Research Article:

SPATIAL OCCURRENCE AND MORPHOLOGICAL COLONY VARIABILITY OF *Bipolaris sorokiniana* ISOLATES CAUSING SPOT BLOTCH OF WHEAT IN NEPAL

Laxman Aryal[a*], Sundar Man Shrestha[b], Hira Kaji Manandhar[b] and Dhruba Bahadur Thapa[c]

[a]National Wheat Research Program, Nepal Agricultural Research Council, Bhairahawa, Rupandehi, Nepal

[b] Department of Plant Pathology, Faculty of Agriculture, Agriculture and Forestry University, Rampur, Chitwan, Nepal

[c]National Plant Breeding and Genetics Research Centre, Nepal Agricultural Research Council, Khumaltar, Lalitpur, Nepal

*Corresponding author: laxmanaryal.ag@gmail.com

DOI: https://doi.org/10.3126/jafu.v7i1.95389

Received date: 27 Feb 2026; **Revised date:** 04 May 2026; **Accepted date:** 14 May 2026; **Published date:** 10 Jun 2026

ABSTRACT

Bipolaris sorokiniana (Sacc.) Shoem. is an important pathogen causing spot blotch in wheat. A study was conducted to explore the occurrence and morphological colony variability of *B. sorokiniana*. A total of 36 isolates from single spore were maintained from the disease sample collected from different wheat growing regions of Nepal. Among them, 27 isolates were from terai region; 7 were from mid hill region and 2 were from high hill region. Radial mycelia growth of all the isolates differed significantly at different days after incubation (DAI). Mean colony diameter at 2 DAI, 4 DAI, 6 DAI and 8 DAI were 2.70 cm, 5.54 cm, 7.82 cm and 8.68 cm respectively. Radial mycelia growth ranged from 7.23 cm to 9 cm after 8 days of incubation. Based on colony color, isolates were categorized into 5 groups. Dull/white greenish black had maximum frequency (33.3%) and white colony had minimum frequency (5.5%). Based on colony growth behavior, 41.6% isolates were suppressed growth; 38.8% were fluffy growth and 19.4% were cottony growth. Variability was also observed in colony growth margin where 66.6% isolates were having regular colony margin and 33.3% isolates were having irregular colony margin. Diverse spatial occurrence and high morphological colony variation suggests that isolates of *B. sorokiniana* causing spot blotch disease might possess different level of virulence profile.

Keywords: Disease, pathogen culture, radial growth, single spore, virulence

INTRODUCTION

Wheat is the third most important cereal crop after rice and maize accounting with the productivity of 2.98 mt/ha (MoALD, 2024) which is lower than the global average productivity of 3.50 mt/ha (FAO, 2024). Spot blotch disease, caused by *Bipolaris sorokiniana* (Sacc.) Shoem., is one of the many biotic constraints responsible for this low productivity of wheat. Globally, an estimated of 25 million hectare wheat area is affected by spot blotch out of which 40% wheat area is grown in Indian subcontinent (Joshi et al., 2007a). Wheat grown in warm and humid conditions is prone to spot blotch disease and cause significant yield loss (Duveiller, 2004). Longer duration of leaf wetness due to high relative is particularly favorable for infection and pathogen growth (Acharya et al., 2011). On the Indian subcontinent, the disease spreads when the temperature is above 26 °C (Chaurasia et al., 2000). Disease is severe in late sown condition

(Duveiller et al., 2005). In the Indo Gangetic plain including Nepal and Bangladesh, where wheat is typically raised after the harvest of the preceding rice crop, severity of the disease is high due to combine effect of disease and terminal heat stress which results in low productivity (Joshi et al., 2007c). Beside, the favorable environmental condition, disease severity is affected by different factors like crop management, soil fertility, planting density, the developmental stage of the plant (Joshi et al., 2007b).

The pathogen first colonizes the older leaves at the base of the wheat plant and then progresses to the upper part of the canopy (Joshi et al., 2002). Spot blotch symptoms initially appear as brown lesions with yellow halos, which enlarge with time to cover larger areas of the leaf. Lesions can turn olive brown in color; especially under humid conditions that promote sporulation of the fungus as a result leaf becomes chlorophyll deficient and eventually dies (Al-Sadi, 2016). When relative humidity is above 90%, temperature is below 30^{o}C and accompanied by light rainfall during heading stage, the spikelets are also affected and dark brown to black discoloration appears around the germinating point of the seed, called 'black point'(Li et al., 2019).

The losses due to the disease in warm regions of Nepal ranged from 23 - 40 % depending on genotypes and other environmental factors (Bhandari &Tripathi, 2005; Sharma & Duveiller, 2006). Up to 100% foliar blight incidence was reported in the field with yield losses up to 52% in eastern plains of Nepal (Sharma & Duveiller, 2006). Yield losses, however, vary with sowing time, years, locations, host cultivar, climatic factors and stress conditions (Duveiller et al., 2005; Sharma & Duveiller, 2006). Complete genetically determined resistance is yet to be identified, since the cultivars that have been classified as resistant still suffer some yield reduction when the disease pressure is high (Joshi & Chand, 2002).

Due to the lack of durable resistant cultivars and ineffective control measures, the disease affected areas is said to increase every year all over the world (Sharma et al., 2007). In addition, due to the effect of climate change, unpredictable weather conditions and development of new races and colony characteristics, the incidence of spot blotch disease has been spread from warm plain areas to comparatively cool mid-hills of Nepal (Bhandari, 2017; Mahto et al., 2012) urging the immediate need to assess pathogen variability through pathogen profiling and virulence studies. Study of morphological characteristics, including colony color, margins, sectoring, exudation and septation, in addition to the use of molecular approaches can be used to identify and investigate the variability among the isolates of *B. sorokiniana* (Jaiswal et al., 2007; Kumar et al., 2002; Poloni et al., 2009).

Moreover, the continuous work has not been carried out to update the occurrence and distribution of spot blotch and study its morphological, pathogenic variability of *B. sorokiniana* isolates causing spot blotch in Nepal. Therefore, the present study was carried out to explore the occurrence of spot blotch disease and assess the morphological colony variability of *B. sorokiniana* which may provide a basis for future virulence studies and as a result enabling their use in upcoming resistant breeding programs.

RESEARCH METHODS

Collection of leaf samples

Spot blotch suspected leaf samples were collected in paper envelope from January to May in the year 2022/23 and 2023/24. From each location 5-6 leaves as a representative sample were collected but the location where disease symptoms looked different, representative sample were doubled. Each sample envelope was marked with location, latitude, longitude, altitude, date of collection.

Isolation of the pathogen

Disease sample processing and isolation activity was conducted at plant pathology laboratory of National Wheat Research Program (NWRP), Bhairahawa, Rupandehi. Symptomatic leaves from each location were cut into a 5 mm segment and surface sterilized with 1% sodium hypochlorite solution (NaOCl) for 1 minute followed by triple rinsing with sterilized distilled water. Sterilized pieces were then transferred to a moisture chamber and incubated at 25 ± 1 °C to allow sporulation for 48 hours. After confirmation of the pathogen growth, a single spore of *B. sorokiniana* was isolated from the incubated sample and transferred to water agar and incubated at 25±1°C. The germination of the spore was observed after 24 hours and then transferred to potato dextrose agar (PDA) media, then incubated at 25 ± 1 °C for eight days for full growth of *B. sorokiniana.* Thirty-six isolates of *B. sorokiniana* from wheat growing areas of Nepal were isolated and maintained (Table 1).

Morphological characterization

Morphological characters such as, colony color, colony growth type and colony growth margin of each isolate were recorded. For this 5 mm mycelia disc of each isolate was cut out from a eight-day-old pure culture with sterilized cork borer, inoculated on the PDA plate by using a sterile needle, and incubated at 25 ± 1°C with a twelve-hour photoperiod for 8 days. Each isolate was replicated four times in Completely Randomized Design (CRD). After 8 days of incubation, colonies from the different isolates were categorized according to I) Colony color characteristics- (A) black colony,(B) brown/dull black colony, (C) grey colony, (D) dull white/ greenish black colony and (E) white colony; II) Colony growth behavior- (A) suppressed, (B) cottony and (C) fluffy; III) Colony growth margin-(A) regular and (B) irregular based on protocol followed by (Chand et al., 2003; S. P. Pandey et al., 2008).

Radial growth

A 5 mm mycelia disc of each isolate was cut out from an eight-day-old pure culture with sterilized cork borer, inoculated on the PDA plate by using a sterile needle, and incubated at 25 ± 1°C with a twelve-hour photoperiod. Radial growth of each isolate at 2 days after inoculation (2 DAI), 4 DAI, 6 DAI and 8 DAI were recorded by using a scale. Each isolate was replicated four times in a Completely Randomized Design (CRD).

RESULTS AND DISCUSSION

Occurrence and distribution of *Bipolaris sorokiniana* causing spot blotch of wheat

Out of 36 *Bipolaris sorokiniana* isolates that were isolated and maintained in Plant Pathology laboratory of National Wheat Research Program (NWRP), Bhairahawa, 27 isolates were from terai origin with elevation ranging from 60-1000 meters above sea level (msl), 7 isolates were from mid hill origin with elevation ranging from 1001- 2000 msl, whereas, 2 isolates were from high hill origin with elevation above 2000 msl (Table 1).

Colony color-based characterization and relative frequency distribution of *B. sorokiniana* isolates

Based on the colony color characteristics, thirty six isolates were categorized into five distinct colony groups. Of the total isolates, 25% of the isolates (N-BS1, N-BS3, N-BS10, N-BS13, N-BS15, N-BS18, N-BS25, N-BS28 and N-BS29) were grouped under black category. Similarly, 16.6% of the isolates (N-BS2, N-BS6, N-BS20, N-BS30, N-BS31 and N-BS34) were grouped under brown/dull black category; 19.4% of the isolates (N-BS5, N-BS8, N-BS12, N-BS14, N-BS19 and N-BS26) were grouped under grey with white spot category; 33.3% of the isolates (N-BS4, N-NS7, N-BS9, N-BS11, N-BS16, N-BS22, N-BS23, N-BS24, N-BS27, N-BS36, N-BS37 and N-BS38) were grouped under dull white/greenish black category whereas only 5.5% of the isolate (N-BS17 and N-BS35) was grouped under white colony category (Table 2, 3).

Colony growth behaviour- based characterization and relative frequency distribution of *B. sorokiniana* isolates

Based on colony growth characteristics, 36 isolates were categorized into three distinct groups. Of them, 41.6% of the isolates (N-BS1, N-BS2, N-BS3, N-BS6, N-BS10, N-BS13, N-BS15, N-BS18, N-BS20, N-BS25, N-BS28, N-NS29, N-BS30, N-BS31, N-BS34) showed suppressed growth (flat growth) of the colony;19.4% of the isolates (N-BS5, N-BS8, N-BS12, N-BS14, N-BS19, N-BS26, BS32) exhibited cottony colony growth and 38.8% of the isolates (N-BS4, N-BS7, N-BS9, N-BS11, N-BS16, N-BS17, N-BS22, N-BS23,N-BS24, N-BS27, N-BS35, N-BS36, N-BS37, N-BS38) produced fluffy type colony growth (Table 2, 4).

Colony growth margin-based characterization and relative frequency distribution of *B. sorokiniana* isolates

Based on the colony growth margin characteristics, 36 isolates were categorized into two distinct groups. Among them, 66.6% of the isolates (N-BS1, N-BS2, N-BS3, N-BS4, N-BS5, N-BS6, N-BS8, N-BS9, N-BS10, N-BS11, N-BS12, N-BS13, N-BS15, N-BS16, N-BS18, N-BS22, N-BS23, N-BS25, N-NS27, N-BS28, N-BS31, N-BS34, N-BS36, N-BS38) showed regular type margin growth of the colony whereas 33.3% of the isolates (N-BS7, N-BS14, N-BS17, N-BS19, N-BS20, N-BS24, N-BS26, N-BS29, N-BS30, N-BS35, N-BS37) showed irregular type margin growth of the colony(Table 2, 5).

Mean colony growth diameter of *B. sorokiniana* isolates at different days after incubation (DAI)

Colony diameter of all 36 *B. sorokiniana* isolates from different ecological zones differed significantly at 2 Days after Incubation (DAI), 4 DAI, 6 DAI and 8 DAI. Sunsari and Dolakha districts having two isolates each from distinct disease sample were named as Sunsari (A, B) and Dolakha (A, B). At 2 and 4 DAI, N-BS38 consistently showed the best mycelial growth whereas at 6 and 8 DAI, N-BS22 and N-BS34 consistently showed the highest mycelial growth (Table 6).

Table 1. *B. sorokiniana* isolates collected and maintained from different wheat growing areas of Nepal during 2023/24

Isolates Code	Location (District)	Elevation (msl)	Latitude	Longitude	Region
N-BS1	Udaypur	304	26.89	86.70	Terai
N-BS2	Palpa	750	27.87	83.59	Terai
N-BS3	Kaski	1,981.85	27.69	86.23	Mid hill
N-BS4	Syangja	560	27.89	83.87	Terai
N-BS5	Salyan	1485	28.25	82.36	Mid hill
N-BS6	Baglung	1,675.49	28.25	83.49	Mid hill
N-BS7	Kailali	1,56.64	28.49	81.09	Terai
N-BS8	Nawalpur	205	27.71	84.41	Terai
N-BS9	Doti	540	29.26	80.93	Terai
N-BS10	Nawalparasi	115	27.53	83.67	Terai
N-BS11	Jhapa	120	26.65	88.02	Terai
N-BS12	Gulmi	815	28.18	83.31	Terai
N-BS13	Dolakha (A)	1,661	27.63	86.14	Mid hill
N-BS14	Rupandehi	112	27.53	83.46	Terai
N-BS15	Lalitpur	1421	27.65	85.33	Mid hill
N-BS16	Banke	157	28.12	81.80	Terai
N-BS17	Sarlahi	1,79.91	27.08	85.58	Terai
N-BS18	Surkhet	580	28.50	81.78	Terai
N-BS19	Mahottari	229	26.98	85.91	Terai
N-BS20	Bardiya	1,36.6	28.42	81.10	Terai
N-BS22	Mustang	2,650	28.34	83.52	High hill
N-BS23	Bara	115	27.11	84.95	Terai
N-BS24	Sunsari (A)	136	26.72	87.28	Terai
N-BS25	Danusha	94	26.80	85.96	Terai
N-BS26	Dang	725	28.10	82.52	Terai
N-BS27	Dolakha (B)	2,567	27.72	85.95	High hill
N-BS28	Rautahat	108	27.10	85.35	Terai
N-BS29	Siraha	131.2	26.83	86.23	Terai
N-BS30	Saptari	125	26.63	86.68	Terai
N-BS31	Sunsari (B)	154	26.53	86.90	Terai
N-BS32	Pyuthan	817	28.62	82.52	Terai
N-BS34	Chitwan	1,69.93	27.65	84.35	Terai
N-BS35	Kanchanpur	210	28.95	80.23	Terai
N-BS36	Bajura	1,390	29.44	81.14	Mid hill
N-BS37	Rukum	1,273	28.67	82.35	Mid hill
N-BS38	Kapilvastu	210	27.67	82.83	Terai

Note: The elevation between 60 and 1000 msl has been considered terai (low land), region falling in between 1001 and 2000 msl has been considered mid hill whereas the region above 2000 msl has been considered as high hill as per Joshi et al. (2017).

Table 2. Morphological colony characteristics of 36 *B. sorokiniana* isolates from different wheat-growing regions of Nepal during 2023/24

Isolate Code	Location (District)	Colony color at Full growth	Growth Behavior	Margin of the colony
N-BS1	Udaypur	Brown	Suppressed	Regular
N-BS2	Palpa	Brown/Dull black	Suppressed	Regular
N-BS3	Kaski	Black	Suppressed	Regular
N-BS4	Syangja	Dull white greenish black	Fluffy	Regular
N-BS5	Salyan	Grey with white spot	Cottony	Regular
N-BS6	Baglung	Brown/Dull black	Suppressed	Regular
N-BS7	Kailali	Dull white greenish black	Fluffy	Irregular
N-BS8	Nawalpur	Grey with white spot	Cottony	Regular
N-BS9	Doti	Dull white greenish black	Fluffy	Regular
N-BS10	Nawalparasi	Black	Suppressed	Regular
N-BS11	Jhapa	Dull white greenish black	Fluffy	Regular
N-BS12	Gulmi	Grey with white spot	Cottony	Regular
N-BS13	Dolakha (A)	Black	Suppressed	Regular
N-BS14	Rupandehi	Grey with white spot	Cottony	Irregular
N-BS15	Lalitpur	Black	Suppressed	Regular
N-BS16	Banke	Dull white greenish black	Fluffy	Regular
N-BS17	Sarlahi	White	Fluffy	Irregular
N-BS18	Surkhet	Black	Suppressed	Regular
N-BS19	Mahottari	Grey with white spot	Cottony	Irregular
N-BS20	Bardiya	Brown / dull black	Suppressed	Irregular
N-BS22	Mustang	Dull white greenish black	Fluffy	Regular
N-BS23	Bara	Dull white greenish black	Fluffy	Regular
N-BS24	Sunsari (A)	Dull white greenish black	Fluffy	Irregular
N-BS25	Danusha	Black	Suppressed	Regular
N-BS26	Dang	Grey with white spot	Cottony	Irregular
N-BS27	Dolakha (B)	Dull white greenish black	Fluffy	Regular
N-BS28	Rautahat	Black	Suppressed	Regular
N-BS29	Siraha	Black	Suppressed	Irregular
N-BS30	Saptari	Brown / dull black	Suppressed	Irregular
N-BS31	Sunsari (B)	Brown / dull black	Suppressed	Regular
N-BS32	Pyuthan	Grey with white spot	Cottony	Irregular
N-BS34	Chitwan	Brown/Dull black	Suppressed	Regular
N-BS35	Kanchanpur	White	Fluffy	Irregular
N-BS36	Bajura	Dull white greenish black	Fluffy	Regular
N-BS37	Rukum	Dull white greenish black	Fluffy	Irregular
N-BS38	Kapilvastu	Dull white greenish black	Fluffy	Regular

Table 3. Colony color characteristics and relative frequency of *B. sorokiniana* isolates collected from different wheat-growing regions of Nepal during 2023/24

Group	Characteristics	No. of Isolates	Isolate Population %	Isolate Designation
A	Black	9	25	N-BS1, N-BS3, N-BS10, N-BS13, N-BS15, N-BS18, N-BS25, N-BS28, N-NS29
B	Brown/dull black	6	16.6	N-BS2, N-BS6, N-BS20, N-BS30, N-BS31, N-BS34
C	Grey with white spot	7	19.4	N-BS5, N-BS8, N-BS12, N-BS14, N-BS19, N-BS26
D	Dull white/ greenish black	12	33.3	N-BS4, N-BS7, N-BS9, N-BS11, N-BS16, N-BS22, N-BS23, N-BS24, N-BS27, N-BS36, N-NS37, N-BS38
E	White	2	5.5	N-BS17, N-BS35

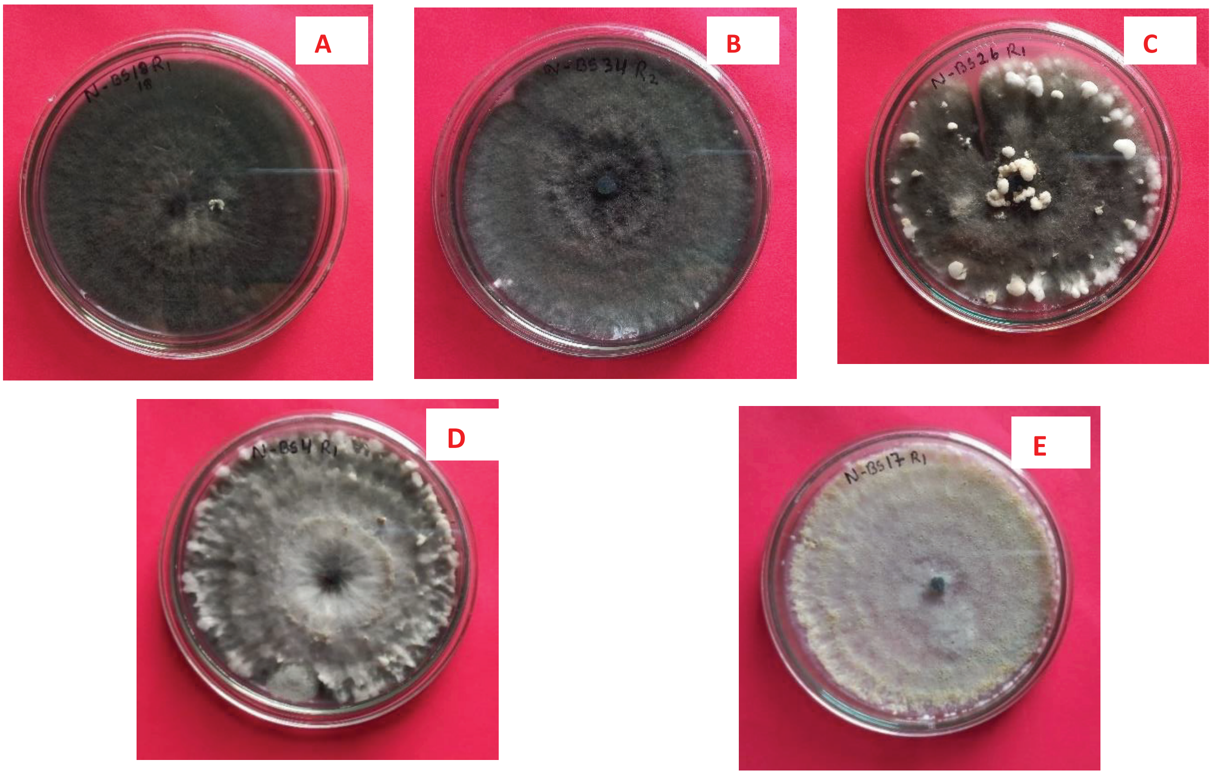


Fig. 1. Grouping of the *B. sorokiniana* isolates based on colony color; Black (A), brown/ dull black (B), grey with white spot (C), dull white/greenish black (D), and white (E)

Table 4. Colony growth behaviour characteristics and relative frequency of *B. sorokiniana* isolates collected from different wheat-growing regions of Nepal during 2023/24

Group	Colony growth behaviour	No. of Isolates	Isolate Population %	Isolate Designation
A	Suppressed	15	41.6	N-BS1, N-BS2, N-BS3, N-BS6, N-BS10, N-BS13, N-BS15, N-BS18, N-BS20, N-BS25, N-BS28, N-NS29, N-BS30, N-BS31, N-BS34
B	Cottony	7	19.4	N-BS5, N-BS8, N-BS12, N-BS14, N-BS19, N-BS26, BS32
C	Fluffy	14	38.8	N-BS4, N-BS7, N-BS9, N-BS11, N-BS16, N-BS17, N-BS22, N-BS23,N-BS24, N-BS27, N-BS35, N-BS36, N-BS37, N-BS38,

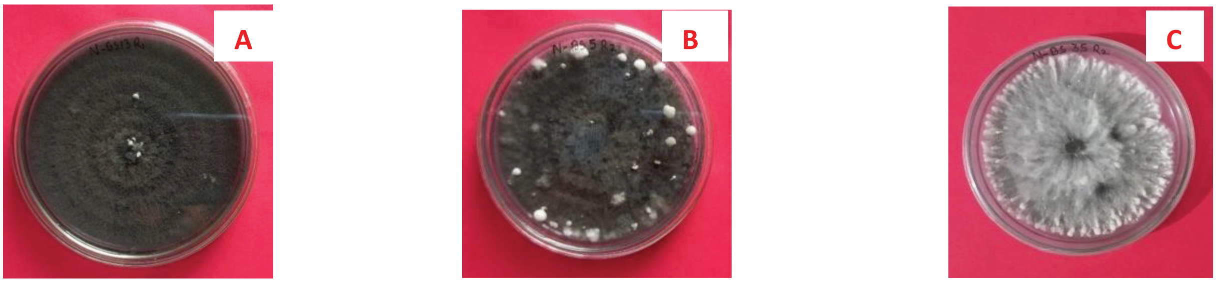
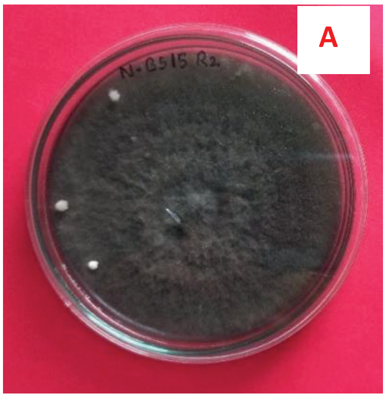


Fig. 2. Grouping of the *B. sorokiniana* isolates based on colony growth behaviour; Suppressed (A), Cottony (B) and Fluffy (C)

Table 5. Colony margin characteristics and their relative frequency of *B. sorokiniana* isolates collected from different wheat-growing regions of Nepal during 2023/24

Group	Colony growth margin	No. of Isolates	Isolate Population %	Isolate Designation
A	Regular	24	66.6	N-BS1, N-BS2, N-BS3, N-BS4, N-BS5, N-BS6, N-BS8, N-BS9, N-BS10, N-BS11, N-BS12, N-BS13, N-BS15, N-BS16, N-BS18, N-BS22, N-BS23, N-BS25, N-NS27, N-BS28, N-BS31, N-BS34, N-BS36, N-BS38
B	Irregular	12	33.3	N-BS7, N-BS14, N-BS17, N-BS19, N-BS20, N-BS24, N-BS26, N-BS29, N-BS30, N-BS35, N-BS37



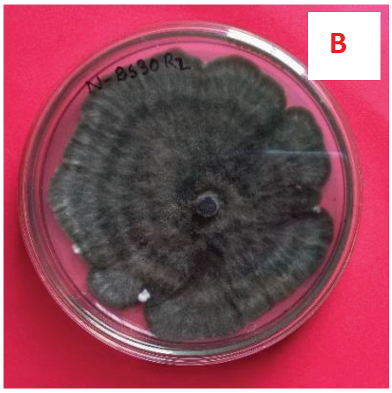


Fig. 3. Grouping of the *B. sorokiniana* isolates based on colony growth margin; Regular (A) and Irregular (B)

Table 6. Mean colony growth diameter of *B. sorokiniana* isolates at different days after incubation (DAI) collected from different wheat growing regions of Nepal during 2023/24

	Mean colony growth diameter (cm)			
Isolates	2 DAI	4 DAI	6 DAI	8 DAI
N-BS1	2.33^{ghi}	4.63^{i}	6.63^{kl}	8.50^{defgh}
N-BS2	2.66^{defg}	5.90^{cdef}	8.26^{bcd}	8.83^{abcd}
N-BS3	2.50^{efg}	5.46^{efg}	8.16^{cd}	8.93^{ab}
N-BS4	2.66^{defg}	5.90^{cdef}	8.26^{bcd}	8.90^{abc}
N-BS5	2.73^{cdef}	5.26^{gh}	8.10^{de}	8.90^{abc}
N-BS6	1.73^{jk}	4.46^{i}	7.46^{ghi}	8.96^{a}
N-BS7	2.80^{cde}	5.50^{efg}	7.26^{hij}	8.50^{defgh}
N-BS8	2.80^{cde}	5.46^{efg}	8.16^{cd}	9.00^{a}
N-BS9	2.50^{efg}	5.50^{efg}	7.90^{def}	8.96^{a}
N-BS10	2.63^{defg}	5.66^{defg}	7.70^{efg}	8.96^{a}
N-BS11	3.03^{c}	6.16^{abcd}	8.26^{bcd}	9.00^{a}
N-BS12	2.13^{hi}	5.70^{defg}	7.63^{fgh}	8.70^{abcdef}
N-BS13	2.80^{cde}	5.90^{cdef}	8.53^{abc}	9.00^{a}
N-BS14	2.53^{efg}	5.40^{fg}	7.00^{jk}	8.03^{j}
N-BS15	2.90^{cd}	5.53^{efg}	7.93^{def}	8.96^{a}
N-BS16	2.73^{cdef}	5.70^{defg}	8.00^{def}	9.00^{a}
N-BS17	2.53^{efg}	5.60^{efg}	8.03^{def}	8.83^{abcd}
N-BS18	2.63^{defg}	5.33^{g}	7.93^{def}	8.90^{abc}
N-BS19	2.40^{fgh}	5.60^{efg}	7.30^{ghij}	8.43^{efghi}
N-BS20	1.53^{k}	3.53^{j}	5.80^{m}	8.23^{hij}
N-BS22	3.50^{b}	6.40^{abc}	8.73^{a}	9.00^{a}
N-BS23	2.53^{efg}	5.43^{efg}	7.43^{ghi}	8.40^{efghij}
N-NS24	2.00^{ij}	4.23^{i}	6.43^{l}	7.23^{k}
N-BS25	2.70^{cdef}	5.53^{efg}	8.00^{ded}	8.90^{abc}
N-BS26	2.43^{fgh}	5.40^{fg}	8.30^{bcd}	$8.56^{bcdefgh}$
N-BS27	2.90^{cd}	5.96^{bcde}	8.66^{ab}	$8.66^{abcdefg}$
N-BS28	2.63^{defg}	5.70^{defg}	7.90^{def}	8.90^{abc}
N-BS29	2.60^{defg}	5.46^{efg}	7.90^{ded}	8.76^{abcde}
N-BS30	2.73^{cdef}	5.46^{efg}	7.70^{efg}	8.30^{ghij}
N-BS31	2.90^{cd}	5.73^{defg}	8.13^{cd}	8.76^{abcde}
N-BS32	2.40^{fgh}	4.76^{hi}	7.40^{ghij}	8.10^{ij}
N-BS34	2.90^{cd}	5.90^{cdef}	8.73^{a}	9.00^{a}
N-BS35	3.40^{b}	6.46^{ab}	8.06^{de}	8.53^{cdefgh}
N-BS36	3.43^{b}	6.33^{abc}	8.53^{abc}	8.73^{abcde}
N-BS37	3.73^{ab}	5.90^{cdef}	8.13^{cd}	8.33^{fghij}
N-BS38	3.90^{a}	6.63^{a}	7.13^{ij}	8.76^{abcde}
p value	<0.0001	<0.0001	<0.0001	<0.0001
LSD	0.35	0.53	0.41	0.38
CV%	8.06	5.92	3.24	2.69

From the study, beside the occurrence of spot blotch disease in terai region, it has also been reported from mid and high hill districts of Nepal (Kaski, Salyan, Baglung, Dolakha, Lalitpur, Bajura, Rukum and Mustang). Occurrence of spot blotch disease has been reported from mid hills district of Nepal with varied morphological colony characteristics (Bhandari, 2017; Mahto et al., 2012). Due to the effect of climate change, unpredictable weather conditions and development of new races, the incidence of spot blotch disease has been slowing appearing in mid hills and higher elevations (Basnet et al., 2022). Previous researchers explained that morphological colony variability of the isolates can be characterized on the basis of color of mycelia, formation of margins, sectoring, exudation, septa of conidia (Jaiswal et al., 2007; Kumar et al., 2002; Poloni et al., 2009). In the present study, only colony color, colony growth behavior and colony growth margin were used to characterize the morphology of *B. sorokiniana* isolates because the characteristics such as sectoring and zonation are highly influenced by media composition, age of culture and environmental conditions such as light, temperature. Five types of colony color were observed for *B. sorokinana* isolates in this study where highest share (33.3%) was obtained by dull white greenish/black colony color. Highest percentage share of dull white greenish/black colony color was also reported by Aggarwal et al. (2009) when 103 *B. sorokiniana* isolates representing different regions of India was categorized into 5 colony color. Although the isolates from five different groups were distinct in their morphology, no relationship was found between these traits and the geographic origin of the isolates. This finding was in agreement with a previous report (Nascimento & van der Sand, 2008). Ahmed et al. (1997) suggested that morphological variability among the isolates of *B. sorokiniana* could be attributed due to the multinucleate condition of mycelium and conidia, with subsequent heterokaryosis. Mean colony radial growth of the isolates differed significantly at different days after incubation (DAI). Isolate (N-BS 38) with highest colony growth at initial period didn't attain highest growth after 8 DAI. In contrast, isolate (N-BS20) with lowest colony growth at initial period also recorded radial growth above than other isolates after 8 DAI. Similar trend was also reported by H. K. Pandey and Adhikari (2025). Morphological variability (colony color, colony growth behavior, colony growth margin and radial colony growth) of the *B. sorokiniana* isolates directly relates to the pathogen's aggressiveness (virulence)**,** host adaptation and survival strategies thus helping to devise disease management strategy. Chakraborty et al. (2024) while evaluating the virulence of twelve *B. sorokiniana* isolates found that the isolate (BSC 11) with grey white spot color, cottony growth behavior and regular margin was much aggressive and obtained highest area under disease progress curve (AUDPC) value during pathogenicity test. Therefore, virulence potential of the collected isolates having varied morphological colony variability can be confirmed by performing pathogenicity test and thus obtained aggressive isolate can be used by plant breeders and plant pathologists in resistant breeding programs.

CONCLUSION

Although spot blotch has been traditionally considered to be more prevalent in warm and humid conditions, the findings from the present study, suggested by the isolation of the pathogen from mid and high hills indicates its occurrence beyond tropical conditions. While the current levels of disease incidence and severity in these areas may not yet be alarming, changing climatic conditions, cultivation of susceptible varieties and the potential emergence of more aggressive pathogen races could increase future risks. The study also revealed significant morphological variability among *B. sorokiniana* isolates in Nepal, particularly in regard to colony color, growth behavior, mycelial growth rate and colony margin. However, no similarities were found between these traits and the geographic origin of the isolates. Further studies involving pathogenicity testing and virulence profiling are necessary to better understand isolate diversity. Such information are essential for identifying highly virulent isolates to be used for screening wheat genotypes against spot blotch resistance.

ACKNOWLEDGEMENTS

The authors are grateful to all the helping hands in laboratory for making this experiment output into research paper. Authors would like to acknowledge Nepal Agricultural Research Council (NARC), Government of Nepal for providing fund to conduct this experiment.

AUTHOR CONTRIBUTIONS

LA: Conceptualization, Methodology, Writing – original draft, Writing – review & editing; **SMS:** Supervision, Writing – review & editing; **HKM:** Formal analysis, Supervision; **DBT:** Supervision, Writing – review & editing.

CONFLICT OF INTEREST

We confirm that this manuscript is original and has not been published before. All authors declare no conflict of interest for publication.

ETHICAL APPROVAL AND PERMITS

Famers were informed about the objective of the study. Prior approval was taken during the disease sample collection from the field.

REFERENCES

Acharya, K., Dutta, A. K., & Pradhan, P. (2011). *Bipolaris sorokiniana* (Sacc.) Shoemaker: The most destructive wheat fungal pathogen in warmer areas. *Australian Journal of Crop Science, 5*(9), 1064–1071.

Aggarwal, R., Singh, V. B., Gurjar, M. S., Gupta, S., & Srinivas, P. (2009). Intraspecific variation among Indian isolates of *Bipolaris sorokiniana* infecting wheat based on morphological, pathogenic, and molecular characteristics. *Indian Phytopathology, 62*(4), 449–460.

Ahmed, A. V., Rahman, M. Z., Bhuiyan, K. A., & Mian, I. H. (1997). Variation in isolates of *Cochliobolus sativus* from wheat. *Bangladesh Journal of Plant Pathology, 13*(1–2), 29–35.

Al-Sadi, A. M. (2016). Variation in resistance to spot blotch and the aggressiveness of *Bipolaris sorokiniana* on barley and wheat cultivars. *Journal of Plant Pathology, 98*(1), 97–103.

Basnet, R., Shrestha, S. M., Bhandari, D., Manandhar, H. K., & Thapa, D. B. (2022). Evaluation of wheat (*Triticum aestivum* L.) genotypes for spot blotch (*Bipolaris sorokiniana*) resistance under Terai conditions of Nepal. *Archives of Agriculture and Environmental Science, 7*(3), 440–449. https://doi.org/10.26832/24566632.2022.0703019

Bhandari, D. (2017). Incursion of Helminthosporium leaf blight disease of wheat in comparatively cool hilly regions of Nepal. *Journal of Medical, Biomedical and Applied Sciences*, 5(12), 1-7.

Bhandari, D., & Tripathi, J. (2005). Intensity of Helminthosporium leaf blight of wheat under different planting methods. In *Proceedings of the 26th National Winter Crops Research Workshop*.

Chakraborty, S., Mahapatra, S., Hooi, A., Bhushan, B. T., Almansour, M. I., Ansari, M. J., & Hossain, A. (2024). Survey, isolation and characterization of *Bipolaris sorokiniana* (Shoemaker) causing spot blotch disease in wheat under the climatic conditions of the Indo–Gangetic Plains of India. *Heliyon, 10*(22), e40398. https://doi.org/10.1016/j.heliyon.2024.e40398

Chand, R., Pandey, S. P., Singh, H. V., Kumar, S., & Joshi, A. K. (2003). Variability and its probable causes in natural populations of spot blotch pathogen *Bipolaris sorokiniana* of wheat (*Triticum aestivum* L.) in India. *Journal of Plant Diseases and Protection, 110*(1), 27–35.

Chaurasia, S., Chand, R., & Joshi, A. K. (2000). Relative dominance of *Alternaria triticina* Pras. et Prab. and *Bipolaris sorokiniana* (Sacc.) Shoemaker in different growth stages of wheat (*Triticum aestivum* L.). *Journal of Plant Diseases and Protection*, *107*(2): 176–181.

Duveiller, E. (2004). Controlling foliar blights of wheat in the rice–wheat systems of Asia. *Plant Disease, 88*(5), 552–556. https://doi.org/10.1094/PDIS.2004.88.5.552

Duveiller, E., Kandel, Y. R., Sharma, R. C., & Shrestha, S. M. (2005). Epidemiology of foliar blights (spot blotch and tan spot) of wheat in the plains bordering the Himalayas. *Phytopathology, 95*(3), 248–256. https://doi.org/10.1094/PHYTO-95-0248

FAO. (2024). *World food situation: FAO cereal supply and demand brief*. Food and Agriculture Organization.

Jaiswal, S. K., Sweta, Prasad, L. C., Sharma, S., Kumar, S., Prasad, R., & Joshi, A. K. (2007). Identification of molecular marker and aggressiveness for different groups of *Bipolaris sorokiniana* isolates causing spot blotch disease in wheat (*Triticum aestivum* L.). *Current Microbiology, 55*(2), 135–141. https://doi.org/10.1007/s00284-007-0035-z

Joshi, A. K., & Chand, R. (2002). Variation and inheritance of leaf angle, and its association with spot blotch (*Bipolaris sorokiniana*) severity in wheat (*Triticum aestivum*). *Euphytica, 124*(3), 283–291. https://doi.org/10.1023/A:1015773404694

Joshi, A. K., Mishra, B., Chatrath, R., Ortiz-Ferrara, G., & Singh, R. P. (2007a). Wheat improvement in India: Present status, emerging challenges and future prospects. *Euphytica, 157*(3), 431–446. https://doi.org/10.1007/s10681-007-9385-7

Joshi, A. K., Ortiz-Ferrara, G., Crossa, J., Singh, G., Alvarado, G., Bhatta, M. R., & Chand, R. (2007b). Associations of environments in South Asia based on spot blotch disease of wheat caused by *Cochliobolus sativus*. *Crop Science, 47*(3), 1071–1081. https://doi.org/10.2135/cropsci2006.07.0477

Joshi, A. K., Ortiz-Ferrara, G., Crossa, J., Singh, G., Sharma, R. C., Chand, R., & Prasad, R. (2007c). Combining superior agronomic performance and terminal heat tolerance with resistance to spot blotch (*Bipolaris sorokiniana*) of wheat in the warm humid Gangetic Plains of South Asia. *Field Crops Research, 103*(1), 53–61. https://doi.org/10.1016/j.fcr.2007.04.010

Joshi, B. K. (2017). Conservation and utilization of agro-biodiversity advanced from 1937 to 2017 in Nepal. In F. Devkota (Ed.), *Krishi Sanchar Smarika* (pp. 181–208). Agricultural Information and Communication Center (AICC), MoAD.

Kumar, J., Schäfer, P., Hückelhoven, R., Langen, G., Baltruschat, H., Stein, E., & Kogel, K. H. (2002). *Bipolaris sorokiniana*, a cereal pathogen of global concern: Cytological and molecular approaches towards better control. *Molecular Plant Pathology, 3*(4), 185–195. https://doi.org/10.1046/j.1364-3703.2002.00120.x

Li, Q. Y., Xu, Q. Q., Jiang, Y. M., Niu, J. S., Xu, K. G., & He, R. S. (2019). The correlation between wheat black point and agronomic traits in the North China Plain. *Crop Protection, 119*, 17–23.

Mahto, B. N., Gurung, S., Nepal, A., & Adhikari, T. B. (2012). Morphological, pathological and genetic variations among isolates of *Cochliobolus sativus* from Nepal. *European Journal of Plant Pathology, 133*(2), 405–417. https://doi.org/10.1007/s10658-011-9914-z

MoALD. (2024). *Statistical information on Nepalese agriculture*. Agri-Business Promotion and Statistics Division, Ministry of Agriculture and Livestock Development, Government of Nepal.

Nascimento, E. J. M., & van der Sand, S. T. (2008). Restriction analysis of the amplified ribosomal DNA spacers ITS1 and ITS2 of *Cochliobolus sativus* isolates. *World Journal of Microbiology and Biotechnology, 24*, 647–652.

Pandey, H. K., & Adhikari, P. (2025). Morphological characteristics of *Bipolaris sorokiniana* causing spot blotch of wheat and its *in vitro* management using different botanicals. *Agriculture Development Journal, 18*, 26–37. https://doi.org/10.3126/adj.v18i1.82086

Pandey, S. P., Sharma, S., Chand, R., Shahi, P., & Joshi, A. K. (2008). Clonal variability and its relevance in generation of new pathotypes in the spot blotch pathogen, *Bipolaris sorokiniana*. *Current Microbiology, 56*(1), 33–41. https://doi.org/10.1007/s00284-007-9034-3

Poloni, A., Pessi, I. S., Frazzon, A. P. G., & Van Der Sand, S. T. (2009). Morphology, physiology, and virulence of *Bipolaris sorokiniana* isolates. *Current Microbiology, 59*(3), 267–273. https://doi.org/10.1007/s00284-009-9429-4

Sharma, R. C., & Duveiller, E. (2006). Spot blotch continues to cause substantial grain yield reductions under resource-limited farming conditions. *Journal of Phytopathology, 154*(7–8), 482–488. https://doi.org/10.1111/j.1439-0434.2006.01134.x

Sharma, R. C., Duveiller, E., & Ortiz-Ferrara, G. (2007). Progress and challenge towards reducing wheat spot blotch threat in the Eastern Gangetic Plains of South Asia: Is climate change already taking its toll? *Field Crops Research, 103*(2), 109–118. https://doi.org/10.1016/j.fcr.2007.05.004