Nepal Agriculture Research Journal
Vol. 17, No. 1, pp. 168-182, 2026
DOI: https://doi.org/10.3126/narj.v17i1.99434

Pathogenic variability and aggressiveness of Nepalese *Bipolaris sorokiniana* isolates causing spot blotch of wheat

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ARTICLE INFO

Research Paper

Received: March 11, 2026

Revised: March 28, 2026

Accepted: June 17, 2026
Published: September 4, 2026

Contents available at
http://www/sasnepal.org.np

Published by Society of Agricultural Scientists Nepal (SAS-Nepal).

ABSTRACT
Spot blotch caused by fungal pathogen *Bipolaris sorokiniana* (Sacc.) Shoemaker is an important fungal disease in warm and humid wheat growing condition. Study on pathogenic variability of *B. sorokinianana* isolates is limited in Nepal. Information based on host × pathogen interaction is a useful tool to devise the disease management strategy. Therefore, aggressiveness of total 36 single spore isolates of *B. sorokiniana* (27 isolates from terai, 7 isolates from mid hill and 2 isolates from high hill) of Nepal were evaluated on 14 days old seedling of RR-21 (susceptible check), Guatam (moderate resistant) and Chirya 3 (resistant check) under polyhouse condition. Results revealed highly significant isolate-by-variety interaction ($P<0.001$) for all the disease development traits, suggesting substantial variability in the pathogenicity of the isolates. Moreover, days to disease appearance, number of lesions per leaf and disease severity was highly correlated to Area Under Disease Progress Curve (AUDPC), illustrating those traits as an important indicator of disease development and pathogen aggressiveness. RR-21 exhibited susceptible to overall isolates response followed by Gautam and Chirya 3. On the basis of AUDPC value, top ten aggressive isolates were identified (N-BS24, N-BS7, N-BS28, N-BS36, N-BS27, N-BS37, N-BS15, N-BS38, N-BS19 and N-BS16). Among them, N-BS24 and N-BS28 were able to show high disease against resistant check Chirya 3, so aggressiveness of these two isolates can be further validated through adult plant screening and genetic study and utilize it for resistant breeding program in Nepal.
Keywords: Diversity, foliar blight, pathogen population, *Triticum aestivum*, virulent

Cited as: Laxman A, SM Shrestha, HK Manandhar and DB Thapa. 2026. Pathogenic variability and aggressiveness of Nepalese *Bipolaris sorokiniana* isolates causing spot blotch of wheat. Nepal Agriculture Research Journal **17**(1): 168-182. DOI: https://doi.org/10.3126/narj.v17i1.99434

INTRODUCTION

Wheat (*Triticum aestivum* L.) is among the most reliable cereal crops globally which supports 35% of the world's population to meet their daily caloric needs (Aditya et al 2024). In Nepal, wheat is the third most important cereal crop after rice and maize with the productivity of 2.98 mt/ha (MoALD 2024). There is increasing demand of wheat in global context to meet food security for increasing population. Limited access to quality seed and fertilizers, untimely availability of labor, inadequate irrigation facilities, and poor access to agricultural machinery were identified as the major constraints to wheat production (Subedi et al 2019). In addition, biotic and abiotic stresses remain major constraints contributing to reduced wheat productivity in Nepal (Khan et al 2020). Successful wheat cultivation is challenged by different biotic factors. Among them Spot blotch, the fungal caused by *Bipolaris sorokiniana* (Sacc.) Shoemaker, is a destructive disease in wheat (Duveiller 2004, Kumar et al 2002). Spot blotch has become particularly important not only in warmer wheat-growing environments with elevated temperature and humidity but due to the effect of climate change in the host pathogen interaction, this disease has been reported in cooler wheat growing region of Mustang, Nepal with elevation of 2650 msl (Aryal et al 2026). Disease 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). This disease occurs at seedling and adult stage and can cause severe losses (Mehta 1993). Losses vary by region, cultivar resistance and environmental conditions. Yield losses vary from 23-40% depending on genotypes and other environmental factors in warm regions of Nepal (Sharma and Duveiller 2006, Bhandari and Tripathi 2005). In severe condition, up to 100% foliar blight incidence was reported in the field with yield losses up to 52% in eastern plains of Nepal (Sharma and Duveiller 2006). Beside this, disease cause qualitative deterioration of grain quality through physical discoloration, reduced kernel weight and also affect the biochemical properties of the wheat plant (Raj et al 2024; Kumari et al 2023).

Spot blotch is said to increase every year all over the world due to the lack of durable resistant cultivars and ineffective control measures (Sharma et al., 2007). In addition, due to the effect of climate change, unpredictable weather conditions and the development of new races, the incidence of spot blotch disease has been spread from warm plain areas to comparatively cool mid-hills and high hills of Nepal (Aryal et al 2026, Bhandari 2017, Mahto et al 2012). Studies on morphological, pathological and molecular approaches have been used to identify and investigate the variability among isolates of *B. sorokiniana* (Poloni et al 2009; Ghazvini and Tekauz 2007, Jaiswal et al 2007, Oliveira et al 1998). High pathological variability among isolates of *B. sorokiniana* has been previously reported (Poloni et al 2009, Ghazvini and Tekauz 2007).

B. sorokiniana is a genetically and phenotypically diverse pathogen. Isolates collected from different geographical regions of Nepal showed different pathogenic behavior and established a significant isolate-by- cultivar interaction (Mahto et al 2012). A cultivar may show different degree of disease severity when challenged with different isolates. A cultivar considered resistant under one pathogen population may exhibit greater susceptibility to another isolate. Study conducted in Nepal highlighted that the isolates from the plains were more aggressive than isolates from the hills (Mahto et al 2012). Spatial distribution and morphological colony variability of Nepalese *B. sorokiniana* isolates also emphasizes to assess pathogen variability through pathogen profiling and virulence studies (Aryal et al 2026).

Despite the importance of spot blotch in Nepal, comparative study on the aggressiveness of geographically diverse *B. sorokiniana* isolates and their differential interactions with wheat cultivars remains limited. Disease development components like days to disease development, number of lesions per leaf, disease severity and disease progress can be used to quantify aggressiveness. Singh et al (2018) found varied phenotypic response of ten *B. sorokiniana* isolates on five wheat cultivars on seedling stage. Comparing the aggressiveness of the isolates under controlled conditions reduces environmental variation and allows more reliable information. Identification of highly aggressive isolates through isolate-by- cultivar interaction guides to develop durable spot blotch resistant variety. Therefore, the main objective of this study was to elucidate the variation in aggressiveness of *B. sorokiniana* isolates collected from different wheat growing regions of Nepal and provide baseline information for future molecular characterization of *B. sorokiniana* populations in Nepal.

MATERIALS AND METHODS

Collection of leaf samples

Typical spot blotch infected leaf samples were collected from Terai and hills wheat-growing districts of Nepal in paper envelope from January to May in the year 2022/23 and 2023/24. From each location, 5-6 leaves were randomly collected as a representative sample, but the location where disease symptoms looked different, representative samples were doubled. Each sample envelope was marked with location, latitude, longitude, altitude and 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 and later rinsed with sterilized distilled water for 3 times. Disease sample pieces were dried in sterilized whatman filter paper in laminar flow and then transferred to a moisture chamber and incubated at 25 ± 1 °C to allow sporulation for 48 hours (Maraite et al 1997). After confirmation of the pathogen growth, a single spore of *B. sorokiniana* was isolated with flame sterilized teasing needle 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 followed by incubation at 25 ± 1 °C for eight to ten days for full growth of *B. sorokiniana*. Fully grown plates of thirty-six *B. sorokiniana* isolates were maintained and stored at 5 ^{O} C for further pathological characterization.

Designation of the maintained Isolates

From the spot blotch disease sample collected from Terai to hilly wheat growing region of Nepal, a total of 36 *B. sorokiniana* isolates were maintained. Isolates were designated as N-BS and numeric letter added at suffix differentiates each isolates with respective districts.

Seedling raising

Three wheat varieties RR-21, Guatam and Chirya 3 were selected for aggressiveness study of the different isolates. Firstly, the soil was autoclaved at 121^{0}C at 15 pound per square inch (psi) for 30 minutes for sterilization of the soil. Then the sterilized soil was filled in the plastic glass with diameter 7cm and length 9.5 cm. For each isolates, five seeds of 3 wheat cultivar were sown in each plastic pot and were replicated 3 times. Each container was considered an experimental unit. Chirya 3 and RR 21 were used as a resistant check and a susceptible check, respectively.

Inoculum preparation

Inoculum of each isolate was prepared by multiplying the isolates in PDA plates. 10 days old fully grown culture plate was taken and 5 mm disc was cut with sterilized cork borer measuring 5 mm diameter and was placed at the center of the solidified PDA plate. Inoculated petridish was incubated at 25±1 ^{0}C for 10 days under continuous fluorescent light for full growth of *B. sorokiniana*. Conidia of the each isolates in the fully grown petridish were harvested by adding distill water with sterilized spatula. The conidial suspension was filtered through four layers of cheese cloth to remove mycelial fragments. Final spore density was measured using a haemocytometer and maintained the spore density of 3000/ ml suspension (Mahto et al 2012).

Inoculation in the selected wheat cultivar

Wheat varieties were arranged in a randomized complete block design (RCBD). One drop of Tween-20 per 100 ml of spore suspension was added as a surfactant to facilitate the dispersion of the inoculum over the wheat leaf surface. Three replicates of 5 plants of tested varieties were inoculated with each isolate spore suspension until runoff at 14 days old seedling. After inoculation, seedlings were sprayed with water mist using hand sprayer to maintain continuous leaf wetness. As method followed by Singh et al (2018), temperature around at 25±1^{O} C and relative humidity of 85-95 per cent was maintained inside the polyhouse.

Disease assessment

The time between the inoculation and first appearance of the spot/lesion on the seedling was considered as day's duration to disease appearance. Appearance of symptom was recorded at 10 a.m. onward every day in every replication. As method followed by Singh et al (2018), number of lesion/spot per seedling leaf was recorded at tenth day of inoculation. After the symptom appearance, disease scoring was done in (0-5) scale as suggested by Rahman et al (2011) to calculate disease severity at different scoring interval. Disease scoring was done 3 times at the interval of 4 days.

0= refers to no visible symptoms

1= flecks or minute lesions

2= small dark lesion, no distinct halo, less than 10% area affected

3= typical leaf with yellow halo covering 10-50% leaf area affected

4= small but coalescing lesion covering more than 50% leaf area

5= necrotic lesions and total leaf collapsed

Statistical analyses

Data collected were tabulated in MS Excel and analyzed using R Studio 4.5.1 package

RESULTS

Maintenance of *B. sorokiniana* isolates causing spot blotch of wheat

Thirty-six *Bipolaris sorokiniana* isolates representing different districts were isolated and maintained in the 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).

Table1. *Bipolaris sorokiniana* isolates collected and maintained from different wheat growing areas of Nepal during 2023 and 2024

Isolates Code	Location (District)	Elevation (msl)	Region
N-BS1	Udaypur	304	Terai
N-BS2	Palpa	750	Terai
N-BS3	Kaski	1,981.85	Mid hill
N-BS4	Syangja	560	Terai
N-BS5	Salyan	1485	Mid hill
N-BS6	Baglung	1,675.49	Mid hill
N-BS7	Kailali	1,56.64	Terai
N-BS8	Nawalpur	205	Terai
N-BS9	Doti	540	Terai
N-BS10	Nawalparasi	115	Terai
N-BS11	Jhapa	120	Terai
N-BS12	Gulmi	815	Terai
N-BS13	Dolakha (A)	1,661	Mid hill
N-BS14	Rupandehi	112	Terai
N-BS15	Lalitpur	1421	Mid hill
N-BS16	Banke	157	Terai
N-BS17	Sarlahi	1,79.91	Terai
N-BS18	Surkhet	580	Terai
N-BS19	Mahottari	229	Terai
N-BS20	Bardiya	1,36.6	Terai
N-BS22	Mustang	2,650	High hill
N-BS23	Bara	115	Terai
N-BS24	Sunsari (A)	136	Terai
N-BS25	Danusha	94	Terai
N-BS26	Dang	725	Terai
N-BS27	Dolakha (B)	2,567	High hill
N-BS28	Rautahat	108	Terai
N-BS29	Siraha	131.2	Terai
N-BS30	Saptari	125	Terai
N-BS31	Sunsari (B)	154	Terai
N-BS32	Pyuthan	817	Terai
N-BS34	Chitwan	1,69.93	Terai
N-BS35	Kanchanpur	210	Terai
N-BS36	Bajura	1,390	Mid hill
N-BS37	Rukum	1,273	Mid hill
N-BS38	Kapilvastu	210	Terai

Based on Joshi et al (2017), 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.

Correlation study

Correlation matrix was studied between disease development components for variety, isolate and interaction between variety and isolate. The correlation matrix exhibited that duration of days to disease appearance was negatively correlated to total AUDPC. Interestingly, total

AUDPC obtained by all the *B. sorokiniana* isolates showed a strong positive correlation with number of lesions per leaf, first disease severity, second disease severity and third disease severity (Figure 1).

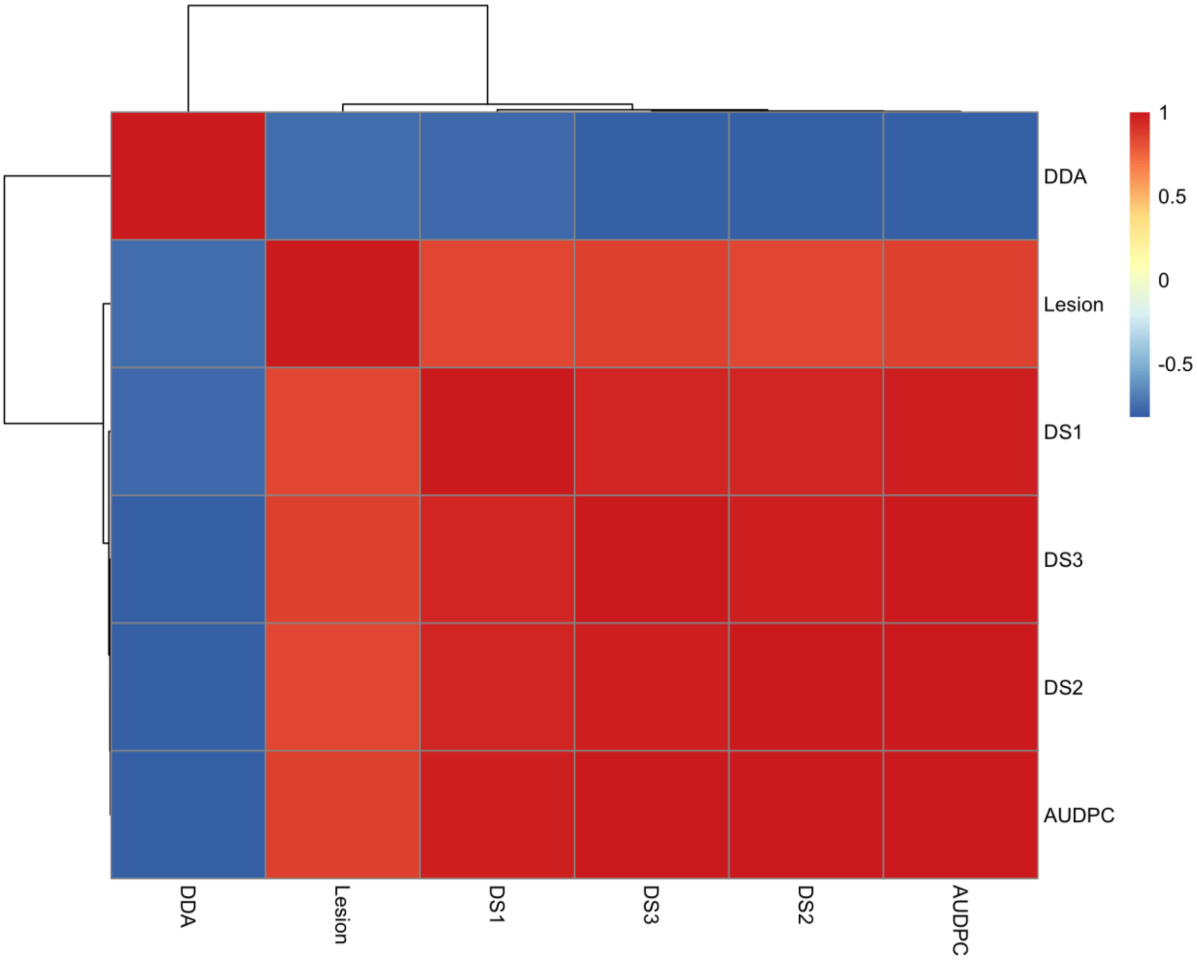


Figure 1. Correlation among the disease development traits

Aggressiveness assessment of the isolates based on disease development components

Days to disease appearance

All the isolates were able to cause disease symptoms on the tested wheat varieties. Days to disease appearance varied highly significantly ($p<0.001$) for the interaction between varieties and isolates (Figure 3). Isolates N-BS17, N-BS 24, N-BS 27, N-BS 28 and N-BS 37 were very aggressive and were able to cause disease symptom early (3) days in the tested wheat varieties. But isolate N-BS 20 was late responsive and took longer days (6) days to cause disease symptom in all the varieties. Among the varieties, RR-21 showed disease symptom in earlier days (3.81) followed by Guatam (4.12) and Chirya 3 (4.5) days respectively (Table 2).

Number of lesions/leaf

Number of lesions per leaf in the seedling of the tested varieties varied highly significantly ($p<0.001$). Wheat varieties responded differently for the isolates producing number of lesions per leaf and proved high host-pathogen interaction (Figure 3). Isolates N-BS7, N-BS 24, N-

BS 27, N-BS 28 and N-BS 38 produced higher number of lesions in RR-21, Gautam and Chirya 3. Even in resistant check Chirya 3, numbers of lesions per leaf were above 10. But N-BS3, N-BS4, N-BS5, N-BS11 produced less than 3 lesions per leaf in RR-21, Gautam and Chirya 3. Minimum number of lesions in the leaf of susceptible variety RR-21 proved that these isolates were less aggressive. Among the varieties, RR-21 produced higher number of lesions (9.36) followed by Gautam (7.46) and Chirya 3 (5.52) (Table 2).

Disease severity

Disease severity of the tested wheat varieties exhibited different values at different date of scoring (P<0.001). Susceptible check RR-21 recorded consistently higher disease severity value DS1, DS2, DS3 (26.3, 35.6 and 42.40) respectively, over time. Similarly, moderate resistant check Gautam recorded intermediate value between RR-21 and Chirya 3. Gautam recorded (24.51, 32.03 and (37.55) for DS1, DS2 and DS3 respectively. Resistant check Chirya 3 recorded lowest disease severity value (21.70, 27.3 and (32.92) respectively for DS1, DS2 and DS3 at different scoring period (Figure 2).

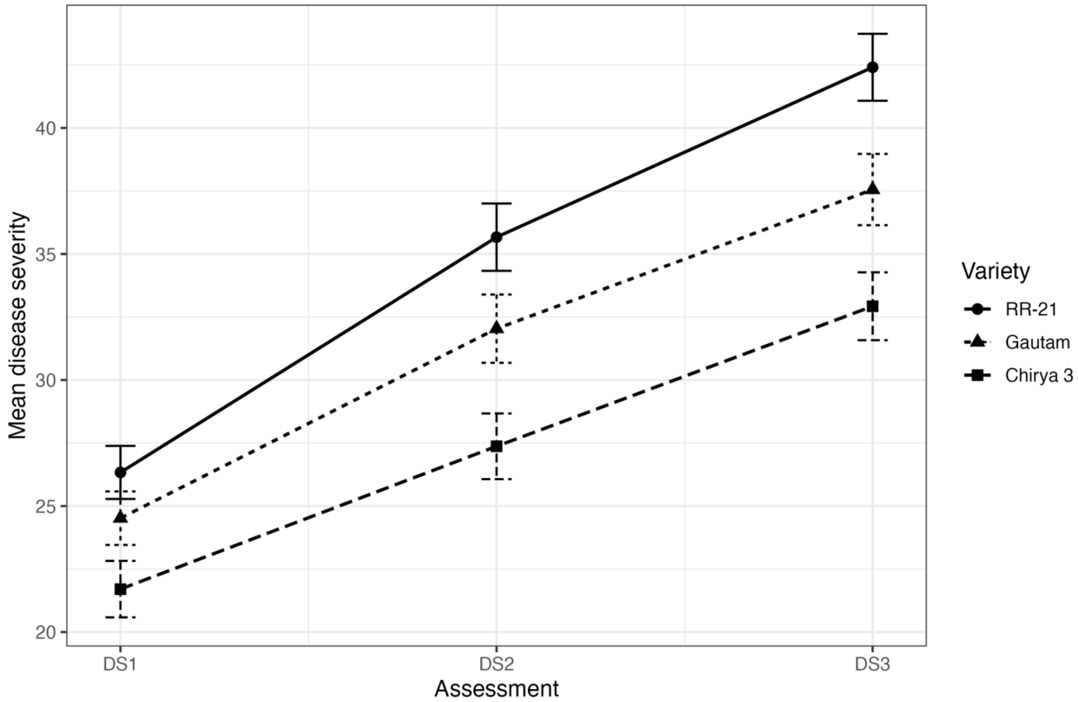


Figure 2. Overall Disease progress by wheat varieties

Total AUDPC

Total AUDPC value varied highly significantly (p<0.001) for the interaction of isolate and variety. Isolates N-BS24, N-BS7, N-BS15, N-BS24, N-BS27, N-BS28 and N-BS36 recorded highest AUDPC value in the tested wheat varieties and showed high aggressiveness to cause the disease. Interestingly, N-BS24 and N-BS 28 recorded highest AUPDC value (424) and (432) respectively even in resistant check Chirya 3. Among the isolates which were inoculated, N-BS25, N-BS26 and N-BS4 produced comparatively lowest AUDPC value in the wheat varieties. AUDPC value of these isolates was lower than 200 even in RR-21 which is susceptible check. Among the varieties, AUDPC value for the susceptible check RR-21, moderate resistant check Gautam and Resistant check Chirya 3 were (280.17), (253.29) and (218.73) respectively (Table 2).

Table 2. Interaction effect of Isolate by variety for disease development parameters

Isolate	Days to disease appearance			No of lesion/Leaf			AUDPC		
	RR-21	**Gautam**	**Chirya 3**	**RR-21**	**Gautam**	**Chirya 3**	**RR-21**	**Gautam**	**Chirya 3**
N-BS1	4.0	5.0	5.0	5.0	1.3	2.0	184.0	136.0	104.0
N-BS2	4.0	5.0	5.0	3.0	2.0	2.0	184.0	184.0	128.0
N-BS3	4.0	4.0	4.0	1.0	3.0	2.0	208.0	224.0	184.0
N-BS4	5.0	5.0	7.0	3.0	2.7	1.0	176.0	152.0	72.0
N-BS5	5.0	6.0	5.0	2.3	1.0	1.3	176.0	96.0	136.0
N-BS6	4.0	5.0	5.0	6.0	4.0	2.7	256.0	192.0	216.0
N-BS7	3.0	3.0	4.0	23.3	18.7	11.7	440.0	413.3	392.0
N-BS8	3.0	4.0	4.0	7.0	4.7	2.7	288.0	256.0	200.0
N-BS9	4.0	5.0	4.0	5.0	3.0	5.0	224.0	160.0	216.0
N-BS10	4.0	4.0	4.0	3.7	7.0	4.7	288.0	256.0	232.0
N-BS11	5.0	4.0	5.0	3.0	3.0	3.0	248.0	256.0	168.0
N-BS12	4.0	3.0	3.0	9.0	12.0	6.0	288.0	304.0	280.0
N-BS13	4.0	4.0	5.0	6.7	7.0	6.0	320.0	328.0	288.0
N-BS14	4.0	6.0	6.0	4.0	2.0	1.0	208.0	128.0	160.0
N-BS15	3.3	3.3	3.3	13.0	11.3	7.3	405.33	400.0	320.0
N-BS16	3.3	3.0	4.3	18.3	16.7	9.0	418.67	344.0	320.0
N-BS17	3.0	3.0	3.0	13.0	8.0	6.7	392.0	336.0	288.0
N-BS18	4.0	3.0	6.0	3.0	5.0	3.0	144.0	168.0	120.0
N-BS19	3.3	3.3	3.3	15.3	11.3	8.0	405.33	360.0	336.0
N-BS20	6.0	6.0	6.0	5.3	3.0	8.0	152.0	128.0	160.0
N-BS22	4.0	4.0	5.0	12.0	9.3	4.0	192.0	216.0	160.0
N-BS23	3.0	3.0	4.0	8.3	5.0	4.0	232.0	264.0	176.0
N-BS24	3.0	3.0	3.0	21.7	17.7	11.7	467.67	448.0	424.0
N-BS25	5.0	4.0	6.0	3.0	4.0	2.0	106.67	146.67	85.3
N-BS26	5.0	5.0	6.0	4.0	2.0	2.0	133.33	160.0	85.3
N-BS27	3.0	3.0	3.0	18.3	15.3	13.3	424.0	400.0	368.0
N-BS28	3.0	3.0	3.0	24.7	17.7	16.3	392.0	392.0	432.0
N-BS29	3.67	5.0	4.3	7.0	7.0	3.0	232.0	200.0	160.0
N-BS30	3.3	6.0	4.3	8.0	2.0	3.0	288.0	128.0	152.0
N-BS31	3.3	4.0	5.3	8.0	5.0	4.3	232.0	168.0	160.0
N-BS32	3.0	5.0	6.0	10.0	6.0	6.0	248.0	168.0	104.0
N-BS34	4.0	5.0	6.0	5.0	4.0	2.0	232.0	160.0	120.0
N-BS35	4.0	4.0	5.0	6.0	6.0	3.0	248.0	216.0	128.0
N-BS36	3.0	4.0	4.0	15.3	11.3	8.7	426.67	453.33	328.0
N-BS37	3.0	3.0	3.0	14.0	13.3	10.3	424.0	368.0	344.0
N-BS38	4.0	3.0	4.0	22.0	16.3	12.3	402.67	373.3	328.0
Mean	**3.81**	**4.12**	**4.55**	**9.36**	**7.46**	**5.52**	**280.17**	**252.29**	**218.73**
p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
CV%		4.87			7.56			5.21	

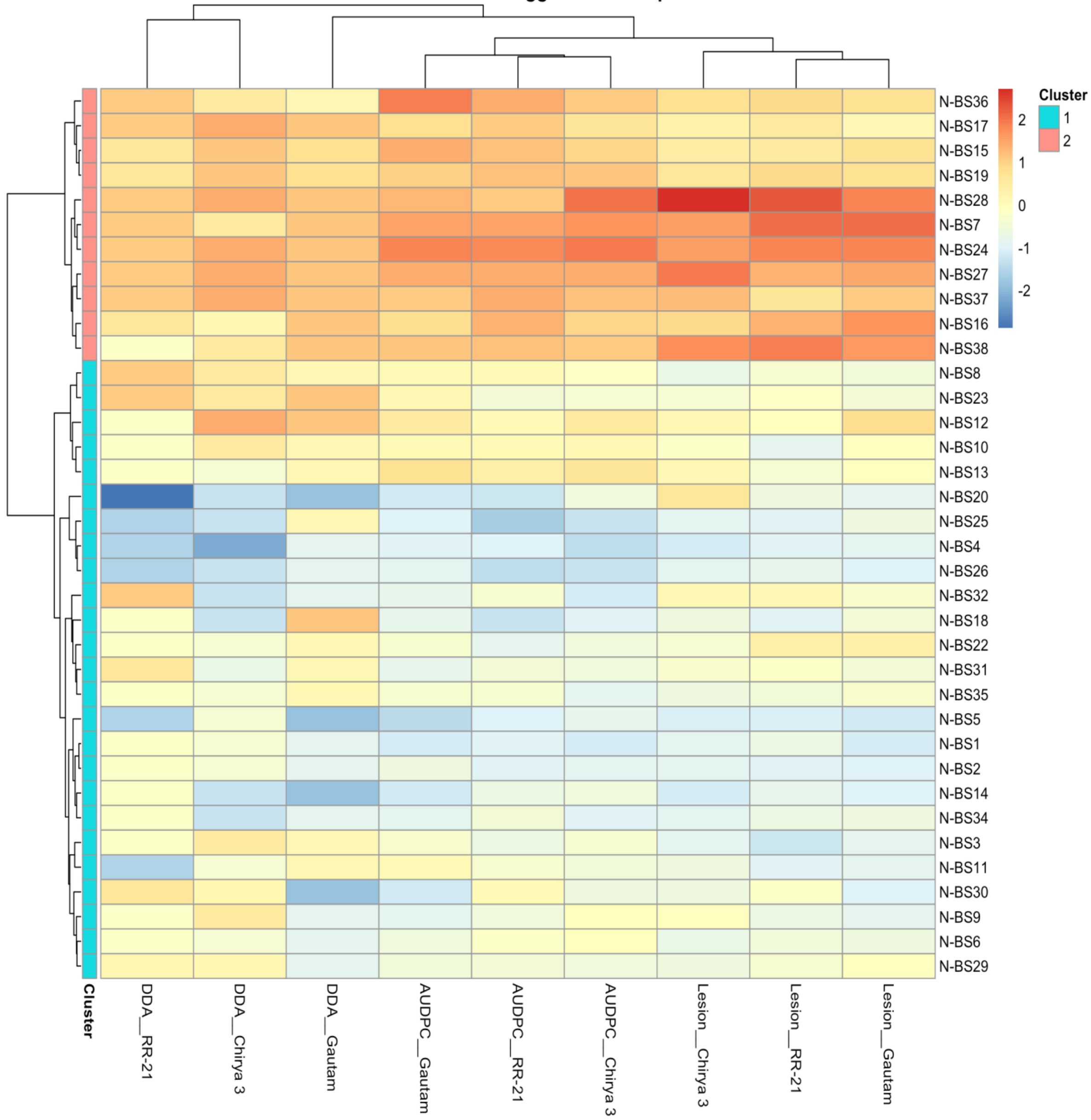
Clustering of the isolates on the basis of aggressiveness for disease development

B. sorokiniana isolates from different origin of Nepal responded differently on the wheat varieties after inoculation on the seedling stage. On the basis of heat map developed by the interaction between isolates and varieties for the different disease development components, isolates were grouped in major two clusters (Figure 3). Among 36 *B. sorokiniana* isolates, 11 isolate (N-BS36, N-BS17, N-BS15, N-BS19, N-BS28, N-BS7, N-BS24, N-BS27, N-BS37, N-BS16, N-BS38) are grouped in cluster 2), representing the aggressiveness of isolates in terms of days to disease appearance (DDA), number of lesions per leaf, disease severity and total AUDPC. The remaining 25 isolates are grouped in cluster 1 as they were low responsive to disease development components (Figure 3).

Figure. 3 Heat map showing multi-trait aggressiveness of the isolates

Arrangement of the *B. sorokiniana* isolates on the basis of AUDPC value

All the *B. sorokiniana* isolates were able to cause disease in the tested wheat varieties comprising susceptible check RR-21, moderate resistant check Gautam and resistant check Chirya 3. The degree of pathogenicity of the isolates varied significantly for AUDPC value. N-BS24 was highly aggressive for disease development in the inoculated wheat varieties and recorded the highest AUDPC value (446.5). Among the isolates N-BS25 was very weak in terms of disease development and recorded the lowest AUDPC value (112.8) (Figure 4). In terms of AUDPC, the isolates were arranged in the following order: N-BS24 > N-BS7 > N-BS28 > N-BS36 > N-BS27 > N-BS37 > N-BS15 > N-BS38 > N-BS19 > N-BS16 (Figure 5).



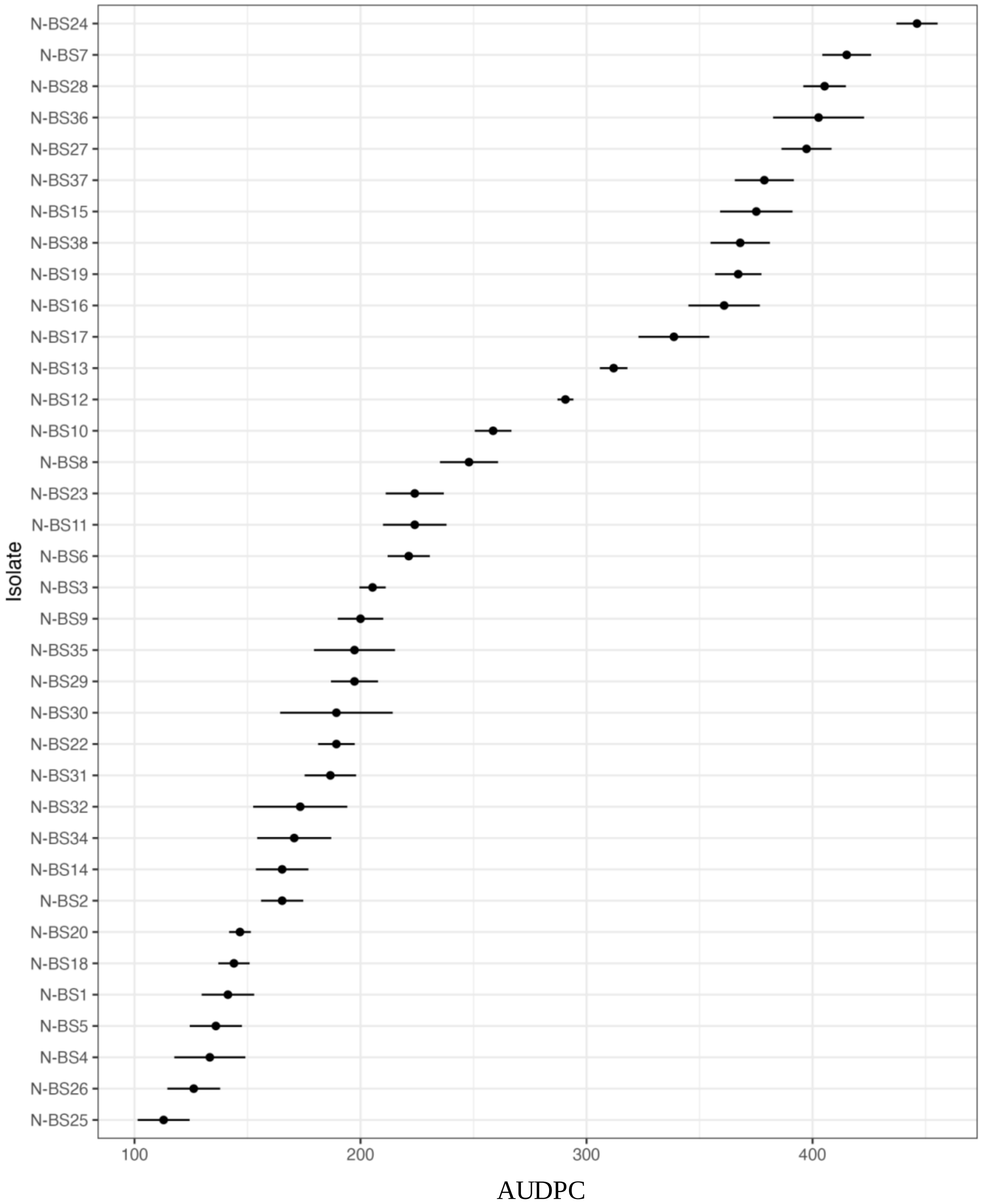


Figure 4. AUDPC value of the *B. sorokiniana* isolates inoculated on different wheat varieties

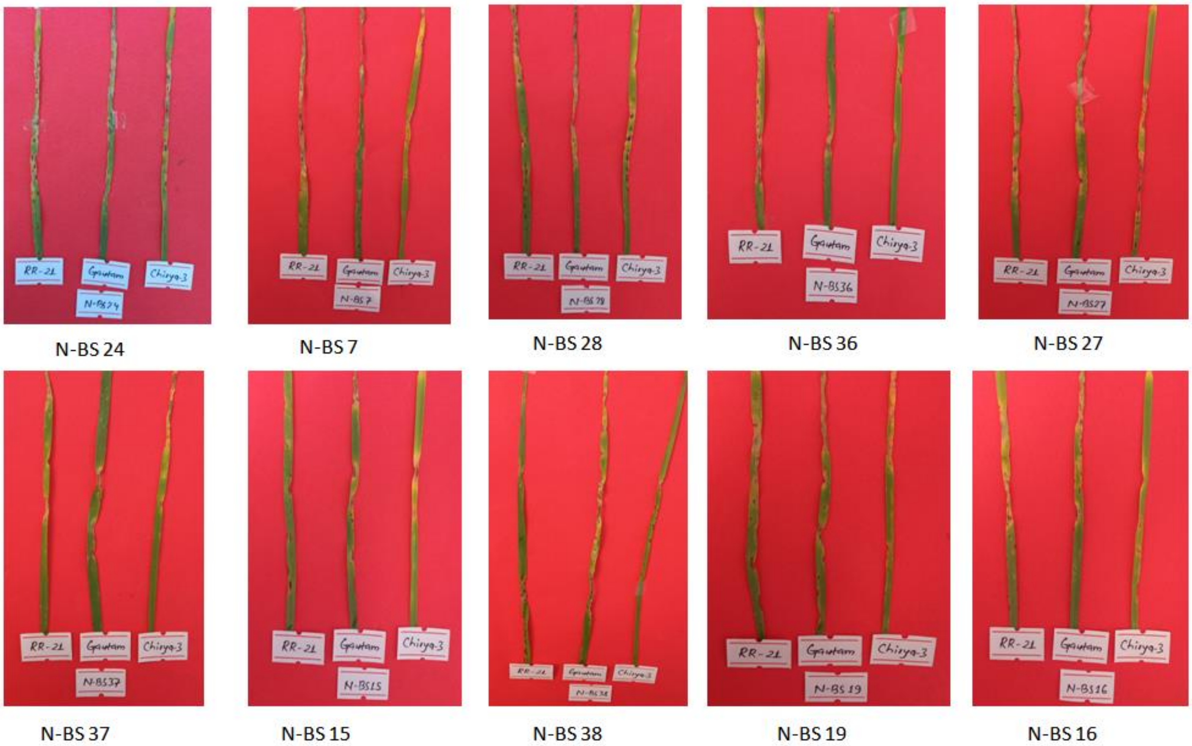


Figure 5. Top ten aggressive *B. sorokiniana* isolates on the basis of disease symptom expressed on seedling of wheat varieties after inoculation

DISCUSSION

Management of spot blotch disease mainly relies on resistant variety, but due to the lack of durable resistant cultivars and ineffective control measures, the disease can cause significant yield loss in severe condition (Sharma et al 2007). Morphological, pathological and molecular characterization of *B. sorokiniana* population of different origin have been used to identify and investigate the variability among isolates to develop a durable resistant spot blotch variety (Poloni et al 2009, Ghazvini and Tekauz 2007, Jaiswal et al 2007, Oliveira et al 1998). In this regard, the study was conducted to collect and isolate the *B. sorokiniana* population from different wheat growing region of Nepal and to categorize the isolates on the basis of their aggressiveness to cause disease. From a two-year field survey and sample collection, a total of 36 *B. sorokiniana* isolates were isolated and maintained for the aggressiveness study. Twenty-seven isolates represented from Terai origin, seven isolates represented from mid hill and two isolates represented from high hill origin of Nepal. Isolation and maintenance of *B. sorokiniana* isolates from mid hill and high hills of Nepal was also reported by Mahto et al (2012).

Thirty-six *B. sorokiniana* isolates were inoculated in 3 sets of wheat variety (RR-21 susceptible, Gautam moderately resistant and Chirya 3 resistant) at 14- day old seedlings. Differential response of pathogen × host interaction was observed in the disease development components like (Days to disease appearance, number of lesions per leaf, disease severity and total AUDPC). Research conducted in Bangladesh, Ahmed et al (1997) suggested that the extreme morphological, pathogenic, and molecular variability in the isolates of *B. sorokiniana* could be attributed to the multinucleate condition of mycelium and conidia, with subsequent heterokaryosis. Aggressiveness of the isolates was broadly grouped on two clusters on the basis of disease development components values developed by pathogen × host interaction. High correlation was obtained between disease development traits. This suggests that higher number of lesions per leaf, high disease severity is positively correlated with AUDPC whereas; delay in symptom appearance is negatively correlated with AUDPC.

Similar correlation between disease development traits was obtained by Chakraborty et al (2024). Interaction effect of isolate × variety varied significantly for days to disease appearance. Days to disease appearance ranged from 3 to 7 days. Isolates N-BS17, N-BS 24, N-BS 27, N-BS 28 and N-BS 37 were able to develop symptoms in 3 days in all wheat varieties and were regarded as very aggressive. But isolate N-BS 20 behaved equally for all the varieties and took 6 days for symptom appearance. Among the isolates N-BS4 took 7 days for symptom appearance in Chirya 3. (Singh et al 2018) also obtained the similar interaction effect of isolate × variety for days to disease appearance. Number of lesions per leaf is an important trait to identify the aggressive isolate. In our study also interaction effect of isolate × variety varied significantly for number of lesions per leaf. Number of lesions per leaf ranged from 1 to 24.7. Isolates N-BS7, N-BS 24, N-BS 27, N-BS 28 and N-BS 38 produced higher number of lesions in all wheat varieties comparative to other isolates. Resistant check Chirya 3, developing a lesion number above 10 per leaf, signifies that these isolates are aggressive. N-BS3 producing only 1 lesion per leaf in susceptible variety RR-21 proved to be less aggressive. Wheat varieties of different resistant level when inoculated with *B. sorokiniana* isolates of different origin produce different number of lesions per leaf (Singh et al 2018, Duiveller and Garcia Altamirano 2000).

As days to disease development, number of lesions per leaf, disease severity over time is highly correlated to Area under Disease Curve (AUDPC) in our study, the major criteria for grouping aggressiveness of the isolate was based on AUDPC value. Each isolate responded differently in wheat varieties for AUDPC value. AUDPC value ranged from lowest (72) to highest (467.67). Isolates N-BS24, N-BS7, N-BS15, N-BS24, N-BS27, N-BS28 and N-BS36 recorded highest AUDPC value in the tested wheat varieties and showed high aggressiveness to cause the disease. Among these isolates, N-BS24 and N-BS 28 recorded highest AUPDC value (424) and (432) respectively even in resistant check Chirya 3. But N-BS25, N-BS26 and N-BS4 produced comparatively lowest AUDPC value in the wheat varieties. AUDPC value of these isolates was lower than 200 even in RR-21 which is susceptible check. Studies conducted to characterize *B. sorokiniana* isolates from distinct geographical areas showed that significant differences exist in host response (Maraite et al 1998, Hetzler 1992, Mehta 1981). Moreover, the large difference in aggressiveness among isolates from the same location confirms the incredible adaptation potential of *B. sorokiniana* which causes fungus population to evolve rapidly and overcome the resistance (Maraite et al 1998, Saari 1998). From our study, we obtained that the aggressiveness of the isolates varied according to geographical origin. Among the top ten isolates, three isolates from terai origin N-BS24, N-BS7 and N-BS28 were most aggressive in terms of disease development followed by moderate aggressive isolates N-BS36, N-BS27, N-BS37 and N-BS15 isolates which were from hill origin. A Similar result on the aggressiveness of Nepalese *B. sorokiniana* isolates according to geographical origin was also reported by Mahto et al (2012).

Our study outlined the results based on the aggressiveness study of the isolates on seedling stage of the wheat varieties under polyhouse conditions. Some research highlighted that tests on adult plants may show different results and some genotypes might possess adult plant resistance, although susceptible in the seedling stage (Duiveller and Garcia Altamirano 2000). Similarly, pathogenicity at the seedling stage is extremely sensitive to experimental conditions (Duveiller et al 1998b). To validate the results obtained in this study, further experiment should be carried out at adult plant stage with ideal experimental conditions.

CONCLUSION

The study concluded that *B. sorokiniana* isolates from different origins of Nepal varied significantly in terms of aggressiveness and confirmed significant isolate-by- variety interaction. Among the wheat varieties, RR-21 was highly susceptible to all disease development traits. Top ten aggressive *B. sorokiniana* isolates were identified, of which N-BS24, N-BS7, N-BS28, N-BS38, N-BS19 and N-BS16 were from Terai origin and N-BS36, N-BS27, N-BS37 and N-BS15 were from midhill and hill origin. Among them, N-BS24 and N-BS28 were highly aggressive as they were able to cause higher disease even in Chirya 3, which is the resistant check variety. These two isolates can be utilized further for isolate × variety interactions at adult stage and also explore the genetic diversity to identify resistant wheat genotypes. Spread of pathogen from Terai to hilly region with varied degree of pathogenicity also highlights the importance of continuous survey and surveillance, identifying the aggressiveness and deploying it for the development of spot blotch resistant wheat varieties in Nepal.

Acknowledgments

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

Authors' Contributions

Laxman Aryal contributed in framing the research concept, designing the methodology and writing the original research paper. SM Shrestha, HK Manandhar and DB Thapa helped in editing and reviewing the original draft. All authors listed have made a substantial, direct and intellectual contribution to the study, and approved it for publication.

Conflicts of Interest

The authors have no relevant financial or non-financial interests to disclose.

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