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Orientation and integration of the classical and molecular genetic maps of chromosome 11 in rice

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Summary

The classical genetic map and molecular map of rice chromosome 11 were oriented to facilitate the use of these maps for genetic studies and rice improvement. Three morphological markers (*d-27, z-2,* and *la*) were crossed to a rice breeding line, IRBB21, which has the *Xa-21* gene for bacterial blight resistance. Three F_2 populations were analyzed with RFLP markers known to be located on chromosome 11. Segregation analysis of molecular markers and morphological markers was used to construct an RFLP map for each population. The recombination frequency between markers varied from population to population although the marker order on the maps was the same for all three populations. Based on a common set of markers mapped in the three populations, an integrated map was generated consisting of both RFLP and morphological markers. The genetic distance between markers on this map was determined by taking a weighted average of the data from the three populations. The oriented map serves as a bridge to understand the relationship between the classical and molecular linkage maps. Based on this information, the location of several genes on the classical map can be approximated with respect to RFLP markers without having to map them directly.

Introduction

To facilitate rice breeding, numerous rice geneticists have, over the past 75 years, studied the inheritance of a large number of marker genes associated with a variety of morphological, physiological, biochemical, and other characters. As a result of these studies, a classical genetic map of rice representing 210 marker genes on twelve linkage groups has been developed (Khush & Kinoshita, 1991). Recently, molecular markers such as isozymes (Wu et al., 1988; Ishikawa et al., 1991), restriction fragment length polymorphisms (RFLP) (McCouch et al., 1988), and polymerase chain reaction (PCR)-based markers (Reiter et al., 1992) have provided hundreds of new markers which can be readily mapped on a single cross. The rapid development of these molecular markers has resulted in the construction of two independent molecular maps of rice (McCouch et al., 1988; Saito et al., 1991). As a result, there are now three independent rice maps, substantially increasing our understanding of the rice genome. However, the information in all three maps cannot be readily cross-referenced, limiting the full exploitation of the information contained in them. Efforts to orient the two molecular maps have been initiated (Xiao et al., 1992), and mapping of morphological mutant phenotypes in relation to RFLP markers is underway (Tanksley et al., 1992; Saito et al., 1991; Ideta et al., 1992; Kishimoto et al., 1992). Because most of the genetic stocks representing morphological mutant phenotypes have only one or a few mutant genes in each, a large number of crosses is required to integrate the morphological and molecular maps.

The integration of the classical genetic and molecular maps would make molecular tagging of agronomically important genes previously located on the classical genetic map relatively straightforward. The amount of time and effort devoted to gene mapping will be minimized because only molecular markers which are located in the region of the target genes need to be eval-

uated. The objective of our work is to orient the classical genetic and the molecular maps of rice. Because of the presence of many genes of agricultural importance on chromosome 11 (Kinoshita, 1990; Khush & Kinoshita, 1991), we oriented the classical genetic and molecular maps of rice on this chromosome.

In this study, a disease resistance locus and three morphological mutant markers served as points of alignment to orient the classical genetic and molecular maps of rice. This was accomplished by mapping genes controlling these four characters onto the molecular map.

Materials and methods

Plant materials

Three morphological mutant markers in japonica background – bunketsuto tillering dwarf (*d-27*) (Iwata & Omura, 1977; Iwata et al., 1978), zebra banding pattern (*z-2*) (Iwata & Omura, 1977; Iwata et al., 1978) and lazy growth habit (*la*) (Nagao & Takahashi, 1963; Iwata et al., 1978) – were crossed with IRBB21, an indica line in IR24 background containing the gene *Xa-21* for resistance to bacterial leaf blight (Ikeda et al., 1990; Khush et al., 1990). F_2 populations were developed from all three crosses (Table 1). These crosses were previously used to locate the *Xa-21* gene in relation to morphological markers on chromosome 11 of the classical genetic map of rice (Ikeda et al., 1991). Sibling F_2 populations were used for RFLP analysis to determine the location of the morphological mutants and *Xa-21* on the molecular map of chromosome 11.

Bacterial leaf blight screening

The parents and the three F_2 populations were grown in a screenhouse. At booting stage, the plants were inoculated with PXO99 (race 6) of *Xanthomonas oryzae* pv. *oryzae* using the leaf clipping method (Kauffman et al., 1973). Leaf lesions from the three youngest inoculated leaves were examined and assigned resistant (R) and susceptible (S) scores.

DNA extraction, restriction digestion, electrophoresis, Southern analysis

DNA was extracted from fresh leaves harvested from the parents and each F_2 plant six weeks after seeding (Dellaporta et al., 1983; Tai & Tanksley, 1990). DNA (5 μg/lane) from the parents was digested with four restriction enzymes, *Eco*RI, *Eco*RV, *Hin*dIII and *Xba*I, and electrophoresed through 0.9% agarose gels using standard protocol (Sambrook et al., 1989). Southern transfer to nylon membranes (Hybond N^+, Amersham Inc.) was done according to manufacturer's instructions. Twenty-eight chromosome 11 RFLP markers (prefix RG and CDO provided by Cornell University, Ithaca, NY) and eight chromosome 11 markers (prefix NpB provided by the National Institute of Agrobiological Resources, Tsukuba, Japan) were PCR-amplified, hexamer-labelled with ^{32}P-dCTP to high specific activities (1-10 $\times$ 10^8 cpm/μg) (Feinberg & Vogelstein, 1983) and used as probes for the parental survey filters. Hybridization, washing, and autoradiography were done using the methods of McCouch et al. (1988).

F_2 DNA was digested with *Eco*RI and *Hin*dIII as clear RFLP could be detected even with just these enzymes. Molecular markers were hybridized with the appropriate F_2 filters, according to the polymorphism patterns observed on parental survey filters.

Linkage analysis

The autoradiograms of the segregating populations were scored according to the inheritance of bands from the parents in the F_2 populations. RFLP analysis was done using the MAPMAKER computer program (Lander et al., 1987). Two-point (LOD = 4.0), three-point (LOD = 3.0), and multipoint (LOD = 3.0) analysis were used to establish marker order and recombination fractions.

Map orientation

The three maps (Fig. 2), each with one morphological mutant marker, were integrated into one map (Fig. 3B) by aligning common RFLP markers used in the three populations. Considering the difference in population size and the use of a common parent, IRBB21, in all three populations, the weighted average of genetic distance between molecular markers was used to create an integrated map. The classical genetic and molecular maps of chromosome 11 were then oriented based on the correspondence of three common morphological markers (*d-27, z-2*, and *la*), one bacterial leaf blight resistance gene (*Xa-21*), one isozyme marker (*Adh-1*) (Magpantay, McCouch & Huang, unpublished data), and seven common RFLP markers.

Table 1. Morphological markers in F_2 populations used to integrate the morphological and molecular maps of rice chromosome 11

Marker	Cross	DNA Polymorphism Level (%)	No. of Plants in F_2			X^2 (3 : 1)
			Normal	Mutant	Total	
Tillering dwarf	*d-27/Xa-21*	83.3	21	14	35	3.44
Zebra banding	*z-2/Xa-21*	86.1	41	22	63	2.80
Lazy growth	*la/Xa-21*	83.3	48	13	61	0.27

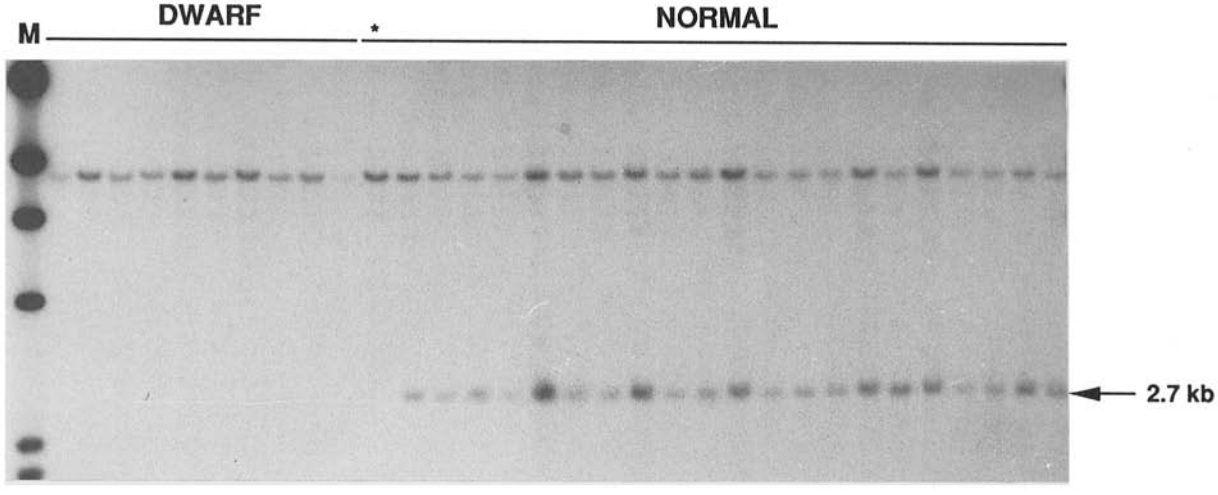

Fig. 1. Autoradiogram of *Hin*dIII digested total DNA of representative F_2 population from the cross *d-27*/IRBB21 hybridized with the probe pTA818. Arrow indicates 2.7 kb band cosegregating with normal phenotype. Dwarf phenotypes cosegregate with null allele. M indicates molecular weight marker Lambda DNA digested with *Hin*dIII. * indicates recombinant individual.

Results

Parental survey

Parental surveys were performed using 36 molecular markers from the entire length of chromosome 11. Extensive polymorphism was observed among the mutant parents and IRBB21 (Table 1). Clear RFLP on 31 markers were detected in the population between IRBB21 and the Zebra banding mutant. No polymorphism was observed with the other five markers, CDO127, RZ141, RZ557, RZ638, and Npb254. Among the polymorphisms found between IRBB21 and the three morphological mutant lines, the banding pattern of 24 markers was identical for all three mutants. A previous study (Ronald et al., 1992) indicated that *Xa-21* was located on the top half of chromosome 11, and Ikeda et al. (1990) showed that *d-27*, *z-2*, and *la* were also located in the same general region as *Xa-21* on chromosome 11. Molecular markers from that region of the chromosome were thus chosen as probes for use with progeny filters.

Segregation of the F_2 populations

The segregation of normal and mutant phenotypes in the F_2 populations followed the expected 3 : 1 ratio (Table 1). Similar Mendelian segregation patterns (3 : 1 for null allele and 1 : 2 : 1 for codominant markers) were also observed among the RFLP probes used. Figure 1 shows an autoradiogram of representative F_2 plants from the *d-27*/IRBB21 population probed with pTA818. The dwarf phenotype cosegregated with a null allele when total DNA was digested with *Hin*dIII. The normal phenotypes cosegregated with a 2.7 kb band. Two recombinants were observed (plant # 4-2-7 and #

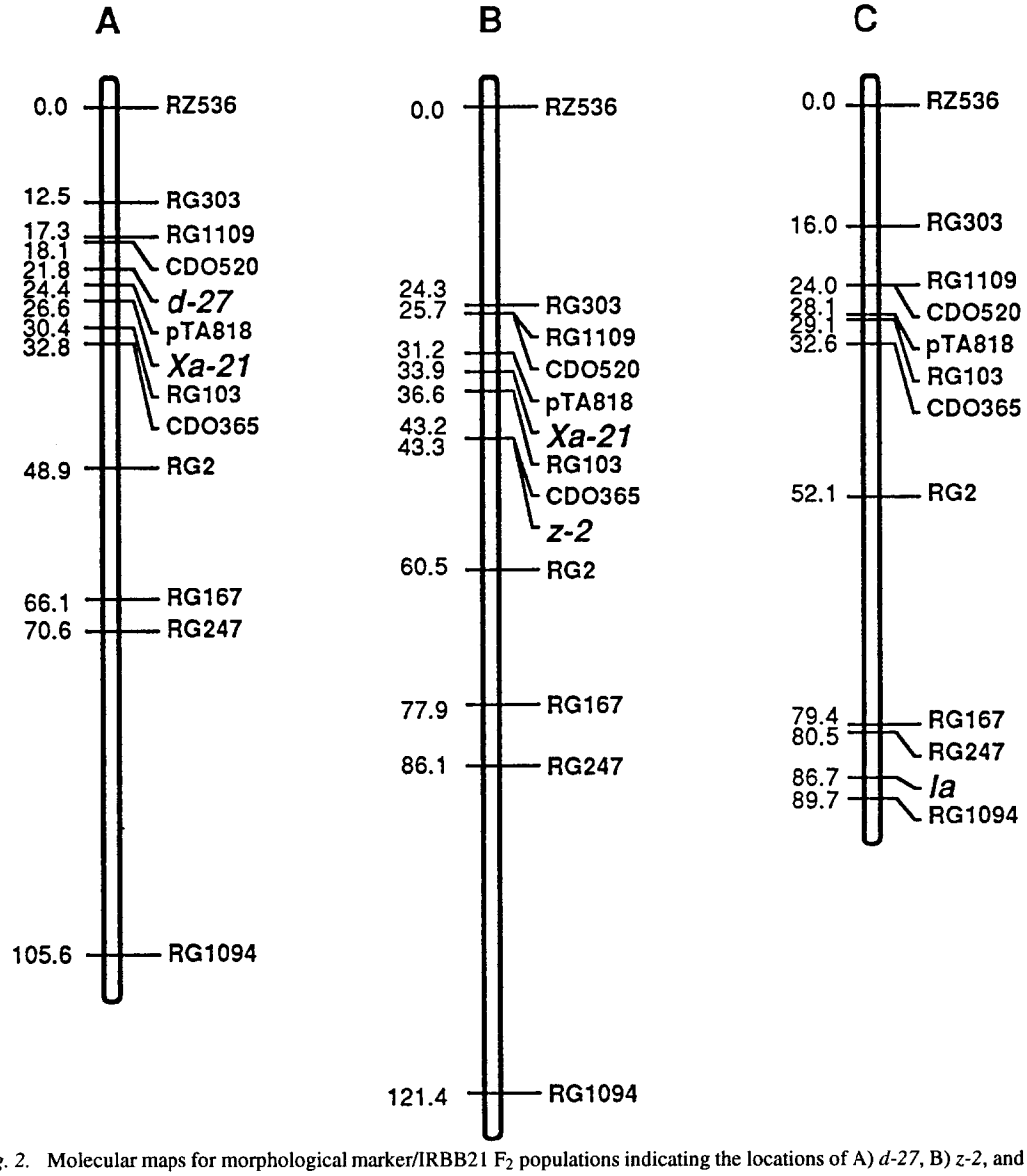

Fig. 2. Molecular maps for morphological marker/IRBB21 F_2 populations indicating the locations of A) *d-27*, B) *z-2*, and C) *la*.

4-5-3); both had normal phenotype but carried the null allele.

Linkage maps of morphological mutant markers

The maps presented in Fig. 2 represent the order of markers based on multipoint linkage analysis. After a trial map was created, the distances were checked using two-point and three-point analysis until a map with the shortest total distance was achieved. The RFLP marker order in each population is in agreement with that of McCouch & Tanksley (1991). The maps in Fig. 2 show the locations of the morphological mutant markers with respect to the RFLP markers. The marker *d-27* is flanked by CDO520 and pTA818, near the top of chromosome 11. CDO365 cosegregated with the *z-2* mutation. The lazy phenotype was mapped between RG247 and RG1094 near the middle of chromosome 11. In a previous study, RG103 and pTA248 were both determined to be within 1.2 cM of *Xa-21*, based on segregation of 386 individuals from a cross between IR24 and IRBB21 which carries a *Xa-21* from wild species *Oryza longistaminata* (Ronald et al., 1992). In the morphological mutant populations used in this

study, pTA818 and RG103 mapped slightly farther from *Xa-21*. In the *d-27*/IRBB21 population, the two markers mapped 2.2 and 3.8 cM from the resistance gene, respectively. In the *z-2*/IRBB21 population, they both mapped 2.7 cM from *Xa-21*. In the *la*/IRBB21 population, the *Xa-21* phenotypic scoring was discarded due to effects of environmental factors.

Integration of morphological mutant maps

To locate the three morphological mutant markers relative to previously mapped RFLP markers, the independent maps derived from the three F_2 populations were aligned as in Fig. 2. The distance between RFLP markers varied from population to population. For example, the recombination frequency between RG303 and RG1109 is 4.8% in the *d-27*/IRBB21, 1.4% in the *z-2*/IRBB21, and 8.0% in the *la*/IRBB21 population. To integrate these maps, a weighted average of genetic distances between RFLP markers was computed, based on the number of individuals in each of the three populations (Table 1). Seven previously mapped RFLP markers were used to align the molecular map (McCouch & Tanksley, 1991) and the integrated map developed in this study (Fig. 3). In the integrated map, the four morphological markers were assigned locations approximating their original positions in the independent population maps. The three mutant markers and the *Xa-21* gene were thus placed in a linear order on the integrated map. An additional isozyme marker, *Adh-1*, was also located in relation to RFLP markers on the integrated map, based on data from another population (Magpantay et al., unpublished data).

Orientation of the classical genetic and molecular maps

Five markers – *d-27, z-2, la, Xa-21*, and *Adh-1* – provided the basis for the orientation of the classical genetic and molecular maps of rice. The order of these markers in the integrated map (Fig. 3B) was consistent with that in the classical map. The integrated map provides a bridge for determining the orientation of the two previously published genetic maps of chromosome 11 (Fig. 3). Common markers are indicated by joining diagonal lines. After examining orientation results of two rice RFLP maps by Xiao et al. (1992; McCouch et al., unpublished data), it is interesting to note that our conclusion of map orientation between the classical genetic map and the RFLP map developed by Tanksley et al. (1992) agrees with that presented by Yoshimura et al. (1992) between the classical genetic map and the RFLP map by Saito et al. (1991).

Discussion

The frequency of polymorphism among the morphological mutant markers and IRBB21 using 36 markers was quite high – 88.33 to 86.11% (Table 1). This high degree of polymorphism facilitated our mapping efforts and was consistent with previous reports of polymorphism for markers with an RG prefix in an indica/japonica cross (McCouch et al., 1988). The three mutant marker stocks, which have a japonica genetic background, exhibited identical banding patterns in the parental survey for the majority of probes used.

After determining the best possible order of molecular and morphological markers on the integrated map, it can be seen that the order of the three morphological mutant loci is consistent with that presented by Khush & Kinoshita (1991). However, the distances between the mutant markers differ on the two maps. In the integrated map (Fig. 3B), the distance between *Xa-21* and *z-2* as well as their distance from *d-27* is much closer than that in Ikeda et al. (1991). On the contrary, the distance between these markers and *la* covers a much larger distance on the integrated map. Though several marker orders were tried during the course of linkage analysis, the shortest map distances (cM) for each population were when the order of the RFLP markers followed the previous map (McCouch & Tanksley, 1991). The collinearity of markers mapped on different populations using different sets of markers suggests that the marker order in the classical genetic and the two available molecular linkage maps are the same. Based on this observation, we speculate that rice chromosome 11 has not undergone any major rearrangements between indica and japonica rices.

The classical genetic map of rice is the product of linkage information from many crosses while the molecular map is made from a single mapping population. Since recombination frequencies are variable in different populations, it is difficult to accurately estimate map distances which involves data from multiple populations. Our emphasis has been the orientation of the morphological and molecular linkage maps for chromosome 11. No attempts have been made to assess the map distances between molecular and morphological markers that were not mapped onto the same population. Extrapolating from the analysis presented here,

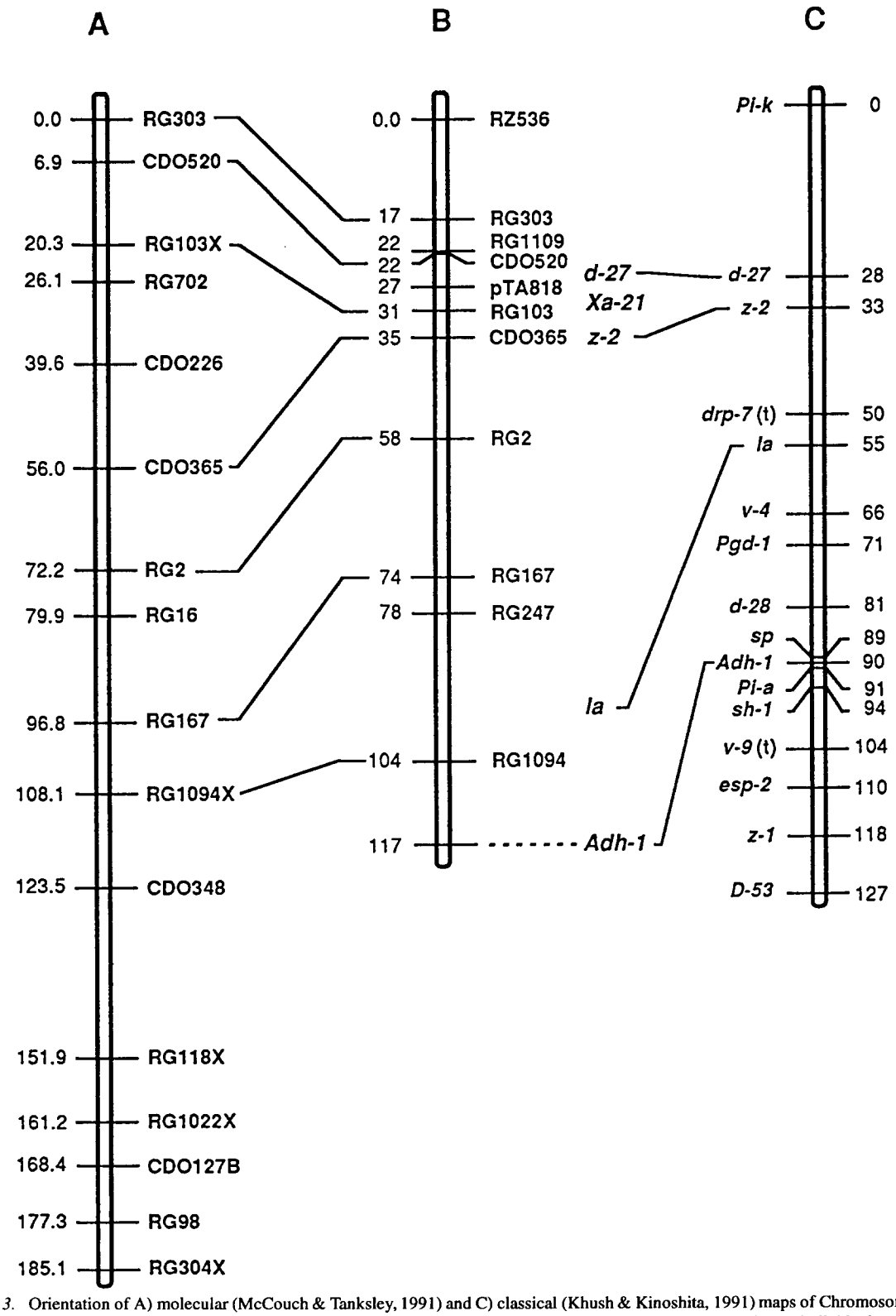

Fig. 3. Orientation of A) molecular (McCouch & Tanksley, 1991) and C) classical (Khush & Kinoshita, 1991) maps of Chromosome 11 using B) integrated molecular and morphological map. Morphological markers on B shown in the approximate location as individual morphological mutant maps. Common markers are connected by diagonal lines.

it is indicated that the blast resistance gene, *Pi-k*, is located in the same region as RZ536 at one end of chromosome 11. Another blast resistance gene, *Pi-l*(t), is also near RZ536 (Yu, Z personal comm.). It is possible that the two blast resistance genes *Pi-l*, or *Pi-k*, reside at the same locus (Inukai et al., 1992). Future attempts to tag the blast resistance genes, *Pi-l*, or *Pi-k*, the isozymes *Pgd-1* or *Adh-1*, or the genes responsible for short panicle (*sp*) and shattering (*sh*), via linkage to molecular markers would involve delimited search area. Furthermore, tagging genes that are known to be linked to the morphological markers on chromosome 11 used in this study (Kinoshita, 1990) will be simplified. For example it is known that *Pi-f* is located on chromosome 11 and linked to *Pi-k*. To map *Pi-f*, RFLP markers in the region of RG303 should be targeted.

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