

Pyricularia pennisetigena and *P. zingibericola* from invasive grasses infect signal grass, barley and wheat¹

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ABSTRACT

Fungal species from the *Pyricularia* genus are associated with blast disease in plants from the Poaceae family, causing losses in economically important crops such as rice, oat, rye, barley, wheat and triticale. This study aimed at characterizing the pathogenicity spectrum of *P. pennisetigena* and *P. zingibericola* to signal grass, barley and wheat, as well as comparing them with those from the species *P. grisea* and *P. oryzae* pathotype *Triticum*, which occur widely in the Brazilian agroecosystem. Twenty isolates of *Pyricularia* spp. were obtained from infected leaf samples of invasive plant species from wheat fields. The isolates classification into distinct *Pyricularia* species was done using molecular phylogeny based on actin and calmodulin genes. *Pyricularia pennisetigena* and *P. zingibericola* inoculated on plant leaves, at a concentration adjusted to 10⁵ conidia mL⁻¹, were pathogenic to signal grass, barley and wheat, with varying levels of aggressiveness.

KEY-WORDS: *Pyricularia grisea*; *Pyricularia oryzae*; blast disease; invasive plant species; inoculum sources.

INTRODUCTION

The *Pyricularia* genus includes several pathogenic fungi species that are associated with blast disease in more than 50 Poaceae plants (Ou 1985). Among these species, *Pyricularia oryzae* is the most important pathogen, due to its global distribution on plants from the Poaceae family. The blast disease caused by *P. oryzae* is responsible for severe losses in crops of high economic importance such as rice (*Oryza sativa*), oat (*Avena sativa*), signal grass (*Urochloa* spp.), rye (*Secale cereal*), barley (*Hordeum vulgare*), maize (*Zea mays*) and wheat (*Triticum aestivum*) (Bailey & Eijnatten 1961, Igarashi et al. 1986, Urashima et al. 1993, Anjos et

RESUMO

Pyricularia pennisetigena e *P. zingibericola* de gramíneas invasoras infectam braquiária, cevada e trigo

Espécies de fungos do gênero *Pyricularia* estão associadas com a doença brusone, em plantas da família Poaceae, causando perdas em culturas de importância econômica como arroz, aveia, centeio, cevada, trigo e triticale. Objetivou-se caracterizar o espectro de patogenicidade de *P. pennisetigena* e *P. zingibericola* em braquiária, cevada e trigo, bem como compará-los com os das espécies *P. grisea* e *P. oryzae* patótipo *Triticum*, de ocorrência generalizada no agroecossistema brasileiro. Foram testados 20 isolados de *Pyricularia* spp. obtidos de amostras de folhas infectadas de plantas invasoras de campos de trigo. A classificação dos isolados em espécies distintas de *Pyricularia* foi efetuada utilizando-se filogenia molecular baseada nos genes actina e calmodulina. *Pyricularia pennisetigena* e *P. zingibericola* inoculados em folhas, à concentração ajustada para 10⁵ conídios mL⁻¹, foram patogênicos a braquiária, cevada e trigo, com índices variáveis de agressividade.

PALAVRAS-CHAVE: *Pyricularia grisea*; *Pyricularia oryzae*; brusone; plantas invasoras; fontes de inóculo.

al. 1996, Couch & Kohn 2002, Goulart et al. 2003, Couch et al. 2005, Marchi et al. 2005).

The adoption of the *Pyricularia oryzae* designation as the species associated with blast disease is recent. Until the early 2000s, *Pyricularia grisea* was considered the pathogen of blast disease on rice, signal grass, barley and wheat (Urashima et al. 1993, Marchi et al. 2005). A molecular phylogenetic reclassification proposed by Couch & Kohn (2002) showed that *P. grisea* was exclusively associated with blast disease on grasses from the *Digitaria* genus, whereas *P. oryzae* was associated with rice, wheat and other cultivated grasses or invasive plants (e.g. goosegrass). Based on this study, a new international classification became necessary.

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The *P. oryzae* fungus is considered a complex species composed of different subgroups distinguished by their distinct mating types, particular phylogenetic characteristics and restricted host range or pathotypes (Tosa & Chuma 2014). The representative pathotypes of *P. oryzae* and their respective range of hosts described so far are: *Oryza* pathotype, pathogenic to rice (*Oryza sativa*); *Panicum* pathotype, pathogenic to common millet (*Panicum miliaceum*); *Setaria* pathotype, pathogenic to foxtail millet (*Setaria italica*); *Triticum* pathotype, pathogenic to wheat (*Triticum aestivum*); *Avena* pathotype, pathogenic to oat (*Avena sativa*); *Eleusine* pathotype, pathogenic to finger millet (*Eleusine coracana*); and *Lolium* pathotype, pathogenic to perennial ryegrass (*Lolium perenne*) (Tosa & Chuma 2014).

The *Triticum* pathotype of *P. oryzae* is associated with wheat blast in South America, especially in Brazil (Tosa & Chuma 2014). Since it was first reported in 1985, in the Paraná State (Igarashi et al. 1986, Goulart et al. 2007), blast disease on wheat ears has become a very important disease, causing crop losses of 40-100 % (Goulart & Paiva 2000, Torres et al. 2009). Due to the lack of varieties with high levels of genetic resistance and efficient chemical control to manage the disease, the pathogen has become widely distributed in all wheat growing fields in Brazil (Maciel et al. 2013, Castroagudín et al. 2014). This disease has also been detected in wheat fields in northern Argentina, Bolivia and Paraguay (Duveiller et al. 2010), but it had never been reported outside South America until 2016. Recently, wheat blast disease was considered one of the most important quarantine diseases and a threat to the wheat crop in the USA (Duveiller et al. 2010) and in Bangladesh (Callaway 2016, Malaker et al. 2016).

In the survey of invasive Poaceae species infected with blast disease described in this study, carried out in wheat fields of the Mato Grosso do Sul and Paraná States, during the 2012 and 2013 growing seasons, an association of *P. pennisetigena* and *P. zingibericola* with several of the grass species sampled, besides *P. grisea* and *P. oryzae*, was detected. Despite the recent report in the Brazilian agroecosystem (Klaubauf et al. 2014), the occurrence of the *P. pennisetigena* and *P. zingibericola* species could be more common and older than assumed. However, the potential of *P. pennisetigena* and *P. zingibericola* as pathogens to economically

important Poaceae hosts in the Brazilian agriculture is still unknown.

Therefore, this study aimed at characterizing the pathogenicity spectrum of *P. pennisetigena* and *P. zingibericola* to signal grass, barley and wheat, as well as comparing them with those from the *P. grisea* and *P. oryzae* species, which occur widely in the Brazilian agroecosystem. The role of invasive plants as possible additional hosts of *P. oryzae* on wheat was also described.

MATERIAL AND METHODS

For the comparative study of the pathogenicity spectrum of *Pyricularia* species, 20 isolates were obtained from leaves or ears of invasive plants sampled from wheat fields in South-Central Brazil (8 isolates of *P. pennisetigena*; 3 isolates of *P. zingibericola*; 5 isolates of *P. oryzae* from invasive plants and 1 from wheat; and 3 isolates of *P. grisea*) (Table 1). The samplings of diseased plants were conducted in wheat fields in the Mato Grosso do Sul (MS), Minas Gerais (MG) and Paraná (PR) States and Brasília (DF), between May and August, during the 2012 and 2013 growing seasons (Table 1).

For fungal genomic DNA extraction, the isolates were grown on potato dextrose broth containing streptomycin (0.050 g L⁻¹) and chloramphenicol (0.050 g L⁻¹), and incubated for 7 days at 25 °C, with constant stirring at 75 rpm. The mycelium obtained from each isolate was filtered, frozen at -20 °C and lyophilized for 24 h. The genomic DNA extraction from the fungal mycelium was performed using a Genelute Plant Genomic DNA Mini Kit (Sigma-Aldrich, Brazil), following the manufacturer's instructions.

The *Pyricularia* species sampled were identified based on amplification via polymerase chain reaction (PCR), and sequencing of ACT (part of actin gene) and CAL (part of calmodulin gene) regions was performed according to Klaubauf et al. (2014). The primers used for the PCR reaction were: ACT: ACT-43F: 5'-CGTCTTCCGTAAGTGCCC-3 and ACT-322R: 5'-GCCCATACCAATCATGATAC-3; CAL: CAL-35F: 5'CTTACCGAAGAGCAAGTTTCCG -3 and CAL-607R: 5'TYTTCTGGCCATCATGGTS-3'.

The amplification program used in the Mastercycler Nexus PCR thermocycler (Eppendorf, Hamburg, Germany) included initial denaturation at 95 °C, for 5 min; followed by 35 cycles at 95 °C,

for 45 s; annealing at 58 °C for the ACT gene and at 55 °C for the CAL gene, for 1 min, with extension at 72 °C, for 1 min; and a final extension at 72 °C, for 8 min. The PCR products were sent for sequencing at the Macrogen Inc. company (Seoul, South Korea), which used the automated PE Applied Biosystems ABI-3730 sequencer. The sequences obtained were analyzed and aligned using the Geneious R 6.7.1 software (Biomatters, New Zealand). The experimental sequences of ACT and CAL genes obtained from the isolates in this study were submitted to the NCBI GenBank (Table 1).

The following DNA sequences were retrieved from the GenBank and used as references to compare with the sequences obtained experimentally: a) for the ACT gene, DQ240877 (*P. grisea*), KJ599796 (*P. oryzae* pathotype *Oryza*, isolated from rice), KM485189 (*P. oryzae* pathotype *Triticum*, isolated from wheat), KM485226 (*P. pennisetigena*) and AB274440 (*P. zingibericola*); b) for the CAL gen, KM485254 (*P. grisea*), AF104986 (*P. oryzae* pathotype *Oryza*, isolated from rice), KM485260

(*P. oryzae* pathotype *Triticum*, isolated from wheat), KM485292 (*P. pennisetigena*) and KM485297 (*P. zingibericola*).

Prior to the phylogenetic analysis, the JModelTest version 2.1.7 software (Darriba et al. 2012) was used to determine the nucleotide substitution model that best fitted the data. The model adopted was K80 + invariable sites. The phylogenetic analysis was performed based on the actin and calmodulin gene sequences aligned and concatenated, based on the maximum likelihood method (ML), using the PhyML algorithm and heuristic searches (Guindon & Gascuel 2003) implemented in the Geneious R 6.7.1 software (Biomatters, New Zealand). The support for the internode branches of the maximum likelihood (ML) tree was tested by bootstrapping the data with 10,000 resamplings.

The isolates of *Pyricularia* spp. were grown on Petri dishes containing oatmeal agar medium and kept under photoperiods of 12 h and at a temperature of 25 °C, for 5 days. Subsequently, 5 mm discs containing mycelium and conidia of *Pyricularia* spp.

Table 1. Isolates and their respective *Pyricularia* species obtained from grass plants invasive to wheat fields in 2012 and 2013, as well as their accession number for the actin (ACT) and calmodulin (CAL) gene sequences deposited at the NCBI GenBank.

Species, isolate	Host	Location	Accession number to the NCBI GenBank	
			ACT	CAL
<i>Pyricularia zingibericola</i> (Pz)				
12.0.149	Signal grass (<i>Urochloa</i> spp.)	Amambaí, MS	KX524119	KX524100
12.0.212	Guinea grass (<i>Panicum maximum</i>)	Aral Moreira, MS	KX524120	KX524101
12.0.595i	Weeping finger grass (<i>Chloris distichophylla</i>)	Londrina, PR	KX524121	KX524102
<i>Pyricularia grisea</i> (Pg)				
12.0.264	Crabgrass (<i>Digitaria sanguinalis</i>)	Aral Moreira, MS	KX524130	KX524111
13.0.002i	<i>D. sanguinalis</i>	Londrina, PR	KX524129	KX524110
13.0.020i	<i>Urochloa</i> spp.	Londrina, PR	KX524131	KX524112
<i>Pyricularia oryzae</i> pathotype <i>Triticum</i> (Po-it) from invasive Poaceae and wheat				
12.0.222	<i>P. maximum</i>	Aral Moreira, MS	KX524118	KX524099
13.0.033i	Star grass (<i>Cynodon</i> spp.)	Londrina, PR	KX524113	KX524094
13.0.017i	Wire grass (<i>Elionurus candidus</i>)	Londrina, PR	KX524114	KX524095
13.0.029i	Natal grass (<i>Rhynchelytrum repens</i>)	Londrina, PR	KX524117	KX524098
12.0.005	Undefined Poaceae species	Brasília, DF	KX524115	KX524096
12.1.005	<i>Triticum aestivum</i>	Patrocínio, MG	KX524116	KX524097
<i>Pyricularia pennisetigena</i> (Pp)				
12.0.002i	<i>Urochloa</i> spp.	Londrina, PR	KX524128	KX524109
12.0.100	Southern sandbur (<i>Cenchrus echinatus</i>)	Amambaí, MS	KU963214	KU963216
12.0.324	<i>C. echinatus</i>	Aral Moreira, MS	KX524127	KX524108
12.0.402	<i>C. echinatus</i>	Aral Moreira, MS	KX524125	KX524106
13.0.012i	<i>C. echinatus</i>	Londrina, PR	KX524123	KX524104
13.0.024i	<i>C. echinatus</i>	Londrina, PR	KX524122	KX524103
13.0.001i	<i>C. echinatus</i>	Londrina, PR	KX524124	KX524105
12.0.358	<i>P. maximum</i>	Aral Moreira, MS	KX524126	KX524107

were transferred to ten other Petri dishes with oatmeal agar medium, aiming at the production of fungal inoculum. The fungal colonies were kept for 15 days under the same incubation conditions (Maciel et al. 2013).

For inoculum preparation, the mycelium was scraped with the help of a sterile spatula and the fungal colonies were washed with distilled water plus the spreader-sticker Tween 80 (2 drops L⁻¹). The concentration of conidial suspensions was determined in a Neubauer chamber, adjusting it to 10⁵ conidia mL⁻¹ for inoculation (Maciel et al. 2013). The conidial suspension volume for the inoculation of young plants was 50 mL, for a total of 15 pots (five for each host).

The pathogenicity spectrum of *Pyricularia* spp. isolates was determined by the inoculation on signal grass (*U. brizantha*) cv. Piatã, barley (*H. vulgare*) cv. BRS Korbel and wheat (*T. aestivum*) cv. Anahuac 75. These plants were sown in two seasons under greenhouse conditions, firstly on June 8 and secondly on June 18, in 2015. About 10 to 12 seeds were sown in 300 mL plastic pots containing Tropstrato HT Hortaliças plant substrate (Vida Verde, Campinas, SP). Prior to the inoculation, the plants were kept in a greenhouse at temperatures ranging 25-30 °C, with a 12 h regimen of light and daily irrigation. A fertilization (2.6 g pot⁻¹) with the N-P-K formula (10-10-10) was performed 10 days after sowing.

Two weeks after emergence, when the plants tested were at the phenological stage of four leaves, thinning was performed, so that only six plants per pot were left. Each experimental plot consisted of one pot with six plants, totaling 30 plants per host. The inoculation was performed subsequently. With the assistance of an air pressure pump spray, the conidial suspension was sprayed onto each plant, until the leaf surface (adaxial and abaxial) was completely wet. After inoculation, the plants were incubated for 24 h in the dark, in a phytotron chamber, at a temperature of 24 °C, under nebulization. The experimental plots were then transferred to an acclimatized greenhouse with automatic control of air temperature (25 °C) and relative humidity (90 %), and with 12 h of natural sunlight. The pots were irrigated daily.

The experimental design adopted for the three host species tested was completely randomized, with five replicates. For each host species, the

experimental treatments were represented by 20 isolates and one check (negative control). This experiment was replicated once and conducted in two seasons.

The plants were assessed 7 days after inoculation. Five leaves with blast symptoms per pot were photographed using an Alpha 5000 Sony digital camera, with 16-50 mm interchangeable lens, attached to a monopod, at a fixed distance of 20 cm. The pathogenicity spectrum of the isolates and the levels of aggressiveness were determined based on the percentage of infected leaf area of plants with the symptoms of leaf blast. The infected leaf area was determined using the Assess Image Analysis Software for Plant Disease Quantification version 2.0 of the American Phytopathological Society (Lamari, Department of Plant Science, University of Manitoba, Winnipeg, Manitoba, Canada).

Analysis of variance was performed using the SAS software version 9.1 (SAS Institute Inc., Cary, North Carolina, US). The PROC MIXED procedure was used to analyze the treatment effect, and the Scott-Knott test ($p < 0.05$) was used for comparison between the groups of isolates.

RESULTS AND DISCUSSION

In general, this study resulted in the phylogenetic identification and in the elucidation of the pathogenicity spectrum of *P. pennisetigena* and *P. zingibericola*, identified by surveying fungi associated with blast disease in invasive plants from wheat fields in South-Central Brazil.

New taxonomic alterations have been recently proposed, such as the definition of the new Pyriculariaceae family, designation of new genera, introduction of new species and the allocation of species previously described in the *Magnaporthe* genus to the *Pyricularia* genus. The *Magnaporthe* genus, previously used to designate the sexual phases of *P. grisea* and *P. oryzae* (i.e. *M. grisea* and *M. oryzae*), has been less used. Furthermore, there was the inclusion of new species of *Pyricularia* previously not described as pathogens of Poaceae, such as *P. pennisetigena* and *P. zingibericola* (Klaubauf et al. 2014).

In the phylogenetic analysis, branch I, with 99.9 bootstrap support, grouped the isolates of *Pyricularia oryzae* obtained from invasive plants [13.0.033i (from *Cynodon* spp.), 13.0.017i (from

Elionurus candidus), 12.0.029i (from *Rhynchelytrum repens*), 12.0.005 (from undefined Poaceae) and the isolate 12.0.222 (from *Panicum maximum*), the isolate of *P. oryzae* from wheat (12.1.005) and two other isolates of *P. oryzae*, whose sequences were retrieved from the GenBank, with one being isolated from wheat (*Triticum* pathotype) and the other from rice (*Oryza* pathotype).

In fact, the latest study elucidating the phylogenetic relationship between several *Pyricularia* species has indicated that isolates obtained from rice and wheat were grouped into the clade *P. oryzae* (Klaubauf et al. 2014). These isolates, although specific to those hosts, potentially have a common evolutionary origin (Tosa & Chuma 2014). Our observations indicated that *P. oryzae* pathotype *Triticum* could also be associated with other Poaceae plant species invasive to wheat fields (Figure 1).

Branch II, with 88.1 bootstrap support, grouped the isolates of *P. zingibericola* obtained from invasive plants [12.0.149 (from *Urochloa* spp.), 12.0.212 (from *Panicum maximum*) and 12.0.595i (from *Chloris distichophylla*)] and the isolate

P. zingibericola, whose sequences were obtained from the GenBank. Only *Zingiber officinale* had been reported as a host of *P. zingibericola*, in the Réunion Island (Klaubauf et al. 2014), and, so far, there have been no reports of this pathogen infecting invasive plant species in Brazil.

Branch III, with 100.0 bootstrap support, grouped the isolates of *P. pennisetigena* (13.0.024i; 13.0.012i; 13.0.001i; 12.0.402; 12.0.358; 12.0.324; 12.0.002i; 12.0.100) and the isolate whose DNA sequences were obtained from the GenBank. The *P. pennisetigena* group was found in the invasive plant species *Urochloa* spp., *Cenchrus echinatus* and *Panicum maximum*. The Poaceae *C. echinatus* had already been described by Klaubauf et al. (2014) as a host of *P. pennisetigena*, as well as *C. ciliaris*, *Echinochloa colona* and pearl millet (*Pennisetum glaucum*).

Branch IV grouped the species *P. grisea*, which included the isolates 13.0.002i, 12.0.264 (from *D. sanguinalis*) and 13.0.020i (from *Urochloa* spp.), with 100.0 bootstrap support, all presenting identical sequences.

Significant differences were detected among *Pyricularia* species according to the blast severity observed in signal grass cv. Piatã (Table 2; Figure 2).

Pyricularia zingibericola was the most aggressive species to signal grass (Figure 3A), causing, on average, 24.1 % of infected leaf area. Among the other species, in decreasing order of aggressiveness, *P. oryzae* from invasive plants and wheat caused an average of 14.8 % disease severity, differing from *P. grisea* (8.8 %) and *P. pennisetigena* (5.2 % of infected leaf area).

One of the factors that could explain the higher aggressiveness of *P. zingibericola* to signal grass is the origin of a few isolates that came from this same host, thereby explaining its better adaptability to this plant species.

All four species of *Pyricularia* tested were pathogenic to barley cv. BRS Korbel (Figures 2B and 3B; Table 2), but *P. zingibericola* was the most aggressive one (mean of 18.7 % of diseased leaf area). There was no significant difference among the groups of isolates from *P. pennisetigena*, *P. grisea* and *P. oryzae*, regarding the disease severity on young barley plants, with an average leaf blast severity corresponding to 12.6 %, 12.0 % and 11.4 %, respectively. Indeed, innumerable barley cultivars have been considered susceptible to pathogens of the *Pyricularia* genus, especially *P. oryzae* (Marangoni et

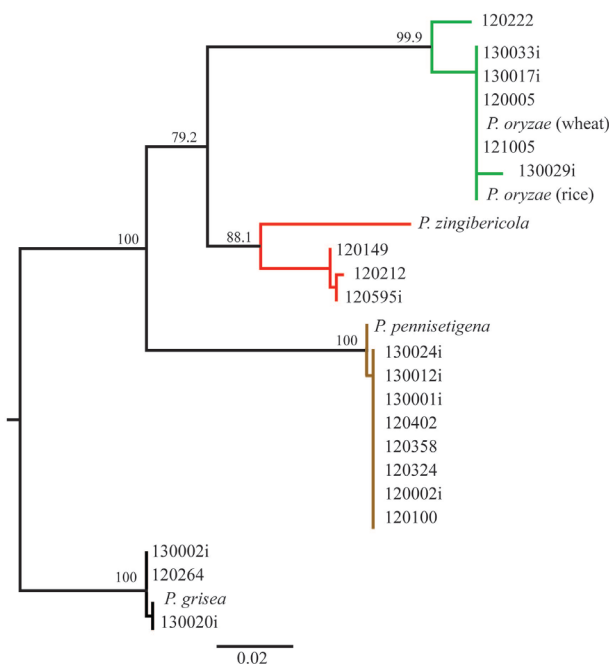


Figure 1. Reconstruction of the phylogenetic relationship among four species of the *Pyricularia* genus, based on sequences of the actin (ACT) and calmodulin (CAL) genes concatenated. Maximum likelihood (ML) implemented in the PhyML algorithm was used. The internode support for the branches was tested by 10,000 bootstrap resamplings of the data.

al. 2013). However, there is variation in the level of blast resistance among genotypes, with some having high levels of resistance to *P. oryzae* (Marangoni et al. 2013).

For wheat cv. Anahuac 75, *P. pennisetigena* was the most aggressive species to young plants, with an average of 26.6 % of diseased leaf area. On the other hand, the group of *P. oryzae* and *P. zingibericola* caused similar damage, with an average disease severity of 20.0 % and 18.3 % of infected leaf area, respectively. *Pyricularia grisea* was not pathogenic to wheat cv. Anahuac 75 (Figures 2 and 3C; Table 2).

Possibly, this is the first report suggesting that *P. pennisetigena* and *P. zingibericola* may cause leaf blast on signal grass, barley and wheat. However, from an evolutionary point of view, it is unknown how recent the adaptation of the two *Pyricularia* species to these distinct hosts is. Yet, from an ecological point of view, it is still unknown the relative importance of *P. pennisetigena* and *P. zingibericola*, in relation to the other species associated with blast disease already described for signal grass, barley and wheat, in the Brazilian agroecosystem.

Finally, this study has also shown that the *P. oryzae* pathotype *Triticum* has a host range wider than wheat, barley, rye and oat (Igarashi et al. 1986, Urashima et al. 1993, Anjos et al. 1996, Goulart et al. 2003).

In particular, considering the host range of the *P. oryzae* pathotype *Triticum*, it is possible to highlight the importance of invasive plants to wheat fields as an initial inoculum source of wheat pathogens, thereby confirming the observations by Urashima et al. (1993). Although ear infection is the most destructive form of wheat blast disease (Goulart & Paiva 2000, Torres et al. 2009), little is known about the origins of the initial inoculum source. It is plausible that the initial inoculum of the pathogen produced in the leaves of invasive grasses has an important epidemiological role in the incidence of wheat blast on ears.

Among the invasive plant species, whether native or not, *Urochloa* spp., *Panicum* spp. and *Cynodon* spp. stood out as important additional hosts, due to their wide geographical distribution as cultivated forages in Brazil (Zimmer et al. 2012). *Urochloa* spp., the most cultivated forage in Brazil, was the host of *P. grisea*, *P. pennisetigena* and *P. zingibericola*, and presented high susceptibility to the *P. oryzae* pathotype *Triticum*. Therefore, extensive grass pasture fields cultivated with plants of the *Urochloa* genus, especially in the Brazilian Savannah, would represent a potential permanent source of initial inoculum between the wheat growing seasons, thereby keeping the inoculum of the *P. oryzae* pathotype *Triticum* active.

Table 2. Variance analysis table for testing the effects of the experiment, species and pathogen isolates on the aggressiveness of *Pyricularia* spp. to signal grass, barley and wheat.

Host	Source of variation	df	SQ	F	P
<i>U. brizantha</i> cv. Piatã	Experiment	1	163.06	2.87	0.0920 ^{ns}
	Isolate (pathogen species)	16	601.04	10.57	< 0.001 ^{***}
	Experiment * species	4	45.41	0.80	0.5274 ^{ns}
	Error	184	56.85		
	Species	4	2,514.65	4.18	0.0166*
	Error for the species factor	16	601.04		
Barley cv. BRS Korbel	Experiment	1	166.35	3.18	0.0764 ^{ns}
	Isolate (pathogen species)	16	218.61	4.17	< 0.001 ^{***}
	Experiment * species	4	75.27	1.44	0.2235 ^{ns}
	Error	184	52.38		
	Species	4	694.46	3.18	0.0423*
	Error for the species factor	16	218.61		
Wheat cv. Anahuac 75	Experiment	1	30.15	0.37	0.5435 ^{ns}
	Isolate (pathogen species)	16	233.43	2.87	< 0.001 ^{***}
	Experiment * species	4	137.38	1.69	0.1546 ^{ns}
	Error	184	81.40		
	Species	4	4,761.41	20.40	< 0.001 ^{***}
	Error for the species factor	16	233.43		

***, * and ^{ns} Significant at $p \leq 0.001$, $p \leq 0.05$ and non-significant, respectively, by the F test.

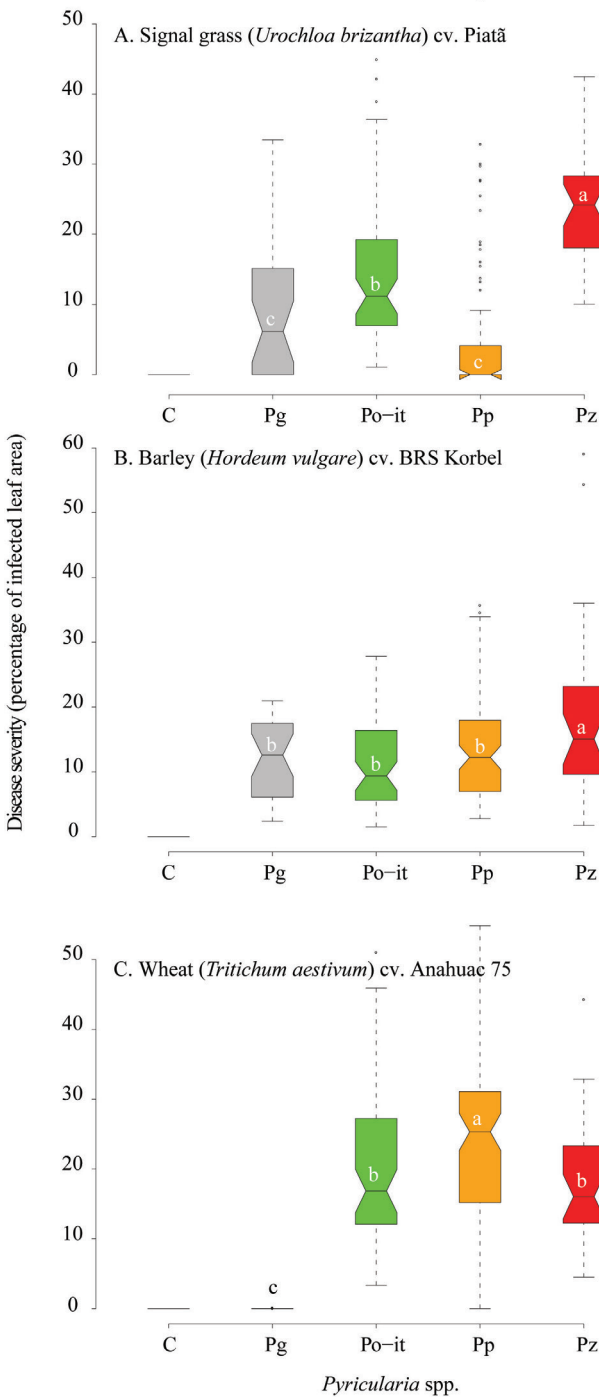


Figure 2. Boxplots representing the pathogenicity of *Pyricularia* species to signal grass (A), barley (B) and wheat (C). Pg = *Pyricularia grisea* (n = 3); Po-it = *Pyricularia oryzae* pathotype *Triticum* (n = 6); Pp = *Pyricularia pennisetigena* (n = 8); Pz = *Pyricularia zingibericola* (n = 3); C (control), with n = number of isolates per species. Each boxplot represents the mean of ten repetitions, combining the two replicas of the experiment. Means followed by the same letter do not differ from each other (p < 0.05), by the Scott-Knott test.

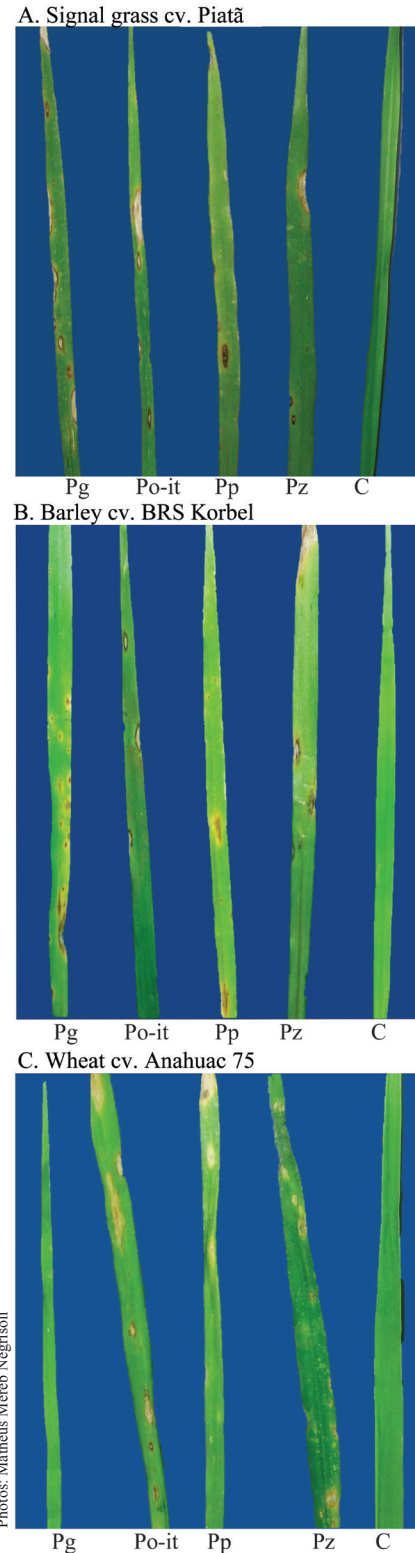


Figure 3. Photographs of leaf blast symptoms caused by different species of *Pyricularia* on the leaves of signal grass cv. Piatã (A), barley cv. BRS Korbel (B) and wheat cv. Anahuac 75 (C). Pg = *P. grisea*; Po-it = *P. oryzae* pathotype *Triticum*; Pp = *P. pennisetigena*; Pz = *P. zingibericola*; C = control.

CONCLUSIONS

1. *Pyricularia zingibericola* and *P. pennisetigena* are pathogenic to signal grass, barley and wheat, with varying levels of aggressiveness between the species. *Pyricularia zingibericola* is the most aggressive species to signal grass and barley.
2. For young wheat plants, *P. pennisetigena* is the most aggressive species, followed by the *P. oryzae* pathotype *Triticum*. On the other hand, *P. grisea* from *D. sanguinalis* or from *Urochloa* spp. do not infect wheat.
3. The phylogenetic analysis of the concatenated ACT and CAL regions sustains the genetic differences among *Pyricularia zingibericola*, *P. pennisetigena*, the *P. oryzae* pathotype *Triticum* and *P. grisea*.
4. *Urochloa* spp. represents a permanent source of initial inoculum of the *P. oryzae* pathotype *Triticum* between wheat growing seasons.

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