FISH-based karyotype of *Gossypium herbaceum* generated with 45S rDNA and gDNA of *Gossypium raimondii* as probes

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Abstract

The formula for FISH-based karyotype of *Gossypium herbaceum* was as 2n = 2x = 26 = 16m + 10sm (6 sat). The ratio between the largest and the smallest chromosomes was 1.63. Three satellite loci were mapped on short arms of the last chromosomes. The two on chromosome 12 and 13 were clearly visualized in both DAPI images and rhodamine/DAPI images and the other one on chromosome 11 was not easily detected in DAPI images.Three NORs were observed and just located following the satellite sites, with one on telomere and two near centromeres. When gDNA from *G. raimondii* was used as probe, GISH-NORs were scored in mitotic chromosomes of *G. herbaceum* with the same numbers, locations and sizes as 45S rDNA NORs. It should be therefore concluded that FISH-based karyotype leaded the analyses more detail than previous karyotype (non-FISH karyotype). Based on this study in conjunction with our other FISH results, there might be great amplifications or pericentric inversions of rDNA in modern A genome species after its contribution to allotetraploid originations, or deamplifications/deletions of tandem repeats like rDNA in extant allotetraploids following their polyploidization. An explanation to D genome specific GISH-NORs is that rDNA contents in D genome species may be much more than those in A genome species. The NORs or GISH-NORs herein may facilitate future locus-specific studies on rRNA gene evolution and function, and also may be useful in developing physical map specific to chromosome order in *Gossypium*.

Media summury

FISH technology is very powerful also to plant karyotype analysis. Here the FISH-based karyotype of *Gossypium herbaceum* should be the standard with its excellent images.

Keywords

Cotton, Chromosome, FISH-based karyotype, NOR, GISH-NOR, rRNA

Introduction

Molecular researches on *Gossypium* developed very fast including comparisons of cotton cp genome to other plants (Ibrahim et al, 2006). FISH (fluorescent in situ hybridization) is an integrated tool on cytogenetic and molecular researches, which become more and more powerful in modern biology sciences (Jiang et al. 2006,

1994). *G. herbaceum* (A_1) is one of the two earliest cultivated diploids (2n=2x=26). However it is still interested because of its germplasm importance relatively easily to improve varieties of *G. hirsutum*, a allotetraploid (2n=4x=52, A_1D_1) and the world leading cotton crop nowadays, or its special role in understanding speciation history of allotetraploids in *Gossypium* possibly as the closest donor to A-sub-genome chromosome of them (Endrizzi et al. 1985; Wendel 2000; Wendel et al. 2003). On *G. herbaceum* karyotype, several papers provided data with photographs (Ruzhi and Maoxue. 1985; Ping et al. 1991; Kunbo et al, 1995) and consistently mapped two pairs of satellites on last pairs of chromosome (11, 12 or 13). The object of this study was to obtain more detail information from FISH-based karyotype of *G. herbaceum*, mainly focusing on physical mapping of satellite, NOR, and even GISH-NOR.

Materials and Methods

Plant material, pretreatment and metaphase preparation. Hongxin Caomian, a variety of *G. herbaceum* developed in China, was used in this study. Chromosome spreads were prepared as described by Kunbo (1994) and Chunying et al (1999). Slides were frozen over liquid nitrogen before use. A line of *G. raimondii* (D_5D_5 or D_5 in brief) was used as probe DNA.

Plasmid DNA of JDH 2-15A (a 8.2 kb XhoI fragment of 45S rDNA which contains almost a full *Arabidopsis* rDNA repeat, kindly provided by Drs Dai Silan and Dr. Wu Rui in Cornell University, USA) was isolated by Chunying et al (1999). Total genomic DNA (gDNA) was isolated by Guoli et al (1998). Whole plasmid and total genomic DNAs were labeled on the DIG-High-Prime System. The procedure of *In situ hybridization* was described by Chunying et al (1999). For karyotype calculations, all images were used to detect hybridized signals (mainly nucleolar organizer region, NOR, probed with 45S rDNA, or GISH-NOR probed with gDNA of *G. raimondii*) but only metaphase images with better morphology of chromosomes, especially with clear centromere and better spreads of chromosomes were used in calculations for karyotype. The procedure for this calculation was described by Kunbo et al (1994, 2001).

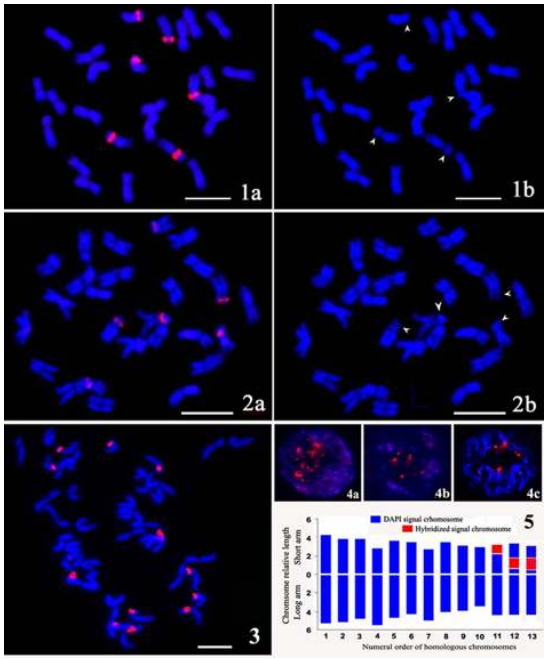
Results

Total 39 FISH images of *G. herbaceum* were recorded with 22 probed from 45S rDNA and 17 probed from gDNA of *G. raimondii*. Within 16 metaphase images, 10 and 6 probed from rDNA and gDNA, respectively, were applied into karyotype calculations. The typical FISH images for karyotype analysis were documented in Figs. 1a, 1b, 2a and 2b. The results of relative length for satellite chromosome, short arm, long arm and whole chromosome, and arm ratio and type for each pair of homologous chromosomes were given in Table 1(omitted). The total or average data of these parameters, ratio of the largest to smallest chromosomes, numeral orders of satellites and karyotype formula for whole karyotype were shown in Table 2. The karyotype formula for the species was as following:

$$2n = 2x = 26 = 16m + 10sm\ (6\ sat).$$

On 45S rDNA probed FISH, we obtained total 22 images, including18 metaphase and 4 interphase cells, respectively. 6 rDNA loci were observed consistently in 15 of the 18 metaphases, accounting for 83.33% (Fig. 1a). In other 3 metaphases signal numbers differed from four to five (no less four. Table 3). Four interphase cells of *G. herbaceum* chromosomes hybridized with 45S rDNA were examined to cluster the signals mainly within nucleoli (Figs. 4a), demonstrating the correspondence of 45S rDNA within nucleolus (nucleolar organizer regions, NORs) in nucleus. On *G. raimondii* (D_5) gDNA probed FISHs, six (or three pairs of) hybridized signals were clearly visible in mitotic FISHs of *G. herbaceum*, with the locations corresponding also to the satellites (Figs. 2a and 2b), and were therefore defined as GISH-NOR, which was an interesting

phenomenon and not reported before in papers concerning cottons, or even other plants (Sanhong et al. 2005). Within 12 enumerable FISH images probed with the gDNA, 11 illustrated six GISH-NORs and only 1 yielded GISH-NORs less than six accounting for 7.69% (Table 3). Fig. 3 was an anaphase spread with 12 GISH-NOR loci and represented a division status of sister chromatids with 6 signals in each set. Figs. 4b and 4c presented the FISH images indicating the clustering of the signals within nucleoli. Besides, the simultaneity between GISH-NORs and NORs also existed in the sizes and distributions on arms and homologs. Fig. 5 was a standard karyogram based on conjunct data from both the rDNA and the gDNA probed FISHs.



Figs. 1a and 1b. FISH images of rhodamine/DAPI(1a) and DAPI(1b) from metaphase chromosomes of *G. herbaceum* hybridized with 45S rDNA as probe, with six red hybridized signals(1a) and white triangles pointing 4 visible satellites (1b).

Figs. 2a and 2b. FISH images of rhodamine/DAPI(1a) and DAPI(1b) from metaphase chromosomes of *G. herbaceum* hybridized with *G. raimondii* gDNA as probe, with six red hybridized signals(2a) and white triangles pointing 4 visible satellites (2b).

Fig. 3. Rhodamine/DAPI image of the anaphase chromosomes of *G. herbaceum* hybridized with gDNA of *G. raimondii* as probe, showing 12 red GISH-NORs.

Figs. 4a, 4b and 4c. Rhodamine/DAPI images of the interphase chromosomes of *G. herbaceum* hybridized with 45S rDNA(4a) and gDNA of *G. raimondii* as probes(4b, 4c), showing red hybridized signals clustered in nucleolus(4a, 4b and 4c) and 6 red distinguishable GISH-NORs(4b).

Fig. 5. A standard FISH-based karyogram of *G. herbaceum*, showing physical mapping to whole chromosomes of the species and positions of NORs or GISH-NORs with red color.

All FISH images were blocked by Salmon perm DNA. Bars = 10μm

Table 2. Major characteristics of FISH-based karyotype of *G. herbaceum*

	Relative length				Arm ratio	LR	NOSC	Karyotype formula
	Satellite	Short arm	Long arm	Whole				
Total	3.51	38.65	57.84	96.49		1.63	11, 12, 13	2n = 26 = 16m + 10sm(6 sat)
Average	1.17	2.97	4.45	7.42	1.61			

Note: Satellites were not included in lengths of whole chromosomes. LR: Length ratio of the largest to smallest chromosome. NOSC: Numeral order of satellite chromosome.

Table 3. NORs (GISH-NORs) detected in mitotic FISHs of *G. herbaceum* blocked with salmon sperm DNA

Probe DNA	Cells of metaphase NOR				Cells of interphase NOR		Cells of anaphase NOR (12 major)	Total
	6 major	5 major + 1 intermediate	4 major + 2 intermediate	less 6 NORs	Signals clustered in nucleolus	6 NORs gathered in nucleolus		
45S rDNA	13		2	3	4			22
D_5 gDNA	8	2	1	1	3	1	1	17
Total	21	2	3	4	7	1	1	39

Discussions

One pair of satellites, mapped in this FISH-based karyotype on chromosome 11, being visibly detected in rhodamine/DAPI images but not in DAPI images, were not provided by previous non-FISH karyotypes, which may demonstrate that it was FISH technology with sensitivity greater enough by which to let the satellite(s) be distinguished more efficiently. It therefore should be reasonable that FISH-based karyotype leaded the analyses more powerful (Jiang et al, 2006; 1994).

From our FISH results, it is most likely that there might be many possibly potential mechanisms, such as great amplifications or pericentric inversions in modern A genome species after its contribution to the tetraploid originations, and as deamplifications or deletions of tandem repeats like rDNA in extant allotetraploids following their polyploidization (Wendel, 2000; Wendel et al. 2003).

The number and position of GISH-NORs in *G. herbaceum* when probed by *G. raimondii* gDNA were the same as those of 45S rDNA NOR but, in another study (Sanhong et al. 2005), there was not any GISH-NOR signal in mitotic FISH of *G. herbaceum var. africanum* ($A_{1\text{-}a}A_{1\text{-}a}$, a wild form of *G. herbaceum*) probed by its own gDNA. Zhao et al (1998) showed $A_{1\text{-}a}$ mitotic chromosomes were covered well by a large number of dispersed repetitive sequences while using as FISH probes. An explanation to the D genome GISH-NOR may be rDNA content in genome D more than in genome A. Wendel et al (1995) argued that rDNA arrays were homogeneous in all allopolyploids and diploids and rDNAs of *G. hirsutum* were homogenized into D genome repeat type. Therefore, comparing to A genome species, gDNAs from D genome species may have advantages to hybridize to whole rDNA sequences including ITS1 and ITS2 of *Gossypium* species whenever were used as probes or blocks in cotton GISH studies. So the gDNAs from D genome species may easily probe GISH-NORs.

Here in this study the 45S rDNA NORs or GISH-NORs were mapped on short arms of the last three pairs of mitotic chromosomes of *G. herbaceum*, indicating that they may be applied future as a useful marker in developing physical map.

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