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Detection of Alien *Oryza punctata* Kotschy Chromosomes in Rice, *Oryza sativa* L., by Genomic *in situ* Hybridization

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Genomic *in situ* hybridization (GISH) using total *Oryza punctata* Kotschy genomic DNA as a probe was applied to detect alien chromosomes transferred from *O. punctata* (W1514: 2n=2x=24: BB) to *O. sativa* Japonica cultivar, Nipponbare (2n=2x=24: AA). Only 12 chromosomes in the interspecific hybrids (2n=3x=36: AAB) between autotetraploid of *O. sativa* cultivar Nipponbare and a diploid strain of *O. punctata* (W1514) showed intense staining by FITC in mitotic metaphase spreads. Only one homologous pair of chromosomes out of the 12 pairs of *O. sativa* chromosomes, which is probably chromosome 8, contained similar repetitive sequences as present in *O. punctata* and were frequently slightly stained by GISH. Monosomic alien addition line(MAAL)s 7 (2n=2x+1=25: AA+7B: Type II) and 10 (2n=2x+1=25: AA+10B) were characterized by an additional single chromosome which had been intensely stained by GISH. Thus, GISH using *O. punctata* total genomic DNA allowed the detection of a specific chromosome transferred into cultivated rice. This method can be useful to search introgressed chromosomal segments or translocated chromosomes in the progenies of the MAALs each carrying an alien chromosome of *O. punctata* in rice.

INTRODUCTION

Repetitive sequences are well known to rapidly amplify in genomes after isolation from a common ancestral species and evolve toward species-specificity in animals (Schmid and Shen, 1986; Batzer and Deininger, 1991) and plants (Walbot and Cullis, 1985; Vedel and Delesny, 1987). These repetitive sequences, therefore, tend to be conservative within a genome, but differentiated among genomes. "Chromosome painting", a recent technique in molecular cytology, has made it possible to detect genome-specific chromosomes using repetitive sequences as probe. Genomic *in situ* hybridization (GISH) using total genomic DNA as a probe is a similar approach because total genomic DNA is rich in repetitive sequences, and can also detect genome-specific chromosomes in hybrids derived from a cross between closely or distantly related species (Anamthawat-Jonsson *et al.*, 1990; Mukai and Gill, 1991; Mukai *et al.*, 1991). This method can be useful in identifying specific alien chromosomes transferred into monosomic alien addition lines (MAALs) carrying a single chromosome of *O. punctata* in addition to normal chromosome complement of *O. sativa*. This study was designed to establish if GISH using total *O. punctata* DNA as a probe would allow for the intense and specific fluorescent staining of *O. punctata* chromosomes in prometaphase spreads and interphase nuclei of the progeny from an interspecific cross between *O. sativa* and *O.*

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punctata. In addition, the chromosome rearrangement of *O. punctata* chromosomes transferred into rice will be discussed.

MATERIALS AND METHODS

Plant materials

The Japonica rice cultivar Nipponbare (2n=2x=24: AA), a diploid strain of *O. punctata* (W1514: 2n=2x=24: BB), their interspecific hybrid (2n=3x=36: AAB), and two MAALs 7 (2n=2x+1=25: AA+7B) (Type II) and 10 (2n=2x+1=25: AA+10B) were used in the present study. The diploid strain of *O. punctata* (W1514) was collected in Kenya in 1964 (Tateoka, 1965) and the seeds have been supplied from the National Institute of Genetics, Japan. This strain has been studied cytologically to determine genomic relationship between *O. sativa* and *O. punctata* (Katayama, 1967; Katayama and Ogawa, 1974; Ogawa and Katayama, 1974). The original interspecific cross was made between Nipponbare autotetraploids (2n=4x=48: AAAA) and W1514. The interspecific hybrids were obtained through embryo rescue. A series of MAALs were developed and isolated from the backcrossed progeny (Yasui and Iwata, 1991). MAAL 7 (Type II) and MAAL 10 had been identified by morphological resemblance to related trisomics (Yasui and Iwata, 1991) and RFLP analysis (Yasui *et al.*, 1992). Total genomic DNA of W1514 was isolated from leaves of a single plant. DNA was extracted from the ground tissue using the slightly modified potassium acetate method (Dellaporta *et al.*, 1983).

Genomic *in situ* hybridization (GISH)

Chromosome preparation: Prometaphase spreads from the plants were prepared from freshly emerged root tip tissues by using a modification of the procedure described by Kurata and Omura (1978). These root tip tissue was fixed in 3:1 ethanol/acetic acid, followed by 0.002 M 8-hydroxyquinoline treatment at 20 °C for 90 minutes, and macerated with the solution containing 4% (w/v) Cellulase Onozuka RS and 1% (w/v) Pectolyase Y-23 in 7.5 mM KCl at 37 °C for 30–45 minutes. Prometaphase spreads were prepared by flame drying following fixation in 3:1 methanol/acetic acid. Well spread chromosomes were stained with 10% Giemsa solution diluted by Sørensen phosphate buffer and observed for karyotype.

Hybridization: Genomic *in situ* hybridization was conducted as described by Hori *et al.* (1990) with a slight modification. Total *O. punctata* genomic DNA was used as a probe to detect the chromosome transferred from *O. punctata*. The DNA in the target cells was denatured by immersion in 70% formamide/0.3 M NaCl/0.03 M sodium citrate (2×SSC) for 3 minutes at 80 °C. The slides were rapidly dehydrated in an ethanol series (70%, 100%) and air-dried. The hybridization mixture [270 ml total volume consisting of 10% formamide, 2×SSC, 20% dextran sulfate, 1 mg sonicated herring DNA per ml and 700 ng digoxygenin (DIG) labeled total *O. punctata* DNA] was then heated as above and applied. Hybridization was at 37 °C overnight (15 hours). Slides were washed in 50% formamide/2×SSC for 15 minutes at 37°C, and rinsed in 2×SSC, 1×SSC, 4×SSC at room temperature each for 15 minutes.

Detection: For detection of hybridization sites, the slides were counterstained using the FITC-PI method. The slides were treated with a solution of a complex of anti-DIG-

FITC in 1% BSA/4×SSC. After rinsing, the slides were counterstained with 1.25 mg/ml propidium iodide for 30 minutes. Photographs of GISH prometaphase spreads were taken with Zeiss Axioplan photomicroscope using Fuji Fujichrome films.

RESULTS

There were no intense signal in metaphase spreads of *O. sativa* cultivar Nipponbare as a result of GISH using total *O. punctata* genomic DNA. The centromeric regions of chromosomes were frequently slightly stained (Data are not shown).

Among the prometaphase spreads of interspecific hybrids, only 12 chromosomes in many nuclei were intensely stained by FITC (Fig. 1a). In some prometaphase nuclei, however, more than 12 (13–14) chromosomes were intensely stained (Fig. 1b). These frequently stained additional chromosomes seemed to be a couple of homologous chromosome 8 of *O. sativa* because of their karyotypic feature.

One chromosome had been intensely stained in each prometaphase and interphase spreads in MAAL 7 (Type Ⅱ) and MAAL 10 (Fig. 2). Both plates a (top and bottom) show GISH nuclei, while both plates b (top and bottom) show Giemsa staining nuclei. The intensely stained chromosome in MAAL 7 (Type Ⅱ) was medium and submetacentric and it was different from chromosome 7 of *O. sativa* which was small and metacentric. The intensely stained chromosome in MAAL 10 was small and submetacentric and was similar

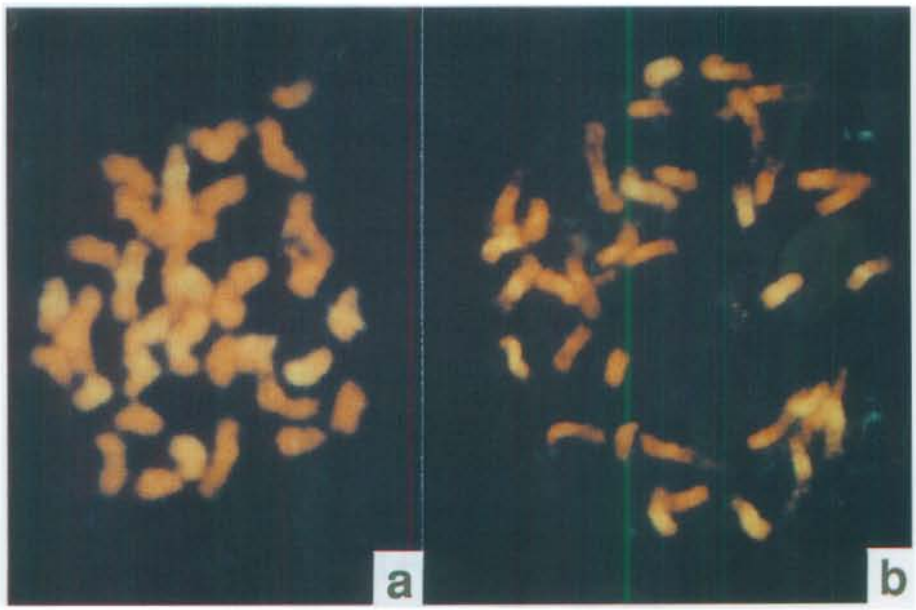

Fig. 1. GISH of the interspecific hybrids (2n=3x=AAB) with *O. punctata* total DNA as a probe, showing the twelve (a) and thirteen (b) chromosomes intensely stained by FITC. Note the intensely stained sites which appear to be heterochromatic regions.

to chromosome 10 of *O. sativa* which was small and with heterochromatic region in the entire short arm (Fig. 3).

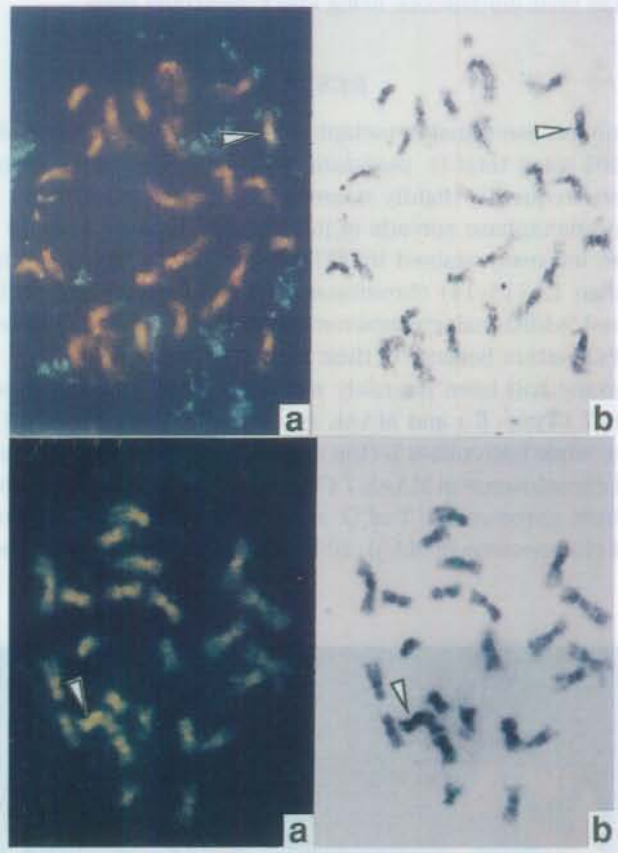

Fig. 2. GISH with *O. punctata* total DNA as a probe (a) and Giemsa staining (b) of MAAL 7 (Type Ⅱ) and MAAL 10, showing the hybridization sites on chromosomes (Arrowheads). Upper and bottom plates show MAAL 7 (Type Ⅱ) and MAAL 10, respectively.

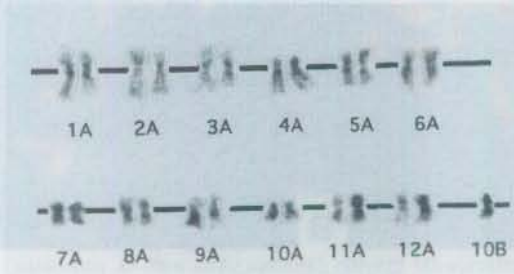

Fig. 3 Karyotype of MAAL 10 revealed by using GISH. Chromosome 10B characterized by its small size, sub-metacentric type and heterochromatic region in entire short arm was similar to *O. sativa* chromosome 10. The capital letters A and B show the chromosome(s) of *O. sativa* and *O. punctata*, respectively.

DISCUSSION

There were no intense signal in metaphase spreads of *O. sativa* cultivar Nipponbare as a result of GISH using total *O. punctata* genomic DNA. This suggests that the genome of *O. punctata* is differentiated from that of *O. sativa*. Only 12 chromosomes were intensely stained by FITC in the interspecific hybrids carrying 24 chromosomes of *O. sativa* and 12 chromosomes of *O. punctata*. Among 12 pairs of *O. sativa* chromosomes, only one homologous pair of chromosomes, presumably chromosome 8, was likely to have the specific repetitive sequences so that this pair of chromosomes were slightly stained by GISH frequently.

MAAL 7 (Type Ⅱ) and MAAL 10 were characterized by their additional chromosomes which were intensely stained by FITC. The intensely stained chromosome in MAAL 7 (Type Ⅱ) was medium and submetacentric and the karyotypic feature was different from chromosome 7 of *O. sativa* (characterized by small size and metacentric type). MAAL 7 (Type Ⅱ) with brown pericarp had been derived from the progenies of MAAL 7 (Type Ⅰ) with red pericarp inherited from *O. punctata* (Yasui and Iwata, 1991). Moreover, RFLP characterization of Type Ⅱ had shown deficiency of several RFLP loci detected in Type Ⅰ (Yasui *et al.*, 1992). These indicate that the alien chromosome in MAAL 7 (Type Ⅱ) had been modified by chromosome rearrangement such as deletion or translocation. The intensely stained chromosome in MAAL 10 was similar to chromosome 10 of *O. sativa*. The results of karyotyping showed that karyological structure of chromosome 10 in both genomes were conservative.

GISH using *O. punctata* total DNA as a probe allowed the detection of a specific *O. punctata* chromosome transferred into cultivated rice. GISH, therefore, can be used in searching introgressed chromosomal segments or translocated chromosomes in the progenies of the MAALs each carrying an alien chromosome of *O. punctata* Kotschy.

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