Rapid Population Analysis of *Magnaporthe grisea* by Using rep-PCR and Endogenous Repetitive DNA Sequences

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ABSTRACT

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DNA samples from *Magnaporthe grisea* isolates were fingerprinted by using repetitive element-based polymerase chain reaction (rep-PCR) with two outwardly directed primer sequences from *Pot2*, an element found in approximately 100 copies in the fungal genome. Variable length fragments, defining the sequences lying between these elements, were generated, and fingerprint patterns specific for individual strains were established. "Long PCR" conditions, including higher pH (9.2) and increased extension time (10 min) were used to amplify DNA fragments ranging from 400 bp to longer than 23 kb. Polymorphisms specific to *M. grisea* strains were generated, allowing inference of their genetic relationships. Segregation analysis was used to confirm single-locus inheritance for the fragments amplified by rep-PCR. Cluster analysis revealed robust groupings that corresponded to previously determined MGR586 restriction fragment length polymorphism lineages of the rice-infecting strains of the pathogen. We have also demonstrated the utility of rep-PCR to differentiate isolates that infect rice from those that infect nonrice hosts. DNA fingerprinting by *Pot2* rep-PCR provides an efficient means to monitor the population dynamics of the blast pathogen. Because of the method's low cost and ease in application, it is now feasible to conduct large-scale population studies to understand the impact of host genotypes on pathogen evolution.

Additional keywords: population genetics, *Pyricularia grisea*, rice blast.

Repetitive element-based polymerase chain reaction (rep-PCR) generates DNA fingerprints by amplifying sequences between randomly dispersed copies of the element in a genome. This technique combines the simplicity of the PCR with the polymorphism detected by restriction fragment length polymorphism (RFLP) and has been used for fingerprinting soil bacteria and plant-pathogenic bacteria and fungi (6,8,12,21,30,31). Primers used in these studies corresponded to conserved motifs in bacteria, such as the repetitive extragenic palindromic sequence from *Escherichia coli* (13), the enterobacterial repetitive intergenic consensus sequences (16), and the BOX element from the gram-positive bacterium *Streptococcus pneumoniae* (22). Because of the conserved nature of these sequences, they allow amplification in many microorganisms, including fungi, and different degrees of polymorphism are detected for different genomes. A higher level of polymorphism and specificity can be achieved when rep-PCR is based on endogenous repetitive elements, such as that developed for the bacterial blight pathogen of rice, *Xanthomonas oryzae* pv. *oryzae* (12).

Numerous repetitive DNA sequences have been identified and cloned from the genome of the rice blast fungus, *Magnaporthe grisea* (T. T. Hebert) Yaegashi & Udegawa (anamorph *Pyricularia grisea* (Cooke) Sacc.) (7,10,14,17,26,27). Among these, the MGR586 element has been the most extensively used probe for characterizing populations of the blast pathogen (3,4,14,20,33,35). *Pot2*, another repetitive element isolated from *M. grisea*, shares structural features with MGR586 and Fot1 from *Fusarium oxysporum* (5,17). These elements have terminal inverted repeats with an internal direct repeat motif and have similar peptide sequences of the putative transposases but no detectable similarity in DNA sequence (9,17). The 1,857-bp *Pot2* element represents one of the major repetitive DNAs shared by isolates of *M. grisea* that infect rice and those that infect nonrice hosts (17). In contrast, MGR586 is present in 45 to 50 copies per genome in the blast pathogen of rice but in only one to three copies per genome in pathogens of other grasses (14).

DNA fingerprinting is central to population structure analysis of *M. grisea* and provides needed information for rational deployment of resistance genes (35,36). The current approach of RFLP analysis requires an enormous amount of time, labor, and resources, making it prohibitive for the analysis of a large number of samples in population studies. Thus, there is a need for a technique that is as discriminating as RFLP analysis yet sufficiently simple for application in laboratories with modest facilities. In this paper, we report the development of a rep-PCR fingerprinting method specific for the blast pathogen.

MATERIALS AND METHODS

Strains and culture media. Two sets of rice-infecting *M. grisea* isolates, previously fingerprinted by the MGR586 RFLP method and phylogenetically analyzed (4,18; J. Kumar, R. S. Zeigler, and R. J. Nelson, *unpublished data*), were used in this study. One set consisted of 71 isolates representing seven MGR586 RFLP lineages from the Philippines maintained at the International Rice Research Institute, Manila. Another set consisted of 35 isolates from the Himalayas of India representing 15 lineages. Dried mycelia from the Indian isolates were kindly provided by J. Kumar of G. P. Pant University of Agriculture and Technology, India. Twenty-six *M. grisea* isolates from eight other host species from the Philippines (3) and India (J. Kumar, R. S. Zeigler and R. J. Nelson, *unpublished data*) were also subjected to DNA fingerprinting (Table 1). To determine the inheritance of DNA fragments amplified by rep-PCR, DNA samples from 36 random ascospore progeny from a

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cross between the laboratory strains Guy 11 and 2539 (19) were fingerprinted.

To determine the specificity of the primers to the blast fungus pathogen, DNA from *E. coli* HB101 and 10 DNA samples each from the bacterial blight pathogen of rice (*X. oryzae* pv. *oryzae),* the sheath blight pathogen of rice (*Rhizoctonia solani*), and rice (*Oryza sativa)* were also subjected to the rep-PCR analysis.

All *M. grisea* isolates were maintained on prune agar slants (19). For DNA extraction, isolates were grown for 5 days with constant shaking at 25°C in Fries broth (29) supplemented with 0.5 g of casein hydrolysate per liter.

Isolation of genomic DNA. Genomic DNA was isolated by the potassium acetate extraction method (25). Mycelia were harvested, freeze-dried, frozen in liquid nitrogen, and ground into fine powder with a mortar and a pestle. About 25 mg of powdered mycelia was suspended in 650 µl of extraction buffer (100 mM Tris, pH 8; 100 mM EDTA; 250 mM NaCl; and 1% sodium dodecyl sulfate, wt/vol) at 65°C for at least 1 h. Cellular proteins were precipitated with 100 µl of potassium acetate (3 M potassium and 5 M acetate, pH 4.8), and then DNA was precipitated by using isopropanol. The DNA was dissolved in 200 µl of sterile distilled water. One microliter of the DNA solution (100 ng/µl) was used as template for PCR.

Oligonucleotide design and synthesis. The primer sequences were based on the sequence of the repetitive element *Pot2* (EMBL accession Z33638), an inverted repeat transposon found in *M. grisea* (17). The two primers (*Pot2-1* 5′ CGGAAGCCCTAAAGCTGTTT 3′ and *Pot2-2* 5′ CCCTCATTCGTCACACGTTC 3′) were designed from each end of *Pot2* in opposite orientation such that the 3′ ends were directed outward from each element. The two primers, whose sequences are internal to the 43-bp inverted terminal repeats, amplify the flanking sequences on both sides of the repetitive element, generating fragments spanning the intervening sequences. The oligonucleotides were designed with the software PC/GENE (IntelliGenetics, Mountain View, CA) and synthesized by Genset Corporation (La Jolla, CA).

PCR. Amplification was performed in a 25-µl volume containing 0.5 µM each of the two opposing primers, 100 ng of genomic DNA, 185 µM each of four dNTPs, and approximately 2.5 units of *Taq* polymerase in PCR buffer no. 9 (10 mM Tris, pH 9.2; 25 mM KCl; 1.5 mM $MgCl_2$; and 15 mM $[NH_4]_2SO_4$). Buffer no. 9 was developed by using the Opti-Prime PCR Optimization Kit (Stratagene, La Jolla, CA). The reaction mixture was overlaid with one drop of mineral oil, initially denatured for 2.5 min at 95°C, and then subjected to four cycles of 1-min denaturation at 94°C, 1-min annealing at 62°C, and 10-min extension at 65°C; 26 cycles of 30-s denaturation at 94°C, 1-min annealing at 62°C, and 10-min extension at 65°C; and a final extension for 15 min at 65°C with a model PTC-100 thermal cycler (MJ Research, Watertown, MA).

To visualize the DNA fingerprints, 10 µl of the PCR products was loaded in a gel containing 0.5% agarose, 0.75% Synergel (Diversified Biotech, Newton, MA), and 0.5× Tris-borate buffer (89 mM Tris, pH 7.8; 89 mM boric acid; and 2 mM EDTA). Gels were run for 7 h at 120 V, stained with ethidium bromide, and then photographed with Polaroid type 665 film. Prints were enlarged to 20 × 25 cm to facilitate the scoring of bands.

To ensure that only reproducible bands were scored, PCR analysis was done at least twice for each DNA sample, and those that were consistently amplified were scored. Resolved bands ranging in size from 400 bp to approximately 23 kb were scored regardless of intensity. Scoring of the amplified bands was based on duplicate PCR products that were loaded separately but side by side in adjacent lanes. A negative control without template DNA was included in all experiments.

Cluster analysis. To determine the genetic relationships among isolates, the presence or absence of bands was converted into binary data (1 for presence and 0 for absence of each band). Similarity matrices were calculated with Dice's coefficient and the SIMQUAL program of NTSYS-pc (Exeter Software, Setauket, NY). Cluster analysis was done within the SAHN program by using the UPGMA (unweighted pair-group method with arithmetic averages) method.

The robustness of the dendrogram was assessed by bootstrap analysis (11) with the WINBOOT program (23,34) and 2,000 repeated samplings with replacement. The bootstrap values, reflecting the frequency with which each group is formed in repeated cycles of dendrogram construction, were used as a measure of the relative stability of the clusters of strains.

Analysis of linkage. Segregation was analyzed by X^2 goodness-of-fit test and random association for all pairwise combinations of markers by X^2 test of independence (1,32). Linkage analysis was

TABLE 1. Number of amplified bands among rice-infecting and nonrice-infecting isolates of *Magnaporthe grisea* from the Philippines and India

	Number of bands in fingerprint	
Natural host	Philippines	India
Oryza sativa	10–26 (71)[a]	9–32 (35)
Cynodon dactylon	20–22 (3)	ND[b]
Digitaria ciliaris	4 (1)	2–7 (3)
Eleusine indica	1 (3)	3–7 (3)
Eragrostis sp.	4–5 (3)	ND
Leersia hexandra	8–21 (3)	ND
Paspalum distichum	12 (1)	ND
Panicum repens	10–28 (3)	ND
Setaria sp.	ND	3 (3)

[a] Number in parentheses is the number of isolates subjected to *Pot2* polymerase chain reaction analysis.
[b] Not determined.

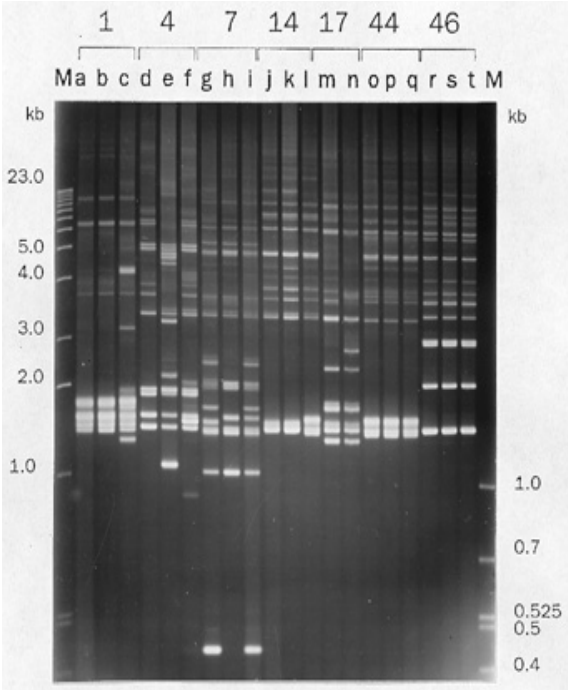

Fig. 1. *Pot2*-based polymerase chain reaction fingerprint patterns from genomic DNA of rice-infecting *Magnaporthe grisea* isolates from the Philippines. Representative fingerprint patterns of isolates C923-11 (a), C923-25 (b), C923-51 (c), C926-2 (d), C9212-14 (e), C9230-38 (f), C9211-2 (g), C9233-19 (h), CBN924-33 (i), CBN9212-5 (j), CBN9212-8 (k), C9232-5 (l), C9225-1 (m), C92337-39 (n), C9240-3 (o), C9240-8 (p), C9240-10 (q), C9228-7 (r), C9238-1 (s), and C9238-4 (t) are shown. The numbers represent the lineage designation of the strains based on MGR586 restriction fragment length polymorphism analysis. The DNA molecular size markers are on the lanes labeled M on the left (1-kb ladder, Bethesda Research Laboratories Life Technologies, Gaithersburg, MD) and right (BioMarker EXT, Bioventures, Murfreesboro, TN). All bands between 400 bp and 23 kb were scored, regardless of intensity.

done with MAPMAKER/EXP 3.0 (Whitehead Institute, Cambridge, MA). Linkage criteria were a maximum recombination fraction of 0.2 and a minimum LOD (log of the odds) score of 4.0. The Kosambi mapping function was used to compute recombination distances in centimorgans.

RESULTS

DNA polymorphisms detected by *Pot2* PCR. Distinct banding patterns were generated by using the two outwardly directed primers in combination with PCR conditions that favored the amplification of long fragments. The amplified bands ranged in length from 400 bp to more than 23 kb (Figs. 1 and 2), although most scorable bands were less than 23 kb long. Reproducible fingerprint profiles can be generated with DNA extracted by different methods, with different sources of *Taq* polymerase enzyme, and with different PCR machines (*data not shown*). However, the same experimental caveats that apply to PCR-based techniques also apply to this method; variations in the concentrations of the PCR components and in the electrophoresis conditions can influence the interpretation of results. Moreover, because the amplification of long fragments of DNA requires very efficient PCR conditions, we recommend that the method be optimized with appropriate positive and negative controls before the amplified bands are scored.

All rice-infecting *M. grisea* isolates showed amplified bands, ranging from 10 to 26 bands in the Philippine isolates and from 9 to 32 in the Himalayan isolates (Table 1). *Pot2* rep-PCR also generated distinct patterns of amplified bands among isolates from different nonrice hosts (Fig. 2). The number of amplified bands in isolates collected from other hosts ranged from 2 to 28 (Table 1).

No bands were amplified with the *Pot2* primers when DNA templates from *E. coli* and *X. oryzae* pv. *oryzae* were used. A few faint bands were amplified with DNA from *Rhizoctonia solani*, another fungal pathogen of rice. However, in contrast to the fingerprints obtained from the blast pathogen, the bands obtained with *R. solani* template DNA were generally monomorphic and remained faint, even when the annealing temperature was lowered to 52°C. No bands were amplified with DNA from rice.

Analysis of rice- and nonrice-infecting *M. grisea* isolates. *M. grisea* DNA from different nonrice hosts showed highly contrasting fingerprint patterns that consisted of intense and faint bands (Fig. 2). The number and intensity of the amplified bands were the same at annealing temperatures of 52 and 62°C. In general, isolates from the same host had comparable numbers of bands with some degree of polymorphism. However, while the number of bands was low in isolates of some hosts, it was within the range of the rice-infecting isolates in others. Isolates infecting *Cynodon dactylon* and *Panicum repens* had the most bands. Isolates from *Eragrostis* sp., *Digitaria ciliaris*, *Eleucine indica*, and *Setaria* sp. had fewer than 10 bands, regardless of country of origin (Table 1).

Cluster analysis of *M. grisea* isolates from the Philippines differentiated the rice-infecting (n = 71) and nonrice-infecting (n = 17) isolates at the 6% similarity level (Fig. 3). The isolates collected from rice shared 33% similarity, while those collected from other hosts were more diverse, sharing only 9% similarity.

Analysis of two collections of rice-infecting *M. grisea* isolates. Cluster analysis of the PCR banding patterns of the isolates from the Philippines (Fig. 4A) and India (Fig. 4B) showed a close correspondence between the groupings based on *Pot2* rep-PCR and those obtained by MGR586 RFLP. At the 70% similarity level, the phenogram of the Philippine isolates showed seven groups of isolates (Fig. 4A). At this level, a one-to-one correspondence was observed between the groups defined by MGR586 and by *Pot2* rep-PCR for the MGR586-defined Philippine lineages 1, 7, 46, and 17. The MGR586-defined lineage 4 was split at the 70% similarity level but formed a single group at the 60% similarity level. MGR586-defined lineages 14 and 44 were grouped together at the 70% similarity level but distinguished at about the 80% similarity level.

The genetic diversity of the Himalayan strains representing 15 MGR586 RFLP lineages was subsumed in 17 clusters at 70% similarity based on *Pot2* rep-PCR (Fig. 4B). One-to-one correspondence with the RFLP lineages was seen in 13 of the clusters. MGR586-defined lineages IHR10 and IHR102 each consisted of two PCR clusters sharing approximately 50% similarity. In contrast to the Philippine isolates, the Himalayan isolates were more diverse, sharing only 14% similarity.

The bootstrap values for the *Pot2* rep-PCR-based groupings ranged from 87 to 100%, except for the cluster corresponding to lineage 46, which had a bootstrap value of 59% (Fig. 4A). For the Himalayan isolates, the bootstrap values of clusters that had at least 80% similarity ranged from 98 to 100% (Fig. 4B).

Segregation of amplified bands and linkage tests. To confirm that the amplified DNA fragments represented single-locus markers, we analyzed DNA samples from 36 random ascospore progeny obtained from a cross between strains Guy 11 and 2539. The two parents differed in their fingerprint patterns, consisting of nine bands (800 bp to 8 kb) in Guy 11 and five (550 bp to 12 kb) in 2539. Goodness-of-fit X^2 tests showed that all 14 loci segregated in 1:1 ratios, suggesting that these sequences flanking the *Pot2* element segregate as single-locus markers (Table 2). Genetic mapping of the amplified loci showed the occurrence of three linkage groups (Fig. 5). The rest of the loci were unlinked.

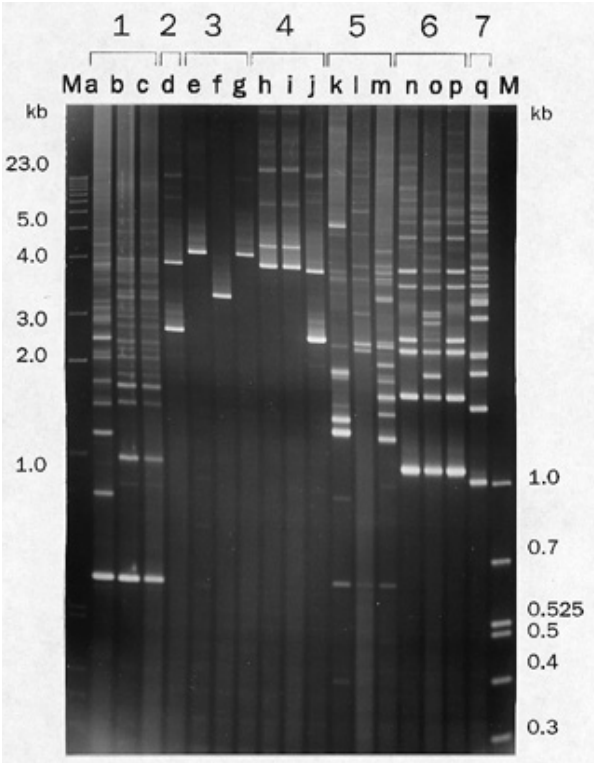

Fig. 2. *Pot2*-based polymerase chain reaction fingerprint patterns from genomic DNA from *Magnaporthe grisea* isolates from the Philippines that infect nonrice hosts. Representative fingerprint patterns of isolates Cd88217 (a), Cd88221-2 (b), Cd88232 (c), Dc9103 (d), EiA8309 (e), Ei88424 (f), Dc83010 (g), Er88271 (h), Er88264 (i), Er9106 (j), Lh88490 (k), Lh902 (l), Lh8865-1 (m), Pr8816-1 (n), Pr8988 (o), Pr9112 (p), and Pd88335-1 (q) are shown. The numbers indicate the hosts from which the isolates were collected: 1, *Cynodon dactylon*; 2, *Digitaria ciliaris*; 3, *Eleusine indica*; 4, *Eragrostis* sp.; 5, *Leersia hexandra*; 6, *Panicum repens*; and 7, *Paspalum distichum*. The DNA molecular size markers are on the lanes labeled M on the left (1-kb ladder, Bethesda Research Laboratories Life Technologies, Gaithersburg, MD) and right (BioMarker EXT, Bioventures, Murfreesboro, TN). All bands between 400 bp and 23 kb were scored, regardless of intensity.

DISCUSSION

RFLP fingerprinting with MGR586 has served as an important tool for understanding the population structure and genetic changes of the blast fungus (3,4,14,20,33,36). However, the high investment in time and resources for RFLP analysis has seriously limited the application of DNA fingerprinting for large-scale studies of pathogen ecology and evolution. Interest in such studies and the significance of the information to rice breeders provided the impetus to develop the rapid and cost-effective PCR-based technique reported here.

Bernardo et al. (2) used the random amplified polymorphic DNA (RAPD) method to fingerprint 120 Philippine isolates of the blast pathogen. The primer J-06 (5′ TCGTTCCGCA 3′) defined groups that corresponded with the groupings generated by MGR586. However, the correspondence between MGR586 and J-O6 PCR appeared to be population specific, and problems inherent with the RAPD technique limited the reproducibility of the fingerprints in different laboratories.

In this study, we used primers specific for the blast pathogen and "long PCR" conditions, including higher pH (9.2) and increased extension time (10 min), to amplify DNA sequences in the genome that lie between copies of the repetitive element *Pot2*. Because *Pot2* occurs at approximately 100 copies per haploid genome and is dispersed among the chromosomes (17), a number of intervening sequences were expected to be within amplifiable distance, generating sufficient polymorphism to detect genetic diversity. The fingerprint profiles consisted of 30 or fewer resolved fragments that are easy to score. Thus, like MGR586 RFLP analysis, *Pot2* rep-PCR provides multilocus haplotypes but with much less effort.

To ensure that the amplified bands are reproducible genetic markers, we determined the inheritance of the PCR products by

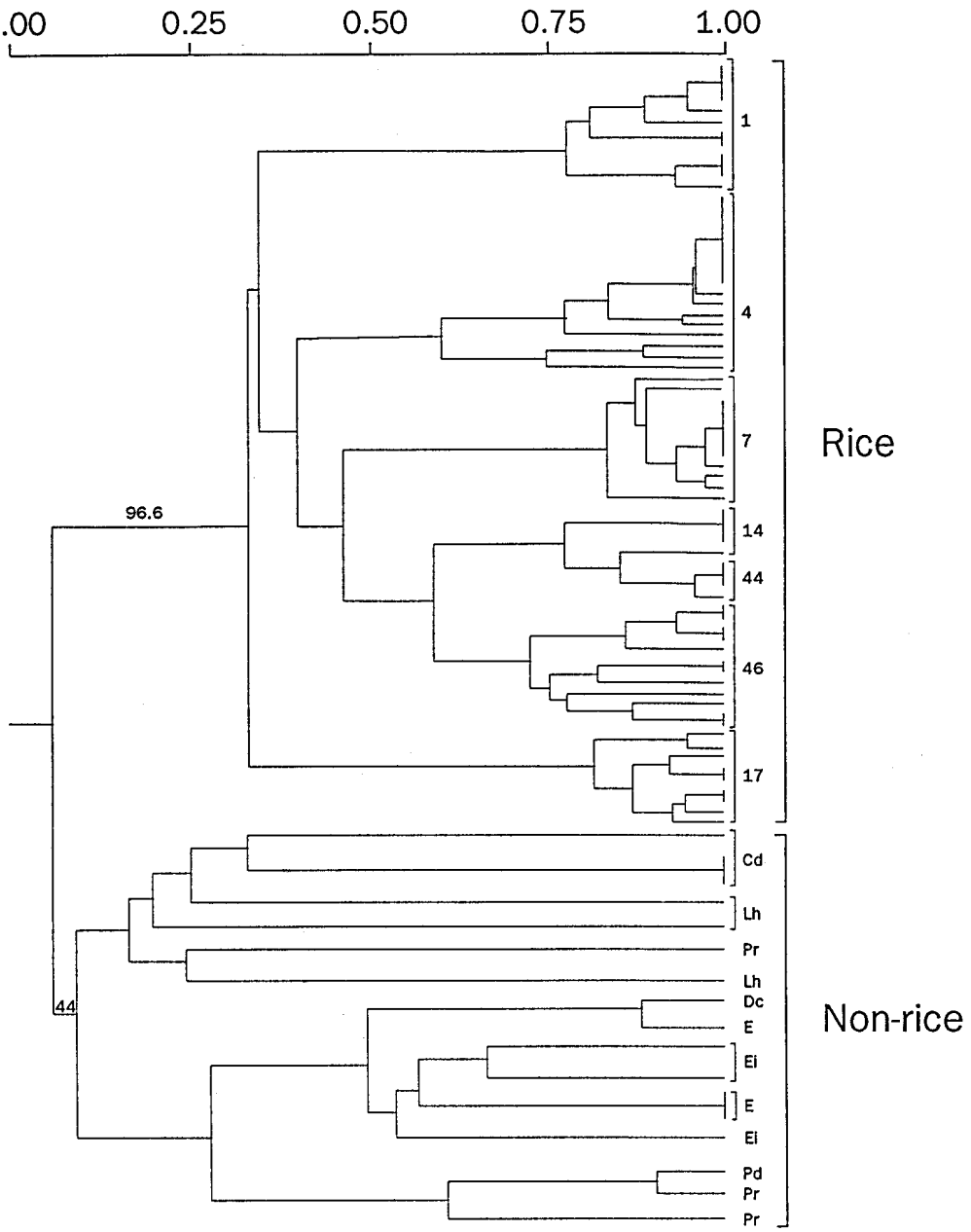

Fig. 3. Dendrogram constructed from *Pot2* repetitive element-based polymerase chain reaction fingerprint data from rice- and nonrice-infecting isolates of *Magnaporthe grisea* from the Philippines. Numbers at the top represent the lineage designation of the strains based on restriction fragment length polymorphism analysis with MGR586 as a probe. Letters indicate the hosts from which the isolates were collected: Cd, *Cynodon dactylon*; Dc, *Digitaria ciliaris*; Ei, *Eleusine indica*; E, *Eragrostis* sp.; Lh, *Leersia hexandra*; Pd, *Paspalum distichum*; and Pr, *Panicum repens*. The bootstrap values of the two major groups are noted as numbers on the main branches of the dendrogram.

segregation analysis. While each amplified band behaved as a dominant, single-locus marker, at least six locus pairs deviated from random association, suggesting possible linkage among these markers. Although the *Pot2* element has been shown to be dispersed among the chromosome-sized DNA molecules of the *M. grisea* genome, there is no information on the distribution of the *Pot2* elements within a chromosome (17). Our segregation data, though based on a relatively small population, suggest that some of the amplified fragments could be linked. This could result from limited recombination between certain regions of the parental chromosomes. Furthermore, the observed linkage may also reflect the unique nature of transposable elements; in at least one instance, a *Pot2* element has been found to locate within another repetitive element (17). This nested arrangement, which is also observed in the MAGGY element (10), may result in the association of DNA fragments amplified by rep-PCR.

The utility of the *Pot2* rep-PCR analysis as a fingerprinting tool is clearly demonstrated by the close correspondence between the lineages defined by rep-PCR and MGR586 RFLP analysis. The polymorphisms detected by the PCR method closely corresponded to those generated by MGR586 in a collection of *M. grisea*

TABLE 2. Results of goodness-of-fit tests for segregation of bands amplified by repetitive element-based polymerase chain reaction in *Magnaporthe grisea*

Amplified band[a]	Parental genotype (a:b)	Ascospore segregant (a:b)	Expected ratio	X^{2}[b]
A0.6	A0.6:null	15:21	1:1	0.69
A1.8	A1.8:null	17:19	1:1	0.03
A1.9	A1.9:null	14:22	1:1	1.36
A2.2	A2.2:null	13:23	1:1	2.25
A2.4	A2.4:null	14:22	1:1	1.36
A2.6	A2.6:null	17:19	1:1	0.03
A3.1	A3.1:null	16:20	1:1	0.25
A4.7	A4.7:null	15:21	1:1	0.69
A4.9	A4.9:null	14:22	1:1	1.36
B1.7	null:B1.7	16:20	1:1	0.25
B2.5	null:B2.6	12:24	1:1	3.36
B3.5	null:B3.5	23:13	1:1	2.25
B3.8	null:B3.8	18:18	1:1	0.03
B4.5	null:B4.5	16:20	1:1	0.25

[a] Numbers are approximate sizes of amplified bands (kb) found in either parental strain.
[b] Not significant at $P > 0.05$.

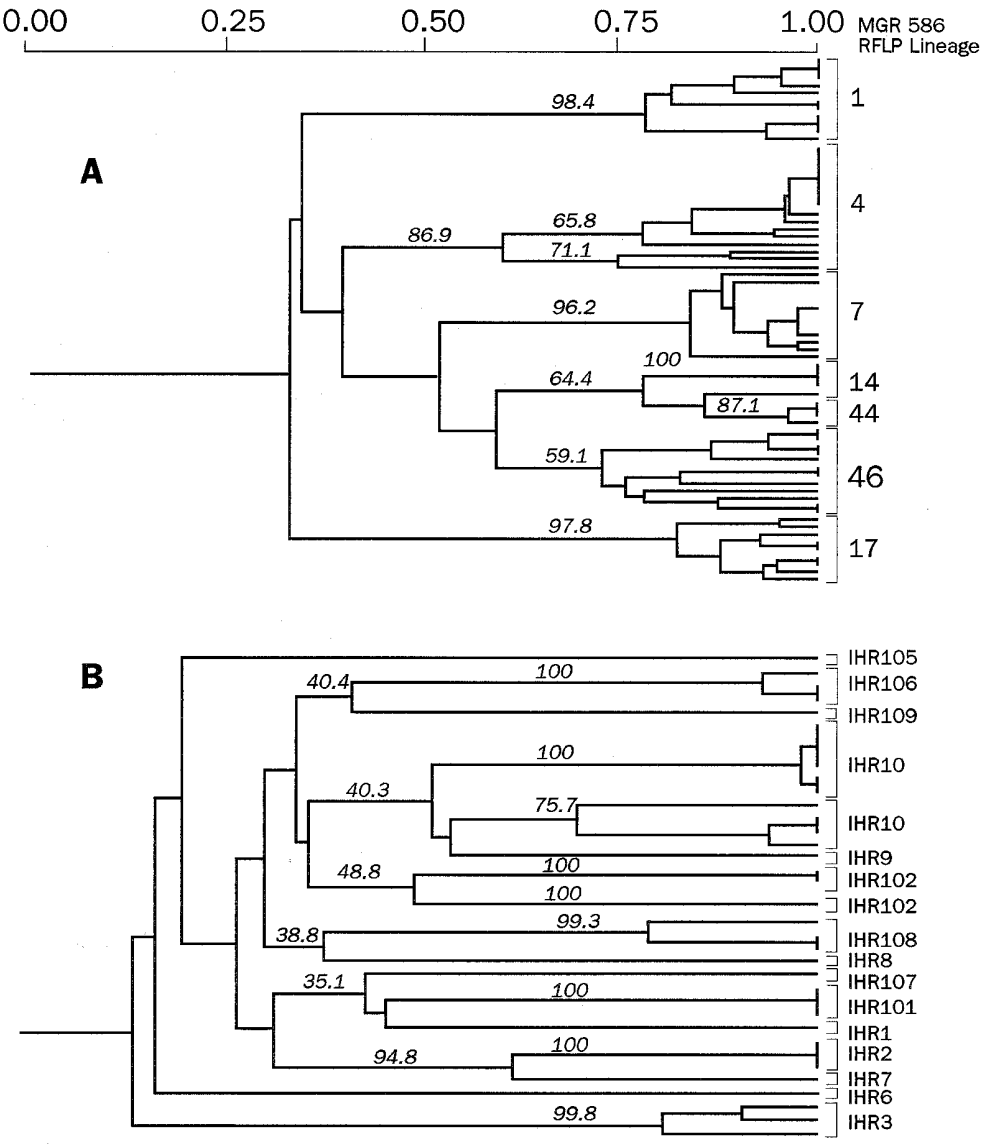

Fig. 4. Dendrogram constructed from *Pot2* repetitive element-based polymerase chain reaction fingerprint data from *Magnaporthe grisea* isolates from the Philippines **(A)** and India **(B)**. Numbers at the top represent the lineage designation of the strains based on restriction fragment length polymorphism analysis in which MGR586 was used as a probe. The bootstrap values are noted as numbers on the main branches of the dendrogram.

isolates that represent clonal lineages from the Philippines as well as highly diverse populations from the Indian Himalayas. General correspondence between MGR586 RFLP and this method was also observed in isolates collected from China (S. Zhang, Guangdong Academy of Agricultural Sciences, *personal communication*), suggesting that DNA fingerprinting by *Pot2* rep-PCR may have a broad application.

We have also demonstrated the utility of rep-PCR to differentiate rice- and nonrice-infecting isolates. Cluster analysis of data from *Pot2* PCR fingerprints distinguished rice- and nonrice-infecting isolates; isolates from rice formed a large and diverse cluster, while isolates from other hosts were much more distantly related. Among the nonrice-infecting isolates, there was divergence in some isolates from the same host. Isolates from *C. dactylon*, *L. hexandra*, and *P. repens* formed one subgroup, while those from *D. ciliaris*, *Eragrostis* sp., *E. indica*, *P. distichum*, and *P. repens* formed another. The fact that *Pot2* is present in both rice- and nonrice-infecting isolates of *M. grisea* at equal copy numbers (17) broadens its utility relative to other host-specific repetitive elements (7). With increasing evidence suggesting that genetic exchanges are occurring among *M. grisea* isolates in certain rice-growing environments (J. Kumar, R. S. Zeigler, and R. J. Nelson, *unpublished data*), fingerprinting with *Pot2* rep-PCR may provide a convenient assay for gene flow in populations that are suspected of having active genetic recombination by sexual or parasexual means.

In addition to serving as a fingerprinting tool, *Pot2* rep-PCR (and rep-PCR in general) can be used to construct linkage maps of *M. grisea*. The MGR sequences have been used to map pathogenicity genes (15,28), to construct a genetic map, and to develop marked strains for mapping new mutations (24). Although the number of fragments detected by *Pot2* rep-PCR is relatively low, it is possible to combine it with other primers to generate more markers. We have produced new banding patterns by pairing each of these *Pot2* primers with outward primers based on the sequence of another repetitive element, *Fosbury* (M. L. C. George, *unpublished data*). This method can be used to rapidly produce a framemap of the fungus by using different mapping populations.

In conclusion, DNA fingerprinting by *Pot2* rep-PCR has excellent potential as a tool for tracking the evolution and population dynamics of the blast pathogen. Through manipulation of the primer combinations, the rep-PCR approach is versatile in generating the number of bands needed for determining clonality or for genetic mapping. Because of the low cost and ease in application of this technique, it may now be feasible to conduct large-scale studies to understand the impact of host genotype on pathogen variability. The availability of a simple technology will enable laboratories with modest facilities to extensively characterize local pathogen populations. The information gathered from broad geographic areas will help to design effective strategies for the deployment of resistant germ plasm in rice-growing countries.

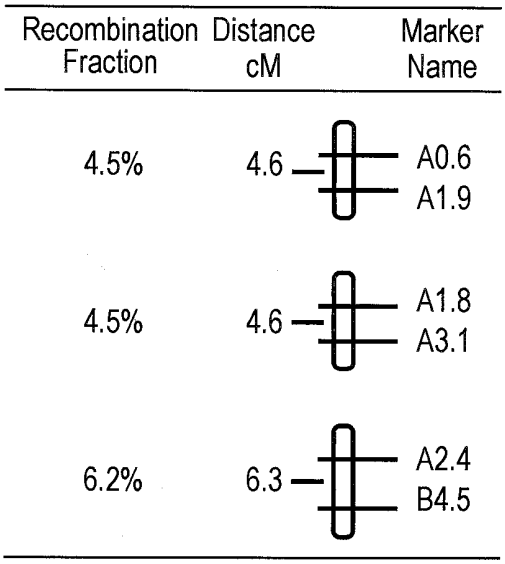

Fig. 5. Linkage relationships of loci flanking copies of the *Pot2* repetitive element based on the segregation of 36 progeny from a cross between strains Guy 11 and 2539. Linkage analysis was done with MAPMAKER/EXP 3.0 (Whitehead Institute, Cambridge, MA).

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