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Centromere mapping and orientation of the molecular linkage map of rice (*Oryza sativa* L.)

(secondary trisomic/telotrisomic/arm location/restriction fragment length polymorphism)

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ABSTRACT **Rice has become a model cereal plant for molecular genetic research. Rice has the most comprehensive molecular linkage maps with more than 2000 DNA markers and shows synteny and colinearity with the maps of other cereal crops. Until now, however, no information was available about the positions of centromeres and arm locations of markers on the molecular linkage map. Secondary and telotrisomics were used to assign restriction fragment length polymorphism markers to specific chromosome arms and thereby to map the positions of centromeres. More than 170 restriction fragment length polymorphism markers were assigned to specific chromosome arms through gene dosage analysis using the secondary and telotrisomics and the centromere positions were mapped on all 12 linkage groups. The orientations of seven linkage groups were reversed to fit the "short arm on top" convention and the corrected map is presented.**

Rice is the world's single most important food crop and a primary food source for one-third of the world's population. It is now becoming a model cereal plant for molecular genetic research, primarily because of its small genome size (4×10^8 bp) (1), well developed linkage maps (2–4), well established transformation and regeneration systems (5), and high level of synteny and colinearity of genes with other cereal genomes (6–8).

The molecular maps of rice consisting of more than 2000 DNA markers are available (3, 4), and provide the most comprehensive maps among all the plant species. No information, however, has been available about the location of these markers to specific chromosome arms, the positions of the centromeres and the orientation of the molecular linkage map. Knowledge of centromere locations is useful (*i*) to ascertain the completeness of the map, (*ii*) to analyze centromeric interference on recombination and the feasibility of map based gene cloning, (*iii*) for map based cloning of rice centromeres, and (*iv*) to firmly establish the synteny and colinearity of rice genome with other cereal genomes.

We have developed novel aneuploid stocks comprising secondary and telotrisomics for all 12 rice chromosomes (9). These stocks proved to be ideal for assigning restriction fragment length polymorphism (RFLP) markers to specific chromosome arms and for mapping the positions of the centromeres. Here we report for the first time the location of RFLP markers to specific chromosome arms, centromere positions on all 12 linkage groups of rice, and hence corrected orientation of the map.

MATERIALS AND METHODS

Plant Material. Secondary and telotrisomics (see ref. 10 for definitions) involving all 12 rice chromosomes were developed in the background of the indica rice variety IR36 (9). These aneuploid stocks and the primary trisomics (also in the background of IR36, ref. 11) were crossed with a tropical japonica variety, MaHae. IR36 and MaHae were known to be highly polymorphic from earlier RFLP analysis. In the F_1, primary trisomics, secondary trisomics, telotrisomics, and disomic sibs were selected following cytological analysis. The parental varieties IR36 and MaHae, F_1 secondary or F_1 telotrisomics, F_1 primary trisomics, and F_1 disomic sibs formed a set of plant material for each of the 12 chromosomes (Table 1).

The designation of trisomic stocks is after Khush (10). For example, for chromosome 1 a primary trisomic is designated as 2n+1S·1L; a secondary trisomic for the long arm as 2n+1L·1L and that for the short arm as 2n+1S·1S, and a telotrisomic for the long arm as 2n+·1L and for the short arm as 2n+·1S.

Molecular Marker Probes. The molecular marker probes belonging to the Cornell molecular linkage map (3) were kindly supplied by Steve Tanksley and Susan McCouch (Cornell University, Ithaca, NY) and those belonging to the Japanese map (4) were obtained from the Rice Genome Research Program (Tsukuba, Japan).

DNA Extraction and Southern Blot Hybridization. DNA was extracted from freshly collected leaves at the preflowering stage (after cytological confirmation) using the potassium acetate method of Dellaporta *et al.* (12) with minor modifications. DNA digestion, electrophoresis, Southern blotting, and hybridization procedures were similar to those described by McCouch *et al.* (13) and Sambrook *et al.* (14). In general, 5–7 μg of DNA was digested with the appropriate restriction endonuclease using the buffer and temperature as described by the manufacturer. Digested DNA was then fractionated on a 0.8% agarose gel in 1× TAE buffer (40 mM Tris acetate/1 mM EDTA). The size fractionated DNA was then transferred to Hybond-N+ membrane (Amersham) using an alkali transfer method as described by the manufacturer. Probes were labeled with ^{32}P by random hexamer priming method (15) and the membranes were hybridized overnight at 65°C in 5× SSPE buffer overnight. The membranes were washed at 65°C once each in 2×, 1×, and 0.5× SSC (each with 0.1% SDS) for 30, 20, and 15 min, respectively. However, stringency was lowered up to only 1× when oat cDNA (CDO) probes were used. Membranes were exposed with an intensifying screen at −80°C for 3–5 days.

Polymorphism Survey. Polymorphism between parents (IR36 versus MaHae) was studied using the following 24 restriction endonucleases from Amersham, Boehringer Mannheim, and New England Biolabs: *Aat*II, *Apa*I, *Bam*HI, *Bcl*I, *Bgl*II, *Cla*I, *Dra*I, *Eco*RI, *Eco*RV, *Hin*dIII, *Hpa*I, *Kpn*I, *Mlu*I, *Nae*I, *Nar*I, *Nco*I, *Nru*I, *Pst*I, *Sac*I, *Sca*I, *Sma*I, *Stu*I, *Xba*I, and *Xho*I. For markers where polymorphism was not observed with these 24 enzymes, a further set of seven enzymes—*Dpn*I, *Hae*III, *Hin*fI, *Msp*I, *Mva*I, *Rsa*I, and *Taq*I—was used.

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Abbreviations: RFLP, restriction fragment length polymorphism; CDO, oat cDNA; cM, centimorgan(s); IRRI, International Rice Research Institute.

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Table 1. Plant material used for RFLP analysis

Chromosome	Primary trisomic	Secondary or telotrisomic
1	2n + 1S·1L	2n + 1S·1S
2	2n + 2S·2L	2n + 2L·2L
3		2n + ·3L
4	2n + 4S·4L	2n + 4S·4S
5	2n + 5S·5L	2n + 5S·5S, 2n + ·5L
6	2n + 6S·6L	2n + 6S·6S
7	2n + 7S·7L	2n + 7S·7S
8	2n + 8S·8L	2n + ·8S, 2n + 8L·8L
9	2n + 9S·9L	2n + 9S·9S
10	2n + 10S·10L	2n + ·10S
11	2n + 11S·11L	2n + 11S·11S, 2n + 11L·11L
12	2n + 12S·12L	2n + 12S·12S

Parental lines IR36 and MaHae and F_1 disomic sibs were included in each set.

Rationale for Arm Location of RFLP Markers and Centromere Mapping. An F_1 disomic plant had one allele each of IR36 and MaHae and the intensities of both the autoradiographic bands in Southern blots were similar. An F_1 primary trisomic had two copies of the IR36 allele and one of the MaHae allele and, thus, the intensity of the IR36 band was expressed as twice that of the MaHae band. An F_1 secondary trisomic had three copies of the IR36 allele (located on the arm for which it is secondary) and one copy of the MaHae allele, and accordingly the intensity of the IR36 band for a marker present on that arm was three times that of the MaHae band (Fig. 1). In contrast, if the marker in question was not located on the arm for which it is secondary trisomic, then the F_1 secondary trisomic showed similar intensity of IR36 and MaHae bands. The telotrisomics behaved the same way as the secondary trisomics except that the intensity of the IR36 band was twice the intensity of the MaHae band. Dosage comparison of the autoradiographs was performed by visual inspection. Based on these arguments RFLP markers were assigned to specific chromosome arms and the positions of the centromeres were mapped between the nearest two markers located on opposite arms of a chromosome.

RESULTS AND DISCUSSION

RFLP Survey of Parents. A total of 211 probes from the 12 linkage groups were used in the polymorphism survey. Of these, 183 were polymorphic with at least 1 of the 24 restriction endonucleases (Table 2). Of the 28 monomorphic probes, 8 were further tested with the second set of 7 restriction endonucleases but none revealed polymorphism.

Table 2. Number of molecular markers used for polymorphism survey and arm location

		Markers	
Chromosome	Markers surveyed	Polymorphic	Monomorphic
1	23	21	2
2	21	16	5
3	25	22	3
4	15	14	1
5	17	16	1
6	22	19	3
7	20	15	5
8	17	14	3
9	11	11	0
10	13	11	2
11	16	14	2
12	11	10	1
Total	211	183	28

Arm Location of RFLP Markers and Centromere Mapping. In the set of plant material for each chromosome, an F_1 disomic and an F_1 primary trisomic were used as controls for comparative intensities of the two polymorphic alleles and to confirm their chromosomal location. Thus, a higher intensity of the IR36 allele in an F_1 primary trisomic confirmed the chromosomal location of the marker and the dosage difference in the F_1 secondary or telotrisomic (Fig. 2) assigned the marker to a specific chromosome arm. In this way about 170 RFLP markers were assigned to specific chromosome arms and centromeres mapped between the two most proximal loci located on opposite arms. The arm locations of RFLP markers, positions of the centromeres, and hence corrected orientation of all 12 linkage groups of Causse *et al.* (3) are presented in Fig. 3.

Chromosome 1. Of the 19 polymorphic probes used for arm location, 12 mapped on the long arm and 7 on the short arm. Markers RZ288 and RZ783, which map toward distal regions of the map, were located on short and long arms, respectively. The centromere was shown to lie between RG811 and RZ413, with RG811 on the short arm and RZ413 on the long arm.

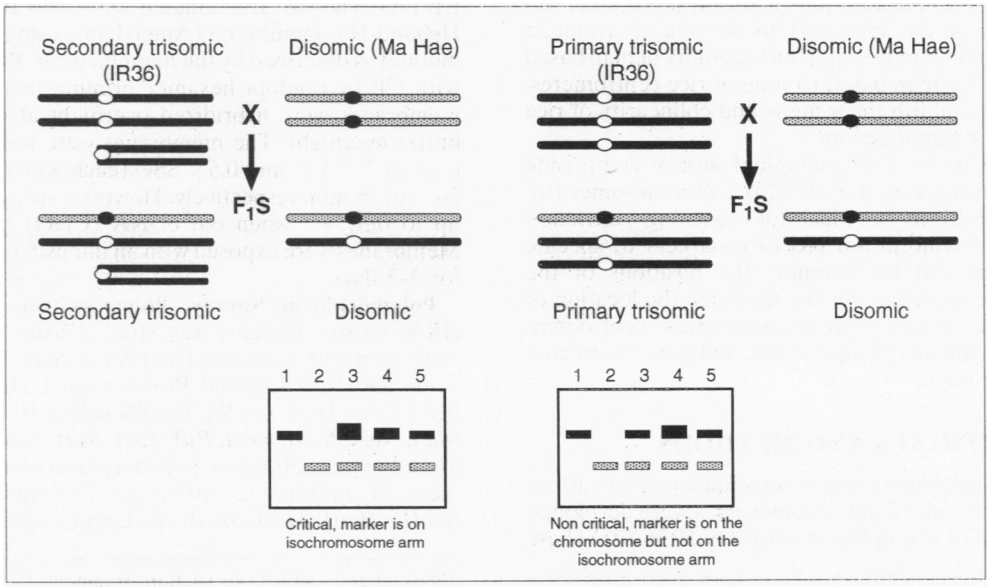

FIG. 1. Diagrammatic presentation of dosage analysis in F_1 primary and secondary trisomics. Lanes 1–5 are IR36, MaHae, F_1 secondary trisomic, F_1 primary trisomic, and F_1 disomic, respectively.

These two RFLP markers lie about 7.0 centimorgans (cM) apart. Marker CDO962 was monomorphic and could not be mapped to a specific arm.

Chromosome 2. Of the 21 markers surveyed for polymorphism, only 16 were polymorphic. Five of these markers mapped on the short arm and 11 on the long arm, with RG365 on the short arm and RG520 on the long arm. Marker RZ643 did not show any polymorphism but was assigned to the short arm by comparing band intensities of RZ643 and RG171 hybridized simultaneously to DNA digested with the restriction enzyme *Bam*HI for which both probes show monomorphic bands. RG171 mapped to the long arm and hence the centromere lies between RZ643 and RG171, which are 10.9 cM apart.

Chromosome 3. Twenty-five probes were surveyed for polymorphism and 22 were polymorphic. An F_1 primary trisomic was not available for this chromosome and only the 2n+·3L

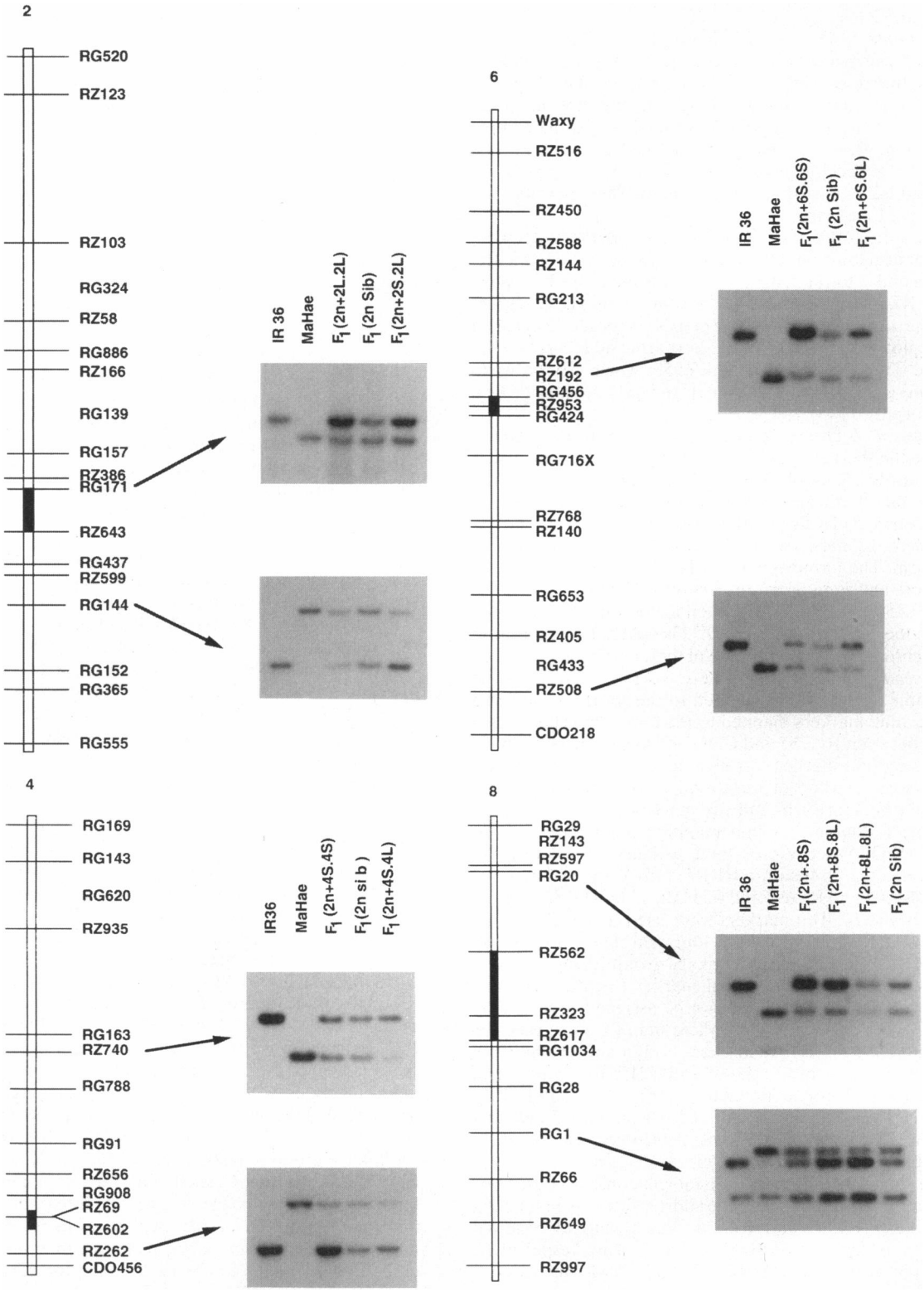

FIG. 2. Dosage versus lack of dosage for various RFLP markers in F_1 secondary trisomics of chromosomes 2, 4, 6, and 8. Solid lines represent positions of centromeres.

was used for analysis. Of the terminal markers studied, RG104 mapped to the short arm and RZ393 to the long arm. The centromere lies in the 5.4-cM gap between RZ394 and RZ576, with the former on the short arm and the latter on the long arm.

Chromosome 4. Thirteen polymorphic markers were used for arm location and only two markers, RZ262 and RZ602, mapped to the short arm. CDO456 showed no polymorphism. The centromere thus lies between the cosegregating loci (3) RZ602 and RZ69.

Chromosome 5. Of the 16 polymorphic markers, 12 were examined for arm location and 8 of these mapped to the long arm with the remaining 4 to the short arm. The terminal markers, RZ390 and CDO202, mapped to the short and long arms, respectively. Marker RG671, which maps in the centromeric region, was monomorphic and hence could not be assigned to a specific arm. The centromere lies between RG182 and RZ945, with the former on the short arm and the latter on the long arm.

Chromosome 6. Of the 19 polymorphic markers, 16 were studied for arm location. Of these 16 markers, 9 mapped to the short arm and 7 to the long arm. The terminal markers waxy (wx) and RZ508 mapped to the short and long arms, respectively. The centromere lies between RG456 and RG424, a 2.8-cM region with RG456 on the short arm and RG424 on the long arm. The arm location of RZ953, which lies between RG456 and RG424, could not be determined because of a lack of polymorphism.

Chromosome 7. Of the 14 markers used for arm location, 6 mapped to the short arm and 8 to the long arm. The centromere lies between RZ272 and RG30, which are 23 cM apart, with the former on the short arm and the latter on the long arm.

Chromosome 8. Twelve markers were studied for arm location. Of these 12 markers, 5 mapped to the short arm and 7 to the long arm. The terminal markers RG29 and RZ997 mapped to the short and long arms, respectively. The centromere lies between RZ562 and RZ617, with RZ562 on the short arm and RZ617 on the long arm. Marker RZ323 could not be mapped to a specific chromosome arm because of the lack of polymorphism.

Chromosome 9. Ten markers were assigned to the specific chromosome arms. RG757 mapped to the short arm and the remaining nine markers mapped to the long arm. The centromere lies between RG757 and CDO590, located 2.9 cM apart. Because only one marker was located on the short arm, we examined some markers of Kurata *et al.* (4). The marker C711 was located on short arm and the marker G103 on the long arm. Using a doubled haploid mapping population derived from the IR64/Azucena cross and available at the International Rice Research Institute (IRRI) (16), G103 was mapped between RZ206 and RZ698 and C711 distal to RG757 (Fig. 3).

Chromosome 10. Ten markers were used for arm location. All except RG257 mapped on the long arm. The map position of RG257, although assigned to linkage group 10, has not been determined. RZ892, the terminal marker (3), did not show polymorphism and hence could not be assigned to a specific arm. Four markers from the Japanese map (4) were used for arm location. Of these four markers G1125 and G1084 were located to the short arm and G1082 and G2155 to the long arm. Using the doubled haploid population (16), G1084 and RG257 were mapped between RZ561 and RZ892 and G2155 between RZ811 and RZ583 (Fig. 3). The centromere is therefore located between RG257 and RZ561.

Chromosome 11. Of the 14 polymorphic markers used for arm location, 8 were located on the short arm and 6 on the long arm. Markers RG304 and RZ536, which map to terminal regions (3), are located on short and long arms, respectively. The centromere lies between RG167 and RZ900, which are 1.7 cM apart.

Chromosome 12. The arm location of 10 polymorphic markers was determined. Of these 10 markers 4 were located on the short arm and 6 on the long arm. Markers RG574 and RG181, which map on terminal regions (3), were located to short and long arms, respectively. The centromere lies between RG869 and RG241, with the former on the short arm and the latter on the long arm. RZ670, which lies between RG869 and RG241, could not be located to the specific arm because of a lack of polymorphism with any of the 31 restriction endonucleases.

Our results on the assignment of approximately 170 RFLP markers to specific chromosome arms clearly reveal that the Cornell map (3) covers both arms of all 12 chromosomes. Earlier reports (17, 18) interpreted this map to be incomplete, and not to span the centromere of chromosomes 2, 3, 4, 6, 7, 9, and 10. Comparison of this map with the rice pachytene karyotype (2), reveals good correspondence, except for the linkage map of the short arm of chromosome 10 and the linkage map of the long arm of chromosome 11. The Cornell linkage map of the short arm of chromosome 10 (3) has very few RFLP markers. However, the map of the short arm of chromosome 10 of the Japanese map (4) corresponds well with the pachytene length of the short arm of chromosome 10. Also the linkage map of the long arm of chromosome 11 of the Cornell map has fewer markers than expected on the basis of pachytene length.

Orientation of the Map. By convention, the short arm of a chromosome is positioned at the top of the diagramatic representation and the most distal marker on the short arm is assigned the 0 map position. With RFLP markers assigned to specific chromosome arms of the 12 chromosomes, it is now possible to show the orientation of all the molecular linkage groups with short arms toward the top as presented in Fig. 3. Thus, the orientation of linkage groups 1, 2, 3, 4, 7, 11, and 12 of Causse *et al.* (3) is reversed. Markers RZ288 (chromosome 1), RG555 (chromosome 2), RG104 (chromosome 3), CDO456 (chromosome 4), RG165 (chromosome 7), RG304 (chromosome 11), and RG574 (chromosome 12), therefore, should be at the 0 cM position.

Correspondence Between Various Linkage Maps. Although the majority of the clones used in this study were from the Cornell map (3), the comparison of the data available in the literature on (*i*) the integration of different molecular maps (19), (*ii*) the integration of classical and molecular maps (20, 21), and (*iii*) the orientation of the classical map (9) with the orientation of the Japanese map (4) can be determined and centromeric regions inferred. For example, in linkage group 1, *d-18* maps between markers Npb107 and Npb269 (22) and *d-18* maps on the short arm of linkage group 1 of the classical map (9). Furthermore, RZ288 maps near Npb107 and RZ288 maps on the short arm of linkage group 1 of the Cornell map (Fig. 3). Thus, the lower portion of linkage group 1 of the Japanese map (ref. 4, Fig. 1) where marker G107 (=Npb107) maps should correspond to the short arm of chromosome 1. Employing the same arguments, the orientations of linkage groups 1, 2, 3, 4, 8, 11, and 12 of the Japanese map (4) need to be reversed. Thus, markers R687 (chromosome 1), C1357 (chromosome 2), R1468A (chromosome 3), P2373 (chromosome 4), P122 (chromosome 8), TCL2B (chromosome 11), and TEL2A (chromosome 12) should occupy the 0- cM position on these linkage groups whereas the other linkage groups remain unchanged. This agrees with the orientations determined by Nonomura *et al.* (23) for linkage groups 1, 4, 9, and 12, but not for linkage group 3. These results establish a linear correspondence between the Cornell and Japanese maps for all 12 linkage groups, thus facilitating the integration of the two maps. Comparisons of the two maps indicate that several marker-deficient regions are common to both maps. More precise comparisons can be made when the centromere positions are established for the Japanese map, which can be facilitated by mapping the centromere-flanking Cornell clones onto the Japanese map.

Comparative mapping of rice with other cereal genomes has progressed rapidly during the past few years. Unlike the Triticeae cereals, the centromeric positions in the rice map were not known. Now however, precise comparisons of colinearity can be made. For example, chromosome 5 of rice shows synteny with homologous group 1 of wheat. Comparison of our results with others (6, 24) established clearly that the short arm of chromosome 5 of rice is orthologous to the short arms of homologous group 1 of Triticeae and their centromeric positions correspond almost exactly. Similar conclusions are drawn

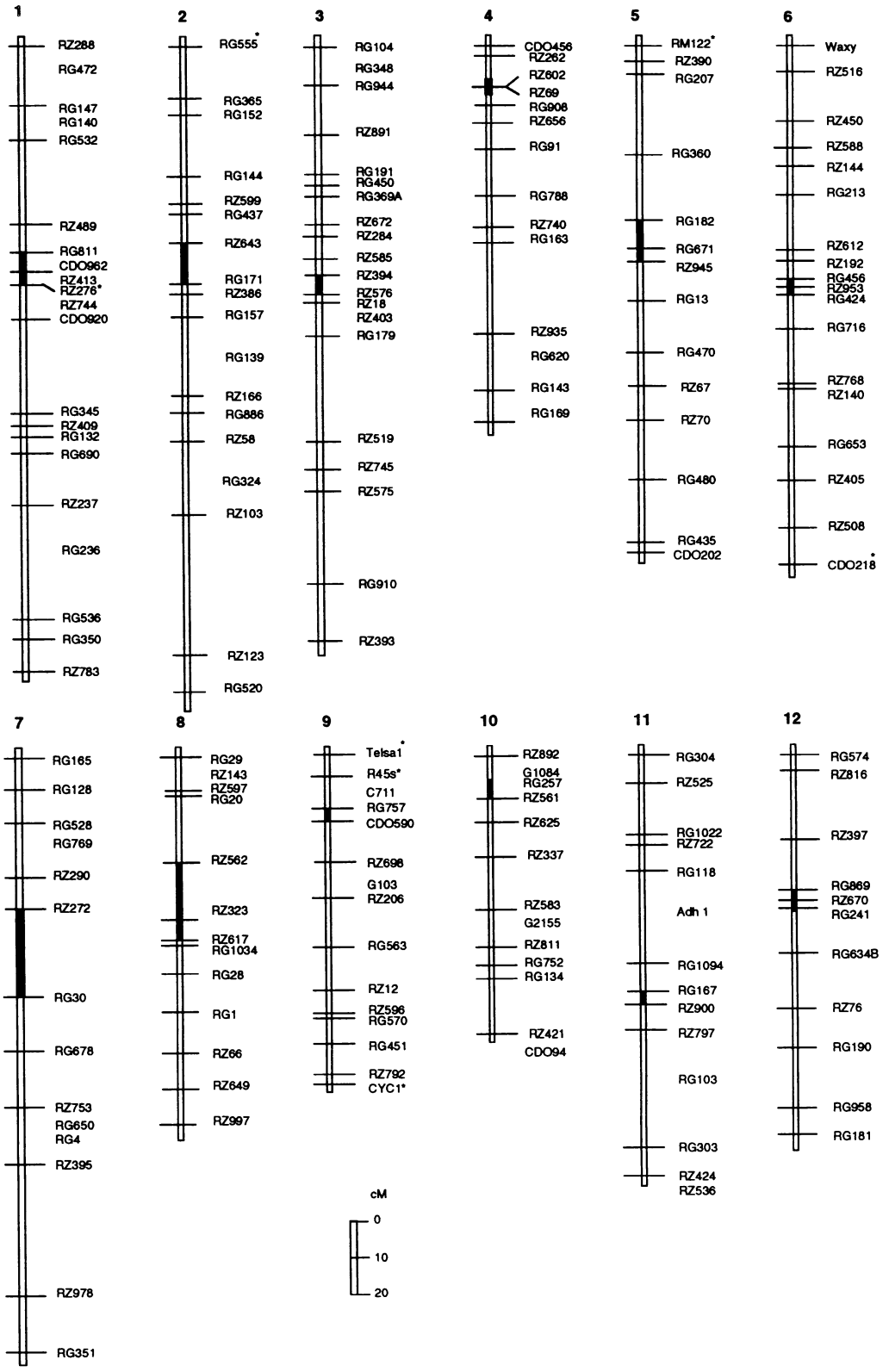

FIG. 3. RFLP map with corrected orientation and centromeric positions. Short arms are at the top and solid lines are the centromeric positions. Markers with an asterisk map on the terminal positions of the linkage group (3), but were not used in the present study. Markers C711 and G103 (chromosome 9) and G1084 and G2155 (chromosome 10) are from the Japanese map (4). Numbers at the top of each linkage group refer to the chromosomes.

for linkage group 1 of rice and homologous group 3 of Triticeae by comparing results presented here with those of Nelson *et al.* (25). Chromosome 4 of rice is subtelocentric and its RFLP map corresponds well with the pachytene karyotype. RFLP markers CDO456 and BCD348 are located on the short arm and RZ69 is located on the long arm of linkage group 4 of rice (Fig. 3), but all of these markers are located on the short arm of homologous group 2 of wheat (6, 26) suggesting that a pericentric inversion occurred after the divergence from the last common ancestor. Such comparisons will enhance our understanding of the chromosome evolution of grass species.

The assignment of RFLP markers to specific chromosome arms, and location of centromere positions, provide a correct orientation of the RFLP map of rice as well as the classical map (9), establishing a linear correspondence between classical and molecular maps. Now with the positions of centromeres mapped both on rice and wheat molecular maps, as well as classical maps, the synteny and colinearity between cereal genomes can be established firmly and manipulated for the improvement of rice and other cereals.

Centromeres play a pivotal role in organization, structure, and evolution of eukaryotic chromosomes, but molecular organization of higher eukaryotic centromeres remains enigmatic (27). Rice can now be used as a model plant for cloning monocotyledonous plant centromeres. With the availability of high density molecular maps (3, 4); yeast artificial chromosome (28) and bacterial artificial chromosome libraries (29), the rice centromeres or centromere specific sequences may be isolated using centromere flanking RFLP markers identified in this study.

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