# Saturated Molecular Map of the Rice Genome Based on an Interspecific Backcross Population

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## ABSTRACT

A molecular map has been constructed for the rice genome comprised of 726 markers (mainly restriction fragment length polymorphisms; RFLPs). The mapping population was derived from a backcross between cultivated rice, *Oryza sativa*, and its wild African relative, *Oryza longistaminata*. The very high level of polymorphism between these species, combined with the use of polymerase chain reaction-amplified cDNA libraries, contributed to mapping efficiency. A subset of the probes used in this study was previously used to construct an RFLP map derived from an inter subspecific cross, providing a basis for comparison of the two maps and of the relative mapping efficiencies in the two crosses. In addition to the previously described *Pst*I genomic rice library, three cDNA libraries from rice (Oryza), oat (Avena) and barley (Hordeum) were used in this mapping project. Levels of polymorphism detected by each and the frequency of identifying heterologous sequences for use in rice mapping are discussed. Though strong reproductive barriers isolate *O. sativa* from *O. longistaminata*, the percentage of markers showing distorted segregation in this backcross population was not significantly different than that observed in an intraspecific $F_2$ population previously used for mapping. The map contains 1491 cM with an average interval size of 4.0 cM on the framework map, and 2.0 cM overall. A total of 238 markers from the previously described *Pst*I genomic rice library, 250 markers from a cDNA library of rice (*Oryza*), 112 cDNA markers from oat (Avena), and 20 cDNA markers from a barley (Hordeum) library, two genomic clones from maize (Zea), 11 microsatellite markers, three telomere markers, eleven isozymes, 26 cloned genes, six RAPD, and 47 mutant phenotypes were used in this mapping project. Applications of a molecular map for plant improvement are discussed.

R ICE is one of the most important food crops in the world. It has also become a model plant among the cereals for molecular genetic studies. It is a diploid with $n = 12$ chromosomes, has the smallest genome of any monocot known (C = 0.45 pg) (ARUMUGANATHAN and EARLE 1991), a large germplasm collection (>120,000 accessions worldwide), can be regenerated from protoplasts [for reviews, see LYNCH *et al.* (1991) and HODGES *et al.* (1991)], and has a relatively high degree of transformation efficiency relative to other cereal species [for review, see HODGES *et al.* (1991) and KOTHARI *et al.* (1993)].

Early versions of a linkage map of rice chromosomes were reported by YAMAGUCHI (1927), CHAO (1927) and JODON (1948). In 1963, NAGAO and TAKAHASHI proposed

the first rice map consisting of 12 linkage groups, corresponding to the haploid number of chromosomes. Work in this area of rice genetics progressed steadily, with updated versions of the rice linkage map published by TAKAHASHI and KINOSHITA (1968, 1977) and annually since 1984 (KINOSHITA 1984–1994). Linkage maps were originally established with morphological mutants, but isozyme markers were subsequently located on the chromosomes [for reviews, see ENDO and MORISHIMA (1983), PHAM *et al.* (1990) and ISHIKAWA *et al.* (1991)]. The cytological basis of linkage groups was first reported by IWATA and OMURA (1971a,b) based on a study of relationships of gene loci with the points of interchange of reciprocal translocations. The development of primary trisomic stocks by several groups resulted in complete trisomic series for both *indica* and *japonica* rice (KURATA *et al.* 1981; KHUSH *et al.* 1984; IWATA and OMURA 1984) and allowed assignment of the linkage groups to their respective chromosomes. The use of morphological mutants in genetic studies has remained limited, mainly because of their deleterious effects and the difficulties encountered when attempting to group a number of these markers in the same genotype. Isozyme markers offer a more versatile set of genetic markers to rice breeders. However, the number of detectable isozyme loci

is still limited (Second 1982; GLASZMANN 1987; PHAM *et al.*, 1990).

The analysis of restriction fragment length polymorphism (RFLP) offers plant geneticists and breeders a powerful set of genetic markers which are abundant, codominant, non-deleterious, and reliable. RFLPs have been successfully used to construct genetic maps in numerous crop species [for review, see O'BRIEN (1993)] and to locate genes of interest along the chromosomes (PATERSON *et al.* 1991; TANKSLEY *et al.* 1989). In addition, high density molecular linkage maps can be used to clone target genes (MARTIN *et al.* 1993).

McCOUCH *et al.* (1988) described the construction of the first RFLP map in rice. This map was constructed from an $F_2$ population derived from a cross between varieties representing the two major subspecies (*indica* and *japonica*) of cultivated rice, *Oryza sativa* L. Primary trisomic stocks (KHUSH *et al.* 1984) were used to assign the 12 linkage groups to their respective chromosomes. The long term usefulness of this segregating $F_2$ population was limited because plants were not maintained vegetatively. In addition, the level of polymorphism in this intraspecific cross was limited, and in order to map a sufficient number of markers it was necessary to screen many probes with a large number of restriction enzymes. Even after mapping nearly 200 markers, several significant gaps still remained in the map which could not be explained by chance alone. It was not clear whether these gaps were due to a bias in the genomic library used for mapping, regions in the rice genome comprised of multicopy sequences that could not be mapped using Southern analysis, or the presence of chromosome segments that were common by descent (and thus monomorphic) in the *indica* and *japonica* mapping parents (McCOUCH *et al.* 1990; our unpublished data). Nonetheless, the map provided the basis for locating a number of agronomically important genes via linkage to RFLP markers, including both single genes and quantitative trait loci (QTL) linked to blast resistance (YU *et al.* 1991; WANG *et al.* 1994), insect resistance (McCOUCH and TANKSLEY 1991; MOHAN *et al.* 1993), bacterial blight resistance (Mc-COUCH *et al.* 1991; RONALD *et al.* 1992), photoperiod sensitivity (MACKILL *et al.* 1993), grain aroma (AHN *et al.* 1992), wide compatibility (LIU *et al.* 1992; YANAGIHARA *et al.* 1994; ZHENG *et al.* 1992), and the semi-dwarf character, *sd-1* (CHO *et al.* 1994), among others.

A second RFLP map of rice based on a different *indica/japonica* cross was reported by SAITO *et al.* (1991). Where the map developed in our laboratory was based on a *Pst*I genomic library from the *indica* variety, IR36, the map developed by SAITO *et al.* (1991) was based on a *Pst*I library developed from the *japonica* variety, Nipponbare. Efforts to integrate the two maps are underway (XIAO *et al.* 1992). An additional map based on an *indica* × *japonica* cross is currently under development in Japan (NAGAMURA *et al.* 1993).

The objective of the mapping project reported here was to generate a well-saturated map of the rice genome. To accomplish this, we broadened the type of markers used in the mapping effort. In addition, we utilized an interspecific mapping population that embodied a high frequency of polymorphism. Such crosses have proven very useful for developing maps in other species including tomato (TANKSLEY *et al.* 1992b), potato (BONIERBALE *et al.* 1988), sorghum (CHITTENDEN *et al.* 1994), and wheat (GILL *et al.* 1991). Moreover, the population we have chosen is readily amenable to long term vegetative propagation.

Phylogenetic studies of the *Oryza* genus, based on isozymes (SECOND 1985) or RFLPs (WANG *et al.* 1990), have shown that, among species with the AA genome, *Oryza longistaminata* A. Chev et Roehr is one of the most distantly related to *O. sativa*. This perennial species propagates in nature by the development of vigorous rhizomes or by outcrossing, enforced by self-incompatibility (NAYAR 1967). GHESQUIÈRE (1985) has described the main features of its population biology. *O. longistaminata* is isolated from the other AA genome species by a strong reproductive barrier (CHU and OKA 1970a) which can nevertheless be overcome (CHU and OKA 1970b; GHESQUIÈRE 1988). Certain traits of this species may be of interest for rice breeding, such as specific disease resistance genes (VALES 1985), allogamic traits (TAILLEBOIS and GUIMARAES 1987), and plant architecture (CAUSSE and GHESQUIÈRE 1991).

The purpose of this report is to describe the construction of an RFLP map based on an interspecific backcross population involving *O. sativa* and *O. longistaminata*. A comparison with the previous map (McCOUCH *et al.* 1988) is presented based on a common set of probes mapped in both studies. A total of 238 markers from the previously described *Pst*I genomic rice library, 250 markers from a cDNA library of rice (Oryza), 112 cDNA markers from oat (Avena), and 20 cDNA markers from a barley (Hordeum) library, two genomic clones from maize (Zea), 11 microsatellite markers (WU and TANKSLEY 1993a), 3 telomere markers (WU and TANKSLEY 1993b), 11 isozymes, 26 cloned genes, 6 RAPD, and 47 mutant phenotypes were used in this mapping project. Levels of polymorphism detected by different types of molecular markers and the frequency of identifying heterologous sequences for use in rice mapping are discussed.

## MATERIALS AND METHODS

**Plant material:** A backcross population of 113 plants, derived from the cross *O. sativa/O. longistaminata//O. sativa* was used as the mapping population (referred to as the SL population). BS125 (*O. sativa*) was an *indica* land race collected in Guinea Bissau. WL02 (*O. longistaminata*) was a unique plant coming from a seed originally collected from a wild population in Botswana (MIEZAN and SECOND 1979). The $F_1$ hybrid (BS125/WL02) and the backcross seeds were obtained by controlled pollination in the ORSTOM (Institut Français de Recherche Scientifique pour le Développement en

Coopération) Research Center in Ivory Coast, by GHESQUIÈRE (1988), who kindly provided them. The 113 backcross plants were grown in either a growth chamber or a greenhouse facility at Cornell University, maintained vegetatively, and propagated by shoot cuttings.

Hybridization of the cDNA probes from oat and barley was evaluated in a preliminary screen using genomic DNA of the rice cultivar IR36. Seeds from this cultivar were provided by the International Rice Research Institute.

The results of this study were compared to the data obtained on the intraspecific $F_2$ population previously used to establish the map (McCOUCH et al. 1988). This population was derived from a cross between an indica breeding line, IR34583-19-3-3, and a japonica landrace from Indonesia, Bulu Dalam (referred to hereafter as the IB population). We have also compared the percent polymorphism detected between the parents of the SL population to that observed between the cultivars, Apura and IRAT177, the parents of a population of doubled haploid lines obtained through anther culture (hereafter referred to as the AI population) (GUIDERDONI et al. 1988, 1990), whose RFLP map is still under construction.

**DNA extraction, Southern hybridization:** The DNA extraction based on the technique proposed by SHURE et al. (1983) has been used in combination with the procedure described by TAI and TANKSLEY (1990), where freshly harvested leaves were dried in a food dryer at 45–50°, for 16–24 hr, before powdering in an electric coffee grinder. The urea-phenol extraction buffer was replaced by a potassium acetate buffer (derived from the procedure of DELLAPORTA et al. 1983). Using both techniques, yields of approximately 250–500 micrograms of DNA per 5–10 g of fresh tissue were obtained.

The DNA digestion, Southern blotting and hybridization procedures are similar to those described in McCOUCH et al. (1988). Five to seven micrograms of total DNA were digested at 37° using one of the following enzymes: EcoRI, EcoRV, DraI, HindIII, ScaI, XbaI, BamHI, and BglII (BRL and NEBiolab) in the buffer recommended by the manufacturer. After digestion, the DNA was fractionated on 0.9% agarose gels, and run in neutral electrophoresis buffer (1 M Tris, 10 mM EDTA, 125 mM NaAc; pH 8.1). The fractionated DNA was then transferred to nylon membrane filters, Genescreen Plus (DuPont) or Hybond N+ (Amersham Corp.), in either 0.5 M NaOH, 1.5 M NaCl or 0.4 M NaOH buffers, following the procedure described by SOUTHERN (1975). Probes were random hexamer labeled (FEINBERG and VOGELSTEIN 1983), and hybridization was performed at 65°, in 5 × SSC buffer, overnight. The filters were washed at 65°, three times for 20 min each time, with successive stringencies of 2 ×, 1 × and a last wash at 0.5 × SSC (each wash 0.1% SDS) for most probes. When mapping known genes, lower stringency washes (2 ×, 1 ×, 1 × SSC) were occasionally necessary for obtaining clear signal with cloned genes from other species, and higher stringency washes (2 ×, 1×, 0.5 ×, 0.05 × SSC) were sometimes used to obtain clear bands where gene families or repeated sequences were involved. The filters were exposed with an intensifier screen at −80° for 1–5 days.

To survey for polymorphism, clones were hybridized onto filters containing DNA from the recurrent indica parent, BS125, and the $F_1$ hybrid, digested by different restriction enzymes. Initially, we included eight restriction enzymes (EcoRV, XbaI, HindIII, ScaI, EcoRI, DraI, BamHI and BglII, those found most efficient by McCOUCH et al. 1988), but due to the high level of polymorphism between the O. sativa and O. longistaminata parents, only five (EcoRV, HindIII, ScaI, XbaI, and DraI) were retained, as they were sufficient to detect polymorphism in most of the clones. For mapping, DNA from the 113 backcross progeny was digested, blotted, and used in Southern hybridization (as described above) for clones showing poly-

morphism between the parents. After examining the molecular weights of the hybridizing bands in survey filters, two or more clones with non-overlapping banding patterns were frequently hybridized together onto the same set of progeny filters, allowing independent scoring and efficient use of filters.

Mapping of telomere sequences was done using CHEF gels as described in WU and TANKSLEY (1993b). Microsatellites were mapped on 6% denaturing polyacrylamide gels as described in WU and TANKSLEY (1993a).

**Clones:** A PstI genomic library from rice (probes designated "RG"), whose construction was previously described in McCOUCH et al. (1988), served as one source of clones. Initially, 100 of these markers were selected at regular intervals from a previous version of the map (McCOUCH et al. 1988; FULTON and TANKSLEY 1990) and transferred to the SL population. An additional 138 probes from this genomic library were also used in this study. Of the previously surveyed rice genomic probes, 30 which were previously unmapped due to monomorphism in the IB combination were mapped onto the SL population. We also mapped markers from three cDNA libraries. These libraries were constructed for use in this study and other RFLP studies involving grass species in this laboratory (O'DONOUGHUE et al. 1992; HEUN et al. 1991; ANDERSON et al. 1992). They were derived from mRNA extracted from etiolated leaf sheath from the cultivars IR36 (rice, O. sativa), Brooks (oat, Avena sativa), and Willis (barley, Hordeum vulgare). cDNA clones from rice are designated "RZ," from oat, "CDO," and from barley, "BCD." Inserts averaging more than 1 kb were cloned into the EcoRI site of the Lambda ZapII vector (rice and barley cDNA) or the EcoRI-XhoI site of the Uni-ZAPXR vector (oat cDNA) (Stratagene). The cDNA inserts were amplified by the polymerase chain reaction (primers were the oligonucleotides GTAAAACGACGGCCAGT and AAAAGCTATGACCATG; 34 cycles: 1 min at 92°, 40 sec at 50°, 1 min at 72°; final extension cycle for 10 min) and then purified through G-50 Sephadex spin columns.

More than 1800 cDNA probes from these three libraries were screened for hybridization signal on filters containing lanes of EcoRI-digested total genomic DNA from five grass species (barley, rice, oat, wheat and sugarcane). Of those presenting a strong signal and a low copy number with rice variety, IR36, 382 clones were utilized in this study.

Telomere sequences were detected by pulsed-field gel electrophoresis using an Arabidopsis telomere probe as described in WU and TANKSLEY (1993b). Microsatellite markers containing the GA, GT, and AT repeat motifs were identified based on a search of all rice sequences in the GenBank and EMBL databases or subcloned from a 15-kb genomic library from cv IR36 as described in WU and TANKSLEY (1993a).

Clones obtained from other laboratories are listed in Table 1. All of these were mapped using Southern analysis, except that the polymorphism for the large subunit of ribosomal DNA (r45s) was read directly from photographs of agarose gels containing DraI-digested genomic DNA stained in ethidium bromide.

**Nomenclature:** Clones were classified as single copy if >90% of the signal was accounted for by only one or two hybridizing bands for at least one enzyme on a survey filter. When a marker detected more than two bands for all enzymes, a suffix (A, B, or X) was added to the probe number to indicate the specific copy that was mapped. If only one band of a multiple copy probe was studied, an X was used as the suffix. If two loci of a multiple copy clone were mapped, the marker was given an A or a B suffix. There were no cases were more than two copies of a particular sequence were mapped.

**Map construction:** Linkage analysis was performed using Mapmaker Version 3.0 (LANDER et al. 1987) on a Sun II workstation, and using Map Manager (MANLY 1993) on a Macintosh

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TABLE 1

Known genes

| Gene | Type | Product/Phenotype | Chromo-some | Reference |
|------|------|-------------------|-------------|-----------|
| *ACC1* | RFLP | ACC synthase | 3 | A. THEOLOGIS, Pl. Gen. Exp. Cntr. (personal communication) |
| *ACC3* | RFLP | ACC synthase | 5 | A. THEOLOGIS, Pl. Gen. Exp. Cntr. (personal communication) |
| *Amy1B* | RFLP | α-Amylase | 1 | HUANG *et al.* (1990); RANJHAN *et al.* (1991) |
| *Amy1C* | RFLP | α-Amylase | 2 | HUANG *et al.* (1990); RANJHAN *et al.* (1991) |
| *Amy1A* | RFLP | α-Amylase | 2 | HUANG *et al.* (1990); RANJHAN *et al.* (1991) |
| *Amy2A* | RFLP | α-Amylase | 6 | HUANG *et al.* (1990); RANJHAN *et al.* (1991) |
| *Amy3A* | RFLP | α-Amylase | 9 | HUANG *et al.* (1990); RANJHAN *et al.* (1991) |
| *Amy3B* | RFLP | α-Amylase | 9 | HUANG *et al.* (1990); RANJHAN *et al.* (1991) |
| *Amy3C* | RFLP | α-Amylase | 9 | HUANG *et al.* (1990); RANJHAN *et al.* (1991) |
| *Amy3D* | RFLP | α-Amylase | 8 | HUANG *et al.* (1990); RANJHAN *et al.* (1991) |
| *Amy3E* | RFLP | α-Amylase | 8 | HUANG *et al.* (1990); RANJHAN *et al.* (1991) |
| *Acp1* | Isozyme | Acid phosphatase | 12 | L. ZHU, Academia Sinica (personal communication) |
| *Acp2* | Isozyme | Acid phosphatase | 12 | L. ZHU, Academia Sinica (personal communication) |
| *Acp4* | Isozyme | Acid phosphatase | 7 | McCOUCH (1990) |
| *Actin 1* | RFLP | Actin | 3 | McCOUCH (1990) |
| *Adh-1* | Isozyme | Alcohol dehydrogenase | 11 | ABENES *et al.* (1994) |
| *ATP* | RFLP | ATPase | 5 | N. CHUA, Rockefeller University (personal communication) |
| *atub* | RFLP | α-Tubulin | 9 | McCOUCH *et al.* (1988) |
| *Bph-10(t)* | Morph | Brown planthopper resistance | 12 | T. ISHII and D. BRAR, IRRI (personal communication) |
| *CALa* | RFLP | Calmodulin | 5 | Z. CHEN, Peking University (personal communication) |
| *CALb* | RFLP | Calmodulin | 7 | Z. CHEN, Peking University (personal communication) |
| *Cl* | Morph | Clustered spikelets | 6 | YU (1991) |
| *cyc1* | RFLP | Cyclophilin | 9 | W. BUCHHOLZ, Texas A&M (personal communication) |
| *cyc2* | RFLP | Cyclophilin | 2 | W. BUCHHOLZ, Texas A&M (personal communication) |
| *d-5* | Morph | Bunketsu-waito tillering dwarf | 2 | YU (1991) |
| *d-11* | Morph | Norin-28 dwarf | 4 | YU (1991) |
| *d-27* | Morph | Bunketsuto tillering dwarf | 11 | ABENES *et al.* (1994) |
| *Dn-1* | Morph | Dense panicle | 9 | YU (1991) |
| *ef* | Morph | Early flowering | 10 | T. ISHII and D. BRAR, IRRI (personal communication) |
| *ESTI-2* | Isozyme | Esterase (isoelectric) | 1 | CHO *et al.* (1994) |
| *EST-2* | Isozyme | Esterase | 6 | NAKAGAHARAand HAYASHI (1976) |
| *fgr* | Morph | Fragrance | 8 | AHN *et al.* (1992) |
| *Gm-2* | Morph | Gall-midge resistance | 4 | MOHAN *et al.* (1993) |
| *gl-1* | Morph | Glabrous leaf | 5 | YU (1991) |
| *Glh* | Morph | Green leafhopper resistance | 4 | L. SEBASTIAN, IRRI and Cornell University (personal communication) |
| *Hbv* | Morph | Hoja blanca resistance | 12 | J. TOHME, CIAT (personal communication) |
| *Hg* | Morph | Hairy glume | 3 | YU (1991) |
| *la* | Morph | Lazy | 11 | ABENES *et al.* (1994) |
| *lax* | Morph | Lax panicle | 1 | YU (1991) |
| *LEC* | RFLP | Lectin | 12 | McCOUCH *et al.* (1988) |
| *Mal I* | Isozyme | Malate dehydrogenase | 1 | McCOUCH (1990) |
| *ZB8* | RFLP | Phenylalanine ammonia lyase | 5 | XHU and LAMB (1991) |
| *Pgd 1* | Isozyme | Phosphogluconate dehydrogenase | 11 | McCOUCH (1990) |
| *Pgi-2* | Isozyme | Phosphoglucose isomerase | 6 | MACKILL *et al.* (1993) |
| *Ph* | Morph | Phenol staining | 4 | McCOUCH (1990) |
| *PHY-A* | RFLP | Phytochrome-A | 3 | KAY *et al.* (1989) |
| *Pi-1(t)* | Morph | Blast resistance | 11 | YU (1991) |
| *Pi-2(t)* | Morph | Blast resistance | 6 | YU *et al.* (1991) |
| *Pi-4(t)* | Morph | Blast resistance | 12 | YU *et al.* (1991) |
| *Pi-5(t)* | Morph | Blast resistance | 4 | WANG *et al.* (1994) |
| *Pi-6(t)* | Morph | Blast resistance | 12 | Z. YU, Cornell University (personal communication) |
| *Pi-7(t)* | Morph | Blast resistance | 11 | WANG *et al.* (1994) |
| *Pi-9(t)* | Morph | Blast resistance | 6 | P. REIMERS and R. NELSON, IRRI (personal communication) |
| *Pi-10(t)* | Morph | Blast resistance | 5 | N. NAQVID and B. CHATOO, University of Baroda (personal communication) |
| *Pi-11* | Morph | Blast resistance | 8 | L. ZHU, Academia Sinica (personal communication) |
| *Pi-?* | Morph | Blast resistance | 4 | J. TOHME *et al.*, CIAT (personal communication) |
| *IPi-(t)* | Morph | Blast resistance | 12 | K. L. ZHENG, CNRRI (personal communication) |
| *IPI-3(t)* | Morph | Blast resistance | 12 | K. L. ZHENG, CNRRI (personal communication) |
| *Pox-2* | Isozyme | Peroxidase | 12 | McCOUCH (1990) |
| *Pr* | Morph | Purple hull | 4 | YU (1991) |
| *R45s* | RFLP | 45S ribosomal | 9 | McCOUCH *et al.* (1988) |
| *R5s* | RFLP | 5S ribosomal DNA | 11 | McCOUCH *et al.* (1988) |
| *Rf* | Morph | Fertility restorer | 10 | YU (1991) |
| *Rc* | Morph | Brown pericarp | 7 | YU (1991) |
| *RCH10* | RFLP | Rice basic chitinase | 3 | XHU and LAMB (1991) |
| *RTSV* | Morph | Rice tungro spherical virus resistance | 4 | L. SEBASTIAN, IRRI and Cornell University (personal communication) |
| *S-5* | Morph | Wide compatibility | 6 | ZHENG (1992); LIU *et al.* (1992); YANAGIHARA *et al.* (1994) |
| *SALT* | RFLP | Salt tolerance | 1 | CLAES *et al.* (1990) |
| *Se-1* | Morph | Photoperiod sensitivity | 6 | MACKILL *et al.* (1993) |
| *sd-1* | Morph | Semi-dwarf | 1 | CHO *et al.* (1994) |

**TABLE 1**

*Continued*

| Gene | Type | Product/Phenotype | Chromosome | Reference |
|---|---|---|---|---|
| *Sdh-1* | Isozyme | Shikimate dehydrogenase | 12 | McCOUCH (1990) |
| *Se-3* | Morph | Photoperiod sensitivity | 6 | M. MAHESWARAN, IRRI (personal communication) |
| *Telsa-1* | RFLP | Telomeres | 9 | WU and TANKSLEY (1993b) |
| *Telsm-3* | RFLP | Telomeres | 11 | WU and TANKSLEY (1993b) |
| *Telsm-1* | RFLP | Telomeres | 8 | WU and TANKSLEY (1993b) |
| *TRYP* | RFLP | Trypsin-inhibitor | 1 | Z. CHEN, Peking University (personal communication) |
| *Wph-1* | Morph | Whiteback planthopper resistance | 7 | McCOUCH (1990) |
| *wx* | RFLP | Waxy | 6 | SHURE et al. (1983); McCOUCH et al. (1988) |
| *Xa-1* | Morph | Bacterial blight resistance | 4 | YOSHIMURA et al. (1992) |
| *Xa-2* | Morph | Bacterial blight resistance | 4 | YOSHIMURA et al. (1992) |
| *Xa-3* | Morph | Bacterial blight resistance | 11 | YOSHIMURA et al. (1992) |
| *Xa-4* | Morph | Bacterial blight resistance | 11 | YOSHIMURA et al. (1992) |
| *xa-5* | Morph | Bacterial blight resistance | 5 | McCOUCH et al. (1991) |
| *Xa-10* | Morph | Bacterial blight resistance | 11 | S. YOSHIMURA and R. NELSON, IRRI (personal communication) |
| *Xa-21* | Morph | Bacterial blight resistance | 11 | RONALD et al. (1992) |
| *z-2* | Morph | Zebra stripe | 11 | ABENES et al. (1994) |
| **OTHERS** | | | | |
| *RRH-18* | RFLP | Linked to Pi-10 (from Tongil) | 5 | N. NAQVID, University of Baroda (personal communication) |
| *RRO-02* | RFLP | Linked to Pi-9 (from O. minuta) | 6 | P. REIMERS, IRRI (personal communication) |
| *RRA-19* | RFLP | Linked to Se-3 | 6 | M. MAHESWARAN, IRRI (personal communication) |
| *pTA248* | RFLP | Linked to Xa-21 (from O. longist.) | 11 | RONALD et al. (1992) |
| *UMC44* | RFLP | From maize map | 4 | J. GARDINER, University Missouri (personal communication) |
| *BNL8.29* | RFLP | From maize map | 3 | B. BURR, Brookhaven National Laboratory (personal communication) |

IIci. All pairs of linked markers were first identified using the "group" command with LOD > 4.0, and recombination fraction ($\theta$) = 0.25 on the Mapmaker program. Cosegregating markers (*e.g.*, no recombination among markers within a linkage group) were identified by scanning two-point linkage data. Framework maps were constructed using only one marker from each set of cosegregating markers. The "orders" and the "compare" commands in Mapmaker, were used to identify the most probable marker order within a linkage group. The "ripple" command was used to verify the order. Markers were retained within the framework map only if the LOD value for "ripple" was >2.5 and if more than 70 individuals had been scored at a locus. The command "uninformative loci" in the Map Manager program was used to identify the number of scored data points at each locus. Following the establishment of a framework map, each interval was scanned for double cross-overs using the "double crossovers" command (Map Manager program). If any single-locus double-crossovers were observed, the primary data was re-checked for accuracy of scoring at the markers in question. Markers responsible for more than three double crossover events in any interval were removed from the framework map and placed in parentheses to the right of the interval most likely to contain them. Additional markers were assigned to intervals within the LOD 2.5 framework using the "try" command, followed by the process of submitting marker orders to "compare," pulling out markers responsible for double crossovers, and reconfirming the LOD > 2.5 framework map using the "ripple" command in Mapmaker. Map units (cM) were derived using the Kosambi function (KOSAMBI 1944).

The software program, Map Manager (MANLY 1993) was used to perform a chi-square test to determine if the allele frequency at individual loci deviated from the expected 1:1 segregation for the BC population (*P* > 0.05).

## RESULTS

**Comparison of copy number and detection efficiency among libraries:** When the rice cDNA library was surveyed for copy number, approximately 66% of clones

were single copy (1–2 bands), 17% were multiple copy (3–9 bands), and 17% were repeated (smear) when filters were washed at 0.5 × SSC at 65° following Southern hybridization (Table 2). This compared with 58% single copy, 20% multiple copy, and 22% repeated sequences observed for a rice genomic library at the same washing stringency (McCOUCH *et al.* 1988). When the oat cDNA library was hybridized onto rice, 18% of the clones gave no signal, but of clones with signal, approximately 64% were single copy, 28% were multiple copy (with 3–8 bands), and 8% were repeated (giving a smear). When the barley cDNA library was hybridized onto rice, 16% of clones gave no signal, but of clones with signal, 48% were single copy, 42% were multiple copy, and 10% were repeated (Table 2). Therefore, the most efficient source of single copy sequences for mapping was the rice cDNA library.

**Comparison of polymorphism in intraspecific and interspecific crosses:** Table 3 shows the percentages of probes detecting polymorphism in three parental combinations for at least one of five restriction enzymes. The parents include the interspecific combination, *O. sativa* and *O. longistaminata* [BS125/WL02//BS125 (SL)], and two intra-*O. sativa* crosses [IR34583-19-3-3/Bulu Dalam (IB) and Apura/IRAT177 (AI)]. The percent polymorphism is compared among parental combinations and for two different libraries of probes: a *Pst*I genomic library from rice and a cDNA library from oat (Avena). Polymorphism in the interspecific parents was higher than for either of the intraspecific combinations for every restriction enzyme tested. In the interspecific cross, 85% of clones from the *Pst*I genomic library detected polymorphism with at least one of the five

## TABLE 2

Frequency of single copy, multiple copy or repeated sequences in cDNA libraries derived from rice, oat, and barley based on Southern hybridization with total rice DNA (washing stringency = 0.5× SSC at 65°)

| Source of cDNA clones | Single copy (%) | Multiple copy (%) | Repeated (%) | No signal (no.) | n |
|---|---|---|---|---|---|
| Rice (cv. IR36) | 66 | 17 | 17 | 0 | 576 |
| Oat (cv Brooks) | 64 | 28 | 8 | 36 | 200 |
| Barley (cv Willis) | 48 | 42 | 10 | 13 | 80 |

enzymes. In comparison, 75% of clones from the same library were polymorphic in the IB combination when the same enzymes were evaluated, and 38% were polymorphic in the AI combination. Statistics are similar with respect to the oat cDNA library, where 87.5% of clones detected polymorphism in the SL combination and 47% detected polymorphism in the AI combination. The IB combination was not tested with cDNA clones, and comparisons of polymorphism with RZ and BCD clones are not available because the IB and AI mapping parents were not surveyed with clones from these libraries.

**Genomic marker analysis:** To determine the usefulness of a rice genomic library for mapping in other *Gramineae* species, 37 clones with clear, single copy signal in rice were hybridized onto filters containing DNA from rice, wheat, barley and oat. Results from this experiment demonstrated that clear hybridization signal was detected for 65% of clones on wheat, 32% on barley, and 11% on oat. This suggests that the wheat genome is likely to contain a large proportion of DNA with homology to rice genomic sequences.

The level of polymorphism detected by a single enzyme for genomic clones in the interspecific combination in this study was in good agreement with the genetic distance between these species observed in an RFLP study by WANG *et al.* (1990). They found 54% of probes were polymorphic between *O. longistaminata* accessions and *indica* varieties of *O. sativa*, using *Eco*RI digests only. This compares with an average of 34% for a single enzyme for the IB combination and 13% for the AI combination. The significant differences in levels of polymorphism observed between the two intraspecific combinations included in this study support the concept that some putative *indica* × *japonica* combinations are genetically much more distant than others. In addition, this work suggests that for rice, as for other crops such as tomato (BERNATZKY and TANKSLEY 1986), potato (BONIERBALE *et al.* 1988) or sorghum (CHITTENDEN *et al.* 1994), the choice of an interspecific cross is an efficient way of detecting high levels of molecular polymorphism.

**Construction of the rice linkage map:** The total length of the map based on the interspecific (SL) population is 1491 cM, which corresponds to approximately one marker every 2.1 cM. It is comprised of 250 rice

cDNAs, 112 oat cDNAs, 20 barley cDNAs, 238 rice genomic clones, 2 maize genomic clones, 26 cloned genes, 11 isozymes, 11 microsatellite markers, 3 telomeric markers, 6 RAPD and 47 morphological mutant loci. The morphological mutant loci, which are underlined and in bold print in Figure 1, were mapped using a variety of plant material but a common set of molecular markers. All underlined markers in Figure 1 represent loci that were mapped on other populations, and whose map position on the SL map has been estimated based on linkage to common markers. Thirty markers, whose location previously had been unambiguously determined by trisomic analysis (McCOUCH *et al.* 1988; YU 1991) were used to assign linkage groups to their respective chromosomes. The linkage analysis was performed using successive thresholds of recombination values of 0.20, 0.25 and 0.30, and a LOD score of 2.5. When the order of adjacent markers could not be established with a LOD higher than 2.5, only one marker was used in the mapping framework (Figure 1), the other one being represented in parentheses at its most probable location to the right of the most closely linked clone on the framework. The LOD 2.5 framework of the map is based on 372 markers and the average interval defined by markers on the framework map is 4.0 cM. The markers that are separated from framework markers by a comma are tightly linked (less than 1 cM) to a framework marker. Two hundred twenty-four additional probes that were mapped with LOD < 2.5 onto the same population are represented in parentheses to the right of the framework markers. Chromosomes are comprised of 20–96 markers, with an average of 50 per chromosome.

There was a good correlation between the relative length of chromosomes measured at pro-metaphase by image analysis (FUKUI and IIJIMA 1991) and the number of probes per chromosome on our map (Figure 2A; $R^2 = 0.91$; $P < 0.01$). The relationship between relative length of pro-metaphase chromosomes and the length of the chromosomes in cM on the RFLP map was also significant (Figure 2B; $R^2 = 0.65$; $P < 0.05$), though lower than for number of markers. This is related to the fact that the recombination distance between markers was not uniform along the chromosomes. Indeed, the average distance between markers differed significantly ($F = 3.01$, $P < 0.001$) between chromosomes. The variation in interval size was notable both between and within chromosomes. The average interval size was smallest for chromosomes *1*, *2* and *3* (the largest and most populated chromosomes), and was largest for chromosomes *7*, *11* and *12* ($P < 0.05$). The distribution of markers along several of the chromosomes was markedly uneven. Two densely populated regions can be noticed on chromosome *3*, punctuated by intervals of 21.5 and 14.0 cM. Several markers place to these intervals but cannot be mapped with precision (*i.e.*, LOD < 2.5). This uneven distribution of markers suggested that the frequency of

## TABLE 3

Percent of genomic and cDNA clones detecting polymorphism in three cross combinations based on a survey of 200 *Pst*I rice genomic clones and 166 oat (Avena) cDNA clones

| Library | Cross combination | Percent probes polym | | | | | Percent probes polymorphic ≥1 restriction enzyme |
|---------|-------------------|------------------------|---|---|---|---|------|
| | | *Eco*RV | *Hind*III | *Xba*I | *Sca*I | *Dra*I | |
| Rice genomic | BS125/WL02//BS125 | 52 | 57 | 57 | 58 | 46 | 85.0 |
| Rice genomic | IR34583-19-3-3 × Bulu Dalam | 39 | 31 | 40 | 30 | 31 | 75.0 |
| Rice genomic | Apura × IRAT177 | 16 | 12 | 9 | 18 | 13 | 38.0 |
| Oat cDNA | BS125/WL02//BS125 | 65 | 64 | 49 | 55 | — | 87.5 |
| Oat cDNA | Apura × IRAT177 | 17 | 25 | 32 | 25 | 23 | 47.0 |

Significant differences in levels of polymorphism ($P < 0.01$ based on a one-way ANOVA) were detected between parents of the SL, IB and AI populations. Differences in levels of polymorphism between libraries were not significant. "—" signifies no data available.

recombination may vary greatly from one region to another on the same chromosome. A similar pattern was observed on chromosomes *1*, *2* and *7* (Figure 1).

Eleven isozyme loci have also been located via linkage to RFLP markers. Currently located on the map are *Mal-1*, *Acp-1*, *Acp-2*, *Acp-4*, *Pgi-2*, *Adh-1*, *Pgd-1*, *Sdh-1*, *EstI-2*, *Est-2* and *Pox-2*. The molecular map developed in this laboratory has been oriented with respect to the classical linkage map of rice (KINOSHITA 1993) based on isozyme and morphological markers (Figure 1) and the rice RFLP map constructed by SAITO *et al.* (1991) and NAGAMURA *et al.* (1993) (XIAO *et al.* 1992) (data not shown). It provides a stable framework for rapidly and efficiently locating new markers on rice chromosomes. The results of this analysis and the RFLP data set associated with the *O. sativa* × *O. longistaminata* BC population are accessible in the Rice Genome Database ("RiceGenes") through the National Agricultural Library in Washington, D.C., or through Gopher (MCCOUCH and PAUL 1994).

**Relationship between molecular and classical linkage map in rice:** Figure 1 summarizes the current status of both the molecular map developed in this laboratory and the classical linkage map of rice (KINOSHITA 1993). Morphological mutant loci in common between the maps are placed to the left of the chromosomes on the classical map. The classical map is constructed from hundreds of different crosses, each segregating for only a few loci. Though the order of loci is expected to be stable, recombination frequency is highly variable among crosses. For example, *sd-1* and *lax* on chromosome *1* are both linked to markers that map within 10 cM of each other on the molecular map, but are much further apart on the morphological map. Similarly, the distances between *pH*, *Pr* and *d-11* on chromosome *4* of the two maps appear quite different. Thus, the genetic distances between markers represented on the classical map cannot be directly compared to those on the molecular map. In a few cases, the order of markers along a chromosome does not agree when the two maps in Figure 1 are compared. This is the case for *Pgd-1* and *la* on chromosome *11*. Additional marker analysis is required in order to resolve these discrepancies. Two of the chromosomes have no markers in common, while seven others

have between one and four markers located on both maps. Chromosome *6* stands out with eight markers in common, followed by chromosomes *11* and *4*, with six and five markers in common, respectively. This reflects interest in specific chromosomal regions containing genes of interest where linkage has been established between target genes and molecular markers.

**Colinearity of the intra and interspecific maps:** A sample of 100 probes, selected at regular intervals from an updated version of the map described in MCCOUCH *et al.* (1988), were initially mapped onto the interspecific population to compare the order of the probes in the two maps. This served to ensure that a map based on an interspecific cross would provide similar information as one based on an intraspecific cross. With few exceptions, probes were found to be located on the same chromosome and in the same order in both populations.

Two types of modifications could be observed: small modifications in the placement of markers and no change in the chromosome assignment, or change of assigned chromosome for a marker or a group of closely linked markers. Changes in chromosomal assignments for a marker or linked cluster of markers occurred in only five cases. In all five cases, the placement of markers to a specific linkage group in the IB $F_2$ population had been based on dosage analysis, rather than linkage analysis, using the trisomic stocks described by KHUSH *et al.* (1984).

The apparent contradictions may be explained by the fact that multiple copy clones, having multiple chromosomal locations, were involved in several cases. These sequences were originally mapped in the IB population based on high stringency washes. When they were mapped in the SL population, it was not clear whether the same or a different allele was segregating, providing an explanation for new locations of some markers. Difficulties in locating markers in distal regions of chromosomes are another source of error. Alternatively, irregularities in the trisomic stocks have been reported (OKA and WU 1988; CHUNG and WU 1990) and small structural rearrangements between related species and subspecies of rice (JENA *et al.* 1992; CHANDRARATNA 1964) may explain other discrepancies.

The five cases of chromosomal discrepancies included *RG365*, previously placed on chromosome *8*, now linked internally on chromosome *2*; *RG136*, previously on chromosome *9*, now linked internally on chromosome *8*; *RG375* and *RG396*, previously mapped onto chromosome *12*, but currently linked in the lower portion of chromosome *4*; *RG29*, previously on chromosome *7*, now located at a distance of 10 cM from one of the telomeres on chromosome *8*; and *RG98*, *RG304A* and *RG235*, three linked, single copy probes, previously reported to reside on chromosome *12*, now placed distally on chromosome *11*. The clearest evidence obtained from this study for the occurrence of a small translocation is related to the placement of *RG190*. In the SL and the IB populations, *RG190* maps to chromosome *12* (LOD > 2.5), supported by trisomic data (McCouch *et al.* 1988). However, in other populations, *RG190* is reported to be linked to *RG375* and *RG396* at the bottom of chromosome *4* (M. Champoux, IRRI, Philippines, personal communication; W. Park and Z. Li, Texas A & M University, Texas, personal communication; A. Saito, NIAR, Japan, personal communication). A study of the extra chromosomes in the IR36 trisomic series by Chung and Wu (1990) suggested that the extra chromosome in Triplo 12 contained a translocated section of chromosome *4*. Further study is necessary to resolve the possibility of structural rearrangements in these regions of chromosomes *4* and *12*.

Based on both genetic and physical mapping, Wu and Tanksley (1993b) demonstrated that *RG29* was linked to a telomere on chromosome *8*, and that *RG98* was linked to a telomere on chromosome *11*. The distance between *RG98* and the chromosome *11* telomere was estimated to be 8 cM, corresponding to a physical distance of approximately 270 kb. This suggests an exceptionally high rate of recombination, such that 1 cM is equivalent to approximately 34 kb in the distal portion of this chromosome. Linkage estimation is more dependable when markers are nested among other markers on a genetic map than when they are located at the ends of chromosomes. Though physical mapping of distal markers based on pulsed field gel electrophoresis (PFGE) offers convincing evidence that markers are actually located near telomeres, only a few probes to date (*RG29* on chromosome *8*, *RG98* on chromosome *11*, and *r45S* on chromosome *9*) have been mapped using th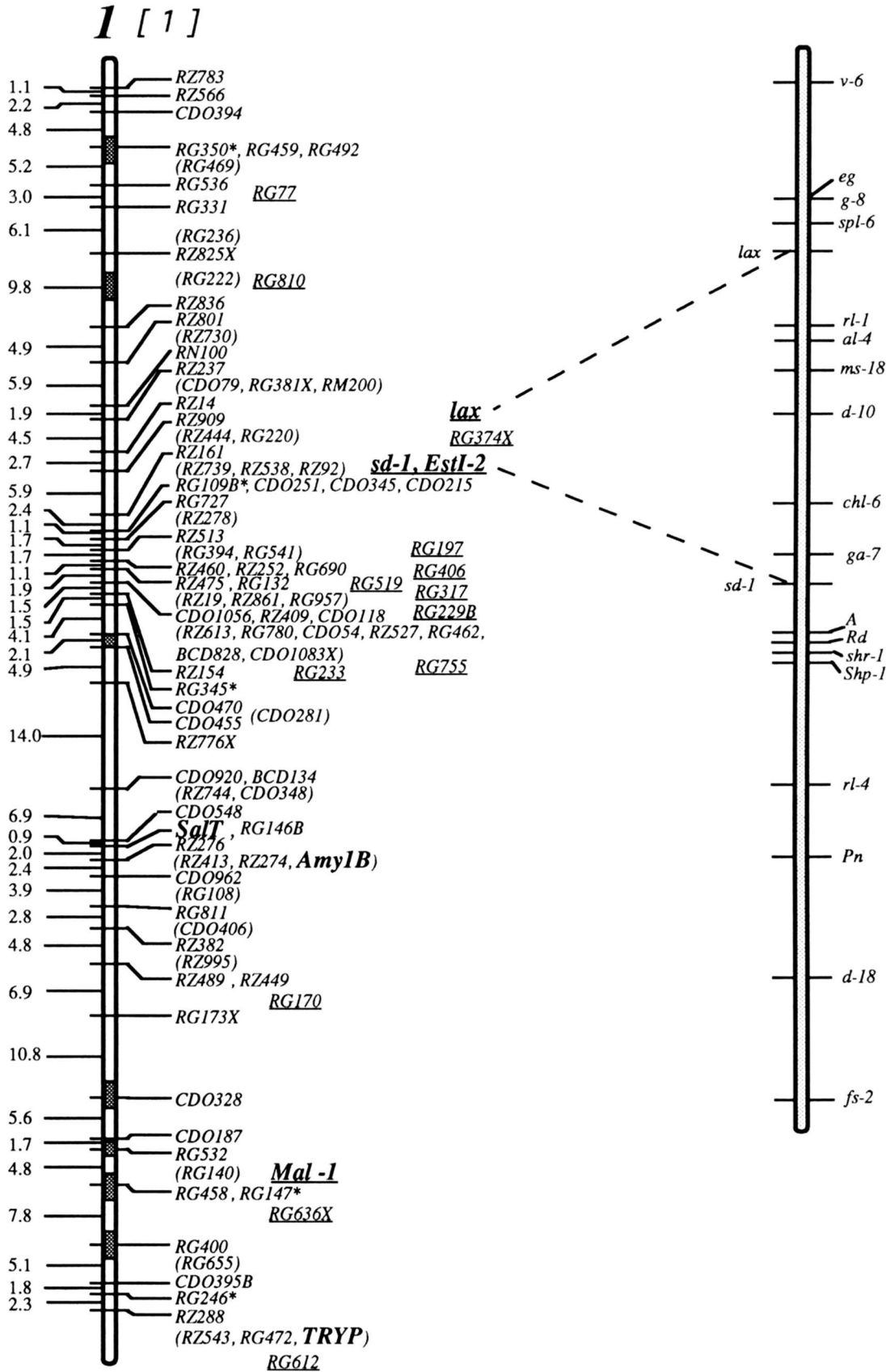is approach in rice due to the difficulty of clearly identifying segregation using PFGE techniques (Wu and Tanksley 1993b). The availability of libraries composed of large DNA fragments, in yeast artificial chromosome (YAC), bacterial artificial chromosome (BAC), or cosmid vectors will facilitate the mapping of distally located markers and provide confirmation of marker order and placement on existing genetic chromosome maps.

**Multiple copy markers:** There are a total of 50 multiple copy markers on the existing RFLP map of rice. In most cases, only one copy could be mapped because segregation could be clearly detected for only one locus. In eight cases (*RG146*, *RG369*, *CDO395*, *CDO507*, *CDO534*, *CDO1387*, *BCD454* and *CDO127*) two copies of a single cloned sequence were mapped. In only one instance (*RG369A* and *RG369B*) did both copies of a clone map to the same chromosome (chromosome *3*). Evaluation of the map positions of other duplicate-copy clones reveals no clear pattern of regional duplication in the rice genome. This situation can be contrasted to that of maize, an ancient polyploid, where almost all chromosomal regions contain contiguous sequences that exist in duplicate elsewhere in the genome (Helentjaris *et al.* 1988; Ahn and Tanksley 1993).

A comparison of copy number of clones mapped in different rice populations revealed no markers that were single copy in one population and highly repeated in another. However, variations in the number and location of members of multiple copy clone families (2–8 copies) among rice populations makes it difficult to use them in comparative mapping studies. This problem is confounded if comparative mapping between species is attempted. The emphasis on cDNA libraries as a source of clones for mapping in this interspecific population provided an efficient way to enrich for single copy sequences. We currently impose stringent selection criteria, targeting only clean, single copy clones for mapping in rice, and find that these are useful in comparative mapping efforts across a range of monocot species (Ahn and Tanksley 1993; Ahn *et al.* 1994; Van Deynze *et al.* 1994).

**Framework mapping kit:** A kit of 96 single copy markers providing good coverage of the rice genome has been assembled for ready distribution to researchers interested in locating genes of agronomic importance in rice. Thirty-five of the clones in this kit are from the rice genomic library, 47 are cDNA clones from rice, and 14

FIGURE 1.—Rice linkage maps: on right is the molecular map based on the interspecific backcross population (*O. sativa/O. longistaminata//O. sativa*); on left is the classical map based on morphological mutants and isozyme markers (reproduced with permission from Kinoshita 1993). The chromosome numbering, indicated at the top of chromosomes, follows the nomenclature established by the Rice Genetics Cooperative Committee in IRRI, May 1990 (Khush 1990). Between square brackets is presented the nomenclature used in McCouch *et al.* (1988), following Khush *et al.* (1984). Probe designation to the right of the chromosomes: cDNA markers are designated as RZ (from rice), CDO (from oat), BCD (from barley), rice genomic markers are designated as RG, maize genomic markers are UMC and BNL, microsatellite markers are RM or RN (Wu and Tanksley 1993a). Probes hybridizing with two or more major bands are indicated with an X if only one locus has been mapped; with A and B if two loci were mapped. Known genes are listed in Table 1. The probes located by trisomic studies are indicated with a star (*). Map distances are presented in centimorgans (Kosambi function) to the left of the chromosomes. Markers located to intervals with a LOD score < 2.5 are represented in parentheses in the appropriate interval. Stippled regions along the chromosomes represent regions containing markers with skewed allele frequencies ($P < 0.05$).

**1** [ 1 ]

| | Left markers | |
|---|---|---|
| 1.1 | RZ783 | |
| 2.2 | RZ566 | |
| 4.8 | CDO394 | |
| 5.2 | RG350*, RG459, RG492 (RG469) | |
| 3.0 | RG536 | RG77 |
| 6.1 | RG331 | |
| | (RG236) | |
| | RZ825X | |
| 9.8 | (RG222) | RG810 |

RZ836
RZ801
(RZ730)
4.9 RN100
RZ237
5.9 (CDO79, RG381X, RM200)
1.9 RZ14
RZ909
4.5 (RZ444, RG220)
RZ161
2.7 (RZ739, RZ538, RZ92)
RG109B*, CDO251, CDO345, CDO215
5.9 RG727
2.4 (RZ278)
1.1 RZ513
1.7 (RG394, RG541)  RG197
1.7 RZ460, RZ252, RG690  RG406
1.1 RZ475, RG132  RG519  RG317
1.9 (RZ19, RZ861, RG957)  RG229B
1.5 CDO1056, RZ409, CDO118
1.5 (RZ613, RG780, CDO54, RZ527, RG462,
4.1 BCD828, CDO1083X)  RG755
2.1 RZ154  RG233
4.9 RG345*
CDO470
CDO455 (CDO281)
14.0 RZ776X

CDO920, BCD134
(RZ744, CDO348)
6.9 CDO548
0.9 **SalT**, RG146B
2.0 RZ276
2.4 (RZ413, RZ274, **Amy1B**)
3.9 CDO962
(RG108)
2.8 RG811
(CDO406)
4.8 RZ382
(RZ995)
RZ489 , RZ449
6.9  RG170
RG173X

10.8

CDO328
5.6
1.7 CDO187
4.8 RG532
(RG140)  **Mal -1**
RG458, RG147*
7.8  RG636X

RG400
5.1 (RG655)
1.8 CDO395B
2.3 RG246*
RZ288
(RZ543, RG472, **TRYP**)
 RG612

**lax**
**RG374X**

**sd-1, EstI-2**

v-6

eg
g-8
spl-6

lax

rl-1
al-4
ms-18

d-10

chl-6

ga-7

sd-1

A
Rd
shr-1
Shp-1

rl-4

Pn

d-18

fs-2

**2** **[ 2 ]**

| | |
|---|---|
| | RG520 |
| 3.5 | RZ217 , RZ20X |
| 1.7 | RG322 , RZ531 |
| 3.8 | RZ213 |
| 5.4 | RZ123 |
| 1.2 | RZ913 |
| 4.9 | RZ962 |
| 5.0 | (RZ446) |
| | CDO1091 |

20.5 — (RG654, RZ681, CDO516, CDO204, RG256, RG151, **Amy1A**)

<u>RG89</u>
<u>RG744, RG95X</u>

| | | |
|---|---|---|
| | RZ103 | |
| 3.4 | RZ273 | |
| 4.5 | RZ906 , RZ976 | |
| 2.1 | (RG73, **Amy1C**) | |
| 3.1 | RZ260 , RZ551, CDO686 | |
| 4.2 | CDO507A, RZ204 | <u>RG324</u> |
| 1.9 | RZ567 | |
| 0.9 | RZ58 | <u>RG188</u> |
| 1.9 | RZ318 , RZ173 | |
| 4.8 | (RZ401, CDO395A) | <u>RG106X</u> |
| | CDO400, CDO32 | |
| 2.5 | (RG905) | <u>RG158</u> |
| 2.1 | RG886 | |
| | RZ668 | |
| 7.4 | RZ166 | |
| | (RG139*, RG252X, RZ701, CDO1380) | <u>RG102</u> |
| | RZ876 | <u>RG25X</u> |
| 12.6 | (RZ961) | |
| | RG157* | |
| 7.3 | (BCD1302) | |
| 2.2 | CDO718, RZ386 | |
| | RG171● | |
| 10.9 | (RRR-6) | |
| | RZ643 | |
| 8.0 | (RZ324) | |
| 2.7 | RG437 | |
| | RZ599 | |
| 6.8 | (RZ476) | <u>RG544</u> |
| | RG144* | |
| 6.5 | (RG83) | |
| 3.8 | CDO534A | <u>RG509</u> |
| | RZ87 | |
| 5.7 | RG152*, CDO196 | |
| 4.4 | (RZ512) | |
| 1.2 | RZ742A | |
| 3.2 | RZ490 , RG365* | |
| | BCD349 , RZ220 | |
| 5.3 | | |
| | **CYC2** | |
| 5.8 | (RZ957) | |
| | RG555X | |
| | (CDO524, RZ742C) | |

Right chromosome markers:
- d-29
- tri
- bc-3
- bl-1
- chl-10
- d-5
- gh-3
- d-32
- d-30
- gh-2
- spl-2

FIGURE 1.—Continued

# 3 [ 4 ]



FIGURE 1.—Continued

**4** [ 12 ]
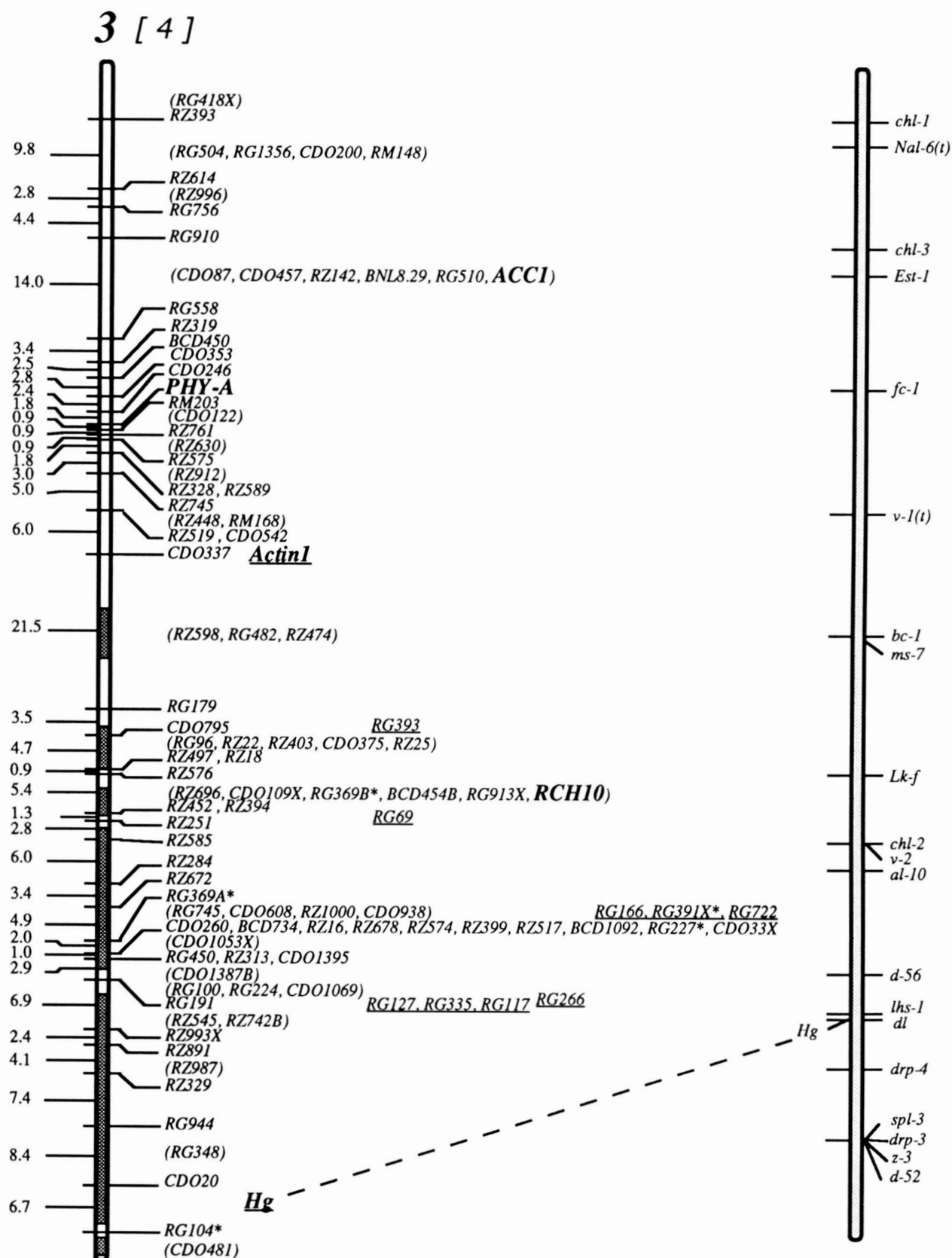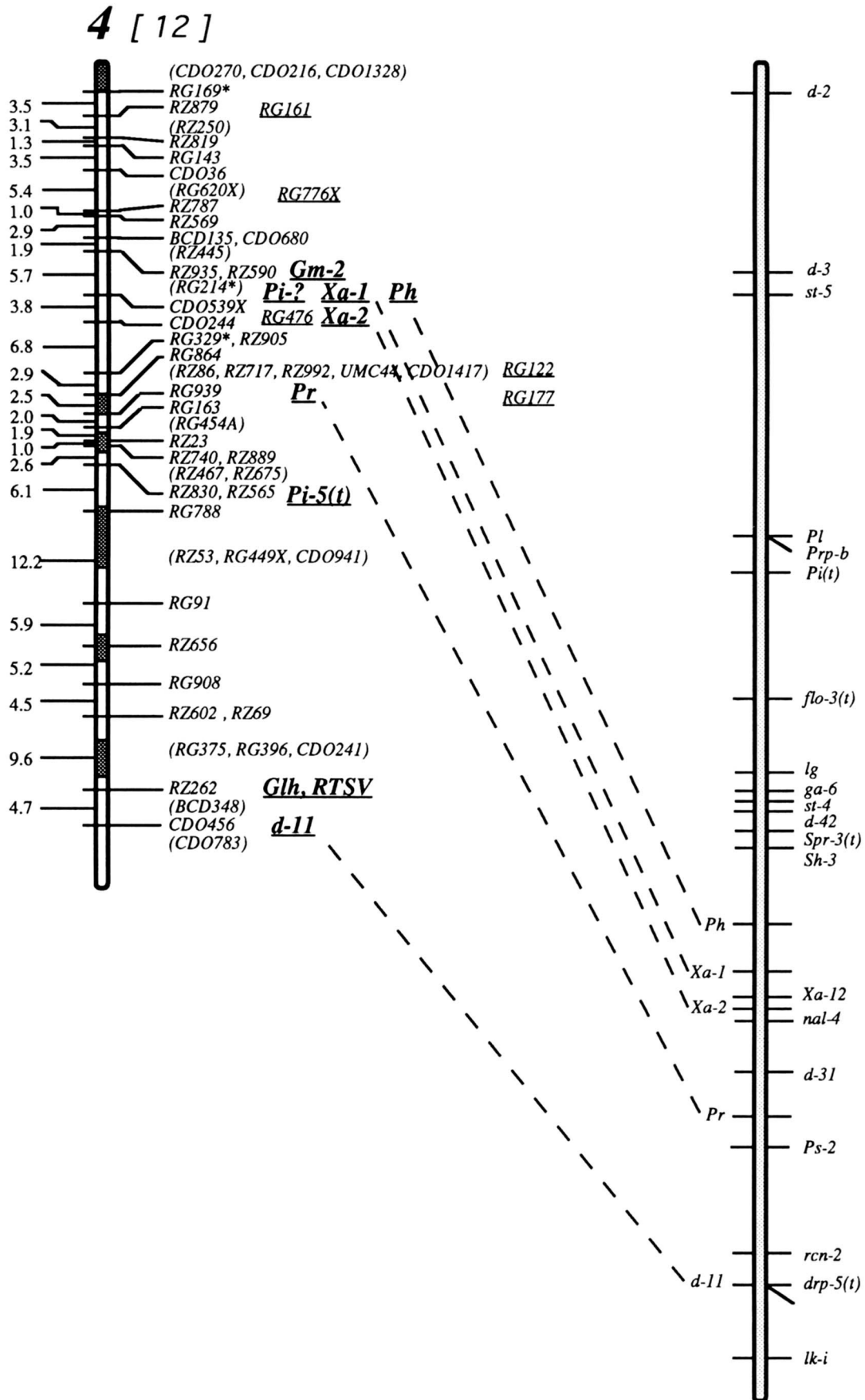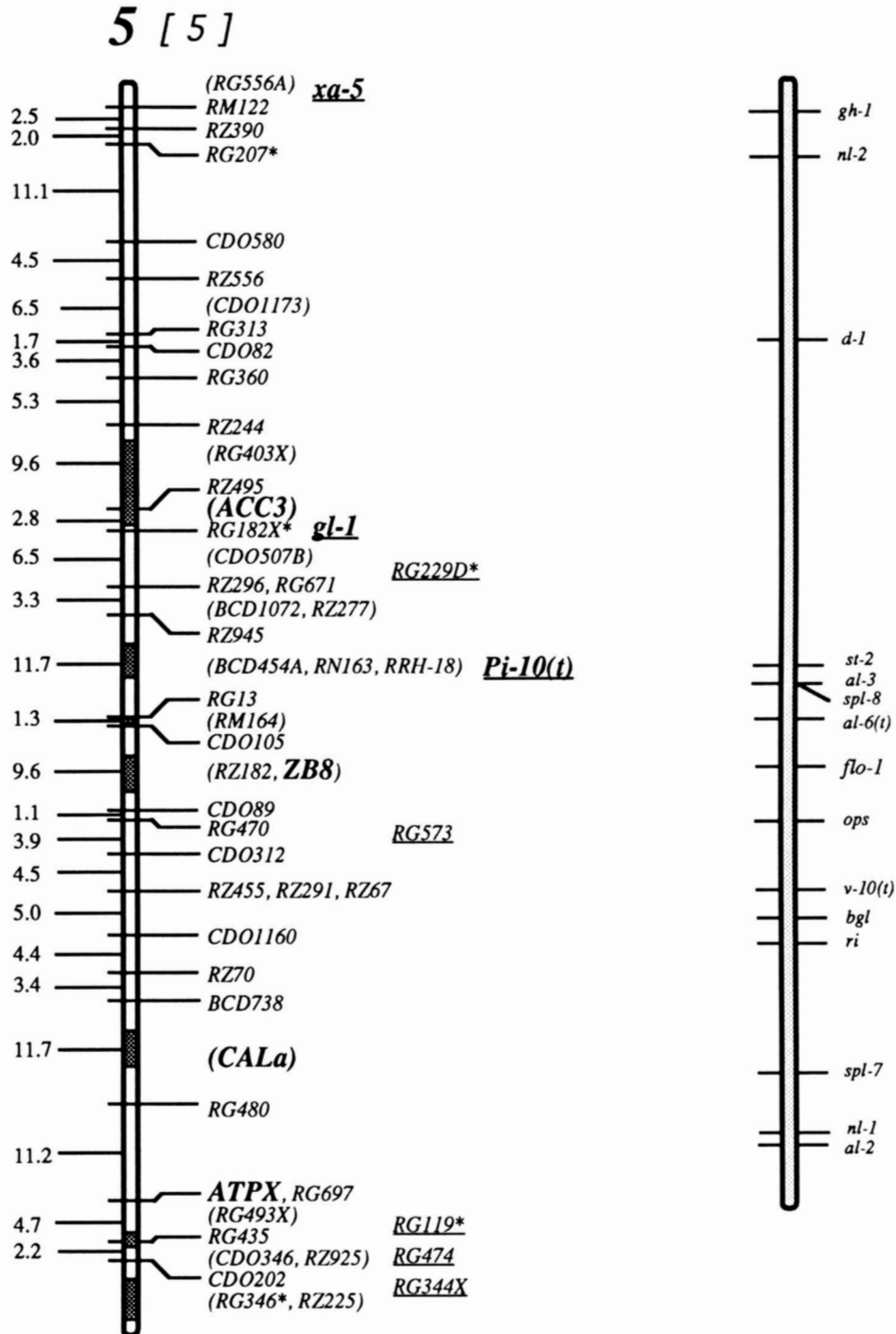


FIGURE 1.—Continued

# 5 [ 5 ]



FIGURE 1.—Continued

are cDNA clones from oat. These and other markers have been distributed to over one hundred researchers worldwide and provide the basis for many gene tagging and QTL studies in rice. For studies involving closely related germplasm, where the general level of polymorphism detectable with RFLP markers may be quite low, it is helpful to avail of multiple markers in a given region. Alternatively, microsatellite markers are proving especially useful in these cases because of their high level of allelic diversity (WU and TANKSLEY 1993a). The wide-

spread use of a common set of publicly available clones has provided a basis for comparison and integration of results in rice genome research and comparative mapping efforts in many parts of the world.

**Comparison of recombination distances:** The recombination fractions between identical pairs of linked markers in the IB population studied by MCCOUCH et al. (1988) and in the SL population studied here were compared to determine whether levels of recombination observed in the intra and interspecific mapping populations were simi-
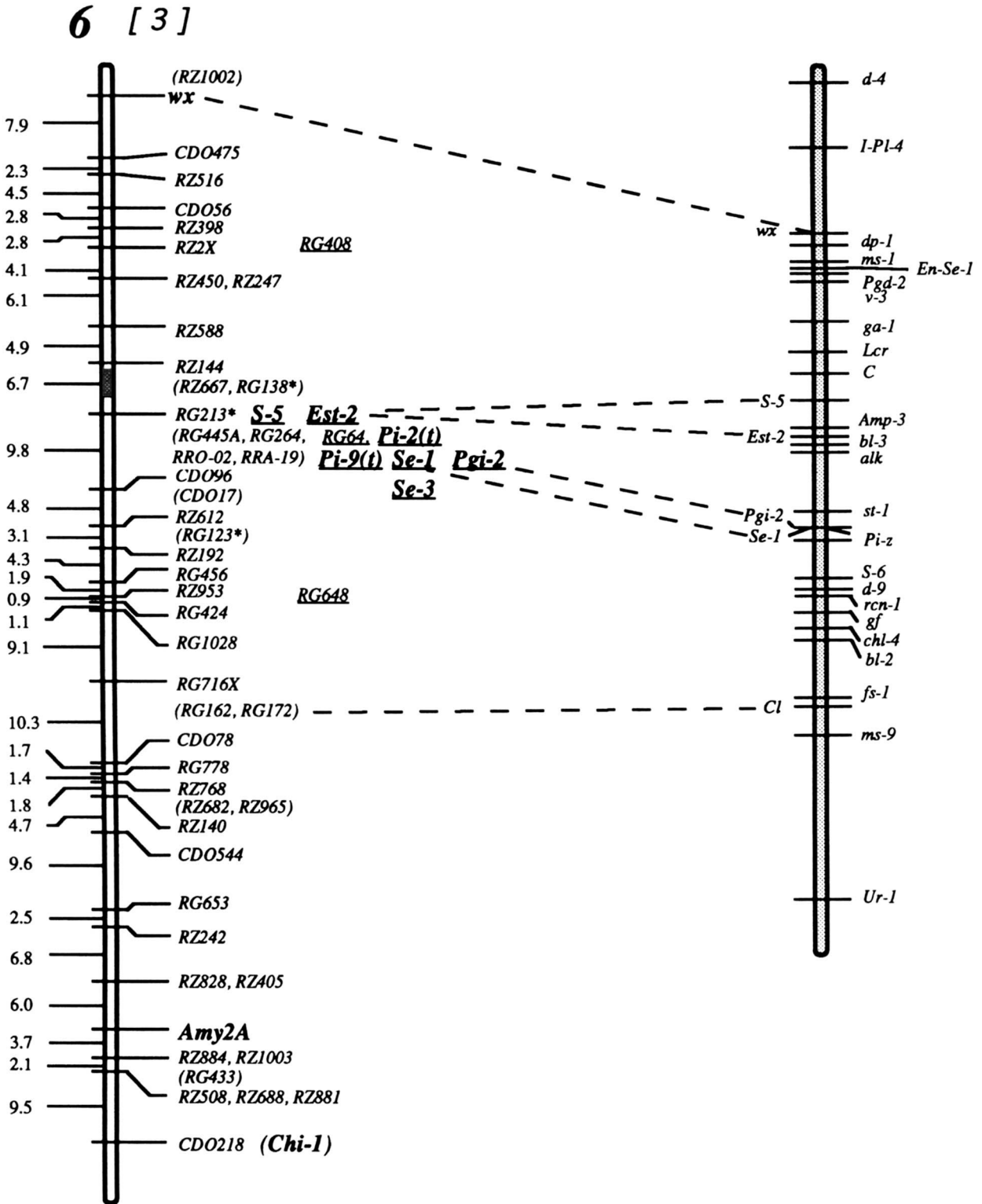
FIGURE 1.—Continued

lar. Based on two-point analysis, 50 intervals located throughout the genome were compared in the two populations. An overall reduction of approximately 25% was observed in the interspecific population. Different selection pressures may act in favor of or against recombination in specific regions. The general reduction of ge-

netic distances between markers observed in this study is in agreement with observations of other interspecific crosses (PATERSON *et al.* 1988; BONIERBALE *et al.* 1988). In interspecific crosses, the homology between DNA strands is reduced, and this is generally related to a reduction in the frequency of chiasmata (GRANT 1958; BORTS and HABER 1987).
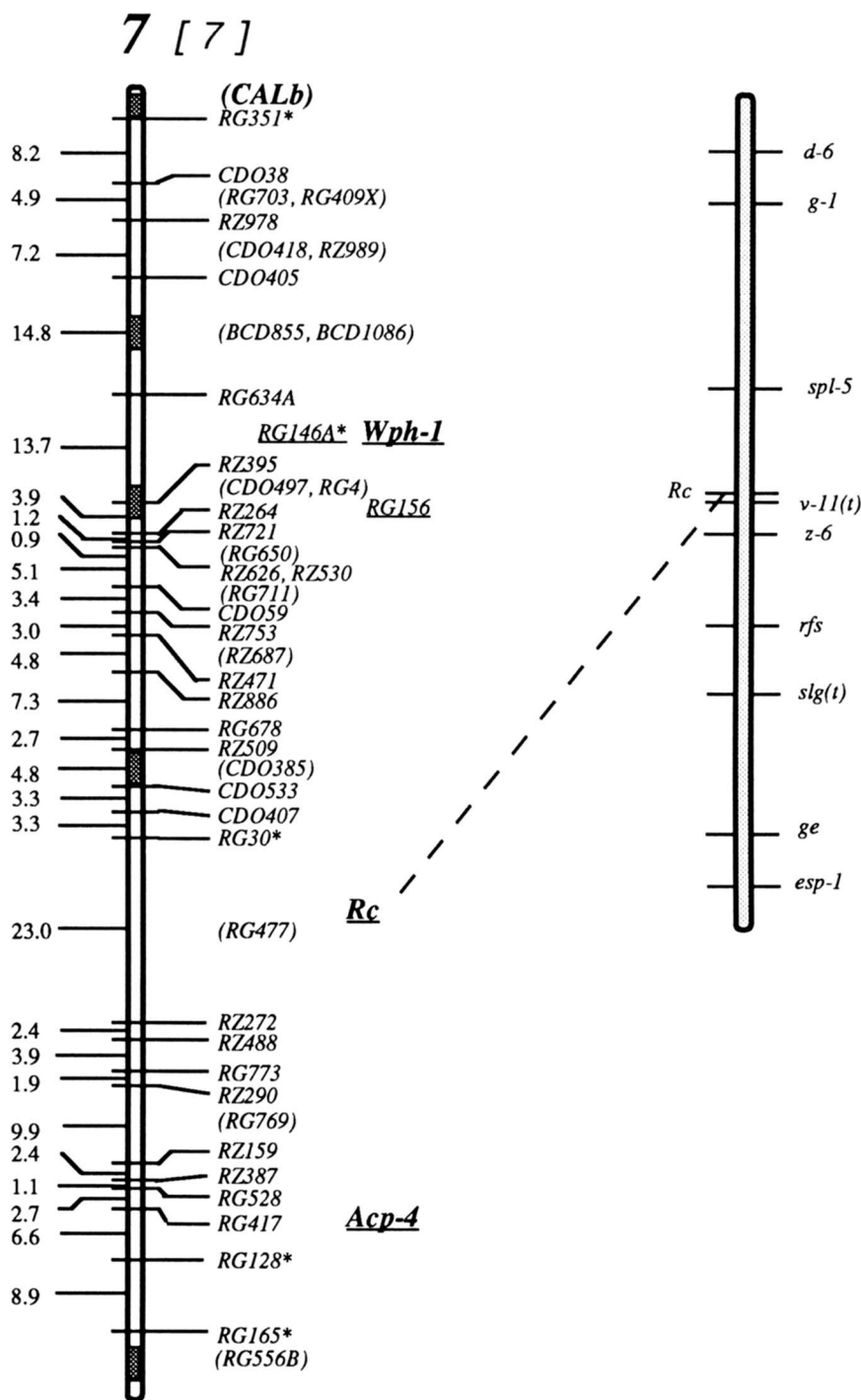
## 7 [7]

```
                    (CALb)
                    RG351*
8.2 ─────
                    CDO38
4.9 ─────           (RG703, RG409X)
                    RZ978
7.2 ─────           (CDO418, RZ989)
                    CDO405

14.8 ─────          (BCD855, BCD1086)

                    RG634A

13.7 ─────          RG146A*  Wph-1
                    RZ395
3.9 ─────           (CDO497, RG4)
1.2 ─────           RZ264        RG156
0.9 ─────           RZ721
                    (RG650)
5.1 ─────           RZ626, RZ530
3.4 ─────           (RG711)
3.0 ─────           CDO59
4.8 ─────           RZ753
                    (RZ687)
7.3 ─────           RZ471
                    RZ886
2.7 ─────           RG678
                    RZ509
4.8 ─────           (CDO385)
3.3 ─────           CDO533
3.3 ─────           CDO407
                    RG30*

23.0 ─────          (RG477)          Rc


2.4 ─────           RZ272
3.9 ─────           RZ488
1.9 ─────           RG773
                    RZ290
9.9 ─────           (RG769)
2.4 ─────           RZ159
1.1 ─────           RZ387
2.7 ─────           RG528
6.6 ─────           RG417        Acp-4

                    RG128*
8.9 ─────

                    RG165*
                    (RG556B)
```

Right chromosome markers: d-6, g-1, spl-5, Rc, v-11(t), z-6, rfs, slg(t), ge, esp-1

FIGURE 1.—Continued

Occasionally, extreme differences in recombination frequency were detected when the SL and the IB maps were compared using two-point analysis between pairs of markers. At one end of the spectrum, three pairs of markers (RG458-RG147 on chromosome 1; RG224-RG100 on chromosome 3; RG125-RG386 on chromosome 9) showing linkage in the IB map appeared completely linked in the SL population. These discrepancies may be the result of differential rates of recombination, or they may be due to small translocations or inversions along the chromosomes of O. sativa and O. longistaminata. The observed lack of recombination may cover long distances on the physical map, as these pairs were separated by as much as 12 cM in the IB map.

At the other end of the spectrum, intervals in which the recombination in the SL population was greater than that observed in the IB population were observed on 6 of the 12 chromosomes. These intervals were not consistently located in any particular region of a chromosome. In two cases, they were associated with regions
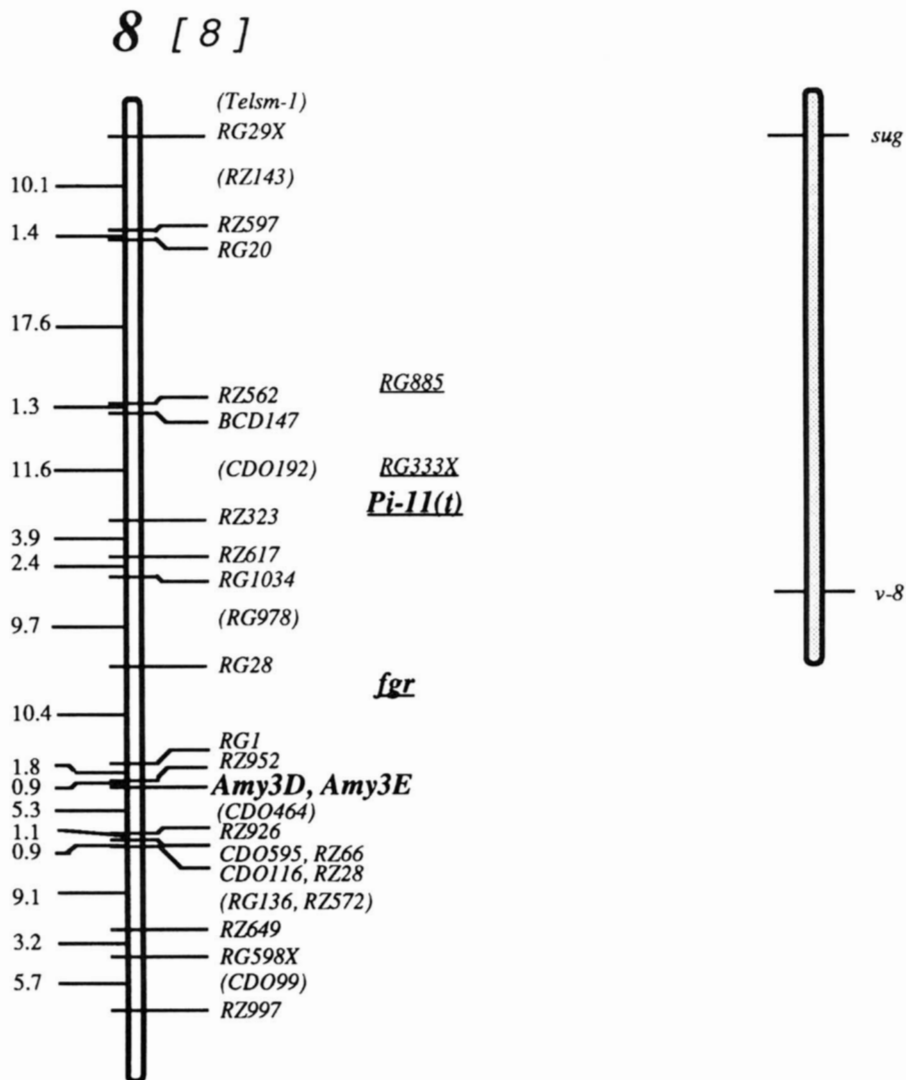
FIGURE 1.—Continued

containing markers that could not be mapped with a LOD > 2.5 (markers in parenthesis on the map), including an interval on chromosome *4* (*RG375-RG396*), and one on chromosome *12* (*RG958-RG323*). In another instance, a higher rate of recombination was observed along a segment of chromosome *6* extending from the *waxy* locus to *RG456*. This region has been reported to have a particularly variable level of recombination. Several workers (YANAGIHARA *et al.* 1992; OKA 1988) have demonstrated drastic differences in estimations of genetic distance between specific markers in this region when crosses between different pairs of *O. sativa* parents are used. Our observations suggest that recombination in the interspecific SL population may exceed that of intraspecific combinations in certain regions.

A consequence of reduced recombination is greater assurance of linkage between markers and ease in recognizing linkage groups in a sparsely populated map. On the other hand, this reduction in map distances lim-

its the ability to precisely order an array of tightly clustered markers when establishing a high density map. For that reason, it is necessary to estimate the precise order of closely linked markers using larger populations and crosses demonstrating a higher rate of recombination in specific regions of interest. Recombinant inbred populations developed via single seed descent offer an alternative way of increasing recombination frequency and simultaneously provide excellent material for mapping quantitative traits of interest (BURR *et al.* 1988; WANG *et al.* 1994). A practical consequence of recombination shrinkage in interspecific crosses is the difficulty in breaking linkage when favorable traits are linked to undesirable ones. However, information about specific regions which are highly recombinagenic in specific cross combinations provides information that can be favorably exploited in a breeding program. Reduction of recombination fractions in interspecific crosses have generally been studied using few morphological markers (RICK 1969; OKA 1988). The use of molecular maps and
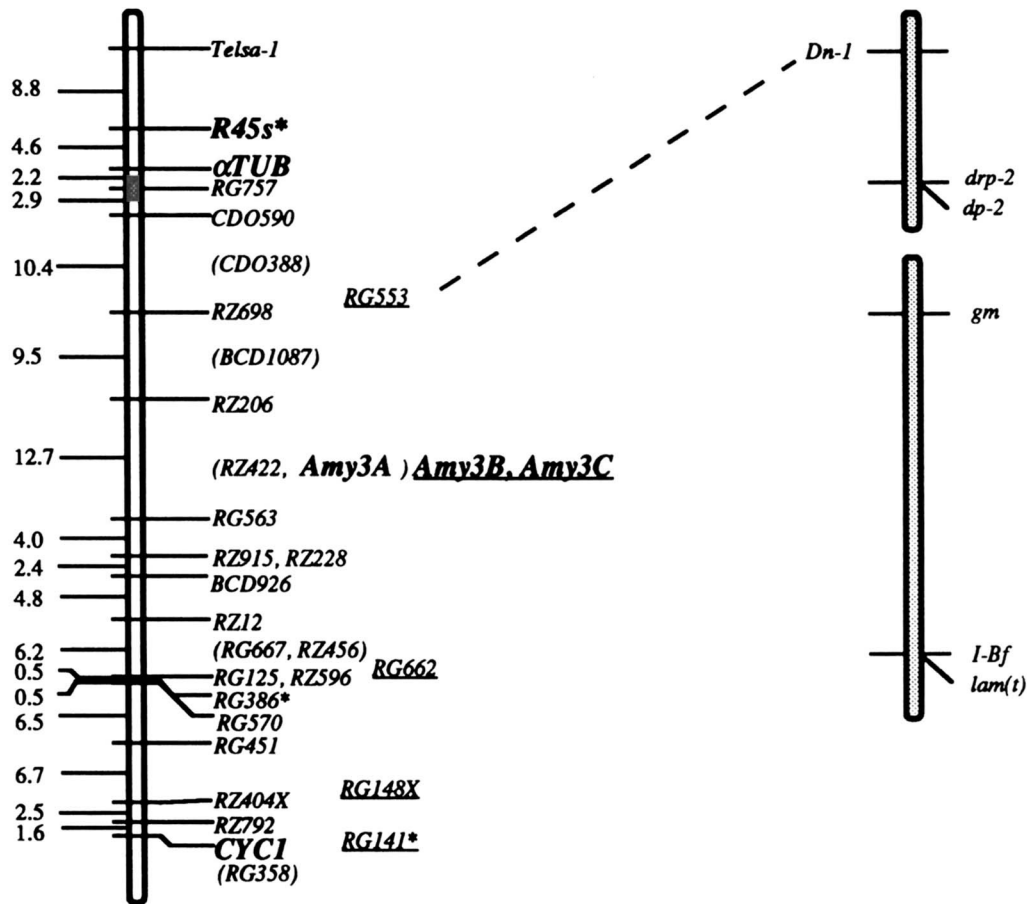
FIGURE 1.—Continued

markers allows us to compare recombination fractions throughout the rice genome for the first time. Fine mapping based on RFLPs or other molecular markers may be helpful in clarifying whether the observed variation is under genetic control and whether it could be exploited to the benefit of plant improvement.

**Genome coverage provided by the interspecific map:** Despite the continuous addition of new markers to an existing map, once a dependable framework has been established for a population, the addition of new markers is not expected to add substantially to the overall length of the map. Indeed, most markers fall within regions already mapped and tend to map very near or on top of markers already on the framework. This would suggest that a map containing 300 markers for a genome the size of rice would provide fairly complete genome coverage. Based on an exchange of 70 markers, a comparison of the maps prepared by SAITO et al. (1991) and that reported by TANKSLEY et al. (1992a) suggested that these two independently constructed rice maps provided very similar genome coverage and that the order of markers along the chromosomes was in good agreement (XIAO et al. 1992). However, both maps contained

several persistent, sparsely populated regions, appearing as large intervals. In some cases, these large intervals fell in comparable regions of the chromosomes, such as the region on chromosome 3 defined by RG96, RG179 and CDO337, or that on chromosome 11 defined by RG1094 and RG118.

Our decision to use an interspecific cross provided us with an opportunity to test the hypothesis that regions of chromosomes might be monomorphic in a cross between two cultivated rice species but polymorphic in a cross between a cultivar and a wild species. The efficiency of this approach was demonstrated, as mapping in the interspecific cross allowed us to readily assemble 12 linkage groups without the unlinked segments that remained in our intraspecific map with the same number of markers. It also allowed us to define a region of approximately 80 cM on chromsome 3 which was mapped for the first time using the SL population.

**Skewing in the interspecific backcross:** Sixteen percent of the markers mapped in the interspecific backcross population exhibited skewed segregation ratios. Deviations from the expected 1:1 allele frequency were
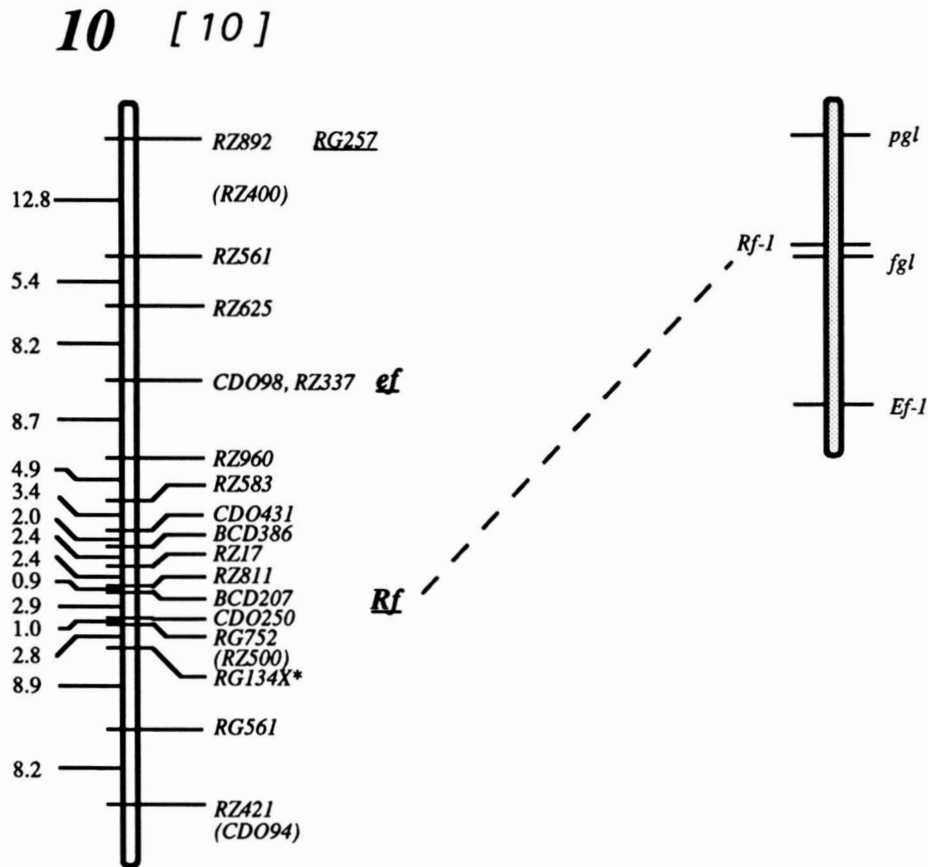
FIGURE 1.—Continued

encountered for markers mapping to nine of the chromosomes (Figure 1). Almost half (46/94) of the skewed markers mapped to chromosome *3*. All of the skewed loci on chromosomes *4* and *5*, and one marker each on chromosomes *2* and *11* (*RZ742C* and *RG98*), deviated in favor of the *O. longistaminata* allele (28/94), while markers on all the other chromosomes deviated in favor of the *O. sativa* allele (66/94). The mean frequency of the *O. longistaminata* alleles in the backcross population, over all the marker loci, was 49%. This statistic was in accordance with observations of allele frequencies at isozyme loci in similar backcross populations (CAUSSE and GHESQUIÈRE 1991, 1992). Distortions are common in interspecific and intersubspecific populations and their origin has been discussed by several authors (IWATA *et al.* 1964; TANKSLEY 1983; ZAMIR and TADMOR 1986; SATO *et al.*, 1990; LIN *et al.* 1992; LIN and IKEHASHI 1993). Because the ability to measure recombination is limited to alleles coming from only one parent in a backcross population, these populations are less sensitive to skewing than are $F_2$ populations. The frequency of markers detecting skewed segregation was similar in both the interspecific population and the previously mapped *indica* × *japonica* population (MCCOUCH *et al.* 1988).

It is interesting to note that markers in the same region of chromosome *3* were reported as skewed by MCCOUCH *et al.* (1988) as on the current map (Figure

1). The segregation distortion in the population studied here can be used to identify the map position of the genetic factor(s) responsible for this phenomenon (Figure 3). NAKAGAHRA (1972) has shown that this region of the genome is involved in the sterility mechanisms which isolate the *indica* and *japonica* subspecies of *O. sativa*. The sterility is due to gametophytic selection, systematically favoring *indica* alleles. Whether an identical mechanism is involved in regulating fertility in this interspecific cross is not clear at this time. The mechanism may be similar, though *O. longistaminata* alleles tended to be favored over *indica* alleles in the population studied here. On the other hand, the sterility may be due to a different mechanism which is controlled by one or several genes residing in the same chromosomal region. The *O. longistaminata* × *O. sativa* $F_1$ hybrid showed very low male fertility; less than 20% of the pollen grains were stainable by ALEXANDER (1969) solution (GHESQUIÈRE 1988). Though backcross seeds could be obtained, the success rate of the back crosses was incomplete, and specific selection could have taken place at this step. In addition, during germination or later development, some plants were weak or developed poorly and were discarded from the mapping population. The reproductive barrier which isolates *O. longistaminata* from the other *Oryza* species represents a possible cause of deviation in the allele frequencies. It would be interesting to find out whether the same ste-
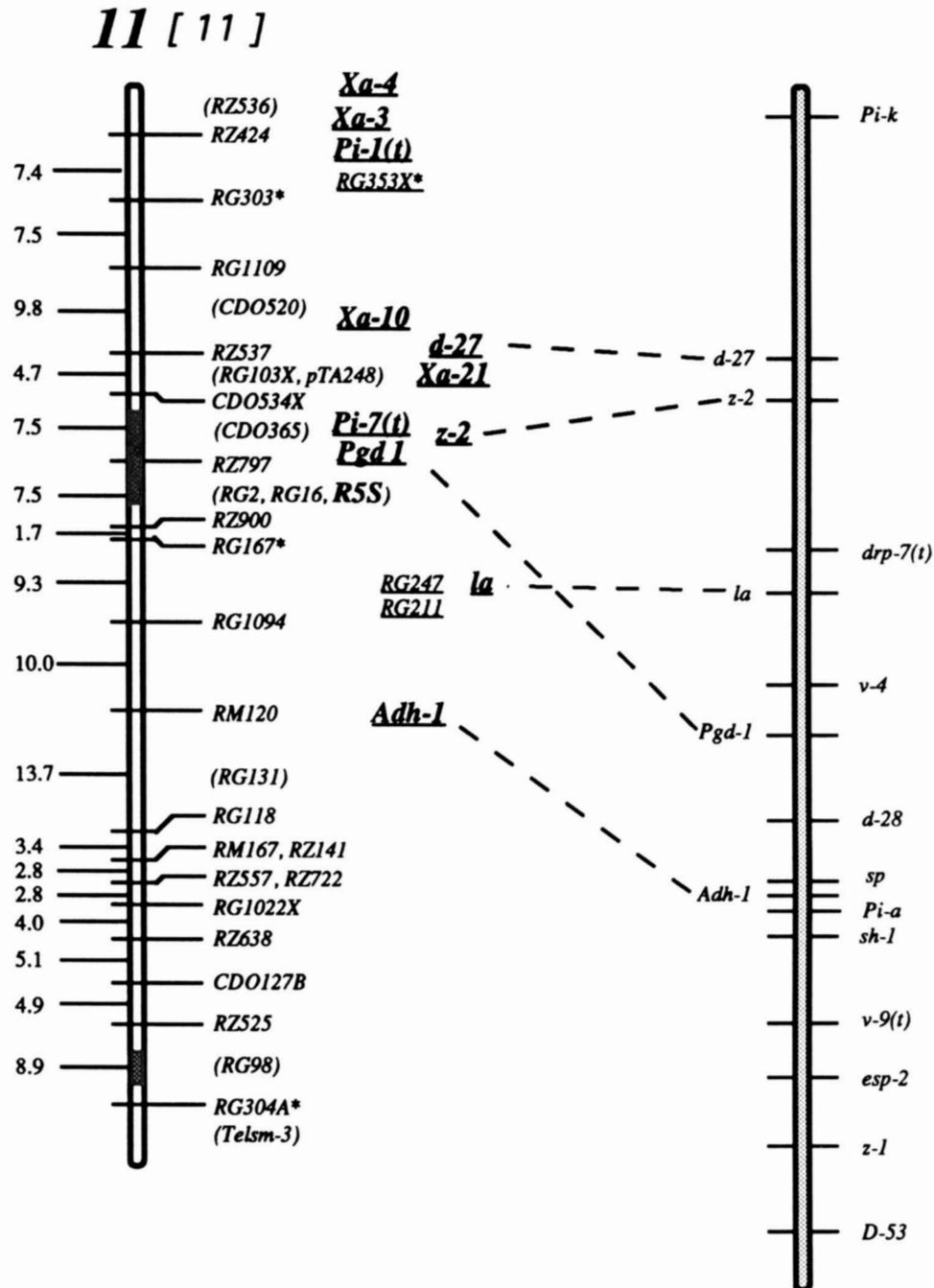
## 11 [ 11 ]



FIGURE 1.—Continued

rility mechanism (outcrossing barrier) is operating in *indica* × *japonica* and in interspecific crosses. PATERSON *et al.* (1990) proposed an approach based on substitution mapping to localize small chromosomal fragments involved in segregation distortions. Studies with subsequent backcross generations from this interspecific cross could be performed to fine map the region associated with the deviations observed here.

### DISCUSSION

The use of cDNA libraries to efficiently construct molecular maps provides a basis for comparative mapping in distantly related species, such as rice, oat, barley,

wheat, maize, and sugarcane. Maps based on common sets of probes constitute a basis for the comparison of genome organization and evolutionary change, as was first demonstrated with *Solanaceae* species (BONIERBALE *et al.* 1988; TANKSLEY *et al.* 1992b), and later in sorghum and maize (HULBERT *et al.* 1990; WHITKUS *et al.* 1992) and wheat, barley, and rye (DEVOS *et al.* 1992, 1993). While genomic clones work well when the species being compared are very closely related, as with tomato and potato or sorghum and maize, genomic sequences are often not well enough conserved to allow comparative mapping across more distantly related genomes. This was demonstrated in the case of rice genomic *Pst*I
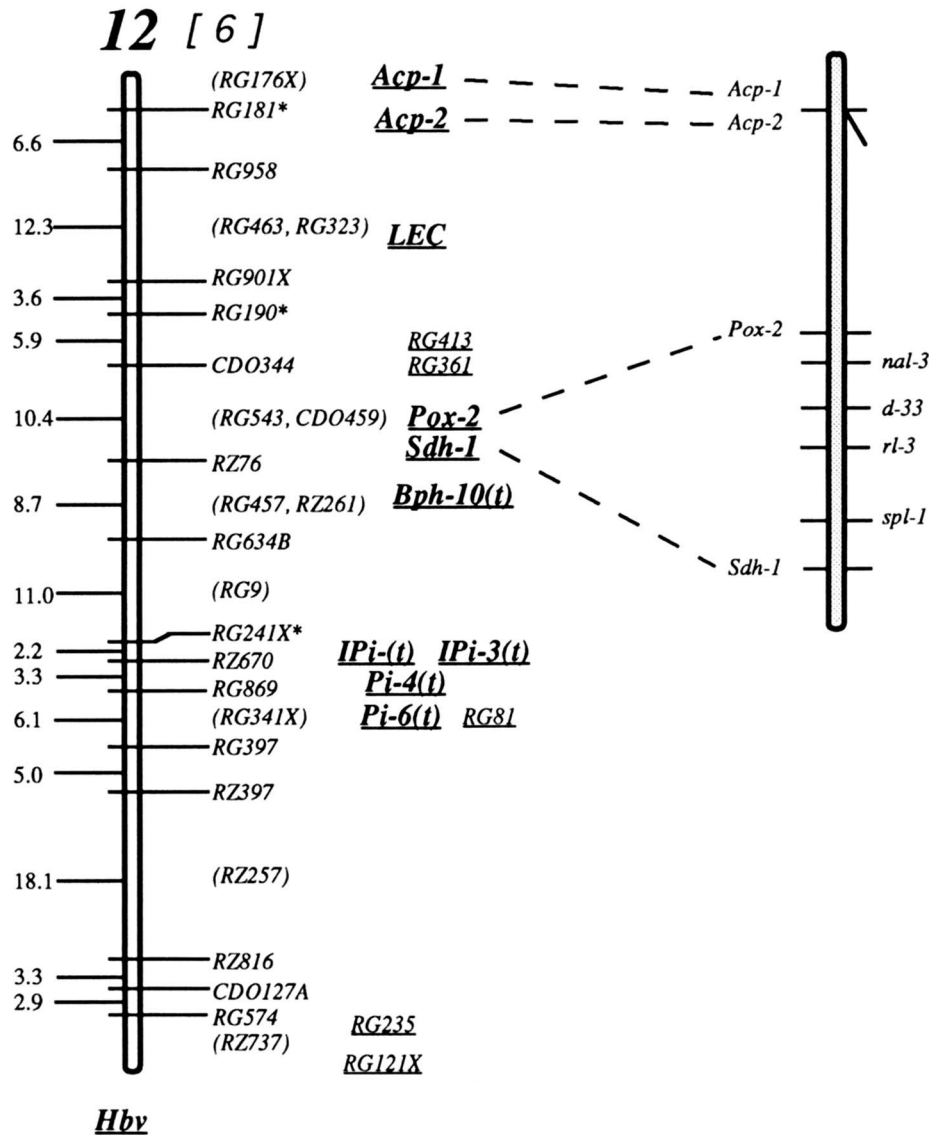
## 12 [ 6 ]

Hbv

*12* chromosome map markers (top to bottom):

(RG176X)
RG181*
6.6
RG958
12.3
(RG463, RG323)   *LEC*
RG901X
3.6
RG190*
5.9
CDO344   RG413 RG361
10.4
(RG543, CDO459)   *Pox-2*
  *Sdh-1*
RZ76
8.7
(RG457, RZ261)   *Bph-10(t)*
RG634B
11.0
(RG9)
RG241X*
2.2   RZ670   *IPi-(t)   IPi-3(t)*
3.3   RG869   *Pi-4(t)*
6.1   (RG341X)   *Pi-6(t)*   RG81
RG397
5.0
RZ397
18.1
(RZ257)
3.3
RZ816
2.9
CDO127A
RG574
(RZ737)   RG235
  RG121X

*Acp-1* — — — — — Acp-1
*Acp-2* — — — — — Acp-2

Pox-2
Sdh-1

nal-3
d-33
rl-3
spl-1

FIGURE 1.—Continued

probes, which frequently gave poor signal on oat and barley. On the other hand, cDNA sequences are sufficiently conserved to cross hybridize with a wide range of species and allowed comparative maps to be developed between rice, wheat, maize and other cereals (AHN and TANKSLEY 1993; AHN *et al.* 1994; KURATA *et al.* 1994; VAN DEYNZE *et al.* 1994). In some instances, cDNAs have been demonstrated to hybridize clearly across much greater evolutionary distance, as is the case with *ATPase* which was cloned out of tobacco (N. CHUA, Rockefeller University, personal communication), or the *Pto* gene which was cloned out of tomato, and gives a clear hybridization signal on a wide range of plants, including rice (MARTIN *et al.* 1993).

The interspecific population has been found to be much more efficient than most intraspecific crosses; on average, one restriction enzyme detected 54% polymorphism, while a single enzyme detected only 34% and 14% in the two intra species crosses examined in this study. The mapping population can be easily multiplied through shoot cuttings, maintained over years, and can be distributed to researchers who would like to map cloned genes. The resolution of our map is limited by the size of the population, but higher resolution mapping in specific regions of interest can be readily accomplished by selecting clones which map to the target region and analyzing them on larger populations, or by pooling individuals for targeted addition of markers (GIOVANNONI *et al.* 1991; MICHELMORE *et al.* 1991).

One of the main uses of the RFLP map of rice is to locate markers linked to genes of interest, both single gene and quantitatively inherited characters. Over 80 genes of agronomic importance have been located on the map (Table 1) and are identified in bold in Figure 1. The SL population has also been used to locate genes related to specific traits of *O. longistaminata*, such as growth habit (T. FULTON, unpublished data) and bacterial blight resistance. This species (though a different accession) was
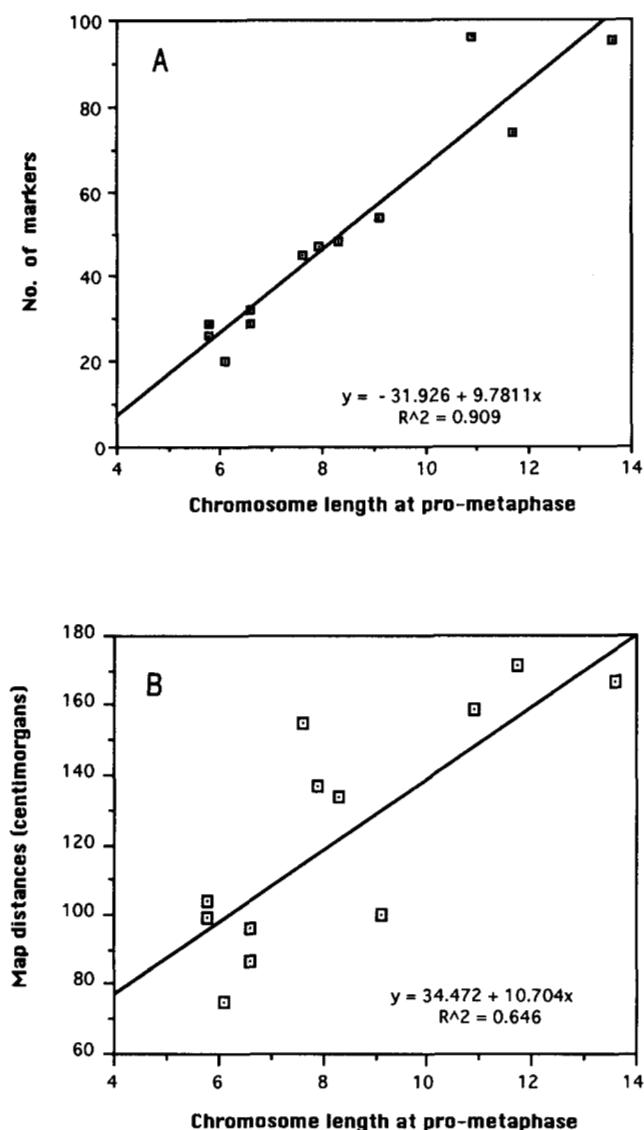
FIGURE 2.—Relation between the relative length (RL) of chromosomes measured at pro-metaphase (FUKUI and IIJIMA 1991) and (A) the number of markers per chromosome; (B) the chromosome length in centimorgans on the RFLP map. The chromosome numbering follows the system established by the Rice Genetics Cooperative Committee, in IRRI, May 1990.

the source of the Xa-21 gene for broad spectrum resistance to bacterial leaf blight (IKEDA et al. 1990; KHUSH et al. 1991). VALES (1985) showed that this species also has genes conferring specific resistance to blast. O. longistaminata has also interested rice breeders for its allogamy traits: it has the longest anthers and stigmas among the Oryza species, and these traits have been found to be correlated with the outcrossing behavior of plants (CAUSSE and GHESQUIÈRE 1991). Production of varieties with long, well exerted stigmas would be valuable in the production of hybrid seeds at a lower cost (VIRMANI et al. 1982).

It is generally argued that reduced recombination and skewed segregation impose limits to the ability to introgress traits from wild species into cultivated forms of rice. We have shown that in spite of an overall reduction of recombination, the actual frequency of recombination in this interspecific cross remained high. Though a strong reproductive barrier isolates the wild species from the cultivars, segregation deviation did not constitute a more important problem than within intersubspecific O. sativa crosses. Unfavorable genes are numerous in wild species and molecular marker-assisted studies of the inheritance of these traits may help to get rid of such deleterious effects. In addition to O. longistaminata, species which are closer to O. sativa, such as O. rufipogon, represent sources of interesting genes for rice improvement. RFLP maps constitute a powerful tool for following introgressions (YOUNG et al. 1988) and separating desirable from unfavorable traits (DE VICENTE and TANKSLEY 1993; PATERSON et al. 1990). PANAUD (1992) and P. REIMERS and R. NELSON (IRRI, personal communication) demonstrated the use of RFLP markers to identify which chromosome had been inherited from a wild species in alien addition lines derived from remote crosses.

Finally, our results offer a foundation for the use of rice as a genetic model among grass species. Recent estimations based on flow cytometry suggest that the amount of DNA in a haploid nucleus (C-value) is about 0.45 pg (ARUMUGANATHAN and EARLE 1991). In addition, it has a large proportion of single copy DNA (approximately 85% at high stringency) (McCOUCH et al. 1988). With a map of 1491 cM, 1 cM corresponds to an average of approximately 300 kb. Physical mapping experiments have demonstrated that this estimate is reasonably accurate (RONALD et al. 1992) and that in telomeric regions, the recombination frequency may be as high as 1 cM:30–50 kb (WU and TANKSLEY 1993b). The relationship between physical and genetic distance estimated from the interspecific map represents a conservative estimate, as the total number of cM in the rice genome is greater when mapping is done on an intraspecific cross, making the estimated kb:cm ratio nearer 1 cM:250 kb. In either case, the ratio of genetic to physical distance in rice is the smallest for any monocot known, and is only slightly higher than that for Arabidopsis (150–200 kb per cM). This estimate can be compared to tomato, which has an average distance of 700 kb per cM, or to other monocots such as maize, with an average of 2000 kb per cM. The first successful map-based cloning gene experiment in any crop plant was recently reported for the Pto gene in tomato (MARTIN et al. 1993), providing evidence that saturated molecular maps can be productively employed in the isolation of genes whose protein product is unknown. Because of its small genome, high proportion of single copy DNA, high recombination frequency, densely populated genetic map, and relative ease of transformation, rice represents an ideal candidate as the basis for comparative mapping studies among monocot species, for

FIGURE 3.—Scatter plot of allele frequencies of markers in highly skewed region of chromosome *3*. Horizontal line represents expected 1:1 ratio in a backcross population. Rectangle at 40 cM region represents cluster of 10 markers showing no recombination in center of highly skewed region where a gene or cluster of genes associated with sterility are putatively located.
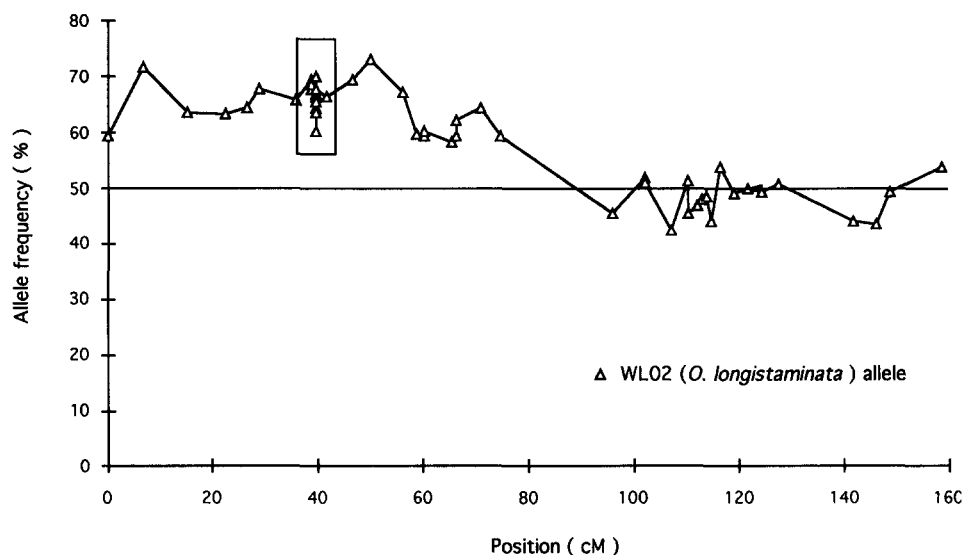
## map-based gene isolation, and for transformation in crop improvement.

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Communicating editor: B. BURR