Blast disease associated with grass weeds in rice production systems of Paraguay †

[*Pyriculariosis* asociada a malezas en sistemas de producción de arroz en Paraguay]

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SUMMARY

Background: Several fungal species within the genus *Pyricularia* are associated with blast diseases affecting Poaceae crops, including wheat, rice, oat, barley, triticale, and various grass weeds. Among these, *Pyricularia oryzae* is recognized as a major pathogen due to its significant impact on cereal production worldwide. The fungus can survive on weed hosts, potentially serving as inoculum reservoirs. **Objective:** To characterize grass weed species acting as alternative hosts for *Pyricularia* spp. in rice agroecosystems in Paraguay. **Methodology:** During the 2020/2021 rice season, symptomatic weed samples exhibiting elongated or elliptical lesions with reddish-brown margins and lighter centers were collected across major rice-producing regions. Samples were processed at the Microbiology and Biotechnology laboratories of the Facultad de Ciencias y Tecnología, Universidad Nacional de Itapúa, and the Instituto Paraguayo de Tecnología Agraria (IPTA). **Results:** Morphological characterization of isolates cultured on oat agar revealed grayish to dark-gray colonies, with conidia hyaline, pyriform to obclavate, and containing two transverse septa. Molecular identification based on ITS region sequencing confirmed isolates from *Cynodon dactylon* as *Pyricularia grisea* (99.8% similarity) and from *Echinochloa* sp. as *Pyricularia oryzae* (100% similarity). Pathogenicity assays in rice seedlings (cv. IRGA 424) reproduced typical blast symptoms for the rice-derived isolate, whereas the *Cynodon dactylon*- and *Echinochloa*-derived isolates elicited differential reactions, including hypersensitive, incompatible, and mild blast-like symptoms. **Implications:** These findings underscore the epidemiological role of grass weeds as alternative hosts and potential inoculum reservoirs of *Pyricularia* spp., emphasizing the need to integrate weed monitoring and management into comprehensive blast disease control strategies in rice cultivation systems. **Conclusion:** Grass weed species present in rice agroecosystems of Paraguay were identified as reservoirs for *Pyricularia* spp., highlighting their potential role in pathogen survival and dissemination within rice fields.

Key words: Alternative hosts; Inoculum reservoirs; blast rice disease.

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RESUMEN

Antecedentes: Diversas especies fúngicas del género *Pyricularia* están asociadas con enfermedades "Blast" que afectan a Poaceaes, incluyendo trigo, arroz, avena, cebada, triticale y diversas malezas gramíneas. Entre ellas, *Pyricularia oryzae* es reconocida como un patógeno de gran importancia debido a su impacto significativo en la producción de cereales a nivel mundial. El hongo puede sobrevivir en hospederos alternativos, que potencialmente actúan como reservorios de inóculo. **Objetivo:** Caracterizar especies de malezas gramíneas que actúan como hospedantes alternativos de *Pyricularia* spp. en agroecosistemas arroceros de Paraguay. **Metodología:** Durante la zafra arrocera 2020/2021, se recolectaron muestras sintomáticas de malezas que presentaban lesiones alargadas o elípticas, con bordes castaño-rojizos y centros más claros, en las principales regiones productoras de arroz. Las muestras se procesaron en los Laboratorios de Microbiología y Biotecnología de la Facultad de Ciencias y Tecnología de la Universidad Nacional de Itapúa y en el Instituto Paraguayo de Tecnología Agraria (IPTA). **Resultados:** La caracterización morfológica de los aislados cultivados en agar avena reveló colonias de color grisáceo a gris oscuro, con conidios hialinos, de forma piriforme a obclavada y con dos septos transversales. La identificación molecular basada en la secuenciación de la región ITS confirmó que los aislados provenientes de *Cynodon dactylon* correspondieron a *Pyricularia grisea* (99,8 % de similitud) y los de *Echinochloa* sp. a *Pyricularia oryzae* (100 % de similitud). Los ensayos de patogenicidad en plántulas de arroz (cv. IRGA 424) reprodujeron síntomas típicos de *Pyricularia* para el aislado proveniente de arroz, mientras que los aislados de *Cynodon dactylon* y *Echinochloa* provocaron reacciones diferenciales, incluyendo respuestas hipersensibles, incompatibles y síntomas leves. **Implicancias:** Estos resultados resaltan el papel epidemiológico de las malezas gramíneas como hospederos alternativos y potenciales reservorios de inóculo de *Pyricularia* spp., enfatizando la necesidad de integrar el monitoreo y manejo de malezas en las estrategias de control integral de la enfermedad en sistemas de cultivo de arroz. **Conclusión**: Las especies de malezas gramíneas presentes en los agroecosistemas arroceros de Paraguay fueron identificadas como reservorios de *Pyricularia* spp., lo que destaca su papel potencial en la supervivencia y diseminación del patógeno dentro de los arrozales.

Palabras clave: Hospedero alternativo; reservorio de inóculos; enfermedad del añublo del arroz.

INTRODUCTION

It is estimated that global rice (*Oryza sativa* L.) production will need to double by 2050 to meet the increasing demand for food driven by global population growth (FAO, Food and Agriculture Organization 2009). As one of the most important cereals, rice provides up to 50% of caloric intake for millions in low-income regions, playing a critical role in global food security (Muthayya *et al*., 2014). In 2021, global production surpassed 513 million tons, with projections of 516 million tons by 2023 (FAO, 2025). In South America, rice is cultivated and consumed extensively in Brazil, Argentina, Uruguay, Paraguay, and Bolivia, accounting for approximately 28.5 million tons, or 5.7% of global output (FAO, 2021).

Rice cultivation faces unique challenges due to its soil and water requirements, as well as the technological inputs necessary for production. Pests and diseases are among the most significant threats. To mitigate these, strategies such as improved agronomic practices, resistant cultivars, and integrated pest management have been adopted to enhance sustainability (Rebolledo-Cid *et al*., 2018).

Fungal diseases are particularly problematic in rice because flooded fields provide optimal conditions for pathogen proliferation. Among them, rice blast, caused by *Pyricularia oryzae* Cavara pathotype *Oryza*, is the most destructive, leading to yield losses of up to 80% (Gowrisri 2019; Farman 2007; Tosa and Chuma 2014). The disease is globally distributed across Asia, the Americas, Oceania, and Europe. Due to its epidemic potential and substantial economic impact, with annual yield losses ranging from 10–30%, it ranks among the ten most devastating fungal diseases worldwide (Asibi *et al*., 2019; Mohanty *et al*., 2013; Thierry *et al*., 2022).

Between 2010 and 2020, Paraguay experienced a sharp expansion in rice cultivation, with harvested area increasing from fewer than 60,000 ha to ~182,000 ha in the 2019/2020 season, positioning the country as an important regional supplier (USDA-FAS, 2024). However, fungal diseases have become major production constraints, especially in years with abundant rainfall and high temperatures that favor fungal development (Quintana *et al*., 2020). The intensification of rice systems, combined with practices such as nitrogen fertilization, chemical control, and improved cultivars, may further modify host-pathogen interactions, favoring the emergence of new fungal threats.

The genus *Pyricularia* affects over 50 cultivated and wild Poaceae species, including cereals such as rice (*Oryza sativa*), wheat (*Triticum aestivum*), oat (*Avena sativa*), barley (*Hordeum vulgare*), and rye (*Secale cereale*), in addition to grasses and weeds associated with these systems (Bastida *et al*., 2019; Gutiérrez and Cúndom, 2015; Perelló *et al*., 2017). Understanding the inter- and intraspecific diversity of this genus is essential to elucidate potential cross-infection pathways and predict disease dynamics in agroecosystems (Chávez *et al*., 2022; Kobayashi *et al*., 2023). These insights are crucial for sustainable management strategies that reduce reliance on chemical inputs while safeguarding crop yields.

Population genomic studies of *P. oryzae* have revealed the expansion of three major clades. Although host resistance (R) genes are central to management, the pathogen's high genetic variability undermines their long-term effectiveness (Inoue *et al*., 2017). Thus, characterizing population structure and mechanisms of pathogen diversification is critical for durable disease control (Gladieux *et al*., 2018; Latorre *et al*., 2020).

In Paraguay, rice blast represents the most serious threat to production, particularly under favorable climatic conditions such as those during the 2015 El Niño, when severe outbreaks were reported in cultivars IRGA 417, Puitá, and the hybrid INOV (Quintana *et al*., 2020). Alternative hosts, including *Echinochloa* spp. and *Oryza sativa* f. *spontanea* (weedy rice), act as reservoirs of inoculum, enabling pathogen persistence across cropping seasons (Chung *et al*., 2020; Qi *et al*., 2019). Recent detection of *P. oryzae* in non-cultivated Poaceae in Paraguay supports the hypothesis that wild grasses are critical to blast epidemiology, complicating disease control efforts (Cazal-Martínez *et al*., 2025).

Climate change, particularly elevated temperatures, has further favored blast epidemics, consolidating it as one of the most destructive diseases in rice agroecosystems (Asibi *et al*., 2019). Throughout the crop cycle, diverse weeds and wild rice persist under similar microclimates as cultivated rice. These non-crop hosts not only compete for resources but also serve as inoculum reservoirs, particularly under favorable environments, imposing yield penalties if not managed adequately (Diagne *et al*., 2025; Ramírez *et al*., 2017).

Although *P. oryzae* has a broad host range, isolates are typically host-specialized and classified into pathotypes according to compatibility with specific hosts (Chung *et al*., 2020). Non-crop Poaceae, especially wild and weedy grasses, may act as interseasonal inoculum sources, facilitating pathogen persistence and interspecific transmission. The co-occurrence of multiple *Pyricularia* species in blast complexes highlights the importance of ecological biodiversity in shaping pathogen emergence and lineage diversification.

Consequently, effective management must transcend cultivar-based resistance and plot-level interventions. Instead, landscape-scale surveillance and identification of non-crop inoculum sources should be integrated into sustainable management frameworks. Despite the recognized epidemiological role of alternative hosts, data on the occurrence of *Pyricularia* spp. in Poaceae weeds associated with rice systems in Paraguay remain lacking. To address this gap, the present study aimed to (i) conduct field surveys in major rice-growing regions to identify grass weed species serving as potential hosts of *Pyricularia* spp. and (ii) characterize the pathogen using morphological traits and phylogenetic analyses.

MATERIALS AND METHODS

The experiments were conducted from February 2020 to September 2021 in Paraguay. Laboratory work was performed at the Laboratorio de Microbiología and the Laboratorio de Biotecnología of the Facultad de Ciencias y Tecnología, Universidad Nacional de Itapúa, Encarnación. Complementary phytopathological evaluations and pathogenicity assays were carried out in growth chambers and phytopathology laboratories of the Instituto Paraguayo de Tecnología Agraria (IPTA), Capitán Miranda and Caacupé research stations.

Sampling and Symptom Observation

Sampling was performed in rice-producing districts of Itapúa, Misiones, and Caazapá. A convenience-based random strategy targeted symptomatic rice plants and grass weeds showing blast-like lesions (elongated, diamond-shaped, dark with gray centers and brown margins). Samples were collected along 50 m transects, with 200 m between points. To induce sporulation, samples were placed in containers with urea solution (10 g in 5 L water). Lesions were examined under a BOECO stereomicroscope (400×) to confirm morphology (Bastida *et al*., 2019).

Isolation and Morphological Identification

Blast-symptomatic tissues (~5 mm) were surface-sterilized in 5% sodium hypochlorite (2 min), rinsed in sterile distilled water, and dried on sterile filter paper. Fragments were plated on potato-dextrose-agar (PDA) in 9 cm Petri dishes (five per plate) and incubated at 25 ± 5°C for 8–10 days. Mycelium resembling *Pyricularia* was subcultured on oatmeal agar (OA) to obtain pure cultures (Chávez and Kohli, 2015). Morphological characterization included colony traits (texture, color, growth) and conidial features (shape, pigmentation, septation). Subcultures were incubated at 25°C for 10 days on OA, and conidia were examined under a ZEISS-Primostar microscope (400×). Fifty conidia per isolate were measured with an ocular micrometer (Klaubauf *et al*., 2014; Bastida *et al*., 2019; French and Hebert 1980).

Molecular and Phylogenetic Analysis

Genomic DNA was extracted from 40 ± 10 mg of mycelium grown on OA (10 days, 25 ± 2°C, 12 h light/dark) using the Wizard® Genomic DNA

Purification Kit (Promega). Mycelium was frozen in liquid nitrogen, macerated in 1.5 mL tubes, and DNA quality was assessed on 1% agarose gels with 0.5× TBE buffer (Sambrock *et al*., 1990).
The ITS region was amplified with primers ITS1 and ITS4. PCR products were visualized on 2% agarose gels and sequenced at Macrogen Inc. (Seoul, South Korea). Consensus sequences were assembled in BioEdit v2 and compared against GenBank via BLAST.

Phylogenetic analyses followed Klaubauf *et al*. (2014). Sequences were aligned with MAFFT v7 (Katoh *et al*., 2002, 2017; Katoh and Standley, 2013) and refined with PhyDE v10.0 (Müller *et al*., 2005). The ITS matrix included 33 taxa and 670 characters. Maximum likelihood analyses were run in RAxML v8.2.10 (Stamatakis, 2014) using GTR+G, with 1000 bootstrap replicates under GTRCAT. Bayesian inference was performed with MrBayes v3.2.6 (Huelsenbeck and Ronquist, 2001). The best-fit model was selected with PartitionFinder (Frandsen *et al*., 2015; Lanfear *et al*., 2014, 2017). Two MC3 runs (10 million generations) were performed until SD ≤ 0.1, convergence checked in Tracer v1 (Rambaut *et al*., 2014). The maximum clade credibility tree was obtained with TreeAnnotator v1.8 (Bouckaert *et al*., 2014), with 25% burn-in.

Pathogenicity Assays

Pathogenicity was tested on 30-day-old IRGA 424 seedlings under controlled conditions. For each *Pyricularia* isolate from weeds, five pots (five plants each) were inoculated. Isolates were cultured on OA, and a conidial suspension (5×10^4 conidia/mL) was prepared and sprayed (50 mL per pot) onto plants fertilized with 10 cc urea.

Three isolates were tested: N12 (*Oryza sativa* cv. IRGA 424), N10 (*Cynodon dactylon*), and N11 (*Echinochloa* sp.). Cultures on PDA were transferred to OA and incubated for 7 days at 28 ± 5°C. Mycelia were crushed with a Drigalski spatula, incubated at >70% humidity for 48 h, and sporulating conidia were harvested in 10 mL sterile water with 0.01% Tween 20, filtered, and adjusted with a Neubauer chamber.

Seeds of IRGA 424 were sown in 20–25 cm pots with commercial substrate (Carolina Soilial). Four plants per pot were grown under growth chamber conditions (28 ± 5°C, 12 h photoperiod, daily watering). Inoculation at the 4-leaf stage was performed with an airbrush compressor (58 PSI). Plants were incubated in a humid chamber (28 ± 5°C, >90% RH, 12/12 h light/dark) for 24 h, then transferred to a growth chamber (28 ± 5°C, >80% RH) for 15 days.

Disease severity was assessed at 10 days post infections (dpi). Five leaves per replicate (five replicates per isolate) were evaluated, totaling 25 units per isolate. Symptom evaluation followed the IRRI blast scale (IRRI, 2013): 0 = no lesions; 1 = brown specks; 3 = necrotic spots (1–2 mm, brown margin); 5 = elliptical lesions (>3 mm); 7 = spindle-shaped lesions; 9 = coalescing grayish lesions. The Disease Severity Index (DSI) was calculated using the equation suggested by (Del Ponte, 2023):

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

Statistical analyses were performed with Microsoft Excel and GraphPad Prism v10.5.0. Data normality was tested with Shapiro-Wilk, followed by ANOVA and Tukey's HSD test (α = 0.05).

RESULTS

Symptomatology and Weed Host Identification

Pyricularia species were detected on Poaceae weeds in Paraguay's rice production systems. Nine weed species were sampled across Itapúa, Misiones, and Caazapá (Table 1).

Table 1. Weed species sampled in rice-producing departments of Paraguay.

		Departments $^{(*)}$	
Weed Species Collected and Identified	**Itapúa**	**Misiones**	**Caazapá**
Brachiaria decumens -Poaceae; perennial grass	0	0	0
Cenchrus echinatus - Poaceae; annual spiny grass	0	0	0
Cynodon dactylon - Poaceae; stoloniferous perennial	3	0	-
Digitaria insularis - Poaceae; perennial rhizomatous	0	0	0
Digitaria sanguinalis - Poaceae; annual tufted grass	0	0	0
Echinochloa spp. - *Poaceae*; annual barnyardgrass complex	3	0	0
Eleusine indica - Poaceae; annual prostrate grass	0	-	0
Eragrostis Pilosa - Poaceae; annual grass	0	0	-
Setaria parviflora - Poaceae; perennial grass	0	0	0
Total isolates obtained per weed species	6	0	0

(*) Numbers indicate colonies obtained; (–) indicates the weed was not found in the region.

Blast-like symptoms included small, dark green oblong spots that evolved into lesions with pale centers and dark margins. With disease progression, lesions elongated into elliptical shapes with reddish-brown borders, frequently coalescing to form large necrotic areas. Advanced stages caused partial or complete drying of the leaf blade. In total, Pyricularia colonies were recovered from two species: *Cynodon dactylon* and *Echinochloa* sp. (six isolates). Representative symptoms from these weeds are shown in Figure 1A–C.

Morphological and molecular characteristics

On oatmeal agar (OA), colonies showed greenish-gray to olive tones with dense cottony white aerial mycelium. Microscopic observation confirmed hyaline, pyriform conidia with two transverse septa on simple septate conidiophores (Figure 1B, D), consistent with *Pyricularia* diagnostic traits.

ITS sequences from isolate N10 (*Cynodon dactylon*) and N11 (*Echinochloa* sp.) showed 98.8–100% similarity with *Pyricularia* spp. using BLAST. Sequences were deposited in GenBank under accession numbers OR573440 (*C. dactylon*, N10) and OR573441 (*Echinochloa* sp., N11). Additionally, an isolate from *Oryza sativa* (N12) was deposited under PV435140.1. Bayesian inference phylogeny (Figure 2) confirmed two distinct species: N10 grouped within *Pyricularia grisea* (from *Cynodon dactylon*), while N11 clustered within *Pyricularia oryzae* (from *Echinochloa* sp.). These represent the first confirmed reports of *Pyricularia* from these weed hosts in Itapúa, Paraguay.

Pathogenicity Test on Rice

The *Oryza sativa*-derived isolate (N12) produced typical blast symptoms, with 100% incidence and a median disease severity index (DSI) of 50% (Figure 3A–B). In contrast, the *Echinochloa*-derived isolate (N11) elicited hypersensitive, incompatible reactions, with <50% incidence (scale 0–1) and a median DSI of 0. Significant differences ($p < 0.01$) confirmed its non-pathogenicity on rice. The *Cynodon dactylon*-derived isolate (N10) induced a variable response: early lesions (scales 0–1) and advanced foliar infections (scales 2–3). Both incidence and DSI differed significantly ($p < 0.05$) from the *P. oryzae Oryza* isolate (N12).

DISCUSSION

The detection of *Pyricularia* spp. on *Cynodon dactylon* and *Echinochloa* sp. in Paraguayan rice fields provides new insights into blast epidemiology in South American agroecosystems. Morphological traits, combined with molecular confirmation, verified the presence of these pathogens on non-crop hosts common in rice systems. These results corroborate preliminary morphological reports by Vigo and Sotomayor (2016) and expand knowledge of *Pyricularia* diversity in Paraguay (Cazal-Martínez *et al.*, 2025). Observed features aligned with the taxonomic framework of Klaubauf *et al.* (2014) and reports by Bastida *et al.* (2019).

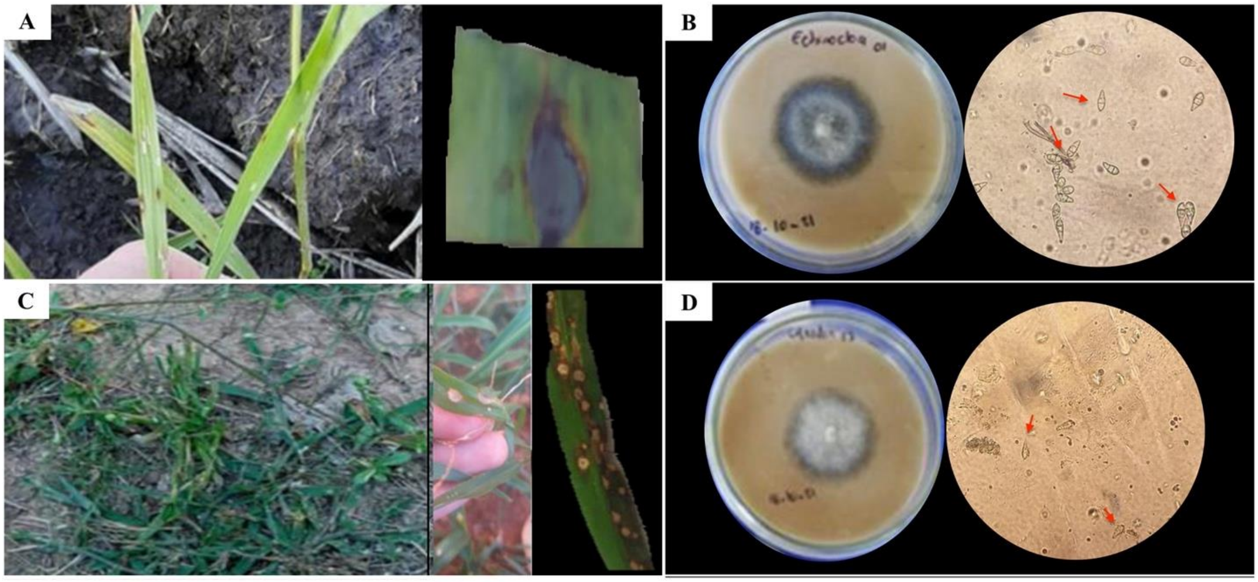

Figure 1. Blast symptoms and colony morphology of *Pyricularia* isolates. (A) Blast symptoms and (B) colony morphology (left) and conidia (right) of isolates from *Echinochloa* sp. (C) Blast symptoms and (D) colony morphology (left) and conidia (right) of isolates from *Cynodon dactylon*. (B and D, right) Microscopy at 400× revealed diagnostic *Pyricularia* features. Red arrows indicate fungal conidia.

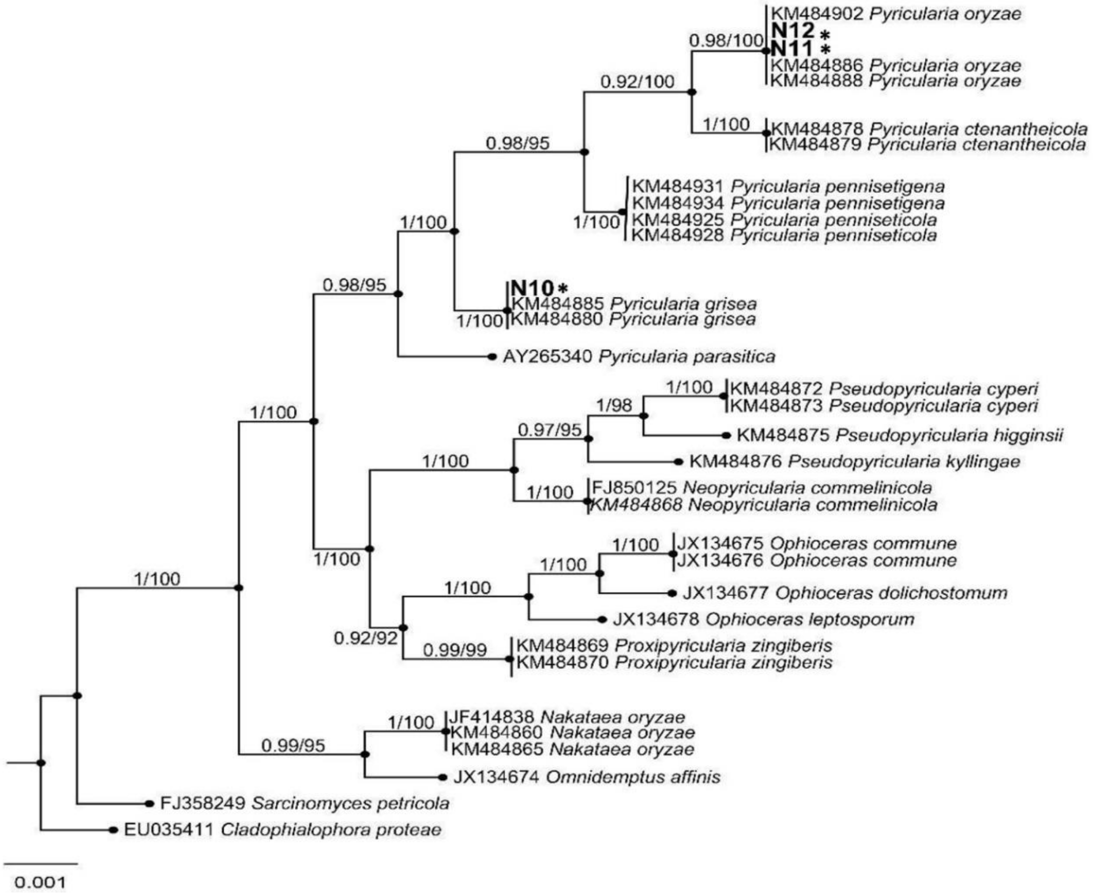

Figure 2 Bayesian inference tree based on ITS sequences. Posterior probabilities (left of slash) and bootstrap values (right) are shown. Study isolates are indicated with asterisks (*). Tree generated with TreeAnnotator v1.8 (Bouckaert *et al.*, 2014) with a 25% burn-in.

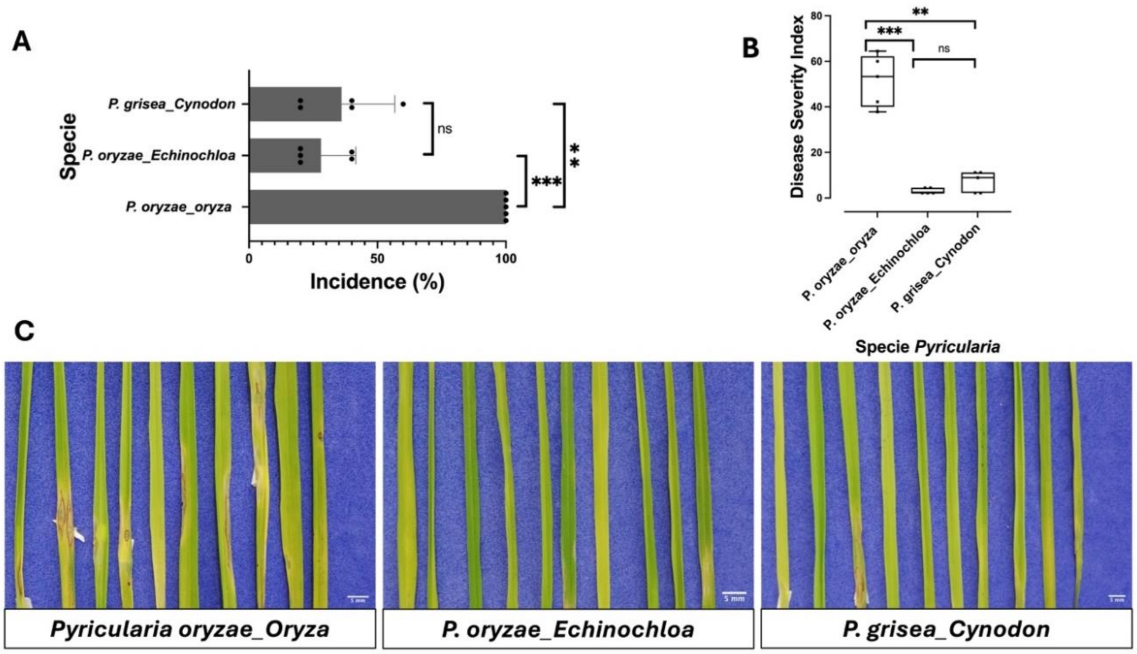

Figure 3. Pathogenicity of *P. oryzae* and *P. grisea* on rice cv. IRGA 424. (A) Incidence (%) of infected leaves (means of five leaves). **p < 0.05; ***p < 0.01. (B) Disease severity index (DSI). (C) Phenotypes of blast symptoms: compatibility between rice and isolates N10 (*C. dactylon*), N11 (*Echinochloa*), and N12 (*O. sativa*) (right to left). Scale bar = 5 mm.

The presence of *Pyricularia* on Poaceae weeds indicates their role as alternative inoculum reservoirs, contributing to disease persistence across seasons. Overlooking these hosts in management programs risks undermining blast control efforts. Weed surveillance and integration into field-level strategies are essential for reducing pathogen carryover and mitigating its impact in rice and wheat systems.

Regionally, weed-associated *Pyricularia* has been documented in wheat systems in Paraguay (Chávez and Kohli, 2015; Cazal-Martínez *et al*., 2025) and Argentina (Perelló *et al*., 2017). Our findings reveal a parallel scenario in rice, where aggressive weeds such as *Echinochloa* sp. and *C. dactylon* act as bridge hosts that sustain inoculum between cropping cycles, particularly in tropical and subtropical contexts with overlapping rice–weed growth. Molecular data confirmed *P. oryzae* (from *Echinochloa*) and *P. grisea* (from *C. dactylon*), consistent with phylogenetic frameworks (Gladieux *et al*., 2018). The detection of *P. oryzae* in *Echinochloa* is especially relevant, as this weed is widespread in rice fields. Although genetically classified as *P. oryzae*, the isolate from *Echinochloa* was non-pathogenic to rice, consistent with host specialization (Vigo and Sotomayor, 2016).

P. oryzae populations comprise >30 host-specialized subgroups, linked to rice blast (Valent *et al*., 1991), wheat blast (Couch and Kohn, 2002; Igarashi *et al*., 1986), and gray leaf spot in grasses (Farman, 2007). Studies show lineage-specific genomic traits and effector repertoires influencing host specificity (Asuke *et al*. 2020; Hyon *et al*., 2012; Inoue *et al*., 2021) Host-driven diversification is largely mediated by Avr gene dynamics, with frequent gain/loss events and polymorphisms generating novel haplotypes (Islam *et al*., 2019; Wang *et al*., 2023; Yoshida *et al*., 2016).

Cross-infection barriers have also documented (Couch *et al*., 2005; Kato *et al*., 2000; Tosa and Chuma, 2014). Our assays confirmed this: the rice isolate produced typical blast lesions, whereas weed-derived isolates were avirulent or weakly symptomatic. This supports the role of weeds mainly as reservoirs, not primary aggressors, in rice systems. The *C. dactylon*-derived *P. grisea* isolate did not cause severe disease but triggered hypersensitive responses (scales 0–3), suggesting latent host–pathogen interactions requiring further study. This highlights the need to characterize weed-derived isolates to understand their epidemiological relevance.

CONCLUSION

Field surveys conducted in the major rice-growing regions of Paraguay identified the grass weed species *Cynodon dactylon* and *Echinochloa* sp. acting as reservoirs of *Pyricularia* spp. Morphological characterization and phylogenetic analyses corroborated the identity of the pathogen isolates. Collectively, these findings highlight the need to expand weed sampling to strengthen population-level studies and to elucidate the ecological role of non-crop hosts within the blast disease complex.

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Conflict of Interest statement. The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Compliance with ethical standards. This study did not involve human participants or animals and therefore did not require approval from an ethics committee. All procedures performed in this study were in accordance with the ethical standards of the institution and with internationally accepted research ethics principles.

Data availability. The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Author Contributions statement (CRediT): B. Morinigo-Gimenez – Conceptualization, Investigation, Validation, Resources, Methodology, Visualization, and Writing – original draft. **A. Urashima** – Conceptualization. **A. Chavez** – Investigation, validation. Methodology, visualization. **M. Fernández-Gamarra** – Investigation, validation. Methodology, visualization. Writing – review and editing. **L. Talavera** – Data curation, Software. Formal analysis. Investigation, Validation, Methodology, Visualization, and Writing – review and editing. **R. Duarte** – Writing – review and editing. **C. Cazal-Martinez** – Data curation, Software. Formal analysis. Funding acquisition. Investigation, Validation. Writing – original draft and Writing – review and editing. **L. Quintana** – Conceptualization, Supervision, Funding acquisition, Investigation, Validation, Resources, Methodology, Visualization, and Writing – original draft and Writing – review and editing.

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