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RESEARCH ARTICLE

Pathological and multi locus sequence analysis in *Bipolaris sorokiniana* inciting spot blotch of wheat reveals the predominant virulent haplotypes in India

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Abstract

Globally, a significant portion of wheat yield is impacted by *Bipolaris sorokiniana*, incitant of spot blotch disease. The current study aimed to unravel the diversity and virulence of *B. sorokiniana* in climate change scenarios. In total, 40 *B. sorokiniana* isolates were established based on the molecular and morphological traits which revealed BS-21 (Pusa, Bihar), BS-27, BS-33 (Sultanpur, UP), and BS-34 (Varanasi, UP) exhibited the highest mycelial growth (90 mm) at 15 DAI. The pathogen population is distributed in two major types, white/greenish black colonies (45%) and brown/dull black suppressed growth (10%). The maximum sporulation was in Pusa, Bihar isolates and the minimum sporulation was in Vidisha, MP isolates (BS-8 and BS-1). BS-21 exhibited the highest ADI (87.97%) while BS-10 showed the lowest ADI (6.86%). Upon phylogenetic analysis, the BS isolates were grouped into six clades using the multigene analysis using TEF*1α*, *GAPDH*, *RPB2*, *BT*, and ITS. The BS isolates from Ujjain, Kanpur and Panipat showed nucleotide variations in *SCD1* gene, correlating with a significant reduction in pathogenicity. *BRN1* gene was highly conserved among all the BS isolates. Among BS isolates, Pusa, and Bihar BS isolates were highly pathogenic. The virulence analysis for the *SCD1* gene was correlated with pathogenicity. The highly virulent isolates were linked to the two most predominant haplotypes *viz*., Hap_2 and Hap_4 was identified from the virulence loci analysis. The present study will enhance our comprehension of *B. sorokiniana*'s pathogenesis and offer valuable perspectives for managing wheat spot blotch.

Keywords: *Bipolaris sorokiniana*, genetic diversity, haplotype, spot blotch, wheat

Introduction

Wheat is among the most extensively cultivated and preferred dietetic cereal grains globally covering 219.15 million hectares area with 808.44 million tonnes and 3.68 metric tons/ha average production and yield, respectively (FAO 2022). In India, it occupies 30.45 million hectares, producing 107.74 million tonnes providing an average yield of 3.53 metric tons/ha (FAO 2022). Uttar Pradesh, ranks first in wheat production in India, followed by Haryana and Punjab. Wheat production is significantly affected by biotic stress, amongst which foliar blight is reported to be one of the most devastating diseases of wheat. The major incitant of foliar blight or spot blotch, *Bipolaris sorokiniana* (Sacc.) Shoem is reported to be among the most destructive pathogens causing yield loss of 2.7% to 100%, varying with severity on wheat varieties (Ayana et al. 2018). In India, losses can reach up to 80%, especially under favorable conditions (Gupta et al. 2018; Sultana et al. 2018). The pathogen is prevalent worldwide such as in Australia, South America, Africa, Canada and Asia, with a significant impact on the Indian subcontinent. The disease commonly takes place in warmer humid places of Madhya Pradesh, Karnataka, Bihar, Maharashtra, Assam, Uttar Pradesh, West Bengal and Orissa

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(Acharya et al. 2011; van Ginkel and Rajaram, 1998). However, a significant increase in mean annual temperatures driven by global heat rise and shifting climate patterns intensifies the risks posed by this pathogen to major wheat-growing regions worldwide. The pathogen has the ability to colonize a diverse range of host plants apart from wheat, namely barley (*Hordeum vulgare*), rye (*Secale cereale*), triticale (Triticosecale), maize (*Zea mays*), tufted airplant (*Guzmania* species, *Tillandsioideae*), pearl millet (*Pennisetum typhoides*), *Panicum* spp. foxtail millet (*Setaria italica*) and many more weedy grasses (Manamgoda et al. 2014).

B. sorokiniana is a hemibiotrophic pathogen that produces an extensive range of symptoms in wheat and barley, notably spot blotch, black point, seedling blight and common root rot. It is crucial to understand pathogen's genetic variability by different housekeeping and virulence genes. Moreover, understanding the diversity patterns of pathogen virulence genes is essential for foreseeing and mitigating the emergence of new pathogenic strains as well as optimizing disease management strategies (Khan et al. 2010). This is particularly important as the pathogen's genetic diversity and the influence of QTL genes of a host on resistance to spot blotch pose persistent challenges for the breeders and the plant pathologists. This variability is the consequence of heterokaryosis and parasexuality within the pathogen population. *B. sorokiniana* infecting wheat cultivars described the occurrence of five pathotypes and SSR-based variability (Aggarwal et al. 2009; Kashyap et al. 2023). A recent study has documented the population dynamics of various *Bipolaris* spp, in the leaf blight complex of wheat (Aditya et al. 2024). It is insufficient to undertake species identification and phylogenetic inference of *B. sorokiniana* purely on morphology due to the presence of a small number of synapomorphic features (Song et al. 2020). PCR-based markers are most frequently used for the genetic characterization and variability investigation of *B. sorokiniana*. The multigene analysis is a very useful tool for diversity analysis and a comprehensive understanding of the pathogen's population structure. The internal transcribed spacer (ITS) region, a primary fungal barcode, is widely used for initial molecular identification but often lacks resolution for precise species delineation (Lucking et al. 2020). To ensure accurate identification of *Bipolaris* isolates, we included glyceraldehyde 3- phosphate dehydrogenase (*GAPDH*), a well-established marker for species differentiation (Manamgoda et al. 2014), along with *TEF1* (translation elongation factor 1 alpha, *RPB2* (RNA Polymerase-II largest subunit), and *BT* (beta-tubulin)—highly conserved genes commonly used in fungal multigene diversity analysis. These genes not only aid in genetic confirmation but also help assess intra-species genetic variability. Additionally, we selected *SCD1* (Scytalone dehydratase) and *BRN1* (1,3,8-naphthalenetriol reductase), key virulence-associated genes (Singh et al. 2022) to determine their indispensability and evaluate the extent of their variability, which may influence the pathogen's interaction with the host.

Keeping in view, the present investigation on the genetic variability of *B. sorokiniana* isolates particularly in reference to the virulence genes, is not only an essential task but also an urgent need in this context. The study involved the evaluation of the recent population structure of *B. sorokiniana* in terms of genetic diversity using the multigene analysis including housekeeping and virulence genes with respect to the BS isolate's pathogenicity.

Material and methods

Survey, collection, isolation, and establishment of B. sorokiniana cultures

Leaf and seed samples showing symptoms of black point and spot blotch respectively were collected from endemic regions of India covering three wheat-growing zones (North-western plains zone, North-eastern plains zone and Central zone) (Supplementary Table S1) during *rabi* season (2021-2022). The fungus was isolated from a solitary discrete lesion from the infected leaf tissue and black tip in the grain near the embryo end of the diseased samples. The infected leaf tissues or seeds were cut into 2 to 3 mm size together with healthy tissue and rinsed with sterile water one or two times. The leaf bits were then surface sterilized for 30 seconds with a 1% (v/v) sodium hypochlorite solution (NaOCl) followed by rinsing with sterile distilled water three times for 2 to 3 minutes and drying on sterilized filter paper for 30 to 45 seconds. The dried small bits (3 bits per plate) were transferred into petri plates (90 mm in diameter) containing PDA media, sealed with parafilm and kept for incubation at 25°C in the BOD incubator for 5 days. A pure culture of fungus was established using the hyphal tip method. The entire procedure was carried out aseptically under the laminar airflow chamber and the purified cultures were regularly maintained by subculturing. Further**,** these fungal cultures were confirmed with the morphological characters and the ITS sequencing.

Morphological and cultural characterization

A mycelial disc from the 7-day-old culture of *B. sorokiniana* isolates was placed onto a sterile Petri dish containing 20 mL of potato dextrose agar medium and kept for incubation at 25°C. Then, the fungal cultural characteristics, such as radial growth, pigmentation, and mycelia color, were recorded at four different time intervals (4^{th}, 7^{th}, 9^{th} and 15^{th} day after inoculation (DAI). For determining conidial characteristics, *B. sorokiniana* cultures incubated for 6 to 7 days were observed under 10X and 40X magnifications and further, the color, shape and septation of conidia were recorded. The dimensions of conidia, including length and breadth were assessed using the NIS Elements imaging software.

The sporulation of the 15-day-old culture was assessed using the hemocytometer.

Isolation of genomic DNA

To isolate DNA, fungal mycelia was cultured in potato dextrose broth (PDB) media. One week after inoculation mycelial mat was extracted, dried out and stored at -20℃ for further study. The CTAB method was used for extracting the genomic DNA of all 40 *B. sorokiniana* isolates (Cullings, 1992). Over 0.5 g of the harvested mycelial mat was pulverized into a fine powder using the liquid nitrogen and 800 µL freshly prepared CTAB buffer was added followed by incubation in the water bath at 65℃ for 1-hour. After an hour, an equal volume of chloroform and isoamyl alcohol (24:1) was added to the mixture and kept for incubation at ambient temperature for 10 minutes. The contents were then centrifuged at 10,000 rpm for 20 minutes followed by collecting the supernatant into a fresh Eppendorf tube. The DNA was precipitated by adding 0.6 volumes of chilled isopropanol and 0.1 mL of 3M sodium acetate and then incubated at -20℃ for 30 minutes or overnight at 4℃. This mixture was then centrifuged at 10,000 rpm for 10 minutes at 4℃, followed by discarding the aqueous layer and washing the pellet with 500 µL of 75% ethanol. The air-dried DNA pellets were dissolved in 50 µL of nucleus-free water or Tris EDTA buffer and stored at -20℃ (deep freezer). The concentration and quality of DNA were ascertained by using the NanoDrop 2000 spectrophotometer (Thermo Scientific).

PCR amplification of ITS region and sequencing

The PCR amplification and the sequencing of the internal transcribed spacer (ITS) region were carried out to identify all 40 *B. sorokiniana* isolates. The primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White et al. 1990) were used for the amplification using the BIORAD thermal cycler. The PCR assay was optimized with a final volume of 50 µL, including 25 µL of Dream Taq Master Mix (2X) (Thermo Scientific), 2 µL (10 pmol/µL) of forward primer, 2 µL (10 pmol/µL) of reverse primer, 2 µL (100 ng/µL) of DNA sample and 19 µL of nuclease-free water. The cycling parameters were at 5 minutes of initial denaturation at 94℃ followed by 35 cycles of denaturation (60 seconds at 94℃), annealing (60 seconds at 55℃) and extension (60 seconds at 72℃) with a final extension step for 10 minutes at 72℃. The PCR amplified products were analyzed on 1.5% (w/v) agarose gel stained with ethidium bromide and exposed to the UV Trans-illuminator or by the gel documentation system (BIORAD) to visualize the amplicon. The Sanger dideoxy sequencing method (Barcode Biosciences Pvt Ltd, Bengaluru) was used for sequencing each gene amplicon.

Pathogenic analysis of B. sorokiniana isolates

B. sorokiniana isolates were analyzed for virulence on the highly susceptible variety 'Sonalika' under the net house conditions. Leaves were categorized on the basis of the area of leaf infected and necrotic spots, and the infection index was calculated (Adlakha et al. 1984).

Selection of housekeeping and virulence genes in B. sorokiniana and PCR amplification

B. sorokiniana ND90Pr genome sequence was taken from the NCBI database (Condon et al. 2013) and analyzed for the housekeeping genes and the virulence genes. A total of four housekeeping genes and two virulence genes that were conserved across isolates were selected for diversity analysis among different *B. sorokiniana* isolates. Four housekeeping genes *viz*. translation elongation factor 1 alpha (*TEF1*), glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), RNA Polymerase-II largest subunit (*RPB2*), and beta-tubulin (*BT*) were selected. Two virulence genes, scytalone dehydratase (*SCD1*) and 1,3,8-naphthalenetriol reductase (*BRN1*), were used. The primers were designed using the Primer3Plus. The designed primers were synthesized from Integrated DNA Technologies, India. The list of genes and primers and the PCR conditions using the ITS amplification protocol with slight changes in the PCR conditions is given in (Supplementary Table S2). The amplicons were visualized and sequenced using the Sanger dideoxy sequencing method(Barcode Biosciences Pvt Ltd, Bengaluru).

Phylogenetic analysis

The nucleotide sequences were aligned to generate the consensus sequences using the Clustal-W (Thompson et al. 1997) and the MEGA 11 software (Tamura et al. 2021) for the phylogenetic analysis. The evolutionary history was reconstructed using the maximum likelihood method and general time reversible model. The heuristic search was conducted automatically by using the maximum parsimony method. The stability of the trees was evaluated using 1000 bootstrap replications. The trees were generated for the pooled gene sequences.

Haplotype analysis

The identification of haplotypes was undertaken for 9 populations representing the various districts of India. The CLUSTAL W algorithm was used to align and concatenate the sequences of housekeeping genes in the order: *TEF1α*, *GAPDH*, *RPB2*, *BT*, and *ITS* using the MEGA11 software (Thompson et al. 1997; Tamura et al. 2021). Similarly, the virulence genes were also aligned and concatenated in the order of *SCD1* and *BRN1* genes. Thereafter, for assessing the genetic diversity various parameters like haplotypes (H), haplotype diversity (H_d), number of segregating sites (S), nucleotide diversity (P_i), total number of mutations (Eta) and minimum number of recombination events (R_m) were estimated using the DNASP 6.0 software (Rozas et al. 2017). Two haplotype networks (housekeeping genes-based

and virulence gene-based) were generated in PopART 1.7 (parameter epsilon set to zero) using the median-joining network algorithm (Bandelt et al. 1999; Leigh and Bryant 2015).

Results

The symptomatic samples of spot blotch and black point were collected during 2021-2022 from the states of Madhya Pradesh, Haryana, Uttar Pradesh, West Bengal, Bihar, and New Delhi representing different wheat growing zones in India. Forty *B. sorokiniana* isolates (7 from the central zone (CZ), 4 from the North-western plains Zone (NWPZ) and 29 isolates from the North-eastern plains zone (NEPZ)) were isolated and established from the diseased samples. Using the hyphal tip method, the pure cultures of *B. sorokiniana* isolates (BS) were established and the cultures were regularly maintained on PDA slants. The PCR amplification using the ITS1 and ITS4 primers for all the *B. sorokiniana* (BS) isolates showed a distinct and clear 590 bp band (Supplementary Fig. 1). The amplicon sequencing of 40 BS isolates was performed. BLAST analysis revealed that the maximum similarity to our sequenced ITS regions for all isolates was shown by *B. sorokiniana* sequences in the NCBI database. After BLAST homology, all the ITS sequences of 40 *B. sorokiniana* isolates were deposited to *GenBank*, and accession numbers were acquired (Supplementary Table S3).

The 40 *B. sorokiniana* isolates exhibited significant variation in radial growth and sporulation across different time points. BS-33 (Sultanpur, UP) showed the fastest growth at 4 DAI (58.11 mm) and remained among the fastest-growing isolates, reaching 90 mm by 15 DAI. In contrast, BS-9 (Ujjain, MP) and BS-40 (Ayodhya, UP) displayed the slowest growth at later stages. Sporulation varied significantly, with BS-16 (Pusa, Bihar) producing the highest spore count (8.4×10^7 spores/mL), while BS-3 (Panipat, Haryana) and BS-8 (Vidisha, MP) had the lowest (0.2×10^7 spores/mL).

Based on colony growth patterns and color, isolates were classified into four groups, with the dull white/greenish black type being the most frequent (45%). Microscopic examination revealed brown, septate conidiophores and olive brown to dark brown conidia, with conidial length ranging from 41.54 to 90.09 µm and width from 17.87 to 26.93 µm. BS-20 (Pusa, Bihar) had the largest conidia (90.09 × 26.74 µm).

Virulence assessment showed a wide range of disease severity [Average Disease Incidence 6.86%-87.97%]. Highly virulent isolates (ADI >80%) included BS-21, BS-22, BS-16, BS-36, BS-37, and BS-20 (Pusa, Bihar), while BS-10, BS-9, BS-5 (Ujjain, MP), BS-3 (Panipat, Haryana), and BS-19 (Kanpur, UP) were the least virulent (ADI <10%) (Supplementary Table 4)

Average Disease Index (ADI)

The ADI ranged from 6.86 to 87.97% among BS isolates, indicating variability in virulence. BS-21 (87.97%), BS-22 (87.10%), BS-16 (86.92%), BS-36 (85.24%), BS-37 (84.38%), and BS-20 (83.48%) from Pusa, Bihar, and BS-34 (83.12%) and BS-35 (74.20%) from Varanasi, Uttar Pradesh, were highly virulent (Supplimentary Table S4). Conversely, isolates BS-10 (6.86%), BS-9 (8.11%), and BS-5 (9.50%) from Ujjain, Madhya Pradesh; BS-3 (8.36%) from Panipat, Haryana; BS-19 (7.39%) and BS-18 (16.64%) from Kanpur, Uttar Pradesh, were less virulent (Supplementary Table S4).

A phylogenetic tree was generated using the ITS sequences of all 40 *B. sorokiniana* isolates, with *B. oryzae* as an outgroup (Accession no: LC494363)**.** On the basis of the phylogenetic analysis, all the BS isolates showed clustering into two clades. Only two isolates *viz.* BS-3 isolate (Panipat, Haryana) isolated from infected seed and BS-13 isolate (Cooch Behar, West Bengal) isolated from infected leaf tissues, clustered in Clade-I, and all remaining 38 BS isolates clustered together in clade-II (Fig. 1).

PCR amplification of genes coding for translation elongation factor 1 alpha protein, *TEF1α* (530 bp); glyceraldehyde-3-phosphate dehydrogenase, *GAPDH* (490 bp); RNA Polymerase II subunit A protein, *RPB2* (520 bp); β-tubulin protein, *BT* (250 bp); Scytalone dehydratase, *SCD1* (520 bp); 1,3,8-napthalenetriol reductase, *BRN1* (515 bp) were performed separately for all 40 *B. sorokiniana* isolates (Supplementary Fig. 2). The homology analysis was conducted using the BLAST tool, revealing that the amplified gene sequences exhibited the maximum similarity to the reference genes of *B. sorokiniana*. A total of 240 gene sequences were submitted in *GenBank* after trimming and curation, and the accession numbers were acquired (Supplementary Table 3). The concatenated sequences of *TEF1α*, *GAPDH*, *RPB2*, *BT*, and *ITS* of the 40 *B. sorokiniana* isolates with 2,058 bp nucleotides, were aligned, and a tree was constructed. The phylogenetic analysis revealed that the 40 BS isolates grouped into six clades (Fig. 2). BS-6, BS-7,

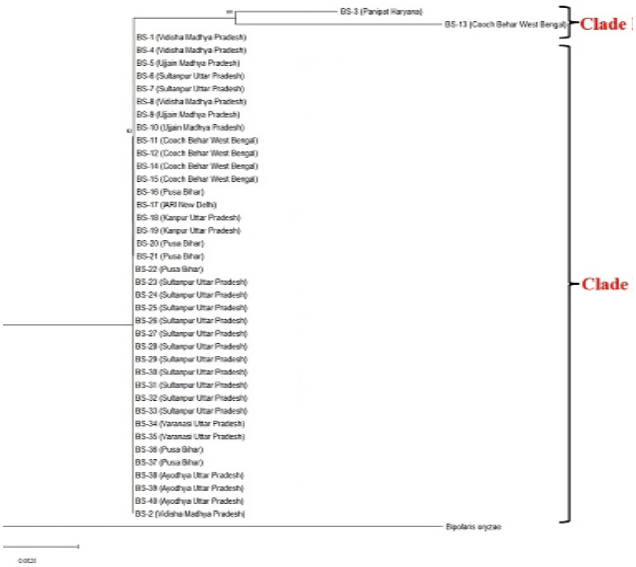

Fig. 1. Phylogenetic tree of *ITS* sequences in 40 *B. sorokiniana*

BS-23, BS-29, BS-33, BS-38, BS-39 and BS-40 *B. sorokiniana* isolates clustered in Clade I. BS-11, BS-13, BS-27, BS-28, BS-32 and BS-37 *B. sorokiniana* isolates grouped in clade II. BS-1, BS-3, BS-4, BS-5, BS-17 and BS-18 *B. sorokiniana* isolates clustered in clade III. BS-8, BS-9, BS-10, BS-12 and BS-14 *B. sorokiniana* isolates clustered in clade IV. BS-15, BS-22, BS-24, BS-25, BS-30 and BS-35 *B. sorokiniana* isolates clustered in clade V. BS-2, BS-16, BS-19, BS-20, BS-21, BS-26, BS-31, BS-34 and BS-36 *B. sorokiniana* clustered in clade VI. The highest number of BS isolates (22.5%) were clustered together in Clade VI, originating from three states (four isolates from Bihar, four isolates from Uttar Pradesh and one isolate from Madhya Pradesh). Clade I comprised 20% of BS isolates belonging to Uttar Pradesh (three isolates from Ayodhya district and five isolates from Sultanpur district). The clade II consisted of 15% of BS isolates from three states (two isolates from West Bengal, one isolate from Bihar and three isolates from Uttar Pradesh). The clade III comprised 15% of *B. sorokiniana* isolates from four states (three isolates from Madhya Pradesh, one isolate from Haryana, one isolate from New Delhi, and one isolate from Uttar Pradesh). The clade IV consisted of 12.5% of isolates from two states (three isolates from Madhya Pradesh and two isolates from West Bengal). The Clade V comprised 15% of isolates from three states (one isolate from Bihar, four isolates from Uttar Pradesh, and one isolate from West Bengal).

Upon *SCD1* gene sequence analysis, all the BS isolates were clustered into two clades. Only six BS isolates *viz*. BS-3 isolate (Panipat, Haryana); BS- 5, BS- 9 and BS-10 (Ujjain, Madhya Pradesh); BS-18 and BS-19 (Kanpur, Uttar Pradesh) clustered in clade II, while all remaining 34 BS isolates were clustered together in clade-I (Fig. 3). BS isolates from Ujjain, Kanpur and Panipat districts showed the distinct variations in the nucleotide sequence of *SCD1* virulence gene. A tree was also generated using the *BRN1* gene sequences**.** It suggested that all the BS isolates were clustered into one single clade (Fig. 4). There was no variation among the *B.*

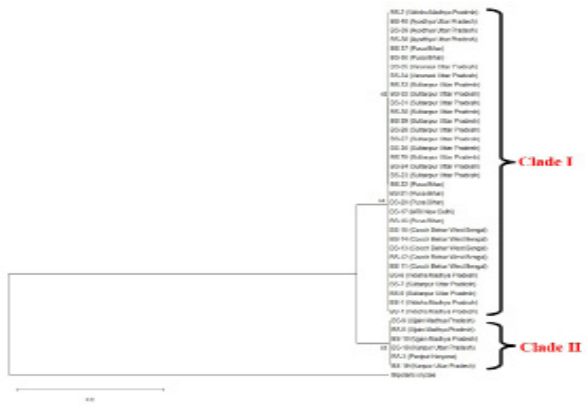

Fig. 3. Phylogenetic tree of *SCD1* gene sequences in 40 *B. sorokiniana* isolates

sorokiniana isolates in terms of the 1,3,8-napthalenetriol reductase (*BRN1*) gene.

For conducting haplotype analysis, the isolates were grouped according to their geographical locations (representing particular districts) and were denoted as Vidisha_MP, Ujjain_MP, Panipat_New Delhi, Samastipur_BH, Sultanpur_UP, Unnao_UP, Ayodhya_UP, Varanasi_UP and Coochbehar_WB. The Panipat and New Delhi BS isolates were grouped together due to their geographic proximity and the fact that each location contained only one BS isolate. Upon analysis of the combined dataset of 5 housekeeping loci (*TEF1α, GAPDH, RPB2, BT,* and *ITS*) of 40 isolates, 25 haplotypes were associated with a haplotype diversity of 0.9615 (Table 1). Hap_3 and Hap_2 were identified as the most predominant, each comprising 12.5% of the total isolates. The Hap_3 was found in four populations comprising 33.33% isolates of Ujjain_MP, 33.33% isolates of Samastipur_BH, 50% isolates of Panipat_NewDelhi and 7.7% isolates of Sultanpur_UP. Hap_2 was also found in four

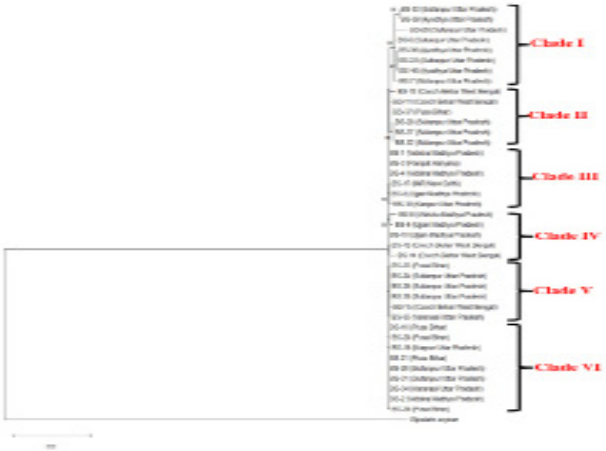

Fig. 2. Phylogenetic tree of concatenated sequences of *TEF1α, GAPDH, RPB2, BT, ITS* genes (2058 bp)

Fig. 4. Phylogenetic tree of *BRN1* gene sequences in 40 *B. sorokiniana* isolates

Table 1. Summary of genetic diversity based on 5 housekeeping genomic loci (*TEF1α*, *GAPDH*, *RPB2*, *BT*, and *ITS*) sequences and 2 genomic virulence loci (*SCD1 and BRN1*) sequences of *B. sorokiniana* representing 9 regions of India

Population	No. of Sequences (N)	Haplotype (h)	Haplotype diversity (H_d)	No. of Segregating sites (S)	Nucleotide diversity (P_i)	Total number of mutations (Eta)	Minimum no of recombination events (R_m)
Housekeeping genomic loci							
Vidisha_MP	4	3	0.83333	7	0.00186	15	2
Panipat_NewDelhi	2	2	1.00000	2	0.00097	10	0
Ujjain_MP	3	3	1.00000	5	0.00162	12	0
Sultanpur_UP	13	10	0.94872	17	0.00254	26	2
Coochbehar_WB	5	5	1.00000	9	0.00194	14	1
Samastipur_BH	6	5	0.93333	8	0.00178	14	1
Unnao_UP	2	2	1.00000	4	0.00194	12	0
Varanasi_UP	2	2	1.0000	5	0.00242	8	0
Ayodhya_UP	3	2	0.66667	5	0.00162	12	0
Total	40	25	0.96154	33	0.00245	39	1
Virulence genomic loci							
Vidisha_MP	4	3	0.83333	5	0.00309	5	0
Panipat_NewDelhi	2	2	1.00000	1	0.00109	3	0
Ujjain_MP	3	3	1.00000	3	0.00218	4	0
Sultanpur_UP	13	5	0.80769	6	0.00227	6	0
Coochbehar_WB	5	4	0.90000	5	0.00306	5	1
Samastipur_BH	6	2	0.33333	2	0.00073	6	0
Unnao_UP	2	2	1.00000	3	0.00328	3	0
Varanasi_UP	2	1	0.00000	0	0.00000	0	-
Ayodhya_UP	3	3	1.00000	2	0.00146	2	0
Total	40	11	0.83205	9	0.00257	9	1

populations comprising 50% isolates of Vidisha_MP, 50% isolates of Panipat_New Delhi, 50% isolates of Varanasi_UP and 33.33% isolates of Ujjain_MP. Most of the haplotypes identified were isolate-specific (Supplementary Table 3). The haplotype diversity (H_d) ranged from 0.667 to 1.000 with the lowest $H_d$in the Ayodha_UP population. The highest H_d was noticed in the populations of Panipat_NewDelhi, Ujjain_MP, Coochbehar_WB, Unnao_UP and Varanasi_UP. The total number of segregating sites/polymorphic (S) sites in BS isolates was found to be 33 with the highest (17) segregating sites observed in a population of Sultanpur_UP and the lowest (2) in Panipat, New Delhi population. The overall nucleotide diversity was found to be 0.00245 with the highest (0.00254) in Sultanpur_UP and lowest in Panipat_New Delhi (0.00097). The total number of mutations (Eta) observed amongst all 40 BS isolates was 39. Within each population, too the total number of mutations were computed which ranged from 26 (Sultanpur_UP) to 8 (Varanasi_UP). The minimum number of recombination events (R_m) was estimated for the whole population (R_m =1) as well as within each population (Range of R_m = 0-2; highest in Vidisha_MP and Sultanpur_MP populations). In the haplotype network, it was observed that Hap_3 serves as a focal point and all other haplotypes radiated from it (Fig. 5).

The analysis of the combined dataset of 2 virulence loci (*SCD1* and *BRN1*) of 40 BS isolates revealed the presence of 11 haplotypes with a haplotype diversity of 0.83205. The highest (5) number of haplotypes was observed for Sultanpur, UP population and the lowest (1) in Varanasi, UP population. The most predominant haplotype was Hap_2 comprising 30% of the isolates representing six populations (Vidisha, MP, Panipat-New Delhi, Sultanpur-UP, Coochbehar-WB, Samastipur-BH and Varanasi-UP). The next most predominant haplotype identified was Hap_4 (40% isolates representing Sultanpur-UP, Ujjain-MP, Samastipur_BH and Coochbehar-WB) which seemed to act as a central point for all other isolates on the haplotype network analysis (Fig. 6). The haplotype diversity (H_d) ranged from 0-1 with the lowest in Varanasi-UP and the highest in Panipat-New Delhi, Ujjain-MP, Unnao-UP and Ayodhya-UP. The total number of segregating sites (S) of all BS isolates was 9 with the highest (6) in Sultanpur, UP population and the lowest (0) in Varanasi, UP population. The overall nucleotide diversity was found to be 0.00257 with the highest (0.00328) in Unnao-UP and the

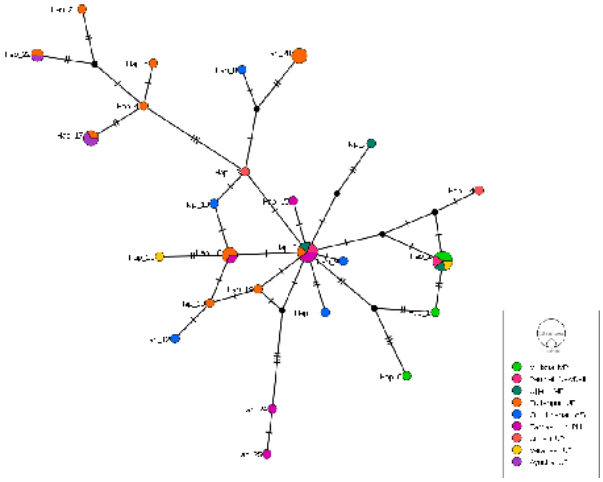

Fig. 5. Haplotype analysis for concatenated sequences of *TEF1a, GAPDH, RPB2, BT, ITS*genes

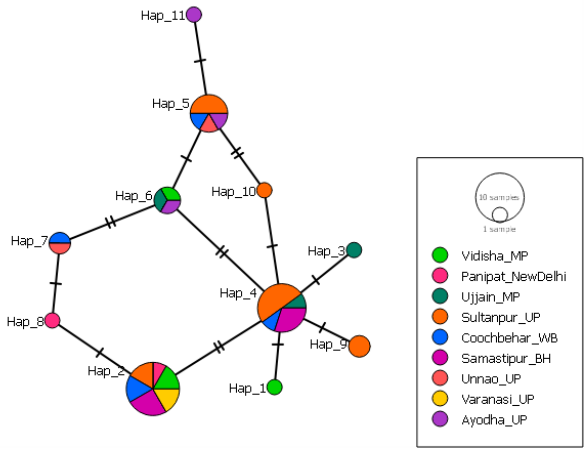

Fig. 6. Haplotype analysis for concatenated sequences of *SCD1* and *BRN1* virulence genes

lowest in Varanasi_UP (0.00000). Similarly, the total number of mutations and the minimum number of recombination events observed in the whole population were 9 and 1 respectively.

Discussion

Wheat is among the most extensively cultivated and consumed cereal grains in the world and holds an important position in the global diet. With the continuous increment in global population, the demand for wheat production is also increasing. Significant challenges in wheat production arise from biotic stresses, with the leaf blight complex, notably *B. sorokiniana*, the primary incitant of spot blotch disease, being one of the most damaging threats. This pathogen affects not only the leaves but also the shoots, roots, and seeds. The disease has a global presence but is particularly significant in hot and humid wheat-growing regions. However, the pathogen's threat to crucial wheat-growing regions worldwide is particularly exacerbated by climate change. Hence, grasping the genetic diversity and virulence of the pathogen is crucial for comprehending its evolution and for mitigating potential losses.

In the climate change scenario, the present investigation was carried out by evaluating a large number of *B. sorokiniana* isolates from different wheat-growing regions of India. Forty *B. sorokiniana* isolates were isolated from the diseased wheat samples from the North-western plains zone (NWPZ), Central zone (CZ), and North-eastern plains zone (NEPZ). The identification of *B. sorokiniana* was confirmed with the morphology and also at a molecular level. The use of ITS as a universal marker for the preliminary identification of fungal pathogens including *B. sorokiniana* has been documented in various studies (Basak et al. 2024; Sharma et al. 2022).

The present study revealed significant morphological and pathogenic variability among *B. sorokiniana* isolates. After 15 DAI, BS-21 (Pusa, Bihar), BS-27, BS-33 (Sultanpur, UP), and BS-34 (Varanasi, UP) exhibited the highest mycelial growth (90 mm), while BS-16 (Pusa, Bihar) had the highest sporulation (8.4×10^7 spores/ml). Isolates from Pusa, Bihar, generally showed faster growth under artificial conditions, highlighting ongoing pathogen evolution. These findings align with previous reports of morpho-pathological variability in *B. sorokiniana* (Aggarwal et al. 2009; Chand et al. 2003).

Conidial size varied significantly, with BS-20 (Pusa, Bihar) having the longest conidia (90.09 µm), followed by BS-12 (Cooch Behar, WB) and BS-35 (Varanasi, UP). Pathogenicity assessment showed no direct correlation between morphological traits and virulence, as highly, moderately, and less virulent isolates were found across all colony types. Virulence varied widely (ADI: 6.86–87.97%), with BS-21, BS-22, BS-16, BS-36, BS-37, and BS-20 (Pusa, Bihar) being the most aggressive. These findings contrast with earlier studies where *B. sorokiniana* isolates from Varanasi, UP, were more pathogenic (Aggarwal et al. 2019).

Although previous studies linked suppressed black mycelial growth to higher virulence (Poloni et al. 2009; Verma et al. 2020), no such correlation was observed in our study. Pathogenic variability may arise from factors like heterokaryosis, hyphal fusion, and nuclear migration (Pandey et al. 2008). Additionally, no strong association was found between geographic origin and morphological traits, suggesting genetic variability is influenced by both genetic and environmental factors rather than location alone (Kang et al. 2021).

The multigene analysis (*TEF1a*, *GAPDH*, *RPB2*, *BT*, *ITS*) on the genetic diversity of *B. sorokiniana* populations revealed six groups, which did not correspond with the geographical origin of the isolates collected. Among all clusters, clade III was the most diverse, containing isolates collected from four different states of India *viz*. Madhya

Pradesh, Haryana, New Delhi and Uttar Pradesh. The clade I was least diverse with all the isolates from Uttar Pradesh only. To our knowledge, this was the first study to analyze the *B. sorokiniana* population with the multigene analysis. The high haplotype diversity indicated genetic variation and the significance of mutation and recombination in determining the population structure of the pathogen (Liu et al. 2023). In our study, the high diversity in haplotypes of housekeeping genes is also evident from the observations generated in the phylogenetic analysis which revealed the formation of 6 clades. This suggests that the use of combined genomic loci will be useful in delineating the diversity of *B. sorokiniana*. Previous studies on housekeeping genomic loci (*ITS*, *GAPDH* and *TEF1*) of *B. sorokiniana* revealed significant genetic variation among BS isolates and also the existence of common haplotypes of wheat and barley (Sharma et al. 2022). In our study, we observed two predominant Haplotypes (Hap_3 and Hap_2) where Hap_3 served as the common ancestor for all other haplotypes. Thus, the presence of common and predominant haplotypes indicated a moderate to high level of gene flow amongst the population.

The virulence genes play a crucial role in pathogenesis in climate change scenarios. The virulence mechanisms evolve continuously and disseminate across microbes through a process known as horizontal or lateral gene transfer. Possible targets for risk assessment and intervention efforts include the virulence genes. The discovery of virulence genes may pave the way for the establishment of fast screening methods. The comparative sequencing analysis provides insights into fungus pathogenic processes, enabling the discovery of known virulence proteins with conserved sequences or motifs, as well as probable novel virulence proteins (Fournier and Raoult, 2017). Melanin is a pigment that is not required for growth and development, but it improves species' survival and competitive capacities in specific conditions (Bell and Wheeler, 1986). They achieve this by protecting against reactive oxygen species (Romero-Martinez et al. 2000), UV radiation (Kawamura et al. 1997), and cell wall-degrading enzymes produced by antagonist microbes (Butler et al. 2001). Melanin synthesis positively correlates with conidiogenesis and virulence in various isolates of the phytopathogenic *B. sorokiniana* (Aggarwal et al. 2011; Bashyal et al. 2010). The scytalone dehydratase gene (*SCD1*) is essential for the production of fungal dihydroxy naphthalene melanin. Similarly, the *BRN1* gene is also reported to be involved in melanin synthesis and production of methyl 5-(hydroxymethyl) furan-2-carboxylate toxin, hence the virulent nature of this gene (Liu et al. 2011). In the present investigation, all *B. sorokiniana* isolates were grouped into two clades using the *SCD1* gene. Only a few BS isolates were clustered in clade II and all remaining 34 BS isolates were clustered together in clade I. *B. sorokiniana* isolates from Ujjain, Kanpur and Panipat districts showed distinct variations in the *SCD1* gene, correlating with the lowest disease severity in these six BS isolates (BS-9, BS-5, BS-10, BS-18, BS-3 and BS-19). In earlier reports, isolates mutant for *SCD1* gene impairing melanin synthesis, exhibited a notable reduction in the pathogenicity of wheat (Singh et al. 2022). This study broadens our understanding and offers a detailed insight into the pathogenesis of *B. sorokiniana*. Additionally, in the present investigation, *BRN1* gene sequence of all 40 *B. sorokiniana* isolates clustered into one single clade. We did not find any variation among all *B. sorokiniana* isolates with respect to the 1,3,8-napthalenetriol reductase (*BRN1*) gene. It means the *BRN1* gene is highly conserved in *B. sorokiniana* isolates indicating its indesponsibillity. The low genetic variation with respect to virulence genes is also evident by haplotype analysis which identified only 11 haplotypes amongst the 40 isolates. This indicates that a high variation in such genes is not maintained by pathogens due to their probable detrimental effects. Previously, the haplotype diversity on *ToxA* gene in *B. sorokiniana* and other related fungi has been reported (Hafez et al. 2022; McDonald et al. 2018; See et al. 2024). Interestingly in our study, the most virulent isolates (BS-16, BS-20, BS-21, BS-22, BS-36 and BS-37) all belonging to the Samastipur_BH population were grouped into either of the most predominant haplotypes (Hap_2 and Hap_4). The next virulent isolate BS-17 which belonged to Hap_8 differed from Hap_2 by only one mutation event. This finding speculates the association of these two haplotypes (Hap_2 and Hap_4) with combined genomic loci of *SCD1* and *BRN1* in governing the high virulence of *B. sorokiniana*. However, additional roles of other virulence loci may also be present. To our knowledge, this is the first report of *SCD1* and *BRN1* gene-based haplotype identification in *B.sorokiniana*. In the future, there is a need to find out the corresponding susceptibility genes in wheat host and then a genome editing approach can be explored to manage spot blotch of wheat.

Supplementary Materials

Supplementary Tables S1 to S5 and Supplementary Figure 1 are provided, which can be accessed at www.isgpb.org

Authors Contribution

Conceptualization of research (MSG, YNR); Designing of the experiments (MSG, YNR, NK); Contribution of experimental materials (YNR, NK, TPK, PB); Execution of field/lab experiments and data collection (NK, YNR, PB); Analysis of data and interpretation (NK, YNR, PB); Preparation of the manuscript (NK, YNR, MSG, TPK, MM, SS, MSS, RA).

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Supplementary Table S1. Collection, isolation and establishment of *Bipolaris sorokiniana* isolatesused in present study

S. No	Location	District	State	Zone*	Designated BS isolates	Source of isolation
1	Vidisha	Vidisha	Madhya Pradesh	CZ	BS-1	Seed
2	Vidisha	Vidisha	Madhya Pradesh	CZ	BS-2	Seed
3	Panipat	Panipat	Haryana	NWPZ	BS-3	Seed
4	Vidisha	Vidisha	Madhya Pradesh	CZ	BS-4	Seed
5	Ujjain	Ujjain	Madhya Pradesh	CZ	BS-5	Seed
6	Domapur	Sultanpur	Uttar Pradesh	NEPZ	BS-6	Leaf
7	Naredrapur	Sultanpur	Uttar Pradesh	NEPZ	BS-7	Leaf
8	Vidisha	Vidisha	Madhya Pradesh	CZ	BS-8	Seed
9	Ujjain	Ujjain	Madhya Pradesh	CZ	BS-9	Seed
10	Ujjain	Ujjain	Madhya Pradesh	CZ	BS-10	Seed
11	Cooch Behar	Cooch Behar	West Bengal	NEPZ	BS-11	Leaf
12	Cooch Behar	Cooch Behar	West Bengal	NEPZ	BS-12	Leaf
13	Cooch Behar	Cooch Behar	West Bengal	NEPZ	BS-13	Leaf
14	Cooch Behar	Cooch Behar	West Bengal	NEPZ	BS-14	Leaf
15	Cooch Behar	Cooch Behar	West Bengal	NEPZ	BS-15	Leaf
16	Pusa	Samastipur	Bihar	NEPZ	BS-16	Leaf
17	IARI	New Delhi	New Delhi	NWPZ	BS-17	Leaf
18	Unnao	Unnao	Uttar Pradesh	NWPZ	BS-18	Leaf
19	Unnao	Unnao	Uttar Pradesh	NWPZ	BS-19	Leaf
20	Pusa	Samastipur	Bihar	NEPZ	BS-20	Leaf
21	Pusa	Samastipur	Bihar	NEPZ	BS-21	Leaf
22	Pusa	Samastipur	Bihar	NEPZ	BS-22	Leaf
23	Lambhua	Sultanpur	Uttar Pradesh	NEPZ	BS-23	Leaf
24	Anapur	Sultanpur	Uttar Pradesh	NEPZ	BS-24	Leaf
25	Bhaidya	Sultanpur	Uttar Pradesh	NEPZ	BS-25	Leaf
26	Sambhuaganj	Sultanpur	Uttar Pradesh	NEPZ	BS-26	Leaf
27	Kathara	Sultanpur	Uttar Pradesh	NEPZ	BS-27	Leaf
28	Chanda	Sultanpur	Uttar pradesh	NEPZ	BS-28	Leaf
29	Rampur	Sultanpur	Uttar Pradesh	NEPZ	BS-29	Leaf
30	Dhapapa	Sultanpur	Uttar Pradesh	NEPZ	BS-30	Leaf
31	Diyra	Sultanpur	Uttar Pradesh	NEPZ	BS-31	Leaf
32	Narharpur	Sultanpur	Uttar Pradesh	NEPZ	BS-32	Leaf
33	Gharwashpur	Sultanpur	Uttar Pradesh	NEPZ	BS-33	Leaf
34	Babatpur	Varanasi	Uttar Pradesh	NEPZ	BS-34	Leaf
35	Jamaalpur	Varanasi	Uttar Pradesh	NEPZ	BS-35	Leaf
36	Pusa	Samastipur	Bihar	NEPZ	BS-36	Leaf
37	Pusa	Samastipur	Bihar	NEPZ	BS-37	Leaf
38	Masaudha	Ayodhya	Uttar Pradesh	NEPZ	BS-38	Leaf
39	Kumarganj	Ayodhya	Uttar Pradesh	NEPZ	BS-39	Leaf
40	Bikapur	Ayodhya	Uttar Pradesh	NEPZ	BS-40	Leaf

*CZ-central zone; NWPZ-North-western plains Zone; NEPZ- North-eastern plains zone

Supplementary Table S2. List of genes and primers used for in the study

				*Annealing Temperature
Housekeeping genes				
1	Translation elongation factor 1 alpha (*TEF1*)	Forward	TTGGTGTCAAGCAGCTCATC	56°C
		Reverse	ACGTTACCACGACGGATCTC	
2	Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*)	Forward	GTCTCAGCATCGAGCACAAC	56°C
		Reverse	CAAGAGGCGTTGGAGAGAAC	
3	RNA polymerase II subunit A (*RPB2*)	Forward	CATGATCAAGGCTGACTTCG	55°C
		Reverse	ACCATCGACGGAGATACTGG	
4	Beta Tubulin (*BT*)	Forward	TTGAGCGCATGAACGTCTAC	54°C
		Reverse	AGTTGTGACCCTTGGTCCAG	
Virulence genes				
5	Scytalone dehydratase (*SCD1*)	Forward	CCAGCCTACGTTTGAGGGTA	56°C
		Reverse	CCTCTGCAAACACCTTGTCA	
6	1,3,8-napthalenetriol reductase (*BRN1*)	Forward	GTTGTGTCTTTCGGCCACTT	55°C
		Reverse	CCTTTCCGTTAACCCAGTCA	

*PCR conditions: Initial denaturation: 94°C for 5 mins, Denaturation: 94°C for 60 seconds, Annealing at primer specific temperature, Extension: 72°C for 60 seconds, Final Extension: 72°C for 10 mins

Supplementary Table S3. Accession numbers of translation elongation factor 1 alpha, glyceraldehyde-3-phosphate dehydrogenase, RNA Polymerase II subunit A, β-tubulin protein, Scytalone dehydratase, 1,3,8-napthalenetriol reductase genes of 40 B. sorokiniana isolates in NCBI database

S. No	Isolates	NCBI accession numbers						
		ITS	TEF1α	GAPDH	RPB1	BT	SCD1	BRN1
1	BS-1	ON891980	ON981195	ON997501	OP006462	ON997461	ON997296	OP006502
2	BS-2	ON891981	ON981196	ON997502	OP006463	ON997462	ON997297	OP142733
3	BS-3	ON891982	ON981197	ON997503	OP006464	ON997463	ON997298	OP006503
4	BS-4	ON891983	ON981198	ON997504	OP006465	ON997464	ON997299	OP006504
5	BS-5	ON891984	ON981199	ON997505	OP006466	ON997465	OP142741	OP006505
6	BS-6	ON891985	ON981190	ON997506	OP006467	ON997466	ON997300	OP006506
7	BS-7	ON891986	ON981191	ON997507	OP006468	ON997467	ON997301	OP006507
8	BS-8	ON891987	ON981192	ON997508	OP006469	ON997468	ON997302	OP006508
9	BS-9	ON891988	ON981193	ON997509	OP006470	ON997469	ON997303	OP006509
10	BS-10	ON891989	ON981194	ON997510	OP006471	ON997470	ON997304	OP006510
11	BS-11	ON891990	ON981185	ON997511	OP006472	ON997471	ON997305	OP006511
12	BS-12	ON891991	ON981186	ON997512	OP006473	ON997472	ON997306	OP006512
13	BS-13	ON891992	ON981187	ON997513	OP006474	ON997473	ON997307	OP142734
14	BS-14	ON891993	ON981188	ON997514	OP006475	ON997474	ON997308	OP006513
15	BS-15	ON891994	ON981189	ON997515	OP006476	ON997475	ON997309	OP006514
16	BS-16	ON891995	ON981180	ON997516	OP006477	ON997476	OP142742	OP142735
17	BS-17	ON891996	ON981181	ON997517	OP006478	ON997477	ON997310	OP142736
18	BS-18	ON891997	ON981182	ON997518	OP006479	ON997478	ON997311	OP006515
19	BS-19	ON891998	ON981183	ON997519	OP006480	ON997479	ON997312	OP006516
20	BS-20	ON891999	ON981184	ON997520	OP006481	ON997480	ON997313	OP006517
21	BS-21	ON892000	ON981170	ON997521	OP006482	ON997481	ON997314	OP006518

22	BS-22	ON892001	ON981171	ON997522	OP006483	ON997482	ON997315	OP006519
23	BS-23	ON892002	ON981172	ON997523	OP006484	ON997483	ON997316	OP006520
24	BS-24	ON892003	ON981173	ON997524	OP006485	ON997484	ON997317	OP006521
25	BS-25	ON892004	ON981174	ON997525	OP006486	ON997485	ON997318	OP006522
26	BS-26	ON892005	ON981175	ON997526	OP006487	ON997486	ON997319	OP006523
27	BS-27	ON892006	ON981176	ON997527	OP006488	ON997487	OP142743	OP142737
28	BS-28	ON892007	ON981177	ON997528	OP006489	ON997488	ON997320	OP006524
29	BS-29	ON892008	ON981178	ON997529	OP006490	ON997489	ON997321	OP006525
30	BS-30	ON892009	ON981179	ON997530	OP006491	ON997490	ON997322	OP006526
31	BS-31	ON892010	ON981160	ON997531	OP006492	ON997491	ON997323	OP006527
32	BS-32	ON892011	ON981161	ON997532	OP006493	ON997492	OP142744	OP006528
33	BS-33	ON892012	ON981162	ON997533	OP006494	ON997493	OP142745	OP142738
34	BS-34	ON892013	ON981163	ON997534	OP006495	ON997494	ON997324	OP006529
35	BS-35	ON892014	ON981164	ON997535	OP006496	ON997495	ON997325	OP006530
36	BS-36	ON892015	ON981165	ON997536	OP006497	ON997496	ON997326	OP142739
37	BS-37	ON892016	ON981166	ON997537	OP006498	ON997497	ON997327	OP006531
38	BS-38	ON892017	ON981167	ON997538	OP006499	ON997498	OP142746	OP142740
39	BS-39	ON892018	ON981168	ON997539	OP006500	ON997499	ON997328	OP006532
40	BS-40	ON892019	ON981169	ON997540	OP006501	ON997500	ON997329	OP006533

Supplementary Table S4. Colony diameter, sporulation, spore size and ADI of different isolates of *B. sorokiniana*

S. No	Isolates	Colony Diameter(mm)				Sporulation/ml ($1x10^7$)	Spore size		Average Disease Index (%)
		After 4 days	After 7 days	After 9 days	After 15 days		Conidial length (µm)	Conidial width (µm)	
1	BS-1	27.30	34.57	35.35	43.98	0.5	72.24	23.03	49.97
2	BS-2	33.31	47.5	59.25	73.50	1.7	51.67	22.78	42.68
3	BS-3	36.54	44.48	48.72	66.94	0.2	73.89	22.80	8.36
4	BS-4	26.09	32.48	35.25	42.95	3.5	41.54	19.30	58.39
5	BS-5	35.84	51.68	72.33	79.60	3.2	77.63	23.04	9.50
6	BS-6	38.23	52.74	69.28	79.50	4.1	68.36	21.59	44.16
7	BS-7	35.21	41.69	43.36	78.49	3.5	54.68	20.46	48.32
8	BS-8	22.00	44.12	64.31	77.87	0.2	44.48	18.51	47.60
9	BS-9	21.10	26.36	31.50	36.28	3.1	57.30	17.87	8.11
10	BS-10	26.27	30.03	34.68	43.55	1.8	82.33	25.19	6.86
11	BS-11	25.77	33.81	40.91	46.58	4.5	77.71	22.32	72.04
12	BS-12	26.32	44.59	57.66	65.76	5.1	89.73	26.56	26.41
13	BS-13	28.17	36.66	40.85	51.56	1.0	86.75	24.64	76.32
14	BS-14	25.11	26.60	35.54	43.58	5.0	87.61	23.61	68.68
15	BS-15	31.00	40.36	45.84	44.89	5.2	68.61	22.21	67.08
16	BS-16	25.30	38.07	42.41	52.55	8.4	74.43	18.27	86.92
17	BS-17	25.14	28.14	35.19	44.60	3.1	66.74	25.34	82.95
18	BS-18	20.68	22.15	32.70	41.85	5.1	51.32	23.31	16.64
19	BS-19	24.92	34.69	40.91	47.64	5.9	63.85	24.57	7.39

20	BS-20	29.12	41.47	50.23	62.12	6.1	90.09	26.74	83.48
21	BS-21	39.01	68.29	80.91	90.00	6.6	81.95	22.61	87.97
22	BS-22	30.32	38.83	46.25	45.07	6.7	67.14	21.15	87.10
23	BS-23	34.17	42.70	50.42	55.72	5.8	48.73	21.07	78.04
24	BS-24	24.91	33.85	36.05	41.86	6.4	67.45	19.47	78.11
25	BS-25	26.79	28.68	32.16	41.80	1.3	50.90	18.01	67.22
26	BS-26	35.20	39.04	43.84	47.48	2.3	75.61	19.25	46.85
27	BS-27	54.90	81.60	87.05	90.00	5.1	71.94	21.77	35.03
28	BS-28	25.88	32.90	42.37	62.20	3.4	69.25	24.36	12.83
29	BS-29	44.68	67.60	74.92	86.55	4.4	76.34	24.03	46.94
30	BS-30	28.76	33.01	36.29	46.43	4.1	44.54	19.18	56.04
31	BS-31	19.37	25.66	36.00	43.68	7.0	68.97	20.72	80.97
32	BS-32	27.15	36.22	41.24	47.85	0.7	86.37	22.54	41.99
33	BS-33	58.11	83.68	87.69	90.00	1.2	54.31	21.71	35.47
34	BS-34	48.92	78.82	84.17	90.00	7.2	66.13	21.90	83.12
35	BS-35	33.11	32.77	44.08	51.67	6.8	88.79	22.63	74.20
36	BS-36	35.12	42.69	50.20	55.93	7.8	76.74	19.68	85.24
37	BS-37	19.05	23.68	36.37	45.89	8.2	79.19	22.30	84.38
38	BS-38	40.56	52.75	63.67	68.98	4.7	75.61	19.49	59.12
39	BS-39	29.54	31.70	35.36	41.14	4.6	41.56	18.21	55.29
40	BS-40	26.59	25.95	30.35	36.35	5.2	58.94	22.32	67.89
C.D.		1.608	2.944	1.340	1.495	0.126	1.353	0.949	2.730
SE (m)		0.570	1.044	0.475	0.530	0.045	0.480	0337	0.968
SE (d)		0.806	1.477	0.672	0.750	0.063	0.679	0.476	1.369
C.V.		3.169	4.377	1.683	1.596	1.805	1.217	2.666	3.083

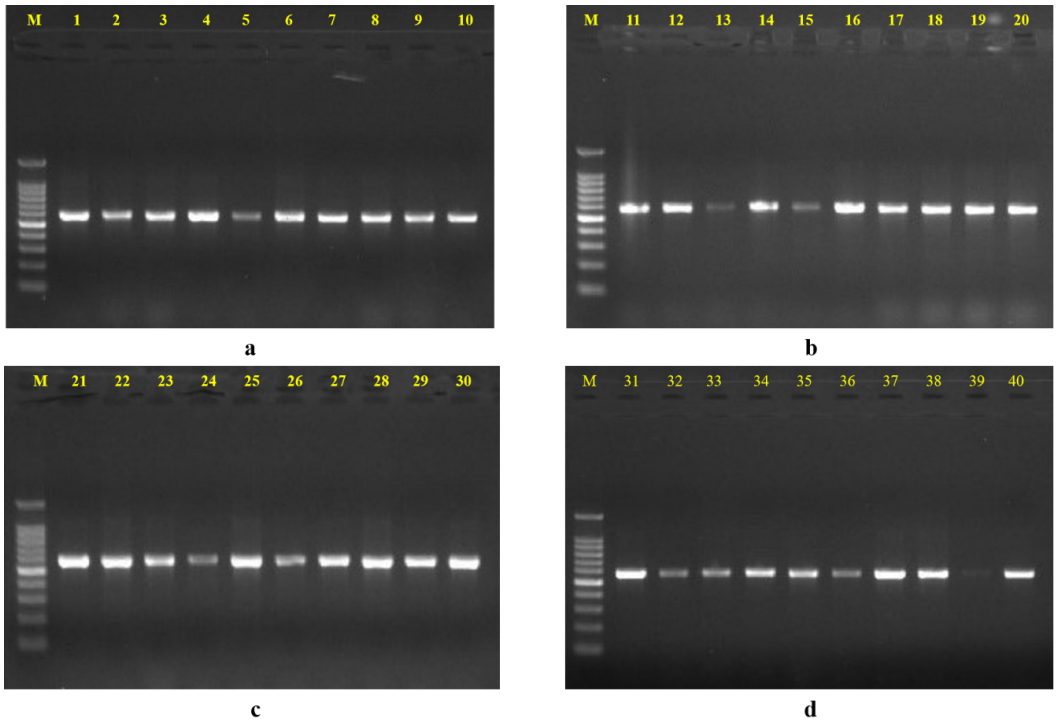
Supplementary Table S5. Haplotypes and their representative isolates identified in the study

Housekeeping genes		
Haplotypes	Number of isolates	Isolates
Hap_1	1	BS-1
Hap_2	5	BS-2 BS-3 BS-4 BS-10 BS-34
Hap_3	5	BS-5 BS-16 BS-17 BS-21 BS-31
Hap_4	1	BS-6
Hap_5	1	BS-7
Hap_6	1	BS-8
Hap_7	1	BS-9
Hap_8	1	BS-11
Hap_9	1	BS-12
Hap_10	1	BS-13
Hap_11	1	BS-14
Hap_12	1	BS-15
Hap_13	1	BS-18
Hap_14	1	BS-19
Hap_15	1	BS-20
Hap_16	3	BS-22 BS-24 BS-30

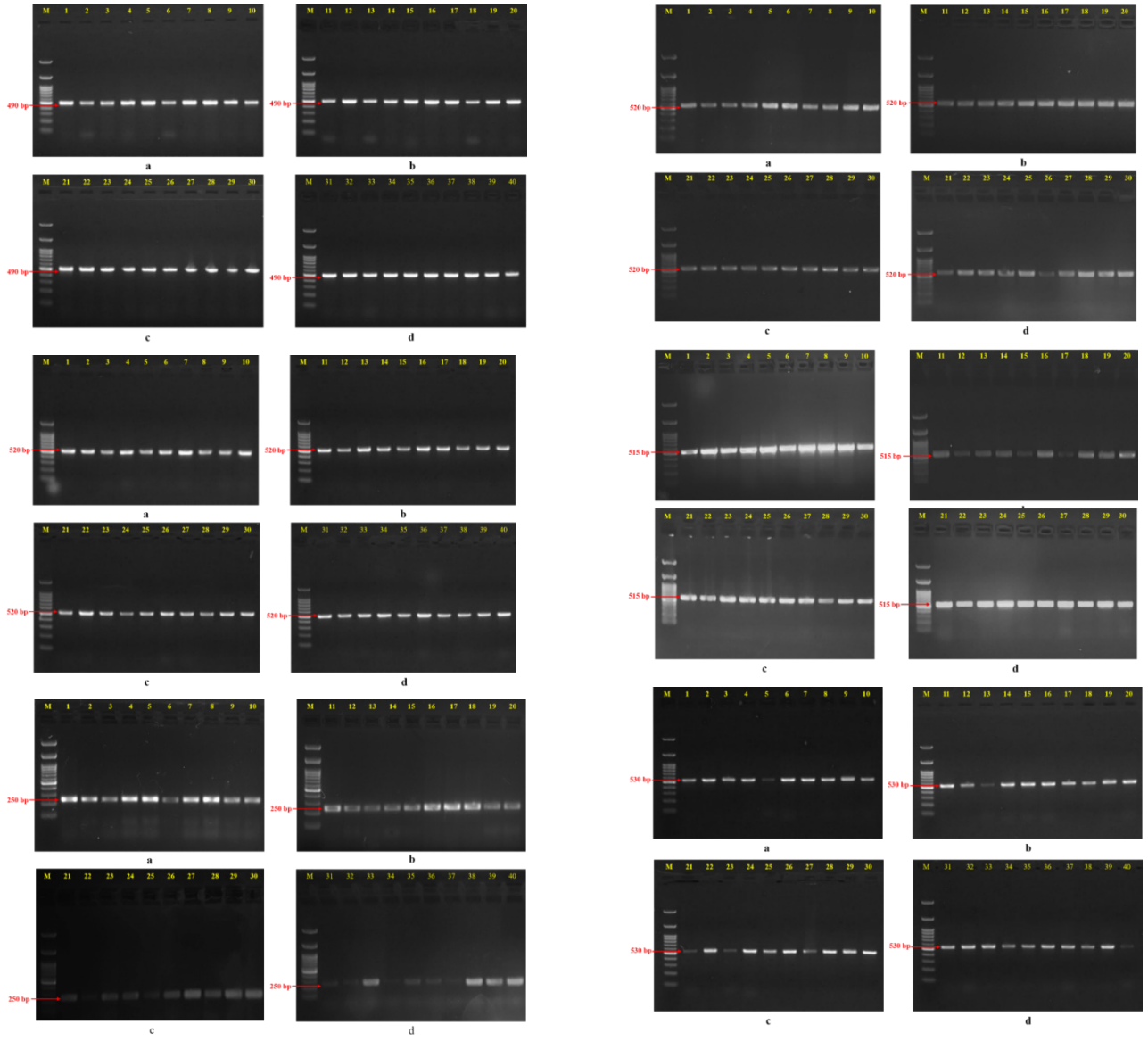
Hap_17	3	BS-23 BS-39 BS-40
Hap_18	1	BS-25
Hap_19	1	BS-26
Hap_20	3	BS-27 BS-28 BS-32
Hap_21	1	BS-29
Hap_22	2	BS-33 BS-38
Hap_23	1	BS-35
Hap_24	1	BS-36
Hap_25	1	BS-37

Virulence genes

Haplotypes	Number of isolates	Isolates
Hap_1	1	BS-1
Hap_2	12	BS-2 BS-3 BS-4 BS-12 BS-14 BS-22 BS-24 BS-30 BS-34 BS-35 BS-36 BS-37
Hap_3	1	BS-5
Hap_4	10	BS-6 BS-9 BS-13 BS-16 BS-20 BS-21 BS-23 BS-25 BS-26 BS-27
Hap_5	6	BS-7 BS-15 BS-18 BS-28 BS-32 BS-39
Hap_6	3	BS-8 BS-10 BS-38
Hap_7	2	BS-11 BS-19
Hap_8	1	BS-17
Hap_9	2	BS-29 BS-33
Hap_10	1	BS-31
Hap_11	1	BS-40



Supplemantary Fig. 1. Amplification of ITS region in *B. sorokiniana* isolates, M – 1kb ladder a) BS-1 to BS-10 isolates, b) BS-11 to BS-20 isolates, c) BS-21 to BS-30 isolates, d) BS-31 to BS-40 isolates.



Supplementary Fig. 2. (A) Amplification of TEF1α gene (530 bp) in 40 B. sorokiniana isolates, M – 1kb ladder (B) Amplification of GAPDH gene (490 bp) (C) Amplification of RPB2 gene (520 bp) (D) Amplification of BT gene (250 bp) (E) Amplification of SCD1 gene (520 bp) (F) Amplification of BRN1 gene (515 bp) a) BS-1 to BS-10 isolates, b) BS-11 to BS-20 isolates, c) BS-21 to BS-30 isolates, d) BS-31 to BS-40 isolates, respectively