DIFFERENTIAL COMPATIBILITY OF *Pyricularia grisea* ISOLATES WITH SOME BRAZILIAN IRRIGATED RICE CULTIVARS

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ABSTRACT

The compatibility of 24 isolates of *Pyricularia grisea* was tested on four commercial irrigated rice cultivars and four standard checks, under greenhouse conditions. Among the isolates tested, seven were compatible with the cultivar 'CICA 8' and 14 with 'Metica 1'. The isolates compatible with 'CICA 8' were incompatible with the cultivars 'Metica 1' and BR-IRGA 409, independent of their origin. Seven pathotypes were identified, and the most predominant, IB-9, was recovered from eight of 11 cultivars. DNA fingerprint analysis using the probe MGR586 showed that the isolates differentially infecting 'CICA 8' and 'Metica 1' belong to two distinct lineages, BZ-A and BZ-10, respectively. Isolates retrieved from 'CICA 8' were compatible with 'Aliança' and *vice versa* and belong to the same lineage, BZ-A. These results indicate high host specificity of the isolates to the cultivars under field conditions. Rice blast resistance of 'CICA 8' and 'Metica 1' appears to be controlled by different genes which can be combined to obtain the desired protection against two common genetic lineages of the pathogen.

Key word: *Magnaporthe grisea*, rice blast, pathotype, molecular characterization.

RESUMO

Compatibilidade diferencial de isolados de *Pyricularia grisea* para algumas cultivares brasileiras de arroz irrigado

Foi estudada a compatibilidade de 24 isolados de *Pyricularia grisea* com quatro cultivares de arroz irrigado e quatro testemunhas como padrões. Entre os isolados testados, sete foram compatíveis com a cultivar 'CICA 8' e 14 com a cultivar 'Metica 1'. Os isolados compatíveis com 'CICA 8' foram incompatíveis com as cvs. Metica1 e BR-IRGA 409, independente do local de coleta. O patótipo predominante, IB-9, foi isolado em oito das onze cultivares. A análise molecular utilizando-se a sonda MGR586 mostrou que os isolados de 'CICA 8' e 'Metica 1' pertencem a duas linhagens distintas, BZ-A e BZ-10, respectivamente, indicando alta especialização dos isolados nas cultivares suscetíveis no campo. Os isolados provenientes da cultivar 'CICA 8' foram compatíveis com a 'Aliança' e *vice versa*, e pertencem à mesma linhagem BZ-A. A resistência nas cultivares 'CICA 8' e 'Metica 1' é, possivelmente, controlada por genes diferentes que podem ser combinados para dar proteção contra as duas linhagens, BZ-A e BZ-10.

Race-specific or vertical resistance to rice blast [*Pyricularia grisea* (Cooke) Sacc.] has been widely employed in breeding in different countries, including Brazil, since it is a practical, effective, and economic measure of disease control, despite its vulnerability to rapid breakdown and to changes in virulence frequency in the pathogen population. Breeding for blast resistance requires selection of segregating progenies and advanced lines in hot-spot breeding sites, where the frequency of matching virulence in the pathogen population to the cultivars to be improved is high. Resistance breakdown was attributed to disease escape of breeding lines due to the absence of compatible pathotype in a population (Zeigler *et al.*, 1995) and to high levels of pathogenic mutation (Ou, 1980). In most of the single crosses, as well as the recurrent selection breeding program of Embrapa Arroz e Feijão, both 'Metica 1' and 'CICA 8' have been included as parents and selection has been made in the early generations. Cultivar development is mainly carried out at the Palmital Experimental Station in the municipality of Brazabrantes, Goiás State. However, there is little information as to the extent of variation in the pathogen population at the test site.

The occurrence of several races has been reported in different parts of Brazil. MGR-DNA fingerprinting is now a tool for population level genetic analysis and recent studies have shown an organization of clonal lineages within and among pathotype groups (Levy *et al.*, 1991, 1993; Correa-Victoria & Zeigler, 1993; Zeigler *et al.*, 1995). The pathotypes within a lineage had a closely related virulence spectrum, differing in single compatibility differences in a subset of international differentials (Levy *et al.*, 1993). The information on isolate-cultivar interaction, coupled with molecu-

lar characterization of pathogen diversity would facilitate the development of lines with stable resistance. The present investigation was undertaken to examine the differences in compatibility among the isolates collected from different cultivars, on a set of genotypes, as well as the overall pathotype diversity in a sample of *P.grisea* rice isolates.

Single conidial isolates of *P. grisea* were established from sporulating leaf lesions of eight rice cultivars ('Metica 1', 'CICA 8', 'Aliança', 'BR-IRGA 409', 'Tetep', 'Mars', 'Oryzica L5', 'CO 39') and three near-isogenic lines (NIL's), 'C101-PKT', 'C101-LAC' and 'C105-TTP-4-L23' developed with 'CO 39' as a recurrent parent (Mackill & Bonman, 1993). Collections were made mainly from two blast screening nurseries located in irrigated and upland experimental stations at Palmital and Capivara, respectively, of Embrapa Arroz e Feijão, Goiânia, GO. Four other isolates were included in this study, one of which was established from the infected panicles of 'CO 39', two others from leaf lesions of 'Metica 1' collected from the experimental fields at Palmital and farmers' fields in the State of Tocantins, and the fourth from 'C105-TTP-4-L23' from the blast nursery at the Experimental Station of IRGA, Cachoeirinha, Rio Grande do Sul State. Stock cultures were maintained on filter paper disks in sterilized butter paper bags at 4 °C ± 1 °C, in the refrigerator.

The compatibility frequency of the isolates was tested using four commercial irrigated rice cultivars ('CICA 8', 'Metica 1', 'Aliança' and 'BR-IRGA 409') and four other genotypes ('Colombia 1', 'IR 50', 'IR 36' and 'C101-A51'). The selection of these four genotypes was based on differential reactions to pathotypes obtained in earlier studies (Correa-Victoria & Zeigler, 1993, Zeigler *et al.*, 1995). Race identification was based on the eight international differentials. The test material was planted in plastic trays (15 x 30 cm) containing 6 kg of soil fertilized with NPK (5g of 5-30-15 + Zn and 3g of ammonium sulfate per 6 kg of soil). Sixteen cultivars were sown (10 to 12 seeds) in 5 cm rows in each tray. For sporulation, the isolates were grown on oatmeal agar in Petri dishes and incubated at 25 °C for 7 days. Inoculum was prepared by flooding culture plates with distilled water and filtering the scrapped mycelial fragments and conidia through cheesecloth. The final conidial suspension was adjusted to a concentration of 3 x10^5 conidia per ml using a haemocytometer. Twenty-two-day-old plants were inoculated by spraying the aqueous spore suspension on the leaves, until run-off, using an atomizer connected to an air compressor. Inoculated plants were incubated in a moist chamber for 24 h at 20 to 24 °C, after which they were kept in the greenhouse, where the temperature ranged from 25 °C – 28 °C, and high humidity. The disease reaction was assessed nine days after inoculation following the visual rating scale varying from 0 to 9 (IRRI, 1988). The isolates that induced typical sporulating lesions were considered compatible (4-9), and necrotic non-sporulating lesions as incompatible (0-3). In case of ambiguous or intermediate reaction, inoculation tests were repeated until a consistent and uniform reaction was obtained.

DNA fingerprinting analysis of the 15 *P. grisea* isolates utilized in the present study was conducted with several other Brazilian isolates at Purdue University, West Lafayette, U.S.A, following the protocol described by Levy *et al.* (1991). DNA was extracted from the mycelia of *P. grisea* isolates grown in liquid cultures and digested with *Eco*R1. DNA blots were hybridized with the repetitive sequence probe, MGR586, which was radioactively labeled by the random primer method. Lineage identification of Brazilian isolates was reported elsewhere (Filippi *et al.*, 1996).

There was a great genetic diversity in compatibility frequency among the 24 test isolates. The results presented in Table 1 showed 14 isolates compatible with 'Metica 1', as compared to seven in 'CICA 8', independent of the collection site or origin. All four isolates retrieved from 'CICA 8' were incompatible with 'Metica 1' and the isolates derived from 'Metica 1' were incompatible to 'CICA 8'. Isolates compatible with 'CICA 8' were also compatible with 'Aliança', but not with BR-IRGA 409. The isolates from 'Aliança' were compatible with 'CICA 8', and *vice versa* . The differential reaction types in 'BR-IRGA 409', 'Colombia-1', 'IR 50', and 'IR 36' distinguished the two isolates of 'Oryzica-L5' collected from Capivara and Palmital. Similarly, the reaction types in 'Metica 1' and 'BR-IRGA 409' distinguished isolates of 'C105-TTP-4L23'. Cultivars 'Metica 1', 'BR-IRGA 409' and 'IR 50' could be used to differentiate the isolates of near-isogenic lines (NIL's) of 'CO39'. Among the standard checks, a high frequency of isolates were compatible to 'Colombia-1'.

Matching virulence for all resistance genes in the cultivars was present in the pathogen population, even though some of them occured at low frequencies. A low frequency of compatible isolates associated with the cultivars, in descending order, was 'C101-A51', 'BR-IRGA 409' and 'IR36'. Both 'IR 50', a susceptible check, and 'IR 36', a partial resistance source in many earlier studies conducted at the International Rice Research Institute (IRRI), showed low reaction types (4-5) to the compatible isolates. No cultivar was susceptible to all isolates tested (Table 1). Compatible reactions were observed on 'C101-A51' from one isolate recovered from 'CO 39', one from 'BR-IRGA 409' and two from 'Metica 1'. No isolate from the NIL's was compatible with 'C101-A51', carrying the resistance gene Pi-2, but the resistance gene was overcome by one of the isolates from its recurrent parent, 'CO 39', which exhibited a high degree of susceptibility in the field. This indicates that the virulence and avirulence genes in the pathogen may be retained in the presence or absence of compatible hosts. However, the assessment of the virulence spectrum of isolates is difficult because of sampling inefficiencies due to low frequency in experimental fields, and requires controlled compatibility assays with isolates collected from large fields.

It has been the common observation that 'Metica 1' and 'CICA 8' are highly susceptible in the blast nursery as well as in the field. However, the results in the present study showed that the isolate from 'CICA 8' was not pathogenic to 'Metica 1', and *vice versa*. Molecular analysis with the probe MGR586 showed that the isolates of 'CICA 8' and 'Metica 1' belong to two distinct lineages, BZ-A and BZ-10, respectively (Fig. 1), indicating that the differential host specificity of the isolates is genetically determined and limited. Consequently, the resistance in 'CICA 8' and 'Metica 1' is likely to be controlled by different genes that could be combined to

TABLE 1 - Leaf blast ratings on eight rice genotypes in greenhouse inoculation tests with 24 *Pyricularia grisea* isolates*.

Isolate**	Pathotype	Lineage	Origin***	Commercial irrigated rice cultivars				Standard checks			
				A****	B	C	D	E	F	G	H
CICA 8^4	IB-45	BZ-A	IRBN PAL 95	7	0	7	0	0	0	0	0
CICA 8^1	IB-38	BZ-A	IRBN PAL 95	9	0	9	0	0	0	0	0
CICA 8^2	IG-2	BZ-A	IRBN PAL 95	7	0	7	0	0	0	0	0
CICA 8^3	IG-2	–	IRBN PAL 95	4	0	5	0	0	0	0	0
Aliança 2^4	IG-2	BZ-A	IRBN PAL 95	7	0	7	0	0	0	0	0
Aliança-1	IB-10	BZ-A	VNB CAP 95	4	0	5	0	0	0	0	0
Aliança-3	IG-2	BZ-A	VNB CAP 95	5	0	5	0	0	0	0	0
Metica 25 $L1^2$	ID-14	–	Field FORM 96	0	9	0	0	7	9	0	4
$Metica^3$	IB-9	BZ-10	Field PAL 95	0	9	0	0	7	4	0	5
Metica 1	IB-9	–	IRBN PAL 95	0	7	0	0	7	0	0	0
CO-39-L^3	IB-9	–	IRBN PAL 95	0	7	0	0	7	0	0	0
CO-39-$P2^4$	IB-9	–	Field PAL 95	0	9	0	0	7	0	0	0
CO-39-$L1^4$	IB-9	–	IRBN PAL 95	0	9	0	0	7	0	0	0
C-101^3-PKT	IB-9	BZ-11	VNB PAL 95	0	7	0	0	5	0	0	0
Mars $L1^1$	IB-9	BZ-12	VNB PAL 95	0	5	0	0	5	0	0	0
Oryzica $L5^2$	IB-9	–	VNB CAP 95	0	5	0	0	5	0	0	0
BR-IRGA 409 $L1^1$	IB-9	BZ-8	VNB IRGA95	0	5	0	9	0	5	0	4
BR-IRGA 409 $L2^1$	IB-10	BZ-10	VNB CAP 95	0	0	0	5	7	4	4	0
CO-39-$L1^1$	IB-9	BZ-10	IRBN PAL 95	0	9	0	0	9	0	0	7
C-105-TTP-4L23	IB-9	–	VNB IRGA 95	0	0	0	7	0	0	0	0
C-105-TTP-4-L-23	IB-9	BZ-11	VNB CAP 95	0	4	0	0	0	9	0	0
C-101 LAC-$L1^1$	IB-9	BZ-20	VNB CAP 95	0	5	0	0	5	9	0	0
Oryzica-$L5^1$	IB-45	BZ-C	VNB PAL 95	0	4	0	5	0	5	4	0
Tetep-L^3	IC-1	–	VNB PAL 95	0	0	0	0	0	5	0	0

* Rice blast ratings 0 to 3 were considered compatible and 4 to 9 incompatible reactions;
** Code name of the isolates was designated based on the cultivar from which it was established;
*** IRBN = International Rice Blast Nursery; VNB = National Rice Blast Nursery;
**** Cultivars: A= CICA 8, B= Metica 1, C= Aliança, D= BR-IRGA 409, E= Colombia 1, F= IR 50, G= IR 36, H= C101 A-51.

obtain desired protection against two genetic lineages of the pathogen. The combination of resistance genes in these two cultivars may be effective against all 24 isolates tested (Table 1). The isolates retrieved from 'CICA 8' were compatible with 'Aliança' and *vice versa*, and belong to the same lineage, BZ-A. We infer that these two cultivars have the same genetic base because one of the parents of 'Aliança' was 'CICA 8'. The isolates from 'Aliança' collected from nurseries located at Palmital and Capivara showed similar host reaction. It is evident from these results that cultivar specificity among field isolates is greatly related to their host of origin. Seven pathotypes were identified among the 24 isolates on the basis of host reaction in eight international differentials, the predominant being IB-9 (Table 1). Pathotype IB-9 was recovered from eight of 11 cultivars. The other pathotypes were IB-10, IB-38, IB-45, IC-1, ID-14 and IG-2. Pathotype analysis based on international differentials showed one predominant race, but exhibited 11 distinct virulence patterns in the other eight test cultivars employed in this study. Some of the isolates that differed in compatibility belong to the same international race, IB-9. The differential set did not provide adequate information concerning pathogen diversity. Isolates with the IB-9 pathotype were found in five genetic lineages, BZ-8, BZ-10, BZ-11, BZ-12 and BZ-20. The commercial cultivars utilized as differentials showed patterns much more useful for resistance breeding. This supports the earlier studies conducted with Philippine isolates (Bonman *et al.*, 1986).

The available genes in the commercial rice cultivars 'Metica 1', 'CICA 8' and 'BR-IRGA 409' do not offer resistance to the prevailing pathogen population in the test fields. New resistance genes or combination of resistance genes are required to be incorporated in these cultivars. The presence of host-specific isolates within the pathogen population from Palmital permits the adoption of appropriate breeding strategies. One approach should be to incorporate resistance in 'Metica 1' by a backcrossing program using 'CICA 8', 'BR-IRGA 409', 'IR 50', 'IR 36', and 'C101 A51' as donors, and 'Metica 1' as the recurrent parent. This study may lead to the development of multilines of 'Metica 1' with different resistance genes. The isolates that showed a differential host reaction in the present study will be useful for screening and selection of progenies for resistance under controlled conditions. For example, inoculation of a segre-

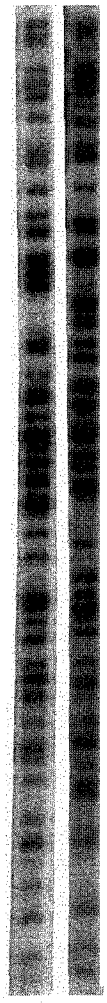

FIG. 1 - MGR-DNA fingerprints from two isolates of *Pyricularia grisea* retrieved from two irrigated rice cultivars a) 'CICA 8 and b) 'Metica 1 exhibiting distinct differences in banding pattern.

gating population of a cross between 'Metica 1' and 'C101-A51' with the isolate from 'C105-TTP-4-L23' should permit the incorporation of Pi-2 gene from 'C101-A51' into 'Metica 1'. Also, it may be possible to obtain a broad spectrum resistance by combining the genes from 'CICA 8' and 'Metica 1' using pathotypes belonging to the lineages BZ-A and BZ-10 for screening the segregating populations for resistance to both lineages, combined with field evaluation for other desirable agronomic traits. The crosses made using 'Aliança' and 'CICA 8' as donors of resistance genes in the backcross program, and the selection using the isolates in this present study may not be desirable, because they probably possess the same genes. Furthermore, other resistant sources developed using the specific pathotypes can be included in an ongoing recurrent selection program. The introgression of known combinations of genes, rather than random combination under natural field conditions, would improve the efficiency of recurrent selection programs for obtaining lines with stable resistance. The genetic and pathotype diversity analysis will improve the process of screening and incorporation of resistance genes into commercial cultivars, and require continuous monitoring of pathogenic variation in the field population at test sites such as Palmital.

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