GENETIC AND PHENOTYPIC CHARACTERIZATION OF ISOLATES OF *Pyricularia grisea* FROM THE RICE CULTIVARS EPAGRI 108 AND 109 IN THE STATE OF TOCANTINS

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ABSTRACT

An epidemic of rice (*Oryza sativa*) blast occurred on cultivars Epagri 108 and 109 in the municipalities of Lagoa da Confusão and Duerê in the State of Tocantins, during the rice-growing season 1998-99. DNA fingerprinting and virulence phenotype analysis were utilized to determine the diversity of *Pyricularia grisea* isolates collected from these cultivars in one epidemic year. Rep-PCR analysis of isolates was done by using two primer sequences from *Pot2*. Two distinct fingerprint groups or lineages were identified among 53 isolates collected from nine different commercial fields. The virulence pattern of isolates retrieved from these two cultivars was analyzed in artificial inoculation tests utilizing 32 genotypes in the greenhouse. A dendrogram constructed from virulence phenotype data showed a single group considering 77% similarity level. The predominant pathotype IB-45 was represented by 47 of the 53 isolates corresponding to 83%. Four other pathotypes (IB-1, IB-9, IB-13 and IB-41) were identified at random among the isolates from these cultivars. There was no relation between rep-PCR grouping and pathotypes. The results showed that the isolates of *P. grisea* recovered from cultivars Epagri108 and 109 in farmers' fields had narrow phenotypic and genetic diversity. The blast outbreak on these two cultivars one year after their introduction could be attributed to the new pathotype IB-45 or its increase, which was hitherto existing in low frequency.

Additional key words: *Magnaporthe grisea*, molecular markers, *Oryza sativa*, rep-PCR analysis, rice blast.

RESUMO

Caracterização genética e fenotípica de isolados de *Pyricularia grisea* coletados em lavouras das cultivares Epagri 108 e 109 no Estado do Tocantins

Foi constatada a ocorrência de uma epidemia de brusone, na safra 1998/99 nas cultivares de arroz (*Oryza sativa*) irrigado Epagri 108 e 109, nos municípios de Lagoa da Confusão e Duerê, do Estado do Tocantins, na safra 1998-99. Foram utilizados "DNA fingerprinting" e análise fenotípica de virulência para determinar a diversidade entre isolados de *Pyricularia grisea* coletados nestas duas cultivares no ano de epidemia. A análise de DNA dos isolados foi realizada utilizando rep-PCR para amplificação das sequências entre os elementos repetitivos *Pot2*. Dois grupos distintos de bandas polimórficas ou linhagens foram identificados entre os 53 isolados coletados em nove diferentes lavouras comerciais. O padrão de virulência dos isolados foi estudado através de inoculações artificiais em 32 genótipos, em casa de vegetação. O dendrograma construído com base nos dados de virulência fenotípica, permitiu distinguir apenas um grupo, considerando o nível de similaridade de 77%. O patótipo predominante IB-45 foi representado por 47 dos 53 isolados correspondendo a 83%. Foram identificados quatro patótipos (IB-1, IB-9, IB-13 e IB-41) entre isolados coletados nestas duas cultivares. Não houve relação entre agrupamento dos isolados por rep-PCR e patótipos. Os resultados ainda demonstraram que os isolados de *P. grisea* provenientes das cultivares Epagri 108 e 109 apresentaram estreita diversidade fenotípica e genética. A epidemia da brusone nestas duas cultivares, um ano após o lançamento, pode ser atribuída ao surgimento do novo patótipo IB-45 ou à sua preexistência em baixa freqüência e rápido aumento no campo.

INTRODUCTION

Rice blast caused by *Pyricularia grisea* (Cooke) Sacc. [= *Magnaporthe grisea* (T.T.Herbert) Yaegashi & Udagawa] is the most destructive disease of irrigated rice (*Oryza sativa* L.) in the State of Tocantins. Even though there are no exact estimates of yield losses, they are considered to be significant by the growers in the blast susceptible cultivar Metica 1. Two rice cultivars Epagri 108 (17719/5738/IR 21015-72-3-3-3-1) and Epagri 109 (CT7347/ IR 21015-72-3-3-3-1) were

introduced from the State of Santa Catarina because of their high yield potential, grain quality and blast resistance. These two cultivars occupied approximately 20.000 hectares in the municipalities of Lagoa de Confusão and Duerê. The occurrence of blast epidemic was recorded, in different farms, at the vegetative phase, during 1998/99 rice-growing season a total breakdown of vertical resistance of these two cultivars in a year after their introduction (Prabhu & Filippi, 1999). The resistance breakdown of commercial rice cultivars was attributed to high pathogenic variability of *P. grisea* (Ou, 1980).

Most of the earlier studies in Brazil were concentrated on determining race composition, frequency of occurrence and compatibility with known resistance genes (Amaral *et al*., 1979; Ribeiro & Terres, 1987; Prabhu & Filippi, 1989; Urashima & Isogawa, 1990; Filippi *et al*., 1999). Pathogenic variation has been reported from single conidial isolates originating from a single lesion (Ou & Ayad, 1968; Bedendo *et al.*, 1979), while some other studies showed that the isolates are pathogenically stable (Giatgong & Frederiksen, 1969; Bonman *et al*., 1987). The pathogenic diversity is generally very high in experimental fields and breeding sites commonly known as "hot spots" where conditions are highly favorable for the pathogen (Correa-Victoria & Zeigler, 1993; Zeigler *et al*., 1995; Filippi *et al*., 1999). However, there is no information in Brazil on the virulence diversity of the pathogen under natural conditions in farmer's fields where there is very little or no allo-infection.

Strategies to incorporate the non-matching resistance genes to the existing pathogen population in the field require studies on virulence and molecular characterization. Characterization of pathogen populations of *P. grisea* is done both by conventional pathotyping based on the reaction on a set of differentials and molecular tools such as DNA-fingerprinting. The MGR586 element has been widely used as a probe for genetic analysis of the blast pathogen population. It has been shown that the isolates of *P. grisea* can be grouped into distinct lineages (Hamer, 1991; Levy *et al*., 1991; Levy *et al*., 1993; Zeigler *et al*., 1995; Filippi *et al*., 1999). Because of difficulties involved in RFLP analysis of large samples of *P. grisea*, George *et al*. (1998) developed a cost effective, simple, repetitive, element-based polymerase chain reaction (rep-PCR) fingerprinting method, specific for monitoring *P. grisea* populations. A close correspondence between the groupings of isolates based on *Pot2* rep-PCR and those obtained by MGR586 was demonstrated.

The present paper reports the results of investigation on pathotyping and genetic diversity in isolates of *P. grisea*, retrieved from the commercial rice cultivars Epagri 108 and 109 planted in extensive areas, in the State of Tocantins.

MATERIALS AND METHODS

Isolates

Isolates of *P. grisea* were collected from nine rice farms planted with cultivars Epagri 108 and 109 during the 1998-99 crop growing season in the municipality of Lagoa de Confusão, in Tocantins. Monoconidial isolates from the sporulating leaf lesions were obtained and maintained on sterilized filter paper discs.

DNA extraction

Isolates were grown on culture medium (10 g of dextrose supplemented with 2 g of yeast extract per liter) in Erlenmeyer flasks for four days without agitation followed by ten days with constant agitation in the dark, at room temperature. One or two mycelial paper discs were transferred to Erlenmeyer flasks (cap. 250 ml) containing 150 ml of culture medium.

DNA extraction was performed utilizing the modified method of Raeder & Broda (1985). The harvested mycelia were freeze-dried, lyophilized and macerated in liquid nitrogen. About 300 mg of powdered mycelia 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 65 ºC for at least 1 h. The cellular proteins were precipitated with 30 µl of potassium acetate (3 M and 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 37 ºC for 30 min. The DNA concentration was estimated by fluorometer and adjusted to 10 ng/µl.

Primers

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* (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 orientation such that the 3´ends were directed outward from each element (George *et al*., 1998). 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 (George *et al*., 1998). The oligonucleotide sequences were synthesized by Federal University of Paraná, Brazil.

DNA Amplification

DNA amplification reactions were performed as described by Williams *et al*. (1990). Each 25 µl reaction contained: 50 ng of DNA, 2.5 µl of 10 X buffer reaction (200 mM Tris - HCl, pH 8.4 and 500 mM of KCl), 0.75 µl of 50 mM $MgCl_2$; 0.5 µl of dNTP (10 mM of each dATP, dGTP, dCTP and dTTP); 0.5 µM each of the two opposing primers; 1.5 units of Taq polymerase, overlaid with 50 µl of mineral oil to prevent evaporation.

In a preliminary test conducted, to determine the conditions of DNA amplification, the method described by

Williams *et al*. (1990) was found to be superior to the protocol adopted by George *et al* (1998). For this reason the following procedure was adopted. The enzymatic amplification was performed in a thermocycler (M.J. Research, Inc.), programmed for 40 cycles. Each cycle was composed of: 15 s at 94 ºC (DNA denaturation); 30 s at 35 ºC (annealing of the primers to the DNA template) and 1 min at 72 ºC (extension of the primers). After 40 cycles an extra extension step was performed for 7 min at 72 ºC. 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 bands were photographed under ultra violet light, utilizing the photo documentation system, Eagle Eye II (Stratagene).

Data analysis

The *Pot2* rep-PCR profiles were analyzed based on the presence (1) or absence (0) of bands of same length. The data were analyzed using the program NTSYS-pc version 2.0/1997. The genetic distances were calculated by the coefficient of similarity of Jaccard. The matrix of genetic distances was used for grouping the isolates based on the method of Unweighted Pair-Group Method with Arithmetic Averages (UPGMA).

Virulence analysis

The phenotypic virulence of 53 isolates retrieved from cultivars Epagri 108 and Epagri 109 were tested on 32 genotypes, including 19 commercial irrigated rice cultivars, five near isogenic lines of CO39 and standard international differentials. Sixteen genotypes per plastic tray (30 x 15 x 10 cm) containing 3 kg of soil fertilized with NPK were planted. Ten to twelve seeds of each genotype were sown in 5 cm rows. Inoculations were made on 21-day-old plants with spore suspension (3 x 10^5 conidia/ml) in the greenhouse. Leaf blast reaction was assessed seven to nine days after inoculation. The isolates that induced typical sporulating lesions were considered virulent (4-9) and non-sporulating necrotic lesions (0-3) as avirulent according to Leung *et al*. (1988). The frequency of virulence was determined on the basis of total number of compatible reactions on 32 genotypes. In case of ambiguous or intermediate reaction, inoculation tests were repeated whenever necessary and the ones that gave consistent and uniform reaction were utilized for analysis. The pathotypes were determined based on the reaction type on eight international differentials.

The virulence pattern was analyzed based on the similarity of reaction type among isolates. A binary matrix indicating compatible reaction (1) and incompatible reaction (0) of each isolate was utilized for constructing a matrix of similarity between all pairs of isolates according to the coefficient of Jaccard. These data were used to calculate similarity coefficients and to construct dendrograms using the unweighted pair-group method with arithmetic averages (UPGMA) employing the SAHN program of the Numerical Taxonomy and Multivariate Analysis (NTSYS-pc version 2.0/ 1997).

RESULTS AND DISCUSSION

A distinct banding pattern was generated utilizing rep-PCR with two primer sequences from *Pot2*. The amplified bands ranged from 200 bp to 1300 bp in length (Figure 1). All isolates of *P. grisea* recovered from Epagri 108 and Epagri 109 showed three to nine polymorphic bands. Three of the 19 isolates, Nos. 5, 8, and 16 identified as pathotypes IB-13, IB-1 and IB-9, respectively, showed differences in DNA profiles compared to the 16 other isolates of the pathotype IB-45 (Figure 1).

Cluster analysis (UPGMA) of 53 isolates showed two distinct groups, one formed by the isolates Nos. 26 and 39, and the other by the rest of the isolates, considering similarity coefficient of approximately 38% (Figure 2). Forty of the 47 isolates of pathotype IB-45 recovered from cultivars Epagri

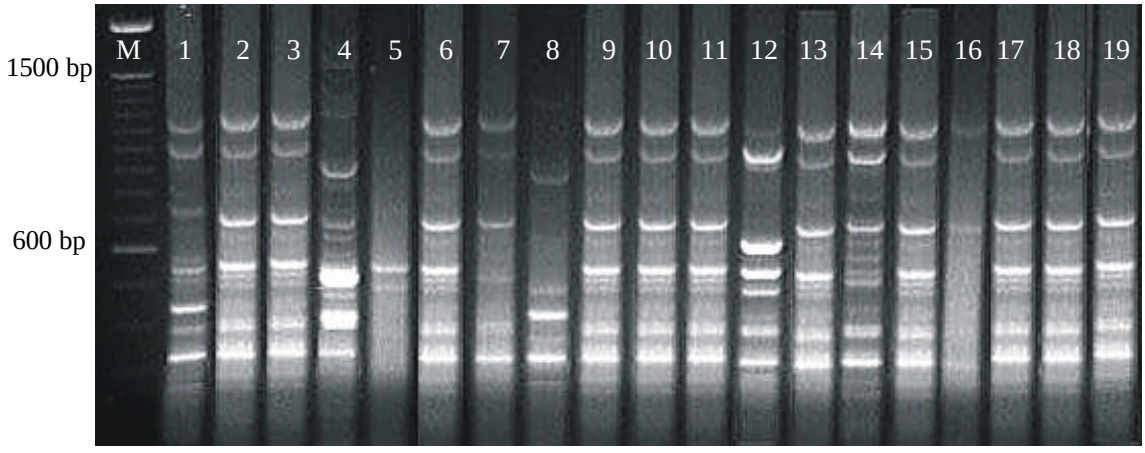

FIG. 1 - *Pot2* rep-PCR analysis showing DNA fingerprinting profiles of *Pyricularia grisea* isolates from rice (*Oryza sativa*) cultivars Epagri 108 and 109. M = Size marker Lambda 100 bp, Gibco BRL. Isolates numbers correspond to the numbers indicated in the dendrogram.

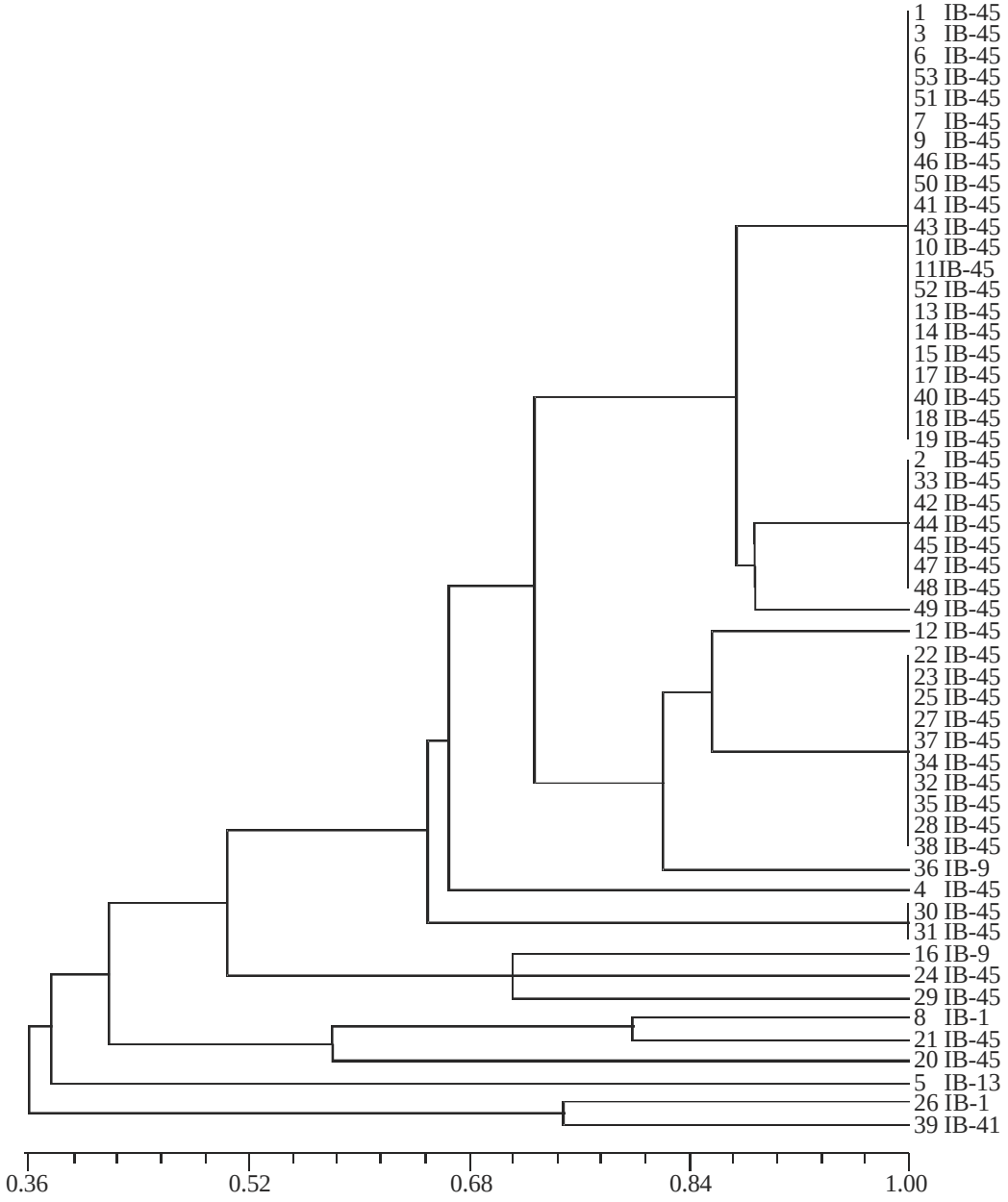

FIG. 2 - Dendrogram of 53 *Pyricularia grisea* isolates from rice (*Oryza sativa*) cultivars Epagri 108 and 109, constructed using UPGMA based an Jaccard's similarity coefficients; data from *Pot2* rep-PCR analysis; scale at the bottom represents coefficients of similarity.

108 and 109 formed a single group (Group I) considering Jaccard coefficient value of 70%. Even though the rest of the isolates did not form into a distinct group they were clustered into Group II. Group II with similarity coefficient values ranging from 0.36 to 1.0 was not well defined and could be considered as six subgroups, each comprised one to three isolates. The pathotypes other than IB-45 such as IB-9, IB-1, IB-13 and IB-41 were included in this group. There seems to be no relation between the pathotypes and rep-PCR based analysis. However, the cluster analysis of the PCR banding pattern of *P. grisea* isolates showed close correspondence between the groupings based on *Pot2* and the phenotypic virulence (Figures 2 and 3). The correlation between Jaccard coefficients of rep-PCR and phenotypic virulence was positive and significant ($r = 0.38$; $p = 0.01$). Among the undefined group of isolates, genetic diversity was evident even though it was not exhibited in phenotypic virulence analysis. These results indicate the utility of rep-PCR analysis for identifying genetic differences among isolates which are not evident otherwise.

The similarities in phenotypic virulence among the 53 isolates of *P. grisea* are shown in the dendrogram (Figure 3). At 77% only one group was distinguished, with the exception of one isolate No. 39 pertaining to the pathotype

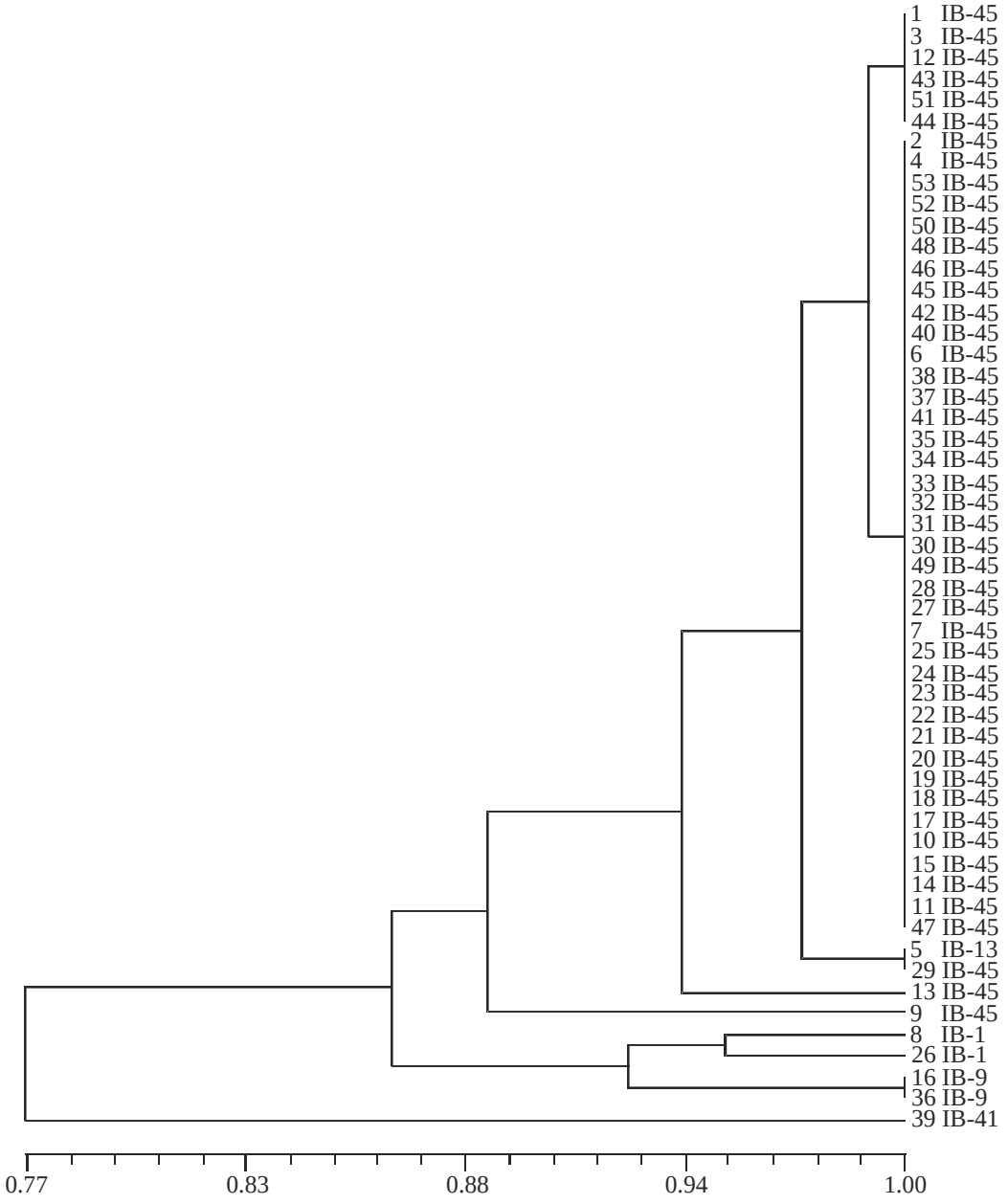

FIG. 3 - Dendrogram of 53 *Pyricularia grisea* isolates from rice (*Oryza sativa*) cultivars Epagri 108 and 109, constructed using UPGMA based an Jaccard's similarity coefficients; data from phenotypic virulence analysis; scale at the bottom represents coefficients of similarity.

IB-41. The predominant pathotype IB-45 was represented by 47 of the 53 isolates corresponding to 83%.

The results of pathotype analysis as well as rep-PCR grouping of isolates collected from nine different farms in the municipality of Lagoa da Confusão during 1998-99 growing season are summarized in Table 1. All 53 isolates collected from cultivars Epagri 108 and Epagri 109 showed a similar reaction pattern on the eight standard international differentials with few exceptions. Forty-seven of the 53 isolates pertained to the pathotype IB-45. Four other pathotypes identified among the isolates collected from these cultivars were in low frequency. They included two each of pathotypes IB-1 and IB-9 and one each of IB-41 and IB-13. These pathotypes were recovered at random in different farms both from Epagri 108 and Epagri 109. It is interesting to observe that the occurrence of these four pathotypes in upland rice cultivars was also reported in earlier studies (Prabhu & Filippi, 1989). Seven pathotypes were identified among the 24 isolates collected from rice blast screening nursery and experimental plots in Goiânia. Pathotype IB-9 was recovered from eight of the 11 cultivars. The predominant pathotype (IB-45) on Epagri 108 and Epagri 109 in farmers' fields in Tocantins was also recovered from CICA-8 and Oryzica-L5 (Filippi *et al.*, 1999). These results showed the preexistence of this

TABLE 1 -Isolates collected from rice (*Oryza sativa*) cultivars Epagri 108 and Epagri 109, their origin, international pathotypes and lineages of *Pyricularia grisea* based on *Pot2* rep-PCR analysis

Number[1]	ACC. NO.[2]	Identification[3]	Origin[4]	Pathotype[5]	Rep-PCR group[6]
1	1634	EP109L2[1] -99	F1	IB-45	I
2	1636	EP109L4[1]-99	F1	IB-45	I
3	1637	EP109L5[1]-99	F1	IB-45	I
4	1638	EP109L5[2]-99	F1	IB-45	II
5	1639	EP109L6[1]-99	F1	IB-13	II
6	1641	EP109L8[1]-99	F1	IB-45	I
7	1642	EP109L9[1]-99	F1	IB-45	I
8	1644	EP109L11[1]-99	F1	IB-1	II
9	1645	EP108L1[1]-99	F2	IB-45	I
10	1647	EP108L3[1]-99	F2	IB-45	I
11	1648	EP108L3[2]-99	F2	IB-45	I
12	1650	EP108L4[1]-99	F2	IB-45	I
13	1652	EP108L4[3]-99	F2	IB-45	I
14	1653	EP108L5[1]-99	F2	IB-45	I
15	1657	EP108L71[1]-99	F1	IB-45	I
16	1658	EP108L1[1]-99	F3	IB-9	II
17	1659	EP108L2[1]-99	F3	IB-45	I
18	1662	EP108L5[1]-99	F3	IB-45	I
19	1664	EP108L1[2]-99	F5	IB-45	I
20	1665	EP108L2[1]-99	F5	IB-45	II
21	1667	EP108L2[3]-99	F5	IB-45	II
22	1669	EP108L3[2]-99	F5	IB-45	I
23	1670	EP108L3[3]-99	F5	IB-45	I
24	1671	EP108L3[4]-99	F5	IB-45	II
25	1672	EP108L3[5]-99	F5	IB-45	I
26	1673	EP108L4[2]-99	F5	IB-1	II
27	1676	EP108L1[1]-99	F6	IB-45	I
28	1680	EP108L5[1]-99	F6	IB-45	I
29	1682	EP108L6[1]-99	F6	IB-45	II
30	1683	EP108L6[2]-99	F6	IB-45	II
31	1684	EP108L6[7]-99	F6	IB-45	II
32	1687	EP108L8[3]-99	F6	IB-45	I
33	1688	EP108L9[1]-99	F6	IB-45	I
34	1689	EP109L1[1]-99	F7	IB-45	I
35	1690	EP109L1[3]-99	F7	IB-45	I
36	1692	EP109L2[1]-99	F7	IB-9	II
37	1693	EP109L2[2]-99	F7	IB-45	I
38	1695	EP109L3[2]-99	F7	IB-45	I
39	1696	EP109L4[1]-99	F7	IB-41	II
40	1697	EP109L4[2]-99	F7	IB-45	I
41	1698	EP109L4[3]-99	F7	IB-45	I
42	1699	EP109L5[1]-99	F7	IB-45	I
43	1700	EP109L1[1]-99	F11	IB-45	I
44	1701	EP109L1[2]-99	F11	IB-45	I
45	1703	EP109L2[1]-99	F13	IB-45	I
46	1704	EP109L2[2]-99	F13	IB-45	I
47	1707	EP109L3[2]-99	F13	IB-45	I
48	1708	EP109L4[1]-99	F13	IB-45	I
49	1711	EP109L5[2]-99	F13	IB-45	I
50	1712	EP109L6[1]-99	F13	IB-45	I
51	1713	EP109L6[2]-99	F13	IB-45	I
52	1714	EP109L7[1]-99	F13	IB-45	I
53	1716	EP109L1[1]-99	F16	IB-45	I

[1]Serial number; [2]Accession number of *P. grisea* culture collection of Embrapa Rice & Beans; [3]EP refers to Epagri, L indicates lesion number, superscript refers to conidial number, two last digits indicate the year of collection; [4]farm number from which the isolates were collected; [5]Pathotypes were identified using eight international differentials; [6]Groups based on clusters analysis of PCR banding patterns.

pathotype, possibly in low frequency, in other cultivars which caused the sudden outbreak of blast when Epagri 108 and Epagri 109 were introduced in the state of Tocantins.

Conidia derived from different lesions L11, L6 and L5[2] originating from the same cultivar Epagri 109 and farm (F1) were identified as pathotypes IB-1, IB-13 and IB-45, respectively. They were all clustered in rep-PCR group II. Furthermore, different monoconidial isolates obtained from the same lesion (EP108L3[2], EP108L3[3], EP108L3[4], EP108L3[5]) and the same farm (F5) were encountered both in rep-PCR groups I and II but classified as the same pathotype IB-45. These results suggest that there is no direct relation between the *Pot2* grouping and pathotypes similar to that observed for the relation between lineages and virulence (Zeigler *et al*., 1995).

The virulence pattern of the isolates clustered into group II by rep-PCR analysis, on 32 genotypes is shown in Table 2. Four pathotypes (IB-1, IB-9, IB-13, IB-41) recovered at random from cultivars Epagri 108 and Epagri 109 from different farms, as well as seven isolates of pathotype IB-45 pertained to this undefined group. They showed no difference in virulence on NIL's. Also, all 53 *P. grisea* isolates tested were avirulent (100%) to the genes Pi-1 (C101 LAC) and Pi-2 (C101 A51) and virulent to Pi-3 (C104PKT), Pi-4^a(C101 PKT) and Pi-4^b (C105 TTP4L). The genes Pi-1 and Pi-2 could be incorporated in susceptible rice cultivars Rio Formoso, Epagri 108 and 109. The reaction of the 13 isolates on nine commercial rice cultivars was similar, except for one isolate (Nº 39) identified as pathotype IB-41. Cultivars Aliança and BR-IRGA 409 among the commercial cultivars and Tetep, utilized as resistant check, showed susceptible reaction.

The pathotype IB-45 was highly virulent on Rio Formoso (17719/5738/IR 21015-72-3-3-3-1), which has the same parentage as Epagri 108 (17719/5738/IR 21015-72-3-3-3-1), whose resistance was overcome one year after its release for cultivation by Embrapa Rice & Beans. The field population of *P. grisea* was avirulent to some test genotypes (Table 2); therefore, they can be utilized as parents in crosses with commercial cultivars.

MGR586-DNA fingerprinting of 64 Brazilian isolates of *P. grisea* collected from irrigated and upland rice cultivars in experimental plots, showed that all isolates pertain to 18 distinct lineages and 15 pathotypes. These studies further showed that the isolates recovered from CICA 8 and Metica 1 belonged to two distinct lineages, BZ-A and BZ-10, respectively, indicating the high host specificity of the isolates to the cultivars, under field conditions (Filippi *et al*., 1996; Filippi *et al*., 1999). The results of the present study suggested some degree of specialization of isolates of *P. grisea* to the cultivar of origin as indicated by greater frequency of pathotype IB-45, although four other pathotypes were also identified. The ability of pathogens to adapt to the cultivar is immense. When a new cultivar with vertical resistance, such as Epagri 108, is introduced, the pathogen too often matches the resistance with the newly accumulated virulence and the cultivar becomes susceptible (Van der Plank, 1982). Furthermore genetic mutation from avirulence to virulence is an appropriate change in the DNA of the pathogen. Thus the rice blast epidemic in the State of Tocantins can possibly be attributed to the occurrence of a new pathotype IB-45.

TABLE 2 - Virulence analysis of *Pyricularia grisea* isolates that showed different *Pot2* rep-PCR banding patterns (group II) on 32 rice (*Oryza sativa*) genotypes

Genotype	Isolates[1]												
	8[2] 1644[3]	5 1639	39 1696	26 1673	21 1667	20 1665	29 1682	24 1671	16 1658	31 1684	30 1683	4 1638	36 1692
Epagri 108	S[5]	S	S	S	S	S	S	S	S	S	S	S	S
Epagri 109	S	S	S	S	S	S	S	S	S	S	S	S	S
Rio Formoso	S	S	S	S	S	S	S	S	S	S	S	S	S
Metica-1	S	S	S	S	S	S	S	S	S	S	S	S	S
Cica-8	R	R	R	R	R	R	R	R	R	R	R	R	R
Cica-9	R	R	R	R	R	R	R	R	R	R	R	R	R
Aliança	R	R	S	R	R	R	R	R	R	R	R	R	R
BR-IRGA 409	R	R	S	R	R	R	R	R	R	R	R	R	R
Javaé	R	R	R	R	R	R	R	R	R	R	R	R	R
IR-8	R	R	R	R	R	R	R	R	R	R	R	R	R
IR-36	S	S	S	S	S	S	S	S	S	S	S	S	S
IR-50	S	S	S	S	S	S	S	S	S	S	S	S	S
Basmati-370	R	R	S	R	R	R	R	R	R	R	R	R	R
Colombia-1	S	S	S	S	S	S	S	S	S	S	S	S	S
Oryzica-5	R	R	R	R	R	R	R	R	R	R	R	R	R
Tetep	R	R	S	R	R	R	R	R	R	R	R	R	R
Yashiro mochi	S	S	S	S	S	S	S	S	S	S	S	S	S
Maratelli	S	S	S	S	S	S	S	S	S	S	S	S	S
C 101 LAC	R	R	R	R	R	R	R	R	R	R	R	R	R
C 101 A 51	R	R	R	R	R	R	R	R	R	R	R	R	R
C 104 PKT	S	S	S	S	S	S	S	S	S	S	S	S	S
C 101 PKT	S	S	S	S	S	S	S	S	S	S	S	S	S
C 105 TTP4L	S	S	S	S	S	S	S	S	S	S	S	S	S
CO 39	S	S	S	S	S	S	S	S	S	S	S	S	S
Raminad	R	R	R	R	R	R	R	R	R	R	R	R	R
Zenith	S	S	S	S	S	S	S	S	S	S	S	S	S
NP 125	S	S	R	S	R	R	R	R	S	R	R	R	S
Usen	S	S	S	S	S	S	S	S	S	S	S	S	S
Dular	S	R	R	S	R	R	R	R	R	R	R	R	R
Kanto	S	R	S	S	R	R	R	R	S	R	R	R	S
Sha tio tsao	S	S	S	S	S	S	S	S	S	S	S	S	S
Caloro	S	S	S	S	S	S	S	S	S	S	S	S	S
Pathotype[4]	IB-1	IB-13	IB-41	IB-1	IB-45	IB-45	IB-45	IB-45	IB-9	IB-45	IB-45	IB-45	IB-9

[1]Isolates retrieved from cultivars EPAGRI 108 and 109 in nine different farms; [2]The numbers refer to isolates indicated in dendrograms; [3]Accession number of *P. grisea* culture collection of Embrapa Rice & Beans; [4]Pathotypes were identified using eight international differentials; [5]S = susceptible reaction; R = resistant reaction.

This pathotype, which pre-existed in low frequency, became widely distributed as a result either of strong selection pressure when Epagri 108 and Epagri 109 were introduced, or the greater fitness of the pathotype IB-45.

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