Morphological variability of rice blast pathogen *Magnaportheoryzae*

ABSTRACT

Rice is the most staple food crop for the human population worldwide. Both biotic and abiotic diseases are known to cause losses to the Rice crop. Mostly, grain yield losses are reported due to blast disease caused by *Magnaportheoryzae* across all crop growing areas of the world. Leaf blast samples were collected from various rice-growing areas in Warangal and samples were tested on different growth media and found that OMA+Rice leaf extract was the most suitable for promoting radial growth, conidial size, and sporulation of the pathogenic isolate. Among the eight isolates studied, the Maheshwaram isolate (M-2) exhibited the highest radial growth, conidial size, and good sporulation. This suggests that this particular isolate might be more aggressive or virulent than the others and could pose a greater threat to rice crops in the region.This could lead to more effective strategies for managing blast disease and minimizing yield losses in rice crops in the specific region.

Keywords: Magnaportheoryzae, Rice blast, Variability, Sporulation

1. INTRODUCTION

Rice (*Oryza sativa* L.) is the most crucial crop worldwide and serves as a primary source of food for over half of the global population.More than 90% of the world's rice is grown and consumed in Asia(1). China is the largest producer, followed by India, Indonesia, Bangladesh, Vietnam, Thailand, and Myanmar (2).

Rice is cultivated on approximately 162.25 million hectares worldwide, leading to an annual production of 787.29 million metric tonsand with average productivity of 4764.2 kilograms per hectare(3).Rice is a nutritionally dense food source, providing protein, carbohydrates, dietary fiber, as well as various essential minerals and vitamins (4). This makes it a vital component of a balanced diet.

In India, rice is grown on a significant scale, covering an area of 46.37 million hectares. The annual production of rice in India is approximately 195.4 million metric tons, with a productivity of about 4213.7 kilograms per hectare, as of FAOSTAT data from 2021. The pathogen attacks all the aerial parts of plant at any stage of crop growth right from germination to harvest (5). It infects the aerial parts of the plant including leaves, nodes, collar, neck and panicle regions which causes yield losses ranging from 10-30% annually (6).

Rice blast caused by a filamentous,ascomycete fungus *Magnaportheoryzae*(syn:*Pyriculariaoryzae* Cav.) is one of the most important diseases of rice worldwide and is one of the major constraint for profitable rice production (7). Efforts to combat rice blast are ongoing, as the fungus has the ability to evolve and overcome resistance mechanisms. Continuous research, monitoring, and the development of resistant rice varieties are essential components of managing this devastating disease and ensuring stable rice production. In this context, research on morphological variability is conducted among the collected isolates.

2. MATERIAL AND METHODS

2.1.Collection of rice blast diseased specimens

Rice blast infected samples were collected from various locations within Warangal district. The eight specific locations are Atmakur, Maheshwaram, Lohitha, Thigarajupally, Kamalapur, Damara, Hasanparthy, and Warangal (RARS). The collected isolates were designated with the labels M-1 to M-8. This likely serves as a systematic way to differentiate and refer to each specific isolate for further study and analysis.

2.1.1. Isolation of mono-conidial isolates of *Magnaportheoryzae*

Rice leaves showing typical symptoms of blast disease were chosen and these leaves were washed with sterile distilled water to remove any surface contaminants.To ensure that only the target pathogen is cultured, a small piece of the diseased tissue along with some healthy tissue was cut from the leaves and the collected leaf tissue was surface sterilized to eliminate any external contaminants. This was done by immersing the tissue in a 1% solution of sodium hypochlorite for one minute.After the sterilization process, the tissue was rinsed three times with sterile water to remove any residual sodium hypochlorite and the sterilized leaf pieces were dried using sterilized filter paper. Later, the sterilized leaf pieces were transferred aseptically onto sterilized Petri dishes containing a suitable growth medium. These plates after inoculation were incubated at 28°C for four days. The incubation period allows the pathogen to grow and form visible colonies.After incubation, the pathogen was further subcultured onto oatmeal agar medium. This step involves transferring a sample of the pathogen from the initial culture to a fresh mediumand was purified using single spore isolation method.The culture was maintained on oat meal agar medium slants and preserved at 4°C for further studies (5).

2.2.. Morphological characteristics

The different isolates of *M. oryzae* were cultured on various growth media, including Potato Dextrose Agar (PDA), Oatmeal Agar (OMA), PDA supplemented with Rice leaf extract, and OMA supplemented with Rice leaf extract. Each isolate was grown separately on these media for 15 days at a constant temperature of 28°C.Morphological characteristics of *M. oryzae*isolates collected from different locations were studied for radial growth (mm), size of conidia, colour, texture and sporulation. Spores of *M. oryzae*of different isolates collected from the infected host tissue were mounted in lacto phenol cotton blue on a clean slide. Spores were measured under high power objective lens (40X) using a precalibrated ocular micrometer. The average size of spore was then determined and shape of the spores were recorded.

3. RESULTS

3.1. Morphological and colony characteristics of *Magnaportheoryzae*

Morphological and colony characteristics of the fungus are the important basic factors for identification of a fungus and its variability. The morphological characteristics such as colour of the mycelium, texture and radial growth of the mycelium of the isolate, size and shape of the conidia and sporulation (number of spores observed per microscopic field) were studied among the isolates of *Magnaportheoryzae*on different media. The isolates were morphologically characterized by measuring the size (length and width) of conidia at a magnification of 40X.

3.1.1. Colour and texture of the colony

Among the eight isolates, six isolates (M-1, M-3,M-4,M-5, M-6 and M-8) were with grey to greyish white colony colour with rough texture, one isolate (M-7) with greyish colony colour with smooth texture and one isolate (M-2) with greyish colour with smooth texture (Table-1)

Table 1. Colour and texture of the different collected isolates

Isolates	Colour and texture of the mycelium			
	PDA	OMA	PDA+Rice leaf extract	OMA+Rice leaf extract

M-1	Greyish white, Rough	Greyish, Smooth	Greyish white, Rough	Greyish, Smooth
M-2	Greyish, Smooth	Dark Greyish,smooth	Greyish, Smooth	Greyish, Smooth
M-3	Greyish white, Rough	Greyish white, Rough	Greyish white, Rough	Greyish white, Rough
M-4	Greyish white, Rough	Greyish white, Rough	Greyish, Rough	Greyish, Rough
M-5	Greyish white, Rough	Greyish white, Rough	Greyish white, Rough	Greyish white, Rough
M-6	Greyish, Rough	Greyish, Rough	Greyish, Rough	Greyish, Rough
M-7	Greyish white, Smooth	Greyish white, Smooth	Greyish white, Smooth	Greyish white, Smooth
M-8	Greyish, Rough	Greyish, Rough	Greyish white, Rough	Greyish white, Rough

3.1.2. Radial growth, conidial size and sporulation of *M. oryzae* isolates

Among different media used such as Potato dextrose agar(PDA), Oat meal agar (OMA), PDA+Rice leaf extract and OMA+Rice leaf extract, OMA+Rice leaf extract was found to be best media for radial growth of the isolates and there is no variation observed in conidial size of the isolates among different media. The radial growth of the isolates varied from 56.13 (M-4) to 87.66 mm(M-2). Highest radial growth at 15 days after incubation was exhibited by M-2 (87.66 mm) isolate (Table-2) and conidial size was ranged from 8-9×3-4 µm (M-7) to 8-12 ×3-4 µm (M-2) among different isolates (Table-3)

Table 2. Radial growth of the mycelium of different isolates on different media

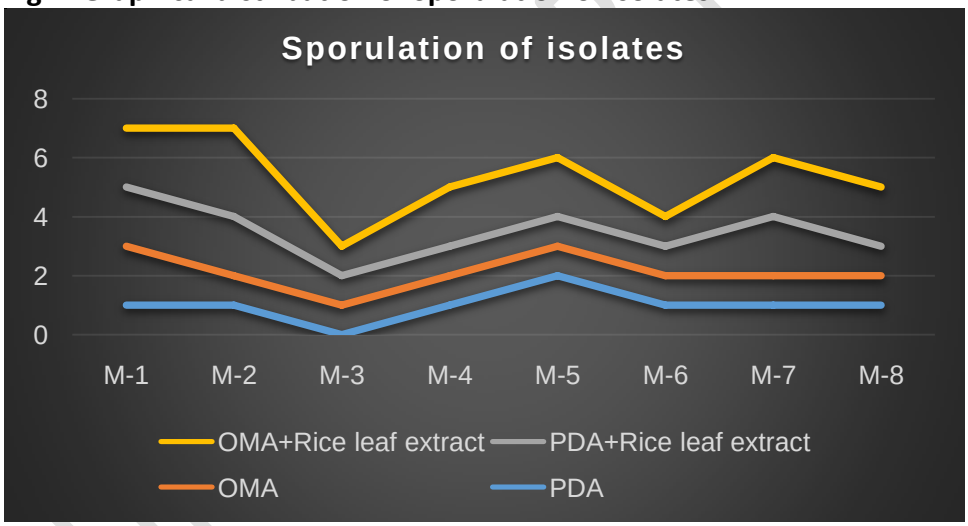
Isolates	**Radial growth of *M. oryzae* isolate (mm)**			
	PDA	**OMA**	**PDA+Rice leaf extract**	**OMA+Rice leaf extract**
M-1	75.31(60.18)	84.83(67.05)	85.33(67.86)	84.98(67.17)
M-2	**76.38(60.90)**	**87.23(69.04)**	**86.41(68.36)**	**87.66(69.41)**
M-3	75(59.97)	75.6(60.37)	75(59.97)	75.16(60.08)
M-4	56.13(48.50)	56.25(48.57)	62.66(52.32)	65.16(53.80)
M-5	75.27(60.16)	65.81(54.19)	81.35(64.39)	77.37(61.57)
M-6	73.9(59.25)	75.55(60.34)	73.26(58.84)	74.83(59.86)
M-7	66.78(54.79)	67.33(55.12)	66.81(54.80)	71.07(57.44)
M-8	71.6(57.78)	72.71(58.48)	73.85(59.22)	75.33(60.20)
C.D	1.437	0.913	1.552	1.101
SE(m)	0.475	0.302	0.513	0.364
SE(d)	0.672	0.427	0.726	0.515
C.V.	1.426	0.874	1.464	1.042

Table 3. Conidia Size of the different isolates on different media

Isolates	Conidia Size (µm) (40x) Range			
	PDA	OMA	PDA+Rice leaf extract	OMA+Rice leaf extract
M-1	9-10×3-4	9-10×3-4	9-10×3-4	9-10×3-4
M-2	**8-10×3-4**	**8-11×3-4**	**8-10×3-4**	**8-12 ×3-4**
M-3	8-10×3-4	8-10×3-4	8-10×3-4	8-10×3-4
M-4	8-10×3-4	8-11×3-4	8-11×3-4	8-11×3-4
M-5	8-11×3-4	8-11×3-4	8-11×3-4	8-11×3-4
M-6	9-10×3-4	9-10×3-4	9-10×3-4	9-10×3-4
M-7	8-9×3-4	8-9×3-4	8-9×3-4	8-10×3-4
M-8	8-10×3-4	8-10×3-4	8-10×3-4	8-12×3-4

Among different media used such as Potato dextrose agar (PDA), Oat meal agar (OMA), PDA+Rice leaf extract and OMA+Rice leaf extract, OMA+Rice leaf extract was found to be best media for sporulation followed by PDA+Rice leaf extract. Among different isolates, M-2 have exhibited “good” sporulation index with a scale of 3 in OMA+Rice leaf extract and no sporulation was observed in M-3 in PDA medium (Fig 1)

Fig 1. Graphical distribution of Sporulation of isolates



Sporulation index (on 0-4 scale) was determined after 15 days after incubation in Petri dishes containing different media incubated at 28°C, wherein Excellent (4)=>30; Good (3)=20-30; Fair (2)=10-20; Poor (1)=<10 and Nil (0)=0 of the number of spores per microscopic field.

4. DISCUSSION

Variability studies among plant pathogens are crucial for several reasons such as they help in assessing the virulence of different pathogen isolates, which is essential for understanding their ability to cause disease.These studies allow researchers to gauge the potential disease severity in various crop-growing regions. In our studies, blast pathogenic isolates showed less variability both in terms of morphology and cultural studies. Several researchers have determined variability among *M. oryzae* isolates at different rice growing areas. Indeed these studies enable to determine and predict the extent of damage by blast pathogenic isolates based on the presence of virulent isolates.

5. CONCLUSION

Rice blast isolates were studied to understand its diversity and characterization. We conclude that *M. oryzae* isolates from various locations of Warangal have not shown much variation among morphological and cultural characteristics.One notable exception was the M-2 isolate, which outperformed the others in all observed characteristics.While the isolates may be relatively uniform, the presence of the high-performing M-2 isolate could have implications for disease control measures in the affected areas.Our study contributes to the knowledge of rice blast pathogen variability in the Warangal region and highlights the potential significance of specific isolates, like M-2, in terms of virulence and pathogenicity. Tailoring disease management strategies based on this information can help mitigate the impact of rice blast on crops in these areas.

REFERENCES

1. Bandumula, N. Rice Production in Asia: Key to Global Food Security. Proceedings of National Academy of sciences, India, Section B: BiologicalSciences. *Springer*. 2017; DOI 10.1007/s40011-017-0867-7

2. Yashaswini Ch, pushpavati B, Madhav MS. Morphological and Molecular variability among riceblast pathogen (Magnaportheoryzae) isolates in Southern India. *Environment and Ecology*. 2017; 35(4B): 3015-3022.

3. FAOSTAT, Food and agricultural organization. statistics database.2021.http.s://www.fao.org/faostat/en/#data/QCL

4. Gopalan C, Rama Sastri BV, Balasubramanian S. Nutritive value of Indian foods, published by National Institute of Nutrition (NIN). *Ind Council Medical Res.* 2007. pp 289.

5. Nidhee Yadav, Netam, R.S, Netam, P.S, and Mehar, N. Evaluation of fungicides against *Pyriculariaoryzae*causing blast disease of rice. *The Pharma Innovation Journal.* 2022; 11(7): 647-652

6. Law J W-F, Ser H -L, Khan TM,Chuah L-H, Pusparajah P,Chan K-G, Goh B-H and Lee L-H. The Potential of *Streptomyces* as Biocontrol Agents against the Rice Blast Fungus, *Magnaportheoryzae* (*Pyriculariaoryzae*). *Frontiers in Microbiology*. 2017; 8(3): 1-10

7. Rahila R, Harish S, Kalpana K and Anand G. Morphological and Pathogenic Variability of *Magnaportheoryzae*, the Incitant of Rice Blast. *International Journal of Current Microbiology and Applied Sciences*.2020; 9(11): 231-238

8. Aishwarya V , Manoranjitham S K , Gopalakrishnan C, Somasundaram E andGeethanjali S. Isolation and Characterization of Rice Blast and Brown Spot Disease in Different Regions of Tamil Nadu, India. *International Journal of Plant & Soil Science.*2023.35(19).975-985.