Variability in rDNA loci in the genus Oryza detected through fluorescence in situ hybridization

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Abstract. The 17s-5.8s-25s ribosomal RNA gene (rDNA) loci in Oryza spp. were identified by the fluorescence in-situ hybridization (FISH) method. The rDNA loci were located on one-to-three chromosomes (two-to-six sites) within the eight diploid Oryza spp. One of the rDNA loci gave the weakest hybridization signal. This locus is reported for the first time in the genus Oryza. The chromosomes containing the rDNA loci were determined to be numbers 9, 10 and 11 in descending order of the copy number of rDNA. The application of image analysis methods, after slide preparation treatments (post-treatments), and the use of a thermal cycler, greatly improved the reproducibility of the results. The evolutionary significance of the variability of rDNA loci among the Oryza spp. is discussed.

Key words: Fluorescence in-situ hybridization (FISH) – Ribosomal DNA – Genus Oryza – Image analysis – NOR variability

Introduction

Rice chromosomes with a large 17s-5.8s-25s ribosomal RNA gene (rDNA) array have been identified as the satellite chromosomes by their characteristics (Fukui and Iijima 1991; Yanagisawa et al. 1991). They are also recognized as the chromosomes with nucleolar organizing regions (NOR chromosomes).

In cultivated rice, Oryza sativa L. ssp japonica, one pair of NOR chromosomes was reported by Kurata and Omura (1978) and Fukui and Iijima (1991). This NOR chromosome was designated as no. 11 (Fukui and Iijima 1991) but according to the new system for numbering rice chromosomes, has now been redesignated as no. 9 (Khush and Kinoshita 1991; Fukui and Iijima 1992). By contrast, two pairs of NOR chromosomes were reported in O. sativa ssp. indica (Wu et al. 1985).

Although the rDNA-containing chromosomes show conspicuous characteristics as satellite chromosomes, they are sometimes difficult to identify morphologically when the copy number of the rDNA units at the locus is small (Leitch and Heslop-Harrison 1992). The in-situ hybridization (ISH) method (Appels et al. 1980; Hutchinson and Miller 1982; Rayburn and Gill 1985) offers a way out of this impasse since it is based on the detection of rDNA loci directly by molecular hybridization. Using this technique one rDNA locus was identified on chromosome 9 in japonica rice. (Fukui et al. 1987; Fukui 1990; Iijima et al. 1991) while two rDNA loci were detected in indica rice (Islam-Faridi et al. 1990).

Although ISH is now widely employed in cytogenetic analysis, it is time consuming and strict experimental protocols are needed for its success. Therefore we have developed a reproducible and convenient fluorescence ISH (FISH) technique in conjunction with imaging methods, the use of a thermal cycler, and various post-treatments. As a result, clear fluorescent signals were reproducibly obtained and a new rDNA locus was detected in two diploid wild rice species.

Materials and methods

Plant materials and cytological procedures

Nine rice species, as listed in Table 1, were obtained either from the gene bank of the National Institute of Genetics (Mishima...
Results

Figure 1a shows the G-light excitation image of the chromosomes of *O. sativa*, ssp, *indica*, cv IR36. Two pairs of fluorescent signals were observed in B light (Fig. 1b). Figure 1c shows the integrated image obtained by image manipulation. The current B or G excitation filter used in the experiment visualized either the yellowish-green fluorescence of FITC/fluorescein or the reddish fluorescence of PI. By image processing, the two fluorescent signals were integrated into a single image with yellowish signals on the reddish chromosomes. For basic information on the size and number of signals on the chromosomes, the visual recognition of the integrated image was markedly improved by image processing as shown in Fig. 1c. The four signal positions of IR36 were more precisely determined by using the integrated image compared with the two original images.