

Characterization of an Alien Chromosome of *Oryza officinalis* Transferred the Genomic and Cytological Environment of *Oryza sativa*

Gang Li · Ming Tang · Wei Hu · Guangcun He ·
Hong Liu · Xuequn Liu · Rui Qin

Received: 2 May 2010 / Revised: 1 June 2010 / Accepted: 14 June 2010 / Published online: 1 July 2010
© The Botanical Society of Korea 2010

Abstract Distant hybridization between rice species and their genome interactions have become the hot points of genetic research. The new formed hybrid was unstable and many of the alien chromosomes would be excluded out in the meiosis procedure. In this study, we investigated the phenomenon that the monosomic alien addition lines (MAALs) of *Oryza officinalis* (CC)–*Oryza sativa* (AA) inherited in the genomic and cytoplasmic environments of cultivated rice. In a series of backcross programs, every time the MAALs were backcrossed with the recurrent parent Hejiang19, we get a portion of the backcross progenies that were similar to the female parent MAALs in morphological characters (with rolled leaves) and chromosome constitutions ($2n+1$). The monosomic alien chromosome exhibited stable inheritance in the genomic and cytoplasmic environments of cultivated rice. Molecular biology and fluorescence in situ hybridization (FISH) analyses on mitotic cells showed that the alien chromosomes in MAALs were inclined to be transferred integrated into the backcross progenies with rolled leaves and no big fragments of alien chromosomes introgressing into the chromosomes of cultivated rice. By FISH analysis on the meiotic cells, we proved that the single alien chromosome performing nonstandard meiosis and behaving like a

dispensable genetic factor, which would be the cytological reasons for the stable inheritance of alien chromosomes.

Keywords Rice · *Oryza officinalis* · Monosomic alien addition line · Genomic interaction · Meiosis

Introduction

Wild species of *Oryza* genus are valuable gene pools for resistance to biotic and abiotic stresses in cultivated rice breeding (Multani et al. 2003; Liu et al. 2004). Distant hybridization was used to transfer valuable genes from wild rice into cultivated rice (Brar and Khush 1997). In the interspecific hybrid of plant, however, two different genomes combining together into one nucleus may result in intergenomic conflicts (Riddle and Birchler 2003). The changes include alterations of gene expressions, epigenetic modifications, activations of transposons (Matzke et al. 1999; Comai et al. 2000; Hanson et al. 2000; Shaked et al. 2001; Jin et al. 2008), and structure rearrangements at the chromosomes and DNA sequences levels (Song et al. 1995; Wendel et al. 1995; Leitch and Bennett 1997). The alien genome, which is embedded in the native cytoplasmic and genomic environment, is often unsteady. Elimination of specific DNA sequences and partial or complete uniparental chromosome occurred commonly in many interspecific hybrids (Feldman et al. 1997; Linde-Laursen and Bothmer 1999; Gernand et al. 2005; Du et al. 2008).

Oryza officinalis is a diploid wild rice species with CC genome, which maintained abundant diversity and is close to the AA genome of *Oryza sativa* (Aggarwal et al. 1997; Vaughan et al. 2003; Ying et al. 2006). Agronomists have produced the hybrids between *O. officinalis* and *O. sativa* in the projects of improving cultivated rice. At the

G. Li · H. Liu · X. Liu · R. Qin (✉)

Key Laboratory of State Ethnic Affairs Commission
for Biological Technology, College of Life Sciences,
South-Central University for Nationalities,
Wuhan 430074, China
e-mail: qin_rui@hotmail.com

G. Li · M. Tang · W. Hu · G. He

Key Laboratory of Ministry of Education for Plant Development
Biology, College of Life Sciences, Wuhan University,
Wuhan 430072, China

International Rice Research Institute, Jena, Brar, and Khush studied the C genome of *O. officinalis* and produced introgression lines containing genes for resistance to BPH and WBPH from the cross between *O. sativa* and *O. officinalis* (Jena and Khush 1990; Brar and Khush 1997). Their studies also indicated that the recombination between chromosomes of cultivated rice and wild species was limited, and no substitution of large chromosome fragment was found. Jin et al. (2006) reported a mosaic chromosome constructed with short arm of chromosome 4 from cultivated rice and long arm from *O. officinalis*. Though the homologous chromosomes of A-genome would recombine freely and exchange genes easily in the hybrids, it was the different thing in the wide hybrids between the A and C genomes. The relationship between A and C genomes was never illustrated thoroughly, and the reasons for recombination barrier between chromosomes of cultivated rice and wild species were poorly known.

Because of intergenomic conflicts, the distant hybrids between *O. officinalis* and *O. sativa* would exclude a portion or all of the alien chromosomes of *O. officinalis*. At some special case, one of the alien chromosomes retained in the nuclear and joined into the formation of the aneuploid plants. The monosomic alien addition lines (MAALs) of *O. officinalis*–*O. sativa* are aneuploid plants holding 12 chromosome pairs of cultivated rice and only one chromosome of *O. officinalis*, and these new materials are convenient for studying relationships between the A and C genomes (Tan et al. 2005; Li et al. 2008). In this study, we investigated the phenomenon that the MAALs of *O. officinalis* (CC)–*O. sativa* (AA) inherited stably in the genomic and cytoplasmic environments of cultivated rice. The single alien chromosome was found performing nonstandard meiosis and behaving like a dispensable genetic factor, so that it can be retained in the new genomic environment rather than be excluded thoroughly out of the native nuclear in the lifecycle of MAALs.

Materials and Methods

Plant Materials

Wild rice *O. officinalis* (Acc. HY018), cultivated rice Hejiang19 and MAAL8 (with an alien chromosome 8 of *O. officinalis*) were used in this study. The aneuploid MAAL8 plant (BC2 generation) was backcrossed to Hejiang19, and the backcross progenies of BC3 generation were obtained. From the BC3 population, we selected the plants with an alien chromosome 8 of *O. officinalis* to be backcrossed again. In such a way, the backcross progenies from BC3 to BC9 generations were obtained. The plants

were planted in field at the Institute of Genetics of Wuhan University under a standard regime for rice plants.

RFLP Analysis

Genomic DNA of each plant was extracted from young leaves using the CTAB method (Murray and Thompson 1980). The DNA restriction digestion and Southern hybridization followed previously described procedures (Huang et al. 2001). The DNA samples were digested with five restriction enzymes: *Dra* I, *EcoR* I, *EcoR* V, *Hind* III, and *BamH* I. Twenty-five restriction fragment length polymorphism (RFLP) probes distributing evenly along the chromosome 8 of cultivated rice were used to identify *O. officinalis*-specific bands from the DNA samples.

Chromosome Preparation

For mitotic chromosome preparation, root tip tissues were collected in the morning from vigorously growing rice plants and fixed immediately in a 3:1 (v/v) mixture of ethanol and glacial acetic acid at 4°C overnight, then stored in a refrigerator until use. After washing thoroughly with distilled water, the fixed root tips were treated with a mixture of 2% pectinase (SERVA, Germany) and 2% cellulase (SERVA, Germany) at 28°C for 3–4 h. Finally, the treated root tips were squashed on slides and dried over a flame (Song and Gustafson 1995). For meiotic chromosome preparation, young panicles of rice plant were treated as described previously by Xiong et al. (2006). Preparation of microsporocytes cell at the early stage of meiosis were kept in a freezer at –20°C before fluorescence in situ hybridization (FISH) analysis.

C_{0t}-1 DNA Preparation

Total genomic DNA of *O. officinalis* was used to prepare *C_{0t}-1* DNA based on the method of Zwick et al. (1997). Genomic DNA was sterilized under 0.14 Mpa for 12–15 min and broken into fragments at about 800–1,500 bp, and then was reannealed at 65°C for the required time calculated according to the formula $C_{0t-1} = C(\text{mol/L}) \times T(\text{s})$. S1 nuclease (Promega, USA) was used to digest the single stretched DNA in a water bath at 37°C for 1 h. The target DNA was purified and stored in TE buffer at –20°C before use.

FISH Analysis

The *C_{0t}-1* DNA of *O. officinalis* was labeled with biotin-11-dUTP using a nick translation kit (Roche, Germany). Blocking DNA was obtained from the *O. sativa* genomic DNA and autoclaved to fragment sizes of 100 bp to 1 kb.

The mixing ratio of probe DNA and blocking DNA was 1:12. In situ hybridization was performed with the procedures described by Yan et al. (1999). Biotin-labeled probes were detected in a three-step detection/amplification procedure: streptavidin-Cy3, biotinylated anti-streptavidin and streptavidin-Cy3 (Vector Laboratories, USA). For each step of the immune reaction, slides were incubated at 37°C for 30 min and washed with PBS at intervals. Finally, the slides were counterstained with 1 µg/mL 4', 6-diamidino-2-phenylindole in Vectashield, an antifade solution (Vector Laboratories, USA). For bicolor FISH analysis, the centromeric DNA (specific sequence from AA genome) was labeled with digoxigenin-11-dUTP (Boehringer Mannheim), and the dig-labeled probes were detected using sheep anti-digoxigenin-fluorescein isothiocyanate and rabbit anti-sheep FITC. Chromosomes were viewed under an Olympus BX51 fluorescence microscope (Tokyo, Japan) equipped with a CoolSNAP fx CCD camera (Roper Scientific, USA). Gray scale images were captured for each fluorescence channel and then merged with V++ Precision Digital Imaging software (Digital Optics, New Zealand). Final images were fine-tuned with Adobe Photoshop 8.0 software.

Results

Production and Morphological Characterization of the Backcrossing Progenies

The monosomic alien addition line 8 (MAAL8; BC2) was a plant with a chromosome 8 of *O. officinalis* added to the entire chromosomes complement of cultivated rice species (Tan et al. 2005). Being an aneuploid plant, the MAAL8 was male sterile and had rolled leaves, which was different from both of the parents. We backcrossed the MAAL8 with the cultivated rice Hejiang19 (Fig. 1) and obtained a backcross population with 99 plants at BC3 generation. Phenotype analysis showed that the BC3 plants were almost the same in plant heights, but they could be obviously discriminated by leaf shapes: 58 plants had rolled leaves (R-leave, Fig. 2a) which were similar to the

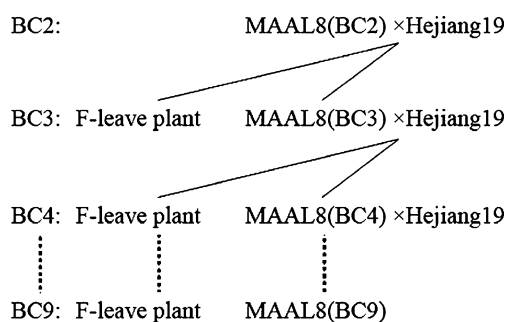


Fig. 1 Experimental schedule for the backcross program



Fig. 2 Morphological characters of two types of plants at BC₃ generation. **a** Plant with rolled leaves (*R-leaf*); **b** plant with flatted leaves (*F-leaf*)

female parent MAAL8; the other 41 had flatted leaves (*F-leave*, Fig. 2b) which were similar to the recurrent male parent Hejiang19. Beside the leaf shapes, other morphological characters among the two types of backcross progenies and their parents were showed in Table 1. Because the plants with *R-leaves* were similar to MAAL8 (BC2) in morphological features and were proved in the following experiments holding a whole alien chromosome 8 of C genome, we named them as MAAL8 (BC3) plants as well. The rolled leaves were used as the visible morphological traits of the aneuploid MAAL plants among the backcross progenies.

In the further experiments, the MAAL8 (BC3) plants were backcrossed with Hejiang19 again, and a population with 53 plants at BC4 generation was obtained. Of these 53 plants, 33 had *F-leaves* and 20 had *R-leaves*, and the latter were also identified as MAAL8 (BC4) plants. The phenotypes of the two typical plants, such as leaf color, heading date and number of panicles, were similar to those of plants at BC3 generation respectively. In this way, the progenies from BC5 to BC9 generations were obtained, and the MAAL8 (BC5), MAAL8 (BC6), MAAL8 (BC7), MAAL8 (BC8), and MAAL8 (BC9) plants were identified at different generations (Fig. 1). The phenotypes of the backcross progenies were stable, i.e., the plants with *R-leaves* of different generations had similar phenotypes and so did the plants with *F-leaves*. The numbers of two

Table 1 Phenotypes of parents and BC3 plants

Plant materials	Leaf shape		Leaf color	Heading date (day)	Number of panicles	Plant height (cm)	Male sterile
	Lengthways section	Transverse section					
Hejiang19	Curve	Flatted	Light green	63.7±2.3	11.6±3.2	65.9±5.2	No
F-leave plant	Curve	Flatted	Light green	72.9±7.1	17.0±1.6	71.3±1.3	Yes
R-leave plant	Erect	Rolled	Dark green	81.3±4.8	13.1±2.3	73.3±3.9	Yes
MAAL8	Erect	Rolled	Dark green	–	–	61.5	Yes

types of plants at different generations were shown in Table 2. The ratios of plants with R-leaves among total plants were variable and segregation ratio of these two types of plants showed no regular tendency. A notable fact was that the MAAL8 plants had been produced at all generations; the single alien chromosome 8 of *O. officinalis* had persisted transmitting through female gametes in the AA genomic background. It seemed that the MAAL8 was “immortal” in these backcross programs.

Chromosome Constitutions and Normal Mitosis in the Backcrossing Progenies

Because of much differences on DNA sequences between the A and C genomes, FISH analyses with C₀t-1 DNA from *O. officinalis* as probes could easily discriminated chromosomes of *O. officinalis* (CC) from those of cultivated rice (AA) in the hybrid nucleus, where the two kinds of chromosomes coexisted (Lan et al. 2006). FISH analyses with C₀t-1 DNA from *O. officinalis* were applied on cell preparations of root tip tissues harvested from the two types of backcross progenies. In the images of cell at metaphase, there were 24 chromosomes detected from the cells of plants with F-leaves (Fig. 3a). Except a little visible hybridized signals showed on the terminals of one pair of chromosomes, all the 24 chromosomes had almost invisible hybridized signals indicating they originated from the cultivated rice. The same experiments have been done on cell preparations from cultivated rice Hejiang19 and we also detected one chromosome pairs having a little visible hybridized signal on the terminals. So, the probed terminals would belong to the chromosomes of cultivated rice (unpublished data). From the images of mitotic cells of

plants with R-leaves, however, 25 chromosomes were detected (Fig. 3b). In order to distinguish the chromosomes of A- or C-gnome, we also performed bicolor FISH analysis: using biotin-labeled C₀t-1 DNA from *O. officinalis* as probes (Texas red) to detected chromosomes from C genome and digoxigenin-labeled centromeric DNA from cultivated rice as probes (green) to detected chromosomes from A-genome. Of these 25 chromosomes, 24 had little hybridized signals of labeled C₀t-1 DNA (Texas red) but many hybridized signals of labeled centromeric DNA (green), indicating they originated from cultivated rice; the other one was entirely covered with strong red hybridized signals and had invisible green hybridized signals, indicating it originated from *O. officinalis*.

In the images of cells at a later metaphase (Fig. 3c, d), the sister chromatids of several chromosomes of cultivated rice were found disjoining initially from the centromere and the alien chromosome (marked with white arrow) was found to have performed the same procedure. This phenomenon implied that the alien chromosome 8 performed normally at the mitosis like the native chromosomes in the hybrid cells.

When we performed FISH analyses on backcross progenies in different generations, we get similar results. Obviously, all the plants with R-leaves had an entire alien chromosome of *O. officinalis*. There were no arms or large fragments of alien chromosome 8 introgressing into the chromosomes of cultivated rice.

RFLP Analysis on the Backcrossing Progenies

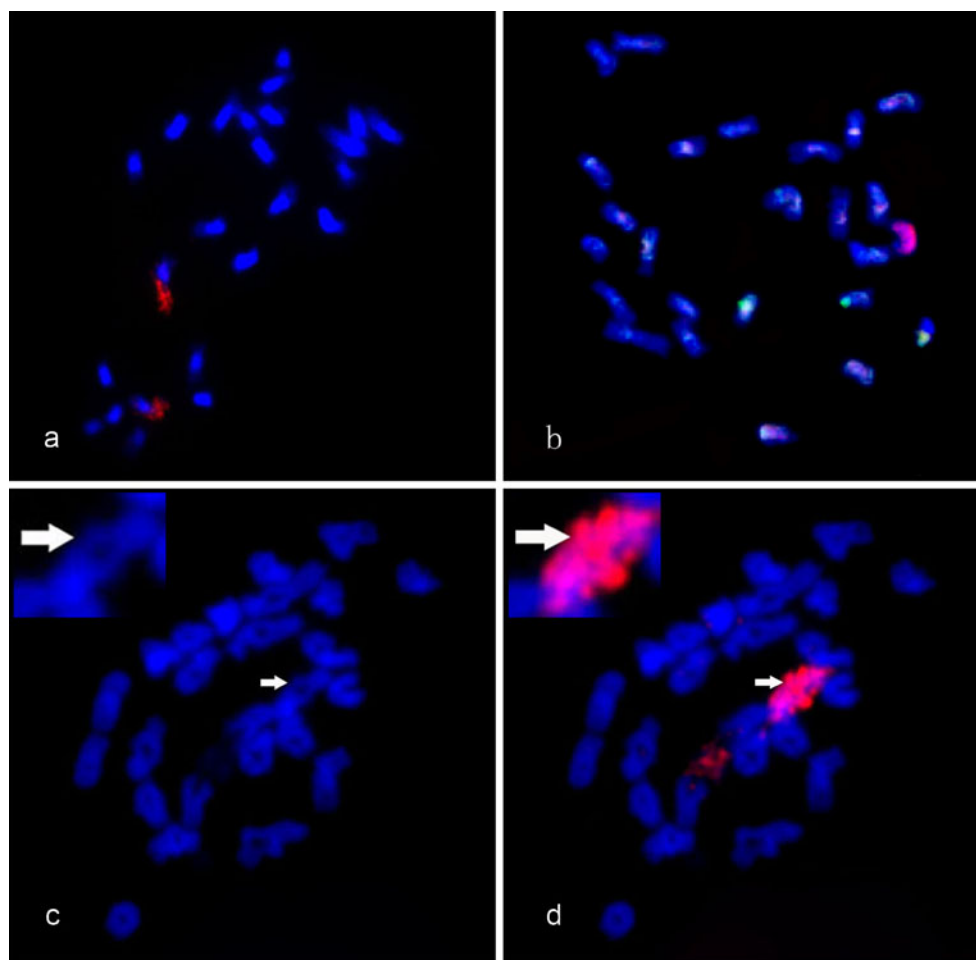
By comparing RFLP analysis, Jena et al. (1994) and Tan et al. (2005) had revealed homologous chromosomes between

Table 2 Numbers of two types of plants at different generations

generations	BC3	BC4	BC5	BC6	BC7	BC8	BC9
Plant with R-leaves	58	65	93	20	28	74	22
Plant with F-leaves	41	117	277	46	25	326	65
Total plants	99	182	370	66	53	400	87
Ratio of R-leave plant (%)	58.6	35.7	25.1	30.3	52.8	18.5	25.3

Fig. 3 Chromosome constitutions of the two types of plants revealed by FISH analysis.

a Mitotic cell from F-leaves plant; **b–d** mitotic cells from R-leaves plant. **b** the strong red signals were showed on the alien chromosome 8 and the green signals were showed on the centromere region of 24 chromosomes of cultivated rice; **c–d** showed the alien chromosomes 8 (pointed out with *white arrow*) disjoining normally at later metaphase as other chromosomes from cultivated rice. The enlarged alien chromosomes were showed in the top left corners



the A and C genomes sharing syntenic RFLP markers. In order to investigate the molecular integrity of the alien chromosome 8, we performed RFLP analysis on backcross progenies at different generations using 25 polymorphic RFLP markers that distributing evenly on chromosome 8 of cultivated rice (Fig. 4b) and could be used to identify the DNA fragments originated from chromosome 8 of *O. officinalis*. We analyzed all the 99 plants at BC3 generation, and found that each of the 25 markers had displayed *O. officinalis*-specific bands on the plants with R-leaves; but none of them displayed any *O. officinalis*-specific bands on the plants with F-leaves (Fig. 4a). These results showed that the molecules of alien chromosome 8 in MAAL8 (BC3) were intact, and the alien chromosome 8 was transferred integrally from the female parental MAAL8 (BC2) into the backcrossing progenies MAAL8 (BC3).

RFLP analyses were also applied on the progenies from BC4 to BC9 generations, and we get the same results like the BC3 generation. Each of the plants with R-leaves was detected with an intact alien chromosome 8, but the plants with F-leaves were detected with not any fragment of alien chromosome 8 by using all the 25 RFLP markers. These results were accorded to above FISH analysis, indicating

the alien chromosome 8 was transmitted from the female parent integrally and stably, so that an entire alien chromosome 8 was detected in the MAAL8 plants. There were no large DNA fragments of alien chromosome

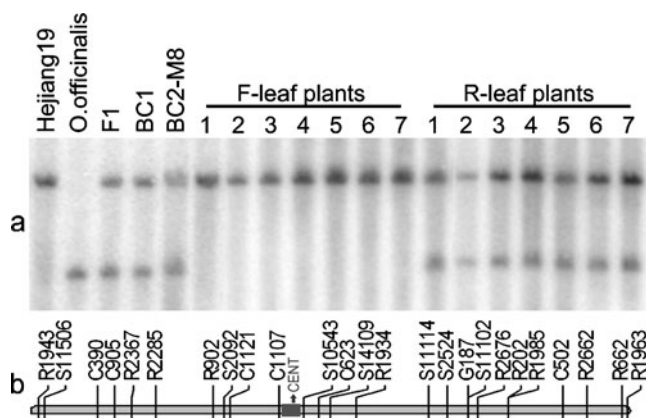


Fig. 4 Southern blotting analysis on parents and two types plants at BC₃ generation. BC2M8, MAAL8 at BC₂ generation. **a** Genomic DNA were digested with *EcoRV* and probed with marker C502 from chromosome 8 of AA genome; **b** the 25 used markers distributing on chromosome 8 of cultivated rice (data originate from <http://rgp.dna.affrc.go.jp>)

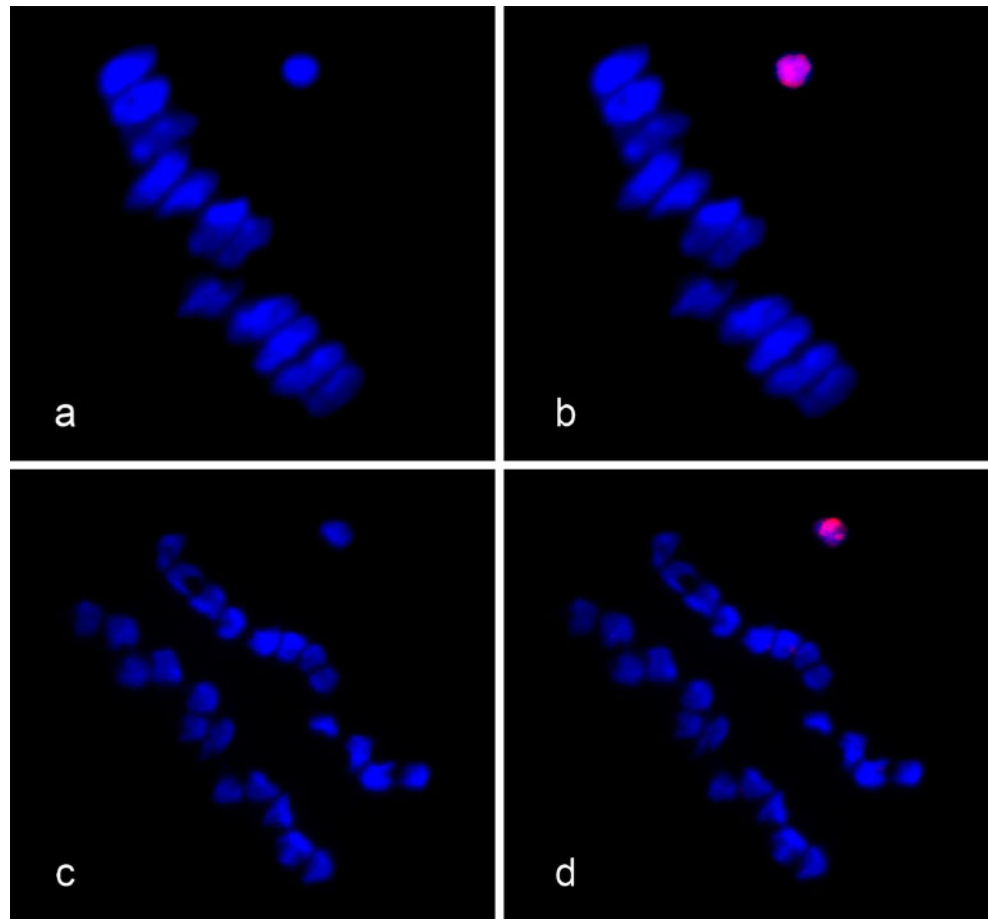
8 covering these 25 markers inserting into the chromosomes of cultivated rice.

Nonstandard Meiosis of Alien Chromosome 8 in the Aneuploid Plants

For the male sterile rice, the pollen abortions were usually found after the uninucleate stage and their meiosis process was normal (Pan 1976; Wu et al. 1994). In this study, we harvested the anther tissues of MAAL8 and studied their meiotic characters by FISH analysis. The meiosis I is crucial in the whole meiosis process because it involve in the chromosomes segregation, number reduction and the creation of genetic diversity (Nonomura et al. 2004). We used the young anther cutting down from the just heading rice for FISH analysis. When the biotin-labeled C0t-1 DNA of *O. officinalis* as probes were hybridized to chromosomes at meiotic metaphase I, 12 bivalents were showing in blue with very weak hybridized signals, suggesting their origination from cultivated rice; and one univalent was sprayed with strong Texas red, suggesting its origination from *O. officinalis* (Fig. 5a, b). The twelve bivalents were arrayed on the metaphase I plate, but the univalent of alien

chromosome 8 was apart from them. When the cells processed into meiotic anaphase I, the 12 bivalents disjoined into 24 chromosomes and arrayed in two groups, which would separated and segregated into the opposite poles. The univalent of alien chromosome 8 moved randomly to one pole (Fig. 5c, d). These results indicated that the meiocytes in aneuploid MAALs underwent a special meiotic process. The 24 chromosomes from cultivated rice performed normally as they paired at metaphase I and then separated at anaphase I, the chromosome number were halved in each secondary spermatocyte; but behaviors of alien chromosome at meiosis I were specific. As there was only one alien chromosome 8, no bivalent of alien chromosome was formed. The alien chromosome 8 was not found pairing with chromosome from cultivated rice, nor was it excluded out from chromosomes constitution of MAALs plants. This phenomenon was different from the newly formed hybrid cells who would exclude many of the alien chromosomes in their meiosis procedures. In these meiosis procedures, it seemed that the alien chromosome 8 just acted like an independent genetic factor and entered randomly into one of the secondary spermatocyte at meiotic anaphase I.

Fig. 5 FISH analysis behaves of the chromosomes at Meiosis I in R-leaves plants. **a** and **b** The 12 bivalents from cultivated rice were distributing on equatorial plate at metaphase I and the univalent of alien chromosome 8 was apart from them; **c** and **d** the homologous chromosomes from cultivated rice disjoined into opposite poles at anaphase I and the alien chromosome 8 move into one pole



Discussions

Distant hybrids between cultivated and wild rice species have been exploited extensively for the improvement of cultivated rice. Because of intergenomic conflicts, the alien genome in the hybrids was always unstable, elimination of partial or complete uniparental chromosome or specific DNA sequences occurred commonly. The formation of MAALs were the results that the wide hybrid cells of triploid BC1(2n+n) plant eliminated the most alien chromosomes but kept only one during their meiosis process, and these new materials are convenient for studying relationships between the A and C genomes. In this study, we used the MAAL materials to investigate actions of the monatomic alien C chromosomes inheriting the new A-genomic environments.

The aneuploid MAAL8 plants were male sterile, but the female gametes were functional and could be fertilized with pollens from recurrent parent Hejiang19 and produced backcross progenies. In a serial of backcross programs, we produced and identified the MAAL8 plants from BC3 to BC9 generations, the alien chromosome 8 have been transferred continuously. It seemed that the MAAL8 was “immortal” in these sexual reproduction programs. In our lab, the MAAL7 and MAL9 materials were also backcrossed with cultivated rice to produce backcross progenies from BC3 to BC5 generations and they get similar results (private communication). The actions of the monatomic alien chromosome inheriting stably in the MAAL plant was much different from those of the new formed hybrids between wild rice and cultivated rice, in which the chromosomes composition in the nuclear was unstable and elimination of alien chromosomes were often happened. Through comparison between MAALs and newly formed distant hybrid, we think the reduction of dosage effect of exogenous genetic material in the MAAL plant might involve its different inheriting characteristics. When there was only one alien chromosome, the intergenomic conflicts would decrease and were sustainable by the nucleus of cultivated rice. So, the alien chromosome was not excluded but was continuously transferred to the female gametes. Though the theoretical ratio of MAAL plants would be half in the backcross progenies, in fact, the MAAL plants were weak on the seedling stage and die easily; the surviving MAAL plants were reduced. The final ratios of MAAL plants among total plants were low and showed no regular tendency.

Usually, the homologous chromosomes in the ordinary hybrids within the AA genome could recombine freely and produce introgression lines easily. But it was a different thing in the distant hybrids between A and C genomes. In previous studies, Brar and Khush had pointed out that the recombination between chromosomes of cultivated rice and *O. officinalis* was limited in their hybrid and no substitution

of large chromosomes fragments was found. The detected introgressions were small in size by an inconvenient method (Brar and Khush 1997). In our lab, Tan et al. had obtained several introgression lines from new formed hybrid between *O. officinalis* and cultivated rice (Tan et al. 2004a, b). But it became difficult once the alien chromosomes were inheriting stably in the aneuploid plants. In this study, we found the alien chromosome 8 in the MAAL8 was transmitted integrally and stably. Molecular analyzing with 25 polymorphic RFLP markers, we checked an intact alien chromosome in each MAAL8 plant and there was not any *officinalis*-specific fragments covering the 25 RFLP markers inserting into the chromosomes of cultivated rice. Using FISH analysis, we also detected an intact alien chromosome 8 in the MAAL8 plant and no fragments of alien chromosome inserting into chromosomes of cultivated rice. In further studies, we investigated the actions of the alien chromosome in meiosis process and found its nonstandard meiosis process. The alien chromosome acted like an independent genetic factor: it did not paired with chromosome from cultivated rice, not participated in composition of bivalent, not join the formation of the metaphase I plate and entered randomly into one of the secondary spermatocyte at meiotic anaphase I. The special meiosis of alien chromosome may be the cytological reason for the recombination barrier between the homologous chromosomes of different genomes.

O. officinalis is a distantly related species of cultivated rice and its CC genome is close to the AA genome of *O. sativa*, so, we could produce their distant hybrid and use this material for improvement of cultivated rice. On the other hand, their intergenomic conflict obstructed our uses of the wild rice materials. The interaction between AA and CC genomes were much complex. Here, we studied the interaction between an alien chromosome of *O. officinalis* and nucleus of cultivated rice. The differentiation between AA and CC genomes, such as CC genome hold abundant specific repeat sequences and is much bigger relatively, would result in the special meiosis of the alien chromosome in MAAL plants. Owing to this nonstandard meiosis, the alien chromosome could be transferred integrally and stably. Further studies were needed to clearly illustrate the relationship between AA and CC genomes, for it is very important for the utility of wild rice materials.

Acknowledgements This study was supported by grants from the Natural Science Foundation of Hubei Province of China (2008CDB392), the Natural Science Foundation of South-Central University for Nationalities (YZZ08005) and National Special Program for Research and Industrialization of Transgenic Plants of China. We sincerely thank the group of plant genetics and development of South-Central University for Nationalities providing laboratory platform, and thank Professor Jin Weiwei in China Agriculture University for providing A-genomic specific centromere DNA.

References

- Aggarwal RK, Brar DS, Khush GS (1997) Two new genomes in the *Oryza* complex identified on the basis of molecular divergence analysis using total genomic DNA hybridization. *Mol Gen Genet* 254:1–12
- Brar DS, Khush GS (1997) Alien introgression in rice. *Plant Mol Biol* 35:35–47
- Comai L, Tyagi AP, Winter K, Holmes-Davis R, Reynolds SH, Stevens Y, Byers B (2000) Phenotypic instability and rapid gene silencing in newly formed Arabidopsis allotetraploids. *Plant Cell* 12:1551–1567
- Du XZ, Ge XH, Zhao ZG, Li ZY (2008) Chromosome elimination and fragment introgression and recombination producing intertribal partial hybrids from *Brassica napus* × *Lesquerella fendleri* crosses. *Plant Cell Rep* 27(2):261–271
- Feldman M, Liu B, Segal G, Abbo S, Levy AA, Vega JM (1997) Rapid elimination of low-copy DNA sequences in polyploid wheat: a possible mechanism for differentiation of homoeologous chromosomes. *Genetics* 147:1381–1387
- Gernand D, Rutten T, Varshney A, Rubtsova M, Prodanovic S, Bruß C, Kumlehn J, Matzk F, Houben A (2005) Uniparental chromosome elimination at mitosis and interphase in wheat and pearl millet crosses involves micronucleus formation, progressive heterochromatinization, and DNA fragmentation. *Plant Cell* 17(9):2431–2438
- Hanson RE, Islam-Faridi MN, Crane CF, Zwick MS, Czeschin DG, Wendel JF, McKnight TD, Price HJ, Stelly DM (2000) Ty1-copia-retrotransposon behavior in a polyploidy cotton. *Chromosome Res* 8:73–76
- Huang Z, He GC, Shu LH, Li XH, Zhang QF (2001) Identification and mapping of two brown planthopper genes in rice. *Theor Appl Genet* 102:929–934
- Jena KK, Khush GS (1990) Introgression of genes from *Oryza officinalis* Will ex Watt to cultivated rice, *O. sativa* L. *Theor Appl Genet* 80:737–745
- Jena KK, Khush GS, Kochert G (1994) Comparative RFLP mapping of a wild rice, *Oryza officinalis*, and cultivated rice, *O. sativa*. *Genome* 37:382–389
- Jin HJ, Tan GX, Brar DS, Tang M, Li G, Zhu LL, He GC (2006) Molecular and cytogenetic characterization of an *Oryza officinalis*-*O. sativa* chromosome 4 addition line and its progenies. *Plant Mol Biol* 62:769–777
- Jin HJ, Hu W, Wei Z, Wan LL, Li G, Tan GX, Zhu LL, He GC (2008) Alterations in cytosine methylation and species-specific transcription induced by interspecific hybridization between *Oryza sativa* and *O. officinalis*. *Theor Appl Genet* 117:1271–1279
- Lan WZ, Qin R, Li G, He GC (2006) Comparative analysis of A, B, C and D genomes in the genus *Oryza* with Cot-1 DNA of C-genome. *Chin Sci Bull* 51(14):1710–1720
- Leitch IJ, Bennett MD (1997) Polyploidy in angiosperms. *Trends Plant Sci* 2:470–476
- Li G, Hu W, Qin R, Jin HJ, Tan GX, Zhu LL, He GC (2008) Simple sequence repeat analyses of interspecific hybrids and MAALs of *Oryza officinalis* and *Oryza sativa*. *Genetica* 134:169–180
- Linde-Laursen I, Bothmer R (1999) Orderly arrangement of the chromosomes within barley genomes of chromosome-eliminating *Hordeum lechleri* × barley hybrids. *Genome* 42:225–236
- Liu L, Lafitte R, Guan D (2004) Wild *Oryza* species as potential sources of drought-adaptive traits. *Euphytica* 138:149–161
- Matzke MA, Mittelsten SO, Matzke AJ (1999) Rapid structural and epigenetic changes in polyploid and aneuploid genomes. *Bioessays* 21:761–767
- Multani DS, Khush GS, Delos Reyes BG et al (2003) Alien genes introgression and development of monosomic alien addition lines from *Oryza latifolia* Desv to rice, *Oryza sativa* L. *Theor Appl Genet* 107:395–405
- Murray MG, Thompson WF (1980) Rapid isolation of highmolecular-weight plant DNA. *Nucleic Acids Res* 8:4321–4325
- Nonomura K, Nakano M, Fukuda T, Eiguchi M, Miyao A, Hirochika H, Kurata N (2004) The novel gene HOMOLOGOUS PAIRING ABERRATION IN RICE MEIOSIS1 of rice encodes a putative coiled-coil protein required for homologous chromosome pairing in meiosis. *Plant Cell* 16:1008–1020
- Pan KQ (1976) Cyto-histological investigations on pollen abortion in malesterile line of the wild rice pollen abortion type. *J Genet Genomics* 6(2):211–215
- Riddle NC, Birchler JA (2003) Effects of reunited diverged regulatory hierarchies in allopolyploids and species hybrids. *Trends Genet* 19:597–600
- Shaked H, Kashkush K, Ozkan H, Feldman M, Levy AA (2001) Sequence elimination and cytosine methylation are rapid and reproducible responses of the genome to wide hybridization and allopolyploidy in wheat. *Plant Cell* 13:1749–1759
- Song YC, Gustafson JP (1995) The physical location of fourteen RFLP markers in rice (*Oryza sativa* L.). *Theor Appl Genet* 90:113–119
- Song K, Lu P, Tang K, Osborn TC (1995) Rapid genome change in synthetic polyploids of *Brassica* and its implications for polyploid evolution. *Proc Natl Acad Sci USA* 92:7719–7723
- Tan GX, Huang Z, Weng QM, Ren X, Zhu LL, He GC (2004a) Two whitebacked planthopper resistance genes shared the same genomic intervals with brown planthopper resistance genes. *Heredity* 92:212–217
- Tan GX, Weng QM, Ren X, Shi ZY, Zhu LL, He GC (2004b) Mapping of a new resistance gene to bacterial blight in rice line introgressed from *Oryza officinalis*. *Acta Genetica Sinica* 31:724–729
- Tan GX, Jin HJ, Li G, He RF, Zhu LL, He GC (2005) Production and characterization of a complete set of individual chromosome additions from *Oryza officinalis* to *Oryza sativa* using RFLP and GISH analyses. *Theor Appl Genet* 111:1585–1595
- Vaughan DA, Morishima H, Kadowaki K (2003) Diversity in the *Oryza* genus. *Curr Opin Plant Biol* 6:139–146
- Wendel JF, Schnabel A, Seelanan T (1995) Bidirectional interlocus concerted evolution following allopolyploid speciation in cotton (*Gossypium*). *Proc Natl Acad Sci USA* 92:280–284
- Wu SB, Zhou KY, Wan JH (1994) Study on the abortive processes of pollen in male sterile rice. *Acta Bot Sin* 36(10):751–754
- Xiong ZY, Tan GX, He GY, He GC, Song YC (2006) Cytogenetic comparisons between A and G genomes in *Oryza* using genomic in situ hybridization. *Cell Res* 16:260–266
- Yan HH, Min SK, Zhu LH (1999) Visualization of *Oryza eichingeri* chromosomes in intergenomic hybrid plants from *O. sativa* × *O. eichingeri* via fluorescent in situ hybridization. *Genome* 42:48–51
- Ying B, Hai FZ, De YH, Song G (2006) Genetic diversity and evolutionary relationships of *Oryza* species with the B- and C-genomes as revealed by SSR markers. *J Plant Biol* 49(5):339–347
- Zwick MS, Hanson RE, McKnight TD, Islam-Faridi MH, Stelly DM, Wing RA, Price H (1997) A rapid procedure for the isolation of *C₀t-1* DNA from plants. *Genome* 40:138–142