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Morphological and Pathogenic Variability of *Magnaporthe oryzae*, the Incitant of Rice Blast

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A B S T R A C T

Rice (*Oryza sativa*) occupies a prime position in the family Poaceae and it is the highly consumable crop of almost half of the world's population. Among the various diseases affecting rice, blast incited by *Magnaporthe oryzae* is the one of the ubiquitous and economically important disease which affects almost all the stages of crop and cause yield loss upto 50%. In the present study, survey was carried out in Nilgiri and Madurai districts of Tamil Nadu for the collection of blast isolates. The blast disease incidence ranged from 8.8 to 42.4 per cent in various rice cultivars. Fifteen isolates of *Magnaporthe oryzae* were isolated from symptomatic tissues and maintained in pure culture. All the 15 isolates produced greyish brown to white colour colony with a diameter which ranged from 80-90 mm in PDA medium. Sporulation was carried out by stem bit inoculation method which produced pyriform shaped conidia with the size range of 8-12 × 3.3-3.6 µm. The pathogenicity tests in cv. ASD 16 by spraying the spore suspension (1×10^6 spores/ml) revealed that, the isolate IS (Kar)-3 was more virulent followed by IS (Aru)-4 and IS (Kul)-6 which was found to be least virulent. Thus, there exists morphological and pathogenic variability among the *Magnaporthe oryzae* isolates within the close proximity.

Introduction

Rice (*Oryza sativa* L.) is an important food crop and it is the staple diet of over three billion people around the world, particularly in Asia which contributes 30% of global food supply (Zeng *et al.*, 2018). More than 90% of world's rice is grown and consumed in Asia, which occupies 60% of world's population (Khush and Jena, 2009). Rice is susceptible to various biotic and abiotic stresses during its cultivation. Among the diseases, rice blast incited by *Magnaporthe oryzae* (anamorph *Pyricularia oryzae* Cav.), a filamentous ascomycete fungus is regarded as one of the most devastating disease which ruin the crops each year, causing huge economic loss to the growers. The disease was probably first recorded as "rice fever" in China in the year 1637 (Gowrisri *et al.*, 2019) and is now present in over 85 countries (Srivastava *et al.*, 2014). Moreover, the pathogen infects more than 50 species of grasses including wheat, barley, oat and millet under the family

Poaceae (Longya *et al.,* 2020). The disease is most serious in temperate regions and moisture stress under upland conditions (Khush and Jena, 2009). 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 (Ashkani *et al.*, 2015). The disease results in yield loss as high as 70-80% when predisposition factors (degree of relative humidity higher than 85-89%, presence of dew, drought stress and excessive nitrogen fertilization) favor epidemic development.

The disease cycle starts with asexual spore (conidia), which spreads from plant to plant through wind, dew drops and rain, that germinates quickly within 2 to 3 hours and invades the leaf tissue (Talbot 2003). The pathogen is a hemibiotroph, which starts the infection process in living host tissue and ends with destructive necrotrophic mode. Appressorium developed from the conidia, adheres tightly to the plant surface which differentiates into germ tube and enter the epidermal cell of leaf using a thin penetration peg. Subsequently, it changes to infectious hyphae and grow inter and intracellularly, resulting in development of lesions (Hasan *et al.,* 2016). The initial leaf infection shows brownish lesion and later develops diamond or spindle shaped symptom with grayish centre and dark brown margin. The size, shape and colour of the lesion are based on the age of lesion, varietal resistance and environmental condition. Each disease lesion from the susceptible host can give rise to more than 20,000 conidia, serving as a source for secondary dispersal of the disease. Under favourable condition, the lesion coalesce leading to drying of the leaves (Hasan *et al.,* 2016). The infection at the nodal region leads to weakening and breakage of nodes. The disease occurrence during panicle development prevents the maturation of rice grain which leads to chaffiness thereby reducing yield. The sexual spore is ascospores in the asci, which is found in the asexual fruiting body called perithecia. The pathogen shows morphological and pathogenic variability within isolates of close proximity and understanding the population structure plays an important role in disease resistance. Various studies have demonstrated the variations that exist among the isolates of blast pathogen within a geographical region (Aruna *et al.,* 2016). In this paper, morphological and pathogenic variability of the rice blast pathogen in Madurai and Nilgiri district of Tamil Nadu was carried out.

Materials and Methods

Survey and collection of samples

A survey was conducted in rice growing areas *viz.*,Madurai and Nilgiri district of Tamil Nadu to assess the incidence of rice blast. Three fields were surveyed in each area and the disease incidence was assessed by calculating the percent disease index. The plant samples showing the symptoms of rice blast was collected and used for further studies.

$$Percent\ Disease\ Index = \left(\frac{sum\ of\ indi\ vidual\ ratings}{total\ number\ of\ plants} X \frac{100}{maximum\ grade}\right)$$

Isolation of pathogen

The pathogen was isolated by tissue segment method, by following the protocol of Patel, 1989. The blast infected portion of the leaves were cut into small pieces using sterile scalpel surface sterilized with 1% Sodium hypochlorite for 30 seconds followed by washing with sterile water thrice for 20 – 30 seconds and placed on sterile filter paper. It was then placed aseptically on sterile Petri plate containing Potato Dextrose Agar (PDA) medium amended with streptomycin and incubated at 23±2°C for 4 days. The pure culture of the pathogen was used for further studies.

Morphological characterization of pathogen

The isolates were grown in PDA medium and the mycelial characters, colony morphology and colour were studied. The sporulation of the pathogen was carried out on pre-sterilized stem bits of alternate weed host (*Digitaria sanguinalis*) and incubated at 22-25°C for 10 – 15 days (Kulkarni and Peshwe, 2019). During this period, the mycelium of the pathogen covers the stem bits and the sporulation was induced. The spores were observed in the microscope and the spore characters were documented.

Pathogenicity test

The pathogenicity test was carried out in green-house condition to identify the virulent isolates. Rice cv. ASD 16, susceptible to rice blast was grown in earthern pots and the seedlings were transplanted. The experiment was conducted in randomized block design and replicated thrice. The spore suspension of the pathogen *M. oryzae* was prepared by pouring 25ml of sterile water into the sporulated stem bits, shacked vigorously and decanted. Using haemocytometer, the spore load in the suspension was adjusted to 1×10^6 spores/ml. A pinch of Carboxy Methyl Cellulose (CMC) was added into the spore suspension. The spore suspension of each isolates was individually sprayed on 20 days old healthy rice seedling. The plants sprayed with sterile distilled water were used as control. The seedling was covered with polythene cover for 24 hrs before and after spray to create humid condition and observed periodically for symptom expression (Srivastava *et al.,* 2014). The expression of symptom was scored using the disease rating scale (SES, 5^{th} edition, 2013). The pathogen was re-isolated from the artificially inoculated plant and compared with those of the original isolates.

Statistical analysis

Experimental data were statistically analyzed using analysis of variance (ANOVA) using the SPSS version 17.0. Prior to statistical analysis of variance (ANOVA) the percentage values of the disease index were arcsine transformed. Data were subjected to analysis of variance (ANOVA) at two significant levels ($P < 0.05$ and $P < 0.01$) and means were compared by Duncan's Multiple Range Test (DMRT).

Results and Discussion

Survey and isolation of pathogen

A roving survey was carried out in Madurai and Nilgiri district of Tamil Nadu to record the incidence of rice blast. The results revealed that, the highest incidence was noticed in Kariapatti village which recorded 42.4% incidence followed by Arumbanur village (39.5%) and least incidence was noticed in Ayilangudi village (8.8%) of Madurai district in Tamil Nadu (Table 1). The typical symptoms of spindle shaped lesion were observed in the leaves which later coalesced and caused death of the plant (Fig. 1). Totally fifteen rice blast pathogen were isolated from different places and maintained in pure culture.

Morphological characterization of *Magnaporthe oryzae*

The isolated pathogen was characterized based on colony colour, colony morphology and conidial characters. The hyphal colour, hyphal septation, conidia colour, conidia size and its septations were observed in a binocular microscope (Labomed) and documented. The colony growth of the pathogen of all the isolates in the petriplate ranged from 82 to 90 mm diameter. The isolates IS (Ynm) – 2, IS (Kar) – 3, IS (Ara) –

7, IS (Che) – 10, IS (Soz) – 11 and IS (Sow) - 13 attained full growth of 90 mm and the least growth was observed in the isolate IS (Por) – 5 which showed 82 mm diameter when observed at 15 days after inoculation (Table 2). All the isolates produced greyish coloured colony with slight difference at dorsal surface (Fig. 2). At ventral surface, brownish black concentric rings appeared in all isolates. The sporulation of the pathogen was observed after 10-15 days of incubation. In all the isolates, the conidia were small and pyriform in shape with 2 septate - 3 celled, middle cell was broader than other two cells with no distinguishing variation in the isolates. However, there was variation in the conidial size of the pathogen with the length ranged from 8 to 12 µm and the width ranged from 3.3 to 3.6 µm (Table 2 and Fig. 3).

Pathogenicity

The pathogenicity test conducted by spraying spore suspension revealed that, IS (Kar) – 3 from Karaiyippatti was found to be more virulent (58.4%) followed by IS (Aru)-4 (52.6%) while the least incidence was noticed in IS (Kul)-6 (Fig. 4).

Rice is one of the most important food crops and is a primary source of energy for more than half of the world population (Mishra *et al.*, 2015) which contributes 30% of global food supply (Zeng *et al.*, 2018).

Table.1 Survey and collection of *Magnaporthe oryzae* isolates from various places of Tamil Nadu

S.No.	Place of collection	District	Variety	Isolate name	Coordinates		Percent Disease Incidence (%)*
					Latitude	Longitude	
1.	Gudalur	Nilgiri	Multilines	IS (Gud) – 1	11°30'N	76°30'E	35.2 (36.43)**
2.	Yanaimalai	Madurai	Akshaya	IS (Ynm) – 2	9°58'N	78°12'E	18.8 (25.57)**
3.	Kariapatti	Madurai	Akshaya	IS (Kar) – 3	10°05'N	78°33'E	42.4 (40.64)**
4.	Arumbanur	Madurai	Akshaya	IS (Aru) – 4	9°59'46.6''N	78°11'37.1''E	39.5 (38.64)**
5.	Porusupatti	Madurai	Akshaya	IS (Por) – 5	10°0'20''N	78°13'53''E	12.5 (20.76)**
6.	Kulamangalam	Madurai	BPT 5204	IS (Kul) – 6	10°0'31''N	78°6'41''E	15.8 (23.51)**
7.	Arittapatti	Madurai	Akshaya	IS (Ara) – 7	10°0'9''N	78°19'56''E	21.5 (27.76)**
8.	Alanganallur	Madurai	Akshaya	IS (Ala) – 8	10°02'46''N	78°05'13''E	24.6 (29.62)**
9.	Ayilangudi	Madurai	BPT 5204	IS (Ayi) – 9	9°59'19''N	78°13'14''E	8.8 (17.25)**
10.	Chettikulam	Madurai	BPT 5204	IS (Che) – 10	9°59'47''N	78°13'32''E	10.2 (18.54)**
11.	Sozhavanthan	Madurai	Akshaya	IS (Soz) – 11	10°1'8''N	78°0'0''E	19.3 (25.89)**
12.	Kilavaneri	Madurai	Akshaya	IS (Kil) – 12	9°48'8''N	77°54'27''E	15.3 (23.09)**
13.	Sowdarpatti	Madurai	Akshaya	IS (Sow) -13	9°48'54''N	77°52'41''E	17.5 (24.73)**
14.	Chellayapuram	Madurai	Akshaya	IS (Chel) – 14	9°50'12.6''N	77°42'2.4''E	23.7 (29.27)**
15.	Usilampatti	Madurai	Akshaya	IS (Usi) – 15	9°58'12''N	77°48'0''E	22.5 (28.22)**
						CD (P=0.05)	**0.81**

*Mean of three replications

**Values in the parentheses are arcsine transformed values

Table.2 Cultural characters of different isolates of rice blast

S.No.	Isolate	Colony character			Conidia size (µm) (40X)	
		Diameter (mm)*	Colour	Texture	Length	Width
1.	IS (Gud) – 1	86	Greyish with concentric rings	Smooth	12	3.5
2.	IS (Ynm) – 2	90	Greyish white	Rough	11	3.3
3.	IS (Kar) – 3	90	Greyish brown	Smooth	9	3.5
4.	IS (Aru) – 4	80	Greyish with white periphery	Smooth	9	3.5
5.	IS (Por) – 5	82	Greyish brown	Smooth	10	3.4
6.	IS (Kul) – 6	88	Greyish	Smooth	10	3.4
7.	IS (Ara) – 7	90	Greyish white	Rough	9	3.5
8.	IS (Ala) – 8	88	Light greyish white	Smooth	8	3.5
9.	IS (Ayi) – 9	88	Greyish white	Rough	10	3.5
10.	IS (Che) – 10	90	Greyish white	Smooth	10	3.5
11.	IS (Soz) – 11	90	Greyish white	Smooth and cottony	9	3.5
12.	IS (Kil) - 12	85	Greyish brown	Smooth	10	3.4
13.	IS (Sow) -13	90	Greyish white	Smooth	10	3.4
14.	IS (Chel) -14	88	Greyish with concentric rings	Smooth	10	3.5
15.	IS (Usi) – 15	88	Greyish white	Smooth	9	3.6

*Colony diameter recorded 15 days after inoculation

Fig.1 Symptoms of rice blast: 1a) Initial infection starts with spindle shaped spot with greyish centre and dark brown margin; 1b) development of several spots on a single leaf; 1c) elongation of spots; 1d) coalescing of spots; 1e) Drying of leaves

Fig.2 Variation in the cultural characteristics of *Magnoporthe oryzae*

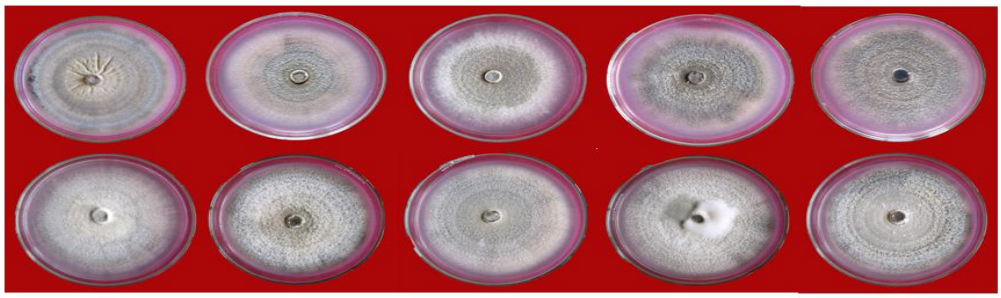


Fig.3 Variation in the shape of conidia of *Magnoporthe oryzae*

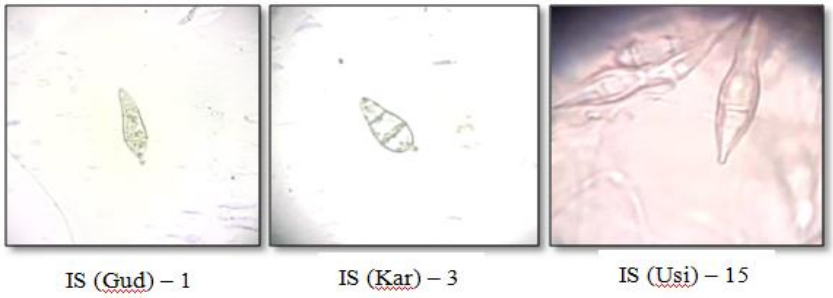
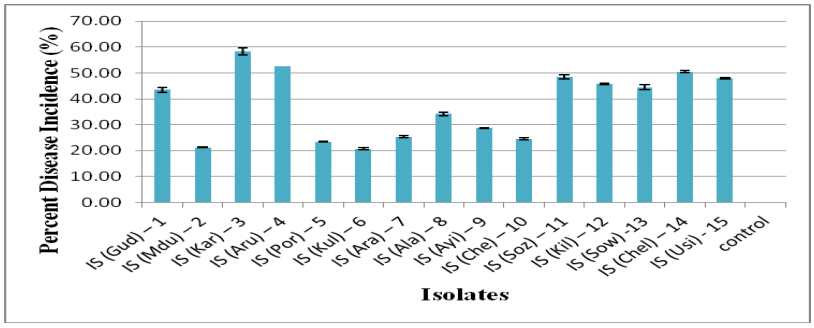


Fig.4 Pathogenicity test of different isolates of rice blast pathogen



During its cultivation, it suffers from various stresses *viz.,* pests, diseases, weeds and disorders. Among the diseases, the pathogen *M. oryzae* is most destructive, which causes huge economic loss. It attacks almost all stages/parts of rice plant *viz.,* leaves, nodes, collar, neck and panicle regions (Ashkani *et al.,* 2015). In severe cases, the fungus can cause death of the rice seedlings, whereas in old plants, it prevent the grain filling or destroy the grain bearing structures of the plant (Li *et al.,* 2010). The pathogen has the ability to overcome the resistance within a short period. Hence, evaluation of variability and virulence of the pathogen is an important prerequisite for the management of this disease (Srivastava *et al.,* 2014). In this study, survey, collection, isolation and characterization of rice blast pathogen was carried out. In our present study, the colony colour varied from light grayish to dark greyish with concentric ring pattern and smooth to rough texture were observed on PDA medium. This is in accordance with Sonah *et al.,* (2009) who reported that grey coloured rough textured isolates produced more number of spores compared to smooth ones. In our study, the pathogen showed varying degrees of growth in colony diameter. Surapu *et al.,* (2017) revealed that, variation in culture growth due to autolysis of mycelium. The isolated pathogen produced pear shaped conidia with rounded base and narrowed towards the tip having 2 septations, thus 3 celled conidia of similar characters was observed in all the isolates in our study. This result was supported by the research conducted by Aruna *et al.,* 2016. But, variation in the number of septations ranged from 1-3 were also reported (Mahboebh *et al.,* 2017). In the present study, the spore size varied between the *P. oryzae* isolates which is in accordance to the research by Gowrisri *et al.,* 2019 who observed similar variations in

the spore characters of the pathogen. The stem bit method of inoculation to induce sporulation was carried out in alternate host of paddy as described by Kulkarni and Peshawe, 2019. In the present study, we could successfully establish the pathogenecity in rice cv. ASD 16 which is susceptible to blast. We conclude that, *M. oryzae* isolates show certain level of cultural diversity in colony colour, colony growth, conidia shape and virulence with respect to geographical distribution. Thus, understanding the pathogenic variation and characterization of *P. oryzae* is one the most efficient ways to manage the disease in a sustainable manner.

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