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Genomic *In Situ* Hybridization in Rice

Somsak Apisitwanich

ABSTRACT

Fluorescence *in situ* hybridization was modified by using genomic DNAs as probes to detect wide cross somatic hybrids. The *A* genome was labelled and visualized as red signal while the *B* and *C* genomes were visualized as blue and green signals, respectively. This technique could distinguishly identify the hybrid SP I line with whole full sets of 6 genomes and the hybrid SP II line with some lost chromosomes in *B* and *C* genomes.

Key words : genomic *in situ* hybridization, interspecific rice, somatic hybrid rice

INTRODUCTION

Nowadays wild plant species are used as the gene pool for insect and disease resistance. Wide hybridization was established to recombine good characters of both cultivar and wild species. The difficulty of this cross is the occurrence of some aneuploid plants. To solve this problem, protoplast fusion method and regeneration technique were used to obtain the somatic hybrids. Escalante *et al*. (1997) succeeded in obtaining some somatic hybrids between tomato and a wild egg plant species. The somatic hybrids of rice, tobacco, *Crocus*, barley and carrot have also been obtained (Hayashi *et al*., 1988; Kenton *et al*., 1993; Kitamura *et al*., 1997 and Kisaka *et al*., 1994). The method primarily used to clarify wide cross somatic hybrids could investigate the chromosome size and morphology of both parental cell lines comparing to those of hybrids such as the hybrids between *Nicotiana tabacum* and *Atropa belladonna* (Babiychuk *et al*., 1992), *Brassica oleracea* and *B. campestris* (Yamashita *et al*., 1989) and *Glycine max* and *Nicotiana glauca* (Kao, 1977). The chromosome in hybrids would be easily observed if the chromosome sets of parental lines were different in sizes and morphologies. However, there were no distinguishable markers of metaphase chromosomes for the hybrids derived from the same genus.

Recently a molecular cytological technique, fluorescence *in situ* hybridization (FISH), could be used for genome identification at every stage of cell division. FISH was developed in 1981 by labelling DNA fragments with fluorescent substances and detection was made under the ultraviolet light. If labelled DNA probe is genomic DNA, this *in situ* hybridization is called genomic *in situ* hybridization (GISH). The GISH technique was used and clear result was obtained for whole chromosome set painting in hybrids between barley and rye (Schwarzacher *et al*., 1989), tomato and *Solanum lycopersicoides* (Escalante *et al*., 1997) and *D* genome of rice (Fukui *et al*., 1997). It is, therefore, quite practical to use GISH to detect three genomes (*A*, *B*, and *C*) of rice in Thailand.

Department of Genetics, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand.

MATERIALS AND METHODS

Somatic hybrid production

Calli were induced from the scutellum of rice seed culture. Somatic hybrids were obtained by the electrofusion between iodoacetamide-treated protoplasts isolated from callus of *Oryza sativa*, *japonica* CV. Kitaake (2n = 2x = *AA* = 24) and irradiated protoplasts of *O. punctata* (2n = 4x = *BBCC* = 48) from the National Institute of Genetics, Shizuoka, Japan according to Mori and Kinoshita (1991).

Genome identification

Chromosome preparation

Root tips of hybrid rice plants were excised and pretreated with 2mM 8-hydroxyquinoline at 15°C for 4 hr then they were transferred to be fixed overnight in the fixative solution (ethanol : acetic acid = 3 : 1). The fixed root tips were macerated and squashed on glass slides according to Fukui's method (1996).

Genomic *in situ* hybridization

Total genomic DNA of *O. sativa* (*AA*) and *O. officinalis* (*CC*) were extracted from leaves and labelled with digoxigenin-11-dUTP and biotin-16-dUTP, respectively, by the nick translation or random primed labelling method. Both labelled DNAs were dissolved in 50% formamide and 2X SSC solution, mixed in the equal volume and denatured. Chromosome spreads were treated with 100 mg/ml RNase A in 2X SSC at 37°C for 1 hr and dehydrated with serial alcohol then air dried. After drying, slides were put on a thermocycler and dropped with 50% formamide and 2X SSC at 70° C for 6 min. The mixture of denatured genomic DNAs were added on slides and kept at 37°C for 1-4 days. After hybridization, the excess probes were removed by washing with 2X SSC, 2X SSC and 4X SSC 2 times at 40°C for 10min in each solution. The mixture of avidin-FITC and antidigoxigenin-rhodamine in conjugating buffer (1% bovine serum albumin + 0.1M sodium hydrogencarbonate + 0.05% tween-20) were poured on slides and incubated at 37°C for 1 hr and the slides were washed in the conjugating buffer. The secondary signal amplification was done by using biotinylated anti-avidine, avidine-fluorescein, antidig-rhodamine and anti-sheep-Texas red and the chromosomes were counterstained with 1 μg/l 4',-6-diamidino-2-phenylindole (DAPI) in Vector Shield antifadant solution. Results of chromosome hybridization were observed under an UV fluorescence microscope. Fluorescent signals were captured and transferred to a computer and analyzed using IPLab Spectrum imaging software.

RESULTS

Protoplast fusion products were obtained and plantlets were regenerated using nurse culture method. Plantlets were transferred to pots and grown in a greenhouse. The SP I and SP II regenerant lines were selected as materials according to their better vigor than parents. Chromosome spreads were stained using conventional and fluorescent methods for preliminary chromosome number checking. Giemsa staining gave dark blue colored chromosomes under tungsten light (Figure 1a) but DAPI staining gave fluorescent blue colored chromosomes under UV light (Figure 1b). The SP I line showed 72 chromosomes but the SP II line showed chromosome number variation from 65 to 72. Hybridization results of *A* and *C* genome probes revealed that the SP I had 6 whole chromosome sets of *AABBCC* genomes. There was a SP II line which presented 2 complete sets of *A* genome and some lost chromosomes of *B* and *C* genomes. Chromosome paintings of SP II are shown in Figure 2a-2d. All chromosomes in Figure 2b were counterstained with DAPI. The target chromosomes

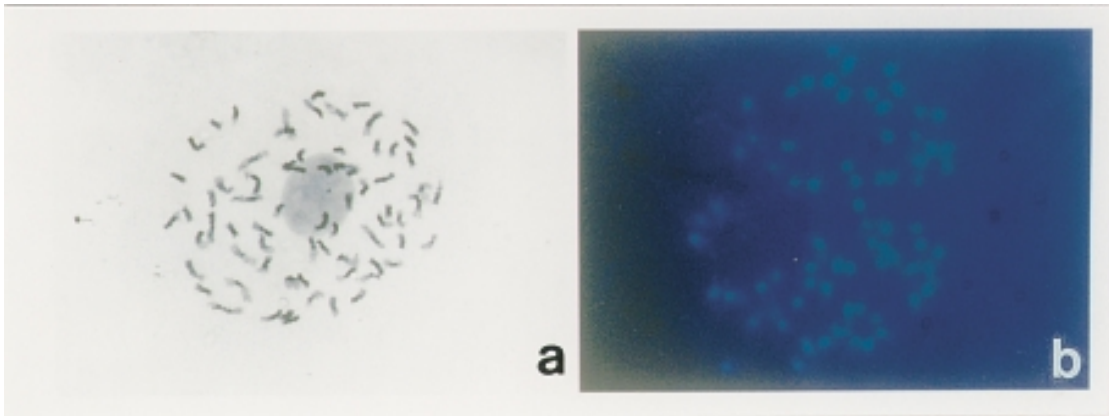

Figure 1 Chromosome complements of somatic hybrid rice.
a. Giemsa staining
b. DAPI staining

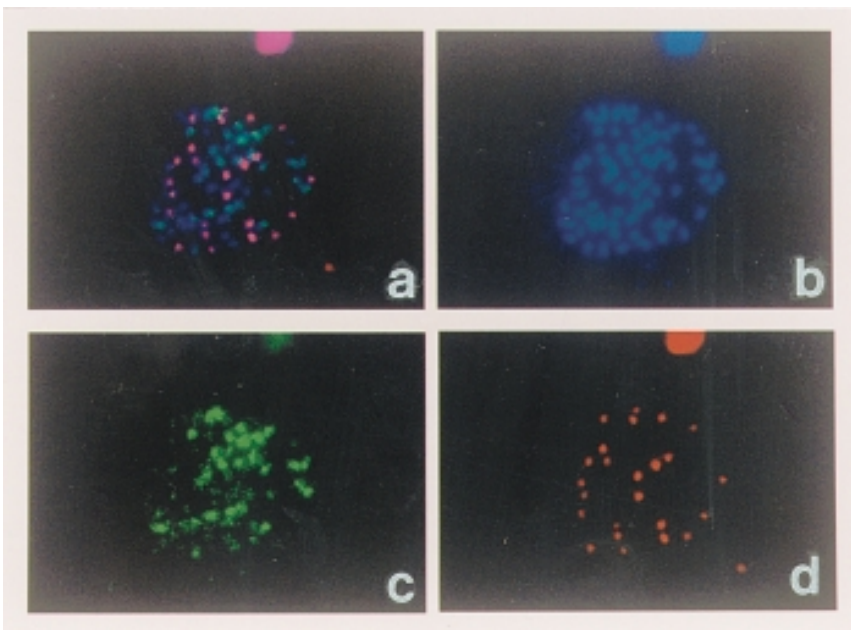

Figure 2 Genomic *in situ* hybridization results.
a. Tricolor discrimination for 3 genomes
(red - *A* genome, blue - *B* genome and green - *C* genome)
b. DAPI counterstaining
c. Fluorescent green color for *C* genome detection
d. Fluorescent red color for *A* genome detection

of *A* and *C* genomes were painted in red and green color (Figure 2d and Figure 2c). The chromosomes in Figure 2a were painted with 3 colors of 3 genomes (red - *A* genome, blue - *B* genome and green - *C* genome).

There were 4 various periods of hybridization duration. After one day of hybridization, only a few parts of chromosomes were painted (Figure 3a). These parts were highly repetitive DNA sequences. Two days of hybridization, around 40% of *A* genome and 50% of *C* genome were painted (Figure 3b). Three days of hybridization, 65% of *A* genome and 80% of *C* genome were painted (Figure 3c). All chromosomes were completely painted after 4 days which was the appropriate time for GISH of rice (Figure 3d).

DISCUSSION

Wild species are genetic resources for some useful agronomic traits such as disease resistance, insect resistance and environmental tolerance (Swaminathan, 1986). Many scientists tried to transfer the desirable genes from wild species to cultivars by conventional breeding methods (Ishii *et al*., 1994; Multani *et al*., 1993). The objective of this experiment was to combine the genomes of *O. sativa*, a cultivar and *O. punctata*, a wild species using asymmetrical protoplast fusion technique. All hybrids obtained were sterile because of the different genomes and the fluctuation of chromosome numbers.

The GISH technique was useful for revealing the hybrid genome. Two genomic probes were simultaneously used to discriminate the chromosomes in three different genomes. The chromosomes of *A* genome were completely maintained whereas some of those *B* and *C* genomes from *O. punctata* were eliminated. Applying the regeneration method of *O. sativa* to the somatic

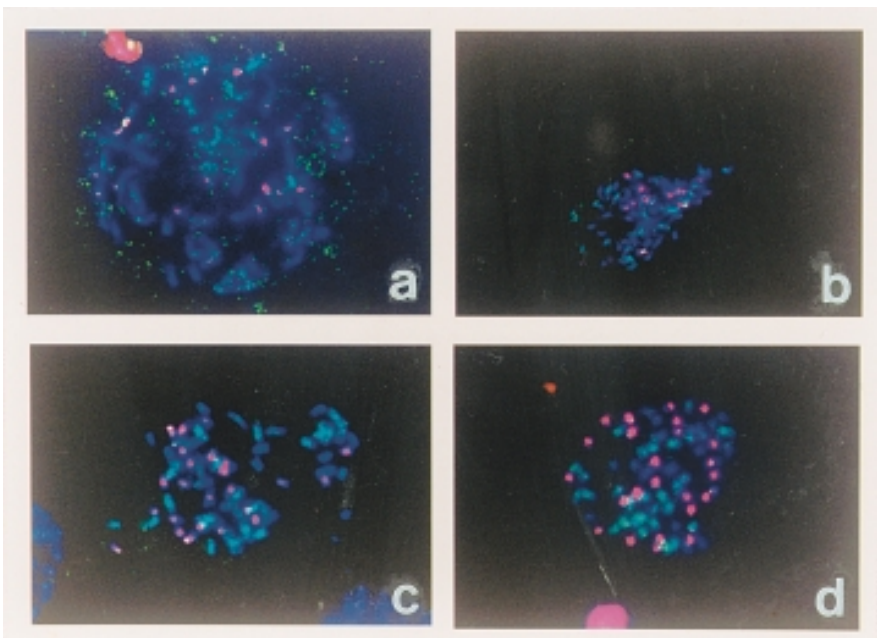

Figure 3 Painted chromosomes in various days of hybridization.
a. One day b. Two days c. Three days d. Four days

hybrids provided a favorable condition for whole *A* genome to be maintained. This GISH technique was also effective for the identification of intergeneric hybrids such as potato/tomato (Jacobsen *et al*., 1995). For the future research on genome evolution or wide hybridization, this technique is considered promising and good for chromosome detection.

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