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PLANT PROTECTION | ORIGINAL ARTICLE

Screening of advance wheat genotypes against spot blotch disease (*Bipolaris sorokiniana*) under varying sowing dates at Chitwan, Nepal

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ABSTRACT

Spot blotch of wheat caused by *Bipolaris sorokiniana* is a problematic biotic constraint that causes 15-80% yield abatement in the Indian subcontinent and other parts of the world. The most effective means of managing crop diseases is to develop resistant varieties against crop diseases. 25 wheat genotypes were evaluated against spot blotch (*Bipolaris sorokiniana*) under natural epiphytotic conditions sown on two dates (26 November and 18 December) at Rampur, Chitwan from November 2015 to April 2016. The experiment was laid out in a split plot design with three replications where dates of sowing were taken as the main plot and wheat genotypes were taken as sub-plots. Genotypes RR-21 and Morocco were taken as a susceptible check. Disease scoring for both sowing date was done 3 times at an interval of seven days. Disease severity and Area Under Disease Progressive Curve (AUDPC) were calculated. Among the tested genotypes, disease severity and AUDPC values varied significantly for both the normal and late sowing dates. The six genotypes were found resistant and eight genotypes were found moderately resistant under normal sowing conditions. None of the genotypes were found to be resistant and moderately resistant under late sowing conditions. This indicates that timely sowing of wheat is important for reducing yield loss caused by spot blotch disease irrespective of wheat genotypes grown. Seed infection percent for normal sowing was lower (25 to 85 percent) than late sowing (31 to 91%). This concluded that if farmers have to use the seed for sowing from their own field they should use the seeds harvested from the normal sowing date. The genotypes BL-4350, BL-4463, NL-1094, Aditya, BL-4316 and NL-971 were found resistant to spot blotch under normal sown condition. These genotypes could be used as donor parents for spot blotch resistance in breeding program or could be released as a variety after evaluating the agronomical traits and quality parameters.

Keywords: Sowing time, AUDPC, spot blotch, seed infection, wheat

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1 Introduction

Wheat is one of the major cereals, which is largely produced and consumed all over the world. It imparts about 20% of total energy and protein to the world population (Poudel and Bhatta, 2017). It is the third most important cereal crop of Nepal after rice and maize in terms of area and production. During 2017-2018, the area under cultivation, production, and productivity were 706843 ha, 1949001 tons, and 2757 kg ha^{-1}, respectively (MoALD, 2018).

Spot blotch [incited by *Cochliobolus sativus* (Ito & Kurib) *Drechslera ex* Dastur; *Biploaris sorokiniana* Sacc.] is one of the major diseases of wheat. It affects around 23% (9 million ha) of wheat producing areas of South East Asia including countries like India, Bangladesh, and Nepal (Joshi et al., 2007). Grain yield loss due to spot blotch ranges between 15 to 25% (Dubin and Ginkel, 1991) whereas under severe epidemic condition it may reach up to 80% (Joshi and Chand, 2002). In Nepal, spot blotch severity under rice-wheat cropping system went up to 100% and 70% in 2004 and 2005, respectively (Sharma and Duveiller, 2007). Dubin and Bimp (1994) reported that spot blotch was predominant only in the later crop season when temperature increased and wheat maturity progressed in south Asia. In Nepal, seed infection was found upto 89% and the germination of the infected seed ranged from 34 to 94% (Shrestha et al., 1998). The loss incurred from seedling infection is not very high but high level of infected seed sowing may cause seedling death and crown root rot (CRR) which is caused by different soil-borne fungal complexes like *Bipolaris*, *Fusarium*, *Pythium* etc.

Management of *B. sorokiniana* is possible through crop rotation, chemical control, managing planting time and use of resistant variety. Genetic resistance is one of the most effective methods of controlling diseases; therefore, researchers have made tremendous efforts in identifying and developing spot blotch resistance genetic resources (Singh et al., 2006). Resistance cultivars such as BH1146, Yangmai 6, Ning 8201, and Chirya 3 have been successfully used as donor parents in many breeding programs to develop desirable resistant cultivars (Gupta et al., 2017; Mujeeb-Kazi et al., 1996a). The genotypes Ning-8319, DL-153-2, Ocepar-7, Annapurna-1, BL-1249, NL-590, and NL-625 were observed as tolerant genotypes to Helminthosporium leaf blight (HLB) while UP-262 and RR-21 were reported as susceptible genotypes (Mahto, 1999). Despite several efforts, wheat cultivars grown in South East Asia regions have limited genetic resistance against spot blotch (Joshi and Chand, 2002; Singh et al., 2015). This gets further complicated with the evolution of new pathogen races (Bhatta et al., 2019).

Bhandari (2001) stated that combination of resistance to seed infection, root rot, and spot blotch was not identified in any of the genotypes, suggesting that these resistance may be governed by different genes. This experiment was conducted to identify the genotypes resistant to moderately resistant against spot blotch and to know the effect of normal and late sowing of wheat genotypes on spot blotch severity under natural epiphytotic conditions. This study also aims to assess the seed infection by B.sorokiniana after harvest.

2 Materials and Methods

2.1 Experimental site

The experiment was conducted in the Agronomy research farm at Agriculture and Forestry University, Rampur, Chitwan, Nepal during November, 2015 to April, 2016. The site is situated at 27°39′21.6″N, 84°21′27.6″E with an elevation of 256 m above mean sea level (Fig. 1).

2.2 Seed materials

Seeds of twenty-three wheat genotypes (released and promising lines) and RR-21 and Morocco (Susceptible checks) were received from National Wheat Research Program (NWRP), Bhairahawa, Rupandehi. The details of wheat genotypes are presented below (Table 1).

2.3 Experimental design and procedures

The experiment was conducted in a split plot design with three replications. The experiment was planted in 26th November, 2015 (Normal Sown condition) and 18th December, 2015 (Late sown condition) in continuous rows with row to row spacing of 25 cm. Organic fertilizer (FYM) @ 6 t ha^{-1} was applied 2 weeks before sowing and chemical fertilizers N, P_2O_5 and K_2O @ 120:60:40 kg ha^{-1} was applied through urea, di-ammonium phosphate and muriate of potash. Nitrogen 100 kg ha^{-1} and full dose of P_2O_5 and K_2O were used as basal dose and remaining 20 kg ha^{-1} nitrogen was used as a split dose at tillering and booting stage. Weeding was done 2 times at 30 d after sowing and tillering stage to suppress weed growth. Two irrigations were provided in both dates of sowing. The seed rate of 120 kg ha^{-1} (6 g/row) was used in the experiment. Morocco was sown uniformly around the experimental field of 1 m width as spreader row of *B. sorokiniana*.

2.4 Climatic condition

The research location represents terai region of Nepal and characterized by subtropical and humid climate. The meteorological data for the experiment period

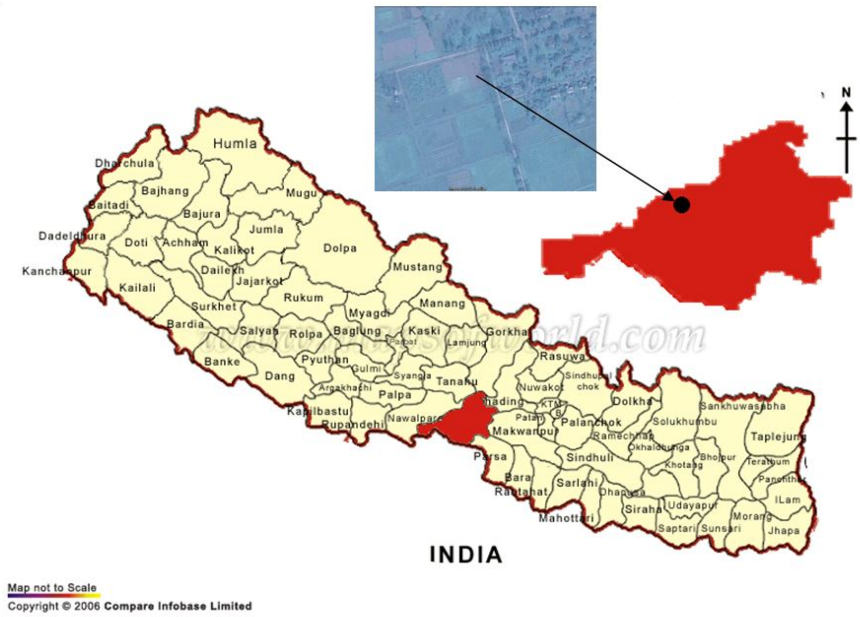


Figure 1. Location of the site

Table 1. Different genotypes used for the experiment

Sl no.	Genotypes	Pedigree	Source
1	Aditya	GS348/NL746//NL748	NWRP, Bhairahawa
2	Bhrikuti	CMT/COC75/3/PLO//FURY/ANA75	NWRP, Bhairahawa
3	BL-3623	XIA-984-10 YAAS KUNMING/BL 1868	NWRP, Bhairahawa
4	BL-3629	XIA-984-10 YAAS KUNMING/BL 1868	NWRP, Bhairahawa
5	BL-4316	BL1981/BL2749	NWRP, Bhairahawa
6	BL-4341	BL2800/BL2801	NWRP, Bhairahawa
7	BL-4347	BL2800/BL2801	NWRP, Bhairahawa
8	BL-4350	BL 1887/BL 2437	NWRP, Bhairahawa
9	BL-4407	FRTL/CHIRIYA-3/PASTOR	NWRP, Bhairahawa
10	BL-4463	KAMBARA1*2/KIRITATI/BABAX/LR42/ BABAX*2/3/VIVITSI	NWRP, Bhairahawa
11	Dhaulagiri	BL1961/NL867	NWRP, Bhairahawa
12	Nepal-297	HD2137/HD2186/HD2160	NWRP, Bhairahawa
13	NL-1055	WAXWING*2/KIRITATI	NWRP, Bhairahawa
14	NL-1064	KIRITATI//2*PBW65/2*SERI.1B	NWRP, Bhairahawa
15	NL-1073	WAXWING*2/VIVITSI	NWRP, Bhairahawa
16	NL-1094	KAUZ//ALTER84/AOS/3/PASTOR/4/ TILHI	NWRP, Bhairahawa
17	NL-1164	NG8201/KAUZ/4/SHA-7/PRL/VEE#6/3/ FASAN/5/MILAN/KAUZ/6/ ACHYUT/7/PBW343*2/KUKUNA	NWRP, Bhairahawa
18	NL-1172	KIRITATI//SERI/RAYON	NWRP, Bhairahawa
19	NL-1177	WHEAR/SOKOLL	NWRP, Bhairahawa
20	NL-1190	WAXWING/PARUS/ WAXWING/KIRITATI	NWRP, Bhairahawa
21	NL-971	MRNG/BUC//BLO/PVN/3/PJB81	NWRP, Bhairahawa
22	Vijay	NL748/NL837	NWRP, Bhairahawa
23	WK-2123	Acc#06272/WK1123//2*WK1204	NWRP, Bhairahawa
24	Morocco (Susceptible check)	Morocco	NWRP, Bhairahawa
25	RR-21	II 54-68/AN/3/YT54/N10B//LR64	NWRP, Bhairahawa

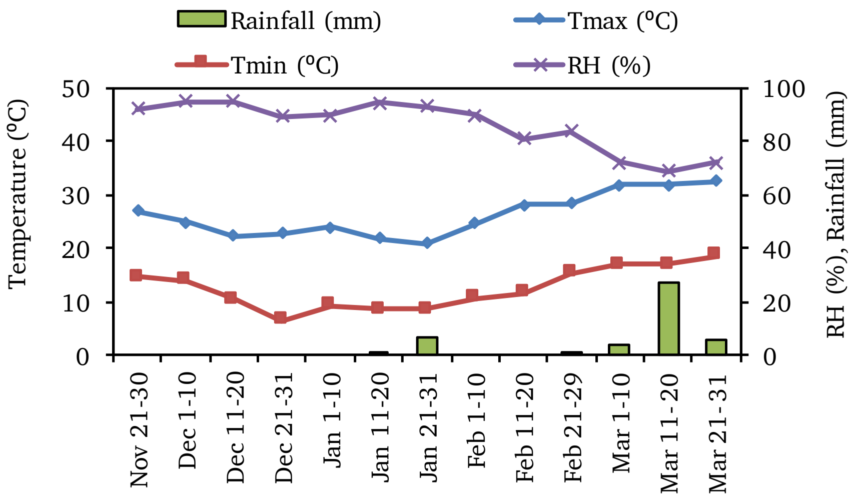


Figure 2. Weather condition during experimental period at, Rampur, Chitwan, 2015/16 (Source: National Maize Research Program, NARC, Rampur, Chitwan)

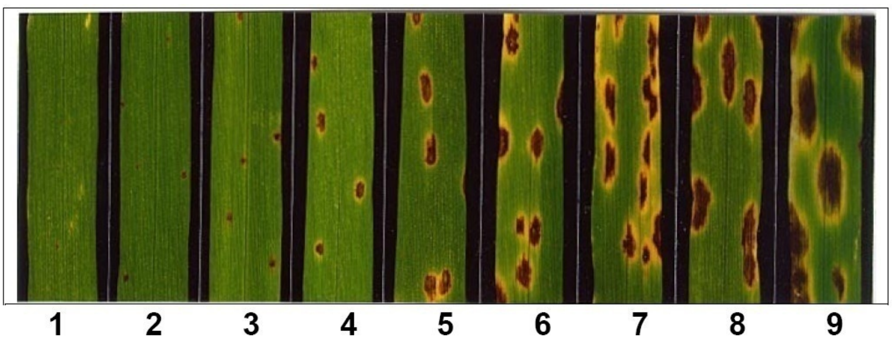


Figure 3. Standard diagram developed by CIMMYT for single digit disease scoring of spot blotch of wheat (Mujeeb-Kazi et al., 1996b)

were obtained from National Maize Research Program (NMRP), Rampur, Chitwan for the year 2015/16 (Fig. 2).

2.5 Disease observations

Percentage of diseased leaf area was scored visually on flag leaf (F) and penultimate leaf (F-1) from 10 randomly selected single tillers per genotype in each replication by using standard diagram developed by International Maize and Wheat Improvement Centre (CIMMYT) (Fig. 3) (Mujeeb-Kazi et al., 1996b).

$$DS = \frac{\sum R_i}{N \times R_{max}} \times 100 \quad (1)$$

where, DS = disease severity (%), $\sum R_i$ = Sum of all numerical ratings, N = total number of sample observed, R_{max} = maximum rating.

2.5.1 Area under disease progress curve (AUDPC)

Area under disease pressure curve (AUDPC) values from flag leaf (F) and penultimate leaf (F-1) were separately calculated by using the following formula given by Das et al. (1992):

$$AUDPC = \sum_{i=1}^{n} \frac{(Y_{i=1} + Y_i)}{2} (T_{i=1} - T_i) \quad (2)$$

where, Y_i = disease scored on first date, T_i = date on which the disease was scored, n = number of dates on which disease was scored.

2.5.2 Resistance and susceptibility of genotypes

The genotypes were grouped into five categories based on the average AUDPC value derived from total AUDPC of flag leaf and penultimate leaf (Table 2) (Aryal et al., 2013).

2.5.3 Seed infection test

Seed harvested from 26 November and 18 December sown crops were tested in- vitro using standard blotter method for the spot blotch pathogen. The petri-plates were sterilized in an oven at 180 °C for 2 h. Four hundred seeds of each genotype were tested by placing 25 seeds per plate arranged in the sterilized petri-plates in 1:8:16 ratios from center to periphery, containing three layers of blotting paper moistened with sterile distilled water. Four plates (i.e. 100 seeds) were considered as an experimental unit and were replicated 4 times in CRD. Then the plates were incubated at 25±1 °C for 3 d and the seeds were observed under a stereo microscope to determine the seed-borne infection by *B. sorokiniana*. The seeds were observed at alternate dates up to 3 times and the percentage of seed infection by *B. sorokiniana* was calculated as below:

$$SI = \frac{S_i}{S_T} \times 100 \quad (3)$$

where, SI (%) = Seed infection (%), S_i = number of infected seeds, and S_T = number of seeds tested.

2.6 Statistical data analysis

The data were processed to fit into R-studio and analyses using agricolae version 1.1-8 R package (Mendiburu, 2014).

3 Results

3.1 Effect of dates of sowing on AUDPC

Analysis of variance (ANOVA) revealed significant difference between the dates of sowing for final AUDPC on flag leaf (F) and penultimate leaf (F-1). Mean value of final AUDPC on flag leaf for 26 November and 18 December sowing was 267.6 and 439.5, respectively (Table 3). Mean value of final AUDPC on the penultimate leaf for 26 November and 18 December sowing was found 405.84 and 581.93, respectively (Table 3).

3.2 Sowing date × genotypes on AUDPC

The interaction between date of sowing and genotypes for AUDPC value was non-significant in flag leaf stage but was highly significant in penultimate leaf stage (p = 0.0084) (Fig. 4). At normal sowing, the susceptible check variety RR-21 had the highest final AUDPC value (593.70) followed by Nepal-297 (523.70) on penultimate leaf. The genotype BL-4350 recorded lowest AUDPC value (286.48) followed by BL-4463 (315.00), NL-1094 (316.29), BL-4316 (329.25), Aditya (331.85) and NL-971 (333.14) (Fig. 4). Under late sown condition, NL-1055 showed the highest final AUDPC value (668.88) on penultimate leaf followed by WK-2123 (659.81). The check variety RR 21 and Morocco showed 662.40 and 657.22, respectively. NL-1094 recorded the lowest final AUDPC value (473.14) followed by NL971(478.33), BL-4350 (493.88) and BL-4463 (500.37) (Fig. 4).

3.3 Categorize based on average AUDPC

Wheat genotypes could be grouped into five categories (resistant, moderately resistant, moderately susceptible, susceptible, and highly susceptible) based on AUDPC value derived from flag and penultimate leaves (Table 4). In the present study, RR-21 and Morocco were taken as susceptible checks. The results indicated that the susceptibility of these genotypes are still maintained as reported by previous

Table 2. Resistant and susceptible categories of genotypes based on AUDPC value

AUDPC	Category	Symbol
>495	Highly susceptible	HS
426-495	Susceptible	S
356-425	Moderately susceptible	MS
286-355	Moderately resistant	MR
<285	Resistant	R

AUDPC = Area under disease progress curve

Table 3. Effect of dates of sowing on AUDPC value of flag leaf (F) and penultimate leaf (F-1) at Rampur, Chitwan, 2015-16

Treatments	Final AUDPC F	Mean AUDPC F	Final AUDPC F-1	Mean AUDPC F-1
26-Nov	267.6b	210.2b	405.8b	317.4b
18-Dec	439.5a	359a	581.9a	487.8a
Mean	353.55	284.58	493.88	402.6
CV(%)	42.7	40.3	40.6	31.1
SEm (±)	2.01	1.52	2.67	1.66
p-value	0.019	0.015	0.032	0.014

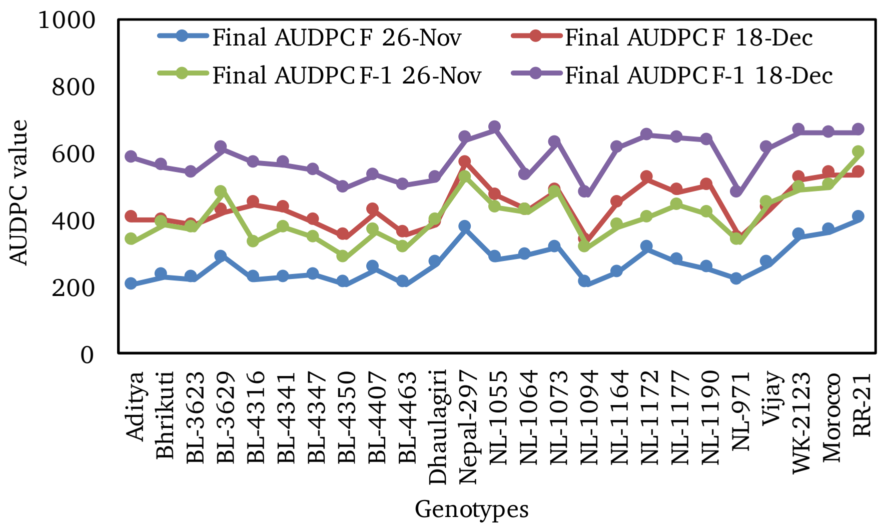


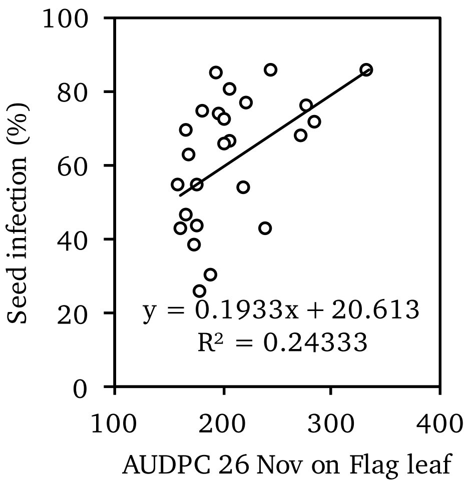
Figure 4. Interaction of dates of sowing and wheat genotypes on AUDPC value of flag and penultimate leaves at Rampur, Chitwan, 2015-16

Table 4. Categories of wheat genotypes under study based on AUDPC value of flag and penultimate leaves at Rampur, Chitwan, 2015-16

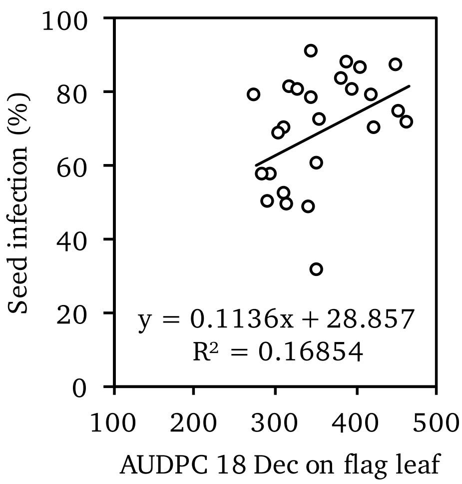
Category	AUDPC value	26 November sowing	18 December sowing
Resistant	<285	BL-4350, BL-4463, NL-1094, Aditya, BL-4316, NL-971	
Moderately resistant	286-355	BL-4347, BL-3623, BL-4341, BL-4407, Bhrikuti, NL-1164, Dhaulagiri, NL-1190	
Moderately susceptible	356-425	NL-1177, Vijay, NL-1064, NL-1055, NL-1172, BL-3629, NL-1073, WK-2123	NL-1094, NL-971, BL-4350
Susceptible	426-495	Morocco, Nepal-297	BL-4463, Dhaulagiri, BL-3623, BL-4347, Bl-4407, Bhrikuti, NL-1064, Aditya
Highly susceptible	>495	RR-21	BL-4341, BL-4316, BL-3629, Vijay, NL-1164, NL-1073, NL-1177, NL-1190, NL-1055, NL-1172, WK-2123, Morocco, RR-21, Nepal-297

Table 5. Percent seed infection of 25 different wheat genotypes after harvest, Rampur, Chitwan, 2015-16

Genotypes	Seed infection (%)	
	26 November sowing	18 December sowing
Aditya	42hij (0.70)	49ij (0.78)
Bhrikuti	74bcd (1.04)	81abcd (1.13)
BL-3623	46hi (0.75)	52ghi (0.81)
BL-3629	53gh (0.82)	60efghi (0.90)
BL-4316	62efg (0.91)	72bcdef (1.04)
BL-4341	43hi (0.71)	48ij (0.76)
BL-4347	25k (0.52)	70cdefg (0.99)
BL-4350	38ij (0.66)	50hij (0.78)
BL-4407	73cde (1.03)	78abcde (1.08)
BL-4463	54fgh (0.83)	57fghi (0.86)
Dhaulagiri	66def (0.95)	68defgh (0.98)
Nepal-297	67de (0.96)	71bcdef (1.02)
NL-1055	80abc (1.12)	83abcd (1.16)
NL-1064	76abcd (1.06)	80abcd (1.11)
NL-1073	85a (1.20)	88a (1.26)
NL-1094	69cde (0.98)	79abcd (1.11)
NL-1164	84ab (1.18)	91a (1.27)
NL-1172	42hij (0.70)	70bcdefg (1.00)
NL-1177	65defg (0.94)	80abcd (1.11)
NL-1190	72cde (1.01)	86abc (1.20)
NL-971	54fgh (0.83)	57fghi (0.86)
Vijay	30jk (0.58)	31j (0.59)
WK-2123	75bcd (1.06)	79abcde (1.10)
Morocco	71cde (1.00)	74bcdef (1.04)
RR-21	85a (1.19)	87ab (1.20)
Mean	61.24 (0.91)	69.64(1.01)
CV(%)	10.4	14.62
SEm(±)	1.96	3.11
LSD value	11.04 (0.13)	17.55(0.21)
p value	<0.001	<0.001



(a) 26 November sowing



(b) 18 December sowing

Figure 5. Estimated linear correlation between the AUDPC value at different date of sowing and seed infection at AFU, Rampur, Chitwan, 2015-16

studies. Aryal et al. (2013) reported the lowest disease severity, mean AUDPC and AUDPC per day in Aditya and the highest in RR-21. Of the 25 genotypes tested, six genotypes were found resistant and eight genotypes were found moderately resistant to spot blotch (Table 4) under normal sowing date (November 26) conditions. But none of the genotypes were found resistant or moderately resistant under late sown conditions (December 18). This suggests that timely sowing of wheat is important for reducing the loss caused by spot blotch disease irrespective of wheat genotypes grown. The results were found to be in line with Rosyara et al. (2008) and Gurung et al. (2012) who also reported increased AUDPC under late sown conditions. The genotypes which were found resistant and moderately resistant under normal sown time were found susceptible under late sown condition. This result is supported by Duveiller (2004) and Aryal et al. (2013) who also reported AUDPC value of resistant genotypes also increased in delayed sowing condition. Increase in AUDPC value of even resistant genotype might be due to combined effect of heat stress and easily available inoculums (spores) from the first date sowing field. Moreover the epidemiological condition might have favored for the high disease in second date of sowing.

3.4 Seed infection

The analysis of variance showed that genotypes differed highly significantly ($p \leq 0.001$) for seed infection (%) at both dates of sowing. Almost all the tested genotypes of wheat were infected by *B. sorokiniana*. The seed infection on 26 November sowing ranged from 25 to 85%. The highest seed infection was found in RR-21 (85%) and NL-1073 (85%). While, the lowest infection was found in BL-4347 (25%) and was statistically at par with Vijay (30%). Similarly, on 18 December sowing, seed infection ranged from 31 to 91%. The highest seed infection was found in NL-1164 (91%) which was statistically at par with NL-1073 (88%), RR-21 (87%), NL-1190 (86%), NL-1055 (83%), Bhrikuti (81%), NL-1177 (80%), NL-1064 (80%), NL-1094 (79%), WK-2123 (79%) and BL-4407(78%). The lowest seed infection was found in Vijay (31%) which was at par with BL-4341 (48%) (Table 5).

3.5 Regression analysis

There was a significant and positive correlation, r = 0.493** and r = 0.411* between the AUDPC value on flag leaf (26 Nov sowing) and seed infection (%) and between AUDPC value on flag leaf (18 Dec sowing) with seed infection (%) respectively (Fig. 5). The seed infection (%) was found higher for 18-December than 26-November sowing time. In late sown condition, final AUPDC value on flag leaf was significantly higher than normal sown condition which favors faster spread of the disease towards the spike. Thus, higher seed infection in late sown condition might be due to the combined effect of high temperature, higher disease severity in the field and heavy rainfall before harvest.

4 Conclusions

The genotypes varied significantly for spot blotch severity, AUDPC value and seed infection under normal and late sowing conditions. Among 25 wheat genotypes, six genotypes were found to be resistant and eight genotypes were found moderately resistant under normal sowing conditions. The genotypes BL-

4350, BL-4463, NL-1094, Aditya, BL-4316 and NL-971 were found resistant under normal sowing conditions. These genotypes could be used as donor parents for spot blotch resistance in breeding programs or could be released as a variety after evaluating the agronomical traits and quality parameters. None of the genotypes were resistant or moderately resistant under late sowing conditions. It suggests that timely sowing of wheat is important for reducing the loss caused by spot blotch disease irrespective of wheat genotypes grown. Genotypes identified and released as resistant may become susceptible after continuous cultivation in the same region over the years. So, farmers should change their varieties from time to time, and screening of wheat genotypes should be done under a wide range of environments.

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Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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