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Full Length Research Paper

Localizing introgression on the chromosome of rice by genomic *in situ* hybridization (GISH)

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Genomic *in situ* hybridization was used to detect introgressed segment from *Oryza australinesis* onto the chromosomes of introgression line derived from the hybrid *O. sativa* x *O. australinesis*. Genomic DNA from *Oryza australinesis* was labeled with biotin and hybridized to the homologous sequences on the *O. sativa* chromosomes. The probe hybridization fluoresced green and non labeled *O. sativa* chromosomes appeared red or blue due to counterstaining with propidium iodide (PI) or 4,6-diamidino-2-phenylindole (DAPI). This differential painting of chromosomes unequivocally detected the introgressed segment. Among the 200 cells analyzed, 6.5% of the cells showed hybridization signal. Signal appeared on one chromosome in 5%, on two homologous chromosomes in 1% and on sister chromatids in 0.5% of the cells. Hybridization was seen on the short arm of the chromosome 12 of the introgression line.

Key words: Genomic *in situ* hybridization, wide hybrid, localizing introgression.

INTRODUCTION

Introduction of alien gene requires hybridization followed by meiotic pairing and recombination between the chromosomes of cultivated and wild species. Successful gene transfer could be greatly facilitated by knowledge of the occurrence and frequency of chromosome pairing (Abbasi et al., 2009) and the identification of the presence of alien chromatin in the recipient progenies. Many important characteristics have been incorporated into rice by alien gene transfer, e.g. grassy stunt resistance from *Oryza nivara* (Khush, 1977), bacterial blight (BB) resistance (*Xa*21) from *Oryza longistminata* (Khush et al., 1990), brown plant hopper (BPH) and white backed plant hopper (WBPH) resistance from *Oryza officinalis* (Jena and Khush, 1990), blast and bacterial blight resistance from *Oryza minuta* (Amante et al., 1992), cytoplasmic male sterility from *Oryza perennis* (Dalmacio et al., 1995) and bacterial blight resistance from *Oryza brachyantha* (Brar *et al.* 1996).

In situ hybridization is a powerful tool for establishing phylogenetic relationship in polyploidy species and physical location of DNA sequences on chromosomes. The technique has been improved to localize sequences as small as 500 base pairs in animals. However, in plants, systems have not been finally resolved: most data have come from mapping highly repetitive sequences. With plants, it appears low mitotic indices and the pre-sence of cell wall materials and associated cytoplasmic debris in chromosome preparations hinder hybridization of low copy number sequences to the chromosome, hin-der their detection and hence, the non specific binding of labeled probes (Gustafson et al., 1990). In this report, the detection of *Oryza australiensis* segment on the chromo-some of the introgression line derived from *Oryza sativa* x *O. australiensis* hybrid using total genomic *O. austral-liensis* DNA as a probe was demonstrated.

MATERIALS AND METHODS

Mitotic chromosome preparation

Newly emerged roots (1 – 2 cm) from field grown F1 hybrid plants

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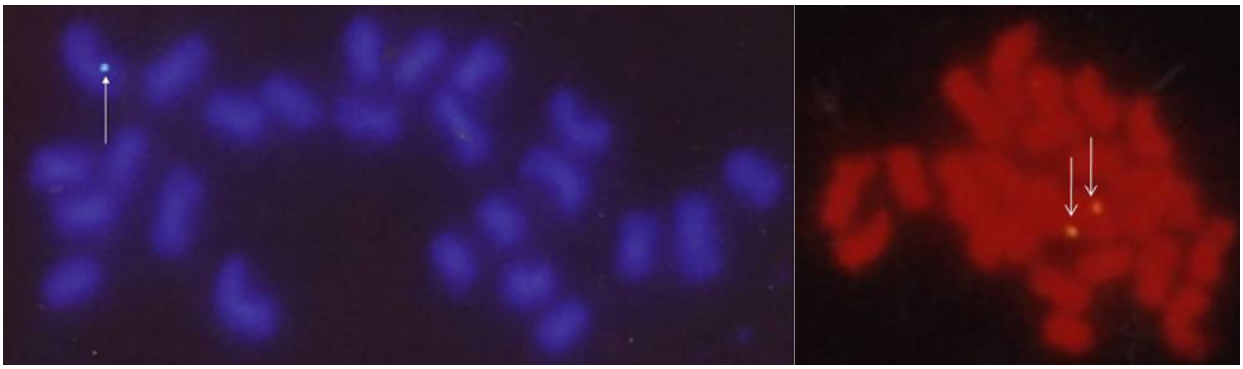

A B

Figure 1. Fluorescence *in situ* hybridization to root tip preparation from an introgression line of rice derived from the cross of *O. sativa* x *O. australiensis.* A) Metaphase cell with green spot (arrow) showing hybridization site on one of the chromosome. B) Metaphase cell with two dots on homologous chromosomes (arrow).

were excised and treated with 5 mM 8-hydroxyquoniline (Sigma) for 30 min. The roots were washed thoroughly with distilled water and fixed in ethanol/glacial acetic acid (3:1) for 24 h at room temperature. To prepare chromosome squashes, the roots were taken out of fixative and thoroughly washed with distilled water and citrate buffer (0.01 M citric acid monohydrate + 0.01 M trisodium citrate dihydrate, pH 4.6). Meristematic portion of root tips were subjected to enzymatic maceration, 3% Cellulase (Onozuka R10) +2% pectolyase (Y-23) at 37°C for 1 h. After enzyme treatment, roots were again thoroughly washed in citrate buffer and distilled water. The cells were spread on the slide in a drop of fixative (3 parts of 95% ethanol + 1 part of acetic acid). The slides were air dried and used for *in situ* hybridization.

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 *EcoR1* and labeled with Biotin-14-dATP by nick translation (Gibco BRL), according to standard nick translation labeling system.

Genomic *in situ* hybridization

The hybridization mixture containing 120 ng of biotinylated probe, 50% formamide, 3 µg SSS DNA, 2xSSC and 2.4 µg unlabeled *O. sativa* DNA was denatured at 80°C for 10 min and immediately quenched in ice for 5 min. An aliquot of 18 µl was dropped on each slide and covered with cover slip, sealed with paper bond and air dried. The chromosomes denatured at 80°C for 10 min, using thermal cycler (Hybaid), followed by incubation at 37°C for 18 h. The cover slips were removed in 2xSSC, and the slides were washed with 2xSSC two times and once with 4xSSC at 42°C for 10 min each. An aliquot of 100 µl of blocking solution, containing 5% bovine serum albumin (BSA) in 4xSSct (4xSSC + 0.05% Tween 20), was dropped on each slide, covered with cover slip and incubated for 5 min at 37°C. An aliquot of 70 µl, fluorescein isothiocynate (FITC)-Avidin (Boehringer Mannheim) in 1% BSA/4xSSct was layered on the slides and incubated for 60 min at 37°C. The slides were washed three 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 2xSSC for 10 min each at 37°C, dehydrated in ethanol series: 70, 95 and 100% for three minutes each at room temperature.

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

RESULTS AND DISCUSSION

Attempts have been made to visualize specific DNA sequences directly on rice chromosome. The segment transferred from *O. australiensis* onto the *O. sativa* chromosomes was determined by analyzing 200 metaphase cells of one of the BPH resistant introgression lines IR65682-136-4-3-2, derived from interspecific cross *O. sativa* x *O. australiensis.* In this line, resistant to BPH has been introgressed from *O. australiensis* and the locus is linked with RG457 located on chromosome12 (Ishi et al., 1994). Labeled genomic DNA from *O .australiensis* was used as a probe. The unlabeled DNA from *O. sativa* was used as blocking DNA at a ratio of 1:20 probe DNA. The probe showed hybridization signals on metaphase and interphase cells. The chromosomes counterstained with DAPI fluoresced blue and the probe signal appeared green (Figure 1A).

The hybridization signals were mostly found on a single chromatid (Figures 1A and B). Twin signals appeared on metaphase chromosomes (Figure 2A) as well as on the interphase nuclei, (Figures 2 B and C). Among 200 cells studied, signals appeared on one chromosome in 5% of the cells, on two homologous chromosomes in 1% of the cells and on sister chromatids in 0.5% of the cells (Table 1).

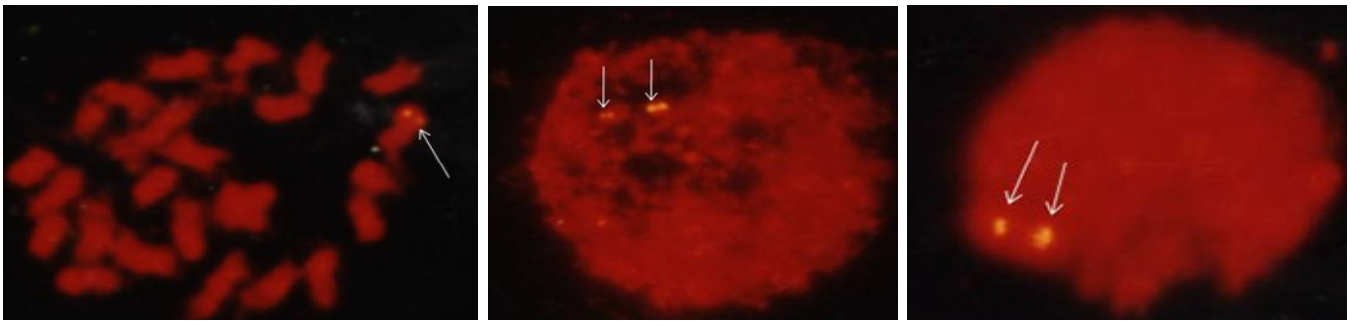

Figure 2. Fluorescence in situ hybridization to root tip preparation from an introgression line of rice derived from the cross of O. sativa x O. australiensis. A) Metaphase cell with twin dots showing hybridization site (arrow) on the chromatids of a chromosome. B and C) Interphase nuclei of introgression line showing hybridization sites (arrow).

Table 1. Detection of hybridization signal through in situ hybridization of O. australiensis labeled genomic DNA onto the O. sativa chromosomes in an introgression line derived from O. sativa x O. australiensis.

Parameter	Cells analyzed	Cell with hybridization	Single chromosome	Two chromosome	Two sister chromatids
Number	200	13	10	2	1
Percent		6.5	5.0	1.0	0.5

It was interesting to identify a small introgressed segment from *O. australiensis* on to the *O. sativa* chromosomes in an introgression line. Only 6.5% of the cells showed hybridization signals. The frequency of signal on one chromosome was higher compared to sister chromatids. Xue et al. (1996) detected the signal for CaM and Ca^{2+}-ATPase gene only on one chromatid of each detected chromosomes and the detection frequency was 6.8%. Melody and Karp (1989) reported that no significant signal appeared on the metaphase chromosomes and clear signals were observed on the interphase nuclei.

The possible reason for this low intensity of signal may be (1) loss of DNA from the slides during denaturation, (2) low accessibility of probe to chromosomes due to cytoplasm or cell wall, (3) high condensation of chromosomes that also makes it difficult to observe the weak signal or (4) over-staining that could mask the hybridization signal.

In this study, when rice chromosomes were counterstained with 2 ug/ml of PI for 2 min, the hybridization signal was completely masked. This indicates that counterstaining is very important to examine the position of labeled and unlabeled chromosomes. High concentration of counterstaining mask the signal and low concentration could not stain the rice chromosome completely. This is especially important for the characterization of introgression lines, because weak signals generated by small DNA sequences could be masked by high concentration of counterstaining. This is the first reproducible result of *in situ* hybridization using total genomic DNA for localizing introgressed segment onto the rice chromosome.

REFERENCES

Abbasi FM, Ahmad H, Sajid M, Inamullah, Brar DS (2009). Detecting Nature of Chromosome Pairing in A and E Genomes of *Oryza*. Cytologia, 74: 229-233

Amante BA, Sitch LA, Nelson R, Dalmacio R, Oliva RD, Aswindnoor NP, Leung H (1992). Transfer of bacterial blight and blast resistance from the tetraploid wild rice *Oryza minuta* to cultivated rice *O. sativa*. Theor. Appl. Genet. 84: 345-354

Brar DS, Dalmacio R, Eloloran R, Aggarwal R, Angels R, Khush GS (1996). Gene transfer and molecular characterization of introgression from wild *Oryza* species into rice. In: Rice Genet. III, pp: 477-486

Dalmacio R, Brar DS, Ishii T, Sitch LA, Virmani SS, Khush GS (1995). Identification, and transfer of a new cytoplasmic male sterility source from *Oryza perennis* into indica rice. Euphytica, 82: 221-225.

Dellaporta SL, Wood J, Hicks JB (1983). A plant DNA mini-preparation, version 11. Plant Mol. Biol. Rep. 1: 19-21.

Gustafson JP, Butler E, McIntyre CL (1990). Physical mapping of low copy DNA sequences, in rye (*Secale cereal* L.). Proc. Natl. Acad. USA, 87: 1899-1902.

Ishi T, Brar DS, Multani DS, Khush GS (1994). Molecular tagging of genes for brown planthopper resistance and earliness, introgressed from *O. australiensis* into cultivated rice, *O. sativa*. Genome, 37: 217-221.

Jena KK, Khush GS (1990). Introgression of genes from *O.officinalis*, to cultivated rice, *O. sativa*. Theo Appl Genet. 80: 737-745.

Khush GS, Bacalangco E, Ohawa T (1990). A new gene for resistance to bacterial blight from *O. longistminata*. Rice Genet. Newslett. 7: 121-122.

Khush GS (1977). Disease and insect resistance in rice. Adv. Agron. 29: 265-341.

Melody C, Karp A (1989). Physical mapping of β -hordein loci on barley

chromosome 5, by *in situ* hybridization. Genome, 32: 925-929.
Xue-Zhi BI, Song YC, Ren N, Liu LH (1996). Physical mapping of CaM and Ca^{2+}-ATPase genes, in rice by *in situ* hybridization. Rice Genet. Newslett., 13: 85-89.