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Cultural and morphological characterization of rice blast pathogen (*Magnaporthe oryzae*) isolated from temperate region of India

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Abstract
Morpho-cultural variability among ten isolates of rice leaf blast pathogen collected from different places of Kashmir region was studied. All the isolates differ in morphological and cultural characters. The isolates were differentiated on the basis of colony characteristics such as, colour, margin, texture and diameter. White colour colonies were produced by three isolates, brownish white by three isolates, two isolates produced greyish coloured colonies and two isolates produced black coloured colonies. Isolates also differed in colony texture, form and diametre. Conidia were pyriform in shape with two septa and size ranged from 20.76 -27.28 × 8.69-10.92 µm. This study will help in identification of *Magnaporthe oryzae* and would enhance the practical application of research in the field of disease control.

Keywords: Blast, isolate, morpho-cultural characterization, management

Introduction
Rice blast is one of the important diseases of rice causing significant yield losses. The annual losses due to diseases were estimated to feed more than 60 million people (Agbowuro *et al*., 2020) [1]. The causal organism of blast is a filamentous heterothallic ascomycete fungus *Magnaporthe oryzae* B.C. Couch (syn: *Pyricularia oryzae* Cavara). The symptoms of rice blast can be seen at any stage of plant development. First spindle shaped lesions are formed on leaves with a wide centre and pointed ends, then the lesions enlarge, coalesce and typically take the shape of a diamond, with a grayish centre and a brown margin, resulting in complete necrosis of infected leaves with a burnt appearance from a distance (Ramesh, 2015) [11]. The disease is known to be highly adaptable to variable environmental conditions. Highest variability has been reported while taking into account cultural, morphological, and pathological characteristics of *Pyricularia oryzae* from India and other parts of the world (Aruna *et al*., 2016) [2].
The fungus has the ability to overcome resistance within a short time after the release of a resistant cultivar and thus has made breeding for resistance a constant challenge. Therefore, to design effective and efficient disease management strategy, knowledge about biology of the pathogen is of utmost importance. Since, less information is available on existence of variability among leaf blast isolates under Kashmir conditions, so, the present investigations were carried out to understand the morphological and cultural variation among the pathogen population under Kashmir conditions.

Materials and Methods
Sample collection and Isolation
Survey of rice growing areas of Kashmir was carried out to collect the diseased samples for the isolation of blast isolates. Isolation was done by cutting leaf discs of 5mm at the zone of advancing decay and surface sterilizing in 2.5 percent sodium hypochlorite for 3 min. The tissues was rinsed with sterile distilled water thrice, dried and then plated on Potato Dextrose Agar (PDA) medium in 90 mm diameter Petri dishes. Cultures were incubated under 12:12-h light: dark cycles at 24±1 ºC for 4 days and pure cultures were obtained by sub-culturing onto fresh PDA medium (Farahanaz, 2015) [4].

Monosporic isolates were obtained by Single Spore Technique in which the conidial suspension of each isolate was prepared and poured onto water agar (agar 20 g in 1L of water). After 12 hours of incubation at room temperature, individual conidia were located using a compound microscope and single conidium will be transferred onto clean PDA medium using sterile mounted needles. The single spore cultures thus obtained were maintained on Potato Dextrose Agar slants at 4 ºC.

Morphological and cultural characterization of pathogen isolates.

Morpho-cultural characterization of blast isolates was determined according to Singh *et al*., (2018) [15]. Single spore isolates obtained as described above were grown on PDA medium at 24±1 ºC. Spore (conidia) characteristics such as shape and size of different isolates was observed. The spore measurements will be recorded under the compound microscope previously calibrated using ocular and stage micrometer. The colony characteristics such as colony texture, form, colony margin of different isolates was assessed on potato dextrose agar medium (PDA). Approximately 20 ml of sterilized media was poured in Petri plates and then allowed to solidify. A 5 mm diameter disc cut with the help of sterilized cork borer from 15 days old cultures of different isolates will be inoculated at the centre of each Petri plate and each treatment will be replicated three times. The inoculated Petri plates were incubated at 25±1 ºC and colony diameter was recorded.

Results and Discussion

Rice leaves showing typical blast symptoms were collected from different regions of Kashmir and the pathogen was isolated for further studies. The isolates are designated as I-1 to I-36.

Cultural and morphological growth characters of *Magnaporthe oryzae* under *in vitro* conditions

The pathogen was identified as *Magnaporthe oryzae* on the basis of colony characters and conidial structures (Ou, 1985) [8]. All the thirty six isolates showed differences in their colony characters (Fig.1.) and size of the conidia (Fig.2.). Based on colony colour the isolates were classified into six groups. Three isolates *viz*; I-2, I-9, I-10, produced white colour colonies, nine isolates *viz*; I-8 and I-3 produced Greyish white colonies, brownish colonies were produced by three isolates i,e I-5, I-6, I-7, and, I-1 and I-4, produced black (Table-1, Fig.1). The results of the experiment were in match Ou (1985) [8] and Mew and Gonzales (2002) [5] who detailed the usage of PDA medium for isolation of the fungus. In view of this study, we presumed that the rice blast fungus could be effectively isolated on PDA medium. Isolates also exhibited variation with respect to colony texture and form, on the basis of colony texture isolates were placed into three groups. Four isolates exhibited raised cottony growth *viz*: I- 2, I-5, I-6 and I-7,Three isolates *viz*: I-8, I-9 and I-10 exhibited flat colony texture while as two isolates *viz*: I-1 and I-4 showed raised fluffy colony texture (Table-1, Fig.1). Majority of isolates were having circular margins *viz* I-1, I-3, I-4, I-5, I-6, I-7, I-9 and I-10 while as I-2 and I-8 exhibited irregular colony form (Table-1, Fig.1). The colony character, sporulation and growth rate of the blast fungus fluctuates with isolates utilized. The variation in colony characters *viz*., colony colour, margin, pigmentation, surface texture, mycelial growth and sporulation were studied in different *M. oryzae* isolates. The outcomes indicated that the cultural characters of *M. oryzae* isolates fluctuates significantly with a different origin. This depends on the age of lesions, varietal resistance and the prevailing environmental conditions (Ou, 1985) [8]. These outcomes were in concurrence with the prior work of Mior *et al*. (2017) [6] on the molecular and morphological characterization of rice blast fungus *M. oyrzae*. They have grown 13 isolates in both PDA and OMA medium and found the variation of colony colour and mycelial characters between the isolates. Also, Variability among 40 neck blast isolates of *Pyricularia oryzae* collected from different agro-ecological zones of Punjab was studied with respect to morphological and cultural traits. The isolates exhibited different colony colours and forms with distinct margins and were categorized in four groups. Blackish grey colour colonies were produced by most of the isolates followed by whitish grey and only one isolate (NB-14) produced grey coloured colonies on oatmeal agar (OMA) medium. Isolates produced variable colony forms as circular, irregular, circular with saltation having colony margins as entire or undulate or entire with concentric rings (Singh *et al*., 2018) [15]. The conidial size ranged from 20.74-24.91µm × 7.53-10.23 µm on OMA medium and 20.85-26.33µm×6.76-8.32 µm on Paspalum grass medium, respectively. Srivastava *et al*. (2014) [16] likewise reported comparable sorts of results in morphological and molecular characterization of *Pyricularia oryzae* causing blast disease in rice wherein variations in colony colour, morphology and conidia shape were noticed between the isolates. Likewise Sahu *et al*., 2018 [12] categorized twenty isolates categorized into three groups based on colony colour i.e., grayish blackish, grayish and white, and in two group based on the texture of the colony as smooth and rough. Similarly, Aruna *et al*., (2016) [2] studied five different isolates on OMA medium and reported diversity in their cultural characteristics.

The data presented in Table-1 represents colony diameter different isolates and showed significant differences among the isolates. The colony diameter of isolates ranged from 73.0 to 90.0mm. Highest colony diameter was exhibited by I-9 followed by I-4 and I-1 and lowest by I-2. These results corroborate the findings of who studied radial growth of 12 different isolates of *P. oryzae* and observed maximum radial growth in Khanpur isolate (88.82 mm) and minimum in isolates collected from Mugad and Sirsi (62.5 mm). Similarly, Ramesh (2015) [11] recorded radial growth rate ranging between 70-90 mm among different test isolates. Based on colony growth rate, the isolates were categorized into two groups and were designated as medium growers and fast growers by Singh *et al*., 2018 [15].

No distinct variations were observed in shape and colour of conidia among different isolates. The conidia were pyriform and hyaline with 2-3 septa. However, the size of conidia (length and breadth) ranged from 20.76 -27.28 × 10.92 µm (Table 2), respectively. These results are in complete agreement with those of Veeraraghavan and Padmanabhan (1965) who reported that the dimensions of conidia produced by *P. oryzae* ranged from 17.6 to 24.0 µm in length and 8.0 to 9.6 µmin width. Similarly, Ramesh (2015) [11] measured size of the conidia that varied between 25.5- 38.5 µm and dimensions of conidia produced by *P. oryzae* ranged from 8.5 to 11.1 µm in length and 3-4 µm in width.

Table 1: The colony characteristics of *M. oryzae* isolates on PDA medium.

Isolates	Colony Colour	Colony texture	Colony form	Mean Colony diameter (mm)
I-1	black	Raised, fluffy	Circular	88.90
I-2	White	Raised, cottony	irregular	73.0
I-3	Greyish white	Flat	Circular	80.0
I-4	Black	Raised, fluffy	Circular	89.3
I-5	Brownish white	Raised, cottony	Circular	87.8
I-6	Brownish white	Raised, cottony	Circular	88.5
I-7	Brownish white	Raised, cottony	Circular	85.6
I-8	Greyish white	Flat	irregular	74.1
I-9	White	Flat	Circular	90.0
I-10	White	Flat	Circular	87.54

Table 2: Spore Characteristics of *M. oryzae*.

Isolates	Length range (µm)	Mean length (µm)	Breadth range (µm)	Mean breadth (µm)
I-1	18.82- 28.51	23.58	7-10- 8.7	8.69
I-2	18- 25.07	20.76	7.03- 12.05	8.97
I-3	22.73-33.87	27.72	7.71- 12.81	10.02
I-4	19.25-29.40	26.46	9.71- 11.11	10.32
I-5	25.57-30.01	27.42	9.75- 12.30	10.08
I-6	21.31-30.20	24.90	9.41- 13.87	10.92
I-7	20.10- 29.66	24.04	8.10- 12.05	10.8
I-8	18.79- 27.77	24.36	8.68- 11.20	9.5
I-9	20.56- 29.63	26.17	7.33- 11.45	9.19
I-10	22.73- 32.49	27.28	9.00- 12.00	10.5

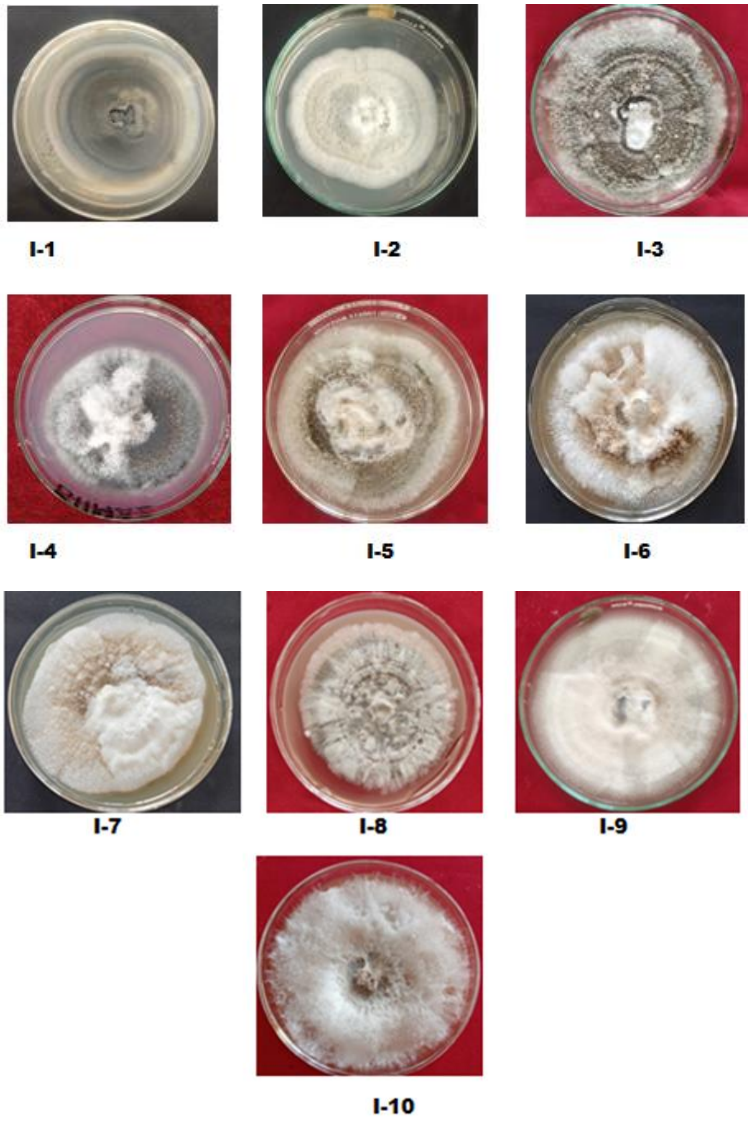

Fig 1: Colony characteristics of *Magnaporthe oryzae* isolates on PDA medium

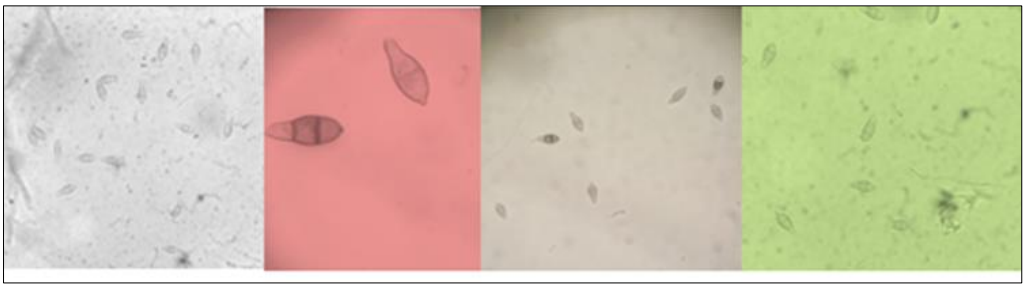

Fig 2: Spores of *M. oryzae*.

Conclusion

Ten fungal isolates of blast infected rice leaves collected from different locations of Kashmir were identified as *Magnaporthe oryzae* based on the cultural and morphological characters. The size and shape of spores are important criteria for classification and identification of *Magnaporthe* species. The present observations on the collected field isolates from rice indicate morphological variation in colony form and spore size.

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