Journal of Crop and Weed, 17(3): 118-129 (2021)

ISSN- O : 2349 9400 ; P : 0974 6315

http://cwssbckv.org
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Variability among *Bipolaris sorokiniana* isolates of wheat from North-Eastern Plain Zones of India

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Received : 11.06.2021 ; Revised : 26.09.2021 ; Accepted : 06.10.2021

DOI: https://doi.org/10.22271/09746315.2021.v17.i3.1500

ABSTRACT

This experiment was conducted to study the morphological, cultural and biochemical variability among fourteen isolates of Bipolaris sorokiniana (Shoem) causing spot blotch of wheat from different locations of northeastern plain zones of India. Colony colour varied in three different media and changed with as the culture became older. Morphological variability was studied by comparing their conidial size (both length and breadth) and number of septations both from the isolates of natural infected leaves and from culture media. Based on α- and β - esterase profiling, the biochemical variability existed among the isolates. Positive activity was reported in case of both α- and β-esterase. α-Esterase enzyme observed the highest enzyme activity in terms of maximum numbers of banding loci as compared to β-esterase. From this study it can be concluded that cultural, morphological and biochemical variability existed among the isolates of B. sorokiniana which may be associated with the pathogenicity within the host plant.

***Keywords*:** Biochemical and morphological variability, *Bipolaris sorokiniana*, spot blotch and wheat

Wheat production played a crucial role in green revolution by securing food security in densely populated regions of the world (Sultana *et al.,* 2017). The demand of wheat has boosted up in last few years due to increase in populations in the countries like, India, Bangladesh, Pakistan and Nepal (Kumar *et al*., 2015; Singh *et al.,* 2015) and now nearly 20% of the total world food requirements are meet by wheat production alone (Uddin *et al*., 2006). There are a number of constrains behind the success in wheat production and the occurrence of diseases as an important biotic cause of reduction in yield (Singh *et al*., 2016, Kumar *et al.,* 2019; Tamang *et al*., 2020).

Spot blotch of wheat caused by *Bipolaris sorokiniana* (BS) is one of the most serious disease which is favored by the warm and humid climatic conditions (Dubin and Ginkel, 1991; Singh *et al*., 2016; Devi *et al.,* 2019). Spot blotch causes severe yield loss of wheat in South Asian countries along with India and under favorable conditions and severe infection, the losses reached up to 80-100% (Joshi and Chand, 2002), even every one unit increase in disease severity there is significant effect on avoidable yield loss of wheat (Devi *et al.,* 2018). *B. sorokiniana* (BS*)* and *A. triticina* can attack singly or together and cause yield loss more than 60% in West Bengal as well as all over Eastern part of India (Prabhu and Singh, 1974; Devi *et al.,* 2012). The disease usually appears at any stage of the crop starting from seedling stage and diseases severity increases with the age of the plant (Devi *et al.,* 2018; Singh *et al.,* 2014).

Almost all released varieties are susceptible to spot blotch of wheat at different degrees due to mutation of new races along with changing climatic conditions but not a single genotype showed highly resistant towards the pathogen due to high variability occurring in pathogenic fungi and narrow genetic spectrum for resistance in currently available wheat cultivars (Kumar *et al.,* 2019). Pathogenic variability of a pathogen has a crucial role in their management (Joshi *et al*., 2007) and the cheapest way to manage the spot blotch of wheat by cultivation of resistant varieties. The new emerging virulent strains and races of BS become global threat which can lower the wheat production and productivity.

In present study, pathogenic variability had been studied among 14 isolates of *B. sorokiniana* (BS) collected from different parts of north-eastern plain zone of India with the aim to determine and compare existence of pathogenic variability based upon the components of spot blotch disease development by BS and aggressiveness of the pathogen associated to agro climatic conditions.

MATERIALS AND METHODS

Collection of samples and isolation

Small bits of the infected materials collected from the infected field were taken and washed thoroughly in distilled water. Surface sterilization was done by immersing these bits in $HgCl_2$ (0.1%) solution for 30 seconds followed by thorough rinsing with distilled

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water to remove the mercury particles and then 1-2 diseased leaf bits were transferred to each Petri plate (sterilized) containing potato dextrose agar (PDA). The Petri plates were incubated at 27±1°C and kept under observation for their periodical growth.

Single spore isolation

Fifteen days old culture was taken to make spore suspension by making a dilution using sterilized distilled water and 10 ml of clear, filtered 2% water agar used to grow the pathogen. One ml of spore suspension was spread on agar plate and were incubated for 12 hr at 27 ± 10°C. These plates were observed under the microscope to find out the single germinated conidium.

Identification

The various isolates were collected from different locations of northeastern plain zones and their details are given in Table 1.

Cultural variability of BS isolates

Cultural variability in respect to colony colour and its margin, mycelium morphology in PDA medium had been shown in fourteen numbers of single spore-cultures of BS isolates collected from different locations.

Morphological variability of BS isolates

Fourteen different BS isolates showed morphological variability both in culture media and diseased plant sample. For this purpose, the slides had been prepared from the spore suspension (infected leaf sample) and selected fungal cultures on PDA media with a view to study the morphology of the fungal pathogen such as length and breadth of conidia, number of septations and length of the beak. The observations were done under phase-contrast microscope. By using micrometric measurements, the photographs and measurements of conidia were taken.

Growth rate on different media

Four different culture media *viz*., potato carrot agar (PCA), potato dextrose agar (PDA), oatmeal agar (OMA) and corn meal agar (CMA) were used to find out the variation among different isolates of BS collected from different symptoms producing wheat leaf in respect to their growth rate.

Biochemical variability

Isozyme (Alpha esterase)

Electrophoretic separation of enzymes, that exploits the polymorphism of detected isozyme forms have been used to generate a large number of markers for the assessment of genetic diversity within the fungal isolates (Clark *et al.*, 1989, Oudemans and Coffey, 1991). So based on α-esterase profiling among BS isolates the study was conducted to determine the biochemical variability.

On native PAGE by following the methods of Davis (1964), electrophoretic separation of the extracts was carried out and gels were stained by using different enzymes. Two separate runs were conducted to determine reproducibility of the bands and also to calculate the relative mobility (Rm) values. Based on the presence or absence of matrix of the bands of each isolate, values of each of the isozyme were determined.

Isozyme (Beta esterase)

To study the biochemical variability based on β-esterase profiling among BS isolates collected from different symptoms of wheat plants, a separate experiment was conducted.

RESULTS AND DISCUSSION

Morphological variability from infected leaf samples

Different isolates from disease specimens showed variability with regard to their conidial size and also showed variability in respect to number of septations. The average conidial length of the isolate BS_5 was found to be larger (154.35 ± 4.16 µm) followed by BS_8 (139.73 ± 3.11 µm) and the difference was statistically significant. The average conidia length of the isolate BS_3 was found to be smallest (64.19 ± 3.28 µm) statistically at par with BS_4 (68.61 ± 4.04µm) followed by BS_9 (71.22 ± 4.16µm), BS_{13} (76.25 ± 2.13µm) and BS_{10} (80.57 ± 4.75µm) and the difference were statistically significant.

All the isolates were significantly differed among themselves in case of breadth of conidia. Maximum breadth was noticed on BS_2 (24.14 ± 1.72 µm) statistically at par with BS_8 (24.11 ± 2.28 µm) followed by BS_5 (23.15 ± 1.31 µm) and their difference was statistically significant (Fig.1).

Average number of septa ranges from 6 ± 1.24 to 10 ± 0.83. Maximum number of septation was noticed in BS_{11} (10 ± 0.83) and minimum in BS_2 (6 ± 1.24) followed by BS_1 (7 ± 1.07), BS_6 (7 ± 1.8) and was statistically significant except the later two isolates. Other isolates were to some extent have similar number of septations (Table 2).

Based on data for morphological variation of fourteen different isolates of BS from direct plant sample, the dendrogram (Fig.3) was constructed. This dendrogram identified two major clusters with 25% Euclidean distance. One cluster comprised of 12 isolates BS_4, BS_9, BS_3, BS_1, BS_{10}, BS_{11}, BS_{13}, BS_2, BS_{12}, BS_7, BS_{14} and BS_6 (group I) while another cluster comprised of remaining two isolates BS_5 and BS_8 (group II). Group I was further sub clustered into two groups, of which seven isolates BS_4, BS_9, BS_3, BS_1, BS_{10}, BS_{11} and BS_{13}

Table 1: Description of *Bipolaris sorokiniana* isolates isolated from different symptoms producing infected wheat crop

Designation	Symptoms	Place	Location (GPS)
BS_1	Infected leaf margin	Chakdaha	N22°58′4.9728″ E88°32′44.214″
BS_2	Infected leaf tip and leaf margin	Petrapol	N23°03′59.532″ E88°87′68.989″
BS_3	Infected leaf margin and also scatter leaf spot	Shukpukuria	N23°4′528.3404″ E88°49′25.408″
BS_4	Infected leaf top, margin and scattered leaf spot	Gobrapur	N23°13′0789″ E88°81′4294″
BS_5	Only scatter leaf spot	Helencha	N23°18′7881″ E88°86′0128″
BS_6	Infected leaf tip and also scatter leaf spot	Chetla	N22°51′8415″ E88°33′1664″
BS_7	Infected half of the leaf tip and also scatter leaf spot	Baranbaria	N23°17′25.1664″ E88°42′9.5976″
BS_8	Infected midrib and also scatter leaf spot	Karimpur	N23°32′41.8488″ E88°32′19.5576″
BS_9	Infected leaf tip and midrib	Jalangi	N24°5′20.724″ E88°41′45.9852″
BS_{10}	Infected leaf tip, midrib and also scatter leaf spot	Madhuban	N24°7′25.0716″ E88°40′16.05″
BS_{11}	Scattered leaf spot with prominent yellow halo	Domkal	N24°7′43.5504″ E88°35′21.7896″
BS_{12}	Leaf completely burnt	Islampur	N24°9′42.6996″ E88°28′42.0204″
BS_{13}	Infected half of the leaf tip and also scatter leaf spot	Dakshin Dinajpur	N25°26′4.9728″ E88°90′44.214″
BS_{14}	Only scatter leaf spot	Akhiriganj	N24°18′16.434″ E88°23′37.4892″

Table 2: Morphological characters of different isolates of *Bipolaris sorokiniana* from direct plant sample

Isolates	Size of Conidia		Average septation
	Length(µm) Average	Breadth(µm) Average	
BS_1	81.82 ± 4.24	16.47 ± 0.73	7 ± 1.07
BS_2	109.67 ± 5.27	24.14 ± 1.72	6 ± 1.24
BS_3	64.19 ± 3.28	14.34 ± 0.94	8 ± 0.85
BS_4	68.61 ± 4.04	14.73 ± 1.39	8 ± 2.04
BS_5	154.35 ± 4.16	23.15 ± 1.31	8 ± 1.95
BS_6	91.04 ± 3.85	17.47 ± 1.27	7 ± 1.28
BS_7	99.16 ± 3.94	15.43 ± 0.94	8 ± 1.17
BS_8	139.73 ± 3.11	24.11 ± 2.28	8 ± 1.09
BS_9	71.22 ± 4.16	14.98 ± 1.33	8 ± 0.80
BS_{10}	80.57 ± 4.75	13.82 ± 0.90	8 ± 0.94
BS_{11}	85.15 ± 5.48	13.73 ± 1.55	10 ± 0.83
BS_{12}	105.59 ± 4.18	20.90 ± 1.23	9 ± 1.09
BS_{13}	76.25 ± 2.13	16.99 ± 1.28	9 ± 1.00
BS_{14}	97.16 ± 4.90	13.69 ± 1.24	8 ± 0.94
SEm(±)	**1.410**	**0.260**	**0.112**
LSD (0.05)	**4.085**	**0.753**	**0.326**

Table 3: Comparative cultural and morphological characters of *Bipolaris sorokiniana* isolates on PDA after 10 days of inoculation

Name of Isolates	Mycelial growth	Colony colour	Margin of colony	Sporulation	Size of the conidia		Septation
					Length (µm)	Breadth (µm)	
BS_1	Thick velvety growth withoutZonation	Greyish black	Regular	++++	56.09 ± 7.32	17.19 ± 2.35	10 ± 0.47
BS_2	Thick growth with zonation	Greyish white	Regular	+++	62.16 ± 8.34	19.96 ± 1.95	9 ± 1.25
BS_3	Thick velvety growth withoutZonation	Greyish white	Irregular	+++	48.95 ± 8.18	18.05 ± 2.28	8 ± 0.82
BS_4	Thick growth without zonation	Greyish white	Irregular	+++	51.45 ± 5.51	19.21 ± 1.41	8 ± 2.16
BS_5	Thick and cottony growthwithout zonation	Whitish	Regular	++	65.08 ± 7.05	20.38 ± 1.17	9 ± 0.47
BS_6	Thick and fluffy growthwithout zonation	Whitish grey	Regular	++	58.33 ± 3.63	18.59 ± 0.92	9 ± 0.47
BS_7	Thick velvety growth withZonation	Greyish white	Regular	+++	60.79 ± 7.27	19.19 ± 2.77	9 ± 0.94
BS_8	Thick velvety growth withZonation	Greyish black	Irregular	++++	64.56±7.13	20.81 ± 2.63	8 ± 0.82
BS_9	Thick growth with zonation	Greyish black	Irregular	++++	52.43 ± 7.50	18.54 ± 2.85	8 ± 0.82
BS_{10}	Thick growth with zonation	Greyish white	Regular	+++	55.07 ± 6.36	19.40 ± 2.40	8 ± 0.47
BS_{11}	Thick growth with zonation	Greyish black	Irregular	++++	56.92 ± 7.05	17.69 ±1.84	8 ± 0.94
BS_{12}	Thick growth without zonation	Dark grey	Regular	++++	61.21± 10.57	20.90 ± 2.14	8 ± 0.47
BS_{13}	Thick growth without zonation	Dark grey	Regular	++++	54.20 ± 7.41	18.94 ± 1.92	9 ± 1.63
BS_{14}	Thick growth withoutzonation	Grayish white	Regular	+++	59.05 ± 8.88	15.52 ± 2.95	9 ± 0.47
SEm(±)					0.816	0.273	0.117
LSD (0.05)					2.364	0.790	0.340

Note: ++++ - Excellent - > 20 conidia per microscopic field, +++ - Good – 15-20 conidia per microscopic field
++ - Fair – 10-15 conidia per microscopic field, + - Poor - < 10 conidia per microscopic field

Table 4: Growth rate of *Bipolaris sorokiniana* isolates on PDA media

Isolates	Growth rate on different media (mm day^{-1})				
	PDA	**PCA**	**OMA**	**CMA**	**Mean**
BS_1	13.38	7.31	11.10	11.87	10.91
BS_2	13.93	6.77	11.17	8.25	10.03
BS_3	13.29	3.89	8.83	9.54	8.89
BS_4	12.94	4.13	8.80	7.23	8.27
BS_5	13.35	3.96	10.46	5.94	8.43
BS_6	13.04	3.81	9.55	8.45	8.71
BS_7	13.27	5.66	9.58	11.73	10.06
BS_8	12.62	3.67	12.75	9.65	9.67
BS_9	13.11	7.63	12.18	8.74	10.41
BS_{10}	12.44	4.94	11.87	8.32	9.39
BS_{11}	13.55	6.42	10.61	8.76	9.83
BS_{12}	13.04	8.75	12.76	10.90	11.36
BS_{13}	13.43	9.98	8.68	7.89	9.99
BS_{14}	12.93	7.04	10.77	8.08	9.71
Mean	13.16	6.00	10.65	8.95	
	SEm (±)		**LSD (0.05)**		
Isolates	0.061		0.170		
Media	0.032		0.091		
Isolates × Media	0.121		0.339		

PDA (Potato dextrose agar), PCA (Potato carrot agar), OMA (Oat meal agar), CMA (Corn meal agar)

were kept under first sub cluster (group IA), while five isolates BS_2, BS_{12}, BS_7, BS_{14} and BS_6 were kept under second sub cluster (group IB). Group IA was further sub divided into two clusters which comprised of three isolates BS_4, BS_9 and BS_3, i.e., group IAa, showing their close relationship while in isolate BS_1, BS_{10}, BS_{11} and BS_{13} group under IAb, whereas isolate BS_{13} formed separate individual cluster. Similarly group IB was further sub divided into two clusters which comprised of two isolates BS_2 and BS_{12}, i.e., group IBa showing their close relationship while in isolate BS_7, BS_{14} and BS_6 group under IBb, showing close relationship. In group II, that was not further sub-clustered and consisted of only two isolates BS_5 and BS_8 showing close relationship.

Variability on culture media

Variability in respect to their size of conidia had also been observed among the different isolates. It was observed that the size of conidia of 14 different isolates in artificial media, size of conidia and septations were shorter than the isolates collected from direct plant sample, the direct plant sample isolates produced comparatively larger size of conidia (Fig. 2)

The maximum average conidial length was found in BS_5 (65.08 ± 7.05µm) statistically at par with BS_8 (64.56±7.13µm) followed by BS_2 (62.16 ± 8.34µm), BS_{12} (61.21± 10.57µm) and BS_7 (60.79 ± 7.27µm) and their difference was statistically similar. The average conidial length of the isolate BS_{14} and BS_6 were 59.05 ± 8.88µm and 58.33 ± 3.63µm, respectively and their difference was statistically significant. Minimum conidial length was noticed in BS_3 (48.95 ± 8.18µm) followed by BS_4 (51.45 ± 5.51 µm) which were statistically at par with BS_9 (52.43 ± 7.50 µm) (Table 3).

In case of breadth of conidia, the maximum value was noticed on BS_{12} (20.90 ± 2.14 µm) statistically at par with BS_8 (20.81 ± 2.63 µm) and BS_5 (20.38 ± 1.17 µm) followed by BS_2 (19.96 ± 1.95 µm), BS_{10} (19.40 ± 2.40 µm), BS_4 (19.21 ± 1.41 µm) and BS_7 (19.19 ± 2.77 µm) and the difference was statistically similar. Whereas, in BS_{14} (15.52 ± 2.95 µm) minimum breadth value was noticed followed by BS_1 (17.19 ± 2.35 µm) and BS_{11} (17.69 ± 1.84 µm) and the difference was statistically significant.

In PDA medium, the average number of horizontal septa of isolates varied from 8 ± 0.47 to 10 ± 0.47 and maximum septum was observed on BS_1 (10 ± 0.47) followed by BS_2, BS_5, BS_6, BS_7, BS_{13} and BS_{14} which produced similar number of horizontal septa (9 ± 1.25, 9 ± 0.47, 9 ± 0.47, 9 ± 0.94, 9 ± 1.63 and 9 ± 0.47, respectively). Horizontal septa produced by the isolates BS_3, BS_4, BS_8, BS_9, BS_{10}, BS_{11} and BS_{12} were almost similar ranging from 8 ± 0.47 µm to 8 ± 2.16 µm (Table 2).

The dendrogram (Fig.4) based on data for morphological variation of fourteen different isolates

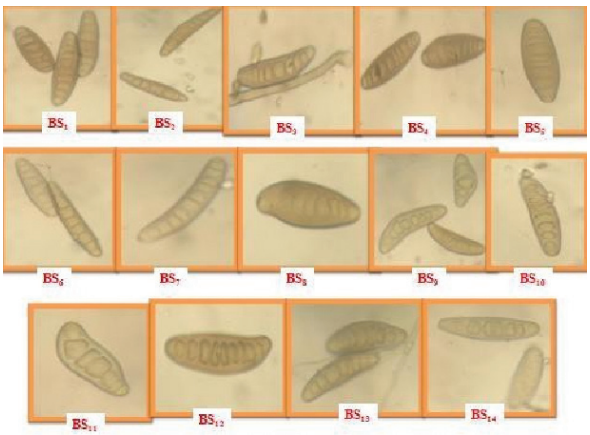

Fig. 1: Morphological characters of BS isolates from wheat leaf sample

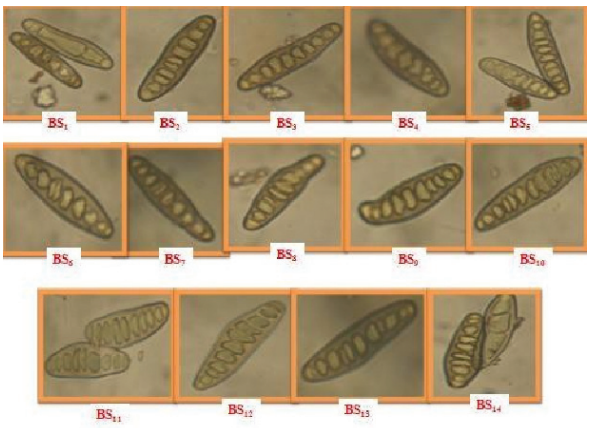

Fig. 2: Morphological characters of BS isolates from wheat leaf sample on Potato dextrose agar (PDA)

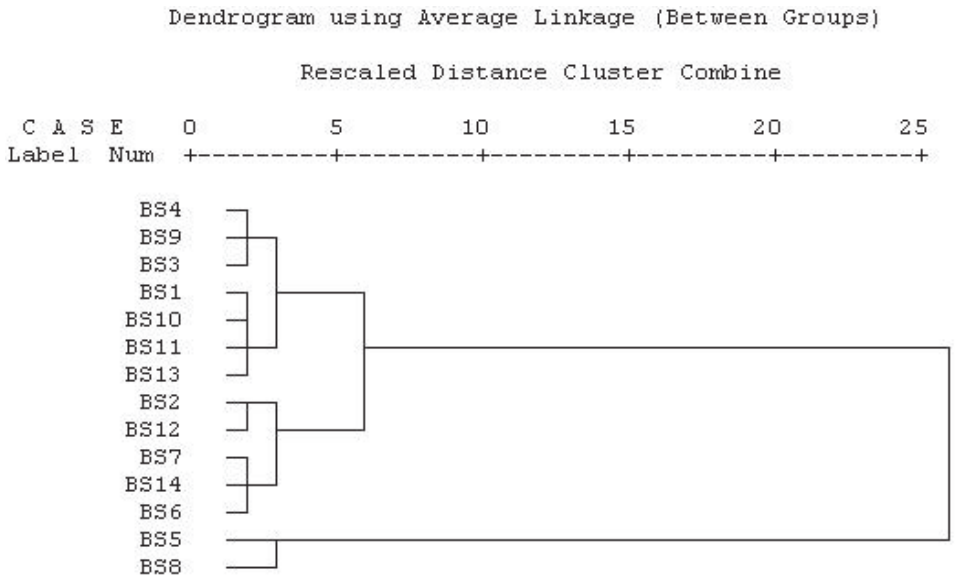

Fig. 3: Morphological dendrogram of different *Bipolaris sorokiniana* isolates from direct plant sample

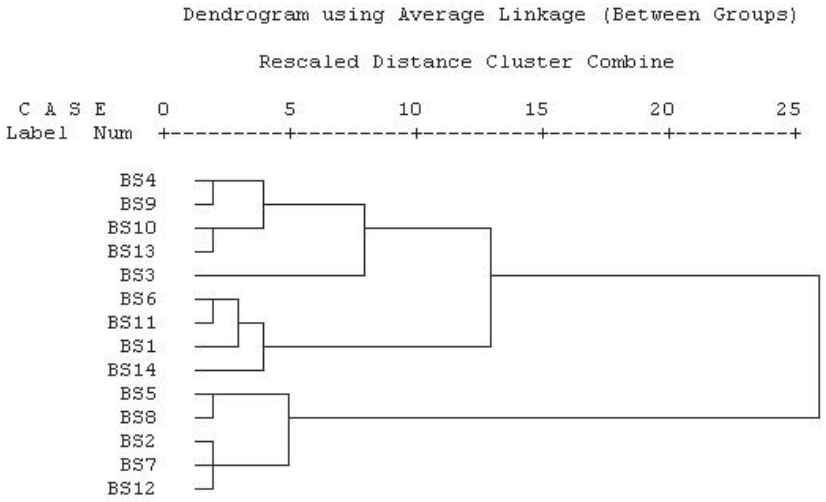

Fig. 4: Morphological dendrogram of different *Bipolaris sorokiniana* isolates from culture media

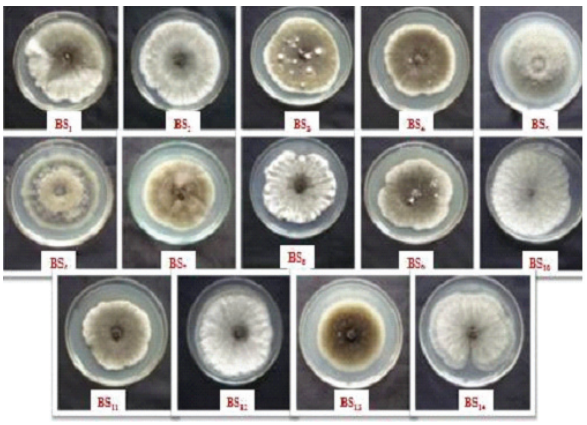

Fig. 5: Cultural characters of BS isolates from leaf blight infected wheat leaf on Potato dextrose agar (PDA) after 10 days of inoculation

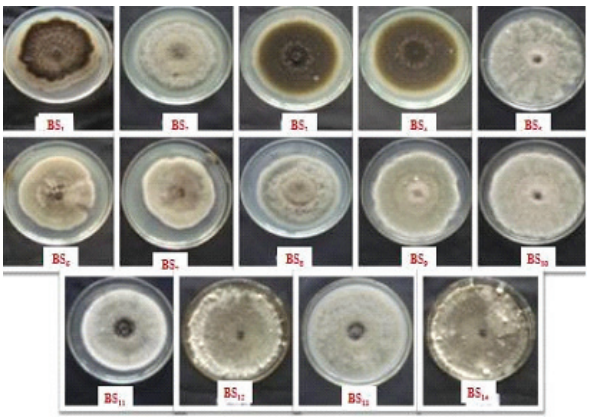

Fig. 6: Cultural characters of BS isolates from leaf blight infected wheat leaf on Potato carrot agar (PCA) after 10 days of inoculation

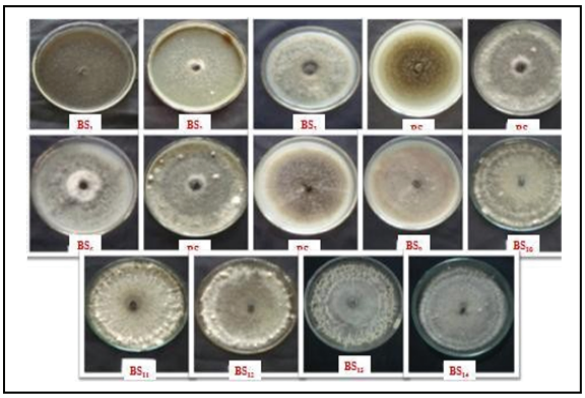

Fig. 7: Cultural characters of BS isolated from leaf blight infected wheat leaf on Oat meal agar (OMA) after 10 days of inoculation

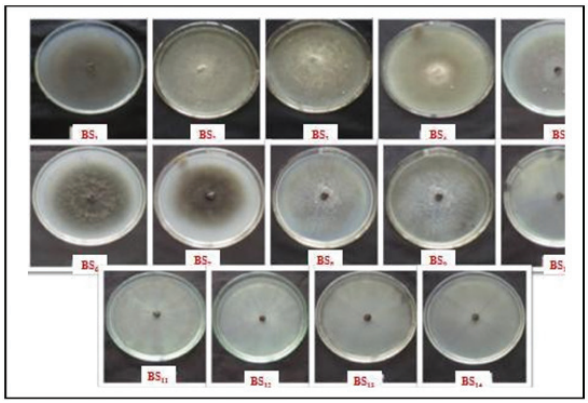

Fig. 8: Cultural characters of *BS* isolated from leaf blight infected wheat leaf on Corn meal agar (CMA) after 10 days of inoculation

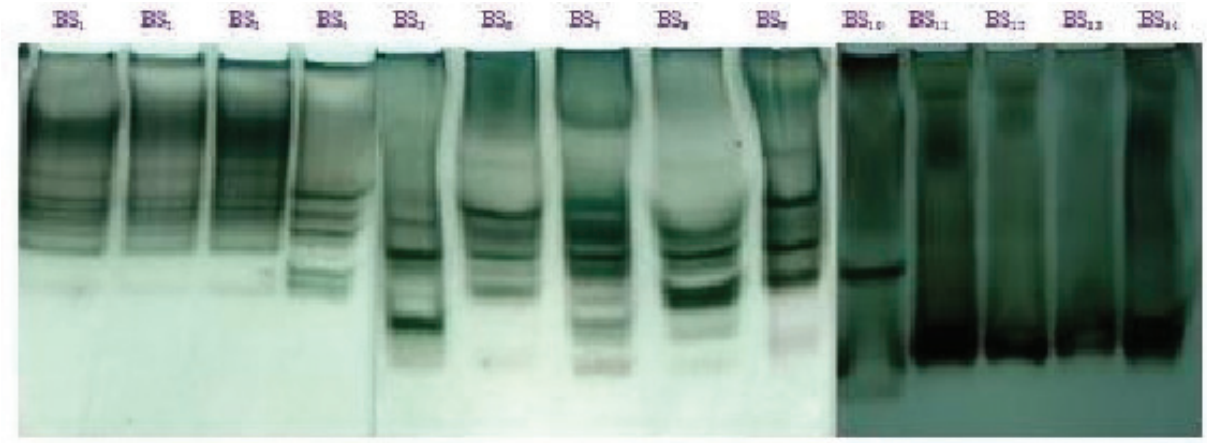

Fig. 9: Alpha esterase isozyme profiling of *Bipolaris sorokiniana* isolates collected from spot blotch infected wheat plants

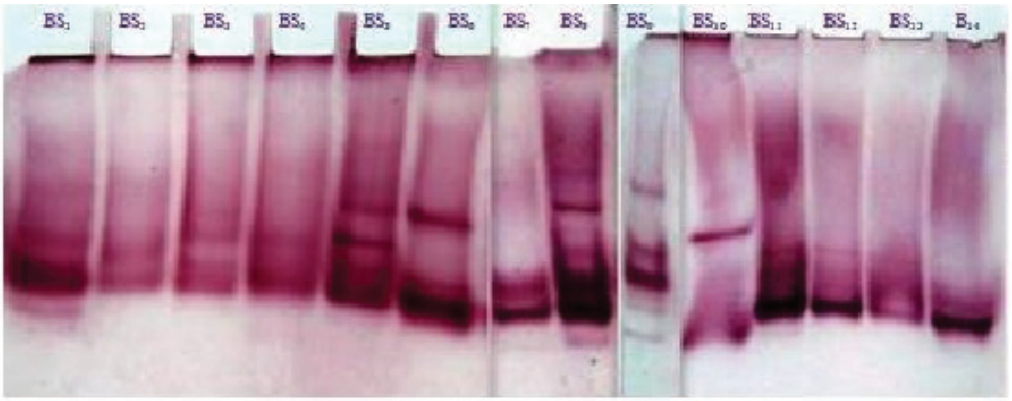

Fig. 10: Beta esterase isozyme profiling of *Bipolaris sorokiniana* isolates collected from spot blotch infected wheat plants

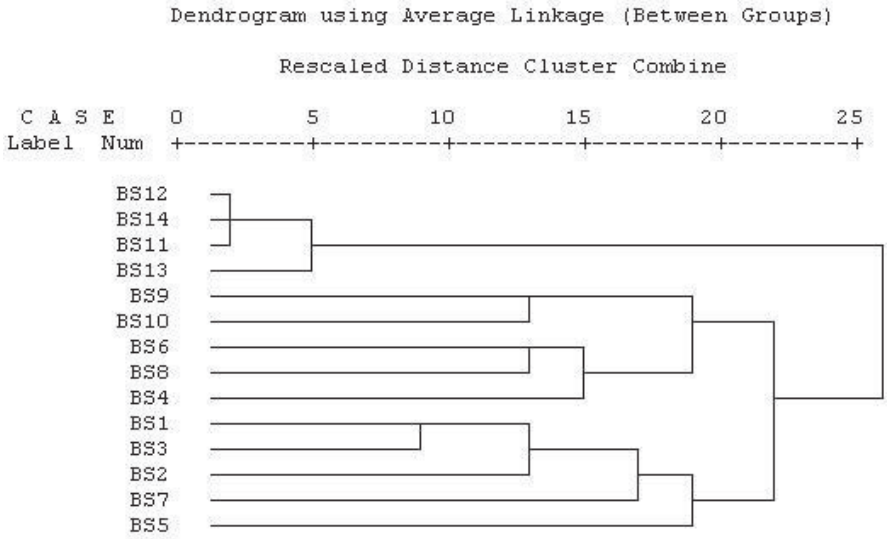

Fig. 11: Dendrogram for Alpha esterase isozyme data, showing relationships among *Bipolaris sorokiniana* isolates

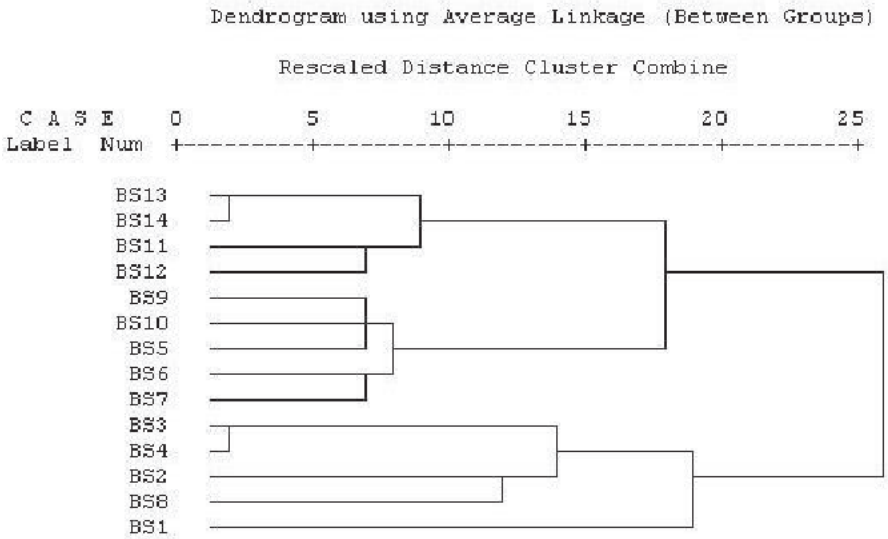

Fig. 12: Dendrogram for Beta esterase isozyme data, showing relationships among *Bipolaris sorokiniana* isolates

of BS from culture media was constructed. The dendrogram had identified two major clusters with 25% Euclidean distance. One cluster comprised of nine isolates BS_4, BS_9, BS_{10}, BS_{13}, BS_3, BS_6, BS_{11}, BS_1 and BS_{14} (group I) While another cluster comprised of remaining five isolates BS_5, BS_8, BS_2, BS_7 and BS_{12} (group II). Group I was further sub clustered into two groups, of which first sub cluster had five isolates BS_4, BS_9, $BS_{10,}$ BS_{13} and BS_3 (group IA) while second sub cluster had four isolates BS_6, BS_{11}, BS_1 and BS_{14} (group IB). Again, group IA was further sub divided into two clusters which comprised of four isolates BS_4, BS_9 and BS_{10} i.e., group IAa, showing their close relationship while BS_{13} formed separate individual cluster and in group IAb consist of only one isolate BS_3 and formed separate individual cluster. Similarly group IB was further sub divided into two clusters which comprised of three isolates BS_6, BS_{11} and BS_1 i.e., group IBa, whereas, BS_1 formed separate individual cluster and in group IBb, isolate BS_{14} formed separate individual cluster. Group II was further sub-clustered into two groups, first sub cluster (group IIA) had two isolates BS_5 and BS_8 showing their close relationship and another sub cluster (group IIB) comprised of three isolates BS_2, BS_7 and BS_{12} showing their close relationship.

It is concluded that isolates of *B. sorokiniana* showed variability in morphological and cultural characters. However, it needs to be confirmed by collecting more isolates from different geographical regions/hosts and studying their pathogenic behavior on different varieties /germplasm lines. The findings of the present investigation clearly showed that cultural and morphological variability exists in *B. sorokiniana*.

Growth rate on different media

Four different culture media viz. potato dextrose agar (PDA), potato carrot agar (PCA), oatmeal agar (OMA) and corn meal agar (CMA)) were used to find out the variation among different isolates of BS collected from different symptoms producing wheat leaf in respect to their growth rate (Fig: 5-8). The results (Table 3-4) revealed that media, isolates and the interaction effect of media and isolates were statistically significant in respect to the growth rate of the isolates. The growth rate of each isolates showed differences on different media.

On PDA media, all the 14 isolates showed different growth rates and their differences were statistically significant. BS_2 exhibited maximum growth rate (13.93 mm day^{-1}) followed by BS_{11} (13.55 mm day^{-1}) and BS_{13} (13.43 mm day^{-1}) statistically at par with BS_1 (13.38 mm day^{-1}) and BS_5 (13.35 mm day^{-1}). Minimum growth rate was found in BS_{10} (12.44 mm day^{-1}) and BS14 (12.93 mm day^{-1}) followed by BS_8 (12.62 mm day^{-1}) and the differences were statistically significant.

On PCA media, maximum growth rate was observed in BS_{13} (9.98 mm day^{-1}) followed by BS_{12} (8.75 mm day^{-1}), BS_9 (7.63 mm day^{-1}) and BS_1 (7.31 mm day^{-1}) and the differences were statistically significant. The minimum growth rate was noticed on BS_3 (5.48 mm day^{-1}) followed by BS_4 (5.61 mm day^{-1}) and BS_7 (5.66 mm day^{-1}) and they were statistically at par with each other.

On OMA media, maximum growth rate was noticed in BS_{12} (12.76 mm day^{-1}) statistically at par with BS_8 (12.75 mm day^{-1}) followed by BS_9 (12.18 mm day^{-1}) and BS_{10} (11.87 mm day^{-1}) and their differences were statistically significant except first two isolates. BS_{13} (8.68 mm day^{-1}) showed the minimum growth rate and was statistically at par with BS_4 (8.80 mm day^{-1}) and BS_3 (8.83 mm day^{-1}).

On CMA media, maximum growth rate was noticed on BS_1 (11.87 mm day^{-1}) statistically at par with BS_7 (11.73 mm day^{-1}) followed by BS_{12} (10.90 mm day^{-1}). Growth of BS_8 (9.65 mm day^{-1}) was statistically at par with BS_3 (9.54 mm day^{-1}) whereas, minimum growth rate was noticed on BS_5 (5.94 mm day^{-1}) followed by BS_4 (7.23 mm day^{-1}) and BS_{13} (7.89 mm day^{-1}) and the difference was statistically significant.

So, among the different culture media, PDA showed maximum influence on growth rate (13.93 mm day^{-1}) followed by OMA (12.76 mm day^{-1}) and minimum on PCA (5.48 mm day^{-1}) irrespective of isolates. Irrespective of media maximum growth rate was shown by BS_2 (13.93 mm day^{-1}) followed by BS_{11} (13.55 mm day^{-1}) and minimum by BS_3 (5.48 mm day^{-1}) followed by BS_4 (5.61 mm day^{-1}) and their differences were statistically significant.

Biochemical variability

Alpha esterase Isozyme

The activity of α-esterase produced distinctive darker bands, whereas, dark pink bands showed the activity of β-esterase and it was easy to score α-esterase and β-esterase according to their band colours. Positive activity was shown for both α- and β- esterase. α- esterase enzyme showed the highest enzyme activity by producing maximum numbers of banding loci among the two isozymes tested (Fig. 9).

All the isolates showed different banding patterns and maximum were observed in isolate BS_7 by producing 8 banding patterns. Two isolates (BS_1 and BS_3) produced 7 banding patterns and three isolates (BS_2, BS_4 and BS_6) produced 6 banding patterns. Isolate BS_8 produced 5 banding patterns. Whereas, two isolates (BS_5 and BS_9) produced 4 banding patterns. It was observed that all the isolates produced loci of Rm value

0.33 except BS_4, BS_5, BS_8, BS_9, BS_{10} and BS_{13}. Another locus of Rm value of 0.49 was also produced except by BS_9, BS_{10}, BS_{11}, BS_{12}, BS_{13} and BS_{14}. Similarly, Rm value of 0.53 was also present in all the isolates except BS_4, BS_{10}, BS_{11}, BS_{12}, BS_{13} and BS_{14}. Loci of Rm value of 0.71 was also produced in all the isolates except BS_2, BS_4, BS_6, BS_8, BS_9 and BS_{10}. Seven isolates produced one loci of Rm value 0.41 except BS_5, BS_8, BS_{10}, BS_{11}, BS_{12}, BS_{13} and BS_{14}. Similarly, 7 isolates produced loci of Rm value 0.58 except isolate BS_1, BS_3, BS_6, BS_{11}, BS_{12}, BS_{13} and BS_{14}. Six isolates (BS_1, BS_4, BS_6, BS_8, BS_9 and BS_{10}) produced same bands on Rm value of 0.63. Isolates BS_3, BS_4, BS_6, BS_7 and BS_8 produced another Rm value of 0.60. The four isolates BS_1, BS_2, BS_3, and BS_4 were also produced one loci of Rm value 0.39. The highest Rm value 0.78 was observed on 6 isolates were BS_7, BS_{10}, BS_{11}, BS_{12}, BS_{13} and BS_{14}. The result indicated that all 14 isolates had different ∝-esterase isozyme pattern.

A dendrogram was constructed by UPGMA clustering as presented in the Fig. 11. This dendrogram identified two major clusters with 25% Euclidean distance. One cluster (group I) comprised of four isolates BS_{12}, BS_{14}, BS_{11} and BS_{13} while other cluster (group II) comprised of 10 isolates BS_9, BS_{10}, BS_6, BS_8, BS_4, BS_1, BS_3, BS_2, BS_7 and BS_5. Group I was further sub-clustered into two groups, of which first sub-cluster had three isolates BS_{12}, BS_{14} and BS_{11} (group IA) and isolate BS_{13} was under separate individual cluster. Group II was further sub-clustered into two, of which five isolates BS_9, BS_{10}, BS_6, BS_8 and BS_4 were under first sub-cluster (group IIA) while five isolates BS_1, BS_3, BS_2, BS_7 and BS_5 were in the second sub-cluster (group IIB). Again, group IIA was further sub divided into two clusters which comprised of two isolates BS_9 and BS_{10} i.e., group IIAa, showing their close relationship whereas three isolates BS_6, BS_8 and BS_4 were group in IIAb and isolate BS_4 was in separate individual cluster formed separate individual cluster. Similarly group IIB was further sub divided into three clusters i.e., group IIBa, which comprised of three isolates BS_1, BS_3 and BS_2 and isolate BS_2 was in separate individual cluster formed separate individual cluster while in group IIBb, isolate BS_7 formed separate individual cluster and in group IIBc isolate BS_5 also formed separate individual cluster.

Beta esterase Isozyme

All the isolates have different banding pattern and maximum was observed on isolate BS_2 producing loci 7. Two isolates (BS_3 and BS_9) produced 6 banding patterns and another seven isolate produced 5 banding patterns (BS_1, BS_4, BS_5, BS_6, BS_7, BS_8 and BS_{10}). Isolate BS_{11} produced 4 banding patterns, whereas, isolate BS_{13} produced 3 banding patterns and isolates BS_{12} and BS_{14} produced two banding patterns. Among the 14 isolates except BS_1, BS_{12}, BS_{13} and BS_{14}, all other isolates produced loci of Rm value 0.69. Similarly, except BS_8, BS_{11}, BS_{12}, BS_{13} and BS_{14} all other isolates produced loci of Rm value 0.64. 8 isolates (BS_1, BS_2, BS_3, BS4, BS_5, BS_7, BS_8 and BS_9) produced loci of Rm value 0.52. Similarly, another 8 isolates (BS_5, BS_6, BS_7, BS_9, BS_{10}, BS_{11}, BS_{13} and BS_{14}) produced another Rm value of 0.74. 8 isolates BS_6, BS_7, BS_8, BS_{10}, BS_{11}, BS_{12}, BS_{13} and BS_{14} produced other loci of highest Rm value of 0.78. Six isolates (BS_2, BS_3, BS_5, BS_9, BS_{10} and BS_{13}) produced loci of Rm value 0.58. Among the 14 isolates, 5 isolates (BS_1, BS_3, BS_4, BS_6 and BS_9) produced loci of Rm value 0.49. Similarly, the 5 isolates (BS_1, BS_2, BS_8, BS_{11} and BS_{12}) of among 14 isolates also produced loci of Rm value 0.60

Four isolates (BS_2, BS_3, BS_4 and BS_8) produced loci of Rm value 0.45. Similarly, two isolates BS_1 and BS_2 produced loci of Rm value 0.39. This indicated that all 14 isolates were different in β-esterase isozyme pattern (Fig. 10).

A dendrogram was constructed by UPGMA clustering as presented in the Fig 12. This dendrogram identified two major clusters with 25% Euclidean distance. One cluster comprised of nine isolates BS_{13}, BS_{14}, BS_{11}, Bs_{12}, BS_9, BS_{10}, BS_5, BS_6 and BS_7 (group I) and another cluster comprised of five isolates BS_3, BS_4, Bs_2, BS_8 and BS_1(group II). Group I was further sub-clustered into two groups, of which first sub-cluster (group IA) had four isolates (BS_{13}, BS_{14}, BS_{11}, Bs_{12}) while isolates BS_{11} and Bs_{12} were in separate individual cluster. Second sub-cluster (group IB) included five isolates BS_9, BS_{10}, BS_5, BS_6 and BS_7 while isolates BS_9, BS_{10} and BS_5 showed their close relationship and isolate BS_6 and BS_7 were in separate individual cluster forming separate individual cluster. Group II was further sub-clustered into two, of which first sub-cluster (group IIA) had five isolates BS_3, BS_4, Bs_2 and BS_8 which again further sub divided into two clusters, i.e., group IIAa, comprised of isolates BS_3 and BS_4 showing their close relationship. In group IIAb, another two isolates BS_2 and BS_8 showed their close relationship. While the second sub-cluster had one isolate BS_1 formed separate individual cluster (group IIB).

B. sorokiniana has a high morpho-pathological, cultural and biochemical variability which has not been so far confirmed in this zone of study, which marked as hot spot for spot blotch of wheat and thus the present work claimed a novel approach.

Different isolates also showed variability in respect to their size and shape of conidia. It was observed that there were differences in the size of conidia of 14 different isolates in artificial media and from direct plant sample. Many reports on the morphological variability within the isolates of BS from wheat based have been

reported from other wheat growing zones of India (Chauhan *et al.,* 2007 and Pandey *et al.,* 2008).

Morphological variability in *B. sorokiniana* isolates from different parts of India has also been reported by Chand *et al.* (2003) and Asad *et al.* (2009). Similar type of experiment was also done by Manjunath *et al.* (2010) with observation of maximum growth of all the isolates of *A. alternata* followed by potato dextrose agar (PDA). Thus, variability of BS isolates can be attributed to the interactions between genetic makeup and environmental conditions. Such edaphoclimatic differences existed in areas from where isolates had been collected.

The fourteen BS isolates showed cultural variability in respect of morphology of conidium and colony colour and margin in PDA medium. With increase of the incubation period, the size of the colony increased. Four isolates produced grayish black colony, six isolates produced grayish white colony and isolate BS_{12} and BS_{13} produced dark grey colony. The remaining two isolates produced whitish and whitish grey colony, respectively. Nine isolates produced regular fungal margin and the remaining isolates produced irregular margin of growth.

Growth rate was different on different media, whereas maximum growth rate was observed on PDA (13.93 mm day^{-1}) followed by OMA (12.76 mm day^{-1}) and minimum on PCA (5.48 mm day^{-1}) irrespective of isolates. Among the isolates, maximum growth rate was exhibited by BS_2 (13.93 mm day^{-1}) followed by BS_{11} (13.55 mm day^{-1}) and minimum growth rate was observed in BS_3 (5.48 mm day^{-1}). Here the differences were statistically significant. Similar type of experiment was done by Poloni *et al.* (2008) with four different culture media viz., potato dextrose agar (PDA), Sabouraud maltose, Sabouraud galactose and Sabouraud glucose. According to the result obtained by Kendra *et al.* (2006), *Bipolaris* colonies grow rapidly in PDA media and reaches maximum diameter of 3 to 9 cm within 7 consecutive days at 25ºC. The morphological and cultural variability sometimes have firm correlation with virulence of the pathogen (Pandey *et al.*, 2008; Jaiswall *et al*., 2007).

Biochemical variability among the 14 isolates of BS from different locations was found in respect of isozyme polymorphism. All the 14 isolates showed higher enzymatic activity for esterase. This might explain the importance of these two enzymes for the initiation of diseases during adherence and invasion of plant tissue by pathogenic fungi (Jaeger and Reetz, 1998). In Brazil, Poloni *et al*., (2009) conducted an experiment where majority of the isolates collected from different locations apart from Brazil showed differences in virulence, morphology and enzymatic activity than those of native isolates which had edaphoclimatic differences among themselves.

This study clearly showed that all the isolates of *Bipolaris sorokiniana* collected from different location of north eastern plain zone of India showed their variability in terms of cultural, morphological and biochemical characterizations. However, more isolates from diverse location needs to be studied further to obtain comprehensive information. Study on molecular variability can also be done on the basis of this basic study.

ACKNOWLEDGEMENT

Authors are very much grateful to Head, Department of Plant Pathology and Director of Research, Bidhan Chandra Krishi Viswavidyalaya for their kind co-operation and support during the research work.

Conflict of interest

Authors declare that they have no any conflict of interest**.**

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