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# Variability in the Population of *Bipolaris sorokiniana* Infecting Wheat

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**ABSTRACT**

*The leaves showing typical symptoms were collected from major wheat growing areas spread over the different agro-ecological zones of India during rabi 2013-14. Cultural characters of 24 isolates of B. sorokiniana studied on PDA revealed lot of variation. Eight (SB-2, 4, 6, 7, 8, 16, 23 and 24) showed excellent growth, 11 (SB- 1, 3, 5, 9, 10, 11, 13, 14, 15, 21 and 22) revealed moderate growth and five (SB- 12, 17, 18, 19 and 20) exhibited poor growth. Five different types of colours of isolates were found on PDA. The frequency of the white (29.2 %), colony type was maximum while both dark gray and light brownish gray colour showed frequency of 25.0 per cent in the population. Light gray displayed 16.7 per cent and lowest frequency (4.2 %) observed was black type. Among these dark gray cultures sporulate profusely and had appressed cottony growth. The whitish type had less or no sporulation. The size of the conidia of 21 isolates varied from 15.3-126.0 µm X 6.3-36.1 µm with 1-6 septa, colour varies from dark brown to olivaceous, Ovate to elliptical in shape and showed germination pattern was by unipolar or bipolar. Sporulation was not observed in the three isolates (SB-17, 19 and 20). On the basis of aggressiveness of the 12 isolates on differentials showing varied response to disease, the isolates of B. sorokiniana were classified into three groups as Highly virulent that comprises three isolates viz., SB-8 (Dharwad), SB-16 (Varanasi (V-2)) and SB-10 (Pantnagar), Moderately virulent that includes five isolates viz., SB-4 (Bijapur), SB-5 (Kamatnur) SB-7 (Arabhavi (A-2)), SB-18 (Indore) and SB-22 (Wellington (W-2)), Least virulent that consist of three isolates viz., SB-9 (Pune), SB-14 (Varanasi (V-1)) and SB-20 (Jammu).*

***Key words:*** *Wheat, B. sorokiniana, agro-ecological zones of India, Cultural characters, PDA*

## INTRODUCTION

*Bipolaris sorokiniana* (Sacc.) Shoem. is the causal agent of common root rot, spot blotch, seedling blight, head blight, black point of wheat and barley. *B. sorokiniana* develops dark brown necrotic lesions on roots, crown, leaves and lower leaf sheaths. It develops oval to elongated light to dark brown blotches on leaf blades and sheaths, when it severely infect the roots and crown portions, the plants dry out without producing any seed. Similarly infected spikelets under favourable conditions produces shriveled grains. Grain yield reductions due to spot blotch are variable but are of great significance in warmer areas of South Asia[18, 19].

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At present, spot blotch of wheat is a major disease 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. Management of this disease through host resistance has become a prime concern. 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[6]. Previously its generic name Bipolaris having fusoid, straight or curved conidia with bipolar germination and characterized by thick-walled, elliptical conidia (60-120um x 12-20 um) with 4–8 septa. The colony of the fungus has interwoven hyphae as a loose cottony mass white or light to grey color depending on the isolates[20].To find out the difference in aggressiveness/ virulence in the pathogenic population on different wheat genotypes to categorize the isolates into least to highly virulent, the quantification of host responses is a prerequisite. In the present study aggressiveness/ virulence of the isolates was characterized on the basis of disease severity observed on 12 wheat genotypes. The present study highlights the cultural, morphological and pathological variability within the *B. sorokiniana* populations of different agro-ecological zones of India.

## MATERIAL AND METHODS

### Collection of isolates

The leaves showing typical symptoms of spot blotch were collected from major wheat growing areas spread over different agro-ecological zones *viz.,* North Western Plains Zone (NWPZ), North Eastern Plains Zone (NEPZ), Central Zone (CZ), Peninsular Zone (PZ), Northern Hills Zone (NHZ) and Southern Hills zone (SHZ) of India during rabi 2013-14 (Table 1). The symptoms observed in these samples were also studied and recorded systematically to find out the variation if any. The presence of *B. sorokiniana* was confirmed by examining infected plant parts under a microscope and by culturing the samples and comparing them with the characteristic features of the fungus. The pathogen was isolated on Potato Dextrose Agar (PDA) and purified by mono conidial isolation[12]. Monoconidial culture maintained from the isolates of different location were accordingly designated for further studies (Table 1) and stored in a refrigerator at 5 °C on PDA. Here after, the monoconidial culture is referred as the isolate. Twenty four monoconidial isolates of *B. sorokiniana* representing six agro-ecological zone cultured on PDA were used for cultural and pathological studies. Multiple isolates collected from Arabhavi (SB- 3 and SB- 7) Varanasi (SB- 14 and SB- 16) and Wellingtone (SB-21, 22, 23 & 24) and was considered as they showed variation in symptoms of disease.

### Growth of isolates on PDA

The cultural characters of 24 isolates of *B. sorokiniana* were studied on PDA. These plates were inoculated at the centre with fungal discs of 10mm diameter of each isolate from the periphery of 10 day old pure culture and incubated at 26 $\pm$ 1 ºC for a period till full growth appeared in any one of the isolate or eight days whichever is early. The colony diameter was recorded by averaging the radial growth of the colony in two directions for each plate. Colony characters *viz.,* colony morphology (colour, growth behavior), radial growth, shape and sporulation were recorded. Photographs were taken to show and to compare the growth characters of the pathogen on PDA. The radial growth was recorded and analyzed statistically by using completely randomized design. Colour of the colony was recorded with help of Munsell's soil colour chart[13].Spore production on 15 day old culture of *B. sorokiniana* was recorded by excising 10 mm diameter colony area using cork borer. It was shaken well, after mixing in 1 ml sterile distilled water, to dislodge the conidia. The average number of spores per microscopic field was counted.

### Conidia morphology

The conidial shape, size, septation and colour was noted and measured according to Sivanesan and Holliday [22]. Microphotographs

were taken to show the spore morphology clearly.

**Pathogenic variability studies**

This study was conducted at MARS, UAS, Dharwad under glass house conditions with 11 isolates (SB-4, 5, 7, 8, 9, 10, 14, 16, 18, 20 and 22) selected based on isolate representing six agro ecological zones.

**Sowing of different genotypes**

Twelve wheat genotypes (six durum, five dicoccum and one bread wheat) selected based on criteria of locally popular genotypes and also showing clear cut reactions of spot blotch symptoms to *B. sorokiniana* and were selected as standard differentials. A mixture of sandy loam and vermi-compost in the ratio of 2:1 was prepared for sowing of differentials. The mixture was then well sieved and filled in 4" earthen pots. Ten healthy seeds of each differential treated with Vitavax power® (@ 2 g/kg seed) were sown in each pot.

**Inoculation and disease score**

**Seedlings inoculation**

One set of each of these genotypes was inoculated separately in the glasshouse sown in earthen pots with conidial suspension of 12 day old culture of each isolate (grown on sterilized sorghum grains) in sterile distilled water having aqueous suspension containing 8-10 spores per microscopic field under $10x^{16}$. The inoculation was done uniformly using hand atomizer on 15 day old seedling. Proper control was maintained by spraying plants with sterile distilled water and covered with polythene bags covered for 24 hrs to maintain high relative humidity. Later bag was removed. The seedlings received only day light. The experiment was replicated twice.

**Disease severity under glass house conditions**

Observations on disease severity was recorded on infected plants seven days after inoculation, when lesions appeared to reached maximum size, using a scale of 0-5[1].

| Disease score | Descriptions of severity levels | Reaction |
|---|---|---|
| 0 | Free of spots | Immune (I) |
| 1 | Necrotic spots without chlorosis up to 5 per cent of the leaf area involved | Resistant (R) |
| 2 | Necrotic spots with light chlorosis, 6-20 per cent of the leaf area involved | Moderately resistant (MR) |
| 3 | Necrotic spots with pronounced chlorosis, 21-40 per cent of the leaf area involved | Moderately susceptible (MS) |
| 4 | Necrotic spots with pronounced chlorosis 41-60 per cent of the leaf area involved | Susceptible (S) |
| 5 | Spots merging, more than 60 per cent of the leaf area involved | Highly susceptible (HS) |

## RESULTS

The symptoms on diseased parts collected from various places are presented in Table 2 and Plate 1.Five different types of colours of isolates were found on PDA (Table 3 and Plate 2). Out of 24, seven isolates exhibited white colour colony (SB-12, 13, 16, 17, 18, 19 and 20), six isolates *viz.*,SB-1, 3, 8, 10, 14 and 24 showed dark gray in colour, light brownish gray colour was noticed in six isolates (SB-9, 11, 15, 21, 22 and 23), four isolates (SB-2, 5, 6 and 7) revealed light gray colour while, SB-4 isolate exhibited black colony colour. The growth behaviour of the colony of different isolates varied from appressed cottony growth with white buttons in the isolates SB-1, 3, 8, 10 and 16, appressed cottony growth without white buttons (SB-9, 14, 20, 21, 23 and 24), cottony growth (SB-5, 6, 7, 11, 15, 18 and 22), Fluffy growth (SB-2, 4, and 13) and suppressed growth (SB-12, 17 and 19). The shape of the colony of different isolates varied

from circular with aerial mycelium at the centre (SB-2, 5, 6, 7, 9 and 15), circular without aerial mycelium (SB-13, 14, 16, 21, 22, 23 and 24), semi-circular with aerial mycelium at the centre (SB-1, 3 and 4), semi-circular without aerial mycelium (SB-17, 18 and 20), circular with zonation along boarder was recorded in SB-11, irregular with aerial mycelium at the centre (SB-8, 12 and 19) while irregular periphery was noticed in SB-10 (Table 3).The results revealed that there was significant difference in mycelia growth among the isolates. The maximum and minimum colony diameter of 89.5 and 39.3 mm respectively was recorded for the isolate SB-16 and SB-17 (Table 3), indicating that isolate SB-16 was the fastest growing attaining the maximum size in eight days on PDA with white colour, appressed cottony growth having white buttons and circular in periphery. This was followed by SB-7 (85.8 mm), SB-23 (83.5 mm), SB-2 and 4 (82.7 mm), SB-8 (80.2 mm), SB-6 (78.8 mm) and were significantly on par with each other. The least radial growth of 39.3 mm was recorded in SB-17 followed by SB-12 (44.0 mm), SB-19 (44.8 mm), SB-18 (46.2 mm), and SB-20 (49.8 mm) were statistically similar. Based on the varying level of radial growth 24 isolates can be grouped in to three categories (Table 3).

**Sporulation**

Among the 24 isolates (Table 3), maximum sporulation was observed in SB-14 and SB-24 (>40 conidia/ microscopic field at 100X) followed by SB-10 (20-40 conidia/ microscopic field at 100X). Sporulation was not observed in the isolates SB-17, 19 and 20.

**Conidial characters of the isolates**

The size of the conidia of 21 isolates varied from 15.3-126.0 µm X 6.3-36.1 µm with 1-6 septa, colour varies from dark brown to olivaceous, Ovate to elliptical in shape and showed germination pattern was by unipolar or bipolar (Table 4 and Plate 3). The maximum length of conidia was observed in the isolate SB-4 (95.4 µm) with 3-6 septa followed by SB-18 (86.7 µm) SB-3 (74.8 µm), SB-8 (70.3 µm), with 3-4 septa and significantly superior over others however, SB-8 was on par with SB-12 (62.6 µm) and SB-16 (57.8 µm). SB-3 and SB-12 were statistically similar. Isolate SB-18 (28.9 µm) recorded significantly higher width of conidia followed by SB-8 (27.9 µm), SB-3 (26.5 µm), SB-10 (24.0µm) and were on par with each other. Septal numbers was significantly greater in isolate SB-4 (4.5) subsequently by SB-3 (4.0) and were statistically similar (Table 4).The colour of the conidia varied from dark brown in SB-3 and SB-8, brown (SB-1, 2, 10, 14, 15 and 21), light brown (SB-4, 9, 11, 12, 16, 18, 22, 23, 24) and olivaceous (SB-5, 6, 7 and 13). The shape of the conidia was elliptical in all the isolates except in isolates SB-14 and SB-21 exhibited ovate in shape of conidia. The isolates, SB-4 and SB-10 showed only bipolar pattern of germination and rest all showed apart from bipolar, unipolar pattern of germination was also noticed (Table 4).

**Pathogenic variability studies**

The result presented in the table 5 revealed that genotype PDW-314 was uniformly resistant to all the isolates except to the isolate SB-16 (Varanasi (V-2)) where it was showed moderately susceptible disease response. Whereas, highly susceptible genotypes A-9-30-1 and Bijaga Yellow exhibited susceptible response to the isolates SB-8 (Dharwad) and SB-10 (Pantnagar). Apart from this, Bijaga Yellow showed susceptible reaction to the isolates SB-18 (Indore) and SB-22 (Wellington (W-2)).

Among the 11 isolates, SB-8 (Dharwad) and SB-10 (Pantnagar) were found highly virulent followed by SB-16 (Varanasi (V-2)) and others by considering the variable reaction pattern of isolates tested on differentials. These isolates can be grouped into three pathogenic groups namely, Highly virulent that comprises three isolates *viz.*,SB-8 (Dharwad), SB-16 (Varanasi (V-2)) and SB-10 (Pantnagar), Moderately virulent that includes five isolates *viz.*,SB-4 (Vijayapur), SB-5 (Kamatnur) SB-7 (Arabhavi (A-2)), SB-18 (Indore) and SB-22 (Wellington (W-2)), Least virulent that consist of three isolates *viz.*,SB-9 (Pune), SB-14 (Varanasi (V-1)) and SB-20 (Jammu).

**Table 1: Characteristics of *Bipolaris sorokiniana* isolates used in phenotypic diversity analysis**

| Isolate code | Location | State | Host source | Collected from wheat species/ genotype | Plant portion used for isolation | Agro-ecological zone (AEZ) |
|---|---|---|---|---|---|---|
| **SB-1** | Mangalagatti | Karnataka | Wheat | *Triticum aestivum* | Leaf | Peninsular Zone (PZ) |
| **SB-2** | Mulmutla | | Wheat | *T. durum* / Bijaga yellow | Leaf | |
| **SB-3** | Arabhavi | | Wheat | *T. durum* | Leaf | |
| **SB-4** | Vijayapur | | Wheat | *T. aestivum* | Neck | |
| **SB-5** | Kamatnur | | Wheat | *T. aestivum* | Leaf | |
| **SB-6** | Ugar | | Wheat | *T. dicoccum* / DDK 1029 | Leaf | |
| **SB-7** | Arabhavi | | Wheat | *T. dicoccum* | Leaf | |
| **SB-8** | Dharwad | | Wheat | *T. durum* | Leaf | |
| **SB-9** | Pune | Maharashtra | Wheat | *T. dicoccum* | Leaf | |
| **SB-10** | Pantnagar | Uttarkhand | Barley | *Hordeum vulgare* | Leaf | North Western Plains Zone (NWPZ) |
| **SB-11** | Karnal | Haryana | Wheat | *T. aestivum* | Leaf | |
| **SB-12** | Delhi | New Delhi | Wheat | *T. aestivum* | Leaf | |
| **SB-13** | Ludhiana | Punjab | Wheat | *T. aestivum* | Leaf | |
| **SB-14** | Varanasi | Uttar Pradesh | Wheat | *T. aestivum* | Leaf | North Eastern Plains Zone (NEPZ) |
| **SB-15** | Kanpur | | Wheat | *T. aestivum* | Leaf | |
| **SB-16** | Varanasi | | Wheat | *T. durum* | Leaf | |
| **SB-17** | Powerkheda | Madhya Pradesh | Wheat | *T. aestivum* / HI 1544 | Leaf | Central Zone (CZ) |
| **SB-18** | Indore | | Wheat | *T. durum* | Leaf | |
| **SB-19** | Anand | Gujarat | Wheat | *T. aestivum* | Leaf | |
| **SB-20** | Jammu | Jammu & Kashmir | Wheat | *T. aestivum* | Leaf | Northern Hills zone (NHZ) |
| **SB-21** | Wellington | Tamil Nadu | Wheat | *T. dicoccum* | Leaf | Southern Hills Zone (SHZ) |
| **SB-22** | Wellington | Tamil Nadu | Wheat | *T. aestivum* | Leaf | |
| **SB-23** | Wellington | Tamil Nadu | Wheat | *T. durum* | Leaf | |
| **SB-24** | Wellington | Tamil Nadu | Wheat | *T. dicoccum* | Leaf | |

**Table 2: Symptoms of *Bipolaris sorokiniana* on wheat from different agro-ecological zones of India**

| Isolate code | Location | Symptoms | State | Agro-ecological zone (AEZ) |
|---|---|---|---|---|
| **SB-1** | Mangalagatti | Spots are oval in shape with ashy gray centre surrounded by black margin without yellow halo. At advanced stage spots coalesce forming larger patches of dead areas on leaves. | Karnataka | Peninsular Zone (PZ) |
| **SB-2** | Mulmutla | Small dark black in point spots observed on leaves, then turn to round to oval in shape with gray centre, later join together to form large elongated brown leaf blotches. | | |
| **SB-3** | Arabhavi | Oval to elongated black spots with ashy gray centre. | | |
| **SB-4** | Vijayapur | Black elongated patch seen on neck region of the ear head. | | |
| **SB-5** | Kamatnur | Elongated light brown patches seen on leaves later on they form elongated blotches. Yellow halo was not seen | | |
| **SB-6** | Ugar | Oval to elongated light brown patches seen with yellow halo seen on leaves. Later on coalesce forming large blotches with dark sporulation at the centre. | | |
| **SB-7** | Arabhavi | Initially irregular spots with gray centre surrounded by black margin seen on leaves that's looks like oily spots. The spots become enlarged in size and they didn't coalesce covering entire leaf area. | | |
| **SB-8** | Dharwad | Elongated brown affected area seen on leaves without yellow halo then coalesce forming large dead areas on leaves. | | |
| **SB-9** | Pune | Irregular spots with distinct zone seen. Light brown at the centre surrounded by dark brown margin. They enlarge in size without merge covering entire leaf area. | Maharashtra | |
| **SB-10** | Pantnagar | Spots having black region at the centre surrounded by light brown margin at later stage they enlarge in size. | Uttarkhand | North Western Plains Zone (NWPZ) |
| **SB-11** | Karnal | Initially black spots seen leaves later on they elongate irregularly with dark sporulation at the centre. | Haryana | |
| **SB-12** | Delhi | Black spots seen on leaves later they exhibit distinct zonation, gray at the centre surrounded by black margin with slight yellow halo. Initially oval in shape and later becomes irregular. | New Delhi | |
| **SB-13** | Ludhiana | Black pin point spots on leaves without yellow halo | Punjab | |
| **SB-14** | Varanasi | Black pin point spots on leaves without yellow halo | Uttar Pradesh | North Eastern Plains Zone (NEPZ) |
| **SB-15** | Kanpur | Black lesions seen on leaves later on enlarge in size. | | |
| **SB-16** | Varanasi | Elongated light brown patches seen with yellow halo seen on leaves. Later on coalesce forming large blotches with dark sporulation at the centre. | | |
| **SB-17** | Powerkheda | Elongated light brown patches seen on leaves, later on they join together covering entire leaf area. Yellow halo not seen | Madhya Pradesh | Central Zone (CZ) |
| **SB-18** | Indore | Light brown spots seen on leaves. They coalesce to form large blotches | | |
| **SB-19** | Anand | Black spots with yellow halo seen on leaves, later on they | Gujarat | |

| | | | | |
|---|---|---|---|---|
| | | join together to form large blotches. | | |
| **SB-20** | Jammu | Light brown spots are seen they elongate and join together covering entire leaf area. Yellow halo not seen | Jammu & Kashmir | Northern Hills zone (NHZ) |
| **SB-21** | Wellington | Light brown patches seen on leaves they enlage in size and cover entire leaf area. | Tamil Nadu | Southern Hills Zone (SHZ) |
| **SB-22** | Wellington | Dark brown patches seen on leaves without yellow halo, coalesce and cover entire leaf area. | Tamil Nadu | |
| **SB-23** | Wellington | Irregular dark patches with yellow halo seen then join together to form large patches covering entire leaf area. | Tamil Nadu | |
| **SB-24** | Wellington | Brown patches seen on leaves without yellow halo join and cover entire leaf area. | Tamil Nadu | |

SB-1= Mangalagatti, SB-2= Mulmutla, SB-3= Arabhavi (A-1), SB-4= Vijayapur, SB-5= Kamatnur, SB-6= Ugar, SB-7= Arabhavi (A-2), SB-8= Dharwad, SB-9= Pune, SB-10= Pantnagar, SB-11= Karnal, SB-12= Delhi, SB-13= Ludhiana, SB-14= Varanasi (V-1), SB-15= Kanpur, SB-16= Varanasi (V-2), SB-17= Powerkheda, SB-18= Indore, SB-19= Anand, SB-20= Jammu, SB-21= Wellington (W-1), SB-22= Wellington (W-2), SB-23= Wellington (W-3), SB-24= Wellington (W-4).

**Table 3: Cultural variability of *Bipolaris sorokiniana* isolates grown on PDA**

| Isolate | Colony morphology | | Shape | Colony diameter (mm) | * Sporulation |
|---|---|---|---|---|---|
| | Color | Growth behavior | | | |
| SB-1 | Dark gray | Appressed cottony growth with white buttons | Semi circular with aerial mycelium at centre | 57.2 | + |
| SB-2 | Light gray | Fluffy growth | Circular with aerial mycelium at centre | 82.7 | + |
| SB-3 | Dark gray | Appressed cottony growth with white buttons | Semi circular with aerial mycelium at centre | 74.3 | ++ |
| SB-4 | Black | Fluffy growth | Semi circular with aerial mycelium at centre | 82.7 | ++ |
| SB-5 | Light gray | Cottony growth | Circular with aerial mycelium at centre | 70.0 | + |
| SB-6 | Light gray | Cottony growth | Circular with aerial mycelium at centre | 78.8 | + |
| SB-7 | Light gray | Cottony growth | Circular with aerial mycelium at centre | 85.8 | + |
| SB-8 | Dark gray | Appressed cottony growth with white buttons | Irregular with aerial mycelium at centre | 80.2 | ++ |
| SB-9 | Light brownish gray | Appressed cottony growth | Circular with aerial mycelium at centre | 67.0 | + |
| SB-10 | Dark gray | Appressed cottony growth with white buttons | Irregular | 53.2 | +++ |
| SB-11 | Light brownish gray | Cottony growth | Circular with zonation along boarder | 64.5 | + |
| SB-12 | White | Suppressed growth | Irregular with aerial mycelium | 44.0 | + |
| SB-13 | White | Fluffy growth | Circular | 57.2 | + |
| SB-14 | Dark gray | Appressed cottony growth | Circular | 69.8 | ++++ |
| SB-15 | Light brownish gray | Cottony growth | Circular with aerial mycelium at centre | 62.8 | + |

| | | | | | |
|---|---|---|---|---|---|
| SB-16 | White | Appressed cottony growth with white buttons | Circular | 89.5 | + |
| SB-17 | White | Suppressed growth | Semi circular | 39.3 | - |
| SB-18 | White | Cottony growth | Semi circular | 46.2 | + |
| SB-19 | White | Suppressed growth | Irregular with aerial mycelium | 44.8 | - |
| SB-20 | White | Appressed cottony growth | Semi circular | 49.8 | - |
| SB-21 | Light brownish gray | Appressed cottony growth | Circular | 71.8 | ++ |
| SB-22 | Light brownish gray | Cottony growth | Circular | 60.7 | ++ |
| SB-23 | Light brownish gray | Appressed cottony growth | Circular | 83.5 | + |
| SB-24 | Dark gray | Appressed cottony growth | Circular | 76.0 | ++++ |
| **S.Em. ±** | | | | 3.2 | |
| **C.D. (p=0.01)** | | | | 12.1 | |

| **Colony diameter (mm)** | | | *** Sporulation** | | **Conidia /microscopic field (100X)** |
|---|---|---|---|---|---|
| 76-90 | Excellent growth | | ++++ | Excellent | >40 |
| | | | +++ | Good | 21-40 |
| 50-75 | Moderate growth | | ++ | Fair | 6-20 |
| | | | + | Poor | <5 |
| <50 | Poor growth | | - | Nil | 0 |

SB-1= Mangalagatti, SB-2= Mulmutla, SB-3= Arabhavi (A-1), SB-4= Vijayapur, SB-5= Kamatnur, SB-6= Ugar, SB-7= Arabhavi (A-2), SB-8= Dharwad, SB-9= Pune, SB-10= Pantnagar, SB-11= Karnal, SB-12= Delhi, SB-13= Ludhiana, SB-14= Varanasi (V-1), SB-15= Kanpur, SB-16= Varanasi (V-2), SB-17= Powerkheda, SB-18= Indore, SB-19= Anand, SB-20= Jammu, SB-21= Wellington (W-1), SB-22= Wellington (W-2), SB-23= Wellington (W-3), SB-24= Wellington (W-4).

**Table 4: Variation in conidial characteristics of different isolates of *Bipolaris sorokiniana***

| **Isolate** | **Conidial characters** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Length (µm)** | | **Width (µm)** | | **No. of septa** | | **Colour** | **Shape** | **Germination pattern** |
| | **Range** | **Average** | **Range** | **Average** | **Range** | **Average** | | | |
| SB-1 | 29.5-68.3 | 48.9 | 15.3-25.3 | 20.3 | 2-4 | 3.0 | Brown | Elliptical | Unipolar/Bipolar |
| SB-2 | 28.1-50.6 | 39.4 | 11.9-16.5 | 14.2 | 2-3 | 2.5 | Brown | Elliptical | Unipolar/Bipolar |
| SB-3 | 64.7-84.9 | 74.8 | 21.4-31.5 | 26.5 | 3-5 | 4.0 | Dark brown | Elliptical | Unipolar/Bipolar |
| SB-4 | 64.7-126.0 | 95.4 | 13.7-21.7 | 17.7 | 3-6 | 4.5 | Light brown | Elliptical | Bipolar |
| SB-5 | 40.1-50.5 | 45.3 | 10.5-15.6 | 13.1 | 3-4 | 3.5 | Olivaceous | Elliptical | Unipolar/Bipolar |
| SB-6 | 35.5-40.2 | 37.9 | 10.2-15.9 | 13.1 | 3-4 | 3.5 | Olivaceous | Elliptical | Unipolar/Bipolar |
| SB-7 | 35.6-41.3 | 38.5 | 10.5-20.6 | 15.6 | 3-4 | 3.5 | Olivaceous | Elliptical | Unipolar/Bipolar |
| SB-8 | 39.6-101.0 | 70.3 | 19.6-36.1 | 27.9 | 3-4 | 3.5 | Dark brown | Elliptical | Unipolar/Bipolar |
| SB-9 | 30.2-41.0 | 35.6 | 10.0-15.5 | 12.8 | 3-4 | 3.5 | Light brown | Elliptical | Unipolar/Bipolar |
| SB-10 | 37.5-68.8 | 53.2 | 18.7-29.3 | 24.0 | 2-4 | 3.0 | Brown | Elliptical | Bipolar |
| SB-11 | 21.4-41.0 | 31.2 | 11.9-20.9 | 16.4 | 2-3 | 2.5 | Light brown | Elliptical | Unipolar/Bipolar |
| SB-12 | 55.5-69.7 | 62.6 | 20.4-25.5 | 23.0 | 2-3 | 2.5 | Light brown | Elliptical | Unipolar/Bipolar |
| SB-13 | 45.0-50.0 | 47.5 | 10.6-15.4 | 13.0 | 3-4 | 3.5 | Olivaceous | Elliptical | Unipolar/Bipolar |
| SB-14 | 15.3-45.2 | 30.3 | 11.8-17.8 | 14.8 | 1-3 | 2.0 | Brown | Ovate | Unipolar/Bipolar |
| SB-15 | 30.5-36.4 | 33.5 | 12.9-14.7 | 13.8 | 1-2 | 1.5 | Brown | Elliptical | Unipolar/Bipolar |
| SB-16 | 57.1-58.4 | 57.8 | 21.4-24.3 | 22.9 | 3-4 | 3.5 | Light brown | Elliptical | Unipolar/Bipolar |
| SB-18 | 80.0-93.4 | 86.7 | 25.3-32.5 | 28.9 | 3-4 | 3.5 | Light brown | Elliptical | Unipolar/Bipolar |
| SB-21 | 25.2-54.0 | 39.6 | 14.4-24.8 | 19.6 | 1-2 | 1.5 | Brown | Ovate | Unipolar/Bipolar |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| SB-22 | 22.4-57.9 | 40.2 | 9.5-13.0 | 11.3 | 2-3 | 2.5 | Light Brown | Elliptical | Unipolar/Bipolar |
| SB-23 | 45.0-50.1 | 47.6 | 10.4-15.6 | 13.0 | 3-4 | 3.5 | Light Brown | Elliptical | Unipolar/Bipolar |
| SB-24 | 23.4-48.4 | 35.9 | 6.3-14.9 | 10.6 | 1-2 | 1.5 | Light Brown | Elliptical | Unipolar/Bipolar |
| **S.Em. ±** | | **4.0** | | **1.2** | | **0.2** | | | |
| **C.D. (p=0.01)** | | **16.1** | | **5.0** | | **0.7** | | | |

SB-1= Mangalagatti, SB-2= Mulmutla, SB-3= Arabhavi (A-1), SB-4= Vijayapur, SB-5= Kamatnur, SB-6= Ugar, SB-7= Arabhavi (A-2), SB-8= Dharwad, SB-9= Pune, SB-10= Pantnagar, SB-11= Karnal, SB-12= Delhi, SB-13= Ludhiana, SB-14= Varanasi (V-1), SB-15= Kanpur, SB-16= Varanasi (V-2), SB-17= Powerkheda, SB-18= Indore, SB-19= Anand, SB-20= Jammu, SB-21= Wellington (W-1), SB-22= Wellington (W-2), SB-23= Wellington (W-3), SB-24= Wellington (W-4).
*SB-17, 19 and 20 showed no spores on PDA.

**Table 5: Disease response of different isolates of *B. sorokiniana* based on wheat genotypes/ differentials**

| **Wheat genotype /differential** | **Disease response of isolates** | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | **SB-4** | **SB-5** | **SB-7** | **SB-8** | **SB-9** | **SB-10** | **SB-14** | **SB-16** | **SB-18** | **SB-20** | **SB-22** |
| B. Yellow (DW) | MR | MR | MR | HS | R | HS | R | MR | MS | MR | MS |
| NP 200 (DIC) | R | R | MR | S | R | S | R | MS | I | I | MR |
| A-9-30-1 (DW) | MR | R | MR | HS | R | HS | I | MR | MR | MR | R |
| UAS 415 (DW) | MS | MR | MS | MS | MR | MS | R | R | MR | MS | R |
| DDK 1025 (DIC) | MS | R | MR | S | R | S | MR | S | MR | R | I |
| DDK 1029 (DIC) | R | MS | MR | MS | R | MS | MR | MS | MS | I | MS |
| DDK 1009 (DIC) | R | R | MS | MS | R | MS | R | MS | R | MR | S |
| DWR 2006 (DW) | I | MS | MS | MS | R | MS | R | MS | R | I | R |
| 0.3% EMS DDK 1001 (DIC) | R | MS | MR | MS | MR | MS | MS | S | MS | R | I |
| PDW 314(DW) | R | R | R | MR | I | MR | R | MS | MR | I | I |
| UAS 448(DW) | R | MR | R | S | I | S | R | MS | R | R | MR |
| DWR 162 (BW) | MS | MR | MS | S | R | S | R | MR | R | R | R |

| **Infection index** | **% leaf area infected** | **Disease response** |
|---|---|---|
| 0 | 0 | Immune ( I) |
| 1 | <5 | Resistant (R) |
| 2 | 6-20 | Moderately Resistant (MR) |
| 3 | 21-40 | Moderately Susceptible (MS) |
| 4 | 41-60 | Susceptible (S) |
| 5 | >60 | Highly Susceptible (HS) |

SB-4= Vijayapur, SB-5= Kamatnur, SB-7= Arabhavi (A-2), SB-8= Dharwad, SB-9= Pune, SB-10= Pantnagar, SB-14= Varanasi (V-1), SB-16= Varanasi (V-2), SB-18= Indore, SB-20= Jammu, SB-22= Wellington (W-2), DW= Durum Wheat, BW= Bread Wheat, DIC= Dicoccum Wheat.

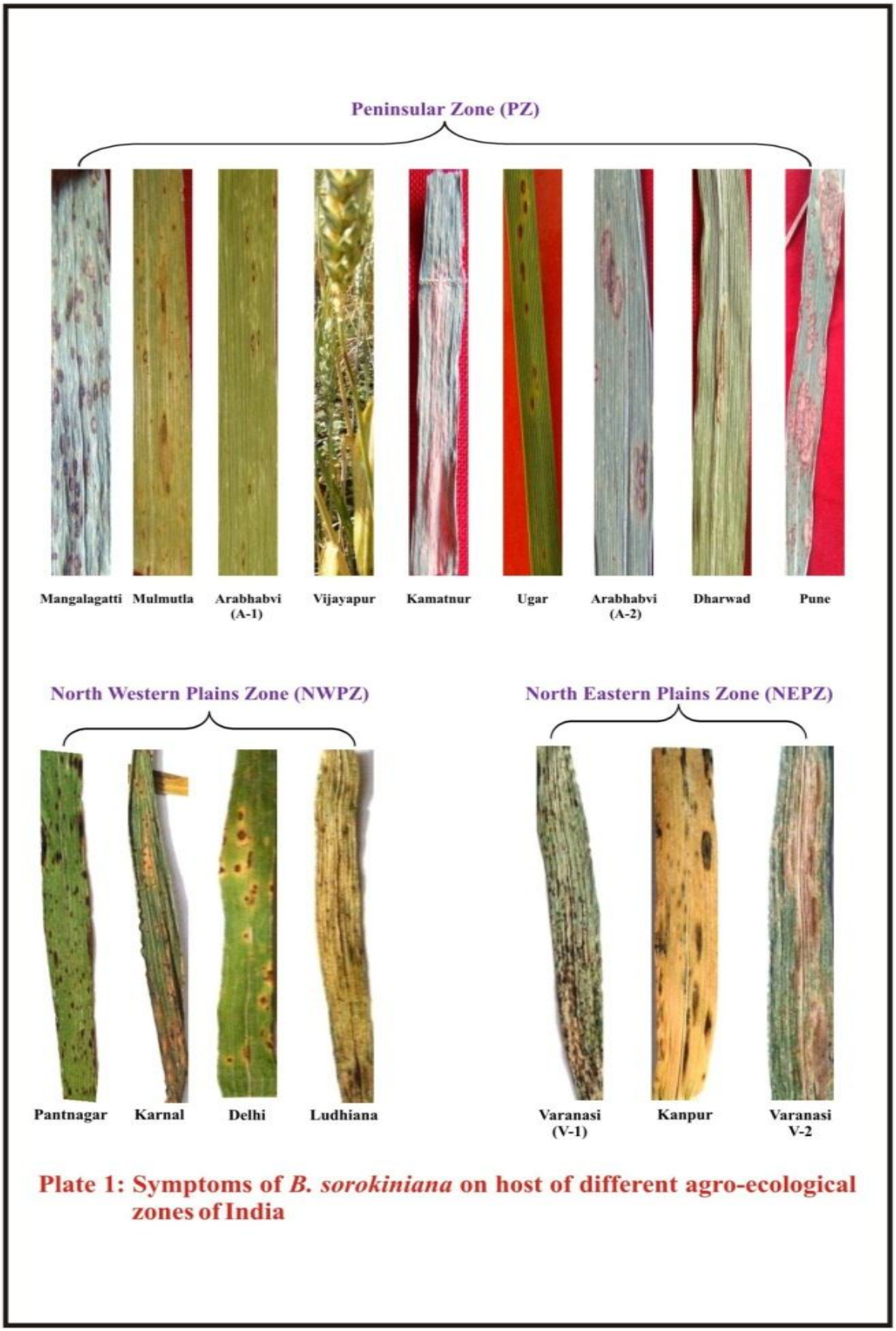


**Plate 1: Symptoms of *B. sorokiniana* on host of different agro-ecological zones of India**


*Plate 1 Contd...*

SB-1= Mangalagatti, SB-2= Mulmutla, SB-3= Arabhavi (A-1), SB-4= Vijayapur, SB-5= Kamatnur, SB-6= Ugar, SB-7= Arabhavi (A-2), SB-8= Dharwad, SB-9= Pune, SB-10= Pantnagar, SB-11= Karnal, SB-12= Delhi, SB-13= Ludhiana, SB-14= Varanasi (V-1), SB-15= Kanpur, SB-16= Varanasi (V-2), SB-17= Powerkheda, SB-18= Indore, SB-19= Anand, SB-20= Jammu, SB-21= Wellington (W-1), SB-22= Wellington (W-2), SB-23= Wellington (W-3), SB-24= Wellington (W-4).

**Plate 2: Cultural variability of *Bipolaris sorokiniana* grown on PDA**

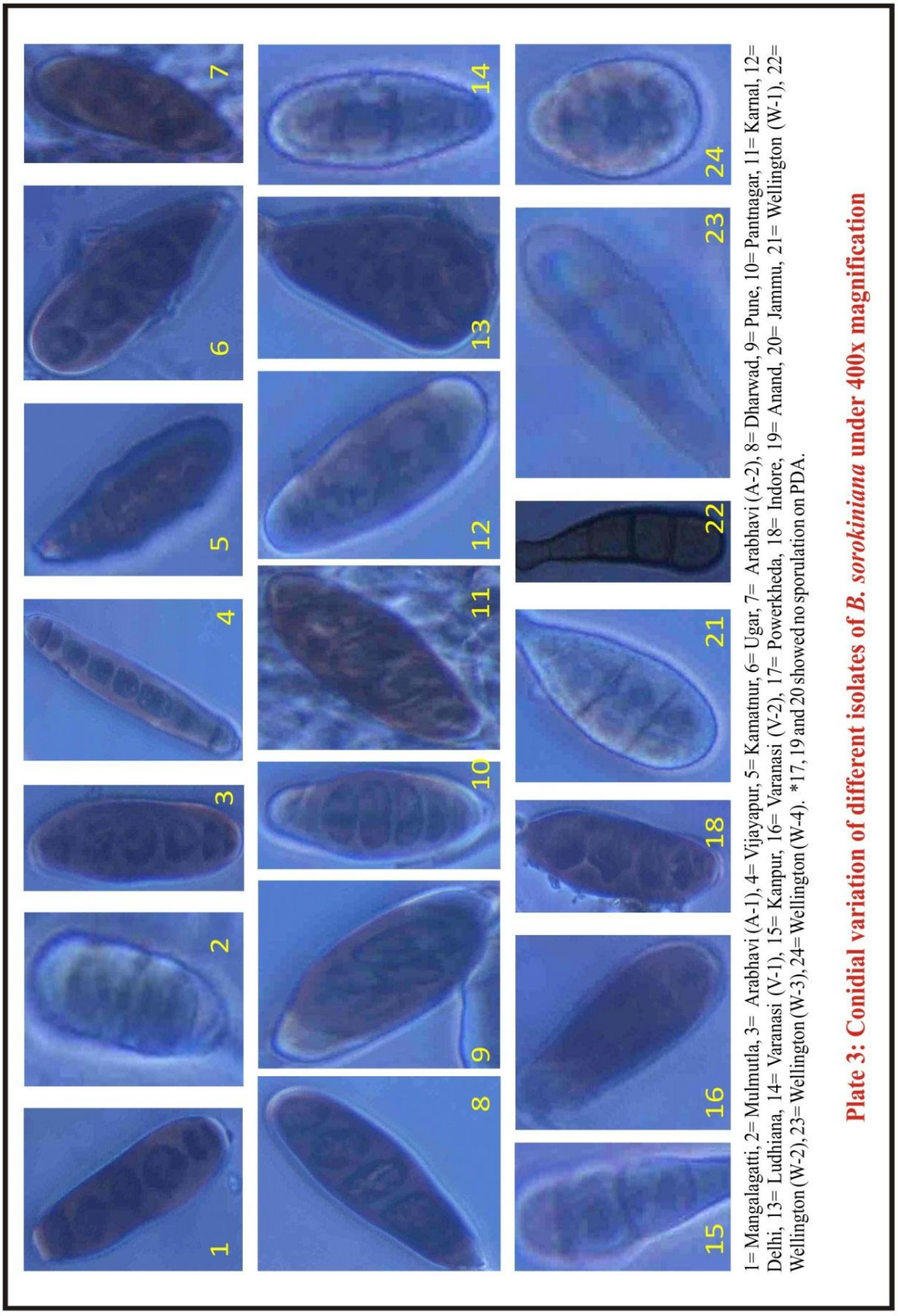

**Plate 3: Conidial variation of different isolates of *B. sorokiniana* under 400x magnification**

1= Mangalagatti, 2= Mulmutla, 3= Arabhavi (A-1), 4= Vijayapur, 5= Kamatnur, 6= Ugar, 7= Arabhavi (A-2), 8= Dharwad, 9= Pune, 10= Pantnagar, 11= Karnal, 12= Delhi, 13= Ludhiana, 14= Varanasi (V-1), 15= Kanpur, 16= Varanasi (V-2), 17= Powerkheda, 18= Indore, 19= Anand, 20= Jammu, 21= Wellington (W-1), 22= Wellington (W-2), 23= Wellington (W-3), 24= Wellington (W-4). *17, 19 and 20 showed no sporulation on PDA.

## DISCUSSION

The mechanisms involved in the variability include heterokaryosis, which occurs chiefly through the anastomosis between adjacent hyphae, enables the parasexual cycle, which is the main source of genetic diversity in fungi with asexual reproduction[7]. Another possible cause of variability in spot blotch pathogen was suggested to be the variable rearrangement of one to six nuclei per cell[5]. This diversity is one cause of the infective success of phytopathogenic fungi in overcoming host resistance[8].Five different types of colours of isolates were found on PDA (Table 3). This confirms the findings of Jaiswal *et al.*[11] studied monoconidial cultures of *B. sorokiniana* isolated from wheat-growing regions in India and showed high morphological variability between the isolates, which formed distinct morphological groups. The frequency of the white (29.2 %), colony type was maximum while both dark gray and light brownish gray colour showed frequency of 25.0 per cent in the population. light gray displayed 16.7 per cent and lowest frequency (4.2 %) observed was black type. The results are in accordance with Aggarwal *et al.*[3] reported the frequency of the dull white/greenish black colony type was maximum (38.83 %), while both black, suppressed type and white fluffy type colonies showed minimum frequency (11.65 %) in the population studied. Among these, dark gray cultures sporulate profusely and had appressed cottony growth. The whitish type had less or no sporulation because of inability to sporulate in PDA or may be due to some other environmental factors like incubation temperature. The dark coloured colony showed a strong correlation with aggressiveness of the pathogen as Chand *et al.*[5] studied the variability in natural populations of the spot blotch pathogen (*B. sorokiniana*) and classified the isolates in to five groups on the basis of colony morphology and reported black type in the natural population were of most aggressive and was identified as the epidemic population as compared to the white coloured having very few conidia. In this study, morphological traits (colour, growth behaviour, and sporulation) were noted to be directly correlated with disease causing ability. The black clones, which displayed greater sporulation and growth, possessed highest disease-causing ability. Morphological characters of conidia and number of septa of 21 isolates were studied for identifying the fungus and to know the existence of variation among the isolates. Sporulation was not observed in the isolates SB-17, 19 and 20. This shows inability of the isolates for spore production on PDA and it also indicates less aggressiveness. The size of the conidia of 21 isolates varied from 15.3-126.0 µm X 6.3-36.1 µm with 1-6 septa, colour varies from dark brown to olivaceous, Ovate to elliptical in shape and showed germination pattern was by unipolar or bipolar (Table 4). The shape and size of conidia are in agreement with findings of Hiremath[10]. Reddy and Biligrami[17] recorded four types of conidial germination *viz.*, unipolar, bipolar, intercalary and purcurrent in *H. sativum*. Studies of Aggarwal *et al.*[2] have shown that the conidia are slightly curved or sometimes straight, fusiform to broadly ellipsoidal, dark olivaceous brown, smooth, thick walled, 3-12 (mostly 6) pseudoseptate. They were about 40-120 µm long and 17-28 µm thick. Zillinsky[23] reported that under low magnification, conidia appeared black and shiny but under higher magnification they were dark olive brown. Earlier studies have also indicated a high level of morpho-pathological variability in the pathogen[5,14]. Based on the reaction on differential hosts three pathogenic groups were identified which were different from groups formed based on morphological variations. The highly virulent isolates can be used for creating artificial epiphytotics while evaluating for resistance in the breeding programme. There was no correlation between the groups identified based on colony characterization and pathogenic variability. Variation and similarity within the species may be attributed to long term influence of weather conditions of location and ability of the pathogen to adapt the varieties developed in a particular

situation[4]. The observations on pathogenic variability studies are in accordance with Singh and Singh[21]. Thus, it is clear that genotype PDW-314 has broad genetic base for resistance, whereas genotype A-9-30-1 and Bijaga Yellow has no gene for resistance. On the basis of aggressiveness of the isolates on differentials showing varied response to disease, the isolates of *B. sorokiniana* were classified into three groups as Highly, moderately and least virulent. Patil[15] categorized isolates of *E. hawaiiensis* causing leaf blight of wheat in to three groups *viz.*, highly virulent (Mahabaleshwar and Ugar Khurd), moderately virulent (Arabhavi, Dharwad and Sangankeri) and less Virulent (Kannur, Digraj and Pune). This fully support the present findings. Singh and Singh[21] tested six wheat genotypes against six isolates of *Bipolaris sorokiniana*, the genotype BOW'S' showed resistance response against three isolates, namely, BS-D-1, BS-DWRK-2 and BS-K-4, whereas moderately resistance response against remaining 3 isolates *i.e.* BS-F-3, BS-P-5 and BS-V-6. The genotype A-9-30-1 showed almost highly susceptible response against each isolate except BS-D-1 which exhibited susceptible reaction on this genotype. Highly virulent isolates exhibited higher infection types on different differentials whereas, some less virulent isolates were unable to produce more infection or pathogenesis as that of virulent isolates. Thus, it clearly indicated the existence of different strains or pathotypes within *B. sorokiniana*. The results are in accordance with Hetzler *et al.*[9] found differences in disease causing ability of different strains on a set of wheat genotypes, which was further confirmed by Maraite *et al.*[12] who reported on morphological variability.

## CONCLUSION

The information on variability existing in this pathogen can be utilized by breeders and pathologists for resistance breeding. There is a need to identify or develop more genotypes resistance to disease as the pathogen exhibits high variability.

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