Physical Mapping of Duplicated Genomic Regions of Two Chromosome Ends in Rice

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ABSTRACT

Two genomic regions duplicated in distal ends of the short arms of chromosomes *11* and *12* in rice (*Oryza sativa* L.) were characterized by YAC ordering with 46 genetic markers. Physical maps covering most of the duplicated regions were generated. Thirty-five markers, including 21 rice cDNA clones, showed the duplicated loci arrayed strictly in the same order along the two specific genomic regions. Regardless of their different genetic distances, the two duplicated segments may have a similar and minimum physical size with an expected length of about 2.5 Mb. However, differences of RFLP frequency for the duplicated DNA copies and recombination frequency for a given homoeologous area between the two regions were observed, indicating that these changes in genome organization occurred after the duplication. Our results establish a good model system for resolving the relationships between gene duplication, expression of duplicated genes, and the frequency of meiotic recombination in small chromosomal regions.

RICE (*Oryza sativa* L.) is one of the most important cereal crops, feeding more than half of the world population. It has become one of the plant models for genome analysis because of its smallest genome size within the cereals, its well-known conventional (phenotypical) genetic map (Kinoshita 1995), and high-density molecular genetic maps (Saito *et al.* 1991; Causse *et al.* 1994; Kurata *et al.* 1994b; Harushima *et al.* 1998). The importance of this plant as a model has become more significant recently since comparative chromosome mapping disclosed the syntenous relationships among rice, wheat, maize, barley, and other cereal crops (Ahn *et al.* 1993; Kurata *et al.* 1994a; Saghai Maroof *et al.* 1996). To accelerate analysis of the rice genome, a first-generation physical map has been completed recently (Antonio *et al.* 1996a; Saji *et al.* 1996; Shimokawa *et al.* 1996; Umehara *et al.* 1996, 1997; Wang *et al.* 1996; Koike *et al.* 1997; Kurata *et al.* 1997; Tanoue *et al.* 1997).

During the construction of a high-density molecular genetic map of rice derived from a single cross between the *japonica* variety Nipponbare and the *indica* variety Kasalath, 33 DNA markers were mapped to the distal ends of short arms of chromosomes *11* (*11S*) and *12* (*12S*). Among these markers, 13 revealed duplicated loci in the two regions, indicating the existence of chromosomal duplication (Nagamura *et al.* 1995). A similar result was also obtained from genetic analysis using different rice mapping populations (Antonio *et al.* 1996b; Tsunematsu *et al.* 1996). The duplicated loci of these markers are distributed within ~10 cM genomic regions in both chromosomes, indicating an event for a large scale chromosomal rearrangement during the evolution of the rice genome. Although a number of YAC clones were already assigned to the two duplicated regions, forming several YAC contigs (Shimokawa *et al.* 1996; Tanoue *et al.* 1997), it was still not enough for intensive analysis and characterization of the chromosome duplication. Precise genetic and physical distances and degree of genomic conservation are still not well known in the duplicated segments. We therefore have made further efforts to construct detailed physical maps of the duplicated genomic regions. This article reports the results of our study upon the distribution and order of DNA markers within the two distal ends of rice chromosomes. The degree of conservation in the composition and structure of these two regions is presented and evolutionary consequences involved in the structural changes of these two regions are discussed.

MATERIALS AND METHODS

DNA probes: A total of 46 genetic markers mapped on the distal ends of rice chromosomes *11S* and *12S* were used as DNA probes for the physical mapping (Harushima *et al.* 1998) (Figure 1). These markers include 37 rice cDNA clones (C, R, and S), six rice genomic clones (G and V) including one telomere-associated DNA sequence (TEL2) and one short in-

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terspersed nuclear element (SINE1-r6), one rice sequence-tagged site (STS) marker (T) and two wheat DNA clones (W). Accession numbers of the clones developed by Rice Genome Research Program in DDBJ are included in Table 1.

YAC screening: A YAC library from the Nipponbare DNA, containing about 7000 clones with an average insert size of 350 kb was used for the construction of a chromosome physical map (Umehara *et al.* 1995). Experiments were carried out with similar methods to those described previously (Umehara *et al.* 1996). For DNA markers (genomic and cDNA clones), Southern colony hybridization to YAC high-density filters was conducted to screen candidate YAC clones. Individual YAC candidates were then confirmed by Southern hybridization with the DNA markers. For STS markers, PCR amplification via a three-dimensional method was used for screening and selecting positive YAC clones (Green and Olson 1990).

Construction and analysis of YAC contigs: Positive YAC clones specific to the duplicated genomic regions were placed to chromosomes after Southern confirmation (see results), and YAC contigs were formed through positioning and overlapping the individual YAC clones. The chromosomal gaps were analyzed by detection of overlapping between the individual YAC clones through isolation and Southern hybridization of YAC end fragments with a similar method to that described before (Umehara *et al.* 1995). For measurement of the physical length of two duplicated chromosomal segments, the inserted fragments of all YAC clones were sized by pulsed-field gel electrophoresis [PFGE; Bio-Rad (Richmond, CA) CHEF Mapper system]. Physical distances for the remaining chromosomal gaps uncovered by YAC clones were determined by the following method: Rice high-molecular-weight DNA was isolated, embedded in low-melting-temperature agarose, digested with a single restriction enzyme (*Not*I, *Sma*I, or *Asc*I), and separated by PFGE. The digested DNA then was blotted and hybridized with those specific DNA markers or YAC-end fragments that flanked the chromosomal gaps. The length of DNA fragment commonly hybridized by the two flanking markers was defined as the maximum physical distance of gaps.

RESULTS

Screening and chromosomal assignment of YAC clones: In the case of V57 that had two genomic copies mapped individually to the two genomic regions duplicated in the distal ends of chromosomes *11S* and *12S*, seven YAC clones were identified as positives (Figure 1). Five clones, Y2049, Y2056, Y2645, Y4310, and Y4738, were positioned to chromosome *12S* as they contained a 4.0-kb DNA band after *Dra*I digestion, which corresponded to the restriction fragment length polymorphism (RFLP) band for locus V57B. The remaining two clones, Y2456 and Y5721, showed a 1.9-kb band corresponding to the other V57 copy in the rice genome. They were assigned to chromosome *11S* because both clones produced a 10-kb DNA band after *Bam*HI digestion, which corresponded to the RFLP band for locus V57A (data not shown). On the other hand, in the case of markers V59 and R1957, they both revealed two genomic copies from Southern analysis but only one from each marker showed RFLP and were mapped to the distal ends of two chromosomes, with V59 to chromosome *11S* and R1957 to chromosome *12S*. A total of six YAC clones were selected commonly by these two markers. Southern hybridization of the *Hin*dIII-digested YAC DNA of clones Y0392, Y0768, Y1256, Y1257, and Y1973 with the DNA probe of marker V59 detected a 2.5-kb band (Figure 1). The remaining clone, Y6028, contained a 16-kb band derived from the other copy within the genome. Similarly, Southern hybridization of the above six YAC clones with the DNA probe of marker R1957 showed that a 4.5-kb band was present in Y0392, Y0768, Y1256, Y1257, and Y1973 and a 2.4-kb band was present in Y6028 (Figure 1). Y0392, Y0768, Y1256, Y1257, and Y1973 were positioned to chromosome *11S* by the RFLP band of markers R2104 and R2954 (Table 1). Chromosomal location of Y6028 was placed to *12S* by the RFLP band of markers R769 and C1116. Consequently, the above results proved that V59 and R1957 hybridized to two chromosomal copies, located on the two duplicated regions of chromosomes *11S* and *12S*, respectively.

Out of 46 markers used in this study, 45 markers identified 38 positive YAC clones. Table 1 presents the detailed results for the YAC clones analyzed. Only one marker, namely the telomere-associated DNA sequence TEL2, did not detect any positive YAC clones. Three markers, C83, S790, and R2104, selected several positive clones, but their locations were confirmed to be outside the duplicated regions according to the Southern analysis.

Physical maps: Seventeen YAC clones formed one contig and three islands for the distal end of chromosome *11S* and covered a total genomic region of 9.1 cM (Figures 1 and 2). The other 14 YAC clones formed one contig and three islands for the distal end of chromosome *12S* and covered a total region of 5.3 cM. The largest YAC contig was constructed for that of chromosome *11S* by 14 clones, covering 8.3 cM (C362B-C83A). For that of chromosome *12S*, the contig formed by clones Y3338 and Y6028 was obtained by confirming the overlapping of YAC-end fragments from the two clones. There were eight YAC-uncovered chromosomal gaps that remained, including the two telomere regions. Physical distances of these gaps were analyzed through hybridization of high-molecular-weight genomic DNA, with the DNA probes of markers flanking the gaps (data not shown). After *Sma*I digestion, a *ca.* 170-kb DNA band was commonly hybridized with TEL2 and S1409. Since only a single band was detected using the TEL2 probe during RFLP mapping and the TEL2B locus mapped to chromosome *11S* was not present in Nipponbare DNA (Kasalath-dominant), this 170-kb band should be derived from the subtelomeric region of chromosome *12S* of the Nipponbare variety, representing the maximum physical distance between the loci for TEL2A and S1409 (Figure 2). On the other hand, hybridization of *Asc*I-digested chromosomal rice DNA with the R2984 DNA probe showed two bands. The larger one was proven to be the same DNA fragment detected by TEL2,

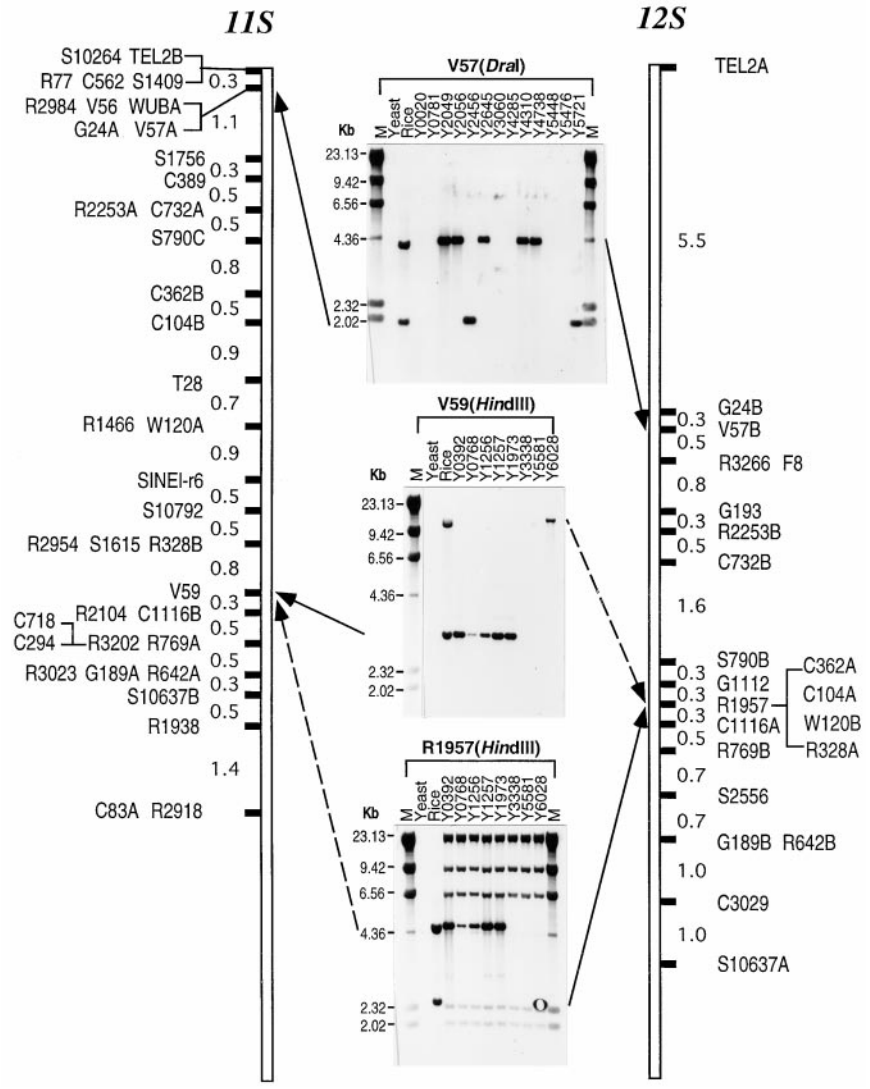

Figure 1.—Linkage maps of the distal ends of rice chromosomes *11S* and *12S* and Southern hybridization results of YAC clones. Numbers along the two chromosomes represent the genetic distance (cM) between markers. Marker number with the letter A, B, or C indicates its duplicated loci. The results of Southern confirmation of candidate YAC clones, after colony hybridization, using DNA probes of markers (V57, V59, and R1957) are shown by the gels between the two linkage maps. Lambda DNA digested by *Hin*dIII is used as the size marker. Solid lines with arrows indicate the DNA band positions (duplicated loci) on the linkage maps of two chromosomes. The band positions that could not be genetically mapped because of no polymorphism are shown by broken lines.

having a size of about 500 kb. This result indicates that the smaller band of about 90 kb hybridized by this probe should be from the duplicated region in chromosome *11S*. Since this smaller DNA band also hybridized to the marker DNA of R2253, the physical distance between these two markers could be estimated to be less than 90 kb (Figure 2). However, analysis of the physical distance for the remaining six gaps was unsuccessful due to a lack of commonly and/or specifically hybridized DNA bands between the flanking markers.

Copies, distribution, and orders of DNA markers: Combining the results of genetic and physical mapping of 46 genetic markers, 35 were found to have their duplicated copies in the two genomic regions (Figures 1 and 2). These markers include those cDNA clones that show strong homology to known function genes such as the Ser/Thr kinase (S1756), the fatty acid desaturase (R77) and the flowering-related protein, TFL1 (R2918) through the similarity search (BLASTN,1.4. 9MP). Eight markers could define only one of their genomic copies on YAC clones (boxed in Figure 2). Two adjacent markers, S10792 and SINE1-r6 (marked by asterisks in Figure 2), seemed to have only one copy within the two duplicated regions because no hybridization signal could be detected from YAC clone Y3338 in chromosome *12S*, where genomic copies of their flanking markers of W120 and R328 in chromosome *11S* were present.

There were no contradictory results between the genetic and physical mapping for the marker orders along the two duplicated genomic regions (Figures 1 and 2). Physical mapping of the DNA markers made it possible to determine the precise chromosomal locations and order of the markers, which cannot be easily achieved only by genetic analysis. Markers C1116 and R2104 were genetically mapped to the same locus in chromosome *11S*. On the basis of the hybridization results of Y0392, Y0768, Y1256, Y1257, and Y1973 with the above two markers, it could be easily determined that R2104 was located proximal to C1116, because this marker hybridized to all of the above six clones while C1116 only hybridized to Y0392 and Y0768.

TABLE 1

Confirmation of positive YAC clones through RFLP markers located in the distal ends of rice chromosomes *11S* and *12S*

Marker	Accession in DDBJ	Enzyme	Bands of Nipponbare genomic DNA (kb)	Positive YAC clones	Bands of YAC DNA (kb)	Locus
TEL2	D16336	*Kpn*I	10			
S10264	D45958	*Bgl*II	6.0, 2.5	Y4972 (chimeric)	2.5	*11S*
C562	D15385	*Bgl*II	4.3, 2.8, 2.4	Y4972	4.3[a], 2.8	*11S*
S1409	D39806	*Dra*I	4.5, 1.8	Y5721	4.5, 1.8	*11S*
				Y2645, Y4738	4.5, 1.8	*12S*
R77	D23756	*Kpn*I	6.0, 5.0	Y5721	6.0[a]	*11S*
				Y2049, Y2056, Y2645, Y4310, Y4738	5	*12S*
WUBA[b]		*Bam*HI	13, 9.0	Y2456 (chimeric), Y5721	13[a]	*11S*
				Y2049, Y2056, Y2645, Y4310, Y4738	9	*12S*
V56[b]		*Bgl*II	20, 6.5	Y2456, Y5721	20[a]	*11S*
				Y2049, Y2056, Y2645, Y4310, Y4738	6.5	*12S*
G24	D13462	*Eco*RI	7.2, 4.3	Y2456, Y5721	7.2	*11S*
				Y2049, Y2056, Y2645, Y4310, Y4738	4.3[a]	*12S*
V57[b]		*Dra*I	4.0, 1.9	Y2456, Y5721	1.9	*11S*
				Y2049, Y2056, Y2645, Y4310, Y4738	4.0[a]	*12S*
R2984	D25053	*Hin*dIII	9.5, 1.8	Y2456, Y5721	9.5	*11S*
				Y2049, Y2056, Y4310, Y4738	1.8	*12S*
R3266	D25130	*Eco*RI	23, 7.5, 6.8, 5.0, 4.6	Y2049, Y2056, Y4310, Y4738	6.8, 4.6[a]	*12S*
F8		*Bam*HI	15, 9.0, 4.5, 3.3, 2.8, 2.1, 1.6, 1.0	Y2049, Y2056, Y4310, Y4738	15	*12S*
G193	D14759	*Hin*dIII	8.5, 1.7	Y4738	8.5, 1.7[a]	*12S*
				Y2049, Y2056, Y4310	8.5	*12S*
S1756	D40033	*Dra*I	10, 8.4, 2.7, 1.0	Y2049, Y2056	10	*12S*
				Y4310, Y4738	10, 2.7, 1.0	*12S*
C389	D15272	*Bgl*II	10, 6.8	Y4738	10[a]	*12S*
R2253	D24613	*Eco*RI	23, 7.0	Y4285	23[a]	*11S*
				Y4738	7	*12S*
C732	D28201	*Eco*RV	15, 7.0	Y4285	15[a]	*11S*
S790	D39455	*Dra*I	5.8, 4.8, 3.5, 2.8, 1.5, 1.0	Y3726, Y3964, Y5727	5.8[a]	*11*[c]
				Y4285	4.8, 3.5, 1.5[a]	*11S*
				Y5581	2.8[a], 1.0	*12S*
C362	D15255	*Dra*I	15, 6.8	Y5351	6.8[a]	*11S*
				U5581	15[a]	*12S*
C104	D28176	*Dra*I	5.2, 4.5, 1.2	Y0613, Y4576, Y5351, Y6864	4.5[a], 1.2	*11S*
				Y2985, Y4064	4.5[a]	*11S*
				Y5581	5.2[a]	*12S*
T28[d]			4.5	Y0613, Y2985, Y4064, Y4576, Y5351, Y6864	4	*11S*
				Y5581	4	*12S*
W120[b]		*Bam*HI	9.8, 7.5, 6.5, 6.0, 2.4	Y2985, Y4064, Y6864	6.5, 6.0, 2.4[a]	*11S*
				Y1256, Y1257	6.5, 6.0	*11S*
				Y0613, Y4576	2.4[a]	*11S*
				Y3338	9.8[a], 7.5	*12S*
R1466	D24172	*Bgl*II	15, 11, 10, 9.5, 9.0, 8.0, 7.0, 3.3	Y2985, Y4064, Y6864	15, 10, 9.0, 7.0, 3.3[a]	*11S*
				Y1256, Y1257	10, 9.0, 7.0	*11S*
				Y0613, Y4576	15, 3.3[a]	*11S*

(*continued*)

TABLE 1

(Continued)

Marker	Accession in DDBJ	Enzyme	Bands of Nipponbare genomic DNA (kb)	Positive YAC clones	Bands of YAC DNA (kb)	Locus
				Y3338	11, 9.5, 8.0	*12S*
SINE1-r6[b]		*Kpn*I	4.5, 3.5	Y1256, Y1257, Y2985, Y4064, Y4576, Y6864	3.5	*11S*
S10792	D46247	*Hin*dIII	4.0, 2.5	Y1256, Y1257	4.0[a]	*11S*
G1112	D25367	*Eco*RV	6.0, 4.0	Y1256, Y1257	6	*11S*
				Y3338	4.0[a]	*12S*
R2954	D25027	*Apa*I	15, 9.5, 5.0, 4.5	Y0392, Y1256, Y1257, Y1973	15, 9.5[a], 4.5	*11S*
				Y3338	5	*12S*
S1615	D39935	*Hin*dIII	5.0, 2.5	Y0392, Y1256, Y1257, Y1973	2.5	*11S*
				Y3338	5	*12S*
R328	D23838	*Hin*dIII	4.8, 4.0	Y0392, Y1256, Y1257, Y1973	4	*11S*
				Y3338	4.8	*12S*
R1957	D28307	*Hin*dIII	4.5, 2.4	Y0392, Y0768, Y1256, Y1257, Y1973	4.5	*11S*
				Y6028	2.4	*12S*
V59[b]		*Hin*dIII	16, 2.5	Y0392, Y0768, Y1256, Y1257, Y1973	2.5	*11S*
				Y6028	16	*12S*
R2104	D24526	*Bgl*II	10, 3.5, 3.2	Y0392, Y0768, Y1256, Y1257, Y1973	10[a]	*11S*
				Y6028	3.2	*12S*
				Y4726	3.5	*8S*[c]
C1116	D15700	*Hin*dIII	3.5, 3.3	Y0392, Y0768	3.3[a]	*11S*
				Y6028	3.5[a]	*12S*
R769	D28290	*Eco*RV	5.0, 2.3	Y0768	5.0[a]	*11S*
				Y6028	2.3[a]	*12S*
R3202	D25111	*Apa*I	5.5, 4.6	Y0768	4.6[a]	*11S*
C294	D15224	*Hin*dIII	5.0, 2.6, 1.8	Y0768	5.0[a], 1.8	*11S*
C718	D15492	*Bgl*II	20, 9.0	Y0768, Y4550	9.0[a]	*11S*
S2556	D40521	*Eco*RV	6.2, 6.0	Y0768, Y4550	6	*11S*
G189	D21838	*Bgl*II	9.5, 9.0, 7.0	Y1236 (chimeric), Y4550	9.5[a]	*11S*
				Y3404, Y5767	9.0, 7.0[a]	*12S*
R642	D23947	*Dra*I	5.0, 3.0, 1.8	Y1236, Y4550	5.0, 3.0[a]	*11S*
				Y3404, Y5767	1.8[a]	*12S*
R3023	D39204	*Dra*I	4.4, 2.8, 2.0, 1.4, 1.2, 1.0	Y1236	4.4, 2.8[a], 2.0	*11S*
				Y1943, Y2038, Y3404, Y4569, Y5767	1.4, 1.2, 1.0	*12S*
C3029	D16086	*Dra*I	15, 4.0	Y1236, Y4889	15	*11S*
				Y1943, Y2038, Y3404, Y4569, Y5767	4	*12S*
S10637	D46160	*Kpn*I	23, 9.0, 3.2	Y1236, Y4889	23, 3.2[a]	*11S*
				Y1943, Y2038, Y3404, Y4569, Y5767	9.0[a]	*12S*
R1938	D24446	*Bam*HI	11, 4.0	Y4889	11	*11S*
				Y2038, Y3404, Y4569, Y5767	4	*12S*
R2918	D24998	*Bam*HI	10, 6.0	Y4889	6.0[a]	*11S*
				Y2038, Y3404, Y5335	10	*12S*
C83	D15113	*Dra*I	7.0, 6.8, 3.7, 1.5	Y2432, Y4946	3.7[a]	*8L*[c]
				Y4889	1.5	*11S*

[a] YAC DNA band corresponding to the rice RFLP band.
[b] DNA clones from outside of RGP (Kurata *et al.* 1994b; Harushima *et al.* 1998).
[c] Locus located outside the two duplicated regions.
[d] STS marker with the primer sequences of 5′-gttgaggcattctacatctg-3′ and 5′-acggggaaaggtttatgaca-3′.

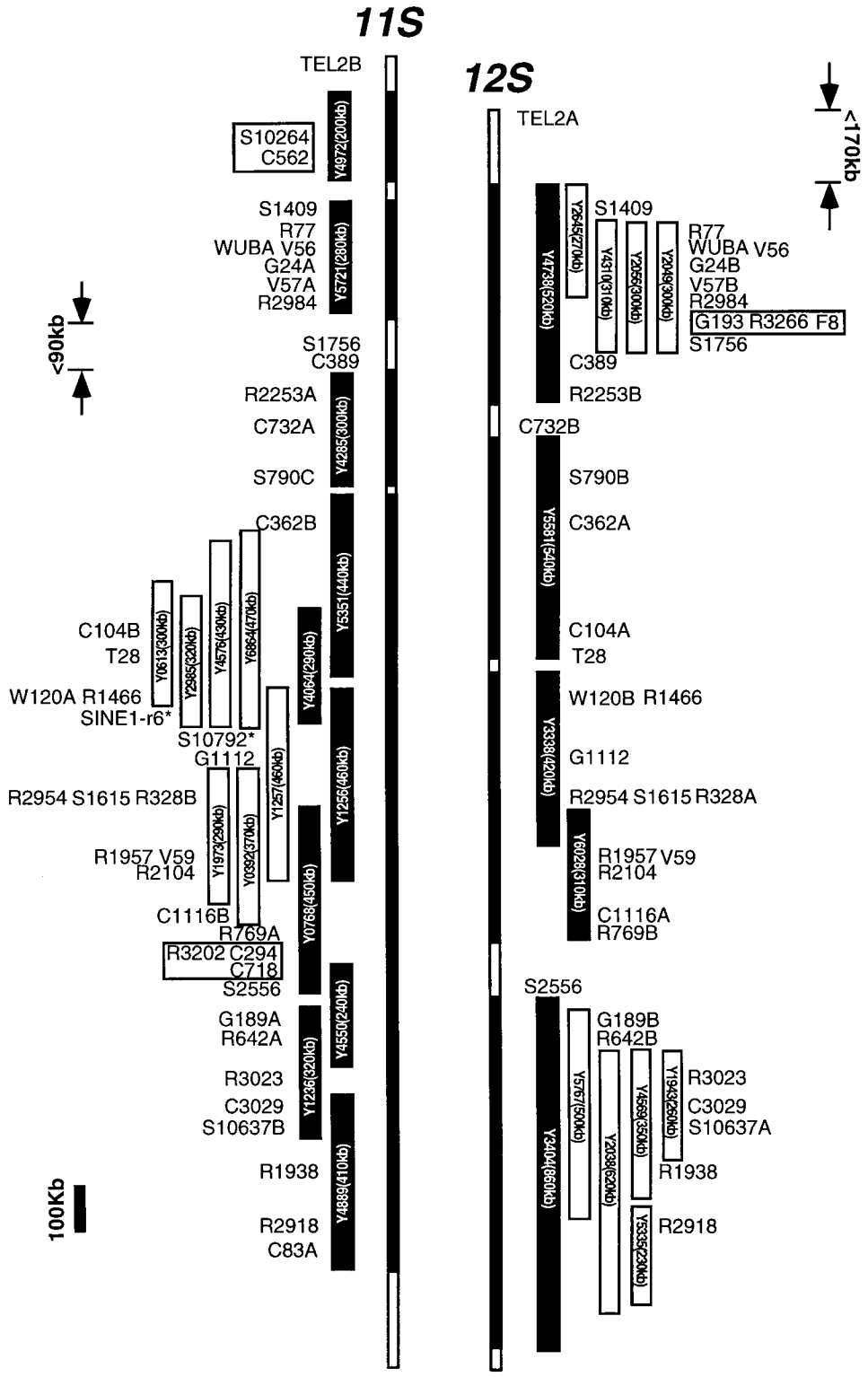

Figure 2.—Physical maps of the two duplicated regions of rice chromosomes *11S* and *12S*. YAC clones are placed to the specific chromosomal regions, on the basis of the Southern confirmation results using DNA probes of genetic markers. Marker positions and orders are decided from the results of genetic mapping and YAC clone overlapping. Rectangles represent the YAC clones whose length is in proportion to the YAC insert size (given as kilobases in parentheses following the YAC name). YAC clones with the black rectangle consist of the minimum overlap YAC array used for the calculation of physical size. White color on the two chromosome bars indicates the chromosome gaps. The markers whose duplicated loci were not detected in this study are boxed.

Discrepancy of genetic distance: RFLP mapping of a new DNA marker, S10637, demonstrated that the genetic distance of duplicated chromosomal segments in chromosomes *11S* and *12S* spanned at least 9.9 and 14.3 cM, respectively (Harushima *et al.* 1998), which was longer than that detected previously (Nagamura *et al.* 1995). Between these two segments, a significant difference in the genetic distance of some corresponding genomic area was present. Genetic distance of the genomic area between markers C362 and C1116 on the middle region of the two duplicated segments showed a distance of 5.1 cM in chromosome *11S* while only 0.3 cM in *12S* (Figure 3). On the other hand, the area near the distal side of the two segments between markers

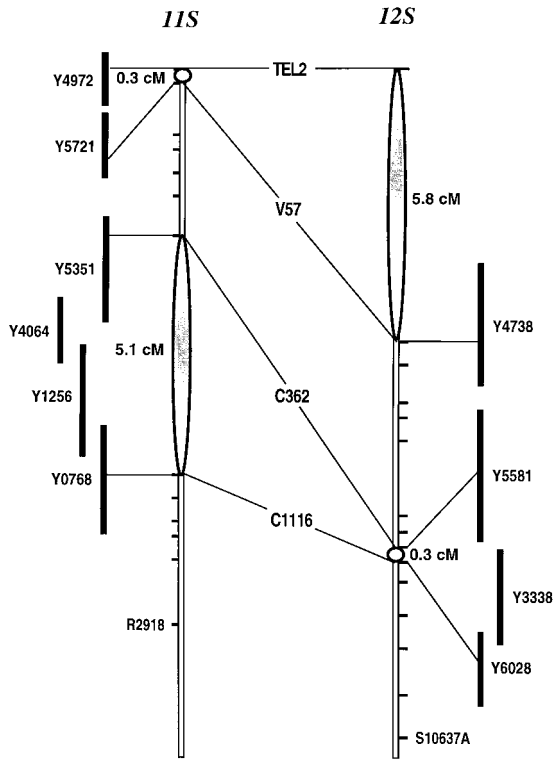

Figure 3.—Differences in the genetic distance of corresponding genomic areas between two duplicated regions in the distal ends of chromosomes *11S* and *12S*. The corresponding genomic areas are thickened, shaded, and joined by lines. Genetic distances and names of YAC clones placed to the corresponding area are also shown along the genomic regions. (See Figure 2 for the insert size of each YAC clone.)

TEL2 and V57 showed a distance of 0.3 cM in chromosome *11S* but 5.8 cM in *12S*.

DISCUSSION

Chromosomal duplications are believed to occur by similar molecular mechanisms in all organisms and are important in genome evolution (Lupski *et al.* 1996). Such studies on rice chromosomes are rare so far because intensive analysis of chromosomal duplications needs a large number of DNA markers, especially in the case of large duplicated segments. In this study, 46 genetic markers were used and led to the identification of 81 DNA loci within the two specific regions (Table 1; Figure 2). Thus, the marker density within the two chromosomal regions is extremely high, with about 3.3 markers per cM (81 loci/24.2 cM) and 5.4 markers per YAC clone (81 loci/15 YAC clones of minimum tiling path in black boxes in Figure 2). The insert of all YAC clones assigned in the duplicated regions has been sized by PFGE. These not only ensured the accuracy of DNA marker placement within the YAC contigs but also provided information regarding the genomic characteristics of the duplicated segments, suggesting that events of chromosome evolution occurred after the duplication.

A large chromosomal duplication involved in the distal ends of rice chromosomes *11S* and *12S*: Two DNA markers, R1938 and R2918, were genetically mapped to the distal end of chromosome *11S*, 0.5 and 1.9 cM from the marker S10637, respectively (Figure 1). These two markers were revealed to have their genomic copies also in the distal end of chromosome *12S* by the present physical mapping (Figure 2). Marker C83, a cDNA clone showing strong homology to the ribosomal protein S25 and cosegregating with R2918, was judged to have four genomic copies according to its hybridization pattern with rice genomic DNA after *Dra*I digestion, one of which, a 1.5-kb band, was confirmed from the YAC clone Y4889 on chromosome *11S*. The second copy of this marker, a 3.7-kb band, was mapped to the long arm of chromosome *8*. Although we were unable to determine the chromosomal locations of the remaining two genomic copies, it seemed that C83 had no copy in the duplicated region of chromosome *12S* because it failed to hybridize to the YAC clones, Y2038, Y3404, and Y5335, that carried the genomic copy of R2918. Physical mapping of other DNA markers located proximal to C83 revealed no homology between the two chromosomes (Shimokawa *et al.* 1996; Tanoue *et al.* 1997). These results indicated that the genomic area between the marker R2918 and C83 might be one of the end points of the duplicated segments. Because duplication of a segment of a genome may often take place in a region flanked by repeated sequences (Lupski *et al.* 1996), further analysis and characterization of genome sequences around these regions in both chromosomes would be interesting. Toward the distal ends, the duplicated segments may extend close to the subtelomeric regions because duplicated copies of markers S1409 and R77 were identified from both end regions of the two chromosomes. We were unable to confirm the copy distribution of markers S10264 and C562 on chromosome *12S* due to a lack of YAC clones covering these sequences. Consequently, the chromosomal duplication involved in the distal ends of two chromosomes might cover the regions from the subtelomere to the marker R2918, with the genetic distance of 11.8 cM in chromosome *11S* and 14.3 cM in chromosome *12S*.

The total size of physical maps can be approximately calculated as the sum of the full insert size of YAC clones (present as a YAC island) and the total size of YAC contigs; the latter is estimated by two different ways: (1) Total size is calculated by assuming 50% overlap for the insert of YAC clones comprising the minimum overlap of YAC array (in black boxes in Figure 2), and (2) total size is calculated by dividing the total YAC size by the average number of hits per marker, as used in the estimation of minimum size of chromosomes in Arabidopsis (Zachgo *et al.* 1996). As a result, the total size of physical maps constructed within the duplicated regions at the distal ends of chromosomes *11S* and *12S* was calculated to be 2.09 and 2.29 Mb, respectively, by the first way

and 2.51 and 2.48 Mb, respectively, by the second way. Taking the gaps into account by using the genetic distance between the two markers bordering the internal gaps and a conversion factor of 280 kb/cM (Harushima *et al.* 1998), we speculate that the two duplicated regions in chromosomes *11S* and *12S* have a similar and minimum physical size of about 2.5 Mb.

Chromosomal evolution in the distal ends of rice chromosomes *11S* and *12S*: By genetic mapping of 46 DNA clones, 15 were found to have their duplicated copies in the distal ends of chromosomes *11S* and *12S*. Physical mapping of these 46 markers in the present study revealed 20 additional markers that have genomic copies in both regions (Table 1 and Figure 2). Eight markers (boxed in Figure 2) had only one of their copies confirmed within the two genome regions, probably due to the lack of YAC clones derived from the duplicated regions as they all showed multiple hybridization bands on Southern blots of rice genomic DNA. There were only two markers, S10792 and SINE1-r6 (marked by asterisks in Figure 2), that seemed to have only one copy within the two duplicated regions. Two markers, S790 and R2104, contained a third DNA copy that was genetically and physically mapped to a region around the centromere of chromosome *11* and to the short arm of chromosome *8*, respectively. These results imply genomic rearrangements that were chromosomal insertions or deletions. With the exception of these markers, distribution of marker copies and their orders within the duplicated regions between the two chromosomes was completely conserved.

This study is the first to intensively analyze the genomic structure and composition within a large chromosomal duplication through the effective physical mapping of DNA markers in a plant genome. Because the degree of genomic conservation is a good measure of chromosome evolution, the above results could be fundamentally important for studies on the mechanisms of rice chromosome evolution. The high degree of genomic conservation between the two chromosomal regions evidently demonstrates an event of long range chromosomal duplication in the rice genome. At present, it is still difficult to speculate when this duplication event took place and to suggest whether it was related to the differentiation of rice chromosomes from those of other plants. Further comparative mapping, with more genomic DNA sequences, for microsynteny analysis of the above duplicated regions between the most distantly related grass genomes will reveal the nature of the evolutionary event of the chromosome duplication observed in the rice genome.

A significant difference in the frequency of polymorphisms between two DNA copies of the duplicated regions was observed. Duplicated copies of 30 DNA markers located on the two genomic regions were characterized by analyzing the YAC clones (Table 1). Among these 30 markers, 13 showed their copies with polymorphism in both regions between Nipponbare (*japonica*) and Kasalath (*indica*). There were 14 markers that showed their copies with the polymorphism only in chromosome *11S*. In contrast, there were only three markers that showed copies with polymorphism only in chromosome *12S*. This is why our genetic mapping of DNA markers resulted in much more loci in the duplicated region of chromosome *11S* than in *12S*. The above finding indicates that, between the rice varieties Nipponbare and Kasalath, more significant changes of DNA compositions have happened to the region in the distal end of chromosome *11S* than that in the chromosome *12S*. This suggests that the genomic region in the distal end of chromosome *12S* might be most similar to the ancestral chromosomal segment for the duplication.

The above speculation is consistent with the significant difference of recombination frequency between a given homeologous area within the two duplicated regions. Regardless of the similar expected physical distance, the genetic distance between the marker C362 and C1116 in chromosome *11S* was about 5.1 cM while that in chromosome *12S* was only about 0.3 cM (Figure 3). The difference of recombination frequency was also present in the two telomere regions. In the latter, however, the lack of fine physical maps on these areas did not allow us to know more about their characteristics and changes of genome organization for further discussion. Genetic mapping using more DNA markers and/or other rice varieties, as well as physical mapping to complete YAC contigs might be needed for further analysis.

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