations that revealed consistent polymorphic patterns.

EST, EST-SSR and SSR analyses were performed as described by Hussein *et al*. (2003) with minor modifications. The annealing temperature ranged from 53 to 60ºC according to the selected primer. The PCR products were resolved on 3% agarose gels. Table (1) shows the 19 selected polymorphic primers that revealed consistent polymorphism between the two parents and F2 individuals. AFLP analysis (Vos *et al.*, 1995) was carried out according to "AFLP Plant Mapping Protocol [Applied Biosystems (ABI)]" employing AFLP Large Plant Genome Kit. Selective amplification was performed with 64 primer combinations involving 8 *Mse*I(M) and 8 *Eco*RI(E) primers for screening both parents for polymorphism, then 10 consistent polymorphic primer combinations were employed with the 60 F2 plants. Fragments were resolved using capillary electrophoresis on an ABI 3130 Genetic Analyzer (ABI) with the data collection software 3.0 (ABI). AFLP fragment analysis was performed with GeneMapper Software v4.0 (ABI) and the data were assembled in binary format. Table (2) shows the 10 selected polymorphic primer combinations that revealed consistent polymorphism between the two parents and F2 individuals.

Table 1: Sequence of the polymorphic EST, EST-SSR and SSR primers.

#	Primer name	Forward sequence (5´-3´)	Reverse sequence (5´-3´)	An. Tm
1	EST 394	TGTTAAATCTCCATGGCTG	GAACACGAAAATGTCTCCTT	55
2	C 3	CATCATGGCTTTCCGTTTTT	CCAGGATTGGTAAACCCGTA	58
3	C 6	ACCCCAATACAACCCCATTT	GCAGAGAAAAGGGACAGAGG	60
4	C 9	TCTCTCAAAATCTCAAACCCAGA	GCTTAGGGCAAACCACTGAA	58
5	C 11	CTGATTCCACTCTCAAAACCAC	CTACTTTCCATCAGATCCCC	58
6	CH 4	TGTTAAGCATACATTAGTTTCACTCG	CCGGCACCACAAAAGTAAAT	55
7	CH 5	CTTCCAAGCCGTCATCAAA	AAGAGCCAACCTTCGACAGT	53
8	CH 20	TCAACTCATACCAAATCAATTCC	CCCTGTTTTGTTCAATGGGT	53
9	CH 21	AGGTGCTTCAGGCATGATTC	CCCTCACACCTAAACCCAAA	54
10	CH 22	TTCATCATTCTAGCCTGAGTCC	GCGATAATCCTTCCAGGGAT	53
11	CH 24	GAAAAACCAAAAAGGAAAATCG	CTCCCTCTCTCTAACCGGCT	53
12	CH 25	TTATTCTCACAGCCGGAACC	TTCACCCTCTCGCTTCTCAT	54
13	CH 26	GCCATTGATGGAAGGTCAGT	CATCCTCGGAATTTTCCAAA	53
14	CH 28	ATCTTGTTGATTTTCTGACTACAGG	CAGACATTCCCCTTCCTTTGA	60
15	CH 29	TGAAGAGCTCGTTGTTGCAC	CGAAAGAGACAAGCAATGCA	60
16	CH 30	ACATTTCCACCCAAGTCCAA	ACTCTATGCCGCCTCTCGTA	59
17	CH 31	GAAATCATTGGAAGAACATATACTACA	TTGCTCCGTATTTTCCAGCT	58
18	CH 33	AGAGATGCAATGGGATCGAC	ATGTGATAATGCGGGGAATG	59
19	CH 35	AGGCTGACCCTTTAAGGAGC	AACCAACTTTTCCAACACCG	60

An. Tm: annealing temperature in degree Celsius.

Table 2. Selective nucleotides of the polymorphic AFLP primer combinations used for constructing the linkage map.

No.	Primer name	Selective primer combinations
1	A_17	E-aca/M-caa
2	A_18	E-aca/M-cac
3	A_19	E-aca/M-cag
4	A_21	E-aca/M-cta
5	A_22	E-aca/M-ctc
6	A_23	E-aca/M-ctg
7	A_43	E-act/M-cag
8	A_44	E-act/M-cat
9	A_46	E-act/M-ctc
10	A_47	E-act/M-ctg

4. The genetic linkage map construction and QTL detection

Markers showing polymorphism between the two parents were employed for constructing the genetic linkage map. Linkage analysis and map construction were performed by Map Manager QTX v1.4 (Manly *et al.*, 2001) employing Kosambi's function with a minimum LOD score of 3.0, followed by ripple command for each linkage group to check the final order of markers. QTLs were detected by single point analysis (SPA) using Windows QTL cartographer v.2.5 (Wang *et al.*, 2007) at significance levels of 5%, 1%, 0.1% and 0.01%**.**

Results and Discussion

One of the genomics–based fields, receiving a growing attention in the last few decades, is the mapping of chromosomal regions affecting qualitative and/or quantitative traits using molecular markers (Adawy *et al*., 2013; Wang *et al*., 2013 and Gore *et al*., 2014). Employing molecular markers in studying polymorphisms between *Gossypium spp*. and cultivars is feasible and well documented (Agarwal *et al.*, 2008 and Kumar *et al.*, 2009). Using several molecular marker types provides a better overall view of cotton genome polymorphism (Noormohammadi *et al*., 2013a). Many researchers employed more than one type of markers in the same time to study cotton genome to have more enriched and informative molecular markers (Abdurakhmonov *et al*., 2007a; Adawy, 2007 and 2013; Hussein *et al*., 2007; Kantartzi *et al.*, 2009 and Mokrani *et al*., 2012). Hence, we employed 4 different markers: EST, EST-SSR, SSR and AFLP in an attempt to develop an enriched molecular linkage map of cotton through an interspecific cross between Giza 45 (*G. barbadense*) and Tamcot Luxor (*G. hirsutum*).

Table 3. Primer name, total number of bands, polymorphic bands and percentage of polymorphism as detected by SSR analysis.

No.	Primer	No. of Bands		% of Polymorphism
		Total	Polymorphic	
1	CH 4	4	4	100
2	CH 5	3	1	33.3
3	CH 20	2	2	100
4	CH 21	4	4	100
5	CH 22	2	2	100
6	CH 24	3	3	100
7	CH 25	4	4	100
8	CH 26	2	2	100
9	CH 28	2	2	100
10	CH 29	2	2	100
11	CH 30	2	2	100
12	CH 31	4	4	100
13	CH 33	2	2	100
14	CH 35	2	2	100
Total		**38**	**36**	**-**
Average		**2.7**	**2.6**	**94.7**

1. SSR analysis

Out of the tested 36 SSR primers, only fourteen primers revealed consistent polymorphic patterns between the two parents. The 14 primers (Table 3) produced a total of 36 fragments and 32 out of them were polymorphic representing 94.7% polymorphism. The total number of fragments per primer ranged from 2 in CH20, CH22, CH26, CH28, CH29, CH30, CH33 and CH35 to 4 in CH4, CH21, CH25 and CH31. The number of polymorphic fragments per primer ranged from 1 in CH5 to 4 in primers CH4, CH21, CH25 and CH31. Microsatellites or SSR were used deeply to study the polymorphisms in diploid and tetraploid cottons (Rong *et al.*, 2004; Zhang *et al.*, 2005a; Blenda *et al.*, 2006; Hoffman *et al.*, 2007; Yu *et al.*, 2012a and Mishra *et al.*, 2013).

2. EST analysis

In this study, out of 50 EST primers tested, only one primer (EST 394) showed consistent polymorphic pattern between the two parents. It is expressed in Boll: BNLGHi12112 Six-day Cotton fiber *Gossypium hirsutum* cDNA 5' similar to (AC003970) germin-like protein [*Arabidopsis thaliana*], mRNA sequence. The primer EST 394 produced 3 fragments in total and they were all polymorphic between the two parents. We used EST because it is an important informative marker, according to Ayeh (2008). ESTs are providing in depth knowledge in plant biology, breeding

and biotechnology and many researchers employed ESTs to study genetic polymorphism in cotton (Chee *et al*., 2004; Kumar *et al*., 2006 and Chen *et al*., 2007).

Table 4. A list of the polymorphic EST-SSR primers showing their products and percentage of polymorphism.

No.	Primer	No. of bands		% of Polymorphism
		Total	Polymorphic	
1	C 3	2	2	100
2	C 6	2	2	100
3	C 9	2	1	50
4	C 11	4	4	100
Total		**10**	**9**	**-**
Average		**2.5**	**2.25**	**90**

3. EST-SSR analysis

In the present study, out of the tested 18 EST-SSR primers, we employed the 4 EST-SSR primers (C3, C6, C9, C11), that revealed polymorphism between the two parents. The 4 EST-SSR primer pairs (Table 4) revealed 9 polymorphic fragments out of total 10 resulting fragments, representing 90% polymorphism. The total number of amplicons per primer ranged from 2 in C3, C6, and C9 to 4 in C11. The number of polymorphic amplicons per primer ranged from 1 in C9 to 4 in C11. According to Agarwal *et al.* (2008), the recently developed markers such as EST-SSR combine the advantages of both markers. Many researchers employed EST-SSR markers to study genetic distances between cotton species. A cost-effective strategy to develop EST-SSR markers by exploiting EST databases is demonstrated by Qureshi *et al.* (2004). Abdurakhmonov *et al.* (2007a) employed 17 SSRs out of 304 markers tested from MGHES (EST-SSR), JESPR (genomic SSR), and TMB (BAC-derived SSR) collections and showed significant linkage associations with lint percentage QTL in a set of recombinant inbred cotton lines segregating for lint percentage. In 2011, Yu *et al*., isolated and mapped 3177 new EST-SSRs from cotton ESTs derived from the A1,D5, (AD)1, and (AD)2 genome. Guo *et al*. (2007) developed and used 2218 EST-SSRs, 1554 from *G. raimondii*-derived and 754 from *G. hirsutum*-derived ESTs, to screen polymorphisms to enhance their backbone genetic map in allotetraploid cotton. They identified 133 EST-SSRs by analyzing 9,948 sequences belonging to *G. hirsutum* L. in GenBank. 19% of the sequences showed considerable sequence similarity with sequences in the *Arabidopsis thaliana* (L.) Heynh. genome.

Table 5. A list of the polymorphic AFLP primer combinations showing their products and percentage of polymorphism.

#	Primer name	Primer combination	No. of bands		% of Polymorph.
			Total	Polymorph.	
1	A_17	E-aca/M-caa	138	34	24.6
2	A_18	E-aca/M-cac	124	31	25
3	A_19	E-aca/M-cag	124	42	33.9
4	A_21	E-aca/M-cta	131	29	22.1
5	A_22	E-aca/M-ctc	42	14	33.3
6	A_23	E-aca/M-ctg	113	27	23.9
7	A_43	E-act/M-cag	98	31	31.6
8	A_44	E-act/M-cat	130	31	23.8
9	A_46	E-act/M-ctc	114	27	23.7
10	A_47	E-act/M-ctg	118	36	30.5
Total			**1132**	**302**	**-**
Average			**113.2**	**30.2**	**26.7**

4. AFLP analysis

Using AFLP we could enrich our map with markers, 10 primer combinations (A_17, A_18, A_19, A_21, A_22, A_23, A_43, A_44, A_46 and A_47), out of the tested 64 AFLP primer combinations, proved polymorphic between the two parents. The highest polymorphic primer combination was A_19 (E-aca/M-cag) with 33.9% polymorphism (Table 5), while the lowest was A_21 (E-aca/M-cta) with 22.1% polymorphism. The average polymorphism per AFLP marker analysis was 26.7% with total number of 1132 fragments including 302 polymorphic fragments. While, Saleh (2012) assessed the genetic variability and chemical components of five upland cotton (*Gossypium hirsutum* L.) varieties grown in Syria using AFLP. Twenty-one AFLP primer combinations yielded 1,017 discernible loci of which 495 (50.569%) were polymorphic. Selected markers/primer pairs were ranged between 22 (E-AGA/T-GAA) and 89 (E-GAA/T-CTT) fragments with an average of 48.429 fragments per primer pair. Marker Index (MI) average for AFLP markers was estimated to be 5.036. Hence, AFLP were being used to study the genetic background of cotton species and cultivars (Murtaza, 2006; Myers *et al*., 2009 and Tiwari *et al.*, 2014).

Table 6. Comparison between the products and polymorphism of the 4 marker types employed.

	Products				
	EST	**EST-SSR**	**SSR**	**AFLP**	**Total**
No. of primers	1	4	14	10	29
Total no. of amplicons	3	10	38	1132	1183
No. of polymorphic amplicons	3	9	36	302	350
% of Polymorphism	100.0	90.0	94.7	26.7	29.6

5. Combined marker analysis

We combined the results of EST, SSR, EST-SSR and AFLP analyses to have more informative and enriched map from the interspecific cross between Tamcot luxor (*G. hirsutum*) and Giza 45 (*G. barbadense*) and to try to cover most of the cotton genome. Analysis of segregation among the resulting 60 F2 individuals was performed using 1 EST, 4 EST-SSR, 14 SSR primers and 10 AFLP primer combinations (29 primer/primer combinations in total) that revealed consistent polymorphic patterns. The total number of resolved amplicons was 1183 (Table 6); out of them 350 were polymorphic, representing 29.6% polymorphism. While using single molecular marker has its own merits, using several molecular marker types provides a better overall view of cotton genome polymorphism (Noormohammadi *et al*., 2013a).

Table 7. Distribution of the different markers on the 26 linkage groups.

LG No.	**Markers**					**Length (cM)**	**cM per Marker**
	AFLP	**EST-SSR**	**SSR**	**EST**	**Total**		
LG1	34	1	1	0	36	386.2	**10.7**
LG2	19	0	0	0	19	275.1	**14.5**
LG3	17	0	0	0	17	343.8	**20.2**
LG4	16	0	0	0	16	178.0	**11.1**
LG5	11	1	1	0	13	341.9	**26.3**
LG6	11	0	0	0	11	206.5	**18.8**
LG7	11	0	0	0	11	191.2	**17.4**
LG8	10	0	0	0	10	158.9	**15.9**
LG9	10	0	0	0	10	248.9	**24.9**
LG10	6	0	1	0	7	111.7	**16.0**
LG11	7	0	0	0	7	127.3	**18.2**
LG12	6	0	0	0	6	163.8	**27.3**
LG13	5	0	0	0	5	73.0	**14.6**
LG14	5	0	0	0	5	53.1	**10.6**
LG15	4	0	1	0	5	123.6	**24.7**
LG16	5	0	0	0	5	107.9	**21.6**
LG17	4	0	0	0	4	75.3	**18.8**
LG18	2	0	1	0	3	33.4	**11.1**
LG19	2	0	1	0	3	50.2	**16.7**
LG20	3	0	0	0	3	44.7	**14.9**
LG21	3	0	0	0	3	63.0	**21.0**
LG22	3	0	0	0	3	63.0	**21.0**
LG23	0	0	2	0	2	14.5	**7.3**
LG24	2	0	0	0	2	17.8	**8.9**
LG25	2	0	0	0	2	25.5	**12.8**
LG26	2	0	0	0	2	25.5	**12.8**
Total	**200**	**2**	**8**	**0**	**210**	**3503.8**	**16.7**

6. **Construction of the linkage map**

Out of the 321 loci detected, 210 loci including 8 SSR, 2 EST-SSR and 200 AFLP markers were mapped in 26 linkage groups covering a total length of 3503.8 cM (Table 7 and Fig. 1). Thus, our map represents about 75.2% of the total recombinational length of the cotton genome, as the estimated map distance of the *G. hirsutum* cotton genome is around 4660 cM (Stelly, 1993 and Reinisch *et al.*, 1994). The average length of linkage groups ranged from 14.5 cM in LG23 to 386.2 cM in LG1. The average map interval ranged from 7.3 in LG23 to 27.3 in LG12 with an average map interval of 16.7cM. The number of markers in each linkage group ranged from 2 to 36. Lin *et al*. (2005) constructed a map covering 5141.8 cM with a mean interlocus space of 9.08 cM. Genetic mapping was performed by Park *et al.* (2005) resulting in 193 loci, including 121 new fiber loci that were not previously mapped. These fiber loci were mapped to 19 chromosomes and 11 LG spanning 1277 cM providing approximately 27% genome coverage. Zhang *et al.* (2005b) constructed a genetic linkage map with 70 loci (55 SSR, 12 AFLP and 3 morphological loci) using 117 F2 plants obtained from a cross between two upland cotton cultivars Yumian1 and T586. The linkage map comprised of 20 linkage groups, covering 525cM with an average distance of 7.5cM between two markers, or approximately 11.8% of the recombination length of the cotton genome. While, Adawy *et al.* (2008), performed an interspecific cross (*G. barbadense* x *G. hirsutum*) between two genotypes, Giza83 (late flowering) and Deltapine (early flowering) to develop F2 segregating population. Analysis of segregation among the 71 F2 individuals was performed using 3 RAPD, 10

SSR, 6 AFLP primer combinations. Their map showed 22 linkage groups with 140 markers covering a total length of 1556.7 cM. The average length of linkage groups ranged from 1.4 to 649.5.

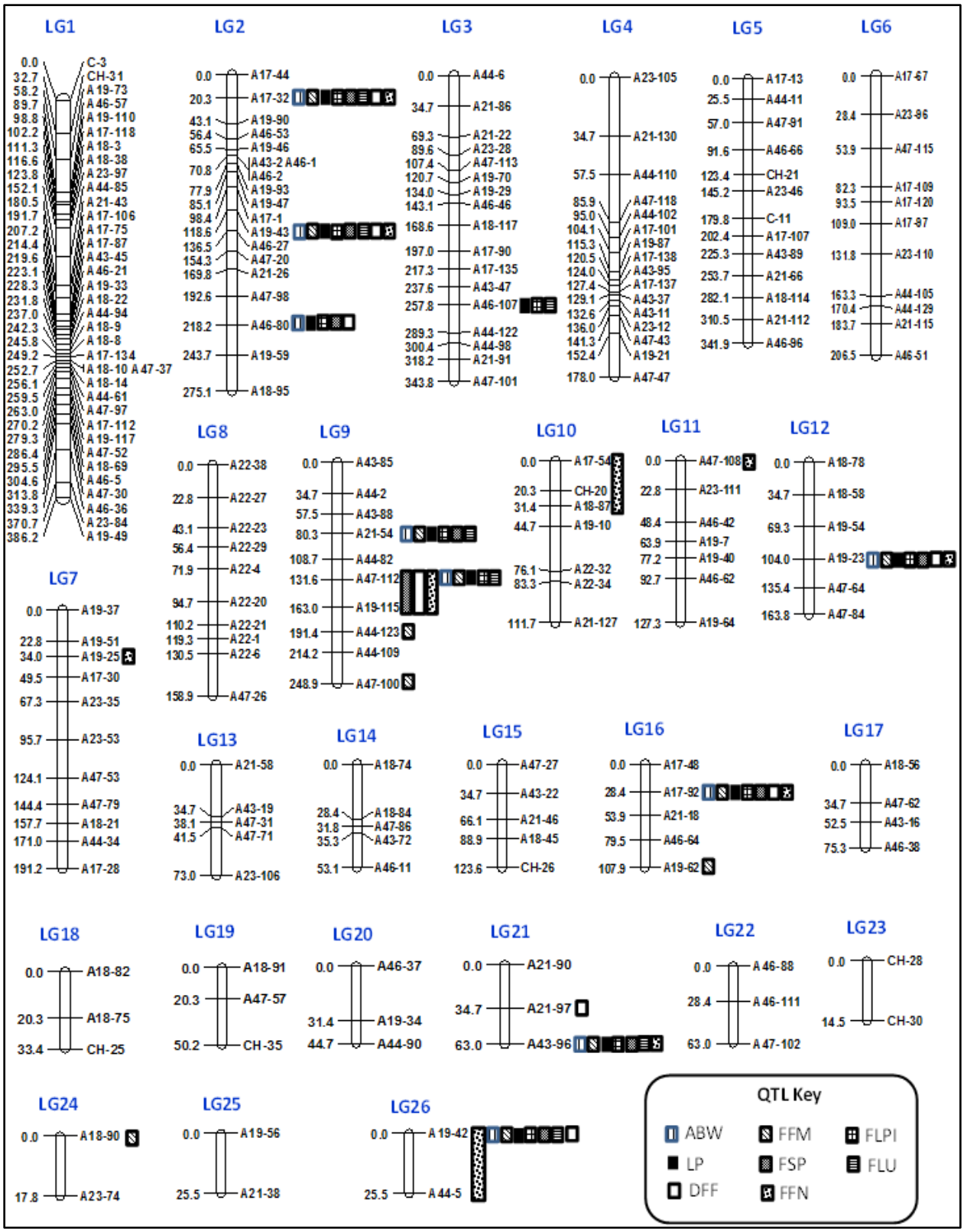

Figure 1. Final map showing QTLs and markers distribution on the 26 linkage groups.

In addition, Adawy *et al.* (2013) studied a segregating F2 population derived from an interspecific cross (*G. barbadense* × *G. hirsutum*) between two genotypes, cvs. 'Giza 83' and 'Deltapine'. They employed different molecular markers: SSR, EST, EST-SSR, AFLP and RAPD to identify markers that reveal differences between the parents. In total 42 new markers were merged with 140 previously mapped markers (Adawy *et al.*, 2008) to produce a new map with 182 loci covering a total length of 2370.5 cM. Among these new markers, some of them were used to assign chromosomes to the produced 26 linkage groups. The LG2, LG3, LG11 and LG26 were assigned to chromosomes 1, 6, 5 and 20 respectively. Yu *et al.* (2011) obtained a final map including 2316 loci on the 26 cotton chromosomes, 4418.9 cM in total length and 1.91 cM in average distance between adjacent markers. Additionally, Shaheen *et al*. (2013) generated 52 polymorphic DNA fragments and used them to construct a linkage map with JoinMap 3.0. A total of 45 loci and 5 phenotypic traits were mapped at a logarithm of odds ratio of ≥3.0 on 10 linkage groups. The total length of the map was 346 cM, and the average distance between adjacent markers was 7.7 cM. Wang *et al*. (2013) used a total of 15,971 markers (gSSRs, EST-SSRs, SRAPs, and SSCP-SNPs) to construct an intraspecific linkage map of *G. barbadense* with 124 F2 individuals derived from the cross (Hai7124 × 3-79). In the F2 population, 412 loci showed polymorphism, giving a polymorphic rate of 2.58%. Three hundred and thirty-seven loci were mapped on 52 linkage groups, and 35 groups were assigned to 20 chromosomes. The full length of the linkage map was 2108.34 cM, and the mean distance between adjacent loci was 6.26 cM. Also, Hou *et al*. (2013) developed 1800 loci from 1347 pairs of polymorphic primers. Of these, 1204 loci were grouped into 35 linkage groups at LOD ≥ 4. The map covered 3320.8 cM, with a mean density of 2.76 cM per locus. Liang *et al*. (2013) constructed a genetic linkage map comprising 421 loci and covering 3814.3 cM, accounting for 73.35% of the cotton genome, using an F2 population.

Table 8. Linkage groups, markers and assigned chromosomes.

#	Linkage Group	Marker	Chromosome No.
1	LG1	CH 31	15
2	LG5	CH21	2
3	LG10	CH 20	6
4	LG15	CH 26	4
5	LG18	CH 25	3
6	LG19	CH 35	23
7	LG23	CH 28	7

7. Assignment of linkage groups to the chromosomes

In this study, 12 primer pairs out of the 14 SSR primers assayed were previously mapped on the cotton chromosomes. Therefore, these SSR markers were used to assign the linkage groups to specific chromosomes. Based on the presence of 7 assigned SSR markers (Table 8), seven linkage groups (LG) were assigned to chromosomes, i.e., LG1, LG5, LG10, LG15, LG18, LG19, and LG23 were assigned to chromosomes 15, 2, 6, 4, 3, 23, and 7, respectively (Table 8). In addition, 5 out of 111 unlinked markers were assigned to chromosomes, too. Markers CH4, CH22, CH24, CH29, and CH33 were assigned to chromosomes 1, 13, 9, 8, and 17, respectively. Park *et al*. (2005) suggested that chromosomes 2, 3, 15, and 18 may harbor genes for traits related to fiber quality. Lin *et al*. (2005) used a total of 238 SRAP primer combinations, 368 SSR primer pairs and 600 RAPD primers to screen polymorphisms between *G. hirsutum* cv. Handan208 and *G. barbadense* cv. Pima90. Sixty-nine F2 progeny from this interspecific cross were genotyped with 749 polymorphic markers (205 SSRs, 107 RAPDs and 437 SRAPs). A total of 566 loci were assembled into 41 linkage groups with at least three loci in each group. Twenty-eight linkage groups were assigned to corresponding chromosomes by SSR markers with known chromosome locations.

8. QTL analysis

In our study, we used single point analysis to identify the genomic regions controlling the eight traits under study. In total, 81 significant QTLs (Table 9 and Fig. 1) were identified for the eight traits on 11 linkage groups. These included 9 QTLs for Average Boll Weight (ABW), 10 QTLs for Lint Percentage (LP), 12 QTLs for Fiber Fineness Micronaire (FFM), 10 QTLs for Fiber Strength Presley (FSP), 10 QTLs for Fiber Length Per Inch (FLPI), 7 QTLs for Fiber Length Uniformity (FLU), 9 QTLs for Date of 1st Flowering (DFF), and finally 14 QTLs for Node of 1st Fruiting Branch (FFN). Fifteen Linkage groups: 1, 4, 5, 6, 8, 13, 14, 15, 17, 18, 19, 20, 22, 23, and 25 did not show any QTLs. Adawy *et al.* (2008) used single point analysis 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. In addition, Adawy *et al.* (2013) used Single point analysis to identify genomic regions controlling traits for

plant height, number of nodes at flowering time, bolling date, days to flowering and number of bolls. In total 40 significant QTL were identified for the five traits on 11 linkage groups (1, 2, 3, 4, 5, 10, 11, 12, 18, 19 and 23). While, Li *et al*. (2012) performed QTL detection for node of first fruiting branch (NFFB) and its height (HNFFB) as the important indicators to measure cotton earliness, within the two F2:3 populations, Baimian2 x TM-1 and Baimian2 x CIR12 by composite interval mapping (CIM) and mixed model CIM (MCIM). Eight QTL for NFFB and six QTL for HNFFB were detected in both populations. The two significant major QTL, qNFFB-17-I for NFFB and qHNFFB-17-I for HNFFB, were detected simultaneously by CIM and MCIM. Single marker-analysis showed that 11 molecular markers, such as CGR5222, are linked with NFFB and/or HNFFB. Additionally, Guo *et al.* (2008b) used F2 population consisted of 251 plants from the cross of a day neutral cultivar Deltapine 61, and a photoperiod sensitive accession Texas 701. Interval mapping and multiple QTL mapping were used to determine QTLs contributing to NFFB. Three significant QTLs were mapped to chromosome 16, 21, and 25; two suggestive QTLs were mapped to chromosome 15 and 16. Four markers associated with these QTLs accounted for 33% of the variation in NFFB by single and multiple-marker regression analyses.

Table 9. Distribution of the 81 QTLs on the 8 traits.

#	Trait		No. of QTLs
	Trait name	**Abbreviation**	
1	Average Boll Weight	ABW	9
2	Lint Percentage	LP	10
3	Fiber Fineness Micronaire	FFM	12
4	Fiber Strength Presley	FSP	10
5	Fiber Length Per Inch	FLPI	10
6	Fiber Length Uniformity	FLU	7
7	Date of 1st Flowering	DFF	9
8	Node of 1st Fruiting Branch	FFN	14
	Total		**81**

Our map revealed that the QTLs related to fiber quality segregate together and they are adjacent on the linkage groups. Eight linkage groups: 2, 3, 9, 12, 16, 21, 24, and 26 contained all the QTLs related to fiber quality (39 QTLs). Three linkage groups: 7, 10, 11 contained 3 QTLs for Node of 1st Fruiting Branch. Nineteen QTLs related to fiber yield (Average Boll Weight and Lint Percentage) mostly segregated together and were distributed on 7 linkage groups: 2, 3, 9, 12, 16, 21, and 26. Twenty-three QTLs related to Earliness (Date of 1st Flowering and Node of 1st Fruiting Branch) were distributed on 9 linkage groups: 2, 3, 7, 9, 10, 11, 12, 16, 21, and 26. In addition the map showed that QTLs related to the studied 8 traits were concentrated on seven linkage groups (LG: 2, 3, 9, 12, 16, 21, and 26) close to each other. According to Abdurakhmonov *et al.* (2007a), interval mapping demonstrated that 9 SSRs with stable critical LOD threshold values at $\alpha = 0.01$ have significant QTL effect. Multiple QTL-mapping (MQM) revealed at least, two highly significant fiber development QTLs. Also, Shaheen *et al*. (2013) mapped 7 quantitative trait loci (QTLs), including 5 for productivity traits and 2 for fiber traits. While, our map is more comprehensive and covered 8 different traits related to fiber, early flowering and yield.

Zhang *et al.* (2005b) used genetic linkage map to identify and map the QTLs affecting lint percentage and fiber quality traits in 117 F2:3 family lines. Sixteen QTLs for lint percentage and fiber quality traits were identified in 6 linkage groups by multiple interval mapping: 4 QTLs for lint percentage, 2 QTLs for fiber 2.5% span length, 3 QTLs for fiber length uniformity, 3 QTLs for fiber strength, 2 QTLs for fiber elongation and 2 QTLs for micronaire reading. Several QTLs affecting different fiber-related traits were detected within the same chromosome region, suggesting that genes controlling fiber traits may be linked or the result of pleiotropy. Additionally, Wu *et al.* (2009) assigned 24 linkage groups (115 SSR loci) to specific chromosomes. Fifty six QTLs (LOD>3.0) associated with 14 agronomic and fiber traits were located on 17 chromosomes. One QTL associated with fiber elongation was located on linkage group LGU01. Nine chromosomes in sub-A genome harbored 27 QTLs with 10 associated with agronomic traits and 17 with fiber traits. Eight chromosomes in D sub-genome harbored 29 QTLs with 13 associated with agronomic traits and 16 with fiber traits. Chromosomes 3, 5, 12, 13, 14, 16, 20, and 26 harbor important QTLs for both yield and fiber quality compared to other chromosomes. Ulloa and Meredith (2000) detected 26 QTLs on 9 linkage groups. Two QTLs were detected for lint yield and three for lint percentage. Three QTLs for fiber strength and two QTLs for fiber 2.5% span length were detected. The QTL positions on the linkage groups suggest that genes conferring fiber quality may cluster on the same cotton chromosome(s). According to Rakshit *et al*. (2010), a number of markers were found to be associated with multiple traits suggesting clustering of QTLs for fiber quality traits in cotton. Rong *et al*. (2007), explained that crosses between closely-related genotypes differing by single-gene

mutants yield profoundly different QTL landscapes, suggesting that fiber variation involves a complex network of interacting genes. Members of the lint fiber development network appear clustered. This is in agreement with our results.

Finally, a genetic map including 210 molecular markers located on 26 linkage groups and spanning a total length of 3503.8 cM, was constructed and it is covering about 75.2% of the total recombinational length of the cotton genome with an average marker interval of 16.7cM. This map was useful in detecting 81 significant QTLs related to earliness, high yield, and fiber quality, which is promising for a better understanding of the cotton crop. To our knowledge, this map represents the first map for the intercross between Giza 45 and Tamcot Luxor and could be considered as a framework map to overcome the limitations of conventional breeding for the improvement of cotton yield, earliness and fiber quality related traits through intra- or interspecific hybridization. Hence, the QTL information will help breeders to understand the important agronomic traits of cotton and will assist in developing new methods of multi-directional selection. Therefore, efforts to place more markers on the population map and conduct in-depth QTL analyses should be continued.

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