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Partial Resistance Components and Morphological Traits Aid Selection of Resistant Wheat Genotypes against Spot Blotch

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Abstract

Spot blotch caused by *Bipolaris sorokiniana* results substantial yield losses (15-80%) in an Indian subcontinent. Wheat varietal improvement through breeding followed by evaluation of elite germplasms against a particular disease is crucial method to manage diseases. Fifty diverse wheat genotypes and two susceptible checks *viz.*, Sonalika and Nepal 297 were evaluated under artificial epiphytotic condition against spot blotch at NWRP, Bhairahawa, Nepal in 2017-18 and 2018-19. Evaluation was based on partial resistance components *viz.*, lesion sizes, lesion types (chlorotic/ necrotic), lesion characteristics (sporulating/ non-sporulating) and area under disease progress curve (AUDPC) and morphological traits *viz.* lesion mimic, leaf angle, leaf tip necrosis and plant height. Statistical analysis revealed that genotypes with smaller lesion size (<1 cm), small dark brown to black lesions with or without chlorosis/ necrosis and non sporulating lesions had lower AUDPC (<225). Similarly genotypes with erect to semi erect leaf (leaf angle 1-2), medium to high leaf tip necrosis (2-4), low percentage of lesion mimic (0-22.5%) were found resistant (AUDPC<225) to moderately resistant (AUDPC value 226-315). Moreover AUDPC showed strong and positive correlation with lesion sizes (0.76), lesion types (0.84) and lesion characteristics (0.54). Twenty genotypes were found resistant (AUDPC<225), could be used as new resistance sources in breeding program. However genotypes *viz.*, KACHU/BECARD//WBLL1*2/BRAMBLING/3/ATTILA*2/PBW65//MURGA, FRET2*2/SHAMA//TNMU/3/FRET2*2/SHAMA/4/UP2338*2/KKTS*2//YANAC/5/FRET2*2/SHAMA//PARUS/3/FRET2*2/KUKUNA, KACHU#1//PI610750/SASIA/3/KACHU/4/MUU#1//PBW343*2/KUKUNA/3/MUU/5/KACHU#1//PI610750/SASIA/3/KACHU, BORL14//KFA/2*KACHU and KFA/2*KACHU//QUELEA were found excellent based on partial resistance components and morphological traits. These genotypes could be further evaluated for yield potential in multi environment and better performing genotypes could be released as resistant varieties for spot blotch.

Keywords: Evaluation, Genotypes, Morphological traits, Partial resistance components, Spot blotch

Introduction

Wheat ranked as the world's second most produced cereal, yielding approximately 765 million metric tonnes after maize in 2019-20 (Shahbandeh, 2020). Wheat is a staple food for 35% of the global population, providing a significant portion of human dietary needs by contributing 20% of the world's calories and protein (Basnet *et al.*, 2023; Poudel and Bhatta, 2017). In the context of Nepal, wheat holds the third most crucial cereal crop cultivated on 0.7 million ha and produced 2 Mt in 2018-19 (MoALD, 2020). The climatic conditions of Eastern Gangetic Plains impose wheat to endure abiotic stress *i.e.*, terminal heat and biotic stress *i.e.*, spot blotch.

The wheat disease known as spot blotch is triggered by the pathogen *Bipolaris sorokiniana* (Sacc.) Shoemaker [formerly

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Cochliobolus sativus (S. Ito & Kurib.)] Drechsler ex Dastur, affect 25 Mha wheat globally (van Ginkel and Rajaram, 1998); whereas, 10 Mha wheat resides in South Asia (Gupta *et al.*, 2018), out of which 0.45 Mha resides in Nepal (NWRP, 2017). Spot blotch of wheat results an average 15.5% to 25% yield losses (Dubin and van Ginkel, 1991), which could reach up to 80% under severe epidemic condition (Duveiller and Gilchrist, 1994). Furthermore, it diminishes grain quality by inducing factors such as shriveling, black point formation and discoloration (Chand and Joshi, 2004). In the warmer region of Nepal, spot blotch typically leads to an average reduction in wheat yield ranging from 23% to 40%, as reported by Sharma and Duveiller (2006), which is a matter of significant concern for developing countries like Nepal, particularly given the prevalence of small landholding farmers (Parlevliet, 1979).

The primary sources of inoculum for diseases such as seedling blight and common root rot are found in or on infected seeds, as observed by Ries and Forcelini (1993), as well as in conidia that can persist on crop residues, as reported by Pandey *et al.* (2005). Additionally, inoculum reservoirs in the soil also contribute to the prevalence of these diseases (Chand *et al.*, 2002; Parlevliet, 1979). Secondary sources of inoculum that contribute to the occurrence of spot blotch and head blight in wheat include alternate hosts and airborne conidia (Duveiller *et al.*, 2005). These factors have been identified as important secondary sources in the development of these diseases. Spot blotch severity is exacerbated by favorable environmental conditions, including terminal heat stress, intermittent rainfall, temperatures exceeding 26 °C, and prolonged dew deposition on leaves during the grain-filling stages, that worsen the intensity of spot blotch (Acharya *et al.*, 2011; Joshi *et al.*, 2007a; Parlevliet, 1979). To effectively manage spot blotch, a range of strategies have been implemented, including crop rotation, timely sowing, and the application of both chemical and organic fungicides (Duveiller and Sharma, 2009; Gupt *et al.*, 2020; Navathe *et al.*, 2020; Parlevliet, 1979).

Breeding for disease resistance is the most cost-effective method for management of spot blotch (Gupt *et al.*, 2021b; Gupta *et al.*, 2018). High-yielding with desirable resistant wheat genotypes have been identified through multi location screening (Joshi *et al.*, 2007b). Resistant wheat genotypes posses three to four resistant alleles (Joshi *et al.*, 2004a), and some of these harbors Leaf tip necrosis (*Ltn*) gene, which serves as a valuable phenotypic markers for spot blotch resistance (Joshi *et al.*, 2004b). Four major QTLs such as *sb1*, *sb2, sb3* and *sb4* conferring spot blotch resistance have been identified in previous studies (Gupt *et al.*, 2021b; Kumar *et al.*, 2015; Lillemo *et al.*, 2013; Lu *et al.*, 2016; Zhang *et al.*, 2020). However, it is essential that continuous efforts should be made towards identifying wheat cultivars characterized by a high degree of disease resistance to cope with the anticipated favorable conditions for spot blotch in future (Gupta *et al.*, 2018).

Development and evaluation of wheat germplasms for resistance against a particular disease is one of the crucial step but a challenging task for researchers. The researcher's aim to select resistant wheat genotypes based on morphological, physiological, biochemical traits and QTLs conferring resistance as well as with phenomenal yield and quality traits. Area under disease progress curve (AUDPC), lesion numbers, lesion sizes, chlorotic/ necrotic lesions and sporulating/ non-sporulating lesions are regarded as partial resistance components for foliar diseases (Bashyal *et al.*, 2011; Clark *et al.*, 2014; Roumen, 1993). Furthermore, morphological traits such as plant height (Joshi *et al.*, 2002), lesion mimic and leaf tip necrosis (Joshi *et al.*, 2004b; Singh *et al.*, 2020) as well as leaf angle (Joshi and Chand, 2002) are found to be associated with spot blotch of wheat.

Thus, the aim of this study was to pinpoint wheat genotypes exhibiting resistance through the evaluation of partial resistance components and the assessment of morphological traits linked to spot blotch.

Materials and Methods

Wheat Genotypes

The experimental materials consisted of fifty (50) diverse wheat genotypes along with two (2) susceptible reference varieties, namely Sonalika and Nepal 297, specifically chosen for studying spot blotch (Table 1). The genotypes were obtained from the 9th Helminthosporium Leaf Blight Screening Nursery (HLBSN) of International Maize and Wheat Improvement Center (CIMMYT), Mexico. These genotypes displayed diversity in terms of their genetic composition, geographical distribution, disease resistance response, as well as morphological and yield traits (Basnet *et al.*, 2023).

Experimental Design, Planting and Cultural Practices

The experiment took place at the Plant Pathology facility within the National Wheat Research Program (NWRP) in Bhairahawa, Nepal, situated at coordinates 27°32′ N and 83°28′ E, with an elevation of 105 meters above sea level (masl). The experiment was organized using an augmented design. Fifty-two (52) wheat genotypes were planted and evaluated against spot blotch for two successive wheat seasons, *i.e.*, 2017-18 and 2018-19. Each genotype was planted in two rows of a 2 m length and 25 cm inter row space on 2nd week of December during both the crop seasons. Field was dressed by 120:60:40 kg ha^{-1} $N:P_2O_5:K_2O$; where 50% of the total nitrogen requirement was administered as a basal dose, while the full doses of phosphorus and potash were also applied at the base; and the remaining 50% of the nitrogen was divided into two split doses, with one applied during the active tillering stage (GS 32-39) and the other during the booting stage (GS 45) (Basnet *et al.*, 2023; Zadoks *et al.*, 1974). On the day following sowing, a pre-emergence weedicide, Pendimethalin 30% EC, was applied @ 2 ml L^{-1} (Basnet *et al.*, 2023), to prevent weed germination, followed

Table 1: Mean value of partial resistance components and morphological traits of 9th HLBSN genotypes across two years (2017-18 and 2018-19)

Sl. No.	Entry	Pedigree	LS [cm]	LT	LC	LM [%]	LA	LTN	PE [cm]	PH [cm]	SL [cm]	TKW [g]	AUDPC	HR
1	9th HLBSN 22	KACHU/BECARD//WBLL1*2/ BRAMBLING /3/ATTILA*2/ PBW65//MURGA	1	1	1	0	2	1	9.1	90.8	11.2	31.1	137	R
2	9th HLBSN 23	KACHU/BECARD//WBLL1*2/ BRAMBLING /3/ATTILA*2/ PBW65//MURGA	1	1	1	0	2	1	11.7	86.8	10.9	30.8	162.9	R
3	9th HLBSN 45	CHONTE*2/SOLALA/5/ BAV92//IRENA/KAUZ/3/ HUITES*2/4/CROC_1/ AE.SQUARROSA(224)// KULIN/3/WESTONIA/6/ KACHU//WBLL1*2/ BRAMBLING	1.5	2	1	2.5	2	2	13.8	86.5	11.3	31	162.9	R
4	9th HLBSN 28	WBLL1*2/KUKUNA/5/PSN/ BOW//SERI/3/MILAN/4/ ATTILA/6/WBLL1*2/KKTS/7/ ROLF07/MUU/8/STLN/ MUNAL#1	1	2	1	10	2	1	18.3	79.8	12	31.7	175.9	R
5	9th HLBSN 29	ATTILA*2/PBW65*2// MURGA/4/MUU#1// PBW343*2/KUKUNA/3/ MUU/5/ATTILA*2/PBW65// MURGA	1	2	1	22.5	1	1	10.8	73.7	12.9	27.3	175.9	R
6	9th HLBSN 40	SAUAL/MUTUS/4/KACHU#1 //WBLL1*2/KUKUNA/3/ BRBT1*2/KIRITATI	2	2	1	7.5	2	1	9.3	86	11	30.1	175.9	R
7	9th HLBSN 5	FRET2*2/SHAMA//TNMU/3/ FRET2*2/SHAMA/4/UP2338 *2/KKTS*2//YANAC/5/ FRET2*2/SHAMA//PARUS/3/ FRET2*2/KUKUNA	1.5	2	1	0	3	4	7.5	81.2	11	29.3	188.9	R
8	9th HLBSN 7	KACHU#1//PI610750/ SASIA/3/KACHU/4/MUU#1// PBW343*2/KUKUNA/3/ MUU/5/KACHU#1// PI610750/SASIA/3/KACHU	1	2	1	0	2	1	15.5	92.3	10.6	37.6	192.6	R
9	9th HLBSN 14	BOKOTA/3/ATTILA*2/ PBW65//MURGA	1	2.5	1	5	2	1	12.8	94.2	11.1	28.8	201.8	R
10	9th HLBSN 16	BORL14//KFA/2*KACHU	1	2.5	1	5	3	1	11.9	89.3	9.9	31.5	201.8	R
11	9th HLBSN 25	CHIRYA.3 (Resistant Check)	2.5	2	1	5	1	4	6.9	89.8	11.5	22.6	201.8	R
12	9th HLBSN 31	UP2338*2/VIVITSI/3/ FRET2/TUKURU// FRET2/4/MISR1*2/5/ KIRITATI/4/2*BAV92// IRENA/KAUZ/3/HUITES	2.5	2.5	2	7.5	1	1	11.7	81.8	10.8	28.9	201.8	R

Table 1: Continue...

Sl. No.	Entry	Pedigree	LS [cm]	LT	LC	LM [%]	LA	LTN	PE [cm]	PH [cm]	SL [cm]	TKW [g]	AUDPC	HR
13	9th HLBSN 49	REH/HARE//2*BCN/3/CROC_1/AE.SQUARROSA (213)//PGO/4/HUITES/5/T. DICOCCONPI94624/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	2	2.5	1	7.5	1	1	10.7	83.2	10.4	31.8	201.8	R
14	9th HLBSN 43	WBLL1*2/KKTS//KINGBIRD#1/3/KACHU#1/KIRITATI//KACHU/4/WBLL1*2/KKTS//KINGBIRD#1	1.5	3	1	7.5	3	4	16.9	83.7	12	30	214.8	R
15	9th HLBSN 3	KFA/2*KACHU//QUELEA	2	2.5	1	2.5	2	2	21.7	82.3	8.6	35.7	218.5	R
16	9th HLBSN 6	UP2338*2/SHAMA/3/MILAN/KAUZ//CHIL/CHUM18/4/UP2338*2/SHAMA*2/5/PBW343*2/KUKUNA*2//FRTL/PIFED	1.5	2.5	1	7.5	2	1	15.2	86	11.1	32.4	218.5	R
17	9th HLBSN 39	BAV92//IRENA/KAUZ/3/HUITES *2/6/TURACO/5/CHIR3/4/SIREN//ALTAR84/AE.SQUARROSA(205)/3/3*BUC/7/PBW343*2 /KUKUNA*2//FRTL /PIFED/8/BAV92//IRENA/KAUZ/3 /HUITES*2/4 /CROC_1/AE.SQUARROSA(224)//KULIN /3/WESTONIA	2	2.5	1	7.5	1	1	11.7	86	11.3	30.5	218.5	R
18	9th HLBSN 1	ATTILA*2/PBW65//KACHU/3/UP2338*2/KKTS*2//YANAC	2	2.5	1	7.5	1	1	9.4	72.7	10.7	31.9	222.2	R
19	9th HLBSN 27	MURGA/KRONSTADF2004/3/PBW343*2/KUKUNA*2//FRTL/PIFED/4/MURGA/KRONSTADF2004	1.5	2.5	1	5	2	1	9.3	85.7	10.8	30.9	222.2	R
20	9th HLBSN 32	MUNAL#1/7/CNO79//PF70354/MUS/3/PASTOR/4/BAV92/5/FRET2/KUKUNA//FRET2/6/MILAN/KAUZ//PRINIA/3/BAV92	2	2.5	1	5	1	1	13.7	86	10.4	30	222.2	R
21	9th HLBSN 12	MUNAL#1/3/ATTILA*2/PBW65//MURGA	1	3	1	5	1	1	11.8	82.8	9.5	31.5	227.8	MR
22	9th HLBSN 50	W15.92/4/PASTOR//HXL7573/2*BAU/3/WBLL1/6/POTCH93/4/MILAN/KAUZ//PRINIA/3/BAV92/5/MILAN/KAUZ//PRINIA/3/BAV92	1.5	3	1	7.5	1	3	18.9	85.7	10.4	37.1	227.8	MR

Table 1: Continue...

Sl. No.	Entry	Pedigree	LS [cm]	LT	LC	LM [%]	LA	LTN	PE [cm]	PH [cm]	SL [cm]	TKW [g]	AUDPC	HR
23	9th HLBSN 37	TACUPETOF2001/6/CNDO/ R143//ENTE/MEXI_2 /3/ AEGILOPSSQUARROSA (TAUS) /4/ WEAVER/5 / PASTOR /7/ ROLF07/ 8/MUU#1 /SAUAL// MUU/9/ TACUPETOF2001 / SAUAL//BLOUK#1	1	3	1	2.5	3	1	13	80.7	11.1	30.7	231.5	MR
24	9th HLBSN 41	KASUKO	2	3	1	7.5	1	1	11.9	84	11.6	33.4	231.5	MR
25	9th HLBSN 35	MURGA/KRONSTADF2004/3/ PBW343*2/KUKUNA*2// FRTL/PIFED	2	3	2	5	3	2	10.2	80	8.8	28	235.1	MR
26	9th HLBSN 36	WBLL1*2/4/YACO/PBW65/3/ KAUZ*2/TRAP//KAUZ* 2/5/ CHUANMAI32 /6/PBW343*2/ KUKUNA*2//FRTL/PIFED	2	2.5	1	2.5	2	1	11	85.7	10.4	33.3	235.1	MR
27	9th HLBSN 38	BAV92//IRENA/KAUZ/3/ HUITES*2 /6/TURACO/5/ CHIR3 /4/SIREN//ALTAR84/ AE.SQUARROSA(205) /3/3*BUC/7 /KINGBIRD#1// INQALAB91*2/TUKURU /8/ BAV92//IRENA /KAUZ/3/ HUITES*2/4/GONDO/TNMU	1.5	3	1	5	2	2	13.9	84.7	10.8	33.1	235.1	MR
28	9th HLBSN 15	ATTILA*2/PBW65// MURGA/3/BORL14	2.5	3	2	7.5	3	1	15.3	92.8	12.5	35.9	240.7	MR
29	9th HLBSN 30	BECARD/AKURI*2//WAXBI	2	3	2	7.5	2	1	12.3	79.2	12.2	28.1	240.7	MR
30	9th HLBSN 24	SERI.1B*2/3/KAUZ*2/BOW// KAUZ*2/4/KINGBIRD#1/6/ KSW/5/2*ALTAR84/ AE.SQUARROSA(221) //3*BORL95/3/URES/JUN// KAUZ/4/WBLL1	2	2.5	1	12.5	2	1	11.4	79.8	9.2	33.8	244.4	MR
31	9th HLBSN 9	PBW343*2/KUKUNA*2// FRTL/PIFED*2/5/UP2338*2/ SHAMA/3/MILAN/KAUZ// CHIL/CHUM18/4/UP2338*2/ SHAMA	1.5	3	1	7.5	1	1	14.6	87.3	10.8	32.6	248.1	MR
32	9th HLBSN 10	UP2338*2/KKTS*2//YANAC*2 /3/WAXBI	2	3	1	5	1	3	17.4	87	11.3	29.9	253.7	MR
33	9th HLBSN 11	WBLL1*2/4/BABAX/LR42// BABAX/3/BABAX/LR42// BABAX/8/TACUPETOF2001/6/ CNDO /R143//ENTE/MEXI_2/ 3/AEGILOPSSQUARROSA (TAUS)/4/WEAVER/5 /PASTOR /7/ROLF07 /9/WBLL1*2/4/ BABAX/LR42//BABAX/3 / BABAX/LR42//BABAX	1.5	3	1	0	4	3	17.5	84	10.3	28.8	257.4	MR

Table 1: Continue...

Sl. No.	Entry	Pedigree	LS [cm]	LT	LC	LM [%]	LA	LTN	PE [cm]	PH [cm]	SL [cm]	TKW [g]	AUDPC	HR
34	9th HLBSN 21	BECARD//ND643/2 *WBLL1/4/ND643/2* WBLL1//ATTILA*2/ PBW65/3/MUNAL	1.5	3	1	2.5	1	1	15.1	83.7	9.7	27	261.1	MR
35	9th HLBSN 33	WHEAR/SOKOLL/8/BOW/ VEE/5/ND/VG9144//KAL/ BB/3/YACO/4/CHIL/6/ CASKOR/3/CROC_1/ AE.SQUARROSA(224)// OPATA/7/PASTOR//MILAN/ KAUZ/3/BAV92	1.5	3	1	2.5	1	1	16.7	86.5	9.5	34.1	261.1	MR
36	9th HLBSN 47	COAH90.26.31/4/2* BL2064//SW89-5124* 2/ FASAN/3/TILHI/5/ UP2338*2/ KKTS*2//YANAC /6/ MUTUS /AKURI	1.5	3	1	7.5	2	1	10.9	86.3	11.7	31.8	261.1	MR
37	9th HLBSN 42	BOKOTA/5/UP2338*2/ VIVITSI/3/FRET2/TUKURU// FRET2/4/MISR1/6/BABAX/ LR42//BABAX*2/3/ KUKUNA/4/CROSBILL#1/5/ BECARD	3	3	2	10	1	1	12.7	87.3	11.7	38.7	270.4	MR
38	9th HLBSN 46	HGO94.7.1.12/2* QUAIU#1// QUAIU#2 /5/ KIRITATI /4/2*BAV92//IRENA/KAUZ/3 /HUITES/6/MUCUY	3	3	2	10	2	1	14.6	79.8	11	41.7	270.4	MR
39	9th HLBSN 26	ALD/CEP75630//CEP75234/ PT7219/3/BUC/BJY/4/CBRD /5/TNMU/PF85487/6/ PBW343*2/KUKUNA/7/ CNO79//PF70354/MUS/3/ PASTOR/4/BAV92/8/ ATTILA*2/PBW65*2// MURGA/9/ATTILA*2/ PBW65//MURGA	1.5	3.5	1	12.5	1	3	6.8	82.8	9.6	32.8	274.1	MR
40	9th HLBSN 34	SUP152/6/OASIS/5*BORL95 /5/CNDO/R143//ENTE/ MEXI75/3/AE.SQ/4/2*OCI	1.5	3	2	7.5	1	1	16.9	80.5	10.4	27	274.1	MR
41	9th HLBSN 44	KINDE*2/SOLALA//2* MUNAL#1	1.5	3.5	1	7.5	1	2	13.5	86.2	10.6	31.2	274.1	MR
42	9th HLBSN 19	SUP152*2/TECUE#1// MUCUY	2	3.5	2	22.5	2	1	13.5	83.8	9.8	29	279.6	MR
43	9th HLBSN 48	CROC_1/AE.SQUARROSA (210)//INQALAB91*2KUKUNA /3/PBW343*2/KUKUNA/5/ SAUAL/3/C80.1/3*BATAVIA //2*WBLL1 /4/SITE /MO // PASTOR /3/TILHI /6/SAUAL#1 /KACHU	2	3.5	1	7.5	2	2	14.4	92.5	11.7	40.1	283.3	MR

Table 1: Continue...

Sl. No.	Entry	Pedigree	LS [cm]	LT	LC	LM [%]	LA	LTN	PE [cm]	PH [cm]	SL [cm]	TKW [g]	AUDPC	HR
44	9th HLBSN 8	SAUAL/3/ SW89.3064// CMH82.17/SERI /4/ SAUAL/5/PBW343*2/ KUKUNA*2//FRTL/ PIFED/6/SAUAL/ KRONSTAD F2004	3	3.5	2	2.5	2	1	11	89.5	9.7	28.8	287	MR
45	9th HLBSN 2	PRL/2* PASTOR // PARUS/5/NAC/ TH.AC//3 *PVN /3/MIRLO/ BUC /4/2* PASTOR /6/ KINGBIRD#1// INQALAB91*2/ TUKURU	2	3.5	1	7.5	1	1	13.1	83.2	10.2	31	290.7	MR
46	9th HLBSN 20	ND643/2* TRCH // MUTUS/3/SUP152/4/ SUP152*2/TECUE#1	2.5	3.5	2	7.5	3	1	16.5	82.2	8.4	30.4	290.7	MR
47	9th HLBSN 4	FRET2*2/KUKUNA// PRINIA /PASTOR/8/ 2*TACUPE TOF2001 /6/CNDO/R143// ENTE/MEXI_2 /3/AEGILOPSS QUARROSA (TAUS) /4/WEAVER /5/ PASTOR /7/ROLF07	2	4	2	5	3	1	19.2	85	9.8	30.9	335.2	MS
48	9th HLBSN 13	BOKOTA/3/ATTILA*2/ PBW65// MURGA	2	4	2	5	2	1	14.3	85.5	11.4	29	335.2	MS
49	9th HLBSN 17	PFAU/WEAVER*2 /4/ BOW/NKT//CBRD /3/ CBRD/5/ATTILA*2/ PBW65*2//KACHU	3	4	2	7.5	3	1	14.8	86.2	11.6	24.3	412.9	S
50	9th HLBSN 18	MUCUY//MUTUS*2/ TECUE#1	3	4	2	5	2	2	12	86.3	10.8	29.8	425.9	S
51	Check 1	Nepal 297	3	5	2	36	3	1	21.1	82	12.5	38	561	S
52	Check 2	Sonalika	5	5	2	1	4	1	16.7	75	11.1	31.6	776	S

Table 1: Continue...

Particulars	LS [cm]	LT	LC	LM [%]	LA	LTN	PE [cm]	PH [cm]	SL [cm]	TKW [g]	AUDPC	HR
Grand Mean	1.97	2.97	-	7.2	-	-	13.7	84.38	10.7	31.55	274.6	
CV (%)	21.1	15.40	-	51.4	-	-	6.4	1.9	2.4	4.20	11.30	
LSD value	1.17	1.29	-	10.5	-	-	2.486	4.6	0.278	3.71	87.45	
P value	<0.001	<0.001	-	<0.001	-	-	<0.001	<0.001	<0.001	<0.001	<0.001	

[NB: LS = Lesion size, LT = Lesion Type, LC = Lesion Characteristic, LM = Lesion Mimic, LA = Leaf Angle, LTN = Leaf Tip Necrosis, PE = Peduncle Extrusion, PH = Plant Height, SL = Spike Length, TKW = Thousand Kernel Weight and AUDPC = Area Under Disease Progress Curve, HS = Host Response] by manual weeding. To control insect infestation, a systemic insecticide called Rogor (Dimethoate 30% EC) was applied twice at a concentration of 1.5 ml L^{-1} during the active tillering stage (GS 32-39) and the booting stage (GS 45) (Basnet *et al.*, 2023). The field received irrigation on three occasions; first at the CRI stage, then at the booting stage (GS 45), and finally at the milking stage (GS 73) (Basnet *et al.*, 2023; Zadoks *et al.*, 1974).

Isolation and Inoculation of Pathogen

Wheat leaves with conspicuous symptoms of spot blotch were collected from farmers' field located nearby National Wheat Research Program (NWRP), Bhairahawa, Nepal. Symptomatic leaves were sterilized by a 1% sodium hypochlorite solution (NaOCl), cut into small pieces having necrotic and healthy parts of leaf. Four to five leaf pieces were kept on moistened Whatman filter paper placed in 9 cm Petridish and incubated in BOD incubator at 25±1 °C for 1 week (Gupt *et al.*, 2021a) to induce conidiogenesis and conidia. Conidia of *B. sorokiniana* were transferred on a 2% PDA and incubated in BOD incubator at 25±1 °C for a week (Gupt *et al.*, 2021a) to promote mycelial growth. Sorghum seeds were processed, inoculated with 5 mm one week old mycelial mat and incubated at 25±1 °C for 4-6 weeks to multiply conidia of *B. sorokiniana*. Infected sorghum grains were washed with distilled water and drained through muslin cloth to collect conidial suspension @ 1×10^4 conidia L^{-1} water. The conidial suspension was sprayed on wheat genotypes at GS 45-51 (Zadoks *et al.*, 1974) during evening hours to take the advantage of dew deposition and high relative humidity during night that favors infection by the pathogen and disease progress.

Phenotyping

Partial Resistance Components

Five plants of similar growth stage per genotype were randomly tagged using red wool for assessments of traits studied. Partial resistance components such as lesion size (LS) was rated on flag leaves of tagged plants at late milking stage to early dough stage, *i.e.*, GS 77-81 (Zadoks *et al.*, 1974) in 1 to 5 scales; score 1 ≤ 0.5 cm, score 2 = 0.5-1 cm, score 3 = 1-1.5 cm, score 4 = 1.5-2 cm and score 5 ≥ 2 cm, as shown in figure 1. The five tagged leaves of each genotype

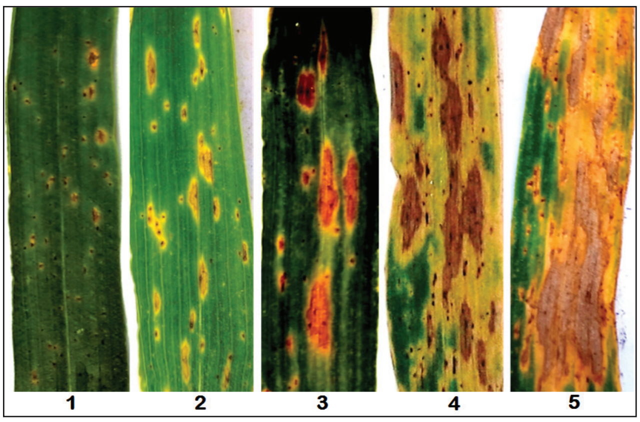

Figure 1: The size of mandibles of the Eri silkworm, *Samia cynthia ricini* Boisd.

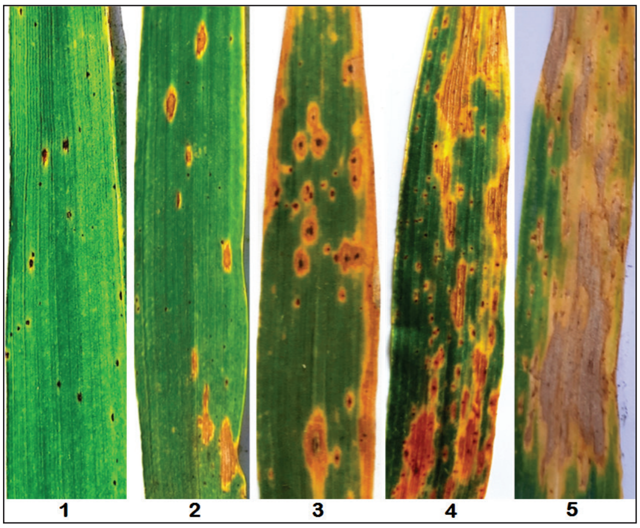

Figure 2: Pictorial representation of scale spanning 1-5 for lesion types on flag leaves

were photographed and lesions size was measured using ImageJ 1.x software (Schneider *et al.*, 2012). Moreover, lesion types (LT) were also assessed on tagged flag leaves at late milking stage to early dough stage, *i.e.*, GS 77-81 (Zadoks *et al.*, 1974), following 1 to 5 scales used by Lamari and Bernier (1989) (Figure 2). The lesion types on flag leaves was rated (Ayana *et al.*, 2018) as 1 = small dark brown to black spots without necrotic or chlorotic surroundings; 2 = small dark brown to black spots with little necrotic or chlorotic surroundings; 3 = small to medium dark brown to black spot (not coalescing) with distinct chlorotic or tan necrotic ring; 4 = medium to large dark brown to black spots with distinct chlorotic and necrotic surroundings, some of the spots coalescing; 5 = large coalescing spots with grey centre and brown to yellow margin, usually causes blight symptoms of leaf (Ayana *et al.*, 2018).

Lesion characteristics (LC) *i.e.*, sporulating/ non-sporulating, specifically for the presence or absence of conidia, were assessed by examining lesions on labeled flag leaves using a 20X magnifying hand lens (Parlevliet, 1979) during the late milking to early dough stage, *i.e.*, GS 77-81 (Parlevliet, 1979; Zadoks *et al.*, 1974) (Figure 3).

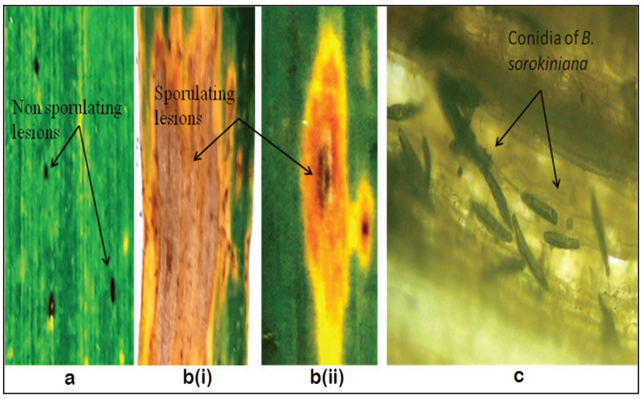

Figure 3: (a) Non-sporulating lesions on flag leaf; b(i-ii) sporulating lesions on flag leaf; (c) conidia of *Bipolaris sorokiniana* on sporulating lesion under 10X binocular microscope

Disease Assessments (AUDPC)

Spot blotch was scored thrice, at GS 55 (50% heading) 7-10 days after inoculation, at GS 69 (anthesis completed) and third at GS 77, *i.e.*, late milk stage (Zadoks *et al.*, 1974). The scoring was done by following double digit scale, *i.e.*, 00-99 basis (Saari and Prescott, 1975). The first digit (D_1) of a score represents upwards progress of disease on plants from ground whereas second digit (D_2) represents percentage of diseased area of leaves (Gupt *et al.*, 2020). Disease severity was calculated by using formula as,

$$\% \text{ Severity} = \left(\frac{D_1}{9}\right) \times \left(\frac{D_2}{9}\right) \times 100$$

The calculation of the AUDPC was performed using the formula given by Shaner and Finney (1977), which involves the percent severity of the corresponding disease ratings, as below.

$$\text{AUDPC} = \sum_{i=1}^{n-1} \left[\left\{ \frac{(Y_i + Y_{i+1})}{2} \right\} \times (t_{i+1} - t_i) \right]$$

Where,

Y_i = disease level at time t_i (first scoring);

$(t_{i+1} - t_i)$ = days between two consecutive disease scores;

n = number of readings.

Fifty (50) wheat genotypes along with two susceptible checks studied were categorized as resistant (R), moderately resistant (MR), moderately susceptible (MS), and susceptible (S) on the basis of the cut off value (lowest AUDPC + LSD value) as described by Sharma *et al.* (2018).

Morphological and Yield Related Traits

Wheat genotypes were scored for five (5) morphological traits *viz.*, lesion mimics (LM), leaf angle (LA), leaf tip necrosis (LTN), peduncle extrusion (PE) and plant height (PH), as well as two yield related traits *viz.*, spikes length (SL) and thousand kernel weight (TKW). Lesion mimic was scored on five tagged leaves per genotypes at late milk stage, *i.e.*, GS 77 (Zadoks *et al.*, 1974), following rating scale of Yao *et al.* (2009). Leaf angle was measured using protractor following protocol of Nigam and Srivastava (1976) at GS 51-55 (Zadoks *et al.*, 1974), as erect (flag leaf making an angle 60° to 90° with respect to horizontal plane), semi-erect (flag leaf making an angle 0° to 60°), semi-drooping (less than half portion of flag leaf drooping) and dropping (more than half portion of flag leaf drooping). Leaf tip necrosis was scored in 0-4 scale (Juliana *et al.*, 2015) at GS 65-69 (Zadoks *et al.*, 1974); where score 1 = slight LTN, score 2 = medium LTN, score 3 = high LTN, and score 4 = very high LTN (Figure 4).

Plant height and peduncle extrusion was measured in tagged plants of all genotypes at GS 87 (Zadoks *et al.*, 1974). The measurement of the height from base to the tip of the spike excluding awns of a plant was recorded as plant height whereas length from base of the auricle of a flag leaf to the base of lowest spikelet of a plant was taken as peduncle extrusion.

Additionally, yield related traits such as spike length were

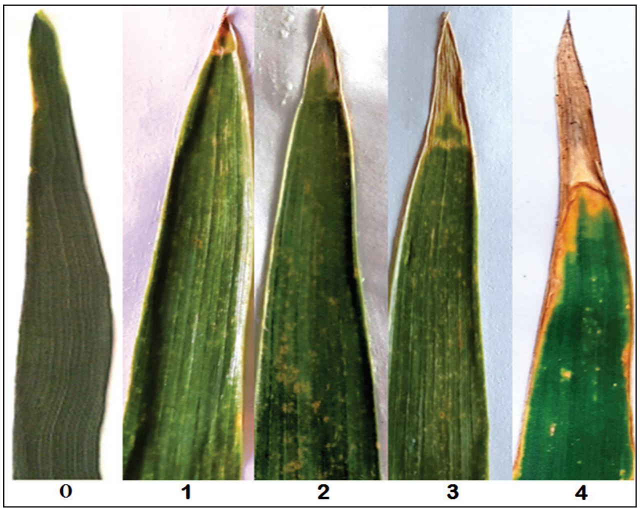

Figure 4: Pictorial representation of scale spanning 0-4 for Leaf Tip Necrosis (LTN)

evaluated in labeled plants at GS 87 (Ullah *et al.*, 2007; Zadoks *et al.*, 1974). To measure thousand kernel weight (TKW), a thousand unbroken seeds were randomly selected for each genotype and weighed in grams (g) (Parlevliet, 1979).

Statistical Analysis

Data entry and processing were conducted using Microsoft Office Excel 2007. For statistical analysis, tasks such as Analysis of Variance (ANOVA), mean estimation and correlation analysis were performed using R (2020) software, with the aid of the Agricolae package version 1.3-3 (de Mendiburu, 2020). A statistical significance threshold (alpha) was set at the 5% level of probability.

Results and Discussion

The statistical analysis indicated significant variations among genotypes across two years for partial resistance components *viz.*, lesion sizes (LS), lesion types (LT), lesion characteristics (LC), area under disease progress curve (AUDPC) and morphological traits *viz.*, lesion mimics (LM), leaf angle (LA), leaf tip necrosis (LTN), peduncle extrusion (PE), plant height (PH) along with yield related traits such as spike length (SL) and thousand kernel weight (TKW) (Table 1). On the basis of cut off value (lowest AUDPC + LSD), fifty-two genotypes were categorized as resistant with AUDPC value < 225; moderately resistant with AUDPC value 225-315; moderately susceptible with AUDPC value 316-400, and susceptible with AUDPC value > 400. Out of the screened genotypes, twenty were found as resistant; twenty-six as moderately resistant; two as moderately susceptible and four genotypes as susceptible (including two susceptible checks) (Table 1 and 2). Twenty resistant genotypes had mean range value for partial resistance components (Table 2). Among twenty resistant genotypes, KACHU/BECARD//WBLL1*2/BRAMBLING/3/ATTILA*2/PBW65//MURGA had lowest AUDPC value (137.0) with <0.5 cm (LS-1), (LT-1 *i.e.*, small and necrotic lesions), (LC-1 *i.e.*, non-sporulating lesions) and 31.1 g as TKW (Table 1).

Moreover, susceptible genotypes (including two susceptible checks) showed mean range value for partial resistance components *viz.*, LS (3-5), LT (4-5), LC (2), AUDPC (412.9-776); whereas, for morphological and yield related traits *viz.*, LM (1-36%), LA (2-3.8), LTN (1-2), PE (12-21.1 cm), PH (75-86.3 cm), SL (10.8-12.5 cm), TKW (24.3-38 g) (Table 2). Susceptible genotypes *viz.*, PFAU/WEAVER*2/4/BOW/NKT//CBRD/3/CBRD/5/ATTILA*2/PBW65*2//KACHU had value for partial resistance components *viz.*, LS (3), LT (4), LC (2), AUDPC value (412.9); whereas for morphological and yield related traits *viz.*, LM (7.5%), LA (3), LTN (1), PE (14.8 cm), PH (86.2 cm), SL (11.6 cm) and TKW (24.3 g) (Table 1). Similarly MUCUY//MUTUS*2/TECUE#1 had value for partial resistance components *viz.*, LS (3), LT (4), LC (2) and AUDPC (425.9); whereas, for morphological and yield related traits *viz.*, LM (5%), LA (2), LTN (2), PE (12 cm), PH (86.3 cm), SL (10.8 cm) and TKW (29.8 g) (Table 1).

Partial Resistant Components

The lowest score for lesion size (LS) 1 was found in seven resistant and two moderately resistant genotypes; whereas, susceptible genotypes had high scores 3 and 5 (Table 1). The low lesion type (LT) score 1 was found in two resistant genotypes; score 2 was found in seven resistant genotypes; whereas, high score 4 and 5 was found in moderately susceptible and susceptible genotypes (Table 1). Furthermore resistant genotypes had non-sporulating lesions *i.e*., LC score 1 whereas moderately susceptible and susceptible genotypes had sporulating lesions (LC-2) (Table 1).

The correlation analysis revealed strong and positive correlation of AUDPC with lesion sizes (0.76), lesion types (0.84) and lesion characteristics (0.54) (Figure 5). Similarly, LT showed highly significant and positive correlation with LC (0.58) (Figure 5).

Morphological and Yield Related Traits

Among fifty two genotypes, nineteen genotypes had erect type flag leaf (score 1), twenty-one genotypes had semi erect

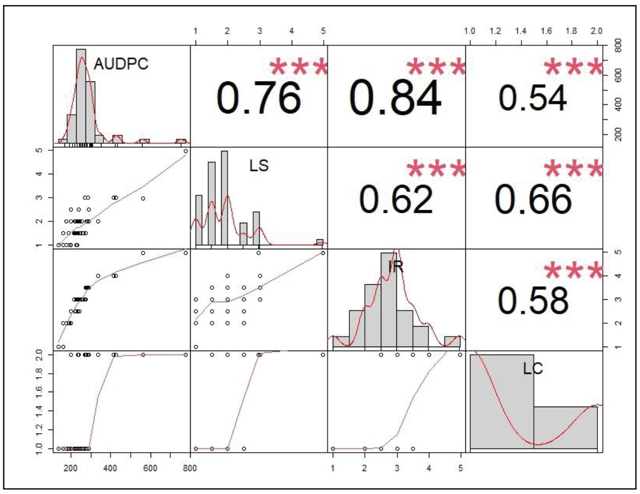

Figure 5: Distribution and correlation matrix of partial resistance components of 9th HLBSN genotypes

type flag leaf (score 2), ten genotypes had semi drooping type flag leaf (score 3) and two genotypes had drooping type flag leaf (score 4) (Table 1). Furthermore out of fifty-two, thirty-eight genotypes had slight LTN (score 1), seven genotypes had medium LTN (score 2), four had high LTN (score 3) and three genotypes had very high LTN (score 4) (Table 1). The mean value of plant height (PH) ranged from 72.7-94.2 cm, spike length ranged from 8.4-12.9 cm and TKW ranged from 22.6-41.7 g (Table 2).

Correlation analysis indicated that morphological traits *viz.*, LA and PE were significant and positively correlated with AUDPC with correlation coefficient value 0.37 and 0.35, respectively; whereas, LM showed non-significant positive correlation with AUDPC (Figure 6). Moreover, LTN and PH showed negative, but non-significant correlation with AUDPC with correlation coefficient value -0.12 and -0.24, respectively (Figure 6). Furthermore, yield related traits *viz.*, SL and TKW showed non-significant and very weak positive correlation with AUDPC with correlation coefficient value 0.063 and 0.076, respectively (Figure 6).

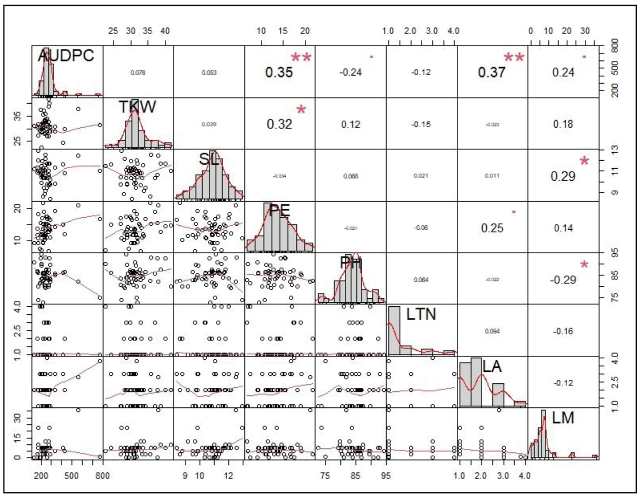

Figure 6: Distribution and correlation of morphological and yield related traits of 9th HLBSN genotypes

Spot blotch affects wheat grown over more than 10 million ha arable land of Indian Subcontinent comprising country like India, Nepal and Bangladesh that causes at least 17.5% yield loss (Gupta *et al.*, 2018; Parlevliet, 1979). The climate in these regions is characterized by elevated temperatures and high relative humidity, and this climatic pattern typically aligns with the flowering to grain-filling stage, exacerbating the severity of spot blotch and resulting in significant yield losses (Joshi *et al.*, 2007a). Partial resistance components play a crucial role in determining the severity of the disease and can be effectively utilized in the development and selection of resistant genotypes (Parlevliet, 1979; Tivoli *et al.*, 2006). This study placed a strong emphasis on the evaluation of partial resistance components and morphological traits linked to spot blotch for the selection of resistant wheat genotypes. This study found that spot blotch resistant genotypes displayed small lesions size (score 1 and

Table 2: Frequency of genotypes and range value of partial resistance components and morphological traits

Host response	AUDPC	Frequency	LS	LT	LC	LM	LA	LTN	PE [cm]	PH [cm]	SL [cm]	TKW [g]	AUDPC	Entry Number
Resistant	<225	20	1-2	1-3	1-2	0-22.5	1-3	1-4	6.9-21.7	72.7-94.2	8.6-12.9	22.6-37.6	137-222.2	9th HLBSN - 1, 3, 5, 6, 7, 14, 16, 22, 23, 25, 27, 28, 29, 31, 32, 39, 40, 43, 45, 49
Moderately resistant	225-315	26	1-3	2.5-3.5	1-2	0-22.5	1-4	1-3	6.8-18.9	79.8-92.8	8.4-12.5	27-41.7	227.8-290.7	9th HLBSN - 2, 8, 9, 10, 11, 12, 15, 19, 20, 21, 24, 26, 30, 33, 34, 35, 36, 37, 38, 41, 42, 44, 46, 47, 48, 50
Moderately susceptible	316-400	2	2	4	2	5	2-3	1	14.3-19.2	85-85.5	9.8-11.4	29-30.9	335.2	9th HLBSN - 4, 13
Susceptible	>400	4	3-5	4-5	2	1-36	2-4	1-2	12-21.1	75-86.3	10.8-12.5	24.3-38	412.9-776	9th HLBSN - 17, 18, NEPAL 297, Sonalika

[NB: LS = Lesion size, LT = Lesion Type, LC = Lesion Characteristic, LM = Lesion Mimic, LA = Leaf Angle, LTN = Leaf Tip Necrosis, PE = Peduncle Extrusion, PH = Plant Height, SL = Spike Length, TKW = Thousand Kernel Weight and AUDPC = Area Under Disease Progress Curve, HS = Host Response]

2), *i.e.*, <0.5 cm to 1 cm on flag leaves. Also LS was highly and positively correlated with AUDPC. Eisa *et al.* (2013) found smaller lesion size (0.23 cm^2) in resistant genotypes Yangmai 6 and larger lesion size (3.43 cm^2) in Sonalika. In addition, they also found high and positive correlation between lesion size (LS) and AUDPC. Similarly, Bashyal *et al.* (2011) also considered lesion size as a partial resistant component for evaluation of barley genotypes against spot blotch. They concluded that resistant genotypes are featured by smaller lesion size on leaves.

For another partial resistant components *i.e.*, lesion type (LT) we found that genotypes with lesion type (LT) 1 (small dark brown to black spots without necrotic or chlorotic surroundings) and 2 (small dark brown to black spots with little necrotic or chlorotic surroundings) had lower AUDPC and categorized as resistant. Similar result was found by Ayana (2017). They had evaluated 294 hard winter wheat genotypes and categorized genotypes manifesting lesion type 1 and 2 as resistant.

In our study, lesion characteristics (LC) *i.e.*, sporulating and non-sporulating lesions was positively and highly correlated with lesion size (LS) and AUDPC with coefficient value 0.66 and 0.54, respectively. The findings revealed that genotypes characterized by larger lesion sizes tend to produce a greater quantity of conidia, thereby intensifying disease severity and progression, *i.e.*, AUDPC. Similar result was found by Bashyal *et al.* (2011), Parlevliet (1979) and Parlevliet and van Ommeren (1975).

For morphological traits our finding revealed that genotypes with erect leaf (LA-1) posture exhibited resistant to moderately resistant response and showed positive and strong correlation with AUDPC (0.37). Joshi and Chand (2002) also found low spot blotch on erect leaf and positive correlation with AUDPC (0.58). Furthermore we found high to very high leaf tip necrosis (LTN) only in resistant to moderately resistant genotypes and negative correlation with AUDPC. Joshi *et al.* (2004b) evaluated 1407 wheat genotypes and found that leaf tip necrosis was only present in resistant to moderately resistant genotypes. In this study we found negative but non-significant correlation between PH and AUDPC (-0.24). Duveiller *et al.* (1997) also reported that association between disease severity and plant height as a complex phenomenon. In a study carried out by Joshi *et al.* (2002) suggested that genetic association between plant height and spot blotch severity is not always true.

Conclusion

This study highlighted the significance of considering partial resistant components and morphological traits as an alternative way for identification of resistant wheat genotypes against spot blotch. This study identified a total of twenty (20) resistant genotypes, which could serve as valuable new sources of resistance in breeding programs. Furthermore, genotype *viz.*, KACHU/BECARD//WBLL1*2/BRAMBLING/3/ATTILA*2/PBW65//MURGA, FRET2*2/SHAMA//TNMU/3/FRET2*2/SHAMA/4/UP2338*2/KKTS*2//YANAC/5/FRET2*2/SHAMA//PARUS/3/FRET2*2/KUKUNA, KACHU#1//PI610750/SASIA/3/KACHU/4/MUU#1//PBW343*2/KUKUNA/3/MUU/5/KACHU#1//PI610750/SASIA/3/KACHU, BORL14//KFA/2*KACHU and KFA/2*KACHU//QUELEA were found excellent. These genotypes can undergo further evaluation for their yield potential across various regions of Nepal. Those exhibiting superior performance could potentially be released as new varieties, contributing to effective spot blotch management. Furthermore, phenotyping a panel of genotypes based on partial resistant components and associated morphological traits could be a useful way of managing spot blotch disease under inadequate molecular facility and at low input cost circumstances.

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