Detecting Nature of Chromosome Pairing in A and E Genomes of *Oryza*

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Received April 26, 2009; accepted August 16, 2009

Summary Genomic *in situ* hybridization (GISH), using genomic DNA probe from *O. australiensis*, was used to study chromosome pairing among AA, EE and AE genomes, in the hybrid *O. sativa* × *O. australiensis*. In the conventional cytogenetic analysis, 0–4 bivalents and 20–24 univalents were recorded. GISH, however, revealed 1–5 bivalents and 19–23 univalents. 3 types of pairing were detected: pairing between A and E genome chromosomes, within AA genome chromosomes and within EE genome chromosomes. The frequency of association between *O. sativa* (AA) and *O. australiensis* (EE) chromosomes (0.98II/cell) greatly exceeded the level of pairing, within *sativa* chromosomes (0.15II/cell) or within *australiensis* chromosomes (0.05II/cell). Results indicated that conventional cytogenetic analysis either underestimates or overestimates the pairing behavior and that GISH is a powerful tool for detecting the nature of pairing in *O. sativa* × *O. australiensis*.

Key words Rice, *Oryza sativa*, GISH, Autosyndetic and allosyndetic pairing.

Wide hybridization is the most important breeding method for transferring agronomically important traits from wild into cultivated rice. The success in the introgression is based on the frequency of recombination in F1 hybrid. It is generally accepted that metaphase-I pairing reflects the chiasma formation, which leads to recombination (Benavente *et al.* 1996). Thus, the successful alien gene transfer could be greatly facilitated by knowledge of the occurrence and frequency of chromosome pairing (Miller *et al.* 1995). Conventional techniques for pairing analysis may overestimate or underestimate the homoeologous pairing. It is difficult to distinguish intergenomic and intragenomic chromosome pairing by conventional meiotic analysis (Abbasi *et al.* 1999). GISH is becoming an important technique for characterizing parental chromosome pairing in wide hybrids, detecting introgressed segment and chromosome rearrangements. GISH has been used for characterizing parental chromosomes in *Triticeae* species (Anamthawat-Jonsson *et al.* 1990, Orgaard and Anamthawat-Jonsson 2001) and *Solanum* and *Lycopersicon* species (Haider *et al.* 2001). We have characterized parental chromosomes in rice wide hybrids (Abbasi *et al.* 1998a, b). Here we used GISH as a tool for studying pairing in *Oryza* and in this study we presented a seminal report.

Materials and methods

Preparation of genomic DNA

The genomic DNA was isolated from 5–10 g fresh leaves from *O. australiensis* and *O. sativa*, using the method of Dellaporta *et al.* (1983). The DNA was digested with EcoRI and labeled with Biotin-14-dATP, by nick translation (Gibco BRL), according to standard nick translation labeling system.

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Meiotic chromosomes preparations

Panicles were collected from the field-grown hybrid, *O. sativa*×*O. australiensis*, and fixed in acetic alcohol (3 : 1). Anthers at suitable stages were squashed in 45% glacial acetic acid and the cells were spread on the slide, with a drop of fixative. For conventional cytogenetic analysis, these slides were examined under a microscope, after staining with aceto-carmin. For GISH analysis, the slides were air dried and dehydrated in ethanol series: 70%, 95% and 100% at room temperature for 1 h.

Genomic *in situ* hybridization

The hybridization mixture, containing 120 ng of biotinylated probe, 50% formamide, 3 µg SSS DNA, 2×SSC and 2.4 µg unlabeled *sativa* DNA, was denatured at 80°C for 10 min and immediately quenched in ice for 5 min. An aliquot of 18 µl, dropped on each slide and covered with a cover slip, was sealed with paper bond and air dried. The chromosomes denatured at 80°C for 10 min, using a thermal cycler (Hybaid), followed by incubation at 37°C for 18 h. The cover slips were removed in 2×SSC, and the slides were washed with 2×SSC 2 times, and once with 4×SSC at 42°C for 10 min each. An aliquot of 100 µl of blocking solution, containing 5% bovine serum albumin (BSA) in 4×SSCt (4×SSC+0.05% Tween-20), was dropped on each slide, covered with a cover slip and incubated for 5 min at 37°C. An aliquot of 70 µl, fluorescein isothiocyanate (FITC)-Avidin (Boehringer Mannheim) in 1% BSA/4×SSCt, was layered on the slides and incubated for 60 min at 37°C. The slides were washed 3 times with BT buffer (Sodium carbonate+Tween 20) for 10 min each at 37°C. After washing, the blocking was carried out by 5% (v/v) goat serum (Cosmo Bio. Ltd.) for 5 min at 37°C. An aliquot of 70 µl biotinylated-anti-avidin solution in 1% BSA was dropped on each slide, and incubated for 60 min at 37°C. The slides were washed thoroughly with BT buffer twice and once with 2×SSC for 10 min each at 37°C, dehydrated in ethanol series: 70%, 95% and 100% for 3 min each at room temperature.

The chromosomes were counterstained with propidium iodide (Sigma), 1 µl/ml in water for 2 min. Each slide was mounted with 15 µl of vectashield. The slides were screened with a fluorescence microscope (Axiophot Zeiss), equipped with filter set no. 05, 09 and 25. Photographs were taken with a Kodak Ektacolor, ASA/ISO 400.

Results and discussion

Conventional chromosome pairing analysis. The interspecific hybrid between *O. sativa*×*O. australiensis* (2n=24, AE), was highly sterile with pollen stainability being 0.05%. The hybrid showed limited chromosome pairing. On an average, 0–4 bivalents and 16–24 univalent were recorded at metaphase I (Table 1). The most frequent configuration was 2II+20I. The bivalents were rod shaped, end-to-end pairing and highly heteromorphic. In the majority of PMCs (55%), the chromosomes were unpaired and appeared as 24 univalents. In 40% of the cells, 1–2 bivalents were observed and a few cells (3.3%) showed 3–4 bivalents. The distribution of chromosomes, at

Table 1. Homoeologous association, among A and E genome chromosomes of *Oryza*, as revealed by conventional cytogenetic analysis

	No. of cell analyzed	Univalents				Bivalents (mean/cell)				
		Range	Mean	Range	Mean	0II	1II	2II	3II	4II
Diakinesis	98	16–24	22.12	0–4	0.79	55	19.38	19.38	4.08	2.04
Metaphase I	150	16–24	22.48	0–4	0.73	56	19.38	21.33	1.33	2.00

anaphase 1, was highly irregular. All the previous meiotic studies, on chromosome associations in the hybrid *O. sativa*×*O. australiensis*, were based on conventional cytogenetic analysis. Some of these studies showed all unpaired chromosomes (Nezu *et al.* 1960), however, other studies detected limited pairing among the chromosomes of A and E genomes (Shastry and Ranga Rao 1961). The limitations in these studies were that the actual chromosome associations between A and E genomes were not established, because the conventional technique was unable to present actual pairing behavior.

GISH for meiotic pairing analysis. Genomic DNA from *O. australiensis* was labeled with biotin-14-dATP and used as probe to meiotic chromosomes of *O. sativa*×*O. australiensis* hybrid. The probe detected with FITC. Chromosomes showing the hybridizational signal, fluoresced yellow green, unlabeled *sativa* chromosomes were red due to counterstaining with propidium iodide (PI). This differential painting of chromosomes, through fluorescent GISH, was used as a tool to discriminate parental chromosomes and *autosyndetic* or *allosyndetic* pairing in the hybrid.

At diakinesis, 191 cells were examined, with a total of 441 bivalents, of which 391 bivalents were involved in allosyndetic chromosome association, 30 bivalents were from pairing within *sativa* chromosomes and 20 bivalents showed pairing within *australiensis* chromosomes. At metaphase-1, 255 cells were analyzed. Of the 300 bivalents scored, an average 0.98 bivalents per cell occurred between *sativa* and *australiensis* chromosomes. Among *autosyndetic* pairing, the frequency of *sativa/sativa* homologous pairing was much higher (0.15 bivalents/cell), compared to the *australiensis/australiensis* pairing: 0.05 bivalents per cell (Table 2). All the bivalents were of open type (Fig. 1).

GISH analysis from our results detected 3 types of chromosomal pairing, like A/A, A/E and E/E. Among the autosyndetic pairing, the frequency of *sativa/sativa* pairing was much higher (0.15

Table 2. Auto-and-allosyndetic pairing among A and E genomes of *Oryza*, as revealed by GISH

	No. of P MCs analyzed	Univalents no. (mean/cell)	Bivalents no. (mean/cell)		
			A/A	A/E	E/E
Diakinesis	191	3702 (19.38)	30 (0.15)	391 (2.05)	20 (0.10)
Metaphase 1	255	5520 (21.65)	39 (0.15)	249 (0.98)	12 (0.05)

Fig. 1. (A and B) Chromosome configurations at metaphase-1: *O. sativa*×*O. australiensis*. Genomic DNA from *O. australiensis* was used as a label probe to the meiotic metaphase chromosomes. The label chromosomes were detected with anti-dig FITC (yellow) and counterstained with propidium iodide (red). Arrows indicate the pairing between A and E genome chromosomes and arrow heads show the pairing between A/A genome chromosomes or within E/E genome chromosomes. A) $2_{II\ A/E} + 1_{II\ A/A} + 1_{II\ E/E}$. B) $3_{II\ A/E} + 1_{II\ A/A}$.

bivalents per cell), compared to *australiensis/australiensis* chromosome pairing (0.05 bivalents per cell) (Table 2). The homoeologous pairing (A–E) was slightly higher (0.79II/cell) than the homoeologous pairing detected by conventional cytogenetic analysis, (0.78II/cell). This indicates that conventional cytogenetic analysis was unable to detect the identity of chromosomes involved in pairing. Autosyndetic pairing in earlier studies using the conventional approach, considered as homoeologous pairing among A and E genomes; and conclusions drawn from such traditional pairing data might need to be reevaluated.

The association between A and E genomes was end-to-end, pseudo-bivalent. The lack of any true allosyndetic bivalents in *O. sativa* × *O. australiensis* may be interpreted as indicating that there is a lack of homology between the chromosomes of *O. sativa* and *O. australiensis* or it may be due to the difference in the meiotic cycle of both the genomes.

The occurrence of autosyndetic bivalents in *O. australiensis* may be due to the exchange of segments during the process of evolution or it may be polyploidy. The autosyndetic pairing involving *sativa* chromosomes may be interpreted as showing that during domestication and improvement for desired traits, some of the segments interchanged among the haploid chromosomes. We were able to discriminate homoeologous pairing by differential painting of chromosomes which unequivocally established the pairing behavior between A and E genomes of *Oryza*. Previous studies, using GISH as molecular cytogenetic tool in other crops, considered it a powerful technique to distinguish the chromosomes of 1 genome from other genomes (Mukai *et al.* 1993, Friebe *et al.* 1994, Cai *et al.* 1998). In the present study, GISH was used successfully with *Oryza* as a cytogenetic technique to characterize inter- and intra- specific chromosome pairing.

The analysis of chromosome pairing has been critical for the determination of the genomic relationships between species. However, collection and interpretation of data varies considerably from one study to another. Some of the early work concluded genomic relationships based on the number of bivalents. Numerical approaches to the analysis of meiosis in hybrids have been developed (Alonso and Kimber 1981). However, numerical analysis can not differentiate between autosyndetic and allosyndetic pairing. Our results showed that GISH offers a reliable means to discriminate between the identity of chromosomes involved in pairing, using genomic DNA as a probe. GISH provides a great opportunity to unravel the secret of chromosome pairing between different species (Cuadrado *et al.* 1997, Benavante *et al.* 1996). GISH analysis showed that the frequency of recombination is very low. Radiation or pairing controlling system has to be used, to facilitate the recombination between A and E genomes chromosomes.

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