Development and Applications of a Complete Set of Rice Telotrisomics

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ABSTRACT

We previously isolated a complete set of primary trisomics along with many other aneuploids from triploid plants derived from an *indica* rice variety "Zhongxian 3037." About 30,000 progeny from these trisomic and aneuploid plants were grown each year from 1994 to 1999. The variants that differed morphologically from both the diploids and the original primary trisomics were collected for cytological identification. From these variants, a complete set of telotrisomics covering all 24 rice chromosome arms was obtained. The identities of the extra chromosomes were further confirmed by dosage analysis of the RFLP markers on extra chromosome arms. The telocentric nature of the extra chromosomes in these stocks was verified by fluorescence *in situ* hybridization (FISH) using a rice centromeric BAC clone as a marker probe. In general, the shorter the extra chromosome arm of a telotrisomic, the stronger the resemblance it bears to the diploid; the longer the extra chromosome arm, the stronger the resemblance to the corresponding primary trisomic. We demonstrated that DNA clones can be rapidly assigned to specific chromosome arms by dosage analysis with the telotrisomics. We also showed that telotrisomics are valuable tools for chromosome microdissection and for developing chromosome-specific DNA markers.

RICE is the staple food of more than half of the world's population. As a self-pollinating diploid species, rice has a relatively small genome, $\sim 4.3 \times 10^8$ bp (Arumuganathan and Earle 1991), and can be easily transformed and regenerated, which makes it a model monocot plant for molecular biology research. However, rice chromosomes are small and it is difficult to consistently recognize individual chromosomes and their variants in somatic cells. Today, both rice physical mapping and molecular genomics require an efficient method for chromosome identification.

Utilization of telotrisomics is a classical method for chromosome identification in plants. As telotrisomics contain an extra telocentric chromosome in each cell, it is often easy to distinguish the extra chromosome from the rest of the chromosome complement. Since Rhoades (1936) discovered the first telotrisomic in *Zea mays*, telotrisomic stocks have been developed in a number of species including *Datura stramonium* (Blakeslee and Avery 1938), *Nicotiana sylvestris* (Goodspeed and Avery 1939), *Triticum monococcum* (Moseman and Smith 1954), *Hordeum vulgare* (Tsuchiya 1960), *Secale cereale* (Kamanoi and Jenkins 1962), *Lycopersicon esculentum* (Khush and Rick 1967), and *Oryza sativa* (Singh *et al.* 1996a,b). However, a complete set of telotrisomics covering the arms of the entire chromosome complement has not been reported in any plant species.

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Before producing telotrisomics for all 24 rice chromosome arms, we first developed a complete set of primary trisomics of Zhongxian 3037, an *indica* rice variety derived from a cross between IR24 and BG90-2 (Cheng *et al.* 1996). Telotrisomics covering all 24 arms of the 12 rice chromosomes have been established from the progenies of these primary trisomics and other aneuploids, all derived from a triploid of Zhongxian 3037. The telocentric nature of the extra chromosomes in the telotrisomics was confirmed by fluorescence *in situ* hybridization (FISH) using a rice centromere-specific bacterial artificial chromosome (BAC) clone, 17p22, as a marker probe. The applications of the telotrisomics in marker assignment and microdissection are demonstrated in this article.

MATERIALS AND METHODS

Plant materials: All 12 primary trisomics and other aneuploids were developed from a triploid of Zhongxian 3037. Approximately 180,000 plants derived from the trisomics and other aneuploids, ~30,000 annually, were evaluated in field from 1994 to 1999. Variants morphologically distinct from the original diploid and trisomic sibs were selected for further cytological analysis.

Chromosome preparation and fluorescence *in situ* hybridization: Young meiotic panicles of the rice variants were harvested and fixed in 3:1 Carnoy's solution supplemented with 0.5% $FeCl_3$. Squashes were prepared in acetic-carmine solution according to Wu (1967). Roots of the rice variants were harvested from field-grown plants. The roots were pretreated in 0.002 M 8-hydroxyquinoline at 20° for 2 hr to accumulate prometaphase cells, fixed in methanol-acetic acid (3:1), and stored at

−20° until use. Root tips were macerated in 2.5% cellulose at 37° for 1.5 hr. Squashes were made in the fixative on a glass slide and flame dried. The chromosomes were stained with 2% Giemsa solution for observation.

The procedure for FISH analysis was as described (Jiang *et al.* 1995) with only minor changes. A rice BAC clone, 17p22, which produces very specific hybridization signals to each rice centromere was used as FISH probe (Dong *et al.* 1998). The hybridization mixture (20 μl for each slide) contained 20 ng of labeled probe DNA, 50% formamide, 10% dextran sulfate, 2× SSC, and 20 μg of sheared salmon sperm DNA. After overnight incubation at 37°, FISH signals were detected by a FITC-conjugated anti-biotin antibody (Vector Laboratories, Burlingame, CA). Chromosomes were counterstained with propidium iodide. Images were captured with a SenSys CCD (charge coupled device) camera (Photometrics, Tucson, AZ) coupled to a Macintosh computer. Gray-scale images were captured individually and merged using IPLab Spectrum v3.1 software.

Southern analysis for dosage effects of restriction fragment length polymorphism (RFLP) markers: Genomic DNA isolation and gel blot hybridization were according to McCouch *et al.* (1988). The amount of DNA in each lane was adjusted to equal amounts using a specific control. All the DNA from the different aneuploids was digested with *Dra*I. Serial volumes of digested DNA were run on a 0.8% agarose gel and stained with ethidium bromide. The gel images were captured with a digital camera and analyzed with molecular analysis software. Consequently, nearly equal aliquots of DNA from the two telotrisomics, one for the extra long arm and the other for the short arm of the same chromosome, could be run on the same agarose gel and transferred to a Hybond-N^+ membrane (Amersham, Buckinghamshire, UK) for Southern analysis. RFLP probes were labeled with ^{32}P by random hexamer priming and hybridized to the above membranes overnight at 65°. The membranes were washed sequentially in 2×, 1×, and 0.5× SSC with 0.1% SDS, 20 min each at 65°, and then exposed on X-ray film with intensifying screens at −70° for 3–7 days. RFLP markers designated as RG#, RZ#, and CDO# were kindly provided by Dr. S. D. Tanksley from Cornell University (Ithaca, NY) and clones of G#, L#, and C# were obtained from the MAFF DNA Bank at NLAR, Japan.

Chromosome microdissection and amplification: To microdissect the extra chromosome arms from the aneuploids, prometaphase chromosomes were prepared as follows. Briefly, roots of the aneuploids were fixed in methanol-acetic acid (5:1) for 10 min and stored in 70% ethanol until use. Squashes were made in the fixative on a coverslip. Microdissection was performed on a Nikon inverted microscope with a Leitz micromanipulator. Telochromosomes were dissected through individual microneedles with a tip of 0.5–1 μm. The microneedle with a telocentric chromosome was inserted into a 0.5-ml Eppendorf tube and the tip was broken off in the 20-μl aliquot of 1× T4 ligase buffer containing 5 ng/μl proteinase K. Ten telochromosomes were collected in a single tube reaction. The microdissected chromosomal DNA was digested with *Sau* 3A, ligated to *Sau* 3A linker adaptors, and amplified by PCR according to the procedures of Chen and Armstrong (1995). The positive control sample containing 10 pg of Zhongxian 3037 genomic DNA and the negative control sample containing no DNA were also amplified using the same protocol. The amplified DNAs were separated in a 1.4% agarose gel and stained with ethidium bromide.

Sequence-tagged site (STS) and microsatellite analyses were conducted to confirm that the PCR products of microdissected chromosomal DNA truly came from the microdissected chromosome arms. The STS primer pairs and microsatellite primer pairs were synthesized according to the sequences reported by Inoue *et al.* (1994) and Chen *et al.* (1997), respectively. Primer pairs for STS and microsatellites on different chromosome arms were selected to amplify the PCR products from both microdissected chromosomes and control samples.

Designation of the trisomics: The trisomic nomenclature used for tomato (Khush 1973) was adopted for this article. For example, a telotrisomic for the short arm of chromosome 1 is designated as 2n+·1S and that for the long arm as 2n+·1L.

RESULTS AND DISCUSSION

Identification of the telotrisomics: Since the extra chromosome in the trisomics, *i.e.*, primary, secondary, or tertiary trisomics, could form a univalent in many of the sporocytes, misdivision of the univalent occurs at a certain frequency. Therefore, it is possible to isolate telotrisomics from the progenies of these trisomics, especially from primary trisomics.

To obtain all possible rice telotrisomics, the progenies of different trisomic types were planted in large populations, up to 30,000 plants each year. The plants showing morphological features different from both of the original trisomics and the normal diploid were collected for further cytological examination. As for the three subtelocentric chromosomes with very short and/or darkly stained heterochromatic short arms, *i.e.*, chromosomes 4, 9, and 10, it is expected that the telotrisomics with these arms may resemble the diploids but differ from the respective primary trisomics, while the trisomics with the long arms of these three chromosomes should resemble the primary trisomics but differ from the diploid. Therefore, all the plants from the progenies of primary trisomics 4, 9, and 10, resembling either the diploid or the primary trisomics, were maintained for further cytological identification.

The somatic chromosomes at prometaphase of all the variant candidates were analyzed to confirm whether or not the variants were telotrisomics. The progenies of the confirmed telotrisomics were planted in the following year. Because a telotrisomic might produce the reverted primary trisomic in its progeny, it was convenient to nominate the involved chromosome according to the morphological traits of the primary trisomics. Using this procedure, we obtained a series of different telotrisomics from whose progenies a complete set of reverted primary trisomics was also recovered.

Morphological evidence showed that the selected telotrisomics cover all 24 arms of the rice genome. To further identify the extra chromosome arms, cytological investigations were conducted on the selected telotrisomic candidates. As the centromere positions in rice molecular linkage maps have been determined by Singh *et al.* (1996a,b) and Harushima *et al.* (1998), we used the dosage effects of the molecular markers in different chromosome arms to distinguish the extra chromosomes of the telotrisomics as belonging to the short arm or long arm. For example, when a ^{32}P-labeled marker from the short arm of chromosome 1 is used

TABLE 1

The tested RFLP markers with dosage effects on the corresponding telotrisomics

Chromosome arms	Tested markers	Chromosome arms	Tested markers
1S	C749, C146, RG811	7S	RZ488, RG128, C1057
1L	RG350, RZ276, G370	7L	G20, C213, RG351
2S	G365, RG509, G357	8S	RG20, C400, G278
2L	RG322, G275, G45	8L	G1073, RG1, RG136
3S	C725, RZ891, RG450	9S	RG757, C152, G36
3L	RG558, RG910, RZ328	9L	CDO590, G385, RG662
4S	CDO456, C708, C820	10S	G1125, C701, L169
4L	RG449, G271, RG620	10L	G291, C16, RG561
5S	RG556, G396, RG360	11S	RG304, RG118, G320
5L	RG13, G81, C246	11L	L190, RG303, G257
6S	RZ450, RG213, RZ2	12S	RG574, RZ816, RZ397
6L	RG716, RZ405, CDO218	12L	RG241, RG190, RG181

to probe a membrane with an equal amount of *Dra*I-restricted DNA from the two telotrisomics related to chromosome 1, the telotrisomic with stronger hybridization bands should be 2n+·1S, while the one with weaker bands should be 2n+·1L. Three markers on each arm were tested in this way (Table 1); thus all 24 telotrisomics were identified by dosage analysis. Figure 1 shows the autoradiographs of three such examples in which G275 on 2L, RG350 on 1L, and C749 on 1S were used to identify 2n+·1L and 2n+·1S. In Figure 1A, the bands in all four lanes revealed by G275 are similar in intensity, indicating that the DNA dosages corresponding to this marker in the two different rice genomes are equal. But in Figure 1, B and C, dosage effects between the two telotrisomics are shown. The marker dosage analysis has also confirmed the identities of all 24 telotrisomics developed from a common triploid with the genetic background of an *indica* rice, Zhongxian 3037.

To confirm the location of the centromeres on the extra chromosomes in the isolated telotrisomic lines, a rice centromeric BAC clone, 17p22, was hybridized to the prometaphase chromosomes of each aneuploid. We found that the hybridization signals on the extra chromosomes were all located at one end (Figure 2). Although the intensities of the FISH signals varied greatly over different chromosomes, we consistently found that the signals in the extra chromosomes were always weaker than those in their corresponding homologous chromosomes in the same metaphase cells, suggesting that the telocentric chromosomes were derived from chromosome breaks within their centromeres. In terms of both location and intensity, the centromeric hybridization signals allow us to conclude that the extra chromosomes in the telotrisomics were all derived from centromere misdivisions. These results also suggest that the functional rice centromeres consist of repetitive units; misdivision-derived rice centromeres, as those in maize (Kaszás and Birchler 1996, 1998), are fully functioned.

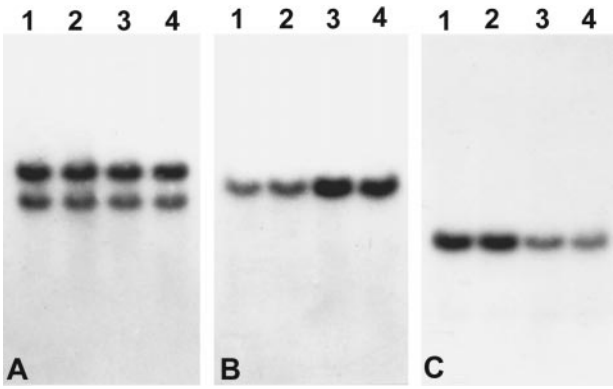

Figure 1.—Dosage analysis of different RFLP markers in the two telotrisomics of chromosome 1; DNA in lanes 1 and 2 from 2n+·1S and DNA in lanes 3 and 4 from 2n+·1L. (A) Probed with G275 that is mapped on 2L; (B) probed with RG350 mapped on 1L; and (C) probed with C749 mapped on 1S.

Morphological and reproductive features of the telotrisomics: Each of the primary trisomics in Zhongxian 3037 has unique morphological features, enabling easy discrimination of all the primary trisomics from one another and from the diploid sib as well (Cheng *et al.* 1996). Most telotrisomics have some characteristics similar to those of their respective primary trisomics. In general, however, telotrisomics with the short arms bear strong resemblance to the diploid while those with long arms bear a stronger resemblance to the corresponding primary trisomic. For the subtelocentric chromosomes 4, 9, and 10, it is difficult to morphologically distinguish the three telotrisomics of their short arms from the diploid, and it is also difficult to detect obvious morphological differences between the telotrisomics of their long arms and their respective primary trisomics. However, for the other nine metacentric or submetacentric chromosomes, all 18 telotrisomics are morphologically distinct from both the diploid and the corresponding

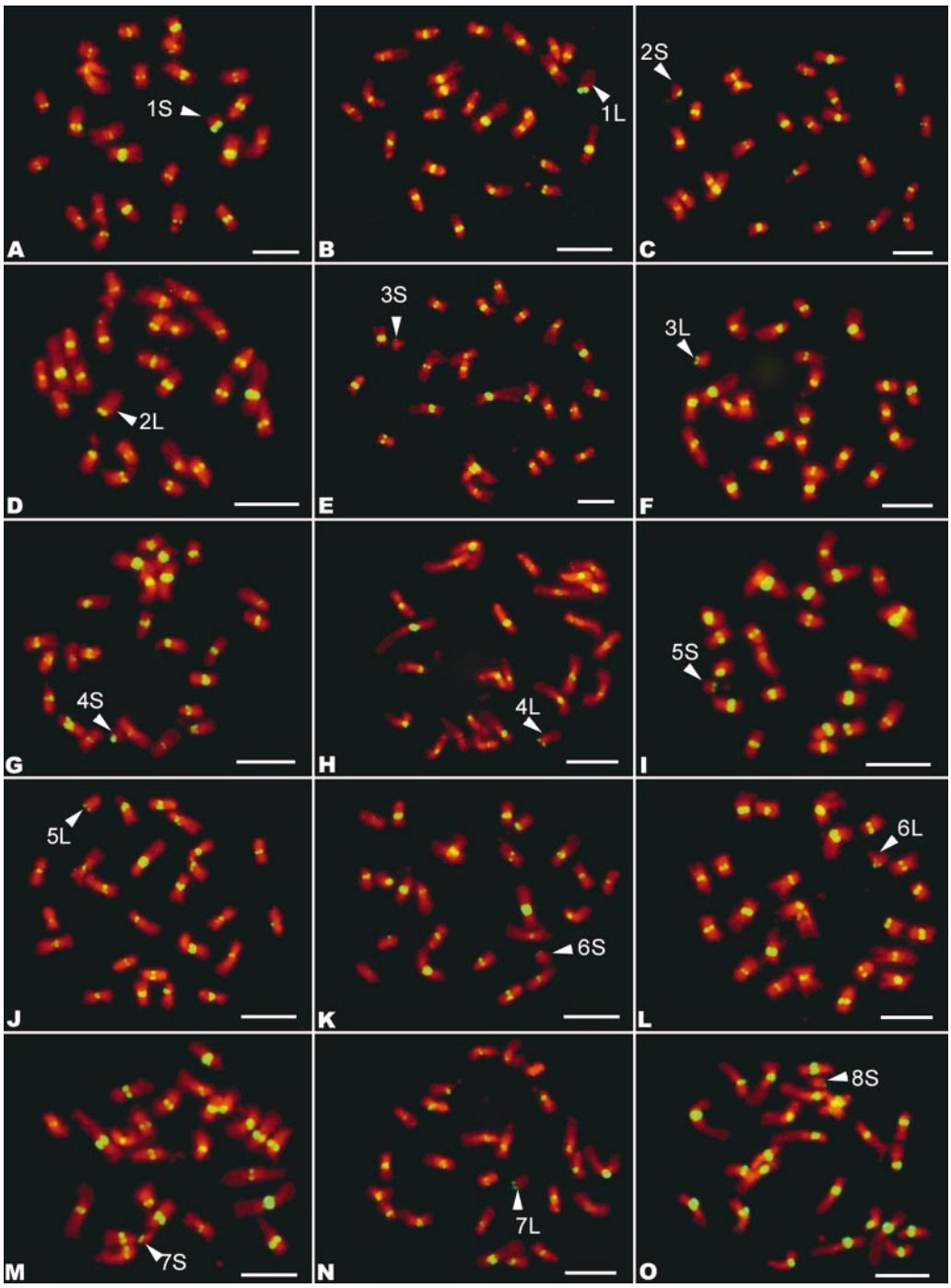

FIGURE 2.—FISH analysis of probe 17p22 on prometaphase chromosomes of different telotrisomics. (A–X) telotrisomics with an extra chromosome arm from 1S to 12L, respectively; arrowheads show the extra telocentric chromosomes. All bars, 5 μm. (Y) 12 individual rice chromosomes and their corresponding chromosome arms presented in the telotrisomics.

primary trisomics to varying degrees and are usually more vigorous than the corresponding primary trisomics. The morphological traits of the 24 telotrisomics and their corresponding primary trisomics are summarized in Table 2. Figure 3 shows the morphological characteristics of the plants and the panicles, respectively, of the diploid sib, 2n+·4S, 2n+·4L, and triplo 4.

The seed-setting behavior of each primary trisomic and telotrisomic was also investigated. In general, telotrisomics have higher seed set than their respective primary trisomics. As shown in Table 2, telotrisomics with short extra chromosome arms displayed relatively higher seed set while those with long extra chromosome arms displayed lower seed set.

SINGH *et al.* (1996a) developed seven telotrisomics from a different rice variety, "IR36," *i.e.*, 2n+·1S, 2n+·2L, 2n+·3L, 2n+·5L, 2n+·8S, 2n+·9S, and 2n+·10S. Most of these are quite similar to the corresponding telotrisomics reported here in terms of both morphology and seed set. However, these two sets of telotrisomics report differences for 2n+·1S and 2n+·10S. For 2n+·1S, the telotrisomic derived from IR36 is earlier in flowering than its counterpart Zhongxian 3037. For 2n+·10S, the telotrisomic derived from

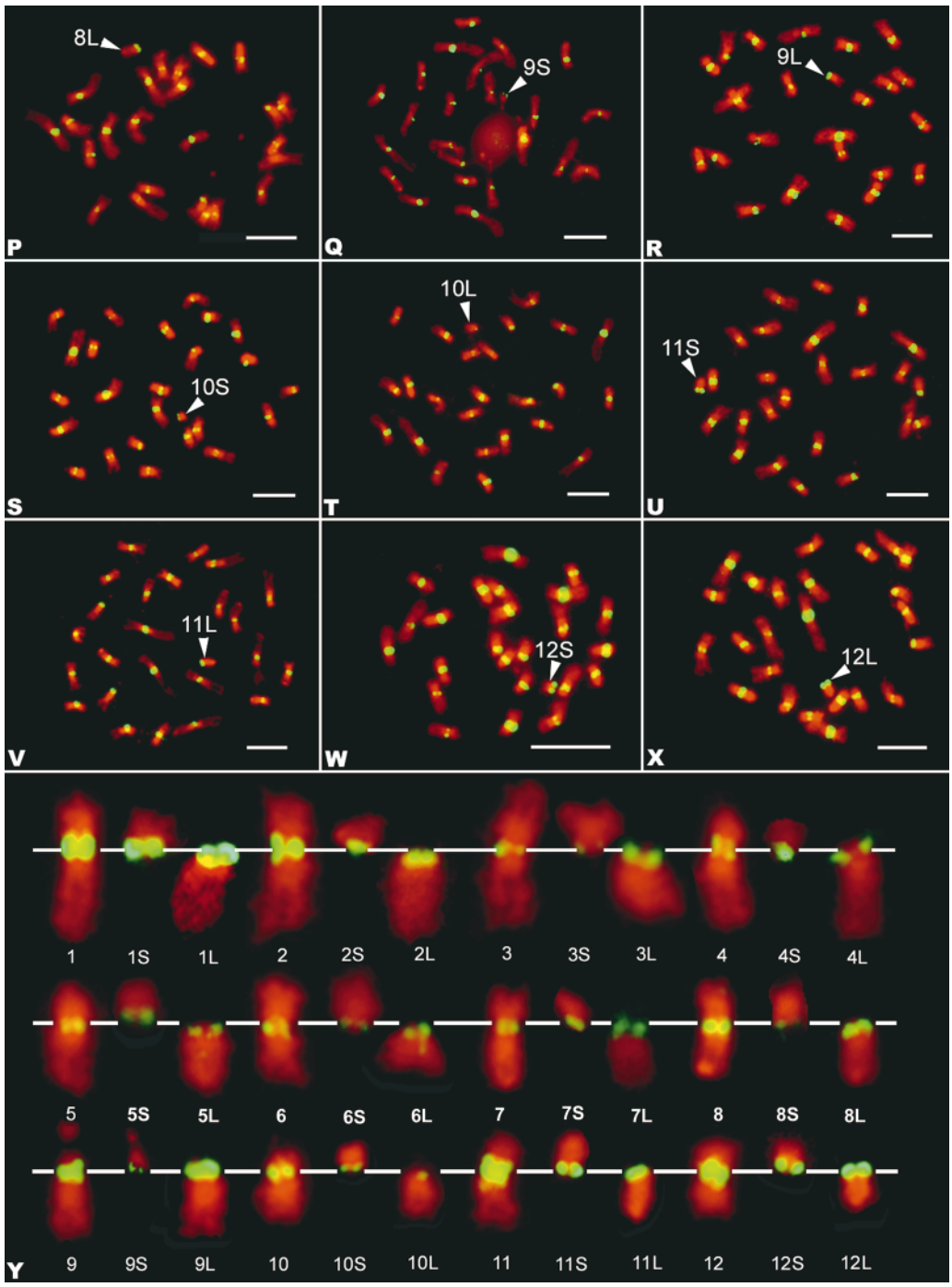

FIGURE 2.—*Continued.*

IR36 is thinner in culm compared to its disomic sib and therefore can be morphologically distinguished. However, the 2n+·10S counterpart derived from Zhongxian 3037 has, rather, normal culm and cannot be distinguished from its disomic sib by this trait. These differences may be attributed to some allelic variations on the respective chromosome arms between the two original varieties (KHUSH *et al.* 1984; CHENG *et al.* 1996).

Using the telotrisomics to assign DNA clones to rice chromosome arms: Linkage analysis is an effective way to localize cloned DNAs to chromosomal linkage maps. However, there are many clones that oftentimes lack polymorphism, have severe distortion segregations in the mapping populations, or include multiple and/or repetitive sequences. Still, their chromosomal mapping is of importance to genomic studies. Here, we present an example of linkage assignment by dosage analysis using a complete set of rice telotrisomics. A multicopy clone, G1073, displayed three bands in a Southern analysis of *Dra*I-digested Zhongxian 3037 genomic DNA. When the membranes, which contain equal amounts of *Dra*I-digested DNAs from the two telotrisomics with the respective extra arms of each chromosome, were probed by G1073, the largest fragment showed a stronger hy-

TABLE 2

Morphological and reproductive features of primary and telotrisomics of the rice variety Zhongxian 3037

Trisomics	Morphological characteristics	Seed setting (%)
Triplo 1	Short, grassy; narrow and pale green leaves; small panicles; top spikelets with curved lemma and palea; late flowering.	17.3
2n+·1S	Slightly longer and narrower leaves, even broader than those of triplo 1; late flowering.	67.6
2n+·1L	Shorter than the diploid, but higher than triplo 1; grassy; pale green leaves; short flag leaves; small grains; top spikelets with curved lemma and palea.	37.7
Triplo 2	Short; dark green, short, and inward-folded leaves; short panicles; long glumes; depressed palea, sometimes with multiple ovaries and multiple stigma.	8.3
2n+·2S	Slightly shorter than the diploid; short and broad leaves; late flowering; normal glume size and normal palea.	32.2
2n+·2L	Tall, vigorous; depressed palea; long glumes.	13.4
Triplo 3	Short, stick stem; dark green, short, and leather-like leaves; late flowering; highly sterile.	1.6
2n+·3S	Slightly shorter than normal diploid, slender stem; dark green and short leaves; thin grains.	41.9
2n+·3L	Stick stem; broad and dark green leaves; big grains, degenerated florets at the tip of panicles.	52.8
Triplo 4	Tall, with only a few tillers; pale green leaves; long ligules; slender grains, the top spikelets always with long awns.	43.3
2n+·4S	Normal, indistinguishable from the diploid sib in morphology.	88.7
2n+·4L	Resemblance to triplo 4, indistinguishable from triplo 4 in morphology.	48.6
Triplo 5	Narrow, pale green, outward-folded, and twisted leaves; short ligules; short flag leaves; short panicles and grains.	20.2
2n+·5S	Normal, slightly later in flowering than diploid sibs.	78.6
2n+·5L	Slender, pale green, and outward-folded leaves; a little earlier flowering; short grains, with short awns at top spikelets.	66.2
Triplo 6	Short; dark green, short, and inward-folded leaves; long ligule; awned panicle; early flowering; short grains.	30.3
2n+·6S	Narrow, inward-folded, dark green leaves; long awns.	66.4
2n+·6L	Slightly shorter than diploid sibs; dark green, short, and broad leaves; short awns.	70.3
Triplo 7	Narrow, dark green, and inward-folded leaves; slender grains, top spikelets with awns.	38.1
2n+·7S	Morphologically normal, with a little shorter leaves and grains than diploid sibs.	76.5
2n+·7L	Like triplo 7, but with higher seed setting.	48.5
Triplo 8	Very narrow, dark green, and rolled leaves; short grains.	29.4
2n+·8S	Resemblance to triplo 8; even broader leaves than those of triplo 8; normal seed fertility.	78.8
2n+·8L	Vigorous; a few tillers; degenerated florets at the tip of panicles.	72.6
Triplo 9	Vigorous; dark green, inward-folded, and thick leaves; long panicles, big grains.	70.0
2n+·9S	Normal, indistinguishable from the diploid sib in morphology.	83.3
2n+·9L	Like triplo 9, indistinguishable from triplo 9 in morphology.	73.4
Triplo 10	Short, thin stem; many tillers; short and slender grains.	55.6
2n+·10S	Normal, indistinguishable from the diploid sib in morphology.	87.6
2n+·10L	Like triplo 10, indistinguishable from triplo 10 in morphology.	63.4
Triplo 11	Normal plant stature, with a bit lower spikelet fertility; gold grains at flowering stage.	57.6
2n+·11S	Normal plant stature; top spikelets with curved lemma and palea.	68.7
2n+·11L	Only a few tillers; long flag leaves, gold grains at flowering stage.	56.4
Triplo 12	Many tillers; pale green leaves; late flowering; degenerated florets at the tip of panicles.	53.9
2n+·12S	Long, inward-folded leaves, with a few degenerated florets at the tip of panicles.	65.3
2n+·12L	Pale green leaves; late flowering, with a few degenerated florets at the tip of panicles.	64.1

bridization signal in 2n+·8L than that in 2n+·8S, and the two smaller fragments showed stronger signals in 2n+·1L than those in 2n+·1S (Figure 4, A and B), while no differences in signal intensity were detected among others. Thus, the largest fragment is located on chromosome arm 8L, and both smaller fragments are on 1L. Using the same approach we assigned RG684, a clone showing severe distorted segregation, to chromosome arm 10L (Figure 4C).

Microdissection and amplification of the two arms of chromosome 5: The techniques of chromosome microdissection and microcloning represent the combination of traditional cytology with modern molecular biology. The microdissection procedure, first performed on Drosophila polytene chromosomes by Scalenghe *et al.* (1981), has been applied to many plant species such as barley (Schondelmaier *et al.* 1993), wheat (Vega *et al.* 1994), rye (Houben *et al.* 1996), oat (Chen and

Figure 3.—Morphology of different trisomic sibs of chromosome 4. (A) Plants: from left to right, diploid, 2n+·4S, 2n+·4L, and 2n+4S·4L, respectively. (B) Panicles.

Armstrong 1995), maize (Ponelies *et al.* 1997), bean (Pich *et al.* 1994), and *Crepis capillaris* (Jamilena *et al.* 1995). However, this technique can hardly be applied to rice due to its small chromosomes and lack of characteristics amenable to chromosome identification. The rice telotrisomics are ideal for identification of individual chromosome arms, which are distinguishable from the normal rice chromosomes. In the present study, two aneuploids, 2n+·5S+·5S, derived from 2n+·5S, and 2n+·5L were used for microdissection of the extra arms of rice chromosome 5. The two extra chromosome arms of 2n+·5S+·5S, which could be easily identified under an inverted microscope at ×400 magnification, were successfully microdissected from the prometaphase cells with a microneedle controlled by a Leitz micromanipulator. Figure 5, A and B, shows the same prometaphase cell before and after microdissection. Using the same procedure, the extra chromosome arms 5L were also microdissected from the prometaphase cells in 2n+·5L. After two rounds of PCR amplification a smear of DNA fragments could be clearly seen in the two dissected chromosomal DNA samples and in the positive control

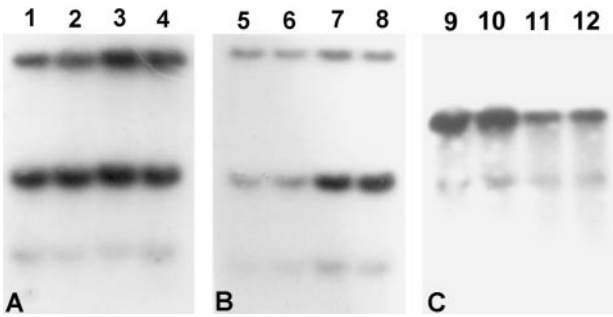

Figure 4.—Assignment of DNA clones to rice chromosome arms by dosage analysis. DNA in lanes 1 and 2 from 2n+·8S, DNA in lanes 3 and 4 from 2n+·8L, DNA in lanes 5 and 6 from 2n+·1S, DNA in lanes 7 and 8 from 2n+·1L, DNA in lanes 9 and 10 from 2n+·10L, DNA in lanes 11 and 12 from 2n+·10S. (A) and (B) probed with G1073, and (C) probed with RG684.

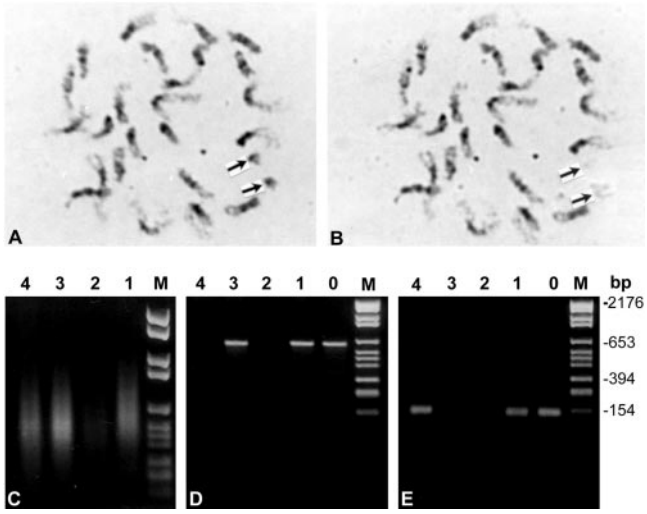

Figure 5.—Microdissection and amplification of the two separate chromosome arms of chromosome 5. (A) Prometaphase cell of 2n+·5S+·5S before chromosome microdissection; arrows show the two extra telochromosomes. (B) Prometaphase cell of 2n+·5S+·5S after chromosome microdissection; arrows point to the areas remaining after dissection. (C) Amplified DNA after the second round of PCR run in agarose gel: 1, positive control sample; 2, negative control sample; 3, microdissected chromosomal DNA from 5S; 4, microdissected chromosomal DNA from 5L. (D) DNA amplified by the STS primer of the molecular marker G396; 0, the genomic DNA of Zhongxian 3037; 1–4, same as in C. (E) DNA amplified by the microsatellite primer RM233 on 5L: 0–4, same as in D.

(Figure 5C). The negative control stained very lightly, which may be attributed to contamination. To verify whether or not the amplified DNAs came from the dissected chromosome arms, the molecular markers on 5S, 5L, and other chromosomes were selected to test the DNA products of the second round of PCR. For example, the STS marker RG396 on 5S and the microsatellite marker RM233 on 5L could be detected only in the amplified DNAs from the microdissected chromosome arm 5S and 5L, respectively (Figure 5, D and E). As expected, all the tested markers on the other chromosomes, *e.g.*, RM23, RM26, RM29, RM44, RM48, RM49, RM53, RM80, RM84, RM205, and RM214, could not be detected in both of the DNA samples from the microdissected 5S and 5L. Thus we conclude that the amplified DNAs from the microdissected chromosome arms came from the extra chromosome arms, 5S and 5L. The amplified DNAs can be used to isolate the chromosome 5-specific DNA sequences for further studies. Because a complete set of rice telotrisomics are available now, all 24 chromosome arms in rice can be microdissected and amplified in this manner.

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