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ORIGINAL RESEARCH ARTICLE



## Evaluation of wheat (*Triticum aestivum* L.) genotypes for spot blotch (*Bipolaris sorokiniana* Sacc) resistance in terai condition of Nepal

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### ABSTRACT

Spot blotch caused by *Bipolaris sorokiniana* is a major disease of wheat in warm and humid regions of Nepal. The fungus has a worldwide distribution but as a pathogen, it is the most aggressive under the conditions of high relative humidity and temperature associated with the low fertility of soils in Nepal. The yield loss due to the disease is very significant in Nepal. This experiment was conducted to identify the genotypes having a good level of resistance against spot blotch. The experiment set was received from CIMMYT comprises 52 genotypes and arranged in alpha lattice design with two replications in 2017/18 at National Wheat Research Program, Bhairahawa, Nepal, and Regional Agricultural Research Station, Parwanipur, Bara, Nepal. Each plot size was 8 rows of 2 meters long. Three times disease scoring was done in the double-digit method and calculated the Area under the disease progress curve (AUDPC). Other data were analyzed by using R software (4.2.2). Heading days, days to maturity, plant height, number of grains per spike (NGPS), number of tillers per meter square (NTPM), mean AUDPC, thousand-grain weight (TGW), and grain yield were found highly significant. The genotype 8HLBSN47 was found the highest yielder (4996kg/ha) with a 304 mean AUDPC value. Seventeen genotypes (15.3%) found the lowest mean AUDPC, Penultimate leaf AUDPC, Flag leaf AUDPC, and the highest number of tillers per square meter, number of grains per spike, thousand-grain weight, and grain yield.

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### INTRODUCTION

Wheat is the most widely cultivated food crop in the world, occupying 17% of the total cultivated land in the world. It is consumed in various forms by more than one thousand million people. Rice-wheat is the major cropping system in South Asia and covers close to 12 million hectares. With the advent of the green revolution in the past 3 decades, significant growth in the production of rice and wheat has occurred. As per production data for 2019, global wheat production was 766 million tons (FAO, 2021). China ranks at the top in wheat production (134

M tons) followed by India (104 M tons) and Russia (74 M tons), whereas Nepal ranks in 37th position (FAO, 2021). Wheat occupies the third position after rice and maize in terms of production and the second position after rice in terms of consumption in Nepal. In Nepal, it is the third most important staple crop next to rice (*Oryza sativa* L.) and maize (*Zea mays* L.) in production but ranks second in consumption after Rice. During 2019-2020, the area under cultivation, production, and productivity were 707505 ha, 2185289 tons, and 3088 kg/ha respectively, (MoALD, 2020/2021). Wheat is widely adapted with its coverage in all the three agro-climatic regions of the

country, ranging from 60 m asl to 4000 m asl. The average productivity of wheat in Nepal is very low than the potential yield. Various diseases and the time of wheat planting are the major yield-limiting factors for wheat production in Nepal. Major diseases of wheat in Nepal are; foliar blight complex (Spot blotch caused by *Bipolaris sorokiniana* Sacc in Sorok. and Tan spot caused by *Pyrenophora tritici repentis* Died). Spot blotch, the number one disease of wheat has been severe in plain areas and foothills and emerging towards mid hills and hills of Nepal. It causes up to 28% grain yield loss in farmer's field conditions (Mahto *et al.*, 2001). Crop management, timely sowing, and good soil fertility are important components of integrated crop management for spot blotch. In Nepal, it has been shown that an application of 30 kg of K<sub>2</sub>O had a similar effect to one fungicide application for spot blotch management (Sharma *et al.*, 2005). However, durable spot blotch-resistant varieties are lacking in Nepal.

Moderate to warm temperatures (18°C to 32°C) favors the growth of *B. sorokiniana*. In Asia, the infection was more rapid and more severe at 28°C than at lower temperatures (Singh *et al.*, 1998). The higher values of AUDPC/day or AUDPC/degree day under late-sown conditions are most likely caused by heat stress, which enhanced spot blotch development. Delayed seeding for wheat, grown after rice in eastern India and Nepal also results in higher losses of grain yield and total kernel weight due to spot blotch (Duveiller *et al.*, 2005). Rice wheat is the predominated cropping pattern of Terai (Plain) of Nepal. Late sowing of wheat in Terai is very common due to many factors viz. excess or loss of soil moisture just after rice harvesting, delay in rice harvesting due to late maturing varieties of rice, or shortage of field manpower during rice harvesting time. Late sowing wheat is always exposed to high temperatures during reproductive and grain filling stages, which forces the premature ripe. The high temperature at the reproductive age favors the spot blotch development and hastens spike development resulting in abortion of late forming florets and reduction in potential kernel number (Pokhrel *et al.*, 2013). Several chemical, cultural and physical methods have been developed to reduce the intensity of this disease but none of them has been found effective for a long time and economic.

The genetic basis of spot blotch resistance has earlier been documented as eight major quantitative trait loci (QTLs), namely, QSb.bhu-2A, QSb.bhu-2B, QSb.bhu-2D, QSb.bhu-3B, QSb.bhu-5B, QSb.bhu-6D, QSb.bhu-7B, and QSb.bhu-7D (Kumar *et al.*, 2009, 2010). Sharma *et al.* (2007a) reported three microsatellite markers (Xgwm67, Xgwm570, and Xgwm469) linked with spot blotch resistance. The QTL QSb.bhu-5B, QSb.bhu-7D, and QSb.bhu-3B have been designated as genes Sb1, Sb2, and Sb3, respectively, in further studies (Lillemo *et al.*, 2013; Kumar *et al.*, 2015;). Lr34 and Lr46, the two broadly used genes conferring leaf rust resistance, have also been reported to contribute to spot blotch resistance. While the Lr34 gene has been reported to be the major locus on chromosome 7D explaining up to 55% phenotypic variation for spot blotch disease resistance, this locus was given the gene designation Sb1

(Lillemo *et al.*, 2013). Cultivar development for resistance to spot blotch is slow due to the quantitative nature of resistance and a limited number of genes are known to have a major effect. Four spot blotch resistance genes with major effects have been named to date, i.e., Sb1, Sb2, Sb3, and Sb4 (Lillemo *et al.*, 2013; Kumar *et al.*, 2015; Zhang *et al.*, 2020). So, the use of disease-resistant variety is one of the best economic methods of disease management for a successful wheat production system.

## MATERIALS AND METHODS

Fifty-two wheat genotypes were received from the international wheat and maize improvement center (CIMMYT), Mexico. The experiments were conducted in two locations; National Wheat Research Program, Bhairahawa, Rupandehi, Bhairahawa, and Regional Agricultural Research Station, Parwanipur, Bara 2016-2017 wheat season. The experiments were laid out in alpha lattice design at both locations with four blocking in two replications. Each plot size was 8 rows of 2 m long. Geographically, Bhairahawa is located at an altitude of 105 m asl (above mean sea level) with 27.54567' North latitude and 83.46084' East longitudes. Parwanipur is located at an altitude of 75m asl with 27.4409'North latitude and 84.56985'East longitude. At the experimental site of Bhairahawa, the maximum and minimum temperatures were recorded on November 30.34°C and 17.53°C respectively, and relative humidity was 71%. In December, the maximum and minimum temperatures were recorded at 22.7°C and 13.2°C respectively, with 0.5 mm precipitation and relative humidity of 82.59%. Similarly, in March maximum and minimum temperatures were recorded at 30.32°C and 18.16°C with 48.2 mm precipitation (Figure 1). At the experiment site of Parwanipur, the maximum and minimum temperatures were recorded in November at 32.33°C and 21.26°C respectively, precipitation was 6.3 mm and relative humidity 61.37%. In December, the maximum and minimum temperatures were recorded at 29.9°C and 11.55°C respectively and relative humidity was 51.18%. Similarly, in March maximum and minimum temperatures were recorded at 25.76°C and 11.28°C and 72.74% relative humidity (Figure 2). For spot blotch, the rate of incubation period completion was described as a linear increase in rate with temperature up to approximately 29°C, then an exponential decline with temperature up to the maximum temperature of approximately 36°C in which disease development checked (Viani *et al.*, 2013).

The severity of the disease was recorded by visually assessing with a double-digit method. Spot blotch severity can be visually scored for each plot at weekly intervals using the double-digit scale (00-99) developed as a modification of Saari and Prescott's severity scale to assess wheat foliar diseases (Saari and Prescott, 1975; Eyal *et al.*, 1987). The first digit (D1) indicates the disease progress in height and the second digit (D2) refers to the severity measured by the diseased leaf area. For each score, the percentage of diseased leaf area (%DLA) was estimated based on the following formula:

$$\% \text{DLA} = ((D1/9) \times (D2/9) \times 100)$$

Individual scores were recorded at weekly intervals over three weeks. The Area Under Disease Progress Curve (AUDPC) was calculated using the percent severity estimates corresponding to the three to four ratings as outlined by (Das et al., (1992) and shown below:

$$\text{AUDPC} = \sum_{i=1}^{n-1} [(X_i + X_{i+1}) / 2] (t_{i+1} - t_i).$$

Where, xi = disease severity on the ith date, ti = ith day, and n= number of scoring dates. The AUDPC measures the amount of disease as well as the rate of progress and has no units.

R (4.2.2) statistical software and a Microsoft spreadsheet were used to analyze the entire data of this experiment.

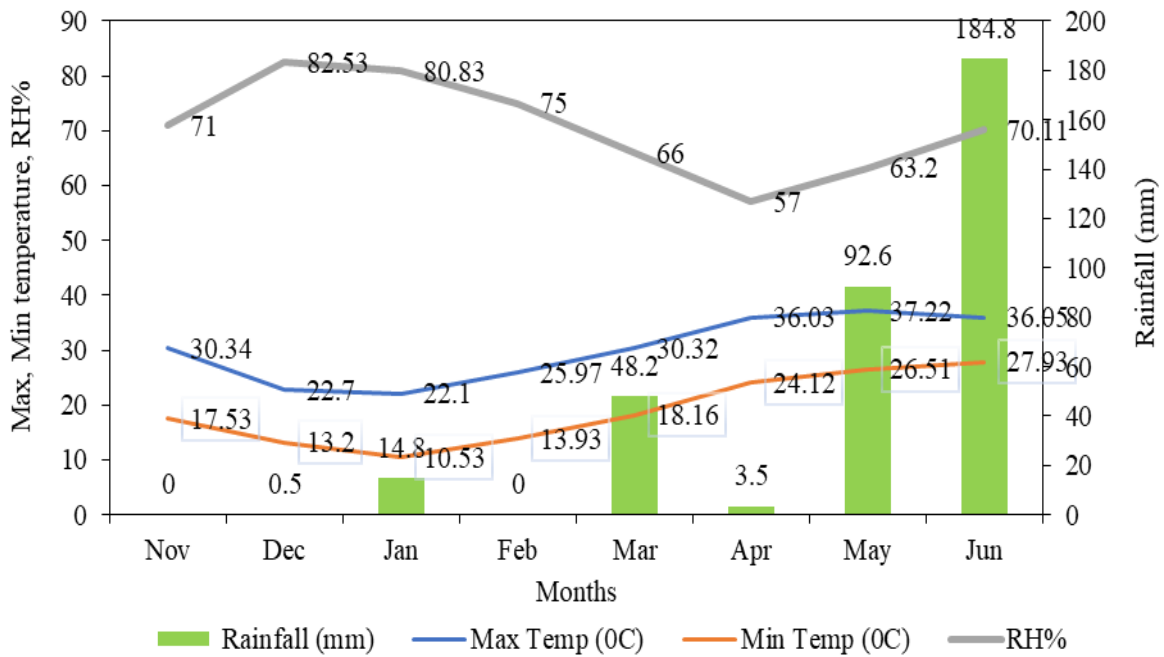


Figure 1. Meteorological information of study site, Bhairahawa, November 2106 to Jun 2017. Source: National Wheat Research Program 2017

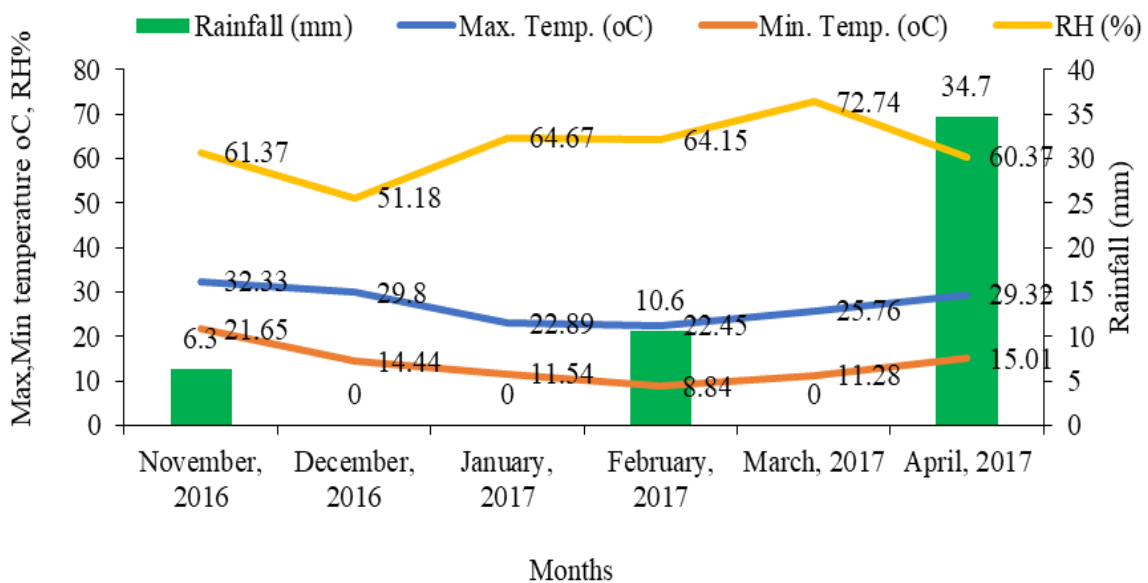


Figure 2. Meteorological information of study site, Parwanipur, November 2106 to Jun 2017. Source: Regional Agricultural Research Station, Parwanipur, 2017

## RESULTS AND DISCUSSION

### Days to heading

Days to heading were recorded when it was observed that 50 percent head appeared of the total population in a plot. Days to heading were found highly significant ( $P \leq 0.05$ ) among the tested genotypes in genotype by location interaction (Table 1). However, it was not statistically different in genotype significance. The first heading was observed in 72 days in 8HLBSN2 followed by 8HLBSN45 (74). The genotypes were recorded with the least mean AUDPC 8HLBSN43 (273), 8HLBSN47 (304), and 8HLBSN28 (320). The maximum mean AUDPC was found in 8HLBSN52 (Table 1). Similar results were also reported by some other authors, late heading results in the lesser development of disease while early heading results in more disease development (Pandey et al., 2018). This result is alike to the findings of Sharma et al. (2007), Shrestha et al. (1998), Duveiller and Dubin (2002). Genotypes late in heading have lower disease severity, it is due to slower plant development and shorter period of exposure of the plant to a pathogen. The difference among wheat genotypes in the duration of the period between sowing and heading days is largely governed by their sensitivity to photoperiod and vernalization (Calderini et al., 1995)

### Days to maturity

Days to maturity were recorded when 75 percent population showed yellowish peduncle. Days to maturity were found highly significantly differ ( $P \leq 0.05$ ) among the tested entries in genotype by location interaction but non-significant in the genotypes. Days to maturity ranged from 108-114 days. The genotypes 8HLBSN45, 8HLBSN19, 8HLBSN46, 8HLBSN19, 8HLBSN33, 8HLBSN10, 8HLBSN34, 8HLBSN17, 8HLBSN52 showed the shortest maturity days (108). Similarly, the longest maturity days (114) were observed in 8HLBSN22 (Table 1). Spot blotch severity increases with the advancement of growth stages (Chaurasiya et al., 2006). However, short and early genotypes appear distinctly more diseased than others; further studies are needed to understand the effect of earliness and plant height on disease development or possible escape (Duveiller et al., 2002). The early maturity was observed maturity duration of any variety plays an important role in the acceptance or rejection of the genotypes. In Nepal, farmers are most frequently reluctant to accept a variety, which has better yields but is late in maturity, mostly in the rice-wheat cropping system (Tewari et al., 1995). The demand of the Nepalese farmers is short-duration, disease tolerant, and high-yielding wheat varieties.

### Plant height

Plant height was measured at the dough stage (Zadoks growth stage 87) (Zadoks, 1974). The combined analysis showed that genotype by location ( $G \times L$ ) interaction was highly significant for plant height, among the tested genotypes in genotypes by location interaction. The shortest plant height was found in 8HLBSN30 (80cm) with a mean AUDPC value of 476 followed

by 8HLBSN44 (81 cm) with a mean AUDPC value of 443. Similarly, the genotypes 8HLBSN24 had 82 cm plant height, 376 mean AUDPC, and 4932 kg/ha yield. The 8HLBSN47 showed 85 cm plant height, with low mean AUDPC (304), and gave a yield of 4996 kg/ha followed by 8HLBSN49 (4990 kg/ha), and 8HLBSN25 (4956 kg/ha) (Table 1). The best leaf blight-resistant wheat in South Asia was reported that the late and tall genotypes, two less desirable agronomic characters, and breeders doubted the possibility to develop early maturing resistant genotypes. Studies reported less spot blotch resistance in short plants with early maturity (Dubin et al., 1998). The tallest genotype was 8HLBSN1 (94 cm) followed by 8HLBSN21 and 8HLBSN29 with AUDPC 484 and 638, respectively. However, 8HLBSN52 was found highest mean AUDPC value (852), although tall and early genotypes appear distinctly more diseased, further studies are needed to understand the effect of earliness and plant height on disease development.

### Number of grains per spike and Number of tillers per meter square

Analysis of variance showed a highly significant difference among the tested genotypes for the number of tillers per meter square and grain per spike was found significant in genotypes by location interaction but non-significant in genotypes. However, the number of effective tillers per meter square was found significantly different among the tested genotypes. The genotypes 8HLBSN31, 8HLBSN28, 8HLBSN29, 8HLBSN48, 8HLBSN20 were found the highest number of tillers per meter square 539, 525, 515, 499, 497 and 487, respectively (Table 1). The genotypes having a greater number of tillers could be used for cyclic breeding to develop high-yielding varieties. The number of tillers/m<sup>2</sup> is important, especially in the case of susceptible wheat cv. Hereward, leaf spot diseases under conducive weather conditions can significantly reduce the number of productive tillers (Ronis et al., 2009). The researcher concluded that the high severity of *Septoria tritici* could reduce the number of spike/m<sup>2</sup> (Simon et al., 2002).

### Grain yield, 1000 grains weight, and mean AUDPC

Analysis of variance showed highly significant differences among the tested genotypes for thousand grains weigh, grain yield, and thousand-grain weight. The combined analysis showed that genotype by location ( $G \times L$ ) interaction was a highly significant mean AUDPC, Thousand-grain weight, and grain yield, mean AUDPC ranged from 273-852. The highest thousand-grain weight (TGW) was produced by genotypes 8HLBSN33 (48 gram) followed by 8HLBSN52 (47 g) and 8HLBSN44 (47 g). The resistant check variety Chirya-3 (8HLBSN1) showed a mean AUDPC value of 382 with a grain yield of 4065 kg/ha. Since this genotype is tolerant to spot blotch grain yield is low than other genotypes due to the genetic characteristics of small grain size. While the susceptible check varieties; RR-21 (8HLBSN51) and NL 297 (8HLBSN52) had mean AUDPC values of 755 and 852 with grain yields of 2537 kg/ha and 2020 kg/ha respectively (Table 1).

**Table 1.** Combined grain yield, mean AUDPC, and other yield attributing character of wheat at Rupandehi, Bhairahawa and Parwanipur, Bara during 2016/17.

E.N.	Genotypes	DTH (Days)	DTM (Days)	PH (cm)	NGPS	NTPM	Mean AUDPC	TGW (gm)	Grain Yield (kg/ha)
47	8HLBSN47	76	110	85	51	447	304	46	4996
49	8HLBSN49	78	111	88	51	431	491	44	4990
42	8HLBSN42	75	109	86	51	383	580	44	4956
25	8HLBSN25	78	110	86	46	485	454	41	4954
24	8HLBSN24	76	110	82	53	464	428	38	4932
45	8HLBSN45	74	108	88	41	431	517	46	4926
20	8HLBSN20	76	111	91	50	497	413	42	4900
23	8HLBSN23	77	108	83	48	460	504	40	4887
38	8HLBSN38	76	109	83	55	381	684	38	4872
19	8HLBSN19	77	108	89	50	482	386	43	4870
13	8HLBSN13	78	109	86	55	414	445	40	4855
21	8HLBSN21	77	112	93	44	412	484	44	4826
37	8HLBSN37	78	111	82	46	468	376	39	4820
39	8HLBSN39	77	109	89	53	446	614	39	4795
28	8HLBSN28	75	112	86	51	525	320	39	4721
27	8HLBSN27	79	113	91	52	431	354	38	4697
29	8HLBSN29	79	113	92	45	515	638	43	4673
22	8HLBSN22	81	114	89	49	456	589	40	4662
48	8HLBSN48	78	112	82	51	499	378	40	4630
46	8HLBSN46	75	108	86	48	447	514	45	4613
43	8HLBSN43	77	111	85	52	429	273	41	4575
4	8HLBSN4	76	111	87	50	429	506	44	4539
40	8HLBSN40	79	113	85	55	358	658	42	4522
33	8HLBSN33	76	108	91	46	420	492	48	4494
36	8HLBSN36	76	110	87	55	320	540	44	4494
16	8HLBSN16	76	109	92	48	334	605	46	4424
15	8HLBSN15	76	109	84	46	453	607	40	4406
31	8HLBSN31	76	110	89	48	539	496	40	4396
12	8HLBSN12	76	109	87	50	396	374	46	4372
2	8HLBSN2	72	111	90	56	367	581	44	4371
8	8HLBSN8	77	109	86	45	438	516	38	4365
14	8HLBSN14	78	111	86	55	390	500	46	4329
41	8HLBSN41	77	111	82	47	430	472	42	4316
32	8HLBSN32	76	109	88	45	449	382	41	4311
5	8HLBSN5	78	111	90	44	328	450	40	4293
44	8HLBSN44	78	112	81	42	379	443	47	4141
10	8HLBSN10	76	108	89	47	487	456	43	4114
34	8HLBSN34	76	108	90	45	419	398	38	4095
1	8HLBSN1	75	112	94	52	476	382	34	4065
11	8HLBSN11	78	112	90	48	434	481	42	4029
7	8HLBSN7	78	111	86	49	317	629	39	3885
3	8HLBSN3	77	112	82	44	464	610	40	3819
9	8HLBSN9	75	111	89	41	416	351	42	3791
35	8HLBSN35	80	113	85	44	483	439	37	3775
6	8HLBSN6	79	113	87	52	430	489	42	3654
17	8HLBSN17	77	108	85	46	383	460	44	3620
18	8HLBSN18	78	112	84	50	283	436	39	3576
30	8HLBSN30	78	111	80	49	207	476	40	3470
26	8HLBSN26	78	111	83	43	415	402	42	3353
50	8HLBSN50	77	109	87	38	435	749	36	2719
51	8HLBSN51	77	109	87	37	320	755	35	2537
52	8HLBSN52	76	108	88	35	310	852	47	2020
Grand Mean		76.8	110.3	86.8	47.8	419.3	495	41.3	4296.9
LSD value		5.61	4.32	8.17	12.84	104	278	5.79	1257.1
CV(%)		2.42	1.59	3.05	12.92	14.37	13.26	4.91	10.21
Genotype significance		0.97	0.249	0.116	0.278	<0.01	<0.01	<0.01	0.0003
Gen x Location		<0.01	<0.01	<0.01	0.001	0.0988	<0.01	<0.01	<0.01

**Table 2.** Correlation matrix for different agronomical and disease traits evaluated at Bhairahawa and Parwanipur for the year 2016/17.

	DTH	DTM	PH	NGPS	NTPM	AUDPC. Penultimate Leaf	AUDPC Flag Leaf	Mean AUDPC	TKW	GY
DTH	1	0.54***	-0.19	-0.02	-0.02	0.13	0.08	0.01	-0.23	-0.09
DTM		1	-0.01	0.22	0.08	-0.29*	-0.22	-0.17	-0.21	0.04
PH			1	-0.04	0.16	-0.26	-0.2	0.05	0.1	0.08
NGPS				1	0.04	-0.33*	-0.49**	-0.29*	0	0.61***
NTPM					1	-0.31*	-0.38***	-0.34*	-0.15	0.45***
AUDPC Penultimate Leaf						1	0.71	0.16	-0.04	-0.43***
AUDPC Flag Leaf							1	0.37	0	-0.57***
Mean AUDPC								1	0	-0.43***
TKW									1	0.16
GY										1

Note: GY: Grain Yield; NGPS: Number of Grains per Spike; TGW: Thousand Grain Weight; PH: Plant Height; NTPM: Number of tiller per square meter; DTH: Days to Heading; DTM: Days to Maturity; AUDPC: Area under Disease Progress Curve. Values are significantly different at 5% level of significance (\*) and highly significantly different at 1% level of significance (\*\*)

**Table 3.** Vector loadings and proportion of variance explained by the first six principal components (PC)

Traits	PC1	PC2	PC3	PC4	PC5	PC6
DTH	0.037	-0.631	0.022	-0.095	0.337	-0.008
DTM	-0.178	-0.574	0.177	-0.280	0.221	-0.016
Plant Height	-0.127	0.268	0.650	-0.117	0.201	-0.650
No. of grains/spike	-0.367	-0.060	-0.388	-0.326	-0.331	-0.385
No. of tillers/sq.meter	-0.315	-0.006	0.230	0.636	0.168	0.215
AUDPC value (Penultimate leaf)	0.418	-0.054	-0.322	0.259	0.137	-0.455
AUDPC value(Flag Leaf)	0.484	-0.025	-0.106	0.111	0.146	-0.188
Mean AUDPC value	0.317	0.076	0.322	-0.386	-0.334	0.285
TKW	-0.015	0.417	-0.226	-0.396	0.710	0.229
Grain Yield	-0.455	0.114	-0.271	0.057	0.062	-0.078
	Loadings					
Standard deviation	1.774	1.3259	1.0908	1.0307	0.90876	0.7953
Proportion of Variance	0.315	0.176	0.119	0.106	0.083	0.063
Cumulative Proportion	0.315	0.491	0.610	0.716	0.798	0.862

The highest grain yield was found in 8HLBSN47 (4996 kg/ha) followed by 8HLBSN24 and 8HLBSN45 (4932 kg/ha and 49826 kg/ha, respectively). Similarly, the highest thousand grain was found in 8HLBSN33 (48), 8HLBSN52 (47), 8HLBSN47 (46), 8HLBSN12 (46) and 8HLBSN16 (46) and 8HLBSN45 (46). Even if the thousand-grain weight is more, the mean AUDPC value of genotypes HLBNS52 (852), 8HLBSN16 (605), and 8HLBSN45 (517) means such genotypes are more susceptible than others are, even though they have genetic characteristics of bold grains. The Lowest mean AUDPC value (273) showed by 8HLBSN43 and followed by 8HLBSN47 (304). Yield losses due to the reduction of productive tillers can be compensated by the increase in grain weight per ear, and thousand-grain weight (TGW).

Grain yield losses of up to 50% have been reported and have been attributed primarily to a reduction in grain size and weight and some cases, to a reduction in the number of grains per unit area (Rees and Platz, 1980). However, (Gilchrist and Pfeiffer, 1992) reported that TKW and grain yield had a positive relation with disease incidence at the later stage of crop development. Reduction in TKW was more closely associated with the HLB score at Feekes large 10.5 wheat growth stage. Both spike and leaf damage by spot blotch had a combined negative effect on

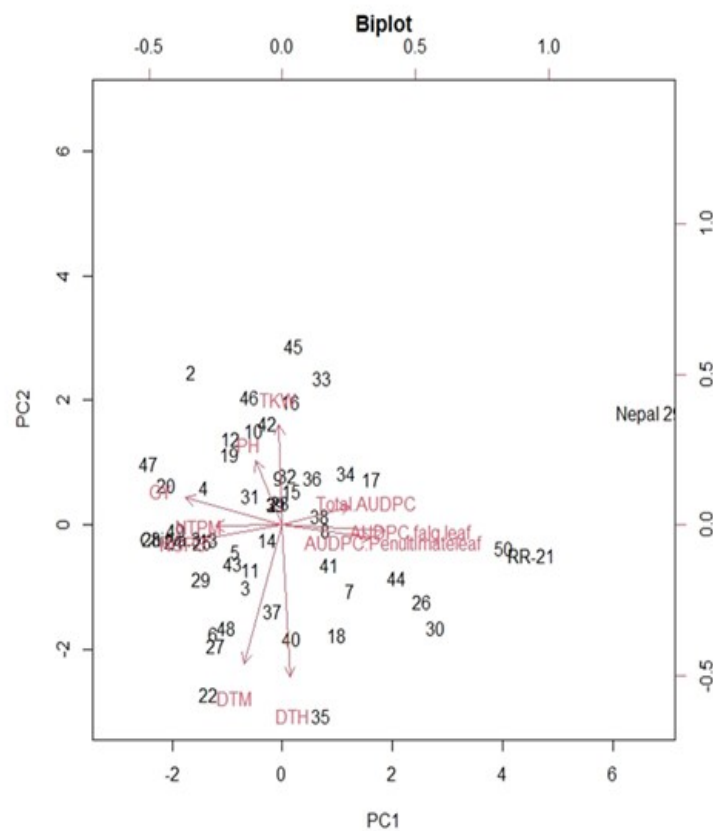
grain yield and TKW. (Gilchrist and Pfeiffer, 1992) reported that genotypes with short grain filling duration had significantly higher leaf and spike damages caused by spot blotch, but lower grain damage compared to genotypes with long grain fill duration. Further, early maturing genotypes had lower grain damage but higher leaf and spike damages due to spot blotch than late maturing genotypes. This result suggested the influence of temperature on disease development. Naitao and Yousan (1998) reported that *Bipolaris sorokiniana* resistance traits in wheat are polygenic and greatly affected by climatic factors such as rainfall, humidity, and temperature. Similarly, Gilbert et al. (1998) reported that a humid environment resulting from high temperature and high rainfall promoted the development of the disease. The number of tillers per square meter and several grains per spike was not affected by the late disease establishment in the fields.

#### Pearson correlation coefficient and principal component analysis (PCA)

The Days to heading showed a highly significant positive correlation with days to maturity (0.54\*\*\*) and a non-significant negative correlation with plant height (-0.19), number of grains/spike (-0.02), number of tillers per square meter (-0.02),

**Table 4.** Mean value of traits for 6 clusters obtained from K-means cluster analysis.

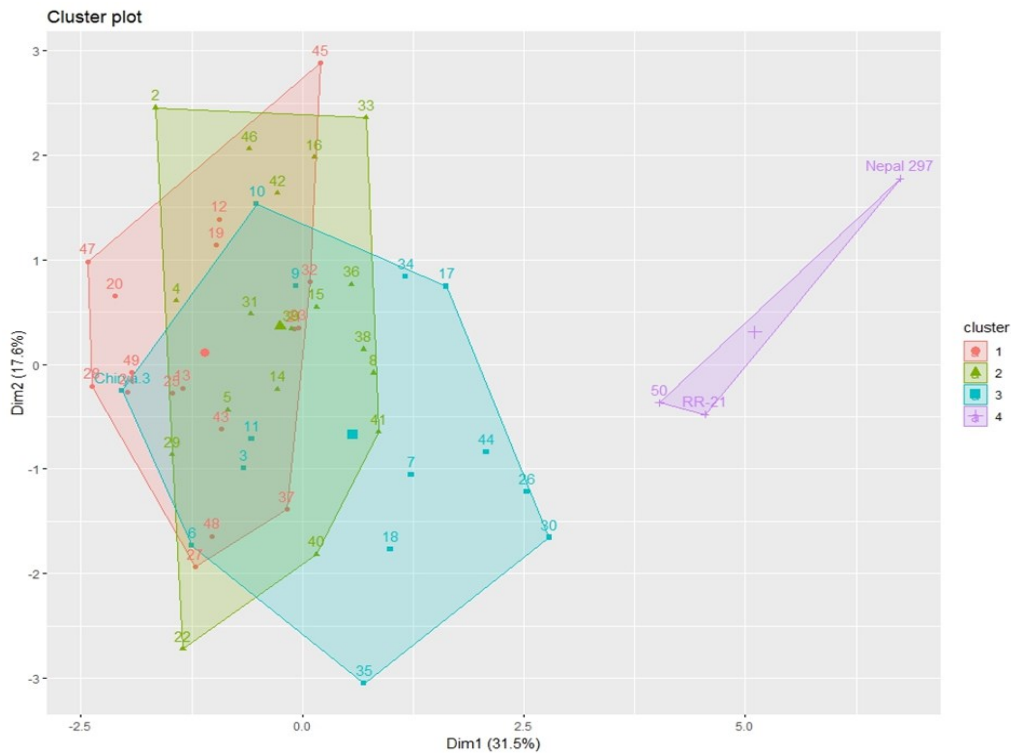
Traits	Cluster I	Cluster II	Cluster III	Cluster IV	Grand Centroid
DTH	76.8	76.7	77.4	76.7	76.9
DTM	110.2	110.3	111.0	108.7	110.0
Plant Height	86.6	87.4	86.1	87.3	86.9
No. of grains/spike	49.2	49.8	46.6	36.7	45.5
No. of tillers/sq.meter	454.1	413.0	399.5	355.0	405.4
AUDPC Value (Penultimate leaf)	637.4	641.6	689.4	818.7	696.8
AUDPC value (Flag Leaf)	280.3	305.1	320.9	508.0	353.6
Mean AUDPC value	404.9	557.9	460.9	785.2	552.2
TKW	41.6	42.4	40.6	39.3	41.0
Grain Yield	4780.1	4528.9	3813.4	2425.3	3886.9

**Figure 3.** Biplot between first and second principal components.

thousand grains weight (-0.23) and grain yield (-0.109). Similarly, days to maturity showed a non-significant positive correlation with the number of grains per spike (0.22), the number of tillers per square meter (0.08), and grain yield (0.04), significant negative correlation with AUDPC (penultimate leaf) (-0.29\*) and non-significant negative correlation with AUDPC (flag leaf) (-0.30\*), mean AUDPC (-0.16) and TGW (-0.21). The grain yield showed a highly significant positive correlation with the number of grains per spike (0.61\*\*\*) and the number of tillers per square meter (0.45\*\*\*), a non-significant positive correlation with TGW, plant height, and days to maturity, and a highly significant negative correlation with AUDPC (penultimate leaf) (-0.43\*\*\*) AUDPC (flag leaf) (-0.57\*\*\*) and mean AUDPC (-0.43\*\*\*) and

non-significant negative correlation with days to heading (Table 2). Similar results were also found by Sharma *et al.* (2007), Tewari *et al.* (2016), and Neupane *et al.* (2013), found a negative correlation between TKW and AUDPC. The number of grains per spike showed a highly significant negative correlation with AUDPC flag leaf (-0.49\*\*) and a significantly negative correlation between n mean AUDPC (-0.29\*) and AUDPC penultimate leaf (-0.33\*). These results are similar to the finding of Tewari *et al.* (2016). This might be due to the higher diseased area and lower assimilation of carbohydrates.

The PCA analysis indicated that the first six components (PC1, PC2, PC3, PC4, PC5, and PC6) explained maximum cumulative variances of 86.2% are important (Table 3). Among all PCs, the



**Figure 4.** K-means clustering of 52 wheat genotypes evaluated at two locations Bhairahawa and Parwanipur in the year 2016.

first PC (0.315) contributed the maximum to the total variance. A similar finding of grouping the bread wheat genotype by principal component analysis were reported by Getachew *et al.*, 2017; Degewione and Alamerew, 2013; Fellahi *et al.*, 2013). The major traits contributing to the first PC are the AUDPC flag leaf, AUPDC penultimate leaf, and grain yield. Similarly, for the second PC, DTH, DTM, and TKW were the major contributors. PH, mean AUDPC, and NGPS were the diversity contributor traits in the third PC. In the fourth PC, max variation was explained by the number of tillers per meter square followed by TGW and mean AUDPC, TKW, DTH, number of grains per spike and mean AUDPC were major contributing traits for PC5. In the last sixth PC, the maximum variation contributors were PH and AUDPC (penultimate leaf).

The biplot explains the relationship of 52 wheat genotypes with component traits (Figure 3). Across the 52 genotypes, grain yield was positively associated with the number of tillers per square meter, the number of grains per spike, thousand grains weight, and plant height and negatively correlated with AUPDC (penultimate leaf), AUDPC (flag leaf), and mean AUDPC.

### Cluster analysis

Genotypes were clustered based on variables: days to heading, days to maturity, number of grains per spike, plant height, number of tillers per square meter, AUDPC Flag leaf, AUDPC penultimate leaf, mean AUDPC, thousand grains weight, and grain yield by K-means clustering method. Cluster analysis sequestered 52 genotypes of wheat into 4 clusters (Figure 4). A similar technique of grouping the diverse bread wheat genotype by cluster analysis was reported by Chatrath *et al.*, 2018; Getachew *et al.*, 2017; Adilova *et al.*, 2020). Cluster I consisted

of 17 genotypes, which represents 15.3 % of total genotypes. It includes 8HLBSN1, 8HLBSN13, 8HLBSN19, 8HLBSN20, 8HLBSN21, 8HLBSN23, 8HLBSN24, 8HLBSN25, 8HLBSN27, 8HLBSN28, 8HLBSN32, 8HLBSN37, 8HLBSN43, 8HLBSN45, 8HLBSN47, 8HLBSN48 and 8HLBSN49. This cluster represents genotypes with the lowest mean AUDPC, AUPDC penultimate leaf, and AUPDC flag leaf and the highest in the number of tillers per square meter and grain yield. The number of grains per spike and thousand grains weight was also found excellent in genotypes under cluster I (Table 4). Cluster II consisted of 18 genotypes, which represents 34.6% of total genotypes. It includes 8HLBSN4, 8HLBSN5, 8HLBSN8, 8HLBSN14, 8HLBSN15, 8HLBSN16, 8HLBSN22, 8HLBSN29, 8HLBSN31, 8HLBSN33, 8HLBSN36, 8HLBSN38, 8HLBSN39, 8HLBSN40, 8HLBSN41, 8HLBSN42 and 8HLBSN46. This cluster represents genotypes with the highest TGW and number of grains per spike, second in NTPM, GY, and lowest AUDPC value of penultimate leaf and flag leaf (Table 4). Cluster III consisted of 14 genotypes, which represents 26.9% of total genotypes. It includes 8HLBSN3, 8HLBSN6, 8HLBSN7, 8HLBSN9, 8HLBSN10, 8HLBSN11, 8HLBSN17, 8HLBSN18, 8HLBSN26, 8HLBSN30, 8HLBSN34, 8HLBSN35 and 8HLBSN44. This cluster represents genotypes with an average value for all traits under study (Table 4). Cluster IV consisted of three genotypes, which represent 5.8% of total genotypes. It includes 8HLBSN50, RR-21, and Nepal 297. This cluster is characterized by genotypes having the highest total AUDPC, AUPDC penultimate leaf, and AUPDC flag leaf, intermediate in plant height, and lowest in the number of grains per spike, thousand grains weight, and grain yield. The mean value of each trait for each cluster was presented in Table 4).



## Conclusion

Among the tested genotypes, seventeen genotypes (8HLBSN1, 8HLBSN13, 8HLBSN19, 8HLBSN20, 8HLBSN21, 8HLBSN23, 8HLBSN24, 8HLBSN25, 8HLBSN27, 8HLBSN28, 8HLBSN32 and 8HLBSN37, 8HLBSN43, 8HLBSN45, 8HLBSN47, 8HLBSN48, and 8HLBSN49) show the lowest mean AUDPC, Penultimate leaf AUDPC, Flag leaf AUDPC and the highest number of grains per spike, the highest number of tillers per meter square, highest thousand-grain weight and highest grain yield. The correlation between grain yield and disease development is negative. These results are of importance for the breeders in developing spot blotch-resistant varieties targeting spot blotch resistance in Nepal where spot blotch is prevalent. However, wheat genotypes, which have shown constantly tolerant reactions against spot blotch in different locations, could be utilized as such or resistance transferred using a cyclic breeding program into commercial varieties to meet the immediate challenge posed by spot blotch in Nepal could benefit from this present study.

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## Conflict of interest

No conflict of interest with the present work.

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