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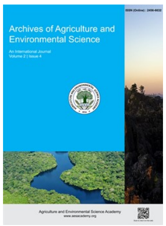

ORIGINAL RESEARCH ARTICLE

CrossMark

Spot blotch disease resistance and heat stress tolerance in spring wheat (*Triticum aestivum* L.)

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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. Canopy temperature measurements using infrared thermometry, to assess variation in foliar blight resistance along with heat tolerance as an integrative selection criterion. The experiment set was comprising 52 genotypes and arranged in alpha lattice design with two replications in 2017/-2018 Directorate of agricultural research center, Parwanipur, Bara, Nepal. Each plot size was 8 rows of 3 meters long. Three times disease scoring was done in Flag leaf, and Penultimate leaf method and calculated the Area under the disease progress curve (AUDPC). Other data were analyzed by using R software (4.2.2). Canopy temperature, heading days, days to maturity, plant height, number of grains per spike (NGPS), number of tillers per meter square (NTPM), thousand-grain weight (TGW), and grain yield were found highly significant. The genotype 32 was found the highest yielder (5141 kg/ha) and canopy temperature 16.5^{0} C with a 279; 610 F; F-1AUDPC respectively.

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INTRODUCTION

Wheat is the world's second most-produced cereal, with worldwide output as 778.6 million metric tons in 2021–2022 (Shahbandeh, 2022). It is consumed by 35% of the world's population and contributes 20% of global human calories and protein (Poudel and Bhatta, 2017). Wheat represents the third most significant cereal crop in Nepal, with 0.71 million ha grown and 2.1 million metric tons production in 2021 (MoALD, 2021). Abiotic and biotic stresses, i.e., terminal heat and spot blotch, respectively, affect almost 23% of wheat-growing land in Southeast Asia's warmer climate zones (Singh *et al.*, 2020). In Southeast Asia, including Nepal, spot blotch of wheat caused by the ascomycetous fungus *Bipolaris sorokiniana* leads to a yield penalty of up to 30% (Kumar *et al.*, 2009). Spot blotch causes shriveling, black points, and discoloration of grains, thus lowering grain quality (Chand and Joshi, 2004). Spot blotch reduces wheat output by 23 to 40% in Nepal's warmer regions (Sharma and Duveiller, 2006). *B. sorokiniana* also causes seedling blight and common root rot of wheat by inoculums present in or on infected seeds (Ries and Forcelini, 1993), conidia surviving on crop leftovers (Pandey *et al.*, 2005), or in the soil (Duveiller *et al.*, 2002). Secondary sources of inoculums, such as alternate hosts or airborne conidia, cause spot blotch and head blight in wheat (Duveiller *et al.*, 2005). At grain filling stages, a favorable environment such as terminal heat stress, intermittent rainfall, temperatures >26 °C, and dew deposition on leaves for an extended period aggravates the severity of spot blotch (Acharya *et al.*, 2011). The eastern part of South Asia, which encompasses the eastern Gangetic Plains (EGP) of India, Nepal, and Bangladesh, is one of the most heavily populated parts of the world. In the EGP, where wheat is grown in about 10 m ha, the two major stresses to the wheat crop are spot blotch (SB) and terminal heat (Al-Khatib *et al.*, 1990). In EGP, SB causes considerable yield loss between 15.5 and 19.6% annually (Gupta *et al.*, 2018). However, when the disease is initiated at the flag leaf stage, losses of grains are estimated to be up to 24.2% (Singh *et al.*, 2015). SB is normally a weak disease that takes advantage of heat stress (Rosyara *et al.*, 2009), nutrient deficiency, and water stress (Sharma *et al.*, 2006) to induce significant grain damage. This disease is favored by cloudy and foggy days during the post -heading stage (Joshi *et al.*, 2007) and is expanding towards non-traditional cooler regions such as India's North West Plain Zone (NWPZ) (Gupta *et al.*, 2018). Further, this disease is predicted to become more severe due to climate change, nutritional and water deficiencies, and increased heat stress (Crespo-Herrera *et al.*, 2021).

Spot blotch resistance and heat stress tolerance are two important breeding objectives of most of wheat improvement programs of South Asia, however progresses have been very limited due to lack of suitable selection criteria. In the case of spot blotch, breeding programs have identified promising resistance sources although none confers immunity (Sivapalan *et al.*, 2003). Deviation of temperature of plant canopies in comparison to ambient temperature, also known as canopy temperature deviation (CTD), has been a good criterion for screening heat stress tolerance (Reynolds *et al.*, 1998). CTD was also found to be negatively correlated with disease severity (Eyal and Blum, 1989; Nutter and Aderman, 1987). CTD has caught breeders' attention because it is easy to calculate and can be used to screen large amounts of material in a single day (Brennan *et al.*, 2007). Studies using CTD for the assessment of heat stress tolerance and spot blotch resistance are few. Thus, the present investigation aims at identifying potential applications of CTD measurements using infrared thermometry, to assess variation in foliar blight resistance along with heat tolerance as an integrative selection criterion

Interestingly, the best grain yield performance is not necessarily obtained by entries scoring a low AUDPC for spot blotch, which underscores the importance of general adaptation to heat stressed environment. However, ongoing efforts to discover wheat genotypes with high disease resistance are required to cope with the expected favorable environment for spot blotch in the future (Gupta *et al.*, 2020). Hence, the objective of this was to identify genetic materials for deploying resistant genotypes against spot blotch disease in the country.

MATERIALS AND METHODS

Genotypes

The experimental materials comprised of 52 wheat genotypes and a susceptible check (RR-21), Nepal 297 and a resistant check (Chirya 3) for wheat season 2017-2018 at Parwanipur, Bara, Nepal. The genotypes were obtained from the breeding unit of National Wheat Research Program (NWRP), Bhairahawa, Nepal. These genotypes were diverse for their genetic makeup, geographical distribution, response to disease resistance, morphological and yield traits. The details of genotypes given in Annexure 1.

Experimental set up and design

The experiment was conducted at the Directorate of agricultural research station, Parwanipur Bara, Nepal. An alpha lattice designed was used for the experiment with four blocks having 13 genotypes in each block in two replications Each plot size was 8 rows of 3 m long. Geographically, Experimental site is located at an altitude of 75m asl with 27.4409' North latitude and 84.56985'East longitude. 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 29.90 °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 1).

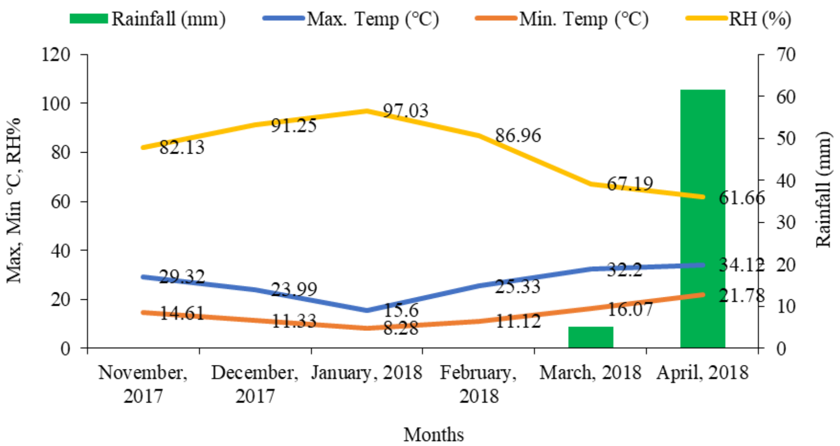

Figure 1. *Meteorological data of the experiment site, Parwanipur, Bara, Nepal during November 2017 to April 2018.*

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). A total of 120:50:40 Kg/ha N: P2O5:K2O was applied to the field, with 50 percent nitrogen and the entire dose of phosphorus and potash applied as a basal dose during sowing and the remaining 50 percent nitrogen applied in two split doses, one at active tillering (GS 32-39) and the other at booting stage (GS 45) (Zadoks *et al.*, 1974). To suppress weed germination, a pre-emergence weedicide (Pendimethalin 30% EC) was administered @ 2 ml /L, on the next day after the sowing and followed by hand weeding. Likewise, for insect management, a systemic insecticide, Rogor (Dimethoate 30% EC), was sprayed twice @ 1.5 ml /L at the active tillering stage (GS 32-39) and booting stage (GS 45). The experimental field was irrigated three times: first at the CRI stage, second at the booting stage (GS 45), and third at the end of the milking stage (GS 73) (Zadoks *et al.*, 1974). Canopy temperature was measured during the anthesis stage and milking stages using a handheld infrared thermometer.

Disease assessment

Percentage of spot blotch diseased leaf area was scored visually on flag leaf (F) and penultimate leaf (F-1); leaf just below the flag leaf, using standard diagram developed by CIMMYT (Mujeeb-Kazi *et al.*, 1996). Leaf blight severity was recorded from 10 randomly selected tillers of all genotypes in both replications. First scoring of the disease was done after heading between the growth stages 69-85 decimal code (DC) (Zadok *et al.*, 1974). Three scorings were done at 7 days intervals at 82DAS, 89DAS and 96DAS to estimate the area under disease progress curve (AUDPC).

$$\text{Disease intensity (\%)} = \frac{\text{Sum of all numeric rating}}{\text{Total numbers of plant observed} \times \text{maximum scale}} \times 100$$

Estimation of area under disease progress curve (AUDPC)

The area under disease progressive curve (AUDPC) was calculated by summarizing the progress of disease severity. The pattern of epidemic in terms of number of lesions, amount of diseased tissue, or number of diseased plants is given by a curve, called the disease progress curve, that shows the epidemic over time, and the area covered by this curve is known as AUDPC. AUDPC values from double digit and AUDPC from flag leaf (F) and penultimate leaf (F-1) were separately calculated by using the formula given by (Das *et al.*, 1992).

$$\text{AUDPC} = \sum_{i=1}^{n} (Y_{i+1} + Y_i) 0.5 (T_{i+1} - T_i)$$

Where,

Yi= disease scored on ith first date

Ti= date on which the disease was scored

n = Number of dates on which disease was scored

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≤0.05) among the tested genotypes (Table 1). It was statistically significantly different. The first heading was observed at 71 days in genotype 3 followed by genotype 9 (73 days) and genotype 47 (74 days). The maximum heading days were found in genotype 5 and 6 (84 days) followed by genotype 27 and 35 (81 days). 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 *et al.* (2005). 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≤0.05) among the tested entries in genotype. Days to maturity ranged from 118-106 days. The genotypes 36 showed the shortest maturity (106 days) (Table 1). Spot blotch severity increases with the advancement of growth stages (Chaurasia *et al.*, 2000). 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 analysis showed highly non-significant for plant height, among the tested genotypes. The shortest plant height was found in genotype 44 (76 cm) with a mean AUDPC (F), (F-1); 580 and 639 respectively with canopy temperature (17.3°C). 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, 1998).

Table 1. Grain yield, AUDPC (F), F-1, canopy temperature and other yield attributing character of wheat at Directorate of agricultural research, Parwanipur, Bara in 2017-2018.

Genotypes (Entry)	DTH (days)	DTM (days)	PH (cm)	NGPS	NTPM	AUDPC (F)	AUDPC (F-1)	TGW (g)	Grain Yield (kg/ha)	Canopy temperature (^{0}C)
32	76	108	86	53	418	279	610	42	5141	16.5
47	74	107	81	53	528	132	441	44	5107	16.4
23	75	109	77	44	484	233	567	39	5098	16.5
4	74	111	81	53	514	146	312	44	5076	16.6
29	74	111	87	41	584	234	555	41	5066	16.1
20	77	111	87	51	566	196	533	41	5065	17.2
25	77	109	79	44	572	257	564	43	5063	16.5
42	74	110	81	51	494	246	682	44	5050	17.7
37	78	111	85	46	527	229	707	36	5043	16.7
28	78	112	83	49	576	171	714	39	5024	17.3
43	77	114	82	56	528	266	616	39	5022	17.1
19	76	108	81	50	591	222	480	43	5018	17.5
45	75	109	88	38	513	260	613	48	4977	16.1
48	79	111	81	50	550	241	562	38	4960	16.5
27	81	115	86	50	481	272	556	35	4950	17.2
41	77	112	80	54	558	266	441	38	4944	16.4
24	76	111	77	50	507	296	537	38	4940	16.5
46	74	110	83	48	495	167	535	43	4938	16.4
26	78	112	83	56	418	229	624	39	4934	17.9
39	76	109	85	49	567	329	680	42	4914	17.1
31	79	112	82	50	603	244	591	41	4906	17.1
49	78	112	91	39	492	227	720	39	4847	17.4
22	82	114	84	49	527	226	630	36	4792	16.1
13	77	111	84	55	433	247	622	36	4790	16.0
9	73	112	80	43	469	269	627	38	4786	17.6
21	77	112	84	37	477	307	609	45	4784	16.5
15	77	109	81	43	501	218	583	39	4752	17.8
8	77	111	80	45	483	400	926	38	4745	17.4
33	73	105	84	48	513	382	823	46	4744	17.7
5	84	118	83	48	372	172	361	37	4684	17.3
38	75	107	77	50	515	363	955	36	4633	16.9
44	78	112	76	38	447	580	639	45	4561	17.3
35	81	114	87	52	583	396	822	39	4489	17.8
2	72	109	83	57	417	236	505	39	4312	18.2
1	75	112	90	51	536	268	576	32	4286	17.8
40	75	112	83	56	411	270	829	44	4122	17.0
3	71	111	79	40	515	288	478	40	4095	18.2
36	77	106	88	57	362	365	883	39	4087	16.9
12	75	111	84	51	459	214	610	42	4065	17.9
16	76	108	90	44	382	331	623	45	3974	16.6
14	76	110	85	53	498	370	733	44	3825	17.9
34	78	111	86	43	498	439	866	37	3694	17.4
6	84	117	85	50	554	259	459	39	3660	17.9
7	80	114	80	51	397	401	631	37	3601	17.7
11	77	111	83	46	503	269	673	40	3351	17.0
10	76	108	85	43	539	416	914	35	3288	17.2
17	76	112	86	49	475	297	794	40	3218	17.3
18	77	111	83	52	215	369	795	36	2983	17.5
30	77	111	79	54	234	378	952	39	2617	17.5
52	76	109	84	38	411	694	748	37	2489	18.7
50	76	109	87	35	335	593	863	38	2484	18.9
51	76	108	82	41	305	611	869	37	2183	18.6
Grand Mean	76.4	110.5	83.2	47.7	479.3	303.3	654.5	39.7	4387.8	17.2
LSD value	2.49	2.78	7.23	11.56	137.94	132.39	288.87	5.26	631.13	1.7
CV %	1.57	1.23	4.33	12.05	14.32	20.87	19.8	6.45	7.16	5.11
Genotype significance	<0.01	<0.01	0.019	0.008	<0.01	<0.01	0.001	<0.01	<0.01	<0.01

DTM= Days to maturity, PH= Plant height (cm), NGPS=Number of grains per spike, NTPM= Number of tillers per square meter, AUDPC= Area under disease progress curve, F= Flag leaf, F-1= Penultimate leaf, TGW=Thousand grain weight, GY= Grain yield and CT= Canopy temperature.

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

The number of grains per spike revealed non-significant among the tested genotypes. However, the highest number of grains was found in genotypes 40 (56) and genotypes 13 (55) with 17.8 °C and 16 °C canopy temperature respectively. The number of tillers per meter square showed highly significant. The genotypes 29 and 20 were found the highest number of tillers per meter square 584 and 566 respectively with canopy temperature 16.1 and 17.2, respectively (Table 1). Similarly, AUDPCF; F-1 234, 555; 196, 533 respectively. The genotypes having a greater number of tillers could be used for cyclic breeding to develop high-yielding varieties. The number of tillers/m2 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^2 (Simmonds *et al.*, 2002).

Grain yield, 1000 grains weight, AUDPC F, F-1 and canopy temperature

Analysis of variance showed highly significant differences among the tested genotypes for thousand grains weigh, grain yield, AUDPC F, F-1 and canopy temperature**.** The highest thousand-grain weight (TGW) was revealed in genotypes 45 (48 g) followed by 33 (46 g) with canopy temperature 16.1^0 C, 17.7^0 C respectively. The highest grain yield was found in genotypes 32 (5144 kg/ha) with canopy temperature 16.5^0 C and followed by genotype 47 (5107 kg/ha) with canopy temperature 16.4°C. The AUDPCF; F-1, also found lowest 279;610 and 132;441 respectively. Resistant check variety Chirya-3 (genotype 1) showed AUDPC F; F-1 268;576 with 17.8°C canopy temperature, and grain yield 4286 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 (32g). (Table 1). Grain yield per ha showed negative and highly significant ($p<0.001$) correlation with AUDPC flag leaf (F) (-0.71***), AUDPC penultimate leaf (F-1) (-0.53***) and canopy temperature (-0.60***). Similarly, positive and significant correlation of grain yield was recorded with thousand grain weight. The non-significant correlation of grain yield per ha recorded with days to heading and maturity, plant height and no. of grain per spike (Table 2). Canopy Temperature showed positive and highly significant ($p<0.001$) correlation with AUDPC flag leaf (F) (0.53***) and significant ($p<0.01$) correlation with AUDPC penultimate (F-1) (0.29*). The negative and highly significant correlation of canopy temperature was found grain yield per ha (-0.60***) and significant with total no. of tiller per square meter (-0.32*). Non-significant correlation of canopy temperature observed with days to heading, days to maturity, plant height, thousand grain weight and no. of grain per spike (Table 2). AUDPC on Flag leaf (F) showed highly significant ($p<0.001$) and strongly positive correlation with AUDPC on penultimate leaf (F-1) (0.62***) and canopy temperature (0.53***). Similarly, AUDPC flag leaf showed negative and highly significant correlation with no. of grain per spike (-0.43**), no. of tiller per square meter (-0.43**), and grain yield per ha (-0.71***). AUDPC on penultimate leaf (F-1) recorded strongly positive and significant correlation ($p<0.01$) with canopy temperature (0.29*). The negative and significant correlation of AUDPC penultimate leaf (F-1) was recorded for days to maturity (-0.33*) and highly significant correlation with no. of tiller per square meter (-0.36**) and grain yield per ha (-0.53***). Thousand grain weight was positively and significantly correlated with grain yield per ha (0.28*). It showed negative and significant correlation with days to heading (-0.34*) and maturity (-0.32*). The non-significant and negative correlation of thousand grain yield was reported with canopy temperature, AUDPC on flag leaf and penultimate leaf, no. of grain per spike and plant height. The phenological traits (days to head and maturity) were positively and highly significant correlated with each other (0.68***). The plant height showed non-significant correlation with all the studied traits. Similarly, no. of grain per spike was non-significant correlation with most of the traits except with AUDPC on flag leaf (F). Deviation of temperature of plant canopies in comparison to ambient temperature, also known as CTD, has been a good criterion for screening heat stress tolerance (Reynolds *et al.*, 1998). CTD was also found to be negatively correlated with disease severity (Eyal and Blum, 1989; Nutter and Aderman, 1987). CTD has caught breeders' attention because it is easy to calculate and can be used to screen large amounts of material in a single day (Brennan *et al.*, 2007).

Table 2. Pearson's Correlation coefficient for different agronomical traits, canopy temperature and disease at Directorate of agricultural research, Parwanipur, Bara in 2017-2018.

	DTM	PH (cm)	NGPS	NTPM	AUDPC F-1	AUDPC F	TGW (g)	GY (Kg/ha)	CT (^{0}C)
DTH	0.68***	0.14ns	0.09ns	0.01ns	-0.05ns	0.01ns	-0.34*	-0.02ns	-0.04ns
DTM	1	0.02ns	0.11ns	0.07ns	-0.33*	-0.22ns	-0.32*	0.07ns	0.03ns
PH		1	-0.07ns	0.01ns	0.13ns	-0.02ns	-0.03ns	-0.12ns	0.05ns
NGPS			1	-0.03ns	-0.12ns	-0.43**	-0.1ns	0.21ns	-0.15ns
NTPM				1	-0.36**	-0.43**	0.16ns	0.63**	-0.32*
AUDPC F-1					1	0.62***	-0.20ns	-0.53***	0.29*
AUDPC F						1	-0.16ns	-0.71***	0.53***
TGW							1	0.28*	-0.23ns
GY								1	-0.60***

DTM= Days to maturity, PH= Plant height (cm), NGPS=Number of grains per spike, NTPM= Number of tillers per square meter, AUDPC= Area under disease progress curve, F= Flag leaf, F-1= Penultimate leaf, TGW=Thousand grain weight, GY= Grain yield and CT= Canopy temperature; Ns p>=0.05, *p<0.05, **p<0.01, and ***p<0.001

Conclusion

Among the tested genotypes, genotypes 33, 47, 23, 4, 29, 20, 25 were found high yielding, low AUDPC F; F-1 and low canopy temperature. It can be also concluded that the genotypes revealed high yielding and low AUDPC and the lower canopy temperature. The correlation between grain yield, disease development is negative. Similarly, the less canopy temperature genotypes showed the less disease severity. 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 with lower canopy temperature against spot, could be utilized as such terminal heat tolerance due to climate change 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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Annexure 1. Genotypic details of wheat lines used for experiment in 2017-2018 Directorate of agricultural research, Parwanipur, Bara, Nepal.

EN	Entry	Genotypes/Cross name	Selection history
1	8HLBSN1	Chirya 3 (Resistant Check)	CIGM87.116-3Y-2M-1PR-3M-2PR-4B-0PR-1Y-0M
2	8HLBSN2	SUP152/3/UP2338*2/KKTS*2//YANAC	CMSS10B00032S-099M-099NJ-099NJ-4WGY-0B
3	8HLBSN3	BL1724*2/3/T.DICOCCON PI272533/ AE.SQUARROSA(458)// CMH81A.1261/ VEE#10/4/2*UP2338*2/KKTS*2//YANAC	CMSS11Y01293T-099TOPM-099Y-099M-5WGY -0B
4	8HLBSN4	KACHU*2/5/WBLL1*2/TUKURU/3/T.DICOCCON PI94624/ AE.SQUARROSA (409)//BCN/4/WBLL1*2/TUKURU	CMSS10B00672T-099TOPY-099M-099Y-10M-0WGY
5	8HLBSN5	WBLL1*2/4/YACO/PBW65/3/KAUZ*2/TRAP//KAUZ/5/ KACHU#1/6/KINGBIRD #1//INQALAB 91*2/TUKURU	CMSS10B00105S-099M-0SY-8M-0WGY
6	8HLBSN6	REH/HARE//2*BCN/3/CROC_1/AE.SQUARROSA (213)//PGO/4/ HUITES/5/ T.DICOCCON PI94624/AE.SQUARROSA (409)// BCN/6/ REH/HARE//2*BCN/3/CROC_1/AE.SQUARROSA(213)// PGO/4/HUITES/7/MUTUS/8/2*UP2338*2/KKTS*2//YANAC	CMSS11Y01281T-099TOPM-099Y-099M-34WGY-0B
7	8HLBSN7	PBW343*2/KUKUNA/5/KAUZ//ALTAR 84/AOS/3/PASTOR/4/ TILHI/6/PBW343/7/TUKURU//BAV92/RAYON/6/NG8201/ KAUZ/4/SHA7//PRL/VEE#6/3/FASAN/5/MILAN/KAUZ/8/ ATTILA*2//CHIL/BUC*2/3/KUKUNA	CMSS10B00961T-099TOPY-099M-099NJ-099NJ -15WGY-0B
8	8HLBSN8	NAVJ07*2//RL6077/AOC-YR	CMSS10B00769T-099TOPY-099M-099NJ-099NJ -8WGY-0B
9	8HLBSN9	YAYE/4/WAXWING/3/PFAU/WEAVER//BRAMBLING/5/KACHU/ SAUAL	CMSS10Y01183T-099TOPM-099Y-099M-099NJ -099NJ-15WGY-0B
10	8HLBSN10	KACHU/SAUAL*2/3/TACUPETO F2001/BRAMBLING//KIRITATI	CMSS10B01029T-099TOPY-099M-099NJ-099NJ -8WGY-0B
11	8HLBSN11	YAYE/4/WAXWING/3/PFAU/WEAVER//BRAMBLING/5/KACHU/ SAUAL	CMSS10Y01183T-099TOPM-099Y-099M-099NJ -099NJ-18WGY-0B
12	8HLBSN12	CHEN/AE.SQ//WEAVER/3/SSERI1/4/TOBA97/PASTOR/5/MUU #1/6/KACHU #1//PI 610750/SASIA/3/KACHU	CMSS10B00473S-099M-0SY-20M-0WGY
13	8HLBSN13	TUKURU//BAV92/RAYON/6/NG8201/KAUZ/4/SHA7//PRL/ VEE#6/3/FASAN/5/MILAN/KAUZ/7/SERI.1B//KAUZ/HEVO/3/ AMAD*2/4/KIRITATI/8/ATTILA*2/PBW65*2//W485/HD29	CMSS10B00988T-099TOPY-099M-0SY-35M-0WGY
14	8HLBSN14	ATTILA*2/PBW65/5/CNO79//PF70354/MUS/3/PASTOR/4/ BAV92/6/TRCH/SRTU//KACHU/7/UP2338*2/KKTS*2//YANAC	CMSS10B01010T-099TOPY-099M-099NJ-099NJ-3WGY-0B
15	8HLBSN15	KAUZ//ALTAR84/AOS/3/MILAN/KAUZ /4/SAUAL/5/SERI.1B// KAUZ/HEVO/3/AMAD*2/4/KIRITATI	CMSS10Y00370S-099Y-099M-099NJ-099NJ-26WGY-0B
16	8HLBSN16	CIRO16/CIRNO C 2008//BOKOTA	CMSS10B01218T-099TOPY-099M-099NJ-099NJ-11WGY-0B
17	8HLBSN17	SERI.1B//KAUZ/HEVO/3/AMAD*2/4/KIRITATI*2/6/BAV92// IRENA/KAUZ/3/HUITES/4/T.SPELTA PI348764/5/BAV92// IRENA/KAUZ/3/ HUITES	CMSS10B00734T-099TOPY-099M-099Y-2M-0WGY
18	8HLBSN18	CIRO16/CIRNO C 2008//BOKOTA	CMSS10B01218T-099TOPY-099M-0SY-32M-0WGY
19	8HLBSN19	CAL/NH//H567.71/3/SERI/4/CAL/NH//H567.71/5/2*KAUZ/6/ WH576/7/WH542/8/ WAXWING/9/ATTILA*2/PBW65//PIHA/3/ ATTILA/2*PASTOR/10/UP2338*2/KKTS*2//YANAC	CMSS10B00824T-099TOPY-099M-099NJ-099NJ-19WGY-0B
20	8HLBSN20	PFAU/WEAVER*2//BRAMBLING/3/DIAMONDBIRD/4/ IWA8600211// 2*PBW343*2/KUKUNA/5/PBW343*2/ KUKUNA*2//FRTL/PIFED	CMSS10B00719T-099TOPY-099M-099Y-5M-0WGY

Annexure 1. *Contd…..*

21	8HLBSN21	ROLF07*2/KIRITATI*2/10/PFAU/WEAVER*2//BRAMBLING/9/ RABE/6/WRM/4/FN/3*TH//K58/2*N/3/AUS-6869 /5/PELOTAS-ARTHUR/7/2*RABE/8/IRENA	Selection History doesn't exist
22	8HLBSN22	WAXWING/KRONSTAD F2004//WHEAR/SOKOLL/3/WAXBI	CMSS10B00806T-099TOPY-099M-099NJ-099NJ-3RGY-0B
23	8HLBSN23	KACHU/3/WHEAR//2*PRL/2*PASTOR/4/BOKOTA	CMSS10B00764T-099TOPY-099M-099NJ-099NJ-12WGY-0B
24	8HLBSN24	BL1724*2/3/T.DICOCCON PI272533/ AE.SQUARROSA(458)// CMH81A.1261 /VEE#10/4/2*UP2338*2/KKTS*2//YANAC	CMSS11Y01293T-099TOPM-099Y-099M-12WGY-0B
25	8HLBSN25	PSN/BOW//SERI/3/MILAN/4/ATTILA/5/KAUZ*2/CHEN//BCN/3/ MILAN/6/WBLL1*2/SHAMA/7/IWA8600211// 2*PBW343*2/ KUKUNA/8/PBW343*2 /KUKUNA*2//FRTL/PIFED	CMSS10B00745T-099TOPY-099M-099Y-14M-0WGY
26	8HLBSN26	SHA7//PRL/VEE#6/3/FASAN/4/HAAS8446/2*FASAN/5/CBRD/ KAUZ/6/MILAN/AMSEL/7/FRET2*2/ KUKUNA/8/2*WHEAR//2*PRL/2*PASTOR	CMSS10B00894T-099TOPY-099M-099NJ-099NJ-9WGY-0B
27	8HLBSN27	WAXWING*2/KRONSTADF2004/3/ PBW343*2/KUKUNA*2//FRTL/ PIFED	CMSS10B00049S-099M-099NJ-099NJ-5WGY-0B
28	8HLBSN28	WAXWING/KRONSTADF2004// WHEAR/SOKOLL/3/WAXBI	CMSS10B00806T-099TOPY-099M-099NJ-099NJ-5RGY-0B
29	8HLBSN29	FRET2/KUKUNA//FRET2/3/TNMU/4/FRET2*2/SHAMA/5/ATTILA*2/ PBW65//TNMU/6/FRET2*2/SHAMA//TNMU/3/FRET2*2/SHAMA	CMSS10B01092T-099TOPY-099M-099NJ-099NJ-26WGY-0B
30	8HLBSN30	PBW343*2/KUKUNA//PIHA/3/PBW343/7/TUKURU//BAV92/ RAYON/6/NG8201/KAUZ/4/SHA7//PRL/VEE#6/3/FASAN/5/MILAN/ KAUZ	CMSS10Y00503S-099Y-099M-099NJ-099NJ-1WGY-0B
31	8HLBSN31	WBLL1*2/SHAMA//KACHU/3/CIRNO C 2008/4/TACUPETO F2001/ BRAMBLING*2// KACHU	CMSS10B01212T-099TOPY-099M-0SY-35M-0RGY
32	8HLBSN32	PRL/2*PASTOR/4/CHOIX/STAR/3/HE1/3*CNO79//2*SERI/8/ NG8201/KAUZ/4/SHA7//PRL/VEE#6/3/FASAN/5/MILAN/KAUZ/6/ ACHYUTA/7/PBW343*2/KUKUNA/9/SUP152	CMSS10Y01233T-099TOPM-099Y-099M-099NJ-099NJ-10WGY-0B
33	8HLBSN33	T.SPELTA PI348599//2*PBW343*2/ KUKUNA/3/WBLL1*2/ KURUKU//HEILO	CMSS11Y00566S-099Y-099M-1RGY-0B
34	8HLBSN34	MUNAL #1/3/KINGBIRD #1//INQALAB 91*2/TUKURU	CMSS10B00004S-099M-099NJ-099NJ-8WGY-0B
35	8HLBSN35	BAV92//IRENA/KAUZ/3/HUITES/4/DOLL/7/PBW343*2/ KUKUNA/5/KAUZ//ALTAR 84/AOS/3/PASTOR/4/TILHI/6/ PBW343/8/BAV92//IRENA/KAUZ/3/HUITES/4/DOLL	CMSS10B00992T-099TOPY-099M-099NJ-099NJ-19WGY-0B
36	8HLBSN36	BAV92//IRENA/KAUZ/3/HUITES/4/DOLL*2/5/FRET2*2/KUKUNA// PIHA/3/FRET2/KURUKU//FRET2	CMSS10B00994T-099TOPY-099M-0SY-7M-0WGY
37	8HLBSN37	FRET2*2/SHAMA*2/4/BOW/URES//2*WEAVER/3/CROC_1/ AE.SQUARROSA (213)//PGO/5/WAXWING/PARUS//WAXWING/ KIRITATI/6/FRET2/KUKUNA//FRET2/3/PARUS/4/FRET2*2/SHAMA	CMSS10B01087T-099TOPY-099M-099NJ-099NJ-13WGY-0B
38	8HLBSN38	KACHU/3/WHEAR//2*PRL/2*PASTOR/4/BOKOTA	CMSS10B00764T-099TOPY-099M-099NJ-099NJ-2WGY-0B
39	8HLBSN39	KAUZ//ALTAR 84/AOS/3/MILAN/KAUZ/4/SAUAL/5/SERI.1B// KAUZ/HEVO/3/AMAD*2/4/KIRITATI/6/KACHU/SAUAL	CMSS10B01027T-099TOPY-099M-099NJ-099NJ-9WGY-0B
40	8HLBSN40	BOKOTA/3/UP2338*2/KKTS*2//YANAC	CMSS10B00295S-099M-099NJ-099NJ-20WGY-0B
41	8HLBSN41	REH/HARE//2*BCN/3/CROC_1/AE.SQUARROSA(213)//PGO/4/ HUITES/5/ T.DICOCCON PI94624/AE.SQUARROSA (409)//BCN/6/ REH/HARE//2*BCN/3/CROC_1/AE.SQUARROSA(213)//PGO/4/ HUITES/7/MUTUS/8/BAV92//IRENA/KAUZ/3/HUITES*2/4/MURGA	CMSS11Y00575S-099Y-099M-2WGY-0B
42	8HLBSN42	SAUAL/MUTUS/3/TACUPETO F2001/BRAMBLING//KIRITATI	CMSS10Y00383S-099Y-099M-099NJ-099NJ-35WGY-0B
43	8HLBSN43	BAV92//IRENA/KAUZ/3/HUITES/4/DOLL/7/PBW343*2/ KUKUNA/5/KAUZ//ALTAR84/AOS/3/PASTOR/4/TILHI/6/ PBW343/8/BAV92//IRENA/KAUZ/3/HUITES/4/DOLL	CMSS10B00992T-099TOPY-099M-099NJ-099NJ-10WGY-0B
44	8HLBSN44	WBLL1*2/4/YACO/PBW65/3/KAUZ*2/TRAP//KAUZ/5/KACHU #1/6/MARCHOUCH*4/SAADA/3/2*FRET2/KUKUNA//FRET2/7/ WBLL1*2/4/YACO/PBW65/3/KAUZ*2/TRAP//KAUZ/5/KACHU #1	CMSS10B00974T-099TOPY-099M-099NJ-099NJ-7WGY-0B
45	8HLBSN45	INQALAB91*2/KUKUNA//PFAU/ WEAVER/3/INQALAB91*2/ KUKUNA /4/TRCH/SRTU//KACHU	CMSS10Y00164S-099Y-099M-099NJ-099NJ-26WGY-0B
46	8HLBSN46	CNDO/R143//ENTE/MEXI_2/3/AE. SQUARROSA(TAUS)/4/ WEAVER/5/ PICUS/6/TROST/7/TACUPETO F2001*2 /8/WBLL1*2/4/ YACO/PBW65/3/KAUZ*2/TRAP//KAUZ/5/KACHU #1	CMSS10Y01126T-099TOPM-099Y-099M-099NJ-099NJ-5WGY-0B
47	8HLBSN47	FRET2*2/KUKUNA//PVN/3/FRET2*2/SHAMA/4/CIRO16	CMSS10Y00446S-099Y-099M-099NJ-099NJ-28WGY-0B
48	8HLBSN48	MUNAL #1/PREMIO	CMSA10B00008S-050ZTM-099Y-4M-0RGY
49	8HLBSN49	CIANO T 79	CM31678-R-4Y-2M-21Y-0M-0MEX
50	8HLBSN50	SONALIKA	II18427-4R-1M
51	8HLBSN51	RR 21 (Susceptible Check)	
52	8HLBSN52	Nepal 297 (Susceptible Check)	

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