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Pyricularia pennisetigena and Pyricularia oryzae isolates from Paraguay's wheat-growing regions and the impact on wheat

Cinthia Carolina Cazal-Martínez [a,b,c,d,*], Yessica Magaliz Reyes-Caballero [e,f], Alice Rocio Chávez [e], Pastor Enmanuel Pérez-Estigarribia [g], Man Mohan Kohli [d,e], Alcides Rojas [a], Andrea Alejandra Arrua [a,c,d], Juliana Moura-Mendes [a,d], Ramón Souza-Perera [h], José Juan Zúñiga Agilar [d,i], Emile Gluck-Thaler [j,k,l], Horacio Lopez-Nicora [d,m], Julio Cesar Masaru Iehisa [d,n]

[a] Centro Multidisciplinario de Investigaciones Tecnológicas, Universidad Nacional de Asunción, San Lorenzo, 111421, Paraguay
[b] Programa de Doctorado de Ingeniería Agraria, Alimentaria, Forestal y de Desarrollo Rural Sostenible. Universidad de Córdoba, Córdoba, España
[c] Departamento de Biotecnología. Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Asunción, San Lorenzo, 111421, Paraguay
[d] Grupo de Investigación en Mejora Genética Vegetal para una Agricultura Sostenible, Paraguay
[e] Departamento de Investigación, Cámara Paraguaya de exportadores y comercilzadores de cereales y oleaginosas, Asunción, 1207, Paraguay
[f] Instituto Paraguayo de Tecnología Agraria, Centro de Investigaciones Capitán Miranda. Capitán Miranda, 6990, Paraguay
[g] Universidad Sudamericana, Facultad de Ciencias de la Salud, Pedro Juan Caballero, 130145, Paraguay
[h] Unidad de Bioquímica y Biología Molecular en Plantas, Centro de Investigación Científica de Yucatán, A.C., Yucatan, 97205, Mexico
[i] Universidad Tecnológica del Usumacinta, Tabasco, 86980, Mexico
[j] Laboratory of Evolutionary Genetics, University of Neuchâtel, Neuchâtel, 2000, Switzerland
[k] Department of Plant Pathology, University of Wisconsin-Madison, Madison, USA
[l] Wisconsin Institute for Discovery, Madison, USA
[m] Department of Plant Pathology, The Ohio State University, Columbus, USA
[n] Departamento de Biotecnología, Facultad de Ciencias Químicas, Universidad Nacional de Asunción, San Lorenzo, Paraguay

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ABSTRACT

The *Pyricularia* genus includes species causing blast disease in monocots, posing significant challenges for disease management due to their ability to infect multiple hosts. This study aimed to identify the pathogenicity and species identity of *Pyricularia* isolates from 11 plant species in wheat-growing regions of Paraguay and assess their capacity to infect wheat. Twenty-four monosporic isolates were analyzed based on macroscopic and microscopic and phylogenetic characteristics. Three phylogenetic clades corresponding to *P. oryzae, P. grisea*, and *P. pennisetigena* were identified through five barcoding genes. For the first time, wheat blast was reported in San Pedro Department, and blast disease was observed in weeds in Cordillera and Central Departments. In greenhouse trials, *P. oryzae* isolates from wheat successfully infected both susceptible and resistant wheat cultivars, whereas isolates from non-wheat hosts did not elicit symptoms. Notably, *P. pennisetigena* isolates derived from *Cenchrus echinatus* were capable of infecting wheat spikes, producing typical blast symptoms, highlighting the potential for cross-species pathogen transmission. This finding suggests *P. pennisetigena* may pose an emerging threat to wheat in Paraguay, as its primary host is prevalent near wheat fields. These results highlight the critical importance of integrated disease management strategies, particularly the identification of inoculum sources, to mitigate cross-species pathogen transmission. This approach aligns with the One Health paradigm by addressing interconnected risks to plant health, food security, and environmental sustainability.

Introduction

Fungal species from the genus *Pyricularia* cause blast disease on several monocotyledonous plant species (Bussaban et al., 2005). Among the most important of these *Pyricularia* spp. is Pyricularia *oryzae* Cavara = *Magnaporthe oryzae* B.C. Couch (synonym: *Magnaporthe oryzae*).

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* Corresponding author.
E-mail address: ccazal@rec.una.py (C.C. Cazal-Martínez).

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P. oryzae is currently subdivided into several phylogenetic lineages that are each preferentially associated with a specific host plant genus, and together, these lineages are responsible for severe losses in agricultural crops such as rice, wheat, barley, millet, and oats worldwide (Couch et al. 2005; Gladieux et al. 2018; Murakami et al. 2003; Qi et al. 2019; Takabayashi et al. 2002).

The complete set of lineages to which different *Pyricularia* species and isolates belong has not been fully resolved, although much progress is being made with the advent of whole genome classification methods (Gladieux et al. 2018). Determining host specificity among different lineages is particularly challenging because field isolates of *Pyricularia* are often considered to be specific to the plant species from which they were isolated; nonetheless, in studies using artificial inoculations, many isolates were able to cross-infect other host species (Choi et al. 2013; Cruz 2008; Hyon et al. 2012; Tosa et al. 2004). For instance, *P. grisea* was previously believed to be the causal agent of blast disease in various monocotyledons, including rice and other grasses. However, genetic analyses and cross-inoculation studies have revealed that *Pyricularia* isolates from rice and wheat specifically belong to *P. oryzae*, distinguishing it from *P. grisea*, which primarily infects other monocotyledons and associated weeds in both cropping systems (Couch and Kohn 2002; Inoue et al. 2017).

While wheat blast disease is now attributed primarily to *P. oryzae* isolates from the *Triticum* lineage (Gladieux et al. 2018), *P. oryzae* isolates from other host-associated lineages (e.g., *Avena* and *Lolium* lineages) have also demonstrated the ability to infect wheat under artificial infection assays (Farman et al. 2017; Oh et al. 2002; Reges et al. 2019). Similarly, isolates from the *Triticum* lineages cause blast disease on barley (*Hordeum vulgare*), oats (*Avena sativa*), rye (*Secale cereale*), signal grass (*Urochloa brizantha,* ex *Braquiria brizanta*), and >10 other grass species (Castroagudín et al. 2016; Urashima et al. 1993). Furthermore, other *Pyricularia* species, such as *P. pennisetigena* and *P. zingibericola* also cause blast symptoms on wheat, sometimes more aggressively compared to isolates from the *Triticum* lineage on younger wheat plants under artificial infection conditions (Reges et al. 2016).

In South America, wheat blast was first reported in Paraná State, Brazil in 1985 (Igarashi et al. 1986) and since then has become an increasingly important disease in the tropical and subtropical wheat regions across the entire continent (Alberione et al. 2008; Dos Anjos et al. 1996; Barea and Toledo 1996; Callaway 2016; Goulart et al. 1990; Tembo et al. 2020; Urashima et al. 2004). In the lowlands of Bolivia, in the Central and South Regions of Brazil, and in Paraguay this pathogen can cause yield losses of up to 100 % (Ceresini et al. 2018; Kohli et al. 2011; Quintana de Viedma 2010). Though initially confined to South America, wheat blast disease has recently spread to Bangladesh in South Asia in 2016 (Callaway 2016; Islam et al. 2016; Malaker et al. 2016) and to Zambia in Africa in 2018 (Tembo et al. 2020). In Paraguay, wheat blast disease was first reported in 2002 (Quintana De Viedma et al. 2004), and the first major outbreak of the disease occurred in 2005 (Viedma 2005).

In Paraguay, wheat blast pathogen infections observed in the field may be caused by different species and lineages of *Pyricularia* that likely originate from different environmental reservoirs (Chávez et al. 2022). The species *P. pennisetigena*, reported by Klaubauf et al. (2014) to infect several grass species, was isolated from invasive Poaceae species in Paraguay (Cazal-Martínez et al. 2021) and in the South Region of Brazil (Reges et al. 2018). *P. pennisetigena* is considered a potential threat to crops of agricultural importance such as forage grasses like *Pennisetum glaucum* (pearl millet) and *Urochloa brizantha syn. Brachiaria brizantha* (palisadegrass) (Klaubauf et al. 2014; Reges et al. 2016). Similarly, it is possible that the initial inoculum of *P. oryzae* in wheat may have originated from other plant species including grasses in the genus *Digitaria* (crabgrass) which are commonly observed weeds present in wheat fields (Durante et al. 2018).

In wheat-*Pyricularia* species systems, the ability of *Pyricularia* species to infect alternative hosts underscores the importance of weedy grass surrounding commercial wheat fields as reservoirs of pathogen inoculum (Chávez et al. 2022). A recent study mentions that the *P. oryzae* population exhibits unusually high nucleotide diversity among individuals and is linked to the introgression of additional sources. When analyzing sequences near two markers (CH7BAC7 and MPG1), it was observed that they matched alleles found in the lineages infecting *Urochloa* (PoU) and *Eleusine* (PoE), respectively. Moreover, both loci displayed alleles closely related to those of *Oryza* (PoO), *Setaria* (PoS), and *Panicum* (PoP) (Rahnama et al. 2023). Therefore, it is essential to monitor the environments in which interactions between different hosts take place, as these areas are conducive to recombination events between distinct populations.

The One Health concept provides a transdisciplinary framework for disease surveillance, prediction, and management, traditionally focused on zoonotic diseases. By integrating insights into pathogen-host interactions, habitat connectivity, and wildlife reservoirs, it facilitates comprehensive epidemic prevention and control. This approach is equally important for managing plant diseases, which significantly impact food security by affecting food availability, quality, and safety. Incorporating plant health within the One Health paradigm highlights the interconnectedness of plant and human health, advancing holistic and sustainable solutions (Singh et al. 2023). The diversity of *Pyricularia* species in Paraguay remains poorly understood. To address this knowledge gap, this study aims to investigate the phylogenetic relationships of 24 *Pyricularia* isolates collected from diverse grass hosts in Paraguay, describe the geographic distribution of these *Pyricularia* species and assessing their pathogenicity on wheat.

Materials and methods

Fungal isolates

Twenty-four monosporic isolates of the genus *Pyricularia* of initially unknown species from different plant hosts were obtained from the “Colección Nacional de Aislados de P*yricularia*'' maintained at the “Instituto Paraguayo de Tecnología Agraria (IPTA)”, Caacupé, and in the “Colección de Cultivos de Microorganismos de la Universidad Nacional de Asunción, Paraguay” (Registration to FELACC, SI-70). These obtained isolates were morphologically identified as belonging to species *P. oryzae* with nine isolates from *Triticum aestivum*, two from *Avena strigosa*, and one isolated each from *Lolium multiflorum, Eleusine indica, Bromus catharticus, Stenothaphrum secundatum, Rynchelyntrum roseum*. Additionally, a set of six isolates of *P. grisea* were identified, distributed as follows: one isolated each from *Digitaria* sp., *Digitaria insularis* and *Urochloa* sp. *(syn. Brachiaria* sp*).*, three isolates from *Digitaria horizontalis*. Moreover, two isolates of *P. pennisetigena,* both isolated from *Cenchrus echinatus*. Depending on the symptoms observed under field conditions, in each instance, the fungus was originally isolated from naturally infected leaves, inflorescences, and/or seeds displaying characteristic symptoms of blast disease (Table 1).

Regarding the geographical location of the collected isolates, they were distributed in five departments: Central (specifically in the city of San Lorenzo [CS]), Cordillera (CC = Caacupe), San Pedro (SP = Guayaibi), Canindeyú (CY = Yhovy), Alto Paraná (AP), and Itapúa (in Tomás Romero Pereira [IT], Pirapo [IP], and Capitán Miranda [IC]) (Fig. 1). The special visualization of isolates was implemented in software R. The map shapefile was obtained with the map_data function, from the ggplot2 package, {ggplot2} can load map data provided by the {maps} package (Kahle and Wickham 2013).

Morphological description

First, the identification of the isolates at the genus level was carried out by macroscopic and microscopic examinations. To examine macroscopic features, a representative sample of 21 isolates were randomly selected, based on the criterion of origin (host) and the similar

Table 1

Pyricularia species used in this study.

					NCBI GenBank accession no.[1]				
Species	**Isolate ID[2]**	**Location**	**Year of collection**	**Host**	**ITS**	**RPB1**	**LSU**	**ACT**	**CAL**
Pyricularia oryzae	ATae039	Alto Parana	2014	*Triticum aestivum*	MN947534.1	MN984725	MN944843	MN917192	MN984702
Pyricularia oryzae	BrTae	Canindeyú, Yhovy	2014	*Triticum aestivum*	MN947538	MN984728	MN944847	-	MN984706
Pyricularia oryzae	CTae005	Canindeyú, Yhovy	2013	*Triticum aestivum*	MN947527	MN984716	MN944835	MN917183	MN984694
Pyricularia oryzae	YTae025	Canindeyú, Yhovy	2014	*Triticum aestivum*	MN947535.1	MN984726	MN944844	MN917193	MN984703
Pyricularia oryzae	YTae028	Canindeyú, Yhovy	2014	*Triticum aestivum*	MN947533	MN984724	MN944842	MN917191	MN984701
Pyricularia oryzae	YEih113	Canindeyú, Yhovy	2018	*Eleusine indica*	MN947525	MN984714	MN944833	MN917181	MN984692
Pyricularia oryzae	SLSsh051	Central, San Lorenzo	2014	*Stenothaphrum secundatum*	MN947536	-	MN944845	MN917194	MN984704
Pyricularia oryzae	IRrh54	Cordillera, Caacupe	2015	*Rynchelyntrum roseum*	MN947520	MN984709	MN944828	MN917176	MN984687
Pyricularia oryzae	IAsh016	Cordillera, Caacupe	2014	*Avena strigosa*	-	MN984720	-	MN917187	MN984697
Pyricularia oryzae	IAsh017	Cordillera, Caacupe	2014	*Avena strigosa*	MN947531	MN984721	MN944839	MN917188	MN984698
Pyricularia oryzae	CTae009	Itapúa, Capitan Miranda	2013	*Triticum aestivum*	MN947529	MN984718	MN944837	MN917185	MN339433
Pyricularia oryzae	CBch004	Itapúa, Capitan Miranda	2013	*Bromus catharticus*	MN947519	MN984708	MN944827	MN917175	MN984686
Pyricularia oryzae	CLmi007	Itapúa, Capitan Miranda	2012	*Lolium multiflorum*	MN947528	MN984717	MN944836	MN917184	MN984695
Pyricularia oryzae	ITTae101	Itapua, Tomas Romero Pereira	2014	*Triticum aestivum*	MN947523	MN984712	MN944831	MN917179	MN984690
Pyricularia oryzae	GTae019	San Pedro, Guayaibi	2014	*Triticum aestivum*	MN947532.1	MN984722	MN944840	MN917189	MN984699
Pyricularia oryzae	GTae021	San Pedro, Guayaibi	2014	*Triticum aestivum*	-	MN984723	MN944841	MN917190	MN984700
Pyricularia grisea	ITDsph107	Itapúa, Pirapo	2018	*Digitaria sp.*	MN947540	MN984729	-	-	-
Pyricularia grisea	YDih056	Canindeyú, Yhovy	2013	*Digitaria insularis*	MN947522	MN984711	MN944830	MN917178	MN984689
Pyricularia grisea	YDhh012	Canindeyú, Yhovy	2013	*Digitaria horizontalis*	MN947530	MN984719	MN944838	MN917186	MN984696
Pyricularia grisea	YDhh014	Itapúa, Capitan Miranda	2013	*Digitaria horizontalis*	MN947539	-	MN944848	-	MN984707
Pyricularia grisea	YDhh105	Canindeyú, Yhovy	2018	*Digitaria horizontalis*	MN947524	MN984713	MN944832	MN917180	MN984691
Pyricularia grisea	YBsph44	Canindeyú, Yhovy	2014	*Urochloa (syn. Brachiaria).*	MN947537	MN984727	MN944846	MN917195	MN984705
Pyricularia pennisetigena	YCeh55	Canindeyú, Yhovy	2015	*Cenchrus echinatus*	MN947521	MN984710	MN944829	MN917177	MN984688
Pyricularia pennisetigena	ITCeh117	Itapúa, Capitan Miranda	2018	*Cenchrus echinatus*	MN947526	MN984715	MN944834	MN917182	MN984693

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[1] **ITS**: internal transcribed spacers and intervening 5.8S nrDNA; **LSU**: partial large subunit (28S) of the nrRNA gene operon; **RPB1**: partial RNA polymerase II largest subunit gene; **ACT**: partial actin gene; **CAL**: partial CAL gene.

[2] Cells in grey indicate the isolates used for pathogenicity testing on wheat.

morphological characteristics observed in preliminary morphological identification assays. Isolates were grown and phenotype was examined on oatmeal agar (OA) prepared as previously described by Antón (2008), and potato dextrose agar (PDA). All media contained 1 % gentamicin. For this assay, a piece of filter paper previously colonized with each isolate was transferred to the center of a Petri plate containing the above media. Plates were incubated at 23–25 °C under a regime of 12 h dark/12 h UV light and examined after 10 days using the taxonomic key provided by Klaubauf et al. (2014). Microscopic details were observed and measured with the software ScopeImage v9.0 associated with a Labomed® model CxL binocular microscope, bulb rating: LED 3.6 V, 1 W, 50/60 Hz frequency where they were scaled according to the

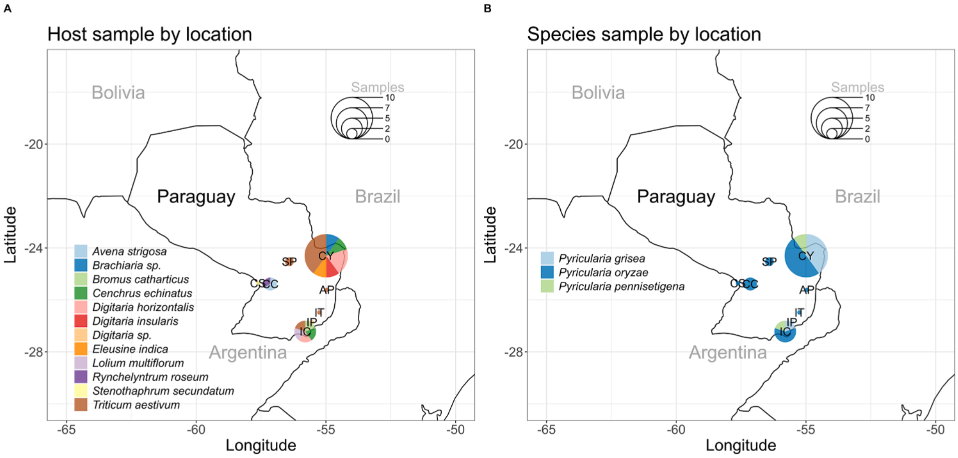

Fig. 1. Map of Paraguay with isolates by (A) plant host of origin and (B) *Pyricularia* species. Size of circles indicate number of samples. Acronyms indicate specific geographic locations (CS = Central Department, city of San Lorenzo. CC = Coordillera, Caacupe. SP = San Pedro, Guayaibi. CY= Canindeyú, Yhovy. AP = Alto Paraná. IT = Itapúa, Tomás Romero Pereira. IP = Itapúa, Pirapo. IC = Itapúa, Capitan Miranda).

graduation of the lenses as indicated in the software manual (Labomed® 2009; ScopeImage V9.3.2 2016). Microscopic preparations were made from colonies on OA medium, obtained from three plates. Measurements were taken on 10 conidia in triplicate.

DNA extraction, amplification, and sequencing

Fragments of five DNA barcode loci were amplified for the 24 isolates according to the protocol presented by Klaubauf et al. (2014): RPB1 (RNA polymerase II largest subunit), ITS (Internal transcribed spacer regions and intervening 5.8S nuclear ribosomal RNA), LSU, (28S large subunit rRNA); ACT (Actin), and CAL (Calmodulin). Briefly, fungal isolates preserved on filter paper were transferred to Petri dishes with OA medium and incubated at 23 ± 5 °C for 7 days. Subsequently, five plugs were collected and placed in potato dextrose broth, incubated at 25 ± 5 °C under constant fluorescent light, and shaken at 130 rpm for 5 days. Mycelia of each isolate were then filtered, weighed, frozen in liquid nitrogen, and stored at −80 °C prior to DNA extraction (Cruz et al. 2010). Mycelium (100 mg) was used for DNA extraction using a buffer containing 100 mM Tris (pH 8.0, HCl), 20 mM EDTA (pH 8.0, NaOH), 1.4 M NaCl, and 2 % CTAB (Cetyl Trimethyl Ammonium Bromide) (modified from Van-Burik et al. 1998). DNA was quantified using a spectrophotometer (DS-11-DeNovix). PCR products were sent for sequencing to Macrogen Inc. (Seoul, South Korea), by using an automated Applied Biosystems ABI-3730xl sequencer. To assess sequence quality, we utilized the Phred quality score to estimate the probability of an incorrect call, thereby removing reads with errors stemming from low Phred-like quality scores (>20). The amplification program used for each locus was the same (Supplementary Table S1). The sequences obtained were analyzed and aligned using the Geneious R 6.7.1 software (Biomatters, New Zealand). The partial sequences of genes obtained from the isolates in this study were submitted to the National Center for Biotechnology Information (NCBI) database (Table 1).

Phylogenetic analyses

We downloaded 59 publicly available genome assemblies of different *Pyricularia* isolates previously classified into phylogenetically distinct lineages using whole genome data and used them as phylogenetic references for our own isolates (Supplementary Table S2; Gladieux et al. 2018). We retrieved the best hit from each assembly to each of the five DNA barcoding genes using BLASTn (-qcov_hsp_perc 80 -perc_identity 80 -evalue 0.001). Nucleotide sequences of each barcode gene from the reference assemblies and from the 24 isolates examined as part of this study were aligned with MAFFT v7.407 (-auto -maxiterate 1000) (Katoh and Standley 2013) and trimmed using ClipKIT default settings (Steenwyk et al. 2020). Alignments were concatenated into a single matrix (Supplementary Data S1) and used as input to generate maximum likelihood phylogenies with 100 replicates of non-parametric bootstrap support using IQ-TREE v2.0.5, where the best-fit substitution model for each gene-based partition was selected using ModelFinder (-m MFP -b 100 -Q) (Kalyaanamoorthy et al. 2017; Nguyen et al. 2015).

Pathogenicity assays and data analysis

Two spring wheat cultivars, Caninde 11 (susceptible to *Pyricularia* spp.) and Milan (resistant), were inoculated with *Pyricularia oryzae-Triticum* ($n = 5$), *P. oryzae*-Other ($n = 4$) and *P. pennisetigena* from *C. echinatus* ($n = 2$), *P. grisea* from *Digitaria* sp. ($n = 2$) (Supplementary Table S3).

The wheat cultivar Milan contains 2NS/2AS translocation and has well documented resistant responses to *Pyricularia* (Juliana et al. 2020). This translocation was also effective against autochthonous isolates from Paraguay (Cardozo-Téllez et al. 2022; Cazal-Martínez et al. 2022). Caninde 11 is a Paraguayan cultivar developed by the “Instituto Paraguayo de Tecnología Agraria, Centro de Investigaciones de Capitán Miranda-Paraguay”, based on the introduction of advanced lines of the CIMMYT for good agronomic yields, for growth in warm regions, for resistance to rusts but it is susceptible to *Pyricularia* and *Fusarium* (Viedma et al. 2013). It was used as a reference susceptible cultivar of the Paraguayan germplasm.

To obtain spikes, four seeds per pot (⌀ = 12 cm � = 14 cm) were sown with a substrate composed of a mixture of peat and gravel at a ratio of 3:1. Urea (46 %) was applied in two phases (80 kg ha^{-1} at tilling and 40 kg ha^{-1} at stem elongation) in a greenhouse under controlled temperature conditions of 16 ± 3 °C with light periods of 12 h. A standard chemical protection was applied in accordance with general recommendations for wheat crops before the anthesis (Kohli et al. 2012). Subsequently, 2 to 3 spikes per pot were taken from different ends of the pot for the inoculations.

Inoculum were obtained following the method suggested by Marangoni et al. (2013). Pieces of filter paper (0.5 cm in diameter) with preserved isolates were placed in Petri dishes with OA medium and incubated for 12 days at 25 ± 3 °C with light periods of 12 h. Later, to force sporulation, the mycelia were crushed with an l-shaped glass rod and incubated under constant light for 72 h. Conidia were removed from the plates by scraping with a sterile glass rod into 10 mL of sterile distilled water with 0.01 % Tween 20 and then shaken for 10 min. The conidia concentration was adjusted using a Neubauer hemacytometer at a concentration of 5.10^4 conidia/ml (Cazal-Martínez et al. 2018; Chavez et al. 2015). The inoculated plants were maintained under controlled temperature (28 ± 2 °C) and humidity (85 ± 5 %) conditions in greenhouses for a period of 72 h using a 1/5 HP 58 PSI oilless airbrush compressor kit. All assays included a negative control for infection using spikes inoculated with distilled water. The susceptible variety Caninde 11 was used as a positive control.

In our analysis, the wheat spikes were used as experimental units; the wheat cultivars and the *Pyricularia* isolates were considered as treatments and the response variable was severity, which itself consisted of the percentage of necrotic spikelet, considering the following scale: (0) No infection (1) Up to 10 % of the necrotic spike (2) Up to 40 % of the necrotic spike, (3) Up to 60 % of the necrotic spike (4)100 % of necrotic spike proposed by Chavez et al. (2017).

The variability in symptom development, ranging from 8 to 22 days post inoculation, reflects differences in pathogen aggressiveness and wheat cultivar susceptibility. Symptoms were first observed as early as 8 days post-inoculation, subsequently, we continued to monitor disease symptoms up to 22 days post-inoculation to capture potential late-appearing symptoms.

Spikes were collected as they reached the ideal phenological stage to favor infection (61 to 65 anthesis) (Lancashire et al. 1991) taking a minimum of 3 spikes and a maximum of 10 spikes per material per isolate per experiment, evaluating all isolates in the study in 2017 and 2018. All *P. oryzae* isolated from wheat were evaluated again in 2020. In the case of *P. pennisetigena* isolate ITCeh117, pathogenicity assay was performed in 2020 because it was collected and isolated in 2018 (Supplementary Table S3). If the infection was absent in all evaluated spikes, the isolate was considered non-pathogenic (as observed in *P. grisea* and *P. oryzae* isolated from other hosts).

To confirm the presence of *Pyricularia* in symptomatic spikes, we randomly selected spikes for pathogen re-isolation. The verification was carried out by examining the conidial morphology under the microscope and performing PCR analysis. Specific primers, MoT3 (specific for *P. oryzae*) and PoT3 (specific for *Pyricularia* species), were used following the thermal cycling conditions described by Pieck et al. (2016).

The infection data were derived from the calculated spike severity values of the spike. The Incidence (I) (%) was determined using the following formula:

$$I = \frac{\sum (number\ of\ infected\ spikes)}{total\ number\ of\ spikes\ observed} \times 100$$

The Disease Severity Index (DSI) (%) can be written as follows formula:

$$DSI = \frac{\sum (Class\ frequency\ in\ spike\ \times\ score\ of\ ranting\ class)}{(n\ total \times\ maximal\ class\ score)} \times 100$$

Both variables were determined through calculations performed in R software (version 4.1.0) utilizing the *R4PDE* package. The *DHARMa* package was employed to assess the normality and homogeneity of variance (Supplementary Figure S1) (Del Ponte 2023).

Data were analyzed using ANOVA Permutation test facilitated by the *permuco* package (Frossard and Renaud 2021). Additionally, we performed a post-hoc test for pairwise comparisons between group levels with corrections for multiple testing using the *MKinfer* package(Kohl 2024).

Results

Taxonomic identification

Morphological description

The morphological observations for each species were described following the taxonomic key provided by Klaubauf et al. (2014).

The classification included isolates from *Triticum aestivum, Avena strigosa, Eleusine indica, Bromus catharticus, Rynchelytrum roseum, Stenotaphrum* sp., and *Lolium multiflorum*, all of which were identified as ***Pyricularia oryzae*** Cavara = ***Magnaporthe oryzae*** B.C. Couch, Mycologia 94(4): 692. 2002 (Couch and Kohn 2002).

Culture characteristics- Colonies on OA with grey to dark grey or with dark green centres, aerial mycelium; sometimes, surface mycelia were white or cream to gold, larger growth with abundant white aerial mycelium, pale grey at the centre. Concentric growth, up to 4.5 cm diam after 10 days; reverse dark grey. Mycelium consisting of smooth, hyaline, branched, septate hyphae, 2–4 μm diam. Conidiophores solitary, erect, straight or curved, unbranched, medium brown, smooth. Conidia solitary, pyriform to obclavate, pale brown, smooth, granular to guttulate, 1–2 septate, 26–34 × 7–10 μm; hilum truncate, protruding, 1–2 μm long, 2–3 μm diam protruding, unthickened, Chlamydospores not observed and just one produced microconidia (ATae039). On PDA colonies with grey to dark grey centres, abundant white aerial mycelium or sparse white mycelial colonies with concentric growth, reaching up to 5.5 cm diam after 10 days; reverse black to dark grey; sometimes with brown to cream margins or black in centre with olivaceous margins. Some isolated were observed to form apical mucilaginous droplets on culture. Morphological data for each isolate were provided in Supplementary Table S4.

Material examined: Isolates in Paraguay from spiks of *Triticum aestivum* (CTae005; CTae009; GTae019; GTae021; YTae025; YTae028; ATae039; ITTae101) (Fig. 2), isolated from leaves of *Avena strigose* (IAsh016; IAsh017), isolated from leaves of *Eleusine indica, Bromus catarthicus, Rynchelyntrum roseum, Stenotaphrum* sp. (YEih113; CBch004, IRrh054, SLSsh051 respectively) and isolated from inflorescences of *Lollium multiflorum* (CLmi007) (Fig. 3).

Isolates obtained from *Digitaria* spp. and *Urochloa* (syn. *Brachiaria*), with morphological characteristics aligning with the descriptions provided in the taxonomic with *Pyricularia grisea* Sacc. = *Magnaporthe grisea* T.T. Hebert (Barr 1977) are detailed in Fig. 4.

Culture characteristics- Colonies on OA with grey to dark grey or with dark green centres; sometimes, surface aerial myceliums were white, grey, or cream. Concentric growth, up to 3.7 cm diam after 10 days; reverse dark grey to black. Mycelium consisting of smooth, hyaline, branched, septate hyphae 2–4 μm diam. Conidiophores solitary, erect, straight or curved, unbranched, medium brown, smooth. Conidia solitary, pyriform to obclavate, pale brown, smooth, granular to guttulate, 1–2 septate, 23–32 × 8–8.5 μm; hilum truncate, protruding, 1–1.5 μm long, 2–2.5 μm diam protruding, not darkened. All isolate produced microconidia, 9– 7 μm × 1.5– 2 μm. On PDA colonies with grey to dark grey centres, sometime white aerial mycelium. Concentric growth, reaching up to 5.4 cm diam after 10 days; reverse black to dark grey; sometimes with brown to cream margins or black in centre with cream margins (Supplementary Table S4).

Material examined: Isolates in Paraguay from leaves on *Digitaria horizontalis*, YDhh012, YDhh014, YDhh105 and from leaves of *Digitaria insularis, Digitaria* sp. and *Brachiaria* spp. YDih056, ITDsph107, YBsph044, respectively (Fig. 4).

Pyricularia pennisertigena Klaubauf, Lebrun & Crous (2014).

The isolates of *P. pennisetigena* used, examined, and evaluated for pathogenicity were previously characterized based on their morphological description and cultural characteristics in Cazal-Martínez et al. (2021).

Material examined: Isolates in Paraguay from leaves on *Cenchrus echinatus* (YCeh55; ITCeh117).

Phylogenetic analyses

We constructed a phylogenetic tree using a concatenated alignment of five DNA barcode genes the RNA polymerase II largest subunit, the internal transcribed spacer regions and intervening 5.8S nuclear ribosomal RNA), 28S large subunit rRNA; actin, and calmodulin to better

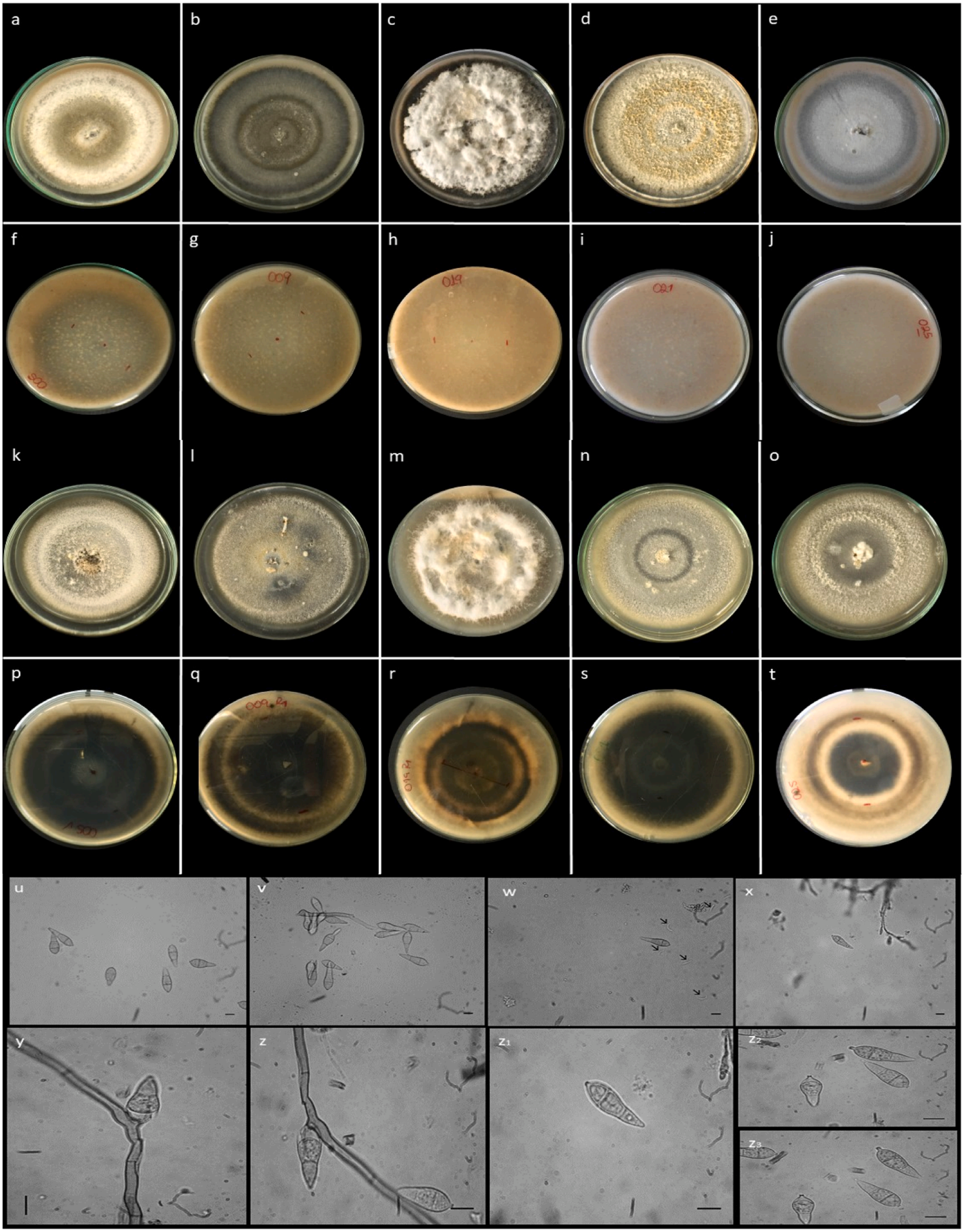

Fig. 2. *Pyricularia oryzae* from *Triticum*. Cultures of isolate grown for 10 days at 12 h photoperiod and 25 °C in Oatmeal Agar (a-j) and Potato Dextrose Agar (k-t). Ctae005 (a, f, k and p), CTae009 (b, g, l and q) GTae019 (c, h, m and r), GTae021 (d, i, n and s), YTae025 (e, j, o and t). Microscope observation of conidia (u-z_3), conidiophores (y and z) and microconidia (w ↘). CTae005 (u), CTae009 (v), ATae039 (w and x) microconidia (w ↘) GTae021 (y, z). a-e and k-*o* = plate font f-j and p-*t* = plate reverse. Scale bars = 10 μm.

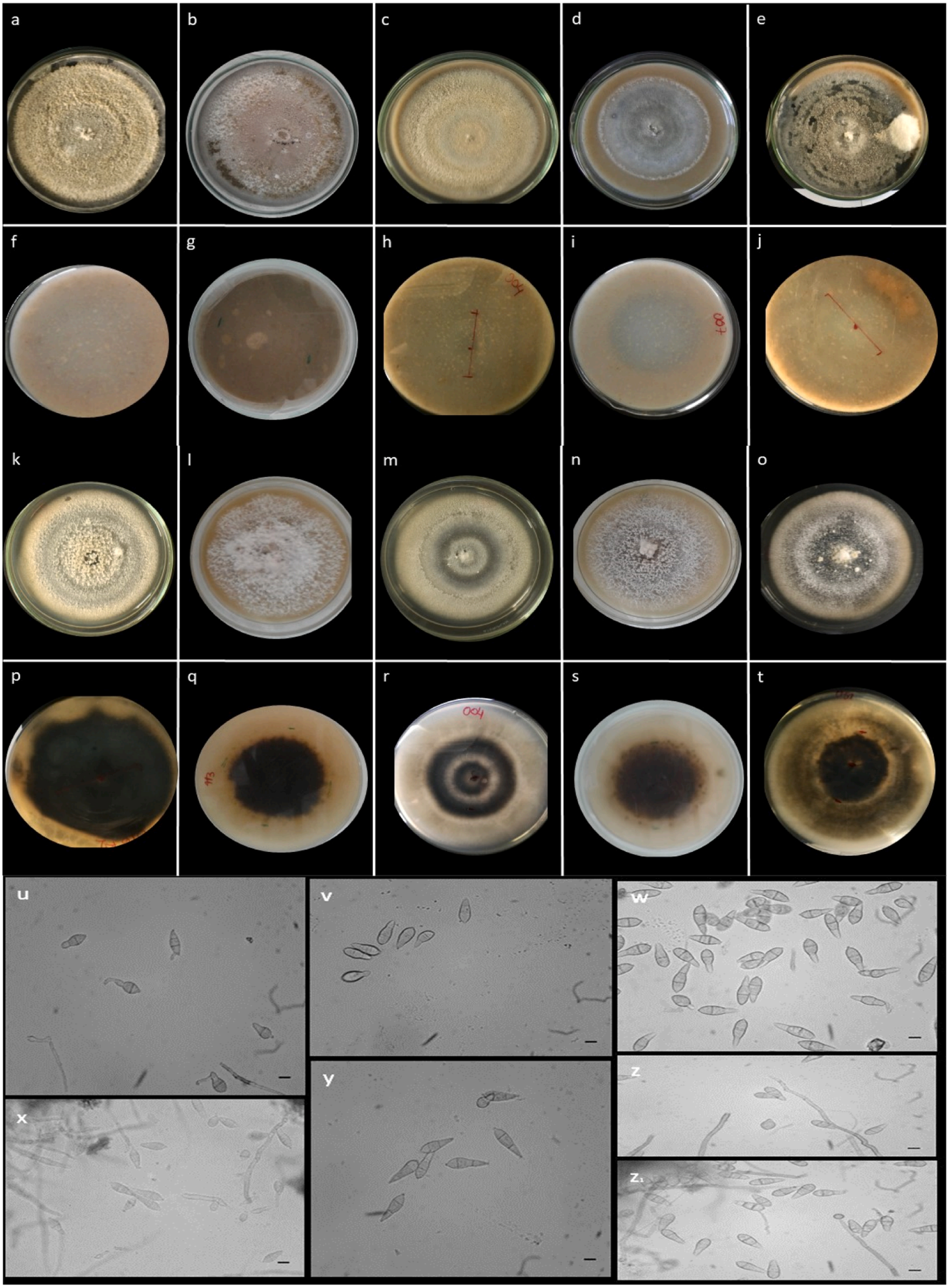

Fig. 3. ***Pyricularia oryzae*** **other hosts. Cultures of isolate grown for 10 days at 12 h photoperiod and 25 °C in Oatmeal Agar (a-j) and Potato Dextrose Agar (k-t).** IAsh016 (a and f); IAsh017 (k and p), YEih113 (b, g, l and q), CBch004 (c, h, r and m), CLmi007 (d, i, n and s), SLSsh051 (e, j, o and t). Microscope observation of conidia (u-z_1), conidiophores (z and z_1). CLmi007 (w), CBch004 (z and z_1), IAsh016 (u), YEih113 (v), IAsh017 (x and y). a-e and k-*o* = plate font f-j and p-*t* = plate reverse. Scale bars = 10 μm.

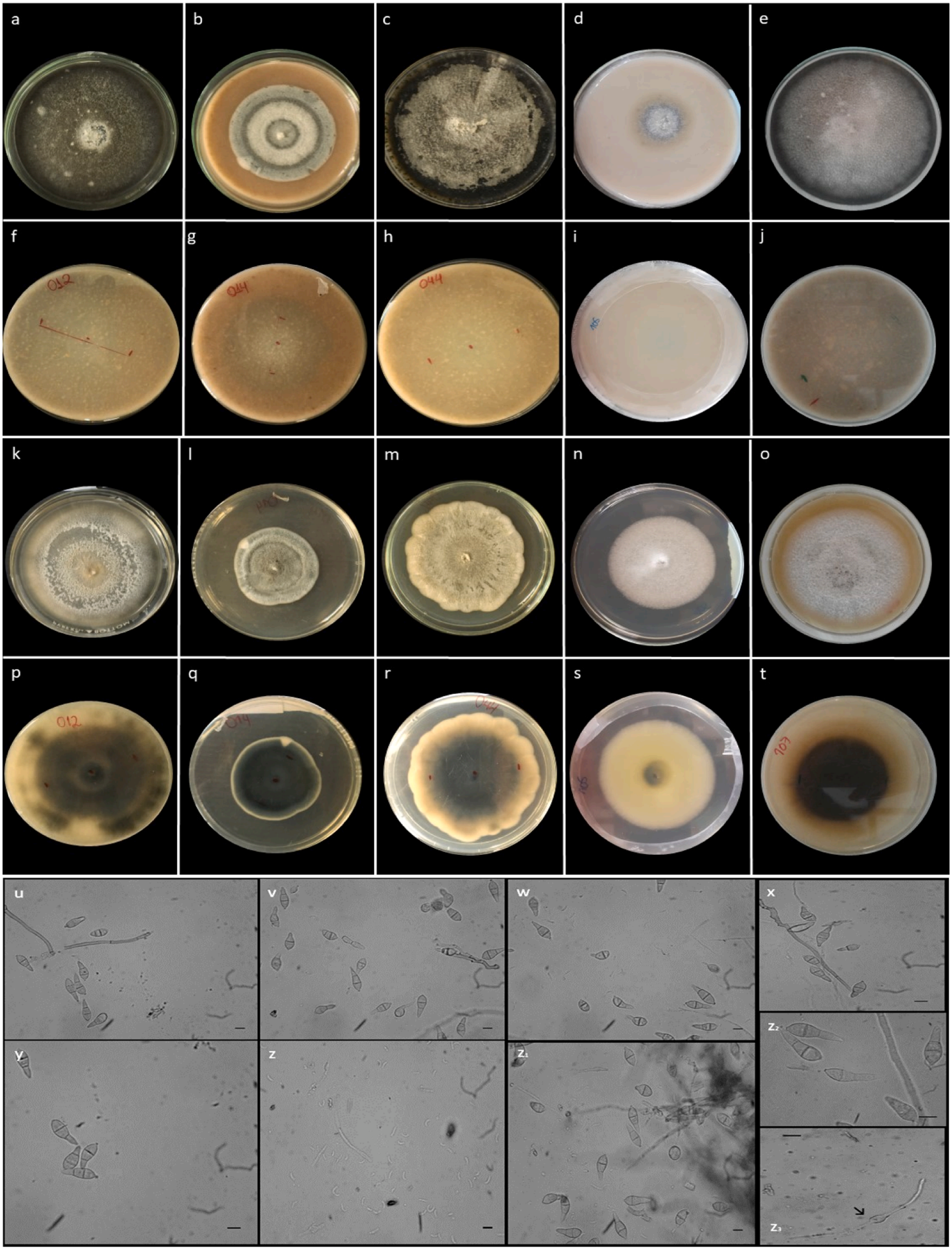

Fig. 4. ***Pyricularia grisea*****. Cultures of isolate grown for 10 days at 12 h photoperiod and 25 °C in Oatmeal Agar (a-j) and Potato Dextrose Agar (k-t).** P13YDhh012 (a,f, k and p); P13YDhh014 (b, g, l and q), P14YBsph044 (c, h, m and r), P18YDhh105 (d, i, n and s), P18ITDsph107 (e, j, o and t). Microscope observation of conidia (u-y, z_1 and z_2), conidiophores (x and z_2), microconidia (z) and chlamydospores (z_3 ↘). ITDsph107 (u, v, w, x, y, z_1), YBsph044 (z_2), YDhh014 (z and z_3). a-e and k-*o* = plate font f-j and p-*t* = plate reverse. Scale bars = 10 µm.

clarify the evolutionary relationships among the 24 isolates examined as part of this study and 59 reference isolates. The 24 isolates from this study fall into three distinct phylogenetic groups that correspond to three different *Pyricularia* species, based on their relationships to the reference sequences of *P. oryzae, P. grisea*, and *P. pennisetigena* (Fig. 5). These sequence data corroborate our morphological observations (Fig. 2–4). The two isolates from *C. echinatus* form a monophyletic clade with the *P. pennisetigena* reference isolate PM1 (bootstrap support = 100). The six isolates from *Digitaria horizontalis, D. insularis, Digitaria* sp., and *Brachiaria* sp. formed a monophyletic clade with the *P. grisea* reference isolates (bootstrap support = 87). The remaining 16 isolates from different grass species, including wheat, grouped with *P. oryzae* reference isolates from outside of the *Oryza* lineage (bootstrap support = 95 for the whole *P. oryzae* clade; 91 for *Oryza* lineage only). Due to a lack of phylogenetically informative sites within the 5 DNA barcode genes (reflected as low branch support within the *P. oryzae* clade), we were unable to distinguish between *P. oryzae* isolates belonging to the *Brachiaria2, Eleusine1, Eleusine2, Eragrostis, Lolium*, and *Triticum* lineages and did not determine the specific lineages to which the 16 Paraguayan *P. oryzae* isolates belong.

Pathogenicity assays

A permutational ANOVA was performed to evaluate the effect of the factors 'Genotype' and 'Species' (Genotype:Species), and 'Species' and 'Host' (Species:Host), as well as their interactions (Table 2). When considering Species:Host, neither factors presented a significant effect on the variables 'Incidence' (I) and 'Disease Severity Index' (DSI) ($P > 0.05$).

When considering Genotype:Species, the factor 'Genotype' presented a significant effect on both variables (I and DSI, $P < 0.05$). The resistant cultivar Milan exhibited a significantly lower DSI respect to that of the susceptible cultivar Caninde 11 ($P < 0.05$) (Fig. 6A). The factor 'Species' demonstrated a highly significant effect on both variables ($P < 0.01$), indicating a strong influence of species on the observed outcomes. The interaction between these two factors showed less pronounced but still significant effect ($P < 0.05$).

Randomly selected spikes with classic symptoms were incubated in a moist chamber for trial periods. After fungal growth and *Pyricularia* conidia developed, they were subsequently subjected to molecular diagnostic testing to confirm the infection (data not shown). *P. oryzae* isolated from hosts other than wheat and *P. grisea* did not infect wheat cultivars Caninde 11 and Milan (Fig. 6B), in contrast to *P. oryzae* isolated from wheat which displayed significantly higher DSI compared to *P. oryzae* isolated from other hosts and *P. grisea*. However, there was no significant difference between the DSI of *P. oryzae* isolated from wheat and that of *P. pennisetigena* ($P = 0.06$). Additionally, multiple pairwise permutation tests were conducted to contrast the Genotype factors while controlling for False Discovery Rates (FDR) (Fig. 7). The susceptible genotype Caninde 11 exhibited significantly higher DSI ($P < 0.05$) compared to that of Milan when infected with *P. oryzae* isolated from *Triticum* and *P. pennisetigena* (Fig. 7, Caninde 11 vs Milan – same species of *Pyricularia*).

Isolates of *P. pennisetigena* from *C. echinatus* caused infections in wheat spikes of both cultivars, with more visible symptoms in the susceptible cultivar, Caninde 11 (Fig. 8). However, DSI was significantly higher in Caninde 11 infected with *P. oryzae* from *Triticum* than in that infected with *P. pennisetigena* (Fig. 7, Caninde 11 vs different species of *Pyricularia*). In contrast, the resistant cultivar Milan showed no significant differences between the infection with *P. pennisetigena* and *P. oryzae* isolated from *Triticum*, with a slightly higher DSI in the latter (Fig. 7, Milan vs different species of *Pyricularia*).

There was no significant effect on the variables studied (I and DSI) when 'year of study' was considered as the only factor (Table 2).

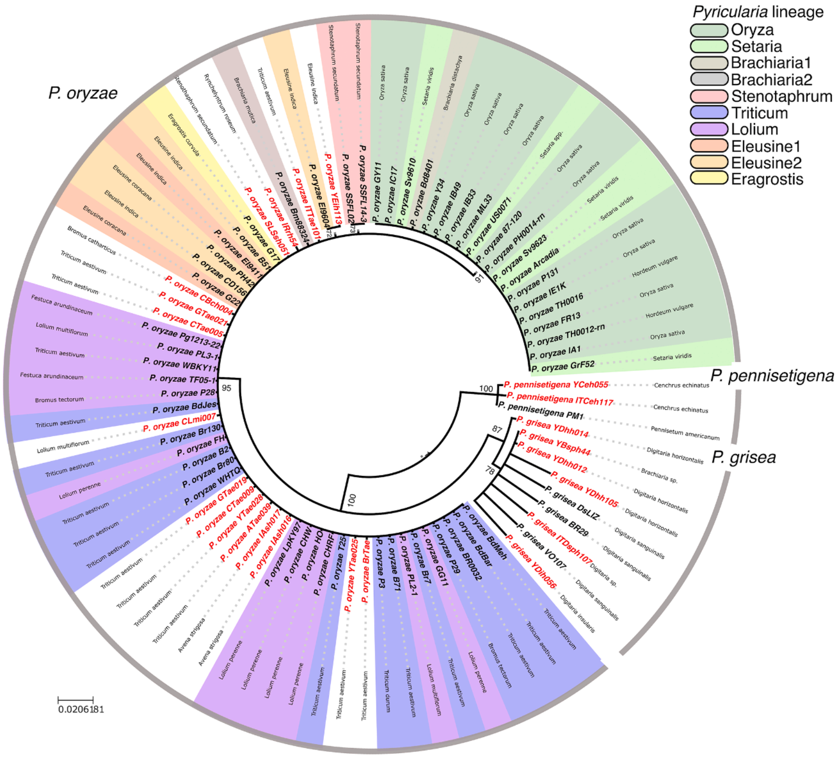

Fig. 5. A Maximum likelihood phylogenetic tree obtained from the concatenated matrix of five barcode genes. The 24 strains in red are from this study, while all others in black are reference isolates from Gladieux et al. (2018). The plant species from which each strain was isolated is indicated next to the strain name. The background of all strains from Gladieux et al. (2018) are colored and named according to their phylogenetic lineage following the original color scheme. Percent bootstrap support (out of 100 replicates) is indicated above internal nodes and all nodes with <70 % support have been collapsed.

Table 2
Analysis of variance for Permutation tests of Genotype, Species, and Host effects on Incidence (I) and Disease severity index (DSI).

Factor[(A)]	V	SS	df	F	parametric P(>*F*)	resampled P(>*F*)	
			Specie:Host				
Specie	I	1037.18	3	0.443	0.724	0.913	ns
	DSI	2900.13	3	1.037	0.391	0.884	ns
Host	I	1430.59	7	0.262	0.964	0.977	ns
	DSI	3756.30	7	0.576	0.769	0.929	ns
Specie:Host	I	2545.15	21	0.155	1.000	0.988	ns
	DSI	5982.99	21	0.306	0.997	0.965	ns
Residuals	I	21,837.46	28				
	DSI	26,093.68	28				
			Genotype:Specie				
Genotype	I	3302.50	1	6.947	0.014	0.014	*
	DSI	3291.89	1	7.704	0.010	0.010	*
Specie	I	52,761.55	3	36.994	7.E-10	1.E-05	**
	DSI	35,311.86	3	27.548	2.E-08	1.E-05	**
Genotype:Specie	I	3559.17	3	2.496	0.080	0.082	*
	DSI	4719.36	3	3.682	0.024	0.023	*
Residuals	I	13,311.40	28				
	DSI	11,963.58	28				
			Year of study				
Y	I	234.11	1	0.914	0.346	0.345	ns
	DSI	1607.05	34				
Residuals	I	74,364.89	1	0.107	0.746	0.743	ns
	DSI	59,798.49	34				

Effects were considered significant when p-values were below 0.05 (*) and highly significant when p-values were below 0.001 (**). ns: indicates non-significant effects.
[(A)] **Host**: refers to the plant species from which the isolate was originally collected. **Genotype:** corresponds to the wheat cultivars evaluated in the study. **Species** refers to the *Pyricularia* sp. species evaluated.

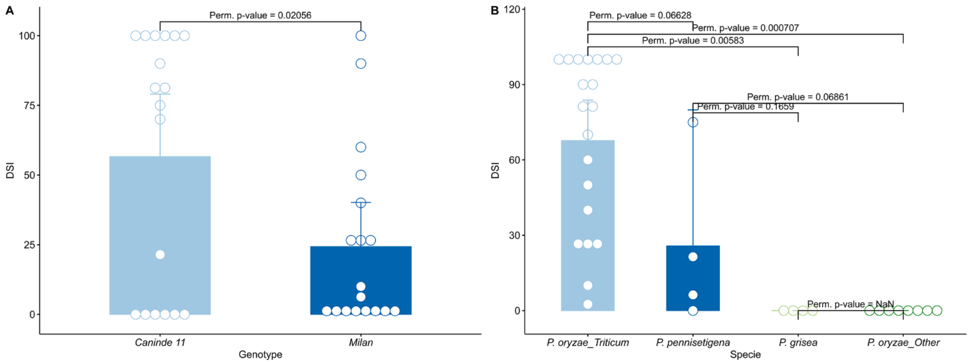

Fig. 6. Disease Severity Index (DSI) in wheat spikes caused by *Pyricularia* species isolated from different hosts in Paraguay. A. DSI comparison between the resistant (Milan) and susceptible (Caninde 11) genotypes. Data point (circle) represents an individual infected spike, with colors distinguishing the two wheat cultivars. **B.** DSI comparison among *Pyricularia* species isolated from different hosts. Data point (circle) corresponds to an infected spike, with colors representing the different *Pyricularia* species analyzed.

Discussion

The genus *Pyricularia* is the causal agent of blast disease in >50 species of cereals and grasses around the world (Zhang et al. 2016). Although many isolates are considered host-specific, there are others capable of infecting multiple plant species, even under natural field conditions (Ascari et al. 2024). This natural occurrence of cross-infections highlights the pathogen's adaptability and its potential to reproduce on alternative hosts. Such reproduction generates additional initial inoculum that, if not accounted for, complicates epidemiological forecasting and challenges effective disease management practices. Given that multiple *Pyricularia* species are found on different grass species within and around wheat fields in wheat-growing regions of Paraguay, there is an urgent need to understand which of these pathogens are capable of infecting wheat to develop a sound and sustainable management strategies. In this study, we examined the ability of different *Pyricularia* species isolated from grass species throughout wheat-growing regions of Paraguay to infect wheat. Notably, isolates of *P. pennisetigena* originally derived from *C. echinatus* were able to successfully infect the susceptible wheat cultivars assessed.

Pyricularia oryzae is the primary agent of blast disease on wheat in Paraguay and may be classified into host-adapted lineages using whole genome data (Gladieux et al. 2018; Latorre et al. 2020). However, there have been no reports on the morphological and morphometrical characterization (Fig. 2–4) and phylogenetic analysis of the isolates of *Pyricularia* in Paraguay (Fig. 5) since blast was first reported in 2002, with the first outbreak was registered in 2005 in the Departments of Itapúa and Alto Paraná (Chávez et al. 2022). To our knowledge, our study

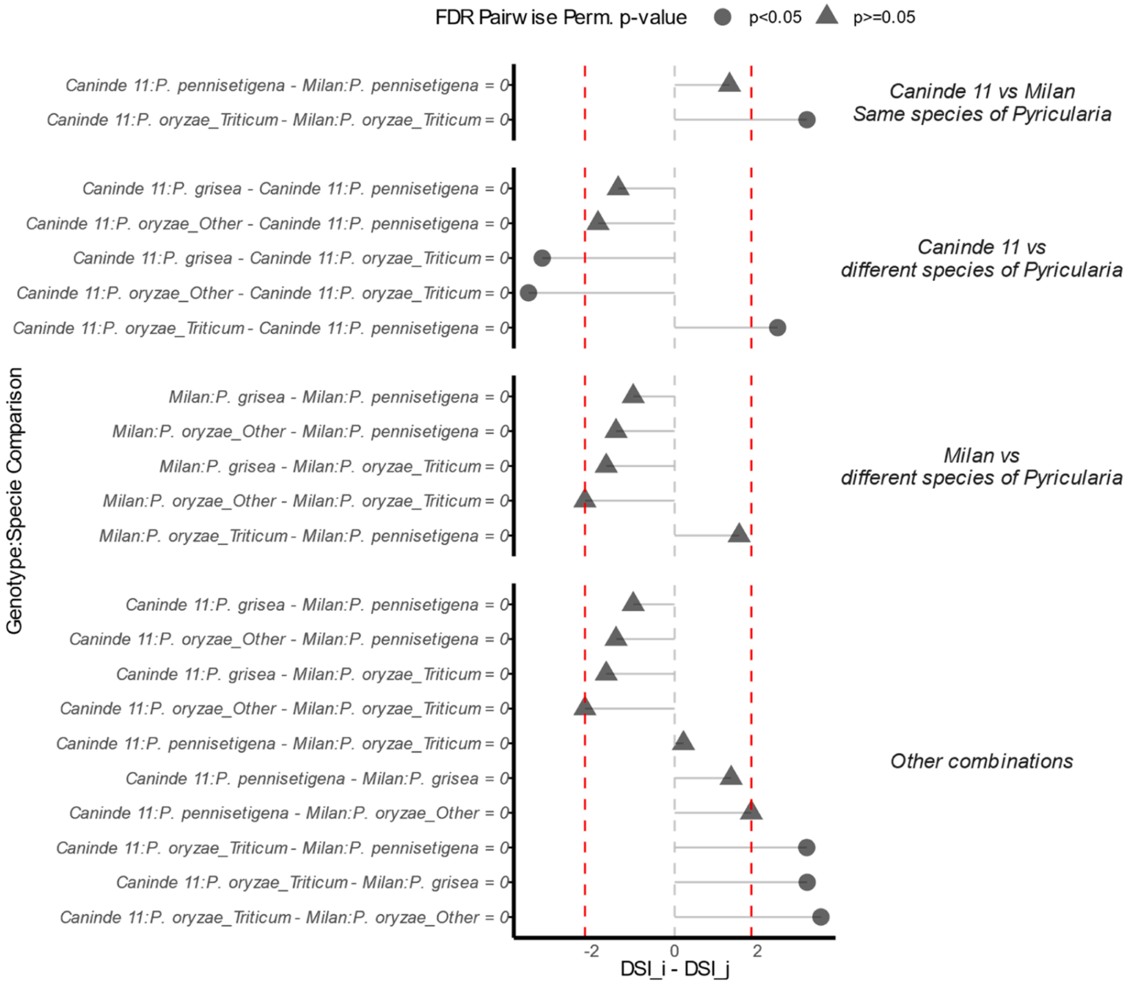

Fig. 7. Pairwise comparison of Genotype:Species. False discovery rate (FDR) was used to adjust *P*-values.

provides the first report of *P. oryzae* isolates capable of causing wheat blast in San Pedro Department, as well as blast disease in weeds in the Central and Cordillera Departments, underscoring the ability of *Pyricularia* to disperse along with its hosts to new regions (Fig. 1). While our findings confirm the presence of blast disease in weeds in these areas, we cannot rule out the possibility that the disease may have been present in these hosts but went unnoticed due to limited surveillance or underestimation of its epidemiological significance. Alternatively, it is also possible that recent changes in agricultural practices, climatic conditions, or pathogen dynamics have increased the visibility and impact of blast disease in weeds. This scenario underscores the need for more comprehensive surveillance and a broader consideration of the disease within agricultural and environmental management frameworks. Our findings underscore the relevance of adopting a One Health approach in agricultural systems, particularly in the management of blast disease caused by *Pyricularia* species.

In this study, all nine isolates of *Pyricularia* that were originally isolated from wheat matched the morphological descriptions of *Pyricularia oryzae* and the sequencing of several housekeeping loci confirmed their grouping with *P. oryzae* reference sequences. Several *Pyricularia* isolates retrieved from *Avena strigosa, Triticum aestivum, Lolium multiflorum, Bromus catharticus, Stenothaphrum secundatum, Eleusine indica* and *Rynchelyntrum roseum* were also classified as *P. oryzae* using the same approach, although we were unable to assign them to specific lineages based on the low phylogenetic resolution of our sequence data. It has been reported that *P. oryzae* survives in the lesions and seeds of weedy grasses during the winter suggesting that the use of alternative hosts is a mechanism by which it survives from one season to the next (Akase and Kusaba 2017). It is unclear, however, whether the pathogen survives in a dormant phase in the seeds of weedy grasses or in wheat stubble during the hot summer months in Paraguay. The inferred phylogenetic relationships among these *P. oryzae* strains sampled from wheat and weedy grasses suggest they belong to a single, highly diverse species, as previously suggested by Gladieux et al. (2018). Such diversity may be explained from the fact that *P. oryzae* lineages from wheat are able to exchange their genetic material with other isolates from different lineages associated with different host plants through recombination (Gladieux et al. 2018; Latorre et al. 2020; Zhong et al. 2018).

The ability of *P. pennisetigena* to infect wheat, as demonstrated in this study, highlights the importance of understanding pathogen spillover from alternative hosts, such as *Cenchrus echinatus*, into major crops like wheat (Reges et al. 2016; Cazal-Martínez et al. 2021; Dorigan et al. 2023). This cross-infection potential complicates disease management, as alternative hosts present in wheat fields or surrounding areas can serve as reservoirs for inoculum, facilitating the re-emergence of the disease and potentially giving rise to more virulent pathogen variants (Durante et al. 2018; Akase and Kusaba 2017). Our finding that isolates of *P. pennisetigena* from *Cenchrus* were able to infect wheat spikes corroborate a previous finding that *P. pennisetigena* isolates from *Urochloa* spp., *C. echinatus* and *Paspalum maximum* can also infect wheat plants (Dorigan et al. 2023; Reges et al. 2016).

Interspecies variability has a role important in the epidemiology of blast disease considering the gene flow between different *Pyricularia* isolates (Gladieux et al. 2018), multi-host pathogenic ability (Chávez

Fig. 8. Blast disease symptoms on wheat spikes caused by *Pyricularia oryzae, Pyricularia pennisetigena* and *Pyricularia grisea*. Wheat from Milan (A) (resistant) and Caninde 11 (B) (susceptible) cultivars are shown, with symptoms categorized according to the *Pyricularia* species responsible for infection.

et al. 2022), and their evolutionary mechanisms by effectors specificity (Inoue et al. 2017).

Currently, the genetic variation in *P. oryzae* is explained by two main mechanisms. The first mechanism is genetic recombination, a process proposed to have given rise to new pathogens infecting wheat.

A preliminary genomic study on Br48 suggests that it is a hybrid between ancestral isolates of *P. oryzae* from the *Triticum* (MoT) or *Lolium* (MoL) pathotypes and an isolate from *Urochloa* (syn. *Brachiaria*). Notably, the genomic region derived from the *Urochloa* strain contains the virulent B-type PWT3 allele, one of the two virulent loci responsible for the recent emergence of MoT (Inoue et al., 2017). In contrast, a more recent investigation sequenced the reference genome B71 and assessed the haplotype divergence (number of SNPs/variant sites) between this genome and five other representative isolates from candidate donor populations, including PoE1/E2 (*Eleusine*), PoLu (*Luziola*), PoO/PoS (*Oryza/Setaria*), and PoSt (*Stenotaphrum*). The study concluded that, in addition to these populations, chromosomal segments contributed by an unidentified population, designated "PoX," further support the hypothesis of a 'multi-hybrid' swarm (Rahnama et al. 2023). The second mechanism is horizontal gene transfer (HGT), which has allowed the incorporation of large DNA fragments from both related and unrelated fungi. In the genomic comparison between *P. oryzae* isolate (Br48 genome) and related species such as *P. pennisetigena* and *P. grisea*, several megabase-scale insertions have been observed. In these insertions, some genes have close homologs in distantly related organisms, such as basidiomycetes or prokaryotes (Kobayashi et al. 2023).

The study of common hosts is crucial, as they may serve as convergence points for *P. oryzae* isolates, potentially facilitating encounters and mating among them. Such interaction might occur not only between closely related species but also with other species coexisting in their environment. These mechanisms could help *P. oryzae* adapt to new hosts and survive under a range of environmental conditions, however, further research is needed to confirm the extent and nature of these interactions (Asuke et al. 2020; Inoue et al. 2017; Tosa et al. 2016).

Results from our pathogenicity assay further suggest that *P. pennisetigena* may be an emerging risk to wheat production in Paraguay, particularly, considering that its primary host *C. echinatus* is a common weed found in wheat fields across Paraguay. Our finding that *P. grisea* isolates were not able to infect either wheat cultivars in this study was also corroborated previously by Reges et al. (2016). Nevertheless, in histopathological analyses of interactions between *D. sanguinalis* and *D. insularis* plants infected by a *Pyricularia* isolate from wheat, fungal penetration of host cells was observed, although the disease fail to make enough progress to cause a cell death response in wheat (Durante et al. 2018), which suggests that weeds can act as alternative hosts and, therefore, sources of inoculum (Chávez et al. 2022). The involvement of multiple Pyricularia species in wheat blast disease aligns with the interconnected principles of One Health, which emphasize the role of environmental biodiversity in disease emergence. Understanding the interactions between cultivated crops, wild plant species, and pathogens is essential for predicting disease dynamics and developing sustainable agricultural practices that reduce chemical inputs while safeguarding crop yields (Kobayashi et al. 2023).

In conclusion, we classified 24 *Pyricularia* isolates into three species using morphological and molecular methods. We updated the geographical distribution of this pathogen by identifying *P. oryzae* causing wheat blast in the San Pedro Department. We also found blast disease in weeds in Central and Cordillera Departments, providing strong evidence that *Pyricularia* is also present in these hosts in these regions. *P. pennisetigena* isolates were capable of infecting wheat spikes, especially in susceptible wheat cultivar. Because the cultivar Milan also presented resistance to *P. pennisetigena,* it suggests that the 2NS/2AS translocation might also contribute in the resistance to this pathogen. Therefore, it is imperative to further investigate the potential of *P. pennisetigena* as an emerging threat to wheat production in Paraguay, given that its primary host, *C. echinatus*, is frequently present in wheat fields from the preplant to the post-harvest stages. The findings from this study not only highlight the importance of continuous pathogen surveillance but also reinforce the need to investigate the mechanisms underlying resistance to emerging pathogens like *P. pennisetigena*. Identifying resistant cultivars, such as Milan with the 2NS/2AS translocation, offers promising avenues for mitigating the impact of this pathogen on wheat production.

CRediT authorship contribution statement

Cinthia Carolina Cazal-Martínez: Conceptualization, Supervision, Formal analysis, Investigation, Validation, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Yessica Magaliz Reyes-Caballero:** Investigation, Validation. **Alice Rocio Chávez:** Investigation, Validation, Writing – review & editing. **Pastor Enmanuel Pérez-Estigarribia:** Data curation, Software, Formal analysis, Methodology, Visualization. **Man Mohan Kohli:** Conceptualization, Supervision, Funding acquisition, Writing – review & editing. **Alcides Rojas:** Data curation, Software, Formal analysis. **Andrea Alejandra Arrua:** Resources, Writing – review & editing. **Juliana Moura-Mendes:** Resources, Writing – review & editing. **Ramón Souza-Perera:** Resources. **José Juan Zúñiga Agilar:** Resources, Writing – review & editing. **Emile Gluck-Thaler:** Data curation, Software, Formal analysis, Writing – review & editing. **Horacio Lopez-Nicora:** Conceptualization, Formal analysis, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Julio Cesar Masaru Iehisa:** Formal analysis, Methodology, Visualization, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.crmicr.2025.100361.

Data availability

Data will be made available on request.

References

Akase, K., Kusaba, M., 2017. Overwintering of *Pyricularia oryzae* in wild infected foxtails. J. General Plant Pathol. 83, 197–204. https://doi.org/10.1007/s10327-017-0722-y.

Alberione, E., Bainotti, C., Cettour, I., Salines, J., 2008. Evaluación de enfermedades en trigos en siembra de verano en el NEA argentino-Campaña 2007/2008. In: 7mo. Congreso Nacional de Trigo. Santa Rosa, La Pampa, Argentina. Instituto Nacional de Investigación Agropecuaria, p. 2.

Dos Anjos, J., Da Silva, D., Charchar, M.J., Rodrigues, G., 1996. Ocorrência de brusone (*Pyricularia grisea*) em trigo e centeio na região dos cerrados do Brasil Central. Pesq. agropec. bras. 31, 79–82.

Antón, W.S., 2008. Efecto de factores físicos y bioquímicos sobre la formación de estructuras infectivas de *Magnaporthe grisea* agente causal de pyriculiarosis en arroz (*Oryza sativa*). Universidad Nacional Autónoma de Nicaragua, León 1, 31–36. https://doi.org/10.5377/universitas.v2i1.1641.

Ascari, J.P., Cazón, L.I., Rahnama, M., Lamour, K., Fernandes, J.M.C., Farman, M.L., Del Ponte, E.M., 2024. Pyricularia are mostly host-specialized with limited reciprocal cross-infection between wheat and endemic grasses in Minas Gerais, Brazil. Phytopathology. 114, 226–240. https://doi.org/10.1094/PHYTO-01-23-0024-R.

Asuke, S., Tanaka, M., Hyon, G.S., Inoue, Y., Vy, T.T.P., Niwamoto, D., Nakayashiki, H., Tosa, Y., 2020. Evolution of an eleusine-specific subgroup of *Pyricularia oryzae* through a gain of an avirulence gene. Molecular Plant-Microbe Interact. 33, 153–165. https://doi.org/10.1094/MPMI-03-19-0083-R.

Barea, G., and Toledo, J. 1996. *Identificación y zonificación de piricularia o bruzone (Pyricularia oryzae) en el cultivo del trigo en el dpto. de Santa Cruz. CIAT. Informe Técnico. Proyecto de Investigación Trigo, Santa Cruz*. Santa Cruz.

Barr, M.E., 1977. Magnaporthe, Telimenella, and Hyponectria (Physosporellaceae). Mycologia 69, 952–966. https://doi.org/10.1080/00275514.1977.12020147.

Bussaban, B., Lumyong, S., Lumyong, P., Seelanan, T., Park, D., Hyde, K., 2005. Molecular and morphological characterization of Pyricularia and allied genera. Mycologia 97, 1002–1011.

Callaway, E., 2016. Devastating wheat fungus appears in Asia for first time. Nature 532, 421–422. https://doi.org/10.1038/532421a.

Cardozo-Téllez, L., Chavez, A., Pérez-, P., Reyes, M., Cazal-Martinez, C., Heesacker, A., Kohli, M.M., 2022. Caninde2 /Milan : promising wheat line to discover novel genes for resistance to wheat blast. Crop Breed Appl Biotechnol 22, 1–9.

Castroagudín, V.L., Moreira, S.I., Pereira, D.A.S., Moreira, S.S., Brunner, P.C., Maciel, J.L.N., Crous, P.W., McDonald, B.A., Alves, E., Ceresini, P.C., 2016. *Pyricularia graminis-tritici*, a new *Pyricularia* species causing wheat blast. Persoonia Molecular Phylogeny and Evolution of Fungi 37, 199–216. https://doi.org/10.3767/003158516X692149.

Cazal-Martínez, C.C., Chávez, A.R., Reyes Caballero, Y.M., Kohli, M.M., Pérez Estigarribia, P.E., 2018. Evaluation of synthetic hexaploid wheats for resistance to wheat blast disease. Mexican J Phytopathol. 37, 1–15. https://doi.org/10.18781/R.MEX.FIT.1807-5.

Cazal-Martínez, C.C., Reyes Caballero, Y.M., Chávez, A., Pérez Estigarribia, P.E., Rojas, A., Arrua, A.A., Moura-Mendes, J., Lopez-Nicora, H.D., Kohli, M.M., 2021. First report of a leaf blight caused by *Pyricularia pennisetigena* on *Cenchrus echinatus* in Paraguay. Plant Dis. 105, 3758. https://doi.org/10.1094/PDIS-02-21-0287-PDN.

Cazal-Martínez, C.C., Reyes-Caballero, Y.M., Kohli, M.M., 2022. Respuesta de Trigos con la presencia de translocación 2NS/2AS, Rmg8 y Rmg7 a *Pyricularia oryzae* patotipo *Triticum* de Paraguay. Summa Phytopathol 48, 32–35. https://doi.org/10.1590/0100-5405/257220.

Ceresini, P.C., Castroagudín, V.L., Rodrigues, F., Rios, J.A., Aucique-Pérez, C.E., Moreira, S.I., Alves, E., Croll, D., Maciel, J.L.N., 2018. Wheat Blast: past, present, and future. Annu Rev. Phytopathol. 56, 427–456. https://doi.org/10.1146/annurev-phyto-080417-050036.

Chavez, A., C, C., Rojas, A., Guillén, A., Núñez, A., Kohli, M., 2015. Evaluación de la concentración de conidios para la inoculación de materiales de trigo con *Magnaporthe grisea*. In: Memorias del I congreso agrario del IPTA. Encarnación. Instituto Paraguayo de Tecnología Agraria, pp. 168–169.

Chavez, A., Cazal, C.C., Kohli, M.M., 2017. Difference in the reaction to *Pyricularia oryzae* of wheat materials in the vegetative and reproductive stages. Investigación Agraria 19, 56–63. https://doi.org/10.18004/investig.agrar.2017.junio.56-63.

Chávez, A.R., Tellez, L.C., Cazal-Martinez, C.C., Kohli, M.M., Carmona, M.A., 2022. Further progress on wheat blast epidemiology: identification of novel alternate hosts of *Magnaporthe oryzae Triticum* pathotype in Paraguay. Eur. J. Plant Pathol. https://doi.org/10.1007/s10658-022-02567-x.

Choi, J., Park, S.-Y., Kim, B.-R., Roh, J.-H., Oh, I.-S., Han, S.-S., Lee, Y.-H., 2013. Comparative analysis of pathogenicity and phylogenetic relationship in *Magnaporthe grisea* species complex. PLoS. One 8, e57196. https://doi.org/10.1371/journal.pone.0057196.

Couch, B.C., Fudal, I., Lebrun, M.H., Tharreau, D., Valent, B., Van Kim, P., Nottéghem, J. L., Kohn, L.M., 2005. Origins of host-specific populations of the blast pathogen *Magnaporthe oryzae* in crop domestication with subsequent expansion of pandemic clones on rice and weeds of rice. Genetics 170, 613–630. https://doi.org/10.1534/genetics.105.041780.

Couch, B.C., Kohn, L.M., 2002. A multilocus gene genealogy concordant with host preference indicates segregation of a new species, Magnaporthe oryzae, from M. grisea 94, 683–693.

Cruz, M.F. 2008. Caracterização do padrão molecular e de virulência de isolados de *Pyricularia grisea* do trigo.

Cruz, M.F.A., Prestes, A.M., Maciel, J.L.N., Scheeren, P.L., 2010. Resistência parcial à brusone de genótipos de trigo comum e sintético nos estádios de planta jovem e de planta adulta. Trop. Plant Pathol. 35, 24–31.

Dorigan, A.F., da Silva Costa Guimarães, S., Vicentini, S.N.C., de Souza Moreira, S., Negrisoli, M.M., Pereira, R.C.M., de Reges, J.T.A., Castroagudín, V.L., Ceresini, P.C., Alves, E., 2023. *Pyricularia pennisetigena* and *Pyricularia urashimae* can also cause wheat head blast. Eur. J. Plant Pathol. https://doi.org/10.1007/s10658-023-02690-3.

Durante, L.G., Bacchi, L.M., Souza, J.E., Graichen, F.A., 2018. Reaction of wheat plants and alternative hosts to *Magnaporthe oryzae*. Arq. Inst. Biol. (Sao Paulo) 84, 1–7. https://doi.org/10.1590/18081657000952017.

Farman, M., Peterson, G., Chen, L., Starnes, J., Valent, B., Bachi, P., Murdock, L., Hershman, D., Pedley, K., Fernandes, J.M., Bavaresco, J., 2017. The *Lolium* pathotype of *Magnaporthe oryzae* recovered from a single blasted wheat plant in the United States. Plant Dis. 101, 684–692. https://doi.org/10.1094/PDIS-05-16-0700-RE.

Frossard, J., Renaud, O., 2021. Permutation tests for regression, ANOVA, and comparison of signals: the permuco package. J. Stat. Softw.

Gladieux, P., Condon, B., Ravel, S., Soanes, D., Maciel, J.L.N., Nhani, A., Chen, L., Terauchi, R., Lebrun, M.-H., Tharreau, D., Mitchell, T., Pedley, K.F., Valent, B., Talbot, N.J., Farman, M., Fournier, E., 2018. Gene flow between divergent cereal- and grass-specific lineages of the rice blast fungus *Magnaporthe oryzae*. American Soc. microbiol. 9. https://doi.org/10.1128/mBio.01219-17 e01219-17.

Goulart, A., Paiva, F., Mesquita, A., 1990. Ocorrência de brusone (*Pyricularia oryzae*) do trigo (*Triticum aestivum*) em Mato Grosso do Sul. Fitopatol Bras 15, 112–114.

Hyon, G.S., Nga, N.T.T., Chuma, I., Inoue, Y., Asano, H., Murata, N., Kusaba, M., Tosa, Y., 2012. Characterization of interactions between barley and various host-specific subgroups of *Magnaporthe oryzae* and *M. grisea*. J. General Plant Pathol. 78, 237–246. https://doi.org/10.1007/s10327-012-0386-6.

Igarashi, S., Utiamada, C.M., Kasuma, A.H., López, R.S., 1986. *Pyricularia* sp em trigo. 1. Ocurrencia de *Pyricularia* sp. no Estado do Paraná. Fitopatología Brasilera 11, 351–352.

Inoue, Y., Vy, T.T.P., Yoshida, K., Asano, H., Mitsuoka, C., Asuke, S., Anh, V.L., Cumagun, C.J.R., Chuma, I., Terauchi, R., Kato, K., Mitchell, T., Valent, B., Farman, M., Tosa, Y., 2017. Evolution of the wheat blast fungus through functional losses in a host specificity determinant. Science (1979) 357, 80–83. https://doi.org/10.1126/science.aam9654.

Islam, M.T., Croll, D., Gladieux, P., Soanes, D.M., Persoons, A., Bhattacharjee, P., Hossain, M.S., Gupta, D.R., Rahman, M.M., Mahboob, M.G., Cook, N., Salam, M.U., Surovy, M.Z., Sancho, V.B., Maciel, J.L.N., NhaniJúnior, A., Castroagudín, V.L., Reges, J.T., de, A., Ceresini, P.C., Ravel, S., Kellner, R., Fournier, E., Tharreau, D., Lebrun, M.H., McDonald, B.A., Stitt, T., Swan, D., Talbot, N.J., Saunders, D.G.O., Win, J., Kamoun, S., 2016. Emergence of wheat blast in Bangladesh was caused by a South American lineage of *Magnaporthe oryzae*. BMC. Biol. 14. https://doi.org/10.1186/s12915-016-0309-7.

Juliana, P., He, X., Kabir, M.R., Roy, K.K., Anwar, M.B., Marza, F., Poland, J., Shrestha, S., Singh, R.P., Singh, P.K., 2020. Genome-wide association mapping for wheat blast resistance in CIMMYT's international screening nurseries evaluated in Bolivia and Bangladesh. Sci. Rep. 10, 1–14. https://doi.org/10.1038/s41598-020-72735-8.

Kahle, D., Wickham, H., 2013. ggmap: spatial visualization with ggplot2. R Journal 5, 144–161. https://doi.org/10.32614/rj-2013-014.

Kalyaanamoorthy, S., Minh, B.Q., Wong, T.K.F., Von Haeseler, A., Jermiin, L.S., 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. Nat. Methods 14, 587–589. https://doi.org/10.1038/nmeth.4285.

Katoh, K., Standley, D.M., 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol. Biol. Evol. 30, 772–780. https://doi.org/10.1093/molbev/mst010.

Klaubauf, S., Tharreau, D., Fournier, E., Groenewald, J.Z., Crous, P.W., de Vries, R.P., Lebrun, M.-H., 2014. Resolving the polyphyletic nature of Pyricularia (Pyriculariaceae). Stud. Mycol. 79, 85–120. https://doi.org/10.1016/j.simyco.2014.09.004.

Kobayashi, N., Dang, T.A., Pham, K.T.M., Gómez Luciano, L.B., Van Vu, B., Izumitsu, K., Shimizu, M., Ikeda, K.I., Li, W.H., Nakayashiki, H., 2023. Horizontally transferred DNA in the genome of the Fungus *Pyricularia oryzae* is associated with repressive histone modifications. Mol. Biol. Evol. 40. https://doi.org/10.1093/molbev/msad186.

Kohl, M. 2024. Package "MKinfer." . https://doi.org/10.1016/S0167-7152(97)00043.

Kohli, M., Mehta, Y.R., Guzman, E., de Viedma, L., Cubilla, L.E., 2011. Pyricularia blast-a threat to wheat cultivation. Czech J. Genet. Plant Breeding 47, 130–134. https://doi.org/10.17221/3267-CJGPB.

Kohli, Viedma, L.de, and Cubilla, L.E. 2012. *Guía para la producción de Trigo. Fortalecimiento de la investigación y difusión del cultivo de trigo en Paraguay*. Paraguay: M. M. Kohli, Lidia de Viedma, L. E. Cubilla.

Labomed®, 2009. CxL User Manual. Labo America, Inc. https://microscopeinternational.com/content/PDF/catalog/labomed-cxl-manual.pdf.

Lancashire, P.D., Bleiholder, H., Boom, T.Van Den, LangelÜDdeke, P., Stauss, R., Weber, E., Witzenberger, a, 1991. A uniform decimal code for growth stages of crops and weeds. Annals Appl. Biol. 119, 561–601. https://doi.org/10.1111/j.1744-7348.1991.tb04895.x.

Latorre, S.M., Reyes-Avila, C.S., Malmgren, A., Win, J., Kamoun, S., Burbano, H.A., 2020. Differential loss of effector genes in three recently expanded pandemic clonal lineages of the rice blast fungus. BMC. Biol. 18, 1–15. https://doi.org/10.1186/s12915-020-00818-z.

Malaker, P.K., Barma, N.C.D., Tiwari, T.P., Collis, W.J., Duveiller, E., Singh, P.K., Joshi, A.K., Singh, R.P., Braun, H.-J., Peterson, G.L., Pedley, K.F., Farman, M.L., Valent, B., 2016. First report of wheat blast caused by *Magnaporthe oryzae* pathotype *Triticum* in Bangladesh. Plant Dis. 100, 2330. https://doi.org/10.1094/PDIS-05-16-0666-PDN.

Marangoni, M.S., Nunes, M.P., Fonseca, J.N., Mehta, Y.R., 2013. Pyricularia blast on white oats - a new threat to wheat cultivation. Trop. Plant Pathol. 38, 198–202. https://doi.org/10.1590/S1982-56762013005000004.

Murakami, J., Tosa, Y., Kataoka, T., Tomita, R., Kawasaki, I., Chuma, Y., Sesumi, M., Kusaba, H., Nakayashiki, H., Mayama, S., 2003. Analysis of host species specificity of *Magnaporthe grisea* toward foxtail millet using a genetic cross between isolates from wheat and foxtail millet. Phytopathology. 93, 42–45. https://doi.org/10.1094/PHYTO.2003.93.1.42.

Nguyen, L.T., Schmidt, H.A., Von Haeseler, A., Minh, B.Q., 2015. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol. Biol. Evol. 32, 268–274. https://doi.org/10.1093/molbev/msu300.

Oh, H.S., Tosa, Y., Takabayashi, N., Nakagawa, S., Tomita, R., Don, L.D., Kusaba, M., Nakayashiki, H., Mayama, S., 2002. Characterization of an Avena isolate of *Magnaporthe grisea* and identification of a locus conditioning its specificity on oat. Canadian J. Botany 80, 1088–1095. https://doi.org/10.1139/b02-101.

Pieck, M.L., Ruck, A., Farman, M., Peterson, G.L., Stack, J.P., Valent, B., Pedley, K.F., 2016. Genomics-based marker discovery and diagnostic assay development for wheat blast. Plant Dis. https://doi.org/10.1094/PDIS-04-16-0500-RE. PDIS-04-16-0500-RE.

Del Ponte, E.M. 2023. *R for plant disease epidemiology (R4PDE)*.

Qi, H., Yang, J., Yin, C., Zhao, J., Ren, X., Jia, S., Zhang, G., 2019. Analysis of *Pyricularia oryzae* and *P. grisea* from different hosts based on multilocus phylogeny and pathogenicity associated with host preference in China. Phytopathology. 109, 1433–1440. https://doi.org/10.1094/PHYTO-10-18-0383-R.

Quintana de Viedma, L., 2010. Manejo integrado de mancha amarilla y la Piricularia en el cultivo de trigo en Paraguay. In: Kohli, Man Mohan, Cubilla, LE, Cabrera, G (Eds.), Tercer Seminario Nacional De Trigo "Del Grano Al Pan". CAPECO/INBIO, Asunción-Paraguay, pp. 31–42.

Quintana De Viedma, L., Pedretti, R., Kohli, M.M., and Gómez, G. 2004. *Avances y Resultados de la Investigacion Del Trigo en El Paraguay*. Bib. Orton IICA /CATIE.

Rahnama, M., Condon, B., Ascari, J.P., Dupuis, J.R., Del Ponte, E.M., Pedley, K.F., Martinez, S., Valent, B., Farman, M.L., 2023. Recent co-evolution of two pandemic plant diseases in a multi-hybrid swarm. Nat. Ecol. Evol. 7, 2055–2066. https://doi.org/10.1038/S41559-023-02237-Z, 2023 7:12.

Reges, J.T.de A., Jesus, M.N.de, Silva, S.D.R.da, Souza, M.H.de, Rodrigues, J.W., 2019. Teste de patogenicidade dos isoaldos *Pyricularia oryzae* nos hospederos de trigo, cevada, arroz e braquiaria. Agronomic crop j. 28, 19–28. https://doi.org/10.32929/2446-8355.2019v28n1p19-28.

Reges, J.T.de A., Santos, I.de J., Rodrigues, J.W., Souza, M.H.de, Silva, S.D.R.da, Jesus, M.N.de, 2018. Caracterização fenotípica de isolados de *Pyricularia oryzae* de trigo e plantas invasoras. Revista de Ciências Agrárias 61, 1–5. https://doi.org/10.22491/rca.2018.2628.

Reges, T.J.D.A., Negrisoli, M.M., Dorigan, A.F., Castroagudín, V., Nunes, M., Ceresini, P., 2016. *Pyricularia pennisetigena* and *P. zingibericola* from invasive grasses infect signal grass, barley and wheat. Pesquica Agropecuaria Tropical 46, 206–214. https://doi.org/10.1590/1983-40632016v4641335.

ScopeImage V9.3.2. 2016. ScopeImage 9.0 professional imaging software. Operation Manual. . https://www.m-shot.com/upload/portal/20210130/202101301134341539.pdf.

Singh, B.K., Delgado-Baquerizo, M., Egidi, E., Guirado, E., Leach, J.E., Liu, H., Trivedi, P., 2023. Climate change impacts on plant pathogens, food security and paths forward. Nature Rev. Microbiol. 21, 640–656. https://doi.org/10.1038/S41579-023-00900-7, 2023 21:10.

Steenwyk, J.L., Buida, T.J., Li, Y., Shen, X.X., Rokas, A., 2020. ClipKIT: a multiple sequence alignment trimming software for accurate phylogenomic inference. PLoS. Biol. 18, 1–17. https://doi.org/10.1371/journal.pbio.3001007.

Takabayashi, N., Tosa, Y., Oh, H.S., Mayama, S., 2002. A gene-for-gene relationship underlying the species-specific parasitism of Avena/Triticum isolates of *Magnaporthe grisea* on wheat cultivars. Phytopathology. 92, 1182–1188. https://doi.org/10.1094/PHYTO.2002.92.11.1182.

Tembo, B., Mulenga, R.M., Sichilima, S., M'siska, K.K., Mwale, M., Chikoti, P.C., Singh, P.K., He, X., Pedley, K.F., Peterson, G.L., Singh, R.P., Braun, H.J., 2020. Detection and characterization of fungus (*Magnaporthe oryzae* pathotype *Triticum*) causing wheat blast disease on rain-fed grown wheat (*Triticum aestivum* L.) in Zambia. PLoS. One 15, 1–10. https://doi.org/10.1371/journal.pone.0238724.

Tosa, Y., Hirata, K., Tamba, H., Nakagawa, S., Chuma, I., Isobe, C., Osue, J., Urashima, A. S., Don, L.D., Kusaba, M., Nakayashiki, H., Tanaka, A., Tani, T., Mori, N., Mayama, S., 2004. Genetic constitution and pathogenicity of *Lolium* isolates of *Magnaporthe oryzae* in comparison with host species-specific pathotypes of the blast fungus. Phytopathology. 94, 454–462. https://doi.org/10.1094/PHYTO.2004.94.5.454.

Tosa, Y., Inoue, Y., Trinh, T.P.V., Chuma, I., 2016. Genetic and molecular analyses of the incompatibility between *Lolium* isolates of *Pyricularia oryzae* and wheat. Physiol. Mol. Plant Pathol. 95, 84–86. https://doi.org/10.1016/j.pmpp.2016.01.007.

Urashima, A., Igarashi, S., Kato, H., 1993. Host range, mating type, and fertility of *Pyricularia grisea* from wheat in Brazil. Plant Dis. 77, 1211–1216.

Urashima, A.S., Martins, T.D., Bueno, C.R.N.C., Favaro, D.B., Arruda, M.A., Mehta, Y.R., 2004. Triticale and Barley: new hosts of *Magnaporthe grisea* in São Paulo, Brazil - relationship with blast of rice and wheat. In: Kawasaki, Shinji (Ed.), Rice Blast: Interaction with Rice and Control. Springer Netherlands, Dordrecht, pp. 251–260.

Van-Burik, J., Schreckhise, R.W., White, T.C., Bowden, R., Myerson, D., 1998. Comparison of six extraction techniques for isolation of DNA from filamentous fungi. Med. Mycol. 36, 299–303, 10075499.

Viedma, L.Q.De. 2005. Wheat blast occurrence in Paraguay. APS Caribbean Division, APS Southern Division, Latin American Association of Plant Pathology Abstracts 95: 2003–2006.

Viedma, L.Q.De, Kohli, M.M., Rodríguez, A., Scholz, R, 2013. Nuevas variedades de trigo de alto rendimiento. Caninde 11, Caninde 12, Caninde 13. Investigación Agraria 15, 51–53.

Zhang, N., Luo, J., Rossman, A.Y., Aoki, T., Chuma, I., Crous, P.W., Dean, R., de Vries, R. P., Donofrio, N., Hyde, K.D., Lebrun, M.-H., Talbot, N.J., Tharreau, D., Tosa, Y., Valent, B., Wang, Z., Xu, J.-R., 2016. Generic names in Magnaporthales. IMa Fungus. 7, 155–159. https://doi.org/10.5598/imafungus.2016.07.01.09.

Zhong, Z., Chen, M., Lin, L., Han, Y., Bao, J., Tang, W., Lin, L., Lin, Y., Somai, R., Lu, L., Zhang, W., Chen, J., Hong, Y., Chen, X., Wang, B., Shen, W.C., Lu, G., Norvienyeku, J., Ebbole, D.J., Wang, Z., 2018. Population genomic analysis of the rice blast fungus reveals specific events associated with expansion of three main clades. ISME J. 12, 1867–1878. https://doi.org/10.1038/s41396-018-0100-6.