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Genetics

Comparative linkage maps of the rice and maize genomes

(genome evolution/chromosomes/synteny/gramineae)

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ABSTRACT **Genetic linkage maps have been constructed for the rice and maize genomes on the basis of orthologous loci detected with a common set of cDNA clones. Conserved linkage groups could be identified, which together account for more than two-thirds of both genomes. In some instances, entire chromosomes or chromosome arms are nearly identical with respect to gene order and gene content. The results also reveal that most of the genes (>72%) duplicated during ancient polyploidization are still present in the maize genome in duplicate copy. The comparative maps of rice and maize provide a basis for interpreting molecular, genetic, and breeding information between these two important species and establish a framework for ultimately connecting the genetics of all grass species.**

The plant family Gramineae contains some 10,000 species, including many of agronomic importance (e.g., maize, rice, wheat, oats, and sugarcane). The haploid DNA content of these species varies greatly, from 0.45 pg in rice to 11.7 pg in oats, and they have different basic chromosome numbers (1). Rice (*Oryza sativa* L.) and maize (*Zea mays* L.) are two of the best characterized species in the family Gramineae, and together they account for the largest output of any agricultural commodity with an estimated annual worldwide value of U.S. $150 billion (2). Rice is a diploid (2n = 24), self-pollinating species. Maize, on the other hand, is an outcrossing and presumably ancient polyploid (2n = 20) (3, 4). Both species have long, but independent, traditions of genetics research.

The advent of technologies for mapping genomes directly at the DNA level has opened the door to determining the relative order of homologous sequences along the chromosomes of distantly related species with a level of detail and accuracy previously unattainable. For example, comparative genetic maps, based on common restriction fragment length polymorphism (RFLP), have revealed that the genomes of tomato and potato are nearly identical in overall gene content and gene order (5, 6). Similarly, conserved linkages have been reported between maize and sorghum (7, 8) and among humans, mice, and cattle (9, 10). Comparative linkage maps not only allow additional insights into chromosome evolution but provide a basis for interpreting genetic information between divergent species. Mice are now a popular model system for studying both single-gene and quantitatively inherited genetic disorders that affect humans, in part because of the availability of comparative maps for these two mammalian species (11).

MATERIALS AND METHODS

An RFLP linkage map of the rice genome, based on both cDNA and genomic clones, has been reported (12). Additional cDNA clones isolated from oats and barley (13) were added to this rice map, which is displayed in Fig. 1. cDNA clones corresponding to single loci in the rice map were screened for hybridization to DNA from maize inbreds T232 and CM37 as well as from other grass species (wheat, barley, sugarcane, and oats). The two maize inbreds are the parents of a recombinant inbred population previously used to construct an RFLP map of maize (14, 15). Eighty-five percent of the cDNA clones tested showed hybridization to maize DNA at moderate-stringency conditions (1× standard saline/citrate, 65°C). By using cDNA probes that correspond to single genes in rice, it was possible to construct a genetic linkage map of maize based on orthologous loci from rice (Fig. 2).

RESULTS AND DISCUSSION

Rice/Maize Comparative Maps. A total of 250 loci were assigned to the 10 maize linkage groups and oriented with respect to the known maize chromosomes by using a previously established data base for the recombinant inbred population (14). A comparison of the two maps reveals many conserved linkage groups between rice and maize. For example, the short arm of maize chromosome 9 is comprised of six contiguous loci [≈27 centimorgans (cM)] from the short arm of rice chromosome 6, and the long arm of the same maize chromosome is comprised of four more contiguous loci (8 cM) from the same rice chromosome, as well as six contiguous loci (60 cM) from chromosome 3 (Fig. 2). Maize chromosome 8 is composed of three conserved linkage blocks from rice chromosomes 1 and 5 (combined total of 120 cM). A total of 32 conserved linkage segments, comprised of two or more loci, could be identified between the rice and maize genomes, ranging in length from 5 cM to 85 cM (Figs. 1 and 2). All together these conserved linkages account for 62% and 70% of the maize and rice genetic maps, respectively.

Gene Duplication and Chromosome Evolution in Maize. It is interesting to note that single-copy loci in rice are almost always duplicated in maize. Duplicate chromosome segments are the predicted outcome of polyploidization, and this result is consistent with earlier findings of duplicate loci in maize based on both isozymes and RFLPs (4, 8, 18). What was not clear from previous studies is (*i*) the extent to which genes duplicated after polyploidization have been retained in the maize genome and (*ii*) the extent to which maize chromosomes were rearranged after polyploidization. Having a comparative map from a related diploid species (rice) makes it possible to deduce the answers to both of these questions.

The clones mapped in both rice and maize were selected to be single-copy in rice. Polyploidization in ancestral maize should have duplicated all of these loci, and, thus, one would predict that all single-copy loci from rice would have been present in two copies in ancestral polyploid maize. On the basis of analysis of 151 cDNA clones determined to be single

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Abbreviations: RFLP, restriction fragment length polymorphism, cM, centimorgan(s); lod, logarithm of odds.

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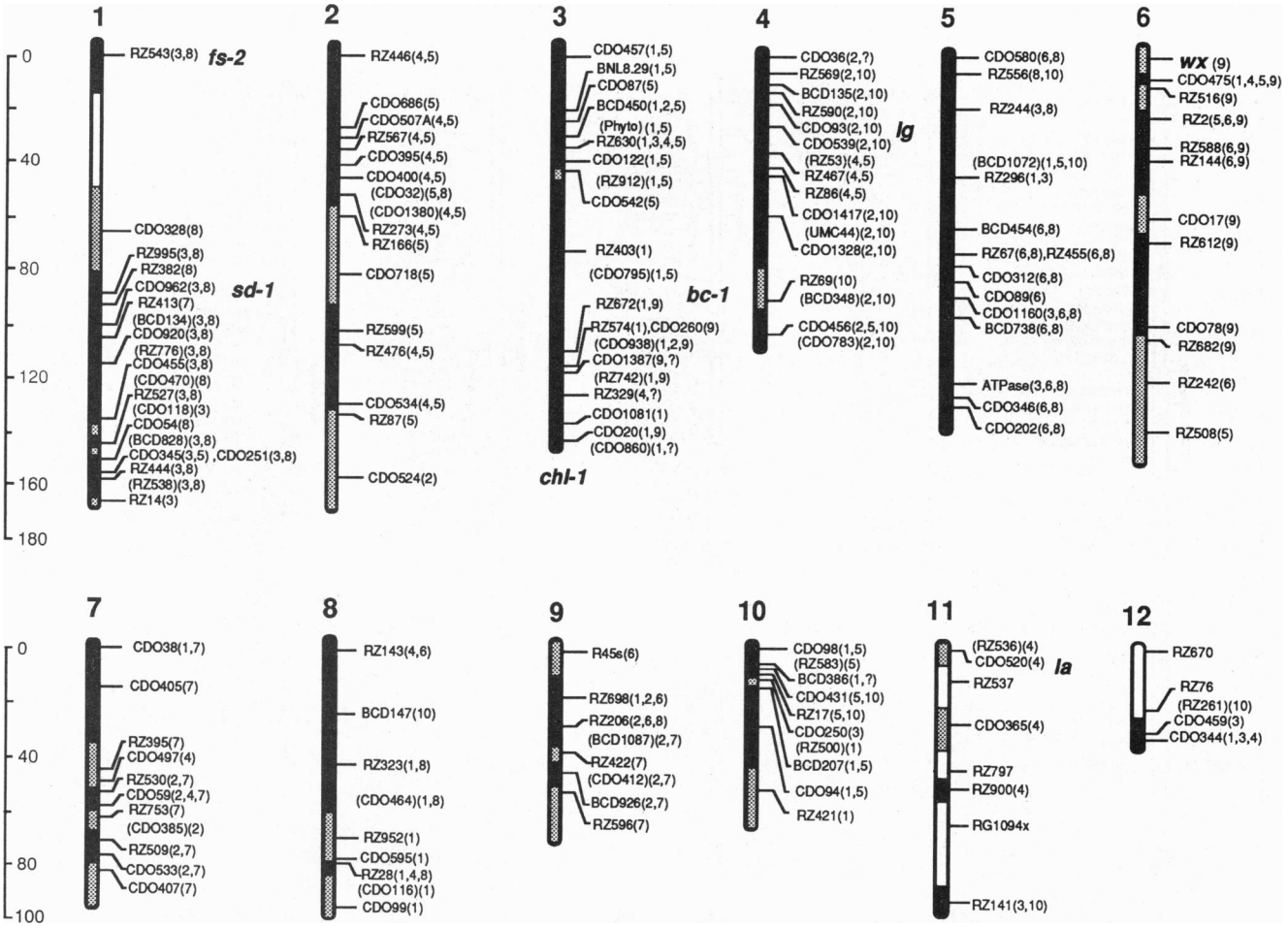

FIG. 1. RFLP linkage map of rice derived from a backcross of 113 plants (*O. sativa* cv. BS125 × *Oryza longistaminata*). Scale in Haldane cM is shown on left. Locus names (corresponding to cDNA probes) are listed to the right of the chromosomes. RZ, rice leaf cDNA; CDO, oat leaf cDNA; BCD, barley leaf cDNA. Loci by tick marks ordered with logarithm of odds (lod) >2, using MAPMAKER software (25). Loci in parentheses have been located to intervals with lod <2. Loci separated by commas cosegregate. Two maize genomic clones, BNL8.29 and UMC44, are located on rice chromosomes 3 and 4, respectively. Chromosomal location(s) of homologous locus/loci in maize is given in parentheses after locus name. Approximate map position of selected morphological markers (boldface) is shown to right of chromosomes (*fs-2*, fine stripe; *sd-1*, semi-dwarf; *bc-1*, brittle culm 1; *chl-1*, chlorina 1; *lg*, liguleless; *wx*, waxy; *la*, lazy growth habit). Dark chromosomal regions contain loci that are single copy in rice but duplicated in maize. Lightly stippled chromosomal regions contain loci that are single copy in both rice and maize. White corresponds to chromosomal regions where no information is available regarding copy number in maize.

copy in rice, the extent of gene duplication in maize (due to ancestral polyploidization) was estimated. Single-copy clones are here defined as those that hybridized to a single restriction fragment on genomic Southern analyses of maize under moderate-stringency conditions (1× standard saline/citrate, 65°C). Clones were classified as detecting duplicate genes in the maize genome when they hybridized to more than one restriction fragment with all restriction enzymes on genomic Southern analyses at the same stringency. In most instances, all segregating restriction fragments were genetically mapped in both the rice and maize genomes, and these results confirmed the classification as single copy or duplicated.

On the basis of these data, we estimate that the majority (72%) of the single-copy loci in rice are duplicated in modern maize. The 28% of loci that are now single copy in maize represent loci for which the duplicate counterparts have been lost from the maize genome or mutated to an extent that they are no longer detectable with Southern hybridization. The loss of duplicate loci may have occurred by several processes, including small, localized deletions or by loss of entire chromosomes or chromosomal segments. The latter process would result in loss of linked groups of loci that should be visible now as linked sets of single-copy loci in the maize genome. Figs. 1 and 2 show the distribution of loci that are single copy in both the rice and maize genomes. Most of the loci appear to be randomly distributed throughout the map, which is consistent with small, localized deletions; however, several pairs of linked, single-copy loci also can be identified in the maize genome. For example, RZ166 and CDO718 are single copy in both rice and maize. In maize these loci are 9 cM apart (chromosome 5), and in rice they are 20 cM apart (chromosome 2). Likewise, the marker pairs RZ952/CDO595 (chromosome 8, rice; chromosome 1, maize) and RZ536/CDO520 (rice chromosome, 11; maize chromosome, 4) are also linked (<20 cM) and single copy in both genomes (Fig. 1 and 2). These results suggest that larger deletions, encompassing several loci, may have played a limited role in the evolution of the maize genome since polyploidization. Such deletions could have resulted from chromosomal rearrangements (e.g., translocations or inversions, ref. 19).

If the maize genome has not undergone substantial chromosomal rearrangements since polyploidization, it should be possible to identify, in the maize genome, pairs of homologous chromosomes. Chromosomes 2 and 10 come closest to meeting this expectation. The short arm of maize chromosome 2 has the same gene order and content as the long arm

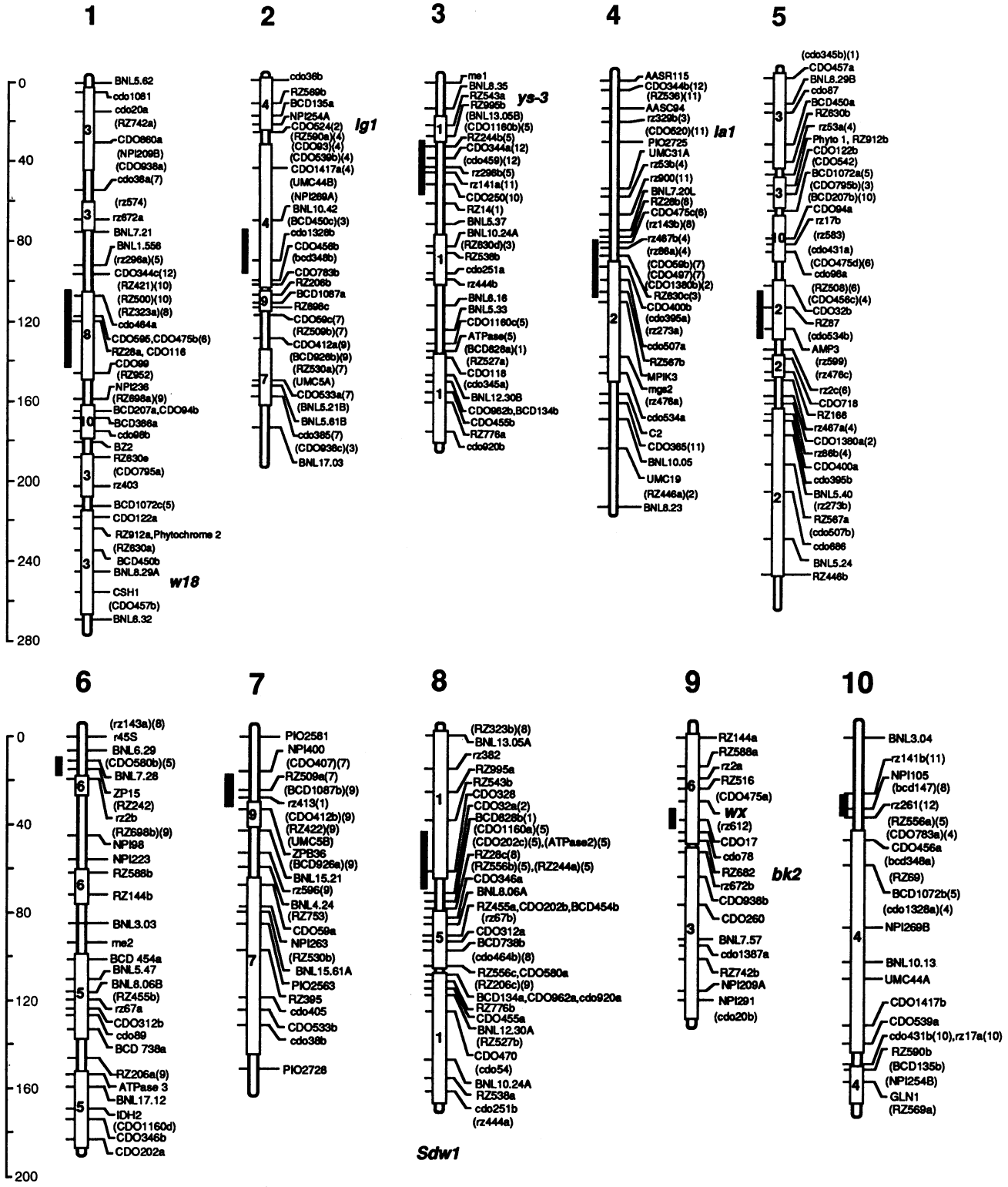

FIG. 2. RFLP linkage map of maize based on a recombinant inbred population of 48 lines derived from a cross between inbreds T232 and CM37 (13). Scale in Haldane cM shown on left was derived from Haldane and Waddington (16). Locus names (corresponding to cDNA probes) are listed at right of chromosomes. RZ, rice leaf cDNA; CDO, oat leaf cDNA; BCD, barley leaf cDNA. BNL represents maize genomic clones that were previously mapped on this population and served as reference points for constructing map (13). Locus names in uppercase letters indicate that all fragments detectable on Southern blots with probe were polymorphic between parents and could be mapped genetically. Loci in lowercase letters correspond to probes for which some fragments were monomorphic between parents and could not be mapped genetically. Loci by tick marks ordered with lod >2 using MAPMAKER software (25). Markers in parentheses have been located to intervals with lod <2. Markers separated by commas cosegregate. Boxed areas in chromosomes are regions where marker order is conserved with rice. The number of homologous rice chromosome is indicated within the box. For other loci, chromosomal location(s) of homologous locus in rice is given in parentheses after locus name. Approximate position of centromeres are indicated by solid bars to left of chromosomes. Approximate map position of selected morphological markers (boldface) is shown to right of chromosomes (*ys3*, yellow stripe; *Sdw1*, semi-dwarf; *bk2*, brittle stalk; *w18*, white seedling stripe; *lg1*, liguleless; *wx*, waxy; *la1*, lazy plant). Putative orthologous morphological loci in rice (based on conserved linkage) are listed in same order in the legend of Fig. 1 and displayed in that figure.

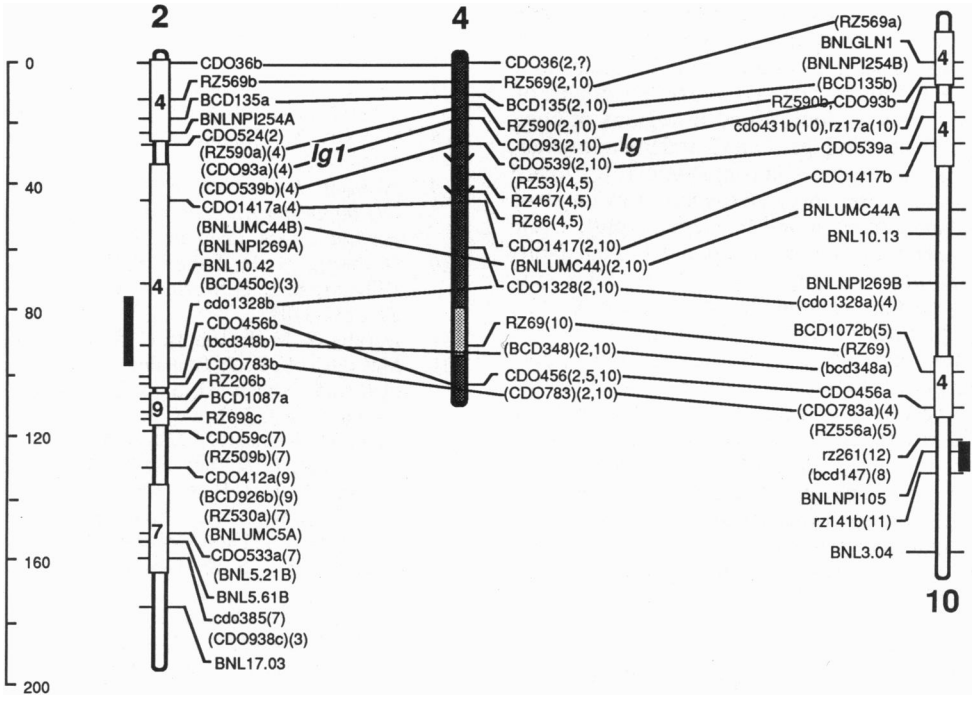

FIG. 3. Conserved linkage between rice chromosome 4 and maize chromosomes 2 and 10. Loci connected by a line are detected by the same clone in both genomes. Maize chromosome 10 is shown in reversed order to clarify the relationship of it with other chromosomes. Approximate position of centromeres are indicated by solid bars to left of chromosomes. Note that the majority of rice chromosome 4 corresponds to a single chromosome arm of both maize chromosomes 2 and 10. Three loci in the middle of rice chromosome 4 (RZ53, RZ467, and RZ86) are not located on either maize chromosome 2 or 10 but, instead, are found on maize chromosomes 4 and 5. The rearrangement(s) leading to this difference between rice and maize likely occurred before polyploidization of maize. See legends of Figs. 1 and 2 for information about locus names and map construction.

of maize chromosome 10, and both correspond in order and gene content to the majority of rice chromosome 4 (Fig. 3, ref. 8). Likewise, most of maize chromosome 8 has the same gene content as maize chromosome 3, which is consistent with earlier findings (Figs. 1 and 2; refs. 8, 18). However, in this instance, the order of the markers on the two putative homologues is not well conserved, suggesting multiple inversions on one or both chromosomes after polyploidization. Overall, these results suggest that a large number of chromosomal rearrangements (both inversions and translocations) occurred after the polyploidization of maize, and the accumulation of these rearrangements may have contributed, not only to the loss of some duplicate loci, but also to the diploidization of the maize genome.

Comparison of Recombination Rates. Maize has approximately 6-fold more nuclear DNA than rice, and the question can be asked whether this has resulted in an overall increase in meiotic recombination. To answer this question, we compared map distances using 14 sets of intervals within areas of conserved linkage. The total map units over all areas compared was 137 cM in rice versus 122 cM in maize (Table 1). A paired *t* test revealed that the 1.2-fold higher value is not statistically significant ($P = 0.55$). These data suggest that the higher DNA content of maize has not resulted in a proportional increase in recombination within conserved regions. This is consistent with results obtained from comparing recombination rates in other divergent pairs of plant species (tomato/pepper and maize/sorghum), which also differ greatly in DNA content (8, 24). Because recombination rates are not significantly different in rice and maize, it is expected that the total map units in maize should be 2-fold greater compared with rice, as maize has an allotetraploid nature compared with the diploid nature of the rice genome. The orthologous loci mapped in rice cover 1055 cM, whereas the same loci in maize define 1723 cM. This value is less than the expected ($2 \times 1055 = 2110$), which may be due to the putative loss of some maize chromosome segments after polyploidization (see previous section).

Applications of Comparative Maps. The rice–maize comparative maps described in this report have a number of applications. For example, it should now be possible to test hypotheses about homologies between loci affecting morphological variation in both species. Currently, >500 mutant genes have been identified in maize, and many of those have been mapped onto the genetic linkage map (20). A comparable number of mutants have also been identified and

Table 1. Comparison of map distance in selected intervals of conserved regions

Interval	Map distance, cM	
	Rice	Maize
CDO455 – CDO920	21.5	10.4
CDO718 – RZ166	21.8	8.8
CDO395 – CDO400	2.9	2.2
CDO20 – CDO1081	6.4	9.6
BCD450 – RZ630	3.2	7.1
RZ67 – CDO312	3.7	3.8
CDO346 – CDO202	2.3	7.1
RZ395 – CDO405	32.0	21.0
CDO99 – RZ28	16.0	26.3
RZ588 – RZ2	10.4	6.3
RZ682 – CDO78	4.3	3.3
BCD386 – CDO98	6.1	7.1
CDO87 – BNL8.29	3.7	2.9
RZ569 – BCD135	2.9	5.8
Total	137.2	121.7

RZ, rice leaf DNA; CDO, oat leaf cDNA; BCD, barley leaf cDNA; BNL8.29, clone BNL8.29.

mapped in rice (21). Many of these mutants are similar in their phenotypic effects, and by comparing map positions of such mutants, it should be possible to deduce which mutant loci are likely to be homologous in rice and maize. Examples of potential homologous mutant loci, found in regions of conserved linkage, are depicted in Figs. 1 and 2 and include genes for loss of ligules (*lg* locus in rice, chromosome 4; *lg1* locus in maize, chromosome 2) and waxy endosperm (*wx* locus in rice and maize, chromosomes 6 and 9, respectively). Crop plants (including maize and rice) are being used extensively for the study of quantitatively inherited traits (22, 23). Comparative maps may provide an opportunity to begin identifying homologous quantitative trait loci for many characters of both biological and agricultural importance, such as disease and insect resistance, heterosis, and yield (17). Finally, comparative maps should allow the position of DNA probes mapped in rice to be predicted in the maize genome and vice versa, allowing cross access of probes and accelerating genome research in both species. The fact that the same cDNA probes used to construct the rice and maize maps also hybridize to orthologous loci in most other grass species (data not shown) opens the way for ultimately connecting the genetic maps of a large number of diverse and agronomically important species in this family of plants.

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