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Morphological, Cultural, Molecular and Pathogenic Variation of *Bipolaris sorokiniana* in Wheat

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Abstract

One hundred and twenty isolates of *Bipolaris sorokiniana* of wheat were isolated, identified and characterized based on morphological, cultural, pathogenic and ITS (Internal Transcribed Spacer) based molecular parameters. Collected isolates differed in respect of radial mycelial growth rate, color of the colony, surface texture of the colony, shape of the colony, conidia production ability and shape and color of conidia. ITS1 and ITS4 based PCR products yielded a species-specific band of 600 bp (base pairs) on agarose gel for the confirmation of *B. sorokiniana*. The mycelial growth rate of the isolates ranged from 1.39 to 4.46 mm/day. The septation of conidia ranged from 1.4 to 8.6. On the basis of conidia morphology, the isolates were grouped into five different groups, whereas the isolates were grouped into 12 cultural groups based on cultural characteristics. Cultural group 8 [CG (Cultural Group)-8] was the most virulent [38.5% LAD (Leaf area diseased)] and [CG (cultural Group)-4] was the less virulent [2% LAD (Leaf area diseased)] group among 12 groups when inoculated at tillering stage of wheat cv. Kachan.

Keywords

B. sorokiniana, Wheat, Morphological Variation, Cultural Variation, Molecular Variation, Virulence

1. Introduction

Wheat (*Triticum aestivum* L.) is one of the most important grain crops in Bangladesh. The production of wheat in Bangladesh has improved tremendously with the expansion of high-yielding varieties and better use of inputs. The causal

agent of spot blotch, root rot, foliar blight, seedling blight, and head blight of wheat and barley is *B. sorokiniana* (teleomorph *Cochliobolus sativus*). The fungus causing this disease affects both crops in warmer growing locations and for this, a significant crop loss has occurred [1]. The outbreak of this disease is particularly dependent on high temperatures and high relative humidity particularly in South Asia's intensive irrigated whole-wheat production system [2]. [3] collected 122 isolates from major wheat growing areas of Bangladesh and found morphological and pathogenic variations among these isolates. Selecting breeding materials should follow some rules to know which pathotype is to be used in the screening process, the process of expression and inheritance and it's adequate and duration periods. Some important factors should be noted like the capacity of the pathogen for change under a controlled environment [4]. Different pathotypes of *B. sorokiniana* significantly differed among themselves in respect of leaf blight severity [5]. Information on morphological and cultural variation of *B. sorokiniana* and their relation with virulence on the host is not well understood. An attempt was therefore made to find out the relationship between morphological and cultural variants and virulence of *B. sorokiniana* on wheat.

2. Materials and Methods

2.1. Collection of Samples from Different Wheat Growing Locations of Bangladesh

Two hundred infected leaves and seed samples were collected from seven districts namely Bogura, Dinajpur, Meherpur, Jashore, Mymensingh, Netrokona and Rajshahi. Leaf samples were collected in a brown paper envelope and seed samples were collected in cotton bags and preserved in the refrigerator at 5˚C temperature. During the sample collection, variety, sample type and specific location were recorded.

2.2. Isolation, Identification, Purification and Preservation of Isolates of *B. sorokiniana*

Infected leaves were cut into 5 mm small pieces including healthy and diseased portions, surface sterilized with mercuric chloride for one minute, rinsed sufficiently with sterile distilled water, blotted dry paper were transferred on PDA (Potato Dextrose Agar) media and incubated at 25˚C ± 1˚C for 7 days. The mycelia grown from planted tissues were transferred to a second PDA (Potato Dextose Agar) plate and incubated at 25˚C ± 1˚C for 14 days for sufficient sporulation. Seeds were surface sterilized by mercuric chloride, rinsed with sterile distilled water thrice and placed on moist blotter paper and incubated under 12-hour light and 12-hour darkness conditions at 25˚C ± 1˚C for sporulation. Seeds were then observed under a stereo microscope and a single conidium was transferred on PDA (Potato Dextrose Agar) plate and incubated in the similar condition described earlier for 14 days. The isolates were transferred to PDA (Potato Dextrose Agar) slants and stored at 5˚C in a refrigerator for morpholog-

ical and molecular study.

2.3. Designation of Collected Isolates

The isolates were designated based on their locations and sources following the procedure of Aminuzzaman *et al*. [6]. For example, an isolate designated by BDKS represents that this isolate was collected and isolated from Bogura district, Dupchanchia Upazila from the variety Kanchon seed (KS) it was isolated.

2.4. DNA Extraction of *B. sorokiniana*

Genomic DNA was extracted using the DNA extraction kit of the Thermofisher company. At first fungal culture was transferred to a sterilized 1.5 µL microcentrifuge tube. One milliliter of FA buffer was added to the cells and resuspended the cells by pipetting. The cells were descended by centrifuging at 5000 ×g for 2 minutes and supernatants were completely discarded. The cells were resuspended in 550 µL of FB buffer and 50 µL of lyticase solution was added and mixed well by vortexing. The samples were incubated at 37˚C for 30 minutes. Eight µL of RNase was added and incubated at room temperature for 2 minutes. The cells were descended by centrifuging at 5000 ×g for 10 minutes and the supernatant was removed. 350 µL of TG1 buffer was added and mixed well by pipetting. The sample mixtures were transferred to a bead tube and mixed well by vortexing for 5 minutes. 20 µL proteinase K was added and mixed well by vortexing and incubated at 55˚C for 15 minutes and vortexed 30 seconds for every 5-minute incubation. The cells were descended by centrifuging at 5000 ×g for 1 minute and 200 µL of supernatant was transferred to a new 1.5 µL microcentrifuge tube. 200 µL of TG2 buffer was added and mixed well by pipetting. 200 µL ethanol (96% - 100%) was added and mixed well by pulse vortexing for 10 seconds. TG mini column was placed in the collection tube and the sample mixtures were transferred to TG mini column and centrifuged at 11,000 ×g for 30 seconds. TG mini column was placed on a new collection tube. A 400 µL W1 buffer was added to the TG mini column and centrifuged at 11,000 ×g for 30 seconds and the flow-through were discarded. TG mini column was placed back into the collection tube. A 750 µL wash buffer was added to the TG mini column and centrifuged at 11,000 ×g for 30 seconds and the flow-through was discarded. TG mini column was placed back into the collection tube. The collection tube with column was centrifuged at 18,000 ×g for an additional 3 minute to dry the column. The TG mini column was placed in an elution tube and 80 µL elution buffer was added to the membrane center of the TG column and the column was kept standing for 3 minutes. The column was centrifuged at full speed (18,000 ×g) for 1 min to elute total DNA. DNA was stored at −20˚C for PCR and future use.

2.5. Polymerase Chain Reaction (PCR)

Universal eukaryotic primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (R 5'TCCTCCGCTTATTG ATATGC-3') were used in PCR [7]. PCR am-

plification was done in a total volume of 25 µL, which contained 10× standard Taq reaction buffer 2.5 µL, 10 mM dNTPs 0.5 µL, 10 µM Forward Primer 0.5 µL, 10 µM Reverse Primer 0.5 µL, template DNA 0.5 µL, Taq DNA polymerase 0.1 µL and nuclease free water 21 µL. In a Perkin-Elmer/Gene Amp PCR System 9700 (PE Applied Biosystems) thermocycler, PCR amplification was carried out in it. The conditions of the PCR amplification were as follows: denaturation at 94˚C for five minutes followed by 40 cycles of denaturation at 94˚C for 30 sec. At 50˚C primer was annealed for 30 seconds. Elongation was done at 72˚C for 1 minute. The primer extension segment was extended at 72˚C for 7 minutes in the final cycle. The PCR product was checked for confirmation of DNA amplification using gel documentation system and a band image of the targeted amplified DNA was taken.

2.6. Morphological Determinations, Growth Study and Grouping of *B. sorokiniana*

The growth of study of *B. sorokiniana* was done on Potato Dextrose Agar (PDA) and the plates were inoculated with 5 mm mycelia block at the middle of the plate and kept at 25˚C [8]. After 14 days of incubation, the radial growth of the isolates on PDA (Potato Dextrose Agar) was measured. The texture, color and colony shape of the isolates were recorded. The conidia produced per unit surface area were estimated using the formula as follows [9]:

$$\text{Conidia produced per Unit surface area} = \frac{\text{Number of conidia/ml of suspension} \times \text{volume of water used to make suspension}}{\text{Total surface area from which conidia suspension was derived}}$$

The isolates were grouped based on their compactness, shape and color of the colony following [6]. The isolates were also grouped based on conidial shape.

2.7. Virulence Test of Different Cultural Groups of *B. sorokiniana* on Wheat

Virulence tests were done on different cultural groups of *B. sorokiniana* on wheat. One isolate of each group was cultured on PDA media for 15 days. Conidia were harvested using sterile distilled water. The spore concentration was adjusted to 2×10^5 conidia/ml of sterile distilled water. The spore suspension was sprayed by hand sprayer on 15 days old wheat seedlings that were sown on sterilized pot soil. Pots were kept at 26˚C, maintaining >90% relative humidity in darkness for 24 hours. Pots were transferred into the shade house. The number of leaves infected the number of spot/leaf and % LAD (Leaf Area Diseased) was recorded at 15 days after inoculation (DAI).

3. Results and Discussion

One hundred and twenty isolates of *B. sorokiniana* of wheat were isolated and identified based on morphological characteristics as well as ITS1 and ITS4 based molecular parameters. These isolates were diversified from seeds and leaves of 7

varieties of wheat collected from 7 major wheat growing regions of Bangladesh. The collected isolates differed significantly in respect of radial mycelial growth rate, color of the colony, surface texture of the colony, shape of the colony, number of conidia production, shape and color of conidia, etc. The mycelial growth rate was recorded from the range of 1.39 (MMPS 66) to 4.46 mm/day (JSBS 88) (data not shown in Table). Seventy-one isolates had blackish white colored colonies. Forty-two isolates had blackish colored colonies and seven isolates had whitish colored colonies. Among one hundred twenty isolates, sixty-six isolates produced regular-shaped colonies and fifty-four isolates had irregularly shaped colonies. Among one hundred twenty isolates, the maximum number of conidia/cm^2 was counted in isolates no BAKS 11 which was 416.17 × 10^4 and the minimum number of conidia/cm^2 was 89.6 × 10^4 found in isolates no JSBS 88 among all the tested isolates. Three different conidia colors, e.g., brown, deep brown and light brown were observed. Among these isolates, ninety-two isolates produced brown colored conidia and seventeen isolates produced deep brown colored conidia and eleven isolates produced light brown colored conidia. A total of 92 (76.7%) isolates produced brown colored conidia, 17 (14.2%) isolates produced deep brown colored conidia and 11 (9.2%) isolates produced light brown colored conidia.

The isolates were grouped into twelve cultural groups (CGs) based on colony morphology and surface texture of the colony (Table 1 and Figure 1). Among

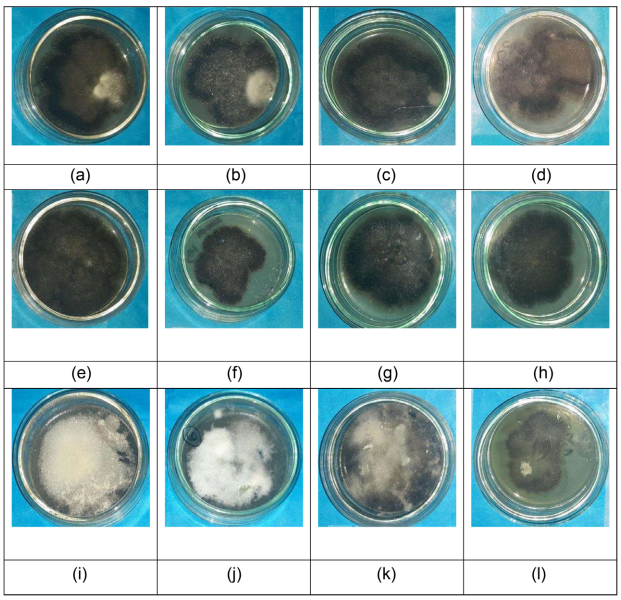

Figure 1. Twelve cultural groups of *B. sorokiniana*. (a). Smoothy and wooly blackish white regular isolates, (b). Smoothy and wooly blackish white irregular isolates, (c). Effuse and rough blackish white regular isolates, (d). Effuse and rough blackish white irregular isolates, (e). Smoothy and wooly Blackish regular isolates, (f). Smoothy and wooly blackish irregular isolates, (g). Effuse and rough blackish regular isolates, (h). Effuse and rough blackish irregular isolates, (i). Smoothy and wooly whitish regular isolates, (j). Smoothy and wooly whitish irregular isolates, (k). Effuse and rough whitish regular isolates and (l). Effuse and rough whitish irregular isolates.

Table 1. Grouping of different isolates of *B. sorokiniana* based on cultural characteristics.

Cultural group (CG)	Cultural characteristics	Number of isolates	Designation of isolates	% Isolates under each group
CG-1	Smoothy and wooly blackish white regular	21	MSBS 35, MSBS 38, MGBS 51, MMPS 52, MMPS 53, MMPS 54, MMPS 62, MTPS 96, MTPS 97, MTPS 98, MTPS 99, MTPS 103, MTPS 104, NSKS 106, NSKS 107, NSKS 108, NSKS 109, NSKS 110, NSKS 114, RSKL 117, RSKL 120.	17.5
CG-2	Smoothy and wooly blackish white irregular	38	BDKS 5, BAKS 11, DSSS 22, DSSS 23, MSBS 31, MSBS 32, MSBS 33, MSBS 34, MSBS 36, MSBS 37, MSBS 39, MSPS 41, MSPS 42, MSPS 43, MSPS 44, MSPS 45, MGBS 46, MGBS 48, MGBS 49, MGBS 50, MMPS 56, MMPS 57, MMPS 63, MMPS 64, MMPS 65, MMPS 66, MMPS 67, MMPS 68, MMPS 69, MMPS 70, MTPS 101, MTPS 102, MTPS 105, NSKS 112, NSKS 113, NSKS 115, RSKL 116, RSKL 118.	31.66
CG-3	Effuse and rough blackish white regular	8	MMPS 55, MMPS 58, MMPS 59, MMPS 60, MMPS 61, MTPS 100, NSKS 111, RSKL 119.	6.66
CG-4	Effuse and rough blackish white irregular	4	BDKS 9, BDKS 10, MSBS 40, MGBS 47.	3.33
CG-5	Smoothy and wooly blackish regular	30	BAKS 16, BAKS 17, BAKS 18, BAKS 19, BAKS 20, DSSS 21, DSSS 24, DSSS 25, DSSS 26, DSSS 27, DSSS 28, DSSS 29, JSBS 71, JSBS 72, JSBS 74, JSBS 75, JSBS 77, JSBS 78, JSBS 79, JSBS 80, JSBS 83, JSBS 84, JSBS 85, JSBS 86, JSBS 89, JSBS 90, JSBS 92, JSBS 93, JSBS 94, JSBS 95.	25
CG-6	Smoothy and wooly blackish irregular	8	DSSS 30, JSBS 73, JSBS 76, JSBS 81, JSBS 82, JSBS 87, JSBS 88, JSBS 91.	6.66
CG-7	Effuse and rough blackish regular	2	BDKS 4, BDKS 6.	1.66
CG-8	Effuse and rough blackish irregular	2	BAKS 12, BAKS 14.	1.66
CG-9	Smoothy and wooly whitish regular	4	BDKS 1, BDKS 2, BDKS 3, BAKS 13.	3.33
CG-10	Smoothy and wooly whitish irregular	1	BDKS 7.	0.83
CG-11	Effuse and rough whitish regular	1	BAKS 15.	0.83
CG-12	Effuse and rough whitish irregular	1	BDKS 8.	0.83

BDKS (Bogura Dupchanchia Kanchan Seed), BAKS (Bogura Adamdighi Kanchan Seed), DSSS (Dinajpur Sadar Shatabdi Seed), MSBS (Meherpur Sadar BARI Gom 30 Seed), MSPS (Meherpur Sadar Prodip Seed), MGBS (Meherpur Gangni BARI Gom 28 Seed), MMPS (Meherpur Mujibnagar Prodip Seed), JSBS (Jashore Sadar BARI Gom 26 seed), MTPS (Mymensingh Trisal Prodip Seed), NSKS (Netrokona Sadar Kanchan Seed), RSKL (Rajshahi Sadar Kanchan Leaf).

these cultural groups, smoothy and wooly blackish white irregular groups contain the highest number of isolates (39 isolates) and that was thirty-nine isolates with 32.5% frequency of the total isolates studied. The different cultural groups of *B. sorokiniana* showed different characteristics based on mycelial growth rate, conidia production and septation number. The radial mycelial growth rate differed from 2.67 mm/day to 4.50 mm/day (Table 2). The highest growth rate was recorded from the group number CG-12 and the lowest growth rate was measured from group number CG-7. The maximum number of conidia/cm^2 was recorded from group number CG-7 which was 248.93 × 10^4 and the minimum number of conidia/cm^2 (88.18 × 10^4) was recorded from group CG-12. The highest septation (7.10) was found in group CG-11 and the lowest (2.44) was found in group CG-1.

4. Molecular Identification (ITS rDNA Gene)

In molecular identification (ITS rDNA gene) PCR using primer pairs ITS1/ITS4 yielded species specific band of 600 bp of amplification product for representative isolates of each cultural group of *B. sorokiniana* (Figure 2) which was previously supported [10]. Here PCR is used to confirm the species. Isolates were grouped into twelve groups. We experimented with each group separately using PCR. But each group showed almost the same as PCR results. Every isolate (one hundred twenty) of *B. sorokiniana* under twelve groups yielded near 600 bp (base pairs) band. So here only two PCR results are shown as the representative isolates. As the PCR results are almost the same, it is almost confirmed that one hundred twenty isolates are of *B. sorokiniana*.

The isolates were grouped into five groups (CS-1 to CS-5) based on their conidial shape (CS) where fifty-seven isolates produced elliptical and straight conidia with 47.50% frequency, thirteen isolates produced elliptical and curved shaped conidia with 10.83% frequency, 28 isolates produced oval shaped conidia with 23.33% frequency, five isolates produced round shaped conidia with 4.17% frequency and seventeen isolates produced pyriform shaped conidia with 14.16% frequency of the collected isolates (Table 2 and Figure 3). Maximum isolates produced straight shaped conidia and some isolates produced curved and round shaped conidia and some isolates produced both straight and curved shaped conidia.

Table 2. Grouping of isolates based on conidia shape.

Groups	Conidia shape	Number of isolates	% Isolates under each group
CS-1	Elliptical straight	57	47.50
CS-2	Elliptical curved	13	10.83
CS-3	Oval	28	23.33
CS-4	Round	5	4.17
CS-5	Pyriform	17	14.16

CS = Conidia shape.

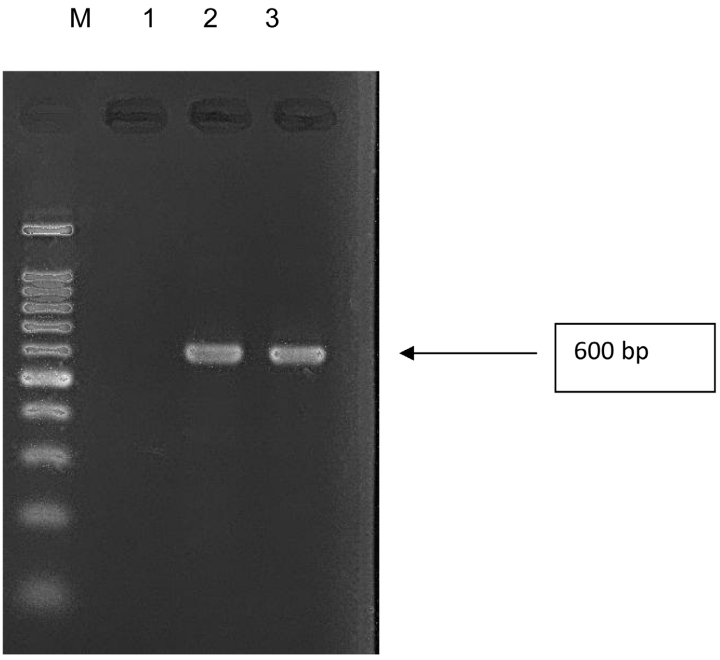

Figure 2. Gel electrophoresis of PCR products from amplification of ITS primer (ITS1F-ITS4R) region of rDNA of isolates. M: 100 bp plus DNA Marker, L1 = Negative sample, L2 and L3 = Two isolates of *B. sorokiniana* under two cultural groups that yielded 600 bp band.

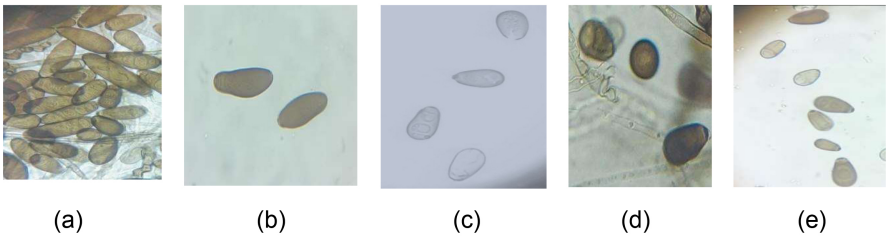

Figure 3. Five conidial shapes of *B. sorokiniana* (a). Elliptical and straight shaped conidia, (b). Elliptical and curved shaped conidia, (c). Oval shaped conidia, (d). Round shaped conidia and (e). Pyriform shaped conidia.

Mycelial growth rate, conidia production and conidial septation also varied among twelve cultural groups (Table 3). Here maximum growth rate was 4.50 mm per day found in CG (Cultural group)-12 and the production of the maximum number of conidia found in CG (Cultural Group)-7, which was 248.93 conidia/cm^2 ($\times 10^4$). Among one hundred twenty isolates, the septation of conidia ranged from 1.4 to 8.6.

Among these 12 groups, the effuse and rough blackish irregular group (CG-8) was the most virulent (38.5% LAD) and the effuse and rough blackish white irregular group (CG-4) was the low virulent group among 12 groups (2% LAD) (Table 4, Figure 4 and Figure 5). All around the world many workers were studying morphological and physiological characteristics of isolates of *B. sorokiniana*. From 1922 to 2020, much work was done by many hard workers. In our present study, the radial mycelial growth rate of isolates of *B. sorokiniana* were

Table 3. Mycelial growth rate, conidia production and septations of conidia of *B. sorokiniana* under 12 cultural groups. (14 days old culture).

Cultural group (CG)	Growth rate/day (mm)	Number of conidia/cm^2 (×10^4)	Number of septation
CG-1	4.35	94.06	2.44
CG-2	4.32	95.62	3.23
CG-3	3.32	161.88	4.2
CG-4	4.28	97.22	3.0
CG-5	3.57	140	3.6
CG-6	4.46	89.6	3.2
CG-7	2.67	248.93	2.6
CG-8	3.14	180.78	6.0
CG-9	3.57	140	4.4
CG-10	4.21	100.54	4.8
CG-11	3.57	140	7.1
CG-12	4.50	88.18	4.6

Table 4. Virulence of different cultural groups of *B. sorokiniana* on wheat.

CG (Cultural groups)	Number of leaves infected	Number of spots/leaf	% LAD (Leaf Area Diseased)
CG-1	5	3.4	19
CG-2	9	3.4	14.44
CG-3	28	2.5	29.46
CG-4	2	1.5	2
CG-5	7	5.7	31.42
CG-6	5	2.0	13
CG-7	18	8.4	32.77
CG-8	10	5.4	38.5
CG-9	4	2.5	11.25
CG-10	2	1.5	7.5
CG-11	15	4.4	18.73
CG-12	8	4.1	21.25

varied from isolates to isolates and ranged from 1.39 mm/day to 4.46 mm/day. [8] recorded the mycelial growth of isolates of *B. sorokiniana* from 29 mm to 78 mm among eighty three (83) isolates tested. Variations in mycelial growth of *B. sorokiniana* isolates were also reported and ranged from 9.26 mm to 24.0 mm [11] and from 20.3 mm to 63 mm [12]. Another study [13] reported the mycelial growth of isolates of *B. sorokiniana* from 4.77 cm. [6] recorded the mycelial growth of isolates of *B. sorokiniana* ranging from 2.77 mm to 9.10 mm/day. The mycelial

Figure 4. Inoculation of isolates of *B. sorokiniana* in wheat, (a). Spore suspension of 12 cultural group of *B. sorokiniana*, (b). (c). (d). and (e). Inoculation of 12 cultural group of *B. sorokiniana* including control where sterile distilled water was sprayed.

Figure 5. Post inoculation plant morphology of wheat after inoculation of 12 cultural groups of *Bipolaris sorokiniana*. (a). CG-1, (b). CG-2, (c). CG-3, (d). CG-4, (e). CG-5, (f). CG-6, (g). CG-7, (h). CG-8, (i). CG-9, (j). CG-10, (k). CG-11 and (l). CG-12 inoculated plant. (m). uninoculated control (sterile distilled water sprayed plant; (n) and (o). typical post inoculation leaf spot symptom caused by *Bipolaris sorokiniana*.

growth rate may vary depending on temperature during the incubation period. Another study [14] reported that the growth of conidia of *B. sorokiniana* was quickly at the temperature of 25˚C ± 1˚C. The maximum temperature was 15˚C to 25˚C due to the germination of the conidia of *B. sorokiniana* [15]. He recorded this temperature from 20 days of the older culture. But above 20˚C the germination growth rate of the isolates of *B. sorokiniana* were decreased slowly and at 35˚C temperature the conidia production was stopped. [16] recorded that the suitable temperature for conidia production of isolates of *B. sorokiniana* was 25˚C and at 35˚C temperature and extreme pH of 4 and 10, it was decreased and inhibited. In our research work, the maximum number of conidia/cm^2 was 416.17 × 10^4 which was found in isolate number BAKS 11 and the minimum number of conidia/cm^2 was 89.6 × 10^4 and which was found in isolate number JSBS 88. In 1992, Variation in conidia production of *B. sorokiniana* isolates were previously reported [8] and they recorded 65 isolates that produced abundant conidia among 83 collected isolates in seven days under UV-light (12/12). The spore production rate of the isolates of *B. sorokiniana* ranged from 3.35 × 10^3 to 122.12 × 10^3 [13]. [12] recorded the highest spore production per colony was 10 × 10^7 and the lowest was 1.0 × 10^7 per colony. In another study, the highest number of conidia production was recorded as 166.84 × 10^3 per cm^2 and the lowest number of conidia/cm^2 was 4.00 × 10^3 per cm^2 [6]. In our present research, maximum isolates produced straight-shaped conidia and some isolates produced curved and round-shaped conidia and some isolates produced both straight and curved-shaped conidia. The results of the present studies were supported by previous work where the shape of the conidia of *B. sorokiniana* were reported as fusoid [17]. [16] studied eighty-six isolates and reported that fifty-two had brown colored conidia and twenty-six had deep brown colored con-

idia, five had light brown colored conidia and two had brown to deep brown colored conidia and the rest one had light brown to deep brown colored conidia. In the present research work, among 120 isolates the septation of conidia ranged from 1.4 to 8.6. [17] recorded the septation number varied from 5 to 9. In 1997, Ahmed studied 27 isolates and reported that the number of cells per conidia ranged from 3 to 10 septa. [18] recorded several celled conidia of *B. sorokiniana*. Whereas [11] reported that the septation number of conidia differed from 2 to 10 septa. In the present research work, 120 isolates were grouped into twelve cultural groups based on colony morphology and surface texture of the colony. Among these cultural groups smoothy and wooly blackish white irregular groups contain the highest number of isolates and that was thirty-nine isolates with 32.5% frequency of the total isolates studied. And among these cultural groups smoothy and wooly whitish irregular, effuse and rough whitish regular and effuse and rough whitish irregular groups contain the lowest (1) isolates with 0.83% frequency of each of isolates. The morphological variation of *B. sorokiniana* isolates were also previously reported [19]. They collected samples from wheat growing locations in West Bengal and found some variation in conidial morphology which they researched on four different media. [20] collected fourteen isolates of *B. sorokiniana* from typical spot blotch lesions of wheat. Colony color was whitish black to black; the shape was oblong and round. They also found three to seven septations and four to eight cells. The results of the present studies are also supported by previous research work [21] [22] [23].

5. Conclusion

The Morphological and cultural variant of *B. sorokiniana* from wheat was also a pathogenic variant on the same host.

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Authors' Contributions

This work was carried out in collaboration among all authors. Author AS conducted the research work. Author FMA designed and supervised the study, inoculated the isolates, extracted genomic DNA and edited the manuscript. Author THA co-supervised the study and author MA performed PCR, gel electrophoresis and took the image of DNA band on the agarose gel. All authors read and approved the final manuscript.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this article.

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