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# Variability and Characterization of *Pyricularia grisea* Causing Blast in *Eleusine coracana* (L.) Gaertn

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## ABSTRACT

**Background:** Finger millet (*Eleusine coracana* L.) is an important small millet crop cultivated widely in South India. Blast disease caused by *Pyricularia grisea* is one of the most destructive diseases affecting finger millet production, leading to significant yield loss. Effective disease management requires an understanding the pathogenicity and variability of the pathogen.

**Methods:** A roving survey was conducted in finger millet growing districts of Tamil Nadu, namely, Tirupattur and Chengalpattu, to record the disease incidence and collect infected samples. The pathogen was isolated using a standard tissue isolation technique on potato dextrose agar (PDA) media. Morphological characterization was carried out based on colony growth, conidial morphology and hyphal characteristics. Pathogenicity tests were performed to confirm Koch's postulate for all the isolates. Molecular identification was performed by conventional polymerase chain reaction (PCR) amplification using universal primers ITS1 and ITS4.

**Result:** Three isolates of *P. grisea* (PG1, PG2 and PG3) were obtained and exhibited variations in colony morphology. Conidia were pyriform, septate and hyaline to pale olive. Pathogenicity tests confirmed the virulence of isolate PG2, exhibiting higher severity. PCR amplification produced an amplicon of 500 to 700 bp, confirming the pathogen's identity as *P. grisea*.


**Key words:** Finger millet (*Eleusine coracana* (L.) Gaertn.), Molecular identification, Morphological characterization, Pathogenicity, *Pyricularia grisea*.

## INTRODUCTION

Finger millet *Eleusine coracana* (L.) Gaertn. is an important small millet crop belonging to the family *Poaceae* (Dida *et al.*, 2008). It is a cereal crop widely cultivated in the arid regions of India, particularly in South India. Grains are rich in calcium, protein, essential amino acids, vitamin A, vitamin B and phosphorus. In India, finger millet is predominantly cultivated and consumed in states including Karnataka Andhra Pradesh, Tamil Nadu, Odisha and Maharashtra. In Tamil Nadu, major cultivation of finger millet is carried out in districts *viz*., Dharmapuri, Krishnagiri, Vellore, Salem and Tiruvannamalai (Kumar *et al.*, 2020). In Karnataka, consumption of finger millet is by preparing ragi balls known as "mudde," leavened dosa and thinner chapatis, whereas in Tamil Nadu it is consumed as ragi mudde, porridge, dosa, idli, puttu, vermicelli and other forms of snacks in other states such as Andhra Pradesh and Telangana, it is prepared as "sangati" in Maharashtra as "bhakri" and in Odisha and Uttarakhand mainly as roti or porridge. The high fibre content of Ragi prevents high blood cholesterol, intestinal cancer and constipation. It is one of the most important small millets in the tropics and is cultivated in more than 25 countries in Africa (eastern and southern) and Asia (from Near East to Far East), occupying 12% of the global millet area. Countries such as Uganda, Sri Lanka, India, Nepal, China, parts of Africa, Madagascar, Malaysia and Japan are major producers of finger millet (Sakamma *et al.*, 2017). Under optimum irrigated conditions, it has a high yield potential of more than 10 t/ha.

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However, finger millet production is seriously constrained by blast disease, which affects the leaves, neck and fingers of the plant. In India, the blast was first reported from the Tanjore delta of Tamil Nadu by McRae (1920). Blast in finger millet is economically important disease in Tamil Nadu (Paramasivan *et al.*, 2024). The disease was observed on leaf, neck and panicles. It occurs in a more destructive form on panicles as compared to leaf and neck (Takan *et al.*, 2012). The disease is highly destructive, causing damage during both vegetative and reproductive growth stages. Disease development is

favoured by environmental conditions such as temperatures ranging from 20°C to 25°C and relative humidity of about 90%. Finger millet blast was earlier reported to cause a yield loss of upto 50% annually (Jadhao *et al.*, 2020).

## MATERIALS AND METHODS

### Survey and symptomatology of samples

The survey was conducted in ragi-growing districts of Tamil Nadu, including Chengalpattu and Tirupattur, from March 2025 to June 2026, including the premises of SRM College of Agricultural Sciences. The standard grade chart suggested by IRRI (1996) was used to compute the Per cent Disease Index (PDI) (Table 1). The symptoms were observed, recorded and monitored. To isolate the pathogen, symptomatic plant samples were collected and stored at 4°C.

### Isolation of pathogen

Isolation of the *Pyricularia grisea* from infected leaf samples was attempted by various methods.

### Standard tissue method

The blast pathogen *P. grisea* was isolated from infected plant samples expressing typical symptoms using standard tissue isolation technique described by Tuite (1969) and Patel *et al.* (2024). The infected leaf samples were washed twice with sterile distilled water to remove the contaminants and dust particles. The samples were pat dried in sterile tissue paper and excised into small segments. The cut pieces were surface-sterilized with 1% sodium hypochlorite for 3 minutes followed by 70% ethanol for 30 sec, followed by three rinses in sterile distilled water. The excess moisture was removed by pat drying in sterile tissue paper and placed on PDA medium and incubated at 25±1°C for 7 days. Emerging fungal colonies were aseptically subcultured onto fresh PDA plates to maintain pure culture following the method outlined by Ou (1985).

### Single spore isolation

*P. grisea* isolates were obtained by incubating infected tissues (1-1.5 cm) on Water Agar (WA) and incubated at 28°C for 24 hours. For obtaining pure cultures, a single spore colony developed on water agar was aseptically transferred onto PDA medium (Akator *et al.*, 2014).

### Spore drop technique

Freshly infected leaves were washed under running tap water and cut into small pieces (4-5 cm). The leaf pieces were fixed on the upper half of a Petri dish using local gum. Sterile moist cotton was placed along the edge of the lid and periodically moistened with sterile water to maintain humidity. Under aseptic conditions, the lower plate was poured with 5 ml of 2% WA and the lid was placed over it and incubated for 48 hours until spore deposition. After 12 hours, the lower plate was examined microscopically for typical.

*P. grisea* conidia. A region containing a single spore was marked and aseptically transferred onto PDA using a sterile cork borer and incubated at 25±1°C to obtain pure cultures (Amoghavarsha *et al.*, 2022).

### Spore dilution method

For isolation by the spore dilution technique, diseased leaf samples were washed under running tap water and cut into small pieces. The pieces were surface sterilized with 1% sodium hypochlorite for 90 seconds, followed by three rinses with sterile distilled water. The sterilized tissues were placed on the lid of a Petri plate lined with moist filter paper and incubated over a base containing sterile distilled water to maintain high humidity. The setup was incubated at 28°C for 48 hours to induce sporulation.

Sporulating tissues were examined under a stereo zoom microscope (S9i: Leica, Germany), transferred to an Eppendorf tube containing sterile water and gently agitated to release conidia. A small aliquot (100 µl) of the spore suspension was spread on 2% WA and incubated at 25±1°C overnight. Germinating single conidia were microscopically identified and aseptically transferred to PDA amended with streptomycin sulfate, followed by incubation at 25±1°C for 7-10 days to obtain pure culture (Amoghavarsha *et al.*, 2022).

**Table 1:** Standard score chart for ragi blast (IRRI System, 1996).

| Scale | Description | Host behavior |
|---|---|---|
| 0 | No lesion observed | Highly resistant |
| 1 | Small brown specks of pin head size | Resistant |
| 2 | Small, roundish to slightly elongated, necrotic grey spots, about 1-2 mm in diameter, with a distinct brown margin. Lesions are found mostly on the lower leaves | Moderately resistant |
| 3 | Lesion type same as in 2, but a significant number of lesions on the upper leaves | Moderately resistant |
| 4 | Typical susceptible blast lesions of 3 mm or longer infecting less than 4% of leaf area | Moderately susceptible |
| 5 | Typical susceptible blast lesions of 3 mm or longer infecting 4-10% of the leaf area | Moderately susceptible |
| 6 | Typical susceptible blast lesions of 3 mm or longer infecting 11-25% of the leaf area | Susceptible |
| 7 | Typical susceptible blast lesions of 3 mm or longer infecting 26-50% of the leaf area | Susceptible |
| 8 | Typical susceptible blast lesions of 3 mm or longer infecting 51-75% of the leaf area | Highly susceptible |
| 9 | Typical susceptible blast lesions of 3 mm or longer infecting more than 75% leaf area affected | Highly susceptible |

### Morphological characterization

Three *P. grisea* isolates were cultured on PDA to study their morphological characteristics. Observations on colony colour, texture, margin type and radial growth (cm) were recorded (Jabbar and Nagaraja, 2018).

The conidial size and hyphal character were observed under compound microscope (Leica DM750 microscope, Germany) and recorded. Conidial masses from a 12-day-old culture were mounted on a glass slide, stained with lactophenol cotton blue and examined under microscope (Palanna *et al.*, 2024).

### Pathogenicity test

Pathogenicity of *P. grisea* causing ragi blast was confirmed by proving Koch's postulates.

Pure cultures of the fungal isolates were established on PDA and incubated for 14 days at 25±1°C. Culture was harvested by washing the culture plates with 10 ml of sterile distilled water and the resulting suspension was filtered through two layers of muslin cloth. The concentration was then standardized to $1.0\times10^6$ conidia/ml by adding Tween 20 (0.2 ml/L) as a surfactant. The suspension was sprayed onto 15-day-old plants, subsequently enclosed in polyethene sheets and misted to maintain high humidity, thereby facilitating infection. Each isolate was tested with three replications. Disease symptoms were recorded seven days post-inoculation and scored on a 1-9 scale based on lesion size. To satisfy Koch's postulates, the pathogen was re-isolated from symptomatic tissues and identified based on morphological features (Radjacommare *et al.*, 2004).

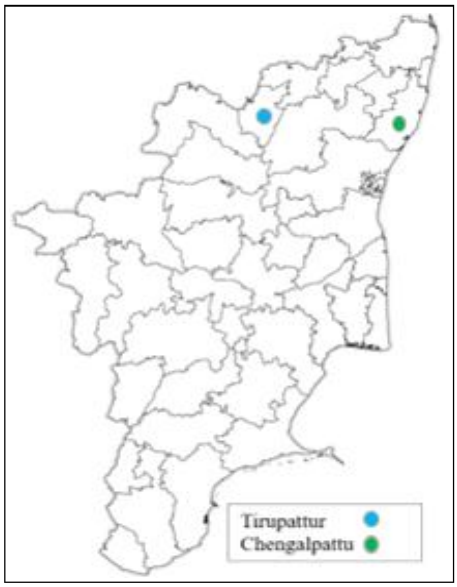



**Fig 1:** Isolates obtained from Tirupattur and Chengalapttu districts.

### Molecular characterization

The mycelial mat of 15-day-old virulent culture was subjected to DNA extraction. Fresh mycelial mat pat-dried onto sterile tissue paper was used for DNA extraction. The genomic DNA was extracted using Cetyl Trimethyl Ammonium Bromide (CTAB) method as described by Prakash *et al.* (2019). PCR was performed using standard primer pair ITS1 and ITS4 at an annealing temperature of 55-56°C for 1 min for 35 cycles. The amplicon was resolved on 1% agarose gel and sequenced at Barcode Biotechnology, Bangalore. The obtained sequence was compared with *Pyricularia* spp. sequences available in the NCBI database. The sequence was submitted to GenBank to retrieve the accession number. Sequence alignment was performed using BioEdit and phylogenetic analysis was carried out using MEGA 12 software (Kumar *et al.*, 2024).

## RESULTS AND DISCUSSION

### Survey and symptomatology of samples

A roving survey was undertaken to assess the blast incidence and collect infected leaf samples from farmers' fields for isolation, following the methodology described by Sharma *et al.* (2024). Three *P. grisea* isolates were obtained from Chengalpattu and Tirupattur districts to evaluate regional variability and disease severity (Fig 1). The highest disease index of 48% was recorded in Chengalpattu (isolate: PG3) (Lat. 12.387413°, Long. 79.736748°), followed by Tirupattur PG1: 44.2% (Lat. 12.48191°, Long. 78.531623°) and PG2: 46.8% (Lat. 12.49626°, Long. 78.539258°) (Fig 2).




**Fig 2:** Survey for the incidence of Ragi Blast disease in Tamil Nadu.

*P. grisea* infects finger millet at all growth stages, from seedling to grain formation. At seedling stage, symptoms appeared as small brown spots that enlarged into spindle-shaped lesions with a greyish centre, a yellow halo and concentric rings. On the lower leaf surface, conidial development is higher (Fig 3). As the disease progresses, abundant conidia and conidiophores develop, often leading to seedling death. In the neck blast stage, infection causes blackening and constriction of the nodal region, resulting in the neck to shrivel and the ear head to droop or break. At later stages, finger blast occurs, with infection typically initiating at the tips of the fingers and spreading downward. Severe infection leads to poor grain development or complete failure or shrivelled and blackened grains (Bhadani *et al.,* 2023).




a) Leaf blast, b) Stem blast.

**Fig 3:** Symptoms of ragi blast.

Similarly, Das *et al.* (2016) reported blast infection across all growth stages, producing elliptical to diamond-shaped lesions on leaves, peduncles and fingers, depending on the crop stage, while Takan *et al.* (2012) observed the disease on leaves, necks and panicles.

**Isolation of pathogen**

Among the four different isolation methods, the blast pathogen was effectively isolated using the standard tissue isolation method. Fungal colonies appeared on PDA within 4-5 days of incubation at 25±1°C and pure cultures were obtained through repeated subculturing (Tuite, 1969). Similar isolation procedures have been reported by Aneja (2005) and Karthikeyan *et al.* (2013) using PDA medium. The isolates produced greyish to olivaceous colonies with aerial mycelium, exhibiting typical morphological characteristics of the blast pathogen. In our experiment, the spore drop technique exhibited contamination similar to the findings of Amoghavarsha *et al.* (2022), who reported negligible contamination compared to higher bacterial contamination in spore dilution method. Contrarily, Jagadeesh *et al.* (2018) and Rajashekara *et al.* (2017) had used the spore dilution technique for the successful isolation of the pathogen.

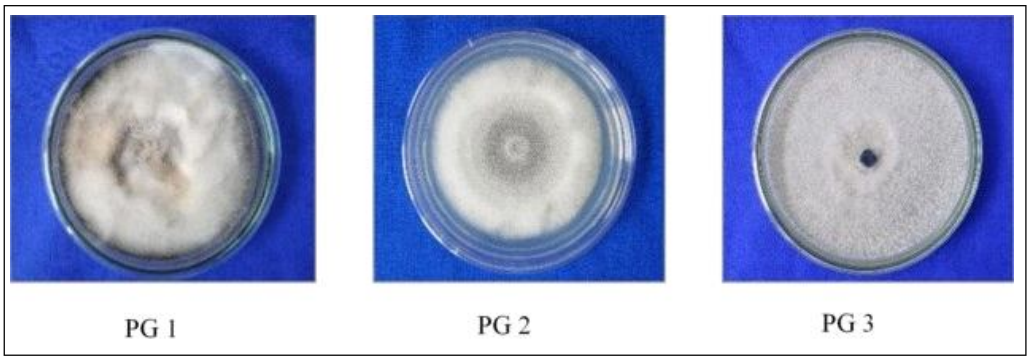



**Fig 4:** Culture morphology of different isolates of *Pyricularia grisea.*

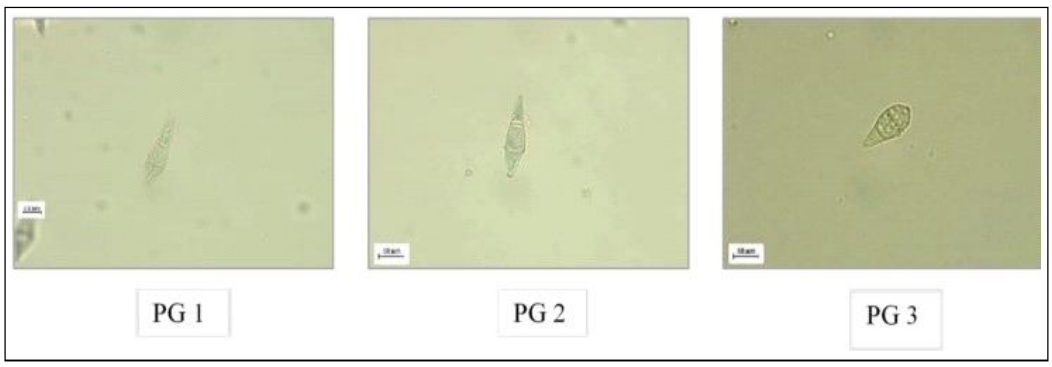



**Fig 5:** Conidial characters of three isolates.

### Morphological characterization

Significant variation in cultural characteristics was observed among the study isolates. PG1 isolate initially surfaced with white mycelial growth and later turned greyish-brown, forming a fluffy to woolly, circular colony with irregular zonation, with slight irregular margins and a raised centre. While PG2 produced slightly elevated cottony whitish-grey colonies with circular zonation and margins ranging from entire to feathery. In contrast, PG3 exhibited grey colonies with a darkened centre, velvety to cottony texture, distinct zonation, covering the entire plate with a convex centre (Fig 4). These variations in morphological traits may be attributed to environmental influences, as reported by Gashaw *et al.* (2014). Similar findings of whitish-grey colonies with raised mycelium on PDA were also reported by Jabbar Sab and Nagaraja (2018) and Soban Babu *et al.* (2021).

The conidia of all isolates were pyriform, hyaline to pale olive, typically 2-septate and 3-celled and varied in size ranges from 24.7 to 29.8 µm, with a round base or a pedicel tapering towards a pointed apex and are similar to the findings of Anjum (2015), Jabbar Sab and Nagaraja (2018) and Poonacha *et al.* (2025) (Fig 5). The hyphae were initially hyaline but later became olivaceous and were septate and branched. Similar observations were reported by Hossain (2000) and Paswan *et al.* (2018), who noted that the mycelium was initially hyaline and subsequently turned olivaceous, measuring 1-5.2 µm in width and exhibited septate and branched characteristics.

### Pathogenicity test

A moderately susceptible finger millet variety ATL 1 was used for the pathogenicity test under pot culture conditions. Typical blast symptoms appeared within seven days after inoculation. Initially the symptom developed as small water-soaked lesions on leaves that later advanced into spindle-shaped spots with brown margins and grey centres. Among the three isolates, PG2 was more virulent were symptom expressed seven days post inoculation (DPI). While in PG1 and PG3 the first symptom was observed 14 DPI. The pathogens were re-isolated and morphology was confirmed. The virulent isolate PG2 was selected for further studies (Fig 6). Poonacha *et al.* (2025) reported that KMR 301, a susceptible finger millet variety exhibited small brown spots within 7 to 12 DPI, while Soban Babu *et al.* (2021) observed the development of symptom within 7 DPI in moderately susceptible variety Paiyur 2.

### Molecular characterization

Genomic DNA was extracted using the CTAB method, followed by PCR amplification with ITS primers, resulting in an amplicon of approximately 500 bp to 700 bp. The PCR products were purified and sequenced at Barcode Biotechnology. The obtained sequence was analysed using BLAST available in the NCBI database and submitted to the GenBank database under the accession number PX945787. Sequence comparison study exhibited high similarity with previously reported *P. grisea* sequences in the database, confirming the identity of the pathogen. Similar amplification of the ITS region in *Pyricularia grisea*

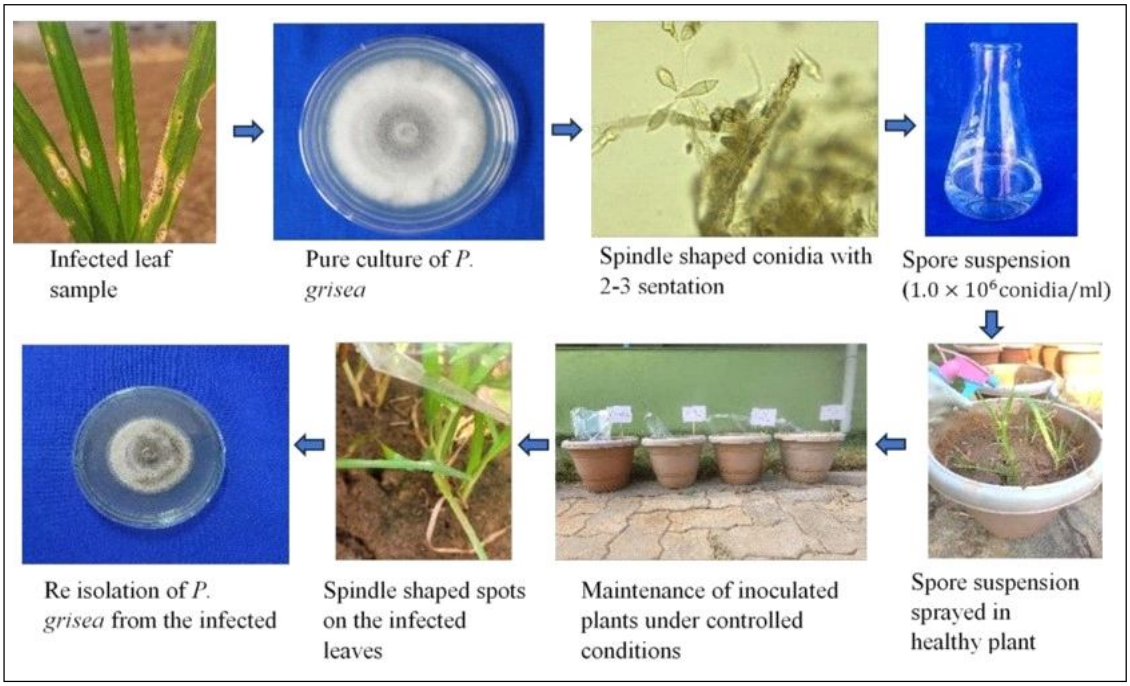



**Fig 6:** Pathogenicity of *Pyricularia grisea.*

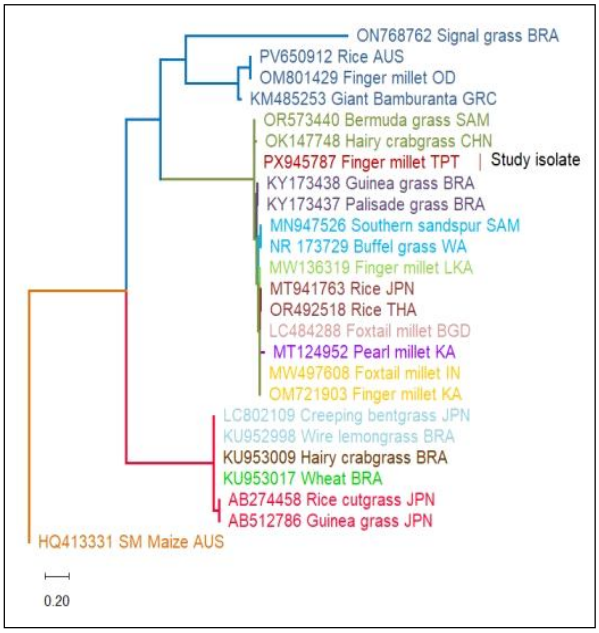



**Fig 7:** Phylogenetic analysis of *P. grisea* using Mega 12 software with a cut-off of 70 per cent and 1000 replications.

using ITS1 and ITS4 primers was reported by Panda *et al.* (2017) and Soban Babu *et al.* (2021). Further, the sequences were aligned with reference *Pyricularia spp*. sequences and phylogenetic analysis was performed using the neighbor-joining tree method in MEGA 12 (Kumar *et al.*, 2024) (Fig 7). All the isolates of *Pyricularia spp*. were grouped into two major groups. The isolates of *P. grisea* were assembled into group 1 and *Sclerophthora macrospora* as group 2. Group 1 diverges into two clusters: A and B. Cluster A is further subdivided into 2 subclusters (I and II). Isolates of *P. grisea* of finger millet (Odissa), rice (Australia), signal grass (Brazil) and giant bamburanta (Greece) have been grouped as subcluster I, whereas subcluster II, encompassing the study isolate PX945787, was found to have high similarity with hairy crabgrass (China) and bermuda grass (South America). Also, isolates of *P. grisea* from finger millet (Karnataka), foxtail millet (India), pearl millet (Karnataka), rice (Thailand), rice (Japan) and *etc.*, were having higher nucleotide similarity (100%). Whereas HQ413331 *Sclerophthora macrospora* Maize (Australia) served as an outgroup.

## CONCLUSION

The present study confirmed the occurrence and variability of blast disease of finger millet in Chengalpattu and Tirupattur districts of Tamil Nadu. Three *P. grisea* isolates (PG1, PG2 and PG3) were successfully isolated and there identity were described both morphologically and molecularly. Noticeable differences were observed among the isolates in colony characters and growth patterns, indicating variability within the pathogen population. All isolates were found to be virulent in pathogenicity testing, whereas, PG2 demonstrated a relatively higher disease severity. The pathogen's identity as *P. grisea* was confirmed by molecular identification using ITS primers, which generated a distinct amplicon of approximately 520 to 560 bp. The results of this study provide valuable baseline information on pathogen variability and confirmation, which will support future research on disease management strategies and the development of blast-resistant finger millet varieties.

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### Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

### Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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