



RESEARCH ARTICLE

Morpho-molecular and pathogenic variability of *Pyricularia* isolates in millets and rice

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Abstract

Blast disease caused by *Pyricularia* species is a significant constraint in millets and rice worldwide. The present research attempted at studying the occurrence, isolation and identification of blast pathogen, with attention on the systematic position in the *Pyricularia* spp. complex and host-related pathotypes. To differentiate between genera and assess host specificity, internal transcribed spacer (ITS) related phylogenetic analysis has been used. During the 2024–2025 cropping seasons, a field assessment was carried out in Tamil Nadu millet and rice-growing districts as well as in a few locations in Andhra Pradesh. Leaf samples exhibiting typical blast symptoms was collected. Fifty blast isolates isolated by standard tissue isolation on potato dextrose agar (PDA) were purified using the single-spore isolation method. Morphological analysis showed a significant variance between the isolates with radial mycelial growth ranging from 62–89 mm with plate cover in 10–16 days. The typical pyriform, 3 celled conidia of *Pyricularia* spp. are produced by abundant sporulation in colony which vary in colour from grey to olivaceous. Detached-leaf inoculation revealed typical spindle-shaped lesions within 3–6 days after inoculation (DAI). Pearl millet isolates were most virulent (0.14–1.38 cm²; MgPM1 highest), followed by barnyard millet (0.04–0.48 cm²; MgBM3), while finger millet (0.06–0.21 cm²; MgFM4) and foxtail millet (0.02–0.18 cm²; MgFoxM3) showed lower virulence. The ITS-based molecular identification yielded ~560 bp amplicons, with 92–100 % similarity to *Pyricularia* spp. In the National Centre for Biotechnology Information (NCBI) GenBank, confirming identity. However, ITS lacked resolution for species and pathotype differentiation, requiring further phylogenetic and pathogenicity analysis.

Keywords: cultural variability; detached leaf assay; internal transcribed spacer sequencing; *Pyricularia*

Introduction

Blast disease associated with *Pyricularia* species (teleomorph: *Magnaporthe*) is known as one of the foremost devastating diseases which affects agriculture crops globally. The pathogen spreads rapidly, infects a wide range of graminaceous hosts including rice and millets which causes severe yield losses under favourable conditions due to its adaptability across diverse agro-climatic regions (1–3). A major turning point in plant pathology was attained when Francesco Cavara (1892) found that *Pyricularia oryzae* from rice was the causative organism of blast disease, which was initially reported on *Digitaria sanguinalis* (L.) Scop (4).

Millets including barnyard millet, foxtail millet, finger millet and pearl millet are now more commonly accepted as climate-resistant crops which are necessary for maintaining adequate nutrition, particularly in semi-arid and marginal regions of Asia and Africa (5, 6). However, an important hindrance to millet production is the rising prevalence and severity of blast disease which affects multiple plant parts, including leaves, nodes, neck and

panicles, leading to reduced photosynthetic efficiency and poor grain filling. Under severe epidemic conditions, yield reductions of 10–30 % have been reported in pearl millet and finger millet (7, 8).

The diverse group of closely related species and host-adapted lineages that make up the genus *Pyricularia* show notable differences in morphological, physiological and genetic characteristics (9). Even though physical characteristics such as colony colour, growth pattern and the development of pyriform, two-septate conidia are utilised in traditional identification, these features are frequently insufficient to distinguish between isolates that are closely associated but derived from distinct host. When it comes to cross-infecting isolates, this limitation is especially noticeable since physical characteristics obscure basic genetic variation. The accuracy of pathogen identification and understanding of their phylogenetic relationships have risen substantially due to advances in molecular biology, such as the use of internal transcribed spacer (ITS) regions and broad genome analysis (10, 11). The significance of studying pathogen behaviour

across different hosts is shown by the high level of genetic similarity and similar host ranges of *Pyricularia* spp. that infect rice and millets. A more accurate understanding of host specificity, cross-infectivity and the evolutionary adaptation of blast pathogens relies on these types of studies. Different virulence patterns within pathogen populations can cause host resistance genes to break down quickly, make disease control measures more difficult (12).

Although millets grow becoming increasingly essential for sustainable agriculture and nutritional security, there are still few extensive studies which combine the morphological, molecular and pathogenic variation among *Pyricularia* isolates across various host species. In this regard, the present study was conducted to: (i) characterise *Pyricularia* isolates from rice and millets using morphological characteristics; (ii) confirm their identity using universal primers; and (iii) evaluate their pathogenic variability using virulence assays. *Pyricularia* isolates related to various host crops are considered to exhibit notable morphological and pathogenic variation because of host-specific adaptation, which leads to genetic diversity within the species group.

Materials and Methods

Survey and documentation of blast disease incidence in millets and rice fields

An intensive roving survey was conducted in 2024–2025 across the millets and rice-growing regions of India to assess the incidence of blast disease. A total of 30 different regions were surveyed and blast severity was recorded using a 0–9 scale with slight modification (13). In every field, 50 plants were selected at random as well as examined for the prevalence of the disease, which ensured a representative sample from all the locations (Table 1). The disease incidence was calculated using the following formula:

Percent disease incidence (PDI) =

$$\frac{\text{Number of infected plants}}{\text{Total number of plants observed}} \times 100$$

Isolation and purification of blast pathogen

Blast-infected leaves of pearl millet, finger millet, foxtail millet, barnyard millet and rice showing typical spindle-shaped lesions with grey centres and brown margins were collected at vegetative, flowering and tillering stages. Sampling was conducted using a random sampling design across multiple fields within each region with all sampling locations geo-referenced using global positioning system (GPS) coordinates (Table 1). The samples were first washed with sterile distilled water and then surface-sterilised using 1 % sodium hypochlorite for 1–2 min, followed by 3 rinses in sterile distilled water. The sterilised tissues were blot dried on sterile filter paper and cut into segments of 2–3 mm. Four leaf bits were aseptically placed on potato dextrose agar (PDA) amended with 50 µg mL⁻¹ streptomycin sulphate. The plates were incubated at 27 ± 2 °C for 7 PDA 10 days. Actively growing fungal cultures were subsequently purified through repeated sub-culturing onto fresh PDA and maintained for further studies.

Cultural and morphological characterisation of the blast pathogen

The cultural characteristics associated with pathogenic isolates from pearl millet, finger millet, foxtail millet, barnyard millet and rice were

evaluated for preliminary identification. Observations on colony texture, growth pattern, margin characteristics and overall colony morphology were recorded at regular intervals by following standard protocol (14). Microscopic observations were carried out to assess conidial characteristics, including shape and dimensions (length and width). Conidial measurements were taken from 10 days old plates culture using Image Focus Plus software (v2 4.9.0) under 400X magnification to ensure accuracy and consistency. Before being observed, spore suspensions were adjusted to a concentration of 1 × 10⁶ spores mL⁻¹.

Assessment of pathogenicity of blast isolates using detached leaf assay

Pathogenicity of blast isolates from pearl millet, finger millet, foxtail millet, barnyard millet and rice was confirmed using detached leaf assay on respective host plant leaves (15). Fresh leaves were collected from blast-susceptible commonly used varieties of each host (pearl millet Co-10, finger millet ATL-1, foxtail millet ATL-1, barnyard millet ATL-1 and rice CO39) and surface-sterilised with 1 % sodium hypochlorite for 1–2 min, followed by 3 rinses with sterile distilled water. The leaves were gently blot-dried and positioned abaxially in sterile petri plates lined with 2 layers of moistened filter paper to maintain humidity. Three replications were maintained. A 9 mm actively growing mycelial disc from each blast isolate was carefully placed on the centre of the leaves and were incubated at 25 ± 2 °C under high humidity. Leaves were monitored daily for symptom expression and lesion diameter was measured 7 days post-inoculation. To confirm pathogenicity, the fungus was re-isolated from representative lesions and cultured on PDA, thereby fulfilling Koch's postulates. Based on lesion size, symptom severity and consistency of infection in the detached leaf assay, the most virulent isolate was identified.

Molecular characterisation of blast isolates

DNA isolation

The genomic DNA of all 50 blast isolates was extracted using the cetyl trimethyl ammonium bromide (CTAB) method (16). 1 mg of the respective fungal mycelia was ground using liquid nitrogen into fine powder. The frozen mycelia were added with the 750 µL of DNA extraction (CTAB) buffer comprised of 0.1 M Tris-HCl (pH 7.5), 0.7 M NaCl, 10 mM (Ethylenediaminetetraacetic acid) EDTA. The suspension was incubated at 65 °C for 1 hr with intermittent shaking at 20 min interval. After incubation, 750 µL of phenol: chloroform: isoamyl alcohol (25:24:1) was added to each tube and centrifuged at 10000 rpm for 15 min at 4 °C. The resulting upper aqueous phase (450 µL) was transferred to a new tube and 2 volumes of ice-cold isopropanol (600 µL) with 150 µL of 5M NaCl was added. The tubes were incubated overnight at -20 °C. After incubation, the tubes were centrifuged at 10000 rpm for 15 min. The supernatant was discarded and the pellet was washed with 70 % ethanol and air-dried. The DNA was suspended in 30 µL Tris-EDTA (TE) buffer (10 mM Tris, 1 mM EDTA, pH 8.0), the volume based on the size of the pellet obtained. The DNA concentration was quantified using NanoDrop and stored at -20 °C.

Polymerase chain reaction amplification using internal transcribed spacer primers

The internal transcribed spacer (ITS) region was amplified from blast isolates obtained from pearl millet (1–30), finger millet (31–35), foxtail millet (36–40), barnyard millet (41–45) and rice (46–50). The ITS region was amplified using the universal primers ITS1

(5'-TCC GTA GGT GAA CCT GCG G-3') and ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3') following the previously known protocol (17). Polymerase chain reaction (PCR) amplification was carried out in a 20 μL reaction mixture containing 10 μL of 2X Ampliqon® Taq DNA Polymerase Master Mix RED, 1 μL each of forward and reverse primers (0.5 μM μL^{-1}), 1 μL of genomic DNA (50 ng μL^{-1}) and 7 μL of nuclease-free water. The PCR was performed in a Qiagen QIA amplifier 96 thermal cycler with an initial denaturation at 95 °C for 2 min, followed by 35 cycles of denaturation at 94 °C for 30 sec, annealing at 56 °C for 1 min and extension at 72 °C for 2 min, with a final extension at 72 °C for 8 min.

Gel documentation and sequencing

The amplified PCR products were separated by electrophoresis in a 1 % agarose gel with 0.5 μgML^{-1} ethidium bromide in 1X Tris-Acetate-EDTA (TAE) buffer (0.4 M Tris, 0.2 M acetic acid, 10 mM EDTA, Ph 8.4) at 70v for 1 hr and photographed in a gel documentation system (UVITECH, Cambridge). A 100 bp ladder serving as a standard marker. The resultant PCR products were sequenced by Sanger's dideoxy sequencing technology at Biokart India Pvt. Ltd. The obtained nucleotide sequences were aligned using ClustalW and compared with GenBank database sequences through the BLASTN search tool. The validated sequences were then submitted to the National Centre for Biotechnology Information (NCBI) GenBank (18).

Phylogenetic tree construction

Phylogenetic analysis was performed for all isolates obtained in this study along with reference sequences from the GenBank database. Multiple sequence alignments were carried out using the MUSCLE algorithm in molecular evolutionary genetics analysis version XI (MEGA XI). The aligned sequences were refined by trimming low quality regions and phylogenetic relationships were inferred using

the neighbour-joining method based on the Kimura 2-parameter model. The robustness of the inferred tree was assessed using bootstrap analysis with 1000 replicates.

Statistical analysis

The experimental data on lesion diameter, disease severity and radial growth rate obtained from different treatments were subjected to statistical analysis using Agres software. The significance of treatment effects was evaluated through analysis of variance (ANOVA) at $p \leq 0.05$. When significant differences were observed, treatment means were separated using Duncan's multiple range test (DMRT) to determine statistically significant differences among treatments.

Results and Discussion

Field survey and assessment of blast disease incidence in millets and rice

A roving survey across millet and rice growing regions revealed significant variation in foliar blast incidence among crops, cultivars and locations. Pearl millet recorded the highest incidence (11.11–55.56 %), followed by finger millet (10.53–17.58 %), rice (10.25–15.58 %), foxtail millet (7.35–16.25 %) and barnyard millet (2.50–7.60 %) (Table 1; Fig. 1). Similar variability in blast incidence across regions and hosts has been reported earlier (19, 20). The higher incidence in pearl millet may be attributed to greater host susceptibility and favourable environmental conditions, particularly during early growth stages which facilitate rapid pathogen establishment (21). In contrast, lower incidence in barnyard millet indicates inherent tolerance and reduced host-pathogen compatibility (22). Variation across locations further shows the influence of host genotype and microclimatic factors on disease development. In addition to improve cultivar susceptibility, desirable

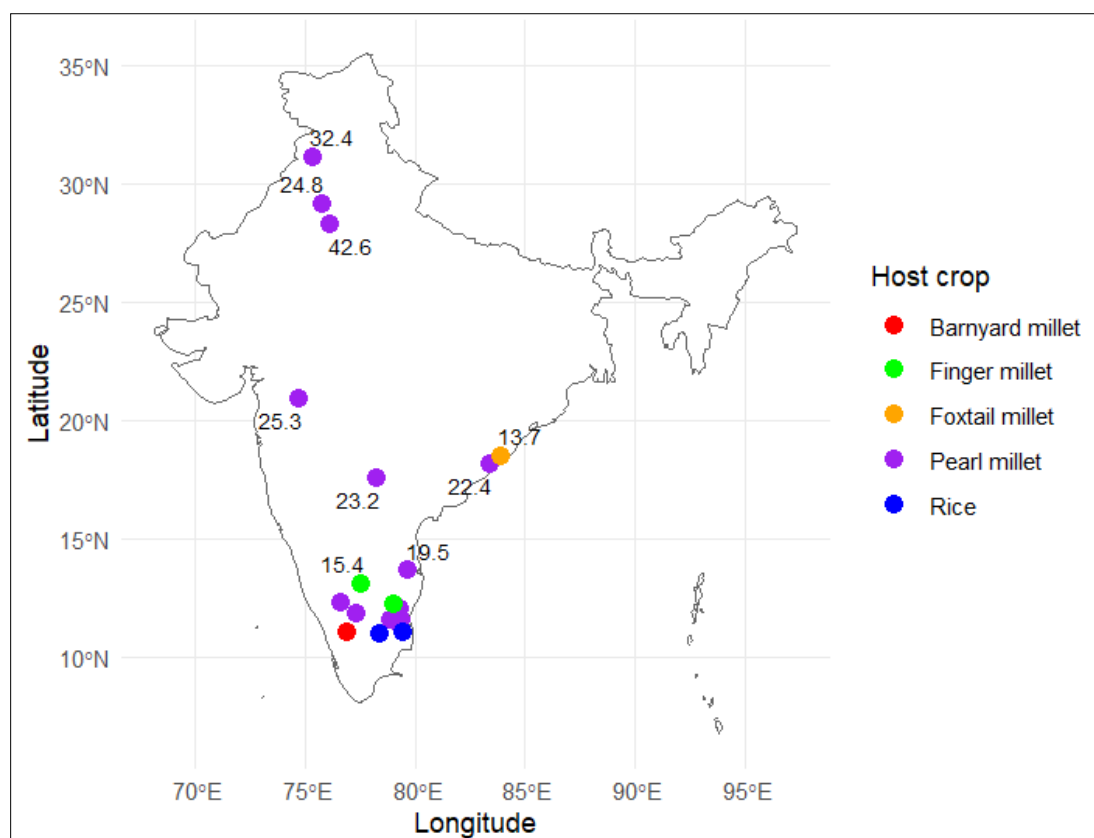


Fig. 1. Spatial distribution of blast disease incidence in major millet and rice growing regions of India [Source: Generated with R (v4.3.1) using ggplot2 and sf packages].

Table 1. Survey and assessment of foliar blast disease in major millet and rice-growing regions of India

Sl No.	District	Block	Village/ Location	Cultivars	GPS coordinates	Area (ac)	Crop stage	PDI %
Pearl millet								
1.	Cuddalore	Vridhachalam	Kuppanatham	Private hybrid	N11°32.434; E079°22.132	2.0	Vegetative stage	55.56 ^a (48.19)
2.		Nallur	Kandapanthurichi	Private hybrid	N11°34.345; E079°11.829	2.0	Heading stage	26.52 ^e (31.00)
3.		Kurinipadi	Iyankurinipadi	Local variety	N11°35.856; E079°37.729	1.0	Vegetative stage	25.50 ^e (30.33)
4.		Mangalur	Alambadi	Private hybrid	N11°59.534; E079°17.369	5.0	Vegetative stage	29.45 ^f (32.87)
5.		Veppur	Memathur	Private hybrid	N11°34.198; E079°12.043	2.0	Heading stage	23.55 ^h (29.03)
6.			Kadaganur	Co9 hybrid	N11°59.312; E079°15.214	1.5	Vegetative stage	23.33 ^h (28.88)
7.	Viluppuram	Mogaiyur	Mogaiyur	Ankur hybrid	N11°58.390; E079°19.250	1.0	Vegetative stage	28.52 ^f (32.28)
8.			Ayandur	Private hybrid	N11°58.206; E079°20.026	1.0	Vegetative stage	33.33 ^e (35.26)
9.			Kaanankaattupattu	Local variety	N11°58.007; E079°21.122	1.0	Flowering stage	30.53 ^f (33.54)
10.		Ulundurpet	Pallavadi	Private hybrid	N 11°48.757; E079°11.276	1.0	Vegetative stage	27.50 ^g (31.63)
11.	Kallakurichi	Rishivandhiyam	Kallipaadi	Local variety	N11°59.746; E079°04.645	1.0	Heading stage	45.56 ^b (42.45)
12.		Chinnasalem	Krishnapuram	Pioneer hybrid	N11°34.798; E078°53.223	1.0	Vegetative stage	23.53 ^h (29.02)
13.		Thirunavalur	Pa. Killanur	Local variety	N11°45.860; E079°19.457	3.0	Flowering stage	11.11 ⁱ (19.47)
14.		Ulundurpet	Pinnalvadi	Pioneer hybrid	N11°43.337; E078°58.250	2.0	Heading stage	35.40 ^d (36.51)
15.	Erode	Bhavanisagar	Maranur	Private hybrid	N11°47.030; E077°20.954	2.0	Vegetative stage	29.56 ^f (32.94)
16.		Sathiyamangalam	Ariyampalayam	Local variety	N11°28.357; E077°12.100	1.0	Heading stage	25.45 ^e (30.30)
17.	Coimbatore	Coimbatore	TNAU Research Farm	Co 9 hybrid	N11°01.480; E076°55.375	1.0	Flowering stage	32.52 ^e (34.77)
18.	Ariyalur	Andimadam	Alagapuram	Murali hybrid	N11°21.294; E079°21.010	2.0	Vegetative stage	28.45 ^f (32.23)
19.	Mysore	Mysore	Mysore	Private hybrid	N12°18.000; E076°39.000	1.0	Booting stage	29.10 ^f (32.65)
20.	Tirupathi	Tirupathi	Tirupathi	Local variety	N13°38.000; E079°42.000	2.0	Heading stage	19.50 ⁱ (26.21)
21.	Vijayanagaram	Vijayanagaram	Vijayanagaram	Local variety	N18°07.920; E083°25.506	1.5	Vegetative stage	22.42 ^h (27.33)
22.	Dhule	Dhule	Dhule	Local variety	N20°54.253; E074°46.494	4.0	Flowering stage	25.34 ^g (28.26)
23.	Ananthapur	Ananthapur	Ananthapur	Private hybrid	N14°41.000; E077°36.000	2.5	Heading stage	30.40 ^f (30.22)
24.	Punjab	Punjab	Punjab	Private hybrid	N31°08.500; E075°20.470	1.5	Heading stage	32.44 ^e (33.46)
25.	Vanniyanaagaram	Vanniyanaagaram	Vanniyanaagaram	Local variety	N12°40.000; E078°40.000	2.0	Heading stage	23.22 ^h (34.72)
26.	Thiruvannamalai	Thiruvannamalai	Thiruvannamalai	Local variety	N12°13.718; E079°03.993	3.0	Flowering stage	18.50 ⁱ (28.81)
27.	Hisar	Hisar	Hisar	Private hybrid	N29°10.000; E075°46.000	1.0	Vegetative stage	24.80 ^g (25.47)
28.	Mohindergarh	Mohindergarh	Mohindergarh	Private Hybrid	N28°16.160; E076°09.153	2.0	Heading stage	42.56 ^c (29.87)
29.	Malnoor	Malnoor	Malnoor	Local varitey	N18°05.000; E079°30.000	1.0	Booting stage	32.44 ^e (40.72)
30.	Telangana	ICRISAT	ICRISAT	Hybrid	N17°31.800; E078°15.870	1.5	Heading stage	23.22 ^h (34.72)

Finger millet								
1.	Coimbatore	Coimbatore	Department of millets	ATL1	N12°07.286; E78°99.044	1.0	Vegetative stage	10.53 ^l (28.81)
2.	Andhra Pradesh	Srikakulam	Srikakulam	KM252	N18°29.49; E83°89.38°	1.0	Flowering stage	12.43 ^k (20.64)
3.	Bangalore	Bangalore	Bangalore	KMR 630	N13°07.81; E77°57.92°	1.0	Vegetative stage	15.44 ^j (23.14)
4.				KMR 316	N13°07.81; E77°57.92°	2.0	Vegetative stage	17.58 ^{ij} (24.79)
5.	Thiruvannamalai	Athiyandhal	Athiyandhal	ATL1	N12°48.5448; E78°96.9599	1.0	Flowering stage	16.50 ^j (23.97)
Foxtail millet								
1.	Thiruvannamalai	Kalasapakkam	Vannianur	ATL1	N12°44.868; E79°07.730	2.0	Flowering stage	13.65 ^k (21.68)
2.		Pudhupalayam	Eraiur	ATL1	N12°28.0573; E78°61.1008	1.0	Vegetative stage	10.02 ^l (18.45)
3.		Kalasapakkam	Elathur	ATL1	N12°44.580; E79°06.128	1.5	Vegetative stage	7.35 ^m (15.73)
4.		Pudhupalayam	Kanthapalayam	ATL1	N12°48.5448; E78°96.9599	1.0	Flowering stage	7.63 ^m (16.04)
5.	Coimbatore	Coimbatore	Department of millets	ATL1	N12°07.286; E78°99.044	1.0	Flowering stage	16.25 ^j (23.77)
Barnyard millet								
1.	Andhra Pradesh	Srikakulam	Srikakulam	VMBC 353 variety	N18°29.49; E83°89.38°	1.5	Vegetative stage	7.60 ^m (16.00)
2.	Coimbatore	Coimbatore	Department of millets	ATL1	N12°07.286; E78°99.044	0.5	Vegetative stage	6.50 ^m (14.77)
3.				ATL1	N12°07.286; E78°99.044	0.5	Vegetative stage	4.25 ⁿ (11.90)
4.	Thiruvannamalai	Athiyandhal	Athiyandhal	ATL1	N12°07.28; E78°99.044	1.0	Vegetative stage	2.50 ⁿ (9.10)
5.				Local variety	N12°07.28; E78°99.044	0.5	Vegetative stage	3.50 ⁿ (10.78)
Rice								
1.	Coimbatore	Coimbatore	Paddy breeding station	CORH 1	N10°99.53; E76°91.65	1.0	Tillering stage	14.58 ^j (22.45)
2.				ADT 43	N10°99.53; E76°91.65	0.5	Tillering stage	15.58 ^j (23.25)
3.			Wetland	IR 50	N10°57.51; E76°45.29.	0.5	Tillering stage	13.70 ^k (21.72)
4.	Ariyalur	Farmers field	Farmers field	CR 1009	N10°54'; 78°40' E79°30'	0.5	Tillering stage	12.68 ^k (20.86)
5.	Thanjavur	Aduthurai	Tamil Nadu Rice Research Institute	BPT 5204	N10°99'; 79°48'E	0.5	Tillering stage	10.25 ^l (18.67)
SEd								0.495
CD (0.05)								0.983

*Values represent the mean of 3 replications.

Means followed by the same letter are not significantly different at the 5 % level as determined by DMRT.

conditions such high humidity and rainfall, which favour pathogen penetration and spread, are the reason for a higher incidence in pearl millet. On the other hand, the lower incidence in barnyard millet shows less ideal climatic factors and inherent resistance to host.

Isolation and cultural characterisation of blast isolates

A total of 50 blast isolates obtained from different hosts exhibited considerable variability in colony morphology, growth, sporulation and conidial characteristics. Radial mycelial growth ranged from 62.00–89.00 mm with plate coverage within 10–16 days. Such morphological variability among *Pyricularia* isolates has been widely documented (23). Sporulation ranged from 2.00×10^6 to 6.00×10^6 spores mL⁻¹, indicating differences in reproductive potential among isolates. Conidia are pyriform to obclavate, hyaline to pale olive, two-septate, measuring

10.31–32.16 $\mu\text{m} \times 4.38$ –16.15 μm , consistent with earlier reports (24, 25). Greater variability in pearl millet isolates suggests strong host-pathogen interaction and higher disease pressure (13), whereas relatively uniform characters in finger millet indicate stable host adaptation (26). Moderate variability in foxtail millet and larger conidia in barnyard millet may reflect partial host compatibility and adaptive survival mechanisms (27, 28). However, rice isolates produced the smallest and most diverse conidia (10.31–23.97 $\mu\text{m} \times 5.44$ –16.15 μm), while barnyard millet isolates formed the biggest conidia (28.98–32.16 $\mu\text{m} \times 7.29$ –8.61 μm), followed by pearl millet and foxtail millet isolates. The Fig. 2 depicts the pairwise distribution and association of growth and conidial characteristics across blast isolates from the present study. Overall, this variability is attributed to genetic diversity and host-driven selection within the *Pyricularia* species complex.

Table 2. *In vitro* evaluation of pathogenicity of blast isolates on millets and rice using detached leaf assay

Sl. No	Isolates	Initial symptom expression	Colour of lesion	Length of lesion (cm)	Breadth of lesion (cm)	Area (cm ²)	Virulence strategy	Reaction type
Pearl millet								
1.	MgPM1	3	Brown with grey centre	2.3 ^a	0.6 ^a	1.38 ^a	Highly virulent	IV
2.	MgPM2	5	Brown with grey center	1.1 ^g	0.3 ^d	0.33 ^k	Less virulent	IV
3.	MgPM3	4	Brown with grey center	1.3 ^e	0.4 ^c	0.52 ^f	Moderate virulent	IV
4.	MgPM4	6	Brown	0.8 ^j	0.3 ^d	0.24 ^q	Moderate virulent	III
5.	MgPM5	5	Brown	0.8 ^j	0.4 ^c	0.32 ^l	Moderate virulent	IV
6.	MgPM6	6	Brown with grey center	0.5 ^m	0.3 ^d	0.15 ^s	Less virulent	II
7.	MgPM7	6	Brown with grey center	0.7 ^k	0.2 ^e	0.14 ^t	Less virulent	II
8.	MgPM8	4	Brown	1.3 ^e	0.4 ^c	0.52 ^f	Moderate virulent	IV
9.	MgPM9	4	Dark Brown	1.4 ^d	0.6 ^a	0.84 ^b	Moderate virulent	IV
10.	MgPM10	6	Brown with grey center	1.5 ^c	0.1 ^f	0.15 ^s	Less virulent	II
11.	MgPM11	4	Brown	1.4 ^d	0.4 ^c	0.56 ^e	Moderate virulent	IV
12.	MgPM12	4	Brown	1.2 ^f	0.4 ^c	0.48 ^g	Moderate virulent	IV
13.	MgPM13	4	Brown with grey center	1.6 ^b	0.4 ^c	0.64 ^c	Moderate virulent	IV
14.	MgPM14	5	Dark Brown	1.5 ^c	0.2	0.30 ^m	Moderate virulent	IV
15.	MgPM15	5	Dark Brown	1.1 ^g	0.4 ^c	0.44 ⁱ	Moderate virulent	IV
16.	MgPM16	6	Brown, grey centre	0.9 ⁱ	0.2 ^e	0.18 ^r	Less virulent	II
17.	MgPM17	4	Dark brown, grey centre	1.6 ^b	0.3 ^c	0.48 ^g	Moderate virulent	IV
18.	MgPM18	5	Dark brown	1.2 ^g	0.2 ^c	0.24 ^q	Moderate virulent	III
19.	MgPM19	6	Brown, grey centre	1.3 ^e	0.2 ^e	0.26 ^p	Moderate virulent	III
20.	MgPM20	5	Dark brown	1.4 ^d	0.2 ^e	0.28 ⁿ	Moderate virulent	III
21.	MgPM21	5	Dark brown grey centre	1.0 ^h	0.3 ^d	0.30 ^m	Moderate virulent	III
22.	MgPM22	4	Brown	1.5 ^c	0.3 ^d	0.45 ^h	Moderate virulent	IV
23.	MgPM23	4	Dark brown	1.2 ^f	0.5 ^b	0.60 ^d	Highly virulent	IV
24.	MgPM24	6	Brown	0.6 ^l	0.3 ^d	0.18 ^r	Less virulent	II
25.	MgPM25	6	Brown, grey centre	0.9 ⁱ	0.3 ^d	0.27 ^o	Moderate virulent	III
26.	MgPM26	6	Brown	0.7 ^k	0.2 ^e	0.14 ^t	Less virulent	II
27.	MgPM27	5	Brown, grey centre	0.7 ^k	0.4 ^c	0.28 ⁿ	Moderate virulent	IV
28.	MgPM28	5	Brown, grey centre	0.6 ^l	0.5 ^b	0.30 ^m	Moderate virulent	IV
29.	MgPM29	6	Dark brown	0.9 ⁱ	0.4 ^d	0.36 ^j	Moderate virulent	IV
30.	MgPM30	5	Brown, grey centre	1.2 ^f	0.4 ^d	0.48 ^g	Moderate virulent	IV
SEd						0.012		
CD (0.05)						0.023		
Finger millet								
1.	MgFM1	6	Brown lesion with yellow halo	0.30 ^c	0.20 ^e	0.06 ^h	Less virulent	I
2.	MgFM2	6	Small brown lesion with yellow halo	0.40 ^b	0.20 ^e	0.08 ^g	Less virulent	I
3.	MgFM3	5	Elliptical Brown lesion with yellow halo	0.50 ^a	0.20 ^e	0.10 ^f	Less virulent	II
4.	MgFM4	4	Elliptical Brown lesion with yellow halo	0.70 ^a	0.30 ^c	0.21 ^d	Highly virulent	III
5.	MgFM5	5	Elliptical Brown lesion	0.50 ^a	0.30 ^c	0.15 ^e	Moderate virulent	II
SEd						0.012		
CD (0.05)						0.027		
Foxtail millet								
1.	MgFoxM1	4	Circular spots with brown centre	0.20 ^c	0.10 ^b	0.02 ^e	Less virulent	I
2.	MgFoxM2	4	Circular brown spots	0.30 ^b	0.10 ^b	0.03 ^d	Less virulent	I
3.	MgFoxM3	3	Circular spots with brown centre	0.60 ^a	0.30 ^a	0.18 ^a	Moderate virulent	II
4.	MgFoxM4	4	Circular brown spots	0.30 ^b	0.20 ^a	0.06 ^b	Less virulent	I
5.	MgFoxM5	3	Circular spots with brown centre	0.50 ^a	0.10 ^b	0.05 ^c	Less virulent	I
SEd						0.014		
CD (0.05)						0.032		
Barnyard millet								
1.	MgBM1	4	Brown lesion	0.80 ^b	0.30 ^b	0.24 ^c	Moderate virulent	III
2.	MgBM2	3	Brown lesion with grey center	0.90 ^b	0.30 ^b	0.27 ^b	Moderate virulent	III
3.	MgBM3	3	Dark brown lesion	1.20 ^a	0.40 ^a	0.48 ^a	Highly virulent	IV
4.	MgBM4	5	Brown lesion with yellow halo	0.20 ^d	0.20 ^c	0.04 ^e	Less virulent	I
5.	MgBM5	4	Brown lesion	0.50 ^c	0.20 ^c	0.10 ^d	Less virulent	II
SEd						0.02		
CD (0.05)						0.046		

*Values represent the mean of 3 replications.

Means followed by the same letter are not significantly different at the 5 % level as determined by DMRT.

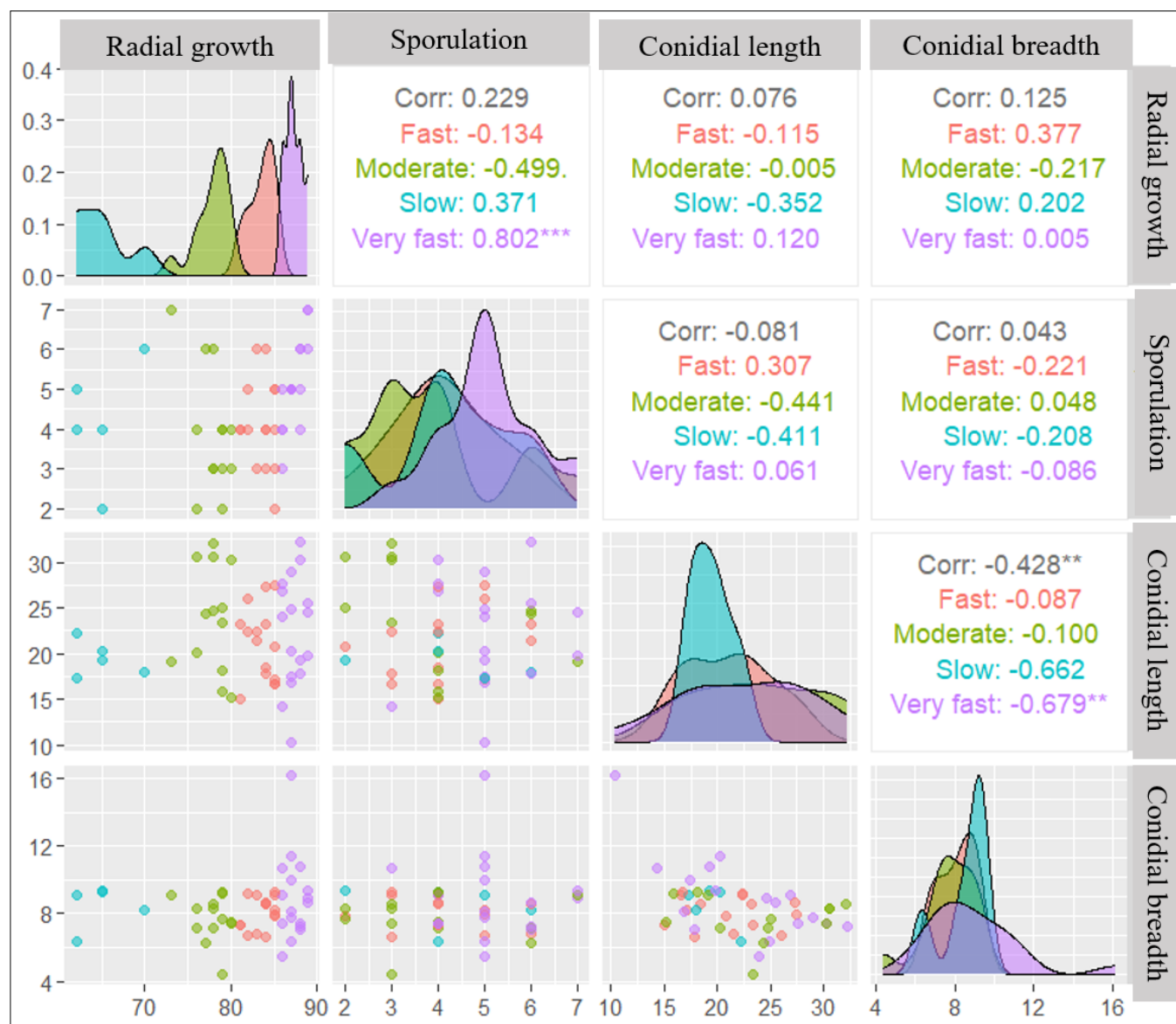


Fig. 2. Pairwise distribution and correlation of growth and conidial characters of blast isolates.

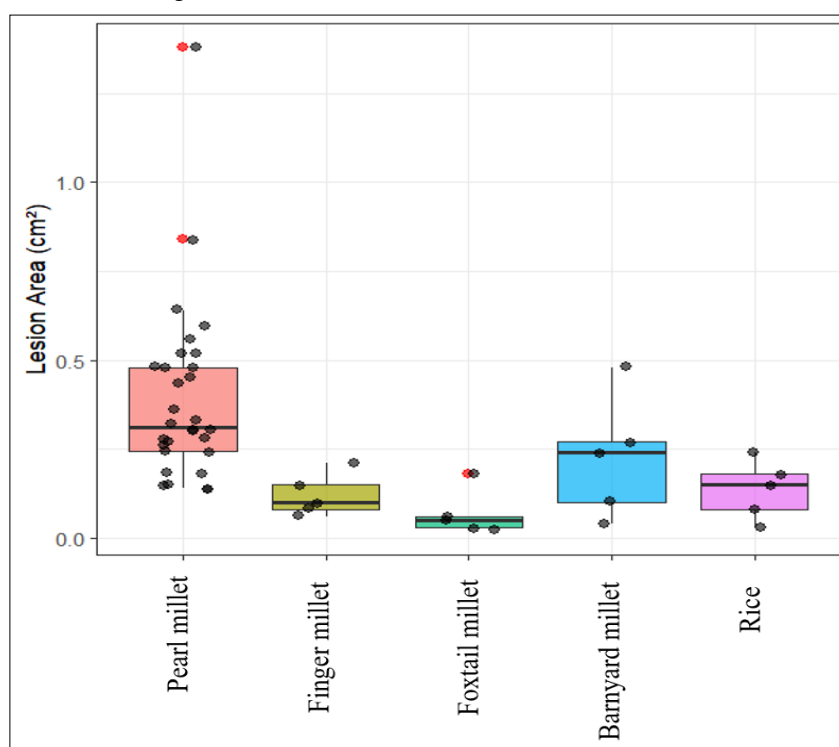


Fig. 3. Host-wise pathogenic variation in lesion area of blast isolates assessed by detached leaf assay.

Detached leaf assay to evaluate pathogenicity of blast isolates

Pathogenicity assays revealed significant variation in virulence with symptom initiation occurring within 3–6 days after inoculation (Table 2; Fig. 3) similar with previous findings (27). Virulence classification was based on lesion area thresholds (high: $>1 \text{ cm}^2$, moderate: $0.3\text{--}1 \text{ cm}^2$, low: $<0.3 \text{ cm}^2$). Pearl millet isolates exhibited higher virulence with lesion areas ranging from $0.14\text{--}1.38 \text{ cm}^2$, indicating strong host compatibility and efficient colonisation (28). In contrast, finger millet ($0.06\text{--}0.21 \text{ cm}^2$) isolates produced smaller lesions, suggesting partial resistance and effective host defence responses (29). Foxtail millet isolates showed low virulence ($0.02\text{--}0.18 \text{ cm}^2$), indicating limited pathogen fitness on this host (30). Barnyard millet isolates exhibited moderate virulence ($0.04\text{--}0.48 \text{ cm}^2$), suggesting that infection may occur under favourable conditions despite general resistance (30). Such variability in virulence across hosts supports the concept of host-specific adaptation and differential infection efficiency among *Pyricularia* isolates.

Polymerase chain reaction amplification and sequencing for molecular identification

Molecular identification using ITS-based amplification produced $\sim 560 \text{ bp}$ amplicon in all isolates, confirming their identity as *Pyricularia* (Fig. 4). Similar amplification patterns using ITS primers have been widely reported for blast pathogen (31). However, the conserved nature of ITS regions limits species-level resolution and differentiation of closely related species within the *Pyricularia* complex.

Phylogenetic analysis of *Pyricularia* isolates

The blast isolates were categorised into 5 well-supported clades depending on species and host origin by maximum likelihood phylogenetic analysis determined by ITS sequences. Clade I comprised *Pyricularia grisea* isolates from pearl millet with high bootstrap support (92–100 %), indicating strong host-associated clustering. Clade II included isolates from finger millet and barnyard millet (85–97 %), forming a mixed-host cluster suggestive of close genetic relatedness and possible cross-host adaptation.

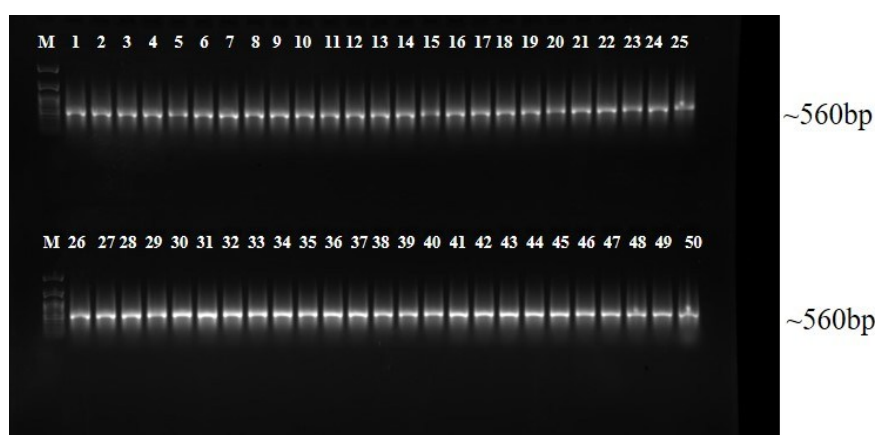
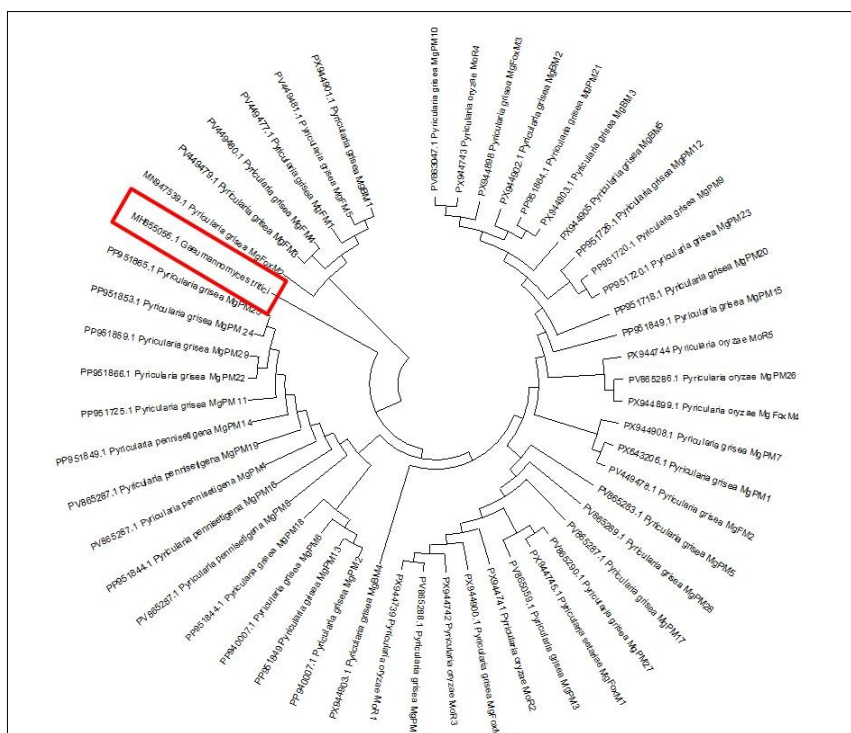


Fig. 4. Polymerase chain reaction (PCR) amplification of *Pyricularia* isolates using the internal transcribed spacer (ITS) region primers ITS1 and ITS4, showing the presence of target DNA fragments.

Lane M: 100 bp ladder; Lane 1–30: Pearl millet isolates; Lane 31–35: Finger millet isolates; Lane 36–40: Foxtail millet isolates; Lane 41–45: Barnyard millet isolates; Lane 45–50: Rice isolates.



Clade III (*P. pennisetigena*) and Clade IV (*P. setariae*) formed distinct host-associated lineages. Clade V contained *P. oryzae* isolates from rice along with one pearl millet isolate (MgPM25) (96–99 %), indicating potential cross-host association. Overall, the results suggest predominant host specificity with limited cross host adaptation among blast isolates. *Gaeumannomyces tritici* was used as an outgroup to root the tree (Fig. 5). Such host-associated clustering has been reported in previous phylogenetic studies of blast pathogen (31). The clustering pattern indicates host-associated genetic differentiation and supports the existence of host-specific lineages within the *Pyricularia* species complex.

Conclusion

This study incorporates field, morphological, pathogenic and genetic information to reveal considerable host-associated variation within *Pyricularia* isolates infected rice and millets. Higher disease incidence, greater morphological and virulence variation in pearl millet suggests significant host-pathogen interactions along with improved pathogenic variability. The identification of highly virulent isolates (MgPM1, MgFM4, MgFoxM3, MgBM3 and MoR2) implies the existence of host-adapted, aggressive lineages that have potential epidemic significance. Phylogenetic differentiation showed distinct species and host-specific clustering, showing continued divergence within the *Pyricularia* species complex, whereas internal transcribed spacer (ITS) based analysis validated genus-level characterisation. The findings emphasise the critical need for multilocus analysis and disease management approaches for prolonged blast suppression in rice and millets through providing new perspectives on specificity of hosts and cross-infectious behaviour.

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Authors' contributions

NHCK drafted the manuscript. JI reviewed the manuscript. KM and PVK reviewed and corrected the manuscript. MBN, AR and PM assisted with manuscript alignment. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare that QuillBot was used solely for paraphrasing and language refinement of the manuscript. No AI tools were used for data generation, analysis and interpretation. All scientific content and conclusions are the original work of the authors, who take full responsibility for the manuscript.

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