MORPHO-MOLECULAR DIVERSITY OF SEED BORNE *BIPOLARIS SOROKINIANA* IN WHEAT IN BANGLADESH

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ABSTRACT

Sultana, A., Rashid, A.Q.M.B., Harun-or-Rashid, M. and Dey, P. 2022. Morpho-molecular diversity of seed borne *Bipolaris sorokiniana* in wheat in Bangladesh. Bangladesh J. Plant Pathol. 38(1&2): 27-36.

Widely cultivated and freshly harvested wheat seed varieties Sonalika, Kanchan, BARI Gom 21, BARI Gom 23, BARI Gom 24, BARI Gom 25, BARI Gom 26 was collected from major wheat growing areas of Bangladesh for identification and isolation of seed borne *Bipolaris sorokiniana*. In this study, a total of 81 isolates of *B. sorokiniana* were isolated from the wheat seed samples and subjected to their morphology which were divided into ten groups. The color of the isolates varied from dark black, dark black with white mycelia edge, dull black, whitish black, white and grayish white. The nature of the isolates was compact, loose and fluffy; the marginal edge of the isolates was regular and irregular shape. Thirty representative isolates from 10 morphological groups were studied for their genetic relationship using 11 primers to analyze the genetic diversity by Random Amplified Polymorphic DNA (RAPD) marker. In cluster analysis, morphological groups were divided into two major genotypic groups from 30 isolates and there were wide genetic distances exist among these two groups. The study found that *B. sorokiniana* isolates from different geographical locations in Bangladesh did not exhibit the same morphological group or phenotype, indicating that geographical origin did not significantly affect their molecular characteristics.

Key words: wheat, seedborne, *Bipolaris sorokiniana,* diversity, phytopathogen, RAPD

INTRODUCTION

Bipolaris sorokiniana (Sacc. in Sorok.) Shoemaker (*Helminthosporium sativum*, P.K. & B.) syn. *Drechslera sorokiniana* (Sacc.) Subram and Jain. perfect state *Cochliobolus sativus* (Ito & Kurib.) Drechs. ex Dastur. is one of the major production constraints in warmer areas worldwide (Gupta *et al.* 2018). According to Sharma *et al.* (2018), fungal diseases are the most ancient and well-known types of plant pathogens. Initial stage of infection, tiny and dark brown scratches which eventually grow into light to dark brown oval or elongated spots. As the process progressed, these lengthy blotches unite to form necrotic lesions, which ultimately lead to cell death. A spot blotch infection causes black-pointed, discolored, shriveled, and deformed seeds, which lowers grain quality and trade. During every stage of growth, the wheat plant is subjected to various damages and pressures that can impact its regular functions and grain yield. According to reports, yield losses in susceptible genotypes range from 15.5 to 19.6% and, under favorable conditions, may reach 100%, lowering the quality of the seed (Gupta *et al.* 2018). The pathogen's extreme genetic diversity is caused by *B. sorokiniana*'s broad host range (Verma *et al.* 2020). Fungicides are not always effective in controlling the spot blotch pathogen because it spreads through seeds and soil. Significant regional variety was observed in the sexual stage of compatible isolates of *B. sorokiniana* (Sultana *et al.* 2018). To comprehend biodiversity, evolution,

ecology, and genomes, phylogenetic analyses are frequently used to represent relationships among gene copies or fungal species (Victorino *et al.* 2021). Breeders face challenges due to the considerable genetic heterogeneity of fungal diseases across a broad range of settings and the role of minor genes in controlling resistance to foliar blight or spot blotch. When choosing effective disease management techniques, knowledge of the genetic composition of fungus might be very beneficial (Esfahani 2020). Deli *et al.* (2019) suggests that a phylogeographic approach provides a more comprehensive comprehension of the influence of past occurrences on genetic diversity patterns within and between species. The goal of the current study was to evaluate the evolutionary relationships between several morphological and pathogenic groups of *Bipolaris sorokiniana* in Bangladesh using the RAPD technique.

MATERIALS AND METHODS

Isolation of *Bipolaris sorokiniana* from wheat seed

Seven wheat varieties *viz.* Sonalika, Kanchan, BARI Gom 21 (Shatabdi), BARI Gom 23 (Bijoy), BARI Gom 24 (Prodip), BARI Gom 25 and BARI Gom 26 were collected from major wheat growing areas of Bangladesh during the year 2014-15. The blotter method (ISTA 1996) was used for identification of pathogen and their growth characters with the help of steriobinocular microscope (Chidaimbaram *et al*. 1973). The identified *B. sorokiniana* was transferred to the centre of potato dextrose agar (PDA) media and incubated at 28±2ºC for a week where monoconidial cultures were prepared to avoid the admixtures of the conidia. Then the identified isolates were retransferred on PDA media and classified into their colony color, shape, compactness, surface, and opacity. and grouped into their morphological characteristics.

Isolation of genomic DNA from *B. sorokiniana* isolates

The CTAB mini-prep method with some minor modifications in the protocol given by Murray and Thompson (1980) was followed to extract DNA from *B. sorokiniana*. In this method, zero point five to two (0.5-2.0) gram mycelium of *B. sorokiniana* isolates was ground in liquid nitrogen and powdered mycelium were then transferred to 100 µl DNA extraction buffer [50 mM Tris, 150 mM NaCl, 20 mM ethylene diamine tetraacetic acid (EDTA)] and 100 µl (1 ml) sodium dodecyl sulfate (SDS) (10%) was added and incubated at 37°C for 1 h. 100 µl of 5 M NaCl was added to the above mixture and mixed well, after that 80 µl CTAB (10%) was added, mixed gently and incubated at 65°C for 20 min. The mixture was centrifuged for 10 minutes with 10,000 rpm. After that, the supernatant was transferred to another eppendorf tube. Equal volume of chloroform: isoamyl alcohol (24:1) was added to each tube followed by centrifugation. After adding chloroform: isoamyl alcohol (24:1), the tubes were inverted 50 times for better mixing. Aqueous, viscous supernatant (collected the upper layer very carefully) was then transferred to a fresh tube. Three times ethanol was added to the supernatant and the DNA was precipitated at 14,000 rpm for 20 minutes at 4°C. After discarding ethanol, the DNA pellet was washed with 70% ethanol (2 times the acquired volume) and centrifuged for 10 minutes with 10,000 rpm at 4°C. The DNA pellet was dried at room temperature. For purification of DNA, RNase solution 10 mg/ml was used. Finally, the pellet was dissolved in 100 µl TE buffer and kept at 4°C and at -20°C for further work. DNA was quantified using spectrophotometer and quality analysis done on 1.0% agarose gel.

RAPD primers selection and their PCR amplification

Initially, 15 RAPD markers were checked with two samples for evaluation of their suitability for amplifying the isolates of *B. sorokiniana*. Finally, 11 subsets of primers OPW-02 (AC CC CG CC AA), OPW-03 (GT CC GG AG TG), OEP-07 (AG AT GC AG CC), OEP-16 (GG TG AC TG TG), OEP-17 (CT AC TG CC GT), OPP-02 (TC GG CA CG CA), OPP-03 (CT GA TA CG CC), OPP-04 (GT GT CT CA GG), OPA-10 (GT GA TC GC AG), OPB-01 (GT TT CG CT CC), OPC-01 (TT CG AG CC AG) exhibiting good quality banding patterns were selected for analysis of the whole sample set and tested for amplification of the monoconidial genomic DNA of *B. sorokiniana* isolates collected from wheat seed samples. All PCR reactions of genomic DNA of *B. sorokiniana* isolates were carried out in a volume

of 20 µl single reaction with PCR Master Mix 10.0 µl, Primer 1.0 µl, DNA 2.0 µl, Molecular grade water 7.0 µl. The PCR reactions were carried out under the following touchdown programmes for 1 initial cycle with 3 min at 94°C followed by 36 cycles of 1 min denaturation at 94°C, 1 min annealing at 32°C and elongation at 2 min at 72°C and 7 min at 72°C to allow complete extension of all amplified fragments. The amplification products were then visualized on 1.0% agarose gel, detected by staining with ethidium bromide and analyzed using the gel documentation system AlphaImager 2200 (Alpha Innotech Corporation, USA). One Kb DNA ladder was used to estimate the size of the amplification products by comparing the distance traveled by each fragment with that of the known sized fragments of molecular weight markers. Fragment sizes for each locus were measured using standard size markers. All PCR (TECHNE TC-512) reactions were repeated at least twice, and only clear and apparently unambiguous bands were scored.

Data scoring and RAPD analysis

All distinct bands were thereby given identification numbers according to their position on gel and scored visually on the the basis of their "0" (absence of the band) and "1" (presence of the band) for each DNA sample with 12 primers in a data matrix. Statistical analysis of the RAPD data was performed using the software NTSYS-pc version 2.2 (Rohlf 2000), through which similarity coefficients were calculated. Clustering of similarity matrices were done by UPGMA (Un-weighted Pair Group with Arithmetic Average) and projection by the TREE programme of POPGENE (Version 1.31).

RESULTS AND DISCUSSION

Morphological variations among the isolates of *B. sorokiniana*

From the Table 1, it can be described that morphological groups I, II, IX was recorded as dark black in color mycelia, group III was dark black in nature with white mycelial edge on periphery, group IV and V were dull black in color and group VI showed whitish black in color. The rest of the groups VII and VIII showed white colony color and group X is grayish white colony color. The shapes of the colony were recorded as regular and irregular. Groups I, III, VI, VII, VIII were regular in shape whereas group II, IV, V, IX, X were recorded as irregular in shape. Compactness of the colony was also varied from each other. Morphological group I, II, III, IV, VI, VII were recorded as compact in nature, where the morphological group V, VIII, X was fluffy in nature. Only the morphological group IX, was recorded as a loose colony. The surfaces of all morphological groups were recorded as rough, elevated, and opaque.

Genetic diversity of *Bipolaris sorokiniana*

The RAPD profile showed genetic variability among the isolates of *B. sorokiniana*. A diverse banding pattern was observed when DNA from thirty fungal isolates was screened by eleven primers which produced reproducible and distinct polymorphic amplified products. The selected primers generated 85 polymorphic bands ranging from 1200 to 100 base pair (bp). The primers OPW-02 and OPA-10 produced the highest number (10) of reproducible and distinct polymorphic amplified banding pattern followed by the primer OEP-07 which produced 9 polymorphic bands. The primer OPB-01 produces the lowest numbers (5) of polymorphic bands.

Cluster analysis

Total 81 isolates of *B. sorokiniana* collected from wheat seed samples were broadly divided into cluster IA and cluster IB. The cluster IA divided into three sub clusters IA_1 and IA_2 and IA_3 and sub cluster IA_1 included of two isolates GaK 5 and DiK 1 (Fig. 12). Sub cluster IA_2 included two isolates, Ja 23.3 and Di 26.1. Sub cluster IA_3 is divided into two major sub sub clusters IA_{3a} and $IA_{3b.}$ The sub sub cluster IA_{3a} consists of two isolates GaS 1 and Ja 23.3. The sub sub cluster IA_{3b} again divided into two groups IA_{3b1} and $IA_{3b2.}$ Group IA_{3b1} belonged to seven isolates which were Ch 24.3, Ga 23.2, Ra 26.3, Di 21.3, Ga 25.4, GaS 3 and Pa 26.2. Group IA_{3b2} belonged to 11 isolates in which (DiK 2, Ra 21.2, Di 24.3, Ch 23.2), (GaS 4, DiK 3), (Ga 25.5, Ma 25.2, Pa 25.1), (Ra 26.1 and Di 21.6) are closely related. The cluster IB is divided into two sub clusters IB_a which included two isolates GaK 3 and Ch 21.5. The sub cluster IB_b included four isolates in which (Ch 24.2 & Ch 24.1) and (GaK 1 & Pa 26.1) are closely related. Between

the two major clusters, the isolates were scatteredly grouped into ten morphological groups. The same morphological groups were not confirmed to the same phenotype rather the different morphological groups were distributed in the major two groups.

Table 1. Groups of isolates of *B. sorokiniana* based on their morphological characteristics

Group No.	Isolate No.	Colony color	Shape	Compactness	Surface	Opacity
I	Ja 21.6, Ma 25.2, Ma 25.3, Ch 21.5, Ch 23.1, Ch 23.2, Ch 24.4, Ra 21.1, GaS 4, Ga 26.1, Ga 24.1, Ga 25.2, Pa 26.5, Ch 24.1, Ch 24.2, Pa 25.3, Ga 23.1, Pa 26.3, Ch 26.3, Di 21.2	Dark black	Regular	Compact	Rough	Opaque
II	Di 21.3, Di 21.4, DiS 2, DiS 5, Ja 21.3, Ma 25.1, Ra 26.2, GaS 3, Ga 25.1, Ga 25.4, Ga 25.5, Ja 23.4, DiK 4, Di 24.1	Dark black	Irregular	Compact	Rough	Opaque
III	Di 21.5, Di 24.2, Ja 21.4, Ch 24.3, Ga 24.2, Ga 25.3, Ch 26.1, Di 26.2, Ja 23.6	Dark black with (white mycelia edge)	Regular	Compact	Rough	Opaque
IV	Pa 25.1, Pa 25.2, Pa 25.4, Ch 21.4, Di 21.6	Dull black	Irregular	Compact	Rough	Opaque
V	DiK 2, Di 24.3, Ga 23.2, Pa 26.1, Pa 26.4, Ch 23.3	Dull black	Irregular	Fluffy	Rough	Opaque
VI	Di 26.1, Di 26.3, Ja 21.5, Ma 25.5, Ra 26.1, Ga 25.6, GaK 1	Whitish black	Regular	Compact	Rough	Opaque
VII	GaK 3, GaK 5, Ja 23.5, GaK 2	White	Regular	Compact	Rough	Opaque
VIII	DiS 3, Ra 26.3, GaS 2, Pa 26.2, Ja 23.1, Ja 23.2, Ja 23.7, DiS 1, DiS 4	White	Regular	Fluffy	Rough	Opaque
IX	DiK 1, DiK 3	Dark black	Irregular	Loose	Rough	Opaque
X	Ma 25.4, GaS 1, Ja 23.3, Ra 21.2, Ga 26.2	Grayish white	Irregular	Fluffy	Rough	Opaque

Here, Ch 21 = Chuadanga BARI Gom 21, Ch 23 = Chuadanga BARI Gom 23, Ch 24 = Chuadanga BARI Gom 24, Ch 26 = Chuadanga BARI Gom 26, DiS = Dinajpur Sonalika, DiK = Dinajpur Kanchan, Di 21 = Dinajpur BARI Gom 21, Di 24 = Dinajpur BARI Gom 24, Di 26 = Dinajpur BARI Gom 26, GaS = Gazipur Sonalika, GaK = Gazipur Kanchan, Ga 23 = Gazipur BARI Gom 23, Ga 24 = Gazipur BARI Gom 24, Ga 25 = Gazipur BARI Gom 25, Ga 26 = Gazipur BARI Gom 26, Ja 21 = Jamalpur BARI Gom 21, Ja 23 = Jamalpur BARI Gom 23, Ma 25= Madhupur BARI Gom 21, Pa 25 = Pabna BARI Gom 25, Pa 26 = Pabna BARI Gom 26, Ra 21= Rajshahi BARI Gom 21, Ra 26= Rajshahi BARI Gom 26.

Table 2: RAPD primers with their polymorphic band size and number

Primer code	Sequences (5´-3´)	Total number of bands	Size ranges (bp)	Proportion of polymorphic loci (%)
OPW-02	AC CC CG CC AA	10	850-100	100
OPW-03	GT CC GG AG TG	6	1000-100	
OEP-07	AG AT GC AG CC	9	1200-300	
OEP-16	GG TG AC TG TG	6	850-200	
OEP-17	CT AC TG CC GT	6	650-100	
OPP-02	TC GG CA CG CA	8	500-100	
OPP-03	CT GA TA CG CC	6	850-100	
OPP-04	GT GT CT CA GG	6	850-300	
OPA-10	GT GA TC GC AG	10	1000-100	
OPB-01	GT TT CG CT CC	5	850-300	
OPC-01	TT CG AG CC AG	8	500-200	
Total	-	85	-	-
Average	-	7.08	-	-

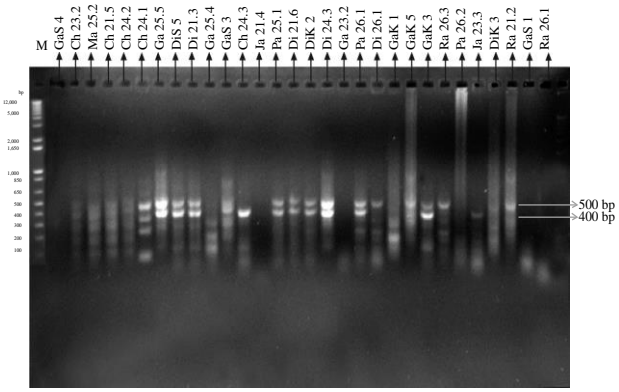

Figure 1. DNA profile of 30 isolates of B. sorokiniana generated with primer OPW-02

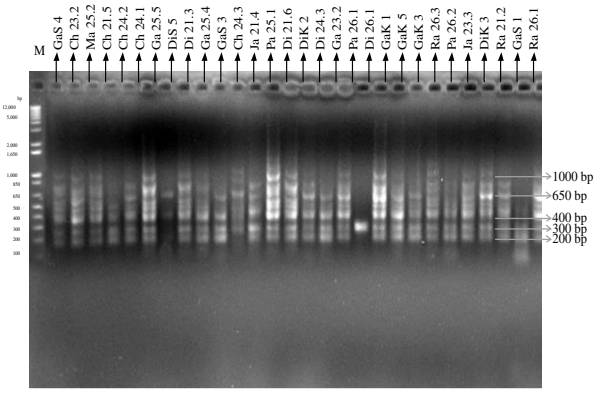

Figure 2. DNA profile of 30 isolates of B. sorokiniana generated with primer OPA-10

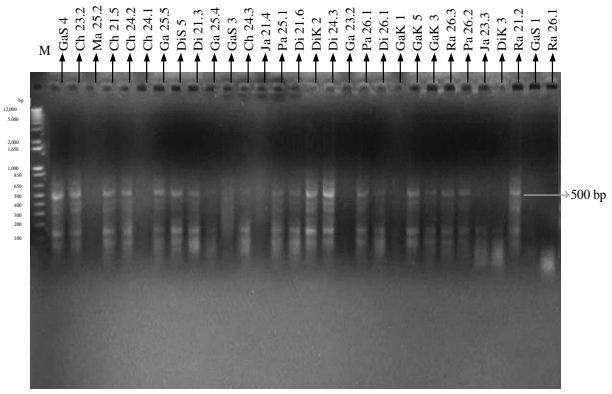

Figure 3. DNA profile of 30 isolates of B. sorokiniana generated with primer OEP-07

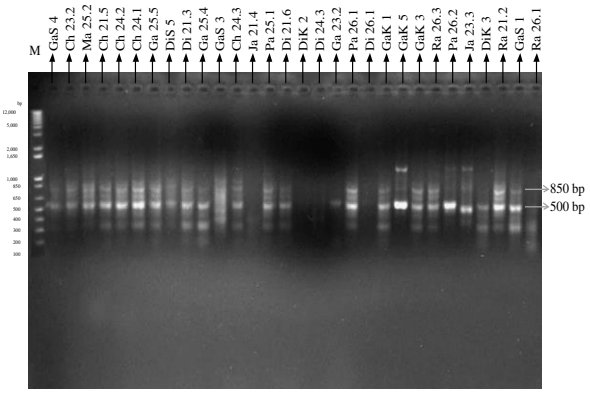

Figure 4. DNA profile of 30 isolates of B. sorokiniana generated with primer OPP-02

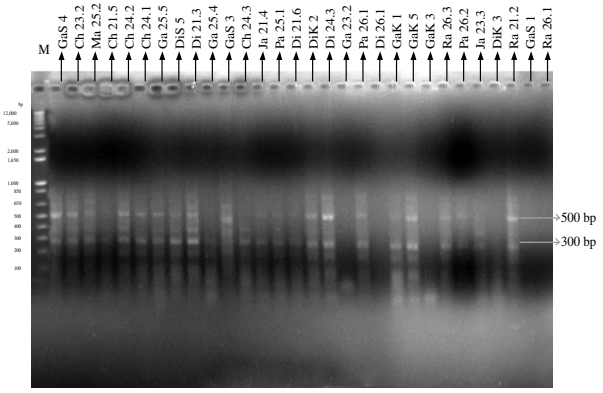

Figure 5. DNA profile of 30 isolates of B. sorokiniana generated with primer OPC-01

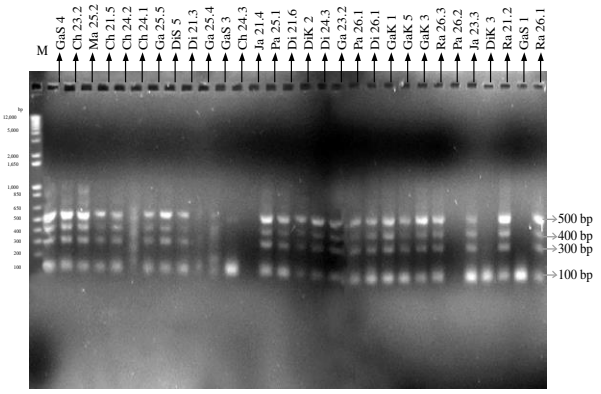

Figure 6. DNA profile of 30 isolates of B. sorokiniana generated with primer OPW -03

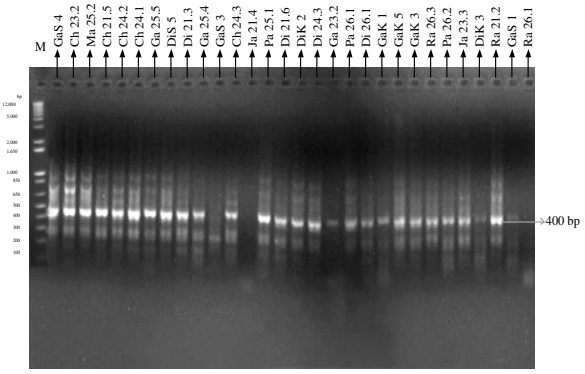

Figure 7. DNA profile of 30 isolates of B. sorokiniana generated with primer OEP-16.

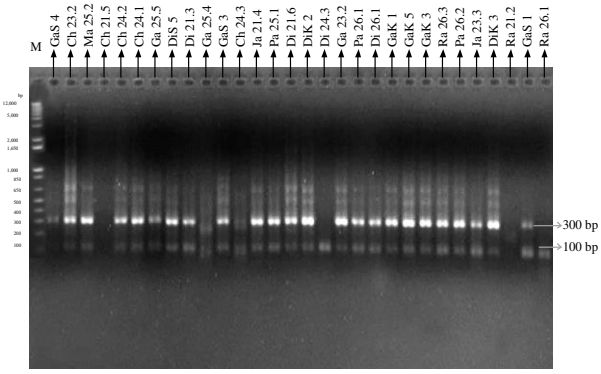

Figure 8. DNA profile of 30 isolates of B. sorokiniana generated with primer OEP-17

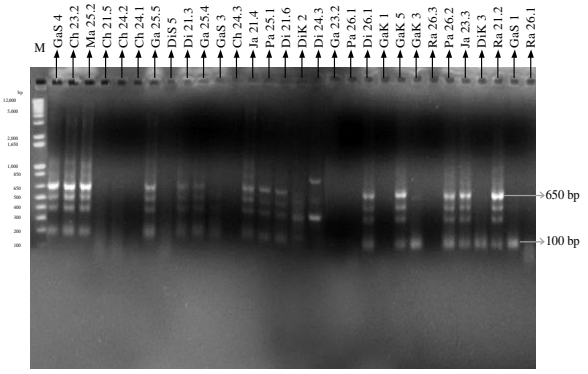

Figure 9. DNA profile of 30 isolates of *B. sorokiniana* generated with primerOPP-03

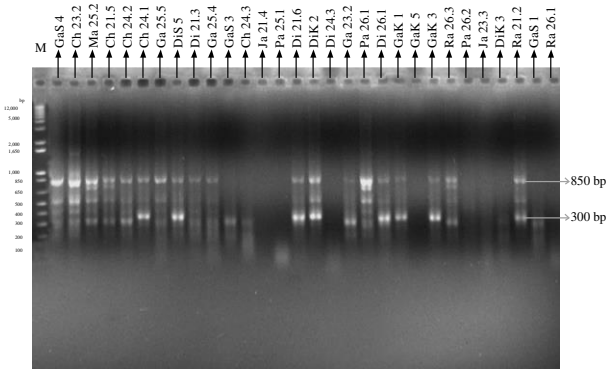

Figure 10. DNA profile of 30 isolates of *B. sorokiniana* generated with primer OPP-04

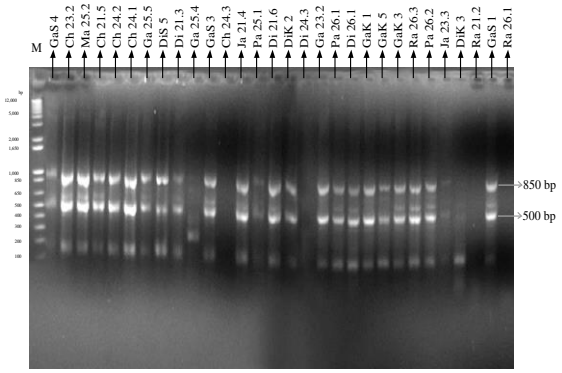

Figure 11. DNA profile of 30 isolates of *B. sorokiniana* generated with primer OPB-01

Here, Lane (1-30): GaS 4 = Gazipur Sonalika (Isolate-4), Ch 23.2 = Chuadanga BARI Gom 23 (Isolate-2), Ma 25.2 = Madhupur BARI Gom 25 (Isolate-2), Ch 21.5 = Chuadanga BARI Gom 21 (Isolate-5), Ch 24.2=Chuadanga BARI Gom 24 (Isolate-2), Ch 24.1= Chuadanga BARI Gom 24 (Isolate-1), Ga 25.5= Gazipur BARI Gom 25 (Isolate-5), DiS 5= Dinajpur Sonalika (Isolate-5), Di 21.3= Dinajpur BARI Gom 21 (Isolate-3), Ga 25.4 = Gazipur BARI Gom 25 (Isolate-4), GaS 3= Gazipur Sonalika (Isolate-3), Ch 24.3 = Chuadanga BARI Gom 24 (Isolate-3), Ja 21.4= Jamalpur BARI Gom 21(Isolate-4), Pa 25.1 = Pabna BARI Gom 25 (Isolate-1), Di 21.6= Dinajpur BARI Gom 21 (Isolate-6), DiK 2= Dinajpur Kanchan (Isolate-2), Di 24.3 = Dinajpur BARI Gom 24 (Isolate-3), Ga 23.2 = Gazipur BARI Gom 23 (Isolate-2), Pa 26.1 = Pabna BARI Gom 26 (Isolate-1), Di 26.1 = Dinajpur BARI Gom 26 (Isolate-1), GaK 1= Gazipur Kanchan (Isolate-1), GaK 5= Gazipur Kanchan (Isolate-5), GaK 3= Gazipur Kanchan (Isolate-3), Ra 26.3= Rajshahi BARI Gom 26 (Isolate-3), Pa 26.2 = Pabna BARI Gom 26 (Isolate-2), Ja 23.3 = Jamalpur BARI Gom 23 (Isolate-3), DiK 3= Dinajpur Kanchan (Isolate-3), Ra 21.2 = Rajshahi BARI Gom 21 (Isolate-2), GaS 1= Gazipur Sonalika (Isolate-1), Ra 26.1= Rajshahi BARI Gom 26 (Isolate-1),

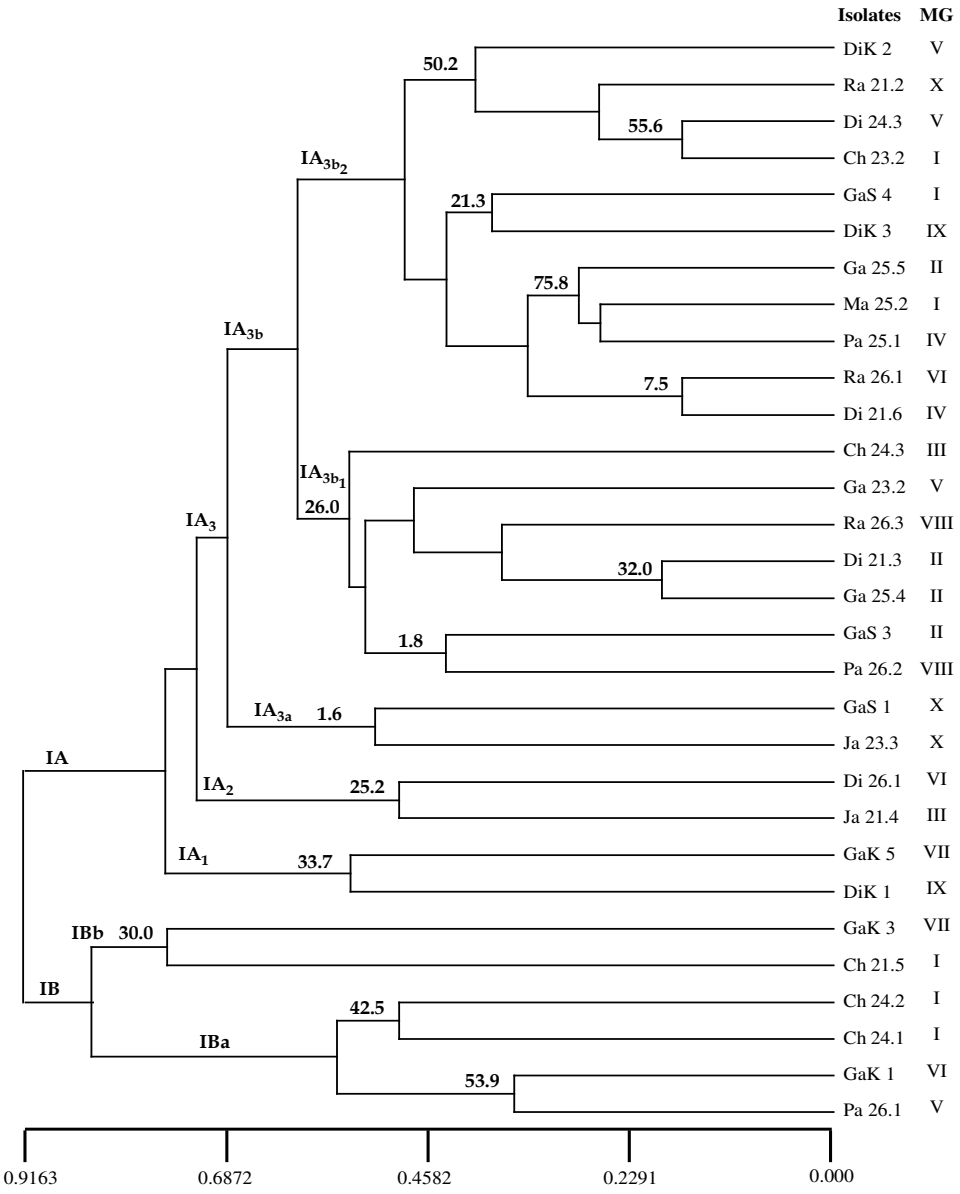

Fig. 12. Dendrogram constructed by Unweighted pair group method of arithmetic mean (UPGMA) using RAPD primers showing correlation of aggressiveness among the 30 representative isolates of *B. sorokiniana* collected from wheat seed

Here, DiK 2= Dinajpur Kanchan (Isolate-2), Ra 21.2 = Rajshahi BARI Gom 21 (Isolate-2), Di 24.3 = Dinajpur BARI Gom 24 (Isolate-3), Ch 23.2 = Chuadanga BARI Gom 23 (Isolate-2), GaS 4 = Gazipur Sonalika (Isolate-4), DiK 3= Dinajpur Kanchan (Isolate-3), Ga 25.5= Gazipur BARI Gom 25 (Isolate-5), Ma 25.2 = Madhupur BARI Gom 25 (Isolate-2), Pa 25.1 = Pabna BARI Gom 25 (Isolate-1), Ra 26.1= Rajshahi BARI Gom 26 (Isolate-1), Di 21.6 = Dinajpur BARI Gom 21 (Isolate-6), Ch 24.3 = Chuadanga BARI Gom 24 (Isolate-3), Ga 23.2 = Gazipur BARI Gom 23 (Isolate-2), Ra 26.3= Rajshahi BARI Gom 26 (Isolate-3), Di 21.3= Dinajpur BARI Gom 21 (Isolate-3), Ga 25.4 = Gazipur BARI Gom 25 (Isolate-4), GaS 3= Gazipur Sonalika (Isolate-3), Pa 26.2 = Pabna BARI Gom 26 (Isolate-2), GaS 1= Gazipur Sonalika (Isolate-1), Ja 23.3 = Jamalpur BARI Gom 23 (Isolate-3), Di 26.1= Dinajpur BARI Gom 26 (Isolate-1), Ja 21.4= Jamalpur BARI Gom 21(Isolate-4), GaK 5= Gazipur Kanchan (Isolate-5), DiK 1= Dinajpur Kamchan (Isolate-1), GaK 3= Gazipur Kanchan (Isolate-3), Ch 21.5 = Chuadanga BARI Gom 21 (Isolate-5), Ch 24.2=Chuadanga BARI Gom 24 (Isolate-2), Ch 24.1= Chuadanga BARI Gom 24 (Isolate-1), GaK 1= Gazipur Kanchan (Isolate-1), Pa 26.1 = Pabna BARI Gom 26 (Isolate-1) ; MG = Morphological group

The colony colors of the identifying groups were exhibited five distinct colors among which dark black color was found the dominating to the other isolates. The present investigations were also agreed with Asad *et al.* (2009) who characterized *B. sorokiniana* based on their morphology and grouped into 4 classes which were black, grayish black, brown and white. In another study of Aggarwal *et al.* (2009), who found white/greenish black color type which was maximum (38.83%), while both black, suppressed type and white fluffy type colonies showed minimum frequency (11.65%) in the population studied. Iftikhar *et al*. (2006) reported the color of *B. sorokiniana* fungal colonies on PDA medium varied from white to light pink and dark green. But there were no light pink and dark green *B. sorokiniana* isolates found in Bangladesh. Climatic variation of agro-ecological zones of Bangladesh might be responsible for the absence of light pink and dark green type isolates.

In the present findings, three types of colony characters e.g. compact, loose and fluffy were observed among the different morphological groups. Out of 82 isolates, the largest numbers (60) of isolating groups were found to be compact in nature. Twenty-four isolates were recorded as fluffy only while two isolates were loose in nature. In Bangladesh, Ahmed (2001) categorized 262 isolates of *B. sorokiniana* from wheat seeds and leaf samples in which 227 were compact and 35 were loose in nature. The present findings were also agreed with the reports of many authors (Iftikhar *et al*. 2006, Asad *et al.* 2009, Aggarwal *et al.* 2009). Out of 82 isolates, 50 isolates were observed as regular in shape while the rest of 32 were irregular shape. In a study of Ahmed (2001) screened 262 isolates of *B. sorokiniana* from wheat seeds and leaf samples, in which 213 were regular and 49 were irregular in shape.

In this present study, surfaces of all the fungal colonies of all were observed as rough and opaque. It was also observed that rough and opaque colony isolated from *B. sorokiniana* from wheat seeds and leaf samples and found a similar result (Ahmed 2001, Iftikhar *et al*. 2006, Asad *et al.* 2009, Aggarwal *et al.* 2009).

The transparent nature of the isolates of *B. sorokiniana* was not found by the other researchers. The diversified colony character indicates the high morphological variation from the isolates of different regions. Out of 82 isolates of *B. sorokiniana,* thirty representative isolates were characterized to observe their diversity by RAPD using 11 primers. The representative isolates showed a high level of polymorphism and grouped into two major groups. But, in case of morphological study, the isolates were in two major groups. This suggested that morphological classification did not reflect total variability. The present finding was also going to the findings of many other researchers (Jaiswal *et al*. 2007, Aggarwal *et al*. 2009) who worked on genetic diversity of *B. sorokiniana*.

CONCLUSION

B. sorokiniana isolates that have been identified from various agro-ecological zones in Bangladesh revealed two significant broad genetic gaps between the ten morphological groups. This study has successfully answered the research question of various morphological groups that did not exhibit comparable genetic respons effect on genetic variation. Surveys should be conducted in the main wheat-growing regions to identify new pathogenic groups on various wheat types for *B. sorokiniana.* Identification, characterization and analysis of the pathogenicity of certain genes would be useful tools for breeders to create resistant varieties.

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