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Molecular linkage map of allotetraploid cotton (*Gossypium hirsutum* L. × *Gossypium barbadense* L.) with a haploid population

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Abstract In the present study, a haploid population from the cross of the two cultivated allotetraploid cottons, *Gossypium hirsutum* L. and *Gossypium barbadense* L., was developed by means of Vsg, a virescently marked semigamous line of Sea island cotton, and some target haploids were successfully doubled with colchicine. A molecular linkage map was constructed with 58 doubled and haploid plants. Among the total of 624 marker loci (510 SSRs and 114 RAPDs), 489 loci were assembled into 43 linkage groups and covered 3,314.5 centiMorgans (cM). Using the monosomic and telodisomic genetic stocks, the linkage groups of the present map were associated with chromosomes of the allotetraploid genome, and some of the unassociated groups were connected to corresponding A or D subgenomes. Through the analysis of the assignment of the duplicated SSR loci in the chromosomes or the linkage groups, ten pairs of possible homoeologous chromosome (or linkage group) regions were identified. Among them, the pairs of Chrs. 1 and 15, Chrs. 4 and 22, and Chrs. 10 and 20 had already been determined as homoeologous by classical genetic and cytogenetic research, and the pair of Chrs. 9 and 23 had also been identified by the ISH method of molecular cytogenetics. But, from present research, it was assumed that Chrs. 5 and 18 might be a new pair of homoeologous chromosomes of the allotetraploid cotton genome detected by molecular mapping of the cotton genome.

Keywords Molecular linkage map · Allotetraploid cotton · SSR · RAPD · Duplicated loci · Haploid · Homoeologous chromosomes

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Introduction

A genetic map is necessary not only for the reliable detection, mapping and estimation of gene effects of important agronomic traits, but also for further research on the structure, organization, evolution and function of the plant genome. Saturated molecular genetic maps based on RFLP markers have been developed for several crops. Cotton (*Gossypium* spp.) is the world's leading fiber crop, and an important source of edible oil as well. Among the four cultivated *Gossypium* species in the world, the American allotetraploid species (*Gossypium hirsutum* L. and *Gossypium barbadense* L.) dominate worldwide cotton production, having almost displaced the old world diploid cultivars (*Gossypium arboreum* L. and *Gossypium herbaceum* L.) (Lee 1984). Variation in ploidy among *Gossypium* spp., together with tolerance of aneuploidy in tetraploid cotton species, has facilitated the use of cytogenetic techniques to explore cotton genetics and evolution research. Among 198 mutants identified in cotton, 61 mutant loci have been assembled into 16 linkage groups, 11 of which have been associated with chromosomes using monosomic and mono-telodisomic stocks (Endrizzi, Turcotte and Kohel 1985). But cotton genomic research is still at the initial step. The first RFLP map of allotetraploid cotton was reported by Reinisch et al. (1994), and after that Brubaker et al. (1999) constructed RFLP maps of the diploid progenitors of allotetraploid cotton and conducted comparative genetic mapping. As the mapping populations of the above-mentioned maps were F_2-derived from the undomesticated allotetraploid cotton races, their use in detecting and mapping the agronomic traits are limited to some extent and could not totally represent the genome of cultivated allotetraploid cottons; however, they are valuable in revealing the organization and evolution of the cotton genome. Shappley et al. (1996, 1998) constructed RFLP maps of *G. hirsutum* L. using the F_2 produced by two upland cotton cultivars. In China, Zuo et al. (2000) constructed a map of 67 marker loci with an F_2 in the upland cotton cultivars.

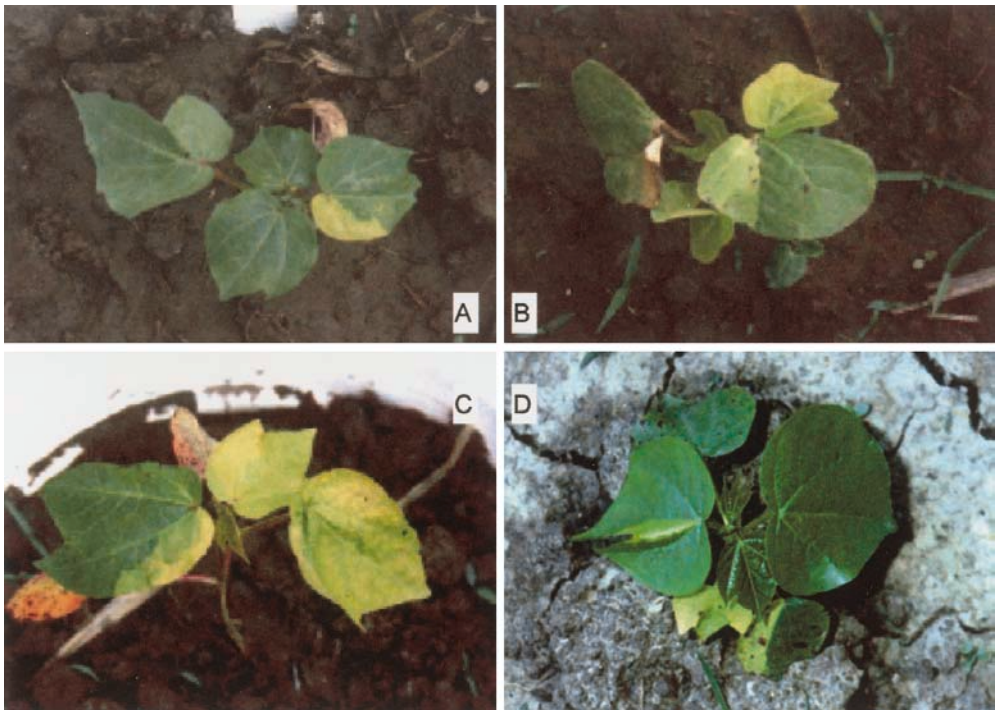

Fig. 1A–D Chimeric plants derived from the offspring of [Vsg × (TM-1 × Hai 7124)]. **A** and **D** show the plants with a major sector of the chimera derived from the male gamate, and the haploid individuals developed from these green sector of this type of chimera are male haploids which are our target haploids. **B** shows about average sectors of male and female gamates of a chimera, which may be equally developed from either male or female haploids. **C** indicates the chimera whose major sectors are female derived and this type of chimera may develop to female haploids in most of the cases. **D** indicates the chimera whose major sectors are male derived and this type of chimera may develop to male haploids

There are many problems in molecular genetic mapping of cultivated cotton. The main one is that all the mapping populations used were tentative (such as the F_2) other than permanent populations (such as DH or RIL) and are not eligible for continuous and cooperative research. Another problem may be the lack of sufficient molecular markers for mapping. Simple sequence repeats (SSRs), also known as microsatellites, are an ideal PCR (polymerase chain reaction)-based DNA marker for genetic mapping because of their abundant high level of polymorphism and wide dispersion in diverse genomes. No SSR map of cotton is reported up to now; however, this type of DNA marker has great usage in research for cotton genome organization and evolution.

We tried to develop a permanent doubled-haploid population from the crossing of *G. hirsutum* L. × *G. barbadense* L. by means of Vsg, a virescently marked semigamic line of island cotton, which was characterized by a cytological mechanism for developing haploids with certain convenience; and thus we constructed an allotetraploid cotton molecular genetic linkage map with SSRs and randomly amplified polymorphic DNA markers (known as RAPD, another PCR-based marker). Through analysis of the assignment of the duplicated marker loci, we have employed the genetic map to investigate the homoeologous chromosomal regions of the A and D subgenomes in the allotetraploid cotton genome.

Materials and methods

Genetic stocks

The genetic standard accession of *G. hirsutum*, TM-1, and the monosomic and telodisomic lines of *G. hirsutum* in a TM-1 background were supplied by Dr. Kohel of the Southern Plain Agricultural Research Center, USDA-ARS, Crop Germplasm Research Unit. *G. barbadense* cv Hai 7124, grown extensively in China, is the offspring of a selected individual in the inheritance research of resistance to cotton. *Verticillium dahliae*. Vsg, a sea island cotton semigamous line with a recessive virescent marker gene was introduced from Nanyang Agricultural Science Institute, Henan province, China. Prior to mapping, self-pollinated progeny from the "TM-1" and "Hai 7124" parents were used for preliminary surveys of DNA polymorphism.

Development of the genetic mapping population

Three types of haploid individuals could be observed in [Vsg×(TM-1×Hai7124)]: (1) were haploids with yellow leaves on the total plant, which might be developed from the egg of Vsg and were called female haploids; (2) were chimeric haploids with two sections independently developed from the egg or sperm cell; and (3) were haploids with green leaves on the total plant, which might be derived from the sperm cell, i.e. the gametes of (TM-1×Hai 7124)F_1, and were termed male haploids (see Fig. 1). The male haploids and the male (green) shoots of chimeric haploids were then treated with 0.3–0.5% colchicine in water to develop the doubled-haploid plants. The self-pollinated seeds of each doubled-haploid were harvested respectively. The genetic mapping population is comprised of 58 haploid and double-haploid individuals from the cross of *G. hirsutum* accession TM-1 and *G. barbadense* cv Hai 7124.

DNA markers

Pairs of SSR primers (216) to amplify cotton SSRs were purchased from Research Genetics, Inc, Huntsville, USA, who devel-

oped the genome library of alloteraploid cotton by screening, and 312 pairs having polymorphism between *G. hirsutum* and *G. barbadense* were synthesized in accordance with Reddy et al. (2001) who reported searching the GenBank (http://www.cotton.org/). The randomly 10-bp primers were purchased from Operon Inc. From a survey of the polymorphism in the two mapping parents, we obtained 624 polymorphic marker loci (510 SSR and 114 RAPD loci).

DNA extraction, PCR reaction and polymorphism detection

DNA extraction from cotton followed Paterson et al. (1993), however without DIECA added in the DNA extraction buffer.

PCR amplification for SSRs was performed in 67 mM of Tris–HCl (pH 8.8), 16 mM of $(NH_4)_2SO_4$, 2.5 mM of $MgCl_2$, 0.2 mM of dNTPs, 0.6 μM of primers, with 0.25–0.5 units of Sangon (Sangon, Shanghai, China) *Taq*ase and 20 ng of genomic DNA per 10 μl using a Thermal Cycler 9600 (Perkin-Elmer). The thermocycler conditions were as follows: one cycle of 95 °C for 2 min, 30 cycles of 94 °C for 45 s, 57 °C for 45 s and 72 °C for 60 s, and one cycle of 72 °C for 7 min. PCR products were run on 8% polyacrylamide gels, using a DYYIII gel apparatus. The gel was pre-run for about 20 min before loading the sample. After electrophoresis, the gel was separated from the plates and treated for 12 min (2-times, each for 6 min) in fixation solution (10% v/v ethanol and 0.5% v/v acetic acid) with gentle shaking. After incubating for 12–15 min in staining solution (0.2% w/v silver nitrate), the gel was washed 2-times with distilled water for 2 min, and with 0.0002% w/v sodium thiosulfate for about 2 min, and then the gel was transferred to developing solution (1.5% w/v sodium hudroxide, 0.4% formaldehyde) to develop the silver-staining DNA bands. The stop and stored solution was 0.75% sodium carbonate. This non-radioactive protocol to detect polymorphisms of cotton SSRs was reported by Zhang et al. (2000).

The PCR reaction and RAPD polymorphism detection was followed by Guo et al. (1997).

Data analysis

Linkage maps and related statistics were determined using MapMaker 3.0 (Lincoln et al. 1992) run on a PC microcomputer. A LOD (base 10-log of the ratio between odds of linkage and odds of non-linkage) score of 3.5 was used to infer linkage in a two-point analysis. Linkage groups were ordered by selecting a subset of five or six loci whose most-likely order was by a LOD of two or more. Remaining loci were added to the initial map using the "try" and "place" functions. The "ripple" function was used to confirm the local order around new loci and identified regions of uncertain order.

Assignment of linkage groups to the chromosomes of allotetraploid cotton

The assignment of chromosome numbers in tetraploid cotton was based on pairing relationships in diplod × tetraploid crosses, with chromosomes 1–13 corresponding to the A subgenome, and chromosomes 14–26 corresponding to the D subgenome (Kimber 1961). Using *G. hirsutum* monosomic and telodisomic lines crossed with *G. barbadense* cultivar Hai 7124, one of our mapping parents, a system used to identify linkage groups to their corresponding chromosomes was developed, which involved Chrs. 1, 2, 3, 5, 6, 7, 9, 10, 11, 12, 14, 15, 16, 17, 18, 20, 22, 23, 25 and 26 out of the total 26 pairs of allotetraploid cotton. Additionally, DNA extracted from identified F_1 aneuploid plants produced between aneuploids involved the same chromosomes and 3–79, a genetic standard in *G. barbadense*, was kindly made available by USDA ARS, Crop Germplasm Research Unit, in cooperation with Texas A&M University, for chromosomal mapping of molecular markers. Two sets of aneuploid hybrids used here were compared for chromosome mapping. The rest were identified compared with the progenitor diploids of allotetraploid cotton, i.e. *G. herbaceum* L and *Gossypium raimondii* L., to the A and D subgenomes respectively.

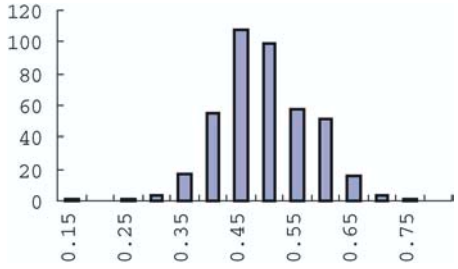

Fig. 2 Distribution of the TM-1 genome ratios in the mapping population. The x-axis is the TM-1 genotype ratio of markers in the population, while the y-axis the number of loci

Results and analysis

Linkage analysis and map construction

From a survey of the two mapping parents using SSRs and random 10-bp primers, we obtained 624 polymorphic marker loci. All the markers were applied for analysis of a total of 58 individuals (lines) of the mapping population in this study. The ratio of the marker-loci segregation in the population was tested with a χ^2-test. The ratio distribution of total counts of the TM-1 genotype of all marker genotypes in the population is given in Fig. 2. The segregation of the SSR marker loci in the mapping population indicated that the population was normal on the whole, although some marker loci were distorted from the expected 1:1 ratio in the haploid or DH population ($P < 0.05$). The data of the distorted marker loci were not used in our map construction.

Among the 624 SSR and RAPD marker loci detected for the mapping population, 489 loci were constructed into 43 linkage groups, totally covering 3,314.5 cM of the allotetraploid cotton; the average genetic distance between two loci was 6.78 cM, the biggest linkage group consisted of 47 marker loci covering 321.4 cM in Chr. 9, and the smallest was a linkage group consisting of two marker loci covering only 9.6 cM. This is the first map presented in cultivated allotetraploid cotton, constructed mainly with SSR markers.

Identification of linkage groups to chromosomes and subgenomes

The monosomic and mono-telosomic stocks with single chromosomes or chromosome arms of *G. barbadense*, and the other chromosomes (arms) pairing with the two allotetraploid cottons, were applied to identify the linkage groups to their corresponding chromosomes or subgenomes. For the co-dominant SSR loci, when observed stocks whose marker genotypes were similar to the ones

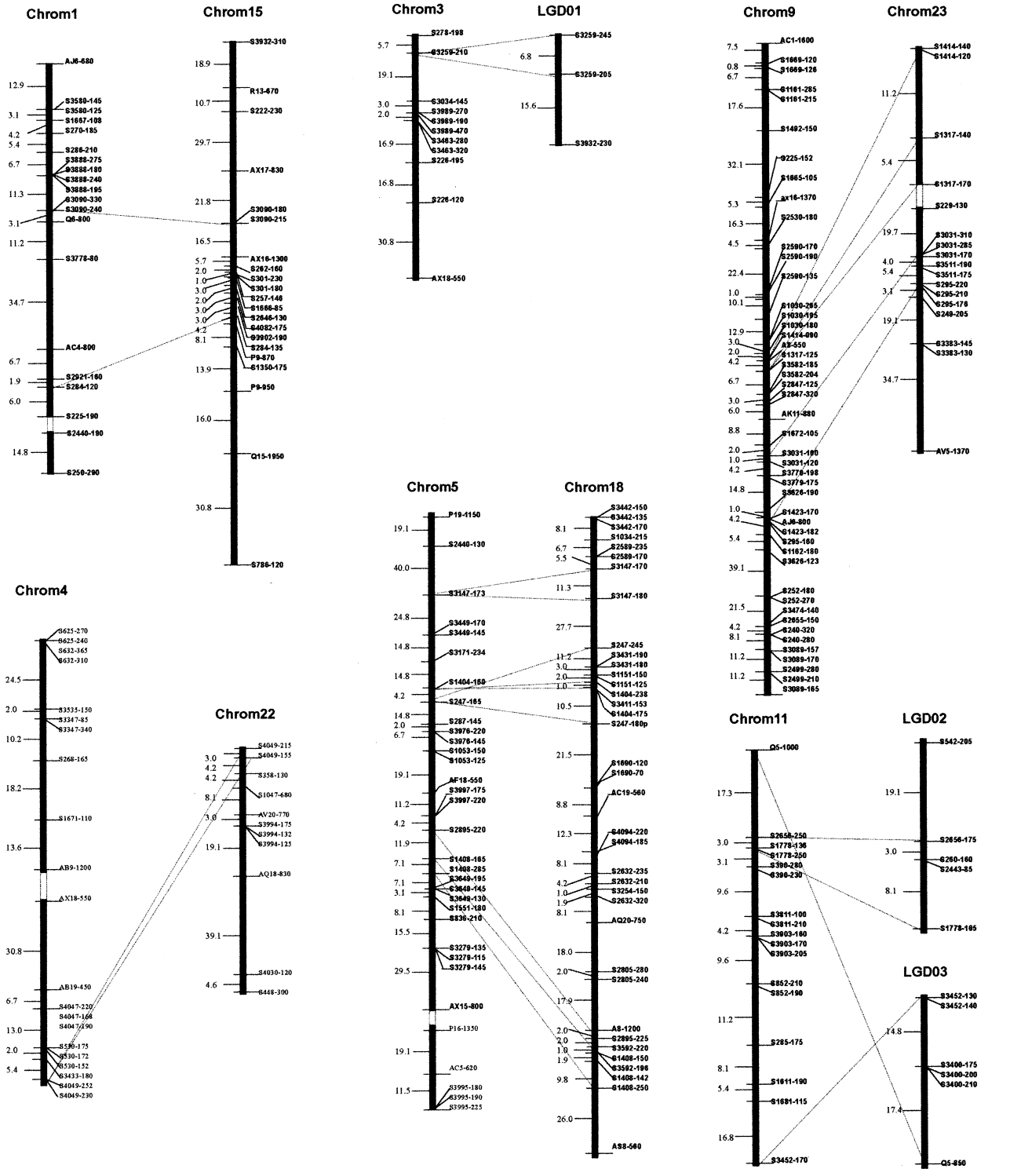

Fig. 3 Molecular linkage map of the allotetraploid genome. The map of presently include 43 linkage groups, and spans 3,314.5 cM, using the Haldane cM function. A total of 25 linkage groups are assigned to the chromosomes. the lines connecting the loci on different chromosome or linkage groups indicate the duplicated marker loci between or among them

of *G. barbadense*, we considered that the involved marker loci be assigned to the corresponding chromosomes or the opposite chromosome arms. Among the total 43 linkage groups presented here, 24 were assigned to their corresponding chromosomes which were Chrs. 1, 3, 4, 5, 9, 10, 11, 12, 15,16, 17, 18, 20, 22, 23, 25 and 26 of the allotetraploid genome, and 13 were associated to the A or D subgenomes by analysis of the marker loci if they existed in the progenitor diploid species the allotetra-

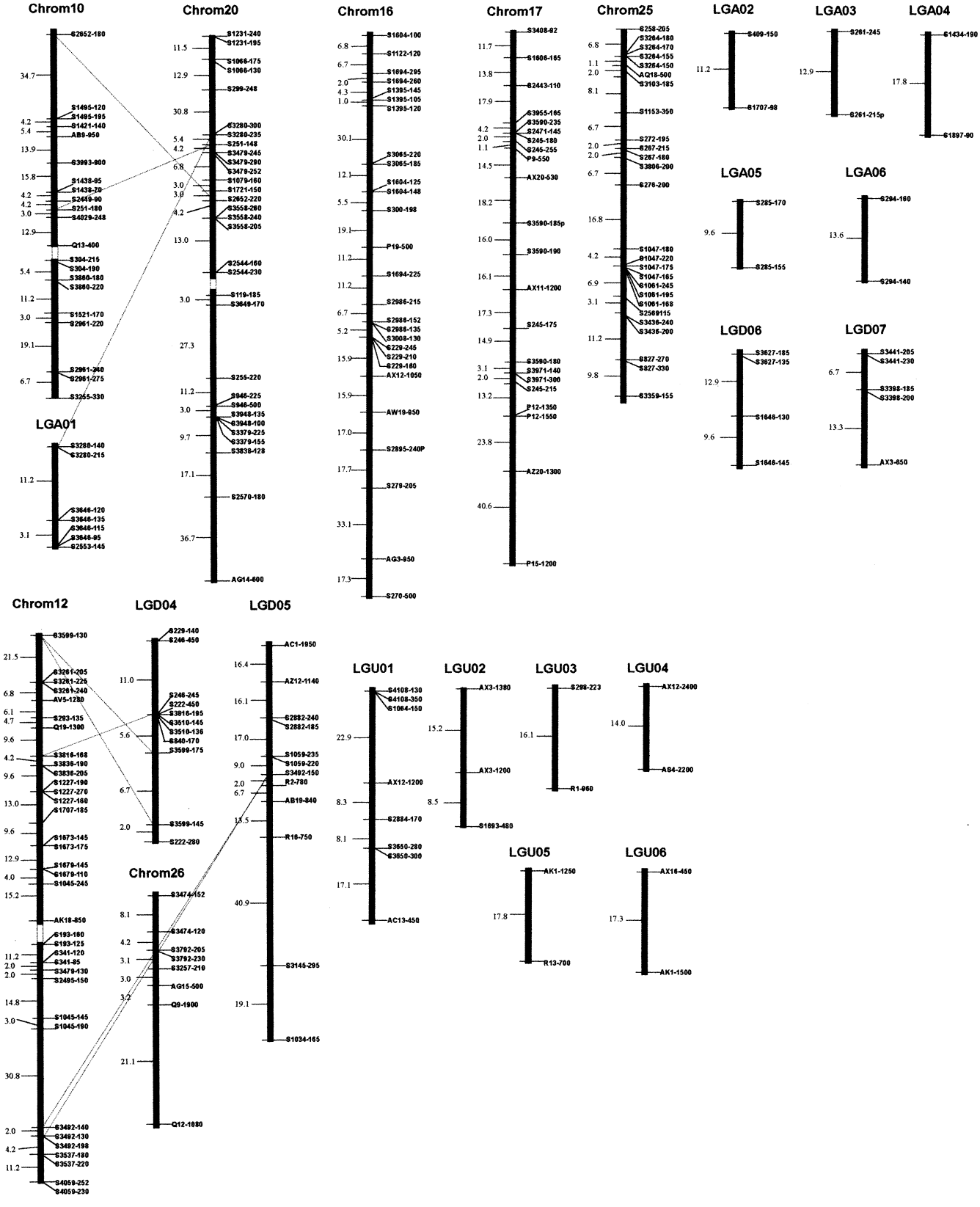

Fig. 3 (continued) Legend see page 1169

ploid cotton. Some linkage groups are still unassociated with any chromosomes or subgenomes.

Distribution of duplicated marker loci and homoeology in the allotetraploid cotton genome

The repeated sequences are one of the characteristics of the plant genome. The molecular markers based on Southern blotting are convenient to reveal the repeat sequence by detecting the sequences of different size, but hybridized with the same DNA probes. In rice (*Oryza sativa* L.), it was discovered that multiple loci generated by a single SSR either co-segregated or were distributed to different chromosomes (Panaud et al. 1996). The majority of SSRs in allotetraploid cotton could amplify multiple loci with a single pair of primers. When the SSRs were used to amplify the genomic DNA of our mapping population, three cases could be observed concerning locus segregation: (1) more than two loci co-segregated in the mapping population; and it was evident that the amplified DNA bands of different molecular size should derive an array or a tandem repeat of single SSRs in the same region of a given chromosome; (2) multiple loci segregated independently from each other, which indicated that a given SSR generated different, but duplicated, loci which were distributed in different chromosomes within an allopolyploid genome; (3) loci derived from a single SSR are genetically linked, which suggested that the SSRs be repeatedly interspersed in a given region of a single chromosome. Through analysis of the assignment of the duplicated SSR loci in the chromosomes or the linkage groups involved in the present molecular linkage map, ten pairs of possible homoeologous chromosome (or linkage group) regions were suggested (Fig. 3):

(1) Chr. 1 (subgenome A) and Chr. 15 (subgenome D): there are two duplicated loci spanning 63.6 cM in Chr. 1 and 35.2 cM in Chr. 15. The pair of Chrs. 1 and 15 had already been determined as homoeologous by classical genetics (Endrizzi, Turcotte, Kohel 1985), and we suggest that this pair of allotetraploid cotton chromosomes be homoeologous; however, no more than two duplicated marker loci were involved in this pair of chromosomes.

(2) Chr. 3 (subgenome A) and LGD01 (subgenome D): these involved three duplicated loci derived from one SSR, and two loci spanning 6.8 cM in LGD01.

(3) Chr. 4 (subgenome A) and Chr. 22 (subgenome D): there are four duplicated loci derived from one SSR and two of which are on Chr. 22 spanning 3.0 cM. The pair of Chrs. 4 and 22 had also been determined as an homoeologous assemblage by classical genetic reseach (Endrizzi, Turcotte, Kohel 1985).

(4) Chr. 5 (subgenome A) to Chr. 18 (subgenome D): these are deemed homoeologous based upon nine duplicated SSR loci, spanning 137.5 cM of Chr. 5 and 187.1 cM of Chr. 18. The order of these duplicated loci is almost the same, except for a single inversion between S1404 and S247, indicating that a homoeologous chromosome region certainly existed between the two chromosomes.

(5) Chr. 9 (subgenome A) and Chr. 23 (subgenome D): these are deemed homoeologous based upon five duplicated loci spanning 51.7 cM of Chr. 9 and at least 49.8 cM of Chr. 23. The homoeologies of Chr. 9 and Chr. 23 had also been identified by the ISH method of molecular cytogenetics (Crane et al. 1994).

(6) Chr. 10 (subgenome A) and Chr. 20 (subgenome D): two linkage groups are assigned to Chr. 10 and Chr. 20 respectively, and there are two duplicated loci spanning at least 82.8 cM in Chr. 10 and 22.4 cM in Chr. 20. An additional linkage group, LGA01, has a locus duplicated in Chr. 20.

(7) Chr. 11 (subgenome A) and LGD02+LGD03 (subgenome D): there are seven loci involving three SSRs in Chr. 11 and LGD02+LGD03; the duplicated loci span at least 71.0 cM in Chr. 11.

(8) Chr. 12 and LGD04: two linkage groups are assigned to Chr. 12, one of which has two loci spanning 48.7 cM duplicated with 3 loci spanning 12.3 cM in LGD04. The morphological traits maps have determined homoeology between Chr. 12 and Chr. 26 (Endrizzi et al. 1985), but could not find any marker loci LGD04 of our map assigned to Chr. 26 using the telo-disomic stock telo26sh, however we have determined this linkage group associated to D subgenome using the diploid cotton species. Since the applied aeuploid genetic stocks are not involving the other arm of Chr. 26, we suggest that LGD04 associate to Chr. 26 in the short arm.

Additional two loci of S3492 in Chr. 12 were found duplicated in another linkage group LGD05, while locus of S1034 in LGD05 is found duplicated in Chr. 18. It indicates that there may be relationship between Chr. 12 and Chr. 18. We also find the locus S3474 in Chr. 9 duplicated with two loci spanning 8.1 cM in Chr. 26 (see Fig. 3).

Discussion

The genetic map constructed with mapping parents of high genetic polymorphism was called a species map, while the map constructed using cultivars among a given plant species was called a cultivar map. The maps presented by Shappley et al. (1996, 1998) and Zuo et al. (2000) were cultivar maps constructed with the F_2 produced by cultivar crosses in *G. hirsutum* L. And this type of map is almost impossible to cover the whole genome. The maps reported by Reinisch et al. (1994) and Brubaker et al. (1999) were constructed with the F_2 derived from the cross of undomesticated races of *G. hirsutum* and *G. barbadense*, so that they could not be applied extensively in genome research. Since cultivated *G. hirsutum* L. cotton is characterized by its high fiber production, and *G. barbadense* L. in particular is of superior fiber quality, the species map is important in researching the genome structure, organization and function, and has a

great role in identifying, detecting and mapping the genes of the critical agronomic traits, including fiber qualities. No linkage map of cultivated allotetraploid cotton has been developed. Additionally, the main molecular markers applied in the above mentioned maps were RFLPs; and no SSR maps have been reported up to now. This is the first paper in which a cultivated allotetraploid cotton molecular linkage map was constructed mainly with SSR markers.

The doubled-haploid mapping population of rice (*Oryza sativa*) was developed from the naturally doubled plants of the regenerated haploid plantlets through pollen culture (Xu et al. 1994). It was not successful to develop haploids from pollen culture because of a certain hindrance in the procedure of cotton pollen culture. Semigamy is a type of reproductive mechanism in which a sperm nucleus penetrates the egg cell but does not fuse with the egg nucleus. The egg and sperm nuclei divide independently, and result in a heterogeneous haploid embryo. Turcotte and Feaster (1963) identified a high-frequency haploid production line from the sea island elite cultivar Pima S-1. It was demonstrated that semigamy was responsible for haploid production (Turcotte and Feaster 1969). In 1969, they obtained semigamy induced haploids and successfully doubled them with colchicine, and transferred the recessive virescent gene (v_7) as a marker into the semigamous line and developed the virescently marked semigamous line (Vsg) (Turcotte and Feaster 1969). It is difficult to double the haploid plant in cotton. We successfully induced target haploids via the Vsg semigamous line and some were doubled in our mapping population of cultivated allotetraploid cotton. By grafting, we will try to produce more haploid plants used for doubling.

The DH population is a permanent mapping population, which has many advantages in genetic mapping relative to tentative populations such as the F_2. Firstly, it can be used for continuous research in the plant genome and may be shared with several laboratories. Secondly, it can be easily used to detect the QTLs for fiber production and quality in cotton, since two cultivars were used as parents. Lastly, the smaller volume of the population could be used to construct a precise linkage map relative to the 3:1 (for dominant loci) or 1:2:1 (for co-dominant loci) segregated F_2 population, because the marker locus segregation is 1:1 in the DH population (Allard 1956). Xu et al. (1994) reported rice (*O. sativa*) RFLP linkage maps using a population of 58 DH lines. The population of the previously reported species molecular map in cotton is comprised of only 57 F_2 individuals (Reinisch et al. 1994). We have compared the linkage groups of our DH mapping results with the F_2 population involving chromosome 16 of allotetraploid cotton. The linkage group constructed using 178 (TM-1 × Substitution 16) F_2 individuals (Ren et al. 2001) is almost the same in SSR marker loci order to Chromosome 16 of the present map. It indicates that our 58-line population is enough to be applied in constructing a species map of allotetraploid cotton.

SSRs are ideal PCR-based DNA markers for genetic mapping and population studies because of their abundance (Weber 1990), high level of polymorphism (Saghai Maroof et al. 1994) and wide dispersion in diverse genomes (Wang et al. 1994). The SSRs primer pairs used in this study were developed by screening the allotetraploid cotton genome library of *G. hirsutum* L. and *G. barbadense* L. elite cultivars (Reddy 1998) and by searching the GenBank. They are convenient for genetic mapping of the high level of polymorphism in allotetraploid cotton. Because the polymorphism of SSRs resulted from the variable number of simple repeat motifs, differences in the SSR PCR production are only slight, so it is difficult in detecting the polymorphisms with general agarose-gel electrophoresis. We have developed a method for fast and effective screening the polymorphisms of cotton SSRs with the PAGE/silver staining protocol (Zhang et al. 2000). It has a great advantage in detecting large numbers of markers in cotton genetic mapping.

The homoeology of chromosome pairs in the allotetraploid cotton genome have been identified by the observation that duplicated mutations in the morphological trait map involved pairs of chromosomes. According to Endrizzi et al. (1985) and Crane et al. (1994), Seven pairs of homoeologous chromosomes have been determined in the tetraploid cotton genome; namely, Chrs. 1 and 15, Chrs. 4 and 22, Chrs. 6 and 25, Chrs. 7 and 16, Chrs. 9 and 23, Chrs. 10 and 20, and Chrs. 12 and 26. The theoretical 13 pairs of homoeologous chromosomes have not been totally identified yet. Molecular genetic mapping of the cotton genome has a great advantage in determining the homoeology between chromosomes. After Reinisch et al. (1994) reported the first RFLP map of cotton, Brubaker et al. (1999) declared that they discovered all 13 homoeologous pairs of the A and D genomes of cotton by comparative mapping of allotetraploid cotton and their theoretical progenitor diploid species; and Jiang et al. (1998) showed evidence of homoeology between the A and D subgenomes in the allotetraploid cotton genome by RFLP mapping and QTL analysis for the production and quality of cotton fiber. A problem generally existing in the genetic mapping of cotton is that there is no way to associate the linkage maps to the total 26 chromosomes of cotton, and this hampers the ability to illustrate the homoeologous relationship of the cotton chromosomes as a whole. Reinisch et al. (1994) assigned only 14 chromosomes for the linkage groups on their RFLP map, and Brubaker et al. (1999) and Jiang et al. (1998) came to the same conclusion in their genetic mapping. The difficulty of chromosome assignment could be attributed to two factors; one is that the discovered monosomic and mono-telosomic stocks can not cover the total 26 chromosomes of allotetraploid cotton, the other is that the homologous loci in different chromosomes may have covered each other when we assigned them to chromosomes using monosomic or telodisomic genetic stocks. We determined 17 chromosomes associated to corresponding linkage groups in the present

map, and identified some of the homoeologous chromosomes using the duplicated marker loci.

Among the homoeologous chromosomes or linkage groups, the pairs of Chrs. 1 and 15, Chrs. 4 and 22, Chrs. 10 and 20 had already been determined as homoeologous assemblages by classical genetic and cytogenetic research (Endrizzi, Turcotte, Kohel 1985). And Chr. 9 has been identified based on the ISH method of molecular cytogenetics on homoeology to Chr. 23 (Crane et al. 1994). Based on the RFLP map developed by *G. hirsutum* L × *G. barbadense* L., Renisch et al. (1994) detected the homoeology of Chrs. 1 and 15, and Chrs. 9 and 23. Among the homoeologous chromosome pairs, they also regarded Chrs. 5 and 20 as homoeologous in the allotetraploid cotton genome. But, according to Endrizzi et al. (1985), Chr. 20 is homoeologous to Chr. 10. Our results presented here supported the Endrizzi et al. (1985) conclusion. The homoeologous pair of Chrs. 5 and 18 has not yet been reported up to now. In the present map, this pair involved nine duplicated SSR loci that was ordered in synteny, except for a single inversion between the duplicated regions in the two chromosomes, and it might be a new homoeologous assemblage of the allotetraploid cotton genome detected by molecular mapping of the genome. In the homoeologous pair of Chr. 12 and LGD04, LGD04 may be a linkage group in Chr. 26, since the homoeology between Chrs. 12 and 26 have already determined by morphological traits maps previously (Endrizzi et al. 1985).

There are still some chromosomes not identified on our map, but we found that some markers could be assigned to Chr. 6, Chr. 7 and Chr. 14; however, these markers should not be added to any linkage groups in our map because of their distorted segregation in the mapping population (data not shown). Chr. 6 is considered homoeologous with Chr. 25, and Chr. 7 is considered homeologous with Chr. 16 (Endrizzi et al. 1985); we can surely find some SSR duplicated marker loci distributed in the two pairs of chromosomes but it is pity that the loci can not be linked (data not shown). We have not discovered any marker assigned to Chr. 2, the possible reason may be that the limited markers we applied could not cover the whole genome.

Among the 624 loci for which segregation has been determined, 489 have been assembled into 43 linkage groups of more than two loci, and 135 marker loci have not yet been linked to the map. The present map spans 3,314.5 cM. Stelly (1993) suggests that the minimum overall map length of tetraploid cotton should be 4,660 cM according to the estimates of recombination in cotton based on chiasma counts, while Reinisch et al. (1994) suggest that a saturated RFLP map should span 5,125 cM at least. According to Tanksley et al. (1988), once a map of 5,125 cM reaches a density of about one marker per 5 cM, or a total of about 1,025 marker loci, there should be fewer than 1% of intervals between marker loci measuring more than 25 cM, and the map of allotetraploid cotton should "link up" into 26 linkage groups corresponding to the 26 gametic chromosomes. We are now mapping RAPD, AFLP and RFLP markers in order to construct the saturated molecular map of allotetraploid cotton, and the mapping population is being perfected by some supplemented new doubled-haploid lines. Since the mapping parents used are the cultivars of allotetraploid cotton and the markers employed are PCR-based, it is our expectation that the appearance of this molecular genetic linkage map may be possible to revolutionize the application of molecular markers to the genetic improvement in cotton and for research on genome organization and evolution.

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