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Variability in *Bipolaris sorokiniana* Causing Spot Blotch in Wheat (*Triticum aestivum* L.) in Jammu Sub-Tropics

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A B S T R A C T

Spot blotch in wheat (*Triticum aestivum* L.) is mainly caused by *Bipolaris sorokiniana* (Sacc.) Shoem in India and south Asian countries and inflected losses in yield up to 50 per cent. The isolates of *B. Sorokiniana* (BS_1 to BS_{10}) isolated from blighted leaf samples collected from different part of Jammu sub-tropics. Cultural and morphological variability of *B. sorokiniana* isolates exhibited that the colonies were effuse grey-white to effuse black and velvety-white mycelial growth with regular to irregular margins. The colour of colonies varied from grey to dark brown and white to light grey. The number of septation in isolates ranged from 3.9 to 6.3 and spore size ranged from 35.07 to 60.53µm in length and 13.20 to 17.60µm in breadth. The shape of the spore was elliptical, slight curved and straight with tapered end. The isolates of *B. sorokiniana* later spray (inoculated) on seedlings of a differential set of wheat genotypes *viz*., Sonalika, PBW-343, HD 2733, PBW 2967 and PBW 660. On the basis of host pathogen interaction, isolates BS_2 and BS_9 were categorised as highly virulent, whereas isolates BS_7 and BS_{10} as least virulent. However, isolates BS_2, BS_3, BS_4, BS_5, BS_6 and BS_8 were categorized as a moderately virulent.

Introduction

Spot blotch or foliar blight caused by *Bipolaris sorokiniana* (Sacc.) Shoem is one of the most concerning disease in warm and humid regions of India and other South Asian countries due to its wide spread prevalence and increasing severity (Joshi *et al.*, 2002). It is an important disease in that mega environment which is characterized by high humid conditions around and after heading stage. It is the major biotic constraint in wheat in the Gangetic plains, especially in the rice-wheat cropping system and is the main limiting factor to growing wheat in South-East Asia (Duveiller *et al.*, 1998). At present spot blotch of wheat is a major pathogen at national level in India and its frequency is highest in north eastern plains zone amongst six agro climatic zones due to prevalence of hot and humid weather conditions. During past two decades, substantial economic loss in wheat

production has occurred due to the severity of spot blotch, affecting the livelihood of millions of small scale farmers (Krishnendu *et al.,* 2011). The fungus (*Bipolaris sorokiniana*) has high morphological, physiological and genetic variability, which make it difficult to identify and also to determine the appropriate measure for disease control (Zhong and Steffenson, 2001). In recent past years, spot blotch was also identified as a major foliar disease along with yellow rust and resistance varieties not performed so good on the farmers' fields due to shifting of the pathogen populations to overcome resistance. Keeping in view of the importance of the crop and losses caused by spot blotch pathogen in recent years, the present study was under taken to find out the variability in the population of *B. sorokiniana* in Jammu sub-tropics.

Materials and Methods

A total of 250 blighted leaf samples of wheat were collected from all over the Jammu sub tropics during survey programme. The isolation of pathogens associated with blighted samples was done on potato dextrose agar (PDA) medium. The pathogen was grown on PDA medium. The cultures were broadly grouped in to 10 isolates from BS_1- BS_{10}. Morphological and cultural characters of the isolates *viz*. colony characters, colony diameter and sporulation were studied. Cultural variability among the 10 isolates was studied on the basis of type and colony colour, growth rate and number and shape of the conidia etc.

Five mm mycelial disc of 7-day old culture of each isolate was transferred to the centre of sterilized Petri plates containing potato dextrose agar medium and incubated at 25 ± 1^0C. Colony character *viz.* colour and margins were recorded after ten days of inoculation. Reverse side of cultural plate of each fungal isolate was also observed to record pigmentation, if any, on under side of the plate**.** To provide a uniform assessment of pathogen growth rate, the fungal isolates were cultured on potato dextrose agar (PDA), Potato carrot agar (PCA) and Czapeck's dox agar (ZDA) medium in 90 mm Petri plates. *B. sorokiniana* isolates from the stored collection were initially inoculated on PDA following suitable growth of each isolates. Five mm fungal disc from the periphery of 7-day old fungal isolates were transferred to the centre of PDA, PCA and CDA poured Petri plates. The isolates were inoculated at 25 ± 1^0C. The colony diameter was recorded after 10 days of incubation by taking two perpendicular measurements and their average calculated.

The morphological variation among various isolates of *B. sorokiniana* was studied on artificial culture under *in vitro* conditions. Monoconidial culture of each isolate was first grown on potato dextrose agar medium and then semi-permanent shades prepared from 10-day old culture, stained with cotton blue in lactophenol. The important characters studied were as under:

Colony character: Growth pattern and colour
Conidia: Septation, size and shape

Characterization of isolates

The colony morphology (colour and growth behaviour), radial growth and sporulation of 10 different isolates of *B. sorokiniana* were studied on PDA. Three plates per isolate were used for characterization. Radial growth was measured by placing a mycelial disc of 5 mm diameter in the centre of the plate and incubated at 25 ± 1^0C for 7 days. Colony colour and growth behaviour were recorded on the 8^{th} day of inoculation. Spore production was recorded by excising 19.62 mm^2 colony area using cork borer. It was shaken well, after

mixing in 1 ml water, to dislodge the conidia. The number of spores/cm^2 was counted with the help of by a haemocytometer.

Pathogenic variability of *Bipolaris sorokiniana* isolates

Ten isolates (BS_1.....BS_9 and BS_{10}) of *B. sorokiniana* and five wheat genotype (Sonalika, PBW-343, HD 2733, PBW-2967 and PBW-660) were used for studying the pathogenic variation against *B. sorokiniana* isolates. Five seeds of each genotype were sown in sterile soil in plastic pots and the pots were maintained in the green house till emergence of 3-4 leaves. These seedlings were spray-inoculated with spore load (4.3 x 10^4spores/ml) of each isolate of *B. sorokiniana* at 3-4 leaf stage under polyhouse at 25±1^0C and relative humidity of 85-95 per cent. The time between the inoculation and first appearance of the spot/lesion on the plant was considered as incubation period, it was recorded at 10 a.m. onward every day, starting from isolate BS_1 to Bs_{10} in sequence to avoid the time difference as inoculation had also been done in same sequence. At tenth day of inoculation, lesion length (mm) was measured. The pathogenic response of these genotypes against isolates of *B. sorokiniana* in relation to infection response (IR), incubation period, average numbers of lesion/leaf and lesion length (mm) were ascertained after tenth days of inoculation. Seedling infection responses (IRs) recorded using four grades i.e., S-susceptible, MS- moderately susceptible, MR-moderately resistant and R-resistant and grade were categorized on the basis of the degree of yellow halo around the necrotic spot and size of necrotic spot. R- no yellow halo, MR- small tinge of yellow around necrotic spot, MS-necrotic area surrounded by thin yellow boundary and S- the yellow halo extended around the necrotic spot and runs parallel to the veins of leaf (Singh *et al.,* 2005). The terminal disease severity was measured on flag and flag-1 leaf using 0-9 scales (Saari and Prescott, 1975) on differential set of wheat genotypes.

Results and Discussion

Variability amongst the isolates of *B. sorokiniana* was recorded with respect to cultural and morphological character A total number of 10 isolates of *B. sorokiniana* were obtained from 10 different locations of the three district surveyed. Isolations and purification were made as per standard procedure. Ten isolates of *B. sorokiniana* obtained from different locations have satisfied Koch's postulates and were named BS_1 to BS_{10} (Table 1). It is revealed from table 1 that the colony colour varied from white, grey, dark brown and white to light grey colour. Mostly, the colonies had effuse grey white to effuse black and velvety white mycelial growth with regular to irregular margins (Table 1). The cottony growth with effuse grey white with regular margin was observed in five isolates *viz*., BS_2, BS_3, BS_4, BS_7 and $BS_{8,}$ while effuse grey white with irregular margin was observed in BS_1 and BS_9 isolates of the fungus. Effuse black with irregular margin at periphery was observed in BS_6 and BS_{10} isolates, whereas, BS_5 showed velvety white with regular margin at periphery. Further, it is also revealed that the isolates BS_2, BS_3, BS_4, BS_5, BS_7 and BS_8 showed grey colony colour, while as dark brown coloured colony was observed in BS_6 and BS_{10} isolates. White coloured colony was observed in BS_1 and white to light grey coloured colony appeared as in BS_9 isolate of the fungus. The fungus BS_6 produced dark brown colony colour.

The isolates of *B. sorokiniana* revealed variation in the radial growth after 10 days of inoculation on three different media *viz*. Potato dextrose agar (PDA), Czapek dox agar (ZDA) and Potato carrot meal (PCA). Data

(Table 3) revealed that isolate BS_9 with radial growth (86.33 mm) was fastest growing fungus followed by BS_8, BS_4, BS_2, BS_1, BS_6, BS_5, BS_7 and BS_{10} having growth of 77.66, 76.00, 70.33, 66.05, 63.00, 61.00, 54.33 and 51.66 mm, respectively on PDA media. The least growth rate (50.66 mm) was observed in BS_3.

Similarly on ZDA medium, maximum radial growth was observed in BS_9 (81.00 mm) and least by BS_3 with radial growth (47.66 mm). Isolate BS_9 again recorded fastest growth in PCA medium with radial growth (78.66 mm) and least growth (41.66 mm) was shown by isolate BS_3. On an average, all the isolates of *B. sorokiniana* showed excellent growth on PDA (65.70 mm) followed by ZDA (60.83 mm) while as least mean growth was exhibited by PCA medium (57.12 mm).

The overall average conidial size of *B. sorokiniana* isolates ranged from 47.75x15.58 µm. The average maximum conidial length (60.53 µm) was observed in BS_6, whereas minimum length (35.07 µm) was recorded in BS_3 isolate (Table 1). Average maximum conidial breadth of 17.60 µm was observed in BS_{10} and minimum conidial breadth of 13.20 µm in BS_9. The number of septa also varied within the isolates of *B. sorokiniana.* The maximum septa were observed in isolate BS_8 (6.3) followed by BS_9 (6.2), BS_2 (6.1), BS_6 (5.8) and BS_4 (5.6). The minimum number of septa was found in BS_3 (3.9).

The isolated of *B. sorokiniana* were differed from each other in respect to spore shape. Six isolates (BS_2, BS_3, BS_4, BS_5, BS_7 and BS_8) had elliptical shaped conidia, while isolates BS_6 and BS_{10} appeared as slightly curved shaped conidia. However, isolates BS_1 and BS_9 were straight with tapering conidia. The data from the Table 3 also showed that all the isolates exhibited varied number of conidia which ranged between 20.39-69.84×10^3/ml. Maximum number of conidia (69.84) was observed in isolate BS_9, while as least number was exhibited by BS_{10}.

Grouping of isolates

Data (Table 2) revealed that the isolates were finally grouped according to colony characteristics, colony colour, margin and shape of spore. The isolates BS_2, BS_3, BS_4, BS_7 and BS_8 were placed in group 'A' exhibiting colony characteristics *viz*., effuse grey white colony with regular margin at periphery, elliptical spore shape, while isolates BS_6 and BS_{10} placed under group 'B' exhibiting colony characteristics of effuse black with irregular margin, slightly curved shaped spores. However, isolates BS_1 and BS_9 were placed in group 'C' exhibiting colony characteristics of effuse grey white colony with irregular margin, straight with tapered end shaped spores. The isolate BS_5 was placed in group 'D' that appeared as velvety white colony with regular margin and had elliptical shaped spores.

The findings of the present study corroborate with the findings of Poloni *et al.*, (2009) who studied the 35 isolates of *B. sorokiniana* and categorized into five morphological groups having black fluffy growth with white sectors, black fluffy growth, gray cottony growth, white cottony growth and white suppressed growth. Further, Morphological variability studies were also carried out by many workers who reported that *B. sorokiniana* is a variable fungus with many morphological and physiological variants attributed due to heterokaryosis and para sexual mechanism (Christensen, 1925 and Chand *et al.*, 2003).

The result achieved is in conformity to those of Christensen (1922) who also observed that spore of four biologic forms of *Helminthosporium sativum* differed slightly in respect of width, length and number of septa.

Table.1 Cultural and morphological features of *Bipolaris sorokiniana* isolates collected from different locations of Jammu province

Isolate	Location	Radial growth (mm)	Number of conidia /ml ($x10^3$)	Size of conidia Length (µ) x Breadth (µ)	Spore septation	Spore shape	Colony character	Colony colour
BS_1	Marh	66.00	57.32	43.20 x 17.02	4.2	Straight with tapered end	EGWI	White to light grey
BS_2	Bishnah	70.33	58.56	43.20 x 15.62	6.1	Elliptical	EGWR	Grey
BS_3	R. S. Pura	50.66	24.42	35.07 x 14.73	3.9	Elliptical	EGWR	Grey
BS_4	Chatha	76.00	39.47	44.16 x 14.76	5.6	Elliptical	EGWR	Grey
BS_5	Vijaypur	61.00	47.59	49.14 x 17.01	4.6	Elliptical	VWR	Grey
BS_6	Sapwal	63.00	28.35	60.53 x 15.54	5.8	Slight curved	EBI	Dark brown
BS_7	Ghagwal	54.33	36.00	48.08 x 13.89	4.3	Elliptical	EGWR	Grey
BS_8	Hiranagar	77.66	55.25	49.44 x 16.38	6.3	Elliptical	EGWR	Grey
BS_9	Chadwal	86.33	69.84	55.22 x 13.20	6.2	Straight with tapered end	EGWI	White to light grey
BS_{10}	Rajbhag	51.66	20.39	49.44 x 17.60	4.7	Slight curved	EBI	Dark brown

EGWR = Effuse Grey White Regular VWR=Velvety White Regular EBI = Effuse Black Irregular EGWI = Effuse Grey White Irregular

Table.2 Groupings of *Bipolaris sorokiniana* isolates on the basis of cultural characteristics

Group	Isolate	Colony characteristics	Spore shape	Colony colour
A	BS_2, BS_3, BS_4, BS_7, BS_8	Effuse grey white regular	Elliptical	Grey
B	BS_6, BS_{10}	Effuse brown irregular	Slight curved	Dark brown
C	BS_1, BS_9	Effuse grey white irregular	Straight with tapered end	White to light grey
D	BS_5	Velvety white irregular	Elliptical	Grey

Table.3 Radial growth of *Bipolaris sorokiniana* isolates on different nutrient media

Isolate	Radial growth (mm)		
	Potato Dextrose Agar	Czapek Dox Agar	Potato Carrot Agar
BS_1	66.05	57.66	43.00
BS_2	70.33	66.33	57.33
BS_3	50.66	47.66	41.66
BS_4	76.00	69.66	67.66
BS_5	61.00	56.33	55.33
BS_6	63.00	60.33	59.33
BS_7	54.33	49.33	49.00
BS_8	77.66	72.00	73.66
BS_1	66.05	57.66	43.00
BS_9	86.33	81.00	78.66
BS_{10}	51.66	48.00	45.66
Mean	65.70	60.83	57.12

Table.4 Incubation period of different isolates of *Bipolaris sorokiniana* on different wheat genotypes

Isolate	Incubation period (days)					
	Sonalika	PBW-343	HD 2733	HD-2967	PBW-660	Mean
BS_1	4.3	4.7	6.3	5.7	6.7	5.54
BS_2	4.0	4.7	6.4	5.3	6.3	5.34
BS_3	4.0	4.3	6.3	5.7	6.7	5.40
BS_4	5.0	4.7	5.3	7.0	7.0	5.80
BS_5	5.3	4.7	6.3	5.7	7.0	5.80
BS_6	5.2	4.7	5.3	6.0	7.7	5.78
BS_7	4.7	5.3	5.0	5.7	7.0	5.54
BS_8	4.0	5.7	6.0	7.0	6.3	5.80
BS_9	3.5	4.3	5.0	5.3	6.0	4.82
BS_{10}	4.3	5.3	5.7	7.3	7.3	5.98
Mean	4.43	4.84	5.76	6.07	6.80	-
CD (0.05)	0.57	0.39	0.39	0.46	0.48	-
SE m ±	0.19	0.13	0.13	0.15	0.16	-

Table.5 Lesions per leaf of wheat genotypes caused by different isolates of *Bipolaris sorokiniana*

Isolate	Average numbers of lesions/leaf					Mean
	Sonalika	PBW-343	HD 2733	HD-2967	PBW-660	
BS_1	5.2	4.2	3.3	1.3	1.2	3.04
BS_2	6.0	5.4	4.0	3.0	2.0	4.08
BS_3	4.2	3.2	3.2	2.1	1.3	2.80
BS_4	4.4	3.0	2.0	1.6	1.3	2.46
BS_5	5.2	3.5	3.0	1.5	1.5	2.94
BS_6	4.1	2.8	2.5	1.5	1.5	2.48
BS_7	4.3	2.9	1.8	2.5	1.6	2.62
BS_8	5.0	4.4	2.6	1.6	1.6	3.04
BS_9	9.0	6.0	5.0	3.4	2.0	5.08
BS_{10}	4.0	1.0	1.8	1.2	1.5	1.90
Mean	5.14	3.64	2.92	1.97	1.55	-
SE(m)±	0.22	0.15	0.15	0.16	0.14	-
CD (0.05)	0.67	0.46	0.46	0.48	0.42	-

Table.6 Development of necrotic lesions on wheat genotypes inoculated with isolates of *Bipolaris sorokiniana*

Genotype	Necrotic lesion size (mm^2)									
	BS_1	BS_2	BS_3	BS_4	BS_5	BS_6	BS_7	BS_8	BS_9	BS_{10}
Sonalika	4.2	6.0	5.2	1.8	2.0	5.4	3.6	4.4	8.5	3.3
PBW-343	2.7	5.2	2.5	0.5	1.5	0.6	5.6	4.2	4.4	1.6
HD 2733	1.6	1.6	1.0	1.8	3.3	0.8	1.4	0.5	2.8	1.3
HD-2967	0.7	1.1	0.4	0.4	0.8	0.7	0.5	0.7	2.4	1.5
PBW-660	1.5	2.2	0.6	0.9	1.9	1.7	0.6	0.4	2.6	2.2
Mean	2.14	3.22	1.94	1.08	1.9	1.84	2.34	2.04	4.14	1.98
SE(m)±	0.13	0.13	0.11	0.08	0.15	0.13	0.13	0.16	0.15	0.15
CD (0.05)	0.42	0.44	0.36	0.25	0.50	0.44	0.44	0.51	0.50	0.48

Table.7 Infection response (IR) of wheat genotypes to isolates of *B. sorokiniana*

Genotype	Infection response (IR)									
	BS_1	BS_2	BS_3	BS_4	BS_5	BS_6	BS_7	BS_8	BS_9	BS_{10}
Sonalika	S	S	S	S	S	S	S	S	S	S
PBW-343	S	MS	MS	MS	MS	MS	MS	MS	S	R
HD 2733	MS	MR	MS	MR	MS	S	MR	S	S	R
HD-2967	MR	R	R	R	R	R	R	R	MS	R
PBW-660	R	S	R	S	R	R	R	R	MS	R

S= Susceptible, MS= Moderately Susceptible, MR= Moderately Resistant, R=Resistant

Table.8 Per cent disease severity on different wheat genotypes with *Bipolaris sorokiniana* isolates

Isolate	Per cent disease severity					Mean
	Sonalika	PBW-343	HD-2733	HD-2967	PBW-660	
BS_1	40.00	24.33	25.10	20.00	10.00	23.88
BS_2	68.50	35.22	15.00	13.50	25.00	31.44
BS_3	35.10	22.50	24.33	10.00	4.50	19.28
BS_4	48.50	25.50	15.00	13.00	25.50	25.50
BS_5	36.22	22.60	24.33	8.66	7.50	19.86
BS_6	38.10	23.20	25.00	9.33	8.33	20.79
BS_7	36.22	22.10	14.33	9.50	8.50	18.13
BS_8	45.10	28.50	28.66	13.20	15.00	26.09
BS_9	69.33	42.50	36.20	24.50	18.00	38.10
BS_{10}	35.00	12.50	12.50	10.00	8.80	15.76

Mathur and Kongsdal (2000) also reported ellipsoid, dark brown to black, smooth, mostly straight or slightly curved conidia having length (40-120µm) and breadth (17-28µm). In another study, Valim *et al.*, (1997) also reported pathogenic and morphologic variation among 10 isolates of *B. sorokiniana* collected from different wheat growing regions in Brazil. Ahmed (2001) also reported 13 physiological groups of *B. sorokiniana* based on their cultural characteristics. Alam *et al.*, (1997) also categorized seven morphological and physiological divergences among 27 isolates of *B. sorokiniana* from Bangladesh. The present result coincides with the finding of Poloni *et al.*, (2008) who studied the morphological variability of monosporic and polysporic *B. sorokiniana* cultures grown in different media and observed high rates of morphological variability in the replicates of polysporic cultures with few differences among monoconidial cultures.

Pathogenic Variability

The mean incubation period for initiation of first symptom of disease ranged from 4.43-6.80 days in wheat genotypes evaluated against *B. sorokiniana* isolates (Table 4). Amongst the isolates, BS_9 was quite fast in developing symptoms in 3.5 days in Sonalika genotype. In PBW-343, the disease development took place from 4.3 to 5.3 days. Minimum incubation period of 4.3 days was recorded in isolate BS_9, whereas BS_7 took the maximum period of 5.3 days. Likewise, in genotype HD 2733, the incubation period ranged from 5 to 6.4 days. In case of genotype HD-2967, isolate BS_9 took the minimum period of 5.3 days for symptom development, while in PBW-660, all the ten isolates took maximum incubation time for symptom initiation. Thus, the minimum average incubation period of 4.82 days was observed in isolate BS_9 and maximum (5.8 days) in isolates BS_4, BS_5, BS_6 and BS_8.

Number of lesions on flag-2 leaf

In polyhouse studies, response to infection by different isolates of *B. sorokiniana* revealed that among the entire wheat germplasm, varieties Sonalika was most susceptible (Table 5) and recorded maximum number of lesions (9.0) in BS_9 followed by PBW-343 (6.0), HD-2967 (3.4) and least in PBW-660 (2.0) followed by isolate BS_2. Two lesions observed in BS_6, BS_7 and BS_{10} isolates. In genotype HD-2967, the maximum number of

lesions (3.4) was observed in isolate BS_9 and minimum (1.2) in isolates BS_{10}. In genotype PBW-660, maximum (2.0) lesions per leaf were observed in case of BS_9 and minimum in 1.2 in BS_1.

Necrotic area (mm^2)

Data (Table 6) revealed that the necrotic area (mm^2) varied from 1.08 to 8.50 in response to *B. sorokiniana* isolates on different wheat genotypes. The maximum area recorded was 8.5 mm^2 in case of isolates BS_9 followed by BS_2 (6.0 mm^2) both in wheat genotype Sonalika. In wheat genotype PBW-343, isolate BS_7 was observed with necrotic area of 5.6 mm^2, while minimum area (0.4 mm^2) was observed in BS_3 and BS_8 isolates in genotype HD-2967 and PBW-660, respectively. The isolate BS_9 again recorded maximum necrotic area of 2.4 mm^2 and 2.6mm^2 on wheat genotype HD-2967 and PBW-660, respectively. It has been inferred from Table 8 that the average necrotic area (4.14 mm^2) was developed in isolate BS_9 followed by BS_2 (3.22 mm^2) and BS_7 (2.34 mm^2), while as the minimum (1.08 mm^2) in case of isolate BS_4.

Infection Response (IR)

Data (Table 7) showed that maximum pathogenic virulence was observed in isolate BS_9, which exhibited maximum frequency of susceptible type (S**)** IRs on 3 genotypes i.e. Sonalika, PBW-343 and HD-2733 and moderately susceptible (MS) response in genotypes HD-2967 and PBW-660. This was followed by BS_6 which showed S-type of IRs on Sonalika and HD-2733, MS type in PBW-343 and R type of IRs in HD-2967 and PBW-660. The isolate BS_{10} was observed as least virulent as all the genotype showed R type of IRs except Sonalika which showed S type of infection response (IR). Isolate BS_4 and BS_2 was the most virulent on resistant genotype PBW-660, which were showed S type IRs, while with the remaining isolates it showed R type IRs.

Terminal disease severity

Data (Table 8) revealed that the maximum disease severity (69.33%) was recorded in isolate BS_9 followed by isolate BS_2 (68.50%) on Sonalika. Minimum score of 4.50, 7.50, 8.33 and 8.50 per cent recorded in isolates BS_3, BS_5, BS_6, and BS_7 on resistant cultivar PBW-660. In wheat genotype HD-2967, isolate BS_5, BS_6 and BS_7 had observed the minimum of 8.66, 9.33 and 9.50 per cent disease severity. The data further revealed that the maximum disease severity has been shown by BS_9 over the wheat genotypes except PBW-660, where the isolate BS_2 was observed as most virulent with the disease severity (25.00%). On the basis of pathogenic response observed in different wheat genotypes, the *B. sorokiniana* isolates were categorized into three groups *viz*., highly virulent, moderately virulent and least virulent. The isolates BS_9 and BS_2 were categorized as highly virulent on the basis of disease severity (31.44-38.10%), less incubation period (4.82, 5.34 days) (Table 6), number of lesions (4.08, 5.08) (Table 7), and size of lesion (3.22,4.14 mm^2) (Table 8), whereas, isolates BS_{10}, and BS_7 were categorized as least virulent which produced disease severity (15.76-18.13%), incubation period (5.54, 5.98 days), number of lesions (1.90, 2.62) and lesion size (1.98, 2.34 mm^2), while rest of the *B. sorokiniana* isolates were in moderately virulent category.

Most of the high yielding varieties which are being grown on commercial scale are found to be more or less susceptible to spot blotch (*B. sorokiniana*) disease. The reasons for lack of substantial durable resistance in the varieties may be attributed to presence of variability in the population (Poloni *et al.,* 2008). In order to develop the disease resistant and high

yielding cultivars, it is imperative to analyse and understand the variability in the pathogen. The host plant resistance depends on the effectiveness of resistance against all biotypes or races of pathogen present in the region. Thus, studies on variability of *B. sorokiniana* have a greater significance in breeding for resistance against spot blotch of wheat. Studies on variability in the pathogen and host are important for documenting resistant sources.

In our study pathogenicity tests revealed significant variation in virulence among *B. sorokiniana* isolates on the test genotypes. All the isolates produced disease on the test host genotypes in a moderate to severe form and none of the test genotypes was free from the disease. The mean disease severity (31.44-38.10%) was reported in isolates BS_2 and BS_9 followed by BS_1, BS_3, BS_4, BS_5, BS_6 and BS_8 in range of 19.28- 26.09 per cent. The isolates BS_7 and BS_{10} exhibited mean disease severity of 15.76-18.13 per cent and categorized as least virulent. The present result also exhibited that maximum pathogenic response noticed in BS_9 having maximum frequencies of susceptible (S) type on Sonalika, PBW-343 and moderately susceptible (MS) type in HD-2967 and PBW-660, whereas isolate BS_{10} observed as least virulent and showed R type of IRs in wheat genotypes except Sonalika (S) type.

On the basis of disease severity, incubation period, number of lesions, and necrotic lesion (mm^2) on wheat genotypes, the isolates of *B. sorokiniana* grouped as three category *viz.* highly virulent, moderately virulent and least virulent. The isolates BS_9 and BS_2 were highly virulent on the basis of mean disease severity (31.44-38.10%), mean number of lesions (4.08-5.08), mean incubation period (4.82-5.34 days) and mean size of lesion (3.22-4.14mm^2), whereas isolates BS_{10}, and BS_7 were categorized as least virulent which produced disease severity (15.76-18.13%), number of lesions (1.90-2.80), incubation period (5.54-5.98 days) and lesion size (1.98-2.34mm^2), while rest of the *B. sorokiniana* isolates were moderately virulent. The present findings gets also support by earlier reports of Chauhan *et al.*, (2007) who had grouped different isolates of *B. sorokiniana* into three categories viz. highly virulent, moderately virulent and least virulent based on pathogenicity test. Aggarwal *et al.*, (2009) reported five pathotypes out of 40 isolates of *B. sorokiniana* tested on 14 wheat genotypes at seedling stage. Akram and Singh (2001) also reported that among 9 isolates, the maximum incubation period (3.4 days), minimum lesion length (2.4 mm) and moderate sporulation (12.2 spore/mm^2 of disease leaf area) was observed in isolate PHS3. They had also observed IP (2.2), the maximum LL (l.5 mm) and highest sporulation (17.3) in isolate PHS3. On the basis of observations, isolate PHS3 was found highly virulent and PHS1 was least virulent on 24 wheat genotypes. Independent of the fact that *B. sorokiniana* has predominantly asexual, haploid and heterokaryotic reproduction, the variability in virulence suggested that extensive genetic exchange occurs in this species (Farias *et al.*, 2005). This may be due to the presence of nuclei in the mycelium and conidia. The variation could be generated when the exchange of nuclei is followed by nuclear fusion, somatic recombination and the consequent chromosomal rearrangement for the haploidization. Thus the present finding of pathogenic variability observed in isolates of *B. sorokiniana* can be very useful in resistance breeding programmes against spot blotch disease of wheat.

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