Int.J.Curr.Microbiol.App.Sci (2018) *7*(2): 161-170

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 7 Number 02 (2018)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2018.702.021

Morphological Characterization of *Bipolaris oryzae* Causing Brown Spot Disease of Rice

P. Valarmathi* and D. Ladhalakshmi

Department of Plant Pathology, ICAR-Indian Institute of Rice Research (IIRR), Hyderabad-500 030, Telangana, India

**Corresponding author*

Keywords

Rice, Brown spot, *Bipolaris oryzae*, Colony morphology

Article Info

Accepted:
04 January 2018
Available Online:
10 February 2018

A B S T R A C T

Brown spot disease caused by *Bipolaris oryzae* results in the yield loss of paddy in the recent years. In the present study, isolates were collected from nine rice growing states of India. Based on colony morphology and growth pattern, the 17 isolates of *B. oryzae* were characterized and formed four groups. These were (Group I) black with fluffy growth, (Group II) grey with fluffy growth and white spots, (Group III) grey with fluffy growth and (Group IV) grey with suppressed growth. Further the morphological characterization was studied on different media and spectral values for all isolates.

Introduction

Rice is one of the most important cereal crops and feeds more than one third of the world's population (Burgos *et al.*, 2013). Rice is susceptible to several leaf spot diseases including blast and brown spot, which cause significant yield losses across the globe. During 1942, Great Bengal Famine occurred wherein the yield loss was upto 90% in epiphytotic form at leaf spot phase (Ghose *et al.*, 1960). Being the disease reported in 1919, extensive research has been carried due to which the disease was controlled. In the recent years, because of the climate change and cultivation practices, disease was found to be severe in dry/ direct seeded rice in the states of Bihar, Chhattisgarh, Madhya Pradesh, Odisha, Assam, Jharkhand and West Bengal. It especially occurs in the environment where scarce water resource combined with nutritional imbalance particularly lack of nitrogen and often referred to as "Poor man's disease'' (Baranwal *et al.*, 2013).

The pathogen attacks the crop from seedling to milky stage. The symptoms appear as minute spots on the coleoptile, leaf blade, leaf sheath and glume, being most prominent on leaf blades and glumes. On leaves, typical spots are brown in colour with grey or whitish centre resembling sesame seed with typical yellow halo over the spot (Sunder *et al.*, 2005). Conidia are 5-10 septate with the oldest

conidium towards base. Typically conidia are slightly curved and widest at the middle. The optimum temperature for growth and conidial germination has been found to be 27-30°C and 25-30°C respectively (Ou, 1985). Conidia are formed between 5-38°C, optimum being 25°C (Ou, 1985; Vinay Kumari *et al.,* 1997). Both light and dark periods were required for sporulation of *B. oryzae*. However, it was stimulated by near-ultra violet light and inhibited by blue light (Ou, 1985). Morpho-pathological and molecular characterization of *B. oryzae* has been carried out for fifty isolates in India (Kumar *et al.,* 2011). Diversity and pathogenicity of the rice brown spot pathogen were investigated earlier by many workers using morphological characteristics as well as genetic fingerprint analysis in India as well as in other rice growing countries (Ouedraogo *et al.,* 2004; Motlagh and Kaviani, 2008; Kamal and Mia, 2009; Motlagh and Anvari, 2010; Burgoss *et al.,* 2013, Archana *et al.,* 2014, Kandan *et al.,* 2014 and Nazari *et al.,* 2015). Morphological, molecular characterization and grouping of 27 isolates of *Bipolaris oryzae* from India were carried out by Singh *et al.,* 2016. Morpho-molecular diversity for 116 isolates of *Bipolaris oryzae* from different rice growing areas of India was studied by Kumar *et al.,* 2016. The present paper emphasised on the morphological characterization of *Bipolaris oryzae* amended with different culture media.

Materials and Methods

Isolation of *Bipolaris oryzae*

A total of 17 samples of rice leaves infected with *Bipolaris oryzae* were collected from Andhra Pradesh, Chattisgarh, Haryana, Himachal Pradesh, Jammu & Kashmir, Punjab, Telangana, Uttar Pradesh and West Bengal of India. The fungus, *B. oryzae* was isolated from single discrete lesion from the infected leaf tissue followed by incubation for 2-3 days and further mycelial growth was sub cultured. The purified cultures were maintained on potato dextrose agar (PDA) slants at 4°C. The colony morphology with respect to colony colour and growth pattern was observed 5 days after inoculation among all the 17 isolates of *B. oryzae* on PDA medium.

Colony morphology in different media

The colony morphology for all the seventeen isolates were carried out in different media *viz.,* Rice extract with potato dextrose agar, Rice extract with oat meal agar, Rice polish agar and Malt extract agar. The radial growth of the fungus was measured 5 days after inoculation for all the 17 isolates on different media. The statistical analysis was carried out with the software OPSTAT developed by CCSHAU, Haryana.

Spectral values of isolates

All the 17 isolates of *B. oryzae* were grown on PDA medium at 25°C. After 5 days of growth, the plates were photographed using a Nikon camera (20.1mp). The spectral RGB (Red, Green, Blue) values for each isolate was calculated using Adobe Photoshop (Chand *et al.,* 2008).

Results and Discussion

Morphological characterization of *B. oryzae* isolates

The pattern of growth of *B. oryzae* among isolates showed significant differences with the range of means varying from 3.6 cm (BO 13 & BO 15) to 2.1 cm (BO 9) with five days after inoculation on PDA (Plate 1a, 1b and Table 1). Based on colony morphology and growth pattern, the 17 isolates of *B. oryzae* were characterized and formed four groups. These were (Group I) black with fluffy

growth, (Group II) grey with fluffy growth and white spots, (Group III) grey with fluffy growth and (Group IV) grey with suppressed growth (Plate 2). Isolates BO 2, 4, 8, 15 and 16 were under group II wherein isolates BO 12, 13 & 14 were under group I. Of all the isolates, those of Group II and III had the highest frequency (29.4 %) in the population, whereas Group I had the lowest frequency (17.6 %) (Table 2). All the seventeen isolates were maintained as pure cultures on PDA slant. One of the major difficulties in isolation of *Bipolaris* is the common contaminant of *Curvularia spp*, as colonies of both the fungus look similar and identification is only possible through examination of spores. Enhancement of sporulation seems difficult in the pathogen and hence only three isolates were found to produce conidial spores.

In the present study, isolates of *B. oryzae* were grouped into four categories based on the colony morphology and growth pattern. Earlier Kumar *et al.,* (2011) grouped fifty isolates of *B. oryzae* into four categories. Similarly Singh *et al.,* (2016) grouped twenty seven isolates of the pathogen into five categories. The fifth category with pink fluffy growth colony morphology was not observed in our isolates and isolates studied by Kumar *et al.,* (2011).

The first group with black colony characteristics was also observed in the isolates of *B. oryzae* in rice reported by Kumar *et al.,* (2011), Singh *et al.,* (2016) and *B. sorokiniana* in wheat by Kumar, 2003 and Aggarwal *et al.,* (2011). On the basis of colony morphology and growth pattern on PDA, 116 isolates were grouped into 8 groups *viz.,* black with suppressed growth, black with cottony growth, black with fluffy growth, grey with suppressed growth, grey with cottony growth, grey with fluffy growth, grey and white mix with cottony growth and white with cottony growth. Majority of the isolates (62.93%) were black followed by grey (33.62%) among the *Bipolaris oryzae* isolates (Kumar *et al.,* 2016).

Conidial characterization

Conidia were usually curved, fusoid or obclavate, occasionally almost cylindrical, pale to mid golden brown, 5 to 6 septate with hillum.

The conidia exhibited bipolar germination pattern and hence named the pathogen as *Bipolaris*. The size of BO conidia were measured with three microscopic fields and average were calculated (Plate 3).

The length and breadth of BO 12 was 113.32 x 27.41 µm, BO 13 was 56.89 x 14.27 µm and BO 14 was 63.42 x 13.75 µm (Table 3).

Spore dimensions of all BO isolates varied from 90.34 µm to 137.48 µm in length while width varied from 14.10 to 23.51 µm was observed by Kumar *et al.,* 2016.

Morphological characterization of *B. oryzae* isolates on different media

The pattern of growth of *B. oryzae* among isolates showed significant differences with the range of means varying from 3.0 cm (BO 11 and BO 13) to 1.5 cm (BO 1 & BO 5) with five days after inoculation on Rice extract+ Potato Dextrose Agar.

The pattern of growth of *B. oryzae* among isolates showed significant differences with the range of means varying from 3.0 cm (BO 13) to 1.5 cm (BO 14) with five days after inoculation on Rice extract+ Oat Meal Agar. The pattern of growth of *B. oryzae* among isolates showed significant differences with the range of means varying from 3.3 cm (BO 13) to 1.8 cm (BO 5) with five days after inoculation on Rice Polish Agar.

Table.1 Radial growth of *Biploaris oryzae* isolates

S. No	Isolates	Place of collection	Radial growth (in cm) 5 days after inoculation on PDA medium		
			R1	R2	Mean
1.	BO 1	Ponnampet, Andhra Pradesh	2.9	3.0	**3.0**
2.	BO 2	Bilaspur, Chattisgarh	2.2	2.3	**2.3**
3.	BO 3	Jagadalpur, Chattisgarh	2.5	2.4	**2.5**
4.	BO 4	Raipur, Chattisgarh	3.0	3.9	**3.0**
5.	BO 5	Rewa, Chattisgarh	2.5	2.6	**2.6**
6.	BO 6	Uchani, Haryana	2.7	2.8	**2.8**
7.	BO 7	Dhaula khan, Himachal Pradesh	2.3	2.3	**2.3**
8.	BO 8	Chatha, Jammu & Kashmir	3.4	3.2	**3.3**
9.	BO 9	Kapurthala, Punjab	2.0	2.1	**2.1**
10.	BO 10	Luthiana, Punjab	2.3	2.2	**2.3**
11.	BO 11	IIRR farm, Telangana	2.3	2.2	**2.3**
12.	BO 12	ARI, Telangana	2.8	3.0	**2.9**
13.	BO 13	IIRR farm, Telangana	3.5	3.5	**3.6**
14.	BO 14	IIRR farm, Telangana	3.4	3.3	**3.4**
15.	BO 15	Nagina, Uttar Pradesh	3.5	3.6	**3.6**
16.	BO 16	Pantnagar, Uttar Pradesh	3.3	3.0	**3.2**
17.	BO 17	Chinsurah, West Bengal	2.6	2.7	**2.7**
				CD* (0.05)	**0.381**
				SE (m)	**0.127**

Table.2 *Bipolaris oryzae* isolates group based on colony colour and growth pattern

Group	Colony morphology	Population		Isolates nos.
		Number	Percentage (%)	
I	Black with fluffy growth	3	17.6	BO 12, 13 & 14
II	Grey with fluffy growth & white spots	5	29.4	BO 2, 4, 8, 15 & 16
III	Grey with fluffy growth	5	29.4	BO 1, 6, 9,10 & 17
IV	Grey with suppressed growth	4	23.5	BO 5, 7, 3 & 11

Table.3 Size of *B. oryzae* conidia

S. no	Isolates	Length (µm)	Breadth (µm)
1.	BO 12	113.32	27.41
2.	BO 13	56.89	14.27
3.	BO 14	63.42	13.75

Table.4 Radial growth of *B. oryzae* isolates on different media

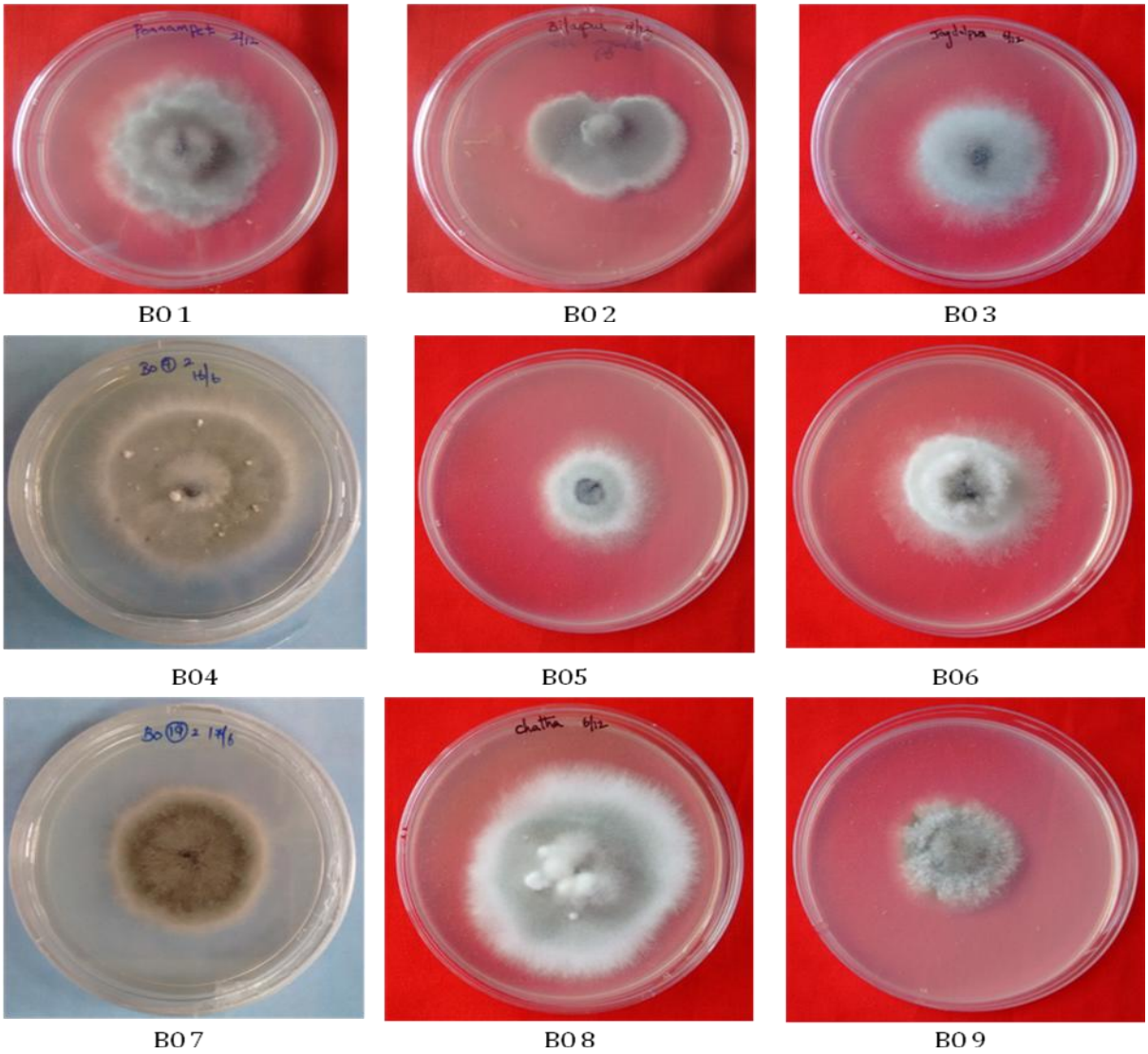
S. No	Isolates	Place of collection	Radial growth (in cm) 5 days after inoculation				
			PDA	RE+PDA	RE+OMA	RPA	MEA
1.	BO 1	Ponnampet, Andhra Pradesh	3.0	1.5	1.8	2.3	1.5
2.	BO 2	Bilaspur, Chattisgarh	2.3	2.5	2.3	2.3	1.8
3.	BO 3	Jagadalpur, Chattisgarh	2.5	2.8	2.5	2.5	1.6
4.	BO 4	Raipur, Chattisgarh	3.0	2.5	2.3	2.5	1.5
5.	BO 5	Rewa, Chattisgarh	2.6	1.5	2.0	1.8	2.0
6.	BO 6	Uchani, Haryana	2.8	1.8	2.5	2.5	1.8
7.	BO 7	Dhaula khan, Himachal Pradesh	2.3	2.5	2.5	2.0	1.8
8.	BO 8	Chatha, Jammu & Kashmir	3.3	2.5	2.8	3.0	2.0
9.	BO 9	Kapurthala, Punjab	2.1	2.3	2.4	2.6	1.8
10.	BO 10	Luthiana, Punjab	2.3	2.8	2.7	2.9	1.5
11.	BO 11	IIRR farm, Telangana	2.3	3.0	2.0	2.2	2.0
12.	BO 12	ARI, Telangana	2.9	2.0	1.8	2.0	1.8
13.	BO 13	IIRR farm, Telangana	3.6	3.0	3.0	3.3	2.5
14.	BO 14	IIRR farm, Telangana	3.4	2.5	1.5	2.5	2.0
15.	BO 15	Nagina, Uttar Pradesh	3.6	2.3	2.0	2.0	1.8
16.	BO 16	Pantnagar, Uttar Pradesh	3.2	2.8	2.3	2.8	2.0
17.	BO 17	Chinsurah, West Bengal	2.7	2.3	2.0	2.0	1.5
CD* (0.05)			**0.381**	**0.303**	**0.213**	**0.346**	**0.367**
SE (m)			**0.127**	**0.101**	**0.071**	**0.115**	**0.122**

RE+PDA= Rice Extract+ Potato Dextrose Agar; RE+OMA= Rice Extract+ Oat Meal Agar; RPA=Rice Polish Agar; MEA=Malt Extract Agar

Table.5 Spectral values of *B. oryzae* isolates

S. No	Isolates	Place of collection	Spectral values (5 days after inoculation)			
			R	G	B	Mean
1.	BO 1	Ponnampet, Andhra Pradesh	106	83	60	**83**
2.	BO 2	Bilaspur, Chattisgarh	128	110	98	**112**
3.	BO 3	Jagadalpur, Chattisgarh	125	110	97	**111**
4.	BO 4	Raipur, Chattisgarh	132	114	95	**114**
5.	BO 5	Rewa, Chattisgarh	83	65	49	**66**
6.	BO 6	Uchani, Haryana	142	128	114	**128**
7.	BO 7	Dhaula khan, Himachal Pradesh	123	106	88	**106**
8.	BO 8	Chatha, Jammu & Kashmir	131	115	98	**115**
9.	BO 9	Kapurthala, Punjab	130	110	90	**110**
10.	BO 10	Luthiana, Punjab	150	131	111	**131**
11.	BO 11	IIRR farm, Telangana	126	105	85	**105**
12.	BO 12	ARI, Telangana	134	116	101	**117**
13.	BO 13	IIRR farm, Telangana	146	128	115	**130**
14.	BO 14	IIRR farm, Telangana	179	155	134	**156**
15.	BO 15	Nagina, Uttar Pradesh	100	78	53	**77**
16.	BO 16	Pantnagar, Uttar Pradesh	113	92	67	**91**
17.	BO 17	Chinsurah, West Bengal	120	95	76	**97**
					CD* (0.05)	**1.666**
					SE (m)	**0.577**

Plate.1a Variations in colony morphology on PDA among *B. oryzae* isolates



BO 1 BO 2 BO 3

BO4 BO5 BO6

BO 7 BO 8 BO 9

Plate.1b Variations in colony morphology on PDA among *B. oryzae* isolates

BO 10 BO 11 BO 12

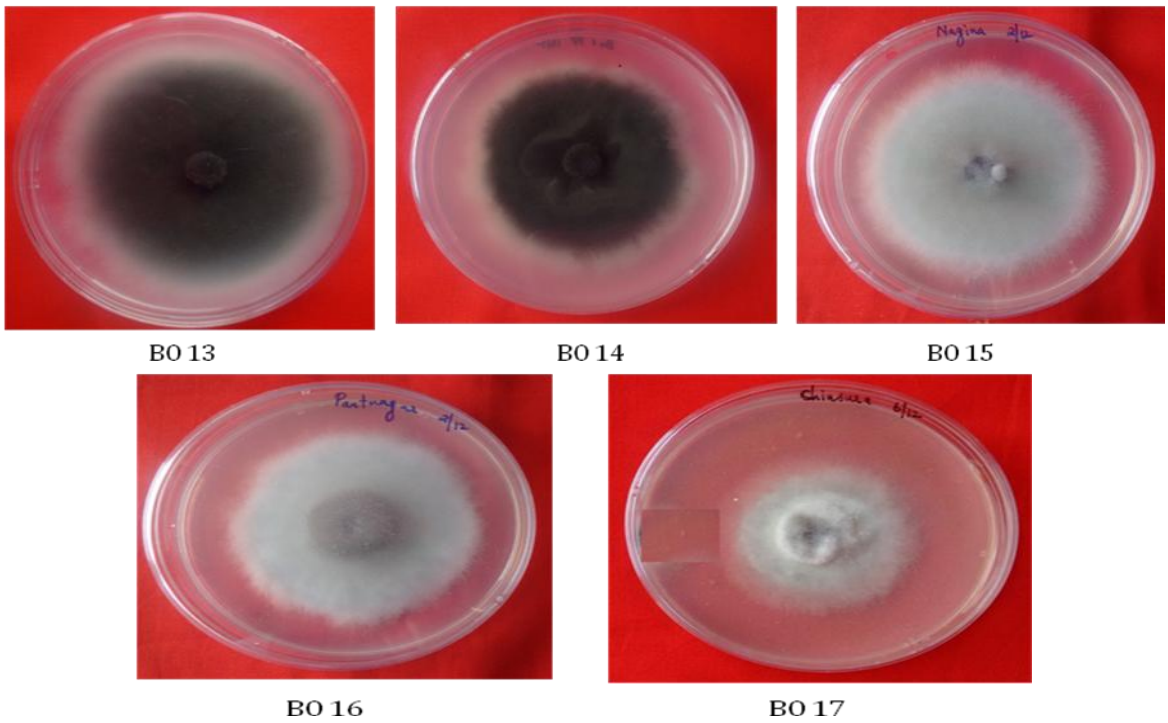
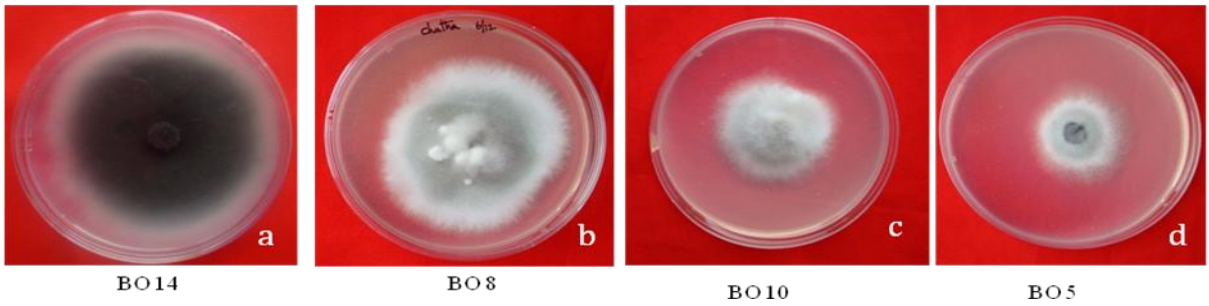
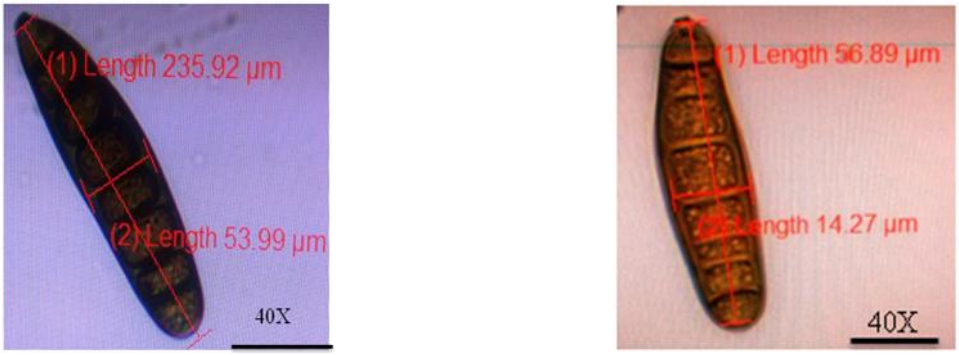


Plate.2 Four different groups based on colony morphology of *Bipolaris oryzae* isolates

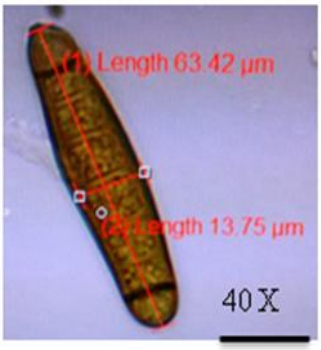


Group I- Black with fluffy growth (a), Group II- Grey with fluffy growth & white spots (b), Group III- Grey with fluffy growth (c) and Group IV- Grey with suppressed growth (d).

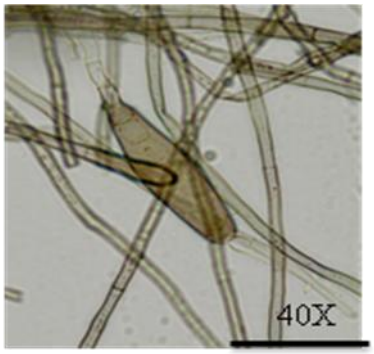
Plate.3 Conidia of *B. oryzae* with length and breadth (µm)



BO 12 BO 13



BO 14



Bipolar germination of conidia

The pattern of growth of *B. oryzae* among isolates showed significant differences with the range of means varying from 2.5 cm (BO 13) to 1.4 cm (BO 4) with five days after inoculation on Malt Extract Agar (Table 4). Vinay Kumari *et al.,* (1997) recorded maximum growth and sporulation of the fungus on corn meal agar medium followed by potato dextrose agar (PDA) and rice leaves agar wherein Arshad *et al.,* 2013 noticed maximum growth of *B. oryzae* on malt extract and PDA after 96 h of incubation with optimum temperature at 28°C. In our study all the four different media supported the growth of the pathogen wherein the Rice polish agar media enhances the sporulation of *Bipolaris oryzae*. Maximum sporulation was observed in the isolates which were black with suppressed growth and minimum sporulation was observed in the isolates which were grey and white mixed with cottony growth (Kumar *et al.,* 2016). Similarly in our study the isolates with black fluffy growth were observed to enhance sporulation (BO 12, 13 and 14).

Spectral values of isolates

The mean spectral values for the isolates ranged from 66 (BO 5) to 156 (BO 14) (Table 5). Group I with black fluffy growth has higher RGB values. Isolates BO 14 with black fluffy growth had higher mean spectral value of 156. Isolate BO 5 with grey suppressed growth had less mean spectral value of 66.

In the present study, the isolates of *Bipolaris oryzae* were characterized based on the colony colour and growth pattern and separated into four groups. Different media were used to study the morphology and its growth pattern. Further in future the research should focus on the enhancement of sporulation in the pathogen *Bipolaris oryzae.* It was observed that the pathogen *Bipolaris oryzae* isolated from seeds seems to produce spores. The sporulated isolates can be used to study its virulence in the susceptible rice cultivar and can be used further for screening the resistance source for the disease.

Acknowledgement

The authors are highly grateful to the Director, ICAR-Indian Institute of Rice Research, Hyderabad for the support and encouragement to carry out the work.

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How to cite this article:

Valarmathi, P. and Ladhalakshmi, D. 2018. Morphological Characterization of *Bipolaris oryzae* Causing Brown Spot Disease of Rice. *Int.J.Curr.Microbiol.App.Sci.* 7(02): 161-170.
doi: https://doi.org/10.20546/ijcmas.2018.702.021