Theor Appl Genet (1997) 94:39–45

M. Maheswaran · P. K. Subudhi · S. Nandi · J. C. Xu
A. Parco · D. C. Yang · N. Huang

Polymorphism, distribution, and segregation of AFLP markers in a doubled haploid rice population

Received: 11 April 1996 / Accepted: 14 June 1996

Abstract We exploited the newly developed amplified fragment length polymorphism (AFLP) technique to study the polymorphism, distribution and inheritance of AFLP markers with a doubled haploid rice population derived from 'IR64'/'Azucena'. Using only 20 pairs of primer combinations, we detected 945 AFLP bands of which 208 were polymorphic. All 208 AFLP markers were mapped and distributed over all 12 chromosomes. When these were compared with RFLP markers already mapped in the population, we found the AFLP markers to be highly polymorphic in rice and to follow Mendelian segregation. As linkage map of rice can be generated rapidly with AFLP markers they will be very useful for marker-assisted backcrossing.

Key words Genetic map · Marker-aided selection · *Oryza sativa* · RFLP markers

Introduction

Rice is the most important crop in the world as it is the major source of calories to more than 50% of the world population. Advances in biotechnology will have great impact in raising as well as stabilizing rice yield potential in the near future. Moreover, rice is becoming a model plant among cereal crops for molecular genetic studies because of its relatively small genome size (4×10^8 bp) (Arumuganathan and Earle 1991), comparatively easy transformability (Hodges et al. 1991) and the collinearity of its genes with those of other cereal crops (Kurata et al. 1994; Moore et al. 1995).

Molecular markers are becoming an essential tool for plant breeding (Tanksley et al. 1989; Rafalski and Tingey 1993). In addition to restriction fragment length polymorphisms (RFLPs), which have been commonly used for linkage analysis, a number of polymerase chain reaction (PCR)-based markers such as random amplified polymorphic DNAs, (RAPDs) (Williams et al. 1990), sequence-tagged sites (STSs) (Olson et al. 1989) and microsatellites (Litt and Luty 1989) have been developed. These PCR-based markers are technically simple and require only a small amount of DNA but can be time-consuming to generate if a large number of primers are used. For crop improvement programs, plant breeders require a marker technology which is technically simple, cost- and time-effective, and which generates a high level of polymorphism. Recently, a new technique called amplified fragment length polymorphism (AFLP) was developed by Vos et al. (1995). AFLP is a powerful, reliable, stable and rapid assay with potential applicationin genome mapping (Thomas et al. 1995), DNA fingerprinting and marker-assisted breeding (Vos et al. 1995). This PCR-based technique permits inspection of polymorphism at a large number of loci within a very short period of time and requires very small amounts of DNA. The reproducibility of AFLPs is ensured by using restriction site-specific adapters and adapter-specific primers with a variable number of selective nucleotides under stringent amplification conditions (Vos et al. 1995).

As a first step of exploiting the utility of AFLP in the rice genome mapping program, and eventually for marker-assisted breeding, we studied the polymorphism and distribution of AFLP markers in the rice genome. Here, we report the generation of an AFLP map with 208 markers derived from 20 primer combinations. We also describe a modified protocol for generating distinct AFLP bands by direct incorporation of [^{32}P]-dCTP.

Communicated by G. Wenzel

M. Maheswaran · P. K. Subudhi · S. Nandi · J. C. Xu · A. Parco · D. C. Yang · N. Huang (✉)
International Rice Research Institute, P.O. Box 933, Manila, Philippines

Materials and methods

Plant material and DNA isolation

A doubled-haploid population was developed from a cross between indica variety 'IR64' and japonica variety 'Azucena' (Guiderdoni

et al. 1992). A linkage map was developed using 135 RFLP markers covering all 12 chromosomes (Huang et al. 1994). A subset of 60 lines with normal fertility was selected based on a 1:1 segregation of RFLP data. Along with both parents, these lines were grown from bagged seeds in a screenhouse, and genomic DNA was isolated from fresh leaf tissue (Dellaporta et al. 1983).

AFLP analysis

The AFLP protocol developed by Vos et al. (1995) was followed, with minor modifications. Genomic DNA (0.5 µg) was restricted with 5 units of *Pst*I and 5 units of *Mse*I (New England Biolab) in a reaction volume of 40 µl at 37°C for 5 h in restriction ligation buffer (10 m*M* Tris-HAc, pH 7.5, 10 m*M* $MgAc_2$, 50 m*M* KAc, 5 m*M* DTT and 0.005% BSA). After complete digestion, 10 µl of solution containing 5 pMol *Pst*I adapter, 50 pMol *Mse*I adapter, 1 µl 10 m*M* ATP, 1 unit T4 DNA ligase and 2 µl of the restriction ligation buffer was added to the same tube, and incubation was continued at 37°C for 4 h. The adapter sequences specific for both enzymes were synthesized according to Zabeau and Vos (1993); they are as follows:

*Pst*I adapter: 5′-CTC GTAGACTGCGTACATGCA-3′
3′-CATCTGACGCATGT-5′
*Mse*I adapter: 5′-GACGATGAGTCCTGAG-3′
3′-TACTCAGGACTCAT-5′

The PCR preamplification of adapter-ligated restriction fragments was performed to generate large quantities of PCR products for subsequent selective amplification. The primers used for the preamplification were *Pst* I primer 5′-CTC GTA GAC TGC GTA CAT GCA-3′ and *Mse* I primer 5′-GAC GAT GAG TCC TGA GTA A-3′. These preamplification primers were designed based on the adapter sequence and restriction sites of *Pst*I and *Mse*I. No selective nucleotide was added to the preamplification primers. The preamplification reaction was performed in 25 µl of reaction mix composed of 50 ng of *Pst* I primer, 50 ng of *Mse* I primer, 5 µl of 1 m*M* dNTPs, 2.5 µl of 10 × PCR buffer (100 m*M* Tris-HCl, pH 8.3, 500 m*M* KCl, 15 m*M* $MgCl_2$), 1 unit of *Taq* polymerase and 2 µl of ligated DNA sample. The amplification profile was 30 cycles of 30 s at 94°C, 30 s at 60°C and 60 s at 72°C followed by final extension at 72°C for 5 min. To confirm the successful preamplification, we electrophoresed 4 µl of the PCR products for each sample on a 2% agarose gel; a low-molecular-weight smear indicated the presence of abundant preamplification products. The PCR product was then diluted 20-fold with sterile water.

Selective amplification was conducted using two AFLP primers specific for *Pst* I and *Mse* I adapters. Each primer contained three selective nucleotides at the 3′ end. The core sequences of selective amplification primers were from Zabeau and Vos (1993). Two *Pst*I and ten *Mse*I selective primers were synthesized.

Pst I (P1): 5′-GACTGCGTACATGCAG **CCA**-3′
Pst I (P2): 5′-GACTGCGTACATGCAG **GTT**-3′
Mse I (M1): 5′-GATGAGTCCTGAGTAA **CAC**-3′
Mse I (M2): 5′-GATGAGTCCTGAGTAA **ACC**-3′
Mse I (M3): 5′-GATGAGTCCTGAGTAA **CCA**-3′
Mse I (M4): 5′-GATGAGTCCTGAGTAA **CAA**-3′
Mse I (M5): 5′-GATGAGTCCTGAGTAA **ACG**-3′
Mse I (M6): 5′-GATGAGTCCTGAGTAA **CAG**-3′
Mse I (M7): 5′-GATGAGTCCTGAGTAA **CAT**-3′
Mse I (M8): 5′-GATGAGTCCTGAGTAA **CGA**-3′
Mse I (M9): 5′-GATGAGTCCTGAGTAA **CGT**-3′
Mse I (M10): 5′-GATGAGTCCTGAGTAA **CCT**-3′

Twenty primer combinations could be derived from these 12 selective amplification primers. AFLP markers were generated in selective amplification step with direct incorporation of alpha-^{32}P-dCTP. The 25 µl of PCR mix was composed of 5 µl of diluted preamplified template, 50 ng of *Pst*I primer, 50 ng of *Mse*I primer, 0.2 m*M* each of dATP, dGTP, dTTP, 4.75 µl of 1 m*M* dCTP, 0.25 µl of alpha-[^{32}P]-dCTP (10 µCi/µl), 1 unit *Taq* DNA polymerase and 2.5 µl of 10 × PCR buffer. The selective amplification profile was 1 cycle of 30 s at 94°C, 30 s at 65°C, 60 s at 72°C, followed by 10 cycles with stepwise reduction of annealing temperature by 1°C to 56°C, followed by 24 cycles at an annealing temperature of 56°C. All amplification reactions were carried out in microtiter plates in a Techne thermocycler.

The PCR products were mixed with an equal volume of formamide dye (98% formamide, 10 m*M* EDTA, 0.005% each of xylene cyanol and bromophenol blue) and were denatured for 3 min at 94°C and then placed on ice. Two microliters of the mix was loaded onto a 6% denaturing polyacrylamide gel and electrophoresed in 1 × TBE at 100 W for2 h. The gel was removed using a 3 MM Whatman filter paper, dried for 2 h at 80°C and exposed to X-ray film for about 14 h depending on signal intensity.

AFLP marker scoring and linkage analysis

Each polymorphic AFLP marker was identified by the primer pair combination with the band number indicated as a suffix. The polymorphic bands were numbered serially in descending order of molecular weight. Only clear and unambiguous bands were scored. Doublets were scored as single markers. Markers were scored for the presence or absence of the corresponding band among the segregating doubled haploid population. Segregating bands were scored as either 1 ('IR64') or 3 ('Azucena'). All markers were tested for the expected 1:1 segregation by the chi-square test at the $P = 0.05$ level. The AFLP marker data set was combined with the RFLP data set (Huang et al. 1994), and linkage analysis was performed using Mapmaker (Lander et al. 1987) (version 2.0) with a Macintosh computer. The data set consists of 48.6% 'IR64' alleles, 47.3% 'Azucena' alleles and 4.1% missing values. All pairs of linked markers were identified using the Group command with LOD score 5.0 and recombination fraction (RF) 0.40. Ordering of markers was done with a LOD score of 4.0 and RF of 0.40. The Ripple command was used to verify the order of the markers on each chromosome. Map units (cM) were derived using the Kosambi function (Kosambi 1944).

Results

AFLP marker polymorphism in rice

The doubled haploid population of rice between the cross 'IR64' × 'Azucena' was used to study the level of polymorphism of AFLP markers in rice and their distribution along the rice genome in order to evaluate the efficiency of this marker technology for use in marker-assisted breeding. Because the RFLP map generated from a subset of 60 lines was similar to the map based on all 135 lines (Huang et al. 1994), this subset was considered to be representative of the population. The use of a smaller but effective population can save time, labor and resources. In this AFLP mapping, 20 available primer combinations were used. AFLP markers were produced for all pairs of primer combinations, and an example is shown in Fig 1.

A survey of different primer combinations (Table 1) indicated that for each primer combination the number of visible bands ranged from 23 to 75 (mean of 47.3). The range of polymorphic bands was from 4 to 21 (mean of 10.4). When all 20 primer combinations were taken into account, 208 out of 945 bands were polymorphic (22%), which is considerably higher than that reported in barley (Becker et al. 1995). The level of polymorphism for each of the primer combinations was quite variable, ranging from 12.8% to 37.5%. A significant correlation

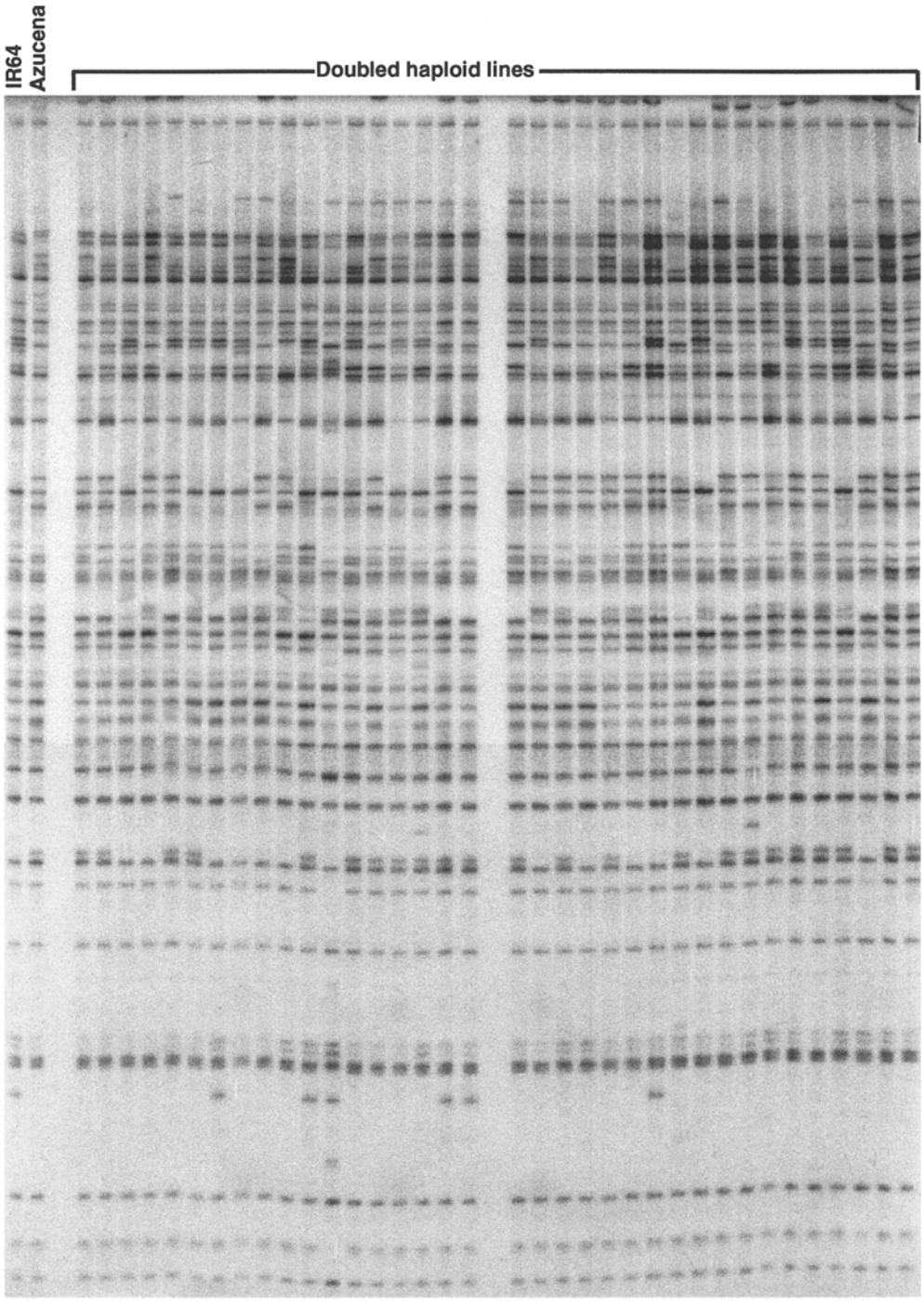


Fig. 1 Autoradiograph showing AFLP markers derived from the selective amplification of restriction fragments by primers P1 and M4. The *first 2 lanes* from the *left* are parents 'IR64' and 'Azucena', respectively; the remaining lanes are doubled haploid lines of the population

($r = 0.69$) between the total number of bands and the number of polymorphic bands was observed. A total of 22 codominant marker pairs was observed for all primer combinations. Because of the modified protocol using direct incorporation of alpha-[^{32}P]-dCTP in the PCR mix during the selective amplification step, a number of doublets occurred and was determined for each primer combination. Only 28 doublets out of 208 markers (13.5%) were observed and scored as single markers (Table 1).

Segregation and distribution of AFLP markers

For the AFLP markers, the overall frequency of the 'Azucena' and 'IR64' alleles was 50.1 and 48.1%, respectively, fitting a 1:1 ratio. This ratio is the same as that of RFLP markers segregating in the same population (Huang et al. 1994). Of the 208 AFLP markers, 45 (21.6%) showed segregation distortion, whereas this value was 20.4% for the RFLP markers in the 60 selected lines. Most of the distorted AFLP markers were

located near the RFLP markers (Fig. 2). The AFLP markers were derived almost equally from both parents (102 from 'IR64' and 106 from 'Azucena') (Table 1).

The 208 AFLP markers and the RFLP markers mapped previously (Huang et al. 1994) were combined to construct a linkage map (Fig. 2). All 208 AFLP markers were mapped and distributed over all 12 chromosomes (Table 2, Fig. 2). The addition of 208 markers to the RFLP map showed no disturbance in the original order of the RFLP markers (Huang et al. 1994) even though all of the markers were used to obtain the best order on the combined map. The correlation between the chromosome length (centiMorgans, cM) and the total number of markers on the combined map was high ($r = 0.97$). The map length of rice increased from 1811 cM (Huang et al. 1994) to 3058 cM, and the average distance between markers was reduced from 13.4 cM to 8.9 cM. The map expansion resulted from a

Fig. 2 AFLP map of rice doubled haploid population derived from cross between 'IR64' and 'Azucena'. The chromosomal locations of the 208 AFLP markers were determined based on linkage to RFLP markers already mapped in the same population (Huang et al. 1994). The *asterisks* (*) after some AFLP and RFLP markers indicate that the segregation of those markers deviated from the Mendelian ratio at the $P = 0.05$ level. The markers in the *boxes* are RFLP markers. Map distances in centiMorgans (cM) are given on the *left*

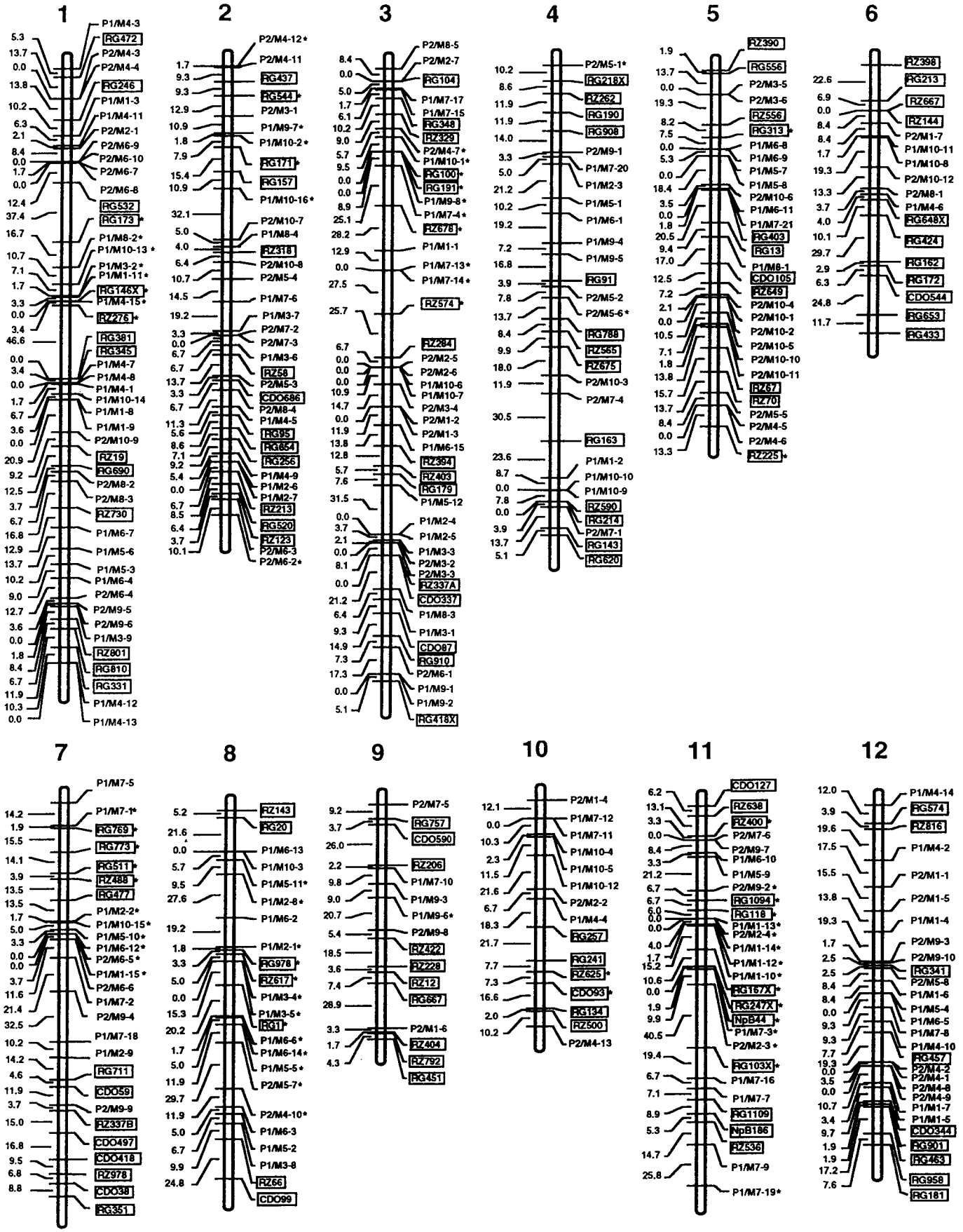
Table 1 Polymorphism, origin and distribution of AFLP markers in the cross 'IR64' × 'Azucena'

Primer pair	Visible bands (no.)	Polymorphic bands (no.)	*Origin of amplification* IR 64	Azucena	Polymorphism (%)	Chromosomes covered (no.)	Doublets (no.)	Codominant markers (no.)
P1/M1	40	15	7	8	37.5	6	3	1
P1/M2	48	9	4	5	18.8	5	1	1
P1/M3	46	9	5	4	19.6	4	0	0
P1/M4	59	15	5	10	25.4	5	0	1
P1/M5	46	12	6	6	26.1	8	0	1
P1/M6	45	15	8	7	33.3	8	0	1
P1/M7	75	21	11	10	28.0	9	3	1
P1/M8	23	4	1	3	17.4	4	1	0
P1/M9	43	8	8	0	18.6	4	1	0
P1/M10	62	16	9	7	25.8	8	0	3
P2/M1	47	7	3	4	14.9	5	1	1
P2/M2	38	7	3	4	18.4	4	4	1
P2/M3	44	6	2	4	13.6	3	0	2
P2/M4	43	13	8	5	30.2	7	2	4
P2/M5	51	8	3	5	15.7	5	2	0
P2/M6	39	10	5	5	25.6	4	0	2
P2/M7	47	6	2	4	12.8	4	2	1
P2/M8	39	5	0	5	12.8	4	4	0
P2/M9	60	10	4	6	16.7	6	2	1
P2/M10	50	12	8	4	24.0	5	2	1
Total	945	208	102	106	–	–	28	22
Mean	47.3	10.4	–	–	21.8	–	–	–
Range	23–75	4–21	–	–	12.8–37.5	–	–	–

Table 2 Comparison of combined AFLP/RFLP map with the RFLP map in the cross 'IR64' × 'Azucena'

Chromosome	RFLP map length (cM)	Combined map length (cM)	RFLP markers (no.)	AFLP markers (no.)	Total no. of markers	Map length (cM) increased by AFLPs
1	222	387	14	34	48	165
2	168	295	13	23	36	127
3	279	394	16	30	46	115
4	163	306	13	16	29	143
5	146	232	11	19	30	86
6	156	173	11	6	17	17
7	170	257	13	14	27	87
8	79	241	7[a]	16	23	162
9	117	153	10	6	16	36
10	63	148	6	9	15	85
11	142	246	12	16	28	104
12	106	226	9	19	28	120
Total	1811	3058	135	208	343	1247

[a] The two RFLP markers RG20 and RZ143 are included, which are now linked to RG978 due to placement of AFLP markers


1
2
3
4
5
6
7
8
9
10
11
12

stretching of most of the chromosomes. The combined AFLP/RFLP map was extended towards telomeric regions in 9 chromosomes, which accounts for 251 cM. The AFLP markers filled a gap between RG20/RZ143 and RG978 on chromosome 8 (Huang et al. 1994), which resulted in an increase in map length of 90 cM (Fig. 2).

The AFLP/RFLP map showed that many AFLP markers were located adjacent to each other (Fig. 2). There were 18 locations where more than 5 AFLP markers were mapped closely together. The most typical example is on chromosome 10. Half of the chromosome was covered by AFLP markers while the other half was covered by RFLP markers. We found very little intermingling of AFLP and RFLP loci. The mapping of these AFLP markers had a stretching effect which was very pronounced on most of the chromosomes (Fig. 2).

Discussion

The AFLP technique allows the detection of polymorphism in multiple loci, generating a vast number of highly reproducible molecular markers within a short period of time. In this study we used only 20 primer combinations and were able to scan 945 loci to generate 208 AFLP markers in a doubled haploid population of rice (Table 1). The efficiency of generating AFLP markers appears to be much higher relative to RFLP mapping in the same population (Huang et al. 1994), while the speed at which they can be generated shows a great potential for application in marker-assisted backcross breeding, where the aim of the breeders is to screen hundreds of loci within a limited time frame. The judicious selection of primer combinations that generate high levels of polymorphism with markers well-distributed over the genome is the key. Such a selection is possible: we observed that some primer combinations produced as many as 21 polymorphic markers distributed over as many as 9 chromosomes (Table 1).

Linkage map construction with AFLP markers is quite efficient and complementary to that based on RFLPs. This can be corroborated in terms of speed of generation of markers and also coverage of genomic regions. In general, the regions covered by RFLPs are also covered by AFLP markers. We believe that the few regions not covered by AFLP markers at present will be so with an increased number of primer combinations. Furthermore, AFLP markers can cover areas lacking RFLPs. This was demonstrated on chromosome 8, where previously unlinked regions were linked by AFLP markers (Fig. 2).

All of the AFLP markers were unambiguously placed on the chromosomes. A great majority of markers (78%) followed Mendelian segregation indicating that AFLP markers are heritable and reliable for genetic analysis. Furthermore, the segregation of AFLP markers was found to be the same as that of RFLP markers (Fig. 2). Comparison between RFLP and AFLP maps indicates a random distribution of AFLP markers along the genome except on chromosomes 6 and 9 where only a few AFLP markers were placed in the present study.

In combining AFLP and RFLP markers, we generated a map of 3058 cM, which is 1247 cM longer than the map generated with RFLP markers alone (Huang et al. 1994). The extension of the map towards telomeric region by AFLPs on many chromosomes and the filling of a gap on chromosome 8 constituted about one-quarter of the increased map distance. The remaining three-quarters was due to a stretching effect, which was pronounced on most of the chromosomes (Table 2). Several causes can account for the stretching effect, which has also been observed in other crops (Becker et al. 1995). The small population size used in this study may be one of the reasons, as it would offer comparatively less genetic resolution. Moreover, the observation of heterozygosity at a number of RFLP loci in the doubled haploid population (unpublished data) might inflate the recombination fractions to great extent, particularly in the case of dominant markers like AFLP. The stretching effect could also be due to AFLP markers targeting different genomic regions than those already marked by RFLPs as very few AFLPs are tightly linked to mapped RFLPs (Fig. 2). However, further experiments are needed to test this hypotheses. Distorted segregation for some markers along the chromosome is another factor for the map stretching. Lorieux et al. (1996) recently showed that linkage map length can be reduced by 27% if the frequency of distorted alleles is corrected for. In this study, we placed all of the markers uniquely on the genome with a high LOD score greater than 4.0. Therefore, misplacement of AFLP markers on the rice chromosome is small. One way to reduce the map distance is by fixing the map length with anchor markers and then placing remaining markers to the interval, as was done by Causse et al. (1994).

Codominant loci are generally the most informative. Most of the codominant amplification products were very close to each other in molecular weight and are readily identifiable. We identified 22 codominant loci, the majority of which were distributed over chromosomes 1, 3 and 5. With the screening of an increased number of primer combinations, more such loci will be evident, which should be useful for gene mapping studies.

To simplify the original AFLP protocol (Vos et al 1995), we labeled the AFLP fragments by direct incorporation of alpha-[^{32}P]-dCTP in the PCR mix during the selective amplification step instead of labeling the primer. As expected, doublets (28/208) were observed due to the unequal mobility of the two complementary strands of the labeled amplified fragments on the denaturing gel (Table 1). These were easily identified and scored as single markers. Therefore, generating AFLPs by the direct incorporation of label is a simple and efficient alternative protocol in rice and perhaps in other cereal crop species as well.

Acknowledgements We would like to thank John Bennett for the helpful discussion, and Allan Bakalinsky, Ramesh Aggarwal and Dahu Chen for their critical review of the manuscript. Somen Nandi was supported by a fellowship from the Jawaharlal Nehru Memorial Fund, India. We thank the German Government and Rockefeller Foundation for financial support.

References

Arumuganathan K, Earle ED (1991) Nuclear DNA content of some important plant species. Plant Mol Biol Rep 9:208–218

Becker J, Vos P, Kuiper M, Salamini F, Heun M (1995) Combined mapping of AFLP and RFLP markers in barley. Mol Gen Genet 249:65–73

Causse M, Fulton TM, Cho YG, Ahn SN, Chunwongse EJ, Wu K, Xiao J, Yu Z, Ronald PC, Harrington SB, Second G, McCouch SR, Tanksley SD (1994) Saturated molecular map of the rice genome based on an interspecific backcross population. Genetics 138:1251–1274.

Dellaporta SL, Wood J, Hick JB (1983) A plant DNA mini preparation; version II. Plant Mol Biol Rep 1:19–21

Guiderdoni E, Galinato E, Luistro J, Vergara G (1992) Anther culture of tropical japonica X indica hybrids of rice (*Oryza sativa* L.). Euphytica 62:219–224

Hodges TK, Peng J, Lyznik LA, Koetje DS (1991) Transformation and regeneration of rice protoplasts. In: Khush G, Toenniessen G (eds) Rice biotechnology. IRRI, Manila, Philippines, pp 157–174

Huang N, McCouch SR, Mew T, Parco A, Guiderdoni E (1994) Development of a RFLP map from a doubled haploid population of rice. Rice Genet Newsl 11:134–137

Kosambi DD (1944) The estimation of map distances from recombination values. Ann Eugen 12:172–175

Kurata N, Nagamura Y, Yamamoto K, Harushima Y, Sue N, Wu J, Antonio BA, Shomura A, Shimizu T, Lin S-Y, Inoue T, Fukuda A, Shimano T, Kuboki Y, Toyama T, Miyamoto Y, Kirihara T, Hayasaka K, Miyao A, Monna L, Zhong HS, Tamura Y, Wang Z-X, Momma T, Umehara Y, Yano M, Sasaki T, Minobe Y (1994) A-300-kilobase interval genetic map of rice including 883 expressed sequences. Nat Genet 8:365–372

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Litt M, Luty J (1989) A hypervariable microsatellite revealed by in vitro amplification of a dinucleotide repeat within the cardiac muscle action gene. Am J Hum Genet 44:391–401

Lorieux M, Petrov M, Huang N, Faure J, Guiderdoni E, Chesquiere A (1996) Aroma in rice is controlled by a major gene and at least two minor QTLs. Theor Appl Genet (in press)

Moore G, Devos KM, Wang Z, Gale MD (1995) Grasses line up and form a circle. Curr Biol 5:737–739

Olson M, Hood L, Cantor C, Botstein D (1989) A common language for physical mapping of the human genome. Science 245: 1434–1435

Rafalski JA, Tingey SV (1993) Genetic diagnostics in plant breeding: RAPDS, microsatellites and machines. Trends Genet 9: 275–280

Tanksley SD, Young ND, Patterson AH, Bonierbale MW (1989) RFLP mapping in plant breeding: new tools for an old science. Bio/Technology 7:257–264

Thomas CM, Vos P, Zabeau M, Jones DA, Norcott KA, Chadwick BP, Jones JDG (1995) Identification of amplified restriction fragment polymorphism (AFLP) markers tightly linked to the tomato *Cf-9* gene for resistance to *Cladosporium fulvum*. Plant J 8:785–794

Vos P, Hogers R, Bleeker M, Reijans M, Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res 23:4407–4414

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey VS (1990) DNA polymorphism amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 18:6531–6535

Zabeau M, Vos P (1993) Selective restriction fragment amplification: a general method for DNA fingerprinting. European Patent Application number: 92402629.7, Publication number 0534858 A 1