African Journal of Biotechnology Vol. 9(40), pp. 6640-6644, 4 October, 2010
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.1008
ISSN 1684–5315 ©2010 Academic Journals

Full Length Research Paper

Characterization of wide cross derivatives in rice *Oryza sativa* L. using genomic *in situ* hybridization (GISH)

Fida M. Abbasi[1]*, M. Afzal[1], F. Perveen[2], R. Masood[1], Safdar H Shah[3], Azhar H. Shah[1], R. Mujaddad[4] , A. Majid[1], M. Sajid[1] and Alia Gul[1]

[1]Department of Genetics, Hazara University Garden Campus, Mansehra, Pakistan.
[2]Department of Zoology, Hazara University Garden Campus, Mansehra, Pakistan.
[3]Institute of Agriculture Biotechnology and Genetic Engineering, Peshawar, Pakistan.
[4]Department of Microbiology, Hazara University Garden Campus, Mansehra, Pakistan.

Accepted 23 August, 2010

Interspecific crosses provide a bridge by which the gene pool of rice can be increased. Introduction of alien genes requires hybridization followed by meiotic pairing and recombination between the chromosomes of cultivated and wild species. Attempts have been made to visualize the genomic constitution of wide-cross derivatives. Genomic *in situ* hybridization was used to detect *Oryza australiensis* chromosomes and introgressed segment from *O. australiensis* into the *Oryza sativa* background. Genomic DNA from *O. australiensis* was labeled with biotin-14-dATP and hybridized to the homologous chromosomes in hybrids, back cross progenies, monosomic alien addition line (MAAL) and introgression line. The probe hybridization fluoresced green and non-labeled *O. sativa* chromosomes appeared red due to counterstaining with propidium iodide (PI). This differential painting of chromosomes unequivocally detected the *O. australiensis* chromatin introgressed into the *O. sativa* genome. The probe produced uniform labeling pattern over the entire length of all the *O. australiensis* chromosomes. Genomic *in situ* hybridization (GISH) detected 12 *O. australiensis* chromosomes in the hybrid, *O. sativa* x *O. australiensis* in BC1 progenies, and a single chromosome in MAAL. Small segment of *O. australiensis* was localized on the chromosome 12 of the introgression line. However, results showed that GISH is a powerful technique to be used as an aid in selecting segregating progenies.

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

INTRODUCTION

Rice (*Oryza sativa)* can be sexually hybridized with many of its wild and cultivated relatives. Such hybrids provide a bridge by which the gene pool of rice can be increased. Introduction of alien genes requires hybridization followed by meiotic pairing and recombination between the chromosomes of cultivated and wild species. Many important characteristics have been incorporated into rice by alien gene transfer, for example, grassy stunt resistance from *Oryza nivara* (Khush, 1977), bacterial blight (BB) resistance (*Xa*21) from *Oryza longistaminata* (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).

*Corresponding author. E-mail: muhammaddr@yahoo.com. Tel: 03429460029.

Abbreviations: MAAL, Monosomic alien addition line; **PI,** propidium iodide; **GISH,** genomic *in situ* hybridization; **BB,** bacterial blight; **BPH,** brown plant hopper; **WBPH,** white-backed plant hopper; **BC1,** backcross 1; **SSS,** sheared salmon sperm; **BSA,** bovine serum albumin; **FITC,** fluorescein isothiocyanate; **RFLP,** restriction fragment length polymorphism.

Successful gene transfer could be greatly facilitated by identification of the presence of alien chromatins in the recipient progenies (Abbasi et al., 2009). *In situ* hybridization is a powerful tool for assessing the genomic relationship (Abbasi et al., 2010), thereby establishing phylogenetic relationship in polyploidy species, physical location of DNA sequences on chromosome and detection of introgression. However, in plant systems, most data have come from mapping highly repetitive sequences. With plants, it appears in low mitotic indices, and the presence of cell wall materials and associated cytoplasmic debris in chromosome preparations hinder hybridization of low copied number sequences to the chromosome, their detection and hence, the non-specific binding of labeled probes (Gustafson et al., 1990). In this report, attempts have been demonstrated to indicate the successful analysis of genomic constitution of wide hybrid, backcross 1 (BC1), monosomic alien addition line (MAAL) and introgression line.

MATERIALS AND METHODS

Mitotic chromosome preparation

Newly emerged roots (1 - 2 cm) from field grown F1 hybrid plants (BC1, MAAL and introgression line) were excised and treated at room temperature 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 the 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

Genomic DNA was isolated from 5 - 10 g of fresh leaves from *Oryza 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 sheared salmon sperm (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, covered with a cover slip, sealed with paper bond and air dried. The chromosomes were 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 twice with 2xSSC and once with 4xSSC at 42°C for 10 min each. An aliquot (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 a cover slip and incubated for 5 min at 37°C. An aliquot of 70µl 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 70µl 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 and dehydrated in an ascending ethanol series (70, 95 and 100%) for three minutes each at room temperature.

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

RESULTS

Alien introgression is possible either at the entire genome level where amphiploids are produced or in the entire individual chromosome level for the production of individual alien chromosome in addition and substitution line or at the level of chromosome segment where introgression lines are produced. In the present investigation, success was achieved at all these three levels through chromosome manipulation. We were able to identify unequivocally the alien chromatin added in the *O.sativa* background using Genomic *in situ* hybridization (GISH) approach. GISH using biotinylated total genomic DNA from *O. australiensis* was hybridized to the chromosomes of F1 (*O. sativa x O. australiensis*), BC1, MAAL and the introgression line derived from this hybrid. The labeled *O. australiensis* chromosomes fluoresced appeared green due to fluorescein isothiocyanate (FITC) and the unlabeled chromosomes appeared red due to counterstaining with propidium iodide (PI). This differential painting of chromosomes was used to look into the wide cross derivatives for discriminating alien chromatin in the *O. sativa* background. GISH unequivocally discriminated 12 *O. sativa* chromosomes in the hybrid of *O. sativa* x *O. australiensis* (Figure 1) and 24 chromosomes in the BC1 progenies (Figure 2). In many cells, prophase chromosomes of *O. australiensis* showed specific organization within the nucleus. As shown in Figure 1 A - C, centromeres are faced towards one pole, while telomeres are faced to the opposite direction.

The *O. australiensis* chromatin was distinguishable at all the stages of the cell cycle. In interphase nuclei, *O. australiensis* chromatin appeared as a distinguished domain and did not intermix with that of *O. sativa* (Figure 1D). The labeled genomic DNA from *O. australiensis* was hybridized with the single alien chromosome. The labeled chromosome appeared yellow and 24 chromosomes from *O. sativa* fluoresced appeared red due to counterstaining with PI (Figure 3). This differential painting of chromosome

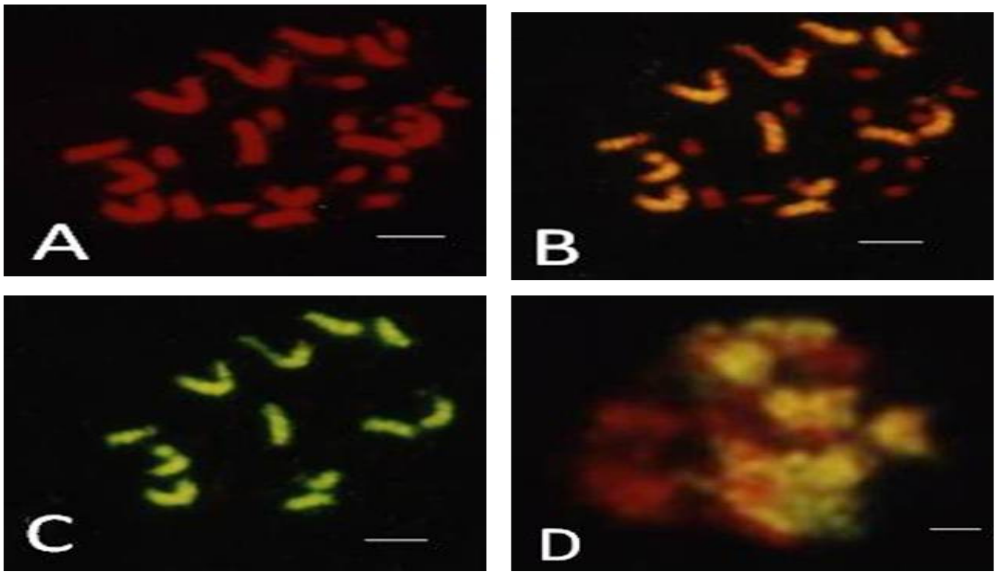

Figure 1. Genomic *in situ* hybridization of root tip chromosomes in the hybrid between *O.sativa* x *O.australiensis*. A, Metaphase cell stained with PI showing 24 chromosomes that are fluoresced red; B, the same cell, as in A, probed with the labeled genomic DNA from *O. australiensis*, where 12 *O. australiensis* chromosomes are fluoresced yellow and 12 *O. sativa* chromosomes are fluoresced red; C, same cell, as in A and B, showing only *O. australiensis* chromosome under blue light excitation; D, interphase nucleus showing separate domain of *O. australiensis* (yellow) and *O. sativa* (red) chromatin. Bars show 10 µm. The probe produced a uniform labeling pattern over the entire length of all *O. australiensis* chromosomes.

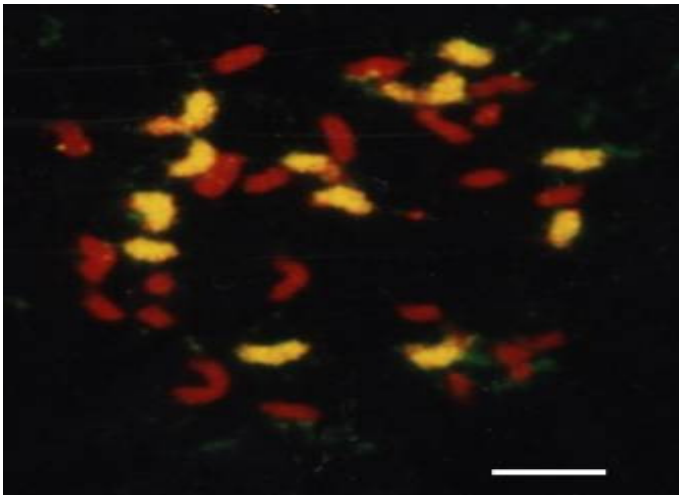

Figure 2. Genomic *in situ* hybridization of root tip chromosomes of the backcross plant derived from the hybrid *O. sativa* x *O. australiensis*. Biotin label genomic DNA from *O. australiensis* was used as a probe. The 12 *O. australiensis* chromosomes fluoresced yellow and 24 *O. sativa* chromosomes appeared red due to counterstaining with PI. Bar shows 10 µm.

confirmed the alien addition line derived from the cross *O. sativa* x *O. australiensis*.

The segment transferred from *O. australiensis* onto the *O. sativa* chromosomes was determined by analyzing the metaphase cells of an introgression line, IR65682-136-4-3-2, derived from the cross *O. sativa* x *O. australiensis*. In this line, resistance to BPH has been introgressed from *O. australiensis* and the locus is linked with RG457 located on chromosome 12. The labeled genomic DNA from *O. australiensis* was used as a probe, while the unlabeled DNA from *O. sativa* was used as a blocking DNA at a ratio of 1:20 probe DNA. The probe showed hybridization signals on metaphase and interphase cells. The chromosomes counterstained with PI appeared as fluoresced red and the probe signal appeared green (Figure 4).

The hybridization signals were mostly found on a single chromatid. Twin signals appeared on metaphase chromosomes as well as on the interphase nuclei. Results showed that GISH is a new and powerful molecular tool for looking into the genomes and could be used as a marker for selecting the segregating progenies in wide crosses.

DISCUSSION

Genomic relationship between *O. sativa* and *O. australliensis* was reported earlier (Abbasi et al., 2010); however, in the present studies, we were able to identify the *O. australiensis* chromatin in the segregating progenies derived from the cross *O. sativa* x *O. australiensis*. Markers may also be used to analyze the

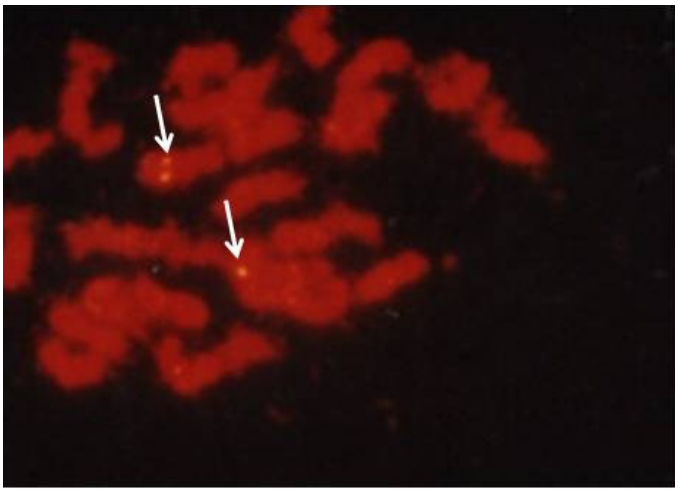

Figure 3. Genomic *in situ* hybridization of root tip chromosomes from monosomic alien addition line (MAAL) derived from the hybrid *O. sativa* x *O. australiensis*. Biotin label genomic DNA from *O. australiensis* was used as a probe. The single *O. australiensis* chromosome fluoresced yellow and 24 *O. sativa* chromosomes appeared red due to counterstaining with PI. As a result, bar shows 10 µm.

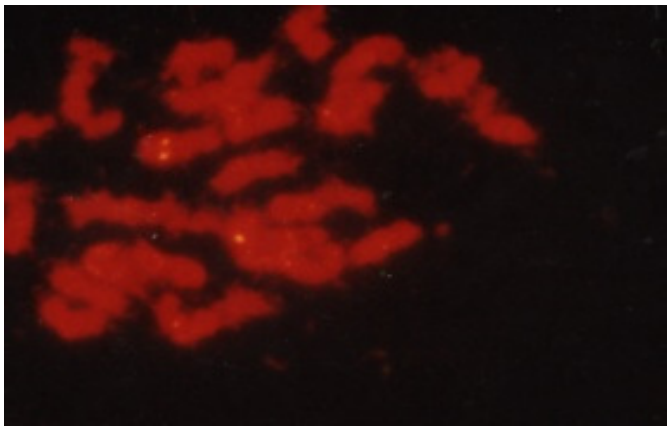

Figure 4. Fluorescence *in situ* hybridization to root tip preparation from an introgression line of rice derived from the cross of *O. sativa* x *O. australiensis*. Metaphase cell with hybridization signal on two chromosomes (arrow).

segregating progenies for the presence or absence of a target gene. However, the presence of a marker does not distinguish between one or two copies of a particular chromosome, demonstrating that GISH is a powerful technique for the analysis of segregating progenies in wide-cross derivatives. Also, the absence of a marker could be the result of a deletion rather than missing a complete chromosome (Dong et al., 1999). For this reason, it was of interest to analyze these materials using the GISH technique. In the present studies, the probe produced a uniform labeling pattern over the entire length of all *O. australiensis* chromosomes. Such a labeling pattern is important particularly when using genomic DNA of wild species as a probe to detect introgression or in representing the entire length of a chromosome (Abbasi et al., 2010). However, the results of the study are in line with Uozu et al. (1997) who described the chromosomes of *O. australiensis* and demonstrated that these chromosomes are larger than that of *O. sativa* and the repetitive DNA sequences that are evenly distributed on all the chromosomes.

The conventional way for identification of alien chromosomes is to study their morphology and karyotyping. In this study, GISH was used to identify the single alien chromosome in the background of *O. sativa,* demonstrating the confirmation of alien addition line having an extra chromosome from *O. australiensis.* Twelve chromosomes of *O. australiensis* detected in F1 and 24 chromosomes were unequivocally discriminated from the 12 chromosomes of *O. australiensis* in BC1. However, restriction fragment length polymorphism (RFLP) analysis could reveal a fine region on chromosomes. A large number of markers that represent different chromosomal regions would have to be used in analyzing a complete chromosome. The classical banding technique does not give a sufficient number of characteristics bands for reliable chromosomal identification (Mok et al., 1974). Therefore, the conventional cytogenetic techniques will not be practical for identifying *O. australiensis* chromosomes in the *O. sativa* background. The GISH was used for the first time in *Oryza* to detect the alien chromatin added to the *O. sativa* genome.

REFERENCES

Abbasi FM, Ahmad H, Perveen F, Inamullah, Sajid M, Brar DS (2010). Assesment of genomic relationship between *Oryza sativa* and *Oryza australiensis*. Afr. J. Biotechnol. 9: 1312-1316

Abbasi FM, Ahmad H, Sajid M, Inamullah, Brar D S (2009). Detecting nature of chromosome pairing in A and E genomes of *Oryza*. Cytologia, 74: 229-233

Abbasi FM, Shah AH, Perveen F, Afzal M, Sajid M, Masood R, Nawaz F (2010). Genomic affinity between *Oryza sativa* and *Oryza brachyantha* as revealed by *in situ* hybridization and chromosome pairing. Afr. J. Biotechnol. 9: 3068-3072

Amante BA, Sitch LA Nelson, 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 Genetics 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.

Dong F, Novy RG, Helgeson JP, Jiang JJ (1999). Cytological characterization of potato-*Solanum etuberosum* somatic hybrids and their backcross progenies by genomic *in situ* hybridization. Genome, 42: 987-992

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

Jena KK, Khush GS (1990). Introgression of genes from *O.officinalis*, to

cultivated rice, *O. sativa*. Theor. Appl. Genet. 80: 737-745.
Khush GS (1977). Disease and insect resistance in rice. Adv. Agron. 29: 265-341.
Khush GS, Bacalangco E, Ohawa T (1990). A new gene for resistance to bacterial blight from *O. longistminata*. Rice Gent. Newslett. 7: 121-122.
Uozu S, Ikehashi H, Ohmido N, Ohtsubo H, Ohtsubo E, Fukui K (1997). Repetitive sequences: Causes for variation in genome size and chromosome morphology in the genus *Oryza*. Plant Mol. Biol. 35: 791-799.