Secondary Trisomics and Telotrisomics of Rice: Origin, Characterization, and Use in Determining the Orientation of Chromosome Map

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ABSTRACT

Secondary trisomics and telotrisomics representing the 12 chromosomes of rice were isolated from the progenies of primary trisomics. A large population of each primary trisomic was grown. Plants showing variation in gross morphology compared to the primary trisomics and disomic sibs were selected and analyzed cytologically at diakinesis and pachytene. Secondary trisomics for both arms of chromosomes *1, 2, 6, 7* and *11* and for one arm of chromosomes *4, 5, 8, 9* and *12* were identified. Telotrisomics for short arm of chromosomes *1, 8, 9* and *10* and for long arms of chromosomes *2, 3* and *5* were isolated. These secondary and telotrisomics were characterized morphologically and for breeding behavior. Secondary trisomics 2n + *1S*·*1S*, 2n + *1L*·*1L*, 2n + 2S·2S, 2n + 2L·2L, 2n + *6S*·*6S*, 2n + *6L*·*6L* and 2n + 7L·7L are highly sterile, and 2n + *1L*·*1L*, 2n + 2L·2L and 2n + 7L·7L do not set any seed even upon backcrossing. Telotrisomics are fertile and vigorous. Genetic segregation of 43 marker genes was studied in the F_2 or backcross progenies. On the basis of segregation data, these genes were delimited to specific chromosome arms. Correct orientation of 10 linkage groups was determined and centromere positions on nine linkage groups were approximated. A revised linkage map of rice is presented.

TWELVE linkage groups of rice were proposed by NAGAO and TAKAHASHI (1963). Independence of the linkage groups was tested by IWATA and OMURA (1975, 1976) through primary trisomic tests. Three linkage groups were combined into one linkage group and another two linkage groups were also combined. IWATA and OMURA (1975, 1976) and ISONO *et al.* (1978) established three new linkage groups, thereby leading to the establishment of 12 independent linkage groups. These linkage groups were associated with cytologically identifiable chromosomes through the use of primary trisomics by KHUSH *et al.* (1984). The chromosome map now consists of about 178 markers (KHUSH and KINOSHITA 1991). However, the distribution of different genes on chromosome arms and, thus, the orientation of linkage groups was not known.

Secondary trisomics and telotrisomics can be employed to determine the arm location of genes and the positions of the centromeres on the chromosome map (KHUSH 1973). However, until recently such aneuploids were not available in rice. This is the first report about the development of secondary and telotrisomic series and their use in determining the chromosome arm location of marker genes and the orientation of linkage groups. In the secondary trisomics, the extra chromosome is an isochromosome for one chromosome arm. In the telotrisomics on the other hand, the extra chromosome is a telocentric composed of centromere and one chromosome arm. As discussed by KHUSH and RICK (1968, 1969), the modified genetic ratios of genes segregating in the progenies of secondary and telotrisomics permit the delimitation of genes to respective chromosome arms. In diploid species like rice and tomato, the secondary (isochromosome) and telocentric chromosomes cannot replace a normal chromosome in the gametes. So any gamete receiving a secondary or a telocentric chromosome in place of a normal chromosome aborts because of a deficiency of part of the chromosome. Consequently n gametes and $2n$ zygotes produced by the secondary or telotrisomics consist only of normal homologues.

When a secondary or telotrisomic is crossed as a female with a recessive mutant stock, one of the two normal homologues of the F_1 trisomic carries the recessive allele, while the other homologue as well as the iso or telo chromosomes carry the normal alleles. In the F_2 or backcross progeny of these trisomics, the disomic portion segregates in a normal 3:1 or 1:1 fashion, and all the trisomics have normal phenotype. This 3:1::All:0 ratio indicates that the gene under study is located on the arm that is duplicated in the trisomic. However, if the gene is not situated in the duplicated arm, the disomic as well as the trisomic portions of the progeny segregate in normal disomic ratios (KHUSH 1973).

MATERIALS AND METHODS

Isolation of secondary and telotrisomics: Isochromosomes and telocentric chromosomes arise as a result of misdivision

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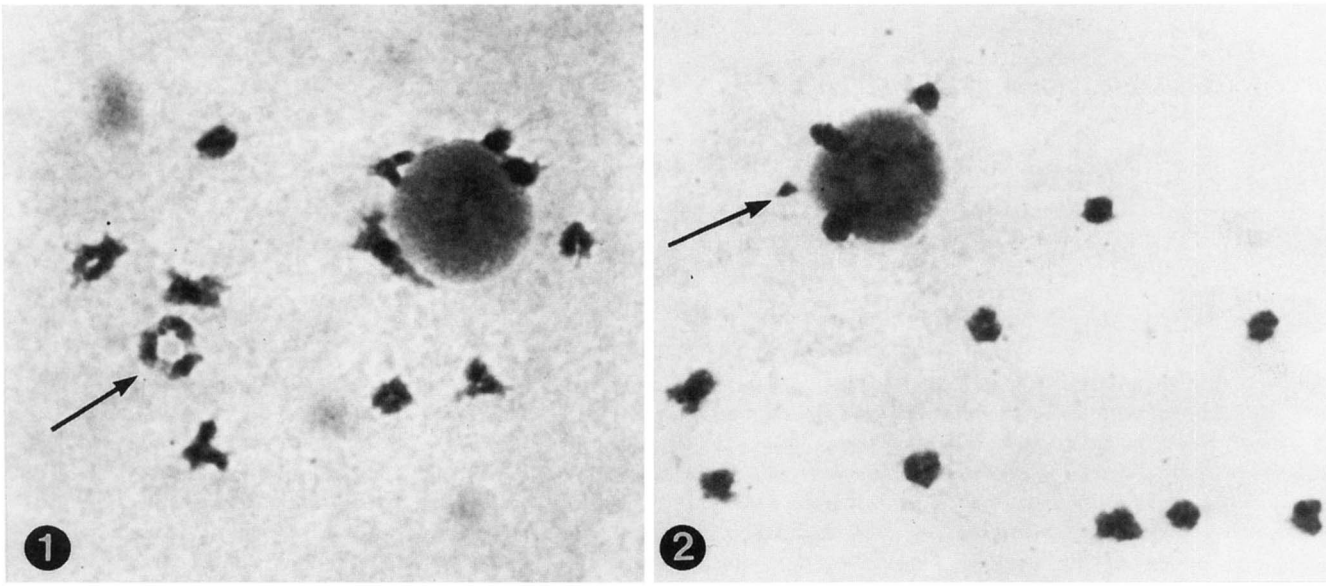


FIGURE 1.—Photomicrograph of a PMC of 2n + 6*L.6*L plant showing 11 bivalents and a ring trivalent at diakinesis stage of meiosis.

FIGURE 2.—Photomicrograph of a PMC of 2n + · *10*S plant showing 12 bivalents and an univalent · *10*S at diakinesis stage of meiosis.

of the univalent. Since in the primary trisomics, the extra chromosome is present as a univalent in many sporocytes, the chances of misdivision are higher. As pointed out by KHUSH (1973), the best sources of secondary and telotrisomics are the progenies of primary trisomics. In fact, secondary and telotrisomics of several species such as Datura (BLAKESLEE and AVERY 1938), maize (RHOADES 1933), wheat (SEARS 1954), tomato (KHUSH and RICK 1968, 1969) and barley (TSUCHIYA 1991) were selected among the progenies of primary trisomics.

We have been growing progenies of primary trisomics of rice since 1970 to locate genes to respective chromosomes (KHUSH *et al.* 1984; SANCHEZ and KHUSH 1994). Whenever, we observed a trisomic with a variant phenotype, it was cytologically examined. We identified three secondary and one telotrisomic in this manner. During the last three years, we grew large populations of selfed progenies of our primary trisomics to isolate secondary and telotrisomics. Plants that looked different from the diploids as well as primary trisomics were selected and were examined cytologically at diakinesis (DK), metaphase I (M1) and pachytene stages of meiosis. Young panicles were fixed in ethanol-acetic acid (3:1) mixture with traces of ferric chloride. Standard squash technique with 1.5% propiono-carmine was used in all cytological preparations. The chromosome number and trivalent configurations in morphological variants were studied at DK and M1 stages with emphasis at DK. A secondary trisomic can form an array of trivalent configurations depending upon number, position and terminalization of chiasmata (KHUSH and RICK 1969). The occurrence of a ring trivalent is the most reliable diagnostic feature of secondary trisomics (Figure 1). The telotrisomics can be initially identified at DK and MI based on the smaller size of the univalent (Figure 2). Also a chain trivalent with one chromosome distinctly smaller is a diagnostic feature of the telotrisomics.

The frequency of secondary and telotrisomics in the progenies of primary trisomics that were specifically grown for isolating these variant types is shown in Table 1. To date we have isolated 15 secondary and seven telotrisomics. Secondary trisomics for both arms of chromosomes *1, 2, 6, 7* and *11* are available. Secondary trisomics for only one arm of chromosomes *4, 5, 8, 9* and *12* could be isolated. Telotrisomics for *2*L, *3*L, *5*L, *1*S, *8*S, *9*S and *10*S were identified. The secondary and telotrisomics were crossed as females with well-defined gene stocks, the trisomic F_1 plants were allowed to set self seed or were backcrossed with the gene stocks. F_2 or backcross populations were classified into diploid and trisomic fractions as well as for the normal or mutant phenotype.

In this paper, the tomato system of designating the trisomics was followed (KHUSH 1973). Thus a primary trisomic for chromosome *5* is designated as 2n + *5*S · *5*L. A secondary trisomic for long arm of chromosome *5* is written as 2n + *5*L · *5*L and that for short arm as 2n + *5*S · *5*S. A telotrisomic for long arm of chromosome *5* is indicated as 2n + · *5*L and that for short arm as 2n + · *5*S.

RESULTS AND DISCUSSION

Cytological identification of secondary and telotrisomics: The morphological variants identified in the progenies of primary trisomics were first examined at DK to determine the nature of trisomy. Once a variant was identified either as a secondary or a telotrisomic, it was further examined at pachytene stage to identify the extra chromosome.

Diakinesis: Various types of chromosomal associations were observed at DK in the secondary and telotrisomics. The frequency of ring, chain and other types of trivalents as well as univalents is shown in Table 2. The frequency of ring trivalents in the secondary trisomics was as low as 0.5% in 2n + *4*S · *4*S and as high as 25.6% in 2n + *8*L · *8*L. Secondary trisomics for the long arms, in general, showed higher frequency of ring trivalents than the secondary trisomics for the short arms. In the 2n + *4*S · *4*S very low frequency of ring trivalent was observed because of extremely small size of *4*S.

In the telotrisomics, cells with 12II + 1I were most

TABLE 1

Frequency of secondary and telotrisomics in the progenies of primary trisomics

Trisomic	Total plants grown	Secondary trisomic		Telotrisomic		Frequency (%)
		Short arm (No.)	Long arm (No.)	Short arm (No.)	Long arm (No.)	
Triplo *1*[a]	—	1	1	1[b]	0	—
Triplo *2*	1632	2	1[b]	0	1	0.18
Triplo *3*[a]	—	0	0	0	1	—
Triplo *4*	1812	1	0	0	0	0.05
Triplo *5*	1536	1	0	0	1	0.13
Triplo *6*	2112	3	1	0	0	0.19
Triplo *7*	3300	2	1	0	0	0.09
Triplo *8*	1608	0	3	1	0	0.25
Triplo *9*	2127	0	3	1	0	0.19
Triplo *10*	1776	0	0	1	0	0.06
Triplo *11*	600	1	1	0	1	0.50
Triplo *12*	1632	2	0	0	0	0.12

[a] Triplo *1* and triplo *3* are highly sterile and large populations could not be grown. 2n + *1S*·*1S* and 2n + *1L*·*1L* and ·*3*L were selected from progenies of primary trisomics before the conscious efforts were made to isolate secondary and telotrisomics.

[b] Telotrisomic 2n + ·*1S* was isolated from the progeny of 2n + *1S*·*1S* and secondary trisomic 2n + 2L·2L was isolated from the progeny of 2n + ·2L.

TABLE 2

Chromosomal associations at diakinesis in secondary and telotrisomics of rice

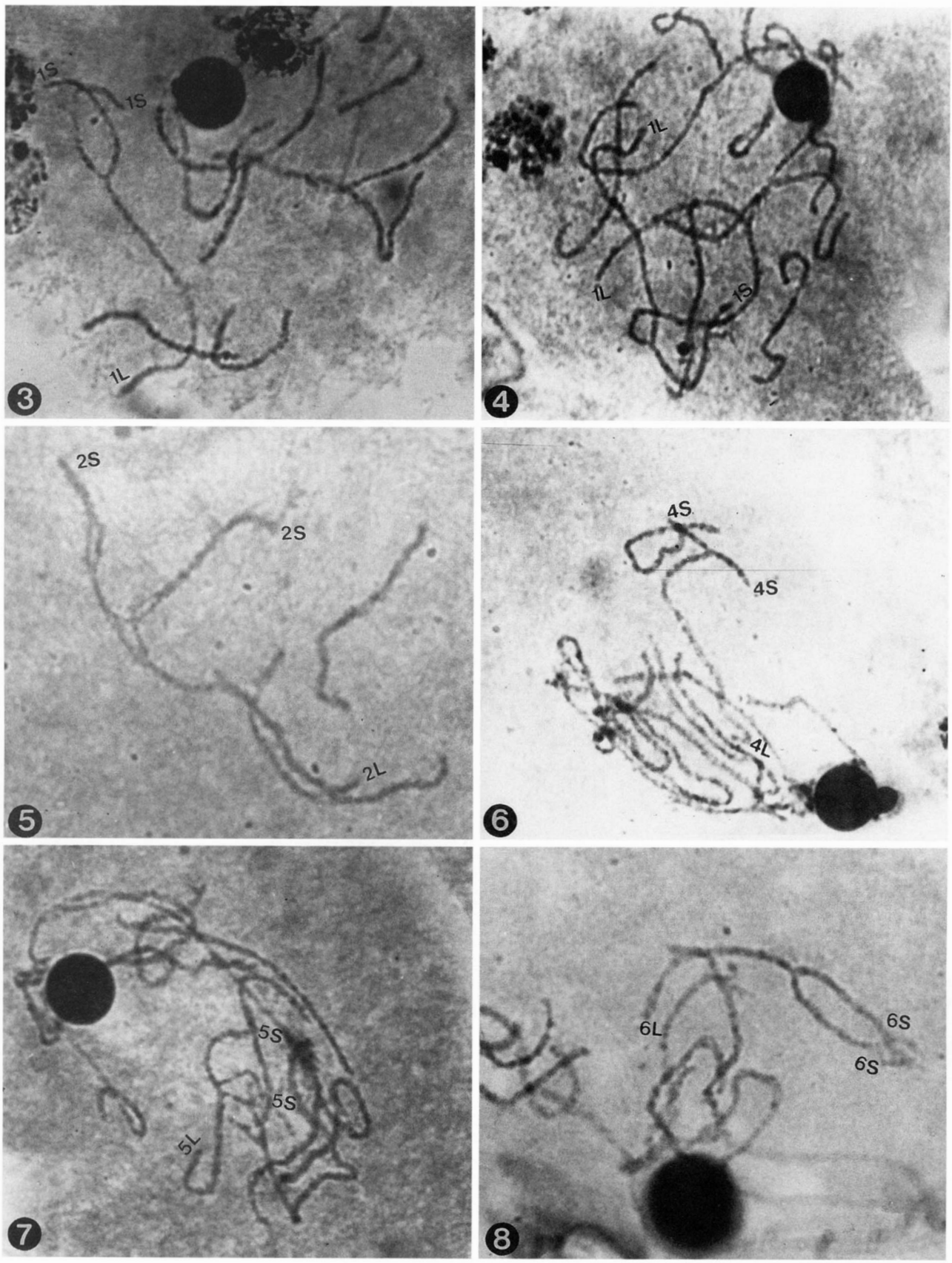
Trisomic	Total cells observed	No. of cells with [a]				
		12II + 1I	11II + 1III			11II + 3I
			Ring	Chain	Others[b]	
2n + *1S*·*1S*	101	47 (46.5)	16 (15.9)	28 (27.7)	8 (7.9)	2 (1.9)
2n + *1L*·*1L*	165	49 (29.7)	41 (24.8)	38 (23.0)	30 (18.2)	7 (4.2)
2n + *2S*·*2S*	147	110 (74.8)	10 (6.8)	10 (6.8)	17 (11.6)	0 (0.0)
2n + *2L*·*2L*	60	29 (48.3)	13 (21.7)	11 (18.3)	6 (10.0)	1 (1.7)
2n + *4S*·*4S*	215	175 (81.4)	1 (0.5)	14 (6.5)	25 (11.6)	0 (0.0)
2n + *5S*·*5S*	127	83 (65.4)	13 (10.2)	18 (14.2)	9 (7.1)	4 (3.1)
2n + *6S*·*6S*	110	64 (58.2)	13 (11.8)	20 (18.2)	6 (5.5)	7 (6.4)
2n + *6L*·*6L*	142	72 (50.7)	28 (19.7)	22 (15.5)	16 (11.3)	4 (2.8)
2n + *7S*·*7S*	156	90 (57.7)	24 (15.4)	28 (17.9)	14 (9.0)	0 (0.0)
2n + *7L*·*7L*	141	78 (55.3)	22 (15.6)	17 (12.1)	17 (12.1)	7 (4.9)
2n + *8L*·*8L*	125	75 (60.0)	32 (25.6)	14 (11.2)	4 (3.2)	0 (0.0)
2n + *9L*·*9L*	118	52 (44.1)	22 (18.6)	20 (16.9)	18 (15.3)	6 (5.1)
2n + *11S*·*11S*	146	95 (65.1)	17 (11.6)	18 (12.3)	15 (10.3)	1 (0.7)
2n + *11L*·*11L*	115	87 (75.6)	8 (6.9)	11 (10.1)	6 (5.2)	3 (2.6)
2n + *12S*·*12S*	93	52 (55.9)	14 (15.1)	13 (14.0)	12 (12.9)	2 (2.1)
2n + ·*1S*	161	106 (65.8)	—	22 (13.7)	30 (18.6)	3 (1.9)
2n + ·2L	122	60 (49.2)	—	30 (24.6)	26 (21.3)	6 (4.9)
2n + ·*3*L	113	73 (64.6)	—	25 (22.1)	11 (9.7)	4 (3.6)
2n + ·*5*L	109	78 (71.6)	—	23 (21.1)	8 (10.3)	0 (0.0)
2n + ·*8*S	141	103 (73.0)	—	25 (17.7)	13 (9.2)	0 (0.0)
2n + ·*9*S	138	120 (87.0)	—	13 (9.4)	5 (3.6)	0 (0.0)
2n + ·*10*S	128	102 (79.7)	—	20 (15.6)	6 (4.7)	0 (0.0)

[a] Values in parentheses indicate the percentage.

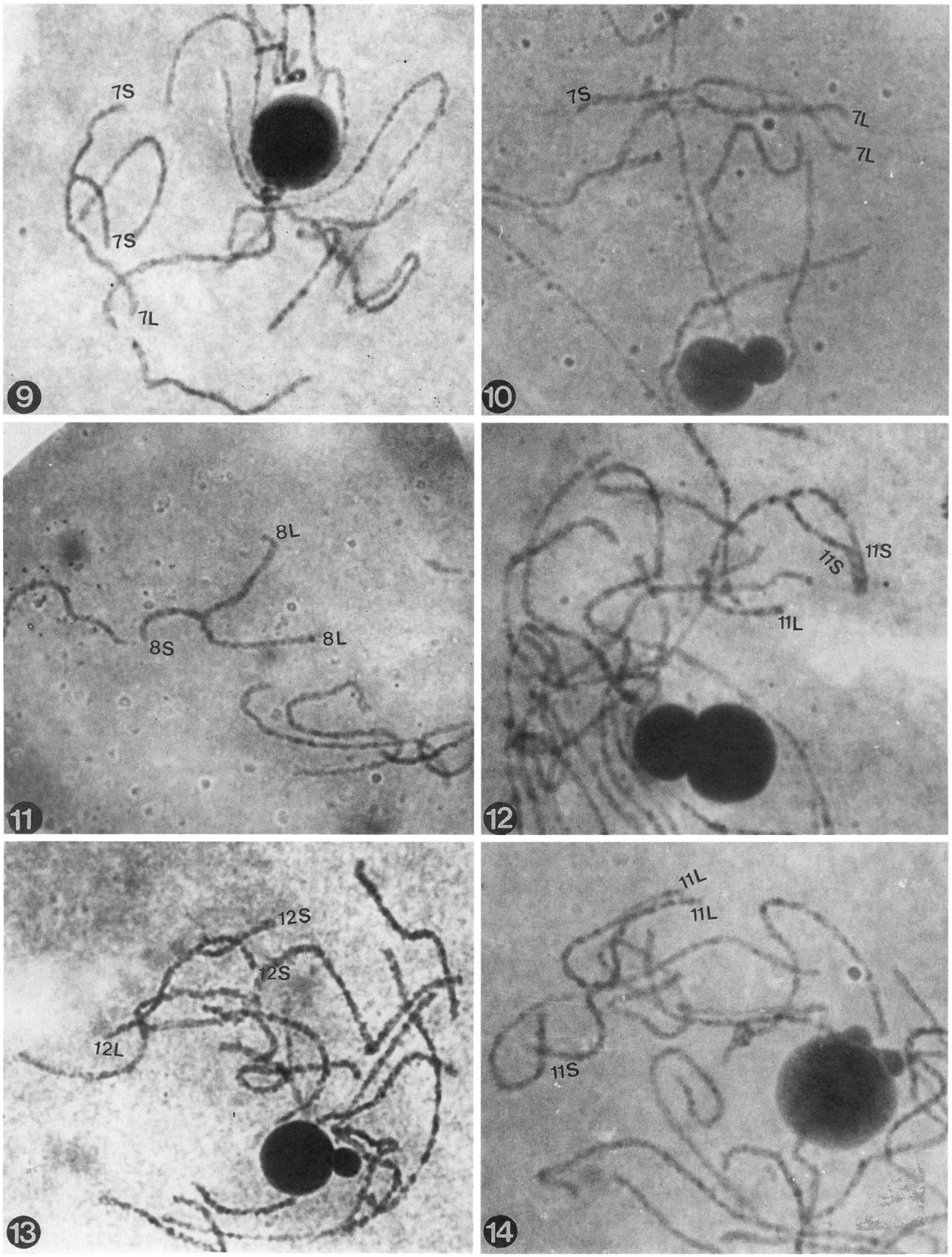
[b] For other types of trivalent configurations, see KHUSH and RICK (1969).

frequent ranging from 49% in 2n + ·2L to 87% in 2n + ·*9*S (Table 2). The frequency of cells with 11II + 1III varied from 13 to 46%. A few cells with 11II + 3I were also observed. In general telotrisomics for the long arms showed higher frequency of trivalents.

Pachytene analysis: Once a secondary or a telotrisomic



FIGURES 3–14.—Photomicrographs showing trivalents of various secondary trisomics at pachytene stage of meiosis. 3, 2n + *1*S· *1*S; 4, 2n + *1*L· *1*L; 5, 2n + *2*S· *2*S; 6, 2n + *4*S· *4*S; 7, 2n + *5*S· *5*S; 8, 2n + *6*S· *6*S; 9, 2n + *7*S· *7*S; 10, 2n + *7*L· *7*L; 11, 2n + *8*L· *8*L; 12, 2n + *11*S· *11*S; 13, 2n + *12*S· *12*S; and 14, 2n + *11*L· *11*L.



FIGURES 3–14.—*Continued*

was identified at DK, pachytene analysis was carried out to identify the extra iso or telocentric chromosomes. As pointed out by KHUSH *et al.* (1984) rice chromosomes and chromosome arms can be identified with certainty at pachytene stage on the basis of distinct cytological features. Before the cytological identification was undertaken, some clues to their identity were available from the plant morphology and the source from where these were isolated. Thus, a secondary or a telotrisomic identified in the progeny of triplo *4* is more likely to be for one of the arms of chromosome *4* although this is by no means the rule.

In a secondary trisomic, the isochromosome can pair internally and stay as a univalent or pair with the homologous arms of the two normal chromosomes to form a trivalent. The Y-shaped trivalent was the most critical for identifying the isochromosomes (Figures 3–14). Other trivalent configurations were also observed. Pachytene karyotype of KHUSH and KINOSHITA (1991) was used as a reference karyotype for identification of extra isochromosomes of the secondary trisomics and telocentric chromosomes of the telotrisomics. The secondary trisomics for the long and short arms of chromosomes *1, 2, 4, 5, 7, 8, 9, 11,* and *12* could be easily identified due to larger size differences in the long and short arms. Chromosomes *3* and *6* are metacentric, and the long and short arms are somewhat difficult to differentiate. The pachytene configurations of the secondary trisomics are shown in Figures 3–14.

The telotrisomics 2n + · *9*S and 2n + · *10*S could be easily identified at pachytene stage due to the association of short arms with nucleolus. The · *9*S was generally completely covered by the nucleolus. The distal end of *10*S was associated with the nucleolus in most cells. The telocentric arms are more difficult to identify at pachytene than isochromosomes.

Confirmation of positions of centromeres: The centromere positions at the pachytene chromosomes of rice cannot be delimited with certainty because the centromeres and the lightly stained chromomeres look alike. However, the pachytene trivalent configurations of secondary trisomics can pinpoint the positions of centromeres with certainty. From the observations of pachytene trivalents of secondary trisomics (Figures 3–14), the centromere positions of 10 chromosomes as shown in the pachytene ideogram (KHUSH and KINOSHITA 1991) were confirmed. However, contrary to locations of centromeres shown in the ideogram, chromosome *6* is more metacentric and chromosome *12* is submetacentric. Thus, the position of centromeres of these chromosomes needs to be revised in the pachytene ideogram.

Morphological and reproductive features of secondary and telotrisomics: All primary trisomics of rice can be distinguished from each other as well as from diploid sibs on the basis of gross morphological and reproductive features (KHUSH *et al.* 1984). Most of the secondary trisomics studied so far resemble their counterpart primary trisomics in many morphological features. Some of the features of primary trisomics are exaggerated in the secondary trisomics while some are absent (Figures 15 and 16). Some of the secondary trisomics have a few features that primary trisomics do not have. One secondary trisomic (2n + *4*S · *4*S) looks like diploid sibs and could be identified only on the basis of cytological examination.

The telotrisomics for the long arms resemble corresponding primary trisomics in many morphological features, and none of them showed any exaggerated traits. The telotrisomics for the short arms, are difficult to differentiate from disomic sibs and could be identified on the basis of cytological examination. All the telotrisomics are more vigorous than the corresponding primary and secondary trisomics (Figures 15 and 16).

The morphological and reproductive features of the secondary, and telotrisomics and corresponding primary trisomics are presented in Table 3. The features of the corresponding primary trisomics are presented for the purpose of comparison. The secondary trisomics in general have slower growth rate and lower fertility than the corresponding primary trisomics. Secondary trisomics 2n + *1*S · *1*S and 2n + *1*L · *1*L have reduced growth rate and are completely self sterile. 2n + *1*L · *1*L does not set any seed even upon backcrossing to IR36. This trisomic is being maintained vegetatively. The telotrisomic 2n + · *1*S on the other hand is very vigorous and highly self fertile. The secondaries 2n + *2*S · *2*S and 2n + *2*L · *2*L do not set any seed upon selfing but 2n + · *2*L gives good seed set upon backcrossing. 2n + *6*S · *6*S and 2n + *6*L · *6*L have reduced growth and fertility than the primary trisomic 2n + *6*S · *6*L.

Secondary trisomic 2n + *7*L · *7*L is very weak with only 10–15 spikelets per panicle. It did not set any seed upon backcrossing and thus could not be utilized in genetic analysis. 2n + *7*S · *7*S on the other has partial fertility. It has open lemma and palea, a character not present in the primary triplo *7*. The secondary 2n + *8*L · *8*L is more vigorous than triplo *8* but is semi-sterile.

Transmission rates of secondary and telotrisomics: The female transmission rates of the extra chromosomes were studied in the selfed progenies of secondary and telotrisomics (Table 4). Since 2n + *1*S · *1*S, 2n + *2*S · *2*S, 2n + · *2*L and 2n + · *3*L are self sterile, their transmission rates were studied in their backcross progenies. The transmission rates of the extra chromosomes of 2n + *1*L · *1*L and 2n + *7*L · *7*L could not be examined as these trisomics do not set any seed even upon backcrossing. Three trisomics 2n + *2*L · *2*L, 2n + *6*L · *6*L and 2n + *7*S · *7*S were isolated only recently, and data on the transmission rates of their extra chromosomes are not yet available. As shown in Table 4, the transmission rates of the extra isochromosomes varied from 8.1% in 2n + *1*S · *1*S to 47.3% in 2n + *4*S · *4*S. These results are not unexpected. Rice is a basic diploid with limited toler-

FIGURE 15.—Plants of rice variety IR36, a secondary, a primary and a telotrisomics of chromosome *1*.
FIGURE 16.—Plants of IR36, a secondary, a primary and a telotrisomic of chromosome *5*.

ance for duplications, particularly at the gametophytic level. Gametes with extra isochromosomes for longer arms are less viable than those with extra isochromosomes for short arms. The short arm of chromosome *1* is several times longer than short arm of chromosome *4*, which is the shortest of the 24 chromosome arms of the rice chromosome complement.

As expected, related primary trisomics also appeared in the progenies of secondary trisomics. Their proportion varied from 1.4% in the progeny of 2n + *1*S · *1*S to 20.7% in the progeny of 2n + *8*L · *8*L (Table 4). As pointed out by KHUSH and RICK (1969), there is a correlation between the frequency of ring formation at DK in the secondary trisomics and the occurrence of related primary trisomics in their progenies; both were highest in 2n + *8*L · *8*L. Male transmission of isochromosomes was observed in 2n + *4*S · *4*S and 2 plants in its progeny had 2 extra *4*S · *4*S isochromosomes.

The female transmission rates of telocentric chromosomes in the progenies of telotrisomics varied from 28.6% for · *2*L to 47.5% for · *9*S, which were higher than the transmission rates of isochromosomes. Moreover, the transmission rates of telocentrics for short arms were higher than those of telocentrics for the long arms. In the progenies of 2n + · *8*S and 2n + · *9*S, about 12% and 20% of the plants respectively had two extra telocentric chromosomes, indicating high rates of transmission of these chromosomes through the male. Transmission rates of these telocentric chromosomes through male were estimated as 26% for · *8*S and 32% for · *9*S. It should be pointed out that *8*S · *8*L and *9*S · *9*L also show high transmission through the male (KHUSH *et al.* 1984).

Arm locations of the genes: For each of the linkage groups a few well-defined recessive marker stocks were crossed with corresponding secondary or telotrisomics as females. Forty-three marker genes belonging to 11 linkage groups were studied (Table 5). For some of the chromosomes such as *8, 9* and *10* only a few markers are known and most were used in crosses. Segregation ratios were studied in the F_2 or backcross populations to determine the arm location of the marker genes. The linkage map of KHUSH and KINOSHITA (1991) and KINOSHITA (1993) is the basis of discussion in this section.

Chromosome 1: The segregation of five genes, *d-18, chl-6, spl-6, z-8* and *gf-2* was studied in their crosses with 2n + · *1*S. Results show that only *d-18* is on the short arm and rest are on the long arm. Two genes *z-8* and *gf-2* are located on chromosome *1* but have not been mapped. Thus, the centromere is between *d-18* and *chl-6*. The orientation of the linkage group thus should be reversed with *fs-2* at the 0 position and *v-6* at the 149 cM position.

Chromosome 2: The segregation of five marker genes, *spl-2, gh-2, z-12, chl-10* and *tri* was studied in their crosses with 2n + *2*S · *2*S, and *bc-3* was studied by crossing with 2n + · *2*L. Of these markers, *spl-2, gh-2* and *z-12* are on the short arm, and *chl-10, bc-3* and *tri* are on the long arm. Therefore the centromere is between *gh-2* and *chl-10*. The marker gene *z-12* was recently located on this chromosome (SANCHEZ and KHUSH 1994) but has not been mapped. The orientation of this linkage group thus should be reversed with *spl-2* at the 0 position and *d-29* at the 201 cM position.

Chromosome 3: The segregation of six marker genes, *spl-3, dl, chl-2, bc-1, v-1* and *chl-1* was studied in their crosses with 2n + · *3*L. Of these, *spl-3, dl, chl-2* and *bc-1* are on the short arm, *v-1* and *chl-1* are on the long arm, and the centromere maps between *bc-1* and *v-1*, which are 20 cM apart. The orientation of the linkage map should be reversed with *d-52* at 0 position and *chl-1* at the 155 cM position.

Chromosome 4: The secondary trisomic 2n + *4*S · *4*S was isolated only recently, and segregation data for *st-4, lg*, and *pl-2* of this linkage group is available. All these are located on the long arm. However, the orientation

TABLE 3

Diagnostic features of primary, secondary and telotrisomics of rice

Primary trisomic	Diagnostic features	Secondary/ telotrisomic	Diagnostic features
Triplo *1*	Short, grassy; narrow and thin leaves; late flowering, narrow and triangular grains, low seed fertility.	2n + *1*S· *1*S	Compared to triplo 1, it has long, narrow, thin outward folded drooping leaves; later in flowering than 2n sib, triangular grains, dull green color, no seed set upon selfing. High seedling mortality; can be identified at seedling stage due to weak seedlings. Pollen fertility is 11.0%.
		2n + · *1*S	Compared to secondary 2n + *1*S· *1*S, it is vigorous and self fertile; leaves are comparatively broader and is early in flowering. Can be identified at seedling stage.
		2n + *1*L· *1*L	Dwarf, grassy; leaves are short and broader than triplo *1*; panicle is very small in size but the spikelets are normal in size; highly male sterile and does not set any seeds even on backcrossing. It is being maintained vegetatively. Pollen fertility is 8.0%.
Triplo *2*	Short, few tillers; short, thick dark green and twisted leaves; small anthers, short panicle, longer glumes, depressed palea and highly self sterile.	2n + *2*S· *2*S	Short, few tillers; dark green and inward folded leaves; short panicle; normal glume size, and normal palea; highly self sterile; high seedling mortality; can be identified at seedling stage. Pollen fertility is 16.8%.
		2n + *2*L· *2*L	Dwarf, very weak; leaves are short, highly twisted and dull in color; depressed palea; long glumes; highly self sterile.
		2n + · *2*L	Tall, vigorous; leaves are broader and twisted and dull colored; depressed palea, long panicle, 15–20% seed set upon selfing. Can be identified at seedling stage. Pollen fertility is 54.0%.
Triplo *3*	Short; slow growth; short thick dark green leaves; late flowering; highly sterile.	2n + · *3*L	Vigorous at seedling stage but the growth slows down after transplanting. Can be identified at seedling stage. Leaves become outwardly folded at maximum tillering stage and are thick and leathery; panicle is compact with little degeneration at the tip, seeds are bold and short compared to 2n sib. Pollen fertility is 78% and sets 30–40% seeds upon selfing.
Triplo *4*	Tall, spreading; long and droopy light green leaves; lax panicles; grains on the upper half of the panicle have tip awns and that of lower half are awnless. Self fertile.	2n + *4*S· *4*S	Hard to differentiate from the disomic plants. However, some minor differences like thinner culm, slightly narrow leaves and lax panicle help in identification of secondary trisomic plants. Pollen fertility and seed set are normal.
Triplo *5*	Outward folded short leaves, short ligule, short anthers, short grains, self fertile; can be identified even at seedling stage.	2n + *5*S· *5*S	Shorter than 2n sib; leaves are short and do not show any outward folding; compact growth; highly reduced ligule and anthers; about 10 days later than 2n sib and triplo *5*. Grains are long and slender. Partially sterile. Pollen fertility is 78.0%.
		2n + · *5*L	Leaves are long; outwardly folded and droopy at maximum tillering stage. Ligules and anthers are of normal size; grains are short, early in flowering and highly self fertile; identifiable at seedling stage. Pollen fertility is normal.

TABLE 3

Continued

Primary trisomic	Diagnostic features	Secondary/ telotrisomic	Diagnostic features
Triplo *6*	Short, short inward folded leaves, lax and awned panicle, long ligule, early flowering, short grains partially sterile.	2n + *6*S · *6*S	Short, narrow and inward folded leaves, long ligule, very lax panicle with tip awns; short grains. Weak seedlings; can be identified at seedling stage. Pollen fertility is 68.8%; very little seed set upon selfing.
		2n + *6*L · *6*L	Short, no inward folding of leaves, normal ligule, short panicle; grains awnless and normal sized; panicles do not exsert well from the flag leaf. Highly self sterile. Seedlings vigorous, difficult to identify at seedling stage. Pollen fertility is 40%.
Triplo *7*	Narrow dark green rolled leaves, lax panicle, long grains with tip awns.	2n + *7*S · *7*S	Erect and compact growth habit; leaves are broad with little inward folding; incomplete panicle exsertion; lemma and palea separated apart; no tip awns; partially sterile; seedlings are weaker than disomic sib, but can be identified. Pollen fertility is 77.0%.
		2n + *7*L · *7*L	Narrow and fully rolled leaves; very lax panicle with only 10–15 spikelets per panicle. Panicle does not exsert out of the flag leaf, long spikelet with tip awns; highly self sterile. Pollen fertility < 1.0%.
Triplo *8*	Short, slow initial growth that recovers at maximum tillering stage; very narrow, dark green rolled leaves; short panicle, short grains; self fertile.	2n + · *8*S	Vigorous; leaves thin and inwardly folded but broader than triplo *8*; grains are longer than those of triplo *8*, self fertile. Trisomic seedlings can be identified easily. Pollen fertility is normal.
		2n + *8*L · *8*L	Vigorous, inwardly folded thick leaves which are much broader than those of triplo *8* and 2n + · *8*S; less tillering; panicle is larger and compact; grains are short and flattened; partially sterile; can be identified at seedling stage. Pollen fertility is 60%.
Triplo *9*	Dark green spreading plant type; thick and inward folded leaves, larger and partially exserted panicle, longer grains, self fertile.	2n + · *9*S	Plants are difficult to distinguish from disomic sibs; cytological examination necessary for identification. Pollen fertility is normal.
		2n + *9*L · *9*L	Plants are dwarf, spreading with stunted growth; short, inward folded dark green leaves; panicle is small and incompletely exerted. Pollen fertility is 27% and does not set seed upon selfing.
Triplo *10*	Short, narrow leaves, thin culm, slender grains, self fertile.	2n + · *10*S	Vigorous and taller than the triplo *10*, can be distinguished from the disomic sibs due to thinner culm. Self fertile; pollen fertility is normal.
Triplo *11*	Plant type is like 2n sibs, but at booting stage plants are slightly golden colored; gold hull color.	2n + *11*S · *11*S	Plant type is like 2n sibs but can be identified at maximum tillering stage due to its dark green color and thin culms; grains are more slender than those of 2n sib; self fertile. Pollen fertility is normal.
		2n + *11*L · *11*L	Plant type is like 2n sibs but at maximum tillering stage, it is yellowish in color, shy tillering; hulls are deep gold in color; self fertile. Pollen fertility is normal.
Triplo *12*	Bushy; many tillers; plants pale green in color; panicles lax; degenerated florets at the tip of panicles; long grains; self fertile	2n + *12*S · *12*S	Plants bushy and green; narrow and inward folded leaves; thinner culms; lax panicles; degenerated florets at the tips of panicles. Long lemma and palea but the grains inside are short, partially sterile. Trisomic plants can be easily differentiated from disomics at seedling stage. Pollen fertility is 87.0%.

TABLE 4

Transmission rates of extra chromosomes in the selfed or backcross progenies of rice

Trisomic	Total plants	Disomics		Secondary/ telotrisomics		Related primary trisomics	
		No.	%	No.	%	No.	%
2n + *1S*·*1S*[a]	74	67	90.5	6	8.1	1	1.4
2n + *2S*·*2S*[a]	96	78	81.2	14	14.6	4	4.2
2n + *4S*·*4S*[b]	53	25	45.5	26	47.3	2	3.6
2n + *5S*·*5S*	181	125	69.1	51	28.2	5	2.7
2n + *6S*·*6S*	41	26	63.4	8	19.5	7	17.1
2n + *8L*·*8L*	116	64	55.2	28	24.1	24	20.7
2n + *11S*·*11S*	103	70	68.0	26	25.2	7	6.8
2n + *11L*·*11L*	124	86	69.3	29	23.4	9	7.3
2n + *12S*·*12S*	123	67	54.5	50	40.6	6	4.9
2n + ·*1S*	80	48	60.0	31	38.8	1	1.2
2n + ·*2L*[a]	56	38	67.8	16	28.6	2	3.6
2n + ·*3L*[a]	150	103	68.7	44	29.3	3	2.0
2n + ·*5L*	136	90	66.2	42	30.9	4	2.9
2n + ·*8S*[c]	324	156	48.1	130	40.1	0	0.0
2n + ·*9S*[c]	80	26	32.5	38	47.5	0	0.0
2n + ·*10S*	105	69	65.7	33	31.4	3	2.9

[a] Transmission rate in the backcross progeny.
[b] In the selfed progeny, 2 plants (3.6%) with a pair of extra isochromosomes were obtained.
[c] In the selfed progeny of 2n + ·*8S* and 2n + ·*9S*, about 12 and 20% plants, respectively, had a pair of extra telocentric chromosomes (2n = 26).

of this linkage can be determined from the data on the linkage of molecular markers with morphological markers. Marker *d-11* shows tight linkage with RFLP marker CDO 456 (YU *et al.* 1992; CAUSSE *et al.* 1994) and CDO 456 is on the short arm (our unpublished data). These conclusions will be confirmed from the tests of more morphological markers underway. Available evidence suggests that the orientation of this linkage group should be reversed with *lk-i* at the 0 position and *d-2* at the 180 cM position.

Chromosome 5: Segregation of seven markers (*bc-2, gh-1, st-2, v-10, bgl, spl-7* and *eui*) was studied in their crosses with 2n + ·*5L*. The results were verified for the two genes (*bc-2* and *spl-7*) from their crosses with 2n + *5S*·*5S*. Three are located on the long arm and the remaining four on the short arm. One telotrisomic with a recessive phenotype was observed in the progeny of 2n + ·*5L*/*spl-7*. This must be the result of chromatid segregation, indicating that *spl-7* is distant from centromere. The centromere is situated between *v-10* and *bgl*. The orientation of this linkage group remains unchanged with *gh-1* at the 0 position. Two genes *bc-2* and *eui* have not been mapped as yet.

Chromosome 6: Segregation of four markers (*dp-1, spl-4, v-3* and *chl-4*) was studied in their crosses with 2n + *6S*·*6S*. Only small F_2 populations could be grown because of poor seed fertility of *6S*·*6S*. Three genes, *dp-1, spl-4* and *v-3* are located on the short arm and *chl-4* on the long arm. The centromere is thus located between *v-3* and *chl-4*. The marker *spl-4*, although not yet mapped, is closely linked to *dp-1*. The orientation of the linkage group remains unchanged with *d-4* at the 0 position.

Chromosome 8: Linkage group 8 has only two genes: *sug* and *v-8*. Segregation of these was studied in their crosses with 2n + ·*8S*. The centromere is situated between these two genes with *v-8* on the short arm and *sug* on the long arm. Thus *v-8* is at the 0 position on the map. Segregation data for *sug* does not fit the expected 3:1 ratio ($\chi^2 = 5.85$) as the recovery of *sug sug* genotypes is generally lower than expected in most crosses.

Chromosome 9: Linkage group nine consists of only three markers; closely linked *drp-2* and *dp-2*, and *Dn-1*. Since 2n + ·*9S* plants cannot be differentiated from 2n sibs, the entire F_2 population was evaluated for a fit to 3:1 ratio. The segregation of *dp-2* agrees with 3:1 ratio. However the segregation of *drp-2* deviates strongly from 3:1 ratio. Such deviation was also observed in the F_2 population of F_1 disomic sib heterozygous for *drp-2*. Additionally, several *drp-2* plants from the F_2 population of its cross with 2n + ·*9S* were examined cytologically and almost 50% were 2n + ·*9S*. These data place *drp-2* and *dp-2* conclusively on *9L*. Since the arm location of *Dn-1* has not been determined, the orientation of this linkage group and the position of centromere remains unknown. The dominant marker *Dn-1*, in the absence of dosage effect, cannot be located on the specific arm through telotrisomic analysis.

Chromosome 10: Linkage group 10 has only four genes. Another gene, *ygl*, has been assigned to this linkage group but has not yet been mapped. The segrega-

TABLE 5

F_2 segregation in the crosses of secondary and telotrisomics of rice with marker genes

Cross	Total plants (No.)	Disomics			Trisomics[a]			Arm location of the gene
		Normal (No.)	Recessive (No.)	χ^2 (3:1)	Normal (No.)	Recessive (No.)	Recessive (%)	
2n + · *1S/d-18*	549	325	122	1.25	102	0	0.0	*1S*
2n + · *1S/chl-6*	461	262	105	2.55	62	32	34.0	*1L*
2n + · *1S/spl-6*	173	91	29	0.04	42	11	20.7	*1L*
2n + · *1S/z-8*	340	186	56	0.45	69	29	29.6	*1L*
2n + · *1S/gf-2*	203	116	54	4.12	24	9	25.7	*1L*
2n + *2S* · *2S/spl-2*	94	62	18	0.29	14	0	0.0	*2S*
2n + *2S* · *2S/gh-2*	231	108	98	0.49[b]	25	0	0.0	*2S*
2n + *2S* · *2S/z-12*	76	31	33	0.06[b]	12	0	0.0	*2S*
2n + *2S* · *2S/chl-10*	172	112	32	0.59	23	5	17.9	*2L*
2n + *2S* · *2S/tri*	38	24	6	0.40	6	2	25.0	*2L*
2n + · *2L/bc-3*	110	57	15	0.66	38	0	0.0	*2L*
2n + · *3L/spl-3*	210	119	32	1.16	47	12	20.3	*3S*
2n + · *3L/dl*	89	39	28	1.80[b]	16	6	27.3	*3S*
2n + · *3L/chl-2*	82	49	13	0.54	16	4	20.0	*3S*
2n + · *3L/bc-1*	280	157	42	1.61	63	18	22.2	*3S*
2n + · *3L/v-1*	110	68	18	0.66	24	0	0.0	*3L*
2n + · *3L/chl-1*	141	71	21	0.23	49	0	0.0	*3L*
2n + *4S* · *4S/pl-2*	185	78	23	0.27	67	17	20.2	*4L*
2n + *4S* · *4S/st-3*[c]	388	305	83	2.69	—	—	—	*4L*
2n + *4S* · *4S/lg*[c]	388	302	86	1.66	—	—	—	*4L*
2n + *5S* · *5S/bc-2*	165	84	24	0.44	57	0	0.0	*5S*
2n + · *5L/bc-2*	328	167	43	1.66	88	30	25.4	*5S*
2n + · *5L/gh-1*	331	184	46	3.07	70	31	30.7	*5S*
2n + · *5L/st-2*	140	73	21	0.35	37	9	19.6	*5S*
2n + · *5L/v-10*	72	37	12	0.01	18	5	21.7	*5S*
2n + *5S* · *5S/spl-7*	240	132	36	1.14	55	17	23.6	*5L*
2n + · *5L/spl-7*	514	264	68	3.61	181	1	0.5	*5L*
2n + · *5L/eui*	193	98	34	0.04	61	0	0.0	*5L*
2n + · *5L/bgl*	164	102	26	1.51	36	0	0.0	*5L*
2n + *6S* · *6S/dp-1*	48	32	8	0.53	8	0	0.0	*6S*
2n + *6S* · *6S/spl-4*	48	31	9	0.10	8	0	0.0	*6S*
2n + *6S* · *6S/v-3*	40	28	5	1.71	7	0	0.0	*6S*
2n + *6S* · *6S/chl-4*	62	39	10	0.55	11	2	15.4	*6L*
2n + · *8S/v-8*[c]	677	265	87	0.02	325	0	0.0	*8S*
2n + · *8S/sug*[c]	351	153	32	5.85	142	24	16.9	*8L*
2n + · *9S/dp-2*[d]	246	181	65	0.27	—	—	—	*9L*
2n + · *9S/drp-2*[d]	524	464	60	51.31	—	—	—	*9L*
2n + · *10S/pgl*	297	160	45	1.02	68	24	26.1	*10L*
2n + · *10S/fgl*	296	161	50	0.19	65	20	23.5	*10L*
2n + · *10S/ygl*	254	142	42	0.46	53	17	24.3	*10L*
2n + *11S* · *11S/z-1*	98	60	17	0.35	21	0	0.0	*11S*
2n + *11L* · *11L/z-1*	91	51	16	0.04	19	5	20.8	*11S*
2n + *11S* · *11S/v-4*	214	100	37	0.25	62	15	19.5	*11L*
2n + *11L* · *11L/v-4*	207	111	28	1.75	68	0	0.0	*11L*
2n + *11S* · *11S/la*	214	100	37	0.29	59	18	23.4	*11L*
2n + *11L* · *11L/la*	207	109	30	0.87	68	0	0.0	*11L*
2n + *11S* · *11S/z-2*	200	119	29	2.31	42	10	19.4	*11L*
2n + *11L* · *11L/z-2*	245	147	39	1.61	59	0	0.0	*11L*
2n + *12S* · *12S/spl-1*	336	158	47	0.47	131	0	0.0	*12S*

[a] A small portion of primary trisomics observed in each F_2 or BC population is not included here.

[b] χ^2 for 1:1.

[c] A high frequency of plants with a pair of extra telocentric chromosomes (ditelotetrasomics) was present and are pooled with telotrisomics.

[d] Since the telotrisomic 2n + · 9S cannot be identified morphologically, the disomics and trisomics are pooled together.

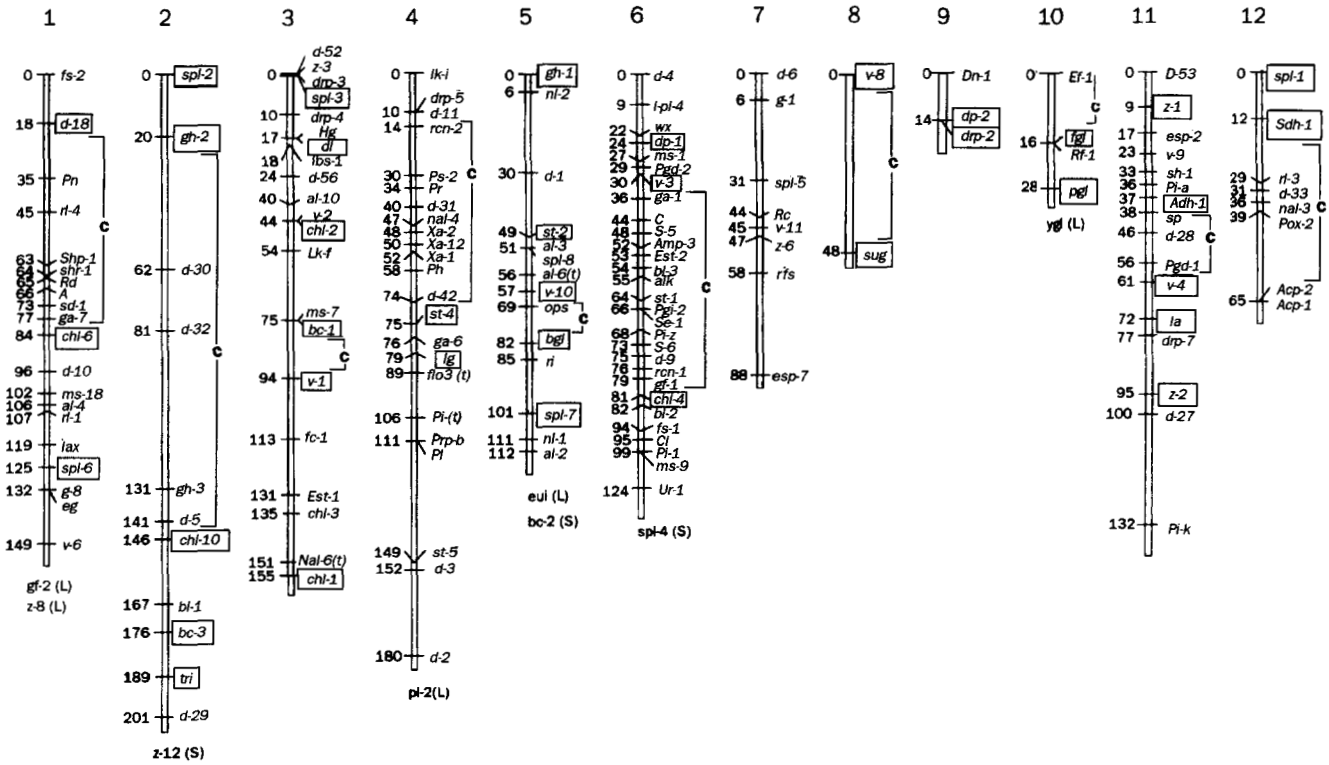


FIGURE 17.—Revised chromosome map of rice showing correct orientation of different linkage groups. Markers in the box are the ones used in this study. Markers below the map are located on respective chromosomes but are not yet mapped; S and L in parentheses indicate their location on short and long arms, respectively. The centromere regions are indicated by a vertical line on the right side of each linkage group.

tion of *ygl, pgl,* and *fgl* were studied in their crosses with 2n + · *10*S. All three genes are located on *10*L. The position of the centromere and the orientation of the linkage group cannot be determined from these data. However, *pgl* has been mapped between *Npb291* and *Npb127* (IDETA *et al.* 1993). Both of these markers are located toward the terminal end of RFLP linkage group 10 (KURATA *et al.* 1994). Our unpublished data show that the two RFLP markers are on the long arm of chromosome *10.* The orientation of this linkage group should thus be reversed with *Ef-1* at position 0 and *pgl* at the 28 cM position.

Chromosome 11: The segregation of four markers (*z-1, v-4, la* and *z-2*) was studied in their crosses with 2n + *11*S · *11*S as well as 2n + *11*L · *11*L. The data show that *z-1* is located on *11*S and the other three genes are located on *11*L. In addition isozyme marker *Adh-1* has been located on *11*S through studying dosage effect of this isozyme in the F_1 progenies of rice variety MaHae and 2n + *11*S · *11*S, which is in the IR36 background. MaHae and IR36 are polymorphic for *Adh-1*; 2n + *11*S · *11*S plants heterozygous for *Adh-1* had 3 dosages of IR36 allele and showed a darker band as compared to disomic sibs, which have one allele of each parent. These data show that the centromere is located between *Adh-1* and *v-4,* and the orientation of the linkage map needs to be reversed with *D53* at the 0 position and *Pi-k* at 132 cM.

Chromosome 12: The secondary trisomic 2n + *12*S · *12*S was isolated only recently and segregation of *spl-1* in the cross of this trisomic was studied. This marker is located on short arm. The isozyme marker *Sdh-1* has also been located on *12*S through gene dosage analysis. Since *spl-1* and *Sdh-1* are at the end of the linkage map and on the short arm, the orientation of the linkage group is reversed with *spl-1* at the 0 position. *Acp-1* and *Acp-2* are linked with RG181 (CAUSSE *et al.* 1994), and our unpublished data show that RG181 is on the long arm. Thus the centromere is located between *Sdh-1* and *Acp-2.*

Orientation of the chromosome map: In this study we examined the segregation of 43 genes belonging to 11 linkage groups in their crosses with secondary or telotrisomics of respective chromosomes. The arm locations of these genes were unequivocally determined from the segregation data. These data also helped determine the centromere positions on eight linkage groups and the orientation of 10 linkage groups. The orientation of eight linkage groups (1, 2, 3, 4, 8, 10, 11, and 12) have been changed. Only the orientations of the linkage group 9 could not be determined because of the paucity of mapped genes. Linkage group 7 has not been studied as yet because 2n + 7L · 7L is completely sterile and 2n + 7S · 7S was isolated only recently. However, crosses between markers of this group and 2n + 7S · 7S have been made and data should be

soon available. The latest chromosome map reflecting the correct orientation of 10 linkage groups, and the positions of centromeres is shown in Figure 17.

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