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ORIGINAL PAPER

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Production and characterization of a complete set of individual chromosome additions from *Oryza officinalis* to *Oryza sativa* using RFLP and GISH analyses

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Abstract Monosomic alien addition lines (MAALs) are valuable materials for comparative analyses of two distinct genomes, for elucidating introgression mechanisms, and for dissecting genes controlling complex traits. In the study reported here, MAALs of rice containing the complete genome of *Oryza sativa* and individual chromosomes of *Oryza officinalis* were produced. Interspecific hybridizations were made between *O. sativa* L. ssp. *Japonica* (CV, Hejiang 19, 2 $n=24$, AA) and *O. officinalis* (Acc. HY018, 2 $n=24$, CC). Two backcrosses were made to the cultivated rice to obtain BC_2F_1 plants. Through RFLP and GISH analyses, 25 MAALs (2 $n=25$, AA + 1C) were identified and divided into 12 syntenic groups, designated MAALs 1–12. MAALs 1, 2, 3, 5, 7 and 10 were each represented by one plant, MAALs 8, 11 and 12 by two plants, MAALs 6 and 9 by four plants, and MAAL 4 by five plants. An ideogram of the C-genome of *O. officinalis* was constructed, based on GISH analysis of the interspecific hybrid and the MAALs. Comparative RFLP maps showed strong syntenic associations between the A-genomes and C-genomes. Chromosomal arrangements such as translocations and duplications were detected in different alien chromosomes of the MAALs. The complete set of *O. officinalis* MAALs generated here provides a novel manipulation platform for exploiting and utilizing the *O. officinalis* genome and carrying out genetic studies.

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Introduction

Monosomic alien addition lines (MAALs) are plants with one chromosome of an alien donor species added to the entire chromosome complement of the recipient species. Constructing MAALs can provide a convenient way to dissect the donor genome into individual chromosome entities in a functional background, facilitating dissection of individual donor chromosomes. In breeding, the prime motive for making alien addition lines is to introgress genes of interest from distantly related wild relatives into the recipient genome of cultivated crops. Since each MAAL carries only a single donor chromosome, genome-wide searches for useful genes are unnecessary. Chromosome segment substitution lines, each carrying an introgressed chromosome segment originating from a donor species in an otherwise uniform elite genetic background were developed in tomato and rice (Ebitani et al. 2005; Eshed and Zamir 1995; Kubo et al. 2002; Kurakazu et al. 2001). The introgression lines were nearly isogenic to the recipient genotype, so all the genetic variations responsible for differences between them could be associated with the introgressed segments. For complex traits, especially traits related to yield and nutritional quality, loci controlling them are numerous, widespread, and intensively interact. Thus, using MAALs can facilitate the identification and localization of loci that control important agronomic traits, and lines carrying desired traits can be used directly for breeding (Zamir 2001). The application of MAALs offers more prospects to dissect these loci and ultimately transform them into simple Mendelian factors via backcrosses, greatly facilitating map-based cloning of the genes in the wild relatives (Fridman et al. 2000, 2004).

The genus *Oryza* consists of more than 20 species, including two cultivated species: *Oryza sativa* and *Oryza glaberrima*. The genomes of the 20 wild *Oryza* species have been classified into 10 distinct types (A, B, C, BC, CD, E, F, G, HJ and HK), representing an enormous gene pool for gene discovery and genetic improvement of rice cultivars. Based on classical taxonomy, isozyme, RFLP and gene sequence studies, all species in the genus *Oryza* except *Oryza brachyantha* (F genome) have been grouped into four main species complexes: *sativa*, *officinalis*, *ridleyi* and *meyeriana* (Ge et al. 1999; Vaughan et al. 2003). Two cultigens, *O. sativa* and *O. glaberrima*, are included in the *Sativa* complex and have A genomes. In the *officinalis* complex, there are nine species, with B, BC, C, CD and E genomes. *Oryza officinalis* is a diploid species with a C genome, which is considered to be the basic genome of the *officinalis* complex and similar to the A genome of *O. sativa* (Aggarwal et al. 1999; Vaughan et al. 2003). The genome of *O. officinalis* (1.45 pg/2C, 697 Mb) is bigger than that of *O. sativa* (Uozu et al. 1997). Genes for resistance to brown planthopper, whitebacked planthopper and bacterial blight have been transferred from *O. officinalis* into cultivated rice (Huang et al. 2001; Jena and Khush 1990; Tan et al. 2004a, b). Genomic libraries of *O. officinalis* have been constructed to facilitate gene isolation and genomics study (He et al. 2003, http://www.omap.org).

The MAALs of *O. officinalis* provide alternative sources for dissecting the C-genome and for improving cultivated rice. In the experiments reported here, MAALs were developed through transferring the chromosomes of *O. officinalis* into cultivated rice lines through wide hybridization and backcrossing, followed by embryo rescue to germinate the resulting seeds. The MAALs were then identified and carefully characterized by Genomic in situ hybridization (GISH) and in morphology. Restriction fragment length polymorphism (RFLP) markers were used to identify the extra C chromosome. Chromosome synteny detected by RFLP makers between A and C genomes is well conserved. These unique plant materials are useful for comparative functional studies on the C genome of *O. officinalis* and A genome of *O. sativa* and will facilitate introgression and identification of developmentally, physiologically and agriculturally important genes in wild rice.

Materials and methods

Plant material

The rice variety 'Hejiang 19' (*O. sativa* L. ssp. *Japonica*) and the wild species *O. officinalis* (Acc. HY018) were used in this study. Accession HY018 of *O. officinalis*, collected in Guangxi Province, China, possesses a number of valuable traits such as resistance to pests and diseases and high protein content in grain. A number of crosses were made between the *O. sativa* line as female and the wild species as the male parent. The hybrids were backcrossed to the recurrent parent *O. sativa* to obtain BC_2 progeny (Fig. 1). In all crosses, the seeds developed imperfectly. So an embryo rescue technique was applied, in which immature florets of all crosses were harvested 15 days after pollination and cultured in vitro to obtain seedlings according to the method of Jena and Khush (1989). The F_1, BC_1, BC_2 and parental plants were planted in pots at the Institute of Genetics, Wuhan University and grown under a standard regime for rice plants.

RFLP analysis

Genomic DNA was extracted from young leaves of the rice plants using the CTAB method of Murray and Thompson (1980). The DNA restriction digestion and Southern hybridization followed previously described

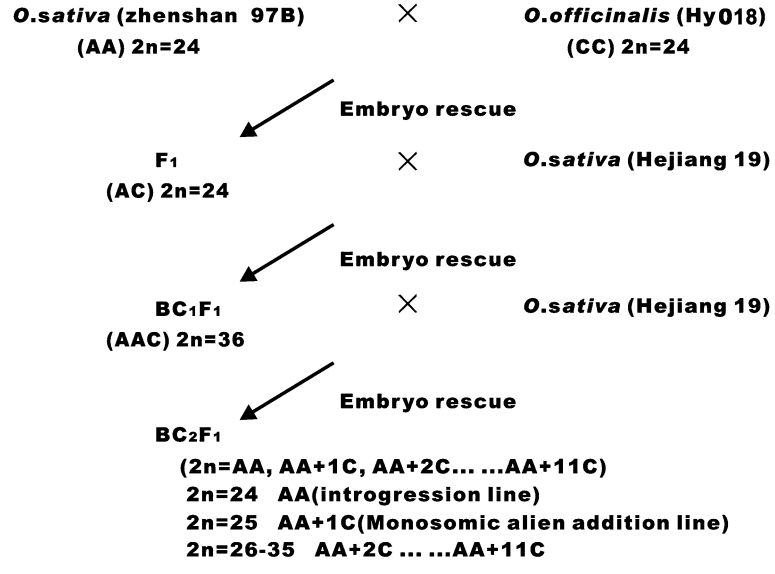

Fig. 1 Experimental schedule for hybridization and production of MAALs

procedures (Huang et al. 2001). The DNA samples were cut by five restriction enzymes: *Bam*HI, *Dra*I, *Hind*III, *Eco*RI and *Eco*RV. One hundred and ninety-two RFLP probes, distributed evenly across the 12 rice chromosomes, were used to identify polymorphisms between the *O. sativa* and *O. officinalis* genomes and the presence of alien *O. officinalis* chromosomes. Comparative maps were developed based on the original rice map from the Japanese Rice Genome Research Project (http://rgp.dna.affrc.go.jp). When multiple loci were detected by a single probe, the loci are designated with lowercase letters (a, b, c, etc.).

GISH

To display the chromosome constitution, the root tips were harvested in the morning from vigorously growing rice plants and fixed immediately in a 3:1(v/v) mixture of ethanol and glacial acetic acid at 4°C for 24 h, then stored in a refrigerator until use. The fixed root tips were hydrolyzed in 1 N HCl at 60°C for 10 min and stained in Schiff's reagent at room temperature for 20 min. The meristem cells were squashed in a drop of 2% (w/v) acetocarmine for chromosome counting. After being thoroughly washed with distilled water, the fixed root tips were treated with a mixture of 2% pectinase (SERVA) and 2% cellulase (SERVA) at 28°C for 3 h. Finally, the treated root tips were squashed on slides and dried over a flame (Song and Gustafson 1995). The slides were kept in a freezer at −20°C before GISH.

The *O. officinalis* genomic DNA was labeled with biotin using a nick translation kit (Sino-American Biotechnology Company, China). Blocking DNA was obtained from the *O. sativa* genomic DNA and autoclaved to fragment sizes of 100 bp to 1 kb. In situ hybridization was performed with the procedures described by Yan et al. (1999). Biotin-labeled probes were detected in a three-step detection/amplification procedure using: goat anti-biotin fluorescein isothiocyanate (FITC) conjugate—rabbit anti-goat biotin conjugate—goat anti-biotin FITC conjugate (Sigma, MO, USA). For each step of the immune reaction, slides were incubated at 37°C for 30 min and washed with PBS at intervals. Finally, the slides were counterstained with 3 μg/ml PI (propidium iodide) in Vectashield, an antifade solution (Vector Laboratories). Chromosomes were viewed under an Olympus BX51 fluorescence microscope equipped with a CoolSNAP *fx* CCD camera. Gray scale images were captured for each fluorescence channel and then merged with V^{++} Precision Digital Imaging software. Measurements were made on the digital images using Meta Imaging Series 4.6 software and final images were fine-tuned with Adobe Photoshop 6.0.

Phenotypic evaluation

The MAALs and parental plants were examined and evaluated at the maturing stage for the following traits: plant height—distance (cm) from the plant base to the tip of the panicle of the tallest tiller; leaf length—distance (cm) from the tip of the flag leaf blade to the point of attachment of the auricles; leaf width—the widest measurement (mm) of the flag leaf blade; ligule length—distance (mm) from the tip to the base line of the ligule of the flag leaf; panicle length—average total length (cm) of panicles per plant. Pollen fertility was determined by the KI staining method.

Results

Development of the MAALs

The 12 chromosomes of the C-genome were recovered as single additions to the normal chromosome complement of the AA genome from a BC_2F_2 population of a cross between *O. sativa* (japonica variety Hejiang 19) and *O. officinalis* (Acc. HY018) (Fig. 1). The inter-genomic F_1 hybrid between *O. sativa* and *O. officinalis* and the backcross progeny BC_1 were completely male sterile. Chromosome counting and GISH analysis showed that F_1 plants possessed 24 chromosomes with an AC-genome constitution (Fig. 2). The BC_1 plants possessed 36 chromosomes and their genome constitutions were AAC (data not shown). After pollinating about 20,000 florets of BC_1 plants with pollen from the recurrent parent Hejiang 19, imperfectly developed seeds numbering 196 were collected. Ninety-one BC_2 plants were generated via in vitro embryo rescue culture and transplanted in the field. We analyzed 84 BC_2 plants for root tip chromosome counting. Root tips were not collected from the remaining 15 plants because of their weak growth. The chromosome numbers of the BC_2 plants ranged from 24 to 35. Twenty-nine plants (34.5%) had 24 normal A chromosomes, among which 14 contained introgressed regions of the C-genome, detected by one or two RFLP markers (data not shown). We obtained 55 aneuploid plants in total, containing the normal 24 A-chromosomes plus 1–11 alien C-chromosomes. In studies by Brar et al. (1996), aneuploids with 1–6 alien C-chromosomes were obtained, and those with more alien chromosomes showed lower viability. Of the 55 aneuploids obtained, 25 had 25 chromosomes, 16 had 26 chromosomes, 6 had 26 chromosomes and 5 had 31 chromosomes. Aneuploid plants with 25 chromosomes were used in further analyses.

Alien chromosomes in the aneuploids could be identified with a suite of RFLP markers, exploiting the syntenic association between the C-genome of *O. officinalis* and the A-genome of *O. sativa*. One hundred and ninety two RFLP markers evenly distributed throughout the 12 chromosomes of cultivated rice were selected from the Rice Genome Project (RGP) map (http://rgp.dna.affrc.go.jp) for polymorphism screening. All the probes revealed polymorphism between the *O. sativa* and *O. officinalis* parents with at least one of the five restriction endonucleases. The high frequency of poly-

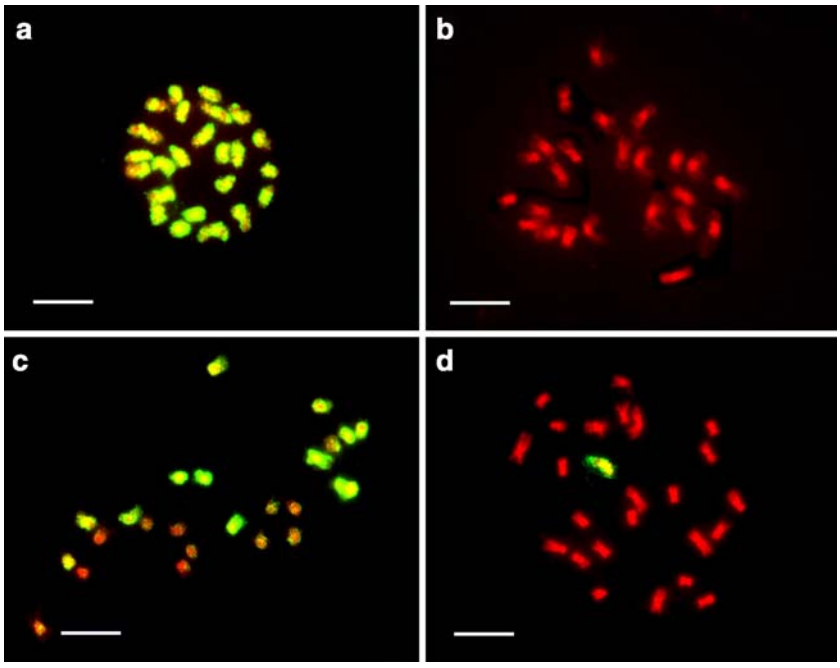

Fig. 2 Characterization of interspecific hybrid and MAALs by GISH. **a** The 24 chromosomes of *O. officinalis* (positive control) fluoresced *yellow–green*. **b** *O. sativa* (negative control) revealed 24 *red* chromosomes by PI counterstaining. **c** Twelve chromosomes in the interspecific hybrid displayed the *yellow–green* hybridization signals. **d** The alien chromosome in MAAL 4 fluoresced *yellow–green*. All *bars* represent 10 μm

morphism reflected the substantial divergence between the A-genome of *O. sativa* and the C-genome of *O. officinalis*. All the aneuploid plants showed characteristic *O. officinalis* band patterns in genomic Southern blot analysis, for at least some RFLP markers, proving that each of them contained a chromosome from *O. officinalis* (Fig. 3). We analyzed the band patterns of 25 MAALs ($2n = 25$, AA + 1C) probed by the polymorphic markers. A majority of the polymorphic RFLP markers of the 12 chromosomes of the A-genome mapped to corresponding syntenic groups of the C-genome. The alien C-chromosomes in the MAALs were identified by exploiting the syntenic association between markers on the C-genome of *O. officinalis* and the A-genome of the cultivated rice. If most RFLP markers from chromosome 1 of cultivated rice displayed *O. officinalis*-specific bands in a MAAL plant, it was assumed to have 1C as an extra chromosome, and thus was designated a MAAL 1 plant; if most RFLP markers from chromosome 2 of cultivated rice displayed such bands in another MAAL plant, it was designated a MAAL 2 plant. In this manner, the 25 plants that contained an additional, alien chromosome were divided into 12 syntenic groups comprising a complete set of MAALs. Of the 12 kinds of MAALs thus designated, MAALs 1, 2, 3, 5, 7 and 10 were each represented by one plant, MAALs 8, 11 and 12 by two plants, MAALs 6 and 9 each by four plants, and MAAL 4 by five plants (Table 1).

The remaining aneuploid plants, each containing more than 25 chromosomes, were analyzed for RFLP markers and the C-chromosomes in each of them were identified accordingly. All the aneuploid BC_2 plants harboring alien chromosomes are listed in Table 1. Alien chromosomes from *O. officinalis* were unequally transmitted from allotriploid BC_1 plants to the BC_2 aneuploids, as indicated by their relative frequencies in the total number of transmitted C-chromosomes. A remarkable feature was that some of the alien chromosomes appeared more frequently than others in the aneuploid population. For example, chromosome 6 of the *O. officinalis* appeared in 19 aneuploid plants tested for this chromosome, and accounted for 16.1% of the alien chromosomes in all the aneuploid plants. In addition, chromosome 4 was found in five MAALs and

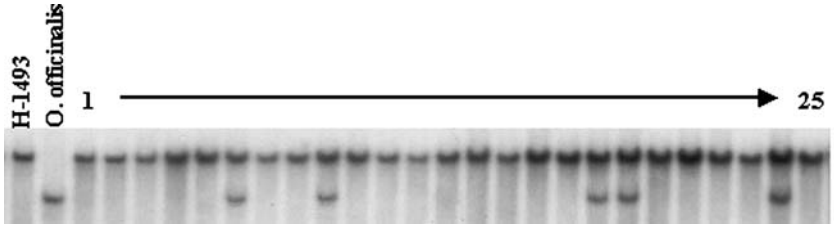

Fig. 3 Southern blot analysis of *O. sativa*,*O. officinalis*, and 25 *O. officinalis* monosomic addition lines. Genomic DNA was digested with *Bam*HI and probed with marker R445 from chromosome 4 of the AA genome. Five out of 25 MAALs showed the *O. officinalis*-specific bands. The patterns indicated that five plants contained a chromosome segment from *O. officinalis* in which marker R445 was located

Table 1 Identification of the alien (*O. officinalis*) chromosome found in *O. sativa* × *O. officinalis* BC_2 aneuploid plants based on root tip chromosome counts and RFLP markers

Chromosome number of aneuploid plants	Added chromosome of C-genome											
	1C	2C	3C	4C	5C	6C	7C	8C	9C	10C	11C	12C
25	1	1	1	5	1	4	1	2	4	1	2	2
26	2	2		4	4	7	2		2	4	4	1
27				3	2	4	1	2	2	1		3
28		1	1				1			1		
31	1	3	2	2	2	3	2	4	2	2	1	4
35	1	1	1	1	1	1		1	1	1	1	1
Sum	5	8	5	15	10	19	7	9	11	10	8	11
Percentage (%)	4.2	6.8	4.2	12.7	8.5	16.1	5.9	7.6	9.3	8.5	6.8	9.3

appeared at a higher frequency (12.7%) than the other ten different chromosomes in the aneuploid population. The exotic chromosomes 1 and 3 occurred at the lowest frequencies (4.2%). Variations in the frequencies of alien chromosomes in an aneuploid population could be analogous to the segregation distortion that commonly occurs in wide crosses of plant species (Zamir and Tadmor 1986; Chetelat et al. 2000), presumably reflecting the homoeologous pairings of corresponding A and C chromosomes or selection at post-zygotic stages (Chetelat et al. 2000).

Morphological characterization of MAALs

There were considerable morphological variations in the BC_2 population. Morphological features have provided a basis for establishing MAALs of wild rice in previous studies (Multani et al. 1994, 2003). In the present experiment, the 12 MAALs exhibited a slow growth habit compared to the *O. sativa* japonica 'Hejiang 19'. The plants differed from each other, as well as from the normal diploid *O. sativa* in fundamental morphological features, such as growth habit, height, shape and length of leaves, size of ligule, presence or absence of awns and pollen fertility (Table 2). The MAAL 1 plants had narrow, light green and droopy leaves, MAALs 2 and 3 were characterized by slow growth, sturdy stem, and dark-green leaves held in an erect position on the stem. In addition, MAAL 2 had awned spikelets and was a dwarf. MAALs 4 and 9 had a spreading growth habit, and MAALs 4 looked similar to *O. sativa* 'Hejiang 19'. MAALs 6 plants were weakly grassy, with leaves greatly reduced in size. MAALs 6 and 4 also had spikelets with long awns. MAAL 7 had light green and rolled leaves. MAAL 8 plants exhibited wide, dark-green and erect leaves. MAAL 10 was the tallest, with long and narrow leaves. Compared with 'Hejiang 19', MAALs 5, 11 and 12 were characterized by very slow growth and a compact habit. MAAL 5 had the shortest ligule, while the longest ligule was observed in MAAL 12. All the MAALs were completely sterile with the exception of MAALs 9 and 12, which produced a few seeds.

Characterization of C-chromosomes in the MAALs

To discriminate between the alien chromosomes and the *O. sativa* chromosomes in the MAALs, biotin-labeled genomic DNA probes of *O. officinalis* blocked with unlabeled *O. sativa* genomic DNA was hybridized in situ to their mitotic metaphase chromosomes. The labeled chromosomes fluoresced green owing to the presence of FITC, while unlabeled chromosomes fluoresced red as a result of counterstaining with PI. Different mixing ratios of probe DNA and blocking DNA were tested. When

Table 2 Morphological traits of *O. officinalis* (Acc. HY018) MAALs in the Hejiang19 (*O. sativa*) background

MAAL	Plant height (cm)	Leaf length (cm)	Leaf width (mm)	Ligule length (mm)	Panicle length (cm)	Awn
1	44	28.0	10.0	0.7	9.5	No
2	27	17.0	9.0	0.5	7.0	Yes
3	34	16.0	12.0	1.0	8.0	No
4	50	22.4	12.2	1.1	13.9	Yes
5	37	22.0	12.0	0.5	9.0	No
6	37	18.0	10.8	1.1	10.1	Yes
7	49	27.0	13.0	1.0	14.0	No
8	54	24.0	17.0	0.7	14.0	No
9	53	27.0	14.0	1.2	15.5	No
10	56	38.0	9.0	1.2	11.0	No
11	40	20.0	11.0	1.2	12.0	No
12	44	22.5	11.0	1.4	13.5	No
O. officinalis	175	28.0	21.0	0.3	35	Yss
O. sativa Hejiang 19	58	26.0	11.0	0.8	12.0	No

the ratio was 1–16, all the 24 chromosomes of *O. officinalis* (positive control) fluoresced green (Fig. 2a), whereas those of the *O. sativa* (negative control) fluoresced red (Fig. 2b). Similarly, in somatic chromosome preparations of the interspecific hybrid (Fig. 2c), 12 chromosomes, originating from *O. officinalis*, showed green coloration while the other 12 chromosomes, originating from *O. sativa*, showed red coloration. To date, a few reports have described the mitotic chromosome morphology of wild rice species, but none have provided quantitative measurements (Uozu et al. 1997). The chromosomes of *O. officinalis* were clearly bigger than those of *O. sativa*. With the blocking DNA present 16 times at the probe concentration, one green *O. officinalis* chromosome and 24 red *O. sativa* chromosomes were clearly visible in all the MAALs (Fig. 2d). Although the intensities of the GISH signals varied greatly among the different alien chromosomes, the green hybridization signals were all associated with the alien chromosomes. An ideogram of the C-genome was constructed through analyses of all the 12 different MAALs (Fig. 4) and the sizes of the chromosomes of *O. officinalis* were found to differ. Chromosomes 1, 2 and 9 were among the largest and chromosomes 4 and 11 the smallest. The karyotype is similar to that of *O. sativa* (Apisitwanich et al. 2001; Cheng et al. 2001).

Synteny analysis using RFLP markers

The aneuploid plants including MAALs were analyzed for 192 RFLP markers distributed throughout the 12 chromosome linkage groups of cultivated rice (Fig. 5, Table 3). Twenty-two markers for chromosome 1 were used. Of these, 11 displayed C-genome-specific band patterns in MAAL 1 and two double monosomic addition lines, but ten markers from the short arm, which had previously exhibited polymorphisms between *O. officinalis* and *O. sativa*, did not (Fig. 3). It is possible that in MAAL 1 the short arm of chromosome 1 of the C-genome was replaced by that of the A-genome during the meiosis of the BC_1 plant. The GISH analysis revealed that MAAL 1 possessed an entire extra chromosome, and the short arm of the exotic chromosome did not show green signals when probed by the genomic DNA of *O. officinalis* (Fig. 4), corroborating the hypothesis. Of 19 markers on chromosome 2, 15 displayed C-genome-specific bands in MAAL 2, but the other four (C560, R1826, G132 and G1327) did not map to MAAL 2 or any of the other MAALs. Five markers on the short arm of chromosome 2 of the A-genome mapped to both MAALs 2 and 3, indicating that the segment linked by these five markers on chromosomes of 2C and 3C might be homologous. In total, 20 markers from chromosome 3 of the A-genome were used, ten of which revealed synteny between chromosomes 3A and 3C. The MAAL 3 did not display *O. officinalis*-specific band patterns for any of the nine markers on the short arm of chromosome 3 of *O. sativa*, except for marker C1135. C1677 did not detect the alien chromosome in MAAL 3, but it revealed a C-genome type band pattern in MAAL 4, indicating translocation between the A-genomes and C-genomes. Chromosome 4 showed the strongest synteny between the A-genomes and C-genomes. All 16 markers for this chromosome displayed C-genome-specific band patterns in the five MAAL 4 plants. Strong synteny was detected for chromosomes 5–7. Of the 17 RFLP markers on chromosome 5, all except marker C246 on the short arm detected the alien chromosome in MAAL 5. Two (R2147 and R2654) of 16 markers on chromosome 6 failed to detect wild rice alleles in MAAL 6. Similarly, markers C39 and C3089 on chromosome 7 of the A-genome did not detect the C-chromosome in MAAL 7. No duplications or translocations were found for markers on chromosomes 5–7. Among 15 markers on chromosome 8, 13 displayed synteny. Alleles were not detected for two markers (G1073 and C347) in MAAL 8. R902 on chromosome 8 also detected a duplicate in MAAL 6. Thirteen markers on chromosome 9 were used and two (R1687 and G385) failed to generate *O. officinalis* band patterns in the corresponding MAAL 9. C1454 detected wild rice alleles in MAAL 6 instead, indicating that a translocation had occurred. Among 11 markers on chromosome 10, all but one showed good synteny between the A-genomes and C-genomes; the exception, marker R1629, displayed wild rice band patterns in MAAL 8. Among 15 markers on chromosome 11, 13 displayed synteny, of which C535

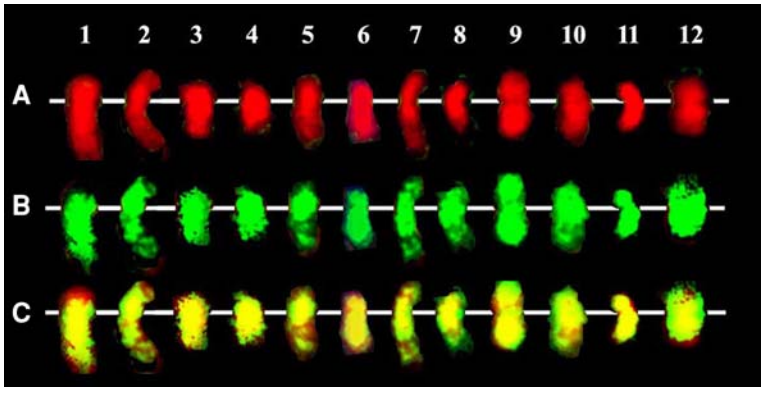

Fig. 4 Ideogram of the C genome of *O. officinalis* based on the results of the GISH analysis of MAALs. The chromosomes *O. officinalis* fluoresced *red* when stained with propidium iodide (**a**), and *green* following GISH of labeled DNA (**b**). All the 12 chromosomes in different MAALs were discriminated and gave signals of varying intensity (**c**). The *bar* represents 10 μm

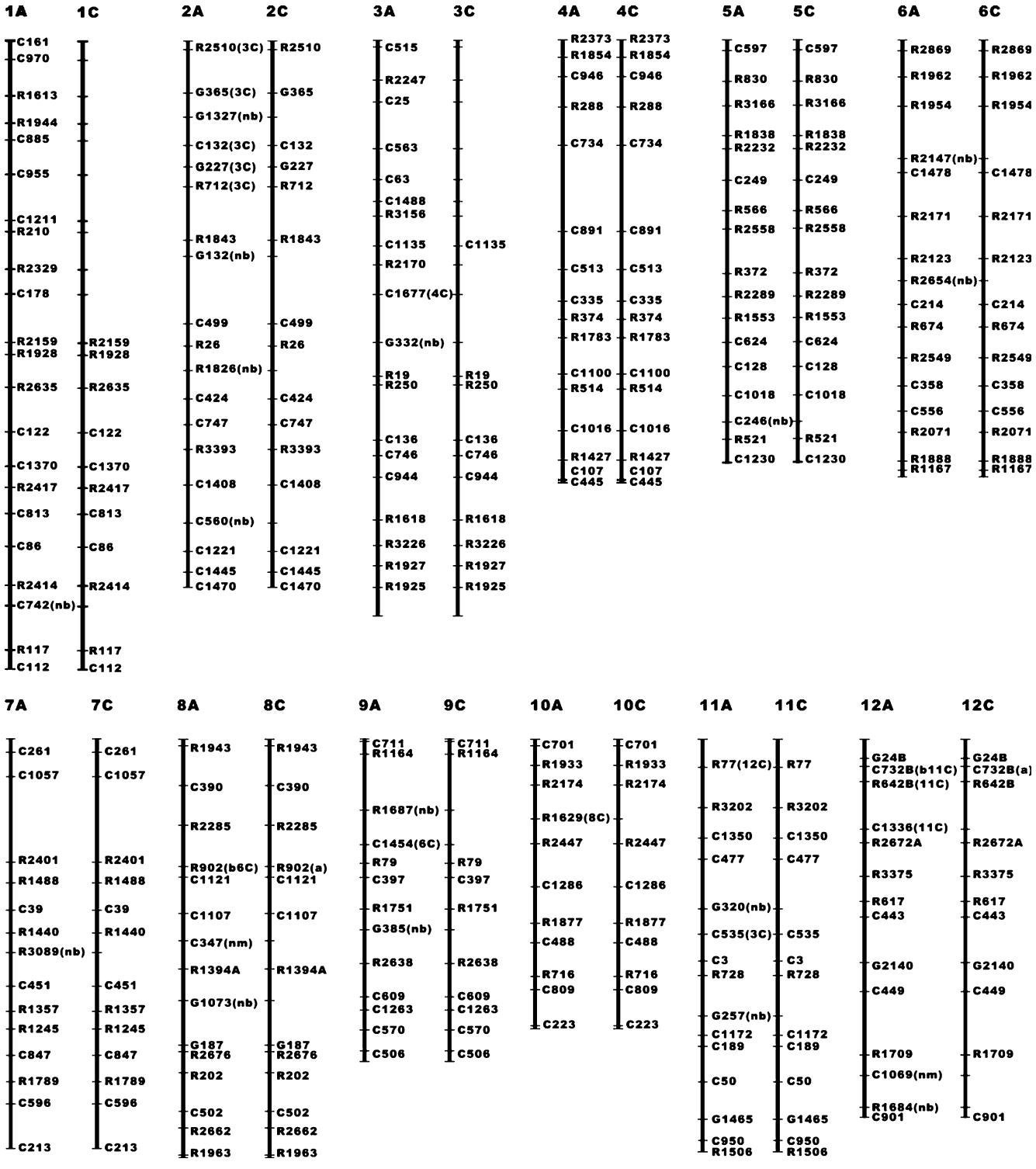

Fig. 5 Comparative map of the A-genomes and C-genomes based on genomic Southern hybridization of the MAALs

and R77 detected duplications in MAALs 3 and 12. The remaining two markers (G257 and G320) did not reveal *O. officinalis* alleles in any of the MAALs. Syntenic association on chromosome 12 was more complex. Among 14 markers used, two (R1684 and C1069) did not display wild rice bands in the MAAL 12 plants. Three markers (C1336, R642B and C732B) on the short arm were detected in MAAL 11 plants, suggesting a segmental duplication in *O. officinalis*.

In all, 151 (78.6%) of the *O. sativa* markers were mapped on the corresponding chromosomes of *O. officinalis*, 10 were detected on multiple chromosomes

Table 3 Classification of 192 RFLP markers based on the band patterns in MAALs

Chromosome	Number of markers				
	Total	Syntenic[a]	Not detected[b]	Duplicated[c]	Translocated[d]
1	22	11	11		
2	19	15	4	5 (3C)	
3	20	10	9		1 (4C)
4	16	16			
5	17	16	1		
6	16	14	2		
7	14	12	2		
8	15	13	2	1 (6C)	
9	13	10	2		1 (6C)
10	11	10			1 (8C)
11	15	13	2	2 (3C, 12C)	
12	14	11	2	2 (11C)	1 (11C)
Total	192	151 (78.6%)	37 (19.3%)	10 (5.2%)	4 (2.1%)

[a] *Syntenic*: the markers showing band pattern of *O. officinalis* in corresponding MAAL
[b] *Not detected*: the markers for which the band of *O. officinalis* was not displayed in corresponding MAAL
[c] *Duplicated*: the markers showing band pattern of *O. officinalis* in more than one MAAL; 3C, 6C et al. in *parenthesis* indicate the extra C chromosome showing the unexpected band
[d] *Translocated*: the markers showing band patterns in non-corresponding MAAL

(indicating duplication), four on different chromosome (indicating translocation), and 37 markers were not detected (indicating deletion) on C chromosomes (Table 3). The fact that most of the RFLP markers mapped to homoeologous chromosomes shows that C chromosomes of *O. officinalis* and A chromosomes of the cultivated rice exhibited good syntenic associations. Sequence searches revealed that most of the RFLP probes used correspond to sequences of low copy genes in the rice genome (data not shown). Therefore, our results provide evidence of conserved gene content and order between the homoeologous chromosomes of the A-genomes and C-genomes.

Discussion

Characterization of the MAALs

A complete set of MAALs is a valuable tool for plant genetic and genomic studies. In rice, MAALs have been reported of *O. officinalis* (Jena and Khush 1989), *O. punctata* (Yasui et al. 1992), *O. minuta* (Amante-Bordeos et al. 1992), *O. australiensis* (Multani et al. 1994), *O. eichingeri* (Yan et al. 1999) and *O. latifolia* (Multani et al. 2003). The MAALs in the cited studies were identified by comparing their morphology with that of primary trisomics of *O. sativa* and, occasionally, by isozyme analysis (Multani et al. 2003). In the set of A–C addition lines generated here, each of the C-chromosomes of *O. officinalis* is represented monosomically in a uniform A-genome background of *O. sativa* japonica cv Hejiang 19. The complete panel of MAALs was characterized using molecular markers, as well as cytogenetic and morphological features. Florescent GISH discriminated between the alien C-chromosome and the 24 A-chromosomes, completely eliminating the possibility of confusing MAALs with A-genome trisomics. Under the microscope, the chromosomes of *O. officinalis* appeared bigger than those of *O. sativa* (Fig. 2). The genome size of C has been estimated to be over 697 Mb (Uozu et al. 1997); much bigger than the 430 Mb A-genome. Therefore, the differences in size between the A-chromosomes and C-chromosomes in the root-tip cell spread of interspecific hybrids may reflect the differences in genome size in nt base pairs. An ideogram of the C-genome was constructed via GISH analysis of the MAALs and the interspecific F_1 hybrid (Fig. 5). The visual size of the individual chromosomes varied, and was largely consistent with the linkage map lengths, in cM, derived from a F_2 population of a cross between two accessions of *O. officinalis* (Jena et al. 1994). RFLP markers for *O. sativa* are extremely useful for identifying MAALs. Syntenic associations between homoeologous chromosomes of the A and C genomes allow us to ascertain the identities of the alien chromosome in the MAALs. Probing with RFLP markers distributed evenly amongst the 12 chromosomes ensures accurate identifications. MAALs were also characterized according to their morphological features. There is good resemblance in morphology of the current set of MAALs to those developed by Jena and Khush (1989), and to the trisomic lines reported by Khush et al. (1984) and Cheng et al. (1996). Variation was also observed since the accessions of *O. officinalis* used to develop the MAALs in the two studies differed, as did the receptor rice varieties (Hejiang 19, used here, is an early season japonica variety, while Jena and Khush used an indica variety).

Synteny between the A-genomes and C-genomes

High-density molecular marker maps are now available for many crop plants, providing useful frameworks for genome studies, gene cloning, quantitative trait locus

(QTL) analysis, variety development, and many other potential applications. Genetic maps have been compared for related species such as rice, maize, sorghum and wheat (Ahn et al. 1993), tomato, potato and pepper (Tanksley et al. 1988; Prince et al. 1993), and Arabidopsis and Brassica (Lagercrantz et al. 1996) species. These studies have revealed a surprisingly high level of marker synteny over large tracts of DNA and conservation of QTLs for agronomic characters in species as divergent as maize and rice. Nevertheless, information is scarce on genomic comparisons between cultivated rice and wild *Oryza* species. Such information would facilitate the identification and utilization of beneficial genes, alleles and QTLs in the unexplored wild germplasm (Tanksley and McCouch 1997).

One hundred and ninety-two polymorphic RFLP markers selected from the molecular map of rice (http://rgp.dna.affrc.go.jp/) were used to analyze *O. sativa*, *O. officinalis*, F_1, BC_1F_1 and 84 BC_2F_1 plants. Most of the markers mapped to the corresponding homoeologous chromosomes of *O. officinalis* as the probes hybridized with the digested DNA of the MAALs, showing that synteny is well conserved for all 12 linkage groups between *O. sativa* and *O. officinalis*. Conservation was strongest for chromosome 4, for which 16 RFLP markers of rice all mapped to MAAL 4. The frequency of recombinant genotypes was also high for this chromosome, indicating that the degree of homology between the genomes may be higher here than for the other pairs of homoeologous chromosomes. However, some differences between the chromosomal structures of the A-genomes and C-genomes were detected in MAALs for chromosomes 2, 3, 8, 9, 10, 11 and 12. Most RFLP markers used in this experiment were developed from cDNA clones and represent sequences coding for genes in the rice genome (data not shown). It has been shown in several groups of plants that the orders of genes in linkage blocks are generally conserved between related species, in spite of karyotype reshuffling during evolution (Ahn and Tanksley 1993; Kowalski et al. 1994). The finding that the locations of genes among chromosomes is highly conserved in the genomes of *O. officinalis* and *O. sativa* is not unexpected, since the overall homosequentiality of the A-genomes and C-genomes is consistent with the karyotype similarity between the two genomes (Kurata and Omura 1984). On the other hand, the degree of synteny of SSR markers between *O. sativa* and *O. officinalis* is reportedly fairly low (Jin et al. unpublished data). Of 21 rice SSR markers on chromosome 4 of *O. sativa*, only seven were convincingly shown to have alleles on the homoeologous chromosome in the genome of *O. officinalis*. The data showed that the SSR markers were located in the intergenic regions and shared low degrees of synteny. The distinguishable state by GISH and SSR and high degree of synteny of RFLP alleles suggest that the A-genomes and C-genomes are well conserved in terms of gene content and allocation on chromosomes, but differentiated with respect to their intergenic, largely repetitive DNA contents.

Structural variation between A-chromosomes and C-chromosomes

Cross-mapping of RFLP probes in a range of cereal crops has led to the widespread identification of chromosomal regions in which marker orders are highly conserved. However, recent genomic comparisons among grass species have shown many exceptions to the general conservation of genes, resulting in the disruption of gene collinearity between closely related species (Bennetzen 2000; Devos and Gale 2000; Feng et al. 2002; Keller and Feuillet 2000).

The MAALs are useful for detecting structural variations in homoeologous chromosomes of related species. Chromosome duplications can be detected and multigene families can be mapped without the need for segregating populations and conventional linkage analysis (Chetelat et al. 1998; Suen et al. 1997). Some RFLP markers from the cultivated rice genome detected duplicated loci on different chromosomes of *O. officinalis*. For example, R77 from chromosome 11 of cultivated rice mapped to the exotic chromosomes of both MAALs 11 and 12. Similarly, markers C732B and R642B of chromosome 12 of *O. sativa* existed in two copies, assigned to chromosomes 11 and 12 of *O. officinalis*, respectively. It seems reasonable to propose that the segment covered by markers R77, R642B and C732B might be duplicated on chromosomes 11 and 12 of the C-genome. In addition, C535 from chromosome 11 of cultivated rice mapped to the exotic chromosomes of both MAALs 11 and 3. Marker R902 from chromosome 8 of cultivated rice appears to be present in two copies in *O. officinalis*, one of which was assigned to chromosome 6 and the other to chromosome 8. Translocation of some chromosomal markers was observed on chromosomes 4, 6, 8 and 11. Probe C1677 from chromosome 3 of the A-genome displayed *O. officinalis*-specific band patterns in MAALs 4, but not in MAAL 3. Similarly, C1454, R1629 and C1336 from chromosomes 9, 10 and 12 of the A-genome, respectively, detected *O. officinalis*-specific band patterns not in MAALs 9, 10 and 12, but in MAALs 6, 8 and 11, respectively.

We finally developed a full set of lines with individual chromosome addition from *O. officinalis* to cultivated rice using RFLP and GISH analyses. Such a set has great potential as a convenient means for preserving the C-genome of *Oryza* in a relatively accessible form, facilitating the chromosomal assignment of dominant genes identified in *O. officinalis*, and the eventual transfer of useful traits into cultivated rice. Synthesis of an IL library for *O. officinalis* would be viable. Genes from *O. officinalis* could be fine-mapped and tagged with closely linked markers. A genomic BIBAC library could be screened using the markers and used to directly transform cultivated rice for complement tests (He et al. 2003). Thus, our results bode well for exploiting the genetic diversity of wild *Oryza* species and expanding the gene pool of cultivated rice.

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References

Aggarwal RK, Brar DS, Nandi S, Huang N, Khush GS (1999) Phylogenetic relationships among *Oryza* species revealed by AFLP markers. Theor Appl Genet 98:1320–1328

Ahn S, Tanksley SD (1993) Comparative linkage maps of the rice and maize genomes. Proc Natl Acad Sci USA 90:7980–7984

Ahn S, Anderson JA, Sorrells ME, Tanksley SD (1993) Homoeologous relationships of rice, wheat and maize chromosomes. Mol Gen Genet 241:483–490

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

Apisitwanich S, Shishido R, Akiyama Y, Fukui K (2001) Quantitative chromosome map of a representative *indica* rice. Euphytica 118:113–118

Bennetzen JL (2000) Transposable elements contributions to plant gene and genome evolution. Plant Mol Biol 42:351–369

Brar DS, Dalmacio R, Elloran R, Aggarwal R, Angeles R, Khush GS (1996) Gene transfer and molecular characterization of introgression from wild *Oryza* species into rice. In: Rice genetics III. IRRI, Manila, Philippines, pp 477–486

Cheng ZK, Li X, Yu HX, Gu MH (1996) A new set of primary trisomics in indica rice, its breeding and cytological investigation. Acta Genet Sini 23:363–371

Cheng ZK, Buell CR, Wing RA, Gu M, Jiang J (2001) Toward a cytological characterization of the rice genome. Genome Res 11:2133–2141

Chetelat RT, Rick CM, Cisneros P, Alpert KB, DeVerna JW (1998) Identification, transmission, and cytological behavior of *Solanum lycopersicoides* Dun. Monosomic alien addition lines in tomato (*Lycopersicon esculentum* Mill.). Genome 41:40–50

Chetelat RT, Meglic V, Cisneros P (2000) A genetic map of tomato based on BC1 *Lycopersicon esculentum*×*Solanum lycopersicoides* reveals overall synteny but suppressed recombination between these homoeologous genomes. Genetics 154:857–867

Devos KM, Gale MD (2000) Genome relationships: the grass model in current research. Plant Cell 12:637–646

Ebitani T, Takeuchi Y, Nonoue Y, Yamamoto T, Takeuchi K, Yano M (2005) Construction and evaluation of chromosome segment substitution lines carrying overlapping chromosome segments of indica rice cultivar 'Kasalath' in a genetic background of japonica elite cultivar 'Koshihikari'. Breed Sci 55:65–73

Eshed Y, Zamir D (1995) An introgression line population of *Lycopersicon pennellii* in the cultivated tomato enables the identification and fine mapping of yield-associated QTL. Genetics 141:1147–1162

Feng Q, Zhang Y, Hao P, Wang S, Fu G, Huang Y, Li Y, Zhu J, Liu Y, Hu X (2002) Sequence and analysis of rice chromosome 4. Nature 420:316–320

Fridman E, Pleban T, Zamir D (2000) A recombination hotspot delimits a wild species QTL for tomato sugar content to 484-bp within an invertase gene. Proc Natl Acad Sci USA 97:4718–4723

Fridman E, Carrari F, Liu YS, Fernie AR, Zamir D (2004) Zooming in on a quantitative trait for tomato yield using interspecific introgressions. Science 305:1786–1789

Ge S, Sang T, Lu BR, Hong DY (1999) Phylogeny of rice genomes with emphasis on origins of allotetraploid species. Proc Natl Acad Sci USA 96:14400–14405

He RF, Wang YY, Shi ZY, Ren X, Zhu LL, Weng QM, He GC (2003) Construction of genomic library of wild rice and *Agrobacterium*-mediated transformation of large insert DNA linked to BPH resistance locus. Gene 321:113–121

Huang Z, He GC, Shu LH, Li XH, Zhang QF (2001) Identification and mapping of two brown planthopper genes in rice. Theor Appl Genet 102:929–934

Jena KK, Khush GS (1989) Monosomic alien addition lines of rice: production, morphology, cytology, and breeding behavior. Genome 32:449–455

Jena KK, Khush GS (1990) Introgression of genes from *Oryza officinalis* Well ex Watt to cultivated rice, *O. sativa* L. Theor Appl Genet 80:737–745

Jena KK, Khush GS, Kochert G (1994) Comparative RFLP mapping of a wild rice, *Oryza officinalis*, and cultivated rice, *O. sativa*. Genome 37:382–389

Keller B, Feuillet C (2000) Colinearity and gene density in grass genomes. Trends Plant Sci 5:246–251

Khush GS, Singh RJ, Sur SC, Librojo A (1984) Primary trisomics of rice: origin, morphology, cytology, and use in linkage mapping. Genetics 107:141–163

Kowalski SP, Lan TH, Feldmann KA, Paterson AH (1994) Comparative mapping of *Arabidopsis thaliana* and *Brassica oleracea* chromosomes reveals islands of conserved organization. Genetics 138:449–510

Kubo T, Aida Y, Nakamura K, Tsunematsu H, Doi K, Yoshimura A (2002) Reciprocal chromosome segment substitution series derived from Japonica and Indica cross of rice (*Oryza sativa* L.). Breed Sci 52:319–325

Kurakazu T, Sobrizal, Ikeda K, Sanchez PL, Doi K, Angeles ER, Khush G.S, Yoshimura A (2001) *Oryza meridionalis* chromosomal segment introgression lines in cultivated rice, *O. sativa* L. Rice Genet Newsl 18:81–82

Kurata N, Omura T (1984) Chromosome analysis. In: Tsunoda ST, Takahashi N (eds) Biology of rice. Japan Scientific Societies Press, Tokyo and Elsevier, Amsterdam, pp 305–320

Lagercrantz U, Putteril J, Coupland G, Lydiate D (1996) Comparative mapping in *Arabidopsis* and *Brassica*, fine scale genome collinearity and congruence of genes controlling flowering time. Plant J 9:13–20

Multani DS, Jena KK, Brar DS, Delos Reyes BG, Angeles ER, Khush GS (1994) Development of monosomic alien addition lines and introgression of genes from *Oryza australiensis* Donin. to cultivated rice *O. sativa* L. Theor Appl Genet 88:102–109

Multani DS, Khush GS, Delos Reyes BG, Brar DS (2003) Alien genes introgression and development of monosomic alien addition lines from *Oryza latifolia* Desv. to rice, *Oryza sativa* L. Theor Appl Genet 107:395–405

Murray MG, Thompson WF (1980) Rapid isolation of high-molecular-weight plant DNA. Nucleic Acids Res 8:4321–4325

Prince JP, Pochard E, Tanksley SD (1993) Construction of a molecular map of pepper and comparisons of syntenic relationships with tomato. Genome 36:404–417

Song YC, Gustafson JP (1995) The physical location of fourteen RFLP markers in rice (*Oryza sativa* L.). Theor Appl Genet 90:113–119

Suen DF, Wang CK, Lin RF, Kao YY, Lee FM, Chen CC (1997) Assignment of DNA markers to *Nicotiana syvestris* chromosomes using monosomic alien addition lines. Theor Appl Genet 94:331–337

Tan GX, Huang Z, Weng QM, Ren X, Zhu LL, He GC (2004a) Two whitebacked planthopper resistance genes shared the same genomic intervals with brown planthopper resistance genes. Heredity 92:212a–217a

Tan GX, Weng QM, Ren X, Shi ZY, Zhu LL, He GC (2004b) Mapping of a new resistance gene to bacterial blight in rice line introgressed from *Oryza officinalis*. Acta Genetica Sinica 31:724b–729b

Tanksley SD, Berantzky R, Lapitan NL, Prince JP (1988) Conservation of gene repertoire but not gene order in pepper and tomato. Proc Natl Acad Sci USA 85:6419–6423

Tanksley SD, McCouch SR (1997) Seed banks and molecular maps: unlocking genetic potential from the wild. Science 277:1063–1066

Uozu S, Ikehashi H, Ohmido N, Ohtsubo H, Ohtsubo E, Fukui K (1997). Repetitive sequences: cause for variation in genome size and chromosome morphology in the genus *Oryza*. Plant Mol Biol 35:791–799

Vaughan DA, Morishima H, Kadowaki K (2003) Diversity in the *Oryza* genus. Curr Opin Plant Biol 6:139–146

Yan HH, Min SK, Zhu LH (1999) Visualization of *Oryza eichingeri* chromosomes in intergenomic hybrid plants from *O. sativa*× *O. eichingeri* via fluorescent *in situ* hybridization. Genome 42:48–51

Yasui H, Yoshimura A, Iwata N (1992) Characterization of monosomic alien chromosomes of *O. punctata* transferred to *O. sativa* using RFLP markers. Rice Genet Newsl 9:139–142

Zamir D (2001) Improving plant breeding with exotic genetic libraries. Nat Rev Genet 2:983–989

Zamir D, Tadmor Y (1986) Unequal segregation of nuclear genes in plants. Bot Gaz 147:355–358