Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(3): 3749-3753
Received: 16-03-2019
Accepted: 18-04-2019

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Morphological characterization of *Pyricularia oryzae* causing blast disease in rice (*Oryza sativa* L.) from different zones of Karnataka

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Abstract
Study was conducted to describe the cultural and morphological characteristics such as colour and texture of the leaf blast pathogen *Pyricularia oryzae* on different solid media *viz.,* Host extract agar, Oat meal agar, Potato dextrose agar and Richard's agar medium. Among all the solid media the highest mean mycelial growth (diameter) of the fungus *Pyricularia oryzae* was recorded on Host extract agar (40.80mm) followed by Oat meal agar (38.33 mm) and least mean mycelial growth of the *P. oryzae* on Rechard's agar (28.4 mm). The highest mean dry mycelial weight(mg) Rechard's agar(300.65mg) followed by Oat meal agar (234.67 mg) and least mean mycelial weight was recorded in Potato Dextrose agar (96.31 mg). In general, among all solid media the Host extract agar media is more appropriate for cultural and morphological study of rice blast fungus *P. oryzae*.

Keywords: Mycelial, richard's agar, blast, pyricularia

Introduction
Rice blast is one of the most spread and the most damageable diseases of rice in India. Rice is a member of the grass family (Poaceae). There are more than 10,000 species of grasses distributed among 600 genera. Grasses occur worldwide in a variety of habitats. Rice is the most important food crop of India covering about one-fourth of the total cropped area and providing food to about half of the Indian population. Rice is unique because it can grow in wet environments that other crops cannot survive in. Such wet environments are abundant across Asia. Rice is life for thousands of millions of people. In Asia alone, more than 2,000 million people obtain 60 to 70 per cent of their calories from rice and its products. Recognizing the importance of this crop, the United Nations General Assembly declared 2004 as the "International Year of Rice" (IYR). The theme of IYR—"Rice is life" reflects the importance of rice as a primary food source, and is drawn from an understanding that rice based systems are essential for food security, poverty alleviation and improved livelihood. 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. The Commonwealth Mycological Institute has recorded its presence from 85 countries throughout the world. Paddy blast is generally considered as the principal disease of rice and is caused by a fungus belonging to the Ascomycete *Pyricularia oryzae* Cavara (Teleomorph= *Magnaporthe grisea* (Hebert) Barr Comb nov.). Losses due to the blast disease may range up to 90 per cent depending upon the component of the plant infected. *Pyricularia oryzae* 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. The pathogen may infect all the above ground parts of a rice plant at different growth stages viz., leaf, collar, node, internodes, base or neck and other parts of the panicle and sometimes the leaf sheath. A typical blast lesion on a rice leaf is gray at the centre, has a dark border and it is spindle-shaped.

Material and Methods
Effect of different media on the growth of *P. oryzae* Culture discs of pathogen (5mm) was inoculated separately on different media and incubated at 28±1ºC for 15 days. The cultural characters and the colony diameter (mm) on each medium were recorded. Fifteen ml of each medium were poured into each of sterilized petriplates. Inoculation was made by transferring the five mm disk of mycelia mat, taken from the periphery of ten days old culture of each fifteen isolates. Each treatments were replicated thrice. The plates were incubated at 28±1ºC.

Observation of colony 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 pathogen was multiplied by transferring a loop full of the stock culture to 250 ml of potato dextrose broth taken in a 1000 ml flask. The inoculated flask was incubated at 28±10 C for fourteen days. The concentration of spore suspension was adjusted to 50 spores/microscopic field by adding sterilized distilled water.

Maintenance of culture

All the fifteen isolates of *P. oryzae* were maintained on PDA slants at 40^0C in the refrigerator and subculture periodically at an interval of 30 days during the course of this study.

Results and Discussion

Growth of *P. oryzae* on different solid media. The experiment was conducted as detailed in material and methods‟ to ascertain the period when the maximum growth of the fungus could occur among all the solid media the highest mean mycelial growth of the fungus *Pyricularia oryzae* was recorded Host extract agar (40.80mm) followed by Oat meal agar (38.33 mm) and least mean mycelial growth of the *P. oryzae* on Rechard's agar (28.4 mm) (Table 3, fig. 2). The highest mean dry mycelial weight(mg) Rechard's agar(300.65mg) followed by Oat meal agar (234.67 mg) and least mean mycelial weight was recorded in Potato Dextrose agar (96.31 mg). (Table 2, Fig. 1). Morphological and cultural studies were carried to find out the variation for the growth and sporulation of isolates (Table 1). Cultural characters of each of the isolates studied on four different solid media at room temperature 28^0C showed the variation among the isolates of *P. oryzae*. Morphological characters *viz.,* size and shape of conidia were studied for identification of the fungus. Conidia were pyriform, almost greyish black, 2-septate and 3 celled. The shape, size, septation and colour characters are in agreement with those described by Nishikado (1926) [6] and Mijan Hossain (2000) [4].

Cultural characteristics studied on different media showed the variation among fifteen isolates of *P. oryzae* with respect to colony characters like type of growth, colour of colony and colony margin (Ou, 1985) [8]. Colour varied from grayish black to dark jet black colour, smooth to irregular margin, medium to good growth of the pathogen were observed (Onofeghara *et al.,* 1973) [7]. These fifteen isolates exhibited considerable variation in colony type and colour when grown on different nutrient media (Table 2).

Potato Dextrose agar

Colonies were buff coloured in isolates Po2, Po3, Po4, Po5, Po6, Po7 and Po8. However isolates Po1, Po9 and Po15 showed grayish black colour, while isolates Po10, Po11, Po12, Po13 and Po14 showed black colour colonies (Akhilesh *et al.,* 2017) [1]. The isolates Po1, Po4 and Po11 only, showed good to medium colony growth.

Richard's agar

Almost all isolates showed grayish colour colonies with smooth and marginal good growth, except Po4, Po7, Po8, Po11, Po12, Po13, Po14 and Po15 showed marginal irregular growth.

Oat meal agar

Colonies were grayish coloured in isolates Po1, Po4, Po5, Po7, Po9, Po10, Po12 and Po13. However isolates Po2, Po3, Po6, Po8 and Po15 showed black colour while only isolate Po11 showed green black colour colonies (Akhilesh *et al.*, 2017) [1]. Except Po3 and Po5 which showed irregular marginal growth, all other isolates showed smooth and good growth.

Host extract agar

Except Po15 (Black colonies) all other isolates showed grayish black colonies. All isolates showed smooth and marginal good growth.

Growth Phase

The experiment was conducted as detailed in 'Material and methods' to ascertain the period when the maximum growth of the isolates could occur. PDA, HEA, OMA and RA were use in the study as a basal media and the dry mycelia weight of each isolated were taken on the 10th day of inoculation. Dry mycelial weight of the isolates Po3, Po6, Po12 and po15 were maximum in HEA media. The dry mycelial weight of the isolates Po3, Po12 and Po15 were maximum in PDA media. The dry mycelial weight of the isolates Po3, Po6, Po10 and Po13 were maximum in RA media and the dry mycelial weight of the isolates Po3, Po5, Po7, Po12 and Po14 were maximum in OMA media. There have been several reports regarding such variation in growth rate among isolates of a pathogen. Simulated observations have been recorded by Kulkarni (1973) [3], Onofeghara *et al.*, (1973) [7] and Akhilesh *et al.* (2017) [1].

Table 1: Source of *Pyricularia* isolates collected from different region of Karnataka

Sl/no	Isolate Number	Location	Plant part	Variety	Zone
1	Po1	Gangavathi	leaf	BPT-5204	Northern Dry Zone 3
2	Po2	Siraguppa	leaf	BPT-5204	Northern Dry Zone 3
3	Po3	Mandya	leaf	Thanu	Southern Dry Zone 6
4	Po4	Shivamogga	leaf	Jyothi	Southern Transition Zone 7
5	Po5	Shivamogga	Neck	Jyothi	Southern Transition Zone 7
6	Po6	Haveri	leaf	Abhilash	Northern transition Zone 8
7	Po7	Mugad	leaf	Abhilash	Northern transition Zone 8
8	Po8	Mugad	Neck	Intan	Northern transition Zone 8
9	Po9	Sirsi	leaf	Intan	Hilly Zone 9
10	Po10	Malagi	leaf	Intan	Hilly Zone 9
11	Po11	Malagi	Neck	Intan	Hilly Zone 9
12	Po12	Mundogadu	leaf	Intan	Hilly Zone 9
13	Po13	Mudigere	leaf	Intan	Hilly Zone 9
14	Po14	Ponnampet	leaf	Intan	Hilly Zone 9
15	Po15	Ponnampet	Neck	Intan	Hilly Zone 9

Table 2: Colony characteristics of different isolate of *Pyricularia oryzae* on different media

Media	Colony Characters						
	Po1	**Po2**	**Po3**	**Po4**	**Po5**	**Po6**	**Po7**
Host extract agar	Grayish black colour smooth colony margin good growth	Grayish black colour smooth colony margin good growth	Grayish black colour smooth colony margin good growth	Grayish black colour smooth colony margin good growth	Grayish black colour smooth colony margin good growth	Grayish black colour smooth colony margin good growth	Grayish black colour smooth colony margin good growth
Oat meal agar	Grayish black colour smooth colony margin medium growth	Black colour smooth colony margin good growth	Black colour irregular margin growth	Grayish black colour margin medium growth	Grayish black colour irregular margin medium growth	Black colour smooth colony margin good growth	Grayish black colour smooth colony margin medium growth
Potato dextrose agar	Grayish black colour smooth colony margin good growth	buff colour smooth margin rised mycelial good growth	buff colour smooth margin rised mycelial good growth	buff colour smooth margin rised mycelial good growth	buff colour smooth margin rised mycelial good growth	buff colour smooth margin rised mycelial good growth	buff colour smooth margin rised mycelial good growth
Richard's agar	Grayish colour smooth colony margin good growth	Grayish colour smooth colony margin good growth	Grayish colour smooth colony margin medium growth	Grayish colour smooth colony margin irrgular growth	Grayish colour smooth colony margin good growth	Grayish colour smooth colony margin good growth	Grayish colour smooth colony margin irrgular growth

Media	Colony Characters						
	p8	**p9**	**p10**	**p12**	**p13**	**p14**	**p15**
Host extract agar	Grayish black colour smooth colony margin good growth	Grayish black colour smooth colony margin good growth	Grayish black colour smooth colony margin good growth	Grayish black colour smooth colony margin good growth	Grayish black colour smooth colony margin good growth	Grayish black colour smooth colony margin good growth	Black colour smooth colony margin good growth
Oat meal agar	Black colour smooth colony margin good growth	Grayish black colour smooth colony margin good growth	Grayish black colour smooth colony margin good growth	Grayish black colour smooth colony margin good growth	Grayish black colour smooth colony margin good growth	Green black colour smooth colony margin good growth	Black colour smooth colony margin good growth
Potato dextrose agar	Buff colour smooth margin rised mycelial good growth	Grayish black colour smooth colony margin good growth	Black colour smooth colony margin good growth	Black colour smooth colony margin good growth	Black colour smooth colony margin good growth	Black colour smooth colony margin good growth	Grayish black colour smooth colony margin good growth
Richard's agar	Grayish colour smooth colony margin irrgular growth	Grayish colour smooth colony margin good growth	Grayish colour smooth colony margin good growth	Grayish colour smooth colony margin irrgular growth	Grayish colour smooth colony margin irrgular growth	Grayish colour smooth colony margin irrgular growth	Grayish colour smooth colony margin irrgular growth

Table 3: Colony diameter of isolates in different media

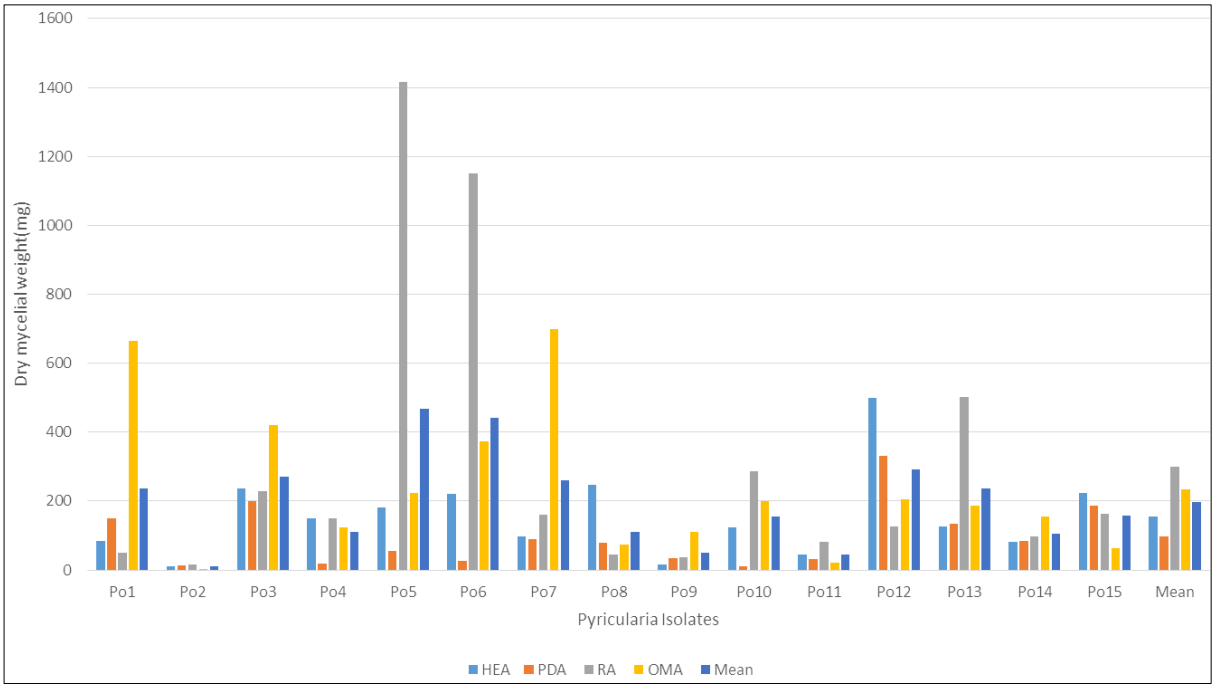
Isolates	Colony diameter in different media (mm)				
	HEA	**OMA**	**PDA**	**RA**	**Mean**
Po1	38	46	40	35	39.75
Po2	44	45	42	42	43.25
Po3	44	39	39	20	35.50
Po4	30	44	33	15	30.50
Po5	35	43	35	35	37.00
Po6	30	46	24	34	33.50
Po7	38	12	37	20	26.75
Po8	45	47	33	20	36.25
Po9	39	37	35	35	36.50
Po10	44	36	38	25	35.75
Po11	44	45	30	35	38.50
Po12	45	32	36	25	34.50
Po13	46	33	38	35	38.00
Po14	46	36	33	30	36.25
Po15	44	34	19	20	29.25
Mean	40.80	38.33	34.13	28.40	35.42
				SEM±	1.07
				CV (%)	11.77

HEA: Host extract agar, OMA: Oat meal agar, PDA: Potato dextrose agar and RA: Richard's agar

Table 4: Dry mycelia weight of Pyricularia isolates grown in different media

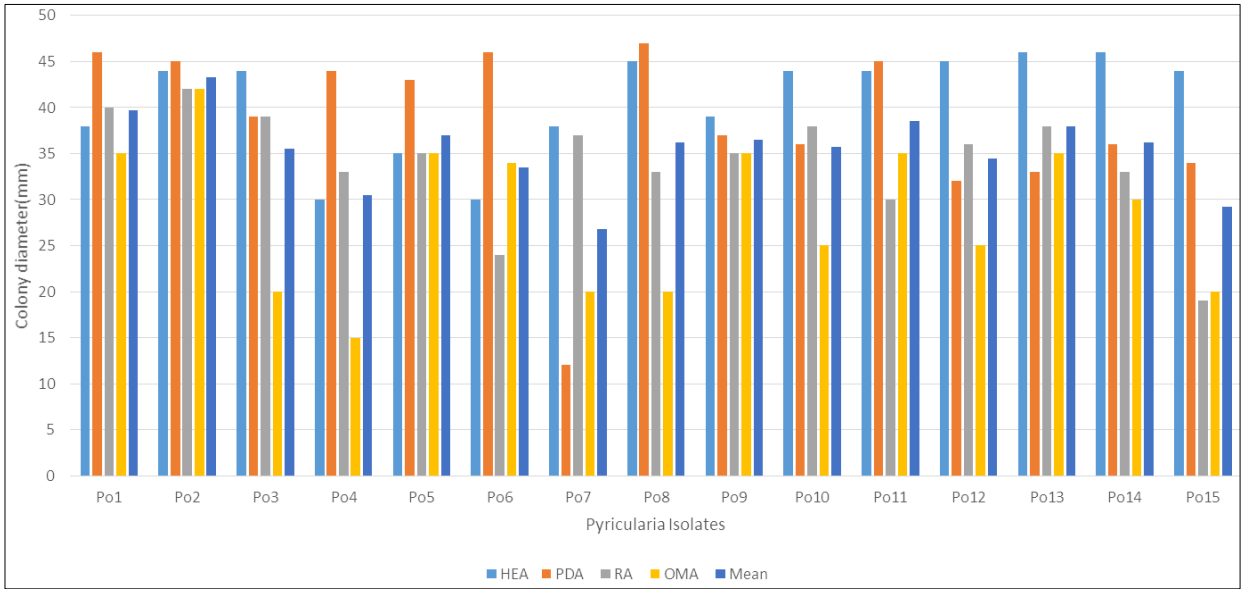
Isolates	Mycelia Dry weight (mg) in different media				Mean
	HEA	PDA	RA	OMA	
Po1	84.6	150.4	49.6	665.5	237.53
Po2	11.5	12.6	14.6	2.9	10.40
Po3	237	200.34	227.4	420	271.19
Po4	150	18.2	150.8	124.6	110.90
Po5	180	56.3	1416.1	223.2	468.90
Po6	220	25.7	1150	373.3	442.25
Po7	96	90.4	160	699	261.35
Po8	245.8	78.3	44.6	74.6	110.83
Po9	15.3	35	38.1	109.7	49.53
Po10	123.5	10.45	287.6	199.1	155.16
Po11	44.5	32.6	80.7	20.9	44.68
Po12	500.45	330	127.4	204.2	290.51
Po13	125.5	134.56	500.87	185.3	236.56
Po14	80.4	82.9	98	155.8	104.28
Po15	222.7	186.9	164	62	158.90
Mean	155.82	96.31	300.65	234.67	196.86
				SEM±	35.32
				CV(%)	69.50

HEA: Host extract agar, OMA: Oat meal agar, PDA: Potato dextrose agar and RA: Richard's agar



HEA: Host extract agar, OMA: Oat meal agar, PDA: Potato dextrose agar and RA: Richard's agar

Fig 1: Effect of different media on mycelial dry weight (mg)



HEA: Host extract agar, OMA: Oat meal agar, PDA: Potato dextrose agar and RA: Richard's agar

Fig 2: Effect of different media on Colony growth

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