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Survey of *Magnaporthe grisea* Isolates around Andhra Pradesh and Telangana States, India

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ABSTRACT

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Rice blast caused by *Magnaporthe grisea* is one of the most destructive diseases of rice causing significant grain yield losses. Keeping in view the importance of disease, studies were conducted on cultural and morphological characters at Indian Institute of Rice Research (IIRR), Hyderabad, Telangana. Survey was carried out to identify and characterize the fungal pathogen associated with rice blast disease in different crop growing regions of Andhra Pradesh and Telangana states. A total of 40 blast disease specimens were collected from different locations of Andhra Pradesh and Telangana and disease severity was recorded. Later the associated pathogen was isolated and identified. In pathogenic studies, considerable variation was found among the isolates. Isolates which showed excellent sporulation index-4 (RBNL 12, RBMU 37, RBPB 38, RBDR 1, RBKM 8 and RBMT 11) has also recorded high PDI (78.13-80) under artificial inoculation on HR-12. The sporulation index-1 of the isolates RBRG 20, RBTP 13 and RBND 24 was poor but they showed high PDI (70.01-80.23) on HR-12 under artificial inoculation.

Introduction

Rice [*Oryza sativa*] is a major staple food and a mainstay for the rural population and their food security. It is widely cultivated in India, China, Indonesia, Bangladesh, Vietnam, Thailand, Myanmar, Japan, Philippines and Brazil. China is the leading rice producer followed by India, Indonesia and Bangladesh in 2013–14 (Commodity profile for rice-January 2015). India was the largest exporter of rice in 2013–14 followed by Thailand, Vietnam and USA. The regions cultivating this crop in India are distinguished as the Western coastal strip, the Eastern coastal

strip, covering all the primary deltas, Assam plains and surrounding low hills, foothills and Terai region along the Himalayas and states like West Bengal, Bihar, Eastern Uttar Pradesh, Eastern Madhya Pradesh, Northern Andhra Pradesh and Orissa. India, being a land of eternal growing season, and the deltas of Kaveri river, Krishna river, Godavari river and Mahanadi river with a thick set-up of canal irrigation, permits farmers to raise two, and in some pockets, even three crops a year (Department of Agriculture, 2015).

Rice blast caused by *Magnaporthe grisea* (Hebert, 1971) Barr (Anamorph: *Pyricularia grisea* (Cooke) Sacc.) a filamentous ascomycetes fungus infecting more than 50 hosts. Rice blast was first recorded in China (1637) later from Japan (1704). In India, the disease gained importance when a devastating epidemic occurred in Thanjavur (Tanjore) delta of Tamilnadu during 1919. In Andhra Pradesh, it was first identified in Chittoor district subsequently at Nizamabad in Telangana (Nagarajan, 1988). The disease is recorded from almost all the rice growing regions of India. However, the intensity of the disease varies in different regions in different years. All aboveground parts viz., the leaf blade, collar region, neck of the panicle and nodes on the culm are attacked by the fungus. The pathogen is adaptable to adverse environmental conditions of widely fluctuating temperatures and relative humidity. It appears in irrigated low land or rain fed upland rice as well as in submerged or deep water rice. Rice was important crop in both Andhra Pradesh and Telangana states and losses due disease was also more. Keeping this in view we are conducting survey in both the states to identify the severity of disease.

Materials and Methods

This experiment was conducted during 2013-2015.

Isolation of mono-conidial isolates of *Magnaporthe grisea*

The fungus was isolated by tissue segmentation method (Bonman *et al.*, 1987). Blast infected leaf tissues stored in refrigerator were cut into small bits. These bits were washed in sterilized distilled water twice, surface sterilized in 0.1% mercuric chloride for 30 seconds, rinsed three times in sterilized water and allowed for sporulation

on sterilized glass slides by incubating in a moist chamber at 25°C for 48 h. 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 and transferred to fresh sterilized Petri plates containing OMA medium. The Petri plates were incubated at 28°C for 7 days and the fungus was identified following mycological description

Cultural and morphological variability among *M. grisea* isolates

Cultural and morphological characters of all monoconidial isolates of *M. grisea* were recorded by growing them on OMA medium for 15 days at 28°C. Cultural characters include color and radial growth (mm) of the fungal mycelium. Morphological characteristics viz., size of conidia, septa formation and sporulation. Spores of *M. grisea* of different isolates were collected from the culture plate mounted in lactophenol on a clean slide. Spores were measured under high power objective (40x) using precalibrated ocular 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.

Sporulation

Sporulation capacity of each isolate was assessed by microscopic observations. For this purpose, spore suspension from each isolate was prepared by harvesting spores into 20 ml of sterile distilled water from a 15-day-old culture plate using camel hair brush. A loopful of spore suspension was then placed on a clean slide and a cover slip was placed on it. The rate of sporulation was recorded in five different microscopic fields.

Sporulation	Number of spores/microscopic field	Index
Excellent	>30	4
Good	20–30	3
Fair	10–20	2
Poor	<10	1

Results and Discussion

Collection, isolation and purification of *M. grisea* isolates

A roving survey was carried out in nine districts of Telangana and ten districts of Andhra Pradesh to assess the incidence of rice blast and to collect the blast infected leaf samples for isolation of *M. grisea* isolates during *rabi* 2013 and *kharif* 2014. The percentage disease index was more during *rabi* 2013 (56.43) over *kharif* 2014 (50.87). Based on microscopic examination the pathogen was identified as *Magnaporthe grisea* (Hebert, 1971) Barr. (Anamorph=*Pyricularia grisea* Sacc.).

A total of 40 blast disease samples from rice were collected from different locations of Andhra Pradesh and Telangana regions during the *rabi* 2013 (21 samples) and *kharif* 2014 (19 samples). The collection sites include hot spots for blast disease in both the regions. These isolates were collected from different locally cultivated rice varieties. The results indicated that disease incidence in different agro climatic regions ranged from 36.66% on MTU 1010 variety (Palem) Mahabubnagar district to 78.88% on HR-12 (IIRR) Rangareddy district. The maximum disease incidence was noticed in IIRR (78.88%) followed by Medak (71.11%), Tirupati and Chirala (67.55%). The PDI of blast among different cultivars and locations was significant. The differences in PDI observed among RBGV 35, RBMU 37, RBPK 40, RBAW 33, RBAV 27, RBNB 16, RBVN 2 and RBNG 7 isolates were non-significant (Table 2).

The results indicate that, the mean blast PDI recorded in Krishna Zone was 55.33, in Godavari Zone 53.13, in North Coastal Zone 46.17, in Southern Zone 55.97, in Scarce Rain fall Zone 61.48, in Northern Telangana Zone 54.80, in Central Telangana Zone 52.39 and in Southern Telangana Zone 51.81 (Table 2).

Among the cultivars highest PDI of 78.88 was recorded on HR-12 variety in RBDR 1 and lowest PDI of 36.66 was recorded on MTU-1010 in RBPM 9. These results indicate variation in PDI which was influenced by the geographical area under cultivation. In BPT-5204, the mean PDI of 41.56 was lowest in North Coastal Zone and highest PDI of 60.03 was in Southern Telangana Zone indicating variation in percent blast disease index was influenced by geographical area under cultivation and the race of *M. grisea* prevailing in these areas.

In MTU-1010, the PDI in Southern Telangana Zone was 36.66, in Central Telangana Zone it was 50. This shows a variation in PDI influenced by geographical area under cultivation. On MTU-1001, the mean PDI in Southern Telangana Zone was 45.1 while in Central Telangana Zone 55.51, in Northern Telangana Zone 57.55, in Godavari Zone 53.13, in Krishna Zone 56.49 and in Scarce Rainfall Zone 61.48.

The variation in PDI may be influenced by geographical area under cultivation or the race prevailing in the region or interaction of the variety and the weather condition in these areas.

In WGL-44645, the mean PDI was 53.89 in Northern Telangana Zone whereas PDI in Central Telangana Zone was 45.44. The isolates (RBMT 11, RBNL 12, RBMU 37, RBPB 38, RBKM 8 and RBDR 1) which produced excellent sporulation (Index-4) recorded variation in PDI on different rice

varieties. In different agro climatic zones the PDI varied from 40 (RBPB 38) to 78.88 (RBDR 1) under artificially inoculated conditions and on susceptible HR-12 variety these isolates showed high disease incidence.

Cultural diversity among the *M. grisea* isolates

Diversity in cultural characteristics of *M. grisea* isolates was studied on oat meal agar medium. Variation was observed in colony characteristics viz., growth, color of the vegetative growth and surface appearance among the isolates of *M. grisea* and results are presented in table 2.

Colony growth of *M. grisea* isolates on oatmeal agar medium revealed significant differences among the isolates from different locations. The colony diameter ranged between 70 mm (RBDR1) to 90 mm (RBWG 19 and RBMD 34). Significant difference was observed in the radial growths of RBDR 1, RBBP2, RBPI 3 and RBMN 10 isolates while the differences among the other isolates were non-significant (Table 2).

The variation among the radial growth may be due to several reasons like autolysis of the mycelium and exhaustion of nutrients in the medium. Lilly and Barnett (1951) opined that the growth in fungi follows a definite pattern and they observed the onset of autolysis after the maximum growth during which cellular enzymes digest the various cell constituents.

Diversity in cultural characters such as color of vegetative growth and texture, were noticed among the isolates, but there was no clear-cut grouping between isolates from different cultivars. The results of the present study grouped 40 isolates into seven categories. First group had greyish white mycelium and smooth surface appearance. This group includes RBGT 4 and RBTN 25. Second group had 28 isolates with greyish

white color mycelium and rough surface appearance. Third group had two isolates RBDR 1 and RBNB 16 which showed grey color mycelium and smooth surface appearance. Fourth group had grey color mycelium with rough surface appearance (RBSP 18). Fifth group had greyish green color mycelium and smooth surface (RBPM 9). Sixth group had greyish brown color mycelium and rough surface (RBWG 19). Seventh group had greyish black colony with rough texture (RBTP 13, RBAV 27, RBPB 36, RBMU 37 and RBPB 38) (Table 2).

Isolates collected from cultivar BPT 5204 in Krishna Zone showed grayish white colour appearance and rough surface on oat meal agar medium and showed good sporulation (Index-3). RBRG 20 isolate collected from BPT 5204 in North Coastal Zone showed greyish white color mycelium and rough surface with poor (Index-1) sporulation while RBSP 18 isolate collected from BPT 5204 in North Coastal Zone showed grey color mycelium and rough surface with good (Index-3) sporulation. RBAC 30 isolate collected from BPT 5204 in South Telangana Zone also showed greyish white color mycelium and rough surface with good (Index-3) sporulation. RBPM 9 isolate collected from MTU-1010 in South Telangana Zone showed greyish green color mycelium and smooth surface with fair (Index-2) sporulation. RBMD 34 isolate collected from MTU-1010 in Central Telangana Zone showed greyish white color mycelium and rough surface with good (Index-3) sporulation. Isolates (RBMU 37, RBPB 38 and RBTP 13) collected from NLR-145 in Southern Zone showed greyish black color mycelium and rough surface with excellent (Index-4) sporulation except RBTP 13, recorded poor (Index-1) sporulation. RBNL 12 isolate collected from NLR-145 in Southern Zone showed greyish white color mycelium and rough surface with excellent (Index-4) sporulation.

Table.1 List of *P. oryzae* isolates collected from Andhra Pradesh and Telangana, their designation codes

S. No	State	District	Isolate	Designation
1	Telangana	Rangareddy	IIRR	RBDR 1
2	Andhra Pradesh	Guntur	Bapatla	RBBP 2
3	Andhra Pradesh	Guntur	Piduguralla	RBPI 3
4	Andhra Pradesh	Guntur	Guntur	RBGT 4
5	Andhra Pradesh	Guntur	Rentachintala	RBRC 5
6	Andhra Pradesh	Krishna	Vijayawada	RBVI 6
7	Telangana	Nalgonda	Nalgonda	RBNG 7
8	Telangana	Khammam	Khammam	RBKM 8
9	Telangana	Mahabhubnagar	Palem	RBPM 9
10	Andhra Pradesh	Kurnool	Mahanandi	RBMN 10
11	Andhra Pradesh	West Godavari	Marteru	RBMT 11
12	Andhra Pradesh	Nellore	Nellore	RBNL 12
13	Andhra Pradesh	Chittoor	Tirupathi	RBTP 13
14	Telangana	Medak	Patancheru	RBMK 14
15	Telangana	Karimnagar	Jagityal	RBJG 15
16	Telangana	Nijamabad	Nizamabad	RBNB 16
17	Telangana	Adilabad	Adilabad	RBAB 17
18	Andhra Pradesh	Srikakulam	S.M Puram	RBSP 18
19	Telangana	Warangal	Warangal	RBWG 19
20	Andhra Pradesh	Srikakulam	Ragolu	RBRG 20
21	Andhra Pradesh	Vijayanagaram	Vizianagaram	RBVN 21
22	Andhra Pradesh	West Godavari	Veeranasaram	RBVR 22
23	Telangana	Adilabad	Basara	RBBS 23
24	Andhra Pradesh	Kurnool	Nadyal	RBND 24
25	Andhra Pradesh	East Godavari	Tuni	RBTN 25
26	Andhra Pradesh	Prakasam	Chirala	RBCH 26
27	Andhra Pradesh	Guntur	Adivi	RBAV 27
28	Andhra Pradesh	Guntur	Amaravati	RBAM 28
29	Andhra Pradesh	Guntur	Karempudi	RBKP 29
30	Telangana	Mahabhubnagar	Atchempet	RBAC 30
31	Andhra Pradesh	Guntur	Mangalagiri	RBMG 31
32	Telangana	Nalgonda	Aleru	RBAL 32
33	Telangana	Khammam	Aswaraopet	RBAW 33
34	Telangana	Khammam	Madhira	RBMD 34
35	Andhra Pradesh	Krishna	Gopavaram	RBGV 35
36	Andhra Pradesh	West Godavari	Poduru	RBPD 36
37	Andhra Pradesh	Nellore	Muttukuru	RBMU 37
38	Andhra Pradesh	Nellore	Penubarti	RBPB 38
39	Telangana	Ranga Reddy	Tandur	RBTD 39
40	Andhra Pradesh	West Godavari	Palakollu	RBPK 40

Table.2 List of *P. oryzae* isolates collected from Andhra Pradesh and Telangana, their designation codes

Cultivar	Isolate	Agro climatic Zone*	Colour	Texture	Sporulation	PDI
BPT-5204	RBBP2	K.Z	Greyish white	Rough	3	50.00
	RBPI 3	K.Z	Greyish white	Rough	3	47.65
	RBGT 4	K.Z	Greyish white	Smooth	2	59.74
	RBRC 5	K.Z	Greyish white	Rough	3	53.28
	RBVI 6	K.Z	Greyish white	Rough	3	58.55
	RBCH 26	K.Z	Greyish white	Rough	3	67.55
	RBAV 27	K.Z	Greyish black	Rough	3	55.40
	RBAM 28	K.Z	Greyish white	Rough	3	53.52
	RBKP 29	K.Z	Greyish white	Rough	3	61.07
	RBMG 31	K.Z	Greyish white	Rough	3	45.41
	RBSP 18	N.C.Z	Grey	Rough	3	43.25
	RBRG 20	N.C.Z	Greyish white	Rough	1	39.87
	RBAC 30	S.T.Z	Greyish white	Rough	3	60.03
	Mean					
MTU-1010	RBPM 9	S.T.Z	Greyish green	Smooth	2	36.66
	RBMD 34	C.T.Z	Greyish white	Rough	3	50.00
	Mean					43.33
NLR-145	RBNL 12	S.Z	Greyish white	Rough	4	59.87
	RBTP 13	S.Z	Greyish black	Rough	1	67.55
	RBMU 37	S.Z	Greyish black	Rough	4	56.49
	RBPB 38	S.Z	Greyish black	Rough	4	40.00
	Mean					55.97
HR-12	RBDR 1	S.T.Z	Grey	Smooth	4	78.88
RGL-2624	RBVN 21	N.C.Z	Greyish white	Rough	2	55.41
MTU-1001	RBNG 7	S.T.Z	Greyish white	Rough	3	55.52
	RBTD 39	S.T.Z	Greyish white	Rough	3	37.60
	RBAL 32	S.T.Z	Greyish white	Rough	3	42.18
	RBMK 14	C.T.Z	Greyish white	Rough	3	71.11
	RBAW 33	C.T.Z	Greyish white	Rough	2	55.41
	RBKM 8	C.T.Z	Greyish white	Rough	4	40.01
	RBAB 17	N.T.Z	Greyish white	Rough	2	57.55
	RBVR 22	G.Z	Greyish white	Rough	3	47.55
	RBMT 11	G.Z	Greyish white	Rough	4	56.49
	RBTN 25	G.Z	Greyish white	Smooth	2	46.49
	RBPD 36	G.Z	Greyish black	Rough	3	58.65
	RBPK 40	G.Z	Greyish white	Rough	3	56.49
	RBGV 35	K.Z	Greyish white	Rough	3	56.49
	RBND 24	S.R.Z	Greyish white	Rough	1	59.70
	RBMN 10	S.R.Z	Greyish white	Rough	2	63.26
	Mean					49.86
WGL-44645	RBJS 15	N.T.Z	Greyish white	Rough	2	48.64
	RBNB 16	N.T.Z	Grey	Smooth	2	56.52
	RBBS 23	N.T.Z	Greyish white	Rough	3	56.52
	RBWG 19	C.T.Z	Greyish brown	Rough	3	45.44
	Mean					51.78

*K.Z (Krishna Zone), G.Z (Godavari Zone), N.C.Z (North Coastal Zone), S.Z (Sothern Zone), S.R.Z (Scarce Rainfall Zone), N.T.Z (Northern Telangana Zone), C.T.Z (Central Telangana Zone), S.T.Z (Southern Telangana Zone)

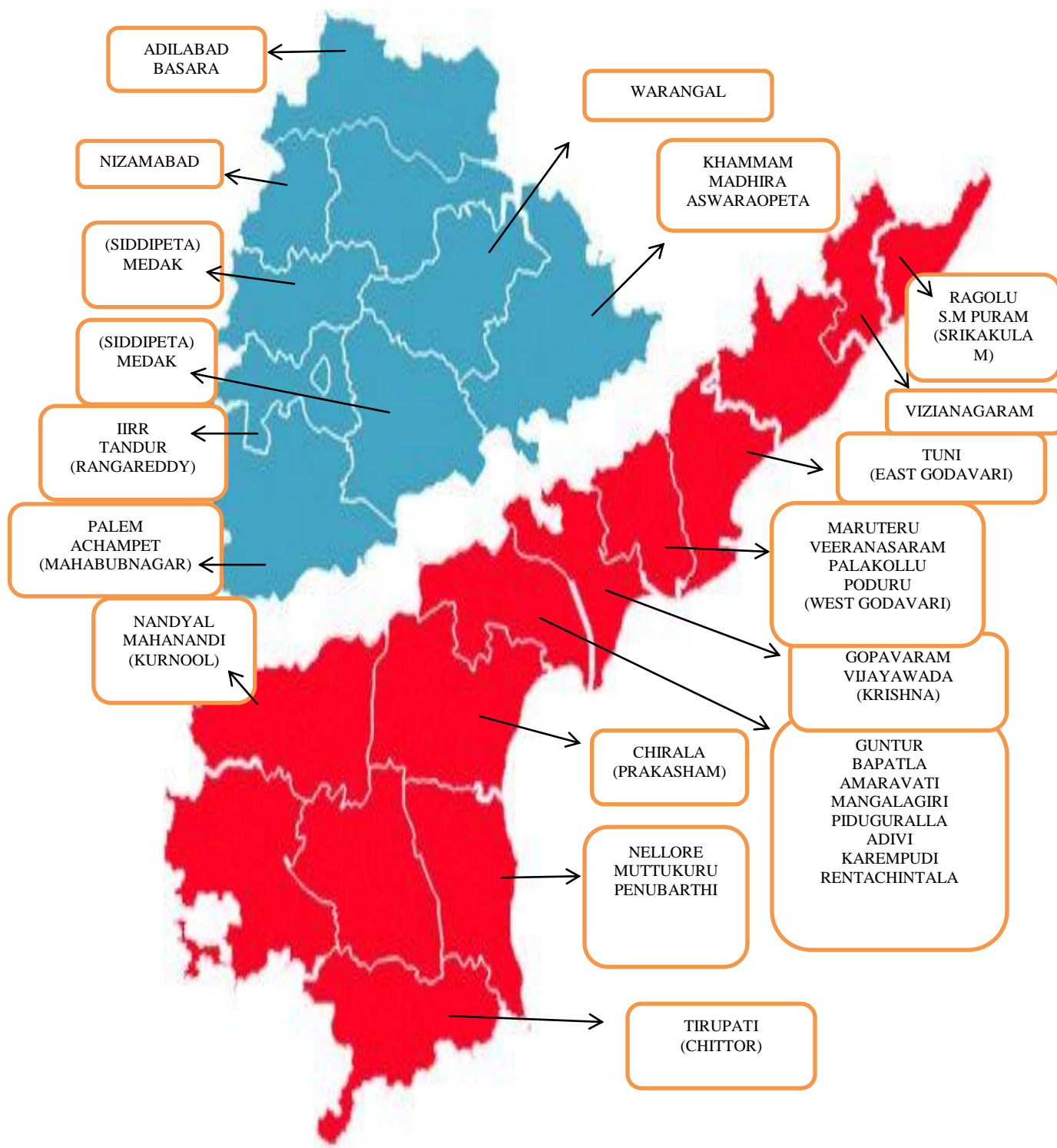
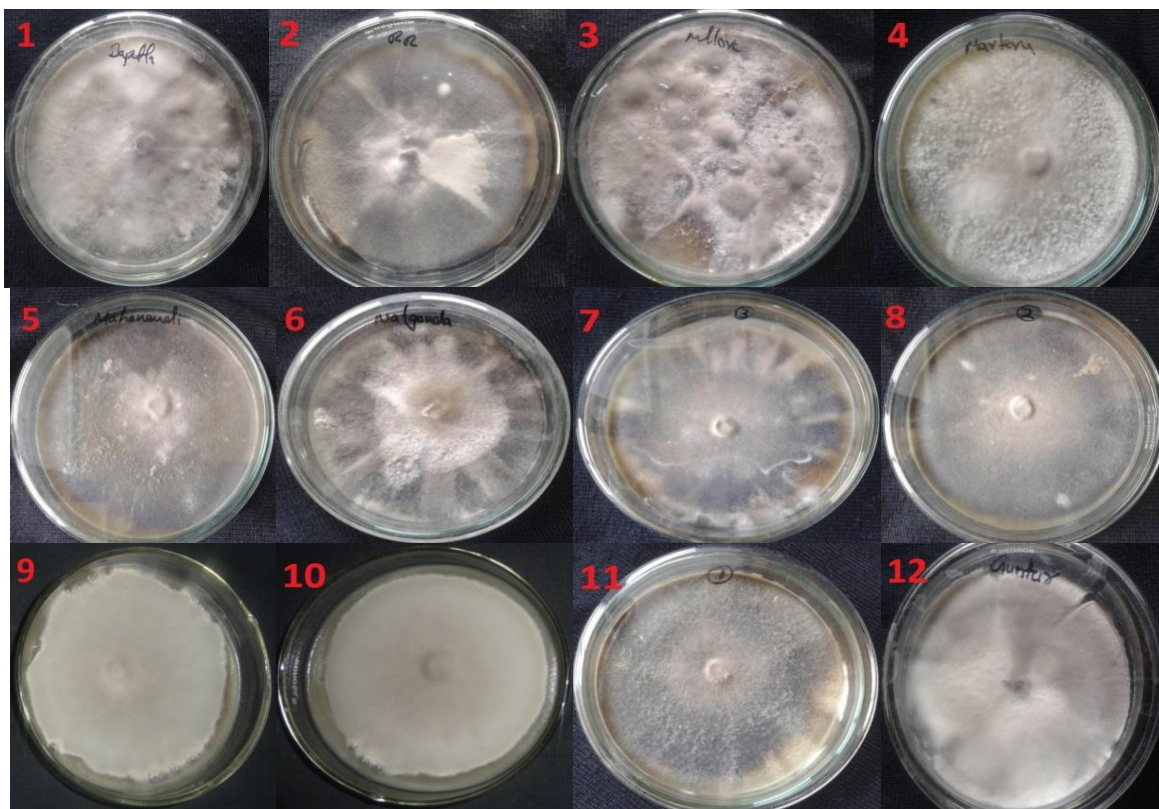


Figure.2 Variation in cultural morphology of *M. grisea* isolates on oat meal agar medium



1. Bapatla, 2. Piduguralla, 3. Nellore, 4. Maruteru, 5. Mahanandi, 6. Nalgonda, 7. Vijayawada, 8. Palem, 9. Nizamabad, 10. IIRR, 11. Medak, 12. Guntur

RBDR 1 isolate collected from HR-12 in South Telangana Zone showed grey color mycelium and smooth surface with excellent (Index-4) sporulation. RBVN 21 isolate collected from RGL-2624 in North Coastal Zone showed greyish white color mycelium and rough surface with fair (Index-2) sporulation. Isolates collected from MTU-1001 in all zones showed greyish white mycelium with rough surface except RBTN 25, which was recorded smooth texture. Sporulation for all these isolates ranged from index 1–4. RBJG 15 and RBBS 23 isolates collected from WGL-44645 in North Telangana Zone showed greyish white mycelium and rough surface with sporulation index 2 and 3 respectively. RBNB 16 isolate collected from WGL-44645 in North Telangana Zone showed grey color mycelium and smooth surface with fair (Index 2)

sporulation. RBWG 19 isolate collected from WGL-44645 in Central Telangana Zone showed greyish brown color mycelium and rough surface with good (Index 3) sporulation (Table 2).

These results are in agreement with Srivastava *et al.*, (2014) who reported existence of variability among the isolates of *M. grisea* with respect to conidial size and was well documented by many workers.

Morphological diversity among the *M. grisea* isolates

Isolates significantly varied in spore morphology. The fungus produced a single bottle-shaped conidiogenous cell bearing 3–5 conidia arranged in a cluster at the active apical tip or they were formed successively

and sympodially in a characteristic pattern, *i.e.* the active apical tip moves to the side to produce next conidium, resulting 3–5 conidia borne sympodially on mature conidiophore. The successive and sympodial bearing of spores was commonly observed with the isolates. Mature conidia of *M. grisea* were pyriform, almost hyaline to pale olive, 2-septate, 3-celled, the middle cell being wider and darker, and exhibit a basal appendage at the point of attachment to the conidiophore. End cells and middle cells germinate giving out germ tubes.

The shape of the conidia was pyriform. The size of the conidia varied among the isolates between 25.5 μm (RBWG 19, RBBS 23, RBMD 34 and RBGV 35) to 38.5 μm (RBNG 7). The length of the conidia ranged from 8 (RBPI 3) to 11 μm (RBNG 7) and width were ranged from 3 (RBMT 11, RBNL 12 etc.) to 4 μm (RBAW 33 and RBPB 38).

The degree of sporulation was compared with the growth patterns of the pathogen. It was observed that isolates that were having rough surface showed more sporulation compared with smooth surface isolates with exception of IIRR isolate. IIRR isolate having smooth surface but produced excellent (Index-4) sporulation. The other isolates having smooth surface (RBGT 4, RBPM 9, RBNB 16 and RBTN 25) produced fair sporulation (Index-2).

The isolates which showed excellent sporulation of index-4 were having greyish white mycelium (RBNL 12 and RBKM 8), greyish black mycelium (RBMU 37 and RBPB 38) and grey colour mycelium (RBDR 1). The isolates which showed poor sporulation of index-1 were having greyish white mycelium (RBND 24 and RBRG 20) and greyish black mycelium (RBTP 13). Vegetative growth of most of the isolates showed greyish white appearance while

RBDR 1, RBNB 16 and RBSP 18 isolate showed the grey appearance. RBPM 9 showed greyish green mycelium and RBWG 19 showed greyish brown appearance. RBDP 36, RBMU 37, RBPB 38, RBAV 27 and RBTP 13 showed greyish black appearance (Table 2 and Figure 2).

With regard to sporulation (Index 1–4), excellent sporulation (Index 4) was noticed in RBDR 1, RBKM 8, RBMT 11, RBNL 12, RBMU 37 and RBPB 38 whereas in RBTP 13, RBRG 20, and RBND 24 isolates it was poor sporulation. Variation in sporulation capacity was also noticed among the isolates.

The size and shape of spores are important criteria for classification and identification of *Pyricularia* species. The results of the present study indicate morphological variation in terms of conidial size and sporulation and also noticed that isolates which had poor sporulation also recorded high PDI.

Shahjahandar *et al.*, (2010) recorded prevalence and distribution of blast in Kupwara district of Jammu and Kashmir and reported 25% disease incidence and 15% severity.

In all the districts of Southern Telangana Zone of Andhra Pradesh (Jagadeeshwar *et al.*, 2014) reported 30–35% incidence of neck blast if the crop was in flowering stage coinciding with North-east monsoon.

It can be concluded that these results indicated that blast isolates were distributed throughout Telangana and Andhra Pradesh. It was also concluded that the disease severity was more in both states.

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