Relationships among the CC, DD, and EE genomes in the *Oryza officinalis* complex detected by two-probe genomic *in situ* hybridization

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The *Oryza officinalis* complex is the largest in the genus *Oryza*, containing diploid species with BB, CC, or EE genomes and tetraploid species with BBCC or CCDD genomes. Several species in this complex such as *O. officinalis*, *O. australiensis*, and *O. minuta* have been intensively used in rice breeding programs because of their useful genes for disease and insect resistance (Brar and Khush 1997). Three closely related tetraploid species, *O. latifolia*, *O. alta,* and *O. grandiglumis,* from Central and South America were reported as allotetraploid with the CCDD genomes, even though no diploid species has been found as the D genome donor in *Oryza*.

Some researchers believe that the DD genome originated from the AA genome, whereas others think it differentiated from the CC genome. The first proposition should be ruled out because the CD-genome species has a greater genetic distance from the A-genome species than species containing other genomes (Wang et al 1992, Aggarwal et al 1999). Recent studies indicated that the DD genome and EE genome (from *O. australiensis*) are most closely related, and that the ancestor of the EE genome might have played an important role in the formation of the CD-genome species (Wang et al 1992, Ge et al 1999). The relationships between the DD and CC or DD and EE genomes, however, still need clarification.

Two-probe genomic *in situ* hybridization (GISH) technology, also called multicolor FISH or bicolor FISH, is an ideal tool for studying relationships among different genomes. This study confirmed the existence of CC and DD genomes in the tetraploid *O. alta* and investigated the relationships among the CC, DD, and EE genomes using the two-probe GISH.

The genomic DNA of three diploid wild rice species, *O. officinalis* (2n = 24, CC genome), *O. eichingeri* (2n = 24, CC), and *O. australiensis* (2n = 24, EE), was used as the probe. Plant materials used in the study are listed in Table 1 with their origins. Chromosome samples of *O. alta* were prepared using an enzymatic maceration and air-dry method. *In situ* hybridization followed that described by Leitch et al (1994). The hybridization stringency and posthybridization washing stringency are shown in Table 2. Stringency is a crucial factor in determining hybridization between homologous sequences or similar sequences. Under low stringency, GISH results reflect the similarity between probe and target sequences, whereas under high stringency, GISH results mainly indicate homologous hybridization. Thus, the identity (homology) and similarity between genomes could be estimated well.

When the chromosomes of *O. alta* were probed with the C-genome DNA from *O. officinalis*, the two genomes in *O. alta* could not be distinguished (Fig. 1a) under conditions of low stringency (50–60%). This result indicates a relatively high similarity of DNA sequences between CC and DD genomes in *O. alta*. Under 78–86% stringency, most chromosomes from the CC genome (labeled with strong green signals) and the DD genome (labeled with weak signals) could be distinguished, but some could not be accurately identified due to minor differences in their fluorescence intensity (Fig. 2a). This suggests that the differentiation between CC and DD genomes is not significant. When hybridized with the C-genome DNA from *O.*

Table 1. Wild *Oryza* species used in this study with their genomes and origins.

Species	Accession no.	2n	Genome	Source/country
O. officinalis	Zhou-198	24	CC	China
O. eichingeri	IRGC 101144	24	CC	Uganda
O. australiensis	IRGC 105263	24	EE	Australia
O. alta	IRGC 100161	48	CCDD	Brazil

Table 2. Chromosome samples, probe constitutions, and stringencies used.[a]

Chromosome sample	Probes	Hybridization stringency	Posthybridization stringency
O. alta	DIG-EE (*O. australiensis*)[b] BIO-CC (*O. officinalis*)	65–75%	50–60%
O. alta	DIG-EE (*O. australiensis*) BIO-CC (*O. officinalis*)	65–75%	78–86%
O. alta	DIG-CC (*O. eichingeri*) BIO-EE (*O. australiensis*)	65–75%	96–100%

[a]Stringency was calculated by using the equation described by Meinkoth and Wahl (1984). The GC content (50–70%) was estimated according to sequencing data of 20 genes (*O. sativa*) from the Genebank. The probe length varies from 200 bp to 500 bp as shown by running a small gel. [b]DIG-EE means the total DNA of the EE genome labeled with digoxigenin; BIO-CC means the total DNA of the CC genome labeled with biotin.

eichingeri and stained with DAPI under conditions of 96–100% stringency, most of the C-genome (violet signals) and D-genome (blue color) chromosomes could be distinguished (Fig. 3a). In most cases, hybridization signals did not homogeneously cover the entire length of the C-genome chromosomes; instead, stronger signals appeared only in certain regions of the chromosomes. Hybridization sites with visible signals were also detected on the D-genome chromosomes. It is difficult to clearly identify all chromosomes of the CC and DD genomes in *O. alta*. Based on these results, we concluded that *O. alta* (including *O. latifolia* and *O. grandiglumis*) is not a strict allotetraploid species.

When chromosomes of *O. alta* were probed by the labeled genomic DNA from *O. australiensis* (EE genome), the red hybridization signals covered all chromosomes (Fig. 1b) under conditions of low stringency (50–60%), suggesting a high similarity in DNA sequences between EE and CC and DD genomes. Under conditions of 78–86% stringency, hybridization signals are small and weak on most chromosomes; stronger signals are mainly located on C-genome chromosomes (Fig. 2b). Under 96–100% stringency, hybridization signals are so weak that further signal amplification had to be made. The amplified hybridization signals (bluish green) were mainly located on chromosomes of the CC genome. Hybridization signals on chromosomes of the DD genome were very weak or invisible (Fig. 3b).

These results together indicate considerable differentiation of the EE genome from CC and DD genomes, and higher affinity of the EE genome to the CC genome than to the DD genome. In conclusion, we believe that the EE genome is not the direct donor of the DD genome. The origin of the DD genome still remains uncertain and needs further study. This study fully demonstrated the advantages of using GISH in identifying genomic constitution and detecting relationships among genomes.

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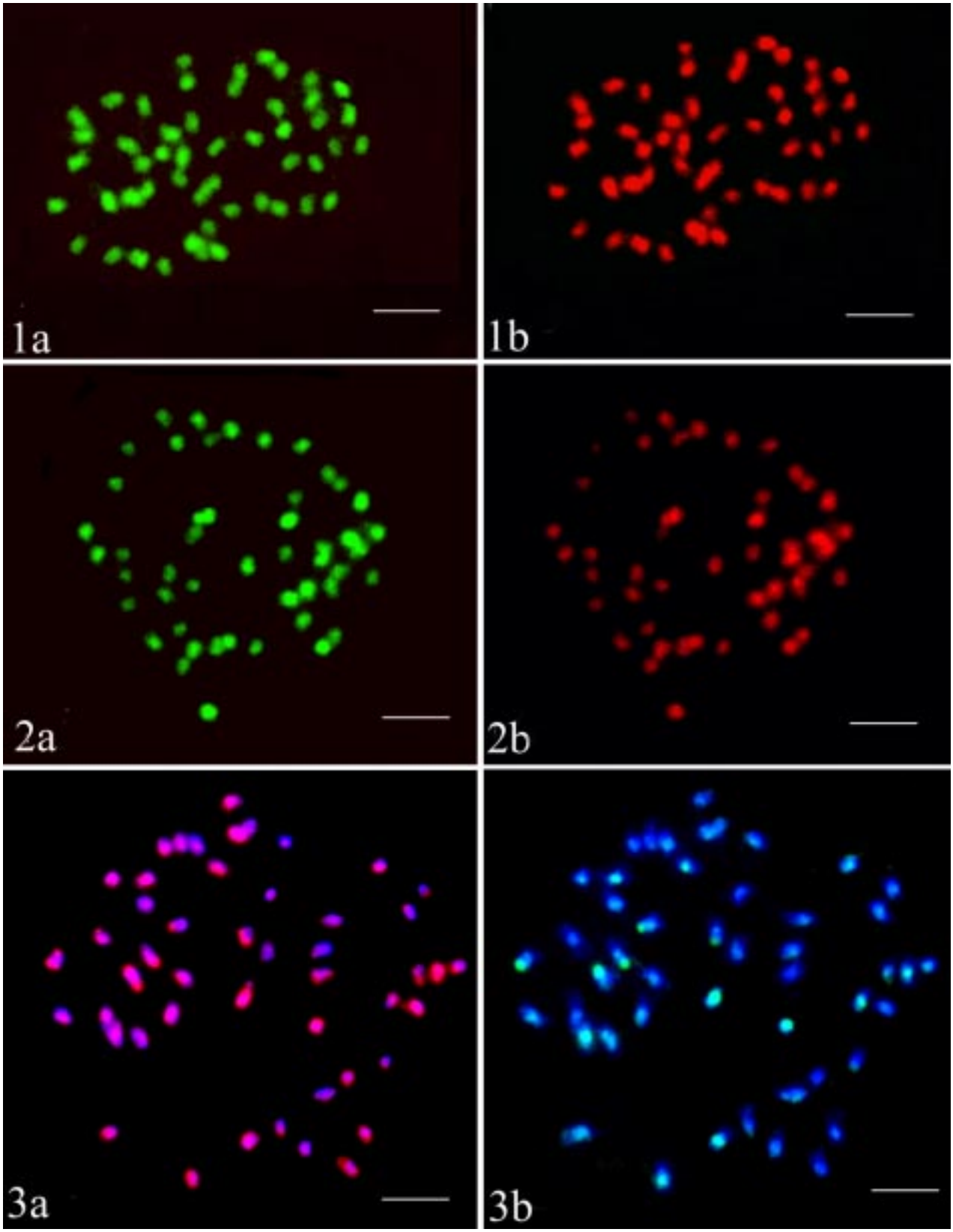

Figs. 1-3. Metaphase chromosomes of *O. alta* after genomic *in situ* hybridization with the C and E-genome species in different posthybridization washing stringencies. Chromosomes hybridized with the C-genome DNA from *O. officinalis* (Fig. 1a) and E-genome DNA from *O. australiensis* (Fig. 1b) under conditions of 50–60% stringency. Chromosomes hybridized with the same probes as in Figures 1a and 1b, but under 78–86% stringency (Fig. 2). Chromosomes probed with C-genome DNA from *O. eichingeri* (Fig. 3a) and E-genome DNA from *O. australiensis* (Fig. 3b) and counterstained with DAPI. The posthybridization washing stringency was 96–100%. The hybridization signals of the E-genome probe were amplified. Scale bar = 5 μm.