PHYSIOLOGICAL, CULTURAL AND MORPHOLOGICAL VARIATION OF *Bipolaris sorokiniana*

MD. MAMUNUR RAHMAN

DEPARTMENT OF PLANT PATHOLOGY
SHER-E-BANGLA AGRICULTURAL UNIVERSITY
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MD. MAMUNUR RAHMAN
REGISTRATION. NO. 05-01805

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Approved by:

Dr. F. M. Aminuzzaman Associate professor Department of Plant Pathology Sher-e-Bangla Agricultural University **Supervisor**	**Prof. Dr. M. Salahuddin M. Chowdhury** Department of Plant Pathology Sher-e-Bangla Agricultural University **Co-supervisor**

Nazneen Sultana
Chairman
Examination Committee
Department of Plant Pathology
Sher-e-Bangla Agricultural University

Dr. F.M. Aminuzzaman
Associate Professor
Department of Plant Pathology
Sher-e Bangla Agricultural University
Dhaka-1207, Bangladesh
Mobile: +8801733717936

Ref: *Date:* ----------

CERTIFICATE

This is to certify that thesis entitled, **"PHYSIOLOGICAL, CULTURAL AND MORPHOLOGICAL VARIATION OF *Bipolaris sorokiniana*)"** submitted to the Faculty of Agriculture, 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 **Md. Mamunur Rahman** bearing **Registration No. 05-01805** 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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SAU, Dhaka

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ABSTRACT

Altogether 69 isolates of *Bipolaris sorokiniana* were isolated from leaf and seeds of 21 varieties of wheat collected from 7 major wheat growing regions of Bangladesh. The collected isolates differed significantly in respect of mycelia growth, conidia production ability, shape, color, size and septation of conidia. The redial mycelia growth rate ranged from 1.96 ± 0.56 mm/day to 5.83 ± 0.02 mm/day. Maximum number of conidia /cm^2 (119.21 ± 41.29 $\times 10^3$) was recorded from isolate JJRPL 01 and minimum (2.79 ± 0.58 $\times 10^3$) from MGMSL 07. Among the isolates, maximum 54 produced deep brown color, 7 brown colored, 3 light brown colored and 3 brown to deep brown colored conidia while 2 isolates fail to produce conidia after 15 days of incubation. Conidia of most of the isolates were straight shaped. The maximum 54 had deep brown colored conidia and 7, 3 and 3 had brown, light brown, brown to deep color conidia, respectively. Highest length of conidia (72.74 ±1.27µ) was recorded in isolate JJRSS 03 where lowest length (36.80 ± 6.03µ) was recorded in isolate PIRPL 08. The highest breadth was 17.65 ± 0.98µ (MGMSL 08) and lowest was 11.42 ±1.29µ in (SNNSL 01) isolates. The highest septation was found in MGMSL 08 (6.50) and lowest in MGKSL 01 (2.66). The isolates were classified into nine cultural groups based on colony morphology and colony color. 23 isolates produced effuse blackish white irregular (EBWI) colony. The isolates of *Bipolaris sorokiniana* under different cultural group**s** also differed significantly in respect of mycelial growth rate, number of conidia/cm^2, size and septation of conidia.

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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

Bipolaris sorokiniana (teleomorph *Cochliobolus sativus*) is the causal agent of root rot, foliar blight, seedling blight, head blight and black point of wheat and barley (Sivanesan 1990). The disease caused by the fungus is one of the constraints for both crops in warmer growing areas and causes significant yield losses (Aftabuddin *et al*. 1991). High temperature and high relative humidity favour the outbreak of the disease particularly in South-Asia's intensive irrigated rice-wheat production systems (Aggarwal *et al*. 2000).

The pathogen causes damage to the crop from seedling to leaf stage (Alam *et al*., 1994). It may attack panicle causing black point of grains The pathogen *B. sorokiniana* has been found to reduce 88.7% grains/ear and produced 87.5% discolored and black pointer grains (Hossain *et al*., 1998). Rashid and Fakir (1998) estimated yield reduction of wheat due to *Bipolaris sorokiniana* as high as 57.6% and 64.5% in Kanchan and Sonalika variety. Increased number of black pointed seed in the seed sample resulted in formation of higher number of seeds having black point infection in the field (Hossain and Hossain, 2001). Fakir (1998) reported that 15-20% seed yield of wheat has been reduced due to shriveled and seriously black point affected grains. Hossain *et al*. (1998) reported that this disease deduced yield up to 40% and 88% over the control under field condition and artificial inoculation, respectively. According to Mehta (1997) spot blotch of wheat caused by *Bipolaris sorokiniana* adversely affect germination, development of the root system or kill the seedling within a few days and capable of causing up to 100% yield losses .

Maraite *et al.* (1998) reported several synonyms of the anamorph viz. *Helminthosporium sorokinianum*, *Drechslera sorokiniana*, and *Helminthosporium sativum*. Shoemaker (1959) proposed the generic name *Bipolaris* for the *Helminthosporium* species with fusoid, straight, or curved conidia germinating by one germ tube from each end (bipolar germination). The former genus *Helminthosporium* was divided into three anamorphic genera: *Bipolaris, Drechslera*, and *Exserohilum* with the teleomorphic stages *Cochliobolus*, *Pyrenophora*, and *Setosphaeria*, respectively. *B. sorokiniana* is characterized by thick-walled, elliptical conidia with five to nine cells. In axenic culture, the mycelium is composed of hyphae interwoven as a loose cottony mass and appears white or light to dark grey depending on the isolates. The fungus is differentiated from other members of the *Bipolaris* genus on the basis of morphological features of conidiophores and conidiospores.

Physiological specialization at the species level was first described by Christensen (1925), who showed that fungal isolates varied considerably in virulence to wheat and barley. Differential reactions of progenies of crosses between isolates that differed in pathogenicity to different grass species indicated complex inheritance involving several genes (Nelson, 1960, 1961). Specificity on the race-cultivar level is indicated by the observation that field populations shift to more aggressive races with long-term continuous wheat cultivation (El Nashaar and Stack, 1989). Valjavec-Gratian and Steffenson (1997) identified three pathotypes from 33 isolates of North Dakota, tested on the basis of interaction phenotypes with three six-rowed barley differentials. There are several additional reports on the genetic variability of *B. sorokiniana* populations (Steffenson *et al.* 1994; Misra, 1979). Recent studies based on large numbers of strains collected on a global basis suggest that *B. sorokiniana* forms a conidium of isolates varying in virulence and aggressiveness with specific and nonspecific interactions (Duveiller and Garcia Altamirano, 2000; Maraite *et al.*, 1998). The mechanism of variability is not well understood.

Fusions (anastomosis) between hyphae, which stem from different conidia, may result in somatic hybridization and the emergence of new fungal variants.

There is no report on physiological races of the pathogen in the world. But variation among isolates of the pathogen were indicated by some workers. Misra *et al*., (1981) observed geographical variation in the pathogenicity of *B. sorokiniana* collected from Dholi (Bihar) and Bhubaneswar (Orissa) on wheat. Pascual and Raymundo (1991) found cultural variation among 20 isolates of *Helminthosporium sativum*. They also reported that the isolates differed in virulence as manifested by incubation period, lesion number per 3 cm^2 leaf area and lesion size. Alam *et al*., (1997) also identified seven morphological and physiological divergences among 27 isolates of *B. sorokiniana,* wheras Adhikary (2000) found variability among 122 isolates collected from all the major wheat growing areas of Bangladesh. While selecting breeding materials for resistance, it is important to know which pathotype to use in the screening process; how the resistance is expressed and inherited; whether it is likely to prove adequate and durable? It is important to ascertain the capacity of the pathogen for change under different selection pressures and the rapidity to with which this may occur (Thakur, 1999). By whatever methods the diverse races or biotypes of *Helminthosporium sativum* arise, they are numerous in nature and complicate the problem of developing and maintaining resistance crop varieties (Wood, 1962).

Information is insufficient on the study of characterization of *Bipolaris sorokiniana* (sacc.) in the Bangladesh agricultural ecosystem. Furthermore, actual status of the pathogenicity and variability of the causal organism is quite unclear and methodological research for increasing accuracy of characterization is also required.

Objective

The present research work was therefore carried out to determine the physiological, cultural and morphological variation of *Bipolaris sorokiniana* isolates collected from Bangladesh.

CHAPTER 2

REVIEW OF LITERATURE

Good number of research work have been carried out on physiological, cultural and morphological variation of *Bipolaris sorokiniana*. However, some important information in respect of physiological, cultural and morphological variation of *Bipolaris sorokiniana* are accumulated in this chapter.

Christensen (1922) described four biological specialization of *Helminthosporium sativum* which differ physiologically as indicated by the rate and growth character on the same and different media and by the facts that they produce different degrees of infection on the same cereal and grasses. He also observed the conidia of four biological forms of *Helminthosporium sativum* differed in respect of length, breadth and number of septa.

Christensen (1925) identified 37 races of *Helminthosporium sativum* based on morphological and pathological characters.

Luttrell (1955) found different measurements of conidia in different isolates of *B. sorokiniana*.

Shoemaker (1959) reported that the conidia of *Bipolaris sorokiniana* are fusoid, straight or cuved with bipolar germination and characterized by thick-walled, elliptical conidia (60-120 µm ×12-20 µm) with 4-8 septa.

Tinline (1962) reported the variability in the asexual populations of the pathogen *Bipolaris sorokiniana* was thought to be due to parasexual recombination.

Anonymous (1975) reported that Initial pH and sucrose concentration of the medium markedly affected sporulation and conidial characteristics in four *Bipolaris* species. *Bipolaris sorokiniana* and *B. zeicola* sporulated well at all pH levels; *B. setariae* and *B. maydis* produced relatively fewer conidia. Most isolates of *B. sorokiniana* sporulated well at all sucrose concentrations; sporulation by *B. zeicola* and *B. maydis* was less abundant, particularly at high sucrose levels. Only one of six isolates of *B. setariae* g/litre.In all produced conidia at sucrose concentrations higher than 5 four species conidium length and number of septa per conidium significantly decreased as the pH of the medium was increased, although some isolates of *B. sorokiniana* showed great variability. Conidium width was less markedly affected. Generally, in *B. sorokiniana* g/litre. In g/litre to 30 conidium length and number of septa decreased as the sucrose concentration was increased from 1 *B. zeicola*, conidia of maximum length and number of septa were produced on sucrose g/litre. Conidial characteristics of concentrations of 5 *B. maydis* were less markedly affected by increases in sucrose concentration.

Mehta (1981) 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* have been reported by Misra *et al.*,1981.

Sivanesan and Holliday (1981) reported more or less of same type of conidia of *Bipolaris sorokiniana* having straight to curved, 3-12 septa with olive brown color.

Chowdhury (1990) observed the variation in colony characteristics and pathogenicity in 10 isolates of *Drechslera sorokiniana* isolated from wheat seeds collected from seven countries.

Bipolaris sorokiniana has a high degree of phenotypic variability; the genetic diversity of this fungus has not been fully studied. Studies have been made of isozyme polymorphisms among isolates. (Matsumura,1991.)

Hetzler *et al*. (1991) found differences in disease-causing ability of different strains of *Helminthosporium sativum* on a set of wheat genotypes.

Pascual and Raymundo (1991) found cultural variation among 20 isolates of *Helminthosporium sativum* when grown in PDA, wheat extract agar and V-8 juice agar. They found a distinct difference in colony morphology among 112, 16 and 118 isolates in PDA. They also reported that the isolates differed in virulence as manifested by incubation period, lesion number per 3 cm^2 leaf area and lesion size in a commercial wheat variety Trigo 3. Of the 20 isolates evaluated 16 and 140 were most virulent while 118 exhibited the least virulence.

Hetzler (1992) suggested a geographical racial differentiation of *Bipolaris sorokiniana* as he found a tendency for isolates from warm and dry regions to be less virulent and most virulent isolates came from southern and central Africa.

Hossain and Azad (1992) collected 83 isolates of *Helminthosporium sativum* from seven locations of Bangladesh and they found variable mycelia growth and conidia production ability of the isolates on PDA in respect of location.

Ahmed *et al*. (1997) obtained physiological and morphological variation among 27 isolates of *Bipolaris sorokiniana* collected from 14 districts of wheat growing regions in Bangladesh. 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 conidium varied from 35-270 µm and 15-65 µm depending on isolates. They classified 27 isolates into

four distinct clusters. Among them three belonged to cluster I, six to cluster II, fourteen to cluster III and four to cluster IV.

Alam *et al*. (1997) identified seven morphological and physiological divergences among 27 isolates of *Bipolaris sorokiniana*.

Debnath (1997) studied the occurrence of chromogenic variant in *Bipolaris sorokiniana* and he designated pigment producing and non pigment producing isolates of *Bipolaris sorokiniana* as chromogenic and noncharomogenic, respectively. The colonies of nonchromogenic isolates were brown to black, compact, more or less regular margined and produced relatively shorted spores than that of chromogenic, one, whereas the chromogenic ones were fluffy, cottony white, more or less irregular and produced relatively longer spores.

Valjavec-Gratian and Steffenson (1997) identified three pathotypes of *B. sorokiniana* from 33 isolates tested, including one from wheat roots, based on their virulence on three differential barley genotypes.

Valim *et al*. (1997) observed pathogenic and morphologic variation among 10 isolates of *Bipolaris sorokiniana* collected from 4 wheat growing regions in Brazil.

Singh *et al*. (1998) found *B. sorokiniana* as one of the pathogenic fungus among the number of the fungi isolated from blighted wheat leaves.

Maraite *et al*. (1998) studying the 27 isolates of *Bipolaris sorokiniana* and found different colours of the colonies on minimal medium varied from white to light pink and dark green. The dark coloured colony showed a strong correlation with aggressiveness of the pathogen. They also reported that large differences in host response after inoculation wheat with isolates from distinct geographical areas, showing a large number of possible gene-for-gene interactions combining both horizontal and vertical resistance types. They also

reported that significant numbers of isolates are able to overcome promising sources of resistance under controlled conditions, suggesting the risk of pathogen adaptation to resistant genotypes, and selection of more specialized or more aggressive pathotypes.

Barnett and Hunter (1999) reported that conidia of *Bipolaris sorokiniana* are brown several celled, elliptical, straight or curved and germinating by on germ tube at each end.

Adhikary (2000) studied the variability among 122 monoconidial isolates of *Bipolaris sorokiniana* collected from all the major wheat growing areas of Bangladesh. He classified the isolates into four clusters based on principal component analysis. The cluster I, II, III and IV contained 22, 8, 14 and 78 isolates, respectively. Eight isolates of cluster II were found to be most virulent and 78 isolates of cluster IV were the least virulent.

Mathur and Kongsdal (2000) reported that the conidia of *Bipolaris sorokiniana* are ellipsoid, dark brown to black, smooth, mostly straight or slightly curved, thick but less so towards the ends, broadest in the middle, ends rounded, 3-12 distoseptate conidia having 40-120 µ length and 17-28 µ breadth .

Ahmed (2001) collected 262 isolates of *Bipolaris sorokiniana* from 16 major wheat growing areas of Bangladesh and he grouped these isolates into 13 physiological groups based on their cultural characteristics namely color, shape and compactness of the colony on PDA. Out of 262 isolates, he tested 43 isolates on five china and six Brazil differential varieties and identified six pathotype.

Kumar *et al.* (2002) reported that the colony of the fungus *B. sorokiniana* has interwoven hypae as a loose cottony mass white or light to gray color depending on the isolates. The fungus is differentiated from others members of

genus of *Bipolaris* on the basis of morphological characters of conidiospore and conidiophores.

Mahto *et al*. (2002) described the pathogenic nature of predominant isolates of *Bipolaris sorokiniana* collected from different agro ecological zones Wheat on cv. Wafaq-2001.

Chand *et al*. (2003) studied the variability in natural populations of the spot blotch pathogen (*B. sorokiniana*) and classified the isolates into 5 groups on the basis of colony morphology. They found that the majority (44.63%) of the isolates of black suppressed type in the natural population were of most aggressive and was identified as the epidemic population as compared to the lowest frequency of the isolates (4.96%) of white coloured having very few conidia. In majority of the zones during second year study, the black coloured cultures were found more as compared to previous year may be due to continuous practicing of same susceptible variety and vigorous establishment of this seed and soil borne pathogen.

Iram and Ahmad (2004) classified Isolates of *B. sorokiniana* according to their aggressive behavior based on disease severity scale. A tree was constructed based on the pattern of bands which highlighted the correlation between morphological, aggressiveness and genetic variations of *B. sorokiniana.*

Aminuzzaman and Hossain (2004-2005) observed the mycelia growth, colony characters, sporilation, size, shape and septaion of conidia varied greatly among 20 isolates collected from barley. Mycelia growth varied from 9.26 mm to 24.0 mm. Colony were effuse, velvety, effuse to velvety, whitish, light brown, brown gray and black having regular or irregular margin where number of conidia/cm^2 of the colony ranged from 3.36×10^3 to 122.12×10^3. Length and breadth of conidia varied from 28.12µm to 75.69µm and 10.64µm to15.04µm respectively. Number of septation varied from 2 to 8. Maximum number of

conidia was straight and light brown in colour though a few number have been recorded as slightly curved and deep brown in color.

Aminuzzaman *et al.* (2005) collected isolates of *Bipolaris sorokiniana* from 17 wheat cultivar of 18 district of Bangladesh. Sixty five physiological races compromising 12 pathotype were identified. Pathotype MS-HS-2-6 exhibited a high infection response (HS) of the range (MS-HS) was most common comprising 18.06% of total isolates collected Pathotype S-HS-2-3-4-5-6-7 was virulent on greatest number of host defferentials and produced 62.96% leaf area diseased on wheat differentials. Twelve pathotypes were identified from each of Dhaka and Rajshahi division, where as 9 from Mymensingh and a single pathotype was identified in each of Feni, Kishorgonj, Kustia, Sirajgong and Thakurgoan. The most virulent pathotype, S-HS-2-3-4-5-6-7 was found in Jamalpur and Pabna. Highest mycelial growth and conidia production ability were recorded in pathotype S-SH-2-3-4-5-6-7.

Pandey *et al.* (2005) reported the virulence, morphological, and physiological variability of 35 *B. sorokiniana* isolates collected from different geographic regions in Brazil and other countries. The isolates were evaluated for their morphological variability, considering mycelium color, sector formation, and growth rate. Based on these morphological characteristics, the isolates were grouped in five different morphological groups. The results obtained from infection of seeds and seedlings showed that isolates from the same geographical region and morphological group had different degrees of virulence.

Iftikhar *et al*. (2006) observed four distinct colors of the colony of the *B. sorokiniana*. Among these the black cultures sporulate profusely and had suppressed type of growth. The others were showing grayish to brownish color and few were of albino (whitish) type having less sporulation. The dimension of the conidia of some of the isolates during (2004) having more width and

length with less number of septa as compared to the isolates collected in 2004. They also found the conidia of isolates of 2004 were slightly curved with brown to olivaceous brown while in 2005 collection the conidia were dark brown, slender and gently curved; few were straight and light brown to brown.

Jaiswal *et al*. (2007) studied one hundred fifty-five isolates of *Bipolaris sorokiniana* of wheat for their morphopathological characterization. These isolates were grouped in five categories-black, brown/dull black, gray cottony growths, dull white/greenish black, and white on the basis of their growth pattern. The frequency of the black suppressed type was maximum (45.63%), whereas the white isolate displayed lowest frequency (6.96%) in the natural population.

Poloni *et al.* (2008) reported that *Bipolaris sorokiniana* is a phytopathogen of great importance on the wheat culture regions, causing diseases such as spot blotch, common root rot and back point of the grain. This fungus is widely distributed over the country and is responsible for a great loss on the production and commercialization of the grain. It has a high physiological and morphological variability that makes the development of control measures a difficult task. They selected 21 isolates of *B. sorokiniana* and from them polysporic 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 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. Polysporic cultures showed a high morphologic variability among the four media.

Pandey *et al*. (2008) collected isolates of *Bipolaris sorokiniana* from the leaves and seeds of field-grown wheat crop at four different sites in eastern Gangetic plains of India. Eighty-six clonal isolates derived from a single isolate (gray with white patches, Group III), which segregated in an equal proportion of parental and nonparental types, were studied. They found morphological variability to be related to the pathological variability.

Poloni *et al.* (2009a) studied the virulence, morphological, and physiological variability of 35 *B. sorokiniana* isolates collected from different geographic regions in Brazil and other countries. The isolates were evaluated for their morphological variability, considering mycelium color, sector formation, and growth rate. Based on these morphological characteristics, the isolates were grouped in five different morphological groups.

Poloni *et al*. (2009b) reported that *Bipolaris sorokiniana* is a phytopathogenic fungus that causes diseases of cereal crops, such as leaf-spot disease, common root rot, and black point of grain. Because of its great morphological, physiological, and genetic variability, this fungus is difficult to control. They study the variability of isolates of *B. sorokiniana* by means of vegetative incompatibility. Thirty-five isolates of *B. sorokiniana* from different geographical regions in Brazil and other countries were used. The vegetative incompatibility between the isolates and the influences of different culture media on these reactions were evaluated. Eighteen of 31 confrontations showed vegetative incompatibility.

Iftikhar *et al.* (2009) collected pathogen from foliar samples of wheat of different agro ecological zones was characterized on the basis of culture/colony colour and texture, conidial morphology and pathogenic nature. 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 had relatively long

and broad slender conidia, while some were uniformly straight and cylindrical and light brown in colour.

Asad *et al*. (2009) characterized *Bipolaris sorokiniana* on the basis of culture/colony colour and texture, conidial morphology and pathogenic nature. They grouped *B. sorokiniana* isolates into 4 classes having black, grayish black, brown and albino (whitish) colony color with profusely sporulated and suppressed type of growth to fluffy and less sporulated type. 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 had relatively long and broad slender conidia, while some were uniformly straight and cylindrical and light brown in color. All the isolates did not show difference in pathogenicity test by producing the symptoms on leaves but their reaction varied in terms of aggressiveness.

Srinivas *et al*. (2009) reported high variability in 103 isolate collected from different geographical zones of India, based on their morphological characters, pathogenicity and DNA fingerprinting. Based on their colony characteristics the isolates were categorized into five groups. The frequency of the dull white/greenish black colony type was maximum (38.83%), while both black, suppressed type and white fluffy type colonies showed minimum frequency (11.65%) in the population studied. A total of 40 isolates from five identified groups were further 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 ($10x10^7$ /colony) and lowest in BS-95 ($1.0x10^7$ / colony). Isolate BS-48 remained non sporulative even after15 days of incubation.

Aminuzzaman *et al*. (2010) isolated 86 monoconidial isolates of *Bipolaris sorokiniana* from leaf and seeds of 17 varieties of wheat collected from 18 major wheat growing regions of Bangladesh. The collected isolates differed

significantly in respect of mycelial growth, conidia production ability, size, septation, shape and color of conidia. The isolates were classified into nine cultural groups based on colony morphology and colony color. Maximum number of 34 isolates produced effuse black regular colony with a frequency of 39.53% of the isolates collected whereas 29 isolates produced effuse black irregular colony with a frequency of 33.72% isolates collected. Cultural group 6 having blackish whitish irregular colony (EBWI) appears to be more aggressive than others following seedling inoculation and detached leaf assay.

Knight *et al.* (2010) revealed differences within Australian *B. sorokiniana* populations by cluster analysis of amplified fragment length polymorphisms in genomic DNA of 48 *B. sorokiniana* isolates collected from the northern grain-growing region of Australia. Cluster analysis of the phenotypic infection response scores grouped isolates into three pathogenicity clusters demonstrating low, intermediate or high pathogenicity. The results of this study suggest divergence within Australian populations of *B. sorokiniana* in relation to host tissue specificity.

Sharma *et al*. (2011)identified *Bipolaris sorokiniana* from foliar blight infected barley leaves. Eight single spore isolates (I_1, - I_8) of pathogen were studied for their morphological characteristics like colony growth, color, sporulation and conidial size. Four isolates (I_1, I_3, I_6 and I_7) were relatively fast growing (80.00 to 85.00 mm) as compared to isolates I_5 and I_8. All isolates were having dirty white colonies with mostly fluffy patterns of growth except I_4 and I_8, with appressed growth. The sporulation was abundant in isolates I_1, I_5 and I_8 where as it moderate in isolates I_2, I_3, I_6 and I_7. The average length of conidia of different isolates varied from 62.00 to 85.25 mm and breadth varied from 25.57 to 27.90 mm with maximum being in isolates I_2 and I_7. The average number of septa per conidium ranged from 3–8. The shape of conidia was ovate-oblong in all the isolates, however, the color of conidia varied from deep olive brown (I_1, I_2, I_3, I_5, I_7 and I_8) to light olive brown (I_4 and I_6).

CHAPTER 3

MATERIALS AND METHODS

3.1. Experimental site

The experiment was conducted in the seed health laboratory, Department of Plant Pathology, Sher-e-bangla Agricultural University, Dhaka.

3.2. Experimental period

The experiment was conducted during the period of April 2010 to June 2012.

3.3. Collection of leaf and seed samples from different wheat growing areas of Bangladesh

Leaves and seed samples having typical disease symptom were collected from seven districts namely Jessore, Kishoregong, Mymensingh, Jamalpur, Sherpur, Dinajpur, and Pabna. The diseased leaves were cut from the plants grown in field randomly and put into a brown paper envelope. Then the brown paper envelopes of each collection were taken to the laboratory, Department of Plant Pathology, Sher-e-bangla Agricultural University, Dhaka, and isolation was done. The seeds were collected by using cotton bags and were sun dried and preserved in refrigerator at 5º C temperature for isolation of *Bipolaris sorokiniana*.

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

Bipolaris sorokiniana was isolated from leaves following the method used by Hossain and Azad (1990-92). The infected leaf tissues along with healthy tissues were cut into 5cm pieces and surface sterilized by dipping in mercuric chloride solution(1:1000) for 30 second. Then the cut pieces were rinsed in sterilized water thrice. The excess water on the surface of the cut leaf pieces

were removed by blotter paper (Whatman No.1) and transferred on to PDA and incubated at 25±1ºc for 7-10 days. At the end of incubation, the mycelium grown from the planted tissues were examined under stereobinocular microscope and mycelial block was transferred on to a second PDA plate and allowed to grow for 7-10 days at 25±1ºc for sporulation. The collected seed samples were surface sterilized by mercuric chloride (1:1000) for 30 second. Then the seeds were rinsed thrice in sterilized water. The seeds (5 seeds/plate) were placed on to PDA plate and plates were incubated at 25º c for luxuriant growth. After 7 days of incubation mycelium growth is observed under stereobinocular microscope then transferred on to second PDA plate and allowed to grow for pure culture. The pathogen was identified following the key of CMI description (Plate. 1 and Plate. 2). The axenic culture of isolates from the PDA were transferred to PDA slants and preserved in refrigerator at 5ºc for further study.

3.5. Designation of collected isolates

The collected isolates were designated based on its location and source following Aminuzzaman *et al*. 2010 (Table 1). For example an isolate designated by MGKSL1 represents that this isolate was collected from district Mymensingh (M), Upazila Gouripur (G) village Kanarampur (K), and from Sonalika leaf (SL) it was isolated. 1 denotes collection serial number. The collected isolates were preserved in test tube culture in refrigerator at $5^{O}C$ (Plate. 3).

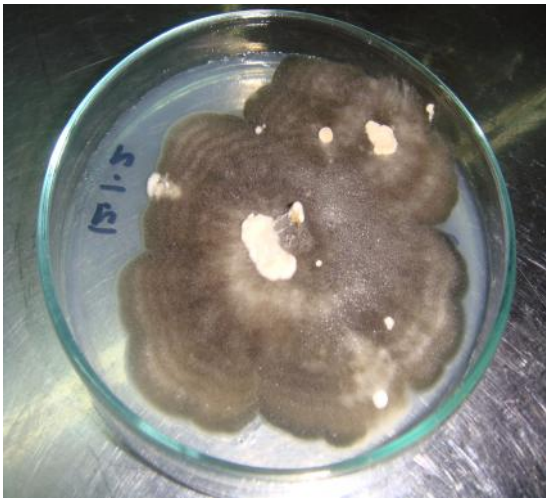

Plate 1. Pure culture of *Bipolaris sorokiniana*

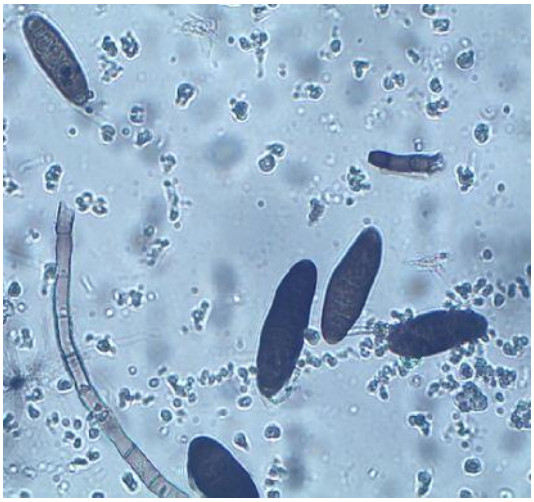

Plate 2. Mycelium and conidia of *Bipolaris sorokiniana* (×40)

Plate 3. Isolates of *Bipolaris sorokiniana* in test tube slant.

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

SL No.	Isolates	Location			Host		Year of collection
		District	Upazila	Village	Variety	Plant part	
1	MGKSL 01	Mymensingh	Gouripur	Kanarampur	Sonalika	Leaf	2010
2	MGKSL 02	Mymensingh	Gouripur	Kanarampur	Sonalika	Leaf	2010
3	MGNSL 03	Mymensingh	Gouripur	Naori	Sonalika	Leaf	2010
4	MGNSL 04	Mymensingh	Gouripur	Naori	Sonalika	Leaf	2010
5	MGTSL 05	Mymensingh	Gouripur	Tachpur	Sonalika	Leaf	2010
6	MGMSL 06	Mymensingh	Gouripur	Madhupur	Sonalika	Leaf	2010
7	MGMSL 07	Mymensingh	Gouripur	Madhupur	Sonalika	Leaf	2010
8	MGMSL 08	Mymensingh	Gouripur	Madhupur	Sonalika	Leaf	2010
9	MIMSL 09	Mymensingh	Iswargonj	Moghtola	Sonalika	Leaf	2010
10	MIMSL 10	Mymensingh	swargonj	Moghtola	Sonalika	Leaf	2010
11	MMBSL11	Mymensingh	Mymensingh sadar	B.A.U, Mymensingh	Sourav	Leaf	2010
12	KKKSL 01	Kishorgonj	Kishorgonj sadar	Katbari	Sourav	Leaf	2010
13	KKKSL 02	Kishorgonj	Kishorgonj sadar	Katbari	Sourav	Leaf	2010
14	KKKSL 03	Kishorgonj	Kishorgonj sadar	katbari	Sourav	Leaf	2010
15	KKDSL 04	Kishorgonj	Kishorgonj sadar	Daukia	Satabdi	Leaf	2010
16	KKDSL 05	Kishorgonj	Kishorgonj sadar	Daukia	Sourav	Leaf	2010
17	KKDSL 06	Kishorgonj	Kishorgonj sadar	Daukia	Sourav	Leaf	2010
18	KKMSL 07	Kishorgonj	Kishorgonj sadar	Mokshedpur	Sourav	Leaf	2010
19	SNNSL 01	Sherpur	Nakla	Nakla	Sonalika	Leaf	2010
20	SSHSL 02	Sherpur	Sherpur sadar	Hatiralga	Sourav	Leaf	2010
21	JJRSS 01	Jessore	Jessore Sadar	R.A.R.S, Jessore	Sonalika	Seed	2011
22	JJRSS 02	Jessore	Jessore Sadar	R.A.R.S, Jessore	Sonalika	Seed	2011
23	JJRSS 03	Jessore	Jessore Sadar	R.A.R.S, Jessore	Sonalika	Seed	2011
24	JJRKS 04	Jessore	Jessore Sadar	R.A.R.S, Jessore	Kanchan	Seed	2011
25	JJRKS 05	Jessore	Jessore Sadar	R.A.R.S, Jessore	Kanchan	Seed	2011
26	JJRKS 06	Jessore	Jessore Sadar	R.A.R.S, Jessore	Kanchan	Seed	2011
27	JJRKS 07	Jessore	Jessore Sadar	R.A.R.S, Jessore	Kheri	Seed	2011
28	JJRKS 08	Jessore	Jessore Sadar	R.A.R.S, Jessore	Kheri	Seed	2011
29	JJRKS 09	Jessore	Jessore Sadar	R.A.R.S, Jessore	Kheri	Seed	2011
30	JJRSS 10	Jessore	Jessore Sadar	R.A.R.S, Jessore	Sheri	Seed	2011
31	JJRSS 11	Jessore	Jessore Sadar	R.A.R.S, Jessore	Sonora	Seed	2011

SL No	Isolates	Location			Host		Year of collection
		District	Upazila	Village	Variety	Plant part	
32	JJRSS 12	Jessore	Jessore Sadar	R.A.R.S, Jessore	Sonora	Seed	2011
33	JJRSS 13	Jessore	Jessore Sadar	R.A.R.S, Jessore	Sonora	Seed	2011
34	JJRAS 14	Jessore	Jessore Sadar	R.A.R.S, Jessore	Akbar	Seed	2011
35	JJRBS 15	Jessore	Jessore Sadar	R.A.R.S, Jessore	Balaka	Seed	2011
36	JJRBS 16	Jessore	Jessore Sadar	R.A.R.S, Jessore	Balaka	Seed	2011
37	JJRBS 17	Jessore	Jessore Sadar	R.A.R.S, Jessore	Balaka	Seed	2011
38	JJRBS 18	Jessore	Jessore Sadar	R.A.R.S, Jessore	Baw-64-2	Seed	2011
39	JJRBS 19	Jessore	Jessore Sadar	R.A.R.S, Jessore	Baw-59-1	Seed	2011
40	JJRAS 20	Jessore	Jessore Sadar	R.A.R.S, Jessore	Agrani	Seed	2011
41	JJRAS 21	Jessore	Jessore Sadar	R.A.R.S, Jessore	Agrani	Seed	2011
42	JJRAS 22	Jessore	Jessore Sadar	R.A.R.S, Jessore	Agrani	Seed	2011
43	JJRAS 23	Jessore	Jessore Sadar	R.A.R.S, Jessore	Agrani	Seed	2011
44	JJRSS 24	Jessore	Jessore Sadar	R.A.R.S, Jessore	Satabdi	Seed	2011
45	JJRSS 25	Jessore	Jessore Sadar	R.A.R.S, Jessore	Satabdi	Seed	2011
46	JJRSS 26	Jessore	Jessore Sadar	R.A.R.S, Jessore	Satabdi	Seed	2011
47	JJRSS 27	Jessore	Jessore Sadar	R.A.R.S, Jessore	Satabdi	Seed	2011
48	JJRSS 28	Jessore	Jessore Sadar	R.A.R.S, Jessore	Satabdi	Seed	2011
49	JJRAS 29	Jessore	Jessore Sadar	R.A.R.S, Jessore	Anando	Seed	2011
50	JJRAS 30	Jessore	Jessore Sadar	R.A.R.S, Jessore	Anando	Seed	2011
51	JJRPS 31	Jessore	Jessore Sadar	R.A.R.S, Jessore	Protiva	Seed	2011
52	JJRSS 32	Jessore	Jessore Sadar	R.A.R.S, Jessore	Sufi	Seed	2011
53	JJRGS 33	Jessore	Jessore Sadar	R.A.R.S, Jessore	Gourav	Seed	2011
54	JJRBS 34	Jessore	Jessore Sadar	R.A.R.S, Jessore	Bijoy	seed	2011
55	JJRPL 01	Jamalpur	Jamalpur sadar	RARS , Jamalpur	Prodip	Leaf	2010
56	JJRSL 02	Jamalpur	Jamalpur sadar	RARS, Jamalpur	Sourav	Leaf	2010
57	JJRSL 03	Jamalpur	Jamalpur sadar	RARS, Jamalpur	Sourav	Leaf	2010
58	JJRSL 04	Jamalpur	Jamalpur sadar	RARS , Jamalpur	Sourav	Leaf	2010
59	JJRTL 05	Jamalpur	Jamalpur sadar	RARS, Jamalpur	Triticale	Leaf	2010
60	DDWSL 01	Dinajpur	Dinajpur sadar	WRC, Dinajpur	Satabdi	Leaf	2010

SL No.	Isolates	Location			Host		Year of collection
		District	Upazila	Village	Variety	Plant part	
61	PIRAL 01	Pabna	Iswardhi	RARS, Iswardhi	Anando	Leaf	2010
62	PIRAL 02	Pabna	Iswardhi	RARS, Iswardhi	Anando	Leaf	2010
63	PIRKL 03	Pabna	Iswardhi	RARS, Iswardhi	Kheri	Leaf	2010
64	PIRKL 04	Pabna	Iswardhi	RARS, Iswardhi	Kheri	Leaf	2010
65	PIRDL 05	Pabna	Iswardhi	RARS, Iswardhi	Duram	Leaf	2010
66	PIRBL 06	Pabna	Iswardhi	RARS, Iswardhi	Bijoy	Leaf	2010
67	PIREL 07	Pabna	Iswardhi	RARS, Iswardhi	Inia	Leaf	2010
68	PIRPL 08	Pabna	Iswardhi	RARS, Iswardhi	Protiva	Leaf	2010
69	PIRGL 09	Pabna	Iswardhi	RARS, Iswardhi	Gourav	Leaf	2010

3.6. Growth study, morphological determinations and grouping of *Bipolaris sorokiniana*

3.6.1. Growth study and morphological determination

The growth study of *Bipolaris sorokiniana* was carried out using the method of Hossain and Azad (1992). The PDA plates were inoculated with 5 mm mycelia block at the center of the plate maintaining three replications. After 15 days of incubation at 25º c temperature the radial growth was measured. Moreover, shape, color and compactness of the colony were recorded. In addition number of conidia produced per unit surface area of colony, length, breadth, septation and colour of conidia at 15 days after inoculation at 25ºC was determined. The conidia produced per unit surface area were estimated using the formula of Chauhan and Panday (1995) as follows:

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

3.6.2. Grouping of *Bipolaris sorokiniana*

The isolates were differentiated into different cultural groups based on their compactness, color and shape of the colony following Aminuzzaman *et al.* (2010).

CHAPTER 4

RESULTS

4.1. Physiological, cultural and morphological variation 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

A total of 69 isolates of *Bipolaris sorokiniana* were isolated from leaves and seeds of wheat from major wheat growing areas of Bangladesh, where 35 isolates were collected from leaves and 34 from seeds. The maximum number of isolates (34) was collected from Jessore followed by Mymensingh (11). A total of 21 varieties of wheat were found to cultivate during the period of *Bipolaris sorokiniana* isolation. The maximum 14 number of isolates were collected from Sonalika and minimum number 1 isolate each from Bijoy, triticle, Duram, Enia, BAw-64-2, BAW 59-1 and Akbar.

4.1.2. Growth study, morphological determination and grouping of *Bipolaris sorokiniana* isolates

4.1.2.1. Growth study and morphological determinations of isolates

The isolates collected from leaf and seed samples were cultured on PDA and readial mycelia growth, number of conidia /cm^2, shape and color of conidia was recorded (Table 2). The redial mycelia growth rate ranged from 1.96±0.56 mm/day (MGTSL 05) to 5.83±0.02 mm/day (JJRBS 18). Maximum number of conidia /cm^2 (119.21±41.29 $\times 10^3$) was counted in isolate JJRPL 01 and minimum (2.79±0.58 $\times 10^3$) from MGMSL 07. Conidia of all isolates produced in PDA culture were straight shaped except the isolates MGMSL 08, JJRAS 29, JJRAS 30, JJRSS 32, JJRGS 33, and JJRPL 01 which produced both straight and slightly curved shaped conidia. Among 69 isolates, the maximum 54 had deep brown colored conidia and 7 and 3 and 3 had brown ,light brown,

brown to deep colored conidia. Highest length of conidia (72.74±1.27µ) was recorded in isolate JJRSS 03 where lowest length (36.80±6.03µ) was recorded in isolate PIRPL 08 (Table 3). The highest breadth was 17.65±0.98µ (MGMSL 08) and lowest was 11.42±1.29µ (SNNSL 01) respectively. The highest septation (6.50) was found in MGMSL 08 and lowest (2.66) in MGKSL 01 (table 3).

4.1.2.2. Grouping of *Bipolaris sorokiniana*

The isolates of *Bipolaris sorokiniana* were differentiated into 9 cultural groups based on their colony morphology and colony color (Table 4 and Plate 4.). The cultural group 1 contains maximum 23 isolates with a frequency of 33.33 of the isolates collected. The cultural group 2, 3, 4, 5, 6, 7, 8 and 9 contained 17.40, 11.60, 8.70, 7.24, 7.24, 5.80, 4.34 and 4.34 % of collected isolates, respectively.

4.1.2.3. Growth study and morphological determinations of *Bipolaris sorokiniana* under different cultural groups

The isolates of *Bipolaris sorokiniana* of different cultural group**s** differed significantly in repect of mycelia growth rate, number of conidia/cm², size and septation of conidia (Table 5). The redial mycelia growth rate ranged from 2.84 ± 0.05 to 4.95 ± 0.09 mm/day, where the highest and lowest rate/day was recorded in cultural groups 6 and 9, respectively. Maximum number of conidia /cm^2 (56.85±15.79 ×10^3) was recorded in cultural group 2 and minimum (20.05±2.71×10^3) in cultural group 9. Highest length of conidia (58.13±8.35µ) was recorded in cultural group 2 where lowest length (48.23±5.14µ) was recorded in group 3. The highest breadth was 14.85±0.61µ in cultural group 9 and lowest was 12.78±1.00µ in cultural group 8 respectively. The highest septation (5.33±1.27) was found in cultural group 2 and lowest (4.30 ±1.05) in cultural group 4.

Table 2. Growth rate, number of conidia, shape and color of conidia of *Bipolaris sorokiniana* (15 days old culture)

Sl. No	Isolates	Growth rate/Day (mm)	Number of conidia/cm² (×10³)	Shape	Color
1	MGKSL 01	3.15 ± 0.21	60.11 ± 26.03	Straight	Deep brown
2	MGKSL 02	2.40 ± 0.34	10.22 ± 0.81	Straight	Deep brown
3	MGNSL 03	2.26 ± 0.1	12.90 ± 1.92	Straight	Brown
4	MGNSL 04	2.23 ± 0.09	47.68 ± 1.53	Straight	Deep brown
5	MGTSL 05	1.96 ± 0.56	50.48 ± 6.15	Straight	Deep brown
6	MGMSL 06	2.88 ± 0.11	17.57 ± 0.42	Straight	Deep brown
7	MGMSL 07	2.54 ± 0.58	2.79 ± 0.58	Straight	Brown
8	MGMSL 08	2.46 ± 0.23	76.31 ± 1.79	Straight/Curved	Light brown
9	MIMSL 09	5.30 ± 0.09	26.28 ± 11.37	Straight	Deep brown
10	MIMSL10	3.10 ± 0.24	16.72 ± 0.92	Straight	Deep brown
11	MMBSL11	3.91 ± 0.29	85.21 ± 27.42	Straight	Deep brown
12	KKKSL 01	2.11 ± 0.17	44.54 ± 2.43	Straight	Deep brown
13	KKKSL 02	2.33 ± 0.09	19.26 ± 1.44	Straight	Deep brown
14	KKKSL 03	4.41 ± 0.12	28.73 ± 9.95	Straight	Brown to deep
15	KKDSL 04	2.58 ± 0.16	9.09 ± 1.18	Straight	Deep brown
16	KKDSL 05	3.16 ± 0.04	21.14 ± 0.44	Straight	Deep brown
17	KKDSL 06	3.03 ± 0.04	25.19 ± 0.44	Straight	Deep brown
18	KKMSL 07	3.13 ± 0.09	30.82 ± 1.23	Straight	Deep brown
19	SNNSL 01	3.13 ± 0.09	51.26 ± 2.06	Straight	Deep brown
20	SSHSL 02	3.15 ± 0.07	30.27 ± 1.89	Straight	Deep brown
21	JJRSS 01	2.89 ± 0.01	80.34 ± 40.17	Straight	Brown
22	JJRSS 02	4.78 ± 0.35	58.65 ± 19.55	Straight	Brown
23	JJRSS 03	2.79 ± 0.19	77.51 ± 36.02	Straight/Curved	Deep brown
24	JJRKS 04	2.93 ± 0.09	51.75 ± 0.01	Straight	Deep brown
25	JJRKS 05	4.76 ± 0.00	46.14 ± 23.56	Straight	Deep brown
26	JJRKS 06	5.28 ± 0.21	42.59 ± 33.27	Straight	Deep brown
27	JJRKS 07	4.46 ± 0.09	44.56 ± 22.28	Straight	Deep brown
28	JJRKS 08	4.03 ± 0.09	27.36 ± 0.00	Straight	Deep brown
29	JJRKS 09	4.73 ± 0.09	26.44 ± 11.43	Straight	Deep brown
30	JJRSS 10	5.26 ± 0.56	28.05 ± 6.93	Straight	Deep brown
31	JJRSS 11	3.15 ± 0.07	44.84 ± 19.31	Straight	Brown to deep
32	JJRSS 12	3.56 ± 0.00	34.96 ± 0.00	Straight	Deep brown
33	JJRSS 13	4.38 ± 0.02	38.57 ± 13.36	Straight	Deep brown
34	JJRAS 14	5.74 ± 0.02	35.85 ± 20.54	Straight	Deep brown
35	JJRBS 15	4.88 ± 0.07	43.48 ± 21.52	Straight	Deep brown
36	JJRBS 16	4.36 ± 0.23	31.09 ± 13.46	Straight	Deep brown
37	JJRBS 17	4.44 ± 0.12	22.44 ± 0.00	Straight	Deep brown
38	JJRBS 18	5.83 ± 0.02	29.31 ± 19.20	Straight	Deep brown
39	JJRBS 19	5.68 ± 0.07	27.52 ± 5.95	Straight	Deep brown
40	JJRAS 20	5.13 ± 0.00	42.46 ± 33.15	Straight	Deep brown

Sl. No	Isolates	Growth rate/Day (mm)	Number of conidia/cm² (×10³)	Shape	Color
41	JJRAS 21	4.36 ± 0.04	38.86 ± 13.46	Straight	Deep brown
42	JJRAS 22	5.68 ± 0.03	64.21 ± 7.94	Straight	Deep brown
43	JJRAS 23	3.73 ± 0.04	31.89 ± 0.00	Straight	Deep brown
44	JJRSS 24	5.71 ± 0.16	77.07 ± 20.77	Straight	Light brown
45	JJRSS 25	5.69 ± 0.04	54.72 ± 13.68	Straight	Deep brown
46	JJRSS 26	5.74 ± 0.12	62.73 ± 15.52	Straight	Deep brown
47	JJRSS 27	5.66 ± 0.04	32.29 ± 7.99	Straight	Brown
48	JJRSS 28	4.83 ± 0.14	38.05 ± 32.96	Straight	Deep brown
49	JJRAS 29	5.74 ± 0.12	89.62 ± 20.53	Straight/Curved	Deep brown
50	JJRAS 30	4.97 ± 0.15	36.04 ± 18.02	Straight/Curved	Deep brown
51	JJRPS 31	4.13 ± 0.04	43.36 ± 15.02	Straight	Deep brown
52	JJRSS 32	5.7 ± 0.14	68.40 ± 13.68	Straight/Curved	Deep brown
53	JJRGS 33	5.46 ± 0.09	54.53 ± 22.72	Straight/Curved	Brown to deep
54	JJRBS 34	5.77 ± 0.09	48.98 ± 20.41	Straight	Deep brown
55	JJRPL 01	3.53 ± 0.52	119.21 ± 41.29	Straight/Curved	Deep brown
56	JJRSL 02	3.13 ± 0.00	23.52 ± 1.89	Straight	Light brown
57	JJRSL 03	3.06 ± 0.19	20.96 ± 0.33	Straight	Deep brown
58	JJRSL 04	4.08 ± 0.82	53.33 ± 11.54	Straight	Deep brown
59	JJRTL 05	3.25 ± 0.13	63.02 ± 31.51	Straight	Deep brown
60	DDWGL 01	4.71 ± 0.18	50.15 ± 8.68	Straight	Deep brown
61	PIRAL 01	4.46 ± 0.14	23.39 ± 0.00	Straight	Deep brown
62	PIRAL 02	2.76 ± 0.14	45.01 ± 1.03	Straight	Deep brown
63	PIRKL 03	5.73 ± 0.00	-	-	-
64	PIRKL 04	5.66 ± 0.04	-	-	-
65	PIRDL 05	3.16 ± 0.15	104.02 ± 25.73	Straight	Brown
66	PIRBL 06	3.20 ± 0.28	58.78 ± 25.45	Straight	Brown
67	PIREL 07	3.98 ± 0.91	42.44 ± 21.22	Straight	Deep brown
68	PIRPL 08	2.69 ± 0.09	35.12 ± 1.14	Straight	Deep brown
69	PIRGL 09	2.63 ± 0.0	21.44 ± 7.28	Straight	Deep brown

- = Conidium was not found.

Table: 3. Length, breadth and septation of conidia of *Bipolaris sorokiniana*

(15 days old culture)

Sl. No	Isolates	Size of conidia		Septation
		Length(µ)	Breadth(µ)	
1	MGKSL 01	39.04 ± 3.73	12.02 ± 1.01	2.66 ± 0.76
2	MGKSL 02	53.02 ± 6.33	12.95 ± 1.17	4.83 ± 0.57
3	MGNSL 03	54.74 ± 7.92	12.99 ± 1.36	5.50 ± 0.86
4	MGNSL 04	45.48 ± 1.90	11.97 ± 0.85	3.90 ± 1.63
5	MGTSL 05	48.70 ± 3.79	13.94 ± 1.25	4.33 ± 0.57
6	MGMSL 06	60.47 ± 9.56	14.56 ± 0.77	5.37 ± 1.04
7	MGMSL 07	46.33 ± 1.82	11.49 ± 0.30	6.37 ± 1.63
8	MGMSL 08	66.31 ± 12.99	17.65 ± 0.98	6.50 ± 2.67
9	MIMSL 09	52.11 ± 8.58	12.87 ± 0.69	5.33 ± 0.28
10	MIMSL 10	58.13 ± 12.72	16.59 ± 1.38	5.50 ± 0.00
11	MMBSL 11	43.47 ± 6.52	11.98 ± 1.11	3.00 ± 0.28
12	KKKSL 01	48.13 ± 7.51	14.01 ± 1.73	3.66 ± 0.57
13	KKKSL 02	66.79 ± 10.03	16.49 ± 0.14	6.25 ± 1.81
14	KKKSL 03	54.09 ± 11.33	13.29 ± 2.36	4.20 ± 1.81
15	KKDSL 04	54.74 ± 6.25	15.63 ± 0.56	5.33 ± 0.28
16	KKDSL 05	52.21 ± 9.38	14.38 ± 0.56	5.50 ± 1.32
17	KKDSL 06	63.9 ± 15.33	14.85 ± 0.28	5.62 ± 0.23
18	KKMSL 07	44.88 ± 4.09	11.87 ± 0.56	4.00 ± 0.50
19	SNNSL 01	45.26 ± 4.33	11.42 ± 1.29	3.66 ± 0.28
20	SSHSL 02	47.22 ± 3.43	11.55 ± 0.54	3.66 ± 0.28
21	JJRSS 01	52.32 ± 3.62	13.41 ± 1.15	5.83 ± 2.46
22	JJRSS 02	69.72 ± 10.73	14.20 ± 0.39	4.78 ± 1.44
23	JJRSS 03	72.74 ± 1.27	14.13 ± 0.94	6.16 ± 1.75
24	JJRKS 04	67.81 ± 4.02	14.92 ± 1.77	4.17 ± 1.89
25	JJRKS 05	48.82 ± 2.96	15.03 ± 0.77	4.83 ± 1.52
26	JJRKS 06	59.14 ± 2.43	14.34 ± 1.55	5.33 ± 1.25
27	JJRKS 07	53.58 ± 4.86	15.23 ± 1.17	5.17 ± 1.60
28	JJRKS 08	52.27 ± 5.67	15.37 ± 0.56	5.17 ± 1.04
29	JJRKS 09	54.71 ± 10.23	15.29 ± 0.90	6.33 ± 1.60
30	JJRSS 10	61.03 ± 0.20	15.33 ± 1.07	5.16 ± 1.25
31	JJRSS 11	47.47 ± 1.58	13.08 ± 0.51	4.50 ± 0.86
32	JJRSS 12	56.80 ± 4.83	13.20 ± 0.91	4.33 ± 0.57
33	JJRSS 13	56.55 ± 9.52	14.09 ± 0.65	4.50 ± 1.32
34	JJRAS 14	47.26 ± 0.98	15.51 ± 0.60	5.50 ± 0.86
35	JJRBS 15	52.33 ± 8.91	14.56 ± 2.87	5.00 ± 0.00
36	JJRBS 16	52.51 ± 0.56	14.25 ± 2.04	4.66 ± 0.76
37	JJRBS 17	55.50 ± 2.60	14.76 ± 0.58	5.66 ± 1.15
38	JJRBS 18	54.93 ± 4.12	12.92 ± 1.28	5.83 ± 1.89
39	JJRBS 19	54.11 ± 15.30	12.09 ± 1.55	5.00 ± 1.73

Sl. No	Isolates	Size of conidia		Septation
		Length(µ)	Breadth(µ)	
40	JJRAS 20	47.15 ± 2.17	13.03 ± 0.40	5.66 ± 0.76
41	JJRAS 21	50.55 ± 0.73	12.52 ± 0.32	4.66 ± 1.60
42	JJRAS 22	54.73 ± 5.73	12.66 ± 0.60	5.33 ± 0.57
43	JJRAS 23	53.71 ± 2.88	12.83 ± 0.74	5.33 ± 1.15
44	JJRSS 24	69.49 ± 5.63	16.10 ± 2.66	5.66 ± 1.60
45	JJRSS 25	64.66 ± 5.66	14.50 ± 1.52	5.50 ± 1.32
46	JJRSS 26	67.75 ± 4.67	14.69 ± 1.44	5.16 ± 1.60
47	JJRSS 27	61.82 ± 5.74	14.91 ± 1.5	6.00 ± 1.32
48	JJRSS 28	60.12 ± 6.91	14.45 ± 1.48	5.83 ± 1.60
49	JJRAS 29	64.18 ± 6.13	13.59 ± 1.18	5.16 ±1.04
50	JJRAS 30	44.25 ± 2.20	11.88 ± 1.54	5.16 ± 1.04
51	JJRPS 31	62.61 ± 0.65	14.77 ± 0.71	4.50 ± 0.50
52	JJRSS 32	65.35 ± 2.00	13.71 ± 1.52	5.00 ± 0.86
53	JJRGS 33	56.42 ± 7.09	15.30 ± 2.89	4.66 ± 0.28
54	JJRBS 34	61.19 ± 14.89	15.83 ± 0.60	5.00 ± 1.73
55	JJRBL 01	54.83 ± 11.72	13.98 ± 0.50	5.57 ± 1.04
56	JJRSL 02	58.35 ± 2.00	13.60 ± 1.15	5.16 ± 1.04
57	JJRSL 03	50.73 ± 9.97	11.88 ± 0.20	5.20 ± 1.20
58	JJRSL 04	51.78 ± 18.19	13.32 ± 3.04	3.91 ± 0.50
59	JJRTL 05	59.34 ± 16.12	14.98 ± 2.03	5.75 ± 1.66
60	DDWGL01	46.68 ± 13.91	13.28 ± 2.25	4.00 ± 1.50
61	PIRAL 01	67.58 ± 12.81	15.08 ± 2.71	6.00 ± 1.00
62	PIRAL 02	48.47 ± 17.57	13.37 ± 1.47	4.62 ± 1.66
63	PIRKL 03	-	-	-
64	PIRKL 04	-	-	-
65	PIRDL 05	48.27 ± 11.28	11.91 ± 0.74	3.90 ± 1.60
66	PIRBL 06	50.45 ± 10.26	12.8 ± 0.60	4.30 ± 1.80
67	PIREL 07	44.79 ± 13.65	12.05 ± 0.66	3.91 ± 1.88
68	PIRPL 08	36.80 ± 6.03	11.87 ± 0.21	3.33 ± 0.02
69	PIRGL 09	44.81 ± 11.41	15.15 ± 0.80	4.35 ± 1.10

- = Conidium was not found.

Table 4. Grouping of isolates of *Bipolaris sorokiniana* on the basis of cultural characteristics

Group	Cultural characteristics	Isolates (Total No.69)	% isolates under each group
G-1	Effuse Blackish White Irregular (EWBI)	MGKSL 02, MGNSL 03 ,MGTSL 05, MGMSL 7, KKKSL 02, KKDSL 04, PIRPL 08, MIMSL 09, PIRKL 04, PIRAL 01, PIRAL 02, JJRSS 01, JJRSS 03, JJRKS 06, JJRKS 09, JJRSS 10, JJRBS 17, JJRBS 19, JJRAS 23, JJRSS 28, JJRAS 30, JJRBS 34, JJRPS 31, (23)	33.33
G-2	Effuse Black Irregular (EBI)	MGMSL 08, JJRKS 04, JJRKS 08, JJRAS 14, JJRBS 15, JJRBS18, JJRSS 25, JJRSS 27, JJRAS 29, JJRSL 04, JJRTL 05, JJRSL 04, JJRPL01, (12)	17.40
G-3	Effuse Black Regular (EBR)	JJRKS 05, JJRKS 07, JJRAS 20, KKKSL 01, KKDSL05, SNNSL01, SSHSL02, MMBSL 11 (8)	11.60
G-4	Velvety Blackish White Regular (VBWR)	JJRSS11, MIMSL10, DDWSL01,PIREL07, KKMSL07,PIRDL 05 (6)	8.70
G-5	Effuse Whitish Black Regular (EWBR)	JJRSS 02, JJRSS 26, MGKSL 01, PIRBL 06, JJRSL 03 (5)	7.24
G-6	Velvety White Regular (VWR)	JJRSS12, JJRBS16, JJRAS 22, JJRSS 32, JJRGS 33 (5)	7.24
G-7	Effuse White Regular (EWR)	JJRSS 13, JJRAS 21, JJRASS 24, KKKSL 03 (4)	5.80
G-8	Effuse Blackish White Regular (EBWR)	MGNSL 04, JJRSL 02, PIRKL 03 (3)	4.34
G-9	Velvety Whitish Black Regular (VWBR)	MGMSL 06, KKDSL 06, PIRGL 09 (3)	4.34

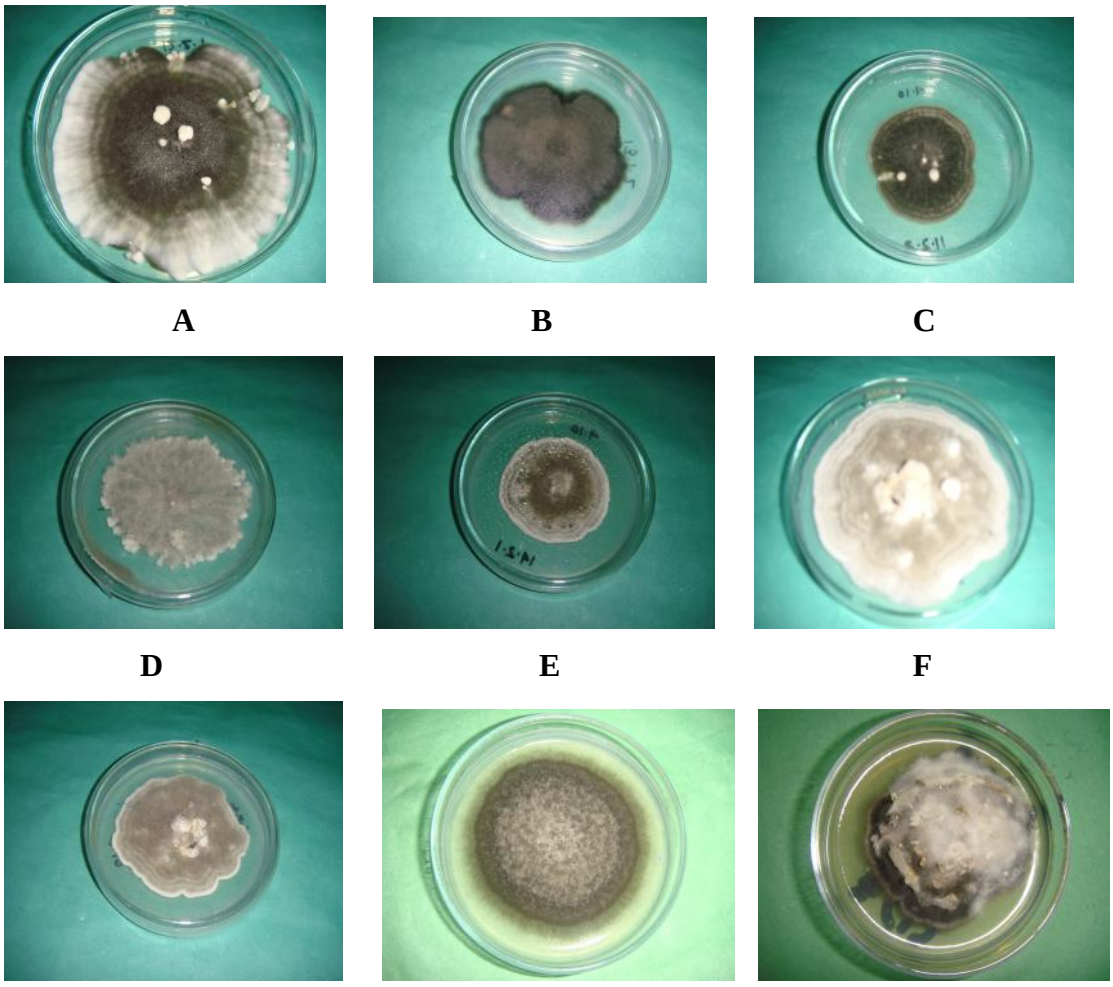

Plate 4. Nine cultural groups of *Bipolaris sorokiniana*.

A. Effuse Blackish White Irregular (EBWI) **B.** Effuse Black Irregular (EBI), **C.** Effuse Black Regular (EBR), **D.** Velvety Blackish White Regular (VBWR), **E.** Effuse Whitish Black Regular (EWBR), **F.** Velvety White Regular (VWR), **G.** Effuse White Regular (EWR), **H.** Effuse Blackish White Regular (EBWR), **I.** Velvety Whitish Black Regular (VWBR).

Table 5. Mycelial growth rate, conidia production, size and septation of conidia of *Bipolaris sorokiniana* under different cultural groups (15 days old culture)

Group	Growth rate (mm)/ day	Number of conidia/cm² (×10³)	Size of conidia		Septation
			Length (µ)	Breadth (µ)	
1	3.88±0.17	33.53±11.62	55.48±6.55	13.89±1.03	5.32±1.17
2	4.48±0.18	56.85±15.79	58.13±8.35	14.68±1.49	5.33±1.27
3	3.72±0.09	45.69±14.24	48.23±5.14	13.32±0.94	4.39±0.82
4	3.53±0.27	48.14±12.80	48.37±9.53	13.13±1.01	4.30±1.05
5	3.98±0.23	52.24±17.37	55.54±7.89	13.11±0.72	4.40±1.36
6	4.95±0.09	50.60±11.56	57.16±4.04	13.82±1.59	4.79±0.60
7	4.71±0.08	45.80±14.38	57.67±6.80	14.00±1.49	4.75±1.58
8	3.68±0.03	35.60±1.71	51.91±1.95	12.78±1.00	4.53±1.33
9	2.84±0.05	20.05±2.71	56.39±12.10	14.85±0.61	5.11±0.79

Data represented the mean of all isolates under each group

CHAPTER 5

DISCUSSION

Sixty nine isolates of *Bipolaris sorokiniana* were isolated from leaf and seeds of wheat collected from major wheat growing regions of Bangladesh. The isolates were cultured on PDA and mycelia growth, number of conidia/cm^2; shape and size of conidia were recorded. Morphology and physiology of isolates of *Bipolaris sorokiniana* were studied by workers all around the world [Christensen (1922), Shoemaker (1959), Hodges, F.C. (1975), Hossain and Azad (1992), Alam *et.al*. (1997), Ahmed *et al*. (1997), Debnath (1997), Maraite *et al. (*1998), Barnett and Hunter (1999), Adhikary (2000) , Mathur and Kongsdal (2000), Ahmed (2001), pandey *et al*.(2005), Iftikhar *et al.* (2009), Srivinas *et al*. (2009) and Aminuzzaman *et al*. (2010)]. In the present study, the redial mycelia growth rate were ranged from 1.96±0.56 (MGTSL 05) mm/day to 5.83±0.02 (JJRBS 18) mm/day. Hossain and Azad (1992) studied 83 isolates *B. sorokiniana* and reported the radial mycelia growth ranged 29-78mm. Aminuzzaman and Hossain, (2005a) reported mycelial growth varied from 9.26 mm to 24.0 mm. Srinivas *et al*. (2009) recorded Colony diameter of *Bipolaris sorokiniana* after seven days of incubation and that was ranged from 20.3 mm to 63 mm . Pandey *et al.* (2005) reported that the range of mean growth varied between 4.77 to 8.27cm on 8th day. Aminuzzaman *et al*. (2010) reported mycelia growth rate of *B. sorokiniana* 2.77±0.23 to 9.10±1.09 mm/day. The differences among the radial mycelial growth of *B. sorokiniana* may be due to difference in temperature during incubation period. Ilija, K. *et* al. (2000) reported that the conidia of *Bipolaris sorokiniana* form quickly at room temperature usually 25±1 °C. Batista, A. (2012) reported that the optimum temperature for mycelia growth and germination of conidia of *B. sorokiniana* were 10-40°C. Hodges, F.C. (1975) reported that the germination of *B. sorokiniana* is maximum at 15- 25°C and 20 days older culture. Above 25°C and 20 days the germination were decreased. At 35°C temperature the conidia

of *B. sorokiniana* were not germinated. Sijam, K. *et al*. (2009) reported that 25 °C is suitable for growth of *B. sorokiniana* but inhibited by 35°C temperature and extreme pH of 4 and 10. In the present research work, Maximum number of conidia/ cm^2 (119.21×10^3) was obtained from isolate JJRPL 01 and minimum (2.79×10^3) from MGMSL 07. Hossain and Azad (1992) reported that among 83 isolates, 65 isolates produced abundant conidia within seven days under near UV-light (12/12). Pandey *et al.* (2005) reported that the range of spore production per cm^2 was 494.80 to 2892.38. Aminuzzaman and Hossain, (2005a) reported that the number of conidia/cm^2 of the colony were ranged from 3.36×10^3 to 122.12×10^3. Srinivas *et al*. (2009) reported highest spore production of *B. sorokiniana* was $10x10^7$ /colony and lowest was $1.0x10^7$/colony. Aminuzzaman *et al.* (2010) reported maximum number of conidia/cm^2 of *B. sorokiniana* was 166.84×10^3 and minimum was 4.00×10^3. In the present research work, Most of the isolates produced straight shaped conidia in PDA except the isolates MGMSL 08, JJRAS 29, JJRAS 30, JJRSS 32, JJRGS 33, and JJRPL 01 which produced both straight and slightly curved shaped conidia. Shoemaker (1959) proposed the conidia of *Bipolaris sorokiniana* were fusoid, straight or curved. Barnett and Hunter (1999) reported conidia of *Bipolaris sorokiniana* were straight or curved. Mathur and Kongsdal (2000) reported most of the conidia were straight or slightly curved. Aminuzzaman and Hossain, (2005a) reported that the maximum number of conidia were straight while few were slightly curved. Iftikhar *et al.* (2009) found the conidia were cylindrical, straight and slightly curved. Aminuzzaman *et al*. (2010) reported that most of the conidia of isolates of *B. sorokiniana* were straight, few were slightly curved. In the present research work, the maximum 54 had deep brown colored conidia, while the rest of the isolates had brown, light brown, brown to deep brown colored. Barnett and Hunter (1999) reported the conidia of isolates were brown. Mathur and Kongsdal (2000) reported the color of conidia were dark brown to black. Aminuzzaman and Hossain, (2005a) reported the color of conidia were light brown in colour though a few numbers have been recorded as deep brown in color. Iftikhar *et al.* (2009) reported the

color of conidia were brown to olivaceous brown and light brown in colour. Aminuzzaman *et al*. (2010) reported that among 86 isolates, the maximum 52 had brown colored conidia and 26, 5, 2 and 1 had deep brown, light brown, brown to deep brown and light brown to deep brown colored conidia. In the present research work, the length and breadth of conidia of the collected isolates of *Bipolaris sorokiniana* ranged from 36.80±6.03µ to 72.74±1.27µ and 11.42±1.29µ to 17.65±.98µ. Christensen (1922) observed the spore of four biologic forms of *Helminthosporium sativum* to differ slightly in respect of width, length and number of septa. Shoemaker reported that the length and breadth of conidia of *Bipolaris sorokiniana* were 60 -120µm × 12-20µm. Ahmed *et al*. (1997) reported the length and width of conidium varied from 35-270 µm and 15-65 µm depending on isolates. Debnath (1997) reported the chromogenic variant in *Bipolaris sorokiniana* were produced longer spore and nonchromogenic variant produced shorted spore. Mathur and Kongsdal (2000) reported the conidia having 40-120µ length and 17-28 µ breadth. Aminuzzaman and Hossain, (2005a) length and breadth of conidia varied from 28.12µm to 75.69µm and 10.64µm to15.04µm. Iftikhar *et al.* (2009) found the conidial average size ranged from 38.3–65.8 µm x 12.3–25 µm. Aminuzzaman *et al*. (2010) reported that highest length of conidia was 75.86µ and lowest was 33.40µ and breadth range was 10.52µ to 22.86µ. Among 69 isolates the septation of conidia ranged from 2.66 to 6.33. Shoemaker (1959) reported that the cell of conidia varied from 5-9. Ahmed *et al*. (1997) reported in 27 isoletes the number of cells per conidium varied from 3-10 septa. Barnett and Hunter (1999) reported the conidia were several celled. Aminuzzaman, and Hossain, (2005a) reported the number of septation varied from 2 to 8. Iftikhar *et al.* (2009) found 2–13 septation in the isolates. Aminuzzaman *et al*. 2010 reported that among 86 isolates the septation of conidia ranged from 2-10septa. In the present study two isolates PIRKL 03 and PIRKL 04 exhibited higher mycelia growth rate but was not sporulated in 15 days on PDA. Srivinas *et al.* (2009). They also found Isolate BS-48 remained non sporulative even after15 days of incubation.

In the present study the isolates of *Bipolaris sorokiniana* were grouped into nine cultural groups based on colony morphology and colony color. Among the nine cultural groups effuse blackish white irregular (EBWI) groups contain highest number of isolates (23) which represents frequency of 33.33% of the total isolates studied again 12 isolates produced effuse black irregular (EBI) colony with a frequency of 17.40% isolates collected. Alam *et.al*. (1997). also identified seven morphological and physiological divergences among 27 isolates of *Bipolaris sorokiniana*. Ahmed *et al*. (1997) classified 27 isolates into four distinct clusters. Among them three belonged to cluster I, six to cluster II, fourteen to cluster III and four to cluster IV. Debnath (1997) classified the collected isolates into two groups chromogenic and noncharomogenic. Chromogenic is pigment producing and noncharomogenic is non pigment producing isolates of *Bipolaris sorokiniana*.

Maraite *et al. (*1998). studied the 27 isolates of *Bipolaris sorokinianaand* found different colors of colony and found the dark colored colony showed a strong correlation with aggressiveness of pathogen. Adhikary (2000) studied the variability among 122 monoconidial isolates of *Bipolaris sorokiniana* collected from all the major wheat growing areas of Bangladesh. He classified the isolates into four clusters based on principal component analysis. The cluster I, II, III and IV contained 22, 8, 14 and 78 isolates and cluster II was most virulent. Ahmed (2001) collected 262 isolates of *Bipolaris sorokiniana* from 16 major wheat growing areas of Bangladesh and he grouped these isolates into 13 physiological groups based on their cultural characteristics. Pandey *et al.* (2005) grouped the collected isolates in five different morphological groups and test the virulence of isolates under the different groups. Iftikhar *et al.* (2009) grouped in 4 classes having black, grayish black, brown and albino (whitish) colony color with profusely sporulated and suppressed type of growth to fluffy and less sporulated type. He reported that the all isolates did not show difference in pathogenecity test. Srinivas *et al*. (2009) classified 103 isolates into five groups based on their morphological characters collected from

different geographical zones of India, The frequency of the dull white/greenish black colony type was maximum (38.83%), while both black, suppressed type and white fluffy type colonies showed minimum frequency (11.65%). They also test the pathogenecity of isolates under different groups. Aminuzzaman *et al.* (2010) classified eighty six isolates into nine cultural groups based on colony morphology and colony color. Maximum number of 34 isolates produced effuse black regular colony with a frequency of 39.53% of the isolates collected whereas 29 isolates produced effuse black irregular colony with a frequency of 33.72% isolates collected. They also test the virulence of isolates under different cultural groups of *Bipolaris sorokiniana* on wheat cv. Kanchan. Isolates under different cultural groups showed significant differences in respect of growth rate, conidia production and size and septation of conidia. The redial mycelia growth rate ranged from 2.84±0.05 to 4.95±0.09 mm/day, where the highest and lowest rate/day was recorded in cultural groups 6 and 9. Maximum number of conidia /cm^2 (56.85±15.79 $\times 10^3$) was recorded in cultural group 2 and minimum (20.05±2.71$\times 10^3$) in cultural group 9. Highest length of conidia (58.13±8.35µ) was recorded in cultural group 2 where lowest length (48.23±5.14µ) was recorded in group 3. The highest breadth was 14.85±0.61µ in cultural group 9 and lowest was 12.78±1.00µ in cultural group 8 respectively. The highest septation (5.33±1.27) was found in cultural group 2 and lowest (4.30±1.05) in cultural group. Aminuzzaman *et al.* (2010) reported the redial mycelia growth rate ranged from 4.21 to 4.8.17 mm/day, where the highest and lowest rate/day was recorded in cultural groups 3 and 8. Maximum number of conidia /cm^2 (136.75 $\times 10^3$) was recorded in cultural group 8 and minimum (19.43$\times 10^3$) in cultural group 3. Highest length of conidia (70.19µ) was recorded in cultural group 5 where lowest length (53.67µ) was recorded in group 1. The highest breadth was 15.95µ in cultural group 9 and lowest was 12.79µ in cultural group 3 respectively. The highest septation (5.90) was found in cultural group 9 and lowest (4.63) in cultural group 2. Grouping is done for

observing the variation of physiological, cultural, morphological and virulence of *Bipolaris sorokiniana* under different cultural group.

CHAPTER 6

SUMMARY AND CONCLUSION

Bipolaris sorokiniana (Teleomorph *Cochliobolus sativus*) is the causal agent of foliar blight, root rot, seedling blight, head blight and black point of wheat and barley. The disease caused by the fungus is one of the constraints for both crops in cool and warmer growing areas that cause significant yield losses. The present research work was carried out to determine the physiological, cultural and morphological variation of *Bipolaris sorokiniana* collected from seven wheat growing areas of Bangladesh.

Initially 69 isolates of *Bipolaris sorokiniana* were isolated from leaf and seeds of 21 varieties of wheat collected from 7 major wheat growing regions of Bangladesh in 2010-2011. The maximum 14 number of isolates were collected from Sonalika .The collected isolates differed significantly in respect of mycelia growth, number of conidia /cm^2 and size of conidia. The isolates of *Bipolaris sorokiniana* were classified into nine cultural groups based on colony morphology and colony color. Isolates of different cultural groups differed significantly in respect of growth rate, conidia production, size and septation of conidia.

The isolates collected from leaf and seed samples were cultured on PDA and radial mycelia growth, number of conidia /cm^2, shape and color of conidia was recorded. The radial mycelia growth rate ranged from 1.96±0.56 mm/day (MGTSL 05) to 5.83±0.02 mm/day (JJRBS 18). Maximum number of conidia /cm^2 (119.21±41.29 $\times 10^3$) was counted in isolate JJRPL 01 and minimum (2.79±0.58 $\times 10^3$) from MGMSL 07. Conidia of all isolates produced in PDA culture were straight shaped except the isolates MGMSL 08, JJRAS 29, JJRAS 30, JJRSS 32, JJRGS 33, and JJRPL 01 which produced both straight and slightly curved shaped conidia. Among 69 isolates, the maximum 54 had deep

brown colored conidia and 7, 3 and 3 had brown, light brown, brown to deep colored conidia, respectively. Highest length of conidia (72.74±1.27µ) was recorded in isolate JJRSS 03 where lowest length (36.80±6.03µ) was recorded in isolate PIRPL 08.The highest breadth of conidia was 17.65±0.98µ (MGMSL 08) and lowest was 11.42±1.29µ (SNNSL 01). The highest septation (6.33) was found in JJRKS 08 and lowest (2.66) in MGKSL 01. The isolates of *Bipolaris sorokiniana* were differentiated into 9 cultural groups based on their colony morphology and colony color. The cultural group 1 contains maximum 23 isolates with a frequency of 33.33 of the isolates collected. The cultural group 2, 3, 4, 5, 6, 7, 8 and 9 contained 17.40, 11.60, 8.70, 7.24, 7.24, 5.80, 4.34 and 4.34 % of collected isolates, respectively. The isolates of *Bipolaris sorokiniana* of different cultural group**s** differed significantly in repect of mycelia growth rate, number of conidia/cm², size and septation of conidia. The radial mycelia growth rate ranged from 2.84±0.05 to 4.95±0.09 mm/day, where the highest and lowest rate/day was recorded in cultural groups 6 and 9, respectively. Maximum number of conidia /cm^2 (56.85±15.79 ×10^3) was recorded in cultural group 2 and minimum (20.05±2.71×10^3) in cultural group 9. Highest length of conidia (58.13±8.35µ) was recorded in cultural group 2 where lowest length (48.23±5.14µ) was recorded in group 3. The highest breadth was 14.85±0.61µ in cultural group 9 and lowest was 12.78±1.00µ in cultural group 8 respectively. The highest septation (5.33±1.27) was found in cultural group 2 and lowest (4.30±1.05) in cultural group 4.

Bipolaris sorokiniana is most destructive pathogenic agent for wheat and barley. This pathogen is highly variable in respect virulence, physiological, cultural and morphologicaly. For control this pathogen it is important to know its physiological, cultural, morphological and genetical variation. So more and more research is needed by collecting more isolates of this fungus from different region Bangladesh to study their morphology, physiology, virulence and evolution.

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