

Original Research Article

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Cultural and Morphological Variability of *Pyricularia grisea* (Cooke) Sacc Isolates from Major Rice Growing Areas of Telangana State, India

K. Aravind^{1*}, B. Rajeswari¹, T. Kiran Babu² and S.N.C.V.L. Pushpavalli³

¹Department of Plant Pathology, ²Rice Research Section, Agriculture Research Institute,
³Institute of Biotechnology, College of Agriculture, PJTSAU,
Rajendranagar, Hyderabad 500030, India

*Corresponding author

ABSTRACT

Rice blast caused by *Pyricularia grisea* (Cooke) Sacc. became one of the most important disease in rice growing areas of Telangana State because of its wide distribution and destructiveness under favourable conditions. However, sometimes resistant varieties may become ineffective due to evolutionary changes in the pathogen population. Keeping in view the importance of disease, studies were conducted on cultural and morphological variability of *P. grisea* isolates. Blast infected samples were collected from different locations of Telangana State were studied for radial growth, colony color, growth pattern, texture of colony, sectoring, zonation and wrinkles formation, dry mycelial weight, time of sporulation and sporulation index. The highest mean radial mycelial growth of the fungus was recorded on OMA (81.7 mm) followed by PDA (77.8 mm) and least mean radial mycelial growth of the *P. grisea* isolates were recorded on HLEA medium (72.5 mm). Colony colour of twelve *P. grisea* isolates were differed from greyish white to greyish black on three solid media tested. All the isolates were circular form and varied with respect to mycelium elevation and texture. Significant differences were also observed among the isolates with the formation of sector, zonation and wrinkles. Among the three different liquid media tested, highest mean mycelial dry weight of the *P. grisea* isolates was recorded on PDB (225 mg) followed by OMB (214 mg) and least mean mycelial dry weight on HLEB(164 mg). Time taken for sporulation of *P. grisea* isolates on OMA medium was 7.9 days followed by HLEA medium for 8 days and PDA medium for 8.2 days. Sporulation index of twelve *P. grisea* isolates were varied from poor to excellent on rating scale of 1 to 4 on three solid media tested. Conidia of the isolates were produced in clusters on long septate, slender conidiophores. The mean conidial size ranged from 18.9 µm to 28.2 µm in length and 6.1 µm to 9.3 µm in width among twelve *P. grisea* isolates. The shape of conidia in all the isolates was pyriform and hyaline to pale olive, 2 septate and 3 celled. Spore germination percentage was high in Pg1 isolate (91.6 %) and least in Pg6 isolate (28.3 %).

Keywords

Rice blast,
Pyricularia grisea,
Rice [*Oryza sativa*]

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Introduction

Rice [*Oryza sativa*] is the most important cereal crop of the world and it is a major

staple food for thousands of millions of people as rice grain contains on an average 7% protein, 62-65 % starch, 0.7% fat and 1.3% fiber. China is the leading rice producer

followed by India, Indonesia and Bangladesh. In India rice crop occupies an area of 47.0 m.ha with a production of 114.47 million tons with a productivity of 2665 Kg ha⁻¹ (INDIASTAT, 2019). In Telangana State, rice is cultivated in area of around 28.3 lakh hectares during *Kharif*, 2019 and *Rabi*, 2019-20. As against the normal area of 6.83 hectares, the actual rice area covered during *Rabi* 2019-20 was 15.69 lakh hectares with an increase of 228.7% over the normal area (www.tsagri.nic.in). This sharp increase in area under rice production due to the improved irrigation facilities and pro-farmer policies being implemented by the State Government and estimated the paddy production may further increase making Telangana the rice bowl of the country. In spite of this phenomenal increase in area and production of rice its productivity is limited by various biotic and abiotic stresses. Among the biotic stresses, blast disease is considered as one of the major recurrent problem in all rice growing regions of the world accounting yield losses to 14-18% (Mew and Gonzales, 2002).

Rice blast caused by *Magnaporthe grisea* (Hebert, 1971) Barr (Anamorph: *Pyricularia grisea* (Cooke) Sacc.) is a filamentous ascomycetes fungus infecting more than 50 hosts and the disease can strike all parts of the plants causing diamond shaped lesions with a grey or white center to appear on leaves or on the panicle which turn white and die before being filled with grain (Scardaci *et al.*, 1997). However, the intensity of the disease varies in different regions and years.

The resulting yield loss as high as 70-80% when predisposition factors with minimum night temperature ranges from 20°–26°C, with the association of >90% of relative humidity, dew deposit, extended leaf wetness period (> 10 h) and cloudy drizzling weather during any crop growth stage of susceptible varieties (Padmanabhan, 1965) favoring

epidemic development and threatens the stability of rice production worldwide.

The use of resistant rice varieties is the most economical and effective means of managing blast disease in rice (Chen, 2001). However, sometimes resistant varieties may become ineffective due to evolutionary changes in the pathogen population (Khadka, 2013). Loss of resistance shortly after variety release is common in many rice growing areas (Kang, 2000). Therefore, understanding variation of *P. grisea* is important in overcoming constraints facing by many rice breeding programs.

Materials and Methods

Collection and isolation of rice blast infected samples

A roving survey was carried out in major rice growing areas of Telangana state during *kharif*, 2019 and collected a total of twelve blast infected rice samples from Karimnagar (Pg1), Rangareddy (Pg2), Mancherial (Pg3), Jagtial (Pg4), Nizamabad (Pg5), Nalgonda (Pg6), Peddapalli (Pg7), Mahabubabad (Pg8), Khammam (Pg9), Mahbubnagar (Pg10), Medak (Pg11), and Warangal (Pg12) districts of Telangana state. The samples were brought to the laboratory for the isolation of test pathogen. Rice plant (leaf and neck) showing typical symptoms of the blast disease were marked and washed with sterile double distilled water. Fine pieces of diseased tissue along with some healthy portion were cut with the help of a sterile scalpel blade and surface sterilized with 1% sodium hypochlorite solution for one min, rinsed thrice in sterile double distilled water and dried on sterilized filter paper. Later, it was transferred aseptically onto the sterilized Petri dishes containing OMA medium and plates were incubated at 25 ± 1°C for 7 days.

Storage of fungal isolates

The fungus was grown on OMA medium

slants for 7 days at $25 \pm 1^\circ\text{C}$ in BOD incubator. The test tubes were filled with mineral oil up to the active mycelial growth of the fungus and stored at 4°C for further studies as short-term preservation.

Pathogenicity test

Pathogenicity test of *Pyricularia grisea* isolates was conducted by using susceptible rice cv. TN1 under glasshouse conditions. Fifteen days old pot grown seedlings were inoculated artificially by spraying the inoculum (1×10^6 conidia /ml) on the foliage using a hand-operated atomizer. All the inoculated plants were covered with polythene covers moistened inside for 24 h with a view to provide appropriate humid conditions during initial stages of infection and incubated at 25°C with $>95\%$ relative humidity. Leaf wetness of 12 h photoperiod for seven days was given with mist sprinklers in glasshouse to enable spore germination. After incubation, observations were made regularly for the appearance and development of symptoms. The fungus was re-isolated from infected leaf and the culture obtained was compared with original culture for further confirmation.

Cultural characteristics of isolates of *Pyricularia grisea*

The cultural characters of all monoconidial isolates of *Pyricularia grisea* were recorded by growing them on OMA, PDA and Host leaf extract + 2 % sucrose agar medium for 14 days at 26°C . Cultural characteristics of *P. grisea* isolates were studied for colour of isolates, growth pattern, texture of colony, sectoring, zonation and wrinkles formation, radial growth (mm), mycelial dry weight, time of sporulation and sporulation index. The colour of *P. grisea* isolates on different media were recorded when the pathogen has attained the maximum growth on the Petridishes 14 days after incubation. The radial growth of

different isolates on different media were measured daily from the first day after incubation until maximum growth on the Petridishes. Time of sporulation of different isolates were recorded for one day interval by growing the *P.grisea* isolates on different media under 14 h light + 8 h dark conditions. The sporulation capacity of each isolate was assessed by microscopic observations.

Morphological characteristics of isolates of *Pyricularia grisea*

Morphological characteristics of *P. grisea* isolates were studied for length, width of conidia and spore germination. Conidia of *P. grisea* of different isolates were mounted in lactophenol cotton blue on a clean slide. The length and breadth of the conidia were measured under high power objective (40X) using a pre calibrated ocular micrometer. The average size of conidia was then determined and shape of the conidia were recorded (Aruna *et al.*, 2016) Spore germination of *P. grisea* isolates were studied by growing them on 2% sucrose solution in cavity slides.

Results and Discussion

Radial mycelial growth

Significant differences were observed in cultural characteristics among the isolates of *P. grisea* on oat meal agar, potato dextrose agar and host leaf extract + 2% sucrose agar medium after 14 days of incubation. The highest mean radial mycelial growth of the fungus was recorded on OMA (81.7 mm) followed by PDA (77.8 mm) and least mean radial mycelial growth of the *P. grisea* isolates were recorded on HLEA medium (72.5 mm). Irrespective of the media the mean radial mycelial growth was found high in Pg1 isolate (90 mm) followed by Pg6 and Pg10 isolates which were found on par with each other in recording the mycelial growth of 84.2 mm and 84.0 mm, respectively.

Radial mycelial growth was ranged from 60.6 mm to 77.6 mm in other isolates of *P. grisea* on three solid media tested. Similarly, Kulmitra *et al.* (2017) also reported that highest mean mycelial growth of *P. grisea* was recorded on oat meal agar (77.6 mm) followed by rice leaf extract media (75.9 mm) and least in sabour d agar media (44.7 mm).

Colony colour

Colony colour of twelve isolates of *P. Grisea* varied from greyish white to greyish black. Among twelve *P. grisea* isolates tested, greyish white colonies were recorded in six isolates *viz.*, Pg1, Pg4, Pg5, Pg7, Pg9 and Pg11 on OMA medium and seven isolates (Pg1, Pg5, Pg6, Pg7, Pg9, Pg11 and Pg12) on PDA medium and five isolates (Pg4, Pg7, Pg8, Pg11 and Pg12) on HLEA medium. Whereas greyish black pigmentation was recorded in Pg2, Pg3, Pg6, Pg8, Pg10 and Pg12 isolates on OMA medium, five isolates (Pg2, Pg3, Pg4, Pg8, Pg9 and Pg10) on PDA medium and seven isolates (Pg1, Pg2, Pg3, Pg5, Pg6, Pg9 and Pg10) on HLEA medium.

Texture of the colony

The results on texture of the colony on three different solid media after 14 days of incubation indicated that isolates of *P. grisea* exhibited the formation of either rough or smooth colonies. Ten isolates of *P. grisea* (Pg2, Pg3, Pg4, Pg5, Pg6, Pg7, Pg8, Pg9, Pg10 and Pg11) produced colonies with smooth margin on OMA medium, nine isolates (Pg2, Pg3, Pg4, Pg5, Pg7, Pg8, Pg9, Pg10 and Pg12) on PDA medium and nine isolates (Pg2, Pg3, Pg5, Pg6, Pg8, Pg9, Pg10, Pg11 and Pg12) on HLEA medium. Colonies with rough margin observed in two isolates (Pg1 and Pg12) on OMA medium, three isolates (Pg1, Pg6 and Pg11) on PDA medium and three isolates (Pg1, Pg4 and Pg7) on HLEA medium. Kalavati *et al.* (2016) also identified the fungal isolates of *P. grisea*

produced circular, irregular mycelium with smooth and rough margin on OMA, PDA, RFA media.

Sector formation was observed in two isolates (Pg4 and Pg12) of *P. grisea* on OMA medium, two isolates (Pg3 and Pg12) on PDA medium and one isolate (Pg11) on HLEA medium. Zonation was noticed in eleven isolates (Pg1, Pg3, Pg4, Pg5, Pg6, Pg7, Pg8, Pg9, Pg10, Pg11 and Pg12) on OMA medium, ten isolates (Pg1, Pg2, Pg3, Pg4, Pg5, Pg6, Pg8, Pg9, Pg10 and Pg11) on PDA medium and eight isolates (Pg1, Pg2, Pg3, Pg5, Pg6, Pg9, Pg10 and Pg12) on HLEA medium whereas wrinkle formation was observed in four isolates (Pg1, Pg6, Pg8 and Pg10) on OMA medium, four isolates (Pg1, Pg4, Pg8 and Pg11) on PDA medium and four isolates (Pg1, Pg4, Pg10 and Pg11) on HLEA medium.

Similarly, Bhaskar, (2018) found that isolates of *P. grisea* varied in the pigmentation and with formation of sectors, zonation and wrinkle.

Mycelial dry weight

Among three different liquid media tested, it was observed that highest mean mycelial dry weight of the *P. grisea* isolates was recorded in potato dextrose broth (225 mg) followed by oat meal broth (214 mg) and least mean mycelial dry weight on host leaf extract + 2% sucrose broth (164 mg).

Irrespective of the media the mean mycelial dry weight was found high in Pg4 isolate (255.0 mg) and lowest mean mycelial dry weight of 162.3 mg in Pg6 isolate. Manjunatha and Kishappa (2019) observed that highest mean mycelial dry weight was recorded in Richards agar (300.6 mg) followed by OMB (234.6 mg) and HLEB (156 mg) and least in PDB (96.3 mg).

Table.1 Radial mycelial growth and mycelial dry weight of twelve isolates of *P. grisea* on three different media tested after 14 days of incubation

S.No.	Isolate	Oat meal agar		Potato dextrose agar		Host leaf extract + 2% sucrose agar	
		Radial mycelial growth (mm)	Dry mycelial weight (mg)	Radial mycelial growth (mm)	Dry mycelial weight (mg)	Radial mycelial growth (mm)	Dry mycelial weight (mg)
1.	Pg1	90.0	229	90.0	197	90.0	173
2.	Pg2	90.0	297	72.5	183	73.6	239
3.	Pg3	72.6	178	87.0	289	70.3	90
4.	Pg4	59.3	254	73.5	217	84.3	294
5.	Pg5	74.6	231	83.5	253	71.3	24
6.	Pg6	90.0	229	90.0	156	72.6	102
7.	Pg7	63.6	193	79.6	201	89.6	189
8.	Pg8	90.0	191	80.0	303	32.6	200
9.	Pg9	86.6	186	85.6	246	60.6	133
10.	Pg10	90.0	172	72.0	189	90.0	195
11.	Pg11	87.5	203	88.0	312	73.0	213
12.	Pg12	86.5	211	33.0	159	62.3	127
13.	Mean	81.7	214.5	77.9	225	72.5	164
		Isolate (A)		Media (B)		Isolate x Media (A x B)	
	C.D. at 5%	1.59	2.09	3.18	4.18	5.51	7.24
	SE(d) ±	0.79	1.04	1.59	2.09	2.75	3.62
	SE(m) ±	0.56	0.74	1.12	1.48	1.95	2.56

Table.2 Cultural characters of twelve isolates of *P. grisea* on three solid media tested after 14 days of incubation

S. No.	Isolate	Oat meal Agar	Potato dextrose agar	Host leaf extract + 2% sucrose agar
1.	Pg1	Greyish whitecolour, elevated mycelium with rough margin and formations of wrinkles and zonations.	Greyish whitecolour, elevated mycelium with rough margin and formations of wrinkles and zonations.	Greyish blackcolour, flat mycelium with rough margin and formations of wrinkles and zonations.
2.	Pg2	Greyish blackcolour, flat mycelium with smooth margin.	Greyish blackcolour, flat mycelium with smooth margin and zonations.	Greyish blackcolour, flat mycelium with smooth margin and zonations.
3.	Pg3	Greyish blackcolour, flat mycelium with smooth margin and zonations.	Greyish blackcolour, flat mycelium with smooth margin and formations of sectors and zonations.	Greyish blackcolour, elevated mycelium with smooth margin and zonations.
4.	Pg4	Greyish whitecolour, elevated mycelium with smooth margin and formations of sectors and zonations.	Greyish blackcolour, flat mycelium with smooth margin and formations of wrinkles and zonations.	Greyish whitecolour, elevated mycelium with rough margin and formations of wrinkles
5.	Pg5	Greyish whitecolour, elevated mycelium with smooth margin and zonations.	Greyish whitecolour, elevated mycelium with smooth margin and zonations.	Greyish blackcolour, flat mycelium with smooth margin and zonations.
6.	Pg6	Greyish blackcolour, flat mycelium with smooth margin and formations of wrinkles and zonations.	Greyish whitecolour, elevated mycelium with rough margin and zonations.	Greyish blackcolour, elevated mycelium with smooth margin and zonations.
7.	Pg7	Greyish whitecolour, elevated mycelium with smooth margin and zonations.	Greyish whitecolour, elevated mycelium with smooth margin	Greyish whitecolour, flat mycelium with rough margin
8.	Pg8	Greyish blackcolour, elevated mycelium with smooth margin and formations of wrinkles and zonations.	Greyish blackcolour, flat mycelium with smooth margin and formations of wrinkles and zonations.	Greyish whitecolour, flat mycelium with smooth margin
9.	Pg9	Greyish whitecolour, flat mycelium with smooth margin and zonations.	Greyish whitecolour, flat mycelium with smooth margin and zonations.	Greyish blackcolour, elevated mycelium with smooth margin and zonations.
10.	Pg10	Greyish blackcolour, flat mycelium with smooth margin and formations of wrinkles and zonations.	Greyish blackcolour, flat mycelium with smooth margin and zonations.	Greyish blackcolour, elevated mycelium with smooth margin and formations of wrinkles and zonations.
11.	Pg11	Greyish white, flat mycelium with smooth margin and zonations.	Greyish whitecolour, elevated mycelium with rough margin and formations of wrinkles and zonations.	Greyish whitecolour, elevated mycelium with smooth margin and formations of sectors and wrinkles
12.	Pg12	Greyish black, elevated mycelium with rough margin and formations of sectors and zonations.	Greyish whitecolour, elevated mycelium with smooth margin and formations sectors.	Greyish white, flat mycelium with smooth margin and zonations.

Table.3 Time taken for sporulation and Sporulation index of twelve isolates of *P. grisea* on three solid media after 14 days of incubation

S.No.	Isolate	Oat meal agar		Potato dextrose agar		Host leaf extract + 2% sucrose agar	
		Time taken for sporulation (DAI)	Sporulation Index (1 – 4 Scale)	Time taken for sporulation (DAI)	Sporulation Index (1 – 4 Scale)	Time taken for sporulation (DAI)	Sporulation Index (1 – 4 Scale)
1.	Pg1	7	3	7	3	12	1
2.	Pg2	10	3	3	4	8	1
3.	Pg3	11	3	5	3	15	2
4.	Pg4	10	2	12	1	13	1
5.	Pg5	13	4	10	2	11	2
6.	Pg6	3	2	12	2	7	2
7.	Pg7	6	4	9	4	3	3
8.	Pg8	10	3	15	2	5	1
9.	Pg9	6	3	13	2	2	1
10.	Pg10	2	4	0	2	8	0
11.	Pg11	13	4	3	3	3	4
12.	Pg12	4	3	0	2	9	0
Mean		7.9	3.1	3.1	2.5	8	1.5
SE(m)±		0.4	0.27	0.27	0.23	1.5	0.25
CD at 5%		1.4	0.79	0.79	0.71	4.4	0.74

Table.4 Size of conidia and Spore germination (%) of twelve *P. grisea* isolates grown on OMA medium 14 days after incubation

S.No.	Isolate	Length (µm)*		Width (µm)*		Spore germination (%)
		Range	Mean	Range	Mean	
1.	Pg1	20.4 – 30.8	25.7	6.4 - 8.5	8.2	91.6
2.	Pg2	15.0 - 22.4	19.3	5.0 – 8.1	6.7	81.6
3.	Pg3	17.0 – 24.1	19.2	5.0 – 7.5	6.7	90.0
4.	Pg4	16.0 – 23.1	19.3	4.2 – 7.2	6.8	46.6
5.	Pg5	22.0 – 32.5	28.2	6.7 – 9.6	8.5	63.3
6.	Pg6	15.6 – 23.4	21.3	4.9 – 7.4	6.8	28.3
7.	Pg7	15.0 – 22.0	18.9	4.5 – 6.8	6.1	68.3
8.	Pg8	22.4 – 29.2	25.1	6.7 – 10.1	9.1	71.6
9.	Pg9	21.8 - 30.7	26.5	5.8 – 10.6	8.6	48.3
10.	Pg10	16.4 – 25.9	22.9	6.7 – 11.6	9.3	81.6
11.	Pg11	18.3 – 26.6	23.8	5.6 – 9.9	8.1	81.6
12.	Pg12	17.8 – 26.4	23.4	6.2 – 9.8	8.6	90.0

Time taken for sporulation and Sporulation index

Time taken for sporulation of *P. grisea* isolates on OMA medium was 7.91 days followed by host leaf extract + 2% sucrose agar medium for 8 days and PDA medium for 8.2 days. Irrespective of the media time taken for sporulation of isolates was found high in Pg4 isolate (11.6 days) and lowest 3.3 days in Pg10 isolate. Sporulation index (1 – 4 scale) of twelve *P.grisea* isolates varied from excellent to poor sporulation. Among the three solid media tested, highest mean sporulation recorded on oat meal agar medium (3.1 score) followed by potato dextrose agar medium (2.5 score) and host leaf extract + 2% sucrose agar medium (1.5 score). Irrespective of the media sporulation of isolates was found excellent in Pg7 and P11 isolates and poor in Pg4 isolate. Similarly, Yashaswini *et al.*, (2016) reported sporulation index of *P. grisea* isolates exhibited from excellent (scale 4) to Poor (scale 1) sporulation.

Conidia size

Rice blast pathogen produced septate and branched mycelium and conidia were produced in clusters on long septate, slender conidiophores. Significant differences were observed with conidial size of *P. grisea* isolates on OMA medium. The mean conidial size ranged from 18.9 µm to 28.2 µm in length and 6.1µm to 9.3 µm in width among different isolates. Conidia of isolate Pg5 was longest (28.2 µm) and that of the isolate Pg7 was shortest (18.9 µm). The width of conidia varied from 6.1 µm to 9.3 µm. Maximum width of conidia (9.3 µm) was recorded in isolate Pg10 whereas the isolate Pg7 showed least width (6.1 µm). The shape of conidia in all the isolates was pyriform and hyaline to pale olive, 2 septate and 3 celled. Dutta *et al.* (2019) also reported that the size of conidia

measured about 17.96 - 26.64 µm in length and 7.36 - 9.22 µm width with an average size of 22.42 × 8.59 µm.

Spore germination percentage

Spore germination percentage was high in Pg1 isolate (91.6 %) and least in Pg6 isolate (28.3 %). In the remaining isolates it was ranged from 90.0 % to 46.6 %. Similarly, Rajput *et al.*, (2017) reported that spore germination of *P. grisea* isolates were varied from 25 % to 75%.

In conclusion, the result of the present study revealed that out of twelve isolates of *P. grisea* only three isolates collected from Mancherial (Pg3), Peddapalli (Pg7) and Khammam (Pg9) districts showed correlation with respect to radial mycelial growth, mycelial dry weight and time taken for sporulation whereas correlation was not existed in other nine isolates of *P. grisea*. Results conclude that isolates of *P. grisea* from various locations of Telangana State consists of variable pathogen populations based on cultural and morphological characteristics. However, isolates were culturally and morphologically varied with respect to geographical location.

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