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Towards rice genome scanning by map-based AFLP fingerprinting

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Abstract Map-based DNA fingerprinting with AFLP markers provides a fast method for scanning the rice genome. Three hundred AFLP markers identified with ten primer combinations were mapped in two rice populations. The genetic maps were aligned and almost full coverage of the rice genome was obtained. The transferability of AFLP markers between *indica* × *japonica* and *indica* × *indica* crosses was tested. The chromosomes were divided into DNA Fingerprint Linkage Blocks (DFLBs) defined by specific AFLP markers. Using these blocks, the degree of similarity or divergence within specific chromosome regions was calculated for nine varieties. Applications of map-based fingerprinting for biodiversity studies and maker-assisted selection are discussed.

Key words Map-based DNA fingerprinting · DNA fingerprint linkage block (DFLB) · Mapping · Genome scan · Rice

Introduction

Amplified fragment length polymorphisms (AFLPs) have been successfully employed for DNA fingerprinting (Zabeau and Vos 1993; Vos et al. 1995) in genetic mapping and biodiversity studies in many plant species including rice (Cho et al. 1996; Mackill et al. 1996; Zhu 1996; Quarrie et al. 1997; Zhu et al. 1998). AFLP markers offer a number of important advantages. They are highly abundant and very efficient for rapid genome coverage since a large number of polymorphic DNA fragments are generated in a single PCR reaction. Thus, AFLPs should be ideal for genotyping a large number of varieties. Their transferability between crosses has been verified in barley (Waugh et al. 1997; Qi et al. 1998) and potato (Rouppe van der Voort et al. 1997).

Although the use of AFLP markers for map-based DNA fingerprinting has been proposed (Zhu 1996; Zhu et al. 1996, 1998), before mapped AFLP markers can be used for assessing genomic variation within every chromosome segment (genome scanning), some theoretical and technical problems must be solved. The first is the question of the transferability of mapped AFLP markers between rice crosses. This concern arises from the technical difficulty involved in identifying the same DNA fragments in different crosses and on different gels, and from the possibility that different DNA fragments may have similar electrophoretic mobilities. The second problem is the adequacy of genome coverage within different rice groups. Map-based DNA fingerprinting will only be practical if it can be achieved using a small number of PCR reactions and gels. Thirdly, an effective analysis strategy has to be developed to quantify genetic divergence more accurately by partitioning the overall variation into variation between specific chromosome regions (genomic variation).

Crop genome scanning – genotyping at the level of defined chromosome segments or even at the gene level – is important for several reasons. Firstly, it should reveal the variation between varieties in different regions of the genome. Secondly, it can be used for tracing genes or chromosome segments through pedigrees. Thirdly, it will help to develop a better strategy for constructing core collections of germplasm that adequately represent each chromosome segment. Fourthly, genome scanning by map-based DNA fingerprinting will make it possible for breeders to select genes in many regions of the genome simultaneously.

RFLP and microsatellite markers are locus specific and usually fully transferable between crosses. However,

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a large number of separate assays must be used to cover the whole genome. Therefore, AFLP markers seem more suitable for genome scanning, while microsatellites can be used for studying genome regions of particular interest. In this paper, map-based AFLP fingerprinting of rice is described using two mapping populations and ten AFLP primer combinations. Examples of assessment of diversity at the chromosome segment level are presented and the possible applications of this technique are discussed.

Materials and methods

Genetic stocks

Two mapping populations were analysed. The first is an F_2 population, consisting of 120 individuals, derived from a cross between IR20 (*indica*) and 63–83 (*japonica*) (Quarrie et al. 1997; Zhu et al. 1998). The second is an F_7 recombinant inbred population of 133 lines, derived from a cross between H359 and 8558 (8558 is a shortened term for variety Acc8558). Both H359 and 8558 were classified as *indica* varieties, but 8558 may contain some genes from *japonica* (Weiming Li, personal communication). Five other rice varieties (IR64, IR4630, IR15324, Bala and Azucena) were also analysed.

Preparation of template DNA for AFLP reactions

Preparation of rice DNA was carried out as described in Zhu et al. (1998). The AFLP procedure followed Vos et al. (1995). The primers used are listed in Table 1.

Genetic map construction and alignment

The framework genetic linkage maps of IR20 × 63–83 and H359 × 8558 were from Quarrie et al. (1997) and Zhu et al. (1998), and Li et al. respectively. From the framework map of IR20 × 63–83, 111 RFLP markers and 31 AFLP markers were used. From the framework map of H359 × 8558, 125 RFLP markers were used. JoinMap V2.0 (Stam 1993) was used for regrouping and constructing new genetic maps incorporating previously scored markers and new AFLP markers. The LOD threshold for grouping was between 4 and 5. The genetic distances in cM were calculated using the Kosambi mapping function.

The two maps were compared and aligned with a published map from the Rice Genome Program, Japan (Harushima et al. 1998) using common RFLP markers. After the common AFLP markers had been identified, they were aligned again. The genetic distances shown in bold face in Fig. 1 are those obtained from each of the three independent maps.

Results

Screening of AFLP primers

To minimize the number of AFLP primer combinations required for mapping and fingerprinting, highly informative primer pairs were needed. To identify such pairs, 52 primer combinations were screened against the parents of the two mapping populations (Table 1). Based on the quality of the fingerprint pattern and the total number of polymorphic bands differentiating the two parental combinations, ten primer pairs were selected for map construction (Table 2).

Adding new AFLP markers to the two independent maps

A total of 186 AFLPs, identified by nine primer combinations were scored in IR20 × 63–83 (Table 2). These AFLP markers were added to the existing map of 142 markers. With a total of 328 markers, the map has a total length of 1239 cM. There are seven gaps of around 20 cM, but no gap is longer than 22 cM.

In all, 154 AFLP markers from seven primer combinations were added to the existing map of H359 × 8558. With a total of 279 markers, this map has a length of 1049.1 cM. The largest gap – 19.7 cM – is located on Chromosome 4.

Alignment of the maps

The two maps were aligned using 120 common RFLP markers (Fig. 1). Most regions of the maps were comparable in genetic distance and the lengths of the linkage groups were similar. In comparison to the reference map (Harushima et al. 1998), IR20 × 63–83 (P-I) and H359 × 8558 (P-III) gave good coverage of the genome.

Table 1 The AFLP primer combinations tested

Primer pairs				
E12/M31	E13/M48	E23/M35	E23/M54	E24/M62
E12/M35	E14/M48	E23/M36	E23/M58	E26/M50
E12 M36	E15/M48	E23/M37	E23/M60	E26/M60
E12/M37	E16/M48	E23/M38	E23/M61	E26/M62
E12/M38	E17/M48	E23/M39	E23/M62	
E12/M39	E18/M48	E23/M42	E23/M63	
E12/M48	E19/M48	E23/M43	E23/M84	
E12/M55	E20/M48	E23/M45	E23/M85	
E12/M74	E21/M48	E23/M47	E23/M86	
E12/M85	E22/M48	E23/M50	E23/M87	
E12/M86	E23/M48	E23/M52	E23/M88	
E12/M87	E24/M48	E23/M53	E23/M89	

Table 2 Numbers of AFLP markers scored

Primer pair	Cross		
	IR20/68-83	H359/8558	Integrated
E12/M35	19	34	48
E12/M38	19	20	37
E12/M48	17	12	28
E12/M74	13	13	23
E22/M48	16	–	16
E23/M50	32	–	32
E23/M61	30	19	43
E23/M63	–	31	31
E23/M84	18	25	36
E26/M60	22	–	22

Fig. 1a–l Two linkage maps P-I (IR20 × 63–83) and P-III (H359 × 8558) were aligned with the help of a reference map (P-II, Nipponbare × Kasalath) constructed by the Rice Genome Program in Japan (Harushima et al. 1998). The markers and genetic distances shown in *bold face* are taken from the original maps. The DNA fingerprint linkage blocks defined for the assessment of genomic diversity are illustrated

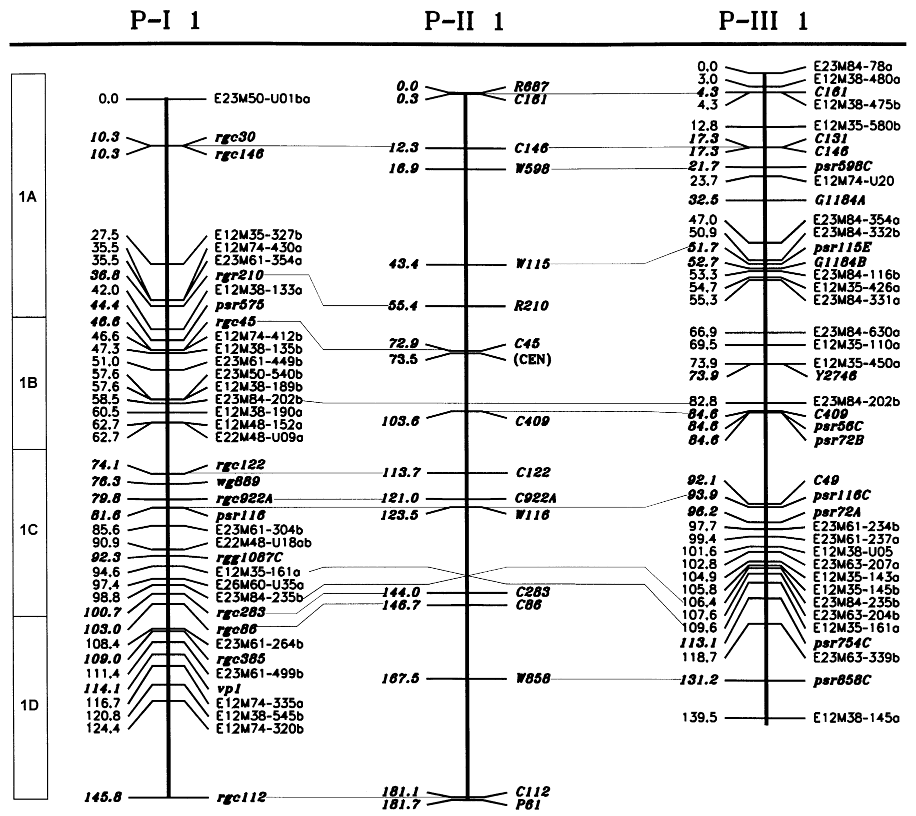
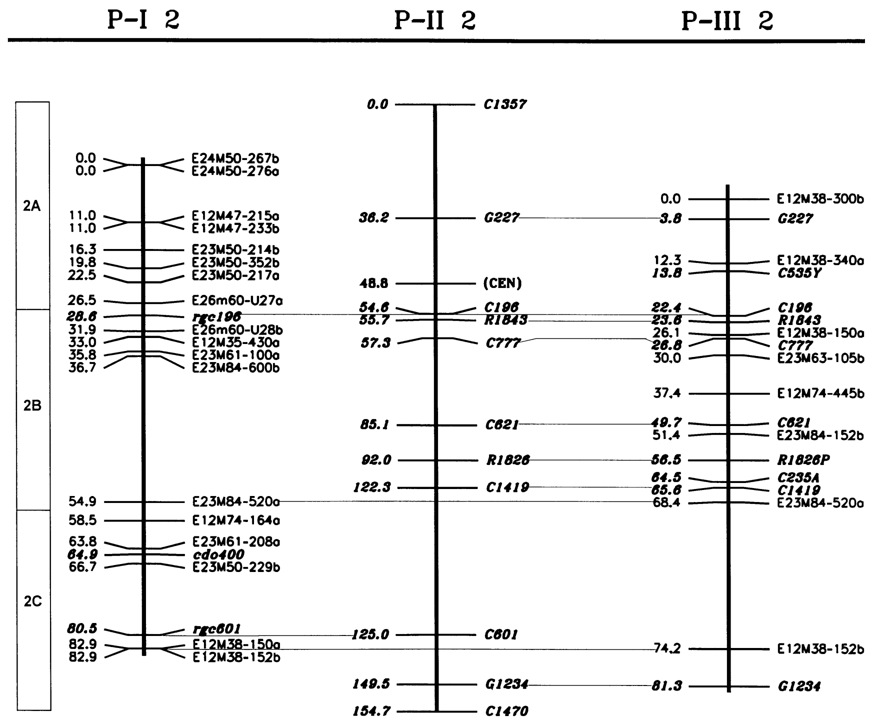


Fig. 1b



The largest missing region, compared with the reference map, was a stretch of about 12 cM at the end of the short arm of chromosome 2. The coverage of the map of IR20 × 63–83 was slightly greater than that of H359 × 8558.

Transferability of AFLP markers

AFLP markers were identified by their mobility and intensity on gels. Fragments having the same mobility and comparable density were designated as candidate

Fig. 1c

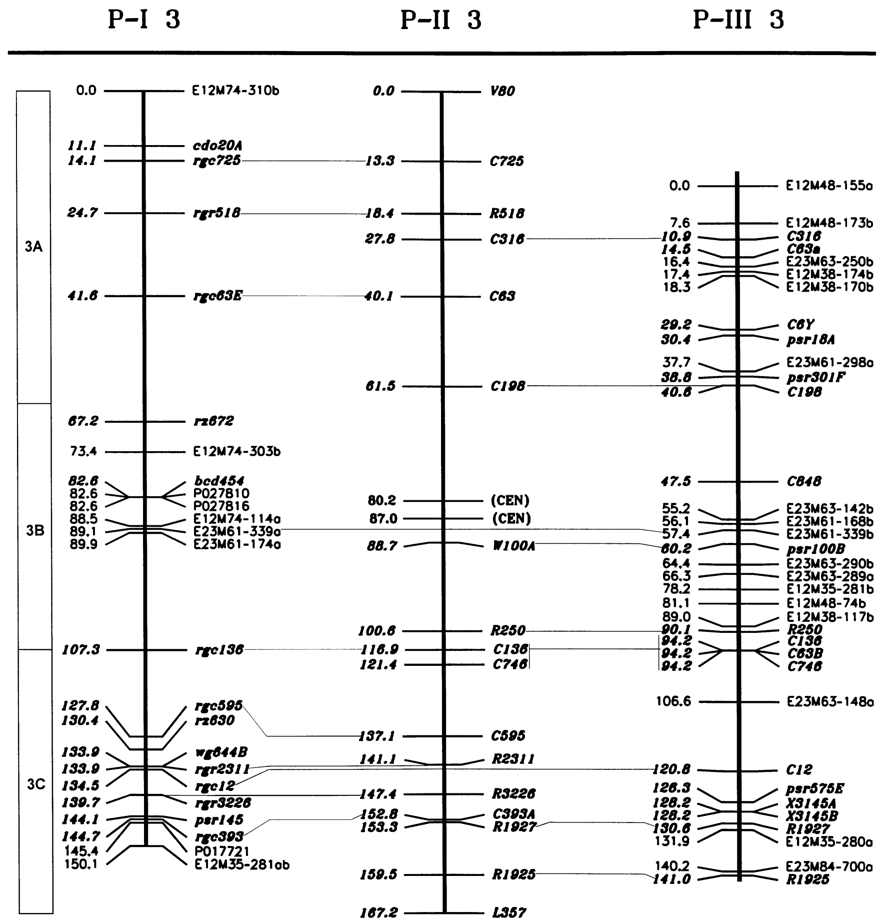


Fig. 1d

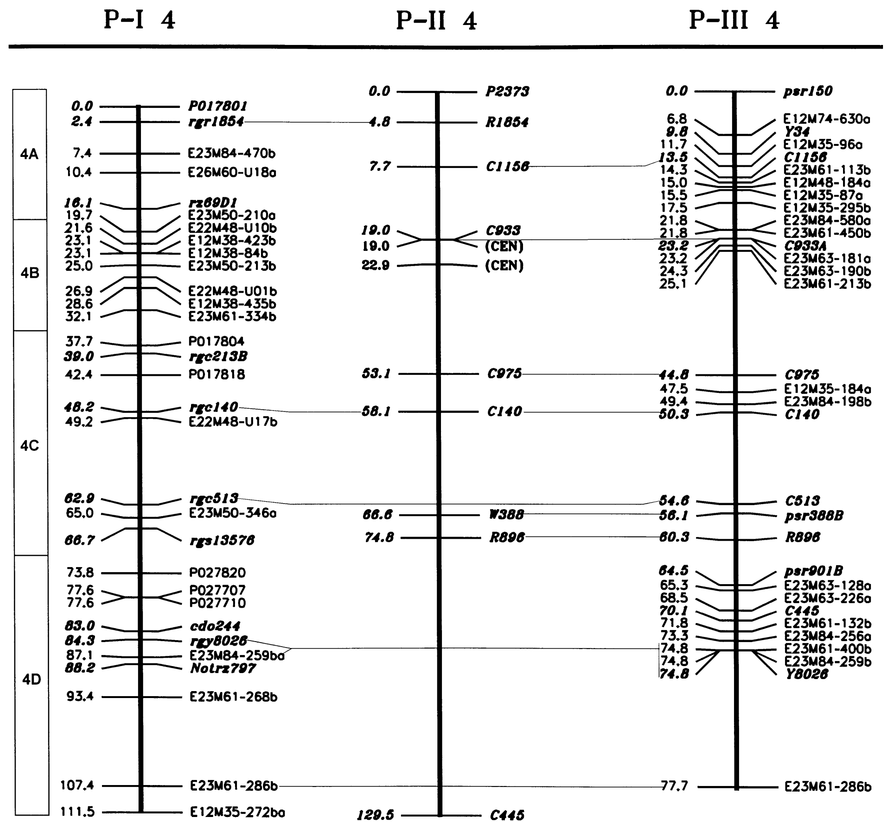


Fig. 1e

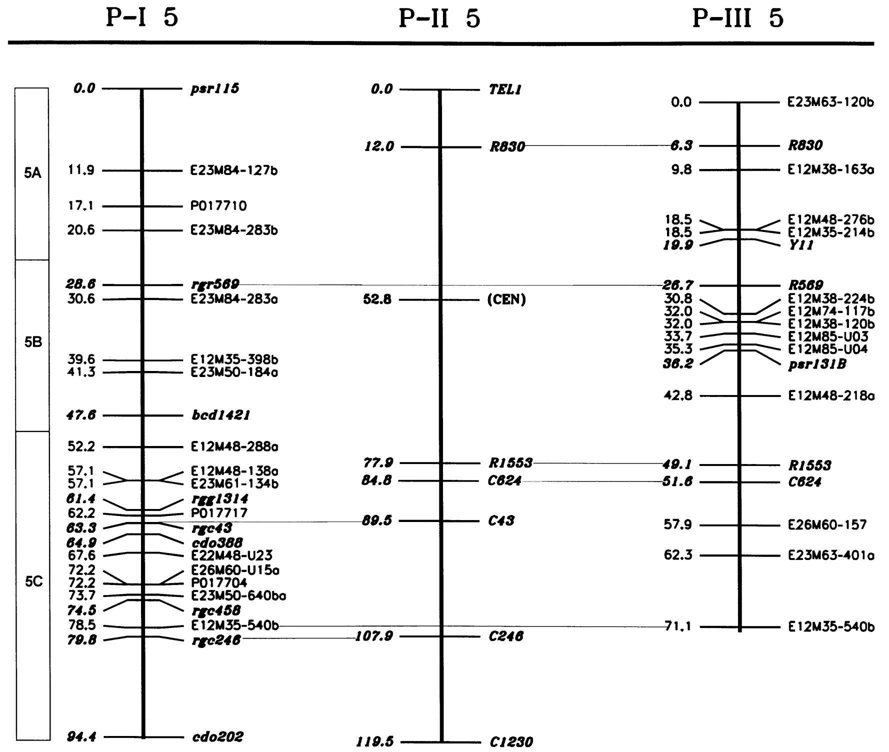


Fig. 1f

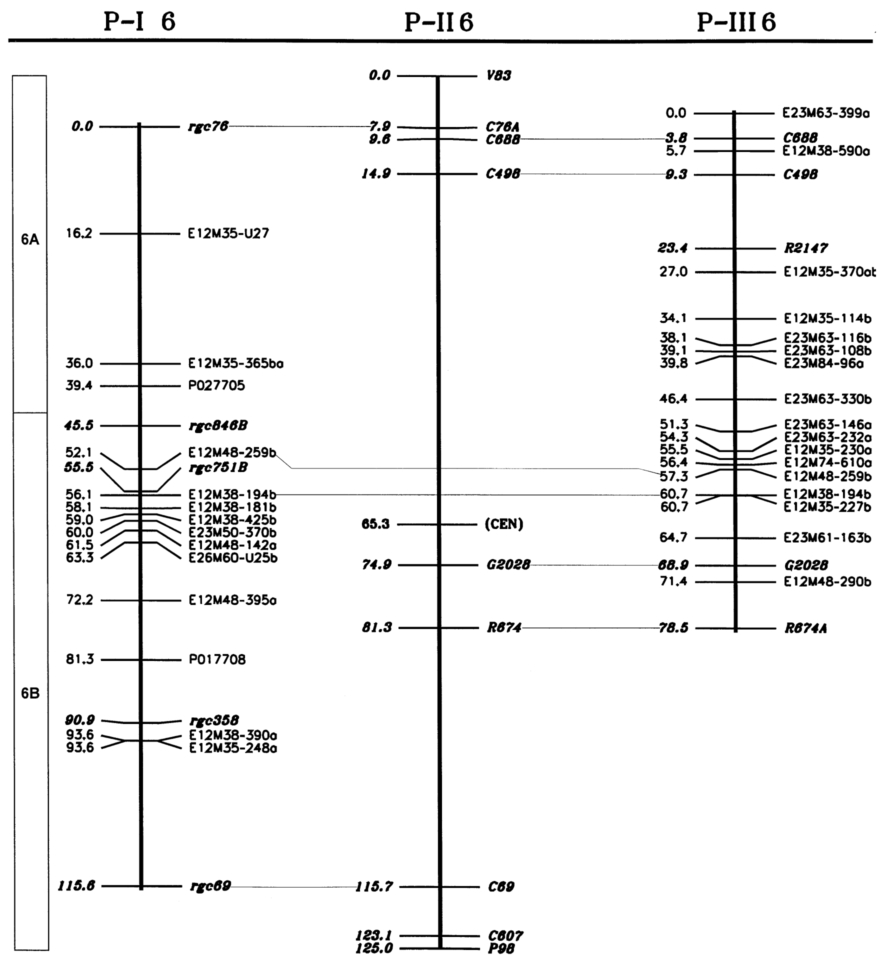


Fig. 1g

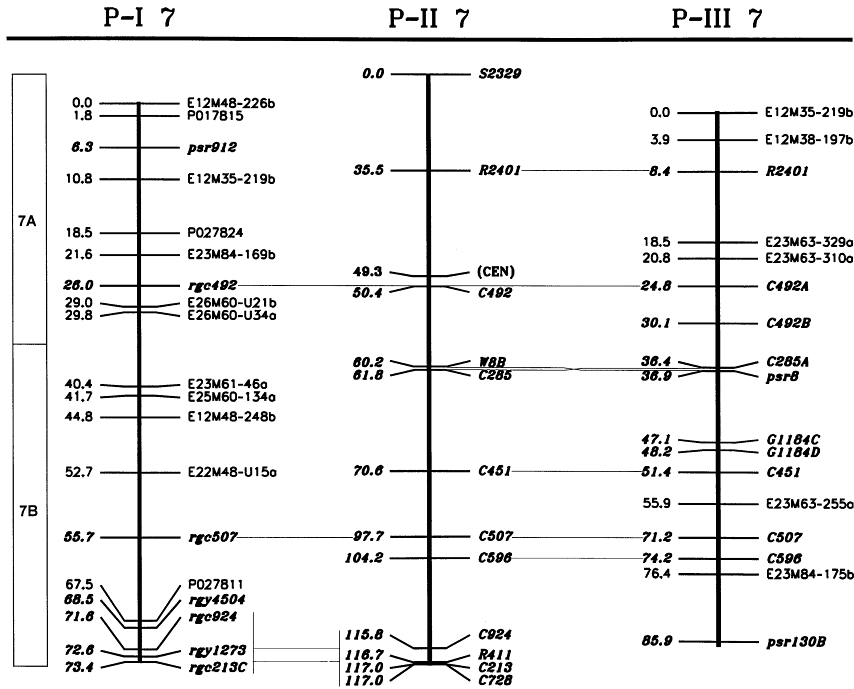
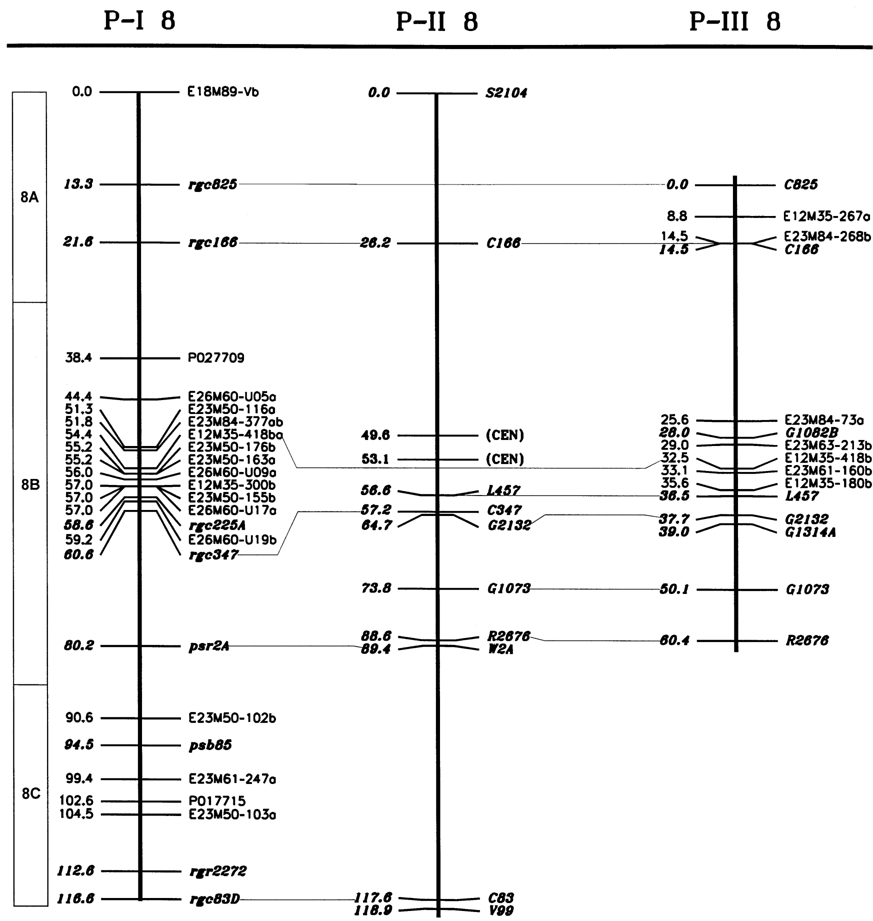


Fig. 1h



common AFLP markers. Double or multiple fragments with similar mobilities were excluded from the analyses because they were liable to mis-scoring. For example, E12M74-303b was mapped on Chromosome 12 (14.9 cM) of the H359 × 8558 map, but in the IR20 × 63–83 population, there were two fragments

Fig. 1i

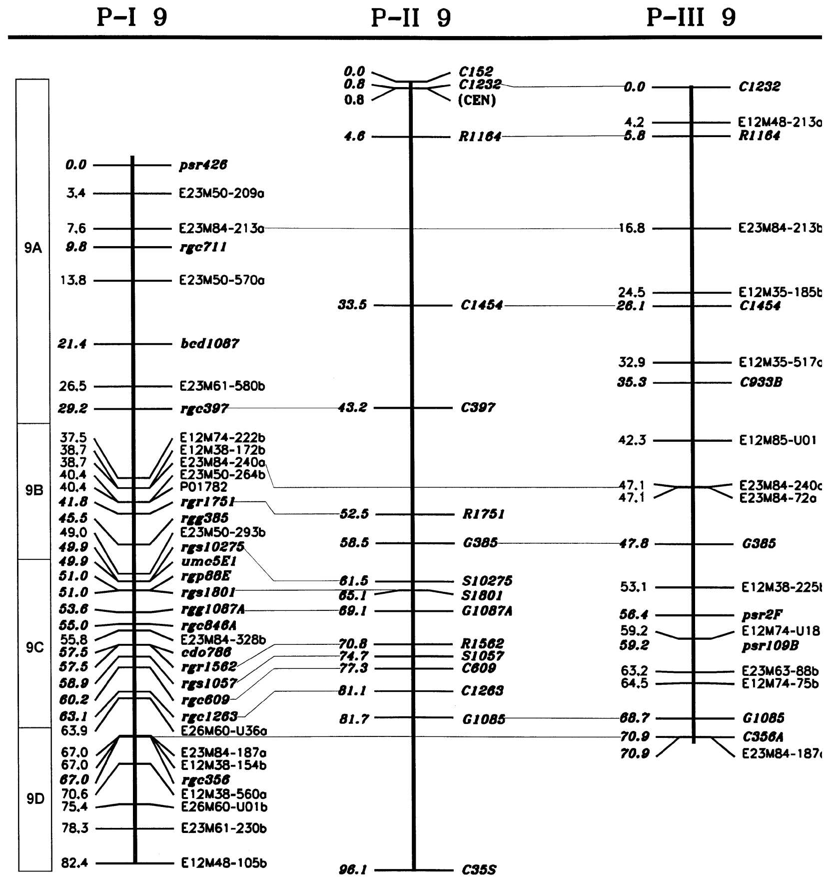


Fig. 1j

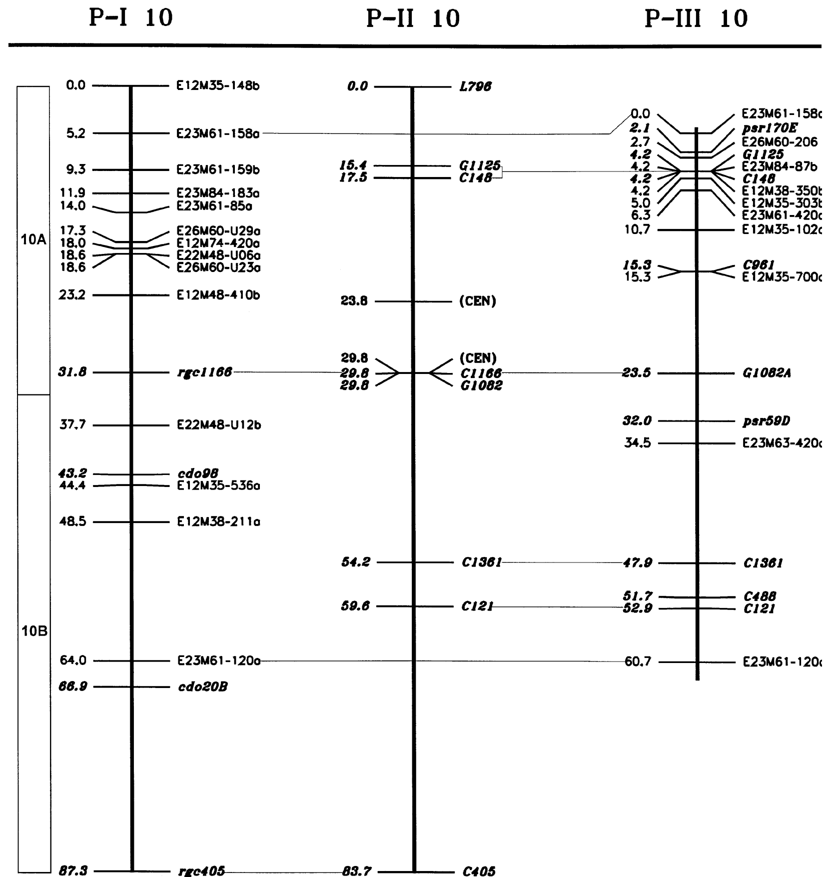


Fig. 1k

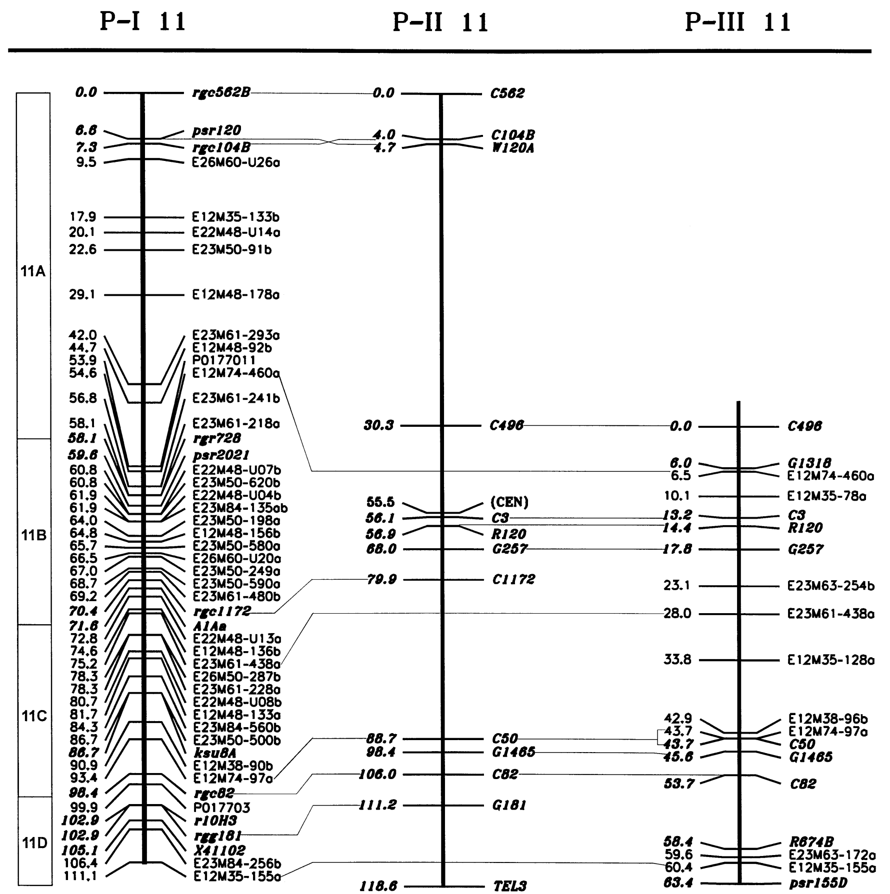


Fig. 1l

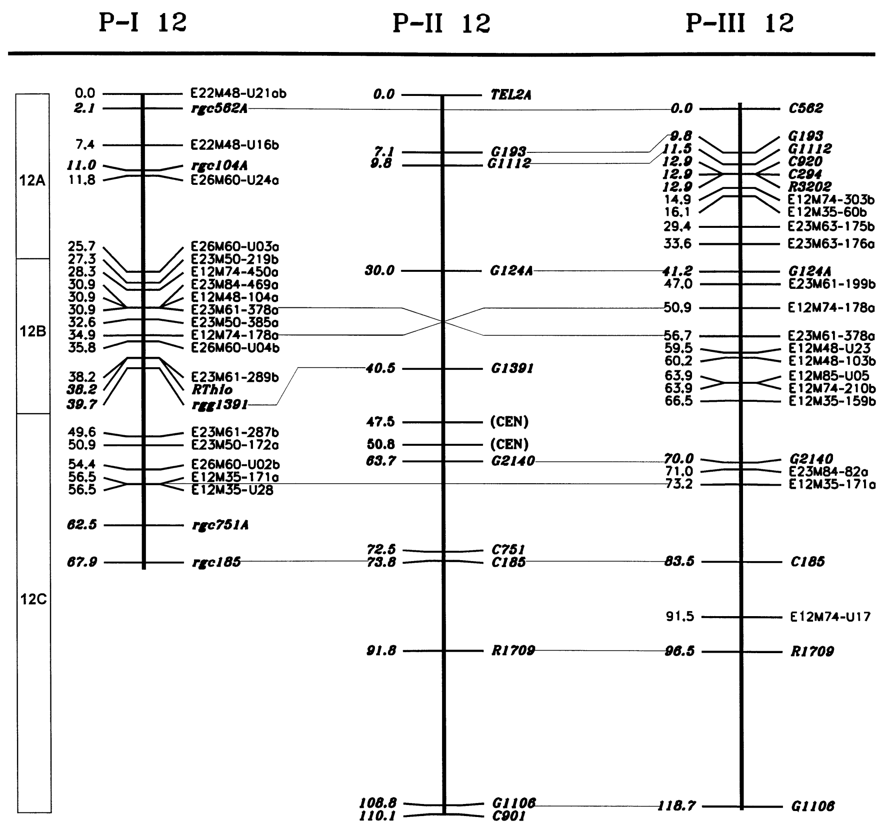


Table 3 Locations of the 24 AFLP markers that segregated in both mapping populations

Marker	IR20/63-83		H359/8558		Anchors
	Chromosome	cM	Chromosome	cM	
E23M84-202	1	−23.1	1	−11.1	psr116
E12M35-161	1	13	1	15.7	psr116
E23M84-235	1	17.2	1	12.5	psr116
E23M84-520	2	26.3	2	46	rgc196
E12M38-152	2	54.3	2	51.8	rgc196
E23M61-339	3	−45.4	3	−63.4	rgc12
E23M84-259	4	2.8	4	0	rgy8026
E23M61-286	4	23.1	4	2.9	rgy8026
E12M35-540	5	49.9	5	44.4	rgr569
E12M48-259	6	52.1	6	55.2	rgc76/C688
E12M38-194	6	4	6	2.7	E12M48-259
E12M35-418	8	32.8	8	18	rgc166
E23M84-213	9	−59.4	9	−54.1	rgc356
E23M84-240	9	−28.3	9	−23.8	rgc356
E23M84-187	9	0	9	0	rgc356
E23M61-158	10	−26.8	10	−23.5	rgc1186/G1082
E23M61-120	10	32.2	10	37.2	rgc1186/G1082
E12M74-460	11	−43.8	11	−47.2	rgc82
E23M61-438	11	−23.2	11	−25.7	rgc82
E12M74-97	11	−5	11	−10	rgc82
E12M35-155	11	12.7	11	6.7	rgc82
E23M61-378	12	−37	12	−26.8	rgc185
E12M74-178	12	−33	12	−32.6	rgc185
E12M35-171	12	−11.4	12	−10.3	rgc185

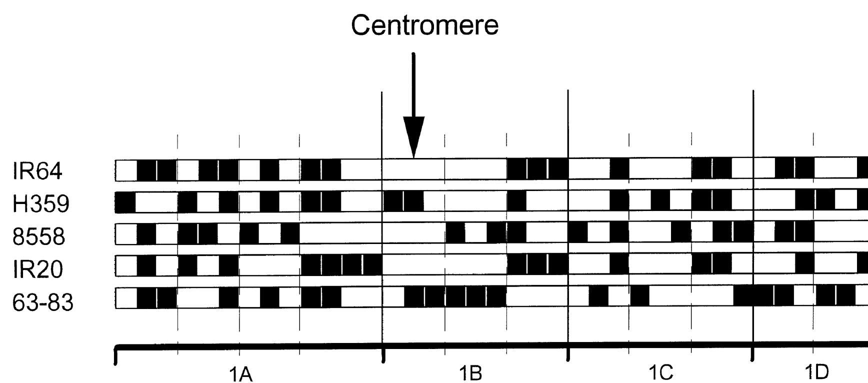


Fig. 2 Linked AFLP fingerprints of Chromosome 1 are illustrated. The *filled boxes* indicate the presence of a given AFLP fragment and the *open boxes* signal its absence. The AFLP markers corresponding to the illustrated squares are listed in Table 4. Two alternative ways of partitioning the AFLP markers into fingerprint linkage blocks are represented by the *solid* and *broken* lines. The larger blocks could be used for genomic similarity/divergence assessment and the smaller ones could be used for pedigree analysis to study recombination within chromosome segments

with similar mobility. This marker was thus eliminated from the IR20 × 63–83 map and was also excluded from the map-based DNA fingerprinting analysis. Besides these fragments, three other markers scored in the IR20 × 63–83 cross were found to be unlinked to any other marker. These were also excluded.

Six primer combinations were common to both crosses, and 24 out of the 116 (21%) segregating AFLP bands which were mapped in the IR20 × 63–83 cross were also mapped in the H359 × 8558 population. All the common AFLPs mapped at similar positions (Fig. 1, Table 3), indicating that the AFLP markers were generally transferable between populations. Therefore, it is reasonable to assume that AFLP fragments of equivalent size and intensity from different varieties will be homologous.

DNA fingerprint linkage blocks (DFLBs)

For map-based analysis, each chromosome was divided into DFLBs. The size and boundaries of the blocks can be varied according to the requirements of any given analysis and the availability of markers in that region. The only possible and necessary rule was that the boundary should be located at an obvious

Table 4 Genotypes of Chromosome 1 in the five varieties tested

AFLP markers[a]	Population	Variety[b]				
		IR64	H359	8558	IR20	63-83
E12M38-480a	P III	0	1	0	0	0
E12M38-475b	P III	1		1	1	1
E23M50-U01b	P I	1	0	0	0	1
E23M50-U01a	P I	0	1	1	1	0
E12M35-580b	P III	1	0	1	0	0
E23M84-354a	P III	1	1	0	1	1
E23M84-332b	P III	0	0	1	0	0
E12M35-327b	P I	1	1	0	0	1
E23M84-116b	P III	0	0	1	0	0
E12M35-426a	P III	1	1	0	1	1
E23M84-331a	P III	1	1	0	1	1
E12M74-430a	P I	0	0	0	1	0
E23M61-354a	P I	0	0	0	1	0
E23M84-630a	P III	0	1	0	0	0
E12M74-412b	P I	0	1	0	0	1
E23M61-449b	P I	0	0	0	0	1
E23M50-540b	P I	0	0	1	0	1
E12M38-189b	P I	0	0	0	0	1
E23M84-202b	P I	0	0	1	0	1
E12M38-190a	P I	1	1	1	1	0
E12M48-152a	P I	1	0	0	1	0
E22M48-U09a	P I	1	0	0	1	0
E23M61-234b	P III	0	0	1	0	0
E23M61-304b	P I	0	0	0	0	1
E22M48-U18a	P I	1	1	1	1	0
E22M48-U18b	P I	0	0	0	0	1
E12M35-143a	P III	0	1	0	0	0
E12M35-145b	P III	0	0	1	0	0
E12M35-161a	P III	1	1	0	1	0
E26M60U35a	P I	1	1	1	1	0
E23M84-235b	P III	0	00	1	0	1
E23M61-264b	P I	0	0	0	0	1
E23M61-499b	P I	1	0	1	0	1
E12M74-335a	P I	1	1	1	1	0
E12M38-545b	P I	0	1	0	0	1
E12M74-320b	P I	0	0	0	0	1
E12M38-145a	P III	1	1	0	1	0

[a] The four blocks of data correspond to those delineated by the solid lines in Fig. 2

[b] The presence of a given AFLP marker is indicated by 1, its absence by 0. Markers with missing data were excluded.

common gap if markers from more than one populations were involved. Thus, ambiguity of the marker order across the DFLB boundaries can be minimized or avoided.

To study genomic variation using DFLBs, the block should be big enough to contain a reasonable number (8–12) of mapped AFLP markers which can be derived from the marker order of a combined map obtained by aligning the maps in Fig. 1. In the present study each chromosome was divided into two to four segments (Fig. 1). For example, the AFLP markers on chromosome 1 were integrated and the genotypes of these markers in five varieties are shown in Table 4.

To study the genotype of, or genetic recombination in, a pedigree, smaller blocks could be defined. As an example, a different division of Chromosome 1 is also demonstrated (Fig. 2). These smaller blocks contain 3–5 markers so that they could have 8–32 possible genotypes, making them suitable for pedigree analysis.

Map-based DNA fingerprinting of nine rice varieties

To develop the concept of AFLP fingerprint linkage blocks (DFLBs), eight rice varieties were compared with the typical indica cultivar IR64 (Fig. 3). The value of Nei's genetic similarity parameter for the eight varieties to IR64 was 83%, 81%, 76%, 73%, 69%, 54%, 43%, 37% and 29% for IR15324, IR20, IR4630, H359, Bala, 8558, Azucena and 63–83, respectively. The advantage of analysis by DFLBs is shown by the fact that some chromosome regions deviated greatly from the overall means. This applies, for instance, to *japonica* and *indica* genotypes. For example, in DFLB 1A (data from 13 AFLP markers), the typical *japonica* varieties 63–83 and Azucena showed 92% and 83% similarity with IR64. A high level of similarity was also found in DFLB 4A. In contrast, the *indica* varieties IR15324 and IR64 differed

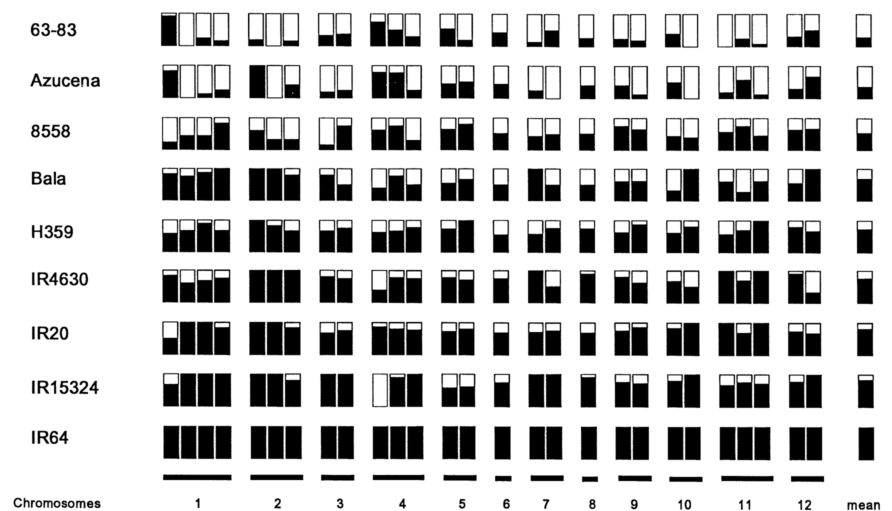


Fig. 3 Eight rice varieties were compared with IR64. Genomic similarity was calculated for every DNA fingerprint linkage block (DFLB) and the degree of similarity is represented by the proportion of the bar coloured black. The Nei's parameter of genetic similarity (mean) was calculated using the data for all the mapped AFLP markers. These DFLBs correspond to those in Fig. 1, except for some merging owning to the reduction in the number of markers caused by missing data. The mergers were made between DFLB 4C and 4D, 5A and 5B, 6A and 6B, 8A and 8B, 8B and 8C, 9A and 9B, 9C and 9D, and 11C and 11D

greatly in DFLB 4A despite a high level of similarity overall (Fig. 3).

Discussion

Possible applications

Map-based DNA fingerprinting revealed considerable variation in the level of similarity in different genomic regions when different rice varieties were compared. This information should be valuable for breeders requiring variation at particular loci. Assessing genomic variation in each chromosome segment provides a more precise view of genetic differentiation and offers a new method for defining and utilizing core collections of germplasm.

Moreover, genome scanning by map-based DNA fingerprinting provides a new approach to marker-assisted breeding. Because the genotype of each chromosome segment in the offspring of a cross can be revealed by the analysis of several PCR reactions, selection can be done simultaneously for all the quantitative and qualitative alleles whose genetic locations are known. Therefore, genome scanning with map-based DNA fingerprinting offers the possibility of *genomic breeding*, in which segregation of the whole genome can be traced by markers.

Genome scanning of the pedigree of breeding programs will provide valuable information on gene flow and reveal strongly selected chromosome segments. The latter would be expected to contain important alleles and would be attractive subjects for further molecular genetic study.

DFLBs

Definition of DNA fingerprint linkage blocks allows one to analyse genomic diversity. However, no fixed, universal size or boundary need be chosen. Each DFLB refers to a particular segment of chromosome based on one or more particular linkage maps. Therefore, DFLBs are arbitrary and temporary, and are referred only to the linkage map used. However, DFLBs provide a means of reducing data complexity to a manageable level which allows the detection of recombinant chromosomes and the assessment of genomic variation. Another advantage of the concept of DFLBs is that each block can have a large number of possible haplotypes ($=2^n$, where n is the number of markers in the block). Therefore, although AFLPs are dominant markers with low polymorphism, DFLBs are variable enough for both pedigree analysis and biodiversity study. Flexibility of size and tolerance of ambiguity of marker order within blocks make the genetic analysis more convenient.

Genomic diversity

The genomic diversity revealed in some DFLBs differs greatly from the mean (genetic diversity). The causes of these differences are not understood although their pedigrees can be traced. For example, IR64 and IR15324 are both *indica* varieties. They share 10 common *ancestral parents* (defined by Souza and Sorrells, 1989), while IR64 has five unique ones and IR15324 has two, one of which is R. Heenati which probably contributes 1/4 of the genome of IR15324 (based on the pedigree). However, the question of whether R. Heenati can possibly explain the observed difference between IR64 and IR15324 in DFLB 4A will only be resolved by genomic scanning of their pedigrees.

Map alignment

Alignment, rather than integration, of the maps was used in this paper because alignment showed the transferability of AFLP markers and their original positions. However, an integrated map is more suitable for defining smaller DNA fingerprint linkage blocks.

Coverage and resolution

AFLP markers vary in frequency in different groups of rice varieties. Some AFLP markers are even specific to one or more groups and therefore give no information on variation within groups (Zhu 1996; Zhu et al. 1998). This is the reason why two types of populations (*indica* × *japonica* and *indica* × *indica*) were used to map AFLP markers. As the number of mapped *indica* × *indica*, *indica* × *japonica* and *japonica* × *japonica* crosses increases, higher coverage of diversity with a minimum number of primer combinations would be obtained. Meanwhile, microsatellites are very polymorphic DNA markers which have a definite genetic location and show full transferability (Panaud et al. 1996; Chen et al. 1997). They can be used to anchor AFLP maps and will greatly assist the study of particular chromosome segments of interest. Therefore, a combined AFLP/microsatellite approach provides a rapid method for high-density marker coverage of the genome.

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