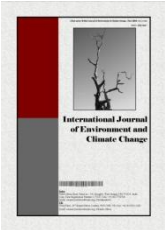

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Morpho-Cultural Variability Characterization of *Bipolaris oryzae* Causing Brown Leaf Spot of Rice in Jammu Sub-Tropics

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Extensive fortnightly surveys were undertaken in major rice growing districts of Jammu division to study cultural and morphological variability amongst the isolates of brown leaf spot disease causing fungus (*Bipolaris oryzae*). Based on disease severity 24 isolates (BO_1 to BO_{24}) were divided into three groups (I, II and III). Group 'I' comprised 11 isolates with disease severity values between 51.22-67.44% and categorized under highly virulent and group 'II' comprised disease severity values between 43.17-48.17% and were categorized under moderately virulent isolates. Group 'III' included eight isolates with disease severity value between 26.11-38.17% and categorized under less virulent isolates. On the basis of colony growth pattern, margin and colour, the isolates were categorized into 4 groups (A, B, C and D). Group A comprised of six isolates had entire margin, circular form, umbonate elevation with smooth surface and black to fluffy growth. Eleven isolates were categorised under group B developed colonies with undulated margin, irregular form, umbonate elevation with grey fluffy growth and greyish colour. Group C comprised three isolates had undulated margin, irregular form, umbonate elevation with smooth surface. Group D contained four isolates developed entire margin, circular form; raised elevation with wrinkled surface, mycelium was grey which turned to black and showed grey to black

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pigmentation. The largest conidial size was recorded in isolate BO_1 (98.43-101×24.54-25.54 µm). The conidia developed mean septa of 4.5-7.4 with straight to curve shaped. The sporulation of different isolates of *B. oryzae* ranged between 6.1-7.6×10^6 spores/ml.

Keywords: Spot disease; Bipolaris oryzae; leaf blade; leaf sheath; rice production; brown leaf spot.

1. INTRODUCTION

In tropical and sub-tropical countries rice production is considered as backbone of agricultural economy, including India, the world's 2nd-largest producer and consumer [1 and 2]. Rice cultivation in Jammu and Kashmir region is mostly mono-cropped, with far higher consumption and importance as a staple grain than other Indian states. Rice is highly essential in the lives of people in the state, despite the fact that the area under the crop cultivation is quite small relative to other Indian states, and hence is very significant in the state economy. In Indian sub-continent including Jammu and Kashmir region, Basmati rice is famous and distinguished all around the world for its appearance and aroma, but unfortunately rice production is reduced because of the biotic and abiotic stresses. Among the biotic constraints, fungal and bacterial diseases play a vital role in preventing normal growth of rice crops and hence reducing yield. Brown leaf spot disease caused by *Bipolaris oryzae* is one of the distinctive and most damaging diseases of rice in the world, because of the extensive distribution of numerous physiological races [3].

Brown leaf spot in rice, is known to cause major qualitative and quantitative losses (up to 90%) in rice crop, especially when the leaf spotting phase reaches epiphytic dimensions, as it did during the Great Bengal Famine of 1942 [4]. The disease has spread throughout the world's rice-growing regions. It has been discovered to be pandemic in locations with high rainfall, such as the Assam, West Bengal, the Malabar Coast and Himalayas [5]. The pathogen attacks the crop from seedling to milky stage. The characteristic 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 with typical yellow halo over the spot [6]. Typically conidia are slightly curved and widest at the middle, conidia are 5-10 septate with the oldest conidium towards base. The causal fungus *B. oryzae* remains in seeds for most of its lifecycle, but can also persist on infested rice stubble and straw. It spreads from plant to plant in the field by the airborne spores. A relative humidity of >89% at 25°C leads to successful inoculation by conidia and infection can be vigorous upon free water on leaf surface [5]. Diversity and pathogenicity of the rice brown spot pathogen were investigated earlier by many researchers using morpho-cultural characteristics as well as genetic fingerprint analysis [7,8,9,10,11]. The present paper emphasised on the morpho-cultural characterization of *B. oryzae* and identifying the most virulent isolates.

2. MATERIALS AND METHODS

2.1 Isolation of Pathogen

Naturally diseased leaves of different rice cultivars infected in varing degrees with brown leaf spot were collected from different locations of Jammu sub-tropics including Jammu, Samba, Kathua and Udhampur districts, and were brought to laboratory of the Division of Plant Pathology, SKUAST-Jammu India, for isolation of the pathogenic fungus. The collected samples were critically observed under microscope, to know the presence of pathogen in infected plant part. After confirming the presence of the pathogen under microscope, the diseased tissues from infected plant parts were subjected to isolation. The pathogen *B. oryzae* was isolated by standard hyphal tip isolation procedure and then the culture was maintained on Potato Dextrose Agar (PDA) slants and kept in a refrigerator at 5°C for further use in all the laboratory studies.

2.2 Pathogenicity Test

The pathogenicity test of *B. oryzae* (isolates BO_1 to BO_{24}) was determined on susceptible cv. Pusa Basmati-1121 under both greenhouse and field experiments. Rice seedlings were sprayed at 25 days old under greenhouse and field conditions with the spore suspension (3 x 10^5 spore ml^{-1}) of the 24 isolates of *B. oryzae* separately. The seedlings were sprayed until runoff occurred. Three replicates were used for each isolate.

2.3 Cultural Variability

Mycelial disc (5 mm) of 10 days old culture of each isolate was transferred to the centre of

sterilized Petri plates containing PDA medium and incubated at 25±2ºC. Colony characters *viz*. colour and diameter were recorded after ten days of inoculation. Reverse side of cultural plate of each fungal isolate was also observed to record pigmentation on under side of the plate.

2.4 Morphological Variability

The morphological variation among different isolates of *B. oryzae* was studied on PDA under *in vitro* conditions. Mono conidial culture of each isolate was first grown on PDA medium and then semi permanent shades were prepared from 10 day old culture were stained with cotton blue in lacto phenol. The growth pattern, progressive colony growth and colony colour was recorded on PDA medium. Morphological features of the pathogen *viz*., type of mycelia, width of mycelia, sporulation pattern, spore size and shape were recorded.

Spores of *B. oryzae* of all the isolates from the culture were mounted on a clean glass slide. Spores were mixed with Lactophenol thoroughly in order to obtain uniform spread, on which cover slip was place. Spores were measured under high power objective using light microscope (400X). The average size of the spores like length, width and number of septa were recorded. Microphotographs were taken to show the typical spore morphology of the pathogen.

3. RESULTS AND DISCUSSION

3.1 Pathogenicity Test

The pathogenicity test of *B. oryzae* (isolates BO_1 to BO_{24}) was determined on susceptible cv. Pusa Basmati-1121 (Table 1). Pathogenicity test was conducted for all the 24 isolates and control pots were maintained by spraying distilled water only. The first symptoms on the affected plant part of rice started with minute pin head shape brown flecks after 4-5 days of inoculation, later such pin head shaped flecks matured into dark brown lesions characteristics of brown spot disease . On the basis of disease severity the isolates were divided into three groups (I, II and III). Group 'I' comprised 11 isolates *viz.,* BO_1, BO_2, BO_3, BO_4, BO_5, BO_8, BO_9, BO_{12}, BO_{14}, BO_{19} and BO_{20} with disease severity value between 51.22-67.44% and were categorized under highly virulent. In group 'II' comprised five isolates *viz.,* BO_6, BO_{11}, BO_{13}, BO_{15} and BO_{16} with disease severity value between 43.17-48.17% and were categorized under moderately virulent isolates. Group 'III' included eight isolates *viz.,* BO_7, BO_{10}, BO_{17}, BO_{18}, BO_{21}, BO_{22}, BO_{23} and BO_{24} with disease severity value between 26.11-38.17% and were categorized under less virulent isolates (Table 2). The pathogen was re-isolated from infected leaves and its morphological characters were compared with the original culture of the pathogen and found similar in all respects i.e., morphological and microscopic. Hence, the causal agent of the brown spot disease was confirmed as *B. oryzae* after confirming of Koch's postulates. However, no such symptoms developed on uninfected leaves even after 12 days of inoculation.

The symptoms observed were in accordance with the description of the pathogen given by Sobanbabu et al. [12] who also reported variation with respect to disease incidence based on pathogenicity test. Similarly, Nazari et al. [9] also conducted pathogenicity test involving 12 isolates of *B. oryzae* under greenhouse conditions and found that isolates were pathogenic to rice seedlings in cv. Tarom. Moreover, Singh et al. [13] also reported variation in virulence among *B. oryzae* isolates based on pathogenicity test on the susceptible variety Pusa-1121.

3.2 Cultural Variability in Different Isolates of *B. oryzae* on PDA Medium

All the 24 isolates, when grown in sterilized Petri plates on sterilized PDA medium, exhibited great variability for colony diameter, growth pattern, colony margin and colour (Table 3) after 15 days of incubation at 25±1 °C. On the basis of colony growth pattern, margin and colour, the isolates were categorized into 4 groups (A, B, C and D); Group A comprised six isolates *viz.,* BO_1, BO_6, BO_9, BO_{11}, BO_{22} and BO_{23} and had colony diameter of 85.67, 78.65, 81.41, 63.51, 70.34 and 64.89 mm, respectively. In addition, the isolates had entire margin, circular form, umbonate elevation with smooth surface and black to fluffy growth. Eleven isolates *viz.,* BO_3, BO_5, BO_7, BO_8, BO_{10}, BO_{14}, BO_{15}, BO_{17}, BO_{18}, BO_{19} and BO_{20} were categorised under group B having colony diameter of 63.51, 70.34, 73.85, 60.32, 65.11, 81.63, 86.11, 70.43, 71.44, 71.56 and 68.24 mm, respectively. Isolates of this group developed colonies with undulated margin, irregular form, umbonate elevation with grey fluffy growth and greyish colour. Group C comprised three isolates *viz.,* BO_4, BO_{16} and BO_{21} and had colony diameter of 86.96, 72.34

and 48.34 mm, respectively. The colonies of the isolates had undulated margin, irregular form, umbonate elevation with smooth surface. Initially, the mycelium was dirty white and later turned to grey in colour. Group D contained four isolates *viz*. BO_2, BO_{12}, BO_{13} and BO_{24} and had colony diameters of 48.34, 78.84, 58.54, and 71.44 mm, respectively. The colonies developed an entire margins, circular form, raised elevation with wrinkled surface, mycelium was grey which turned to black and showed grey to black pigmentation.

The results obtained in the present investigation showing variation in colony colour of *B. oryzae* corroborate with the findings of Kumar et al. [14] who reported that color of the isolates of *B. oryzae* showed huge variation i.e., black with fluffy growth, grey with fluffy growth, grey with fluffy growth and white spots, grey with suppressed growth. Kumar et al. [10] grouped *B. oryzae* isolates into 5 categories on the basis of morphology and growth pattern i.e. black with fluffy growth, black with suppressed growth, grey with cottony growth, grey and white mix with cottony growth and white with cottony growth. The margins of colonies varied from circular, undulated, irregular having umbonate to raised elevation along with smooth to wrinkled surface. Nayak and Hiremath [15] also reported variations with respect to colony colour (Dark greyish black, light greyish, greyish to white, greyish black to white, black to white colour), mycelial growth (cottony and flat), margin (regular to irregular), sporulation and conidial morphology among *B. oryzae* isolates.

Table 1. Pathogenicity test of different isolates of *B. oryzae* on *cv.* Pusa Basmati-1121

District	Location	Isolate of *Bipolaris oryzae*	Disease severity (%)	Virulence pattern
Jammu	Bishnah	BO_1	51.33 (45.74)	++++
		BO_2	62.53 (52.23)	++++
	Marh	BO_3	59.67 (50.55)	++++
		BO_4	64.20 (53.22)	++++
		BO_5	58.07 (49.62)	++++
		BO_6	44.17 (41.63)	+++
		BO_7	26.11 (30.71)	++
Samba	Vijaypur	BO_8	54.08 (47.32)	++++
		BO_9	51.22 (45.68)	++++
		BO_{10}	38.00 (38.03)	++
		BO_{11}	48.17 (43.93)	+++
	Ghagwal	BO_{12}	53.22 (46.82)	++++
		BO_{13}	43.17 (41.05)	+++
		BO_{14}	52.11 (46.19)	++++
Kathua	Hiranagar	BO_{15}	44.89 (42.04)	+++
		BO_{16}	47.44 (43.51)	+++
	Bilawar	BO_{17}	35.44 (36.52)	++
		BO_{18}	38.17 (38.14)	++
	Kathua	BO_{19}	67.44 (55.19)	++++
		BO_{20}	52.50 (46.41)	++++
Udhampur	Udhampur	BO_{21}	35.00 (36.25)	++
		BO_{22}	32.55 (34.77)	++
	Tikri	BO_{23}	34.44 (35.92)	++
	Manwal	BO_{24}	35.17 (36.35)	++
CD (p=0.05)		-	1.28	-

Table 2. Grouping of isolates of *Bipolaris oryzae* on the basis of pathogenicity test

Group	Isolate	Disease severity (%)	Virulence pattern
Group-I	BO_1, BO_2, BO_3, BO_4, BO_5, BO_8, BO_9, BO_{12}, BO_{14}, BO_{19} and BO_{20}	>50	Highly virulent
Group-II	BO_6, BO_{11}, BO_{13}, BO_{15} and BO_{16}	40-50	Moderately virulent
Group-III	BO_7, BO_{10}, BO_{17}, BO_{18}, BO_{21}, BO_{22}, BO_{23} and BO_{24}	<40	Less virulent

Table 3. Cultural variation of isolates of *Bipolaris oryzae* on potato dextrose agar medium

Isolate	Radial growth (mm)	Group	Colony characteristics	Pigmentation
BO_1	85.67		Entire margin, circular form, umbonate elevation with smooth surface, black with fluffy growth	Black
BO_6	78.65			
BO_9	81.41	A		
BO_{11}	63.51			
BO_{22}	70.34			
BO_{23}	64.89			
BO_3	63.51		Undulated margin irregular, umbonate elevation with grey fluffy growth	Greyish
BO_5	70.34			
BO_7	73.85			
BO_8	60.32			
BO_{10}	65.11			
BO_{14}	81.63	B		
BO_{15}	86.11			
BO_{17}	70.43			
BO_{18}	71.44			
BO_{19}	71.56			
BO_{20}	68.24			
BO_4	86.96		Undulated margin, irregular form, umbonate elevation, smooth surface, mycelium initially dirty white and later turning to grey	Mixture of grey
BO_{16}	72.34	C		
BO_{21}	48.34			
BO_2	48.34		Entire margin, circular form, raised elevation with wrinkled surface, mycelium grey turning to black.	Grey to black
BO_{12}	78.84			
BO_{13}	58.54	D		
BO_{24}	71.44			

Table 4. Morphological variation of conidia of different isolates of *Bipolaris oryzae*

Isolate	Mean		Size of conidia (um)	Number of septa	Number of conidia/ml (1×10^6)	Shape of Spore
	Length (µm)	Breadth (µm)	Range			
BO_1	99.71	25.04	55.34-57.32 x 12.22-15.23	7.1	7.4	Curved
BO_2	56.33	13.72	59.08-60.43 x 10.25-11.23	6.6	6.9	Straight
BO_3	59.75	10.74	98.54-99.43 x 23.44-26.43	4.5	6.6	Curved
BO_4	98.98	24.93	82.56-84.34 x 15.63-33.21	5.6	7.2	Curved
BO_5	83.45	24.42	57.21-58.44 x 12.54-14.33	6.9	7.6	Straight
BO_6	57.82	13.43	89.43-90.11 x 14.32-15.43	5.9	7.2	Curved
BO_7	86.77	14.87	38.87-39.54 x9.54-10.43	6.4	6.3	Curved
BO_8	39.20	9.98	95.87-99.32x18.45-21.32	4.9	6.5	Curved
BO_9	97.59	19.88	51.39-55.34x14.32-16.43	6.1	7.2	Curved
BO_{10}	53.36	15.37	80.32-85.39x19.20-20.23	6.3	6.1	Curved
BO_{11}	82.85	19.71	89.21-93.33x23.54-24.32	5.2	7.2	Curved
BO_{12}	91.27	23.93	59.23-60.12x 12.32-14.34	6.9	6.4	Curved
BO_{13}	59.67	13.33	89.33-92.12x12.56-14.32	5.0	7.4	Curved
BO_{14}	90.72	13.44	69.32-71.65x12.23-15.77	4.8	7.6	Curved
BO_{15}	70.48	14.00	83.34-87.33x11.34-13.34	5.6	6.9	Straight
BO_{16}	85.33	12.34	78.44-89.34x9.54-10.55	4.9	6.2	Straight
BO_{17}	83.89	10.04	80.34-81.44 x12.33-14.32	5.0	6.9	Curved
BO_{18}	80.89	13.32	39.33-42.56x12.33-14.33	7.4	7.5	Curved
BO_{19}	40.94	13.33	59.23-60.12x12.32-14.34	7.3	6.5	Curved
BO_{20}	59.67	13.33	89.33-92.12x12.56-14.32	6.7	7.2	Straight
BO_{21}	90.72	13.44	69.32-71.65x12.23-15.77	7.1	7.2	Curved
BO_{22}	70.48	14.05	89.21-93.33x23.54-24.32	5.8	7.2	Curved
BO_{23}	91.27	23.93	98.43-101 x 24.54-25.54	5.4	7.2	Curved
BO_{24}	59.67	13.33	55.34-57.32 x 12.22-15.23	6.4	6.5	Curved

3.3 Morphological Variability in Different Isolates of *B. oryzae*

The isolates of *B. oryzae*, grown on PDA culture medium, developed conidial length ranged between 39.20-99.71 µm and breadth between 9.98-25.04 µm. The largest conidial size was recorded in isolate BO_1 (98.43-101×24.54-25.54 µm), followed by BO_4 (98.54-99.43×23.44-26.43 µm) and it was minimum in isolate BO_8 (38.87-39.54 × 9.54-10.43 µm). The conidia developed mean septa of 4.5-7.4 with straight to curved shaped (Table 4). The sporulation of different isolates of *B. oryzae* ranged between 6.1-7.6×10^6 spores/ml.

Existence of variability among the isolates of *B. oryzae* with respect to conidial size was well documented in earlier studies. Ou [16] reported that size of conidia of *B. oryzae* isolates varied from 45-106 x 14-17 µm in India. Jaiganesh and Kannan [17] reported that the colour of the conidia was brown to light brown and it was slightly curved with a bulge in the middle and tapering towards the ends. They also reported that the size of the conidia varied from 29.3-33.2 µm length and 13.5-14.8 µm width.

4. CONCLUSION AND RECOMMENDA-TIONS

The pathogenicity test of *B. oryzae* isolates was determined on susceptible rice cultivar Pusa Basmati-1121. The disease severity ranged between 26.11-67.44 per cent, 11 isolates of *B. oryzae*, with higher disease severity (51.22-67.44%) were categorized as highly virulent, whereas five and 8 isolates with disease severity between 43.17-48.17 and 26.11-38.17% were moderately and less virulent, respectively.

All the 24 test isolates exhibited great variability when cultured on PDA medium with colony colour varying from black, grey and whitish grey. The margins of colonies were circular, irregular and undulated and the colonies showed umbonate and raised elevation along with smooth and wrinkled surface. On the basis of colony growth pattern, margin and colour, the isolates were categorized into 4 groups (A, B, C and D). Group A comprised six isolates *viz.,* BO_1, BO_6, BO_9, BO_{11}, BO_{22} and BO_{23} whereas, 11 (BO_3, BO_5, BO_7, BO_8, BO_{10}, BO_{12}, BO_{14}, BO_{15}, BO_{17}, BO_{19} and BO_{20}), 3 (BO_4, BO_{16} and BO_{21}) and 4 (BO_2, BO_{13}, BO_{23} and BO_{24}) isolates were categorized under group B, C and D, respectively.

The isolates of *B. oryzae*, grown on PDA culture medium, developed conidial lengths ranging between 39.20-99.71 µm and breadth between 9.98-25.04 µm. The conidia developed mean septa of 4.5-7.4 with straight to curve shaped. The sporulation of different isolates of *B. oryzae* ranged between 6.1-7.6×10^6 spores/ml.

Investigating the response of the varieties to the isolates will enable us to appreciate the level of resistance of those varieties. To well understand the level of resistance of these varieties to brown leaf spot disease, it would be important to test them in field conditions and to understand the effects of abiotic factors on their behavior. Selecting genotypes with horizontal resistance could help us in taking part in achieving the set goals of global food security.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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