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ORIGINAL PAPER

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Complete assignment of the chromosomes of *Gossypium hirsutum* L. by translocation and fluorescence in situ hybridization mapping

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Abstract Significant progress has been made in the construction of genetic maps in the tetraploid cotton *Gossypium hirsutum*. However, six linkage groups (LGs) have still not been assigned to specific chromosomes, which is a hindrance for integrated genetic map construction. In the present research, specific bacterial artificial chromosome (BAC) clones constructed in *G. hirsutum* acc. TM-1 for these six LGs were identified by screening the BAC library using linkage group-specific simple-sequence repeats markers. These BAC clones were hybridized to ten translocation heterozygotes of *G. hirsutum*. L as BAC-fluorescence in situ hybridization probes, which allowed us to assign these six LGs A01, A02, A03, D02, D03, and D08 to chromosomes 13, 8, 11, 21, 24, and 19, respectively. Therefore, the 13 homeologous chromosome pairs have been established, and we have proposed a new chromosome nomenclature for tetraploid cotton.

Introduction

Cotton is the leading natural fiber crop and an important edible oil crop in the world (Lee 1984). The cotton genus *Gossypium* is composed of about 45 diploid and 5 allotetraploid species (Fryxell 1992). Diploid *Gossypium* species fall into eight different genome types designated as A through G, and K, based on meiotic pairing behavior (Beasley 1942; Endrizzi et al. 1985; Percival et al. 1999). Only four are cultivated species: *G. hirsutum* L. [$n=2x=26$, $(AD)_1$], *G. barbadense* L. [$n=2x=26$, $(AD)_2$], *G. herbaceum* L. ($n=x=13$, A_1), and. *G. arboreum* L. ($n=x=13$, A_2). Of these, the New World allotetraploid species, *G. hirsutum* L. and *G. barbadense* L., dominate worldwide cotton production (Lee 1984). Both allotetraploids originated in the New World from interspecific hybridization between diploid species closely related to *G. herbaceum* L. (A_1) and *G. raimondii* L. (D_5) (Wendel 1989) or *G. gossypioides* (Ulbrich) Standley (D_6) (Wendel et al. 1995). *G. herbaceum* and *G. raimondii* are generally regarded as the best exemplars of the A and D-subgenome progenitors (Endrizzi et al. 1985; Wendel et al. 1995; Zhao et al. 1998), respectively.

In cotton, chromosomes are numbered in the order of discovery based on some distinguishing cytological feature associated with a particular chromosome (Kohel 1973). Numbers 1 through 13 are reserved for the A subgenome and numbers 14 through 26 are reserved for the D subgenome. Kohel (1973) suggested that when chromosome homology has been established, the A subgenome chromosomes should be identified as A1 through A13, and the homeologous D subgenome chromosomes should be designated as D1 through D13 in the cotton genetic nomenclature.

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. In cotton, significant progress has been made in the development of genetic maps (Reinisch et al. 1994; Rong et al. 2004; Nguyen et al. 2004; Han et al. 2006). In the meantime, other genetic maps have been developed from intraspecific or interspecific populations (Shappley et al. 1998; Brubaker et al. 1999; Zhang et al. 2002; Mei et al. 2004; Ulloa and Meredith 2000; Ulloa et al. 2002; Song et al. 2005). The DNA markers currently available in cotton are mainly based

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on restriction fragment length polymorphisms, random amplified polymorphic DNAs, amplified fragment length polymorphisms, simple-sequence repeats (SSR), and sequence tagged sites. A new kind of DNA marker, sequence-related amplified polymorphism, has also been used in the construction of the maps (Li and Quiros 2001; Han et al. 2006).

Previously, LGs were assigned to chromosomes mainly using monosomic and mono-telosomic stocks of cotton (Stelly 1993; Reinisch et al. 1994). However, due to the lack of a similar complete set of aneuploid stocks in cotton, there remained six LGs that were not associated with specific chromosomes. These LGs were assigned to the A subgenome (A01, A02, and A03) or the D subgenome (D02, D03, and D08) by analysis of marker loci in two progenitor diploid species (*G. herbaceum* and *G. raimondii*) of the allotetraploid cotton (Reinisch et al. 1994).

Because aneuploid stocks are presently unavailable, other methods were developed to achieve this goal. In cotton, 25 of the 26 chromosomes of *G. hirsutum* have been distinguished and numbered based on a set of 62 homozygous translocation lines (Brown 1980). Chromosome 26 has not been involved in a known translocation and is identified through process of elimination, although it is now marked by a monotelodisome (Endrizzi et al. 1984). The translocation stocks constitute the only complete set of cytogenetic markers for the *G. hirsutum* genome. Meiotic fluorescence in situ hybridization (FISH) methods, using these translocation lines, have been used to physically map rDNA sites to specific chromosomes or chromosome arms (Hanson et al. 1995; Ji et al. 1999). Therefore, meiotic FISH and translocation lines can be used to locate DNA markers, which represent the LGs, on the exact chromosomes. Although most DNA markers are too small to be used directly as a probe in FISH, FISH using genomic DNA clones provides an alternative method to map small DNA probes. A large genomic DNA clone could be isolated by screening a genomic DNA library using the small DNA probes selected as the linkage group-specific marker. This large genomic DNA clone used as the linkage group-specific marker could be mapped using FISH. Based on a PCR-based genetic map in tetraploid cotton constructed in our laboratory, we used an SSR marker-based BAC-FISH strategy and assigned the six LG A01, A02, A03, D02, D03, and D08 to chromosomes (chr.) 13, 8, 11, 21, 24, and 19, respectively.

Materials and methods

Selection of SSR markers and isolation of linkage group-specific BAC clones

The BAC clones used in this study all came from a TM-1 BAC library constructed at USDA-ARS, Crops Germplasm Research Unit, Texas, USA. SSR markers on the eight LGs (A01, A02, A03, D02, D03, D08, and chr. 3 and 6) from three maps (Song et al. 2005; Han et al. 2004; Lacape et al. 2003) were used to screen the library. To ensure that the SSR markers were truly located on the specific LG, two factors were considered. One was that all markers must be mapped on the same LG of the three maps, and secondly, that these markers must represent a single locus on the maps. The selected markers were used to screen the library based on a PCR library screening approach (Wang et al. 2005), and positive individual clones were re-identified by corresponding markers by PCR. Positive BAC clones were then used as FISH probes to hybridize to cotton chromosomes in both mitotic and meiotic cells. Only BAC clones that consistently produced a single strong and unambiguous signal, meaning that they clearly represented the corresponding LGs, were selected as linkage group-specific BAC clones (Fig. 1). Markers and clones on two LGs that were previously assigned to chr. 3 and 6 were used to confirm the reliability of the screening strategy.

Translocation heterozygotes and chromosome preparation

One set of homozygous translocation lines in *G. hirsutum* were kindly made available by USDA-ARS, Crop Germplasm Research Unit/Texas A&M University, in 1983. Ten reciprocal translocation lines involving eight chromosomes (3, 6, 8, 11, 13, 19, 21, and 24) in *G. hirsutum* L. were used to associate our unassigned LGs to chromosomes. These lines were crossed with TM-1 at Nanjing Agricultural University (NAU) to produce the translocation heterozygotes (NTs) (Table 1). Meiotic chromosome spreads were prepared as described by Crane et al. (1993) with several modifications. Upon removal of the calyx and corolla, floral buds from the NTs were fixed in ethanol–acetic acid (3:1) fixative for 2–24 h at 4°C. Next, the buds were screened for metaphase I, and several anthers from selected bud were placed on an ethanol-washed glass slide with a drop of 45% acetic acid (v/v), freed of debris, and squashed. Mitotic chromosomes were prepared as described by Hanson et al. (1995) and Wang et al. (1999). Cotton TM-1 root tips were harvested from germinated seeds, pretreated with 25 μg/ml cycloheximide at room temperature for 2 h to accumulate metaphase cells, and fixed in methanol–acetic acid (3:1) fixative. Root tips were macerated in 2% cellulose and 0.5% pectolyase at 37°C for 1.5 h and squashed with 45% acetic acid. All slides were stored at −70°C. After removing the coverlips, slides were dehydrated through an ethanol series (70, 90, and 100%; 5 min each) prior to use in FISH.

Fluorescence in situ hybridization

BAC-DNA was isolated using an alkaline-lysis method (Sambrook et al. 1989), and labeled with

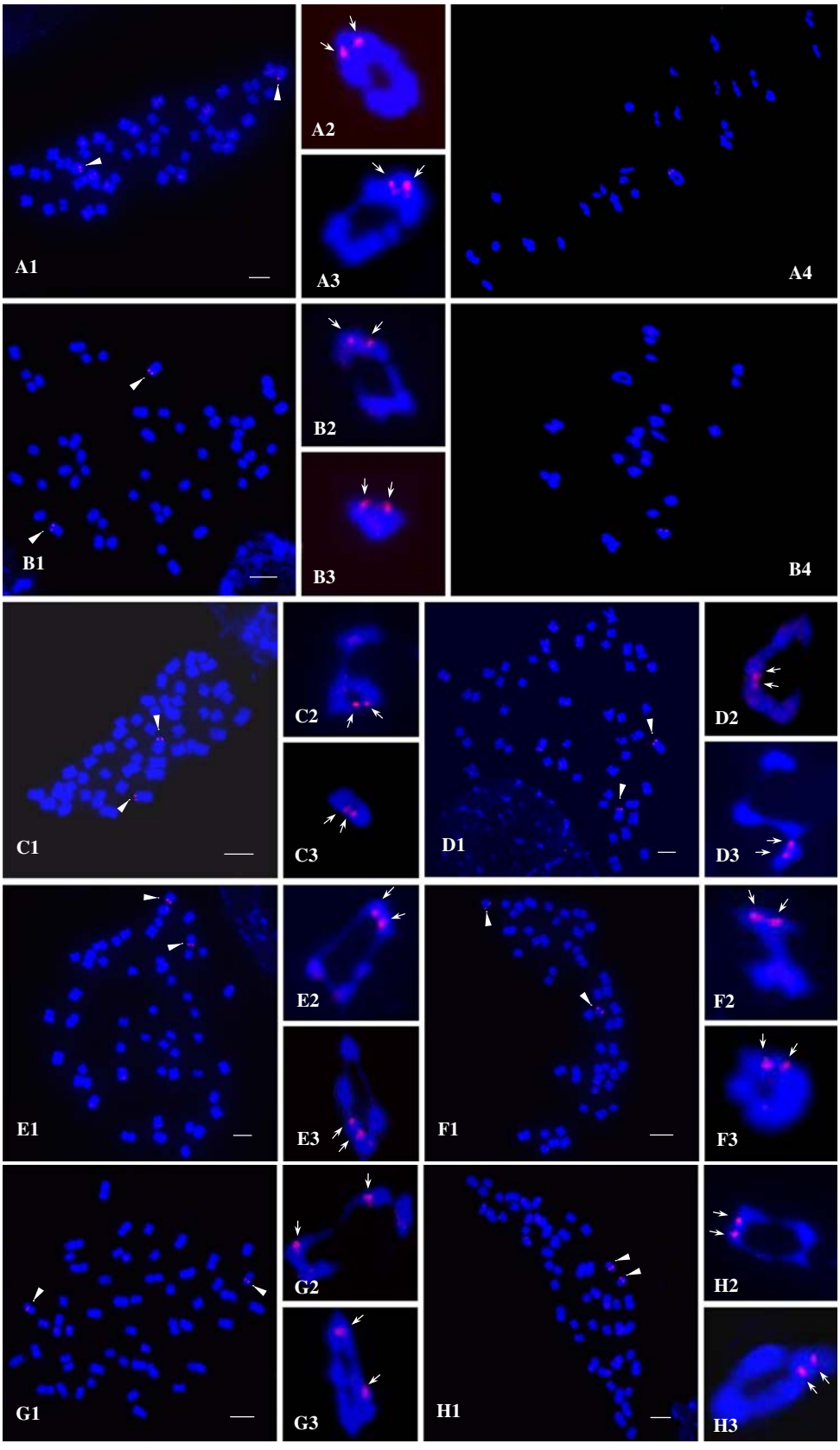

Fig. 1 Eight chromosome-specific BAC clones of cotton identification and chromosomal and subchromosomal location by FISHing to *Gossypium hirsutum* NTs. A1 to H1 show the hybridization of BAC probes (Table 1) to eight chromosomes in mitotic cells to identify chromosome-specific BAC clones, respectively: (A1) BAC clone 104O10, (B1) 62K03, (C1) 98H10, (D1) 35J07, (E1) 14G14, (F1) 59B08, (G1) 37F17 and (H1) 50D03. Arrowheads point the dual chromosome-specific FISH signals (*red*). All *bars* are 5 μm. A2, A3 to H2, H3 show the chromosomal and subchromosomal location of FISH sites. *Arrows* indicate the dual FISH signals (*red*). All the figures of IVs and bivalents were derived from the integrated metaphase cell images. For example, A4 and B4 show the integrated cell images of A2 and B3, respectively

digoxygenin-11-dUTP (Roche Diagnostics, Mannheim, Germany) by standard nick translation reactions. To block the hybridization of repetitive DNA in the BAC probes, cotton Cot-1 DNA was used in the hybridization mixture. Cotton genomic DNA was isolated from TM-1 and the Cot-1 fraction of the genomic DNA was

Table 1 BAC clones, SSR markers and translocation heterozygous (NTs) used in this study, and their chromosomal and subchromosomal assignment of LGs

LGs	SSR markers	BAC clones	Signal sites location	Translocation lines	Corresponding figures in Fig. 1
A01	BNL1495	98H10	13L[a]	T13R-19R	C2
				T4L-19R	C3
A02	BNL3627	35J07	8	T1L-8L	D2
				T8R-19R	D3
A03	BNL4094	14G14	11sh[b]	T11R-12L	E2
				T11L-15L	E3
D02	NAU694	59B08	21L[a]	T7R-21R	F2
				T20R-21L	F3
D03	BNL2655	37F17	24	T14R-24R	G2
				T19R-24R	G3
D08	BNL0390	50D03	19	T4L-19R	H2
				T8R-19R	H3
Chr. 3	BNL3441	104O10	3L[a]	T2R-3L	A2
				T3L-6L	A3
Chr. 6	BNL1064	62K03	6	T3L-6L	B2
				T2R-3L	B3

[a]Left arm of the chromosome
[b]Short arm of the chromosome

prepared according to Zwick et al. (1997). FISH was performed using a combination of the protocols of Hanson et al. (1995) and Jiang et al. (1995) with significant modifications. Slides were immersed in 2× SSC containing 100 μg/ml RNase A at 37°C for 1 h and washed twice with 2× SSC (37°C, 10 min each wash). Chromosomal DNA was denatured by placing slides in 50 ml of 70% formamide in 2× SSC at 78°C for 1 min and immediately dehydrated in an ethanol series at −20°C and air-dried. Fifteen microliters of a mixture containing 25 ng labeled BAC-DNA, 10% w/v dextran sulfate, 10 μg sheared salmon sperm DNA, an appropriate amount of cotton Cot-1 DNA (see Discussion), and 1.5 μl 20× SSC was denatured at 97°C for 10 min, chilled on ice, reannealed at 37°C for 1 h, and applied to a dried slide. After overnight incubation at 37°C and washing at 42°C in 2× SSC, probes were detected with 20 μg/ml rhodamine-conjugated anti-digoxygenin antibody (Roche Diagnostics). Slides were stained in 4′, 6-diamidino-2-phenylindole (Roche Diagnostics) for 10 min at room temperature, and finally, antifade (Vector, USA) was applied under a coverslip. Slides were examined under an Olympus BX51 fluorescence microscope. Chromosome and FISH signal images were captured using an Evolution VF CCD camera (Media Cybernetics, USA) and merged using Image-Pro Express software.

Assignment of linkage groups by BAC-FISH

The LG assignment strategy is derived from the translocation-based strategy for mapping meiotic FISH loci to chromosomes according to Price et al. (1990) and Crane et al. (1993). Here, the linkage group-specific BAC clones were first developed to represent the corresponding LGs. Once the BAC clones used as FISH probes were located on specific chromosomes, the LGs were assigned. As described in Crane et al. (1993), unrelated NTs are screened until one is found with the FISH signal site on its quadrivalent (IV). Each of the two involved chromosomes is then examined in other translocations to determine which of them bears the probed locus. For example, one linkage group-specific BAC clone was probed and FISHed to all the NTs. If FISH signals occurred on the NT1-2's IVs (formed by chr. 1 and 2), this indicates that the BAC clone is located on either chr. 1 or 2. If the signals are not found on the IVs with a related NT having one chromosome in common with NT1-2, e.g., NT2-3, but can be found on the normal bivalents, this indicates that the BAC clone is located on chr. 1. However, the signals re-occurring on the IVs of NT2-3 indicate that the BAC clone is located on chr. 2. Linkage group-specific BAC clones of chr. 3 and 6 were selected and FISHed to two NTs involving chr.3 and 6 to test this system (see Results).

The positions of BAC-FISH signals on IVs of NTs at metaphase I were also used for subchromosomal localization (left and right arm) relative to translocation breakpoints (Brown et al. 1981; Menzel et al. 1985). The strategy for mapping meiotic FISH loci to left or right arms of chromosomes in this study is as described in Price et al. (1990) and Crane et al. (1993).

Results

Selection of SSR markers and isolation of linkage group-specific BAC clones

Fifteen SSR markers mapped on the same LG available in three cotton genetic maps (Song et al. 2005; Han et al.

2004; Lacape et al. 2003) were selected to screen the BAC library. An additional five markers of LGA02 and D02 not mapped to the map in Lacape et al. (2003) were also selected because there were only a few common markers, and they produce distinct single-copy bands when screening the library by PCR. Of these, 16 positive BAC clones screened by 16 SSR markers were used as FISH probes to hybridize to TM-1 chromosomes in both mitotic and meiotic cells. Finally, eight SSR markers whose BAC probes generated only one clear signal in mitotic and meiotic FISH (Fig. 1) were selected, among which six markers came from all the three maps. The markers BNL3627and NAU694 not mapped on the map in Lacape et al. (2003) were also selected because other markers mapped on the three maps were not associated with positive clones or the positive clone probes did not generate a single distinct FISH signal. The eight BAC clones that consistently yielded strong and unambiguous signals were selected as linkage group-specific BAC clones (Table 1) to be further used in meiotic FISH.

Interestingly, all eight selected SSR markers mapped to relatively high recombination regions. All BAC clones screened using these markers also yielded a single distinct hybridization signal site. Two BAC clones screened by JESPR-308 and BNL3452 that were mapped to the low recombination regions of LGD03 and D08, respectively, were also labeled and FISHed. These produced dispersed signals over all the chromosomes, but when a large amount of blocking DNA (100-200-fold Cot-1 DNA) was used, several weak signals could be detected (data not shown). Additionally, we found that all selected BAC probes except 14G14 could generate distinctive FISH signals without a Cot-1 pre-annealing procedure, suggesting that there was little dispersed repetitive DNA within these BAC clones. The BAC clone 14G14 could also generate one unambiguous signal when little blocking DNA (25-fold Cot-1 DNA) was used. In sorghum [*Sorghum bicolor* (L.) Moench], Kim et al. (2002) selected markers in apparently high recombination regions of LGs to increase the likelihood of finding BAC clones with relatively low repetitive sequence content. Therefore, the strategy could also be adapted to cotton (*G. hirsutum* L.).

Identification of the chromosomes for six LGs by BAC-FISH

Identification of chr. 3 and 6 by BAC-FISH

Two chromosome-specific BAC clones, 104O10, and 62K03, identified through screening the TM-1 BAC library by SSR marker BNL3441 for chr. 3 and BNL1064 for chr. 6, respectively, were selected and FISHed to the same two NTs, NT2R-3L, and NT3L-6L. For BAC clone 104O10 for chr.3, FISH signals were found on the IVs of both NT2R-3L (Fig. 1A2) and NT3L-6L (Fig. 1A3). Because the clone was chromosome-specific, this information was sufficient to map it to chr. 3 since there was only one common chromosome between these two independent translocations. For BAC clone 62K03 for chr. 6, dual FISH signals were detected only on the IVs of NT3L-6L (Fig. 1B2), and not on the IVs of NT2R-3L (Fig. 1B3), but were detected on one normal bivalent. We deduced that the SSR-based BAC clone 62K03 was on chr. 6. Therefore, our BAC-FISH assignment procedure is reliable for cotton chromosome identification.

LGA01 is assigned to chr. 13

Using the same strategy, ten NTs involving chr. 8, 11, 13, 19, 21, and 24, which were not previously correlated with any LGs in cotton genetic maps (Lacape et. al. 2003; Song et. al. 2005; Rong et al. 2004) and six linkage group-specific BAC clones (Table 1) were used to assign the six LGs to corresponding chromosomes. The linkage group-specific BAC clone 98H10 for LGA01 identified by the SSR marker BNL1495 (Table 1) was hybridized to NT13R-19R and NT11R-12L. FISH signals occurred on the IVs of NT13R-19R (Fig. 1C2) and on the bivalents of NT11R-12L. From these results, we can deduce that the LG may be chr. 13, since the LGA01 is in the A-genome LG. NT4L-19R was further used as a probe for FISH. As expected, all dual FISH signals were detected on one bivalent of NT4L-19R (Fig. 1C3), so LGA01 was assigned to chr. 13.

LGA02 is assigned to chr. 8

When the BAC 35J07 for LGA02 was hybridized to NT8R-19R and NT11R-12L, signals occurred on the IVs of NT8R-19R (Fig. 1D3) and the bivalents of NT11R-12L, indicating that chr. 8 was a possibility. When NT1L-8L was further hybridized to 35J07, signals were found only on its IVs (Fig. 1D2), so LGA02 was assigned to chr. 8.

LGA03 is assigned to chr. 11

Because two above LGs of the A subgenome were assigned, it was likely that LGA03 would be chr. 11. To test this, the BAC clone 14G14 for LGA03 was directly FISHed to NT11R-12L and NT11L-15L. One pair of signals occurred only on the IVs of these two NTs (Fig. 1E2, E3), respectively. Therefore, the presumed assignment of LGA03 was correct.

This result was also confirmed by monotelodisomic stock analysis. The aneuploid hybrids crossed between the monotelodisomic lines of chr.11 in the TM-1 (*G. hirsutum*) genetic background as the female parent and 3–79 (*G. barbadense*) was used in this analysis. As the monotelosomic stock has a single chr. 11 short arm of *G. barbadense* with a co-dominant molecular marker

locus, if one marker genotype is similar to that of *G. barbadense* and the counterpart marker allele is not observed, the involved marker loci would be assigned to the short chromosome arm. Among the six selected SSR markers from LGA03, BNL1231 (data not shown), NAU539 (data not shown), and NAU1014 (Fig. 2b) generated a hybrid genotype (Fig. 2b), and BNL3442 (data not shown), NAU2118 (Fig. 2a), and BNL4094 (data not shown) generated only the 3–79 genotype (Fig. 2a). This implies that the latter three SSR markers are located on the short arm of chr. 11.

LGD02 is assigned to chr. 21

For the three D-subgenome LGs, BAC clone 59B08 for LGD02 was first hybridized to NT14R-24R and NT8R-19R, but signals were only detected on the bivalents of these two NTs rather than on their IVs. This suggested that the LGD02 might be chr. 21 rather than chr. 14, 19, or 24. To test this, 59B08 was further hybridized to NT7R-21R and NT20R-21L. As expected, signals occurred only on the IVs for these two NTs (Fig. 1F2, F3). Therefore, LGD02 was assigned to chr. 21.

LGD03 is assigned to chr. 24

NT14R-24R and NT8R-19R were used again in FISH with BAC clone 37F17 as a probe for LGD03. Signals were found on the IVs of NT14R-24R (Fig. 1G2) and bivalents of NT8R-19R, indicating that it might be located on chr. 24. The same probe was further hybridized to NT19R-24R and signals were found only on the IVs (Fig. 1G3), so LGD03 was assigned to chr. 24.

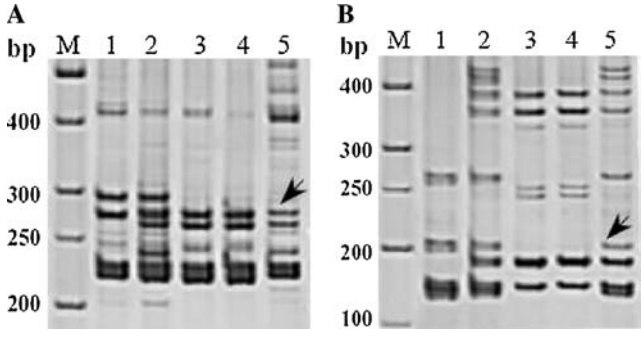

Fig. 2 Simple-sequence repeats profile of primer NAU2118 (**a**) and primer NAU1014 (**b**) in the aneuploid chromosome assignment system. In both **a** and **b**, Lane 1–5 were TM-1, F_1(TM-1 × Hai7124), *Gossypium barbadense* cv. Hai7124, 3–79 and (Te11Lo × 3–79)F_1 monotelodisomic plants, respectively. *M* DNA size marker. *Arrows* point the different patterns of Te11Lo hybrids according to primer NAU2118 and NAU1014. From the monotelodisomic test, we can conclude that $NAU2118_{275}$ was mapped on the short arm of chr. 11 (**a**), and $NAU1014_{200}$ was not on the short arm of chr. 11 (**b**), may be on the long arm on the chromosome

LGD08 is assigned to chr. 19

As the assignment of LGD08 could theoretically be the same as for LGA03, BAC clone 50D03 for LGD08 was hybridized to two NTs involving chr. 19, NT4L-19R (Fig. 1H2), and NT8R-19R (Fig. 1H3). Dual FISH signals were all located on both IVs. As a result, LGD08 was assigned to chr. 19.

These six LGs unassigned to specific chromosomes have been associated with the A or D subgenome by analyzing marker loci in the representative A and D diploid species, i.e., *G. herbaceum* L. and *G. raimondii* in several cotton maps (Lacape et. al. 2003; Rong et al. 2004; Song et al. 2005). Our meiotic BAC-FISH results are consistent with the conclusion that LGA01, A02, and A03 are all assigned to the A subgenome (chr. 13, 8, and 11) and LGD02, D03, and D08 are assigned to D subgenome (chr. 21, 24, and 19). Additionally, it is well known that the A subgenome chromosomes (1–13) are larger than the D subgenome chromosomes (14–26) in cotton. As shown in Fig. 1 A1–H1, the five A subgenome chr. 3, 6, 8, 11, and 13 are clearly larger than the three D subgenome chr. 19, 21, and 24, which also confirmed our results.

Subchromosomal localization of hybridization sites

According to Price et al. (1990) and Crane et al. (1993), three FISH signal sites were located on the corresponding chromosome arms (left and right) (Table 1). As shown in Fig. 1, a pair of FISH signals was located on both sides of NT2R-3L (A2) ring IV, indicating that the signals were on the same arm with the breakpoint. This implied that the probe was on the left arm of chr. 3. A pair of FISH signals was located on the exterior of NT13R-19R (Fig. 1C2) frying-pan IV (also a megaphone IV, which the small ring opened), indicating that the signal site was opposite to the breakpoint, i.e. on the left arm of chr. 13. A pair of FISH signals was found opposite to the breakpoint of NT7R-21R (Fig. 1F2) on the barbell IV, indicating that the signals were on the left arm of chr. 21. The ring IVs and normal bivalents could not give us exact information on the location of the sites. To obtain unambiguous results of FISH signals mapping, only the easily distinguishable IVs configurations such as the ring were selected. The megaphone and barbell IVs that are not easily recognized but indispensable in determining subchromosomal location were almost not selected. Therefore, other FISH signals were not mapped to chromosomal arms.

Discussion

Price et al. (1990) and Crane et al. (1993) described the principles of single FISH site locations and mapped rDNA loci in *G. hirsutum*. Therefore, screening linkage group-specific BAC clones that may lack repetitive-

Table 2 Chromosome identification in tetraploid cotton

Proposed assignment of A-genome chromosome	Former assignment of A-genome chromosome	Proposed assignment of D-genome chromosome	Former assignment of D-genome chromosome
A1	Chr. 1	D1	Chr. 15
A2	Chr. 2	D2	Chr. 14
A3	Chr. 3	D3	Chr. 17
A4	Chr. 4	D4	Chr. 22
A5	Chr. 5	D5	LGD08/Chr. 19
A6	Chr. 6	D6	Chr. 25
A7	Chr. 7	D7	Chr. 16
A8	LGA02/Chr. 8	D8	LGD03/Chr. 24
A9	Chr.9	D9	Chr.23
A10	Chr.10	D10	Chr.20
A11	LGA03/Chr.11	D11	LGD02/Chr.21
A12	Chr.12	D12	Chr.26
A13	LGA01/Chr.13	D13	Chr.18

sequences and generate one site signal is critical to the success of BAC–FISH. Both Hanson et al. (1995) and Zwick et al. (1997) used cotton genomic and Cot-1 DNA Southern hybridization with BAC-DNA to pick BAC clones with few or no repetitive sequences. In sorghum, Kim et al. (2002) selected markers that mapped to regions of apparently high recombination near the ends of LGs to increase the likelihood of finding BAC clones with relatively low repetitive sequence content and relatively high gene content, or unique sequence content. In this study, we found that all eight SSR markers mapped to relatively high recombination regions. This suggests that the strategy may be also adapted to cotton (*G. hirsutum* L.), an allotetraploid species that contains approximately 40% repetitive sequences (Baker et al. 1995). This will also facilitate the application of BAC-FISH in cotton, though the location of all markers on the LG is not near the ends on these maps.

Chromosome-specific FISH markers are effective tools for chromosome identification, analysis of genetic stocks, and physical mapping. In some plant species, a set of BAC clones were isolated and used as chromosome-specific cytogenetic FISH markers for chromosome discrimination and physical mapping (Dong et al. 2000; Cheng et al. 2001; Kim et al. 2002). For cotton, because of the large number and small size of the chromosomes, as well as the absence of suitable cytogenetic markers like bands, routine and unambiguous identification of individual chromosomes based on their morphology is almost impossible. Therefore, the cytological identification of the individual chromosome has been hitherto limited. FISH signals derived from chromosome-specific BAC clones can be used as reliable cytological markers for chromosome identification in cotton or other species with a large number of chromosomes and small chromosomes. Here, we developed eight chromosome-specific BAC clones of cotton, which are excellent cytological markers for chromosome identification in both meiotic and mitotic cells (Fig. 1). Especially in mitotic cells, clear signals were detected in more than 90% of all the metaphase cells, so well-spread metaphase cells that are difficult to obtain are not always necessary. The development of chromosome specific-BAC clones for the remaining cotton chromosomes is underway in our laboratory, which will provide an accurate tool to discriminate between individual cotton chromosomes and promote the development of cotton cytogenetics.

Kohel (1973) established a genetic nomenclature for cotton and proposed that when chromosome homology was established, the A subgenome chromosomes would be identified as A1 through A13, and the homeologous D subgenome chromosomes would be designated D1 through D13. Since all chromosomes were identified in the present research, a new homeologous chromosomes-based nomenclature of tetraploid cotton was proposed. The A subgenome chromosomes were assigned as A1 through A13, the same as the former assignment, but the D subgenome chromosomes were reassigned as D1 through D13 to comply with the order of the A homology (Table 2). For example, chr. 15 was renamed D1 because chr. A1 and 15 are one pair of homeologous chromosomes. This preserved the nomenclature of the A subgenome chromosomes and mainly reordered the D subgenome chromosomes, clearly identifying the homeologous chromosome pairs. This will facilitate chromosome identification, ease communication between research groups, and facilitate data usage across genome maps.

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