MORPHOLOGICAL, CULTURAL AND MOLECULAR VARIATION AND VIRULENCE OF *BIPOLARIS SOROKINIANA* IN WHEAT

SHARMIN AKTER

DEPARTMENT OF PLANT PATHOLOGY
SHER-E-BANGLA AGRICULTURAL UNIVERSITY, DHAKA-1207

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MORPHOLOGICAL, CULTURAL AND MOLECULAR VARIATION AND VIRULENCE OF *BIPOLARIS SOROKINIANA* IN WHEAT

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SHARMIN AKTER

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Approved by:

Dr. F.M. Aminuzzaman	**Dr. Tahmid Hossain Ansari**
Professor	Principal Scientific Officer
Department of Plant Pathology	Plant Pathology Division
Sher-e-Bangla Agricultural University	Bangladesh Rice Research Institute
Supervisor	Co-Supervisor

Dr. Fatema Begum
Chairman
Examination Committee
Department of Plant Pathology
Sher-e-Bangla Agricultural University

Dr. F.M. Aminuzzaman

Professor

Department of Plant Pathology

Sher-e-Bangla Agricultural university

Dhaka-1207, Bangladesh

Mobile: +8801733717936

Ref. Date:.....

CERTIFICATE

This is to certify that the thesis entitled **"MORPHOLOGICAL, CULTURAL AND MOLECULAR VARIATION AND VIRULENCE OF *BIPOLARIS SOROKINIANA* IN WHEAT"** submitted to the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE in PLANT PATHOLOGY, embodies the result of a piece of bona-fide research work carried out by **SHARMIN AKTER** bearing **Registration No. 18-09284** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

I further certify that such help or source of information, as has been availed of during the course of this investigation has duly been acknowledged.

Dated:
Dhaka, Bangladesh

Dr. F.M. Aminuzzaman
Supervisor

DEDICATED TO

MY

BELOVED PARENTS

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The Author

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ABSTRACT

Monoconidial one hundred and twenty isolates of *Bipolaris sorokiniana* of wheat were isolated and identified based on morphological and ITS1 and ITS4 based molecular parameters. These isolates were isolated from seeds and leaf 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 colony, surface texture of colony, shape of 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). Maximum (73) isolates had blackish white colored colonies. Forty-two isolates had blackish colored colonies and five isolates had whitish colored colonies. Among 120 isolates, 65 isolates produced regular shaped colonies and 55 isolates had irregular shaped colonies. One hundred twenty isolates showed smoothy and wooly surface texture of colony and 18 isolates showed effuse and rough surface texture of colony. Among 120 isolates, maximum number of conidia/cm² was counted in isolates no BAKS 11 which was 416.17×10^4 and minimum number of conidia/cm² was 89.6×10^4 found in isolates no JSBS 88. Among these isolates, 92 isolates produced brown colored conidia and 17 isolates produced deep brown colored conidia and 11 isolates produced light brown colored conidia. Among 120 isolates, the septation of conidia ranged from 1.4 to 8.6. The isolates were grouped into five groups based on their conidial shape where 57 isolates produced elliptical and straight conidia with 47.50% frequency, 13 isolates produced elliptical and curved shaped conidia with 10.83% frequency, 28 isolates produced oval shaped conidia with 23.33% frequency, 5 isolates produced round shaped conidia with 4.17% frequency and 17 isolates produced pyriform shaped conidia with 14.16% frequency. Again the 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 39 isolates with 32.5% frequency of the total isolates studied. The different cultural groups of *Bipolaris 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. The highest growth rate counted from the group number G-12 and the lowest growth rate counted from the group number G-7. Maximum number of conidia/cm² was recorded from the group number G-7 and that was 248.93×10^4 and the minimum number of conidia/cm² was recorded from group G-12. The highest septation (7.1) was found in group G-11 and the lowest (2.44) was found in group G-1. Among these 12 groups, effuse and rough blackish irregular group (G-8) was most virulent (38.5% LAD) and effuse and rough blackish white irregular group (G-4) was low virulent group among 12 groups (2% LAD).

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATE

LIST OF ABBREVIATED TERMS

ABBREVIATION	FULL WORD
AEZ	Agro-Ecological Zone
et al.	And others
BARI	Bangladesh Agricultural Research Institute
BAU	Bangladesh Agricultural University
BSMRAU	Bangabondhu Sheikh Muzibur Rahman Agricultural University
CIMMYT	International Maize and Wheat Improvement Center
Cm^2	Centimeter Square
CV.	Cultivar
°C	Degree centigrade
CMI	Commonwealth Mycology Institution
DLB	*Drecheslera* leaf Blight
etc.	Etcetera
J.	Journal
No.	Number
LAD	Leaf Area Diseased
LSD	Least Significant Difference
PDA	Potato Dextrose Agar
%	Percent
µ	Micrometer
Res.	Research
SAU	Sher-e-Bangla Agricultural University
SD	Standard Deviation

CHAPTER 1

INTRODUCTION

Wheat (*Triticum aestivum* L.) which is one of the most important grain crops in our Bangladesh. It provides nearly 20% of the total world food requirement. In Bangladesh it is considered as the second staple food crop and production of this staple food crop has been increasing day by day. The contribution of wheat to total food grain is impressive. 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, head blight of wheat and barley is *Bipolaris sorokiniana* (teleomorph *Cochliobolus sativus*). Fungus causing this disease both crops in warmer growing locations and for this a significant crop loss has occurred (Aftabuddin *et al.*, 1991). Outbreak of this disease is particularly dependent on high temperature and high relative humidity particularly in South-Asia's intensive irrigated whole-wheat production system (Aggarwal *et al*., 2000).

This destructive pathogen damages the crop from the stage of seedling to leaf (Alam *et al*., 1994). It attacks the panicle of the crops and black point of grains is caused by this pathogen. Almost 88.7% grains are lost by the pathogen *Bipolaris sorokiniana* and produced 87.5% discoloured and black pointed grains (Hossain *et al*., 1998). In Kanchan and Sonalika varieties 57.6% and 64.5% yield reduction has been found by Rashid and Fakir (1998) of wheat production and Hossain (2001) reported that infected seed samples were shown in formation of higher numbers of black pointed infections in the field. Hossain *et al.,* (1998) also reported that about 40% and 88% reduction of yield were found over the controlled environment by the attacking of *Bipolaris sorokiniana.* Fakir (1998) found that about 15 to 20% yield reduction for the attacking of black pointed grains. 100% yield reduction could happen for *Bipolaris sorokiniana* attacking wheat production. This pathogen slowly attacks the root system of wheat and causes 100% yield losses.

Another synonym was reported by Maraite *et al.* (1998) of the anamorph viz. *Helminthosporium sorokiniana, Drechslera sorokiniana* and *Helminthosporium sativum.* Shoemaker (1959) proposed the generic name of *Bipolaris* for the *Helminthosporium* species with fusoid, straight or curved conidia germination by one

germ tube each end. *Helminthosporium* genus was categorised into three categories like *Bipolaris, Drechslera* and *Exserohilum* with *Cochliobolus, Pyrenophora* and *Setosphaeria teleomorphic* stages respectively. The pathogen *Bipolaris sorokiniana* is thick-walled, elliptical conidia with five to nine cells. Depending on the isolates, the mycelium of this pathogen is composed of hyphen interwoven as a loose wooly or cotton mass and looks like white or blackish or light to dark grey in colour in axenic culture. Sometimes *Bipolaris* genus showed different variations among their cultural and morphological characteristics features of conidia and conidiospores.

Christensen (1925) first reported that these fungal isolates showed virulent characteristics to both wheat and barley. In 1960 and 1961, Nelson (1960) found that different reactions of progeny of crosses between isolates showed different reactions in pathogenicity to different grass species indicating complex inheritance involving several genes. By race cultivation more aggressiveness was observed by EI Nashaar and Stack (1989) with long term continuous wheat cultivation. Valjavec-Gratian and Steffenson (1997) collected 33 isolates and from it they identified three different pathotypes. These were tested on the basis of interaction phenotypes with three six-speed barely differentials. Duveiker and Altamirano (2000) reported that when large numbers of strains were collected on a global basis then these were shown variability between violence and aggressiveness with both specific and nonspecific interactions. The variation was not cleared. Hyphal fusion may result in somatic hybridization and may cause the growth of growing new fungal varieties. Although no reports were found about physiological races of pathogens, some workers or researchers found some variation among isolates of the pathogen.

Misra *et al.,* (1981) collected samples from Dholi and Bhubaneswar and identified some variation in the pathogenicity of *Bipolaris sorokiniana*. In 1991, some reports were published by Pascual and Raymundo that they found cultural variation of *Helminthosporium sativum* among 20 isolates during incubation period and that were lesion number, lesion size and areas of affected leaves. Alam *et al.,* collected 27 isolates of *Bipolaris sorokiniana* and found some morphological and physiological divergences in 1997.

Adhikary*et al.,* (2000) collected 122 isolates from major wheat growing areas of Bangladesh and found some variation among these isolates.

Selecting breeding materials should follow some rules as to know which pathotype is to be used in the screening process, process of expression and inheritance and it's adequate and duration periods. Some important factors should be noted: the capacity of the pathogen for changing under controlled environment (Thakur, 1999).

In Bangladesh, the study of characterization of *Bipolaris sorokiniana* is insufficient. Information on pathogenicity and variation of causal organism variation is so unclear. So proper methodological research should increase for characterising *Bipolaris sorokiniana*.

Objectives

1. To isolate and identify *Bipolaris sorokiniana* based on morphological and ITS based molecular parameters.
2. To study morphological and cultural variations and virulence of *Bipolaris sorokiniana*.

CHAPTER 2

REVIEW OF LITERATURE

A lot of research works have been done upon *Bipolaris sorokiniana*. Among these a good number of work has been done which is about physiological, cultural and morphological variation of *Bipolaris sorokiniana*. In this chapter some accumulated information about physiological, cultural and morphological variation of *Bipolaris sorokiniana* are discussed slightly.

In 1955, Luttrell found different measurements and characteristics of conidia from different isolates of *Bipolaris sorokiniana*.

In 1959, Shoemaker submitted a report about the conidia of *Bipolaris sorokiniana* and that's conidia was straight and curved and thick walled elliptical conidia (60-120-12-20) with 4-8 septa.

In 1962, Tinline reported the asexual variation of *Bipolaris sorokiniana* due to para sexual recombination.

In 1975, Anonymous reported about the affection of pH and sucrose concentration for changing the sporulation and conidial characteristics of four species of *Bipolaris*. He found that in all pH levels *Bipolaris sorokiniana* and *Bipolaris zeicola* sporulated good but the species *Bipolaris setariae* and *Bipolaris maydis* produced a little amount of conidia. At all sucrose concentration,only *Bipolaris sorokiniana* sporulated well but *Bipolaris zeicola* and *Bipolaris maydis* sporulated less than *B. sorokiniana* when sucrose concentration level became so high.

In 1981, Mehta identified a total of 32 different races of *Helminthosporium sativum* out of 96 monoconidial isolates obtained from 41 municipal regions comprising five different states and 51 cultivars in Brazil. Morphological variability of *B. sorokiniana* has been reported by Misra et al., (1981).

In 1990, Chowdhury found variation of colony characteristics in 10 isolates of *Drechslera sorokiniana* in which seed samples were collected from seven countries.

In 1991, Matsumura found a high degree of phenotypic variability of *Bipolaris sorokiniana*. But its genetic diversity has not been studied properly. Only isozyme polymorphisms were done among isolates.

In 1991, Hetzlar *et al* found differences in disease-causing ability of different strains of *Helminthosporium sativum* on a set of wheat genotypes.

In 1991, Pascual and Raymundo found that, in PDA media they found variation in 20 isolates of *Helminthosporium sativum*that was colonial differences among 112, 16 and 118 isolates in PDA media. They also found differences in incubation period, lesion number per 3 cm^2 leaf area and lesion size in a wheat variety Trigo 3 of the 20 isolates.

In 1992, Hetzler reported that a geographical racial differentiation has found of *Bipolaris sorokiniana* from warm and dry regions which is more or less virulent and came from central Africa.

In 1992, Hossain and Azad reported about 83 isolates of *Helminthosporium sativum* from Bangladesh from its seven districts. They found differentiation among mycelium growth and production of conidia. And the isolates were observed in PDA media.

In 1997, Ahmed *et al.*, collected samples from fourteen districts of wheat growing locations of Bangladesh. He found physiological and morphological differences among 27 isolates of *Bipolaris sorokiniana*. Colonies were ash brown, olive green, light green or dark green in color with regular or wavy margins, fluffy, spread or velvety texture and with or with or without sector. Number of cells per conidium varied from 3-10 and length and width of the conidium varied from 35-270 μm and 15-65 μm depending on isolates.He also classified it into four clusters. There three belonged to cluster 1, six to cluster 2, fourteen to cluster 3 and four to cluster 4.

In 1997, Alam *et al.*, prepared 27 isolates of *Bipolaris sorokiniana* and he identified seven morphological and physiological differences among these 27 isolates.

In 1997, Debnath reported about chromogenic and nonchromogenic characteristics of *Bipolaris sorokiniana*and he designed pigment and nonpigment producing isolates of *Bipolaris sorokiniana*. He found that the chromogenic isolates were more or less irregular, slightly fluffy, cottony and white in colour and they produced longer spores whereas the colonies of nonchromogenic isolates were more or less regular marined, compact and brown to black in colour and produced shorter spores.

In 1997, Valjavec-Gratian and Steffenson studied wheat roots, based on its virulence on three barley genotypes. He identified three different pathotypes of *Bipolaris sorokiniana* from 33 isolates of *Bipolaris sorokiniana*.

In 1997, Valim *et al.,* identified 10 isolates of *Bipolaris sorokiniana* as their morphologic and pathogenic variation. From four wheat growing regions in Brazil, he collected these samples and research work was done over it.

In 1998, Singh *et al.,* found a super most pathogenic fungus which was isolated from blighted wheat leaves. And that was *Bipolaris sorokiniana*.

In 1998, Maraite *et al.,* studied the fungus *Bipolaris sorokiniana*. He identified 27 isolates of *Bipolaris sorokiniana*. The colony colour of isolates were different from each other. And that was white to light pink and dark green. The dark coloured colony showed a strong correlation with aggressiveness of the pathogen. And that was the host responding. They showed a large number of possible gene-for-gene interactions combining both horizontal and vertical resistance types. They also found that some isolates were able to protect under controlled conditions and selection of more specialized or more aggressive pathotypes.

In 1999, Barnett and Hunter found some different cultural characteristics of the conidia of *Bipolaris sorokiniana*. Conidia were brown in colour and several celled, elliptical, regular and irregular and straight or curved on the germ tube at each end when germination was done well.

In 2000, Adhikary collected samples from the wheat growing regions of Bangladesh. He reported about 122 mono-conidial isolates of *Bipolaris sorokiniana* and found some variation among these isolates. He also grouped it in four clusters where 22 isolates in the no of 1 cluster, 8 isolates in the no of 2 cluster, 14 isolates in the no of 3 cluster and 78 isolates in the no of 4 cluster. And here 2 no cluster which contains 8 isolates were the most virulent and 78 of cluster 4 were the least virulent.

In 2000, Mathur and Kongsdal found some cultural variation of the conidia of *Bipolaris sorokiniana*. The conidia were ellipsoid, colour was dark brown to black, mostly straight and slightly curved. Broadest in the middle and end rounded. He found 3-12 distoseptate conidia which were 40-120 macro length and 17-28 macro breadth.

In 2001, Ahmed collected samples from 16 major wheat growing locations of Bangladesh. And he collected 262 isolates of *Bipolaris sorokiniana* from these samples. He grouped it in 13 physiological groups based on their cultural identities. And that was the colour,shape and compactness of the colony.He did it on PDA media.From 265 isolates,he tested 43 isolates on 5 china and 6 Brazilian varieties. He found and identified six pathotypes from this work.

In 2002, Kumar *et al.,* studied the colony of the fungus *Bipolaris sorokiniana*. He found that it has interwoven hype as a loose cotton mass with white or grey color depending on the isolates. And this fungus showed different morphological characteristics of conidia and conidiospores than other members of the genus *Bipolaris.*

In 2002, Matho *et al.,* collected samples from different agro ecological zones wheat on cv.Wafaq-2001. He reported the pathogenic characteristics of predominant isolates of *Bipolaris sorokiniana*.

In 2003, F. Ahmed, I. Hossain and F. M. Aminuzzaman published that a total of representative eleven isolates of six different pathotypes of *Bipolaris sorokiniana* were compared for their effect on leaf blight severity and yield contribution characters by inoculating plants of wheat cv. Kanchan at maximum tillering stage. Then disease severity scored from 61 to 81.

In 2003, Chand *et al.,* found different morphological characteristics of *Bipolaris sorokiniana* and he classified it into five groups on the basis of colony morphology. In their research work they found that 44.63% isolates which were black in colour were suppressed type and were identified as the epidemic population whereas 4.96% isolates which were white in colour were least aggressive. And also produced very few conidia. During second year studies, he found that, black coloured and least conidia produced isolates were produced more conidia and became aggressive due to conditions practicing of the same susceptible variety. And also got vigorous establishment from this pathogen.

In 2004, Iram and Ahmed studied about different aggressive behaviour of isolates of *Bipolaris sorokiniana* based on disease severity scale. For this research work he

constructed a tree on the pattern of bands which highlighted the correlation between aggressiveness and morphological characteristics and genetic variation of *Bipolaris sorokiniana*.

In 2004-2005, Aminuzzaman and Hossain found some different morphological characteristics by observing the mycelia growth, colony characters, sporulation, size, shape and separation of conidia from 20 isolates which were collected from barley. The growth of mycelia varied from 9.26 mm from 24.0 mm. Colonies were effuse, velvety, effuse to velvety, whitish, brown gray and black having regular or irregular margin where the number of conidia/cm² of the colony ranged from 3.36×10^3 to 122.12×10^3. Length and breadth of conidia varied from 28.12 to 75.69µm and 10.64 to 15.04µm, respectively. Conidia were 2 to 8 septed and maximum were straight. They were light brown in colour and some curved conidia were found which were deep brown in colour.

In 2005, Aminuzzaman and Hossain collected 17 wheat cultivars of 18 wheat growing districts of Bangladesh and he collected isolates of *Bipolaris sorokiniana*. He identified 65 physiological races with 12 pathotypes. MS-HS-2-6 pathotype showed a high infection response (HS) of the range (MS-HS)was most common compromising 18.06% of sixty five isolates collected pathotype S-HS-2-3-4-5-6-7 was virulent on greatest number of host differentials and produced 62.96% leaf area diseased on wheat differentials. From these 12 pathotypes were identified from each of Dhaka and Rajshahi divisions. 9 pathotypes from Feni, Kishorgonj, Kushtia, Sirajgonj and Thakurgaon. From Jamalpur and Pabna, the most virulent pathotype, S-HS-2-3-4-5-6-7 was found. In pathotype S-HS-2-3-4-5-6-7, the highest mycelial growth and conidia production ability were recorded from this pathotype.

In 2005, Pandey *et al.*, collected samples from different geographic locations in Brazil and other countries. He found different virulence, physiological, morphological variations from 35 isolates of *Bipolaris sorokiniana*. Morphological characteristics were recorded based on morphological variability, mycelium colour, sector formation and the radial growth of pathogens. The isolates were grouped into five different morphological groups. The results from infection of seeds and seedlings showed that isolates from the same geographical regions and morphological group had different degrees of virulence.

In 2006, Iftekhar *et al.,* found four different colony colours of the *Bipolaris sorokiniana*. These were black, grayish, brownish and albino or whitish in colour. Black culture had suppressed type growth and sporulated profusely. And the other three colours showed less sporulation growth. They also found the conidia of isolates of 2004 were slightly curved with brown to olive colour whereas in 2005 collection, the conidia were dark brown and slightly curved. Few were light brown to brown and slightly straight.

In 2007, Jaiswal *et al.,* reported about different morphological characteristics from 155 isolates of *Bipolaris sorokiniana*. He grouped it into five groups on the basis of its growth level. These were black, brown, gray cotton, dull white/greenish black and white. Suppression of the blackish isolates was maximum (45.63%) whereas white isolates showed lowest frequency (6.96%) in the normal population.

In 2008, Pandey *et al.,* collected seed and leaves samples from four different wheat growing areas in Eastern Gangetic plains of India. He collected isolates of *Bipolaris sorokiniana* from these samples. From a single isolate, he recultured 86 clonal isolates which were gray and having white patches. He also found morphological and pathological variation from it.

In 2009, Poloni *et al.,* selected 21 isolates of B. sorokiniana and from them poly sporic and monosporic cultures were obtained. The morphological aspects such: coloration, edge, superficial texture aerial mycelium and color, and shape of the sectors; and the growth rate of the isolates were analyzed in four different media: potato dextrose agar (PDA), Sabouraud maltose, Sabouraud galactose and Sabouraud glucose. The monosporic cultures did not present a significant difference in the growth rate among the different media. However, a small morphologic variation, as well as on the repetitions of the isolates in the same medium was obtained. Poly sporic cultures showed a high morphologic variability among the four media.

In 2009, Iftikhar collected samples from wheat growing areas from different agro ecological zones based on cultural and morphological nature. The size of conidia was 38.3–65.8 μm x 12.3–25 μm with slightly curved, brown to olivaceous brown with 2–13 septa. Some isolates had longer and broader conidia and some were light brown in colour and curved in shape.

In 2009, Asad *et al.,* reported the cultural and morphological characteristics of *Bipolaris sorokiniana* based on its colony colour, shape, texture and conidial characteristics. He classified it into four categories based on colour like black, grayish black, brown and albino or whitish. The conidial average size ranged from 38.3–65.8 µm x 12.3–25 µm with slightly curved, brown to olivaceous brown with 2–13 septa. Some isolates were straight and cylindrical and colour was light brown and some were long and broad in size. In the pathogenicity test, all isolates showed the same result as well as by producing the symptoms on leaves. But their reaction varied with their aggressiveness.

In 2009, Srinivas *et al.,* collected 103 isolates from different regions of India based on their different characteristics and pathogenicity and DNA fingerprinting. He categorized it into 5 categories. Maximum amounts like 38.83% were dull white or greenish black colony type. And these showed minimum frequency like 11.65% in the population studied. From 103 isolates, among them only 40 isolates were again studied for growth rate, sporulation pathogenic and molecular variability. Colony diameter after seven days of incubation ranged from 20.3 mm (BS-95) to 63 mm (BS-63). Highest spore production was observed in BS-69 (10x107/colony) and lowest in BS-95 (1.0x107/colony). Isolate BS-48 remained non sporulating even after15 days of incubation.

In 2010, Aminuzzaman *et al.,* collected pathogens from leaf and seed samples from 17 varieties of wheat from 18 major wheat growing locations of Bangladesh. He isolated 86 monoconidial isolates of *Bipolaris sorokiniana* from it. He found different cultural and morphological characteristics from it based on their mycelial growth, conidia production ability, size, shape, separation and colour of conidia. He categorized it into 9 categories based on colony colour and morphology. From this category's maximum number like 34 isolates produced effuse black regular colonies. And its frequency was 39.53%.From these isolates 29 isolates produced effuse black irregular colonies with 33.72% frequency.

In 2010, Knight and Lehmensiek collected 48 isolates of *Bipolaris sorokiniana* from the northern wheat growing areas of Australia. He grouped it into three pathogenicity clusters based on phenotypic infection response like low, intermediate and high

pathogenicity. This provided a result of Australian *Bipolaris sorokiniana* in relation to host tissue specificity.

In 2011, Sharma *et al.,* worked with foliar blight infected barley leaves and he identified *Bipolaris sorokiniana* from it. For finding morphological characteristics like colony growth, colour, sporulation and conidial size, he studied with 8 single spore isolates of pathogens. And four isolates were studied for fast growing. The sporulation was abundant for some isolates. The average length of conidia of different isolates varied from 62 to 85.25 mm and breadth were from 25.57 to 27.90 mm. And the average number of septa per conidia were from 3 to 8 septa. Conidia was oval-oblong shaped. And the conidia colour varied from deep olive brown to light olive brown.

In 2013, M. M Rahman, F. M. Aminuzzaman and M. S. Chowdhury submitted that the collected isolates differed significantly in respect of mycelia growth, conidia production ability, shape, color, size and septation of conidia. The radial mycelia growth rate ranged from 1.96 ± 0.56 mm day-1 to 5.83 ± 0.02 mmday-1 while the highest mycelia growth rate was found in JJRBS 18. Maximum number of conidia cm-2 (119.21 ± 41.29 $\times 10^3$) was counted from isolate JJRPL 01 and minimum (2.79 ± 0.58 $\times 10^3$) from MGMSL 07. In terms of conidia color, most of the isolates produced deep brown colored conidia while 2 isolates failed to produce conidia after 15 days of incubation. Conidia of most of the isolates were straight shaped. Highest length of conidia (72.74 ±1.27 µm) was recorded in isolate JJRSS 03 while lowest (36.80 ± 6.03 µm) in isolate PIRPL 08. The isolate MGMSL 08 showed highest breadth (17.65 ± 0.98 µm) while lowest (11.42 ±1.29 µm) in isolate SNNSL 01. The isolates were classified into nine cultural groups based on colony morphology and colony color. Maximum 23 isolates showed effuse blackish white irregular (EBWI) colony with a frequency of 33.33% of the collected isolates

In 2016, Chowdhury and Bhattacharya collected isolates of *Bipolaris sorokiniana* from different wheat growing areas of West Bengal. They identified some morphological and physiological variations among these isolates. Conidial length was high and more septations were identified with aggressiveness of the pathogen. They also found correlation between toxic production and aggressiveness of the isolates.

In 2017, Chauhan and Singh identified spot blotch caused by *Bipolaris sorokiniana*. They collected 560 blighted leaf samples of wheat from different locations. They found different morphological variations during the incubation period of pathogens.

In 2018, Ankita Biswas and Srikanta Das reported about the morphological characteristics of *Bipolaris sorokiniana*. They collected samples from wheat growing locations of West Bengal and found some conidial morphology which they researched on four different media. Different media showed different characteristics.

In 2020, Ashwini and Patil collected spot blotch affected leaves from wheat growing areas. They collected 14 isolates of *Bipolaris sorokiniana*. They found some morphological changes among these isolates like colony colour, shape, growth rate etc. Colony colour was whitish black to black, shape was obloned and rounded. They also found three to seven septations and four to eight celled.

CHAPTER 3

MATERIALS AND METHODS

3.1. Experimental site

The experiment was carried out in the Laboratory, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka and Laboratory, Plant Pathology Division, Bangladesh Rice Research Institution, Gazipur.

3.2. Experimental period

The experiment was conducted during the period from October 2019 to February 2021.

3.3. Collection of samples from different wheat growing locations of Bangladesh

Some disease affected and symptoms carried leaves and seeds were collected from seven districts namely Bogura, Dinajpur, Meherpur, Jashore, Mymensingh, Netrokona and Rajshahi. The affected and symptom carried leaves were cut from the plants from the disease affected wheat field randomly and put in a brown paper envelope. Then all samples were taken to the laboratory, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka. Seeds were also collected and put in cotton type bags and also were sun dried and preserved at refrigerator under 5°C temperature. At last isolation was done from both samples.

3.4. Isolation, identification, purification and preservation of isolates of *Bipolaris sorokiniana*

Isolation of *Bipolaris sorokiniana* was done from diseased seeds and leaves following standard protocol. Leaf isolates of *Bipolaris sorokiniana* were collected from the Laboratory of SAU. Then these were transferred to PDA media and incubated at 25±1°c for 7 to 14 days. Ending the incubation, the mycelia grown from planted tissues were examined under stereo binocular microscope and these mycelium blocks were again transferred to a second PDA plate and incubated at 25±1°C for 14 days.

Again the collected seeds were stabilized by mercuric chloride (1:1000) for 30 to 40 seconds. These were rinsed twice in sterilized water. Five seeds per plate were taken and placed on to a PDA plate and were incubated at 25°C for growing well. After 5 to

7 day, mycelium was grown and these were observed under a stereo binocular microscope. Then that was transferred to a second PDA plate and allowed to grow for pure culture for 14 days. The pathogen was identified and the culture of isolates from the PDA were transferred to PDA slants or plates and these were preserved in the refrigerator at 5°c for further study.

3.5. Designation of collected isolates

The isolates were designed on its location and sources following procedure of Aminuzzaman *et al.*, 2010 (Table 1). As for example an isolate designed by BDKS represents that this isolate was collected from Bogura district, Dupchanchia Upazila from Kanchan seed (KS) it was isolated.

3.6. ITS based identification of *Bipolaris sorokiniana*

3.6.1. Molecular studies

Molecular identification of *Bipolaris sorokiniana* was done using Internal Transcribed spacer (ITS1 and ITS4) (White *et al*., 1990). This research work was done in the Laboratory, Plant Pathology Division, Bangladesh Rice Research Institute, Gazipur.

3.6.2. Extraction and purification of DNA

Genomic DNA was extracted using the DNA extraction kit of Thermofisher company. At first fungal culture was transferred to a sterilized 1.5 µL microcentrifuge tube. One milliliter of FA fubber was added to the cells and resuspended the cells by pipetting. The cells were descended by centrifuging at 5000 ×g for 2 minute 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^{O}C for 30 minute. Eight µL of RNase was added and incubated at room temperature for 2 minute. The cells were descended by centrifuging at 5000 ×g for 10 minute 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 minute. 20 µL proteinase K was added and mixed well by vortexing and incubated at 55^{O}C for 15 minute and vortexed 30 second for every 5 minutes 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 11000 ×g for 30 second. 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 11000 ×g for 30 second and the flow-through were discarded. TG mini column was placed back to the collection tube. A 750 µL wash buffer was added to the TG mini column and centrifuged at 11000 ×g for 30 second and the flow-through was discarded. TG mini column was placed back to the collection tube. The collection tube with column was centrifuged at 18000 ×g for an additional 3 minute to dry the column. The TG mini column was placed to an elution tube and 80 µL elution buffer was added to the membrane centre of the TG column and the column was kept stand for 3 minute. The column was centrifuged at full speed (18000 ×g) for 1 min to elute total DNA. DNA was stored at -20^{O}C for PCR and future use.

3.6.3. Polymerase Chain Reaction (PCR) using ITS

By using universal eukaryotic primers ITS1 (5′- TCCGTAGGTGAACCTGCGG -3′) and ITS4 (R 5′TCCTCCGCTTATTG ATATGC-3′) according to (White et al., 1990), the first round of amplification was done.

3.6.4. PCR components

Performance of PCR amplification was done in a total volume of 25 µL, which contained 10X 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.

3.6.5. PCR condition (thermal profile)

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 annealing for 30 Sec. 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.

3.6.6. Gel documentation and image of PCR product

The PCR product was checked for confirmation of DNA amplification using gel documentation system and band image of the targeted amplified DNA was taken.

3.7. Morphological determinations, growth study and grouping of *Bipolaris sorokiniana*

3.7.1. Morphological determinations and growth study

By using the method of Hossain and Azad (1992), the growth of study of *Bipolaris sorokiniana* was done. Maintaining the replications, the PDA plates were inoculated with a little mycelia block at the middle of the plate at 25°c. And after 14 days of incubation, the radial growth of these were measured. Then it's texture, colour, shape was recorded properly. The conidia produced per unit surface area were estimated using Chauhan and Panday's (1995) formula as follows:

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

3.7.2. Grouping of *Bipolaris sorokiniana*

One hundred and twenty (120) isolates were grouped into 12 groups based on their compactness, shape and colour of the colony following Aminuzzaman *et al*., (2010).

3.7.3. Grouping of isolates of *Bipolaris sorokiniana* based on conidia morphology

One hundred and twenty isolates (120) isolates were grouped into 5 groups based on their conidial shape.

3.7.4. Virulence test of different cultural groups of *Bipolaris sorokiniana* on wheat

Virulence tests were done of different cultural groups of *Bipolaris sorokiniana* on wheat. One isolate of each group was cultured on PDA media for 15 days. Conidia was harvested using sterile distilled water. The spore concentration was adjusted to conidia/ml of water. The spore suspension was sprayed by hand sprayer on 15 days

old wheat seedlings sown on sterilized pot soil. Pots were kept in 26°C maintaining >90% relative humidity in darkness for 24 hours. After that pots were transferred to shade houses. Number of leaf infected, number of spot/leaf and % LAD were recorded at 15 DAI.

CHAPTER 4

RESULTS

4.1. Variations of physiological, cultural and morphological characteristics of *Bipolaris sorokiniana* on wheat in Bangladesh

4.1.1. Isolation, identification, purification and preservation of isolates of *Bipolaris sorokiniana* collected from the leaf and seed samples

One hundred twenty isolates of *Bipolaris sorokiniana* were isolated from wheat leaves and wheat seeds from the major wheat growing locations of Bangladesh, where 115 isolates were collected from seeds and 5 isolates from leaves. The maximum number of isolates (40) were collected from Meherpur district followed Rajshahi district (4). The maximum 35 number of isolates were collected from Kanchan variety. (Table 1).

4.1.2. Determinations of growth study, morphological characteristics and grouping of isolates of *Bipolaris sorokiniana.*

4.1.2.1. Growth study and morphological determinations of isolates

The isolates were collected from different wheat growing areas of Bangladesh and these were cultured on PDA media. Radial mycelial growth per day, number of conidia production, colour of colony, surface texture of colony and shape of colony was recorded clearly (Table 2). The radial mycelial growth rate ranged from 1.392 mm/day to 4.46 mm/day. Maximum growth rate per day counted in isolates no JSBS 88 which was 4.46 mm and minimum growth rate per day were counted in isolates no MMPS 66 which was 1.392 mm. Maximum number of conidia /cm^2 was 416.17×10^4 which was found in isolates no BAKS 11 and minimum number of conidia/cm^2 was 89.6 mm and which was found in isolate no JSBS 88. Among 120 isolates, 73 isolates were shown blackish white colour and 42 isolates were shown blackish colour and rest of all 5 isolates were shown whitish in colour. The surface texture of the colony of maximum isolates were smooth and wooly textured. About 102 isolates were produced smoothy and wooly textured and 18 isolates were produced effuse and rough textured. The maximum shape of isolates was regular.

Among these isolates 92 isolates produced brown coloured conidia and 17 isolates produced deep brown coloured conidia and 11 isolates produced light brown coloured conidia. 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. Among 120 isolates, 65 isolates were regular in shape and 55 isolates were irregular in shape. The highest septation was found in isolate number MMPS 53 and that was 8.6 and the lowest septation was found in isolate number JSBS 74 and that was 1.4. (Table 3).

4.1.2.2 Molecular identification (ITS rDNA gene)

PCR using primer pairs ITS1/ITS4 yielded specific species band (around 600 bp) of amplification product for the isolates of *Bipolaris sorokiniana* (Fig. 1).

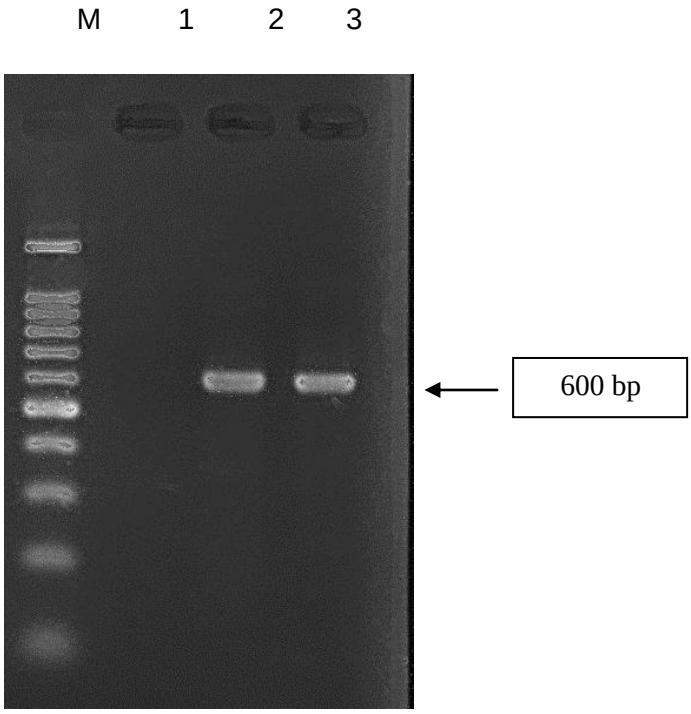


Fig. 1. Gel electrophoresis of PCR products from amplification of ITS primer (ITS1F-ITS4R) region of rDNA of isolates. M: 100bp plus DNA Marker, L1=Negative sample, L2 and L2= Two isolates of *Bipolaris sorokiniana* under two cultural groups that yielded 600 bp band.

4.1.2.3. Grouping of isolates of *Bipolaris sorokiniana* based on morphological and cultural characteristics

The isolates of *Bipolaris sorokiniana* were grouped into 12 groups based on their morphological characteristics variation (Table 4). The cultural group 1 contains 22 isolates with 18.33% frequency. The group 2 contains maximum isolates 39 with 32.5% frequency of the isolates collected. And other cultural group 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 contained 8, 4, 30, 8, 2, 2, 2, 1, 1, 1 isolates with 6.66, 3.33, 25, 6.66, 1.66, 1.66, 1.66, 0.83, 0.83, 0.83 % frequency of collected isolates, respectively.

4.1.2.4. Grouping of isolates of *Bipolaris sorokiniana* based on conidial shape

One hundred twenty isolates were grouped into 5 groups based on their conidial shape (Table 5). 57 isolates produced elliptical and straight conidia with 47.50% frequency, 13 isolates produced elliptical and curved shaped conidia with 10.83% frequency. 28 isolates produced oval shaped conidia with 23.33% frequency, 5 isolates produced round shaped conidia with 4.17% frequency and 17 isolates produced pyriform shaped conidia with 14.16% frequency all above of isolates.

4.1.2.5. Determinations of growth study and morphological characteristics of *Bipolaris sorokiniana* under 12 cultural groups

One hundred twenty isolates were grouped into 12 groups based on their different morphological characteristics (Table 6). The radial mycelial growth per day ranged from 4.5 mm to 2.67 mm per day.The maximum growth rate per day was found in the group number 12 and lowest was in the group number 7.00. The maximum number of conidia per cm^2 was 248.93×10^4 which was found in the group number 7 and lowest 88.18 was found in the group number 12.The maximum septation was found in the group number 11 and that was 7.4 and lowest number of septations found in the group number 1 and that was 2.44 respectively.

4.1.2.6. Virulence test of different cultural groups of *Bipolaris sorokiniana* on wheat

Virulence test was done among 12 cultural groups which groups were previously made (Table 7). Among these 12 groups effuse and rough blackish irregular group was most virulent with 38.5% frequency and effuse and rough blackish irregular group was low virulent group among 12 groups with 2% frequency of above virulence.

Table 1. Collection sites and sources of isolates of *Bipolaris sorokiniana*from wheat

Sl No.	Isolates	Location		Host		Year of collection
		District	Upazila	Variety	Plant part	
1.	BDKS 01	Bogura	Dupchanchia	Kanchan	Seed	2019
2.	BDKS 02	Bogura	Dupchanchia	Kanchon	Seed	2019
3.	BDKS 03	Bogura	Dupchanchia	Kanchan	Seed	2019
4.	BDKS 04	Bogura	Dupchanchia	Kanchan	Seed	2019
5.	BDKS 05	Bogura	Dupchanchia	Kanchan	Seed	2019
6.	BDKS 06	Bogura	Dupchanchia	Kanchan	Seed	2019
7.	BDKS 07	Bogura	Dupchanchia	Kanchan	Seed	2019
8.	BDKS 08	Bogura	Dupchanchia	Kanchan	Seed	2019
9.	BDKS 09	Bogura	Dupchanchia	Kanchan	Seed	2019
10.	BDKS 10	Bogura	Dupchanchia	Kanchan	Seed	2019
11.	BAKS 11	Bogura	Adamdighi	Kanchan	Seed	2020
12.	BAKS 12	Bogura	Adamdighi	Kanchan	Seed	2020
13.	BAKS 13	Bogura	Adamdighi	Kanchan	Seed	2020
14.	BAKS 14	Bogura	Adamdighi	Kanchan	Seed	2020
15.	BAKS 15	Bogura	Adamdighi	Kanchan	Seed	2020
16.	BAKS 16	Bogura	Adamdighi	Kanchan	See	2020
17.	BAKS 17	Bogura	Adamdighi	Kanchan	Seed	2020
18.	BAKS 18	Bogura	Adamdighi	Kanchan	Seed	2020
19.	BAKS 19	Bogura	Adamdighi	Kanchan	Seed	2020
20.	BAKS 20	Bogura	Adamdighi	Kanchan	Seed	2020
21.	DSSS 21	Dinajpur	Dinajpur Sadar	Shotabdi	Seed	2020
22.	DSSS 22	Dinajpur	Dinajpur Sadar	Shatabdi	Seed	2020
23.	DSSS 23	Dinajpur	Dinajpur Sadar	Shatabdi	Seed	2020

Continue...

Sl No.	Isolates	Location		Host		Year of collection
		District	Upazila	Variety	Plant part	
24.	DSSS 24	Dinajpur	Dinajpur Sadar	Shatabdi	Seed	2020
25.	DSSS 25	Dinajpur	Dinajpur Sadar	Shatabdi	Seed	2020
26.	DSSS 26	Dinajpur	Dinajpur Sadar	Shatabdi	Seed	2020
27.	DSSS 27	Dinajpur	Dinajpur Sadar	Shatabdi	Seed	2020
28.	DSSS 28	Dinajpur	Dinajpur Sadar	Shatabdi	Seed	2020
29.	DSSS 29	Dinajpur	Dinajpur Sadar	Shatabdi	Seed	2020
30.	DSSS 30	Dinajpur	Dinajpur Sadar	Shatabdi	Seed	2020
31.	MSBS 31	Meherpur	Meherpur Sadar	BARI GOM 30	Seed	2020
32.	MSBS 32	Meherpur	Meherpur Sadar	BARI GOM 30	Seed	2020
33.	MSBS 33	Meherpur	Meherpur Sadar	BARI GOM 30	Seed	2020
34.	MSBS 34	Meherpur	Meherpur Sadar	BARI GOM 30	Seed	2020
35.	MSBS 35	Meherpur	Meherpur Sadar	BARI GOM 30	Seed	2020
36.	MSBS 36	Meherpur	Meherpur Sadar	BARI GOM 29	Seed	2020
37.	MSBS 37	Meherpur	Meherpur Sadar	BARI GOM 29	Seed	2020
38.	MSBS 38	Meherpur	Meherpur Sadar	BARI GOM 29	Seed	2020
39.	MSBS 39	Meherpur	Meherpur Sadar	BARI GOM 29	Seed	2020
40.	MSBS 40	Meherpur	Meherpur Sadar	BARI GOM 29	Seed	2020
41.	MSPS 41	Meherpur	Meherpur Sadar	Prodip	Seed	2020
42.	MSPS 42	Meherpur	Meherpur Sadar	Prodip	Seed	2020

Continue...

Sl No.	Isolates	Location		Host		Year of collection
		District	Upazila	Variety	Plant part	
43.	MSPS 43	Meherpur	Meherpur Sadar	Prodip	Seed	2020
44.	MSPS 44	Meherpur	Meherpur Sadar	Prodip	Seed	2020
45.	MSPS 45	Meherpur	Meherpur Sadar	Prodip	Seed	2020
46.	MGBS 46	Meherpur	Gangni	BARI GOM 28	Seed	2020
47.	MGBS 47	Meherpur	Gangni	BARI GOM 28	Seed	2020
48.	MGBS 48	Meherpur	Gangni	BARI GOM 28	Seed	2020
49.	MGBS 49	Meherpur	Gangni	BARI GOM 28	Seed	2020
50.	MGBS 50	Meherpur	Gangni	BARI GOM 28	Seed	2020
51.	MGBS 51	Meherpur	Gangni	BARI GOM 28	Seed	2020
52.	MMPS 52	Meherpur	Mujibnagor	Prodip	Seed	2020
53.	MMPS 53	Meherpur	Mujibnagor	Prodip	Seed	2020
54.	MMPS 54	Meherpur	Mujibnagor	Prodip	Seed	2020
55.	MMPS 55	Meherpur	Mujibnagor	Prodip	Seed	2020
56.	MMPS 56	Meherpur	Mujibnagor	Prodip	Seed	2020
57.	MMPS 57	Meherpur	Mujibnagor	Prodip	Seed	2020
58.	MMPS 58	Meherpur	Mujibnagor	Prodip	Seed	2020
59.	MMPS 59	Meherpur	Mujibnagor	Prodip	Seed	2020
60.	MMPS 60	Meherpur	Mujibnagor	Prodip	Seed	2020
61.	MMPS 61	Meherpur	Mujibnagor	Prodip	Seed	2020
62.	MMPS 62	Meherpur	Mujibnagor	Prodip	Seed	2020
63.	MMPS 63	Meherpur	Mujibnagor	Prodip	Seed	2020
64.	MMPS 64	Meherpur	Mujibnagor	Prodip	Seed	2020

Continue...

Sl No.	Isolates	Location		Host		Year of collection
		District	Upazila	Variety	Plant part	
65.	MMPS 65	Meherpur	Mujibnagor	Prodip	Seed	2020
66.	MMPS 66	Meherpur	Mujibnagor	Prodip	Seed	2020
67.	MMPS 67	Meherpur	Mujibnagor	Prodip	Seed	2020
68.	MMPS 68	Meherpur	Mujibnagor	Prodip	Seed	2020
69.	MMPS 69	Meherpur	Mujibnagor	Prodip	Seed	2020
70.	MMPS 70	Meherpur	Mujibnagor	Prodip	Seed	2020
71.	JSBS 71	Jashore	Jashore Sadar	BARI GOM 26	Seed	2020
72.	JSBS 72	Jashore	Jashore Sadar	BARI GOM 26	Seed	2020
73.	JSBS 73	Jashore	Jashore Sadar	BARI GOM 26	Seed	2020
74.	JSBS 74	Jashore	Jashore Sadar	BARI GOM 26	Seed	2020
75.	JSBS 75	Jashore	Jashore Sadar	BARI GOM 26	Seed	2020
76.	JSBS 76	Jashore	Jashore Sadar	BARI GOM 26	Seed	2020
77.	JSBS 77	Jashore	Jashore Sadar	BARI GOM 26	Seed	2020
78.	JSBS 78	Jashore	Jashore Sadar	BARI GOM 26	Seed	2020
79.	JSBS 79	Jashore	Jashore Sadar	BARI GOM 26	Seed	2020
80.	JSBS 80	Jashore	Jashore Sadar	BARI GOM 26	Seed	2020
81.	JSBS 81	Jashore	Jashore Sadar	BARI GOM 26	Seed	2020
82.	JSBS 82	Jashore	Jashore Sadar	BARI GOM 26	Seed	2020

Continue...

Sl No.	Isolates	Location		Host		Year of collection
		District	Upazila	Variety	Plant part	
83.	JSBS 83	Jashore	Jashore Sadar	BARI GOM 26	Seed	2020
84.	JSBS 84	Jashore	Jashore Sadar	BARI GOM 26	Seed	2020
85.	JSBS 85	Jashore	Jashore Sadar	BARI GOM 26	Seed	2020
86.	JSBS 86	Jashore	Jashore Sadar	BARI GOM 26	Seed	2020
87.	JSBS 87	Jashore	Jashore Sadar	BARI GOM 26	Seed	2020
88.	JSBS 88	Jashore	Jashore Sadar	BARI GOM 26	Seed	2020
89.	JSBS 89	Jashore	Jashore Sadar	BARI GOM 26	Seed	2020
90.	JSBS 90	Jashore	Jashore Sadar	BARI GOM 26	Seed	2020
91.	JSBS 91	Jashore	Jashore Sadar	BARI GOM 26	Seed	2020
92.	JSBS 92	Jashore	Jashore Sadar	BARI GOM 26	Seed	2020
93.	JSBA 93	Jashore	Jashore Sadar	BARI GOM 26	Seed	2020
94.	JSBS 94	Jashore	Jashore Sadar	BARI GOM 26	Seed	2020
95.	JSBS 95	Jashore	Jashore Sadar	BARI GOM 26	Seed	2020
96.	MTPS 96	Mymensingh	Trisal	Prodip	Seed	2021
97.	MTPS 97	Mymensingh	Trisal	Prodip	Seed	2021
98.	MTPS 98	Mymensingh	Trisal	Prodip	Seed	2021
99.	MTPS 99	Mymensingh	Trisal	Prodip	Seed	2021
100.	MTPS 100	Mymensingh	Trisal	Prodip	Seed	2021

Continue...

Sl No.	Isolates	Location		Host		Year of collection
		District	Upazila	Variety	Plant part	
101.	MTPS 101	Mymensingh	Trisal	Prodip	Seed	2021
102.	MTPS 102	Mymensingh	Trisal	Prodip	Seed	2021
103.	MTPS 103	Mymensingh	Trisal	Prodip	Seed	2021
104.	MTPS 104	Mymensingh	Trisal	Prodip	Seed	2021
105.	MTPS 105	Mymensingh	Trisal	Prodip	Seed	2021
106.	NSKS 106	Netrokona	Netrokona Sadar	Kanchan	Seed	2021
107.	NSKS 107	Netrokona	Netrokona Sadar	Kanchan	Seed	2021
108.	NSKS 108	Netrokona	Netrokona Sadar	Kanchan	Seed	2021
109.	NSKS 109	Netrokona	Netrokona Sadar	Kanchan	Seed	2021
110.	NSKS 110	Netrokona	Netrokona Sadar	Kanchan	Seed	2021
111.	NSKS 111	Netrokona	Netrokona Sadar	Kanchan	Seed	2021
112.	NSKS 112	Netrokona	Netrokona Sadar	Kanchan	Seed	2021
113.	NSKS 113	Netrokona	Netrokona Sadar	Kanchan	Seed	2021
114.	NSKS 114	Netrokona	Netrokona Sadar	Kanchan	Seed	2021
115.	NSKS 115	Netrokona	Netrokona Sadar	Kanchan	Seed	2021
116.	RSKL 116	Netrokona	Rajshahi Sadar	Kanchan	Leaf	2010
117.	RSKL 117	Rajshahi	Rajshahi Sadar	Kanchan	Leaf	2010
118.	RSKL 118	Rajshahi	Rajshahi Sadar	Kanchan	Leaf	2010
119.	RSKL 119	Rajshahi	Rajshahi Sadar	Kanchan	Leaf	2010
120.	RSKL 120	Rajshahi	Rajshahi Sadar	Kanchan	Leaf	2010

Table 2. Mycelial growth, growth rate, color of colony, surface texture of colony, shape of colony of *Bipolaris sorokiniana* (14 days old culture)

Sl No.	Isolates	Radial mycelial growth (mm)	Growth rate/day (mm)	Color of colony	Surface texture of colony	Shape of colony
1.	BDKS 01	50	3.57	Whitish	Smoothy & Wooly	Regular
2.	BDKS 02	50	3.57	Whitish	Smoothy & Wooly	Regular
3.	BDKS 03	42	3.00	Whitish	Smoothy & Wooly	Regular
4.	BDKS 04	50	3.57	Whitish	Smoothy & Wooly	Regular
5.	BDKS 05	29.5	2.10	Blackish White	Smoothy & Wooly	Irregular
6.	BDKS 06	37.5	2.67	Whitish	Smoothy & Wooly	Regular
7.	BDKS 07	59	4.21	Whitish	Smoothy & Wooly	Irregular
8.	BDKS 08	50	3.57	Whitish	Rough	Irregular
9.	BDKS 09	70	5.00	Blackish White	Rough	Irregular
10.	BDKS 10	60	4.28	Blackish White	Rough	Irregular
11.	BAKS 11	29	2.07	Blackish White	Smoothy & Wooly	Irregular
12.	BAKS 12	50	3.57	Whitish	Smoothy & Wooly	Regular
13.	BAKS 13	42	3.00	Whitish	Smoothy & Wooly	Regular
14.	BAKS 14	37.5	2.67	Whitish	Smoothy & Wooly	Regular
15.	BAKS 15	63	4.50	Whitish	Rough	Regular
16.	BAKS 16	32.5	2.32	Blackish	Smoothy & Wooly	Regular
17.	BAKS 17	34	2.42	Blackish	Smoothy & Wooly	Regular
18.	BAKS 18	37.5	2.67	Blackish	Smoothy & Wooly	Regular
19.	BAKS 19	37.5	2.67	Blackish	Smoothy &Wooly	Regular

Continue...

Sl No.	Isolates	Radial mycelial growth (mm)	Growth rate/day (mm)	Color of colony	Surface texture of colony	Shape of colony
20.	BAKS 20	42.5	3.03	Blackish	Smoothy & Wooly	Regular
21.	DSSS 21	35	2.50	Blackish	Smoothy & Wooly	Regular
22.	DSSS 22	47.5	3.39	Blackish White	Smoothy & Wooly	Irregular
23.	DSSS 23	40	2.85	Blackish White	Smoothy & Wooly	Irregular
24.	DSSS 24	45	3.21	Blackish	Smoothy & Wooly	Regular
25.	DSSS 25	32.5	2.32	Blackish	Smoothy & Wooly	Regular
26.	DSSS 26	42.5	3.03	Blackish	Smoothy & Wooly	Regular
27.	DSSS 27	45	3.21	Blackish	Smoothy & Wooly	Regular
28.	DSSS 28	42.5	3.03	Blackish	Smoothy & Wooly	Regular
29.	DSSS 29	37.5	2.67	Blackish	Smoothy & Wooly	Regular
30.	DSSS 30	32.5	2.32	Blackish	Smoothy & Wooly	Irregular
31.	MSBS 31	36	2.57	Blackish White	Smoothy & Wooly	Irregular
32.	MSBS 32	25.5	1.82	Blackish White	Smoothy & Wooly	Irregular
33.	MSBS 33	34.5	2.46	Blackish White	Smoothy & Wooly	Irregular
34.	MSBS 34	36.5	2.60	Blackish White	Smoothy & Wooly	Irregular
35.	MSBS 35	40	2.85	Blackish White	Smoothy & Wooly	Regular
36.	MSBS 36	31	2.21	Blackish White	Smoothy & Wooly	Irregular
37.	MSBS 37	40.5	2.89	Blackish White	Smoothy & Wooly	Irregular

Continue...

Sl No.	Isolates	Radial mycelial growth (mm)	Growth rate/day (mm)	Color of colony	Surface texture of colony	Shape of colony
38.	MSBS 38	40	2.85	Blackish White	Smoothy & Wooly	Regular
39.	MSBS 39	35.5	2.53	Blackish White	Smoothy & Wooly	Irregular
40.	MSBS 40	39.5	2.82	Blackish White	Rough	Irregular
41.	MSPS 41	37.5	2.67	Blackish White	Smoothy & Wooly	Irregular
42.	MSPS 42	40	2.85	Blackish White	Smoothy & Wooly	Irregular
43.	MSPS 43	40	2.85	Blackish White	Smoothy & Wooly	Irregular
44.	MSPS 44	40.5	2.89	Blackish White	Smoothy & Wooly	Irregular
45.	MSPS 45	39.5	2.82	Blackish White	Smoothy & Wooly	Irregular
46.	MGBS 46	34.5	2.46	Blackish White	Smoothy & Wooly	Irregular
47.	MGBS 47	33.5	2.39	Blackish White	Rough	Irregular
48.	MGBS 48	33.5	2.39	Blackish White	Smoothy & Wooly	Irregular
49.	MGBS 49	40.5	2.89	Blackish White	Smoothy & Wooly	Irregular
50.	MGBS 50	38.5	2.75	Blackish White	Smoothy & Wooly	Irregular
51.	MGBS 51	42.5	3.03	Blackish White	Smoothy & Wooly	Regular
52.	MMPS 52	34.5	2.46	Blackish White	Smoothy & Wooly	Regular

Continue...

Sl No.	Isolates	Radial mycelial growth (mm)	Growth rate/day (mm)	Color of colony	Surface texture of colony	Shape of colony
53.	MMPS 53	29.5	2.10	Blackish White	Smoothy & Wooly	Regular
54.	MMPS 54	38	2.71	Blackish White	Smoothy & Wooly	Regular
55.	MMPS 55	40.5	2.89	Blakish White	Rough	Regular
56.	MMPS 56	35.5	2.53	Blackish White	Smoothy & Wooly	Irregular
57.	MMPS 57	28.5	2.03	Blackish White	Smoothy & Wooly	Irregular
58.	MMPS 58	38.5	2.75	Blackish White	Rough	Regular
59.	MMPS 59	40	2.85	Blackish White	Rough	Regular
60.	MMPS 60	37.5	2.67	Blackish White	Rough	Regular
61.	MMPS 61	35	2.50	Blackish White	Rough	Regular
62.	MMPS 62	37.5	2.67	Blackish White	Smoothy & Wooly	Regular
63.	MMPS 63	37.5	2.67	Blackish White	Smoothy & Wooly	Irregular
64.	MMPS 64	37.5	2.67	Blackish White	Smoothy & Wooly	Irregular
65.	MMPS 65	42.5	3.03	Blackish White	Smoothy & Wooly	Irregular
66.	MMPS 66	32.5	2.32	Blackish White	Smoothy & Wooly	Irregular
67.	MMPS 67	35	2.50	Blackish White	Smoothy & Wooly	Irregular

Continue...

Sl No.	Isolates	Radial mycelial growth (mm)	Growth rate/day (mm)	Color of colony	Surface texture of colony	Shape of colony
68.	MMPS 68	32.5	2.32	Blackish White	Smoothy & Wooly	Irregular
69.	MMPS 69	36	2.57	Blackish White	Smoothy & Wooly	Irregular
70.	MMPS 70	30.5	2.17	Blackish White	Smoothy & Wooly	Irregular
71.	JSBS 71	50	3.57	Blackish	Smoothy & Wooly	Regular
72.	JSBS 72	44.5	3.17	Blackish	Smoothy & Wooly	Regular
73.	JSBS 73	44	3.14	Blackish	Smoothy & Wooly	Irregular
74.	JSBS 74	42.5	3.03	Blackish	Smoothy & Wooly	Regular
75.	JSBS 75	50	3.57	Blackish	Smoothy & Wooly	Regular
76.	JSBS 76	45	3.21	Blackish	Smoothy & Wooly	Irregular
77.	JSBS 77	50	3.57	Blackish	Smoothy & Wooly	Regular
78.	JSBS 78	40	2.85	Blackish	Smoothy & Wooly	Regular
79.	JSBS 79	45	3.21	Blackish	Smoothy & Wooly	Regular
80.	JSBS 80	45	3.21	Blackish	Smoothy & Wooly	Regular
81.	JSBS 81	45	3.21	Blackish	Smoothy & Wooly	Irregular
82.	JSBS 82	50	3.57	Blackish	Smoothy & Wooly	Irregular

Continue...

Sl No.	Isolates	Radial mycelial growth (mm)	Growth rate/day (mm)	Color of colony	Surface texture of colony	Shape of colony
83.	JSBS 83	37.5	2.67	Blackish	Smoothy & Wooly	Regular
84.	JSBS 84	37.5	2.67	Blackish	Smoothy & Wooly	Regular
85.	JSBS 85	40.5	2.89	Blackish	Smoothy & Wooly	Regular
86.	JSBS 86	47.5	3.39	Blackish	Smoothy & Wooly	Regular
87.	JSBS 87	55	3.92	Blackish	Smoothy & Wooly	Irregular
88.	JSBS 88	62.5	4.46	Blackish	Smoothy & Wooly	Irregular
89.	JSBS 89	50	3.57	Blackish	Smoothy & Wooly	Regular
90.	JSBS 90	45	3.21	Blackish	Smoothy & Wooly	Regular
91.	JSBS 91	52.5	3.75	Blackish	Smoothy & Wooly	Irregular
92.	JSBS 92	47.5	3.39	Blackish	Smoothy & Wooly	Regular
93.	JSBS 93	50	3.57	Blackish	Smoothy & Wooly	Regular
94.	JSBS 94	45	3.21	Blackish	Smoothy & Wooly	Regular
95.	JSBS 95	42.5	3.03	Blackish	Smoothy & Wooly	Regular
96.	MTPS 96	47	3.35	Blackish White	Smoothy & Wooly	Regular
97.	MTPS 97	45	3.21	Blackish White	Smoothy & Wooly	Regular

Continue...

Sl No.	Isolates	Radial mycelial growth (mm)	Growth rate/day (mm)	Color of colony	Surface texture of colony	Shape of colony
98.	MTPS 98	61	4.35	Blackish White	Smoothy & Wooly	Regular
99.	MTPS 99	45.5	3.25	Blackish White	Smoothy & Wooly	Regular
100.	MTPS 100	46.5	3.32	Blackish White	Rough	Regular
101.	MTPS 101	60.5	4.32	Blackish White	Smoothy & Wooly	Irregular
102.	MTPS 102	49	3.5	Blackish White	Smoothy & Wooly	Irregular
103.	MTPS 103	35	2.5	Blackish White	Smoothy & Wooly	Regular
104.	MTPS 104	57.5	4.10	Blackish White	Smoothy & Wooly	Regular
105.	MTPS 105	60.5	4.32	Blackish White	Smoothy & Wooly	Irregular
106.	NSKS 106	47.5	3.39	Blackish White	Smoothy & Wooly	Regular
107.	NSKS 107	62	4.42	Blackish White	Smoothy & Wooly	Regular
108.	NSKS 108	46.5	3.32	Blackish White	Smoothy & Wooly	Regular
109.	NSKS 109	46	3.28	Blackish White	Smoothy & Wooly	Regular
110.	NSKS 110	47	3.35	Blackish White	Smoothy & Wooly	Regular
111.	NSKS 111	46.5	3.32	Blackish White	Rough	Regular
112.	NSKS 112	49	3.50	Blackish White	Smoothy & Wooly	Irregular

Continue...

Sl No.	Isolates	Radial mycelial growth (mm)	Growth rate/day (mm)	Color of colony	Surface texture of colony	Shape of colony
113.	NSKS 113	61.5	4.39	Blackish White	Smoothy & Wooly	Irregular
114.	NSKS 114	45.5	3.25	Blackish White	Smoothy & Wooly	Regular
115.	NSKS 115	49	3.50	Blackish White	Smoothy & Wooly	Irregular
116.	RSKL 116	60.5	4.32	Blackish White	Smoothy & Wooly	Irregular
117.	RSKL 117	47	3.35	Blackish White	Smoothy & Wooly	Regular
118.	RSKL 118	49	3.50	Blackish White	Smoothy & Wooly	Irregular
119.	RSKL 119	46.5	3.32	Blackish White	Rough	Regular
120.	RSKL 120	62	4.42	Blackish White	Smoothy & Wooly	Regular

Plate 1. Pure culture of 120 isolates of *Bipolaris sorokiniana* from wheat

Table 3. Conidia production and conidial characteristics of isolates of *Bipolaris sorokiniana* (14 days old culture)

Sl No.	Isolates	Number of conidia/cm ^{2} × (10^4)	Color of conidia	Shape of conidia	Number of Septations	
					Range of Septations	Average number of septations
1.	BDKS 01	140.00	Brown	Straight	3.0-5.0	4.4
2.	BDKS 02	140.00	Brown	Straight	3.0-5.0	3.8
3.	BDKS 03	198.41	Brown	Straight	4.0-7.0	5.0
4.	BDKS 04	140.00	Brown	Straight	4.0-7.0	5.0
5.	BDKS 05	402.29	Brown	Straight	3.0-5.0	3.8
6.	BDKS 06	248.93	Deep brown	Straight	1.0-5.0	3.6
7.	BDKS 07	100.54	Brown	Straight	3.0-8.0	4.8
8.	BDKS 08	140.00	Brown	Straight	7.0-8.0	7.4
9.	BDKS 09	71.42	Deep brown	Straight	2.0-5.0	4.2
10.	BDKS 10	97.22	Brown	Straight	2.0-5.0	3.0
11.	BAKS 11	416.17	Brown	Straight	1.0-9.0	5.0
12.	BAKS 12	140.00	Brown	Straight	3.0-7.0	4.8
13.	BAKS 13	198.41	Brown	Straight	1.0-5.0	3.2
14.	BAKS 14	248.93	Brown	Straight	2.0-6.0	3.2
15.	BAKS 15	88.18	Brown	Straight	3.0-7.0	4.6
16.	BAKS 16	331.43	Light brown	Straight	3.0-7.0	4.8
17.	BAKS 17	302.76	Brown	Curved	3.0-9.0	6.0
18.	BAKS 18	248.93	Deep brown	Straight	3.0-6.0	4.2
19.	BAKS 19	248.93	Brown	Straight	3.0-6.0	4.6
20.	BAKS 20	193.79	Brown	Straight	4.0-6.0	4.8
21.	DSSS 21	285.71	Light brown	Straight	1.0-4.0	2.6

Continue...

Sl No.	Isolates	Number of conidia/cm ^{2} × (10^4)	Color of conidia	Shape of conidia	Number of Septations	
					Range of Septations	Average number of septations
22.	DSSS 22	155.14	Brown	Curved	2.0-5.0	3.6
23.	DSSS 23	218.75	Brown	Straight	3.0-6.0	4.2
24.	DSSS 24	172.83	Brown	Straight	2.0-4.0	3.0
25.	DSSS 25	331.43	Brown	Straight	1.0-3.0	2.2
26.	DSSS 26	193.79	Brown	Curved	1.0-3.0	2.2
27.	DSSS 27	172.83	Brown	Straight	2.0-3.0	2.4
28.	DSSS 28	193.79	Brown	Straight	3.0-6.0	4.6
29.	DSSS 29	248.93	Light brown	Straight	4.0-7.0	5.4
30.	DSSS 30	331.43	Light brown	Straight and curved	1.0-3.0	2.2
31.	MSBS 31	270.06	Brown	Straight	2.0-4.0	3.0
32.	MSBS 32	277.77	Brown	Straight	2.0-4.0	2.8
33.	MSBS 33	294.11	Brown	Straight	2.0-5.0	3.8
34.	MSBS 34	262.76	Deep brown	Straight	2.0-3.0	2.8
35.	MSBS 35	218.75	Brown	Straight	2.0-3.0	2.8
36.	MSBS 36	364.20	Brown	Straight	1.0-5.0	2.8
37.	MSBS 37	213.41	Deep brown	Straight	1.0-4.0	2.6
38.	MSBS 38	218.75	Deep brown	Round	2.0-4.0	2.8
39.	MSBS 39	270.06	Brown	Straight	2.0-5.0	3.6
40.	MSBS 40	224.35	Brown	Straight	2.0-5.0	3.6
41.	MSPS 41	248.93	Brown	Straight	3.0-4.0	3.4
42.	MSPS 42	218.75	Brown	Straight	2.0-4.0	3.2
43.	MSPS 43	218.75	Brown	Curved	1.0-4.0	2.6

Continue...

Sl No.	Isolates	Number of conidia/cm ^{2} × (10^4)	Color of conidia	Shape of conidia	Number of Septations	
					Range of Septations	Average number of septations
44.	MSPS 44	213.41	Brown	Straight	2.0-4.0	2.8
45.	MSPS 45	224.35	Brown	Straight	1.0-3.0	2.2
46.	MGBS 46	294.11	Brown	Straight	2.0-3.0	2.6
47.	MGBS 47	311.94	Brown	Straight	3.0-9.0	4.6
48.	MGBS 48	311.94	Brown	Curved	3.0-4.0	3.4
49.	MGBS 49	213.41	Brown	Straight	2.0-4.0	2.8
50.	MGBS 50	236.16	Deep brown	Straight	3.0-5.0	4.4
51.	MGBS 51	193.79	Deep brown	Straight	3.0-5.0	3.8
52.	MMPS 52	294.11	Brown	Straight	4.0-7.0	5.0
53.	MMPS 53	402.29	Brown	Curved	8.0-9.0	8.6
54.	MMPS 54	242.38	Brown	Straight	3.0-5.0	3.8
55.	MMPS 55	213.41	Brown	Straight	1.0-5.0	3.6
56.	MMPS 56	270.06	Light brown	Straight	3.0-8.0	4.8
57.	MMPS 57	231.03	Brown	Straight	7.0-8.0	7.4
58.	MMPS 58	236.16	Brown	Straight	2.0-5.0	4.2
59.	MMPS 59	218.75	Brown	Straight	2.0-5.0	3.0
60.	MMPS 60	248.93	Brown	Straight	3.0-4.0	3.8
61.	MMPS 61	285.71	Brown	Straight	4.0-7.0	5.4
62.	MMPS 62	248.93	Brown	Straight	5.0-8.0	6.4
63.	MMPS 63	248.93	Brown	Curved	4.0-5.0	4.8
64.	MMPS 64	248.93	Brown	Straight	1.0-6.0	3.4
65.	MMPS 65	193.79	Brown	Straight	7.0-8.0	7.6

Continue...

Sl No.	Isolates	Number of conidia/cm ^{2} × (10^4)	Color of conidia	Shape of conidia	Number of Septations	
					Range of Septations	Average number of septations
66.	MMPS 66	331.43	Brown	Round	3.0-5.0	4.0
67.	MMPS 67	285.71	Deep brown	Straight	2.0-4.0	3.0
68.	MMPS 68	331.43	Brown	Straight	3.0-7.0	4.8
69.	MMPS 69	270.06	Brown	Straight	2.0-4.0	3.8
70.	MMPS 70	376.34	Brown	Straight	3.0-7.0	4.6
71.	JSBS 71	140.00	Brown	Straight	3.0-4.0	3.6
72.	JSBS 72	176.76	Brown	Straight	1.0-3.0	2.4
73.	JSBS 73	180.78	Brown	Straight	5.0-7.0	6.0
74.	JSBS 74	193.79	Deep brown	Straight	1.0-2.0	1.4
75.	JSBS 75	140.00	Brown	Curved	3.0-5.0	4.2
76.	JSBS 76	172.83	Brown	Straight and curved	3.0-4.0	3.2
77.	JSBS 77	140.00	Deep brown	Straight	2.0-4.0	3.0
78.	JSBS 78	218.75	Brown	Straight	5.0-7.0	6.4
79.	JSBS 79	173.83	Brown	Straight	2.0-4.0	3.4
80.	JSBS 80	172.83	Brown	Straight	3.0-7.0	4.4
81.	JSBS 81	172.83	Light brown	Straight	4.0-7.0	6.0
82.	JSBS 82	140.00	Brown	Straight	4.0-7.0	5.6
83.	JSBS 83	248.93	Brown	Straight	1.0-4.0	2.6
84.	JSBS 84	248.93	Light brown	Straight	5.0-7.0	6.4
85.	JSBS 85	213.41	Brown	Straight	1.0-3.0	2.2
86.	JSBS 86	155.14	Brown	Straight	1.0-3.0	2.4
87.	JSBS 87	115.70	Brown	Straight	1.0-3.0	2.2

Continue...

Sl No.	Isolates	Number of conidia/cm ^{2} × (10^4)	Color of conidia	Shape of conidia	Number of Septations	
					Range of Septations	Average number of septations
88.	JSBS 88	89.06	Deep brown	Straight	2.0-4.0	3.2
89.	JSBS 89	140.00	Deep brown	Curved	2.0-5.0	4.0
90.	JSBS 90	172.83	Light brown	Straight	2.0-5.0	3.2
91.	JSBS 91	126.99	Brown	Straight	2.0-7.0	4.2
92.	JSBS 92	155.14	Brown	Straight	4.0-6.0	4.8
93.	JSBS 93	140.00	Brown	Straight	3.0-6.0	3.8
94.	JSBS 94	172.83	Brown	Straight	1.0-5.0	3.0
95.	JSBS 95	193.79	Brown	Straight	1.0-5.0	3.6
96.	MTPS 96	158.44	Brown	Straight	3.0-7.0	4.8
97.	MTPS 97	172.83	Brown	Straight	1.0-5.0	2.8
98.	MTPS 98	94.06	Brown	Straight	1.0-5.0	2.4
99.	MTPS 99	179.08	Light brown	Straight	1.0-5.0	3.4
100.	MTPS 100	161.88	Light brown	Straight	1.0-7.0	4.2
101.	MTPS 101	95.62	Deep brown	Straight	1.0-3.0	2.2
102.	MTPS 102	145.77	Deep brown	Straight	1.0-2.0	1.4
103.	MTPS 103	285.71	Brown	Straight	1.0-9.0	5.0
104.	MTPS 104	105.86	Brown	Straight	3.0-7.0	4.8
105.	MTPS 105	95.62	Brown	Straight	1.0-5.0	3.2
106.	NSKS 106	155.14	Brown	Curved	2.0-6.0	3.2
107.	NSKS 107	91.05	Brown	Straight	3.0-7.0	4.6
108.	NSKS 108	161.88	Deep brown	Straight	3.0-7.0	4.8
109.	NSKS 109	165.40	Deep brown	Straight	3.0-9.0	6.0

Continue...

Sl No.	Isolates	Number of conidia/cm ^{2} × (10^4)	Color of conidia	Shape of conidia	Number of Septations	
					Range of Septations	Average number of septations
110.	NSKS 110	158.44	Brown	Straight	3.0-6.0	4.2
111.	NSKS 111	161.88	Brown	Straight	3.0-6.0	4.6
112.	NSKS 112	145.77	Brown	Straight	4.0-6.0	4.8
113.	NSKS 113	95.54	Brown	Straight	1.0-4.0	2.6
114.	NSKS 114	169.08	Light brown	Straight	2.0-5.0	3.6
115.	NSKS 115	145.77	Brown	Straight	3.0-6.0	4.2
116.	RSKL 116	95.62	Brown	Straight	2.0-4.0	3.0
117.	RSKL 117	158.44	Brown	Straight	1.0-3.0	2.2
118.	RSKL 118	145.77	Brown	Straight	1.0-3.0	2.2
119.	RSKL 119	161.88	Brown	Straight	2.0-3.0	2.4
120.	RSKL 120	91.05	Brown	Round	3.0-6.0	4.6

Fig. 2. Pure culture of *Bipolaris sorokiniana*

Fig. 3. Conidia of *Bipolaris sorokiniana*(100X)

Table 4. Grouping of different isolates of *Bipolaris sorokiniana* based on cultural characteristics

Sl No.	Group	Cultural characteristics	Number of isolates	% Isolates under each group
1.	G-1	Smoothy and wooly blackish white regular	22	18.33
2.	G-2	Smoothy and wooly blackish white irregular	39	32.5
3.	G-3	Effuse and rough blackish white regular	8	6.66
4.	G-4	Effuse and rough blackish white irregular	4	3.33
5.	G-5	Smoothy and wooly blackish regular	30	25
6.	G-6	Smoothy and wooly blackish irregular	8	6.66
7.	G-7	Effuse and rough blackish regular	2	1.66
8.	G-8	Effuse and rough blackish irregular	2	1.66
9.	G-9	Smoothy and wooly whitish regular	2	1.66
10.	G-10	Smoothy and wooly whitish irregular	1	0.83
11.	G-11	Effuse and rough whitish regular	1	0.83
12.	G-12	Effuse and rough whitish irregular	1	0.83

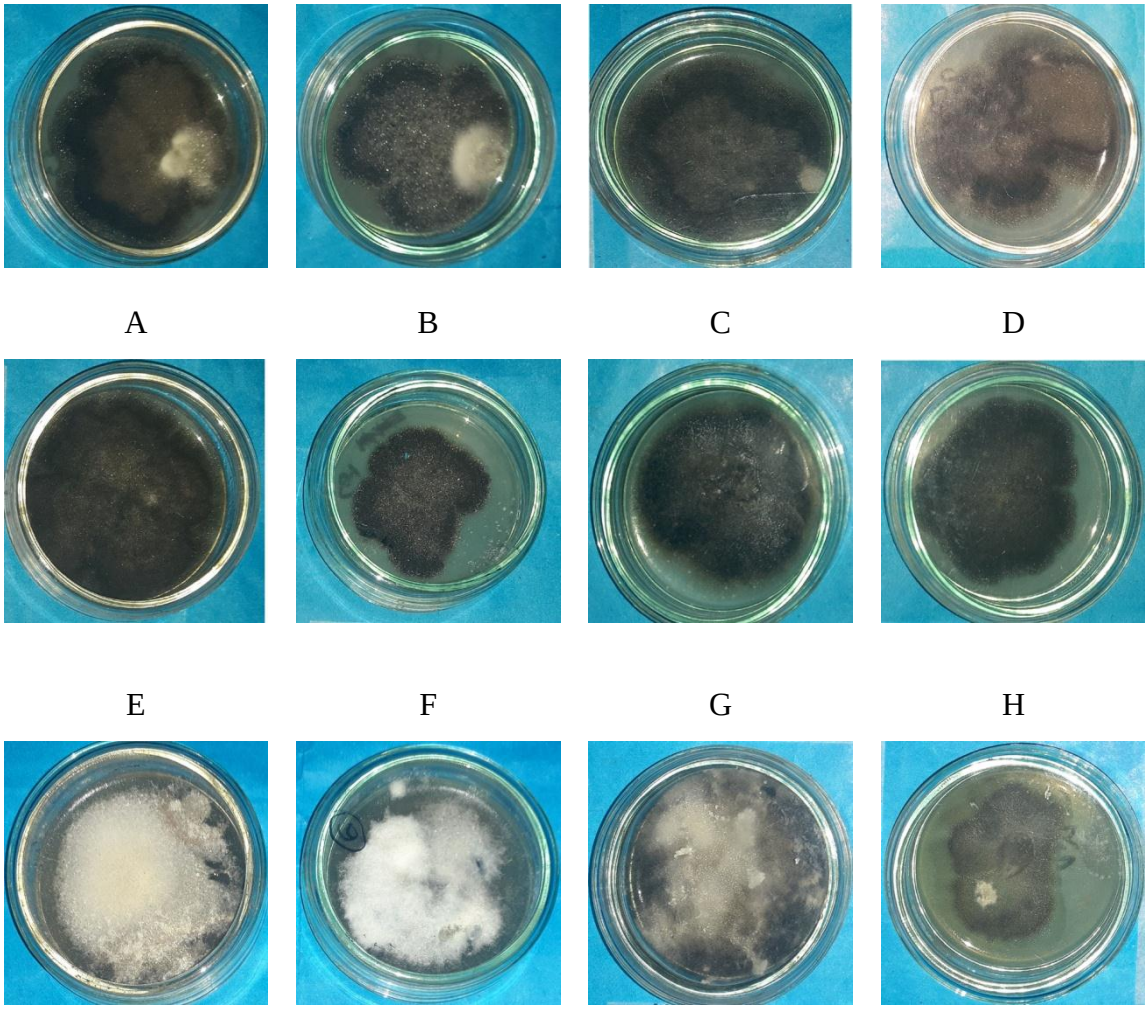
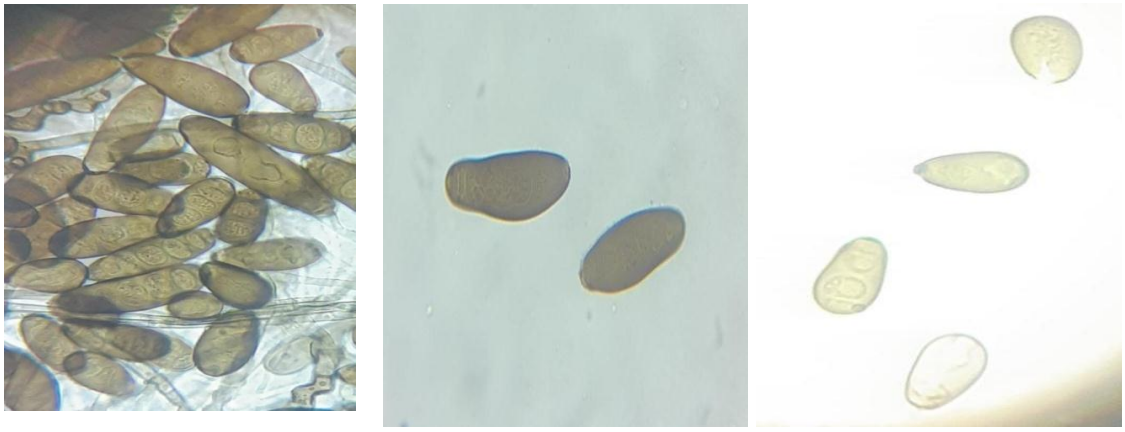


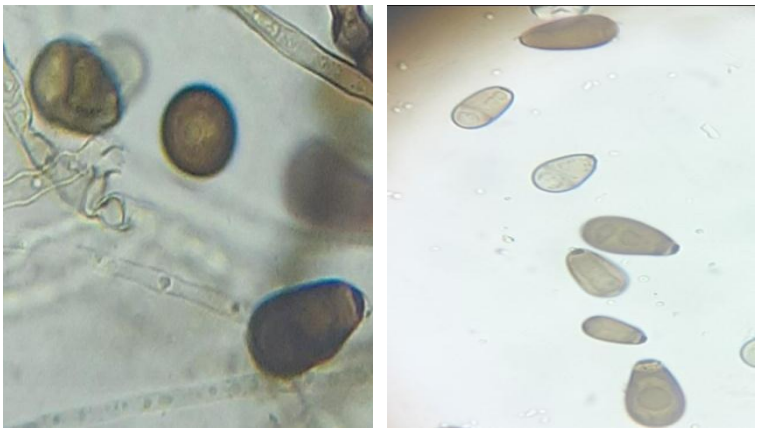
Plate 2. Twelve cultural groups of Bipolaris sorokiniana. A. Smoothy and wooly blackish white regular isolates, B. Smoothy and wooly blackish white irregular isolates, C. Effuse and rough blackish white regular isolates, D. Effuse and rough blackish white irregular isolates, E. Smoothy and wooly Blackish 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 5. Grouping of isolates based on conidial shape.

Sl No.	Group	Conidial shape	Number of isolates	% isolates under each group
1.	G-1	Elliptical straight	57	47.50
2.	G-2	Elliptical curved	13	10.83
3.	G-3	Oval	28	23.33
4.	G-4	Round	5	4.17
5.	G-5	Pyriform	17	14.16



A B C



D E

Plate 3. Five conidial shapes of *Bipolaris sorokiniana* A. Elliptical andstraight shaped conidia, B. Elliptical and curved shaped conidia, C. Oval shaped conidia, D. Round shaped conidia and E. Pyriform shaped conidia.

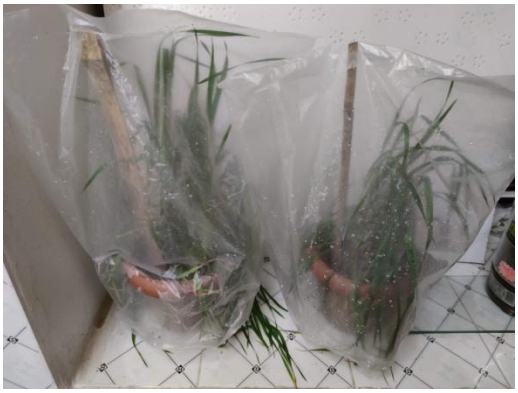
Table 6. Mycelial growth rate, conidia production and septations of conidia of *Bipolaris sorokiniana* under 12 cultural groups. (14 days old culture)

Sl No.	Cultural group	Growth rate/day (mm)	Number of conidia/$cm^2 \times 10^4$	Number of Septation
1.	G-1	4.35	94.06	2.44
2.	G-2	4.32	95.62	3.23
3.	G-3	3.32	161.88	4.2
4.	G-4	4.28	97.22	3.0
5.	G-5	3.57	140	3.6
6.	G-6	4.46	89.6	3.2
7.	G-7	2.67	248.93	2.6
8.	G-8	3.14	180.78	6.0
9.	G-9	3.57	140	4.4
10.	G-10	4.21	100.54	4.8
11.	G-11	3.57	140	7.1
12.	G-12	4.50	88.18	4.6

Table 7. Virulence of different cultural groups of *Bipolaris sorokiniana* on wheat

Sl No.	Cultural group	Number of leaf infections	Number of spot/leaf	% LAD
1.	G-1	5	3.4	19
2.	G-2	9	3.4	14.44
3.	G-3	28	2.5	29.46
4.	G-4	2	1.5	2
5.	G-5	7	5.7	31.42
6.	G-6	5	2.0	13
7.	G-7	18	8.4	32.77
8.	G-8	10	5.4	38.5
9.	G-9	4	2.5	11.25
10.	G-10	2	1.5	7.5
11.	G-11	15	4.4	18.73
12.	G-12	8	4.1	21.25

A



B

C

D

E

Fig. 4. Inoculation of isolates of *Bipolaris sorokiniana* in wheat, A. Spore suspension of 12 cultural group of *Bipolaris sorokiniana*, B.C.D. and E. Inoculation of 12 cultural group of *Bipolaris sorokiniana* including control where sterile water was sprayed.

A

B

C

D

E

Fig. 5. Post inoculation plant morphology of wheat after inoculation of 12 cultural groups of *Bipolaris sorokiniana* (A, B, C) and leaf spot symptom on wheat leaf after inoculation of *Bipolaris sorokiniana* (D, E).

CHAPTER 5

DISCUSSION

Infected leaves and seeds of wheat were collected from different wheat growing locations of Bangladesh and 120 isolates of *Bipolaris sorokiniana* were isolated from these collected samples. Isolates were cultured on PDA media and then growth rate, conidia production and different cultural, physiological and morphological characteristics were recorded, isolates were also identified by ITS based molecular identification tools. All around the world many workers were studying morphological and physiological characteristics of isolates of *Bipolaris sorokiniana*. From 1922 to 2020, many work was done by many hard workers. In our present study the radial mycelial growth rate of isolates of *Bipolaris sorokiniana* were varied from isolates to isolates and ranged from 1.39 mm/day to 4.46 mm/day. Hossain and Azad (1992) recorded the mycelial growth of isolates of *Bipolaris sorokiniana*from 29 mm to 78 mm from 83 isolates. Aminuzzaman and Hossain (2005) reported mycelial growth of isolates ranged from 9.26 mm to 24.0 mm. Srinivas *et al.,* (2009) recorded the growth rate of isolates from 20.3 mm to 63 mm. In another study Pandey *et al.,* (2005) reported the mycelial growth of isolates of *Bipolaris sorokiniana* from 4.77 cm. Aminuzzaman *et al.,* (2010) recorded the mycelial growth of isolates of *Bipolaris sorokiniana* ranging from 2.77 mm to 9.10 mm/day. The mycelial growth rate may vary depending on temperature during the incubation period. Ilija *et al.,* (2000) reported that the growth of conidia of *Bipolaris sorokiniana* was quickly in the temperature of 25$\pm$1°C. The maximum temperature was 15 to 25°C due to germination of the conidia of *Bipolaris sorokiniana* and that was recorded by Hodges (1975). He recorded this temperature from 20 days of older culture. But above 20°C the germination growth rate of the isolates of *Bipolaris sorokiniana* were decreased slowly and at 35°C temperature the conidia production was stopped. Sijam *et al.,* (2009) recorded that the suitable temperature for conidia production of isolates of *Bipolaris 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 minimum number of conidia/cm^2 was 89.6×10^4 and which was found in isolate number JSBS 88. In 1992, Hossain and Azad studied 83 isolates and they reported

among 83 isolates, 65 isolates produced many conidia in seven days under UV-light (12/12). Pandey *et al.,* (2005) recorded the spore production rate of the isolates of *Bipolaris sorokiniana* from 3.35×10^3 to 122.12×10^3. Srinivas *et al*., (2009) recorded the highest spore production per colony was 10×10^7 and lowest was 1.0×10^7 per colony. Aminuzzaman *et al.,* (2010) reported the highest number of conidia production was 166.84×10^3 per cm^2 and lowest number of conidia/cm^2 was 4.00×10^3. 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. Shoemaker (1959) reported that the shape of the conidia of *Bipolaris sorokiniana* were fusoid, straight or curved. Burnett and Hunter (1999) recorded the shape of conidia were straight or curved. Mathur and Kongsdal (2000) reported that most of the conidia of *Bipolaris sorokiniana* were straight or slightly curved. Aminuzzaman and Hossain (2005) reported that the maximum conidia were straight shaped and some were slightly curved. Iftekhar *et al.,* (2009) found cylindrical, straight and slightly curved conidia of *Bipolaris sorokiniana*. Aminuzzaman *et al.,* (2010) reported that most of the conidia of *Bipolaris sorokiniana* were straight but few were slightly curved. In the present research, 92 isolates produced brown coloured conidia and 17 isolates produced deep brown coloured conidia and 11 isolates produced light brown coloured conidia. Barnet and Hunter (1999) recorded the colour of conidia were brown. Mathur and Congsdal (2005) reported that the colour of conidia were dark brown to black. Aminuzzaman and Hossain (2005) reported that some isolates produced deep brown coloured conidia and some produced light brown coloured conidia. Iftekhar *et al.*, (2009) reported that the isolates of *Bipolaris sorokiniana* produced olivaceous brown and light brown coloured conidia. Aminuzzaman *et al.,* (2010) studied 86 isolates and reported that 52 had brown coloured conidia and 26 had deep brown coloured conidia, 5 had light brown coloured conidia and 2 had brown to deep brown coloured conidia and rest 1 had light brown to deep brown coloured conidia. In the present research work, among 120 isolates the septation of conidia ranged from 1.4 to 8.6. In 1997, Shoemaker 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. Burnett and Hunter (1999) recorded several celled conidia of *Bipolaris sorokiniana*. Aminuzzaman and Hossain (2005) 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 39 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 lowest (1) isolates with 0.83% frequency of each of isolates. Alam *et al.,* (1997) identified seven groups based on morphological and physiological divergences among 27 isolates of *Bipolaris sorokiniana*. Ahmed (1997) studied 27 isolates and he classified it into four different clusters. He kept them three into cluster 1, six to cluster 2, fourteen to cluster 3 and four to cluster 4 significantly. Debnath (1997) grouped the isolates into 2 groups. These were chromogenic and nonchromogenic. Chromogenic groups produced pigment isolates and nonchromogenic groups produced non pigment isolates of *Bipolaris sorokiniana*. In present study, effuse and rough blackish irregular group was most virulent (38.5) and effuse and rough blackish irregular group was low virulent group among 12 groups. Maraite *et al.,* (1998) collected 27 isolates and found different morphological characteristics that showed a strong correlation with aggressiveness of pathogen of *Bipolaris sorokiniana*. Ahmed (2001) studied 262 isolates of *Bipolaris sorokiniana* from 16 wheat growing areas of Bangladesh and he classified these isolates into 13 physiological groups based on their cultural identities. Pandey*et al.,* (2005) grouped his studied isolates into five different cultural groups based on their virulence. Srinivas*et al.,* (2009) grouped 103 isolates into five groups based on their morphological characteristics which he collected from wheat growing zones of India. The dull white or greenish black colony contains maximum frequency and that was 38.83% and black suppressed type and white fluffy type colony showed minimum frequency and that was 11.65%. The pathogenicity test was also done by him over different groups. Aminuzzaman *et al.,* (2010) studied 86 isolates and he grouped it into nine cultural groups based on colony morphology and colony colour. 34 isolates produced effuse black regular colonies with maximum frequency and that was 39.53%. 29 isolates produced effuse black irregular colonies with minimum frequency and that was 33.72%. They also reported the virulence of the pathogen of *Bipolaris sorokiniana* under different cultural groups on wheat cv. kanchan. Based on growth rate, conidia production, size and septation of conidia, these cultural groups showed different characteristics. The highest growth rate found in the group of 6 and that was

4.95 mm/day and lowest was found in the group 9 and that was 2.84 mm/day. In cultural group 2, they found maximum conidia production and that was 56.85×10^3 and minimum in the group of 9 and that was 20.05×10^3 The highest septation was found in group 2 and that was 5.33 and lowest was 4.30. Aminuzzaman *et al.*, (2010) recorded the range of mycelial growth rate of *Bipolaris sorokiniana* was from 4.21 to 4.81 mm/day where the maximum growth rate found in cultural group number 3 and lowest in cultural group number 8. In cultural group number 8, maximum conidia production was recorded and minimum was in the group number 3 that ranged from 136.75×10^3 to 19.43×10^3 conidia/cm^2. This grouping was done by observing the variation of physiological, cultural and morphological characteristics and virulence of *Bipolaris sorokiniana*. PCR using primer pairs ITS1/ITS4 yielded specific species band (around 600 bp) of *Bipolaris sorokiniana* which was supported by Abo-Ghazala et al., (2019).

CHAPTER 6

SUMMARY AND CONCLUSION

Infected leaves and seeds of wheat were collected from seven districts namely Bogura, Dinajpur, Meherpur, Jashore, Mymensingh, Netrokona and Rajshahi.The isolates from collected samples were cultured on PDA media. Then cultural, physiological and morphological characteristics and virulence were recorded based on their radial mycelial growth rate, colour of colony, surface texture of colony, shape of colony, number of conidia production, shape and colour of conidia etc. The maximum mycelial growth rate was recorded ranges from 1.39 (MMPS 66) to 4.46 mm/day (JSBS 88). Maximum isolates had blackish white coloured colonies (73 isolates). 42 isolates had blackish coloured colonies and 5 isolates had whitish coloured colonies. Among 120 isolates, 65 isolates showed regular shaped colonies and 55 isolates had irregular shaped colonies. 102 isolates showed smoothy and wooly surface texture of colony and 18 isolates showed effuse and rough surface texture of colony. Among 120 isolates, maximum number of conidia/cm^2 was counted in isolates no BAKS 11 which was 416.17×10^4 and minimum number of conidia/cm^2 was 89.6×10^4 found in isolates no JSBS 88. Among these isolates 92 isolates produced brown coloured conidia and 17 isolates produced deep brown coloured conidia and 11 isolates produced light brown coloured conidia. 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. Among 120 isolates the septations of conidia ranged from 1.4 to 8.6. 120 isolates were grouped into 5 groups based on their conidial shape. 57 isolates produced elliptical and straight conidia with 47.50% frequency, 13 isolates produced elliptical and curved shaped conidia with 10.83% frequency. 28 isolates produced oval shaped conidia with 23.33% frequency, 5 isolates produced round shaped conidia with 4.17% frequency and 17 isolates produced pyriform shaped conidia with 14.16% frequency all above of isolates. In molecular identification (ITS rDNA gene), PCR using primer pairs ITS1/ITS4 yielded specific species band (around 600 bp) of amplification product for the isolates of *Bipolaris sorokiniana*. 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 39 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 lowest (1) isolates with 0.83% frequency of each of isolates. The different cultural groups of *Bipolaris 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. The highest growth rate counted from the group number G-12 and lowest growth rate counted from the group number G-7. Maximum number of conidia/cm^2 was recorded from the group number G-7 and that was 248.93×10^4 and the minimum number of conidia/cm^2 was recorded from group G-12. The highest septation was found in group G-11 and that was 7.1 and lowest was found in group G-1 and that was 2.44. Among these 12 groups, effuse and rough blackish irregular group was most virulent (38.5% LAD) and effuse and rough blackish irregular group was low virulent group among 12 groups(2% LAD).

Wheat is the second main crop in our country next to rice. It is affected by the pathogen *Bipolaris sorokiniana* which is the most destructive causal agent for both wheat and barley. This pathogen shown different physiological, cultural and morphological characteristics and also virulence. To save the crop and to control this pathogen, we need to know its physiological, cultural and morphological and genetic variations. Thus more investigation on varieties of *Bipolaris sorokiniana* collecting more and more isolates from different agro-ecological zone of the country are suggested to formulate a paper approach for its effective management.

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