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Integrated map of AFLP, SSLP and RFLP markers using a recombinant inbred population of rice (*Oryza sativa* L.)

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Abstract A molecular map of rice consisting of 231 amplified fragment length polymorphisms (AFLPs), 212 restriction fragment length polymorphisms (RFLPs), 86 simple-sequence length polymorphisms (SSLPs), five isozyme loci, and two morphological mutant loci [phenol staining of grain (*Ph*), semi-dwarf habit (*sd-1*)] has been constructed using an F_{11} recombinant inbred (RI) population. The mapping population consisted of 164 RI lines and was developed via single-seed descent from an intercross between the genetically divergent parents Milyang 23 (M) (*tongil* type) and Gihobyeo (G) (*japonica* type). A subset of previously mapped RFLP and SSLP markers were used to construct the map framework. The AFLP markers were derived from ten *Eco*RI(+2) and *Mse*I(+3) primer combinations. All marker types were well distributed throughout the 12 chromosomes. The integrated map covered 1814 cM, with an average interval size of 3.4 cM. The MG map is a cornerstone of the Korean Rice Genome Research Program (KRGRP) and is being continuously refined through the addition of partially sequenced cDNA markers derived from an immature-seed cDNA library developed in Korea, and microsatellite markers developed at Cornell. The population is also being used for quantitative trait locus (QTL) analysis and as the basis for marker-assisted variety development.

Key words Amplified fragment length polymorphism (AFLP) · Simple-sequence length polymorphism (SSLP) · Restriction fragment length polymorphism (RFLP) · Rice (*Oryza sativa* L.) · Molecular map

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Introduction

Molecular maps are now available for many of the higher plants used in crop production (for current information see the World Wide Web: http://probe.nalusda.gov:8300). These maps, largely composed of restriction fragment length polymorphism (RFLP) markers, serve many purposes including gene localization, gene isolation, marker-aided selection, and evolutionary studies.

The construction of truely saturated maps requires not only the analysis of large numbers of DNA markers, but also the analysis of large numbers of individuals. Of the conventional marker types in use today, RFLP markers generally represent single- and low-copy sequences, due to the nature of Southern analysis. Microsatellites (or SSLPs) are derived from repetitive stretches of DNA, but can be detected as discrete loci because of the unique sequences flanking the microsatellite motifs (Weber and May 1989). Amplified fragment length polymorphism (AFLP) analysis complements other marker systems in that these markers may be derived from any portion of the genome that can be digested with restriction enzymes. Because of variability at the sequence level, a combination of molecular markers capable of sampling all types of sequence configurations will provide the best genome coverage.

AFLP maps are currently being developed in a variety of plant species (Becker et al. 1995; Pot et al. 1996; Maheswaran et al. 1997). This approach to molecular mapping promises great efficiency because of the ability to screen large numbers of DNA fragments in a single lane of a polyacrylamide gel, thus increasing the possibility of identifying polymorphisms and expediting the construction of high-density linkage maps.

The use of a recombinant-inbred (RI) or doubled-haploid (DH) population as the basis for mapping provides an important mapping resource (Burr et al. 1988). Obtaining DNA is not a limiting factor and genetically pure seed can be reliably reproduced from each line. RI and DH populations are especially advantageous for the genetic analysis of quantitative traits because experiments can be replicated over years and environments using identical genotypes. If the parental combination selected for RI or DH population development and subsequent QTL analysis is of direct interest in a breeding program, molecular marker-assisted breeding, guided by the QTL analysis, could feasibly be used to efficiently produce varieties from the experimental population.

We have developed a recombinant inbred population to construct a saturated molecular map of rice. The RFLP framework map is based on the cDNA and genomic DNA markers (Cho et al. 1998) previously mapped by Causse et al. (1994) and Kurata et al. (1994). We have integrated microsatellite and AFLP markers into the RFLP map, which served both to fill gaps and enhance the level of saturation. The map presented here can be expected to provide a reasonable starting point for high resolution QTL analysis, gene isolation, and molecular breeding.

Materials and methods

Plant material

An F_{11} recombinant inbred population consisting of 164 lines was developed from a cross between Milyang 23, (an *indica*/*japonica* derivative known as the *tongil* type), and Gihobyeo (*japonica* type) (hereafter referred to as the MG RI population) at the National Institute of Agricultural Science and Technology in Korea. The parents were crossed in 1988 and F_2 plants were selfed, with generations progressing via single-seed descent (selected randomly) until the F_6 generation. F_7 seeds were planted in single rows in the field, and seeds were harvested from single plants until the F_{11} generation.

Crosses among *indica*and *japonica* rice varieties have been very successful in Korea, where 39 *tongil* varieties have been released by the Rural Development Administration during 1970–1985. Milyang 23, the maternal parent, was released in 1976 and, though no longer planted commercially, it contains many favorable genes and is frequently used as a parent in rice breeding programs. It was derived from a cross between the *japonica* variety, Suwon 232 and the indica variety, IR24. Gihobyeo, the paternal parent, is a temperate *japonica* variety developed from a cross between two *japonica* parents, Fuji 280 and BL 1. These two parents represent genetically divergent types, providing ample segregation of molecular marker alleles, agronomically important genes and quantitative trait loci (QTLs).

DNA extraction and RFLP analysis

Three-week-old leaf tissue was harvested from over 100 seedlings of each F_{11} line and bulked for DNA extraction based on the technique described by Cho et al. (1994). For RFLP mapping, DNA was digested with 8 restriction enzymes (*Bam*HI, *Dra*I, *Eco*RI, *Hin*dIII, *Eco*RV, *Sca*I, *Xba*I, *Kpn*I) and 8 μg per lane was used to make mapping filters. Southern blotting and hybridization procedures were similar to those described in Cho et al. (1994).

Source of the DNA clones and the microsatellite primers

Genomic and cDNA markers from the molecular maps of rice reported by Causse et al. (1994) and Kurata et al. (1994), together with the microsatellite primer pairs described by Chen et al. (1997) were used to develop an evenly distributed molecular framework map.

Adapters and primers used in AFLP analysis

The principle of the AFLP method has been described by Vos et al. (1995). DNA was cut with restriction enzymes, and double-stranded (ds) adapters were ligated to the ends of the DNA fragments to create amplicons. In this way, the sequence of the adjacent restriction site and the adapters served as primer-binding sites for subsequent amplification of the restriction fragments (Vos et al. 1995). Selective nucleotides were added to the 3′ ends of the PCR primers, which resulted in their priming only a subset of the restriction sites. Where the nucleotides flanking the restriction site matched the selective nucleotides, restriction fragments were amplified. The adapters and primers used in this study were synthesized on a Biotronic Synostat D DNA synthesizer (Eppendorf GmbH, Maintal, Germany) or an Expedite 8909 DNA-Synthesizer (Millipore Corp. Bedford, Mass. USA). The sequences of adapters, primers, and primer combinations are summarized in Table 1.

DNA digestion and ligation of adapters for AFLP analysis

To create amplicons, two different enzymes, *Eco*RI and *Mse*I, were chosen. Genomic DNA (0.1–0.5 mg) was incubated for 1 h at 37°C with 5 units of *Eco*RI and 5 units of *Mse*I, 8 μl of 5 × RL ± buffer in a 40-μl reaction volume. Ten microliters of a solution containing 5 pMol of *Eco*RI-adapters, 50 pMol of *Mse*I-adapters, 1 μl of 10 mM ATP, 2 μl of 5 × RL ± buffer, and 1 unit of T4 DNA ligase was added, and the incubation was continued for 3 h at 37°C. Adapters were prepared by adding equimolar amounts of both strands, but were not phosphorylated. After ligation, the reaction mixture was diluted to 500 μl with 10 mM Tris-HCl, 0.1 mM EDTA pH 8.0, and stored at −20°C.

Amplified fragment length polymorphism

Amplified fragment length polymorphism (AFLP) was carried out with two oligonucleotide primers, one corresponding to the *Eco*RI-end and the other corresponding to the *Mse*I-end. As summarized in Table 1, a total of ten primer combinations with different overhangs were used to amplify AFLP bands. One of the two primers was end-labeled, usually the *Eco*RI-primer, using [γ–33P]ATP and T4

Table 1 The sequences of adapters, primers, and primer combinations

(1) The sequences of *Eco*RI and *Mse*I adapters:
*Eco*RI adapter: 5′-CTCGTAGACTGCGTACC-3′
3′-CATCTGACGCATGGTTAA-5′
*Mse*I adapter: 5′-GACGATGAGTCCTGAG-3′
3′-TACTCAGGACTCAT-5′

(2) The core sequences of primers for *Eco*RI and *Mse*I amplicons:
*Eco*RI: 5′-GTAGACTGCGTACCAATTC-3′
*Mse*I : 5′-GATGAGTCCTGAGTAA-3′

(3) Primer overhangs for selectivity of specific bands:

13 = AG	48 = CAC
23 = TA	49 = CAG
24 = TC	50 = CAT
25 = TG	59 = CTA
26 = TT	60 = CTC
47 = CAA	

(4) The ten primer combinations used:

E13/M59	E13/M60	E23/M50	E24/M50	E25/M48
E25/M50	E25/M59	E25/M60	E26/M47	E26/M49

polynucleotide kinase. The labeling reactions were performed in 50 µl of 25 mM Tris-HCl pH 7.5, 10 mM MgCl2, 5 mM DTT, 0.5 mM spermidine-3HCl using 500 ng of oligonucleotide primer, 100 µCi of [γ–33P]ATP and 10 units of T4 polynucleotide kinase. Fifty microliter PCR reactions were performed containing 5 µl of template-DNA (RL mix), which was diluted 20-fold in TE buffer, 1.5 µl of primer-1 (+ 0, 50 ng/µl = 75 ng), 1.5 µl of primer-2 (+1, 50 ng/µl = 75 ng), 2 µl of 5-mM dNTPs (0.2 mM), 20 µl of water, 0.2 µl of *Taq*-polymerase (5 units /µl), 5 µl of 10 × PCR-buffer, and 14.8 µl of water.

Prior to the AFLP reaction, DNA was selectively pre-amplified using an *Eco*RI primer without extension (+0) and an *Mse*I primer with a single-C extension (+1). The pre-amplification reactions were performed for 20 cycles with the following cycle profile: a 30-s DNA denaturation step at 94°C, a 30-s annealing step at 56°C, and a 1-min extension step at 72°C. The AFLP reactions with primers having two or three selective nucleotides were performed for 36 cycles with the following cycle profile: a 30-s DNA denaturing step at 94°C, a 30-s annealing step, and a 1-min extension step at 72°C. The annealing temperature was 65°C in the first cycle, subsequently reduced by 0.7°C per cycle for the next 12 cycles, finally stabilizing at 56°C for the remaining 23 cycles. All amplification reactions were carried out in a PE-9600 thermocycler (Perkin Elmer Corp., Norwalk, Conn., USA).

Denaturing polyacryamide gel analysis and detection of AFLP bands

The AFLP reaction products were mixed with an equal volume (20 µl) of formamide dye (98% formamide, 10 mM EDTA pH 8.0, and with bromophenol blue and xylene cyanol as tracking dyes). The mixtures were denatured for 3 min at 90°C, and then quickly cooled on ice. Each sample (2 ml) was loaded on a 5% denaturing polyacrylamide gel (Maxam and Gilbert 1980; Cho et al. 1996). The gel matrix was prepared using 5% acrylamide, 0.25% methylene bisacrylamide, 7.5 M urea in 50 mM Tris/50 mM Boric acid/1 mM EDTA. To 100 ml of gel solution, 500 µl of 10% ammonium persulfate and 100 µl of TEMED were added and gels were cast using a SequiGen 38 × 50-cm gel aparatus (BioRad Laboratories Inc., Hercules, Calif., USA). Running buffer consisted of 100 mM Tris/100 mM Boric acid/2 mM EDTA. Electrophoresis was performed at a constant power, 110 W, for 2 h. After electrophoresis, gels were exposed onto Fuji phosphoimage screens for 16 h. Fingerprint patterns were visualized using a Fuji BAS-2000 Phosphoimager (Fuji Photo Film Company Ltd., Japan).

Nomenclature

The nomenclature for an AFLP marker is derived from the enzyme combination, the primer combinations and the relative molecular weight. A typical AFLP marker name, E13M60.320-P2 at the top of chromosome 1, indicates that the marker was amplified with the primer combination E13 (E for *Eco*RI, 13 for the specific selective bp combination) and M60 (M for *Mse*I, 60 for the specific selective bp combination) (Table 1). The three-digit number following the dot gives the relative molecular weight of the AFLP band, 320 nucleotides, as measured against a 10-base ladder (SequaMarkTM). The designation following the dash (-P2) indicates that this marker was scored by the presence of a band amplified from the paternal (P2 = Gihobyeo) parent.

Data analysis

The segregation ratio at each marker locus was analyzed for deviation from the expected Mendelian segregation ratio (1 : 1) by $\chi 2$ tests using Map Manager version 2.5 (Manly 1993).

Linkage analysis

Linkage analysis was performed using Mapmaker Version 2.0 (Lander et al. 1987) on a Macintosh Power PC 8100/80AV. All pairs of linked markers were first identified using the "group" command with LOD > 4.0 and recombination fraction (q) = 0.25 on the Mapmaker program. Co-segregating markers were identified by scanning two-point linkage data. Framework maps were constructed using only one marker from each set of co-segregating 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 > 2.0. Additional markers (indicated in parentheses) were assigned to intervals within the LOD > 2.0 framework using the "try" and "ripple" commands. Map units (cM) were derived using the Kosambi function (Kosambi 1944).

Results

Construction of the RFLP map

Of the eight enzymes used to detect RFLP between Milyang23 and Gihobyeo, *Eco*RV was the most efficient, detecting polymorphism in 53.1% of markers surveyed. *Hin*dIII, *Sca*I, *Dra*I, *Eco*RI, and *Bam*HI each detected over 30% polymorphism, while *Xba*I and *Kpn*I detected 29% and 23%, respectively (Table 2). Based on this survey, the level of polymorphism between the parents was approximately 80%.

A subset of the RFLP markers previously mapped by Causse et al. (1994) and Kurata et al. (1994) were used to construct a framework map. This framework

Table 2 Percent RFLP polymorphism observed between mapping parents, Milyang 23 and Gihobyeo, when surveyed with eight restriction enzymes

Restriction Enzyme	Recognition site	Polymorphism (%)
*Eco*RV	GATATC	53.1
*Hin*dIII	AAGCTT	41.4
*Sca*I	AGTACT	40.6
*Dra*I	TTTAAA	39.8
*Eco*RI	GAATTC	37.5
*Bam*HI	GGATCC	31.3
*Xba*I	TCTAGA	28.9
*Kpn*I	GGTACC	22.7

consisted of 212 RFLP markers (Fig. 1). The marker order on the RFLP map was in good agreement with that of the two maps reported previously. A few exceptions were observed: (1) RG143 and RZ879B on chromosome 4 were in reverse order on the MG map, (2) the single-copy marker, RG303, which was mapped to the long arm of chromosome 11 on the SL map (Causse et al. 1994), was assigned to the middle of chromosome 1 on the MG map, (3) RG1028, which was mapped on chromosome 6 on the SL map, was located to the long arm of chromosome 1 on the MG map, and (4) G329, G12 and G122, which were mapped on chromosome 6 on the Nipponbare/Kasalath map (Kurata et al. 1994), were located to the long arm of chromosome 5 on the MG map.

Addition of SSLP markers to the RFLP framework map

One hundred and thirty one microsatellite markers previously reported by Chen et al. (1997) were evaluated for polymorphism between Milyang 23 and Gihobyeo. One hundred (76%) of them detected polymorphism and 86 SSLP loci (detected by 80 primer pairs) have been mapped onto the MG RI population. The microsatellite markers were well distributed throughout the 12 rice chromosomes. All positions on the MG map were in good agreement with those reported by Chen et al. (1997) except for RM259, which was located on chromosome 2 on the MG population but was present on the short arm of chromosome 1 on the IR64/Azucena DH population map (Chen et al. 1997). The integration of SSLPs onto the RFLP map generated a map with a total of 1649 cM, and an average interval size of 5.5 cM along the framework. Before adding microsatellite markers, there were 25 intervals greater than 15 cM. Microsatellite markers helped to fill six of these gaps on four different chromosomes, often due to monomorphism of RFLPs between Milyang 23 and Gihobyeo using the eight enzymes tested. This demonstrates the specific value of these hypervariable markers and the more general advantage of having a variety of marker systems for the construction of a high-density linkage map.

Residual heterozygosity detected by RFLP and SSLP markers

Of the 298 RFLP and SSLP loci tested, 52 marker loci (17.4%) were homozygous in all 164 lines, 193 markers (64.8%) were heterozygous in up to ten RILs, while 53 markers (17.8%) were heterozygous in 11–22 of the MG RI lines (Fig. 2). The average frequency of heterozygous loci in the F_{11} RI lines used as the mapping population in this study was 3.6%, as detected by RFLP and SSLP markers. This level of heterozygosity is higher than that of the F_7 generation reported by Xiao et.al. (1996) and is also higher than would be expected theoretically (0.1%). Of the 298 RFLP and SSLP markers tested, 73% of the loci showed heterozygosity in fewer than eight (5%) lines, while 16 loci were heterozygous in 10–13% of the lines. Three of sixteen loci (RM24, RZ744, RG303) were located near a hybrid-yield QTL on Chr.1 (Xiao et al. 1996; unpublished data from this MG RI population), two loci (RM24 and RZ744) were located in close proximity to the SalT and Amy1B genes on the CU map, and six (RG100, RZ313, RZ598, CDO337, RZ745, RG1356) were dispersed along chromosome 3, with CDO337 and RZ745 mapping near a QTL for "days-to-heading" in this population (unpublished data). Two of the heterozygous loci mapped to chromosome 4 (RM226, RG143) and the others all mapped to different chromosomes (RM25 on chromosome 8, CDO94 on chromosome 10, RG797 on chromosome 11, and RG901 and RG181 on chromosome 12).

Number of bands detected and polymorphism rates of AFLP markers

A single enzyme combination (*Eco*RI and *Mse*I) was used to generate the AFLP data. The five *Eco*RI(+2) and *Mse*I(+3) primers gave ten different primer combinations with different overhangs. The number of bands generated by each primer combination ranged from 73 to 134, with a mean of 101 bands visible on the polyacrylamide gels. Of the 1011 AFLP bands from the ten primer combinations, 269 (26.6%) were polymorphic in the MG RI population (Fig. 3, Table 3). This level of polymorphism was higher than that observed for the mapping parents used in barley (Becker et al. 1995) or in a previous study of rice (Maheswaran et al. 1997). The best two primer combinations were E13/M59 and E26/M47, which showed over 30% polymorphic bands. A total of 231 out of the 235 AFLP markers scored were integrated into the

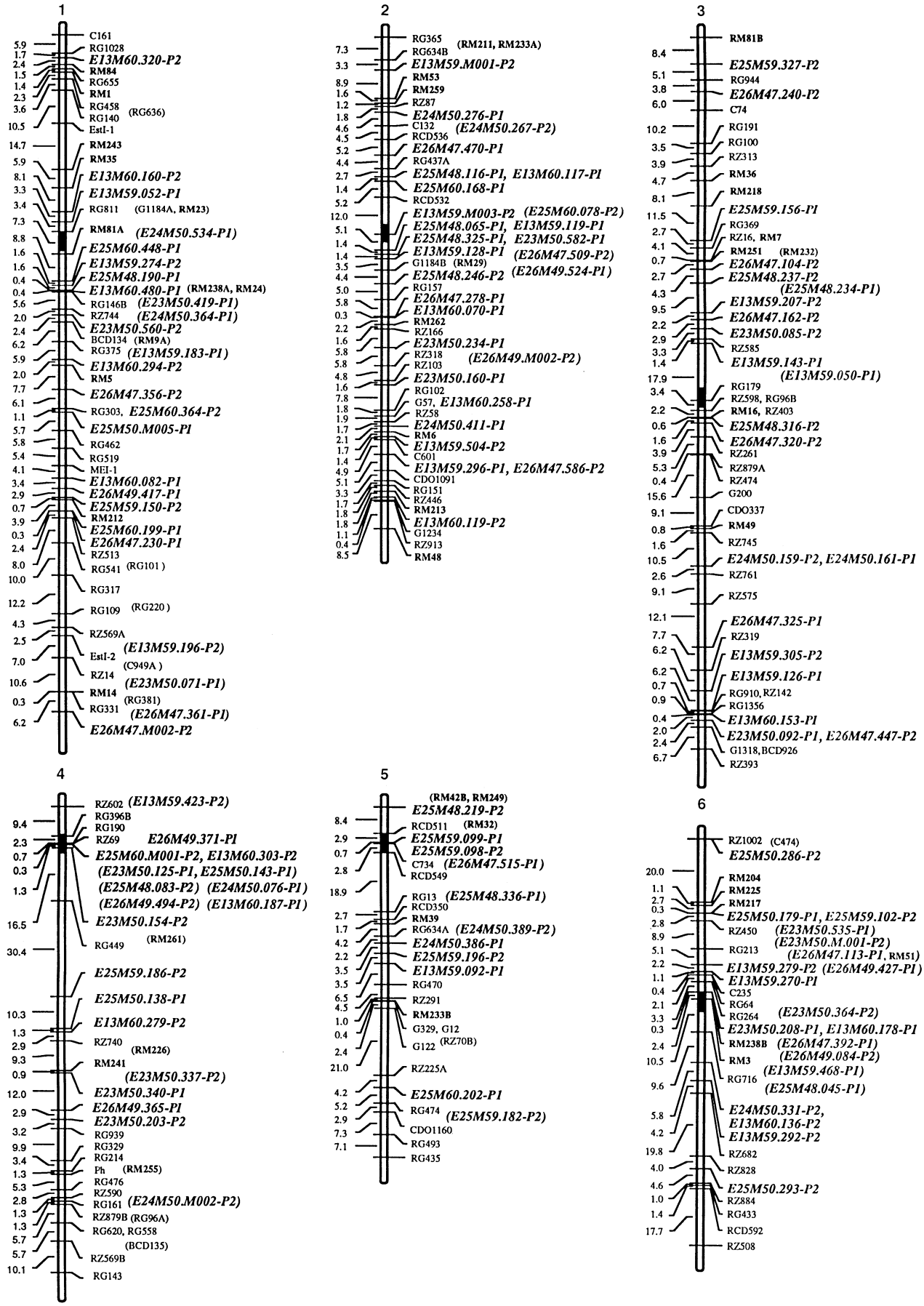

Fig. 1 See page 375 for legend

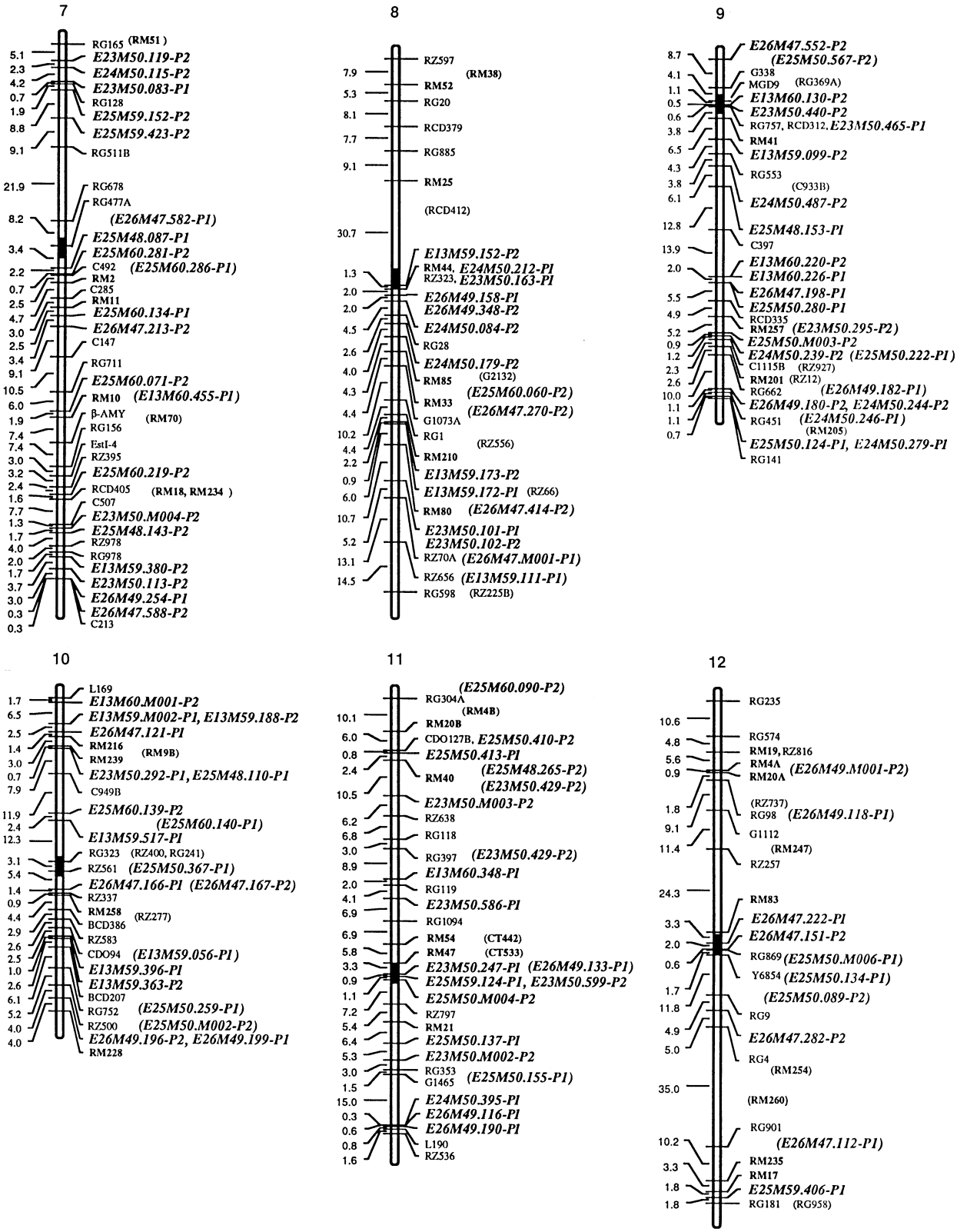

Fig. 1 The integrated map based on AFLP, SSLP, and RFLP markers. Map distances (on the left) are given in cM (Kosambi function). AFLP markers are designated as in the following example: E13M60.320-P2, which is mapped at the top of chromosome 1, indicates that the marker was amplified with the primer combination E13 (E for *Eco*R1, 13 for the specific bp overhang) and M60 (M for *Mse*I, and 60 for the specific bp overhang). AFLP markers are highlighted in *italics*

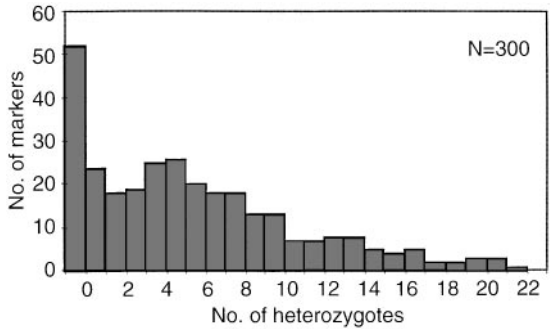

Fig. 2 Frequency of residual heterozygosity based on RFLP/SSLP analysis in the MG RI population

RFLP/SSLP map. Four loci were not mappable, possibly due to cryptic miscoring. The *Eco*RI(+2)/*Mse*I(+3) enzyme combination proved useful for a small-genome crop such as rice.

Segregation and skewness

The percentage of alleles inherited from each parent in the recombinant inbred population was estimated by Hypergene (Young and Tanksley 1989). Deviation from the expected 1 : 1 segregation ratio was significant for 147/536 (27.4%, $P \leq 0.05$) of the markers mapped. This figure was lower than that of a previous report by Xiao et al. (1996). The percent of loci detecting the Milyang 23 allele in each RI line ranged from 10% to 80%, with a mean of 49.5%, as shown in Fig. 4. The mean of this distribution was not significantly different from the expected 50% ($P \leq 0.05$). There were no individuals in this MG RI population that were completely non-recombinant.

Overall, 32.5% of AFLP markers showed distorted segregation. Fifty seven (24.7%) of the 231 AFLP markers were skewed in favor of Milyang 23 allele and 18 markers (7.8%) were skewed in favor of Gihobyeo. This can be compared to a total of 24% of the RFLP and SSLP markers showing segregation distortion in the same population; with 52/298 (17.4%) in favor of Milyang 23 alleles and 20/298 (6.7%) in favor of Gihobyeo alleles. Milyang 23 alleles were 2–3 times more frequent at loci showing distorted segregation, regardless of the marker type.

Distribution of AFLP markers

A total of 231 AFLP markers were integrated into the MG map. The AFLP markers were distributed throughout the 12 chromosomes, as can be seen in Fig. 1. The integrated map embodied a total of 1814 cM with an average interval size of 4.4 cM along the framework. The map based on RFLP and SSLP markers contained 57 intervals greater than 10 cM. AFLP markers helped fill 14 of these gaps on eight different chromosomes. 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 (Fig. 5 a; $r = 0.90$; $P < 0.01$). The relationship between the relative length of the pro-metaphase chromosomes and the genetic length of the chromosomes in cM on the RFLP map was also significant (Fig. 5 b; $r = 0.72$; $P < 0.01$), though lower than for the total number of markers. This is related to the fact that the recombination distance between markers was not uniform along the chromosomes.

Overall, adding the AFLP markers generated 164 new loci on the framework map and caused small changes in the genetic length of the chromosomes ranging from 5 to 25 cM (Table 4) – an average increase of 0.96 cM per marker. For example, on chromosome 10 the total map distance increased by 5 cM following the addition of 19 AFLP markers. On chromosome 9, the total map distance increased by 12 cM following the addition of 22 AFLP markers. On chromosome 2, 28 AFLP markers were mapped evenly throughout the entire length of the chromosome and reduced the average interval size from 5.0 to 2.7 cM (Table 4). The final length of each chromosome ranged from 96 to 229 cM. The total length of the integrated AFLP, SSLP and RFLP map was 1814 cM, compared to 1649 cM before the AFLP markers were added. In summary, the addition of AFLP markers to the RFLP/SSLP map resulted in an increase of 157 cM (9.5%) and an overall reduction of the average interval size from 5.4 cM to 3.4 cM.

Discussion

The MG RI population offers a good foundation for the development of a highly saturated map of rice. The fact that individual RI genotypes are inbred and can be propagated indefinitely by seed makes this population especially useful for replicated experiments to localize, fine-map, and eventually clone major genes and QTLs. The population is also immediately useful for marker-aided selection and near-isogenic line development, as both parental genotypes are of agronomic interest.

The construction of a high-density map requires the analysis of large numbers of DNA markers. Amplified fragment length polymorphism (AFLP) analysis was used in this study to complement other marker systems because it provided an effective way to evaluate large numbers of restriction fragments in a single reaction (Vos et al. 1995). While the ability to directly transfer information between mapping populations using AFLPs has not been conclusively demonstrated, the fact that AFLP fragments can be readily cloned from

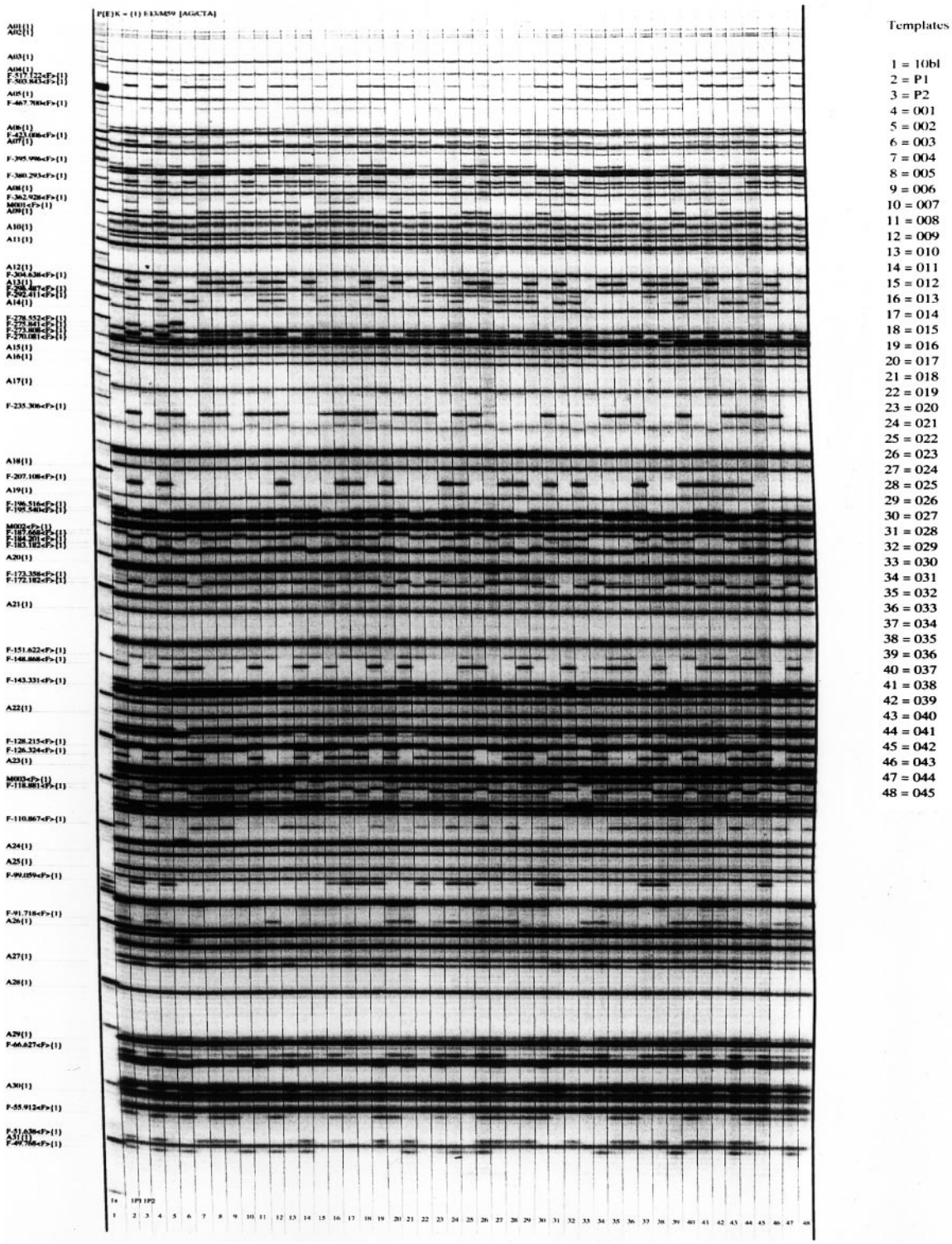

Fig. 3 Autoradiogram obtained with E13/M59 primer combination. Marker names and alignment bands are indicated in left margin. Template identity is indicated in right margin. *10BL* is the 10-base ladder; *P1 and P2* indicate the parents, Milyang 23 and Gihobyeo, followed by plant numbers from the MG RI population

Table 3 Comparison of ten primer combinations for their capacity to generate AFLP marker polymorphisms between the parents Milyang 23 and Gihobyeo

Primer pair	Number of resolvable bands	Number of polymorphic bands	Polymorphism (%)	Number of scored AFLP bands
E13/M59	111	39	35.1	33
E13/M60	79	22	27.8	21
E23/M50	131	37	28.2	35
E24/M50	101	25	24.8	23
E25/M48	77	22	28.6	17
E25/M50	110	33	30.0	22
E25/M59	106	16	15.1	13
E25/M60	73	17	23.3	16
E26/M47	134	37	27.6	34
E26/M49	89	21	23.6	21
Totals	1011	269	26.6	235

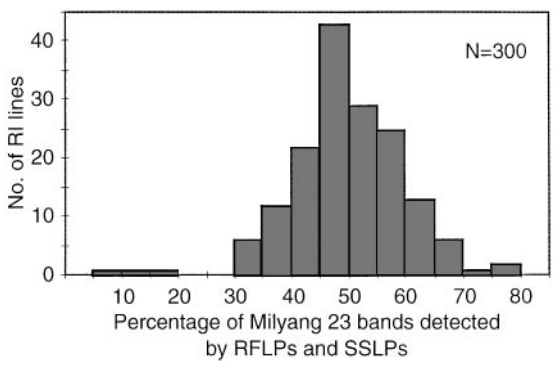

Fig. 4 Frequency distribution of the percentage Milyang 23 alleles by RFLP and SSLP analysis in the F_{11} generation of the MG RI population

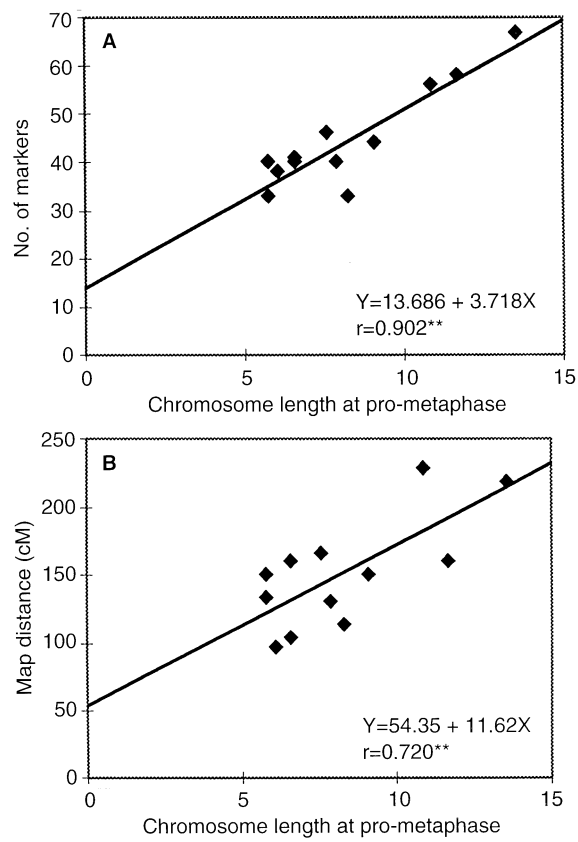

Fig. 5 Relation between the relative length (RL) of chromosomes measured at pro-metaphase (Fukui and Iijima 1991) the number of markers per chromosome (**A**) and the chromosome length in centimorgans on the RFLP map (**B**). The chromosome numbering follows the system established by the Rice Genetics Cooperative Committee, in IRRI, May 1990

silver-stained polyacrylamide gels, and subsequently sequenced and mapped (Cho et al. 1996; Chalhoub et al. 1997), demonstrates that they can be converted into specific markers that serve as anchor points for studies with other populations.

To generate the AFLP data presented in this study, we used the enzyme combination *Eco*RI and *Mse*I. This allowed us to generate more scoreable bands than was reported for the *Pst*I/*Mse*I combination in the IR64/Azucena DH population (Maheswaran et al. 1997). In that study, 945 scoreable AFLP bands were generated from 20 primer combinations, and 208 (21.8%) of them were polymorphic. In comparison, we generated 1011 scoreable bands from ten primer combinations, with 269 (26.6%) of them polymorphic in the MG RI population. The statistics summarizing the percent of polymorphic AFLP fragments between the *indica* and *japonica* rice varieties were very similar in the two studies, and were also in agreement with the results from a study by Mackill et al. (1996). In comparison, the AFLP polymorphism rate in the permanent DH mapping population of barley was only 11.3% (Becker et al. 1995). While the percent of scoreable AFLP bands that can be expected to be polymorphic between divergent germplasm is much smaller than for RFLP or SSLP markers, the efficiency of

Table 4 Effect of integrating AFLP markers into the SSLP/RFLP map of the MG RI population on the number of marker loci per chromosome and the chromosome length

Item	Loci defined by chromosome												Total loci
	1	2	3	4	5	6	7	8	9	10	11	12	
RFLP markers	26	21	27	19	17	14	17	16	15	14	12	14	212
AFLP markers	25	28	21	19	11	20	20	16	22	19	20	10	231
SSLP markers	12	9	8	5	5	6	7	8	4	5	8	9	86
Other markers	4	0	0	1	0	0	2	0	0	0	0	0	7
Totals	67	58	56	44	33	40	46	40	41	38	40	33	536
Length (cM)	219	160	229	151	114	131	166	161	104	96	133	150	1814
Fr. markers added[a]	20	23	19	10	7	11	17	11	15	13	14	4	164
Increase in cM	23	25	17	8	15	16	13	6	12	5	7	10	157

[a] Number of framework-AFLP markers added

AFLP lies in the fact that it allows the simultaneous analysis of a large number of bands per gel.

Adding 231 AFLP markers generated 164 new framework loci on the map and caused small changes in the genetic length of chromosomes. The total length of the integrated AFLP, SSLP and RFLP map was 1814 cM, compared to 1649 cM before the AFLP markers were added. In barley, the total map distance increased from 1096 to 1873 cM (41%) after adding 116 AFLP markers to an existing 166-marker RFLP map based on a DH population of 113 lines (Becker et al. 1995). A similar increase in total map distance was observed in rice using 60 DH lines to integrate 208 AFLP markers into a 135-marker RFLP map; the total map distance expanded from 1811 cM to 3058 cM (41%) (Maheswaran et al. 1997). The large increases in genome length in these DH populations may come either from the better genome coverage provided by the AFLP markers or from the expansion of recombination fractions due either to scoring errors or to small population size coupled with the conscions elimination of heterozygous data points (Becker et al. 1995; Maheswaran et al. 1997).

The percent of previously mapped RFLP clones that detected polymorphism between Milyang 23 and Gihobyeo with at least one of the eight enzymes surveyed (approximately 80%) was similar to the percent polymorphism of random genomic clones detected with nine enzymes between IR87 34583-19-3-3 (*indica*) and Bulu Dalam (*japonica*) (McCouch et al. 1988). This number was slightly higher than the approximately 70% polymorphism detected when surveying large numbers of random cDNA clones with eight enzymes between Nipponbare (*japonica*) and Kasalath (*indica*) (Kurata et al. 1994).

Despite ten generations of inbreeding, the level of residual heterozygosity per marker detected in the bulked DNA samples from this F_{11} MG RI population (3.6%) was higher than would be expected theoretically (0.1%). This figure was higher than that of an F_7 generation of RI lines reported by Xiao et al. (1996). It is tempting to suggest that there may be a selective advantage to the retention of heterozygosity at some loci. To avoid the perpetuation of heterozygosity or mixtures that would confound future genetic analysis using this population, marker-based selection will be imposed to select pure lines for each of the putatively heterozygous loci.

The proportion of markers showing distorted segregation in the MG RI population (27.5%) was similar to that observed in other *indica/japonica* RI, DH and BC populations (Guiderdoni et al. 1989; Xiao et al. 1996; Huang et al. 1997; Xu et al. 1997). Of the entire set of 536 markers mapped, 109 (20.4%) were skewed in favor of Milyang 23 and 38 (7.1%) were skewed in favor of Gihobyeo.

A total 536 AFLP, SSLP, RFLP, isozyme, and morphological markers have been mapped onto the MG map reported here, providing the foundation for locating genes and QTLs governing traits of commercial and agronomic interest. The addition of 231 AFLP marker loci to the framework map consisting of 212 RFLP and 86 SSLP markers was an efficient way to increase the marker density and help fill in the gaps. In the future, end-sequenced cDNA markers from the Korean Rice Genome Research Program will be added to increase the level of marker saturation and to enhance the information content of the map as a resource for molecular breeding and gene isolation.

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