Virulence and rep-PCR Analysis of *Pyricularia grisea* Isolates from Two Brazilian Upland Rice Cultivars

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ABSTRACT

The phenotypic and genetic diversity of 77 isolates of *Pyricularia grisea* collected from two upland rice cultivars, Maravilha and Primavera, was studied. Isolates exhibiting compatible reaction to cv.Primavera were incompatible to cv.Maravilha and *vice versa,* with the exception of six isolates that were compatible to both cultivars. The virulence of isolates from cv. Maravilha on 32 test genotypes of rice was significantly higher ($t = 9.09$, $p \leq 0.0001$) than the isolates from cv.Primavera. A phenogram constructed from virulence data showed two main groups, one constituted mainly of isolates from cv.Primavera (97.6%) and the other of isolates from cv.Maravilha (91.17%). Rep-PCR analysis of isolates using two primers designed from sequences of *Pot2* showed that isolates could be clustered broadly into two groups. The average similarity within a cluster of isolates from cv.Primavera was significantly greater than the average similarity among the isolates of cv.Maravilha ($t = 5.37$, $p \leq 0.0001$). There was close correspondence between clusters based on PCR and virulence data ($r = 0.48$, $p \leq 0.011$). The results showed that isolates of *P. grisea* were cultivar specific and had low phenotypic and genetic diversity.

Additional keywords: *Oryza sativa,* rice blast, *Magnaporthe grisea*, pathotypes, genetic diversity.

RESUMO

Análise de virulência e rep-PCR de isolados de *Pyricularia grisea* de duas cultivares brasileiras de arroz de terras altas

A diversidade fenotípica e genética de *Pyricularia grisea* foi avaliada em uma amostra de 77 isolados coletados das cultivares de arroz de terras altas Maravilha e Primavera. Isolados que apresentaram reações compatíveis em cv. Primavera foram incompatíveis em cv.Maravilha e *vice versa*, com exceção de seis isolados que foram compatíveis nas duas cultivares. A virulência de isolados provenientes da cv. Maravilha, em 32 genótipos de arroz foi significativamente maior ($t = 9,09$; $p \leq 0,0001$) que dos isolados da cv. Primavera. O fenograma construído a partir dos dados fenotípicos mostrou dois grupos, um constituído principalmente por isolados da cv. Primavera (97,6%) e outro de isolados da cv. Maravilha (91,17%). A análise de rep-PCR dos isolados, utilizando os primers *Pot2.*, revelou dois grupos distintos. A similaridade média dos isolados da cv. Primavera dentro de grupo foi maior do que a similaridade média dos isolados da cv. Maravilha ($t = 5,37$; $p \leq 0,0001$). Observou-se uma correspondência entre a análise de agrupamento baseada em PCR e virulência fenotípica ($r = 0,48$; $p \leq 0,011$). Os resultados mostraram ainda uma alta especificidade de isolados de *P. grisea* para cada cultivar e baixa variação fenotípica e genética.

Palavras-chave adicionais: *Oryza sativa*, brusone, *Magnaporthe grisea*, patótipos, diversidade genética.

INTRODUCTION

Rice blast caused by *Pyricularia grisea* Sacc. [teleomorph *Magnaporthe grisea* (T.T. Hebert) M.E. Barr] is the major yield constraint in upland rice in Brazil. It is widely recognized that when a cultivar with moderate resistance is extensively grown, pathotypes matching their resistance genes will increase in frequency resulting in greater susceptibility (Filippi & Prabhu, 2001). The susceptibility of the widely grown upland rice cultivars Maravilha and Primavera has increased since their release, and currently the grain yield losses are considered significant.

Studies leading to determine the virulence diversity of pathogen populations isolated from widely grown cultivars is important for blast resistance breeding. Rice blast fungus populations are composed of pathotypes or physiological races with distinct virulence characteristics. These pathotypes are identified based on reaction types on a set of eight standard international differentials (Atkins *et al*., 1967**)**.

In Brazil and many other countries, a great number of physiological races have been reported indicating changes in virulence frequencies (Ou, 1980; Bonman *et al*., 1987). In an earlier investigation, the pathotypes IC-1 and IB-9 were found to be predominant in a sample population of *P. grisea* isolates collected from the cv. Primavera. In contrast, a relatively high frequency of isolates from the cv. Maravilha were identified as pathotypes IB-41 and IB-9 (Prabhu *et al*., 2003). The predominance of the pathotypes IB-9 and

IB-41 among isolates of Maravilha was also encountered among isolates of *P. grisea* retrieved from other upland rice cultivars (Filippi & Prabhu, 2001). The pathotype IC-1 was encountered at a high frequency only among isolates of cv. Primavera, whereas the pathotype IB-9 was commonly found among isolates of both cultivars Primavera and Maravilha. The pathotypes representing other groups (IA and ID) were obtained from the cultivars Primavera and Maravilha at low frequency (Prabhu *et al*., 2003).

As the information about virulence and genetic diversity of isolates retrieved from widely grown commercial cultivars is available their value and potential use in breeding for blast resistance increases. The genetic diversity of *P. grisea* is commonly assessed employing molecular techniques. Distinct DNA fingerprint groups or lineages were identified using the probe MGR586 (Filippi *et al*., 1999; Hamer, 1991; Zeigler *et al*., 1995). Many of these clonal lineages have been shown to exhibit restricted diversity (Levy *et al*., 1993; Xia *et al*., 1993; Chen *et al*., 1995; Roumen *et al*., 1997; Don *et al*., 1999; Park *et al*., 2003; Rathour *et al*., 2004). Because of difficulties in large scale use of RFLP analysis, a rep-PCR technique with two primer sequences from *Pot2* has been developed. Comparison of groupings of isolates based on *Pot2* rep-PCR and those obtained using MGR586 showed a close correspondence (George *et al*., 1998). Analysis of *P. grisea* isolates from irrigated rice cultivar Metica-1 using rep-PCR with two primer sequences from *Pot2* generated fingerprint profiles ranging from one to nine bands (Filippi *et al*., 2002). Two distinct fingerprint groups or lineages were identified among 53 isolates collected from nine different commercial irrigated rice fields grown with cultivars Epagri 108 and 109 in the State of Tocantins, Brazil (Prabhu *et al*., 2002). Javan-Nikkhah *et al*. (2004) reported a low level of genetic diversity in the Iranian *P. grisea* population similar to that reported elsewhere, using *Pot2* rep-PCR analysis. The present paper reports the genetic and virulence diversity of populations of *P. grisea* from two cultivars sampled from 1997 to 2001.

MATERIALS AND METHODS

Isolates

A collection of isolates of *P. grisea* was obtained from sporulating lesions on leaves and panicles of the cultivars Primavera and Maravilha, grown in experimental plots located at the Embrapa Arroz e Feijão, Santo Antonio de Goiás and in farmers' fields in the State of Mato Grosso, during 1997/01. Single conidial isolates were maintained on sterilized filter paper discs at 4 °C $\pm$ 1 °C in the refrigerator. Forty-six isolates of *P. grisea* collected from cv. Primavera and 31 from cv. Maravilha were used in inoculation tests.

Virulence analysis

The virulence of each isolate was tested under controlled greenhouse conditions, based on inoculation of 32 rice genotypes, including six elite upland rice breeding lines (CNA_s 8711, CNA_s 8934, CNA_s 8812, CNA_s 8170, CNA_s 8540, CNA_s 8983), twelve commercial rice cultivars (Aimoré, Bonança, Canastra, Carisma, Carajás, Confiança, Maravilha, Primavera, Progresso, Caiapó, IAC-47, IAC-201), a somaclone of cultivar Araguaia (SC 09) with a known gene as a control for vertical resistance, besides eight standard international differentials ('Raminad Str 3', 'Zenith', 'NP125', 'Dular', 'Kanto 51', 'Usen', 'Caloro', 'Sha-tia-tsão') and five near isogenic lines of the cultivar 'CO 39' ('C 101 LAC', 'C101A 51', 'C104 PKT', 'C101 PKT', 'C101- TTP-4L-23'). The test material was planted in plastic trays (30 x 15 x10 cm) containing 3 kg of soil fertilized with NPK (5g of 5-30-15 + Zn and 3g of ammonium sulfate per 3 kg of soil). An additional 2g of ammonium sulfate was applied 20 days after planting. Ten to twelve plants of each entry were sown in 0.4 m long rows, totaling 16 rows per tray.

Mycelial growth, sporulation on culture medium and inoculation procedure were adapted as described earlier (Filippi & Prabhu, 2001). Leaf blast reactions were assessed as compatible and incompatible, seven to nine days after inoculation. Lesion types 0, 1, 2, and 3 were considered incompatible and 4 to 9 as compatible in a 0-9 scale (IRRI, 1988). Inoculation tests were repeated twice and in case of ambiguous or intermediate reactions, the test was repeated again and the ones that gave consistent and uniform reaction were utilized. A tray containing international and Brazilian differential hosts was maintained as non-inoculated control to ensure that no contamination occurred during the inoculation.

DNA extraction and amplification

The molecular characterization of *P. grisea* isolates was done employing the DNA-fingerprinting protocol described by George *et al*. (1998). DNA extraction was performed using the modified method of Raeder & Broda (1985). Isolates were grown on culture medium (10 g dextrose supplemented with 2 g yeast extract per liter) in Erlenmeyer flasks for four days without agitation followed by 10 days with constant agitation in the dark, at room temperature. One or two mycelial paper discs were transferred to Erlenmeyer flasks containing 150 mL of culture medium.

The harvested mycelia were freeze-dried, lyophilized and macerated in liquid nitrogen. About 300 mg of powdered mycelium was suspended in 700 µl of extraction buffer (50 mM Tris-HCl, pH 8.0; 50 mM EDTA; 3% sodium dodecyl sulfate, wt/vol and 1% of mercaptoethanol) at 65ºC for at least one hour. Proteins were precipitated with 30 µl of potassium acetate pH 5.2. DNA was precipitated in 200 µl of cold isopropanol, washed with 70% ethanol, dried under vacuum and resuspended in TE buffer (10 mM Tris-HCl, pH 8.0; 1.0 mM EDTA) containing 10 mg/mL of RNase A and incubated at 37ºC for 30 minutes. The DNA concentration was estimated by fluorometer and adjusted to 10 ng/µL.

The oligonucleotide sequences were based on the primer sequence of the repetitive element *Pot 2* (EMBL accession Z33638), an inverted repeat transposon found in approximately 100 copies in *M. grisea* genome (Kachroo *et al*., 1994). The two primers (*Pot2-1* 5′ CGGAAGCCCTAAAGCTGTTT 3′ and *Pot2-2* 5′CCCTCATTCGTCACACGTTC 3′) were designed from each end of *Pot 2* in opposite directions so that the 3′ends were directed outward from each element.

DNA amplification reactions were performed as described by George *et al*. (1998), with the following modification: each 25 mL reaction contained: 50 ng DNA, 2.5 µl 10X buffer reaction (200 mM Tris - HCl, pH 8.4 and 500 mM of KCl), 2.0 µl 50 mM $MgCl_2$; 0.5 µl dNTP (10 mM each dATP, dGTP, dCTP and dTTP); 1.25 µl of each primer (100mM); 5 units of Taq polymerase.

Amplification products were separated by gel electrophoresis on 1.4% agarose gel in TBE buffer (90 mM Tris-borate and 2 mM EDTA) containing 10 mg/mL of ethidium bromide. DNA fragments were photographed under ultra-violet light, utilizing the photo documentation system, Eagle Eye II (Stratagene).

Data analysis

The virulence frequency of each isolate was calculated as the percentage of compatible reactions on 32 test genotypes. A binary matrix was created from phenotypic virulence analysis by assigning 1 to compatible reaction and 0 to incompatible reaction on test varieties before constructing a matrix of similarity between all pairs of isolates according to the Simple Matching coefficient (Sokal & Michener, 1958). The phenograms of the isolates were constructed based on the matrix of similarity using the unweighted pair groups with arithmetical averages (UPGMA) algorithm with NTSYS-pc version 2.2/1997 (Rohlf, 1997).

Fingerprinting patterns were determined based on the presence (1) or absence (0) of each fragment. The data were analyzed using the program NTSYS-pc version 2.0/1997. Genetic distances were calculated by Jaccard′s coefficient of similarity (Clifford & Stephenson, 1975). The matrix of genetic distances was used for cluster analysis using the UPGMA algorithm.

To determine the association between virulence and rep-PCR patterns, the similarity matrices based on virulence and rep-PCR data were compared by correlation analysis using the matrix comparison (MXCOMP) program of the NTSYS-pc. The significance of correlation was tested by Mantel test with 1,000 random permutations.

RESULTS

The virulence frequency of *P. grisea* isolates from the cultivars Primavera and Maravilha ranged from 25 to 75 percent and 46 to 75 percent, respectively (Table 1). The mean virulence of the isolates from cv. Maravilha (63.58%) was significantly higher ($t = 9.09$, $p = 0.0000$), as compared to the isolates from cv. Primavera (41.80%). The majority of isolates exhibiting compatible reaction with cv.Primavera were incompatible with cv. Maravilha and *vice versa* (Table 2). However, six isolates from Primavera and 11 isolates from cv. Maravilha showed compatible reaction with both cultivars. Two isolates from cv.Primavera and three from cv. Maravilha were not found to be compatible with the cultivars of their origin under greenhouse test conditions.

The virulence frequency of isolates of *P. grisea* from Maravilha, on twelve commercial upland rice cultivars, was also greater compared to the ones from Primavera (Figure 1). Similarities in phenotypic virulence on 32 rice genotypes are shown in phenogram (Figure 2). All isolates were associated at 58% Simple Match similarity coefficient. At 74% similarity, two main groups were recognized: one constituted mainly of isolates from cv. Primavera (97.61%) and the other of the isolates from Maravilha (91.17%). There was no correlation between pathotype and virulence structure determined based on test genotypes for cultivar Primavera ($R^2 = 0.045$, $p \leq 0.3843$) and Maravilha ($R^2 = 0.1167$, $p \leq 0.3495$).

The rep-PCR fingerprint profiles of 26 of the 77 isolates tested are shown in Figure 3. The isolates exhibited differences and similarities in banding pattern varying from 4 to 15 fragments of 300 bp to 3.5 Kb in length. The banding pattern of twenty sample isolates from cv.Primavera (#27 to 46) was distinctly different from six isolates (#47 to 52) retrieved from cv.Maravilha.

The similarity coefficients varied from 0.3 to 1.0 and could be clustered broadly into two groups or lineages (Figure 4). At 24% similarity, two main groups were recognized; one was constituted only of isolates from cv. Primavera and the other of isolates from cv. Maravilha (95.65%). The average similarities within a cluster of isolates from cv. Primavera (0.50±0.020) were significantly greater than the average similarities among isolates of cv. Maravilha (0.33±0.019), $t = 5.37$, $p = 0.0000$. Forty-four of 46 isolates retrieved from cv. Primavera, with the exception of two isolates (#38 and 9) were clustered into one group. The similarity coefficients within the group constituted by isolates from cv. Primavera were greater than 0.50. The correlation coefficient between matrixes of rep-PCR and virulence was of 0.48, $p \leq 0.011$ indicating significant association.

DISCUSSION

Most of the field isolates tested in this study were compatible with the cultivars from which they were collected. However, two isolates from cv. Primavera and

TABLE 1 - Isolates, collection site, year of collection and virulence frequency of *Pyricularia grisea* collected from the rice cultivars Primavera and Maravilha

Number [a]	Identification [b]	Location/year [c]	Virulence frequency [d]	Number [a]	Identification [b]	Location/year [c]	Virulence frequency [d]
1	Py 1300 -L1 [2]	Capivara/1997	56	40	Py 2713 -L19 [1]	Jaciara/2001	34
2	Py 2379 -L3 [1]	Capivara/2000	38	41	Py 2538 -L2 [3]	Capivara/2000	38
3	Py 1303 -L2 [1]	Capivara/1997	44	42	Py 2623 -L1 [1]	Palmital/2001	69
4	Py 2376 - L1 [1]	Capivara /2000	38	43	Py 2758 -L16 [1]	Palmital/2001	32
5	Py 2389 -L4 [5]	Capivara/2000	32	44	Py 2768 -L18 [1]	Palmital/2001	32
6	Py1309 -P1 [1]	Capivara /1997	47	45	Py 2704 -L17 [1]	Palmital/2001	38
7	Py 2385 -L4 [1]	Capivara/2000	34	46	Py 2634 -L2 [1]	Palmital/2001	32
8	Py 2380 -L3 [2]	Capivara/2000	55	47	Py 1383 -L1 [1]	Capivara/1997	59
9	Py 1302 - L1 [4]	Capivara/1997	56	48	Py 1388 - L1 [6]	Capivara/1997	56
10	Py 1310 -P1 [4]	Capivara/1997	50	49	Py 1389 - L2 [1]	Capivara/1997	46
11	Py 2388 -L4 [2]	Capivara/2000	53	50	Py 1390 - L2 [2]	Capivara/1997	59
12	Py 1306 -L2 [4]	Capivara/1997	47	51	Py1600 - P1 [1]	Capivara/1998	56
13	Py 2650 -L7 [1]	Jaciara/2001	44	52	Py 1601 - P1 [2]	Capivara/1998	59
14	Py 2651 -L8 [1]	Jaciara/2001	28	53	Py 1604 - P2 [2]	Capivara/1998	53
15	Py 2636 -1L31	Palmital/2001	47	54	Py 1607 - P4 [3]	Capivara/1998	53
16	Py 2644 - L1 [1]	Jaciara/2001	32	55	Py 2002 - L1 [2]	Capivara/1999	69
17	Py 2653 -L9 [1]	Jaciara/2001	50	56	Py 2003 - L1 [3]	Capivara /1999	65
18	Py 2648 -L6 [1]	Jaciara/2001	32	57	Py 2004 - L2 [1]	Capivara/1999	63
19	Py 2672 -L7 [1]	Jaciara/2001	38	58	Py 2006 - L2 [4]	Capivara/1999	71
20	Py 2721 -L2 [1]	Jaciara/2001	44	59	Py 2007 - L2 [5]	Capivara/1999	59
21	Py 2726 -L3 [1]	Jaciara/2001	38	60	Py 2434 - L1 [2]	Capivara/2000	46
22	Py 2734 -L7 [1]	Jaciara/2001	41	61	Py 2437 - L2 [2]	Capivara/2000	63
23	Py 2660 -L2 [1]	Jaciara/2001	38	62	Py 2441 - L3 [1]	Capivara/2000	71
24	Py 2732 -L4 [1]	Jaciara/2001	75	63	Py 2447 - L4 [3]	Capivara/2000	71
25	Py 2662 -L3 [1]	Jaciara/2001	41	64	Py 2584 - L3 [1]	Capivara/2001	71
26	Py 2667 -L5 [1]	Jaciara/2001	34	65	Py 2586 - L4 [1]	Capivara/2001	65
27	Py 2664 -L4 [1]	Jaciara/2001	34	66	Py 2588 - L5 [1]	Capivara/2001	75
28	Py 2669 -L6 [1]	Jaciara/2001	38	67	Py 2594 - L7 [1]	Capivara/2001	71
29	Py 2656 - L1 [1]	Jaciara/2001	38	68	Py 2595 - L8 [1]	Capivara/2001	71
30	Py 2689 -L12 [1]	Jaciar/2001	25	69	Py 2597 - L9 [1]	Capivara/2001	75
31	Py 2753 -L15 [1]	Jaciara/2001	25	70	Py 2600 - L10 [1]	Capivara/2001	65
32	Py 2684 -L11 [2]	Jaciara/2001	69	71	Py 2603 - L11 [1]	Capivara /2001	69
33	Py 2694 -L13 [4]	Jaciara/2 001	34	72	Py 2606 - L12 [1]	Capivara/2001	75
34	Py 2745 -L12 [1]	Jaciara/2001	34	73	Py 2608 - L13 [1]	Capivara/2001	63
35	Py 2741 -L11 [1]	Jaciara/2001	28	74	Py 2614 - L16 [1]	Capivara/2001	63
36	Py 2739 -L10 [1]	Jaciara/2001	47	75	Py 2615 - L17 [1]	Capivara/2001	59
37	Py 2748 -L13 [1]	Jaciara/2001	41	76	Py 2618 - L1 [1]	Capivara/2001	65
38	Py 2698 -L15 [2]	Jaciara/2001	65	77	Py 2621 - L2 [1]	Capivara/2001	65
39	Py 2754 -L15 [2]	Jaciara/2001	38				

[a]Serial number indicated in the phenogram; 1-46= isolates from cv. Primavera, 47-77= isolates from cv. Maravilha; [b]accession number of *P. grisea* culture collection of Embrapa Arroz e Feijão, code number is followed by lesion number (L) and the conidial number as an exponent; [c]Capivara = experimental station of Embrapa Arroz e Feijão, GO, Jaciara= experimental station of Empaer, MT, Palmital= experimental station of Embrapa Arroz e Feijão, GO; [d]virulence frequency was calculated as the percentage of the total number of compatible reactions in relation to 32 test genotypes.

three from cv. Maravilha were not found to be compatible to cultivars of their origin. The extent of compatibility of isolates with cultivar of origin is variable. Chen *et al.* (1995) observed that only 41% of isolates were fully compatible to their hosts of origin and 42% were unable to infect the rice lines or cultivars from which they had been isolated. Similar observations were made by Correa-Victoria & Zeigler (1993) and Levy *et al.* (1993) in studies conducted in Colombia

TABLE 2 - Compatibility frequency of *Pyricularia grisea* isolates to the cultivars Primavera and Maravilha

Cultivar	Number of compatible isolates[a]	
	Primavera (n=46)[b]	Maravilha (n=31)[c]
Primavera	44	11
Maravilha	6	28

[a]Six isolates from Primavera and 11 isolates from Maravilha were compatible to both cultivars. [b]Number of isolates from Primavera utilized in inoculation tests. [c]Number of isolates from Maravilha utilized in inoculation tests.

with widely prevalent isolates of *P. grisea* and by Prabhu & Filippi (2002) in Brazil. These were considered to be opportunistic infections caused by members of the resident pathogenic lineages present in the field and might have exhibited partial compatibility to the host cultivars (Correa-Victoria & Zeigler, 1993). Ou (1980) attributed to variation in virulence pattern of conidia produced in culture media.

The greater virulence of *P. grisea* isolates from the cv. Maravilha on twelve commercial upland rice cultivars as compared to the ones from cv. Primavera can possibly be attributed to the years of cultivation, different lineage origin and area planted. Earlier studies have indicated that an increase in a certain pathotype as well as highly adapted isolates of blast pathogen in farmers' fields was closely correlated with the area planted with a particular rice cultivar (Kiyosawa & Shiyomi, 1976). The cultivar Maravilha was released two years earlier than cv. Primavera. In the past, the rapid breakdown of resistance of newly released cultivars has been over-attributed to pathotype diversity, instability in isolates, disease escape in breeding nurseries (Ou, 1980; Zeigler *et al*., 1995), rather than to an increase in frequency of preexisting pathotypes with the release of a new rice cultivar. The extensive and intensive use of a resistance gene in a determined cultivar exerts selection pressure on preexisting population of *P. grisea*, favoring the increase of certain pathotypes with corresponding virulence gene (Ahn, 2000).

Population structure analyses of *P. grisea* using molecular markers have contributed to the knowledge on evolutionary dynamics. Studies on genetic structure of *P. grisea* population using MGR-DNA fingerprinting showed that even though each isolate had a unique fingerprint, they could be grouped into distinct lineages The results of the analysis of isolates from Primavera and Maravilha using rep-PCR in the present study are in accordance with the observations made in earlier investigations (Levy *et al*., 1991; Correa-Victoria & Zeigler, 1993; Levy *et al*., 1993; Xia *et al*., 1993; Chen *et al*., 1995; Roumen *et al*., 1997; Don *et al*., 1999; Park *et al*., 2003; Rathour *et al*., 2004). Information on the interactions between cultivars and lineages can be used to improve the diversity of pathogen populations at screening sites (Chen *et al*., 1995). Furthermore, the results suggested some degree of specialization of isolates of *P. grisea* to the cultivar of origin, even though most isolates were obtained from breeders' fields in different years. Different resistance genes present in the host exert selection on the pathogen population. This effect was clearly observed in an earlier investigation where sampling of isolates was done from the cultivars CICA-8 and Metica-1 which were severely affected by leaf blast. DNA fingerprinting analysis using the probe MGR586 showed that the isolates differentially infecting cv. CICA-8 and cv. Metica belong to two distinct lineages, BZ-A and BZ-10, respectively (Filippi *et al*., 1999). The rep-PCR is cost effective and may serve as an important tool to genetically differentiate distinct

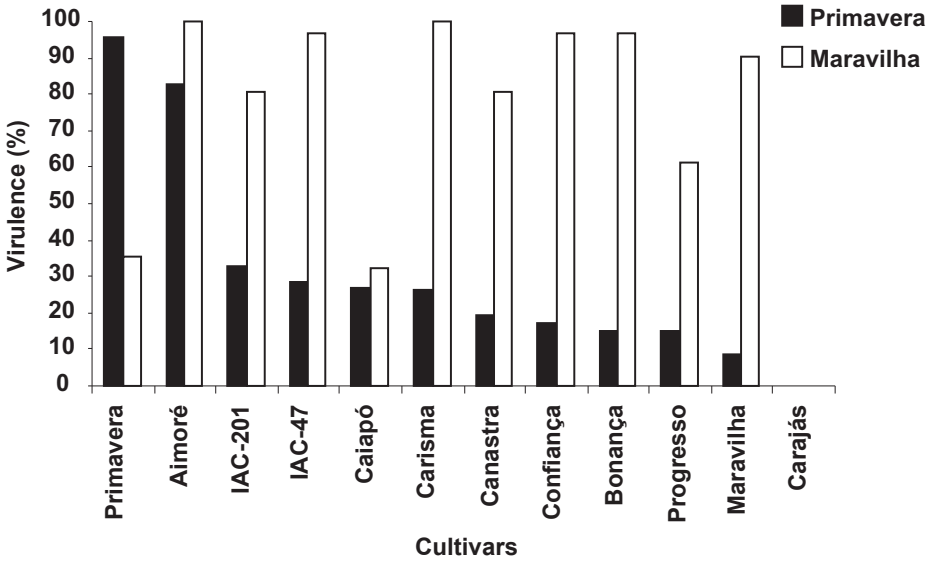

FIG. 1 - Virulence frequency of isolates of *Pyricularia grisea* on commercial rice cultivars.

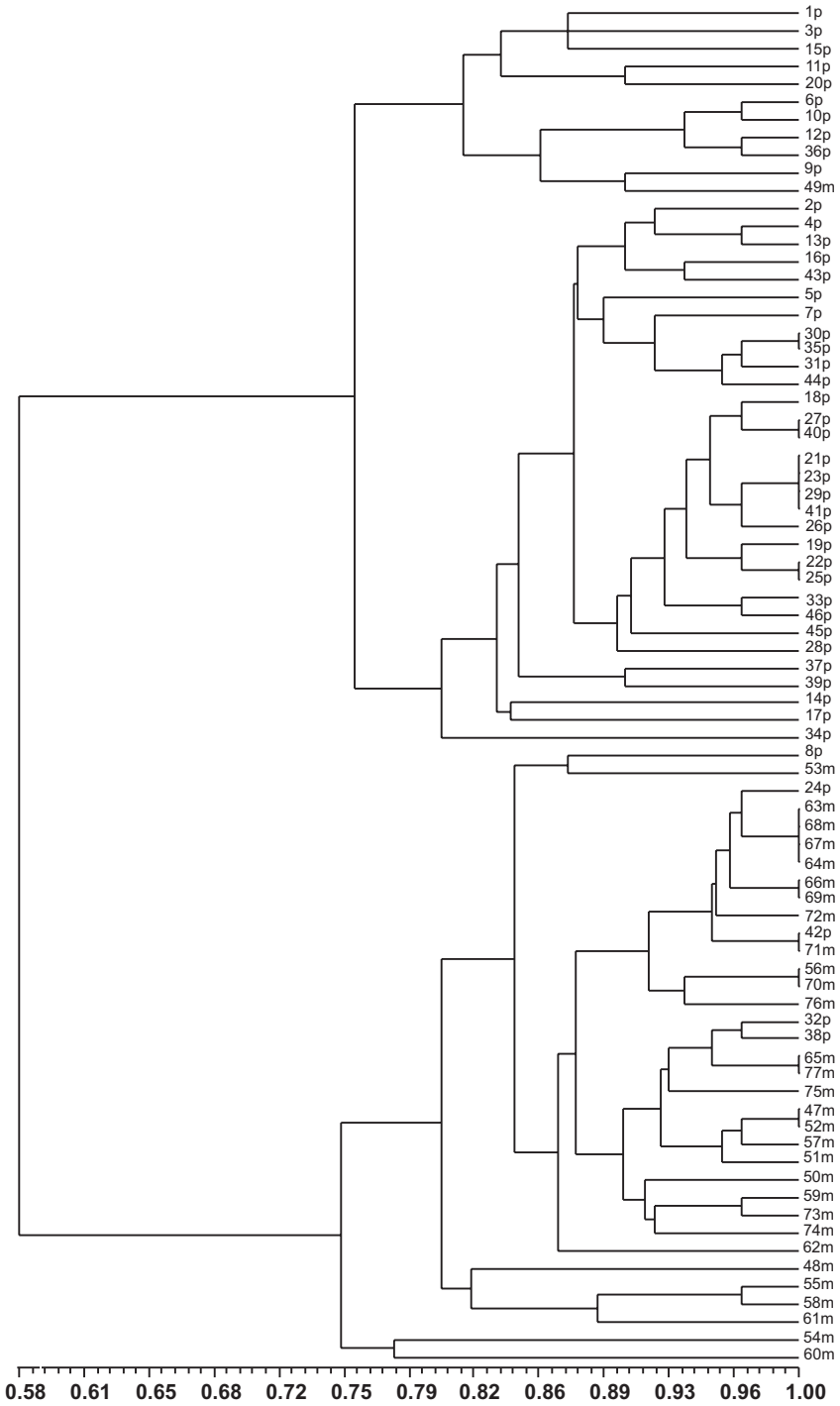

FIG. 2 - Phenogram of 77 *Pyricularia grisea* isolates from rice cultivars Primavera and Maravilha, constructed using UPGMA based on Simple Match's similarity coefficients; data from phenotypic virulence analysis; scale at the bottom represents coefficients of similarity. Co-phenetic coefficient of the dendogram was 0.91, $p \leq 0.002$. The serial numbers in the phenogram from 1 to 46 represent isolates from the cultivar Primavera and 47 to 77 the isolates from Maravilha.

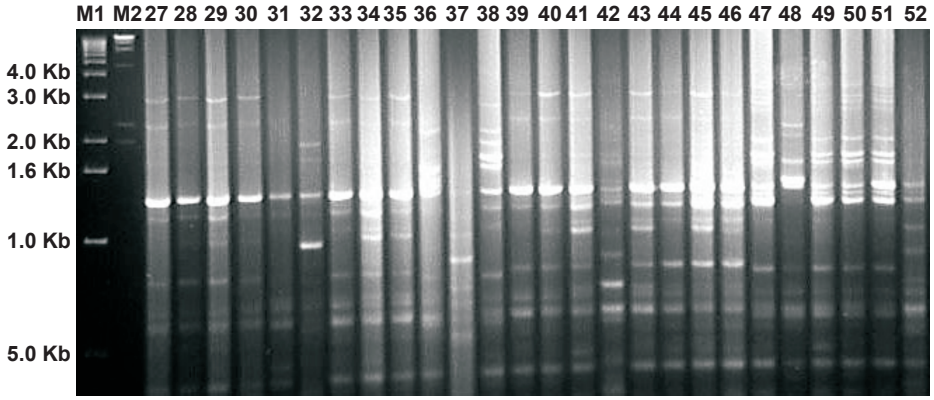

FIG. 3 *Pot2* rep-PCR analysis showing DNA fingerprinting profiles of *Pyricularia grisea* isolates from cultivars Primavera (#27 to 46) and Maravilha (#47-52). The DNA molecular size markers are on the lanes labeled M on the left M1 = Size marker Lambda 1 kb, Gibco BRL; M2 = λ DNA/Hind III fragments. Isolates numbers correspond to the numbers indicated in the phenogram.

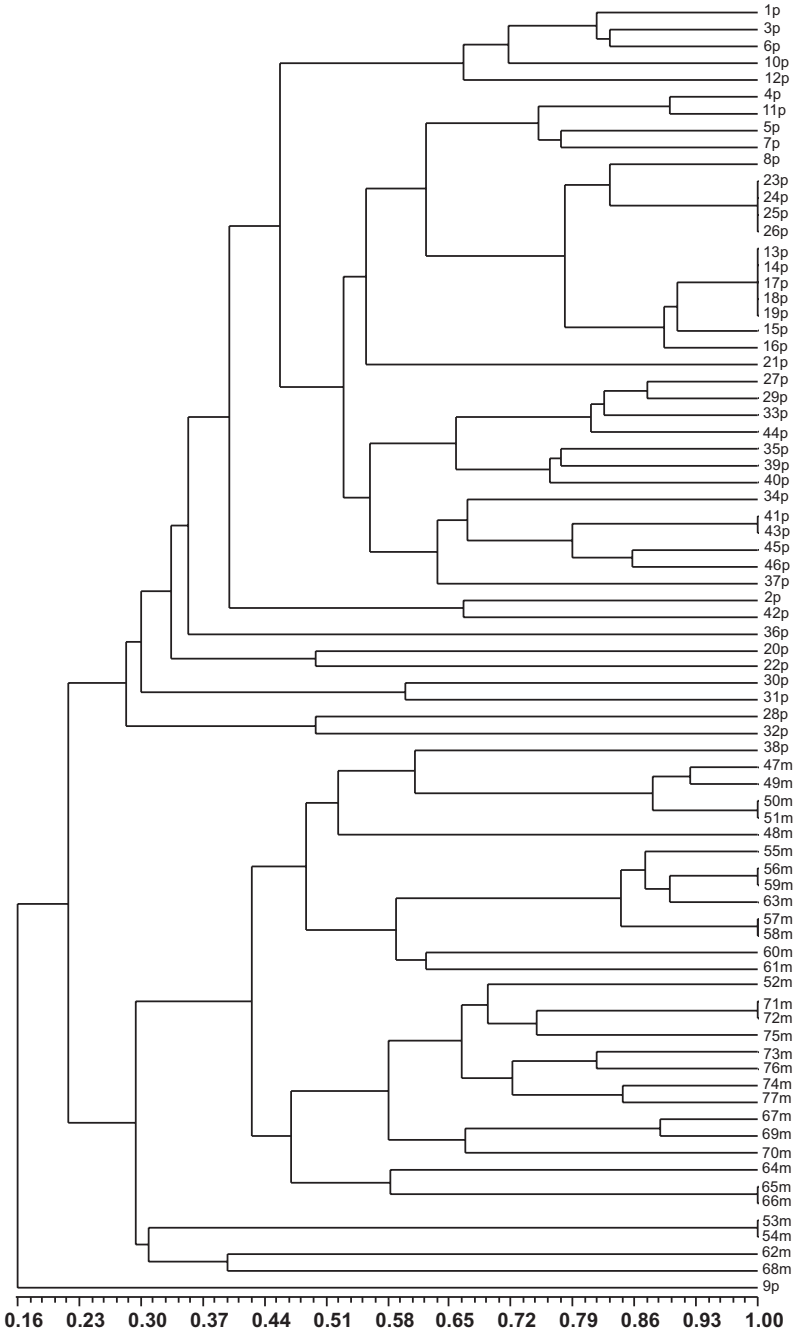

FIG. 4 - Phenogram of 77 *Pyricularia grisea* isolates from rice cultivars Primavera and Maravilha, constructed using UPGMA based on Jaccard's similarity coefficients; data from *Pot2* rep-PCR analysis; scale at the bottom represents coefficients of similarity. Co-phenetic coefficient of the dendogram was 0.86, $p \leq 0.0004$. The serial numbers in the phenogram from 1 to 46 represent isolates from cv. Primavera and 47 to 77 the isolates from cv.Maravilha.

cultivar-specific populations or lineages with a well defined virulence pattern, because there was close correspondence between clusters based on rep-PCR and phenotypic virulence data based on 32 rice genotypes.

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