



Analysis of Cultural and Pathogenic Diversity in *Bipolaris sorokiniana* Causing Spot Blotch of Bread Wheat in North India

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Authors' contributions

This work was carried out in collaboration among all authors. Authors RVS and RS designed the study. Author AC performed the statistical analysis, Authors AC and LKM wrote the protocol, and wrote the first draft of the manuscript. Author AC and Author LKM managed the analyses of the study. Authors AC and LKM managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Present study was conducted to analyse the cultural and pathogenic diversity in different isolates of *Bipolaris sorokiniana* the causal organism of spot blotch in bread wheat. Six isolates of *Biploris sorokiniana* (BS-F-5, BS-D-2, BS-K,4, BS-DWR-K-1, BS-V-6, and BS-P-3) were evaluated simultaneously for cultural and pathogenic variability on the basis of diverse characteristics against seven different genotypes of wheat grown in the region viz: BOW'S', HS 375, HUW 234, PBW 343, PBW 443, K9107 and A9-30-1. The results indicated that isolates varied significantly among themselves for all the characters analysed. The isolate BS-F-5 exhibited the maximum colony diameter, maximum average dimension (length and width) of conidiophore, maximum number of septa in conidiophore and conidia. Variations were also observed in texture of the colony. Among the seven genotypes tested against six isolates, BOW'S' showed resistance against three isolates (BS-D-2, BS-K,4, BS-DWR-K-1) and genotype A-9-30-1 showed high susceptibility against all isolates except BS-D-2. The isolates BS-F-5 and BS-P-3 exhibited maximum pathogenic virulence among the isolates analysed in the present investigation.

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1. INTRODUCTION

Wheat production in India constantly suffers from numerous threats caused by diverse categories of plant pathogens which results in huge losses in production and precious economic loss to the farmers. Spot blotch is an important disease of wheat caused by *Bipolaris sorokiniana* [(Sacc. in Sorokin) Shoem. syn. *Helminthosporium sativum* Pammel, King & Bakke; teleomorph: *Cochliobolus sativus* (Ito and Kurib.) Drechs. Ex. Dastur] in major wheat growing regions [1,2,3,4].

The importance of spot blotch also popularly known as foliar blight or *Helminthosporium* leaf blight has been highlighted in South Asian countries like India, Bangladesh and Nepal adequately in earlier reports [5,6,7]. It is a soil and seed borne pathogen and is considered an important pathogen of wheat in regions with warm and humid climate as prevailing in South Asian countries [8,9]. *Bipolaris sorokiniana* initially shows its impact on the older leaves at the base of the wheat plant and then progresses to the upper part of the canopy [10].

Disease severity is affected by several factors such as crop management, soil fertility, planting density, the developmental stage of the plant, virulence of the isolates involved in infection, susceptibility of the host genotype and the weather conditions experienced by the host during later parts of the life cycle [11]. The extent of grain yield losses caused by foliar blight, particularly in South Asia has been widely reported by several studies reported previously [12,13]. Yield losses are significant and depend on genotype, sowing time, year, location and stress conditions [14]. They are estimated to be 15–20% on average [15], but might reach 40–70% in susceptible genotypes [16].

Several interventions are being adopted in modern agriculture to minimize losses due to such pathogens. Management of plant pathogens using chemicals has economic and environmental costs [17,18]. Management of the disease through host resistance is considered as the most economical, low cost, sustainable and environmentally favorable method of controlling diseases [19,20]. Managing any pathogen through host resistance requires a comprehensive knowledge of prevalent races of the target pathogen, which can be achieved by

exploring the virulence diversity as well as the genetic diversity of host. Adopting these strategies is a viable option for improving yield and reducing economic losses especially under stresses and future climate change conditions [21].

Variability in the spot blotch pathogen *Bipolaris sorokiniana* at pathogenic, morphological and physiological level have been reported previously [22,23,24]. The spot blotch resistance gene(s) in wheat are not known to interact in gene for gene manner, but resistant genotypes are known to show significant reduction in disease development as compared to the susceptible cultivars Joshi and Chand 2000; Joshi et al., [25]; Kumar et al., [26]. Spot blotch can be controlled effectively using chemicals but that involves high economic and environmental costs. Considering this drawback of the chemical control method of spot blotch incorporation of genetic resistance into cultivars against the pathogen is widely accepted as a cost effective and environment friendly strategy [27].

In order to screen potential spot blotch resistance in wheat cultivars and its importance in controlling the disease the present investigations has been planned and executed to highlights the cultural, morphological and pathogenic variability within the *B. sorokiniana* population of North India and explore seven genotypes of wheat for their resistance against spot blotch pathogen that may be useful in overall management of this disease in future.

2. MATERIALS AND METHODS

2.1 Cultural and Morphological Variability

Wheat leaves showing spot blotch symptoms were collected from different wheat growing areas of north India (Table 1). The pathogen was isolated on Potato dextrose agar (PDA) and purified through monoconidial isolation [24]. Monoconidial culture maintained from the isolates of different places were accordingly designated for further studies (Table 1) and stored in a refrigerator at 5°C under Varanasi conditions (Udai Pratap Autonomous PG College, Varanasi) in two consecutive growing seasons 2017-18 and 2018-19. To study the colony characters, the *Bipolaris sorokiniana* culture was grown on Potato Dextrose Agar

Table 1. Isolates of *B. sorokiniana* collected from different places of north India

Isolate	Place of collection	Wheat variety
BS-DWR-K-1	Directorate of Wheat Research, Karnal (Haryana)	PBW 343
BS-D-2	Indian Agriculture Research Institute, New Delhi	PBW 443
BS-P-3	Rajendra Agriculture University, Pusa (Bihar)	UP 262
BS-K-4	C.S. Azad Univ. of Agril. & Tech., Kanpur (U.P.)	Sonalika
BS-F-5	N.D. Univ. of Agril. & Tech., Kumarganj, Faizabad (U.P.)	PBW 443
BS-V-6	Banaras Hindu University, Varanasi (U.P.)	HUW 234

(PDA) in petri dishes. Colony characters viz., colony diameter, colour, texture and shape of each isolate were recorded after 8 days of incubation at 28±1°C. Conidiophore and conidial characters i.e. size (length and width), number of septa, colour and shape of conidia were observed and recorded. The colony diameter, size of conidia and conidiophore and sporulation were recorded with the help of linear scale, ocular and stage micrometer and haemocytometer, respectively. Colour of the colony, conidia and conidiophore were observed and recorded with the help of Munsell's soil colour chart [28] used previously by Jang et al., [29] for *Trichoderma harzianum*.

2.2 Pathogenic Variability

Experiments on pathogenic variability of six isolates of *B. sorokiniana* were carried out in glass house on seven wheat genotypes viz., BOW'S', HUW 234, HS 375, PBW 343, PBW 443, K 9107 and A-9-30-1 at 42 to 45 days old plants. These varieties were selected because they exhibit varying degree of reactions (Resistant to highly susceptible) under local environmental conditions. Five seedlings of each genotype were raised in plastics pots. Two hours before inoculation, the plants were kept in humidity chamber, fitted with mistifier to maintain film of water on leaf surface. The plants were inoculated with spore suspension of each test isolate after pin pricking the leaves. The inoculum was obtained from 20 days old culture of each test isolate, which was grown as mass culture on sterilized sorghum grains [30]. Plants were sprayed with inoculum containing about 70000 to 80000 conidia per milliliter of water and two drops of Tween 20 (polyoxyethylene sorbitan monolaurate) per 100 ml for adherence of spore suspension on plant surface. After inoculation, plants were kept in a moist chamber maintained at 90 to 100% RH for about 48 hours. Subsequently, plants were transferred to another chamber of the glass house where temperatures may have been 20-30°C (month of February 1st fortnight). Disease rating was done, 21 days after

inoculation, when lesions attained its maximum size using the rating scale given by Dubin [31] which categorized the response of wheat genotypes against *B. sorokiniana* into six distinct categories Immune, Resistant, Moderately resistant, Moderately susceptible, Susceptible and highly susceptible having scores of >0, >1-10, >10-30, >30-50, >50-70 and >70 above respectively based on the percent lesion area of leaf on the flag leaf at the dough stage.

2.3 Statistical Analysis

Standard error mean were calculated for variations in colony characters, sporulation, conidiophore and conidial characteristics of *Bipolaris sorokiniana* and Data were subjected to ANOVA by using Microsoft excel 2007 software and significant difference among the treatments was calculated at probability levels of $p = 0.05$ by Tukey's test.

3. RESULTS AND DISCUSSION

3.1 Colony Characters

Colony of isolate BS-F-5 exhibited fastest growth with brown to black colour, appressed texture, circular periphery. It attained a diameter of 86.63 mm in eight days (Fig. 1). This was followed by BS-P-3 in terms of growth speed. The isolate BS-P-3 possessed brownish to black colour with gray margin, appressed texture, circular periphery. Three isolates i.e. BS-DWR-K-1, BS-V-6 and BS-K-4 were almost intermediate type in growth, whereas isolate BS-D-2 was comparatively slow growing which attained the colony diameter of 49.5 mm in eight days and had olivaceous brown with whitish growth, fluffy texture and irregular periphery (Table 2). Aggarwal et al., [32] found high variability in 103 isolates of *Bipolaris sorokiniana* collected from different geographical zones of India. Based on colony characteristics they categorized the isolates into five groups. Similar findings with respect to variability in growth characteristics, colony colour, texture and shape were reported by Dinesh et al., [33].



Fig. 1. Morphological variation in colony characters, conidial shape and size of different isolates of *Bipolaris sorokiniana*

3.2 Sporulation

Among the six isolates (Table 2), maximum sporulation was observed in BS-F-5 (22.00×10^4) followed BS-P-3 (18.33×10^4), BS-V-6 (15.67×10^4), BS-DWR-K-1 (14.67×10^4) and BS-K-4 (13.33×10^4) isolates (Fig. 1). Isolate BS-D-2 exhibited least sporulation (6.33×10^4). Based on

radial growth and sporulation these six isolates can be grouped into three categories, as given below:

Dinesh et al., [33] found that sporulation varied from fair to excellent in ten monoconidial isolates of *Bipolaris sorokiniana* collected from different regions of North India.

Table 2. Variation in colony characters and sporulation of *B. sorokiniana* isolates

Isolates	Colony characters				Sporulation per 5 mm disc x 10 ⁴
	Size (mm)	Colour	Growth	Shape	
BS-DWR-K-1	68.44	Gray with whitish dots	Semi appressed	Irregular	14.67
BS-D-2	49.50	Olivaceous brown with whitish growth and whitish margin	Fluffy cottony	Irregular	6.33
BS-P-3	82.14	Brownish to black with gray margin	Appressed	Circular	18.33
BS-K-4	65.08	Olivaceous with whitish margin	Fluffy	Irregular	13.33
BS-F-5	83.63	Brown to dark black	Appressed	Circular	22.00
BS-V-6	66.33	Black whitish growth, light brownish margin	Semi appressed	Irregular	15.67
SEm ±	1.55				2.23
CD at 5%	3.37				4.86

Table 3. Categories of isolates of *Bipolaris sorokiniana*

Category	Characteristics	Isolate
Category I	Fast growing and high sporulating	BS-F-5 and BS-P-3
Category II	Moderate growth and sporulation	BS-DWR-K-1, BS-K-4 and BS-V-6
Category III	Slow growing and less sporulating	BS-D-2

3.3 Conidiophore Characters

The measurement of conidiophore and conidia also varied considerably and can be grouped in three categories. The maximum average length of conidiophore was found in BS-F-5, which was 146.64 µm followed by BS-D-2 (139.68 µm), BS-P-3(137.58 µm),BS-K-4 (136.96 µm), BS-V-6 (134.78 µm) and BS-DWR-K-1 (131.42 µm), whereas maximum average width was noticed in BS-K-4 isolate which was 6.97 µm followed by BS-DWR-K-1(6.85 µm), BS-F-5 (6.83µm), BS-P-3 (6.80 µm), BS-D-2 (6.69 µm) and BS-V-6 (6.61 µm) isolates. Isolates BS-F-5 and BS-P-3 had maximum number of septa followed by BS-DWR-K-1, BS-V-6 and BS-D-2. Least number of septa was found in BS-K-4 isolate. Regarding colour of the conidiophore, light brown colour was observed in isolates BS-F-5 and BS-P-3, dark olivaceous in isolates BS-K-4 and BS-V-6, olivaceous and light olivaceous in BS-DWR-K-1 and BS-D-2, respectively (Table 4). High morpho-pathological variability in *B. sorokiniana* has been reported earlier also by Poloni et al., [34]. They reported five morphological groups without any relationship to geographical regions. This variability could be attributed to the interactions between genetic information, where isolates from same species differ from one another in their genetic composition, and environmental conditions, such as the edaphoclimatic variations in the areas from

where the isolates were obtained. Similar morphological variability in *B. sorokiniana* has been reported by Nasir et al., [35].

3.4 Conidial Characters

The maximum average length of conidia was observed in the isolate BS-V-6 (76.07 µm) followed by BS-F-5 (71.15 µm), BS-DWR-K-1 (70.98 µm), BS-P-3 (69.47 µm), BS-D-2 (60.91 µm) and BS-K-4 (60.29 µm), whereas maximum average width of conidia was in BS-V-6 (19.90 µm) followed by BS-K-4 (16.50 µm), BS-D-2 (16.40 µm), BS-DWR-K-1 (15.90 µm), BS-P-3 (15.80 µm) and BS-F-5 (15.10 µm) isolates. Similar variations in the average length of the conidia were reported by Bandyopadhyay et al., [36] isolates from 12 different locations of India. The isolates BS-F-5 and BS-P-3 had maximum number of septa with slightly curved elliptical shaped conidia. In other isolates, shape of conidia were ovate to either slightly curved elliptical or slightly curved (Table 5).

Findings of present investigation are in accordance and support the findings of earlier workers like Akram and Singh [37], Mahto et al., [38], Chand et al., [39] and Kumar et al., [40] even though the physiologic forms of *B. sorokiniana* may be different. Tinline [41] reported that variability in *B. sorokiniana* was due to anastomosis and nuclear migration.

Table 4. Variation in conidiophore and conidial characteristics of *B. sorokiniana* isolates

Isolates	Conidiophore characters				Conidial characters				
	Length (µm)	Width (µm)	No. of Septa	Colour	Length (µm)	Width (µm)	No. of septa	Colour	Shape
BS-DWR-K-1	131.42	6.85	4-11	Olivaceous	70.98	15.90	3-10	Dark olivaceous	Ovate
BS-D-2	139.68	6.69	4-10	Light olivaceous	60.91	16.40	2-6	Light olivaceous	Curved ovate
BS-P-3	137.58	6.80	6-11	Light brown	69.47	15.80	4-10	Light to dark brown	Slightly curved elliptical
BS-K-4	136.96	6.97	4-9	Dark olivaceous	60.29	16.50	2-8	Dark olivaceous	Slightly curved ovate
BS-F-5	146.64	6.83	6-12	Light brown	71.15	15.10	4-11	Light to dark brown	Slightly curved elliptical
BS-V-6	134.78	6.61	4-11	Dark olivaceous	76.07	19.90	2-7	Dark brown	Slightly curved ovate
S. Em±	1.69	NS			1.996	0.66			
CD at 5%	3.69				4.35	1.43			

Table 5. Disease severity and reaction of genotypes of wheat inoculated with six isolates of *B. sorokiniana*

Wheat genotypes	Per cent disease severity*/reaction					
	BS-DWR-K-1	BS-D-2	BS-P-3	BS-K-4	BS-F-5	BS-V-6
BOW 'S'	8.87(R)	4.99(R)	24.89(MR)	9.51(R)	28.45(MR)	11.57(MR)
HUW 234	67.10(S)	29.58(MR)	67.98(S)	46.85(MS)	80.79(HS)	50.95(S)
HS 375	32.41(MS)	24.51(MR)	43.10(MS)	32.74(MS)	40.15(MS)	28.45(MR)
PBW 343	52.92(S)	30.45(MS)	58.88(S)	29.64(MR)	64.39(S)	47.60(MS)
PBW 443	49.28(MS)	30.40(MS)	68.25(S)	30.74(MS)	61.85(S)	55.91(S)
K 9107	25.84(MR)	25.96(MR)	68.52(S)	70.55(HS)	70.92(HS)	48.35(MS)
A-9-30-1	74.92(HS)	58.32(S)	91.53(HS)	75.09(HS)	96.85(HS)	80.75(HS)
Mean	44.48	29.17	60.45	42.16	63.34	46.22

*Mean of two seasons (2017-18 and 2018-19); R = Resistant (1-10%), MR = Moderately resistant (10-30%), MS = Moderately susceptible (30-50%), S = Susceptible (50-70%), HS = Highly susceptible (70%-above)

3.5 Pathogenic Variability

To determine the virulence diversity in *B. sorokiniana* isolates, total seven genotypes of wheat were artificially inoculated with six isolates of *B. sorokiniana* and it was observed that the isolates BS-F-5 and BS-P-3 were found to be more or less similar and highly virulent followed by BS-V-6, BS-DWR-K-1 and BS-K-4. Isolate BS-D-2 was least virulent and distinct as compared to other five isolates evaluated in the present investigation. It was found that isolates of *B. sorokiniana* differed drastically in their pathogenic capabilities. Two isolates viz., BS-F-5 and BS-P-3 were highly virulent, three isolates i.e. BS-DWR-K-1, BS-K-4 and BS-V-6 were moderately virulent whereas one isolate i.e. BS-D-2 was found to be least virulent, rather avirulent against all genotypes except one i.e. A-9-30-1, against which BS-D-2 showed susceptible reaction. On the basis of disease severity, the six isolates of *B. sorokiniana* were grouped in three categories i.e. highly virulent, moderately virulent and least virulent.

Historically, the spot blotch pathogen *B. sorokiniana* has been described as a variable fungus with many morphological [42] and physiological [41] variants. Part of the variability has been attributed to heterokaryosis and parasexual mechanism [43]. The variability and aggressiveness of this pathogen seems to increase over time [44,45]. Further, the two most virulent isolates in the present investigation BS-F-5 and BS-P-3 exhibited light brown conidiophores and light to dark brown conidial characters. Differences among the fungal isolates behavior with respect to pathogenicity was reported by Asad et al., [46]. Similar to their findings our results also indicate that dark coloured isolates had highest pathogenicity as

compared to light colored isolates. The dark color of the isolates may be due to presence of melanins which are darkly pigmented polymers protects the isolates against environmental stress and enhances their ability to survive in adverse conditions. It has been reported earlier that some phytopathogenic fungi produce melanized appressoria for host invasion through natural wounding, stomata or with the use of an appressorium like structures through the cell wall of the leaves [47]. The findings of the present investigation are in accordance with the earlier reports by Duveiller et al., [19], who identified 15 pathotypes out of 40 isolates, from different countries, on the basis of resistance reactions in a 12 member differential set of genotypes. Singh et al., [2] who reported that the Faizabad strain of *B. sorokiniana* has been found to be the most virulent among strains collected from across the country fully support the present findings.

3.6 Response of Wheat Genotypes

Among the seven genotypes tested, the genotype BOW'S' showed resistant reaction against BS-DWR-K-1, BS-D-2 and BS-K-4 and moderately resistant reaction against remaining isolates i.e. BS-F-5, BS-P-3 and BS-V-6. The wheat genotype HUW 234 was found to be moderately resistant against BS-D-2, moderately susceptible against BS-K-4, susceptible against three isolates, namely, BS-DWR-K-1, BS-P-3 and BS-V-6 and highly susceptible against BS-F-5 isolate. Genotype HS 375 was found to be moderately resistant against BS-D-2 and BS-V-6, while remaining four isolates viz., BS-DWR-K-1, BS-P-3, BS-K-4 and BS-F-5 exhibited moderately susceptible reaction on this genotype. The genotype PBW 343 was found to be moderately resistant against BS-K-4, moderately susceptible against BS-D-2 and BS-

V-6 and susceptible against remaining three isolates i.e. BS-DWR-K-1, BS-P-3 and BS-F-5. The genotype PBW 443 was found to be moderately susceptible against BS-DWR-K-1, BS-D-2, and BS-K-4, whereas found to be susceptible against rest three isolates i.e. BS-P-3, BS-F-5 and BS-V-6. The genotype K 9107 was found to be moderately resistant against BS-DWR-K-1 and BS-D-2, moderately susceptible against BS-V-6, susceptible against BS-P-3 and highly susceptible against BS-K-4 and BS-F-5 isolates. The genotype A-9-30-1 was found to be susceptible against BS-D-2 and highly susceptible against remaining five isolates. It is clear that only one genotype i.e. BOW'S' exhibited resistant to moderately resistant reaction against all isolates, while isolate BS-F-5 was highly virulent. It can be said that BOW'S' genotype has a broader genetic base for resistance as it is showing almost resistant reaction. Similarly, the genotype A-9-30-1 seems to have no gene for resistance as it exhibited susceptible to highly susceptible reaction against all isolate even the BS-D-2 which seems to be almost avirulent. This study also gave the indication for existence of type of virulence i.e. highly virulent, virulent, moderately virulent and less virulent (Table 5). Verma et al., [48] carried out extensive research to characterize the fungus and its pathogenicity and screen the genotype under field conditions for spot blotch susceptibility in the Rabi season of 2016–2017 at Borlaug Institute for South Asia. They reported significant variability among the 32 genotypes of *B sorokiniana* isolates. The mean pathogenicity value showed that isolates were more pathogenic on Sonalika (3.7) than Chirya 3 (2.5) wheat genotypes. They found that colony color and level of exudations were related to the level of pathogenicity and aggressiveness. Area under disease progress curve of isolates on Chirya 3 varied from 198.77 (white group) to 730.25 (black group) in both natural and polyhouse condition, while for Sonalika it varied from 458.02 (white group) to 134.83 (black group) in natural condition, whereas from 458.02 (white group) to 1374.07 (black group) in polyhouse condition. The seven genotypes studied in the present investigation represent a nominal range of variability for disease and agronomic features. This provides an opportunity to select most suitable wheat genotype among these for spot blotch resistance. The findings of this study have potential influence on integrated foliar blight management to reduce yield losses under the weather conditions prevalent in wheat growing

regions of South Asian countries by selecting a proper cultivar.

4. CONCLUSION

Efficient management of spot blotch in wheat is pivotal for sustained wheat production to feed the increasing global population. Use of disease resistant varieties in different agro-climatic zones is key to achieve this task. The present investigation analysed seven wheat genotypes that exhibit varying degree of reactions against *Bipolaris sorokiniana* under local environmental conditions. Results indicate that one genotype BOW-S showed high to moderate resistance against most of the isolates analysed. These findings may further be used for breeding programs for development of wheat varieties having significant resistance against spot blotch particularly for Northern India.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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