



Diversity of virulence of *Pyricularia oryzae* isolates obtained from a single lesion of wheat

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ABSTRACT

The objective of this study was to evaluate the virulence of *Pyricularia oryzae* isolates obtained from a single leaf blast lesion on wheat. The wheat cultivars (BRS Louro, BR 18, BRS Tarumã, BRS Parrudo, BRS 208) at the four-leaf stage were inoculated with a suspension of 100.000 conidia/mL. A significant difference was observed in the severity in the cultivars. Among the evaluated isolates, it was possible to observe differences in the susceptibility of the cultivars. Severity data ranged from 3.3 to 99.54% among treatments. Isolates 1 and 2 were the most aggressive for all cultivars tested. The greatest disease severity occurred in the interactions BRS 208 x isolate 1 (99.54%), BRS Louro x isolate 2 (99.34%), and BRS 208 x isolate 2 (99.09%). The lowest disease severity was observed in BRS Tarumã (68%) and BR 18 (75%) cultivars for the isolates 1 and 2, respectively. Isolate 3 presented medium aggressiveness in which the cultivars presented between 44 to 58% of wheat blast severity. The isolate 4 was the least aggressive for all cultivars with severity indexes ranging from 3 to 12%. These data indicate the existence of virulence diversity in *P. oryzae* isolates obtained from a single lesion.

Keywords: genetic resistance; genetic variability; monosporic isolates; wheat blast.

INTRODUCTION

Wheat blast disease was first discovered in the state of Paraná, Brazil in 1985 (Igarashi *et al.*, 1986). Since then, the disease has been recorded in different Brazilian states and in South America (Maciel *et al.*, 2014). Known as one of the most important plant pathogenic fungi, *Pyricularia oryzae* has an unusual capacity of rapidly changing its genetic makeup resulting in new pathogenic variants (Dean *et al.*, 2012). In February 2016, wheat blast was observed in eight districts in Bangladesh, and caused losses in the yield, reaching up to 100%. The phylogenetic and population genomic analyses revealed that the wheat blast outbreak in Bangladesh was most likely caused by a wheat-infecting South American lineage of the blast fungus *Magnaporthe oryzae* (Islam *et al.*, 2016). It had been already pointed out that, under field conditions, most of the genetic variations in *P. oryzae* were induced by

mutation and parasexual recombination (Noguchi *et al.*, 2006). Consequently, the fungus exhibits a high degree of genetic variability; however, the main question of how the genetic makeup of the fungus changes so rapidly remains unanswered. Therefore, the objective of this study was to evaluate the virulence of *P. oryzae* isolates obtained from a single lesion when inoculated on five different wheat cultivars. The hypothesis of this work is as follows: if the monosporic isolates of *P. oryzae* from the same lesion have genetic diversity, different levels of severity will be observed in the group of plants tested.

MATERIAL AND METHODS

For the experiment, the wheat cultivars BR 18 (resistant), BRS 208 (susceptible), and BRS Louro, BRS Tarumã, BRS Parrudo (no information about blast resistance) were selected according to the Technical

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Recommendations for Wheat and Triticale Crop 2018 (RCBPPT, 2018). Wheat seeds were surface-sterilized in 10% NaOCl for 2 min, washed in sterilized water for 3 min, and sown in 500-mL plastic pots, filled with substrate composed of biosynthesized *Pinnus* bark, vermiculite, charcoal mill, water and phenolic foam. The plants were fertilized with modified nutrient solution of Hoagland & Arnon (1950) on a week basis. Initially, to obtain the isolates, susceptible cultivar BRS 208 was inoculated with the monosporic isolate of *P. oryzae* UFV-DFP-01 (monosporic isolate obtained from the rachis of cultivar BR18, Viçosa, MG, BR). After inoculation, samples of young leaves of cultivar BRS 208 were selected, and kept in a humid chamber for 24 hours. A typical blast lesion was selected and monosporic isolates were obtained from this sample. In the present experiment, four of these isolates were used.

The isolates were preserved in filter paper strips on silica gel at 4°C. Fifteen days before inoculation, pieces of these strips were plated in Petri dishes containing oat-agar medium. When the colony of the fungus reached 3 cm in diameter, it was peeled into new Petri dishes containing oat-agar, and maintained for twelve days in a growth chamber (25 °C and photoperiod of 12 hours light) until inoculation. Plants in stage 13 (Zadoks *et al.*, 1974) with four leaves fully expanded were inoculated with a conidial suspension of *P. oryzae* at 100.000 conidia / mL. The inoculum was sprayed on the adaxial and abaxial leaves surface until runoff using a manual atomizer. After inoculation, the plants were submitted to an initial 24-h dark period. After that, the photoperiod was adjusted to 12-h light. The plants were maintained in a growth chamber at a temperature 25±2 °C and the relative humidity was 90±5%. Five days after inoculation, the severity of the wheat blast was evaluated in the third leaf from the base to the top of each plant using the diagrammatic scale with severity values ranging from 0 to 100% (IRRI, 1996).

The experiment consisted of 5 × 4 factorial with five cultivars and four isolates, arranged in a completely randomized design, with five replications. Each repetition consisted of six plants in a plastic pot. The severity data were submitted to analysis of variance (ANOVA) and the means of the treatments were compared by Tukey's test ($P \leq 0.05$) using the SISVAR software (Ferreira, 2008).

RESULTS AND DISCUSSION

It was observed in this study a significant difference ($p = 0.001$) in the cultivar and isolate factors and also in the interaction between the factors. According to the data shown in Table 1, the severity ranged from 3.3 to 99.54% among treatments. Isolate 4 was the least aggressive for the tested cultivars, with severity ranging from 3.3 to 11.9%. For this isolate, no difference was found in blast

severity among cultivars. The mean values of the severity for isolate 3 ranged from 43.86 to 58.18% among cultivars. Isolate 3 presented medium aggressiveness, and no significant difference was observed in blast severity among cultivars. The isolates 1 and 2 were the most aggressive for the cultivars. The mean values of severity for isolate 1 ranged from 67.72 to 99.54%. The lowest severity was verified in the cultivar Tarumã, which did not differ significantly from the cultivar BR 18 (88.66%). The severity averages for isolate 2 ranged from 74.77 to 99.34%. The cultivar BR18 presented the lowest disease severity, to this isolate, differing statistically from the other cultivars. In this study, all cultivars were considered susceptible to isolates 1, 2 and 3, and resistant to isolate 4. In a preliminary work carried out with the parental isolate (UFV-DFP-01), the cultivars BRS Louro, BR 18 and BRS 208 showed the following severity levels, respectively: 85%, 60% and 35% (unpublished data).

Although sexual reproduction may confer a greater genetic variability among microorganisms, the importance of asexual reproduction for this pathogen should not be disregarded. In the present experiment, the isolate from a single lesion allowed the identification of different levels of severity (low, medium and high) in a reduced number of cultivars (five). That is, even in a short time and in a reduced leaf area, the pathogen may be undergoing changes in its genome, which result in different levels of blast severity in wheat cultivars. These changes may contribute to the production of *P. oryzae* genotypes with higher plasticity, aggressiveness, and greater resistance to environmental conditions and fungicides.

To explain the high genetic variability presented by *P. oryzae*, studies have pointed out both for the importance of asexual and sexual reproduction, even though the latter has not been evidenced in field conditions. Pagliaccia *et al.* (2018), when analyzing the genetic structure of *P. oryzae* from isolates collected from the initial identification of the disease in 1990 to more recently sampled isolates, evidenced a significant increase in genotypic diversity over the time, but without evidence of sexual reproduction. However, the high genetic variability in *P. oryzae* in wheat, which contributes to the lack of blast durability of resistance in wheat cultivars, was related to the sexual reproduction capacity between wheat isolates and weeds such as *Brachiaria plantaginea* and *Setaria geniculata* (Bruno & Urashima, 2001). According to Maciel *et al.* (2014), the pathogen performs both sexual and asexual reproduction in the field. New genotypes of pathogens produced by sexual recombination may be favored by selection and increased frequency via asexual reproduction within each wheat growing area, spreading like clones over short distances through conidia. Different from those presented by these authors, the present work

indicates that even in a small and unique lesion, the fungus presents genetic variability. When the severity levels of three of these cultivars are compared with the response to parental isolate (UFV-DFP-01), it is observed that the tendency of susceptibility of the cultivars is maintained, with alteration in the severity levels for 3 of the 4 isolates. Probably, these facts may contribute to the understanding of the variation in the results presented in different studies evaluating the fungus aggressiveness on different cultivars since the identification of the disease in Brazil (Urashima *et al.*, 2004; Arruda *et al.*, 2005; Prestes *et al.*, 2007; Cruz *et al.*, 2009, 2010; Maciel *et al.*, 2014).

The cultivar BR 18, for example, is considered as resistant or partially resistant to wheat blast in the heading stage (RCBPTT, 2018; Prestes *et al.*, 2007; Rios *et al.*, 2016). However, for leaf resistance, differentiated responses were observed in the literature (Urashima *et al.*, 2004; Cruz *et al.*, 2009). In the present work, the cultivar BR 18 was considered susceptible to 3 of the 4 isolates tested, with severity ranging from 55 to 88.86%. Similar results were observed by Cruz *et al.* (2009), in a study carried out with 18 monosporic isolates of *P. oryzae* obtained from different geographical regions in Brazil. Using a greater number of isolates (72) obtained from the Mato Grosso do Sul and Paraná States, Urashima *et al.* (2004) verified that cultivar BR18 exhibited a broad resistance spectrum in relation to the rest of the tested cultivars, showing resistance to more than 50% of the isolates of the both States. Cruz *et al.* (2009, 2010) observed the susceptibility of BRS 208 and BRS Louro cultivars for 17 monosporic isolates of *P. oryzae*, similar to that observed in this study, where the highest severity indices were observed in these cultivars. The BRS Tarumã is a cultivar with double purpose (forage and grains), considered moderately susceptible to leaf spots (Fontanelli *et al.*, 2016). The BRS Parrudo is a cultivar characterized as moderately resistant to leaf spots (*Pyrenophora tritici-repentis* and *Bipolaris sorokiniana*) (Scheeren *et al.*, 2014). Information on the reaction of BRS Tarumã and BRS Parrudo cultivars to wheat blast has not been found in the literature.

According to this study in relation to the response of the tested cultivars, the fungus simply undergoes periodic changes, even within a single lesion, without the pressure of a selection factor. Ou & Ayad (1968), observed that from monoconidial cultures isolated from one typical leaf rice blast lesion, and respective monoconidial subcultures of the original isolates allowed the identification of different pathogenic races of *P. oryzae*. For these authors, the cells of both mycelium and conidia are multinucleated and anastomosis as well as nucleus migration could contribute to the production of heterodiploid forms of the pathogen. This would explain the genetic variability of the *P. oryzae* in rice, and the different pathogenicity results found in the set of the differential varieties. Pathogenic variability among monoconidial isolates of *Colletotrichum graminicola* from single lesions on sorghum and from monoconidial cultures was observed by Casela & Fredriksen (1994). From each lesion, a total of 20 monoconidial isolates were obtained, followed by the isolation of 20 monoconidial sub-cultures from one isolate of each set of 20 plus an additional generation of 20 monoconidial sub-subcultures from one isolate of each sub-set. Fifteen isolates per lesion were selected, and inoculated onto plants of five sorghum cultivars. The authors confirm the occurrence of pathogenic instability in monoconidial cultures of *C.graminicola* derived from single lesions and from monoconidial cultures. Despite the possibility that some variability within the single lesion results from infection of different genotypes, these data were helpful in explaining part of the aggressiveness observed in *C.graminicola* natural populations, as changes in the cultivar host-specificity (Casela & Fredriksen, 1994). In addition, variation in the virulence and aggressiveness was reported by Scharen & Krupinsky (1970) in a study with single conidia from *Stagonospora nodorum* onto wheat.

The molecular analysis of the conidia onto single lesion or monoconidial culture is useful for the genetic characterization of pathogen populations (Linde *et al.*, 2002; Maciel *et al.*, 2014). The spatial distribution of 15

Table 1: Severity of wheat cultivars submitted to inoculation with four monosporic isolates of *Pyricularia oryzae* obtained from a single BRS 208 blast lesion

Cultivar/Severity	Isolates			
	1	2	3	4
BRS Louro	94.31 C ¹ c ²	99.34 Bc	58.18 ABCb	3.3 Aa
BR18	88.86 BCcd	74.77 Ac	55 ABb	4.4 Aa
BRS Tarumã	67.72 ABb	95.9 Bc	61.36 ABCb	4.8 Aa
BRS Parrudo	96.36 Cc	95 Bc	43.86 Ab	6.8 Aa
BRS 208	99.54 Cc	99.09 Bc	56.81 ABCb	11.9 Aa

^{1,2}Means followed by the same letter, lowercase in the row and upper case in the column, did not differ statistically by the Tukey's test at the 5% probability. CV=28.62%.

Mycosphaerella graminicola genotypes found among 158 isolates sampled from five individual lesions was used to determine across a hierarchy of spatial scales using molecular markers. At the smallest spatial scale, each lesion had two to six different genotypes including both mating types in four of the five lesions, but in most cases, a lesion was composed of one or two genotypes that occupied the majority of the lesion, with other rare genotypes interspersed among the common genotypes. Low population differentiation was indicated among populations, suggesting a corresponding high degree of gene flow among these populations. At the largest spatial scale, populations from Switzerland, Israel, Oregon, and Texas were compared. Population differentiation among these populations was low, and genetic identity between populations was high. The hierarchical gene diversity analysis included 1,098 isolates from seven field populations. According Linde *et al.* (2002), the high levels of gene flow in *M. graminicola* populations on a regional level indicates a significant potential risk for the regional spread of mutant alleles that enable fungicide resistance or the breakdown of resistance genes. Maciel *et al.* (2014) used 11 microsatellite loci to elucidate the population structure of the wheat blast pathogen in wheat fields in central-western, southeastern, and southern Brazil. However, no subdivision was found among the wheat-infecting populations, consistent with high levels of gene flow across a large spatial scale.

The diversity of virulence and aggressiveness observed in this study and in other works with *P. oryzae* where the evaluated cultivars showed resistance to a restrict number of the patotypes of the pathogen (Urashima *et al.*, 2004; Cruz *et al.*, 2010; Maciel *et al.*, 2014) may be related to the information presented by Peng *et al.* (2019). The authors observed in the genome of *P. oryzae* strains, seven cromossomes and mini-cromossomes with highly repetitive and enriched transposons most frequently at the core chromosome ends. Transposons in mini-chromosomes lack the characteristic signature for inactivation by repeat-induced point (RIP) mutation genome defenses. Thus, mini-chromosomes and core-chromosome ends are coupled as a mobile, fast-evolving effector compartment in the wheat pathogen genome, which may explain the great flexibility of the fungus in adapting to different hosts (Peng *et al.*, 2019). Wicaksono *et al.* (2017), analyzing the *P. oryzae* genetic diversity through the Repeating Element-based Polymerase Chain Reaction (rep-PCR), collected several samples of the fungus in different regions of Indonesia and observed that, although collected from very close regions, different fungus samples do not always own high genetic similarity. These results show the high capacity of the fungus to make fast and random mutates,

designating high genetic diversity. Further studies for the genetic characterization of *P. oryzae* isolates may contribute to the identification of the most frequent mutations in this genome and to the development of more efficient strategies to reduce the damage caused by blast epidemics.

CONCLUSION

The results of the present work indicate the existence of virulence diversity among *P. oryzae* isolates obtained from a single lesion on leaves of wheat. Thus, further studies with a greater number of isolates and cultivars and using molecular tools may contribute to the understanding of the genetic diversity of the *P. oryzae* population.

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