Molecular Diversity, Haplotype Distribution and Gene Flow of Bipolaris Sorokiniana Fungus Causing Spot Blotch Disease in Different Wheat Growing Zones

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Abstract

*Bipolaris sorokiniana* (BS) is an economically important fungal pathogen causing spot blotch of wheat (*Triticum aestivum*) and found in all wheat growing zones of India. Very scanty and fragmentary information is available on its genetic diversity. The current research is the first detailed report on the geographic distribution and evolution of BS population in five geographically distinct wheat growing zones [North Western Plain Zone (NWPZ), North Eastern Plain zone (NEPZ); North Hill Zone (NHZ), Southern Hill Zone (SHZ) and Peninsular Zone (PZ)] of India, studied by performing nucleotide sequence comparison of internal transcribed spacer region of 183 isolates. A moderate to high levels of haplotypic diversity was noticed in different wheat growing zones. Phylogenetic analysis suggests that *B. sorokiniana* exist in two distinct lineages as all isolates under study were grouped in two different clades and found analogous to the findings of haplotypic and median joining network analysis. The genetic parameters revealed the existence of 59 haplotypes with three major haplotypes (H_2, H_3, and H_25) which showed star-like structure network surrounded by several single haplotypes, revealing high frequency of the mutations (\( \text{Eta} = 2 - 437 \)) in total analyzed population. H_3 was observed as a predominant haplotype and prevalent in all the five zones. Moderate level of genetic differentiation was found between NEPZ and PZ (\( F_{st} = 0.563 \)), whereas it was low between NEPZ and NHZ (\( F_{st} = -0.062 \)). High level of gene flow was noticed between NWPZ and NEPZ (\( N_m = 14.32 \)), while it was found minimum between SZ and NHZ (\( N_m = 0.50 \)). Moreover, negative score of neutrality statistics (Tajima's D and Fu's FS test) for NWPZ, PZ and SHZ populations, suggested recent population expansion in these zones. However, positive score for both the neutrality tests observed in NEPZ and NHZ indicated the dominance of balancing selection in structuring their population. Recombination events were observed in the NWPZ, NEPZ and NHZ population, while it was absent in SHZ and PZ population. Thus, the lack of any specific genetic population structure in all the zones indicates for the expansion history only from one common source population i.e. NWPZ, a mega zone of wheat production in India. Overall, it seems that the predominance of individual haplotypes with a moderate level of genetic variation and men mediated movement of contaminated seed and dispersal of inoculum, mutations and recombination as prime evolutionary processes play essential role in defining the genetic structure of BS population.

Introduction

Wheat (*Triticum aestivum* L.) is acknowledged as a major cereal crop and staple food source for billions of people all over the globe. Spot blotch (SB) caused by *Bipolaris sorokiniana* (Sacc.) Shoem is documented as one of the most important diseases that affects wheat production worldwide (Devi et al. 2018; Ayana et al. 2018; Singh et al. 2014). The disease starts as small, dark brown lesions without chlorotic margins on leaves at initial stage of attack (Chand et al. 2003). Later on, these lesions turned oblong to elongated and light brown to blackish brown areas. In addition to lesions and blotches on foliage, the fungus is documented to cause seedling blight, root rot, and seed rot or black point on the embryo (Hudec and Muchova 2008; Kumar et al. 2002; Wildermuth et al. 1997). It has been estimated that the pathogen may cause up to 80% yield loss (Aggarwal et al. 2019), but it can also lead to a total
crop loss in conducive environment (Sharma and Duveiller 2007; Mehta et al. 1992). Joshi and Chand (2002) reported 15.5% yield loss due to leaf blotch in India. Leaf blotch pathogen is difficult to manage by fungicides because of its seed and soil borne nature. At present, host resistance is the only viable and practical approach for minimizing economic losses caused by BS in wheat. Thus, understanding genetic diversity of BS will be useful for breeding durable resistant wheat cultivars.

A large number of studies have revealed the significance of internal transcribed spacer (ITS) region (a nuclear rDNA repeat unit) in resolving their fungal taxonomic status at the genus and species level (Kashyap et al. 2017; Zhang et al. 2017; Kashyap et al. 2016; Katoch et al. 2016; Rai et al. 2016; Nilsson et al. 2009; Wickert et al. 2012). Glass and Donaldson (1995) reported high rate of evolution in ITS1 and ITS2 spacers and provided strong evidence for their deployment as an evolutionary marker for dissecting inter- and intra-specific variations. The rDNA sequences display variation within species, generally as a consequence of insertion or deletion or nucleotide substitutions without major alterations in their functionality (Kashyap et al. 2020a). Several researchers explored rDNA sequences to construct phylogeny for the determination of genetic relatedness among related taxa (Raja et al. 2017; James et al. 2006; Karol et al. 2001; Medina et al. 2001; Woese et al. 1990). Thus, the comparative analysis of ITS sequences is advantageous for understanding the phylogenetic linkages among various groups of fungi including Bipolaris species (Sonavane et al. 2015; Manamgoda et al. 2014).

Bipolaris sorokiniana has a wide host range (e.g. Avena sativa, Sorghum bicolor, Zea mays, Hordeum vulgare, Vigna radiata, Vigna mungo, Lens culinaris, Glycine max, Brassica komestris, Sesamum indicum, and Pennisetum amaricanum) and massive variation in pathogenic isolates (Verma et al. 2020; Sultana et al. 2018; Mann et al. 2014; Pandey et al. 2008; Iftikhar et al. 2001). A number of reports on the pathogenic variability of BS isolates derived from wheat hosts in various parts of the globe have been published (Sultana et al. 2018; Muller et al. 2005; Adhikary and Mian 2005; Oliveira et al. 1998; Ahmed et al. 1997). Despite huge economic importance of disease in India, sparse information is available on the genetic variability in Indian population of B. sorokiniana. Aggarwal et al. (2009) documented the occurrence of five pathotypes of B. sorokiniana on wheat in India. Later on, several reports on the intraspecific variations in B. sorokiniana isolates affecting wheat on the basis of morphology, virulence and molecular characters have been published (Ashwini and Patil 2018; Yadav et al. 2013; Aggarwal et al. 2010). However, there is still no clear picture regarding the genetic variation of B. sorokiniana in different wheat growing zones of India. Such analysis would also be important to reveal eventual gene flow among populations, as well as better understanding the biology of the pathogen (e.g. sexual recombination).

Distribution and diversity of pathogenic population over a large area is an essential criterion for disease management (Kumar et al. 2019; Mahapatra and Das 2013; Rampersad et al. 2013; McDonald and Linde 2002). Therefore, the current research is the first attempt to (i) analyze the genetic and phylogenetic relationships between five different populations of B. sorokiniana isolates from wheat growing zones of India and (ii) elucidate their demographic expansion by generating and comparing ITS gene sequence data.
Materials And Methods

Plant samples and molecular analysis

Wheat leaves showing typical spot blotch symptoms were gathered from five major agro ecological wheat growing zones [North Western Plain Zone (NWPZ), North Eastern Plain zone (NEPZ); North Hill Zone (NHZ), SZ: Southern Hill Zone (SHZ) and Peninsular Zone (PZ)] of India during regular annual wheat crop health surveys. Sampling was done using stratified random sampling method (transect sampling by walking through the field) from seven to ten transect, at least 10 m apart, in each field. Samples in the form of diseased leaf tissue were gathered around flowering time, dried in blotting paper and brought to the laboratory for isolation. Surface sterilization of a small section of infected leaf tissue was executed in 2% solution of sodium hypochlorite (NaOCl) for 15 seconds followed by three consecutive washes with sterilized water under laminar air flow. The treated plant tissue was dried with the help of a sterilized filter paper and shifted to water agar (1%) slants before sub-culturing mycelium on potato dextrose agar (PDA; HiMedia, India) to obtain pure cultures. The inoculated glass tubes were placed in a biological oxygen demand (BOD) incubator at 25±2 °C for 7 days. Single spore isolation technique (Zhang et al. 2013) was employed for the purification of fungal isolates and growth initiated from single spore was cultivated on PDA medium and used for the research experiments.

DNA extraction, PCR amplification and rDNA sequencing

Mycelia from 7 days old potato dextrose broth (25 ± 1 °C) were collected by passing through a double layer of the filter paper. The collected filtrate of fungal mass was dried by placing between double layered Whatman's sterilized filter paper in laminar air flow cabinet and stored at -20 °C for later use. Total genomic DNA of twenty seven BS isolates collected from different zones (Table S1) was extracted using CTAB method (Kumar et al. 2013) with minor modifications. The quantification of total genomic DNA was done by using UV/VIS spectrophotometer (Smart Spec 3000, Bio-Rad) and stored at -20 °C until use. The amplification of fungal rDNA was performed in Biometra Trios (Analytic jena, Germany). The composition of PCR master mix (25 µl) include:12.5 µl of Go Taq Green master mix (Promega Biotech India Pvt. Ltd), 1 µl of template DNA (50 ng µl⁻¹) and 1 µl of ITS1 and ITS4 primers (10 µM) (White et al. 1990). Double distilled sterilized water was added to adjust the total volume at 25 µl. The thermal amplification profile was: initial denaturation at 95 °C for 120 seconds followed by 35 cycles at 95 °C for 60 seconds, 60 °C for 30 seconds, 72 °C for 60 seconds and a final extension at 72 °C for 10 min. The PCR product was resolved in 1.2 % agarose gel in 1X Tris-acetate-EDTA buffer for 50 min at 80 V with 100 bp DNA ladder (Bangalore Genei, India). The amplification image was taken in a gel documentation system (Alpha imager 2200, USA). The generated amplicon (~500 bp) was freeze dried and customized sequencing was performed by using same set of forward and reverse primers (Eurofins, India). Twenty seven ITS sequences generated in the current study were combined with 156 ITS sequences of BS obtained from National Center for Biotechnology (http://www.ncbi.nlm.nih.gov) for the analysis (Table S1).
Phylogenetic analysis

The sequences of BS isolates submitted from India were retrieved from the NCBI genbank and matched with BS isolates sequenced in current study (Table S1). Analysis of the nucleotide sequences was executed with BLAST sequence algorithms and sequences alignment was done with BioEdit (Hall 1999) using Clustal W algorithm (Thompson et al. 1994). The phylogenetic relationship was inferred by maximum Likelihood (ML) algorithms implemented in MEGA (molecular evolutionary genetic analysis) software version 7 (Kumar et al. 2016). The best-fit model of sequence evolution was chosen on the basis of Akaike Information Criterion (AIC) from Model Test (Posada and Crandall 1998). Bootstrap analysis (1000 replications) was executed to evaluate the confidence level of each node.

Gene genealogies analysis

The population genetic analysis was performed with 183 ITS nucleotide sequences of BS isolates. All the sequences either retrieved from GenBank or sequenced in present study belonged to the five wheat growing zones (Fig 1). Population diversity indicators, such as haplotype (h), haplotype diversity (Hd), nucleotide diversity (π), number of segregating sites (S), average number of pairwise nucleotide differences within population (K) and recombination events (Rm) were estimated by DnaSP version 5 software (Librado and Rozas 2009). The neutrality statistics of Fu's F test (Fu 1997) and Tajima's D (Tajima 1989) in each population was also calculated. Statistical tests and confidence intervals for D and F's were based on a coalescent simulation algorithm. The Tajima's D test (Tajima 1989) is based on comparison of the allelic frequency of segregating nucleotide sites. A positive value of this test indicates a bias towards intermediate frequency alleles, while negative value indicates a bias towards excess of the number of rare alleles and the latter being reflected a case of recent population expansion. Fu's FS test (Fu 1997) is based on the alleles or haplotypes distribution, where negative values indicate an excess number of alleles, reflecting a recent population expansion or from genetic hitchhiking. Nucleotide substitution per site (Dxy), average number of pairwise nucleotide differences (Kxy), and net nucleotide substitution per site (Da) between populations were also calculated (Librado and Rozas 2009). Gene flow (Nm) between pairs of populations was calculated based on the Fst values using the formula: Nm = (1 - Fst)/4 Fst). Haplotype network analysis was performed for detecting various links between haplotypes and finding probable missing mutational links. The median-joining method was employed to construct networks with Network 5.0 software (Bandelt et al. 1999). All the test aspects were evaluated against the anticipated score under the assumption of a recent population expansion and bootstrap analysis was performed with 1000 replications.

Results

Phylogenetic analysis

Phylogenetic analysis based on ITS nucleotide sequences of BS isolates revealed 98-100% similarity among each other and to those of reference B. sorokiniana isolates from wheat deposited in the NCBI
database. The ITS gene sequences of Indian isolates of BS (Table 1) were employed to draw a phylogenetic tree (Fig. 2). Cluster I consisted of 156 isolates, representing all the five wheat growing zones of India. Several sub-groups were noticed within this cluster, indicating the existence of genetic variability within isolates. Cluster II included 27 isolates representing populations from NWPZ, NHZ, PZ and NEPZ zones (Fig. 2). No geographic structuring of the BS isolates was observed.

**Genetic divergence**

Different measures were employed to decipher the genetic variability of BS population in each zone (Table 2). BS population representing NEPZ had maximum segregating sites in ITS sequences (S=312) than those from NHZ (S = 297), PZ (S = 275) and NWPZ (S = 272). The maximum number of mutations was observed in NEPZ (Eta = 437) followed by NWPZ (Eta = 398), NHZ (Eta = 376), PZ (Eta = 292) and SHZ (Eta=2). Haplotype diversity was highest for NHZ (Hd = 0.95238) followed by NEPZ (Hd = 0.88235), PZ (Hd = 0.75758), NWPZ (Hd = 0.6332) and SHZ (Hd = 0). Only one haplotype (H_3) was shared among all the populations followed by H_25 and H_2, which was shared by the populations of three (NEPZ, PZ and NWPZ) and two zones (PZ and NWPZ), respectively (Table 1). High rate of recombination events (R_m) were observed in NEPZ (R_m = 23), NWPZ (R_m = 20) and NHZ (R_m = 11) populations, recombination events were absent in SHZ and PZ populations (Table 2).

The BS population from NEPZ showed maximum number of nucleotide differences (k = 145.5) followed by PZ (k = 130.667), NHZ (k = 119.381), NWPZ (k = 24.289) and SHZ (k = 0.80). Similarly, higher nucleotide diversity (Pi) was observed in NEPZ (Pi=0.35575) in comparison to NHZ (Pi=0.29697), PZ (Pi = 0.29496), NWPZ (Pi = 0.06980) and SHZ (Pi = 0.00142) populations. The values of Watterson's theta (θ sequence⁻¹ and site⁻¹) were found maximum for NHZ followed by NEPZ, PZ, NWPZ and SHZ (Table 2).

The maximum average number of nucleotide substitutions site⁻¹ (D_xy=0.395) and inter population nucleotide differences (K_xy= 123.778) in BS populations were found between NHZ and SHZ, whereas these were minimum between NWPZ and SHZ (Table 3). The higher rate of genetic differentiation based on haplotype frequency was observed between NHZ and SHZ populations (G_st = 0.243), whereas it was minimum among NWPZ and NEPZ populations (G_st = 0.019) (Table 3). The genetic distance (F_st) between different BS populations varied from -0.062 (NEPZ and NHZ) to 0.563 (NEPZ and SHZ). Moreover, no significant difference was detected in these populations. Similarly, gene flow (N_m) ranged from 0.54 (NEPZ and SHZ) to 14.32 (NEPZ and NWPZ) (Table 4).

Neutrality (Tajima's D and Fu and Li's F* tests) estimates were calculated to understand the population expansion of BS population in different zones. In this study, score of Tajima's D was negative for NWPZ, NHZ, SHZ and PZ populations, but statistically non-significant except NWPZ (P < 0.05 and P < 0.01). Only NEPZ and SHZ had non-significant and positive Tajima's D values. Similarly, F* statistic was also found non-significant and negative for NWPZ, SHZ and PZ populations. The overall negative values resulting from both tests for NWPZ, NHZ, SHZ and PZ populations signifies an excess of low frequency haplotypes relative to expectation, indicating BS population size expansion and positive selection. Contrarily, positive
F* statistic of NEPZ and NHZ (Table 2) indicated low levels of both low and high frequency haplotypes, indicating BS population size decline and balancing selection.

**Genealogic relationships**

The genealogic relationships among BS isolates determined by DnaSP and network software detected one dominant haplotype (H_3). All the remaining haplotypes were significantly less frequent (Fig. 3). The H_3 haplotype was reported in NWPZ (60.56%), NHZ (14.28%), PZ (25%), SHZ (100%), and NHZ (35.27%) populations. Similarly, H_2 haplotype was noticed in NWPZ (60.56%), PZ (25%) and NEPZ (11.76%) populations, while H_25 was observed in NWPZ (0.70%) and PZ (41.66%) populations, only. The Hap_36 was reported from NWPZ (2.12%), NEPZ (9.09%) and NHZ (16.66%). Majority of the haplotypes occur once in the same populations. Only four haplotypes (H_3, H_2, H_25 and H_36) shared among populations. H_3 haplotype shared wide geographical distribution and prevalent in all the zones; H_25 was observed in three zones (NWPZ, NEPZ and PZ), whereas the Hap_2 was noticed only in two zones (NWPZ and PZ). The three major haplotypes (H_2, H_3 and H_25) displayed several individual haplotypes in a star-like pattern, revealing high frequency of the distinctive mutations (Fig. 3).

**Discussion**

*B. sorokiniana* fungus causing spot blotch raised serious concerns especially with regards to wheat production because BS invasions are well documented in all the wheat-producing zones of India (Mahapatra et al. 2020; Gupta et al. 2018; Singh et al. 2014; Jaiswal et al. 2007). Several approaches including host resistance are employed to manage the spot blotch pathogen in wheat. However, these measures have not produced adequate results so far. The information on genetic variation of BS is highly valuable in identifying and characterizing resistant germplasm, deploying cultivars with different resistance genes and analyzing the occurrence of new pathotypes/races. It is worth to mention here that an effective resistance breeding programme relies heavily on the use of genetically diverse pathogen population instead of an individual isolate. Hence, it becomes vital to decipher the genetic variation of BS in natural population in search of good management tactics. Therefore, in the current study, intra-specific genetic analysis of *B. sorokiniana* was performed to determine the evolution and phylogeography in five different wheat growing zones of India. ITS based phylogenetic analysis suggests that all the 183 BS isolates grouped in two major clades. This observation is well correlated with extrapolations drawn from median joining network and haplotypic data generated in the present study. Haplotype diversity represents a collective effect of mutation, marker ascertainment, recombination and demography (Zhang et al. 2017; Stumpf 2004). In the present study, 59 haplotypes have been observed in a group of 183 isolates based on ITS sequences with largest haplotype H_3 comprised of 101 individuals (55.19 % of total population) followed by H_2 (3.27% of total population) and H_25 (3.27% of total population) with distinct geographical origin. These results clearly established the frequent occurrence of gene flow (N_m) among the ecological divergent BS populations.
The results of median joining network analysis reveals that majority of BS isolates are unique and zone-specific. Although, there is an indication of specific evolution of haplotypes (H_3, H_2, H_25 and H_36) with high gene flow in BS populations, the H_2 and H_25 haplotypes of PZ and NEPZ seem to have been originated from the common haplotype H_3, since the identical haplotype was also observed in the BS population from these areas. Similarly, H_47-54 and H_56-59 were found unique and specific to NEPZ and NHZ populations, respectively. Thus, there is possibility that these populations might have idem from H_2 haplotype, signifying BS population from PZ, NEPZ and NWPZ due to the close geographic proximity of the zones. Further, there is also significant probability of recent human driven movement of the fungus through contaminated wheat material and dispersal of inoculum originated from other hosts like barley and weeds, followed by rapid host adaptation with a probability for wide spatial dissemination through airborne conidia (Gupta et al. 2018; Malaker et al. 2008) to the present structure of BS haplotypes.

The values of haplotype diversity (H_d) ranged from zero (no diversity) to 1.0 (high levels of haplotype diversity) in any population (Rampersad et al. 2013). In the current study, value of H_d revealed high level of diversity in all the zones. It is worth to mention here that several haplotypes showed only a different at one site compared to their genetically closest haplotype, confirming an important role of mutation in creating haplotype diversity as also reported by earlier workers in case of different fungal crop pathogens (Kashyap et al. 2019; Yang et al. 2018; Brunner et al. 2008). Moreover, presence of minimum number of recombination events in NWPZ, NEPZ and NHZ, reflects intragenic recombination, which may lead to variability in pathogen population in these zones. Similar observations have been reported by Zhang et al. (2017). It is important to mention here that B. sorokiniana is a heterothallic fungus and reproduces asexually in most of the places except Zambia (Raemakers 1991). The existence of two distinct mating types was suggested by Wen and Lu (1991) in isolates of B. sorokiniana. So far, there is no scientific evidence advocating the occurrence of two different mating types in B. sorokiniana isolates and their role in sexual reproduction in India.

Tajima's D values reveal how much variation in BS population can be sustained over span of time (Roussel et al. 2014). The significant and negative score of two neutrality (Fu's Fs and Tajima's D) test statistics of NWPZ, PZ and NHZ populations highlighted that the BS population do not follow neutrality model and therefore, discard the assumption of constant population size. However, positive Tajima's D reflected the role of population reduction and balancing selection (Pichler 2002). The positive neutrality indices were observed in case of NEPZ and NHZ population, pointing to different demographic histories of these populations relative to other zones. Similar observations regarding the geographic distribution pattern of Plasmopara viticola causing downy mildew on cultivated and wild grape have been reported from China and North America (Zhang et al. 2017; Rouxel et al. 2014). A star like shape of median joining network observed in the present study also provides an evidence to support that the assumption of deviation from neutrality for constant or uniform population size (Fu's Fs and Tajima's D) due to contemporary population expansion of BS fungus. Thus, domestication, introduction, and continuous cultivation of host plants of B. sorokiniana could be the plausible explanation for topical population expansion in different wheat growing zones of India. Such type of fungal population movement has been documented
in different types of fungal pathogens (Kashyap et al. 2020b; Dietzel et al. 2019; Katoch et al. 2016; Linde et al. 2010; Zaffarano et al. 2008).

Mutational processes, recombination and gene flow from different populations could generate new pathotypes with better pathological and biological fitness traits (Mahalingam et al. 2020; Kashyap et al. 2020c; Eschenbrenner et al. 2020; Mishra et al. 2006). The present study clearly documented a high rate of gene flow ($N_m$) and absence of population distinctiveness among BS population in different zones. At the same time, genetic differentiation and geographic distance were not interrelated, so isolation by distance does not seem to be a barricade for gene flow in different wheat growing zones of India.

Moreover, the lack of population structure in B. sorokiniana indicates that gene flow is presently occurring or has occurred historically between the BS populations from wild and cultivated weeds hosts due to its wide host range (Singh et al. 2016; Manamgoda et al. 2011; Sultan and Ahmad 2009; Hobbs and Morris 1996) and the secondary spread through air-borne spores or conidia (Duveiller et al. 2005). The present study also provides evidence regarding the role of recombination and population admixture leading to rapid adaptation of BS population in NWPZ, NEPZ and NHZ, as minimum number of recombination events at ITS loci has been observed in these zones. However, the absence of recombination events in the SZ and PZ populations indicates the role of the main gene flow between populations causing the mixing of populations as the BS fungus spreads from one zone to another.

With respect to genetic population structure of B. sorokiniana, topical population expansion in different wheat growing zones was identified. The findings also suggest the probability of dissemination of B. sorokiniana in recent past along with wheat seed movement as one of the haplotypes found prominent across the wheat growing zones. Therefore, fungicide-treated seed transport across zones could be advantageous to reduce BS severity and limit gene flow. In addition, identification and characterization of new sources of resistance will help the breeding efforts against BS from India.

Declarations

Author Contributions

PK and SK conceived and designed the work and drafted the manuscript. PK, SK, SM and PJ performed the sampling survey. PK and AS executed computational analysis of the data sets. AS and RSK executed laboratory experiments. SK, PK, SM and GS performed the final editing and proofing of the manuscript. All authors contributed to the article and approved the submitted version.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Tables
Table 1  Accession numbers of nucleotide sequences of internal transcribed spacer (ITS) region of *Bipolaris sorokiniana* isolates collected from different wheat growing zones of India

| Agro climatic zone | Isolate | Region/State | NCBI Accession No. | Year of collection | Haplotype(Hap) |
|--------------------|---------|--------------|-------------------|--------------------|----------------|
| A. NWPZ (N=142)    | BHURC 8 | Uttar Pradesh | MH209057          | 2018               | H_3            |
|                    | B-1-1  | Uttar Pradesh | MH209002          | 2018               | H_3            |
|                    | A16     | Uttar Pradesh | MK676001          | 2019               | H_3            |
|                    | A14     | Uttar Pradesh | MK676000          | 2019               | H_3            |
|                    | UASBW   | Uttar Pradesh | MH209071          | 2018               | H_3            |
|                    | BHURC 9 | Uttar Pradesh | MH209068          | 2018               | H_3            |
|                    | S-13    | Uttar Pradesh | MH209052          | 2018               | H_3            |
|                    | HP-1744 | Uttar Pradesh | MH209051          | 2018               | H_3            |
|                    | BS-27   | Uttar Pradesh | DQ229950          | 2005               | H_3            |
|                    | K 1012  | Uttar Pradesh | MH208976          | 2018               | H_3            |
|                    | WR 109  | Uttar Pradesh | MH208977          | 2018               | H_4            |
|                    | D5      | Uttar Pradesh | MH208978          | 2018               | H_3            |
|                    | PUSA 2  | Uttar Pradesh | MH208979          | 2018               | H_4            |
|                    | BHURC 3 | Uttar Pradesh | MH208980          | 2018               | H_3            |
|                    | W2      | Uttar Pradesh | MH208981          | 2018               | H_3            |
|                    | HUW206  | Uttar Pradesh | MH208982          | 2018               | H_3            |
|                    | BD 2503 | Uttar         | MH208983          | 2018               | H_4            |
| BHURC 5 | Uttar Pradesh | MH208984 | 2018 | H.4 |
|---|---|---|---|---|
| B-4-2-12 | Uttar Pradesh | MH209050 | 2018 | H.3 |
| OASIS | Uttar Pradesh | MH208989 | 2018 | H.4 |
| BHURC 7 | Uttar Pradesh | MH208985 | 2018 | H.3 |
| K8020 | Uttar Pradesh | MH208986 | 2018 | H.3 |
| B-1-3 | Uttar Pradesh | MH208987 | 2018 | H.3 |
| N11538 | Uttar Pradesh | MH208988 | 2018 | H.3 |
| B-8-3 | Uttar Pradesh | MH208992 | 2018 | H.6 |
| UBS 12 | Uttar Pradesh | MH208993 | 2018 | H.3 |
| UBS 3 | Uttar Pradesh | MH208994 | 2018 | H.3 |
| HC-B | Uttar Pradesh | MH208995 | 2018 | H.3 |
| BHURC 6 | Uttar Pradesh | MH208996 | 2018 | H.3 |
| K1913 | Uttar Pradesh | MH208997 | 2018 | H.3 |
| MDSN222 | Uttar Pradesh | MH208998 | 2018 | H.7 |
| GW 433 | Uttar Pradesh | MH208999 | 2018 | H.8 |
| S-11-3 | Uttar Pradesh | MH209000 | 2018 | H.6 |
| HUW 55 | Uttar Pradesh | MH209001 | 2018 | H.9 |
| UBS 14 | Uttar Pradesh | MH209003 | 2018 | H.3 |
| WBI-4 | Uttar Pradesh | MH209004 | 2018 | H.10 |
| Code     | Description         | State  | Code     | Description         | State  |
|----------|---------------------|--------|----------|---------------------|--------|
| HD2403   | Uttar Pradesh       |        | MH209005 | 2018               | H_3    |
| BHURC 2  | Uttar Pradesh       |        | MH209006 | 2018               | H_3    |
| SUJATA   | Uttar Pradesh       |        | MH209007 | 2018               | H_3    |
| DBW14    | Uttar Pradesh       |        | MH209008 | 2018               | H_3    |
| BHURC 10 | Uttar Pradesh       |        | MH209009 | 2018               | H_11   |
| PBW 352  | Uttar Pradesh       |        | MH209010 | 2018               | H_3    |
| MDSN 86D | Uttar Pradesh       |        | MH209011 | 2018               | H_3    |
| SONALIKA | Uttar Pradesh       |        | MH209012 | 2018               | H_12   |
| HD 3094  | Uttar Pradesh       |        | MH209013 | 2018               | H_13   |
| LOK 31   | Uttar Pradesh       |        | MH209014 | 2018               | H_3    |
| PBW 671  | Uttar Pradesh       |        | MH209015 | 2018               | H_3    |
| DD-1-3   | Uttar Pradesh       |        | MH209016 | 2018               | H_3    |
| S-7-3    | Uttar Pradesh       |        | MH209017 | 2018               | H_3    |
| SEED 28  | Uttar Pradesh       |        | MH209018 | 2018               | H_11   |
| HUW 648  | Uttar Pradesh       |        | MH209019 | 2019               | H_11   |
| HUW 234  | Uttar Pradesh       |        | MH209020 | 2018               | H_3    |
| BLACK isolate | Uttar Pradesh |        | MH209021 | 2018               | H_14   |
| S 13-3   | Uttar Pradesh       |        | MH209022 | 2018               | H_15   |
| SEED R2  | Uttar Pradesh       |        | MH209023 | 2018               | H_3    |
| HD 3091  | Uttar Pradesh       |        | MH209024 | 2018               | H_3    |
| Variety     | Origin      | Accession | Year | Status |
|------------|-------------|-----------|------|--------|
| BARLEY-3   | Uttar Pradesh | MH209025 | 2018 | H_3    |
| 70B-1002   | Uttar Pradesh | MH209026 | 2018 | H_16   |
| S-13-4-C25 | Uttar Pradesh | MH209027 | 2018 | H_17   |
| CG80001    | Uttar Pradesh | MH209028 | 2018 | H_3    |
| HD 2329    | Uttar Pradesh | MH209029 | 2018 | H_3    |
| B 7        | Uttar Pradesh | MH209030 | 2018 | H_3    |
| S-913-14   | Uttar Pradesh | MH209031 | 2018 | H_3    |
| BR 3705    | Uttar Pradesh | MH209032 | 2018 | H_3    |
| MP-1266    | Uttar Pradesh | MH209033 | 2018 | H_3    |
| K1013-1    | Uttar Pradesh | MH209034 | 2018 | H_18   |
| BHU RC 1   | Uttar Pradesh | MH209035 | 2018 | H_3    |
| HUW 16     | Uttar Pradesh | MH209036 | 2018 | H_19   |
| LOK 67-19-2| Uttar Pradesh | MH209037 | 2018 | H_20   |
| UBS 1      | Uttar Pradesh | MH209038 | 2018 | H_3    |
| RSP 561    | Uttar Pradesh | MH209039 | 2018 | H_3    |
| MDSN 76 D  | Uttar Pradesh | MH209040 | 2018 | H_21   |
| HD 2888    | Uttar Pradesh | MH209041 | 2018 | H_3    |
| T-2-3      | Uttar Pradesh | MH209042 | 2018 | H_22   |
| T-1        | Uttar Pradesh | MH209043 | 2018 | H_23   |
| RAJ 4252   | Uttar         | MH209044 | 2018 | H_24   |
| Strain   | Origin   | Accession  | Year | Type |
|----------|----------|------------|------|------|
| HP-1493  | Uttar Pradesh | MH209045    | 2018 | H_3  |
| D-6-1-6  | Uttar Pradesh | MH209046    | 2018 | H_25 |
| MP1261   | Uttar Pradesh | MH209047    | 2018 | H_3  |
| HI 1538  | Uttar Pradesh | MH209048    | 2018 | H_3  |
| B-10-A   | Uttar Pradesh | MH209049    | 2018 | H_3  |
| HI 1461  | Uttar Pradesh | MH209053    | 2018 | H_26 |
| KO-5803  | Uttar Pradesh | MH209054    | 2018 | H_27 |
| BARLEY 356 | Uttar Pradesh | MH209055    | 2018 | H_28 |
| W        | Uttar Pradesh | MH209056    | 2018 | H_29 |
| RAJ 3975 | Uttar Pradesh | MH209058    | 2018 | H_30 |
| UBS 5    | Uttar Pradesh | MH209059    | 2018 | H_31 |
| UBS 4    | Uttar Pradesh | MH209060    | 2018 | H_3  |
| UBS 9    | Uttar Pradesh | MH209061    | 2018 | H_3  |
| UBS 7    | Uttar Pradesh | MH209064    | 2018 | H_32 |
| D6899    | Uttar Pradesh | MH209065    | 2018 | H_33 |
| K0911    | Uttar Pradesh | MH209066    | 2018 | H_3  |
| MDSN24 D-16 | Uttar Pradesh | MH209067    | 2018 | H_3  |
| T7-11    | Uttar Pradesh | MH209069    | 2018 | H_21 |
| UAS390   | Uttar Pradesh | MH209070    | 2018 | H_34 |
|    |          |          |       |     |
|----|----------|----------|-------|-----|
| 112 | Uttar Pradesh | KU201275 | 2015  | H_35 |
| HD 3065 | Uttar Pradesh | MH208990 | 2018  | H_5  |
| RAJ 3972 | Uttar Pradesh | MH208991 | 2018  | H_4  |
| UBS - 2 | Uttar Pradesh | MH209063 | 2018  | H_3  |
| UBS-10  | Uttar Pradesh | MH209062 | 2018  | H_3  |
| WLB-18-43 | Uttar Pradesh | MN535888 | 2006  | H_36 |
| WLB-10-3 | Uttar Pradesh | MK809553 | 2019  | H_1  |
| WLB-18-6 | Uttar Pradesh | MK809554 | 2019  | H_2  |
| WLB-17-13 | Uttar Pradesh | MN535891 | 2006  | H_37 |
| WLB-18-24 | Uttar Pradesh | MK809544 | 2019  | H_1  |
| PSWBSs-11 | Haryana   | KT884113 | 2015  | H_3  |
| PSWBSsb-23 | Haryana   | KT884125 | 2015  | H_3  |
| PSWBSb-21 | Haryana   | KT884123 | 2015  | H_3  |
| PSWBSb-16 | Haryana   | KT884118 | 2015  | H_3  |
| PSWBSb-17 | Haryana   | KT884117 | 2015  | H_3  |
| PSWBSb-14 | Haryana   | KT884116 | 2015  | H_3  |
| PSWBSb-7  | Haryana   | KT864933 | 2015  | H_3  |
| PSBSb-1   | Haryana   | KT864928 | 2015  | H_3  |
| PSBSb-4   | Haryana   | KT864930 | 2015  | H_3  |
| PSBSb-5   | Haryana   | KT864931 | 2015  | H_3  |
| PSBSb-3   | Haryana   | KT864929 | 2015  | H_3  |
| PSWBSb-8  | Haryana   | KT864934 | 2015  | H_3  |
| Code     | State         | Accession | Year | Strain Code |
|----------|---------------|-----------|------|-------------|
| PSWBSb-9 | Haryana       | KT864935  | 2015 | H_38        |
| PSWBSb-12| Haryana       | KT884114  | 2015 | H_3         |
| PSWBSb-13| Haryana       | KT884115  | 2015 | H_3         |
| PSWBSb-17| Haryana       | KT884119  | 2015 | H_39        |
| PSWBSb-18| Haryana       | KT884120  | 2015 | H_3         |
| PSWBSb-19| Haryana       | KT884121  | 2015 | H_3         |
| PSWBSb-20| Haryana       | KT884122  | 2015 | H_3         |
| PSWBSb-22| Haryana       | KT884124  | 2015 | H_3         |
| BS-5     | Haryana       | DQ286764  | 2005 | H_3         |
| BS-7     | Haryana       | GU345084  | 2009 | H_3         |
| BS-03    | Haryana       | HM195250  | 2010 | H_3         |
| PSWSb-6  | Haryana       | KT864932  | 2015 | H_3         |
| Strain 53| Delhi         | GU480767  | 2010 | H_40        |
| B28      | Delhi         | KF725803  | 2013 | H_44        |
| B 41     | Delhi         | KF725816  | 2013 | H_41        |
| B 39     | Delhi         | KF725814  | 2013 | H_43        |
| B 7      | Delhi         | KF725782  | 2013 | H_42        |
| BS 50    | Delhi         | HM195254  | 2010 | H_3         |
| 64       | Delhi         | GU480768  | 2010 | H_3         |
| WLB-18-14| Delhi         | MK809548  | 2019 | H_45        |
| ASW 3    | Jammu & Kashmir| MK075017  | 2018 | H_46        |
| AS 1     | Jammu & Kashmir| MK075008  | 2018 | Hp_46       |
| BS-9     | Rajasthan     | DQ229952  | 2005 | H_3         |
| BS-18    | Rajasthan     | DQ242475  | 2005 | H_3         |
| BS-25    | Rajasthan     | DQ286763  | 2005 | H_3         |
### B. PZ (N=12)

| Location | State      | Accession   | Year | Code |
|----------|------------|-------------|------|------|
| Dharwad  | Karnataka  | KJ562719    | 2010 | H_3  |
| BS 72    | Karnataka  | HM195258    | 2010 | H_3  |
| A        | Maharastra | KJ562718    | 2014 | H_25 |
| L        | Maharastra | KJ562717    | 2014 | H_25 |
| D2       | Maharastra | KJ562716    | 2014 | H_25 |
| J        | Maharastra | KJ562715    | 2014 | H_25 |
| HD 3069  | Maharastra | KJ562714    | 2014 | H_25 |
| WLB-18-20| Maharastra | MK809545    | 2019 | H_55 |
| WLB-18-5 | Maharastra | MK809546    | 2019 | H_2  |
| WLB-18-8 | Maharastra | MK809555    | 2019 | H_2  |
| WLB-18-7 | Maharastra | MK809556    | 2019 | H_2  |
| BS-69    | Maharastra | HM195257    | 2010 | H_3  |

### C. SHZ (N=5)

| Accession | Year | Code |
|-----------|------|------|
| BS-75     | 2010 | H_3  |
| BS-77     | 2010 | H_3  |
| BS 79     | 2010 | H_3  |
| BS 92     | 2010 | H_3  |
| SHZ-Bs 3  | 2019 | H_3  |

### D. NEPZ (N=17)

| Location | State      | Accession   | Year | Code |
|----------|------------|-------------|------|------|
| WLB-18-11| Bihar      | MK809562    | 2019 | H_47 |
| WLB-18-12| Bihar      | MN535890    | 2019 | H_36 |
| WH.PBW.IP.04 | West Bengal | KM066949 | 2014 | H_3  |
| WLB-18-13| West Bengal| MK809551    | 2019 | H_48 |
| WLB-17-2 | West Bengal| MK809564    | 2019 | H_53 |
| WLB-17-1 | West Bengal| MK809563    | 2019 | H_52 |
| Bs 41    | West Bengal| HM195251    | 2010 | H_3  |
| BS-42    | West Bengal| HM195252    | 2010 | H_3  |
| WLB-18-31| West Bengal| MK809558    | 2019 | H_50 |
| Code   | Region       | Accession  | Year | Zone |
|--------|--------------|------------|------|------|
| WLB-18-9 | West Bengal | MK809561   | 2019 | H_51 |
| WLB-17-47 | West Bengal | MN535889   | 2019 | H_54 |
| WLB-18-22 | West Bengal | MK809565   | 2019 | H_2  |
| WLB-18-33S | West Bengal | MK809566   | 2019 | H_2  |
| WLB-18-10 | West Bengal | MK809557   | 2019 | H_49 |
| BS-47   | West Bengal | DQ367884   | 2010 | H_3  |
| BS-48   | Assam       | GU480766   | 2010 | H_3  |
| BS-49   | Assam       | HM195253   | 2010 | H_3  |
| **E. NHZ (N= 7)** | | | | |
| WLB-18-1 | Uttarakhand  | MK809549   | 2019 | H_59 |
| WLB-18-55 | Uttarakhand  | MK809552   | 2019 | H_56 |
| WLB-10-03 | Uttarakhand  | MK809550   | 2019 | H_58 |
| WLB-10-6  | Uttarakhand  | MK809559   | 2019 | H_56 |
| WLB-18-3  | Uttarakhand  | MK809560   | 2019 | H_57 |
| BS-55    | Uttarakhand  | DQ242476   | 2005 | H_3  |
| WLB-17-55 | Uttarakhand  | MN535892   | 2006 | H_36 |

Bold letters indicate the gene accession number generated in this study; NWPZ: North Western Plain Zone; NEPZ: North Eastern Plain zone; NHZ: North Hill Zone; SHZ: Southern Hill Zone; PZ: Pennisular Zone
Table 2 Summary of the DNA divergence values and neutrality tests calculated for the nucleotide sequences of *B. sorokiniana* isolates population representing different wheat growing zones of India

| Parameter                                      | NWPZ  | NEPZ  | PZ    | SHZ   | NHZ   |
|-----------------------------------------------|-------|-------|-------|-------|-------|
| Number of isolates, N                         | 142   | 17    | 12    | 5     | 7     |
| Number of haplotypes, h                       | 47    | 11    | 4     | 1     | 6     |
| Haplotype diversity, *H*<sub>d</sub>          | 0.63320 | 0.88235 | 0.75758 | 0.000 | 0.95238 |
| Polymorphic (segregating) sites, *S*          | 272   | 312   | 275   | 2     | 297   |
| Total number of mutations, *Eta*              | 398   | 437   | 292   | 2     | 376   |
| Nucleotide diversity, *Pi*                    | 0.06980 | 0.35575 | 0.29496 | 0.00142 | 0.29697 |
| Theta (*site<sup>-1</sup>), *Pi*              | 0.07696 | 0.67674 | 0.48615 | 0.00143 | 0.49163 |
| Theta (*site<sup>-1</sup>), *S*               | 0.24613 | 0.42537 | 0.34190 | 0.00171 | 0.63136 |
| Theta (*site<sup>-1</sup>), *Eta*             | 0.28029 | 0.48688 | 0.28766 | 0.00171 | 0.66627 |
| Theta-W (*site<sup>-1</sup>)                  | 0.14135 | 0.22564 | 0.20556 | 0.00171 | 0.30155 |
| Theta-W (sequence<sup>-1</sup>)               | 49.191 | 92.288 | 91.063 | 0.960 | 121.224 |
| Average number of nucleotide differences, *k* | 24.289 | 145.500 | 130.667 | 0.800 | 119.381 |
| Tajima's *D*                                   | -2.185** | 0.542 | -1.307 | -0.972 | 1.633 |
| Fu and Li'F*                                   | -0.724 | 0.367 | -1.195 | -0.954 | 1.596 |
| Recombination events, *R*<sub>m</sub>         | 20    | 23    | 0     | 0     | 11    |

NWPZ: North Western Plain Zone; NEPZ: North Eastern Plain zone; NHZ: North Hill Zone; SHZ: Southern Hill Zone; PZ: Peninsular Zone; Statistical significance: * P < 0.05; ** P < 0.01; A negative Tajima's D signifies an excess of low frequency polymorphisms relative to expectation, indicating population size expansion or positive selection (Tajima 1983); A negative and significant Fu and Li'F* statistical value provides strong evidence for past population expansion, and rule out the possibility of genetic hitching or background selection, and evolutionary forces that produce a pattern similar to population expansion (Fu 1997)
Table 3 Genetic differentiation measurement between subpopulations from pairwise comparison of *B. sorokiniana* isolates from different zones

| Population 1 | Population 2 | $K_{xy}$ | $D_{xy}$ | $D_{a}$ | $G_{st}$ |
|--------------|--------------|----------|----------|---------|----------|
| NWPZ         | NEPZ         | 119.182  | 0.381    | 0.180   | 0.019    |
| NWPZ         | NHZ          | 123.365  | 0.394    | 0.184   | 0.047    |
| NWPZ         | PZ           | 66.652   | 0.213    | 0.037   | 0.048    |
| NWPZ         | SHZ          | 11.179   | 0.036    | 0.002   | 0.050    |
| NEPZ         | NHZ          | 100.954  | 0.323    | -0.020  | 0.024    |
| NEPZ         | PZ           | 105.647  | 0.338    | 0.030   | 0.036    |
| NEPZ         | SHZ          | 119.294  | 0.381    | 0.215   | 0.111    |
| NHZ          | PZ           | 109.009  | 0.348    | 0.031   | 0.067    |
| NHZ          | SHZ          | 123.778  | 0.395    | 0.219   | 0.243    |
| PZ           | SHZ          | 61.583   | 0.197    | 0.055   | 0.231    |

$K_{xy}$: Average proportion of nucleotide differences between populations; $D_{xy}$: The average number of nucleotide substitutions per site between populations; $D_{a}$: The number of net nucleotide substitutions per site between populations; $G_{st}$: Genetic differentiation index based on the frequency of haplotypes.

Table 4 Pairwise genetic distance ($F_{st}$ in above diagonal) and gene flow ($N_m$ in lower diagonal) between different populations of *B. sorokiniana* calculated from nucleotide sequence of ITS region

| Zone(s) | NWPZ | NEPZ | NHZ | SHZ | PZ   |
|---------|------|------|-----|-----|------|
| NWPZ    |  -   | 0.473**| 0.467**| 0.047**| 0.175 |
| NEPZ    | 14.32 | -     | -0.062| 0.563| 0.087* |
| NHZ     | 5.29 | 10.7  | -   | 0.554| 0.088** |
| SZ      | 4.64 | 1.38  | 0.54 | -   | 0.280* |
| PZ      | 5.37 | 6.67  | 3.83 | 0.90 | -     |

NWPZ: North Western Plain Zone; NEPZ: North Eastern Plain zone; NHZ: North Hill Zone; SHZ: Southern Hill Zone; PZ: Peninsular Zone; $F_{st}$: A coefficient of gene differentiation or fixation index, which measures inter-population diversity; Statistical significance: * P < 0.05; ** P < 0.01

Figures
Figure 1

A map showing five geographically distinct wheat growing zones [North Western Plain Zone (NWPZ), North Eastern Plain zone (NEPZ); North Hill Zone (NHZ), Southern Hill Zone (SHZ) and Peninsular Zone (PZ)] from where samples of the B. sorokiniana were obtained and studied in present research. Number of isolates (N) obtained from each zone indicated in parenthesis.
Figure 2

Molecular phylogenetic analysis of *B. sorokiniana* isolates from different wheat growing zones of India by employing maximum likelihood method based on the Kimura 2-parameter model (Kimura 1980). The percentage of trees in which the associated taxa clustered together is shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. All positions containing gaps and missing data were eliminated. Evolutionary analyses were conducted in MEGA7 (Kumar et al. 2016). Branches with <50% bootstrap support are not shown. Different colour indicates different states/union territory from where *B. sorokiniana* isolates collected. Different colour triangle
represents state/region from where B. sorokiniana isolates were collected. The tree was rooted by outgroup taxon Urocystis agropyri FLS1.

Figure 3

Haplotype network of ITS gene of B. sorokiniana isolates from different wheat growing zones of India. Major circles represent predominant haplotypes. The size of the each circle is proportional to the frequency of the haplotypes. NWPZ: North Western Plain Zone (Yellow circle); NEPZ: North Eastern Plain zone (red circle); NHZ: North Hill Zone (green circle); SHZ: Southern Hill Zone (purple circle); PZ: Pennisular Zone (blue circle). Common haplotypes at the center of a network are inferred to be ancestral, while tip haplotypes at the periphery are derived or descendant from ancestral haplotypes. The occurrence of star-like patterns radiating from the major haplotypes indicates BS population has undergone significant population size expansions in the relatively recent past.