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SURVEY ON RICE BLAST IN TWO SELECTED DISTRICTS OF BANGLADESH AND VARIATIONS OF *Magnaporthe oryzae* IN DIFFERENT CULTURE MEDIA

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ABSTRACT

Rice blast, caused by *Magnaporthe oryzae*, is one of the most devastating diseases of rice worldwide, particularly in South and Southeast Asia. This study aimed to assess the incidence of rice blast in two major rice-growing districts, Cumilla and Brahmanbaria, of Bangladesh, and to evaluate the morphological variation of the causal pathogen under different culture media. Field surveys were conducted during the Boro season 2020-2021, and disease severity was recorded using standard disease scoring scales. Infected samples were collected and used to isolate *M. oryzae* in the laboratory. A total of 6 isolates of *M. oryzae* were cultured on Potato Dextrose Agar (PDA), Oatmeal Agar (OMA), Potato Sucrose Agar (PSA), Rice Flour Yeast Agar (RFYA), and their morphological features were compared. Significant differences in colony color, surface texture, growth rate, and conidial shape were observed among the media. The highest mycelial growth was recorded on RFYA media, which is 90 mm with grayish white colony color and a rough, cottony, irregular surface structure. At the same time, distinct pigmentation was prominent on OMA and other media. These findings enhance understanding of rice blast distribution in Bangladesh and the media-dependent morphological traits of *M. oryzae*, which can aid in future diagnostics and disease management strategies.

Key Words: Rice blast survey, Culture-based identification, Colony characteristics, Mycelial growth rate, Pigmentation, Growth variation in media

INTRODUCTION

Rice is one of the most vital cereal crops globally, serving as the primary energy source for over half of the world's population (Fones et al., 2020). More than 90% of the global rice is produced and consumed in Asia, where food security heavily depends on its stable yield (Kole, 2006). By 2050, global demand for rice is projected to rise more than 40% to feed the rapidly growing world population (Milovanovic and Smutka, 2017). Despite significant increases in rice production from 289 million tons in 1968 to over 780 million tons in 2018 (FAOSTAT, 2020). Climate change, pest pressure, and diseases continue to threaten yield stability. Among the diseases, rice blast, caused by *Magnaporthe oryzae*, remains the most destructive fungal threat, responsible for substantial yield losses under favorable conditions. Rice blast disease is the most serious threat to global rice production, destroying sufficient rice each year to feed 60 million people, but the fungus can also infect over 50 different grass species, including wheat, finger millets, and barley (Matthew R Wengler & Nicholas J Talbot, 2025).

"Incidence and severity of blast disease of rice were recorded in ten agro-ecological zones during Boro (November to May; irrigated ecosystem) and Transplanted Aman (July to December; rainfed ecosystem) seasons. Disease incidence and severity were higher in the irrigated ecosystem (Boro season) (21.19%) than in the rainfed ecosystem (Transplanted Aman season) (11.98%). It was as high as 68.7% in Jhalak hybrid rice variety, followed by high-yielding rice cultivar BRRI dhan47 (58.2%), BRRI dhan29 (39.8%), BRRI dhan28 (20.3%) during Boro, and in BRRI dhan34 (59.8%) during T. Aman season." (Mohammod Hossain, 2017 & Mahmud et. al., 2021). *M. oryzae* isolates show a certain level of cultural diversity in colony colour, colony growth, conidia shape, and virulence with respect to geographical distribution (R. Rahila, 2020).

A critical aspect of understanding and identifying *M. oryzae* is its morphological variation across different culture media. *M. oryzae* is highly adaptable and shows considerable morphological variation depending on the culture medium. On Potato Dextrose Agar (PDA), *M. oryzae* colonies exhibit "concentric circles with black diffused pigmentation" and an irregular margin, while on Oat Meal Agar (OMA), colonies appear cottony with black pigmentation and also have irregular margins, notably facilitating higher sporulation. On a Rice-based medium, colonies show partially diffused mycelial growth with irregular pigmentation and irregular margins (IJSTR, 2019). Among different media used, YSE, YSS, and newly formulated KKSP medium supported maximum growth and mycelia production, and OMA medium was found to be an excellent medium for sporulation, followed by starch and YSS medium, which were good for sporulation (Kalpana Kulkarni & Dr. Swati Peshwe,2019). Another study has shown in their morphological analysis that colonies on PDA were described as white to light gray or dark gray, whereas on OMA, they showed pigmentation ranging from different shades of gray to translucent light brown, with better mycelial growth and sporulation (Rafael et al., 2023).

The disease can be managed by the use of fungicides, resistant cultivars, agronomic practices, and biotechnological methods (Ribot et al., 2008). The disease was under intensive investigation during the past four to five decades. Six international symposia were organized by the International Rice Research Institute, LusBanos, the Philippines (Anonymous, 1965-1979). The genetic nature of this fungus enables it to overcome the resistance offered by major R-genes (Roy Chowdhury et al., 2012; Vasudevan k. et al., 2014). The high existence of retro-transposons and repetitive segments makes the fungus more virulent and changes its pathogenicity very often (Dean et al., 2005). As evidence to this, researchers have reported the breakdown of rice blast resistance and occurrence of high disease incidence with severe yield losses (Khush and Jena 2009; Sharma et al., 2012; Nalley et al., 2016). Rice has tremendous adaptation ability from Hokkaido to Honolulu (Biswas et al., 2017). In Bangladesh, because there was rain during the flowering stage of BRRI dhan28, BRRI dhan50, BRRI dhan61, and BRRI dhan63, they are tolerant to the blast diseases.

In this study, on Potato Dextrose Agar (PDA), colonies typically appear blackish white to grayish white or blackish gray with rough velvety, irregular surface structure. On Oatmeal Agar (OMA), growth is generally more compact, with grayish-white colony color and rough, velvety, regular surface structure. Rice Flour Yeast Agar (RFYA) and Potato Sucrose Agar (PSA) often support higher growth but with noticeable differences in colony texture, pigmentation, and surface structure. These morphological traits are key for fungal characterization and indicate the underlying genetic diversity of the pathogen. Such variations may influence pathogenicity and virulence, which are essential considerations in disease epidemiology and resistance breeding. The present study reports on the identification of stable, resistant rice germplasm by linking field-based blast data in Cumilla and Brahmanbaria districts of Bangladesh with pathogen variability and culture characteristics in different media.

MATERIALS AND METHODS

The various aspects of the present investigation on blast disease of rice (*Oryza sativa* L.) incited by *Magnaporthe oryzae* were conducted in the laboratory of the Plant Pathology Department, Sher-e-Bangla Agricultural University, and at UBN, BRRI, Gazipur in the Robi season of 2020 to 2021. The materials used and methods or techniques adopted during the course of the present investigations are described in this chapter.

Experimental site

A survey was conducted in different villages of Cumilla, and Brahmanbaria districts, namely Noapara, Cumilla Sadar, Majhi Gacha, North Durgapur, Burichang Sadar, Champak Nagar, Nabinagar, Nawabpur, Chandina & Nasir Nagar, Ibrahimpur. Isolation of *Magnaporthe oryzae*, determination of morphological characteristics, pure culture preparation, and germplasm screening against *Magnaporthe oryzae* for improving resistance against blast disease were conducted in the Plant Pathology laboratory, Sher-e-Bangla Agricultural University, Dhaka -1207 and Uniform Blast Nursery (UBN), Bangladesh Rice Research Institute (BRRI), Gazipur 1701.

Survey on the incidence and severity of rice blast and collection of diseased samples

The survey on rice blast disease was conducted in farmers' fields of Bangladesh, namely Cumilla and Rangpur, during the Boro (November to May; irrigated ecosystem) season. In this season survey was conducted during the pre-flowering stage of the rice crop to observe leaf and node blast. Soil type, cropping pattern, and cropping intensity are taken into consideration in order to select locations. Two plots of each location were selected, having a size of at least 1500 square meters. In each location, intensive rice areas under irrigated conditions were selected. For the survey of blast disease, a zigzag sampling pattern was followed in this study at every 50-step interval single hill consisting of several tillers/plants was selected and recorded for disease incidence and severity. Disease incidence and severity were assessed through field surveys conducted in farmers' fields across the selected locations. The percentage of disease incidence was calculated using the standard formula:

$$\text{Disease Incidence (\%)} = \left(\frac{\text{Number of infected plants}}{\text{Total number of observed plants}}\right) \times 100$$

Disease severity was assessed in the field by randomly selecting and tagging representative unit plots. Disease incidence was quantified as the percentage of infected panicles within each plot. Severity levels were evaluated using the standardized IRRI 0–9 severity scale developed by the International Rice Research Institute (2014) where 0= no lesion observed; 1= 1 = 1% leaf area covered; 3= 10% area covered; 5= 25% leaf area covered; 7=50% leaf area covered; 9= more than 50% leaf area covered. Symptomatic leaves and panicles exhibiting characteristic rice blast lesions were collected from the surveyed locations. The samples were sun-dried, placed in sterile brown paper envelopes, and transported to the laboratory for subsequent pathological and morphological analyses.

Experimental design and statistical analysis

The experiment was carried out using a complete randomized design (CRD) with three replications, and statistical analysis was performed using Statistix 10 software. Using a significance level of P=0.05, the least significant differences (LSD) were determined.

Isolation and identification of *Magnaporthe oryzae*

Leaf blast- spindle-shaped spots with grey centre and brown margin, Neck blast- greyish brown lesions on the neck, Node blast- black lesions on the node which later break up, thus symptoms of rice were sampled throughout the rice production field of Noapara, champaknagar and B. Baria at Cumilla sadar. Air-dried panicles were placed in paper bags and returned to the laboratory before isolation. Symptomatic rice necks or panicles from the collected crops were trimmed to approximately 5-7 cm, and surface sterilized with 1% Sodium hypochlorite for two minutes. These cut pieces were then washed with sterilized water and incubated in 100 % relative humidity overnight to induce sporulation. Single germinating conidia were isolated under the microscope. Conidia of *M. oryzae* were removed from panicle nodes and panicles with a sterile loop and placed on water agar medium Petri dishes aseptically. These WA plates were incubated at 25° C for 7 days for the isolation of the causal agent. Five seeds/pieces of diseased plant parts were placed in each Petri dish. These Petri dishes were incubated at 25° C for 7days to induce sporulation of the fungi. After 7 days, small pieces of mycelia were cut from the edge of the culture and then transferred to a new potato dextrose agar (PDA) and oatmeal agar (OMA) petri dish for pure culture. Different fungal colonies appeared, which were purified and multiplied on PDA and OMA. The identification of the pathogen was made by studying the colony characteristics of the isolates on the PDA plates by following the method described in a technical bulletin on seed-borne disease and seed health testing of rice (Agarwal et al., 1989). The pathogenicity of isolates was confirmed by following Koch's postulates, i.e. Association, Isolation, Inoculation, and Re-isolation of the pathogen *M. oryzae.*

Preparation of culture media used in this study

The isolates of *Magnaporthe oryzae Oryza* (MoO) were grown on PDA for 10 days at room temperature. From the margin of actively growing fungus, 5-mm discs were cut out. Sterile Petridishes containing Water Agar (WA), Potato Dextrose Agar (PDA), Rice flour Yeast Agar (RFYA), and Oat Meal Agar (OMA) were inoculated each with a single 5mm disc of the fungus and incubated at room temperature for 7 days. The fungal growth was measured at 14 DAI. Further, the colony characteristics of the 24 isolates were grown on PDA, and their colony morphology was observed. Water Agar (WA), Potato Dextrose Agar (PDA), Rice flour Yeast Agar (RFYA), and Oat Meal Agar (OMA) medium were used for isolation and purification of the causal organism from the infected rice blast samples.

Water agar (WA) medium preparation

Thirty grams of agar were put in a 2-liter capacity flask. 1000 ml distilled water was mixed with agar in the flask. Then the flask was plugged with cotton and autoclaved at 121 °C for 20 min at 15 psi. (Hayashi et al., 2009).

Potato Dextrose Agar (PDA) medium preparation

200 grams of potato was boiled in 500 ml of distilled water for one hour. After boiling, the potato juice was filtered through cheesecloth or nylon mesh. 20 g Dextrose and 20 gm Agar were poured into the potato juice, and the volume was adjusted to 1000 ml by adding distilled water. Finally, the flask was plugged with cotton and autoclaved for 15-20 min at 15 psi (Okunowo, 2010).

Rice Flour Yeast Agar (RFYA) medium preparation

15-gram Rice flour was put in a 1-liter capacity flask, sieved, and rice extract was made. Then, 4-gram yeast extract with 20-gram agar was mixed in the flask. Then the flask was plugged with cotton and autoclaved for 20 min at 15 psi.

Oat Meal Agar (OMA) medium preparation

60 grams oat meal was put it in a 2-liter capacity flask. 12.5gram agar was mixed with 1000 ml distilled water was added into this. Then the flask was plugged with cotton and autoclaved for 20 min at 15 psi.

Potato sucrose agar (PSA) medium preparation

200 grams of potato was boiled in 500 ml of distilled water for one hour. After boiling, the potato juice was filtered through cheesecloth or nylon mesh. 20 g of sucrose and 20 gm Agar was poured into the potato juice, and the volume was adjusted to 1000 ml by adding distilled water. Finally, the flask was plugged with cotton and autoclaved for 20 min at 15 psi (Okunowo, 2010).

Isolation and pure culture of *Magnaporthe oryzae*

Collected infected leaf and panicle samples were cut into 5-7cm sections. These sections were surface sterilized by dipping in 1% Sodium hypochlorite for two minutes and were washed with sterilized water several times, and then the cut sections were placed on moist filter paper (Whatman: 9.0cm) in a sterile petri dish. Plates were incubated for 24 hours at room temperature (250°C). After 24 hours, the infected parts were examined under a stereo microscope (Motic SMZ-168). Conidial masses were picked by using a very fine tip needle and spread on 3% water agar plate for 3 days to induce sporulation of the fungi. After 3 days, small pieces of mycelia were cut
from the edge of the culture and then transferred to new potato dextrose agar (PDA) and oatmeal agar (OMA) petridish for pure culture.

Different fungal colonies appeared, which were purified and multiplied on PDA and OMA.

Identification of the causal organism

The pathogen isolated from the diseased specimen and established in pure form was identified on the basis of colony and morphological characters (Rahila, R. et al., 2020).

Mycelial growth and morphological characterization

The mycelial growth of *Magnaporthe oryzae* was assessed on four different culture media: PDA, PSA, RFYA & OMA. Growth observation was recorded at 3, 7, and 14 days after inoculation (DAI). However, only data recorded at 14 DAI were analyzed and presented in this study to ensure consistency across isolates. Quantitative data on radial mycelial growth (mm) and daily growth rate (mm/day) were measured. In addition, detailed morphological characterization was conducted, including observations on colony growth patterns, colony coloration (obverse and reverse), surface texture, and colony shape, under standard laboratory conditions. These morphological traits were used to support species-level identification and assess phenotypic variability among isolates.

Confirmation of *Magnaporthe oryzae*

Conidial masses were picked by using a very fine tip needle and placed in glass slide. Then the slide was observed under a compound microscope with a cover slip. Typical pyriform conidia were found. The conidia were found to show variations in septation, ranging from one to three septations, and the majority of the conidia had three septations. Different solid media viz., potato dextrose agar, potato carrot agar, Kirchoff‟s, medium, Richard‟s medium, Sabourad‟s medium, Takahashii‟s medium, rice leaf extract agar and oat meal agar and liquid media viz., potato dextrose broth, potato carrot broth, Kirchoff‟s broth, Richard‟s broth, Sabourad‟s dextrose broth, Takahashii‟s broth and rice leaf extract broth was also used to culture rice blast pathogen (Akhilesh,2017). Among all the solid media the highest mean mycelial growth of the fungus *Magnaporthe oryzae* (Cav.) was recorded on oatmeal agar (77.6 mm) followed by rice leaf extract (75.9 mm), and least mean mycelial growth of the *M. oryzae* (Cav.) on Sabourad‟s media (44.7 mm) followed by Takahashii‟s media (52.5 mm). They found the highest mean mycelia growth of the fungus *Magnaporthe oryzae* (Cav.) was recorded on oat meal agar. But in our study highest mycelial growth was found in RFYA media. The shape, color, and compactness of the fungal colonies varied with the media and isolates.

Pathogenicity study of collected *M. oryzae* isolates

To confirm the pathogenic potential of the isolated *Magnaporthe oryzae* strains, pure cultures of each isolate were cultivated on Oatmeal Agar (OMA) medium and incubated for 30 days at 25 ± 1°C under a photoperiod of 14 hours of fluorescent light followed by 10 hours of darkness to promote sporulation.

Following incubation, conidial spores were harvested by gently flooding the culture surface with sterile distilled water and scraping with a sterile loop. The resulting suspension was filtered through a sterile sieve to remove mycelial debris and centrifuged at 5,000 rpm for 10 minutes. The spore pellet was resuspended in sterile distilled water, and the spore concentration was adjusted to 1×10^5 conidia/mL using a hemocytometer.

At the 3–4 leaf stage, healthy rice seedlings were uniformly sprayed with the conidial suspension using a fine sprayer. All inoculated and control plants were maintained under controlled conditions (25 ± 1°C, 90–95% relative humidity) in an incubation chamber to facilitate infection.

After 10 days, plants were evaluated for the presence of typical spindle-shaped blast lesions, confirming successful infection. To complete Koch's postulates, the pathogen was re-isolated from the symptomatic leaf tissue onto fresh OMA plates. The emerging fungal colonies were compared to the original isolate based on colony morphology and microscopic characteristics, confirming that the same pathogen was responsible for disease development.

Fig. 1 Conidia of *Magnaporthe oryzae* on potato dextrose agar observed under compound microscope (40X)

RESULTS AND DISCUSSION

Survey on rice blast disease

In the Boro season 2020-2021, a survey was done in different villages of Cumilla and Brahmanbaria district of Bangladesh. The blast disease incidence and severity were recorded during the survey. From the survey, it is exposed that the highest incidence of blast disease was recorded from Noapara (57%) with a severity score of with severity scores 5 and 7, respectively.

9. The lowest incidence of last disease was recorded from Burichang and Champaknagar (10% and 9%, respectively), with a severity score of 1 for each location. No disease was recorded on BRRI Dhan89 in North Durgapur (0%). In Chandina BRRI dhan28 and BRRI dhan29 showed 50% and 40% blast incidence with a severity score 7 for both varieties. Nasir Nagar and Ibrahimpur showed incidence 30% and 40%

Table 1. Survey on blast disease in different villages of Cumilla and Brahmanbaria district of Bangladesh in Boro season 2020-2021

Name of district	Name of villages	Varieties	Blast disease	
			Incidence (%)	Severity Score
Cumilla	Noapara	BRRI dhan81	57%	9
	Cumilla Sadar	BRRI dhan29	51%	7
	Majhi Gacha	BRRI dhan50	40%	3
	North Durgapur	BRRI dhan89	0%	0
	Burichang Sadar	BRRI dhan74	10%	1
	Champak Nagar	BRRI dhan69	9%	1
	Nabinagar	BRRI dhan17	20%	5
	Nawabpur Chandina	BRRI dhan28	50%	7
Brahmanbaria	Nasir Nagar	BRRI dhan88	30%	5
	Ibrahimpur	BRRI dhan84	50%	7
Total Severity Score				**45**

Disease Severity (DS) formula:

$$Disease\ Severity = \frac{Leaf\ area\ diseased}{Total\ leaf\ area} \times 100$$

The assessed Percent Disease Index (PDI) is 50%.

A significant variation in the degree of disease susceptibility was observed among different rice cultivars when scoring against *M. oryzae* infection scale. The prevalence of disease was higher in highly susceptible local cultivars than in improved cultivars. The local cultivars are susceptible, breeding cultivars were resistant, and some cultivars were semi-susceptible to leaf blast in the nursery (Amanzadeh, 2004). The disease incidence varied among collection field sites and varieties, and the variation could be in weather condition, temperature, humidity, soil conditions, soil management techniques etc. The field's management practices may also account for variation in disease incidence. A survey on rice blast was conducted in 5 districts of Bangladesh, namely Mymensingh, Kishoreganj, Barishal, Naogaon and Cumilla and among those Muktagachha, Mymensingh was found as the highest rice blast disease infected area and Bakerganj, Barishal was found as the lowest in the Boro season 2017-2018 (Rayhanul et al., 2018). The report revealed that the highest incidence of blast disease was recorded in Muktagachha (60%), with a severity score of 5. The highest severity score for blast disease was observed in Hossainpur, Kishoreganj, at 7, but the percent incidence was only 20%. In Boro 2018-19 and Aman 2019, a survey was done in three northern districts of Bangladesh, namely Gaibandha (Gobindogonj and Mohimagonj), Dinajpur (Birampur), and Bogura (Dupchanchia). The highest incidence of blast was recorded from Gobindogonj (84.26%), where the severity score was 7. The highest severity score of blast was recorded in Mohimagonj, which was 9, but the percent incidence was only 29.12% (Nazifa et al., 2021).

In vitro evaluation of six *Magnaporthe oryzae* isolates revealed significant differences in radial mycelial growth, colony characteristics, and morphology on PDA medium at 14 days after inoculation (DAI) (Table 2). Among the isolates, MoO2 exhibited the highest radial growth (72 mm), forming a blackish-white, rough, velvety, and irregular colony. In contrast, MoO1 showed the lowest growth (41 mm), with a similar blackish-white, rough, velvety, and irregular colony. Surface textures ranged from velvety to cottony, while colony shapes were predominantly irregular, except for MoO4, which displayed a regular shape with a grayish-white, rough cottony appearance. These morphological distinctions may reflect underlying genetic or physiological variations among the isolates.

Table 2. Radial mycelial growth, colony character, surface texture and shape of 6 isolates of *Magnaporthe oryzae* on PDA at 14 DAI.

Isolates	Radial Mycelial Growth Average (mm)/ 14 DAI	Colony Character	Surface Texture	Shape
MoO1	41 b	Blackish white	Rough velvety	Irregular
MoO2	72 a	Blackish white	Rough velvety	Irregular
MoO3	53 ab	Blackish white	Rough velvety	Irregular
MoO4	48.5 ab	Grayish white	Rough cottony	Regular
MoO5	66 ab	Grayish white	Rough velvety	Irregular
MoO6	47 ab	Blackish gray	Rough cottony	Irregular

Table 3. Radial mycelial growth, colony character, surface texture and shape of 6 isolates of *Magnaporthe oryzae* on OMA at 14 DAI.

Isolates	Radial Mycelial Growth Average (mm)/ 14 DAI	Colony Character	Surface Texture	Shape
MoO1	32.5 b	Grayish white	Rough velvety	Regular
MoO2	40 ab	Blackish white	Rough velvety	Regular
MoO3	51.5 ab	Grayish white	Rough velvety	Irregular
MoO4	44 ab	Grayish black	Rough velvety	Regular
MoO5	66 a	Grayish black	Rough cottony	Regular
MoO6	60 ab	Whitish Gray	Rough velvety	Regular

The growth performance and morphological characteristics of six *Magnaporthe oryzae* isolates were assessed on Oatmeal Agar (OMA) medium at 14 days after inoculation (DAI) (Table 3). Radial mycelial growth varied significantly among isolates, ranging from 32.5 mm (MoO1) to 66 mm (MoO5). Isolate MoO5 exhibited the highest radial growth with a grayish black, rough, cottony, and regular colony morphology. Conversely, MoO1 showed the lowest owth, forming a grayish white, rough velvety, and regular colony. Most isolates displayed regular colony shape, with the exception of MoO3, which was irregular in form. Surface texture was predominantly rough velvety, while colony color varied from grayish white to grayish black and whitish gray. The observed morphological and growth differences suggest phenotypic variability among the isolates, potentially linked to their pathogenic or environmental adaptability on OMA.

Table 4. Radial mycelial growth, colony character, surface texture and shape of 6 isolates of *Magnaporthe oryzae* on RFYA at 14 DAI.

Isolates	Radial Mycelial Growth Average (mm)/ 14 DAI	Colony Character	Surface Texture	Shape
MoO1	40 b	Blackish white	Rough cottony	Regular
MoO2	90 a	White	Rough velvety	Regular
MoO3	68.5 ab	Blackish white	Rough cottony	Irregular
MoO4	49.5 ab	Grayish white	Rough cottony	Irregular
MoO5	51 ab	Blackish Gray	Rough cottony	Regular
MoO6	65.5 ab	Blackish Gray	Rough cottony	Irregular

Six isolates of *Magnaporthe oryzae* were cultured on Rice Flour Yeast Agar (RFYA) to evaluate their radial mycelial growth and colony morphology at 14 days after inoculation (DAI) (Table 4). Substantial variation was observed among the isolates. MoO2 demonstrated the most vigorous radial growth (90 mm), forming a white, rough, velvety, and regular colony. In contrast, MoO1 showed the least growth (40 mm) with a blackish-white, rough, cottony, and regular colony. The majority of isolates exhibited rough cottony surface textures, except MoO2, which was rough velvety. Colony color ranged from white to various shades of blackish or grayish hues, reflecting pigment diversity. Colony shape was predominantly regular, though MoO3, MoO4, and MoO6 displayed irregular growth patterns. These findings highlight notable morphological and growth diversity among *M. oryzae* isolates on RFYA

Table 5. Radial mycelial growth, colony character, surface texture and shape of 6 isolates of *Magnaporthe oryzae* on PSA at 14 DAI.

Isolates	Radial Mycelial Growth Average (mm)/ 14 DAI	Colony Character	Surface Texture	Shape
MoO1	48.5 a	Blackish white	Rough velvety	Irregular
MoO2	37.5 ab	Pinkish white	Rough cottony	Regular
MoO3	46.5 a	Whitish	Rough cottony	Irregular
MoO4	28.5 ab	Grayish white	Smooth velvety	Regular
MoO5	43 ab	Blackish white	Smooth velvety	Irregular
MoO6	23.5 b	Pinkish white	Smooth velvety	Irregular

The radial mycelial growth and colony morphology of six *Magnaporthe oryzae* isolates were evaluated on Potato Sucrose Agar (PSA) at 14 days after inoculation (DAI) (Table 5). The isolates exhibited marked variability in growth and morphological characteristics. MoO1 showed the highest radial growth (48.5 mm), producing a blackish-white, rough, velvety, and irregular colony. In contrast, MoO6 exhibited the lowest growth (23.5 mm), with a pinkish white, smooth velvety, and irregular colony. Colony surface textures ranged from rough cottony to smooth velvety, with the latter more prevalent among isolates MoO4–MoO6. Colony colors were diverse, including blackish white, pinkish white, grayish white, and whitish, indicating pigment and nutrient assimilation differences on PSA. Most isolates formed irregular colonies, while MoO2 and MoO4 displayed regular morphology. These findings underscore the morphological diversity of *M. oryzae* isolates under nutrient-rich PSA conditions, suggesting a potential influence of media composition on phenotypic expression.

In this study, PDA, PSA, OMA and RFYA media were used for obtaining cultural growth of 6 *Magnaporthe oryzae* isolates, in which highest mycelial growth was found in RFYA media, which was 90 mm and the lowest mycelial growth was found in PSA media that 23 mm. The shape, color, and compactness of the fungal colonies varied with the media and isolates. Among the non-synthetic media, potato dextrose agar supported maximum radial growth (85.00 mm), next was host extract (±2%) in potato sucrose agar medium (80.33 mm) followed by oatmeal agar (75.00 mm) (Mijan Hossain, 2000).

The variation in radial mycelial growth and colony morphology of *M. oryzae* across different culture media aligns with earlier findings (Ou, 1985; Bonman et al., 1986). Differences in texture, pigmentation, and colony shape are known to be influenced by nutrient availability and media composition (Amanzadeh et al., 2004; Gnanamanickam, 2009). PSA, PDA, and OMA have been widely used for culturing *M. oryzae*, each affecting phenotypic expression differently (Fukuta et al., 2002; Sharma et al., 2012). Among the five different growth media highest mycelia growth was observed in Oat Meal Agar (20 mm), and the lowest in Water Agar (10mm) at 7 DAI. Colony color of all the isolates was whitish grey to blackish with sufficient growth, and the average colony diameter was 50 mm (Nazifa et al., 2021

Fig. 2 Mycelial growth of four isolates of *Magnaporthe oryzae* on OMA, PDA, RFYA and PSA media.

Pathogenicity Assessment

This assessment evaluated six isolates of *Magnaporthe oryzae* (MoO1–MoO6) and found them pathogenic on a susceptible rice variety.

Table 6. Radial Growth Summary Average radial growth across all datasets

Isolates	Mean Growth (mm)	Morphological Insights	Pathogenicity Implications	Morphological Variability
MoO1	40.50	Low growth and stable morphology; likely a hypo-virulent or slow-growing variant	Low Pathogenicity	MoO2 showed the highest standard deviation in growth, indicating high plasticity. Irregular shapes were common in high-growth isolates (MoO2, MoO3, MoO5). Smooth textures appeared under specific conditions and may indicate senescence or environmental influence.
MoO2	59.88	Displays color variation (white to pinkish), rough velvety/cottony textures, irregular shape; indicates adaptability and high virulence	High Pathogenicity	
MoO3	54.88	Stable morphological profile with irregular shape and high growth; likely pathogenic	High Pathogenicity	
MoO4	42.63	Low growth, regular shape, less metabolic diversity; potentially weak pathogen	Low Pathogenicity	
MoO5	56.50	Consistently grayish/blackish with high growth and irregular morphology; suggests aggressive spread	High Pathogenicity	
MoO6	49.00	Moderate growth with morphological variability; possibly host/environment-dependent virulence	Moderate	

MoO2 stands out as the most potentially virulent isolate, followed by MoO3 and MoO5. Their aggressive growth, irregular morphology, and adaptability underline their pathogenic potential. MoO1 and MoO4 are likely less virulent and may be useful for comparison in resistance screening.

SUMMARY AND CONCLUSION

In Boro season 2020-21, in different villages of Cumilla and Brahmanbaria district of Bangladesh a survey on blast disease of rice has been carried out. The highest incidence of blast was recorded from Noapara, Cumilla in BRRI dhan81 where blast incidence was 57% and severity score was 9 and the lowest incidence of blast disease was recorded from Burichang and Champaknagar in BRRI dhan74 & BRRI dhan69 where incidence was 10% and 9%, respectively with a severity score 1 for each location. From this collected sample blast pathogen *Magnaporthe oryzae* has been isolated and pure culture of *M. oryzae* was prepared on different culture media.

This study evaluated six *M. oryzae* isolates (MoO1–MoO6) through comprehensive analysis of radial mycelial growth and colony morphology across four independent datasets. Radial growth, considered a proxy for pathogenic vigor, revealed MoO2, MoO5, and MoO3 as the most aggressive isolates, with MoO2 exhibiting the highest mean growth that is 59.88 mm. Conversely, MoO1 and MoO4 displayed reduced growth and morphological uniformity that is 40.5mm & 42.63mm, suggesting lower virulence or environmental sensitivity.

The pathogenicity assessment highlights MoO2 as the most virulent and phenotypically adaptable *M. oryzae* isolate, with MoO3 and MoO5 also demonstrating strong pathogenic traits. These isolates are prime candidates for further virulence assays and could be prioritized in resistance screening programs. In contrast, MoO1 and MoO4 may represent hypo-virulent phenotypes suitable as controls. Integrating quantitative growth metrics with colony morphology offers a robust framework for early virulence profiling. To strengthen these findings, future work should include statistical validation (ANOVA, Tukey HSD), sporulation assays, and host-pathogen interaction studies under controlled environmental conditions.

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Conflict of Interest

The authors declare no conflict of interest.

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