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Characterization of *Magnaporthe grisea* strains and their morphological variability isolated from different agroclimatic zones of India

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Abstract
Pearl millet (*Pennisetumglaucum*), a C4 species, is one of the most significant global millet crops that is majorly grown in the states of Rajasthan, Gujarat, Maharashtra, Uttar Pradesh, Haryana, and Karnataka in India. The blast, also referred to as leaf spot caused by *Magnaporthe grisea*, has been reported as a devastating disease that causes severe losses of forage as well as grain yields of the host plant. With the advent of time, the pathogen has evolved at a very fast pace to adapt to the different agro-climatic zones of India. This study aimed to explore the cultural and morphological variations among isolates obtained from different agro-climatic regions. The prevalence of blast disease was higher in Jamnagar, Jaipur and parts of Karnataka which was evident for its diversified symptoms in cultivated pearl millet plants of farmer fields. Among the isolates collected from field survey, 17 isolates exhibited significant diversity in cultural and morphological variability namely, mycelial growth, colony diameter, pigmentation, and conidia production under *in vitro* conditions. The present investigation provides an insight into the emergence of various strains of *M. grisea* existing in India and their morphological changes will help us to understand the severity of the disease spread in various agroclimatic zones of India.

Keywords: pearl millet; *Magnoporthe grisea*; disease severity; morphology; variability

1. Introduction

Pearl millet (*Pennisetum glaucum*) is one of the staple cereal crops in the arid and semi-arid zones of India. It is the main cereal crop that is capable of producing dependable yield minor agro-climatic conditions. It is also gaining importance as a nutritional feed for domesticated animals and poultry farms [1]. In India, pearl millet is cultivated across 7.4 million hectares with a production of 9.13 million tons, and production index of 1237 kg/ha [2] Rajasthan, Gujarat, Maharashtra, Uttar Pradesh, and Haryana are the major pearl millet cultivating states which account for 90% of pearl millet land in India [2]**.** However, the yield potential has been substantially challenged by plethora of biotic and abiotic stresses, of which the infection of *Magnaporthe grisea* that causes blast disease has been reported to cause huge economic losses [3, 4] Pearl millet blast, caused by *M. grisea*, has emerged as one of the most devastating diseases in India. The other reported hosts of the blast pathogen are foxtail millet, wheat, finger millet, rice, and many kinds of grass. The blast pathogen *M. grisea* exhibits high variability that is conferred to the different varieties of pearl millet grown across different climatic conditions. Pathogenic variation of *M. grisea* populations adapted to pearl millet, wheat, foxtail millet, rice, finger millet, and numerous weed hosts have been noted [5, 6, 7]. The blast pathogen infection on Napier grass was first recorded in Singapore and the United States of America [8, 9]. In India, the blast disease pathogenic variation was first documented in 1942 in Kanpur, Uttar Pradesh [10], and subsequently the reports on incidence of pearl millet blast disease were recorded across different regions of India [11, 12]**.** In Karnataka state, the highest severity of blast disease was registered in Koppal district whereas in Bagalkot district, the severity of blast disease was comparatively low [13]. The disease has been accounted to influence different growing stages of pearl millet seedlings to tillering stage on stem, leaves, and boot leaf, which eventually affect cereal yield [14]. The severity of blast disease is accelerated during raining season that is associated with high moisture conditions. The disease is characterized by the appearance of typical lesions that are water-soaked with gray coloured centers, which subsequently turn into brown color and flanked by a chlorotic halo. The lesions eventually turn into necrotic tissue that gives the appearance of concentric rings. The initial infection is usually visualized as pale green to the greyish green lesion, which later turns yellow to grey as the disease progresses [15].Thus, based on this rationale, the present work was framed to: (i) isolate and characterize *M. grisea* from different agro-climatic regions of India (ii) pathogenicity of 17 isolates on local cultuvar; (iii) examination of cultural and conidial morphology variation under *in vitro* conditions.

2. Materials and methods

2.1. Survey for blast diseases in pearl millet growing areas of India

Pearl millet crops roving survey was conducted in the major pearl millet farming farmers' fields of various agro-climatic zones like Bagalkot, vijayapur, Gadag, Koppala, Raichur, Belagavi, Dharwad, Kalaburgi, Mysore and Chamarajnagar districts of Karnataka, Jamnagar (Gujarat) Jaipur (Rajasthan), and Kolhapur (Maharashtra) states in India during the Kharif season of 2018-2019.

2.2. Host and Pathogen

Pearl millet plants that showed typical disease symptoms were collected from the surveyed regions to isolate blast pathogen from randomly selected farms. Additionally, crops at different stages of their growth, i.e., from tillering to maturity, were also examined for disease progression. The pearl millet cultivated land was evaluated for a minimum of 200 plants in each quadrant (2 rows x 5 m).One quadrant was located in each of the four corners as well as one in the center of the pearl millet field [16]. The pearl millet blast severity was recorded using the scale o-9 scale of [17].

2.3. Isolation of *Magnaporthe grisea* from infected leaves

Pearl millet blast infected leaves along with healthy parts were cut into small bits with the help of sterilized scissor and rinsed well in tap water, then surface sterilized with 2% sodium hypochloride solution for 30 seconds and rinsed in sterile distilled water at least three times and transferred to petri plate containing potato dextrose agar (PDA) medium. The plates were incubated at room temperature (28±10 ^{0}C) for 7 days and the plates were observed for fungal growth periodically. The colonies' characters were recorded which developed from the tissue bits [18].

2.6. Cultural morphological variation of blast pathogen *Magnaporthe grisea*

The cultured isolates were investigated for variations in morphological characters. The characteristics under consideration were: colony characters, morphology, colony color, colony size, sporulation radial colony growth, and colony diameter. The variability studies were done for each of the three replicates of each culture.

2.5. Single spore isolation

Identified well-developed lesions, washed with running tap water for 15 minutes, and the excised sections were surface sterilized in 2% sodium hypochlorite then washed with sterilized distilled water. The sections were then placed on clean glass slides in moist chamber at room temperature for 48 h to induce sporulation. The sporulated leaves were then flooded with sterile distilled water and collected. The spores were then observed different magnifications of light microscope. The spore suspension was also cultured on PDA slants, and analyzed for conidial characters. All the cultures were maintained on PDA and stored under refrigerated conditions.

2.7. Pathogenicity test

The spores obtained from were utilized to artificially inoculate two sets of pearl millet Kundur isolate (Mysore District) seedlings. The first set was undertaken to prove pathogenicity of the isolated *M. grisea* pathogens. Briefly, the pearl millet seedlings were sown in the pot containing sterilized soil/sand/FYM (farmyard manure) mix (2:1:1 by volume; 550 g/pot) and the 12-day-old seedlings of pearl millet crop were inoculated with fungal mycelia plugs and conidial suspension ($1x10^5$ spores/ml) by foliar spraying. The sprayed seedlings were covered with polythene bags to avoid cross-contamination and to generate high (>90%) relative humidity for a period of 7 days and grown in a greenhouse to study blast disease progression [19]. After the development of characteristic blast symptoms on the lower surface of the pearl millet blast susceptible genotype ICMB95444, the pathogen was re-isolated and the culture thus obtained was compared with the original for confirmation. The experiment were conducted in a completely randomized design (CRD) with three replicates; 1 pot/replicate with 10 seedlings. Blast severity was examined 8 days post inoculation using a 1-to-9 progressive scale as proposed by [20]. Further, these scales were converted to Percent Disease Index (PDI) using the formula given by [21].

3. Results

3.1. Symptoms of Blast disease

During the field survey, 357 farmer's fields covering a distance of 1750 kms comprising north and south India were survey for the blast disease incidence during kariff season (2018 and 2019). The blast disease samples were accurately observed for any of the typical symptoms in the cultivated varieties or hybrids grown in that particular field. It was noticed that prevalence of blast disease was higher in Jamnagar, Jaipur and parts of Karnataka which was evident for its diversified symptoms in cultivated pearl millet plants of farmer fields (Fig. 1). Diseases plants usually appeared as water-soaked gray leaf spots on the surface of leaves. The initial infection was usually visualized as pale green to grayish green, which later turned yellow to grey with progression in disease. The severity of the disease increased in humid weather conditions and crowded plant stands. Typical lesions were diamond-shaped with dimensions of 1–3.5 mm long and 0.3–0.7 mm wide. The centers of the spots were gray, which turned into brown color and edged by a chlorotic halo that eventually turns into necrotic tissue that gave the appearance of concentric rings. Symptoms may also appear on the nodal region of the stem as spindle shaped spots with the grayish-white center with brown margin spots and coalesced to form huge patches appeared as a blast on stem region.

Fig 1: Blast disease symptoms of pearl millet leaves during field survey. a cultivar Kaveri boss in Bagalkot field, b. GHB 354 in Jamnagar field, c. MH727 in Matgaon field, d. MRB2262 in Dharwad field, e. Local cultivar in Chamrajnagar field.

3.2. Screening of disease severity from field survey

In the survey conducted, disease severity was reported in various agro-climatic zones during kharif season of 2018-2019. Among the 127 fields, the highest mean percent disease index was reported in farmer's field of Southern India at Vijayapur, Karnataka with severity or incidence of 7.9. This intensity of the severity was correlated with Jamnagar and Jaipur of Gujarat and Rajasthan of Northern India where the severity of the disease 7.2 and 7.7 was observed. Interestingly the out of the surveyed field one third of the farmers field produced one or the other typical symptoms of pearl millet blast disease. It was also noticed that Southern Karnatka the prevalence of the pathogen is moderate and also the same pathogen load was recorded for Kolhapur district of Maharashtra.

3.3. Isolation of diseased samples from various agroclimatic zones of India

The diseased samples were collected from 17 farmers' fields that were severely infected and showed any of the typical symptoms of pearl millet blast disease. The isolates belonging to Karnataka mainly involve the fields of Bagalkot (Bagalkot District), Vijayapur (Vijayapur District), Hungund (Bagalkot District), Badimanhal (Koppala District), Kallur (Darwad District), Hegsanhalli and Bevinur (Raichur District), Gangapur (Gadag District), Sulebhavi (Belagavi District), Hadagali (Gadag District), Kundur and Nandikeshwar (Mysore District), Alurkere (Chamarajanagar District), Pardarmotakpalli (Kalaburgi District). Maharashtra state Matgaon (Kolhapur District). Rajasthan state in Jaipur (Jaipur District) and Gujarat state in Jamanagar (Jamanagar District). These strains were considered to investigate the variability in pearl millet blast populations (Table 1). The infected samples were collected in brown paper bags and were used to study the cultural and conidial morphology of the pathogen under microscope.

Table 1: Isolates of *Magnoporthe grisea* collected from field survey

Sl No	Place of collection	District	*Magnoporthe grisea* isolates
1	Bagalkot	Bagalkot	*BgkMg*1
2	Badimanhal	Koppala	*BdmMg*2
3	Hungund	Bagalkot	*HgdMg*3
4	Kallur	Darwad	*KlrMg*4
5	Hegsanhalli	Raichur	*HglMg*5
6	Gangapur	Gadag	*GgrMg*6
7	Sulebhavi	Belagavi	*SlbMg*7
8	Hadagali	Gadag	*HdgMg*8
9	Kundur	Mysore	*KdrMg*9
10	Pardarmotakpalli	Kalaburgi	*PtkMg*10
11	Matgaon	Kolhapur	*MtgMg*11
12	Bevinur	Raichur	*BvrMg*12
13	Nandikeshwar	Mysore	*NdkMg*13
14	Alurkere	Chamarajanagar	*AlkMg*14
15	Vijayapur	Vijayapur	*VjpMg*15
16	Jaipur	Jaipur	*JprMg*16
17	Jamanagar	Jamanagar	*JmnMg*17

3.4. Pathogenicity test

For the pathogenicity test, the 17 strains were artificially inoculated on the highly susceptible pearl millet cultivar (ICMV155). After two weeks of inoculation, the inoculated plants showed typical blast symptoms as explained earlier confirming the disease infection on the host pearl millet. From these experimental infected plants on which the 17 strains were established were re-isolated on the PDA plates and further examined for cultural and morphological characteristics under microscope. It was observed that the re-isolated pathogen showed similar characters to the original isolate in its cultural and conidial morphology (Fig. 2).

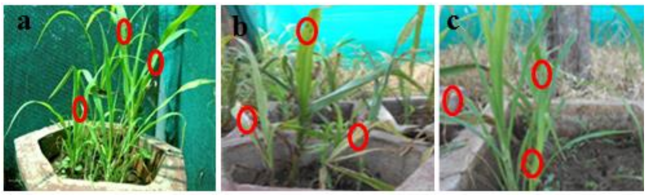

Fig 2: Pathogenicity test on highly susceptible host cultivar ICMV 155 showing typical symptoms of blast disease, a. challenge inoculated with isolate (JmnMg17), b. challenge inoculated with isolate (MtgMg11) and **c**. challenge inoculated with isolate (JprMg16).

3.5. Cultural morphological variability

The cultural variation of the 17 isolates revealed colony color ranged from white to black, gray in color, whitish pale pink, mycelia growth from flat to raised, margin varied from irregular to regular, and variability of growth rate (Fig. 3).

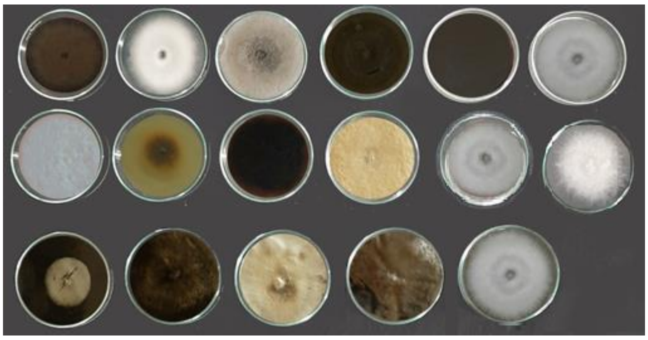

Fig 3: Cultural variability of 17 isolates of *Magnoporthe grisea* used in this study

The number of septa in conidia are about two septa with three celled, excellent sporulation with pyriform shaped spores, measuring about from 14-24 ×4.3-6.5 µm to 15-37×4.2–5.9 µm with 1.7 µm, with 1.7 µm as well as the width of the basal appendage measured1.4–1.9 µm (Fig. 4; Table 2).

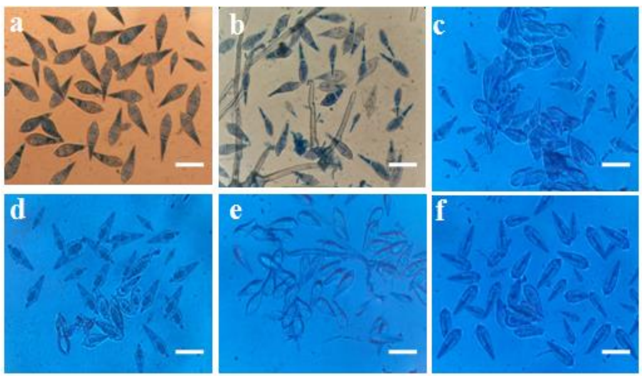

Fig: 4. Variation in the condia size and structure of *Magnoporthe grisea* isaolated from fields. Scale bar: 20 µM.

Table 2: Cultural and morphological variability in isolates of *Magnoporthe grisea*

Isolate	Colony Colour	Colony diameter (mm)	Type of margin	Mycelial growth	Texture	Mean conidial size (µm) Length ×Width	Colour	Sporulation
*BgkMg*1	Black	92	Regular	Flat	Smooth	15-37×4.2–6.9	Hyaline	++++
*BdmMg*2	White	86	Regular	Raised	Smooth	15-25×3.5–7.5	Hyaline	+++
*HgdMg*3	Ashy grey	84	Regular	Flat	Smooth	14-24 ×4.3-6.5	Pale olive	++++
*KlrMg*4	Black	89	Regular	Raised	Smooth	15-41×5.2–4.9	Hyaline	+++
*HglMg*5	Black	87	Irregular	Flat	Coarse	14-28 ×3.9-5.9	Pale olive	++
*GgrMg*6	White	88	Regular	Raised	Smooth	14-32 ×5.1-6.5	Pale olive	+
*SlbMg*7	Brown	83	Regular	Raised	Smooth	15-30×4.9–3.2	Pale olive	+
*HdgMg*8	Black	90	Irregular	Flat	Coarse	14-43 ×4.3-3.5	Hyaline	+++
*KdrMg*9	Whitish brown	87	Irregular	Raised	Coarse	15-63×5.3–7.2	Hyaline	++
*PtkMg*10	White	89	Regular	Raised	Coarse	15-24×5.2–3.6	Hyaline	+
*MtgMg*11	White	90	Irregular	Raised	Coarse	14-32 ×4.5-4.6	Hyaline	+++
*BvrMg*12	White	82	Regular	Flat	Smooth	15-32×5.3–4.7	Pale olive	+
*NdkMg*13	Black	85	Regular	Raised	Smooth	14-24 ×4.3-6.5	Hyaline	++
*AlkMg*14	White	87	Regular	Flat	Smooth	14-34 ×6.3-4.5	Pale olive	+
*VjpMg*15	White	94	Irregular	Raised	Smooth	15-27×5.6–2.9	Hyaline	++++
*JprMg*16	Black	88	Regular	Raised	Coarse	14-35 ×5.3-4.5	Hyaline to pale olive	++++
*JmnMg*17	Whitish pink	89	Irregular	Raised	Smooth	14-31×4.8-5.7	Hyaline to pale olive	++++

*All isolates have pyriform shape and two septate conidia

Table 3

Per microscopic field (100X)		
Low	20 spore	+
Medium	21-40	+ +
Good	41-60	+ + +
Excellent	>60	+ + + +

3.6. Sporulation
All the isolates were observed for sporulation using single leaf tissue in the microscopic field. Tremendous sporulation (> 60 conidia) was noticed among blast isolates, Bgk*Mg1*, Hgd*Mg3*, Vjp*Mg15*, Jpr*Mg16*, and Jmn*Mg17*. Good sporulation (40-60 conidia) was recorded in Bdm*Mg2*, Klr*Mg4*, Hdg*Mg8*, and Mtg*Mg11*. Minimum sporulation (20-40 conidia) was observed in Hgl*Mg5*, Ndk*Mg13*, Slb*Mg7*, Kdr*Mg9*, and the least sporulation (< 20 conidia) was observed in *PtkMg10*, Bvr*Mg12*, Ggr*Mg6* and Alk*Mg16* isolates (Fig. 5).

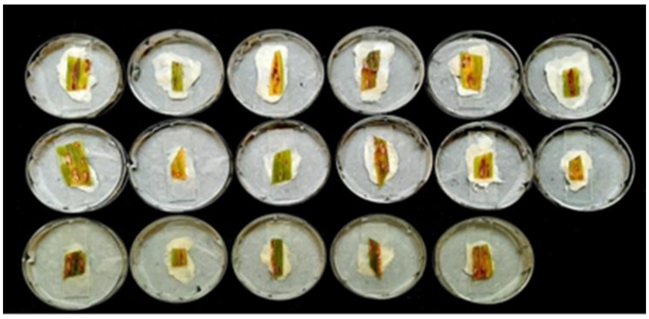

Fig 5: Single spore Isolation from infected pearl millet leaves

4. Discussion
Pearl millet is fourth generally significant among grains and second most significant coarse grains. Pearl millet production is constrained by several biotic and abiotic stresses. Among the biotic stresses, diseases like downy mildew, rust, blast *etc.* cause significant reduction in yield. Pearl millet growing is compelled by a few biotic and abiotic stresses. Among the biotic stresses, diseases like blast, rust, downy mildew *etc*so on reason huge decrease in yield. Pearl millet blast caused by *Pyricularia grisea* (Cooke) Sacc is one of the significant illnesses influencing both forage and grain production in pearl millet and has been noticed all through India. Recently, the blast disease severity on pearl millet commercial hybrids has been rapidly increasing in India owing to the development of susceptibility of pearl millet cultivars [22]. The illness shows up as grayish, water- soaked foliar lesions that enlarge and become necrotic, bringing about broad chlorosis and untimely drying of young leaves [23]. It causes dark brown, appear boat shaped lesions on the lower surface of the leaves. At extreme disease conditions, leaves become totally dry. This disease turns out to be more severe during humid climate conditions particularly with thick plant stands. Study on the severity of blast disease helps to gather information on the predominance, severity and distribution of disease, pathogen diversity in specifically agro-climatic region [15]. The present investigation aimed to understand the level of variations among different *Magnaporthe grisea* isolates collected from various pearl millet growing places of India. A total of 17 districts fields were surveyed for incidence of pearl millet blast and *M. grisea* isolates were collected. The cultured isolates were subjected to variability studies. The highest blast severity was reported 7.9% in Vijayapur district in Karnataka state, and 7.7 % severity was Jaipur of northern India. The severity levels have been have been grouped into low, moderate, and high blast areas, with severity levels of 1–10 %, >10– 50 %, and >50–100 [24]. All 17 isolates collected from the different agro-climatic zone were subjected to cultural and morphological variability investigation on potato dextrose agar medium (PDA). The major variation in the colony color ranged from white to black, mycelial growth from flat to raised, margin varied from irregular to regular, slow to excellent sporulation with pyriform shaped spores, and slow to excellent sporulation (10 to 60 spores/ microscopic field under 100 X).The conidia were two septate, three celled, and middle cell-wide than other cells. The conidial size ranged from 15-37×4–9 µm. In an independent study, conidial size on various host of *Pyricularia grisea* in rice ranged from 15.2–24 × 4.2–8 µm, in pearl millet 12–36.7 × 6–12 µm, in finger millet 10.2–30.5 × 2–10 µm, and 10–35 × 5–12 µm in foxtail millet was documented [25]. The conidial morphology such as size and shape are in agreement with those reported by several other authors [26, 27, 28, 29, 30]. The infestation of

pathogen in host plant leads to development of defense responses. These defense mechanisms are extremely complicated and intricate. They include different responses like death of the plant cells (HR) [31]. A plant's successful protection against attacking pathogens relies upon early acknowledgment of the pathogens and commencement of the proper signaling cycles to activate the multi-cascade defense responses. Interaction between pathogen and their hosts include a persistent replace of data between the two living beings. A systematic understanding of the host–pathogen interaction would require clarification of the biomolecular pathways, which are required for pathogenesis or resistance [32]. Disease resistance mechanisms in plants involve prior physical and chemical obstructions just as inducible defense responses as enlistment of defense related compounds that are triggered upon pathogen infection [33, 34]. Taken together, pathogenicity of the emerging of 17 pearl millet blast isolates fulfill the Koch's postulates which provided a significant variation in terms of disease scale on inoculated plants. This study has crucial implications in detection of pearl millet blast populations across India and the cultural or morphological variability observed in this study not only provide the possible impact of its field survival but will also route a way for elucidation of genetic diversity among 17 isolates of *M. grisea* that needs further investigation by high through molecular markers for breeding high yielding pearl millet blast cultivars or hybrids.

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Conflict of Interest
The authors do not have any conflict of interest to declare in this work.

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