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Cultural and morphological studies on Ponnampet leaf and neck blast isolates of *Magnaporthe grisea* (Herbert) barr on rice (*Oryza sativa* L.)

Kalavati Teli*, M.K. Prasannakumar, V. Jyothi, S.C. Chandrashekar, M. Bhagyashree, M. Raviteaz and N. Amrutha

Department of Plant Pathology, University of Agricultural Sciences, Gandhi Krishi Vigyan Kendra, Bengaluru - 560065 (Karnataka), INDIA

*Corresponding author. E-mail**:** agriunit.kalavati@gmail.com

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Abstract: The study was carried out to standardize the optimal growth, sporulation and production of perfect stage of pathogen on different media. Among different media used such as Potato dextrose Agar (PDA), Oat meal Agar, Ragi flour agar, yeast extract + 2% soluble starch, Host extract + 2% soluble sucrose agar, Potato dextrose agar + Biotin + Thiamine and Rice flour agar, Oat meal agar and potato dextrose agar was found to be best media for radial growth and sporulation of *M. grisea*. Maximum conidia length (9.46µm) and breadth (7.36µm) was recorded in Oat meal agar followed by Potato dextrose agar and least conidia length (6.15 µm) and breadth (5.11 µm) was recorded in ragi flour media after 20 days of inoculation. Conidial size varied in leaf and neck blast isolates, the maximum mean colony diameter of 88.00mm and 89.16mm in neck and leaf blast was recorded in Oat meal agar respectively. The maximum sporulation mean index was observed in Oat Meal agar of 3.15 µm in leaf and 3.20 µm in neck blast was recorded. The best growth of the pathogen was recorded at optimum pH range from 6.0 - 7.0 and temperature of 27^{o}C. Therefore oat meal agar media was found to be best among all the media used for growth, sporulation, conidial size and colony characters of *M. grisea*.

Keywords: Blast, Fungus, Isolates, Resistance, Rice

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important cereal crop of the family Poaceae. Presently the rice production is insufficient to cover the needs and hence several countries have been importing rice. In 2008, Sub-Saharan Africa imported more than US $ 3.6 billion worth of rice, mainly from Asia, to fill the gap between production and consumption. India is the largest rice growing country accounting for about one third of the world acreage under the crop. Rice crop occupies about 44.3 mha in the country with a record production of 103.41 mt and productivity of 2125 kg/ hectare as estimated during 2011-12. However, in Karnataka rice is cultivated in about 15.39 lakh hectare with an annual production of 42.97 lakh tonnes and productivity of 2938 kg/hectare during the year 2010-11 (Anonymous, 2011). Rice suffers from many diseases caused by fungi, bacteria, viruses, phytoplasma, nematodes and other non-parasitic disorders. Among the fungal diseases, blast is considered as a major threat to rice production because of its wide spread distribution and its destructiveness under favourable conditions. Losses due to the blast disease may range up to 90% depending upon the component of the plant infected (Padmanabhan, 1963).

M. grisea infects above ground parts of the plant, but neck blast and the panicle blast are the most damaging phases of the disease and have been shown to significantly reduce yield, grain weight and milling quality. A typical blast lesion on a rice leaf is gray at the centre, has a dark border and it is spindle-shaped. Under favourable conditions, leaf lesions enlarge and coalesce, eventually blighting the entire leaf. Neck blast results in a girdled neck with grayish brown lesions (Pinnschmidt *et al*., 1994). The report of the perfect stage of a non-pathogenic species of *Pyricularia* (Webster, 1965) lead to the possibility of the successful development of perithecia in pathogenic species of *Pyricularia*. The formation of perfect stage of *Pyricularia grisea* was observed in culture media by mating two isolates of blast fungus obtained from crabgrass (*Digitaria sanguinalis* (L.) Scopoli) (Hebert, 1971). Therefore in this study, the extensive work was carried out to standardize the optimal growth, sporulation and production of perfect stage of pathogen on different media.

MATERIALS AND METHODS

Collection and isolation of the fungus: Infected leaf and neck plant samples collected from Ponnampet during *kharif* 2011 were used for isolation of

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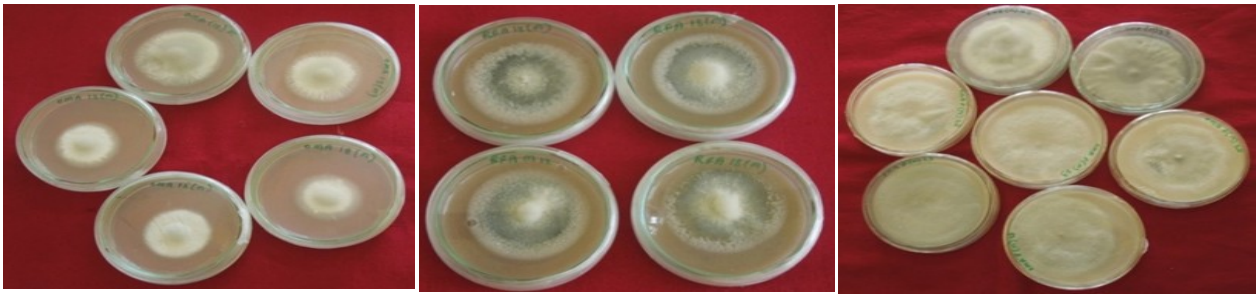


Oat Meal Agar (Himedia) Ragi flour agar Oat Meal Agar

Fig. 1. *Magnaporthe grisea* leaf isolate (Ponnampet) culture on different media.

Table 1. Criteria used to index the sporulation frequency of *M. grisea.*

Sporulation	No. of spores/ microscopic field	Index
Excellent	>30	4
Good	20-30	3
Fair	10-20	2
Poor	<10	1
Nil	0	0

Magnaporthe oryzae by following the standard tissue isolation technique. The infected leaf and neck portions were split open longitudinally with the help of sterilized scalpel and washed well with running tap water. These bits were surface sterilized with 1: 1000 mercuric chloride ($HgCl_2$) solution for 30 sec and washed three times in sterile distilled water before transferring them to oat meal agar plates. They were incubated at 28 ± 1°C for two weeks.

Monoconidial isolation: This method was followed for maintaining pure culture of *M. oryzae,* since the fungus is heterokaryotic in nature. Hyphal tip isolation for the leaf isolates was done on 2 per cent water agar plates. Well developed lesions were identified, excised and washed in running water for 2 hrs. The leaf bits were surface sterilized with mercuric chloride then washed serially with sterile double distilled water and allowed for sporulation on sterilized glass slides by incubating in a moist chamber at 28°C for 48 hrs. Well sporulated lesions were placed in double distilled water in the test tubes and vortexed for 1 min. About 1 ml of spore suspension was added to sterilized plates and 2% agar was added. Single spores were located and picked up microscopically. Each spore was eventually transferred to potato dextrose agar slants. The slants were incubated at 28°C for 2 days and stored at 4°C in refrigerator.

Maintenance of isolates of *Magnaporthe grisea*: Pure culture of *M. grisea* so obtained from hyphal tip isolation method was sub cultured on Oatmeal agar slants and kept at 28±1°C for 15 days. Subsequently sub culturing of isolates was done at an interval of 20 days. Such slants were stored in a refrigerator at 5°C. The culture was renewed every two months.

Pathogenicity test: Pathogenicity test was carried out to confirm the ability of the fungus in culture to produce typical symptoms of the disease under artificial conditions on rice leaves of healthy plants. The susceptible paddy variety (HR-12) was sown in sterilized soil. At seedling stage, they were sprayed with spore suspension obtained from culture of *M. grisea* grown

Table 2. Cultural characteristics of *M. grisea* on different media.

Media	Isolate colour on media	Growth	Margin	Sporulation
Oat meal agar	Off white	Good and uniform	Smooth	Excellent
Potato dextrose agar medium	Dull brown in the centre and gray white at margins	Good and uniform	Smooth	Good
Ragi flour medium	Light grayish brown in the centre and grey white at margins	Good and uniform	Irregular	Poor
Yeast extract + 2% soluble starch	Dull brown in the centre and gray white at margins	Good and uniform	Irregular	Poor

Table 3. Colony characteristics of Ponnampet leaf and neck blast isolates of *M. grisea* on different media.

S. N.	Media	Colony characters	
		Leaf isolate	Neck isolate
1	Oat meal agar	Buff colour, smooth margin and good growth	Buff colour, irregular margin, concentric ring pattern and good growth
2	Potato dextrose agar medium	Grayish black colour, irregular margin and good growth	Buff colour, smooth margin and good growth
3	Ragi flour medium	Black colour, smooth colony margin and poor growth	Greyish black colour, smooth colony margin and good growth
4	Yeast extract + 2% soluble starch	Black colour, irregular margin and good growth	Buff white colour, irregular margin and medium growth

Table 4. Mean colony diameter of Ponnampet leaf and neck blast isolates of *M. grisea* on different media.

Different media	Colony diameter five observation (mm)	
	Leaf blast	Neck blast
Oat meal agar	89.16	88.00
Potato dextrose agar	74.63	73.50
Ragi flour medium	60.60	58.30
Yeast extract+ 2% soluble starch	57.56	55.00
Mean	70.48	68.7
SEm±	0.54	0.58
CD at 1%	1.63	1.78

Table 5. Sporulation index of Ponnampet Leaf and neck blast isolates of *M. grisea* on different solid media.

Different media	Sporulation (Mean index of five observations)	
	Leaf	Neck
Oat meal agar	3.15 (27 spores)	3.20 (29 spores)
Potato dextrose agar medium	2.83 (18 spores)	2.88 (20 spores)
Ragi flour medium	1.77 (16 spores)	1.72 (18 spores)
Yeast extract+ 2% soluble starch	0.00	0.00

on oat meal agar. Control plants were maintained for which no inoculum was added. Inoculated plants were covered with polythene bags to ensure high humidity and periodical observations were made for the development of symptoms. Re-isolations were made from infected plants and cultures thus obtained were compared with original cultures to confirm identity of the pathogen**.**

Morphological and cultural variations among the leaf and neck blast isolates of *M. grisea*

Morphological variability: Spores of *M. grisea* of leaf and neck isolates were measured directly from the infected host tissue mounted in lactophenol on a clean slide. Spores were mixed with lactophenol thoroughly so that, a uniform spread is obtained and then a cover slip was placed over it. Spores were measured under high power objective (40x) using ocular and stage micrometer. The average size of spore was then determined and shape of the spores were recorded. Microphotographs were taken to show the typical spore morphology of the pathogen.

Oat meal agar (Himedia) Potato dextrose agar

Fig. 2. *Magnaporthe grisea neck isolate (Ponnampet) culture on different media.*

Cultural variation

Growth characters on solid media: The growth characters of and neck isolates were studied on four solid media *viz*., Yeast extract +2% soluble starch, Oat meal agar, Potato dextrose agar and Ragi flour agar medium. Fifteen ml of each of the medium was poured into each of sterilized Petri plates. Inoculation was made by transferring the five mm disc of mycelial mat, taken from the periphery of 10 days old culture. Each treatment was replicated thrice. The plates were incubated at 27 ± 1°C. Observation on colony radial growth was taken when the maximum growth was attained in any one of the media tested. Other cultural characters *viz*., rate of growth, type of margin, colony colour and sporulation were also recorded. The composition and procedures for preparation of the media used in this experiment were followed as explained by Ainsworth (1971) and Tuite (1969).

Sporulation variation: The sporulation capacity of isolate on different media was assessed by microscopic observations. A loopful of culture was transferred to a

Table 6. Spore morphometry of Ponnampet leaf and neck blast isolates of *M. grisea* on different media.

Different media	Measurement of leaf and neck blast conidia of five observations (µm)			
	Leaf blast spore		Neck blast spore	
	Length	Breadth	Length	Breadth
Oat meal agar	9.60	7.30	9.46	7.36
Potato dextrose agar	8.63	6.75	8.53	6.81
Ragi flour medium	6.83	5.76	6.15	5.11
Mean	8.35	6.60	8.04	6.42
SEm±	0.82	0.50	0.86	1.12
CD at 1%	2.49	1.52	2.61	3.37

clean slide and mixed well with lactophenol and a cover slip was placed on it. The Criteria used to index the sporulation frequency was followed as per the standard technique given by Henry and Andersen (1948) as below (Table 1).

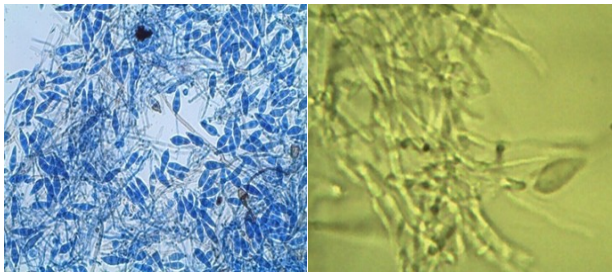
RESULTS AND DISCUSSION

The identification of fungal pathogen in the present study as *M. grisea* was based on the principal morphological and cultural characters as described by Nishikado (1926). The typical blast symptoms were observed on 10th day after inoculation. The initial leaf blast symptoms appeared as small, water soaked, greyish dots which subsequently enlarged into spindle shaped spots with greyish white centre with a brown margin. In case of neck blast, the panicle infected was blackened and shrivelled leading to chaffy earhead in early stage. Later, panicle hanged down at the neck. In case of nodal blast, infected nodes turned black. The symptoms observed are in accordance with the description of Manibhushan Rao (1994) and Srivastava *et al*. (2014).

Cultural characteristics studied on different media to find out the variation for the growth and sporulation of leaf and neck blast isolates. Among the different media *M. grisea* grown on Oat meal agar showed good and uniform growth with smooth margin and excellent sporulation. While in case of Ragi flour medium and Yeast extract + 2% soluble starch medium the *M. grisea* growth was good and uniform but there was poor sporulation (Table 2).

The isolates were grown on different media like, oat meal agar, potato dextrose agar, ragi flour medium and yeast extract + 2% soluble starch. On the basis of morphological features like Buff colour colony with irregular margin, concentric ring pattern and medium to good growth the fungus was characterised and identified as *M. grisea* (Table 3 and Fig 1& 2). Similar results were recorded by Srivastava *et al*. (2014) where various isolates of *M. grisea* produced ring like, circular, irregular colonies with rough and smooth margins on oat meal agar media having buff color, grayish black to black color. Ou, (1985a) recorded colony colour as greyish black to dark jet black color, smooth to irregular margin, medium to good growth of the pathogens on oat meal agar media.

In case of leaf blast, the maximum mean colony diameter was recorded in Oat meal agar (89.16 mm) followed by Potato dextrose agar (74. 63 mm) and minimum colony diameter was observed in Yeast extract + 2% soluble starch (57.56 mm). In case of neck blast, the maximum mean colony diameter was recorded in Oat meal agar (88.00 mm) followed by Potato dextrose agar (73. 50 mm) and minimum colony diameter was observed in Yeast extract + 2% soluble starch (55.00 mm) (Table 4). The similar results were obtained by Awoderu *et al.* (1991) and Getachew Gashaw *et al*. (2014) which showed that Oat meal agar and Richard Agar media had maximum mycelial growth of *P. grisea* with 87.3mm and 88.2mm in isolates of Pg 41 and Pg 26, respectively.



Leaf blast isolate Neck blast isolate

Fig. 3. *Photomicrograph of mycelium and conidia of leaf and neck blast isolates.*

In case of leaf blast isolate, the maximum sporulation mean index was observed in Oat Meal agar (3.15µm) followed by Potato Dextrose Agar (2.83 µm) and minimum sporulation was observed in ragi flour media (1.77 µm) and there was no sporulation on yeast extract + 2% Soluble starch (Fig 3). In case of neck blast isolate, the maximum sporulation mean index was observed in Oat Meal agar (3.20 µm) followed by Potato Dextrose Agar (2.88 µm) and minimum sporulation was observed in ragi flour media (1.72 µm) and there was no sporulation on yeast extract + 2% Soluble starch (Table 5). Similarly, Arunkumar and Singh (1995) reported good growth and sporulation of 2.95 µm in leaf blast and 3.00 µm in neck blast in oat meal agar followed by host extract agar media. The isolates varied in their ability to grow and sporulate on different media. Same results were recorded by Sun guochang and Sun shuyuan (2001) cornmeal and rice straw agar medium supported maximum sporulation of the rice blast fungus. Rice leaf and potato agar medium, rice straw agar medium and oatmeal agar medium were also found to be effective.

Studies on morphological character of different isolates of *M. grisea* revealed the variation with respect to conidial size. *M. grisea* isolated from leaf region was grown on three different media. From each medium, six plates were selected and in each plate *M. grisea* spore morphometry was recorded. Maximum conidia length (9.6µm) and breadth (7.3µm) was recorded in Oat meal agar followed by Potato dextrose agar and least conidia length (6.83 µm) and breadth (5.76 µm) was recorded in ragi flour media. The observation was recorded 20 days after inoculation.

Whereas slightly small conidial size was observed in neck blast isolate (9.46 and 7.36 µm). However, no variation with respect to conidial shape was noticed where conidia was pyriform, almost hyaline to pale olive, 2-septate, 3-celled (Table 6). These results are in agreement as described by Shirai (1896) who recorded that, mycelium in cultures was first hyaline in colour, then changed to olivaceous, 1 – 5.2 µm in width, septate and branched. The spore measurements were

15 – 22 µm x 4 – 7 µm (Average, 17.4 µm x 5.2µm). These results are also in confirmation with Meena (2005) and Getachew *et al*. (2013) where in all the isolates, the shape of the conidia was typically pyriform with base rounded, apex narrowed, 2-3 septate, 2-4 celled, and middle cells were broader than others in fingermillet crop. Existence of variability among the isolates of *P. grisea* with respect to conidial size ie., the average length of the isolate ranged from 21.2 to 28.4µm, and the average width from 7.3 to 9.0µm which was well documented by many workers also (Aoki, 1935., Tochinai and Shimamura, 1932). The isolates of fingermillet Pg.41 and Pg.40 had the longest conidia of 26.91 – 35.43 µm and 24.36 – 29.48 µm respectively. The lowest conidial length was for isolates Pg.22 of 15.66 – 24.37 µm and Pg.20 of 18.01 – 24.03 µm. Highest conidial width was observed for isolates Pg.40 and Pg.22 with 8.35-11.92 µm and 7.70-12.90 µm respectively as reported by Getachew Gashaw *et al*. (2014).

Conclusion

M. grisea grown on Oat meal agar showed good and uniform growth with smooth margin and excellent sporulation. While in case of Ragi flour medium and Yeast extract + 2% soluble starch medium the *M. grisea* growth was good and uniform but there was poor sporulation.In Spore morphometry study, the size of conidia was 9.6 x 7.3 µm on Oat meal agar and least conidial size of 6.15 x 5.11 was recorded in ragi flour medium. The fungal radial growth of leaf isolate was maximum on Oat meal agar (89.16 mm) and minimum on Yeast extract+ 2% soluble starch (57.56 mm). A similar trend was observed with neck isolate with 88.00 mm and 55 mm on Oat meal agar and Yeast extract+ 2% soluble starch respectively. The maximum sporulation indices for leaf and neck blast isolates were 3.15 µm and 3.20 µm on Oat meal agar respectively. Therefore oat meal agar media found to give best results on growth, sporulation, conidial size and colony characters of *M. grisea* as compared to all other media used.

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