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Assessment of Morphological and Cultural Variability of *Pyricularia grisea* (Cooke) Sacc. Isolates Associated with Finger Millet Blast Disease

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ABSTRACT

Cultural and morphological studies were carried out to understand the biology of *Pyricularia grisea* causing finger millet blast disease. Of the 10 solid and liquid media tested, carrot agar and ragi yeast lactose agar (RYLA) among the solid media supported the best growth and sporulation of the fungus; and in liquid media highest dry mycelial weight was found in ragi yeast lactose broth (490 mg) but no growth was found in Richards's broth. Morphological characters of 68 *P. grisea* isolates from different regions were studied; highest colony diameter of 8.93 cm was recorded in BHullubele (L) isolate and least colony diameter of 6.23 cm was found in BHR374 (L) and majority of the isolates formed whitish grey to greyish black colony. While 53 isolates formed coarse texture remaining formed smooth textured colonies. In most of the isolates regular margins were seen but some exhibited irregular margins. The isolates produced dull to shiny lustre with erratic topography with aerial, raised and fluffy to flat growth with most of them produced concentric rings. Time taken for sporulation in different isolates of *P. grisea* varied from 7-13 days with fast sporulation (7 days) in RHUduru mallige (N), RHUduru mallige (L), MPR202 (L), BUduru mallige (L), BIndaf15 (L), BL5 (L), BIndaf9 (L), BPR202 (L) and BIndaf3 (L) isolates. Delayed sporulation (13 days) was observed in MGPU 45 (L), JBR36 (L), VGPU28 (L), BGPU48 (L), AGPU45 (L), AVL352 (L) and APRM2 (L) isolates. Conidia per microscopic field varied from 5-40; with the size of the conidia ranging from 20.80 × 7.60 to 3.56×1.59 ìm but the largest conidia (20.80 ìm × 7.60 ìm) observed in the isolate BGPU48(L).

Keywords: *Pyricularia grisea*, morphological variability, finger millet, blast

FINGER millet [*Eleusine coracana* (L.) Gaertn.] also known as *ragi* (India), African millet, *Wimbi* (Swahili), *Bulo* (Uganda) and *Telebun* (Sudan) is a cereal that belongs to the grass family, Poaceae. It is an important staple food in parts of eastern Africa, central Africa and India. It is the principal cereal grain in northern Uganda, parts of western Uganda and north eastern Zambia. The grains are malted for making beer. Finger millet can be stored for long periods without insect damage. Finger millet is a staple food for many farming communities in south India due to its nutritional value *viz*., high calcium and iron, excellent malting qualities, can be stored for up to two years without pesticides and acts as a food reserve during the lean season. In India it is grown in all the regions, it ranks next to pearl millet and grown over an area of 1.138 m ha with an average production of 1.82 m t (Anon., 2015a). Karnataka stands first in the production of finger millet with an area of 0.705 m ha and production of 1.188 mt (Anon., 2015b).

Major constraints in finger millet production include blast disease and abiotic stresses such as drought and low soil fertility. Blast is widely distributed and also a most destructive disease in almost all finger millet growing regions of the world. In India, blast was first reported from Tanjore delta of Tamil Nadu by Mc Rae in 1920. The disease is seen on leaf, neck and on panicles, on panicles it occurs in most destructive form as compared to leaf and neck (Takan *et al*., 2012); causes losses as high as 80-90 per cent.

Netam *et al*. (2013) conducted the cultural studies of *P. grisea* on different solid culture media on the mycelial growth and sporulation; highest mycelial growth and sporulation were observed on ragi meal agar medium. The morphological variability studies of the six isolates were carried out on host seed extract sucrose agar, oat meal agar, potato dextrose agar and Richard's agar culture media. All

the isolates of *P. grisea* showed constantly good growth on oat meal agar than other media (Gashaw *et al*., 2014). Anjum (2015) examined 12 different growth media for *P. grisea* and found RYLA was best medium for growth and sporulation of *P. grisea*. Looking into this the present investigation was carried out for assessing the morphological and cultural characteristics of *P. grisea* on 10 solid and liquid media so as to understand its biology.

Material and Methods

Collection of samples

Leaf and neck blast samples were collected from different cultivars of finger millet grown at GKVK, Bangalore; V.C. Farm Mandya; College of Forestry and Hill Agriculture Ranichauri; Birsa Agricultural University, Kanke, Ranchi; Agricultural Research Station, Vizianagarum and Vivekananda Parvitiya Krishi Anusandhana Sanstha, Almora. The collected samples were packed in paper bags and stored at 4 °C for further use.

Isolation of *P. grisea* and maintenance of isolates

P. grisea was isolated from leaf and neck regions of finger millet plants with blast symptoms in the laboratory by adopting standard procedures. Samples were saturated in distilled water for 2h. The steeped samples were incubated in a humid chamber at 28 ^{0}C for 24 h to induce sporulation. The spore mass from individual lesion was streaked on 4 per cent water agar, incubated for 12 h at 25 ^{0}C and single germinating conidium was transferred to ragi yeast lactose agar (RYLA)medium and the pure culture was maintained (Srivastava *et al*., 2009).

Composition of RYLA: 20 g L^{-1} ground ragi powder, 20g L^{-1} agar, 5 g L^{-1} lactose, 1 g L^{-1} yeast extract. Colonies were grown at 28 ^{0}C and stored at 4 ^{0}C (Anjum, 2015).

Effect of different media on the growth of *P. grisea*

Solid media : The pathogen was cultured on 10 different cultural media *viz*., Carrot Agar, RYLA, Yeast extract agar, Potato dextrose agar, Sabouraud's agar, Richard's agar, Oat meal agar, Tochinai's agar, Czapek's Dox agar and host leaf extract sucrose. All these media were sterilized at 121 ^{0}C for 15 minutes, 5 mm mycelial discs were transferred to the centre of each media and each treatment was replicated thrice. The colony growth, morphology, texture, colour and conidial production on different media was examined after 10 days of incubation at 28 ^{0}C. Colony characters were observed for colour of the mycelium, growth of the fungus such as growth patterns, appearance such as rough and smooth.

Cultural variability

On liquid media : The growth characters of *P. grisea* were studied on 10 liquid media *viz.*, carrot broth, ragi yeast lactose broth, yeast extract broth, potato dextrose broth, Sabouraud's broth, Richard's broth, oat meal broth, Tochinai's broth, Czapek's Dox broth and host leaf extract sucrose broth. Different broths were sterilized at 121^{0}C temperature and 1.1 $kgcm^{-2}$ pressure for 15 min. For the study, 20 ml of each medium was poured into 100 ml conical flasks. These flasks were inoculated with 5 mm disc of actively growing culture and incubated at 27±1 ^{0}C with three replications per treatment. Observations were taken 10 days after incubation. The dry mycelial weight was recorded by averaging the mycelial weight of three replications. The data obtained was analyzed statistically.

Morphological variability among the isolates of *P. grisea*

Morphological characteristics of 68 different *P. grisea* isolates were studied for its radial growth (cm), colony texture and colour, type of margin, size and shape of conidia and its production by growing on the RYLA medium. The conidia were measured and micro photographed under high power objective (40X) using Motic Image Analyzer. The spores were observed on slides after staining with lacto phenol or

Table I

Sporulation index of P. grisea

Sporulation	No. of spores/ microscopic field	Index
Excellent	> 30	4
Good	21 - 30	3
Fair	10 - 20	2
Poor	< 10	1
Nil	0	0

cotton blue under light microscope for their number and index numbers from 0-4 were assigned as per the descriptions of Meena (2005).

Results and Discussion

Cultural characteristics *viz.*, colony diameter, colony colour, morphology, texture and sporulation rate of *P. grisea* isolated from infected leaf of Uduru mallige land race on 10 different media is presented in Table II.

Colony diameter varied considerably from 1.37 cm to 8.58 cm (Table II, Fig. 1). Significantly higher mycelial diameter of 8.58 cm was observed in host leaf extract +2 per cent sucrose agar. However significantly lower diameter was on Tochinai's agar (1.37 cm). Among the other characters, colony colour was whitish grey on Czapeck's dox agar, Tochinai's agar, Richards agar, Potato dextrose agar and RYLA, but greyish black on host leaf extract +2 per cent sucrose agar, white on oat meal agar, greyish on yeast extract agar and black on carrot agar (Fig. 1).

Fig.1: Growth of *P. grisea* on solid media

1= Host leaf extract +2 per cent sucrose agar, 2= Oat meal agar 3= Potato dextrose agar, 4= Tochinai's agar, 5= Carrot agar, 6= RYLA, 7= Richard's agar, 8= Yeast extract agar, 9= Sabouraud's agar and 10= Czapek's Dox agar

Table II

Cultural characteristics of P. grisea *on different solid media*

Media	Colony diameter (cm)	Colony colour	Morphology	Texture	Sporulation	No. of conidia per microscopic field
Carrot Agar	8.13	Black	Raised mycelium at the margins with concentric rings	Coarse	+	>15
Host leaf extract +2% sucrose agar	8.58	Greyish black	Flat mycelium	Smooth	+	8
Ragi yeast lactose agar	7.78	Whitish grey	Fluffy and Slightly raised mycelium at the edges	Smooth and coarse	+	>12
Yeast extract agar	7.23	Greyish	Fluffy mycelium with concentric rings	Coarse	-	0
Potato dextrose agar	8.15	Whitish grey	Raised mycelium	Smooth	-	0
Sabouraud's agar	5.50	Greyish	Flat mycelium	Smooth	-	0
Richard's agar	1.78	Whitish grey	Flat mycelium	Coarse	-	0
Oat meal agar concentric rings	7.05	White	Flat mycelium with	Smooth	-	0
Tochinai's agar	1.37	Whitish grey	Flat mycelium	Smooth	-	0
Czapek's Dox agar mycelium	2.08	Whitish grey	Slightly raised	Coarse	-	0
S.Em±	0.03					
CD (P0.01)	0.14					
CV (%)	2.51					

Carrot agar and RYLA were the next best media for culturing different isolates of *P. grisea* as higher growth and fair amount of sporulation were recorded. *P. grisea* produced flat to raised mycelium growth with concentric rings on oat meal, carrot agar and yeast extract agar medium, while the texture / surface appearance was smooth on many media *viz*., carrot agar, yeast extract agar, Richards's agar and Czapek's Dox agar and both coarse and smooth texture was found on RYLA medium and in other media it was coarse. The results are in agreement with Anjum (2015) who reported that RYLA was best for culturing different isolates of *P. grisea*; further Srivastava *et al.* (2009) also found ragi flour agar medium as ideal for the growth and sporulation of *P. grisea*. According to Netam *et al.* (2013) mycelial growth of the fungus was significantly higher on ragi meal agar medium, followed by potato dextrose agar medium, ragi leaf medium.

Significantly highest dry mycelial weight was found in ragi yeast lactose broth (490 mg) but no growth was seen in Richards's broth (Table III, Fig. 2).

TABLE III

Dry mycelial weight on different liquid media

Media	Dry mycelial weight (mg)
Carrot Agar	193.0
Host leaf Extract +2% sucrose broth	190.0
Ragi yeast Lactose broth	490.0
Yeast extract broth	45.0
Potato dextrose broth	90.0
Sabouraud's broth	43.3
Richard's broth	0.0
Oat meal broth	96.7
Tochinai's broth	14.0
Czapeck's Dox broth	16.7
S. Em±	0.2
C.D. (P0.01)	1.0
C.V. (%)	4.9

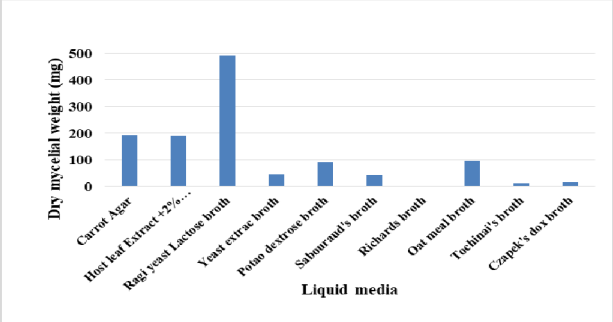

Fig. 2: Graph showing the growth of *P. grisea* on different liquid media

Thus, ragi yeast lactose broth is best for mass multiplication of *P. grisea* as also supported by Anjum (2015), who reported that RYLA as best for multiplication of the fungus.

Morphological characters of different isolates on ragi yeast lactose agar medium

Morphological characters of 68 isolates were recorded as mentioned in materials and methods and presented in Table IV. Highest colony diameter of 8.93 cm was found in BHullubele (L) and lowest colony diameter of 6.23 cm was found in BHR374 (L).

Thirty six isolates formed whitish grey colony and 25 isolates formed greyish black colonies; while three isolates *viz*., RGPU45(N), RVL352(N) and RGPU67(L) isolates formed blackish grey colony, two isolates *viz*., RVR708(N) and JCGR2(L) formed grey coloured colony and two isolates *viz*., RHGPU67(L) and MGPU67(L) formed buff whitish grey colony. Dar *et al.* (2011) reported greyish to pale olive and light grey colony colour, effuse and slow growth, submerged and thin growth with concentric rings on different media; Anjum (2015) also found whitish grey, greyish back/blackish grey and grey colour colonies on RYLA. The texture / surface appearance of 53 isolates was coarse whereas the remaining15 isolates formed smooth texture. Gashaw *et al*. (2014) concluded that the different environmental conditions under which the various isolates are growing, exert a significant influence upon the morphological characters (Texture / colour /conidial size / margins) of *P. grisea* isolates.

Most of the isolates produced regular margins except isolates RGPU67(L), RHGPU67(L), MGPU67(L), APRM2(L), JHR911(L), MGPU28(L),

TABLE IV

Morphological characters of P. grisea *isolates*

Isolates*	Region	Colony diameter (cm)	Colony Colour	Texture/surface appearance	Topography	Margin	Lustre
RVR847(N)	Ranichauri	8.10	Whitish grey	Coarse	Raised And Aerial mycelium, tuft	Regular	Dull
RPR202(N)	Ranichauri	8.35	Whitish grey	Coarse	Fluffy Mycelium with concentric rings	Regular	Dull
RGPU45(N)	Ranichauri	8.33	Blackish grey	Smooth	Aerial mycelium at the edges	Irregular	Dull
RVL352(N)	Ranichauri	8.13	Blackish grey	Coarse	Raised aerial mycelium with concentric rings	Irregular	Dull
RVR708(L)	Ranichauri	7.56	Whitish grey	Coarse	Raised aerial mycelium with concentric rings	Irregular	Dull
RGPU67(L)	Ranichauri	8.32	Blackish grey	Coarse	Raised mycelium with regular edges and concentric rings	Regular	Dull
RGPU28(L)	Ranichauri	7.14	Whitish grey	Smooth	Raised aerial mycelium with concentric rings	Regular	Dull
RVL352(L)	Ranichauri	8.06	Whitish grey	Coarse	Raised aerial mycelium with concentric rings	Regular	Dull
RGPU45(L)	Ranichauri	8.45	Whitish grey	Coarse	Raised mycelium at the edges	Regular	Dull
RVR708(N)	Ranichauri	7.82	Grey	Smooth	Flat mycelium with concentric ring	Regular	Dull
RVR936(L)	Ranichauri	8.21	Whitish grey	Smooth	Flat mycelium without concentric ring	Regular	Shiny
RVR936(N)	Ranichauri	8.19	Whitish grey	Coarse	Raised aerial mycelium with concentric rings	Regular	Dull
RHGPU67(L)	Ranchi	7.70	Buff whitish grey	Coarse	Raised aerial mycelium , Tuft	Regular	Dull
RHUduru mallige (N)	Ranchi	7.68	Whitish grey	Coarse	Fluffy Mycelium and mycelium raised at the edges	Regular	Shiny
RHGPU28(L)	Ranchi	8.20	Whitish grey	Coarse	Fluffy Mycelium raised uniformly	Regular	Shiny
RHUduru mallige (L)	Ranchi	8.13	Whitish grey	Coarse	Raised mycelium at the edges	Irregular	Dull
RHA404(L)	Ranchi	8.15	Whitish grey	Coarse	Fluffy Mycelium and mycelium raised at the edges with larger concentric rings	Regular	Shiny
RHVR936(L)	Ranchi	7.50	Whitish grey	Coarse	Raised mycelium at the edges Grey colour at the centre	Regular	Dull
RHBM10 (L)	Ranchi	7.98	Greyish black	Coarse	Raised whitish mycelium at the periphery	Regular	Dull
RHBM1 (L)	Ranchi	7.57	Greyish black	Smooth	Flat mycelium with concentric ring	Irregular	Shiny
MGPU67(L)	Mandya	7.43	Buff whitish grey	Coarse	Raised mycelium with concentric rings	Regular	Dull
MGPU 45(L)	Mandya	7.86	Whitish grey	Coarse	Raised aerial mycelium at the edges, tuft	Regular	Dull
MPR202(L)	Mandya	7.98	Greyish black	Coarse	Raised aerial mycelium at the edges with concentric rings	Regular	Dull
MGPU28(L)	Mandya	8.05	Greyish black	Smooth	Raised mycelium with concentric rings	Regular	Dull
JGPU67(L)	Jagdalpur	7.65	Whitish grey	Coarse	Raised mycelium at the edges	Regular	Dull

Table IV contd.

Isolates*	Region	Colony diameter (cm)	Colony Colour	Texture/surface appearance	Topography	Margin	Lustre
JVR936(L)	Jagdalpur	8.03	Greyish black	Coarse	Raised mycelium thread like appearance	Irregular	Dull
JHR911(L)	Jagdalpur	8.15	Greyish black	Coarse	Raised mycelium at the edges	Regular	Dull
JBR36(L)	Jagdalpur	7.47	Greyish black	Coarse	Raised mycelium with concentric rings	Regular	Dull
JCGR2(L)	Jagdalpur	7.26	Grey	Smooth	Flat mycelium sectored with concentric rings	Regular	Dull
VGPU28(L)	Vizianagaram	8.15	Whitish grey	Coarse	Raised aerial mycelium at the edges	Irregular	Dull
VVR936(L)	Vizianagaram	6.50	Whitish grey	Coarse	Fluffy raised mycelium evenly distributed	Regular	Shiny
VVR762(L)	Vizianagaram	7.75	Greyish black	Coarse	Raised mycelium at the edges	Regular	Dull
VVR708(L)	Vizianagaram	8.12	Greyish black	Coarse	Slightly Raised mycelium with concentric rings	Regular	Dull
VVR900(L)	Vizianagaram	8.15	Greyish black	Coarse	Raised mycelium at the edges	Regular	Dull
BUduru Mallige(L)	Bengaluru	8.30	Greyish black	Smooth	Flat mycelium grey colour at the centre	Regular	Dull
BGPU67 (L)	Bengaluru	8.42	Greyish black	Smooth	Flat mycelium raised at the edges	Regular	Dull
BGPU28(L)	Bengaluru	7.52	Greyish black	Coarse	Slightly Raised mycelium with concentric rings	Regular	Dull
BINDAF7(L)	Bengaluru	7.82	Greyish black	Coarse	Raised mycelium at the edges with concentric rings	Regular	Dull
BKarikaddi ragi (L)	Bengaluru	7.75	Whitish grey	Coarse	Raised mycelium at irregular intervals	Regular	Dull
BMR2(L)	Bengaluru	7.93	Greyish black	Coarse	Raised mycelium at the edges	Irregular	Dull
BGIDD RAGI(L)	Bengaluru	7.62	Whitish grey	Coarse	Raised mycelium all over except centre	Regular	Dull
BINDAF 15(L)	Bengaluru	7.63	Whitish grey	Coarse	Slightly Raised mycelium with concentric rings	Regular	Dull
BL5(L)	Bengaluru	7.72	Whitish grey	Coarse	Raised whitish mycelium at the periphery grey at the centre	Regular	Dull
BHR374(L)	Bengaluru	6.23	Whitish grey	Coarse	Raised mycelium	Regular	Dull
BMR6(L)	Bengaluru	7.52	Whitish grey	Coarse	Raised whitish mycelium	Regular	Dull
BHAMSA(L)	Bengaluru	6.35	Whitish grey	Coarse	Raised whitish mycelium	Regular	Dull
BINDAF9(L)	Bengaluru	7.88	Greyish black	Coarse	Slightly Raised mycelium with concentric rings	Regular	Dull
BKMR204(L)	Bengaluru	8.05	Whitish grey	Coarse	Fluffy whitish raised mycelium at the edges	Regular	Dull
BMR1(L)	Bengaluru	7.87	Whitish grey	Coarse	Slightly raised fluffy mycelium	Regular	Dull
BPR202(L)	Bengaluru	7.45	Whitish grey	Coarse	Slightly raised fluffy mycelium	Regular	Dull

Table IV contd.

Isolates*	Region	Colony diameter (cm)	Colony Colour	Texture/surface appearance	Topography	Margin	Lustre
BPURNA(L)	Bengaluru	7.45	Whitish grey	Coarse	Raised mycelium with concentric rings	Regular	Dull
BGPU26(L)	Bengaluru	8.02	Whitish grey	Coarse	Fluffy whitish raised mycelium at the edges	Regular	Dull
BHR374(L)	Bengaluru	8.33	Whitish grey	Coarse	Raised mycelium at the edges	Regular	Dull
BINDAF3(L)	Bengaluru	8.12	Whitish grey	Coarse	Raised mycelium with concentric rings	Regular	Dull
BHBP76(L)	Bengaluru	8.53	Whitish grey	Coarse	Raised mycelium	Regular	Shiny
BHR911(L)	Bengaluru	8.12	Whitish grey	Coarse	Raised mycelium at the edges	Regular	Dull
BINDAF5(L)	Bengaluru	8.15	Greyish black	Coarse	Flat mycelium with concentric rings	Regular	Dull
BGPU48(L)	Bengaluru	8.42	Whitish grey	Coarse	Flat mycelium with concentric rings	Regular	Dull
BBilikaddi Ragi(L)	Bengaluru	8.52	Greyish black	Smooth	Flat mycelium with concentric rings	Irregular	Dull
BGPU66(L)	Bengaluru	8.55	Greyish black	Coarse	Flat mycelium with concentric rings	Regular	Dull
BINDAF8(L)	Bengaluru	8.87	Greyish black	Coarse	Slightly Raised mycelium with concentric rings	Regular	Dull
BHullubele(L)	Bengaluru	8.93	Whitish grey	Coarse	Raised fluffy mycelium	Irregular	Dull
AGPU45(L)	Almora	8.60	Greyish black	Smooth	Flat mycelium	Regular	Shiny
AVl149(L)	Almora	8.62	Whitish grey	Coarse	Fluffy raised mycelium	Irregular	Dull
AGPU67(L)	Almora	8.23	Greyish black	Smooth	Flat mycelium with concentric rings	Regular	Shiny
APR202(L)	Almora	8.08	Greyish black	Smooth	Flat mycelium with irregular black colour	Irregular	Shiny
AVL352(L)	Almora	8.08	Greyish black	Smooth	Flat mycelium with irregular black colour	Irregular	Shiny
APRM2(L)	Almora	7.63	Greyish black	Smooth	Flat mycelium with concentric rings	Irregular	Shiny
	S.Em±	0.180					
	CD (P0.01)	0.633					
	CV(%)	3.819					

BGPU28(L), VVR936(L), BHR374(L), BPURNA(L), BHAMSA(L), BMR1(L), BPR202(L) and BINDAF15(L) which produced irregular margin. Similarly, the isolates produced dull to shiny lustre with erratic topography with aerial, raised, and fluffy to flat growth with most of them with concentric rings (Table IV). This type of variations in morphological characters is due to environmental conditions in which the isolates were grown(Gashaw *et al*. 2014).

Variations in sporulation by *P. grisea* were also noticed from different isolates (Table V). Isolates of different areas took 7-13 days for sporulation with earliest sporulation (7 days) observed in RHUduru mallige (N) RHUduru mallige (L) MPR202(L) BUduru Mallige(L), BINDAF15(L), BL5(L), BINDAF9(L), BPR202(L) and BINDAF3(L) isolates, while late sporulation (13 days) was observed in MGPU 45(L), JBR36(L), VGPU28(L), BGPU48(L),

TABLE V

Conidial characters of P. grisea *isolates*

Isolate	Region	No. of days to Sporulation	No. of conidia per microscopic field (40X)	Index	Conidial size (LxBμm)
RVR847(N)	Ranichauri	12	18-20	2	8.88x3.34
RPR202(N)	Ranichauri	8	30-35	4	7.40x3.92
RGPU45(N)	Ranichauri	13	10-15	2	9.00x3.38
RVL352(N)	Ranichauri	8	25-30	3	8.90x3.23
RVR708(L)	Ranichauri	8	30-35	4	9.30x3.51
RGPU67(L)	Ranichauri	8	30-35	4	9.32x3.50
RGPU28(L)	Ranichauri	13	18-20	2	12.32x6.18
RVL352(L)	Ranichauri	12	30-40	4	3.56x1.59
RGPU45(L)	Ranichauri	10	5-10	1	12.33x6.12
RVR708(N)	Ranichauri	8	25-30	3	9.21x4.88
RVR936(L)	Ranichauri	10	25-30	3	9.20x4.87
RVR936(N)	Ranichauri	10	20-25	3	10.31x3.00
RHGPU67(L)	Ranchi	10	25-30	3	9.24x3.15
RHUduru mallige (N)	Ranchi	7	35-40	4	4.20x2.11
RHGPU28(L)	Ranchi	12	15-20	2	15.65x6.18
RHUduru mallige (L)	Ranchi	7	30-40	4	9.85x3.90
RHA404(L)	Ranchi	10	22-26	3	11.04x3.05
RHVR936(L)	Ranchi	8	25-30	3	9.35x3.72
RHBM10 (L)	Ranchi	10	20-30	3	9.90x2.76
RHBM1 (L)	Ranchi	10	20-30	3	10.01x3.61
MGPU67(L)	Mandya	8	25-30	3	12.32x6.14
MGPU 45(L)	Mandya	13	10-15	2	15.68x6.15
MPR202(L)	Mandya	7	30-40	4	4.22x2.21
MGPU28(L)	Mandya	12	15-20	2	12.32x6.16
JGPU67(L)	Jagdalpur	11	25-30	3	8.15x3.66
JVR936(L)	Jagdalpur	11	20-30	3	9.32x3.52
JHR911(L)	Jagdalpur	12	20-25	3	6.03x3.60
JBR36(L)	Jagdalpur	13	18-20	2	10.02x3.56
JCGR2(L)	Jagdalpur	10	15-20	2	12.34x6.15

Isolate	Region	No. of days to Sporulation	No. of conidia per microscopic field (40X)	Index	Conidial size (LxBμm)
VGPU28(L)	Vizianagaram	13	5-10	1	15.72x6.20
VVR936(L)	Vizianagaram	9	15-20	2	12.30x6.17
VVR762(L)	Vizianagaram	9	20-30	3	10.52x3.60
VVR708(L)	Vizianagaram	10	30-40	4	8.51x3.40
VVR900(L)	Vizianagaram	10	20-30	3	7.26x3.94
BUduru Mallige(L)	Bengaluru	7	30-35	4	8.72x3.85
BGPU67 (L)	Bengaluru	8	25-30	3	11.00x3.48
BGPU28(L)	Bengaluru	12	15-20	2	12.30x6.10
BINDAF7(L)	Bengaluru	8	30-35	4	8.34x3.09
BKarikaddi ragi (L)	Bengaluru	8	25-30	3	11.02x3.50
BMR2(L)	Bengaluru	10	25-30	3	10.65x3.60
BGIDDRAGI(L)	Bengaluru	8	25-30	3	7.24x3.85
BINDAF15(L)	Bengaluru	7	30-40	4	4.02x1.56
BL5(L)	Bengaluru	7	30-40	4	4.50x1.58
BHR374(L)	Bengaluru	10	18-20	2	18.50x3.40
BMR6(L)	Bengaluru	9	18-20	2	17.48x4.48
BHAMSA(L)	Bengaluru	9	25-30	3	9.18x4.88
BINDAF9(L)	Bengaluru	7	30-35	4	7.49x3.90
BKMR204(L)	Bengaluru	10	25-35	3	11.03x3.51
BMR1(L)	Bengaluru	10	18-20	2	20.14x7.65
BPR202(L)	Bengaluru	7	35-40	4	8.32x3.05
BPURNA(L)	Bengaluru	8	25-30	3	12.18x6.01
BGPU26(L)	Bengaluru	8	18-20	2	15.10x2.48
BHR374(L)	Bengaluru	8	18-20	2	15.12x2.51
BINDAF3(L)	Bengaluru	7	25-30	3	4.02x1.61
BHBP76(L)	Bengaluru	10	25-30	3	7.20x3.80
BHR911(L)	Bengaluru	12	25-30	3	7.19x3.80
BINDAF5(L)	Bengaluru	8	35-40	4	4.00x1.62
BGPU48(L)	Bengaluru	13	5-10	1	20.80x7.60
BBilikaddi Ragi(L)	Bengaluru	12	15-20	2	17.45x4.44
BGPU66(L)	Bengaluru	8	15-20	2	17.50x4.51
BINDAF8(L)	Bengaluru	9	25-30	3	7.22x3.90
BHullubele (L)	Bengaluru	9	30-35	4	7.49x3.88
AGPU45(L)	Almora	13	15-20	2	14.28x2.78
AVl149(L)	Almora	12	25-30	3	12.22x6.04
AGPU67(L)	Almora	12	25-30	3	12.25x6.06
APR202(L)	Almora	8	30-40	4	4.05x1.55
AVL352(L)	Almora	13	18-20	2	15.14x2.52
APRM2(L)	Almora	13	15-20	2	20.78x7.60

AGPU45(L), AVL352(L) and APRM2(L) which may be attributed to the adaptability to location or the genotype or to both.

Conidia per microscopic field also varied from 5-40 numbers. High index of 4 was observed in 17 isolates with more than 30 conidia per microscopic field indicating excellent sporulation (Table V). Good sporulation was witnessed in 28 isolates and fair sporulation in 20 isolates, while poor sporulation with lowest index of 1 was seen in three isolates *viz*., RGPU45(L), VGPU28(L) and BGPU48(L) with only 5-10 conidia per microscopic field. Correlation between sporulating capacity and aerial growth was reported by Sonah *et al*. (2009) and Srivastava *et al.* (2014). There were no variations with respect to conidial shape; regardless of the number of days taken for sporulation, the conidia of all the isolates were pyriform; almost hyaline to pale olive, 2-septate, 3-celled; either large, medium to small in size with rounded base or the pedicel narrowed towards the pointed tip and are in similarity with the findings of Anjum (2015).

Size of the conidia (L×B) varied from 20.80µm × 7.60µm to 3.56µm×1.59 µm (Table V) with the largest conidium (20.80µm × 7.60 µm) observed in the isolate BGPU48(L) followed by APRM2(L) (20.78 µm × 7.60 µm), BMR1(L) (20.14 µm × 7.65 µm) and smallest conidium of 3.56µm×1.59 µm in RVL352(L) isolate.

Presence of variability among the isolates of *P. grisea* with respect to conidial size is well known (Mc Kenzie *et al.*, 2010; Gashaw *et al*., 2014 and Anjum, 2015). According to Gashaw *et al*. (2014) the different environmental conditions under which the various isolates are growing, exert a significant influence upon the size of conidia of *P. grisea* isolates. The results are supported by the descriptions of Kiran Babu (2011) who recorded spores of size 15-22 µm × 4-7 µm and Anjum (2015) also observed conidia (L × B) from 23.20 × 6.40 to 3.80 × 1.50 µm.

The present investigation explains the best media for growth and sporulation of *P. grisea* useful for mass multiplication of the pathogen for artificial screening studies; further the differences in morphological and cultural characters of the isolates collected from different regions has helped in better understanding of the biology of *P. grisea.*

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