四倍体栽培棉与二倍体野生棉杂种PMC-FISH研究

凌 键[1*]，邹美娟[1,2*]，宋国立[1]，王春英[1]，彭仁海[1,3]，刘 方[1]，黎绍惠[1]，张香娣[1]，王玉红[1]，王坤波[1**]

(1. 中国农业科院棉花研究所，农业部棉花遗传改良重点实验室，河南 安阳 455000；
2. 中国科学院植物研究所，北京 100093；3. 安阳工学院，河南 安阳 455000)

摘要：首次将PMC-FISH(花粉母细胞荧光原位杂交)技术应用于四倍体栽培棉与二倍体野生棉杂交所形成的三倍体杂种棉F_1中，成功的鉴定了目标染色体中的单价体、双价体、多价体，还发现在非目标染色体上有星状和片段状的杂交信号。在以A组棉基因组DNA作为探针的PMC-FISH中，分别对三倍体杂种棉F_1及其母本四倍体栽培棉的减数分裂中期Ⅰ的染色体构型进行了鉴定。结果显示，四个三倍体杂种棉F_1的减数分裂时期染色体的构型分别是：陆地棉$(AD)_1$×雷蒙德氏棉(D_5)为1ⅢIaad＋1ⅡIaa＋4ⅡIad＋7ⅡIdd＋5ⅠIa＋7ⅠId；海岛棉$(AD)_2$×旱地棉(D_4)为1ⅣIaaaad＋1ⅢIadd＋2ⅡIad＋8ⅡIdd＋6ⅠIa＋5ⅠId；陆地棉$(AD)_1$×澳洲棉(C_3)为2ⅢIadd (adc or acc)＋1ⅡIaa＋9ⅠIa＋4ⅡIcc (dc or dd)＋14ⅠId (c)；陆地棉$(AD)_1$×南岱华棉($C_{1\text{-}n}$)为：1ⅡIaa＋1ⅡIad (ac)＋10ⅠIa＋6ⅡIcc (dc or dd)＋13ⅠId (c)。然而，两个四倍体棉染色体的构型都是13ⅡIaa＋13ⅡIdd。上述结果还显示，AD×D的两个杂种组合中，二价体多，单价体少，AD×C的两个杂种组合中，二价体少，单价体多；并且AD×D型杂种中A亚组染色体单体的数目比其在AD×C型杂种中的更少。这说明，与C染色体组相比较，D染色体组与四倍体棉种的亲缘关系更近。同时，我们的结果也证明PMC-FISH技术在分析三倍体杂种棉F_1的亲缘关系中起着不可取代的作用。

关键词：棉属；种间杂交；PMC-FISH；减数分裂染色体构型

中图分类号：S562.03 文献标识码：A

文章编号：1002-7807(2009)02-0141-09

PMC- FISHs in Hybrids of Tetraploid Cultivated and Diploid Wild *Gossypium* Species

LING Jian[1*], ZOU Mei-juan[1,2*], SONG Guo-li[1], WANG Chun-ying[1], PENG Ren-hai[1,3], LIU Fang[1], LI Shao-hui[1], ZHANG Xiang-di[1], WANG Yu-hong[1], WANG Kun-bo[1**]

(1. *Key Lab of Cotton Genetic Improvement of Ministry of Agriculture, Cotton Research Institute of Chinese Academy of Agricultural Sciences, Anyang, Henan* 455000, *China*; 2. *Institute of Botany, Chinese Academy of Sciences, Beijing* 100093, *China*; 3. *Anyang, Henan Institute of Technology, Anyang, Henan* 455000, *China*)

Abstract: PMC-FISH (pollen mother cell-fluorescence in situ hybridization) technology was firstly applied to identify probed chromosomes in univalent, bivalent and multivalent, as well as to distinguish star- or fragment-like hybridization signals from non-probed chromosomes in four triploid F_1 hybrids produced by cultivated allotetraploid cotton crossed with different diploid cotton species in the genus *Gossypium*. Using genomic DNA from A genome species as probe, the meiotic chromosome configurations in metaphase Ⅰ of four triploid F_1 hybrids were identified as 1Ⅲaad＋1Ⅱaa＋4Ⅱad＋7Ⅱdd

收稿日期：2008-09-03 作者简介：凌键(1977-)，男，博士

* The two authors contributed equally to this work; ** Corresponding Author (E-mail: wkbcri@cricaas.com.cn)

基金项目：国家自然科学基金(30170501)

+ 5 Ⅰa + 7 Ⅰd for $(AD)_1D_5$ (*G. hirsutum* × *G. raimondii*), 1Ⅳaaaad +1Ⅲadd + 2Ⅱad + 8Ⅱdd + 6 Ⅰa + 5 Ⅰd for $(AD)_2D_4$ (*G. barbadense* × *G. aridum*), 2Ⅲadd (adc or acc) + 1Ⅱaa + 9 Ⅰa + 4Ⅱcc (dc or dd) + 14 Ⅰd (c) for $(AD)_1C_3$ (*G. hirsutum* × *G. australe*), and 1Ⅱaa + 1Ⅱad (ac)+ 10 Ⅰa + 6Ⅱcc (dc or dd) + 13 Ⅰd (c) for (AD)1C1-n (*G. hirsutum* × *G. nandewarense*), respectively. While the two cultivated tetraploid species, *G. hirsutum* $(AD)_1$ and *G. barbadense* $(AD)_2$, which were used for the triploid female parents, displayed the same chromosome configuration as 13Ⅱaa + 13Ⅱdd. It is obvious that there were more bivalents but less univalents in ADD [$(AD)_1D_5$ and $(AD)_2D_4$] type than that in ADC [$(AD)_1C_3$ and $(AD)_1C_{1\text{-}n}$] type of triploid F_1 hybrids and with fewer A-subgenome univalents and more A-subgenome combined pairings in ADD than in ADC type of triploid F_1 hybrids. The results indicated that specific relationship of AD cultivated tetraploid species with D genome may be much closer than with C genome, and that the PMC-FISH technology may play an irreplaceable role in the specific relationship analyses of triploid F_1 hybrids in *Gossypium*.

Key words: *Gossypium*; interspecific hybrid; PMC-FISH; meiotic chromosome configurations

CLC: S562.03　　**Document Code**: A

Article ID: 1002-7807(2009)02-0144-09

Introduction

There are 51 species in the genus *Gossypium* including 46 diploids (2n=2x=26), designing genomes as A to G and K, and five tetraploids (2n = 4x = 52), designing genome as AD [1]. The tetraploid cotton is usually considered as the amphidiploid composed of A- and D-subgenome [2]. Now, many new technologies have been brought to bear on the problem of the origin and evolution of tetraploid cotton, and some basic theory has gotten the same conclusions [3-4]. Yet few data address the question of the actual diploid ancestor of tetraploid D-subgenome, the relationship between the diploid and the tetraploid genome, and homologous degree among diploid taxa [5].

The integrated events of genome may occur immediately after synthetic polyploidy formation [6-7] and the investigation to the chromosome paring in polyploidy is a good way to study the relationship among polyploidy genomes [8-9]. The previous reports about interspecific relationship mainly focused on studies of chromosome paring based on traditional meiotic morphology or mitotic karyotypes. Chen et al [10] had chosen the interspecific hybridization between *Cucumis hystrix* and *Cucumis sativus* to investigate the chromosome pairing and found that the interspecific relationship of the above species is relatively far. Rodrigues et al [11] investigated the interspecific hybridization among several *Triticum* species and found that the relationship between G and S genome of *Triticum* is closer than that between B and S genome.

It also has become a common method in cytobiology to utilize the chromosome pairing in meiotic metaphase Ⅰ to investigate interspecific relationship of *Gossypium*. However, it is difficult to distinguish the source of the chromosome in interspecific hybrids by using the traditional cytological methods, which has limited to investigate the interspecific relationship more exactly.

Fluorescence in situ hybridization (FISH), which integrates the advantages of cyto- and molecular biology, has been widely applied in many aspects, especially in studying the interspecific relationship. The pollen mother cell FISH (PMC-FISH) by using the genomic DNA as probes, which can reflect the hybridization signal of whole genome and obtain more comprehensive genetic information, has become a reliable method for investigating the interspecific relationship of different genome in hybrids. The PMC-FISH in cotton was established by the laboratory led by Stelly in Texas A&M University [12-20] and developed well currently in

Zhang's laboratory in Nanjing Agricultural University with the application in conjunct with BAC library [21-22]. However it may be a powerful tool more accurately to probe the nature of chromosome pairing in the hybrids, there has been no report of PMC-FISH based on interspecific hybrids in *Gossypium*[23-24].

This paper firstly reported the application of PMC-FISH on detecting the meiotic chromosome configurations in triploid F_1 hybrids produced by cultivated allotetraploid cotton crossed with different diploid cotton species to investigate the interspecific relationship in the genus *Gossypium*.

1 Materials and Methods

1.1 Plant materials

Nine accessions were used in this study, belonging to the four cultivated species and five wild species in *Gossypium*, respectively (Table 1). CCRI 12 and CCRI 16 were upland cotton cultivars (*G. hirsutum*, $(AD)_1$) developed by Cotton Research Institute, Chinese Academy of Agricultural Sciences (CRI-CAAS, or Chinese Cotton Research Institute, CCRI). Hai-7124 (*G. barbadense*, $(AD)_2$) and Shixiya-1 (*G. arboreum* A_2) were cultivars developed in China and provided by CCRI. The *herbaceum var. africanum* ($A_{1\text{-}a}$) was a wide accession and provided by CCRI. The wild species accessions 530888 D4-3 (*G. aridum*, D_4), 5308998 D5-2 (*G. raimondii*, D_5) were introduced from USDA-ARS, Crop Germplasm Research Unit in College Station, Texas, USA, and C_1-n-01 (*G. nandewarense*, $C_{1\text{-}n}$), 464482 C3-7 (*G. australe*, C_3) were introduced from Australia.

Table 1 The parent materials and their genomes

Accession / PI number	Species	Genome	Usage
CCRI-12	*G. hirsutum*	$(AD)_1$	Female parent
CCRI-16	*G. hirsutum*	$(AD)_1$	Female parent & target DNA
Hai-7124	*G. barbadense*	$(AD)_2$	Female parent & target DNA
—	*G. herbaceum var. africanum*	$A_{1\text{-}a}$	Probe
Shixiya-1	*G. arboreum*	A_2	Probe
530888 D4-3	*G. aridum*	D_4	Male parent
5308998 D5-2	*G. raimondii*	D_5	Male parent & blocking
C_1-n-01	*G. nandewarense*	$C_{1\text{-}n}$	Male parent
464482 C3-7	*G. australe*	C_3	Male parent

Table 2 shows four F_1 triploid hybrids used in this study. The hybrids were all made by crossing diploid wild species as male parents to tetraploid cultivars as female parents in 1990s in National Wild Cotton Plantation, Hainan Island, China, which is managed by CCRI. All plant materials including F_1 hybrids and their wild species parents were maintained in living plants in the plantation. The cultivated parents were maintained in Anyang City (CCRI), China.

Table 2 The combinations of interspecific hybrids

Accession combination	Species combination	Genome	Hybrid type	Year
CCRI-16 × 464482 C3-7	*hirsutum* × *australe*	$(AD)_1 \times C_3$	ADC	1998
CCRI-12 × C_1-n-01	*hirsutum* × *nandewarense*	$(AD)_1 \times C_{1\text{-}n}$	ADC	1999
Hai-7124 × 530888 D4-3	*barbadense* × *aridum*	$(AD)_2 \times D_4$	ADD	1994
CCRI-16 × 5308998 D5-2	*hirsutum* × *raimondii*	$(AD)_1 \times D_5$	ADD	1998

1.2 DNA probes and labeling

The genomic DNA (gDNA) prepared from *G. arboreum* and *G. herbaceum var. africanum* were used as probes. The procedures to label probes of the gDNA were according to Wang et al [24].

1.3 Blocking DNA

The gDNA prepared from *G. raimondii* was used as blocking DNA in all experiment. The procedure of preparing blocking DNA was according to Wang et al [24].

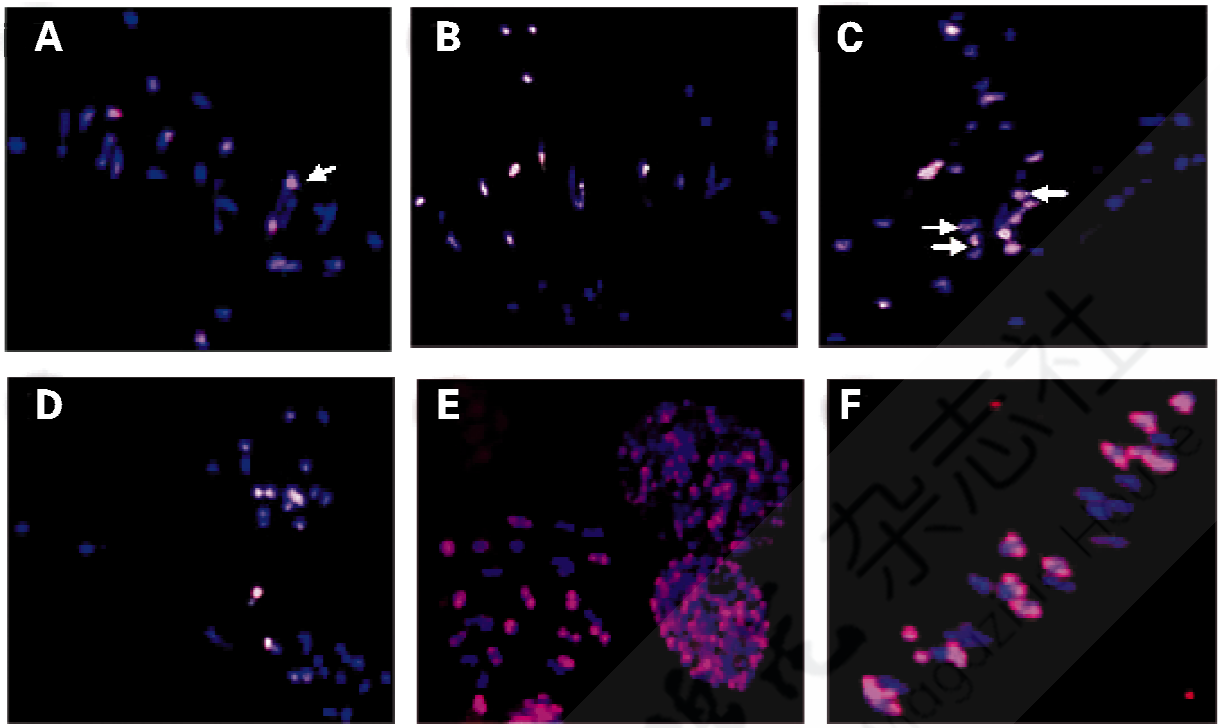

Figure explanation:

Figs. A and B, FISH images of ADD type triploid hybrids using gDNA from A genome (*G. arboreum*, A_2) as probes and gDNA from D genome (*G. raimondii*, D_5) as blockings. Hybridization signals (red Rhodamine fluorescence) are visualized on 4′,6-diamidino-2-phenylindole (DAPI) stained chromosomes (blue).

Fig. A, the triploid F_1 hybrid of *G. barbadense* ($(AD)_2$) × *G. aridum* (D_4), with chromosome configuration as 1Ⅴaaaad +1Ⅲadd + 2Ⅱad + 8Ⅱdd + 6Ⅰa + 5Ⅰd. The arrow indicates a pentavalent.

Fig. B, the triploid F_1 hybrid of *G. hirsutum* [$(AD)_1$] × *G. raimondii* (D_5), with chromosome configuration as 1Ⅲaad + 1Ⅱaa + 4Ⅱad + 7Ⅱdd + 5Ⅰa + 7Ⅰd.

Figs. C and D, FISH images of ADC type triploid hybrids using gDNA from A genome (*G. arboreum*, A_2) as probes and gDNA from D genome (*G. raimondii*, D_5) as blockings. Hybridization signals (red Rhodamine fluorescence) are visualized on 4′,6-diamidino-2-phenylindole (DAPI) stained chromosomes (blue).

Fig. C, the triploid F_1 hybrid of *G. hirsutum* ($(AD)_1$) × *G. nandewarense* ($C_{1\text{-}n}$), with chromosome configuration as 1Ⅱaa + 1Ⅱad (ac)+ 10Ⅰa + 6Ⅱcc (dc or dd) + 13Ⅰd (c). The arrows indicate the fragment-like signals.

Fig. D, the triploid F_1 hybrid of *G. hirsutum* ($(AD)_1$) × *G. australe* (C), with chromosome configuration as 2Ⅲadd (adc or acc) + 1Ⅱaa + 9Ⅰa + 4Ⅱcc (dc or dd) + 14Ⅰd (c).

Figs. E and F, FISH images of meiotic metaphase Ⅰ or prophase spreads of two AD tetraploid species, *G. hirsutum* (E) and *G. barbadense* (F), using gDNA from A genome (*G. herbaceum var. africanum*, A_1-a) as probes and gDNA from D genome (*G. raimondii*, D_5) as blockings, showing chromosome configuration of 13Ⅱaa (hybridization signals (red Rhodamine fluorescence) + 13Ⅱdd (non-hybridization blue DAPI stained fluorescence) for both species.

1.4 Chromosome preparation

Meiotic chromosome spreads were prepared according to the procedure of Wang et al [24] with some modification. Young buds were selected and the appropriate prophase was determined firstly. A few anthers from a bud were squashed in 45% acetic acid on a slide and checked under a phase microscope. The buds checked to be at meiotic metaphase Ⅰ were fixed overnight in fixative (ethanol: acetic acid: chloroform = 5:3:2) at room temperature. After two washes in deionized water, the anthers were squeezed with a surgical knife to extrude the chromosomes in 60% acetic acid. Then immediately, the slide was briefly immersed in absolute ethanol, and left to dry. The slides could be used directly for FISH experiment or stored at -20℃ for up to several months.

1.5 Fluorescence in situ hybridization (FISH) and detection

The FISH of meiotic chromosomes were performed according to Wang et al [24]. The slides were pretreated with 100 g • L^{-1} DNAase-free RNAase in 2×SSC at 37℃ for 1 h and then washed three times for 5 min each in 2 ×SSC. For each slide, total 20 μL hybridization mixture including 50% formamide, 2×SSC, 10% sodium dextran sulphate, 50 mmol • L^{-1} phosphate buffer with pH 7.0, 1～2 mg • L^{-1} probe DNA and 50～100 mg • L^{-1} block DNA were used. In situ hybridization was allowed to proceed at 37℃ overnight, followed by post-hybridization washes for 3×5 min in 50% formamide at 37℃, 3×5 min in 2×SSC at room temperature, and 3×5 min in 0.2×SSC at room temperature. The slides were blocked with 5% BSA at 37℃ for 30 min and then covered with corresponding antibody (Roche) at 37 ℃ for 1 h each. After washing with 1×PBS for 3×5 min, the slides were counterstained with 1 mg • L^{-1} 4′, 6-diamidino-2-2-phenylin-dole (DAPI) and then washed with 1×PBS for 3×3 min. And finally the slides were mounted in 10% Vectashield antifade mounting.

The hybridization signals were observed using a fluorescence microscope (Leica MRA2). Images were captured by the Imaging System Software and brought together to make the plate using Adobe Photoshop 7.0 software.

2 Results

There are two types of triploid F_1 hybrids based on their genome combinations, ADD type includes an AD genome (tetraploid, 2n=4x=52) and a D genome species (diploid, 2n=2x=26), and ADC type includes an AD genome and a C genome species (diploid) (Tab. 2). Probes hybridized to the two type hybrids were the same as gDNA prepared from a diploid species *G. arboreum* (A_2) but the FISH patterns differences existed between them.

2.1 ADD type triploid hybrids

The identification of chromosome configuration and translocation was primarily according to Zhang et al [25]. Figs. A and B represented FISH images of meiotic chromosomes (metaphase Ⅰ) of two hybrids of the ADD type, *G. barbadense* $(AD)_2$× *G. aridum* (D_4) [fig. A as $(AD)_2D_4$] and *G. hirsutum* $(AD)_1$ × *G. raimondii* (D_5) [fig. B as $(AD)_1D_5$], respectively, with *G. arboreum* gDNA as probes to both triploid F_1 hybrids. The chromosome configurations differed from 1Ⅳaaaad +1Ⅲadd + 2Ⅱad + 8Ⅱdd + 6Ⅰa + 5Ⅰd for $(AD)_2D_4$ to 1Ⅲaad + 1Ⅱaa + 4Ⅱad + 7Ⅱdd + 5Ⅰa + 7Ⅰd for $(AD)_1D_5$. The highest valent was pentavalent in $(AD)_2D_4$ and trivalent in $(AD)_1D_5$, respectively. There were 2 multivalents, 10 bivalents and 11 univalents in former, and 1 trivalents, 12 bivalents and 12 univalents in the latter. Clearly, bivalent number in $(AD)_1D_5$ was more than that in $(AD)_2D_4$, and the translocation number in $(AD)_1D_5$ (5) was more than $(AD)_2D_4$(3), demonstrating the closer relationship of *G. raimondii* (D_5) than *G. aridum* (D_4) to AD genome cottons. While generally, both hybrids showed more bivalents or multivalents than univalents with their primarily corresponding locations in the PMCs at equatorial plane and bipolar (figs. A and B).

One of the most advantages of FISH on intespecific hybrids with the cognition to genome or subgenome chromosome derivations in bi- and multivalent configurations, such as A- and/or D-subgenome, and D genome origins in ADD type triploid F_1 hybrids, was well confirmed in today's study. Based on hybridization signals in figs. A and B, bivalents could be distinguished as following three combinations, aa, dd, and ad, with the corresponding numbers of 1, 7 and 4 in $(AD)_1D_5$ and 0, 8 and 2 in $(AD)_2D_4$, respectively, showing much more dd than aa, or aa plus ad combinations, and thus indicating close relationships of the wild diploid D genome with two tetraploid cultivated cottons, *G. hirsutum* $(AD)_1$ and *G. barbadense* $(AD)_2$. Chromosome univalents in the two triploid F_1 hybrids of the

ADD type were also distinguished as A or D genome origins clearly, 5 a and 7 d in $(AD_1)D_5$, and 6 a and 5 d in $(AD)_2D_4$, respectively. The pentavalent in $(AD)_2D_4$ would be of 4 a and 1 d genomic origins.

It was obviously detected that most univalent chromosomes with red hybridization signals were bigger in size than those with blue non-hybridization signals. Star-like or fragment-like red hybridization signals existed in some blue non-hybridization bivalents and univalents, but reverse could not be observed in our experiments. Referring to A genome species gDNA probes, the red hybridization and blue non-hybridization signals would be reflected to the A- and D-subgenome or D genome sequences, respectively. The existing of star- or fragment-like hybridization signals in non-probed chromosomes indicated a unidirectional introgression of DNA sequence from A-subgenome into D-subgenome chromosomes, which could be difficult to be observed by the traditional cytological (non-FISH) technology.

Above results were basically or largely in accordance with the previous cytological and cytogenetic studies on cotton interspecific hybrids [1,3,26-27], but hybrid PMC-FISH researches here provided the direct evidence.

2.2　ADC type triploid hybrids

Two C genome species, *G. australe* (C_3) and *G. nandewarense* ($C_{1\text{-}n}$), are diploid wild cotton distributed on Australia. Figs. C and D represented FISH images of meiotic chromosomes (metaphase Ⅰ) of two hybrids of the ADC type, *G. hirsutum* ($(AD)_1$) × *G. nandewarense* ($C_{1\text{-}N}$) [fig. C as $(AD)_1C_{1\text{-}N}$] and *G. hirsutum* ($(AD)_1$) × *G. australe* (C) [fig. D as $(AD)_1C$], respectively. The probes and the blocked DNAs used here were the same as above, but a rather different result was obtained. The chromosome configurations for the two hybrids were as 1Ⅱaa + 1Ⅱad (ac) + 10Ⅰa + 6Ⅱcc (dc or dd) + 13Ⅰd (c) for $(AD)_1C_{1\text{-}n}$(*G. hirsutum* × *G. nandewarense*) and 2Ⅲadd (adc or acc) + 1Ⅱaa + 9Ⅰa + 4Ⅱcc (dc or dd) + 14Ⅰd (c) for $(AD)_1C_3$(*G. hirsutum* × *G. australe*), respectively, with corresponding translocation numbers of 11 and 8. Comparing to ADD type, there were fewer multivalents [only 2 trivalents in $(AD)_1C_3$], less bivalents (8 and 5 in former and latter) but more univalents (23 in both), thus indicating that, to AD tetraploid species, C genome was less relative than D genome. The similarity with ADD type was the locations at equatorial plane of bivalents and bipolar of univalents in the PMCs, as well as bigger size of chromosomes with red hybridization signals than those with blue non-hybridization signals (figs. C and D). It was easy to distinguish A-subgenome derivated chromosomes but difficult to discriminate D-subgenome from C genome derivated chromosomes. However there were two and three valent combinations in $(AD)_1C_{1\text{-}n}$ and $(AD)_1C_3$, respectively, related to A-subgenome derivated chromosomes, suggesting that there were, though not very close, certain relationships between the two Australian species to *G. hirsutum*.

Similarly to ADD type, the star- or fragment-like hybridization signals could be detected on part of univalents or bivalents, which existed blue non-hybridization signals and should be taken as D-subgenome or C genome origins in the ADC type PMCs of the two triploid F_1 hybrids. Higher frequency of the existing of star- or fragment-like hybridization signals in non-hybridization chromosomes than in ADD type (fig. C, the white arrows) may indicate, with more possibility, a unidirectional introgression of DNA sequence from A-subgenome into D-subgenome or even to C genome chromosomes.

2.3　PMC-FISHs of two cultivated tetraploid cottons

The PMC-FISH of two cultivated tetraploid cottons, *G. hirsutum* $(AD)_1$ and *G. barbadense* $(AD)_2$, used as female parents for producing triploid hybrids with D or C genome species (to form ADD and ADC type F_1 hybrids), by using

gDNA from *G. herbaceum var. africanum* as probes, showed the same chromosome configuration as 13 Ⅱ aa + 13 Ⅱ dd in their FISHs of meiotic PMCs, being obviously visualized as probed and non-probed chromosome pairs, respectively (figs. E and F). The result of the PMC-FISH used gDNA from *G. herbaceum var. africanum* as probes in our study was actually similar with that used gDNA from *G. arboreum* as probes because *G. herbaceum var. africanum* and *G. arboreum* are phylogenetically sister to each other and hence are genealogically equidistant from the A-genome of the allopolyploids [16]. The FISH based bivalent chromosome pairing could be distinguished very clearly but could be deduced in traditional cytology (non-FISH) studies mainly according to their sizes, in which bigger and smaller pairs were thought as A- and D-subgenome derivations, respectively, in tetraploid cottons [3,27]. There were two cells in interphase stage, showing many copies of red Rhodamine fluorescent hybridization signals which dispersed dottily in whole cells (fig. E on right). It was prospected that, almost in all A gDNA probed aa bivalents of both species, hybridization signals did not cover whole chromosomes but tended to being clustered mainly in bipolars of their centromere regions, demonstrating hetero- and eu-chromatin distributions on centromere- and telomere-near regions in plants[13,28].

3 Discussion

It has been long worried for cytologists, with traditional (non-FISH) technologies, how to identify meiotic chromosome derivation in triploid F_1 hybrids in cotton, especially for bivalent and multivalent combinations which may have different genome or subgenome origins [29], thus leading to possibly misunderstanding for chromosome pairing in meiotic metaphase and then for interspecific homogeneity estimating among genomes or subgenomes of cotton triploid F_1 hybrids. It is expected that PMC-FISH technology, developed in this study, has well brought its advantages to identify probed chromosomes both in univalents such as a or d univalents and in bivalents or multivalents like aa, ad and dd bivalent combinations in ADD type of triploid F_1 hybrids. The technology let us distinguish the star- or fragment-like hybridization signals in non-probed chromosomes in both ADD and ADC types of triploid F_1 hybrids, which is very difficult or even impossible to do so by traditional (non-FISH) cytology techniques. Here in the ADD type of triploid F_1 hybrids, the bivalent combination of aa would be derive from A-subgenome of AD tetraploid parent only [(just from $A_{(AD)}$, taking as $a_{(AD)}\,a_{(AD)}$], while dd from D genome parent and/or D-subgenome origin [(from $D_{4(or\,5)}$ and/or $D_{(AD)}$, taking possibly as $d_{(AD)}\,d_{(AD)}$, $d_{(AD)}\,d_{D4(or\,5)}$, and/or $d_{D4(or\,5)}\,d_{D4(or\,5)}$], and ad from A- and D-subgenome and/or D genome origin [(from $A_{(AD)}$ and $D_{(AD)}$, and/or $A_{(AD)}$ $D_{4(or\,5)}$, taking possibly as $a_{(AD)}\,d_{(AD)}$ and/or $a_{(AD)}\,d_{D4(or\,5)}$]. It was similarly difficult or impossible to distinguish D-subgenome [$D_{(AD)}$] from C genome origin in the chromosome pairing including bivalents and even univalents in ADC type of triploid F_1 hybrids. It may be hopeful to solve the problems by using genome (or subgenome) specific probes simultaneously in this kind of hybrid PMC-FISH experiments.

Based on the comparison of the two types of triploid F_1 hybrids, there were less univalents but more bivalents in ADD than in ADC, as well as some trivalents or even a pentavalent in ADD but no multivalents in ADC. The result were well corresponding to previous studies with non-FISH cytology [1,3,26-27], and further demonstrating, to AD genome, much closer specific relationship of D genome than C genome. The univalents in the ADC triploid F_1 hybrids would be consist of A-, D-subgenome and C genome. However the A-subgenome univalents could be well distinguished by probed chromosomes from the other two genomes or subgenomes (figs. C and D). There were 10 and 9 of A-subgenome u-

nivalents, in $(AD)_1C_{1-n}$ and $(AD)_1C_3$, respectively, just close to total 13 chromosomes each. On the other hand, fewer A-subgenome chromosomes [only 1 and 2 in $(AD)_1C_{1-n}$ and $(AD)_1C_3$, respectively] did behave to pair to other two genome chromosomes (D-subgenome or C genome). In contrast, there were fewer A-subgenome univalents in ADD triploid F_1 hybrids [5 and 6 in $(AD)_1D_5$ and $(AD)_2D_4$, respectively] and more A-subgenome combined pairings [5 and 4 in $(AD)_1D_5$ and $(AD)_2D_4$, respectively] did behave to pair to D genome or D-subgenome chromosomes. These investigations indicate that specific relationship of AD cultivated tetraploid species with D genome may be much closer than with C genome, and that the meiotic FISH technology may play an irreplaceable role in the analyses of interspecific relationship in *Gossypium*.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (Grant No. 30170501)

References:

[1] WANG Kun-bo, Du Xiong-ming, Song guo-li, et al. Status and prospect for cotton germplasm enhancement [J]. Plant Genetic Resources, 2004, 5(suppl): 23-28.

[2] WENDEL J F. Genome evolution in polyploids[J]. Plant Mol Biol, 2000, 42:225-249.

[3] ENDRIZZI J E, Turcotte E L, Kohel R J. Genetics, cytogenetics, and evolution of *Gossypium* [J]. Adv Genet, 1985, 23:271-375.

[4] WENDEL J F, Cronn R C. Polyploidy and the evolutionary history of cotton[J]. Adv Agro, 2002, 78: 139-180.

[5] SENCHINA D S, Alvarez I, Cronn R C, et al. Rate variation among nuclear genes and the age of polyploidy in *Gossypium*[J]. Mol Biol Evol , 2003, 20:633-643.

[6] ADAMS K L, Cronn R C, Percifield R, et al. Genes duplicated by polyploidy show unequal contributions to the transcriptome and organ-specific reciprocal silencing [J]. Proc Natl Acad Sci USA, 2003, 100: 4649-4654.

[7] ADAMS K L, Percifield R, Wendel J F. Organ-specific silencing of duplicated genes in a newly synthesized cotton allotetraploid [J]. Genetics, 2004, 168: 2217-2226.

[8] MASTERSON J. Stomatal size in fossil plants: evidence for polyploidy in majority of angiosperms[J]. Science, 1994, 264: 421-424.

[9] ADAMS K L, Wendel J F. Polyploidy and genome evolution in plants[J]. Curr Opin Plant Biol,2005, 8: 135-141.

[10] CHEN Jin-feng, Staub J E, Tashiro Y, et al. Successful interspecific hybridization between *Cucumis sativus* L. and *Cucumis hystrix* Chakr[J]. Euphytica, 1997, 96: 413-419.

[11] RODRIGUEZ S, Maestra B, Perera E, et al. Pairing affinities of the B-and G-genome chromosomes of polyploid wheats with those of Aegilops speltoides[J]. Genome, 2000, 43: 814-819.

[12] CRANE C F, Price H J, Stelly D M, et al. Identification of a homeologous chromosome pair by *in situ* DNA hybridization to ribosomal RNA loci in meiotic chromosomes of cotton (*Gossypium hirsutum*) [J]. Genome, 1993(6), 36:1015-1022.

[13] JI Yuan-fu, Raska W A, Donato M D, et al. Identification and distinction among segmental duplication-deficiencies by fluorescence in situ hybridization (FISH)- adorned multivalent analysis[J]. Genome, 1999, 42:763-771.

[14] CRONN R C, Zhao Xin-ping, Paterson A H, et al. Polymorphism and concerted evolution in a tandemly repeated gene family: 5S ribosomal DNA in diploid and allopolyploid cottons [J]. J Mol Evol, 1996, 42:685-705.

[15] CRONN R C, Small R L, Haselkorn T, et al. Rapid diversification of the cotton genus (*Gossypium*: Malvaceae) revealed by analysis of sixteen nuclear and chloroplast genes [J]. Am J Bot, 2002, 89: 707-725.

[16] HANSON R E, Islam-Farid M N, Percival E A, et al. Distribution of 5s and 18s-28s rDNA loci in a tetraploid cotton (*Gossypium hirsutum* L.) and putative diploid ancestors [J]. Chromosoma, 1996, 105:55-61.

[17] HANSON R E. Evolution of interspersed repetitive elements in *Gossypium* (*Malvaceae*) [J]. Am J bot,1998,85:1364-1368.

[18] ZHAO Xin-ping, Wing R A, Paterson A H. Clo-

ning and characterization of the majority of repititive DNA in cotton (*Gossypium* L.) [J]. Genome, 1995, 38:1177-1188.

[19] ZHAO Xin-ping, Ji Yuan-fu, Ding Xiao-ling, et al. Macromolecular organization and genetic mapping of a rapidly evolving chromosome-specific tandem repeat family (B77) in cotton (*Gossypium*) [J]. Plant Mol Bio, 1998a, 38:1031-1042.

[20] ZHAO Xin-ping, Si Yang, Hanson R E, et al. Dispersed repetitive DNA has spread to new genome since polyploid formation in cotton[J]. Genome Res, 1998b, 8:479-492.

[21] WANG Kai, Song Xian-liang, Han Zhi-guo, et al. Complete assignment of the chromosomes of *Gossypium hirsutum* L. by translocation and fluorescence in situ hybridization mapping [J]. Theor Appl Genet, 2006, 113:73-80.

[22] WANG Kai, Guo Wang-zhen, Zhang Tian-zhen. Development of one set of chromosome-specific microsatellite-containing BACs and their physical mapping in *Gossypium hirsutum* L. [J]. Theor Appl Genet, 2007, 115:675-682.

[23] JIANG Ji-ming, Gill B S. Current status and the future of fluorescence in situ hybridization (FISH) in plant genome research [J]. Genome, 2006, 49: 1057-1068.

[24] WANG Kun-bo, Wang Wen-kui, Wang Chun-ying, et al. Investigation of *Gossypium babardense* L. by FISH and karyotype analysis [J]. Acta Genetica Sinica, 2001, 28:69-75.

[25] ZHANG Xiang-di, Wang Kun-bo, Li Mao-xue, et al. Observing behavior of monosome and telosome chromosomes of *Gossypium hirsutum* and their identification approach [J]. Cotton Science, 2000, 12: 302-305.

[26] WANG Kun-bo, Li Shao-hui, Ye Wu-wei, et al. Cytological Studies of Hybrids between *G. hirsutum* and two wild cotton species from Australia[J]. Cotton Science, 1994, 6(1):23-28.

[27] WANG Kun-bo, Li Mao-xue, Zhang Xiang-di, et al. Cytological studies on hybrids of *G. hirsutum* and seven wild cotton species[J]. Acta Agronomica Sinica, 1996, 22(1):27-35.

[28] CHENG Zhu-Kuang, Gu Ming-hong. Karyotype analysis for pachytene chromosome of indica, japonica rice and their hybrid [J]. Acta Genetica Sinica, 1994 , 21 (5):383-392.

[29] REYES-VALDES M H, Ji Yuan-fu, Crane C F, et al. ISH-faciliated analysis of meiotic bivalent pairing [J]. Genome, 1996, 39:784-796.