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Assessment of Spot Blotch Resistance and Yield Traits in Wheat Genotypes in a Subtropical Agro-Ecology of Nepal

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ABSTRACT

A field and laboratory study was conducted from November 2021 to April 2022 at Debchuli-2, Nawalpur (East), Nepal, to evaluate 50 wheat genotypes for resistance to spot blotch (caused by *Bipolaris sorokiniana*) and associated traits. The experiment followed an alpha-lattice design with three replications. Disease severity was assessed using single and double-digit scales at four intervals post-heading, and area under disease progress curve (AUDPC) was computed to categorize genotypes into resistant, moderately resistant, susceptible, and highly susceptible groups. Laboratory seed health testing was performed using the blotter method to assess seed infection by *B. sorokiniana*. AUDPC values varied significantly among genotypes, ranging from 178.4 (resistant) to 487.5 (highly susceptible). Genotype YMI#6 had the lowest AUDPC, while RR 21 exhibited the highest, indicating loss of previously reported resistance. Root rot index (RRI) ranged from 17% to 44% and positively correlated with AUDPC. Significant variation was observed in yield and yield-attributing traits, including thousand kernel weight (TKW), grain yield, and spike characteristics. Grain yield showed a significant negative correlation with AUDPC (r = -0.280*), while TKW was positively correlated with grain yield. Phenological observations revealed that susceptible genotypes had earlier booting, heading, and maturity. A negative correlation was found between AUDPC and days to maturity (r = -0.57*), indicating accelerated development in diseased plants. Plant height also differed significantly among resistance categories. The study highlights genetic variability among genotypes in response to spot blotch and identifies promising resistant lines for wheat improvement programs in subtropical regions.

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KEYWORDS: AUDPC, *Bipolaris sorokiniana*, Genotypes, Resistance, Screening, Susceptible

INTRODUCTION

Wheat (*Triticum aestivum* L.), the third most important cereal crop in the country after rice and maize, makes a significant contribution to the national economy and the livelihoods of millions of farmers. It is cultivated on an area of 697,762 hectares, with a production of 2,098,462 metric tons and a productivity rate of 3.01 metric tons per hectare (MoALD, 2024). However, its productivity is heavily affected by various biotic and abiotic stresses. Among these, spot blotch, caused by the fungal pathogen *Bipolaris sorokiniana*, is a major foliar disease thriving in warm, humid conditions. The disease manifests as brown to dark brown necrotic lesions on leaves, which eventually merge, causing premature leaf aging and decreased photosynthesis (Duveiller & Gilchrist, 1994). In Nepal's Terai and inner Terai regions, environmental conditions are especially favorable for the disease, resulting in substantial yield losses (Duveiller & Sharma, 2009). In addition to foliar symptoms, *B. sorokiniana* also causes root rot, further weakening plant health (Navathe et al., 2022). The pathogen's development is closely associated with weather parameters, particularly when night temperatures exceed 18°C and relative humidity is above 80% (Kaur et al., 2008). Moreover, seed-borne infection acts as a primary source of inoculum, initiating early disease outbreaks in the field. The severity of root rot is evident through darkened roots and infected crowns, and is particularly damaging under dry conditions (Singh et al., 2023). Yield losses due to spot blotch vary widely depending on genotype, environment (Paulitz, 2006; Singh et al., 2023; Xiong et al., 2023), and sowing time (Toledo & Guzman, 1998), with disease incidence reported as high as 90% in susceptible cultivars like RR21 in the

Terai region (Shrestha et al., 1998). Given this context, the present study aims to screen wheat genotypes for resistance or susceptibility to spot blotch and root rot, examine the role of seed-borne inoculum in disease progression, and evaluate the influence of climatic factors on disease severity to support the development of effective management strategies.

MATERIAL AND METHODS

Field experiment

Experimental site

The experiment was conducted in an upland at Debchuli-2; Nawalpur-east, during November 2021 to April 2022. Climate of the location was humid and subtropical type. Rice-Wheat- Maize cropping patterns were adopted in the field during previous years.

Seed collection

Wheat seed samples of 50 genotypes were obtained from National Wheat Research Program (NWRP), Bhairahawa (Table 1). Varieties Gautam and RR 21 were used as resistant and susceptible checks, respectively.

Table 1. List of wheat genotypes included in the field study at Dibyapuri, Nawalpur, 2021/22

SN	Local genotypes	SN	Exotic genotypes
1	Rasuwa	26	Sabuf
2	LGP masino wheat (Red)	27	Ning 8201
3	Masino wheat (Red)	28	Ning 8319
4	Chavage local(Red)	29	G162
5	LGP AR (WG)	30	CHIRYA-3
6	LGP 1 AR (WG)	31	CHIRYA-1
7	LGP 2 AR(WG)	32	NL922
8	LGP 3 AR(WG)	33	NL923
9	LGP 2 ABR (WG)	34	YMI#6
10	LGP 2 AR (WG)	35	JINMAL 4058
11	LGP 2 AOB R(WG)	36	MILAN/SHA-7
12	LGP 2 AOB (WG)	37	CHIRYA-7
13	LGP 4 AOR (WG)	38	BL 3623
14	LGP 120 AOR (WG)	39	BL 3635
15	LGP 116 AOB (BG)	40	BL 3624
16	LGP 114 AOB (WG) Amber	41	DL 803-2
17	LGP 74 AOR (WG)	42	BL 3621
18	LGP54 AOR (BG)	43	BL 3819
19	LGP 64 AOR (WG) Amber	44	NL 1073
20	LGP 54 AOR (WG)	45	NL 1064
21	LGP 25 AOR (BG)	46	NL 971
22	LGP 61AOR (WG)	47	Bhirkuti
23	LGP 44 AOR (WG)	48	Gautam(resistant check)
24	LGP 51 AB (WG) Amber	49	RR 21 (susceptible check)
25	LGP 42 AOB (BG) Amber	50	BL 2884

Design of the experiment

The experiment was conducted in an Alfa-lattice design. There were 5 blocks, 12 plots per block (2 plots for border effect, thus one plot per genotype), and 3 rows/plot of 100 cm length with 3 replications. Inter-block, inter-plot and inter-replication spacing were 50 cm, 25 cm and 100 cm, respectively with a total length of 30.90 m, breadth of 6.80 m and total area of 210.12 m^2.

Land preparation, sowing and crop management

Land preparation was done by plowing with a tractor. Seeds were sown on November 24, 2021 continuously in rows 25 cm apart at the rate of 120kg ha^{-1}. Soil was fertilized with FYM at the rate of 10 tha^{-1}. Chemical fertilizers (N: P: K) were applied @120:60:40 kg ha^{-1}, respectively. One hand weeding was done 35 days after seeding. Irrigation was given at crown root initiation at 21 DAS.

Disease assessment

Single and double digit disease scoring

The percentage of diseased leaf area by spot blotch for single digit disease scoring was assessed visually on flag and penultimate leaves using standard diagram developed by CIMMYT. Six randomly selected main tillers were tagged and used for disease scoring. Four scorings were done at 7 days intervals, i.e 75, 82, 89 and 96 days after sowing. The same plants used for single digit disease scoring were used for double digit disease scoring and the disease severity data were used to estimate the area under progress curve (AUDPC).

Severity (%) = (D_1\9) x (D_2\9) x100

Where,

D_1 =first digit (height of infection) or vertical disease progress and

D_2 =Second digit (Severity of infection)

The severity values were used to compute AUDPC values.

Root rot assessment

The score was transformed into root rot index by using the following formula (Tinline et al., 1975).

$$\text{Root rot index (\%)} = \frac{\text{Sum of (category value x number of root samples in category)}}{\text{Total number of roots rated x maximum rating grade}} \text{x } 100$$

Observation on agronomical traits

Days to booting, heading and maturity were calculated from the day of sowing.

Harvesting and threshing

Harvesting was done manually with sickles after the maturity of the crop, and the plants were sun-dried in the field for 3 days.

Yield and yield attributing characters

Grain yield, thousand kernel weight (TKW) and days to flag leaf maturity were taken from all three rows of each plot. The final grain yield was adjusted at 12% moisture level.

Laboratory experiment

The experiment was carried in laboratory of Ramnagar Technical and Management College during 5thJuly to16thAugust 2022.

Seed infection test *in vitro*

Four hundred seeds of each genotypes were tested in laboratory for seed infection by *B. sorokiniana* using standard blotter method. Twenty-five seeds per plate were placed in sterilized petriplates containing triple layers of blotting paper. One hundred seeds were taken as one replication. The seeds were incubated at 25 $^{\circ}$C for 48-72 hours for sporulation, and observed under a stereomicroscope. Seed infection percent was calculated as below

$$\text{Seed infection (\%)} = \frac{\text{Number of infected seeds}}{\text{Total number of seed observed}} \text{x } 100$$

Resistance and susceptibility of the genotypes were differentiated based on Bhandari (2002).

Statistical Analysis

The statistical analysis included analysis of variance (ANOVA) to test the main and interaction effects, multivariate analysis and regression analysis. Normality of data was tested, mean comparison and correlation and regression analyses were done. Statistical analysis was done by using statistical software program Crop stat 7.2 version; Minitab 14 versions, SPSS and Microsoft excel.

RESULTS AND DISCUSSION

Disease incidence

The appearance of spot blotch disease in the field differed among the genotypes. Out of 50 genotypes, the mean days of disease appearance on leaves was 94, 85, 70 and 55 days after sowing (DAS) in resistant, moderately resistant, susceptible and highly susceptible genotypes, respectively (Table 2).

Table 2. Categorization of wheat genotypes based on mean days of spot blotch appearance in field at Dibyapuri, Nawalpur, 2021/22

Resistance Categories	Code	Spot blotch appearance (DAS)
Resistant	R	94
Moderately Resistant	MR	85
Susceptible	S	70
Highly susceptible	HS	55

DAS = Days after showing

Disease intensity

The Value of AUDPC ranged from 178.4 to 487.5, which were highly significantly different from each other (Table 3). Eight Resistant genotypes had AUDPC values from 178.4 to 196.5 with a mean of 189.30, thirty moderately resistant genotypes had values from 200.40 to 305.10 with a mean of 256.30, seven susceptible genotypes had values ranging from 315.40 to 361.00 with a mean of 335.50, and five highly susceptible genotype had 369.00 to 487.50 with a mean of 410.70.

Root rot

There was a significant difference among 50 genotypes for root rot index (RRI). Correlation between root rot index and AUDPC of spot blotch was highly significant and positive (Table 3). Mean root rot index of resistant, moderately resistant, susceptible and highly susceptible genotypes was 24%, 33%, 38% and 39%, respectively.

Yield and yield attributing characters

Thousand kernel weight and grain yield

The genotypes were significantly different in 1000 kernel weight (Table 3) and grain yield. Mean kernel weight and grain yield in resistant (R), moderately resistant (MR), susceptible (S) and highly susceptible (HS) were 42.33 g and 1.40 tha^{-1}, 33.18 g and 1.50 tha^{-1}, 28.60 g and 1.20 tha^{-1}, 33.42 g and 1.10 tha^{-1}, respectively. The result showed that there was slightly negative correlation between spot blotch severity and thousand kernel weight (r = -0.225) (Figure 1). The result was similar to that of Gilchrist et al. (1992), Sharma et al. (1997) and Dubin et al. (1998). Similarly, there was a clear negative correlation (r = -0.280*) between AUDPC and grain yield at 5% level of significance. Sharma et al. (2006) also reported that grain yield loss due to spot blotch in South Asia ranged from 4% to 38%.

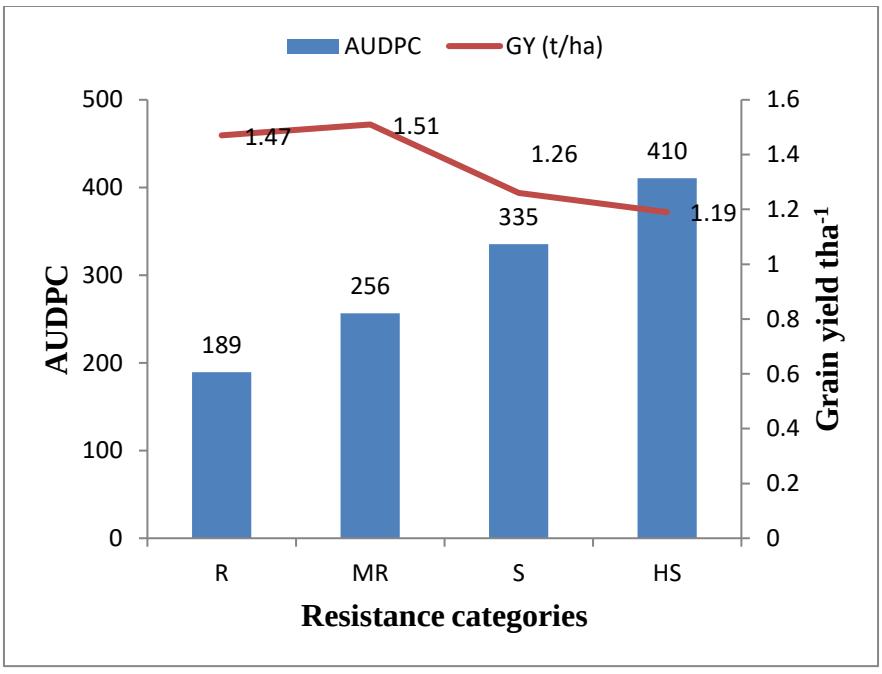

Figure 1. Effect of AUDPC on yield of wheat genotypes in field at Dibyapuri, Nawalpur, 2021/22

Agronomical characteristics

Days to booting and heading

Analysis of variance (ANOVA) revealed highly significant difference among the genotypes for days to booting. The range of days to booting varied from 59-90 days after sowing (Figure 3). Dubin et al. (1998) reported that there was a negative correlation between spot blotch and heading days.

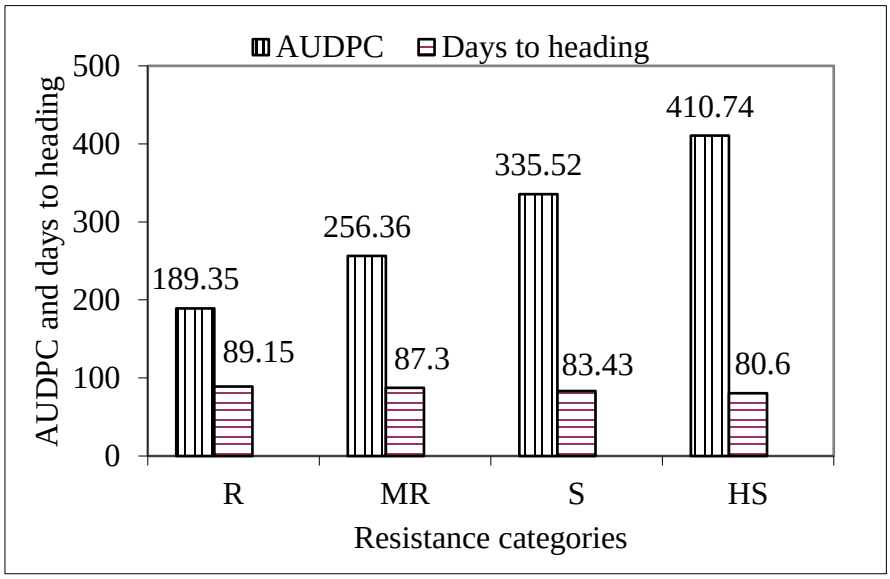

Figure 2. AUDPC and heading duration of wheat genotypes in field at Dibyapuri, Nawalpur, 2021/22

Table 3. Disease severity of spot blotch and yield and yield attributing characters of 50 wheat genotypes in field at Dibyapuri, Nawalpur, 2021/22

Genotypes	AUDPC	RRI	Seed infection (%)	AUSDC	TKW	Grains per spike (No.)	Grain yield (t/ha)	Resistance categories
YMI#6	178.40	19.40	5.71	206.80	38.33	39	1.50	R
BL 3819	182.60	16.60	5.71	194.50	57.11	34	1.80	R
G 162	186.90	27.70	17.14	185.70	39.72	52	1.20	R
Gautam	188.50	30.50	12.57	187.20	30.48	57	1.90	R

BHIRKUTI	196.50	30.50	11.42	194.70	42.36	43	1.50	R
Mean	**189.30**	**23.90**	**10.21**	**193.20**	**42.34**	**44**	**1.40**	**R**
NL 923	200.40	19.40	10.28	166.40	36.90	48	1.90	MR
NL 1064	204.60	22.20	8.57	175.80	26.10	41	1.40	MR
BL 3624	214.60	25.00	12.57	222.00	51.70	61	1.60	MR

LGP AR(WG)	302.50	43.51	21.14	160.70	27.30	32	0.90	MR
LGP 42 AOB (BG) Amber	305.00	37.96	25.14	174.90	28.20	45	1.10	MR
LGP 64 AOB (WG) Amber	305.10	40.74	8.00	159.90	32.80	29	0.80	MR
Mean	**256.30**	**33.10**	**17.54**	**172.4**	**33.14**	**44**	**1.50**	**MR**
LGP 61 AOR (WG)	315.40	43.50	25.14	166.00	29.20	41	1.60	S
LGP 2 AOB R (WG)	324.30	37.96	25.14	188.30	36.00	47	0.90	S

LGP 3 AR (WG)	361.00	35.18	20.00	171.90	20.30	47	1.10	S
Mean	**335.50**	**37.50**	**20.56**	**172.50**	**28.10**	**42**	**1.20**	**S**
LGP4 AOR(WG)	369.00	31.48	50.85	167.50	33.30	58	1.00	HS
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RR 21/ Sonalika	487.50	43.51	52.57	170.40	44.90	38	1.60	HS
Mean	**410.70**	**38.60**	**34.62**	**168.70**	**34.40**	**49**	**1.10**	**HS**

AUDPC= area under disease progress curve, AUSDC= area under spad decline curve, RRI= root rot index, TKW= test kernel weight

Days to maturity

The genotypes, which had latest maturity (133 DAS) were G 162 and LGP 74 AOR (WG), belonged to resistant (R) and moderately resistant (MR) categories, respectively, and the genotype which had earliest maturity (112 DAS) was RR 21 from highly susceptible (HS) group. This result was similar to the result of Dubin et al. (1988), Shrestha et al. (1998), and Mahato (2001).

Plant height

Highest plant height was found in genotype LGP 2 ABR (WG) (153.3cm), which fell into susceptible (S) category and lowest plant height in genotype NING 8319 (89.3cm) which fell into moderately resistant (MR) group.

Area under SPAD retreat curve

There was significant difference among the 50 genotypes for area under SPAD decline curve (AUSDC). The values of AUSDC ranged from 139.40 to 222.00 Mean AUSDC value was highest in BL3624 (Figure 3). This result was also similar to the result obtained by Rosyara et al. (2009).

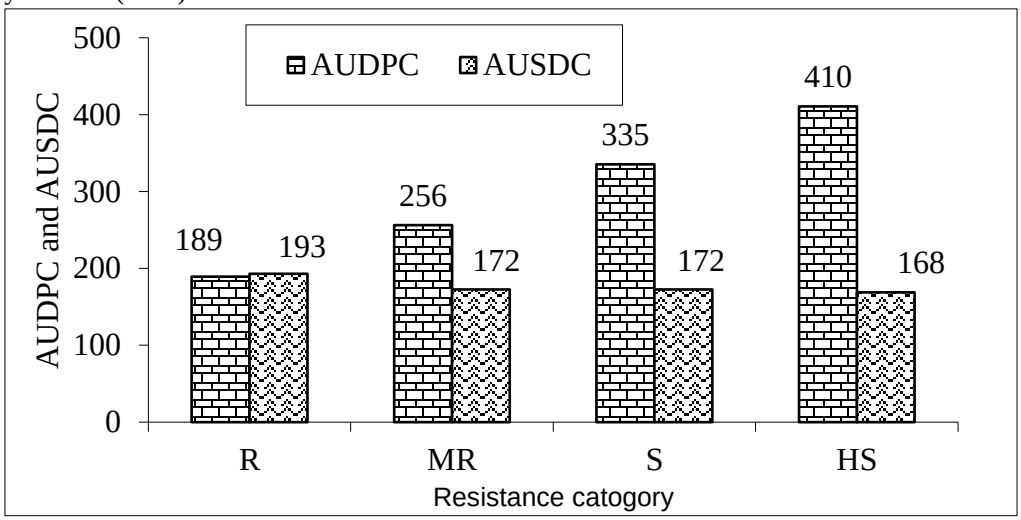

Figure 3. AUDPC and AUSDC values on 50 wheat genotypes at 4 resistance categories in field at Dibyapuri, Nawalpur, 2021/22

Table 4: Pearsons correlation coefficient of different variates of 50 wheat genotypes in field at Dibyapuri, Nawalpur during Nov. 2021/22

	DB	DH	DM	PH	TKW	PY	SL	GPS	AUDPC	AUSDC	RRI	Seed infection (%)
DB (days)	1	.948**	.848**	.318*	-.085	-.412**	-.322*	-.165	-.166	-.127	-.067	-0.107
DH (days)			.871**	.267	-.087	-.340*	-.335*	-.160	-.174	-.111	-.074	-0.078
DM (days)				.364**	-.064	-.445**	.218	-.146	-.057	-.088	.082	-0.074
PH(cm)					-.240	-.540**	.103	-.208	.326*	-.325*	.482**	0.217
TKW(g)						.057	.164	.103	-.255	.210	-.291*	-0.069
PY (g)							.073	.048	-.280*	.284*	-.264	-0.163
SL (cm)								.423**	.128	-.013	-.093	0.101
GPS									.027	.259	-.123	0.289*
AUDPC										-.303*	.704**	0.652**
AUSDC											-.303*	-0.242
RRI												0.438**

* Significantly different at 5% level and * *significantly different at 1% level, DG= days to germination, DB= days to booting, DH= days to heading, DM= days to maturity, PH= plant height, TKW= test kernel weight, PY= plot yield, SL= spike length, GPS= grain per spike, AUDPC= area under disease progress curve,
AUSDC= area under SPAD decline curve, RRI= root rot index

Correlation of spot blotch to seed infection

There was a significant, positive correlation (r = 0.652**) between foliar infection and seed infection (Table 5). This study revealed that the genotypes with high foliar infection had also severe seed infection and *vice versa*. Raemakers (1987) reported a significant correlation between foliar infection of spot blotch and seed infection. However, the direct correlation of seed infection with the leaf infection is still not clear (Gilchrist et al, 1992).

Weather effect on disease development

Foliar blight severity increased with rise in temperature and fall in relative humidity. Highest spot blotch severity (27.68%) was found at maximum temperature 28.8˚C, minimum temperature 9.53 ˚C and relative humidity 81.3% on 10th March 2012 (Figure 4). At that time, the plants were in flowering stage. Lowest spot blotch severity (1.56) was found at maximum temperature 19.80 °C, minimum temperature 7.50)C and relative humidity 92.17% on 17thFeb 2012, at that time the plants were in heading stage. First occurrence of disease was found on 3rd week of December, but severity increased after first week of February, 2012. Spot blotch was most common and widespread in the coolest month (January) as temperature was higher than 12.5 ˚C and high relative humidity (Dubin & Van Ginkel, 1991).

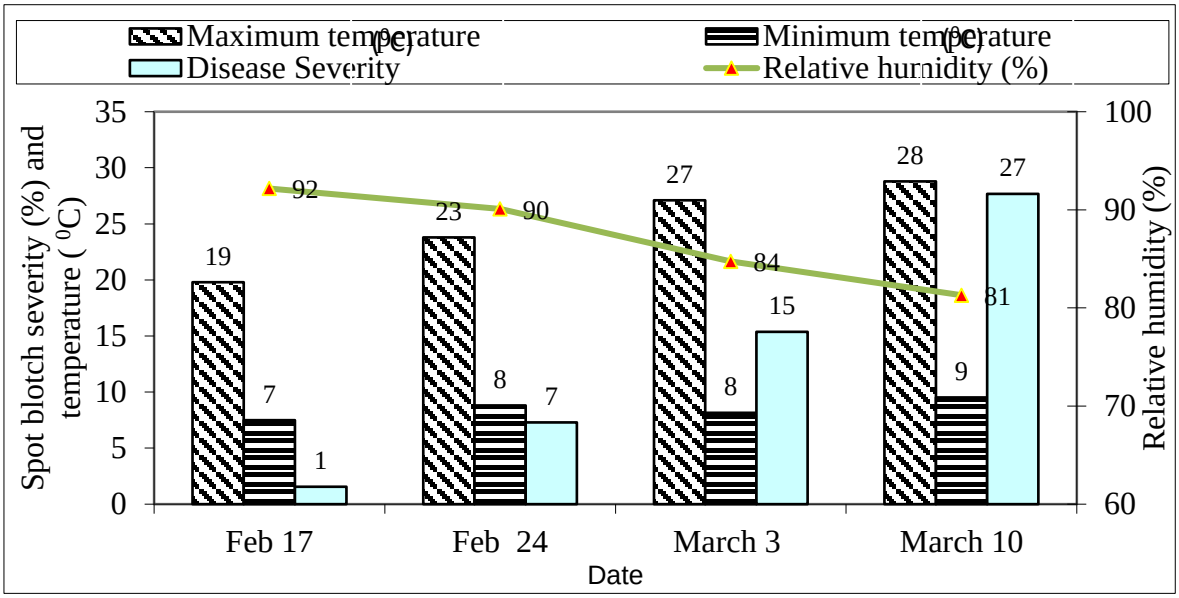

Figure 4. Effect of weather on spot blotch severity of 50 wheat genotypes in field at Dibyapuri, Nawalpur during November 2021/22

CONCLUSIONS

A study conducted in Debchuli-2, Nawalpur (East), Nepal, evaluated 50 wheat genotypes for resistance to spot blotch (*Bipolaris sorokiniana*) from November 2021 to April 2022. Using an alpha-lattice design, disease severity was measured through AUDPC, which ranged from 178.4 (YMI#6) to 487.5 (RR 21). Laboratory tests showed a positive correlation between foliar blight and seed infection. Root rot index also correlated with disease severity. Grain yield was negatively associated with AUDPC, while thousand kernel weight showed a positive correlation with yield. Susceptible genotypes matured earlier and had reduced plant height. The study identified resistant genotypes for use in wheat improvement programs.

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