

# Disease Scenario and Virulence Pattern of Major Wheat Pathogens Occurring in Indo-Gangetic Plains of West Bengal, India

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**Abstract:** Owing to climate change, the disease dynamics have been altered across important wheat growing belts of our country. Considering the importance of wheat in West Bengal, a survey was conducted across different districts of the state in the year 2019-20 and 2020-21 for monitoring the present status of foliar diseases of wheat. In the year 2019-20, seven locations across two districts, Nadia and Murshidabad, were surveyed while twenty locations across six districts were surveyed in the following year. In 2019-20, high spot blotch severity (43.21%) was observed in four locations of Nadia district, while Karimpur of Nadia (24.69%) and Jalangi in Murshidabad district recorded lower severity (24.69). Highest leaf rust severity score was recorded in Jalangi, Murshidabad (100 S), while lowest severity was observed in Karimpur Parisha in Nadia district (20 S). In 2020-21, highest spot blotch severity (59.26%) was recorded in two locations (Jalangi, Dhanirpur-Bhaduripara) of Murshidabad district, while lowest disease severity (20 S) was observed in Bara Aduliya (Krishnanagar) of Nadia. Highest rust severity (80 S) was observed in Nadia District while lowest severity (20 S) was recorded in four locations of Nadia (Bara Aduliya, Krishnanagar, Magurakhod, Bhajanghat), one in Birbhum (Tarapith) and Murshidabad (Raninagar). Pathogen identification revealed rust pathotype 77-9 (121R60-1) was most abundant in both the consecutive years, while isolate WB7 of *Bipolaris sorokiniana (*Accession no. MT804348) was most aggressive among the isolates collected. The identified pathotypes can be used further for screening of genotypes.

#### Keywords: Leaf rust, Spot blotch, Survey, Variability, Wheat

Wheat (Triticum aestivum L.) is one of the most significant cereal crops and is the staple for almost 2.5 billion people worldwide. Being blessed and equipped with a diverse agroecological environment, India is the world's second-largest producer of wheat, providing food and nutrition security to the majority of its population through production and consistent supply, notably in the recent past (Sharma and Sendhil 2015). With a share of 13.53% in the global total production of wheat, India is the world's second-largest producer behind China (PIB 2021). Approximately 32 million hectares of wheat were cultivated in India, yielding 110 million tonnes at productivity of 3467 kg per hectare (FAOSTAT 2021). By reaching record and surplus wheat output, coordinated research and multiple food security and development-based programmes in various stages have brought the country closer to realising "food and nutrition for everyone." However, wheat is susceptible to a plethora of biotic stresses, which includes rust and spot blotch.

Spot blotch disease of wheat (caused by *Bipolaris sorokiniana*), and leaf rust (caused by *Puccinia triticina*), are common in warm, humid regions of the world where wheat is grown (Joshi et al 2007 Kumar et al 2020). It causes significant yield loss in warm and humid regions of the world such as China, Africa, Eastern India, Bangladesh, Latin

America and Nepal (Devi et al 2018, Gupta et al 2018). Typically, B. sorokiniana causes symptoms on the stem, sheath, and leaf (Roy et al 2023). Severe infection may also penetrate the spikes, producing low weight, shrivelled grains with a black tip at the embryo end of kernels (Gupta et al 2018). The pathogen heavily relies on favourable environmental factors for disease development (Devi et al 2018, Tamang et al 2021). Extensive research has been conducted to identify and develop resistant and tolerant genotypes against the disease (Kumar et al 2019, Mahapatra et al 2020). Leaf rust solely affects foliage, in the form of dusty, reddish-orange to reddish-brown fruiting structures that emerge on the surface of the leaf are indicative symptoms. In case of severe infection, numerous spores from these lesions can almost completely cover the upper leaf surface, which leads to defoliation and yield reduction. There is a 15-20% estimated annual yield loss of wheat in South Asia as a result of this disease (Duveiller and Sharma 2009). Early infection may cause plants to become weak and have poor root and tiller growth (Heidarian et al 2020). For assessing the disease severity, survey is a foremost tool. With an alteration in climatic conditions, the disease dynamics have changed (Debnath et al 2021), which necessitates the conduction of surveys. Further, the

variability study of a fungus and its aggressiveness are important to determine a wholesome picture of pathogen. Hence, the present investigation was carried out to develop a comprehensive idea of the disease severity and variability of associated pathogen in the Indo-Gangetic plains of West Bengal.

#### MATERIAL AND METHODS

Survey and data recording: For the present investigation, the disease severity of spot blotch and leaf rust across different districts of West Bengal were recorded from 2019-20 to 2020-21. Regular visits to farmers' fields as well as trial nurseries were conducted to assess the severity of the diseases and identify the disease hotspots. Monitoring for leaf rust and spot blotch was conducted during crop season as well as off-season to examine the survival of the pathogen on collateral hosts. The surveys were conducted from November in Nadia, Birbhum, Murshidabad, Malda and Hooghly districts of West Bengal. For assessing the severity of leaf rust, the infected plants were evaluated using modified Cobb's scale by using 0-9 scale (Kaur et al 2018). For spot blotch, the disease severity was assessed by visually scoring the flag (F) and penultimate (F-1) leaves, as per the double digit scale (00-99), Disease severity (Diseased leaf area DLA%) was calculated using the following Osman et al (2015). Here, D1 indicates the disease scored in F leaf and D2 indicates the diseases scored in F-1 leaf.

Disease Severity (DLA%) = (D1/9) x (D2/9) × 100,

**Pathogen identification:** The collected rust samples were sent to Rust laboratory, Flowerdale, Shimla for identification of the collected pathotype (Nayer et al 1997). The recorded disease severity for the consecutive years were further analysed to determine their dependence on weather

parameters and shift in prevalent pathotype. For isolation of *Bipolaris sorokiniana* isolates, naturally infected wheat leaves exhibiting characteristic symptoms of *Bipolaris sorokiniana* were collected from 10 different locations of West Bengal, which exhibited high disease severity (Table 1). Fragments of infected leaves were then surface sterilized and incubated for 3 d (12 h photoperiod, 25°C). After the growth of mycelia on the incubated fragments, the pathogen was isolated using single spore technique (Aregbesola et al 2020). The morphological and microscopic characteristics, described by Mew and Gonzales (2002) were used to identify the pathogen. The culture was then multiplied on Potato Dextrose Agar media and incubated in BOD incubator at  $26\pm1°C$ .

For molecular characterization of the pathogen, amplification of ITS genomic region was done using primer pair ITS1/ITS4. The PCR cycle included initial denaturation at 94 °C for 1 min, followed by 35 cycles of denaturation, annealing and extension at 94 °C for 30 s, at 55 °C for 30 s, and 72 °C for 1 min, respectively, and final extension at 72 °C for 10 min. The amplicon was subjected to a 1.2% gel electrophoresis. A 100bp ladder was used for confirming the amplicon size and the product was visualized using Gel Doc XR System (Bio-Rad Laboratories). The sequence homology was conducted by BLAST analysis at NCBI. The identified sequences were aligned using Clustal W multiple alignment software. The generated amplicons were freez dried and then sent to Eurofins Genomics, India for sequencsing.

Virulence assessment of *Bipolaris sorokiniana* isolates: K-4015, a popularly grown genotype was used for virulence assessment of the isolates. For the assessment under greenhouse conditions, the pure culture of pathogen, was harvested by flooding the plates with sterilized distilled water,

Table 1.	Prevalence	of foliar	diseases	of wheat in	different	districts c	of West	Bengal	durina 2	2019-20

Location	Geographical location (GPS)	Spot blotch (Disease severity)(%)	Leaf rust (Cobbs Scale)
Nadia district			
Bara Anduliya, Krishnanagar	23.62° N, 88.53° E	24.69 (29.79)	20 S
Thana rd. Chapra	23.54°N, 88.55°E	43.21 (41.10)	30 S
Chhitka Daspara	23.84°N, 88.49° E	43.21 (41.10)	40 S
Karimur cha	23.97° N, 88.62° E	43.21 (41.10)	80 S
Jamtala	22.11°N, 88.57° E	43.21 (41.10)	80 S
Karimpur, Parisha	23.97° N, 88.62° E	24.69 (29.79)	20 S
Murshidabad district			
Jalangi, Domkol	24.13° N, 88.69° E	24.69 (29.79)	100 S

and rubbing the culture with a sterilized glass rod. 1% TWEEN®20 solution was added to the suspension to aid in emulsification of the spores. The obtained suspension was filtered through three-layered muslin cloth, to obtain a dirt free, uniform suspension of fungal spores/mycelial bits. Three weeks old plants were sprayed with a pathogen solution. The concentration of the inoculum was adjusted at  $2 \times 10^5$  spores/ml with the aid of hemocytometer prior to inoculation. After inoculation, the plants were covered with clear plastic bags for 24 h, to help in pathogen establishment. The pots were arranged in completely randomized block design, with 3 replications. The average temperature of the greenhouse was  $25^{\circ}$ C, with RH >95%. Irrigation was provided on a regular basis to maintain the humid microclimate.

#### **RESULTS AND DISCUSSION**

Disease scenario: For identifying the major diseases of wheat, a survey was conducted across different districts of West Bengal in the year 2019-20 and 2020-21 (Fig. 1). While recording spot blotch severity, characteristic spot blotch symptoms in the form of elongated brown elliptical to oval lesions were observed on the leaves, sheath, glumes and nodes (Fig. 2). In the year 2019-20, seven locations across two districts, Nadia and Murshidabad, were conducted. The wheat varieties mostly cultivated were HD2967, HD 3086, PBW 343 and in few places still growing Sonalika. The size of the farmers field are mostly very small 2-3 bighas and farmers were mostly growing wheat for their own consumption only. But when survey were conducted in Murshidabad district then few farmers were growing wheat for commercial perpus and overall crop health were good enough. They were mostly growing HD 2967, DBW187, HD 3086 etc. and few farmers even don't had knowledge about variety they were using. Spot blotch and leaf rust was reported in all the locations surveyed, with varying severity (Table 1). High spot blotch severity (43.21%) was observed in four locations of Nadia district (Thana rd. Chapra, Chhika Daspara, Karimur cha, Jamtala), while Karimpur of Nadia (24.69%) and Jalangi in Murshidabad district recorded lower severity (24.69%). The rust severity, in the year 2019-20, ranged between 20S to 100 S across varying locations. The highest leaf rust severity score was recorded in Jalangi, Murshidabad (100 S), while the lowest severity was observed in Karimpur, Parisha and Bara Anduliya, Krishnanagar in Nadia district (20 S). In Nadia, the highest severity (80S) was observed in Karimur cha and Jamtala. In the following year, twenty locations across six districts were surveyed (Table 2). Among the locations surveyed, highest spot blotch severity (59.26%) was recorded in two locations (Jalangi, DhanirpurBhaduripara) of Murshidabad district, followed by Raninagar (51.85%) in Murshidabad. In Nadia district, spot blotch disease severity ranged from 9.88-51.85%, with Bara Aduliya (Krishnanagar) and Magurakhod recording lowest and highest disease severity respectively. A disease severity of 43.21% was recorded in Kalvani, Shukpukuria, Chhitka (Krishnanagar-Karimpur road side) and Jamtala, while relatively lower scores were recorded in Bhajanghat (37.04%), followed by Chhika-Daspara, Karimur cha and Thana rd. Chapra .The disease severity was maximum in in Malda (43.21%) followed by Birbhum, Hooghly and North 24 Parganas. Lowest disease severity (9.88%) was observed in Bara Aduliya (Krishnanagar) of Nadia district. In both the year diseases severity data not varied much as the prevailing weather and it is a good indication that diseases were under control due to use of tolerant newly recommended varieties used by the farmers of survey locations (Table 3). Among the locations surveyed for leaf rust severity, highest severity (80 S) was observed in Karimur cha and Jamtala of Nadia District while lowest severity (20 S) was in four locations of Nadia (Bara Aduliya, Krishnanagar, Magurakhod, Bhajanghat), one in Birbhum (Tarapith) and Murshidabad (Raninagar). No rust incidence was recorded in North 24 Parganas and Hooghly Districts. Among the twelve locations of Nadia highest leaf rust severity (80S) was in Karimur cha and Jamtala followed by Kalyani, Shukpukuria, Chhika-Daspara. Among the four districts of Murshidabad highest leaf rust incidence in Domkol-taraf (60 S) followed by Dhanirpur-Bhaduripara, Jalangi and Raninagar.

Identification of samples: Following survey of disease prevalence across different districts of West Bengal, the samples were kept for further identification. The collected rust samples were sent to Rust laboratory, Flowerdale, Shimla for identification of the collected pathotype (Table 4). In 2019-20, the occurrence of Pathotype 77-9 (121R60-1) was highest in the collected samples (21), followed by 77-5 and 77-9+Raj1555. The pathotypes 12-3 (49R37) and 12-4 (69R13) were identified in one sample each. In the following year, pathotype 77-9 (121R60-1) was most abundant (11 samples) followed by 77-9+Raj1555 (121R60-1,7), 77-5 (121R63-1) and 12-3 (49R37). During the cropping season 2019-20 and 2020-21, ten isolates of B. sorokiniana were collected from different fields of West Bengal (Table 5). The isolates were subjected to molecular identification by conducting PCR, using primer pairs ITS1/ITS4. Species specific bands were observed at around 600 bp, which was measured using the representative ladder (Fig. 3). The DNA sequences were sent to NCBI to get the accession numbers (Table 5). The sequences were searched in NCBI-BLAST and their identity percentage with reported ITS sequences

was obtained (Table 6). While determining the aggressiveness among the isolates of *Bipolaris sorokiniana*, isolate WB7 (Accession no. MT804348) was the found to be the most aggressive among the ten isolates when evaluated on DBW 90 (70.99%). Among the 10 isolates highest severity was noticed in WB7 (70.99%), while lowest severity was observed in WB3 and WB4 (7.02 and 7.86%, respectively).

The disease severity in the isolates WB1 (39.88%), WB2 (39.24%) and WB5 (41.69%) were observed to be statistically at par with each other. Similarly, the difference in disease severity between WB8 (25.02%) and WB9 (24.24%) were statistically at par with each other. Different disease severity was observed in WB10 (55.18%) and in WB6 (36.28%), though they were not significantly different. The

Table 2. Prevalence of foliar diseases of wheat in different districts of West Bengal during 2020-21

District	Location	Geographical location (GPS)	Spot Blotch (Disease severity %)	Leaf rust (Cobbs Scale)
North 24 Parganas	Bagda	23.21° N, 88.89° E	14.81 (22.63)	No rust
	Jaypur, Chandanpukur	23.38°N, 88.56°E	24.69 (29.79)	No rust
Nadia	Bhajanghat	23.38° N, 88.74° E	37.04 (37.49)	20s
	Kalyani	22.97° N, 88.43° E	43.21 (41.10)	40s
	Shukpukuria	23.61° N, 88.36°E	43.21 (41.10)	40s
	Magurakhod	25.25°N, 88.08°E	51.85 (46.06)	20s
	Chhitka (Krishnanagar-Karimpur road side)	23.84°N, 88.49°E	43.21 (41.10)	No rust
	Chhika-Daspara	23.84°N, 88.49°E	24.69 (29.79)	40 S
	Karimur-cha	23.97°N, 88.62°E	24.69 (29.79)	80 S
	Bara Aduliya, Krishnanagar	23.62° N, 88.53° E	9.88 (18.32)	20 S
	Thana rd. Chapra	23.54°N, 88.55°E	24.69 (29.79)	30 S
	Jamtala	22.11°N, 88.57°E	43.21 (41.10)	80 S
Malda	Sultanganj	24.85° N, 88° E	43.21 (41.10)	40s
Birbhum	Tarapith	24.11° N, 87.79° E	29.63 (32.98)	20s
	Mallarapar	24.08° N, 87.71° E	24.69 (29.79)	40s
looghly	Bhedia, Sekampur	23.58°N, 87.74°E	43.21 (41.10)	No rust
Murshidabad	Raninagar	24.22° N, 88.55° E	51.85 (46.06)	20s
	Jalangi	24.13°N, 88.69°E	59.26 (50.34)	40s
	Domkol-Taraf	24.12° N, 88.54° E	43.21 (41.10)	60s
	Dhanirpur-Bhaduripara	24.88° N, 88.56°E	59.26 (50.34)	40s

<b>Tuble 0.</b> Wedner parameters of the districts surveyed during residury-march of 2010-20 and 2020-2	Table 3. Weather	parameters of the	districts surveyed	during Februar	y-March of 2019-20 and 2020-21
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Year	T max (°C)	Tmin (°C)	Rainfall (mm)	RH max (%)	RH min (%)	DLA (%)
2019-20	27.09	11.81	0.18	87.37	66.93	35.27
2020-21	29.46	14.99	0.00	85.58	60.00	38.14

result therefore indicated that most aggressive isolate which produced maximum disease severity was WB7 followed by WB10 whereas WB3 and WB4 were least virulent with respect to their symptom development in host.

In this era of changing climatic conditions, an array of new pathotypes has emerged in different wheat growing zones of the world. Although North-Eastern Plain Zone is a major wheat growing belt in the country, the disease dynamics have changed drastically, owing to a drastic climate change,

 Table 4. Pathotype distribution of brown rust (Puccinia triticina) in West Bengal during 2019-20 and 2020-21

Pathotype observ	ed	No. of samples
2019-20	12-3 (49R37)	1
	12-4 (69R13)	1
	77-5 (121R63-1)	5
	77-9 (121R60-1)	21
	77-9+Raj1555	3
2020-21	12-3 (49R37)	1
	77-5 (121R63-1)	3
	77-9 (121R60-1)	11
	77-9+Raj1555 (121R60-1,7)	9

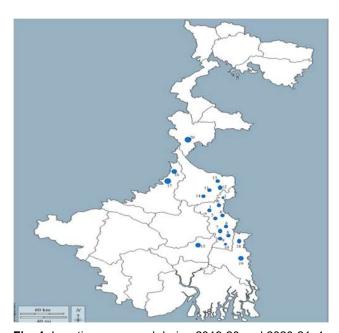


Fig. 1. Locations surveyed during 2019-20 and 2020-21. 1-Karimur Cha, 2- Karimur, Parisha, 3- Jamtala, 4- Bara Anduliya, 5-ChikkaDaspara, 6-Thana Rd., 7-Mungrakhod, 8-Shukpukuria, 9-Bhajanghat, 10-Kalyani, 11- Domkol, 12- Jalangi, 13- Raninagar, 14-Bhaduripara, 15-BhediaSekampur, 16-Mullarpur, 17-Tarapith, 18-Bagda, 19-Chandanpukur, 20-Sultanganj

occasional drizzles and warm temperature during winter (Debnath et al 2021). Considering the importance of wheat in the eastern zone of the country, it becomes imperative to identify the prevalent diseases in the wheat belts of NEPZ. Hence the present study was conducted to identify the major foliar diseases of wheat across different districts of West Bengal, as well as to identify the pathotypes of the brown rust collected during the survey. In the present study, spot blotch and leaf rust have been observed to be the most prevalent in the districts surveyed. Different frequencies of Bipolaris sorokiniana have been found throughout the wheat-growing regions in India as well as in neighboring nations. Devi et al (2021) already established the variability among the isolates collected from the same study area. If tikhar et al (2010) discovered that in Pakistan, the Sindh zone had the highest prevalence of spot blotches (75% in 2005) followed by Foot hill areas of Gilgit and Sukardu (62.5%), and central Punjab area (60%). A minimum yield loss of 30% has been reported under favourable conditions in susceptible varieties in India (Sharma-Poudyal et al 2016), while 18-50% yield loss was



Fig. 2. Characteristic disease symptoms observed during field survey A. Rust B. Spot blotch

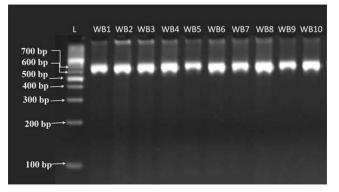


Fig. 3. PCR amplification products from the ITS region (ITS1, 5⋅8S, ITS2) of 10 *Bipolaris sorokiniana* isolates. Lane L, DNA ladder (100 bp); WB(1-10): Amplicons of *Bipolaris* isolates

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observed in warm and humid regions of the world (Gurung et al 2014). In study, high spot blotch severity (43.21%) was observed in four locations of Nadia district (Thana rd. Chapra, Chhika Daspara, Karimur cha, Jamtala), while Karimpur of Nadia and Jalangi in Murshidabad district recorded lower severity (24.69%) in 2019-20. In the following year, the highest spot blotch severity was recorded in two locations of Murshidabad district, followed by Raninagar in Murshidabad. The variations in disease severity might be due to varying weather conditions and differences in pathogen inoculum density across different districts. Leaf rust has been considered as the most severe and widely distributed among the three rusts of wheat (Huerta-Espino et al 2011). In study, the highest leaf rust severity score was recorded in Jalangi, Murshidabad in 2019-20, while highest leaf rust severity was d in Karimur cha and Jamtala of Nadia District in the following year. The difference of intensity might be due to difference in degree of resistance among cultivars, as well as prevalent weather conditions (Pasquini et al 2003). In both the year except few locations diseases data of both spot and leaf rust were under control as because of the farmers using the recent NEPZ recommended varieties. Except HD 3086 showed high leaf rust score as it susceptible to leaf rust. Even in both the years winter season not received must rain so disease severity were low. Overall diseases status of the both year were food along with good crop health.

The collected samples of leaf rust were sent to Rust laboratory, Flowerdale, Simla for identification of the collected pathotype, which revealed the prevalence of Pathotype 77-9, 77-577-9+Raj1555, 12-3 and 12-4 in the year 2019-20. In the following year, the collected pathotypes were identified as 77-9, 77-9+Raj1555. 77-5) and 12-3. In the same year, the pathotype 77-5 was identified in two samples from Tamil Nadu, while the pathotype 77-9 was identified in four samples from Himachal Pradesh and one sample from Tamil Nadu (Prasad et al 2020). In a subsequent survey conducted in India in 2020, the pathotype 77-5 was discovered in five samples each from West Bengal and Gujarat, two samples each from Himachal Pradesh, Punjab, and Uttar Pradesh, one sample each from Jammu & Kashmir and Bihar, six samples each from Uttarakhand, seventeen samples each from Madhya Pradesh, eleven samples from Maharashtra, and twelve samples each from Karnataka and sixty two samples of Tamil Nadu. The same pathotype was identified in multiple samples from Himachal Pradesh, Punjab, and Uttar Pradesh, as well as three samples each from West Bengal, Maharastra, Karnataka, Tamil Nadu, and Madhya Pradesh in 2021 (Prasad et al 2021). The pathotype 77-9 was identified in West Bengal, Himachal Pradesh, Jammu & Kashmir, Punjab, Uttar Pradesh, Uttarakhand, Madhya Pradesh, Bihar, Gujrat, Maharastra, Karnataka, and Tamil Nadu in 2020. In 2021, the pathotype 77-9in a number was observed from Himachal Pradesh, Punjab, Haryana, Uttar Pradesh, Uttarakhand, Maharastra, Madhya Pradesh, Bihar, Maharastra, Karnataka, and Tamil Nadu in addition to eleven samples from West Bengal. The pathotype 77-9+Raj1555 was identified from Uttar Pradesh (3 samples) and from Punjab (1 sample) in 2020 (Prasad et al 2020), while it was

Table 5. Details about the isolates of Bipolaris sorokiniana collected from survey locations

Isolates	NCBI accession	No. Location	Geographical location (GPS) Mean disease s		
WB1	MT804342	Sultanganj	24.85° N, 88° E	39.88 ± 1.89°	
WB2	MT804343	Bhajanghat	23.38° N, 88.74° E	39.24± 1.55°	
WB3	MT804344	Tarapith	24.11° N, 87.79° E	7.02± 1.67ª	
WB4	MT804345	Mallarapar	24.08° N, 87.71° E	7.86± 1.01ª	
WB5	MT804346	Domkol-Taraf	24.12° N, 88.54° E	41.69± 1.82°	
WB6	MT804347	Shukpukuria	23.61° N, 88.36°E	36.28± 1.10 <sup>bc</sup>	
WB7	MT804348	Jalangi	24.13°N, 88.69°E	70.99 ± 1.99°	
WB8	MT804349	Jamtala	22.11°N, 88.57°E	25.02± 1.43 <sup>b</sup>	
WB9	MT804350	Chhitka (Krishnanagar-Karimpur road side)	23.84°N, 88.49°E	24.24 ± 1.93 <sup>b</sup>	
WB10	MT804351	Raninagar	24.22° N, 88.55° E	55.18 ± 1.16 <sup>d</sup>	

Means with the same letter are not significantly different according to DMRT's significant difference test ( $\alpha$ =0.05)

Sample	BLAST match	NCBI accession no.	Query coverage (%)	Max identity (%)
WB1	Bipolaris sorokiniana strain WH.PBW.IP.04	KM066949.1	100	100
WB2	Bipolaris sorokiniana isolate PJC5	OM419194.1	100	100
WB3	Bipolaris sorokiniana strain BS-47	DQ367884.1	100	100
WB4	Bipolaris sorokiniana isolate WLB-18-10	MK809557.1	100	100
WB5	Bipolaris sorokiniana isolate WLB-18-13	MK809551.1	100	100
WB6	Bipolaris sorokiniana isolate A35	OQ225221.1	100	100
WB7	Bipolaris sorokiniana isolate 01	MT254730.1	100	100
WB8	Bipolaris sorokiniana isolate WLB-18-22	MK809565.1	100	100
WB9	Bipolaris sorokiniana isolate WB9	MT804350.1	100	100
WB10	Bipolaris sorokiniana isolate WLB-17-47	MN535889.1	100	100

Table 6. Identification of *Bipolaris sorokiniana* isolates of ITS region

identified from Himachal Pradesh, Punjab, Uttar Pradesh, Uttarakhand, Madhya Pradesh, and Maharashtra in 2021 (Prasad et al 2021). Pathotypes 77-5, 77-9 and 77-9+Raj1555 were discovered in nearby countries like Nepal in two, twenty seven, and twelve samples, respectively (Prasad et al 2021). Upon conduction of molecular studies, ten different isolates of Bipolaris sorokiniana were identified. Verma et al. (2020) had identified different isolates of Bipolaris sorokiniana based on Two Internal transcribed spacer (ITS) region, from Maharashtra. In the present study, the isolates were also observed to vary based on the aggressiveness. Isolate BS7 (Accession no. MT804348) was most aggressive among the ten isolates when evaluated on wheat genotype DBW 90 (70.99%), while BS3 was least virulent. Both morphological and pathological variations among B. sorokiniana isolates have been documented (Jaiswal et al 2007). Previously, Bandopadhyay et al. (2016) and Devi et al. (2021) collected 10 isolates from different parts of India, which varied significantly in aggressiveness among themselves. Kashyap et al. (2022) had identified a moderate to low haplotype diversity among 528 isolates of Bipolaris sorokiniana collected from different parts of the country.

#### CONCLUSION

The disease prevalence of wheat has been changing due to changes in weather parameters and emergence of new virulent pathotypes in the pathogens. In the present study, the prevalence of spot blotch and leaf rust has been assessed in different districts of West Bengal. The prevalent virulent pathotypes have also been identified. Hence, the results of this study will help the plant pathologists and breeders for screening genotypes against existing virulent pathotypes.

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