NOTAS CIENTÍFICAS

Variation in virulence in the rice blast fungus *Magnaporthe grisea* in São Paulo State(1)

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Abstract – Resistant varieties have been the preferred means to control *Magnaporthe grisea*, the causal organism of the rice blast disease. The objective of this study was to examine the degree of diversity of the pathogen in different rice growing regions of São Paulo State, Brazil. Blast samples collected from rice varieties in three different regions (Tremembé, Mococa and José Bonifácio) were analyzed for race structure employing the Japanese rice differentials. The highest degree of virulence diversity was observed in Tremembé with 22 different races in three different varieties. Furthermore, no resistance gene in the Japanese differentials was effective to all isolates of *M. grisea* from São Paulo State.

Index terms: *Pyricularia*, genes, disease resistance, pathogenicity.

Variação na virulência na brusone do arroz *Magnaporthe grisea* no Estado de São Paulo

Resumo – Variedades resistentes têm sido o método preferido de controle de *Magnaporthe grisea*, agente causal da brusone do arroz. O objetivo deste estudo foi examinar o grau de diversidade do patógeno em diferentes regiões produtoras de arroz do Estado de São Paulo. Amostras de brusone obtidas de diferentes variedades das regiões de Tremembé, Mococa e José Bonifácio foram analisadas quanto à estrutura das raças, empregando-se as diferenciais Japonesas de arroz. A maior diversidade da virulência foi observada em Tremembé com 22 raças, em três variedades distintas. Não foi identificado nenhum gene de resistência nas diferenciais japonesas que fosse efetivo contra todos os isolados de *M. grisea* do Estado de São Paulo.

Termos para indexação: *Pyricularia*, genes, resistência a doenças, patogenicidade.

The rice blast disease caused by *Magnaporthe grisea* (T.T. Hebert) Yaegashi & Udagawa [anamorph *Pyricularia grisea* (Cooke) Sacc.] is a major disease of rice, limiting yield in many parts of Brazil. Development of resistant varieties has been the most employed means to protect the rice crop from the pathogen. However, the breakdown of resistant varieties has been reported in

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the field shortly after varieties were released (Yamada,1985; Bonman et al., 1986; Correa-Victoria & Zeigler, 1993).

The exact cause of resistance breakdown is still a mystery, it is not known whether the ability of the pathogen to overcome resistant variety reflects shifts in the frequency of formerly rare pathotypes or the frequent occurrence of genetic changes to new virulent forms or a combination of both phenomena (Levy et al., 1993; Zeigler et al., 1995). Therefore, the knowledge on the diversity of virulence among isolates collected from rice in different regions in the State of São Paulo is an important step towards a successful strategy to reduce the impact of the rice blast through the use of resistant varieties.

The isolates of *M. grisea* are grouped into physiologic races based on the reaction type on a set of eight international differentials and a standardized system of numbering the races was proposed to avoid confusion (Ling & Ou, 1969). Later, Yamada et al. (1976) proposed a new set of nine differential varieties for Japan on the basis of known resistance genes adopting the Gilmour's octal system of numbering the races.

This work was undertaken to assess the degree of diversity of *M. grisea* in different rice growing regions of São Paulo State.

All isolates were collected in 1999 in different rice-growing regions of São Paulo State. The isolate code, region of origin, number of isolates, the variety from which *M. grisea* were obtained are shown in Table 1. The method of single spore isolation from diseased plants and storage of isolates were described previously (Urashima et al., 1993). Twenty six isolates were established from rice fields in Mococa, 35 from Tremembé and six from José Bonifácio.

The Japanese rice differential varieties (Yamada et al., 1976) were used in this work because each differential variety has only one vertical resistance gene. Seeds were soaked for three days and 3-5 germinated seeds were sown in plastic pots in uniformly fertilized soil. They were kept at 23-28°C until three- to four-leaf stage when they were inoculated. Two replicates were prepared for each isolate.

Spores for inoculation were prepared as described (Urashima et al., 1993). The conidial suspension was adjusted to 1×10^5 spores per milliliter in Tween 20 at a concentration of 10^{-4}. Inoculation was made using 25 mL of conidial suspension sprayed on rice plants using a manual sprayer. Inoculated plants were placed inside plastic bags under darkness for 18 hours and temperature of 23±5°C. The pots were subsequently placed on greenhouse bench in a randomized order until disease assessment.

Disease reaction was evaluated after seven days. A variety was considered susceptible when more than 50% of the inoculated plants exhibited either lesion 4, typical compatible lesions (elliptical with gray center) or lesion type 3, small eyespot shaped lesion with gray center (Urashima et al., 1993).

The race identification was done using the Japanese rice differential varieties that adopts the Gilmour's octal notation (Yamada et al., 1976). Under this scheme, a code number is given to each differential variety corresponding to each vertical resistance gene. The number of each race is the sum of code

Table 1. Identification code, origin, number of isolates, variety, plant growth stage, plant part infected, number of physiologic races, and race designation of *Magnaporthe grisea*.

Isolates code	Origin	Number of isolates	Variety	Host growth stage	Plant part infected	Number of races	Race identification[1]
MC02-	Mococa	12	IAC4440	Mature	Neck	1	200
MC03-	Mococa	14	IAC4440	Milk	Neck	2	200;210
JB01-	José Bonifácio	7	Caiapó	Mature	Rachis	6	1;22;135;142;145;177
VP01-	Tremembé	3	IAC101	Milk	Neck	3	533;577;637
VP02-	Tremembé	2	IAC101	Tillering	Leaf	2	3;33
VP03-	Tremembé	8	IAC103	Milk	Neck	8	232;336;433;437;477;537;573;637
VP04-	Tremembé	5	IAC101	Milk	Neck	4	212;413;433;437
VP05-	Tremembé	5	IAC101	Milk	Neck	5	12;430;533;537;737
VP07-	Tremembé	12	IAC242	Milk	Neck	11	120;232;310;332;432;433;437;533;637;733;737

[1] Races were identified based on nine Japanese differentials (Yamada et al., 1976).

number of each resistance gene, accordingly of each differential variety, to which the race is virulent.

Twenty-six isolates of *M. grisea* pertaining to two races (race 200 and 210) were identified. Samples from Mococa region were collected from one rice variety (IAC4440) from different fields at different host developmental stages (Table 1). However, a great majority of samples (25 isolates, representing 96.2% of the total) were identified as race 200. Those isolates could infect only one variety (PiNo.4), which carries the resistance gene *pi-ta*^{2}. The only exception was the isolate MC03-08, identified as race 210 because this isolate was virulent to two varieties, PiNo.4 and Kanto51, which carry the resistant genes *pi-ta*^{2} and *pi-k*, respectively.

Among the *M. grisea* isolates collected from José Bonifácio, diversity in virulence was observed even though the number of isolates examined was small. Six different physiologic races were identified out of seven isolates from this region. The race 01 was capable of overcoming the resistance of one gene (*pi-k*^{s}) while the race 177 overcame the resistance conferred by seven different genes. Other races observed in José Bonifácio included races 22, 142, 145 and 135, infecting varieties with 2, 3, 4 and 5 distinct resistance genes, respectively. Only race 177 was detected more than once and varieties PiNo.4 and Toride1, with resistance genes *pi-ta*^{2} and *pi-z*^{t}, were not infected.

Compatibility with resistance to all differential varieties was observed in the pathogen population collected from Tremembé. Twenty-two different races were identified among 35 blast isolates. Race 433 was most frequently detected (four times), followed by races 437, 533, 637 (three times each) and 232, 332, 537, and 737 (twice). With reference to isolates from Tremembé, the same race was recovered from the same rice variety, race 332 was identified in two isolates from IAC242 (field VP07) and race 433 in two isolates from IAC101 (field VP04). In contrast, different rice fields were infected by the same blast race (races 232, 433, 437, 533, 637, and 737). Also, different races were recovered from the same field (VP03). The distribution frequency of none of the isolates was higher than 11.4%. The high diversity of *M. grisea* from Tremembé is also confirmed by the range of isolates capable of infecting different Japanese rice differentials. Races 03, 12 and 120 (isolates VP02-01, VP05-07, and VP07-06, respectively) were virulent to only two varieties. On the other hand, races 477, 537, 573 were capable of overcoming seven resistance genes, and one isolate (VP01-07) infected eight varieties, it did not overcome only one resistance gene of the differential set.

Table 2 shows the frequency of virulent phenotypes of *M. grisea* isolates from different regions of São Paulo. This value was calculated by the total number of compatible reaction for each rice differential variety divided by the number of isolates used for inoculation on that particular variety. Isolates from Mococa showed narrowest spectrum of virulence. All isolates from this region were compatible on two varieties, PiNo.4 (*pi-ta*^{2}) and Kanto51 (*pi-k*). Fungal isolates compatible to all resistance genes of the Japanese differential varieties have been identified in the samples collected from Tremembé although the frequency of virulence to each gene varied. The virulence frequency of isolates from Tremembé to the genes in the Japanese differentials varied from 8.6% to 94.3%. Another pattern of frequency of virulent phenotypes, different from the former two, was observed in blast isolates from José Bonifácio. Despite the small number of isolates (7), seven out of nine differential varieties exhibited compatible reaction, and frequency of virulent phenotypes ranged from 42.9 to 71.4%. Two varieties, PiNo.4 (*pi-ta*^{2}) and Toride1 (*pi-z*^{t}) were resistant to all isolates in this region.

A great variation in race composition and virulence spectra of *M. grisea* according to the region was observed in the present work. Only two races among isolates from Mococa were identified, the most prevalent one was race 200. Twenty two different physiologic races were obtained from blast isolates of Tremembé with no apparent predominance of any.

As the rice variety IAC4440 was the sole source of isolates in Mococa, the results in the present study indicate that the host from which the isolate originated was important and that race prevalence is influenced by rice variety planted in a specific area. Similar results were obtained in earlier studies (Yamada, 1979; Yamada, 1985; Bonman et al., 1986). However, this strong host specificity was not observed in other regions, multiple races in a single field were common in Tremembé and José Bonifácio. Each one of the eight blast isolates (VP03), from IAC103, was classified as different race, while six races were recovered from seven isolates from rice variety Caiapó in José Bonifácio. All isolates (VP01, VP02, VP04, VP05) collected from different fields but from the same rice variety showed 13 different physiologic races. The common races were, however, rare. The races 136 and 337 identified earlier by Urashima & Isogawa (1990) in blast isolates collected from IAC4440 from Paraíba Valley were not detected in this variety grown in Mococa. These results demonstrate that besides the varietal origin, ecological factors possibly play a role with reference to the prevalence of a specific fungal race in a determined region.

The virulence spectra also varied according to the region. The virulence spectra of isolates from Mococa was simple compared to the spectra of isolates from Tremembé and José Bonifácio where isolates were compatible to at least seven different resistance genes. Mococa is one of the sites for rice breeding program in São Paulo. Therefore, the narrow spectrum of virulence

Table 2. Percentage of isolates of *Magnaporthe grisea* collected from three regions of São Paulo State virulent to the Japanese differential rice varieties(1).

Rice variety	Resistance gene	Mococa	José Bonifácio	Tremembé
Shin2	*pi-k*^{s}	0.0	71.4	68.6
Aichi-Asahi	*pi-a*	0.0	57.1	91.4
Ishikarishiroke	*pi-i*	0.0	57.1	37.1
Kanto51	*pi-k*	3.8	42.9	94.3
Tsuyuake	*p-k*^{m}	0.0	57.1	85.7
Fukunishiki	*pi-z*	0.0	57.1	8.6
Yashiromochi	*pi-ta*	0.0	71.4	42.9
PiNo.4	*pi-ta*^{2}	100.0	0.0	31.4
Toride1	*pi-z*^{t}	0.0	0.0	68.6

(1)The number of *M. grisea* isolates from Mococa, José Bonifácio and Tremembé were, respectively, 26, 7, and 35.

observed in this locality deserves a more detailed study. It is necessary, for instance, to study the behavior in Mococa of rice varieties grown in Tremembé. An accurate definition of pathogen diversity at this site is crucial to the success of newly released rice varieties. According to Correa-Victoria & Zeigler (1993) virulent pathogen strain that do not occur in the experimental site poses a potential threat to varieties/lines that are resistant and may be a source of cryptic error in breeding resistant varieties.

Resistance genes that were effective in one region were not found to be effective in other regions. Matching virulence to all resistance genes analyzed in this work was already present in blast population of São Paulo State. Thus, a search for other resistance or unknown gene in Brazilian varieties needs to be investigated for successful blast resistance breeding.

The virulence diversity assay on a different set of varieties might reveal the presence of undetected races. However, the Japanese differentials for *M. grisea* isolates utilized in this study were found useful because the reactions were predominantly either highly susceptible (type 4 lesions) or completely resistant (type 0 lesions). Nevertheless, for an extensive breeding program, attempts to create a differential set of rice varieties for rice blast isolates occurring in Brazil are necessary.

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