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GROWTH BEHAVIOUR AND CULTURAL CHARACTERISTICS OF NECK BLAST ISOLATES OF *Magnaporthe oryzae* ON DIFFERENT CULTURE MEDIA

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ABSTRACT

Neck blast of rice, caused by the filamentous fungus *Magnaporthe oryzae* (syn. *Pyricularia* oryzae), remains the most devastating biotic constraint to global rice production. The present study evaluated the growth behaviour and cultural characteristics of five neck blast isolates (NB1-NB5) on four different culture media. Mean radial growth of the cultures varied significantly across media and isolates during incubation. At 3 DAI, growth ranged from 0.4 to 1.6, with PDA generally supporting the highest values, while growth increased steadily at 6 and 9 DAI and approached maximum levels by 12 DAI. Among the media tested, PDA consistently promoted the highest radial growth, followed by OMA, whereas Richards and RLEA supported comparatively lower growth. NB2 showed the most vigorous early growth, while NB4 and NB1 were generally slower in the less favourable media. Overall, the results indicate that PDA was the most suitable medium for radial expansion of the test isolates under the conditions studied. Colony morphology varied from cottony, fluffy and velvety with radial and concentric growth patterns. The results indicate that nutrient composition strongly influences fungal growth and morphology. PDA and OMA were identified as suitable media for culturing, sporulation and maintaining neck blast isolates for pathological studies.

Keywords **:** Rice blast, *Magnaporthe oryzae*, culture media, colony morphology, radial growth

Introduction

Rice (*Oryza sativa* L.) is one of the world's most important staple crops and a primary source of food for more than half of the global population (FAO n.d.; International Plant Protection Convention [IPPC], n.d.). Among the diseases affecting rice, blast caused by *M. oryzae* is considered the most destructive fungal disease and can result in severe yield losses under favourable environmental conditions (Dean *et al*., 2012; Wilson & Talbot, 2009). The pathogen infects leaves, nodes, panicles, and neck tissues, making it a major constraint to rice production in many rice-growing regions (Dean *et al*., 2012). Cultural and morphological variability among *M. oryzae* isolates is an important feature for pathogen characterization and epidemiological studies (Saharan *et al*., 2023). In addition, different culture media can influence colony morphology, pigmentation, sporulation, and radial growth rate, which makes medium selection important for laboratory studies and culture maintenance (Saharan *et al*., 2023; "Rice Blast (*M. oryzae*) Management: A Review," 2020). Therefore, evaluating the growth behaviour of *M. oryzae* isolates on different media is essential for understanding isolate variability and for maintaining pure cultures under laboratory conditions (Wilson & Talbot, 2009). Several studies have reported considerable cultural and morphological variability among *M. oryzae* isolates collected from different rice-growing regions, indicating the existence of physiologically diverse populations of the pathogen (Kumar *et al*., 2021; Singh *et al*., 2020). Such variability influences pathogen adaptation, sporulation, virulence and epidemiological behaviour under different environmental conditions (Fernandez and Orth, 2018). Identification of suitable culture media is essential for routine isolation, growth & sporulation,

maintenance and pathological studies of the rice blast pathogen under laboratory conditions.

Materials and Methods

A total of five neck blast isolates of *M. oryzae* were selected and designated as NB1, NB2, NB3, NB4 and NB5 were used in the study. The isolates were maintained in the Division of Plant Pathology at ICAR-IIRR, Rajendranagar, Hyderabad. Culture media preparation and growth assessment were performed following standard protocols described by Kumar *et al.* (2021) and Singh *et al.* (2020). Potato Dextrose Agar (PDA), Oat Meal Agar (OMA), Richard's Agar, Rice Leaf Extract Agar (RLEA) were used for evaluation. Mycelial discs (5 mm diameter) from actively growing cultures were placed at the centre of petri plates containing sterilized culture media. The plates were incubated at 27 ± 2 °C and observations were recorded periodically. Colony diameter was measured at 3, 6, 9 and 12 days after incubation (DAI). Each treatment was replicated three times and mean radial growth was calculated.

Results and Discussion

Significant variation in radial growth and colony morphology of *M. oryzae* isolates was observed on different culture media (Tables 1 and 2; Fig. 1). Among the different media tested, Potato Dextrose Agar (PDA) supported maximum mycelial growth of most isolates followed by Oat Meal Agar (OMA) and Richard's agar, whereas comparatively reduced growth was observed on Rice Leaf Extract Agar (RLEA). Similar observations were reported by Ashrafi *et al.* (2021), found that PDA and OMA supported better growth of blast isolates compared to other synthetic media.

At 3 DAI, the radial growth of isolates ranged from 0.4 to 1.6 cm. Among the isolates, NB2 recorded comparatively higher growth on PDA (1.6 cm) and Richard's agar (1.4 cm), while NB4 exhibited minimum growth on RLEA (0.4 cm). The growth rate increased considerably at 6 and 9 DAI, with maximum radial growth recorded on PDA medium. At 12 DAI, most isolates nearly covered the Petri plates on PDA and OMA media, recording radial growth between 7.0 and 7.8 cm. Among the isolates, NB2 and NB5 showed comparatively faster growth across all media, indicating higher vigour and adaptability under in vitro conditions. Similar results were reported by Li *et al.* (2019), who stated that nutrient composition and carbon–nitrogen balance of culture media significantly influence the growth behaviour of rice blast pathogen isolates. Overall, the results indicate that PDA was the most favourable medium for radial growth of the test isolates, followed by OMA, while Richards and RLEA were less supportive.

Table 1 : Growth behaviour of neck blast isolates of *Magnaporthe oryzae* on different culture media

Medium	Isolate	Mean Radial growth of the cultures			
		3 DAI	6 DAI	9 DAI	12 DAI
PDA	NB1	1.3	3.2	4.7	7.8
	NB2	1.6	3.8	5.9	7.7
	NB3	1.2	3.2	5.3	7.6
	NB4	1.4	3.5	5.6	7.6
	NB5	1.5	3.6	5.8	7.7
OMA	NB1	1.2	2.6	4.2	7.6
	NB2	1.3	3.2	5.2	7.6
	NB3	1.3	2.7	4.4	7.2
	NB4	0.6	2.2	3.7	7.0
	NB5	1.4	3.1	5.2	7.5
Richards	NB1	0.8	2.4	3.6	7.8
	NB2	1.4	3.5	5.4	7.8
	NB3	0.9	2.8	4.4	7.0
	NB4	0.6	2.3	4.1	7.2
	NB5	1.2	2.9	4.9	7.4
RLEA	NB1	0.7	2.4	3.9	7.3
	NB2	1.1	3.0	4.6	7.1
	NB3	0.9	2.6	4.4	6.8
	NB4	0.4	2.1	3.8	6.5
	NB5	1.4	3.3	4.8	6.9
		Factor(A)	Factor(B)	Factor (A X B)	
C.D.		0.127	0.142	0.284	
SE(d)		0.063	0.07	0.14	
SE(m)		0.044	0.049	0.099	

Factor A- Media; Factor B- Isolate; A X B- Interaction

The reduced growth observed on Rice Leaf Extract Agar (RLEA) may be attributed to the limited availability of readily utilizable nutrients and the presence of host-derived inhibitory compounds. Similar observations were reported by Gayatonde *et al.* (2016), observed comparatively slower mycelial growth of blast isolates on host extract-based media.

Colony colour varied from bluish white to greyish white with cottony to fluffy mycelial texture. These results indicate that nutrient composition of the culture medium strongly influences fungal growth behaviour. Fernandez and Orth (2018) reported that nutritional and environmental factors strongly influence the physiological behaviour and infection biology of the blast pathogen. Similarly, Sharma *et al.* (2021) observed that media composition significantly affects colony growth, pigmentation and sporulation of rice blast isolates under laboratory conditions. Variability in colony pigmentation and texture among isolates may reflect underlying physiological and genetic diversity within pathogen populations. Such cultural variability has been widely used as a preliminary indicator for differentiation of fungal isolates in plant pathological studies (Singh *et al.*, 2020). Considerable variation in colony morphology was also observed among the isolates on different media (Table 2). Colony colour ranged from whitish gray to dark gray with cottony, fluffy, velvety and rough mycelial texture. Radial, concentric and zonate growth patterns were observed depending on the isolate and medium. Sector formation was observed in isolates NB4 and NB5 on PDA and in NB2 and NB4 on Richard's agar. Sporulation was comparatively better on OMA and RLEA media, whereas poor sporulation was observed on PDA and Richard's agar. The variation in colony morphology and sporulation may be attributed to differences in nutrient composition and physiological variability among the isolates.

The superior growth observed on PDA may be due to the availability of readily utilizable carbohydrates and nutrients which favour rapid mycelial development of the fungus. Similar findings were reported by Ashrafi *et al.* (2021), who observed enhanced growth of blast isolates on PDA and OMA media. Comparable results were also reported by Gayatonde *et al.* (2016), who found significant differences in growth and sporulation of *M. oryzae* on different nutrient media. Variability in colony morphology, pigmentation and growth pattern among isolates of the blast pathogen has also been documented by several workers, indicating the existence of considerable cultural variability within pathogen populations (Singh *et al.*, 2020; Kumar *et al.*, 2021). The observed sector formation in some isolates may indicate physiological instability or saltation during fungal growth on artificial media. Sectoring in fungal cultures has been associated with genetic variability, mutation and adaptation to nutrient conditions. Similar observations were reported by Raghavendra *et al.* (2019), documented sector formation and morphological variability among rice blast isolates collected from different geographical regions.

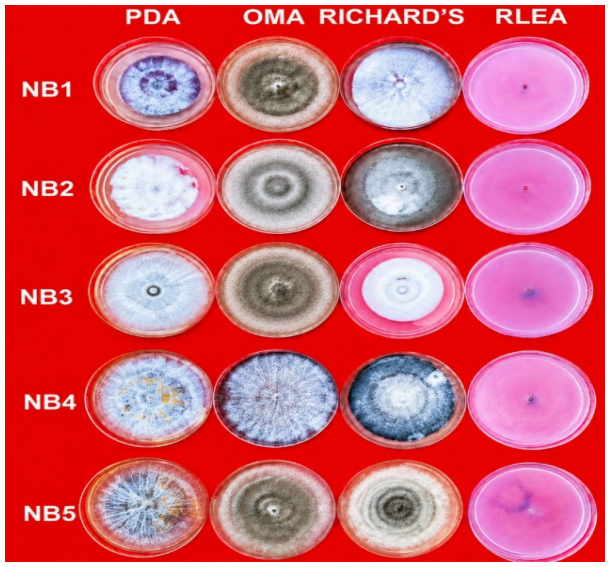

Fig. 1 : Morphological and Cultural Characteristics of neck blast isolates of *Magnaporthe oryzae* on different culture media

Table 2 : Cultural characteristics of neck blast isolates of *Magnaporthe oryzae* on different culture media

Medium	Isolate	Growth pattern	Texture / Surface	Colony colour	Sector formation	Sporulation
PDA	NB1	Circular radial growth with concentric pattern	Cottony / fluffy	Grayish white	No	No
	NB2	Circular spreading growth	Cottony / fluffy	White to grayish white	No	No
	NB3	Circular radial growth	Cottony	grayish white	No	No
	NB4	Circular radial growth	Cottony, rough	Grayish white	Yes	No
	NB5	Radial spreading growth with zonation	Cottony, rough	Grayish white	Yes	No

OMA	**NB1**	Circular zonate growth	Cottony / velvety	Grayish with whitish margin	No	Yes
	NB2	Circular growth with concentric zonation	Cottony / velvety	Grayish white	No	Yes
	NB3	Circular zonate growth	Cottony / velvety	Grayish with whitish periphery	No	Yes
	NB4	Dense radial growth	Cottony, filamentous	Grayish white	No	Yes
	NB5	Circular concentric zonate growth	Cottony / velvety	Grayish brown / olive	No	Yes
Richards Agar	**NB1**	Circular radial growth	Cottony, filamentous	Grayish white	No	No
	NB2	Circular growth	Cottony / compact	Grayish white with dark gray margin	Yes	No
	NB3	Compact circular growth	Smooth-cottony	White to grayish white	No	No
	NB4	Circular growth with dark peripheral ring	Cottony, rough	Grayish white	Yes	No
	NB5	Concentric zonate growth	Cottony / velvety	Creamy white to grayish	No	No
RLEA	**NB1**	No visible growth/restricted growth	Smooth surface	Very scanty Mycelium	No	Yes
	NB2	No visible growth/restricted growth	Smooth surface	Very scanty Mycelium	No	Yes
	NB3	Very restricted central growth	Smooth surface	Scant gray central growth	No	Yes
	NB4	Very restricted central growth	Smooth surface	Scant central growth	No	Yes
	NB5	Restricted diffuse central growth	Smooth surface	Scant gray diffuse growth	No	Yes

Conclusion

The present investigation revealed considerable variability in growth behaviour and cultural characteristics among neck blast isolates of *M. oryzae* on different culture media. Among the tested media, Potato Dextrose Agar (PDA) supported maximum radial growth and vigorous colony development, followed by Oat Meal Agar (OMA) and Richard's agar, whereas comparatively restricted growth was observed on Rice Leaf Extract Agar (RLEA). Significant differences in colony colour, texture, growth pattern and sporulation were also observed among the isolates, indicating the existence of cultural variability within the pathogen population. The study demonstrated that nutrient composition of the medium plays a crucial role in influencing fungal growth and morphology. PDA and OMA were found suitable for routine culturing, maintenance and further pathological studies of rice neck blast isolates. These findings may be useful for further studies on pathogen variability, sporulation, pathogenicity and screening of rice genotypes against blast disease.

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