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RESEARCH

Assessing dual resistance to stripe rust and powdery mildew in wheat germplasm through molecular and field studies across the north-western Himalayas

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Abstract Wheat production in cooler regions like the north-western Himalayas, is significantly impeded by devastating diseases, namely stripe rust (SR) and powdery mildew (PM). Genetic resistance against SR and PM loses effectiveness over time which underscores the importance of periodic disease screening. This study aims to assess resistance to SR and PM in 81 wheat genotypes across multiple locations over three years (2019–20, 2021–22 and 2022–23); and detect candidate genes (*Yr5*, *Yr10* and *Pm24*) for resistance using respective molecular markers viz., SSR/STS primers (STS7/8, Xp3000 and Xgwm337). The resistance towards SR and PM under natural epiphytotic conditions was displayed by eight and twelve genotypes respectively, across all locations. Notably, four genotypes (DH 202, HPW 368, HPW 373 and DH 114) were found resistant to both diseases. The phenotypic disease reaction for SR and PM was further validated through molecular markers. Genotypes DH 202, DH208, DH 217, CIMMYT Entry no. 23 and VL 829 emerged as high yielding disease resistant genotypes. Agrometeorological parameters specifically, precipitation and relative humidity exhibited significant positive correlations with disease incidence, leading to reduced grain yields. Genotype and genotype by environment interaction (GGE) biplot identified stable genotypes with less disease incidence over locations. Additionally, Kukumseri may serve as the optimal test site for screening wheat germplasm against SR, while Palampur and Kukumseri could be ideal for PM screening. Genotypes exhibiting combined disease resistance to both SR and PM, alongwith superior agronomic traits, hold promise for immediate deployment as wheat varieties or as potential donors for breeding resistant cultivars.

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Introduction

Wheat, (*Triticum aestivum* L., 2n = 6x = 42) is an important cereal grain crop that serves as a staple food for millions of people worldwide. According to the latest data from FAO (2022), it is cultivated over 219.15 million hectares worldwide, with a production of 808.44 million tonnes. Globally, China leads wheat production with 17.04%, followed by India with 13.33%. Wheat stands as a cornerstone of global food security and contributing approximately 20% of the calories and proteins essential for human nutrition. The grains primarily consist of carbohydrates (~ 70%–80%) and proteins (~ 8%–22%) (Slafer et al. 2021). Rapidly growing global population, shrinking arable land, and exacerbating effects of climate change increases the frequency of abiotic and biotic stresses, which significantly constrain wheat productivity (Valizadeh et al. 2014).

A variety of diseases such as rusts, bunts, powdery mildew and smuts, along with abiotic stresses like heat, drought and salinity pose significant obstacles to wheat crop production (Chatrath et al. 2007). Notably, among these diseases, stripe rust (SR) and powdery mildew (PM), caused by *Puccinia striiformis* f. sp. *tritici* and *Erysiphe graminis* f. sp. *tritici* (syn. *Blumeria graminis* f. sp. *tritici*), respectively, are particularly devastating. These pathogens are well adapted to temperate areas with cool-humid weather, which are generally associated with higher elevations (Bennett 1984; Roelfs et al. 1992; Han et al. 2020; Mehta et al 2022). Specifically, SR pathogen initiates primary infection in December and January in the North hill zone (NHZ) of India, where conditions are conducive. Subsequently, winds transport the urediospores to the surrounding foothills, further leading to the spread of infection to the major wheat-producing north west plain zone (NWPZ) (Nagarajan 1977).

Globally, wheat cultivation has experienced recurring epidemics of SR, capable of causing total crop loss (Bhardwaj et al. 2019; Hovmøller et al. 2016). In severe cases, PM can cause wheat yields to drop by 50% (Morgounov et al. 2012; Xu et al. 2015). To address these challenges, plant breeders consistently seek for germplasm with a diverse array of genes/alleles to improve resilience, which involves exploring a range of potential germplasm to uncover new sources of alleles and selecting lines with greater adaptability (Verma et al. 2024). Researchers across the globe have identified various SR and PM resistance genes in wheat germplasm, among which *Yr5*, *Yr10*, *Yr15*, *Yr24/Yr26*, *Yr32*, *YrSp* (against *P. striiformis*) (Rani et al. 2019) and *Pm1a*, *Pm2*, *Pm3/Pm8/Pm17*, *Pm5e*, *Pm21/Pm12*, *Pm24*, *Pm33*, *Pm41*, *Pm51*, *Pm60*, *Pm64*, *Pm69*, *MlZec1* and *MlAB10* (against *E. graminis*) are still effective (Gupta et al. 2022).

Grain yield in wheat is a complex trait influenced by various genetic and environmental factors. Thus, the stability of genotypes for their performance can be best analyzed under multi-environment evaluations, providing a clear picture of their adaptability across locations (Sharma et al. 2022). In breeding programs, understanding genotype × environment interaction (GEI) is important for effectively optimizing host-plant resistance across environments and pathosystem dynamics (Sankar et al. 2021). Among various statistical methods used for GEI analysis, genotype and genotype by environment interaction (GGE) biplot analyses are widely utilized for assessing multi-environment data (Abraha et al. 2019).

So, in the present study a diverse set of wheat germplasm comprising of doubled haploids, exotic collections, landraces and popular cultivars were evaluated for adult plant response against SR and PM under natural epiphytotic field conditions at four different locations depicting diverse agroclimatic zones of the north-western Himalayan region. Further, allelic differences among wheat genotypes were characterized at genetic level by molecular markers associated with SR and PM resistance genes.

Materials and methods

Experimental material

The study involved 81 diverse germplasm accessions, comprising 23 established doubled haploids, 11 promising landraces from the north-western Himalayas, 40 outstanding exotic collections from CIMMYT, Mexico and 7 elite Indian wheat varieties (Supplementary Table S1). The experiment was

conducted at four different locations namely, CSK HP Agricultural University, Palampur; Krishi Vigyan Kendra (KVK), Sundernagar; Hill Agricultural Research and Extension Centre (HAREC), Bajaura; and HAREC Kukumseri, situated in different agro-climatic zones of the north-western Himalayan region for three years (2019–20, 2021–22 & 2022–23) (Fig. 1 and Table 1). Germplasm was sown in randomized complete block design (RCBD) with three replicates, during mid-November at all locations, except at HAREC Kukumseri, where sowing was done in April. Two rows of each entry were grown in 1 m length with inter- and intra-row distance of 20 cm and 10 cm, respectively. To ensure the uniform spread of inoculum, two infector rows of susceptible varieties like Sonalika and Agra Local were sown after every nine rows within the plot and around the experimental trial. Weather parameters for each cropping season at each location were recorded by NASA POWER DAVe (https://power.larc.nasa.gov/data-access-viewer/) (Fig. 2 and Supplementary Table S2).

Fig. 1 **a** Disease incidence under field conditions, **b** Symptoms of Stripe rust, and **c** Powdery mildew on wheat plant

Phenotyping and disease scoring

Germplasm was screened for resistant genotypes against stripe rust (SR) and powdery mildew (PM) at adult plant stage, under natural epiphytotic conditions. The disease reactions were recorded on 10 random plants at heading stage for each genotype in each replication. Disease reactions for SR was recorded in accordance to, 0–100 modified Cobb's Scale (Peterson et al. 1948). The host responses were graded: S = susceptible (> 30), abundant mycelium development and uredia surrounded by necrotic tissues; MS = moderately susceptible (21–30), development of mycelium and small uredia surrounded by necrotic tissues; MR = moderately resistant (11–20), slight uredia surrounded by necrotic tissues; R = resistant (6–10), very slight uredia surrounded by necrosis and chlorosis; and HR = Highly resistant, slight flecking of necrotic/chlorotic spot (1–5) (Akin et al 2008). While, PM was monitored in accordance to, Saari-Prescot 0–9 scale (Bennett and Westcott 1982), where '1' represents small flecks (HR), '2' small chlorotic flecks (R), '3' large flecks with chlorosis and necrosis (MR), '4–6' mycelium and conidia barely detectable with small and moderately sized lesions (MS) and '7–9' increased amount of mycelium and conidia production (S).

At each location, the data for flag leaf area (FLA) (cm^2) and grain yield per plant (GY) (g) were

Table 1 Geographical locations for conducting germplasm evaluation during the experiment

Sr.	Code	Location	Altitude	Latitude	Longitude	Seasons
1	E_1-E_3	CSKHPKV, Palampur	1290.8	32.10°N	76.55°E	Rabi (2019–20, 2021–22, 2022–23)
2	E_4-E_6	KVK, Sundernagar	914	31.54°N	76.90° E	Rabi (2019–20, 2021–22, 2022–23)
3	E_7-E_9	HAREC, Bajaura	1090	31.85°N	77.16°E	Rabi (2019–20, 2021–22, 2022–23)
4	E_{10}-E_{12}	HAREC, Kukumseri (Lahaul & Spiti)	2672	32.70° N	76.69°E	Summer (2019, 2021, 2022)

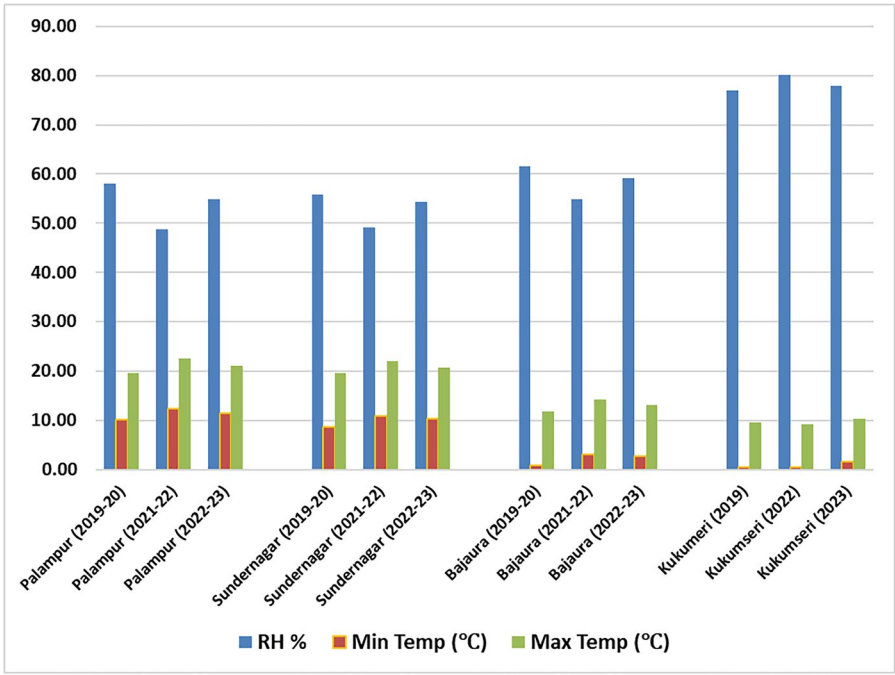

Fig. 2 Variations in temperature and relative humidity during cropping seasons at different environments

recorded in triplicates for each genotype and in each replication five random competitive plants were chosen. To further estimate the effect of disease on agro-morphological traits, total chlorophyll content (TCC) (mg/g) was estimated using Witham et al. (1986) at Molecular Cytogenetics and Tissue culture laboratory, Department of Genetics and Plant Breeding, CSKHPKV, Palampur.

Molecular studies

Molecular studies were performed in the Molecular Cytogenetics & Tissue Culture Lab, Department of Genetics and Plant Breeding, CSKHPKV, Palampur. Genomic DNA was extracted from juvenile leaves of 10–15-day-old seedlings using the CTAB method described by Doyle and Doyle (1987), with slight modifications. DNA quantity was determined with 0.8% agarose gel and quality was checked using Eppendorf BioSpectrometer® basic.

Specific SSR/STS primers linked to SR resistance genes *Yr5* (STS7/8) and *Yr10* (Xpsp3000) were used (Rani et al 2019). Similarly, SSR primers associated with PM resistance genes *Pm24* (Xgwm337) was employed (Cheng et al 2022) (Table 2).

DNA amplification was carried out using 12.5 µl reaction mixture consisting of 1 µl template DNA (50 ng), 1.25 µl 10X PCR buffer, 1.25 µl 25 mM $MgCl_2$, 1.25 µl 2 mM dNTPs, 0.5 µl 10 µM each of forward and reverse primers, 0.25 µl Taq DNA polymerase and 6.5 µl sterilized doubled distilled water in Eppendorf thermal-cycler. The PCR regimen consisted of initial denaturation step at 94 °C for 5 min, followed by 35 cycles of denaturation (94 °C), annealing (X°C) and extension (72 °C) each for 45 s. The final extension step was carried out at 72 °C for 5 min. Subsequently, the PCR products were subjected to electrophoresis on a 3.5% agarose gel at 120 V for 120–150 min. The size of the amplicons was determined using a 100 bp DNA ladder and visualized with a gel documentation system (UVITEC, Cambridge).

Data analysis

The average values from the 81 genotypes for FLA, TCC, SR, PM and GY from different environments and pooled over environments were subjected to analysis of variance (ANOVA) using Microsoft Excel Data Analysis tools. Simultaneously, Bartlett's test was applied for testing homogeneity of variance and validating ANOVA. To assess the average performance of genotypes across all environments, phenotypic data were analyzed using best linear unbiased

Table 2 List of primer sequences used for screening stripe rust and powdery mildew resistance among the wheat germplasm

S. No.	Primer	Linked with	Chromosomal location	Primer sequences (5'–3')	Annealing temperature	Product size (bp)	Reference
1	STS7/8-F	*Yr5*	2B	GTACAATTCACC TAGAGT	45 °C	500 (+)	Murphy et al (2009)
	STS7/8-R			GCAAGTTTTCTC CCTATT			
2	Xpsp3000-F	*Yr10*	1BS	GCAGACCTGTGT CATTGGTC	52 °C	240 (–), 220 (–), 260 (+), 285 (–)	Bariana et al (2002); Elkot et al (2016)
	Xpsp3000-R			GATATAGTGGCA GCAGGATACG			
3	Xgwm337-F	*Pm24*	1DS	CCTCTTCCTCCC TCACTTAGC	55 °C	204 (+), 184 (–)	Zhao et al. (2010); Cheng et al. (2022)
	Xgwm337-R			TGCTAACTGGCC TTTGCC			

predictions (BLUP) modeling. The BLUP values obtained were analyzed and interpreted in comparison to the best check(s) for each trait in each pooled environment. BLUP values, least significance difference (LSD) test, heritability (H^2) and genetic advance as % of mean (GA) were worked out using the META-R program (Alvarado et al. 2020).

The Pearson's correlation coefficient (*r*) between agro-morphological traits and disease scores was determined across locations and combined. These results are graphically represented by using R version 4.0.5 (R Core Team 2021; Peterson and Carl 2020). Pooled correlation was also worked out with agrometeorological data including temperature, precipitation, relative humidity (RH%) and root zone soil wetness (RZSW%). The GGE Biplot analysis for all environments was performed using the package GGE Biplots of R studio (R Core Team 2021).

Results

Mean performance and analysis of variance

ANOVA was performed for all the traits per individual environments and pooled over years for all the locations, which indicated that the mean sum of squares of genotypes were significantly different for FLA, TCC, SR, PM and GY across all the four locations over three years, suggesting ample genetic variability for the studied traits in the germplasm (Supplementary Table S3). Bartlett's test confirmed that variances were uniform for studied traits. Consequently, pooled ANOVA for four locations combined over three years revealed that the mean squares attributed to genotypes and genotype × year interaction differed significantly; suggesting morpho-genetic variability among treatments throughout the experiments (Table 3).

BLUP values for FLA ranged from 17.34–31.98 cm^2 with an average of 24.29 cm^2. GY ranged from 5.56–8.83 g with an average of 7.05 g. Five genotypes namely, DH 202, DH 210, DH 217, CIMMYT Entry no. 23 and Chamba landrace 3, showed significantly higher yields over their respective check varieties (VL829, HPW373 and DH114) across all the environments. Additionally, the average GY is highest in Sundernagar and lowest in Kukumseri.

High heritability (95%) coupled with moderate genetic advance (23.63%) was observed for FLA, while moderate heritability (72%) combined with low genetic advance (19.34%) was observed for GY (Table 4, Fig. 3, Supplementary Table S4 and S5).

Disease screening under field conditions

Eight genotypes (9.88%) viz., DH 202, DH 208, DH 217, CIMMYT Entry No. 133, CIMMYT Entry No. 278, HPW 368, HPW 373 and DH 114 showed consistent SR resistant reactions over years at all the locations. For powdery mildew 12 genotypes (14.81%) viz., DH 195, DH 198, DH 202, DH 219, CIMMYT Entry no.23, CIMMYT Entry no.242, CIMMYT line

Table 3 Analysis of variance for four locations combined over three years

Source of Variation	DF	FLA	SR	PM	GYPP	Source of Variation	DF	FLA	SR	PM	GYPP
Palampur						**Bajaura**					
Year	2	8,185.7*	2,685.6*	282,160.9*	3,796.6*	Year	2	5,517.5*	3,600.9*	1,545.1*	315.8*
Rep within Year	6	10.2	0.6	2,298.3	14.9	Rep within Year	6	37.1	0.788	220.0	17.0
Genotype	80	32.8*	4,122.6*	90.6*	11.3*	Genotype	80	28.8*	4,697.4*	34.9*	8.3*
Year × Genotype	160	6.0*	675.4*	47.4*	10.4*	Year × Genotype	160	4.4*	254.0*	7.5*	6.9*
Pooled Error	480	1.0	1.0	1.0	1.0	Pooled Error	480	1.0	1.0	1.0	1.0
Total	728					Total	728				
Sundernagar						**Kukumseri**					
Year	2	14,645.7*	897.0*	0.1*	483.4*	Year	2	63,718.6*	973.7*	0.04*	113.3*
Rep within Year	6	7.6	1.8	6.4	36.4	Rep within Year	6	10.4	0.5	4.9	24.2
Genotype	80	32.4*	2,898.1*	0.9*	6.5*	Genotype	80	299.1*	2,206.8*	0.3*	5.9*
Year × Genotype	160	9.4*	273.3*	0.1*	6.0*	Year × Genotype	160	22.7*	188.2*	0.1*	2.3*
Pooled Error	480	1.0	1.0	0.02	1.0	Pooled Error	480	1.0	1.0	0.001	1.0
Total	728					Total	728				

(*Significant at 5% level of significance)

Table 4 Parameters of variability and promising genotypes for four locations pooled over three years

Traits	Location	Mean	GCV (%)	Heritability	GA (%) mean	Best check(s)	Promising genotypes (3)
FLA (cm^2)	Palampur	24.13	13.58	0.87	26.09	HPW 360	DH 217*, CIMMYT Entry no.106*, Chamba landrace 15
	Sundernagar	26.00	12.41	0.85	23.60	VL 829	Chamba landrace 15*, DH 217*, DH 208
	Bajaura	24.68	11.60	0.87	22.27	C 306	Chamba landrace 15*, DH 217*, DH 198
	Kukumseri	22.34	14.79	0.98	30.14	VL 829	Chamba landrace 15*, DH 217*, DH 198*
	Pooled	24.29	11.79	0.95	23.63	VL 829	Chamba landrace 15*, DH 217*, DH 208*
GY (g)	Palampur	6.13	5.99	0.14	4.56	VL 829	CIMMYT Entry no.23, CIMMYT Entry no.13, CIMMYT Entry no.278
	Sundernagar	10.23	9.40	0.35	11.50	DH114	DH 217, DH 210, DH 209
	Bajaura	7.65	10.23	0.32	11.88	VL829	DH 202, CIMMYT Entry no.23, DH 216
	Kukumseri	4.21	11.84	0.60	18.83	HPW373	DH 217, CIMMYT Entry no.23, Chamba landrace 3
	Pooled	7.05	11.08	0.72	19.34	VL 829	CIMMYT Entry no.23*, DH 217, DH 210

(* Significantly superior to the best check)

30–16, Chamba landrace 1. HPW 368, HPW 373, VL 829 and DH 114 demonstrated consistent PM resistant reactions over years at all the locations.

Moreover, 11 and 28 genotypes were found resistant to SR and PM, respectively in pooled over 12 environments (Table 5 and Supplementary Table S6). Overall, the disease intensity for SR is highest at Kukumseri, while for PM, it is higher at Kukumseri and Palampur than rest of the locations.

Screening at molecular level

The STS marker, STS7/8 (resistant amplicon size 500 bp), used in the study is the dominant type marker, indicating the presence of the SR resistance gene *Yr5*, amplified in 71 lines (87.65%), while the remaining 8 wheat genotypes failed to amplify indicating the absence of the gene. On the other hand, the SSR marker Xpsp3000 (resistant amplicon size 260 bp) was detected in 18 genotypes (22.22%), indicating the presence of SR resistance *Yr10* gene, while it was absent in the remaining 62 entries tested (Fig. 3). Eleven genotypes, namely DH 198, DH 200, DH 207, DH 208, DH 216, CIMMYT Entry no.278, CIMMYT line 60-35, CIMMYT line 30-7, Chamba landrace 16, Chamba landrace 17 and DH 114 each possessing both SR resistance genes (*Yr5* and *Yr10*) demonstrated resistant reactions in fields. However, seven genotypes, DH 202, DH 217, CIMMYT line 60-44, CIMMYT line 30-10, HPW 360, HPW 368 and HPW 373 exhibited resistance to SR in the fields without amplifying either or both of the genes (*Yr5* and *Yr10*), whereas two genotypes (DH196 and CIMMYT line 30-5) displayed moderately susceptible reactions despite possessing both genes (Fig. 3 and Table 6).

PM resistance gene *Pm24* was not found at all in the whole germplasm as maker Xgwm337 failed to amplify.

Correlation studies

Correlation studies conducted across all locations (Fig. S1), pooled results showed a notable positive correlation between GY and TCC, conversely both morpho-biochemical traits were negatively associated with SR and PM occurrences (Fig. 4a). Additionally, the correlation between the studied traits, disease prevalence and agrometeorological highlighted significant positive correlations between precipitation and RH% with SR and PM diseases, while showing negative correlations with GY (Fig. 4b).

Mega-environment analysis of genotypes

The GGE biplot revealed that PC1 (disease score) and PC2 (resistance stability) accounted for 58.66% & 16.73% and 40.42% & 20.33% of the total variation for SR and PM, respectively (Figs. 6).

The 'which-won-where' perspective of the GGE biplot demonstrated that genotypes i.e. G-5 (DH 198), G-9 (DH 202), G-15 (DH 208), G-19 (DH 216),

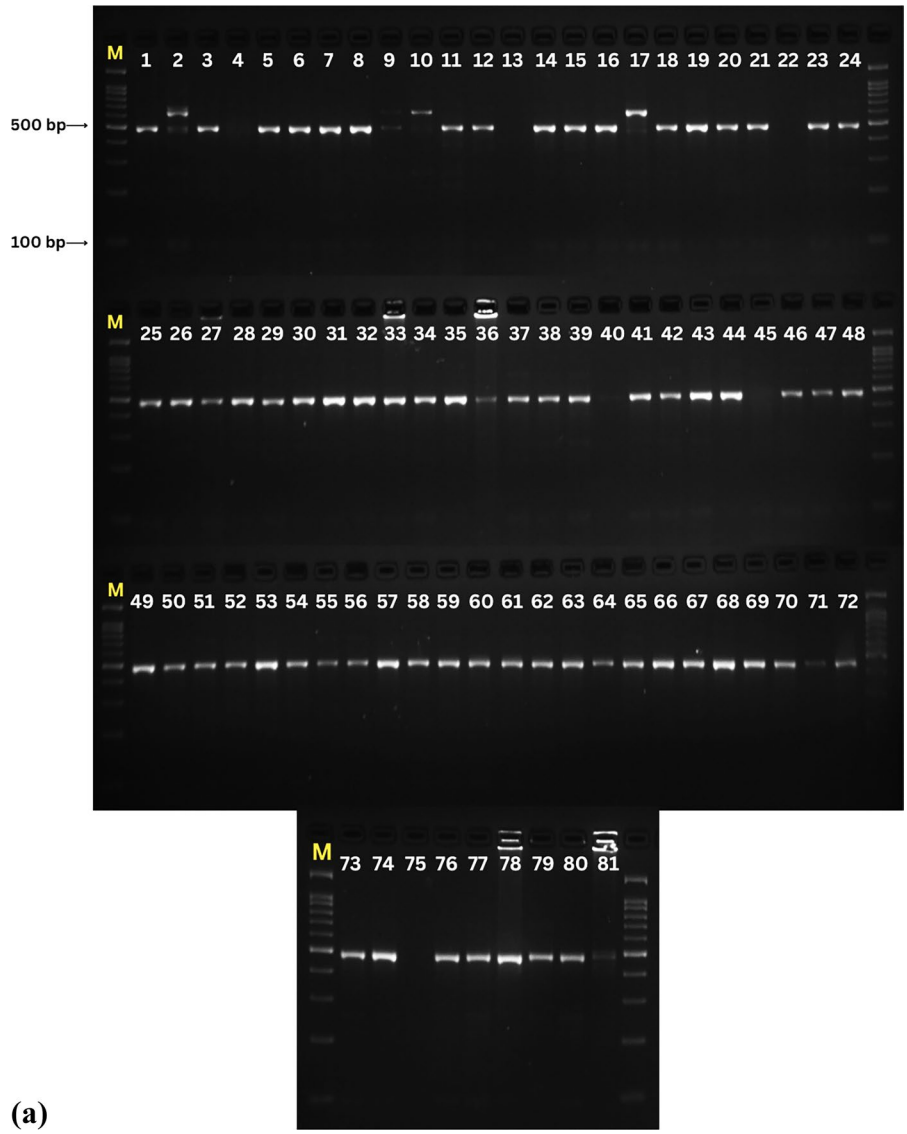

Fig. 3 PCR amplification profile of marker **a** STS7/8, associated with SR resistance gene *Yr5*, and **b** Xpsp3000 linked with SR resistance gene *Yr10*

G-20 (DH 217), G-36 (CIMMYT Entry no.133), G-40 (CIMMYT Entry no.240), G-41 (CIMMYT Entry no.242), G-43 (CIMMYT Entry no.278), G-49 (CIMMYT line 60-44), G55 (CIMMYT line 30-24), G-58 (CIMMYT line 30-10), G-75 (HPW 360), G-76 (HPW 368), G-77 (HPW 373), G-79 (VL892) and G-81 (DH 114) had low levels of SR intensity by being the farthest to the right side of the origin of biplot. While Genotypes, G-13 (DH 206), G-21 (DH 218), G-29 (CIMMYT Entry no.92), G-30 (CIMMYT Entry no. 95), G-31 (CIMMYT Entry no.98), G-32 (CIMMYT Entry no.101), G-45 (CIMMYT line 60-24), G-68 (Chamba landrace 2), G-70 (Chamba landrace 13) and G-72 (Chamba landrace 15) constantly showed higher disease score for SR and were located outermost to the left side of the origin of the biplot (Fig. 5a).

The genotypes present at the left side of the hull showed more PM susceptibility and those on the right side had stable resistance across the environments. Genotypes i.e. G-2 (DH 195), G-4 (DH 198), G-5 (DH 198), G-29 (CIMMYT Entry no.92), G-41 (CIMMYT Entry no.242), G-56 (CIMMYT line 30-16), G-64 (Chamba landrace 1), G-70 (Chamba landrace 13), G-76 (HPW 368), G-77 (HPW 373), G-78 (VL 829) and G-81 (DH 114) had low levels

Fig. 3 (continued)

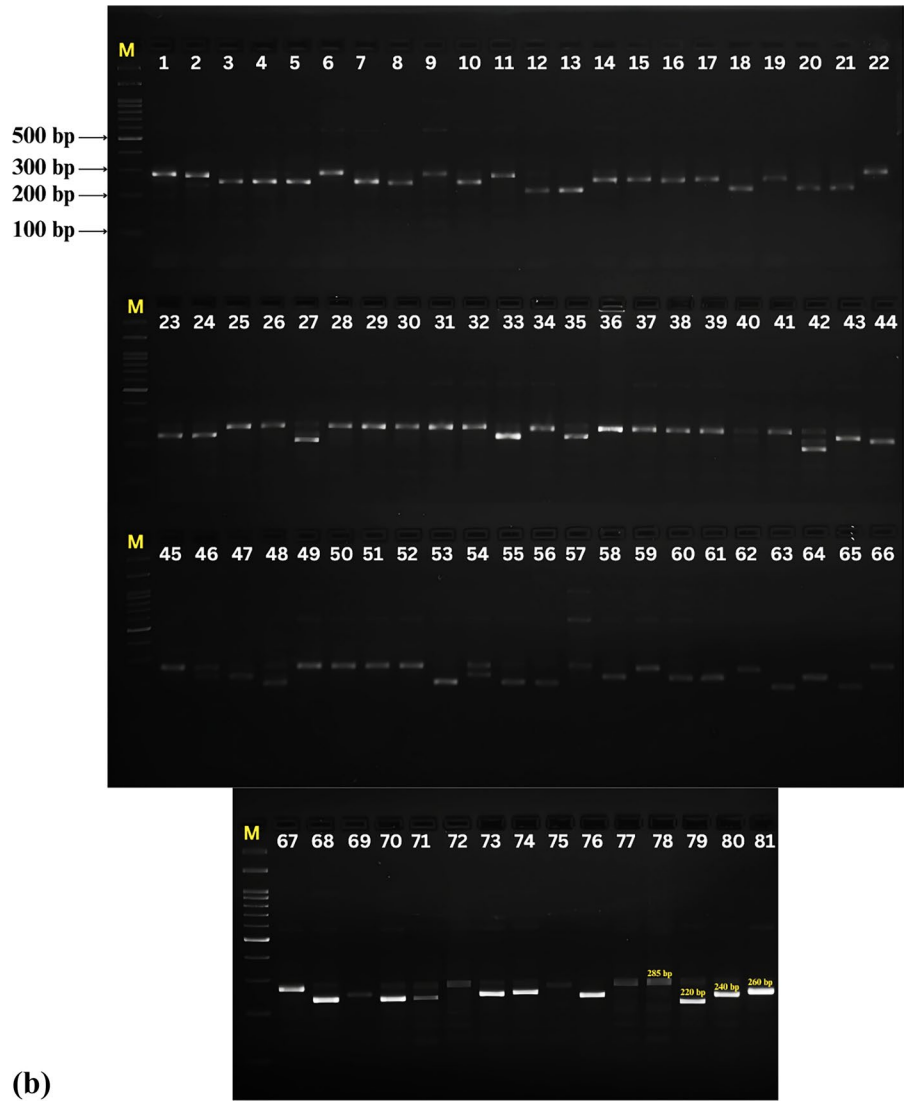

Table 5 Categorization of resistant wheat genotypes based on their disease responses to SR and PM pooled over all locations

	Genotypes response to SR reaction	Genotypes response to PM reaction
Immune	–	–
Highly Resistant	CIMMYT Entry no.133 (1)	–
Resistant	DH 202, DH 208, DH 216, DH 217, CIMMYT Entry No.278, CIMMYT Line 60-44, CIMMYT Line 30-10, HPW 360, HPW 368, HPW 373, DH 114 (11)	DH 194, DH 195, DH 197, DH 198, DH 202, DH 205, DH 218, DH 219, CIMMYT Entry no.13, CIMMYT Entry no.23, CIMMYT Entry no.74, CIMMYT Entry no.92, CIMMYT Entry no.242, CIMMYT line 60-35, CIMMYT line 60-37, CIMMYT line 60-15, CIMMYT line 30-16, Chamba landrace 1, Chamba landrace (1)2, Chamba landrace 3, Chamba landrace 13, Chamba landrace 16, Chamba landrace 17, HPW 368, HPW 373, VL 829, VL892, DH 114 (28)

Table 6 Detection of *Yr5* and *Yr10* genes through tagged markers on wheat germplasm

Sr.no	Genotypes	STS7/8 (*Yr5*) 500 bp (+)	Xpsp3000 (*Yr10*) 260(+)
1	DH 194	+	–
2	DH 195	–	–
3	DH 196	+	+
4	DH 197	–	+
5	DH 198	+	+
6	DH 199	+	–
7	DH 200	+	+
8	DH 201	+	–
9	DH 202	–	–
10	DH 203	–	+
11	DH 204	+	–
12	DH 205	+	–
13	DH 206	–	–
14	DH 207	+	+
15	DH 208	+	+
16	DH 209	+	–
17	DH 210	–	+
18	DH 215	+	–
19	DH 216	+	+
20	DH 217	+	–
21	DH 218	+	–
22	DH 219	–	–
23	DH 776	+	–
24	CIMMYT Entry no.7	+	–
25	CIMMYT Entry no.12	+	–
26	CIMMYT Entry no.13	+	–
27	CIMMYT Entry no.23	+	–
28	CIMMYT Entry no.74	+	–
29	CIMMYT Entry no.92	+	–
30	CIMMYT Entry no.95	+	–
31	CIMMYT Entry no.98	+	–
32	CIMMYT Entry no.101	+	–
33	CIMMYT Entry no.105	+	–
34	CIMMYT Entry no.106	+	–
35	CIMMYT Entry no.107	+	–
36	CIMMYT Entry no.133	+	–
37	CIMMYT Entry no.164	+	–
38	CIMMYT Entry no.237	+	–
39	CIMMYT Entry no.238	+	–
40	CIMMYT Entry no.240	–	–
41	CIMMYT Entry no.242	+	–
42	CIMMYT Entry no.277	+	–
43	CIMMYT Entry no.278	+	+
44	CIMMYT line 60-3	+	–
45	CIMMYT line 60-24	–	+
46	CIMMYT line 60-35	+	+
47	CIMMYT line 60-36	+	–
48	CIMMYT line 60-37	+	–
49	CIMMYT line 60-44	+	–
50	CIMMYT line 60-47	+	–
51	CIMMYT line 60-50	+	–
52	CIMMYT line 60-15	+	–
53	CIMMYT line 60-34	+	–
54	CIMMYT line 30-30	+	–
55	CIMMYT line 30-24	+	–
56	CIMMYT line 30-16	+	–
57	CIMMYT line 30-13	+	–
58	CIMMYT line 30-10	+	–
59	CIMMYT line 30-7	+	+
60	CIMMYT line 30-6	+	–
61	CIMMYT line 30-1	+	–
62	CIMMYT line 30-5	+	+
63	CIMMYT line 30-8	+	–
64	Chamba landrace 1	+	–
65	Chamba landrace 18	+	–
66	Chamba landrace 19	+	–
67	Chamba landrace (1)2	+	–
68	Chamba landrace 2	+	–
69	Chamba landrace 3	+	–
70	Chamba landrace 13	+	–
71	Chamba landrace 14	+	–
72	Chamba landrace 15	+	–
73	Chamba landrace 16	+	+
74	Chamba landrace 17	+	+
75	HPW 360	–	–
76	HPW 368	+	–
77	HPW 373	+	–
78	VL 829	+	–
79	VL892	+	–
80	C 306	+	–
81	DH 114	+	+

of PM intensity by being farthest to the left side of the origin of the biplot (Fig. 5b). Genotypes, viz., G-13 (DH 206), G-14 (DH 207), G-15 (DH 208), G-30 (CIMMYT Entry no.95), G-33 (CIMMYT Entry no.105), G-34 (CIMMYT Entry no.106), G-35

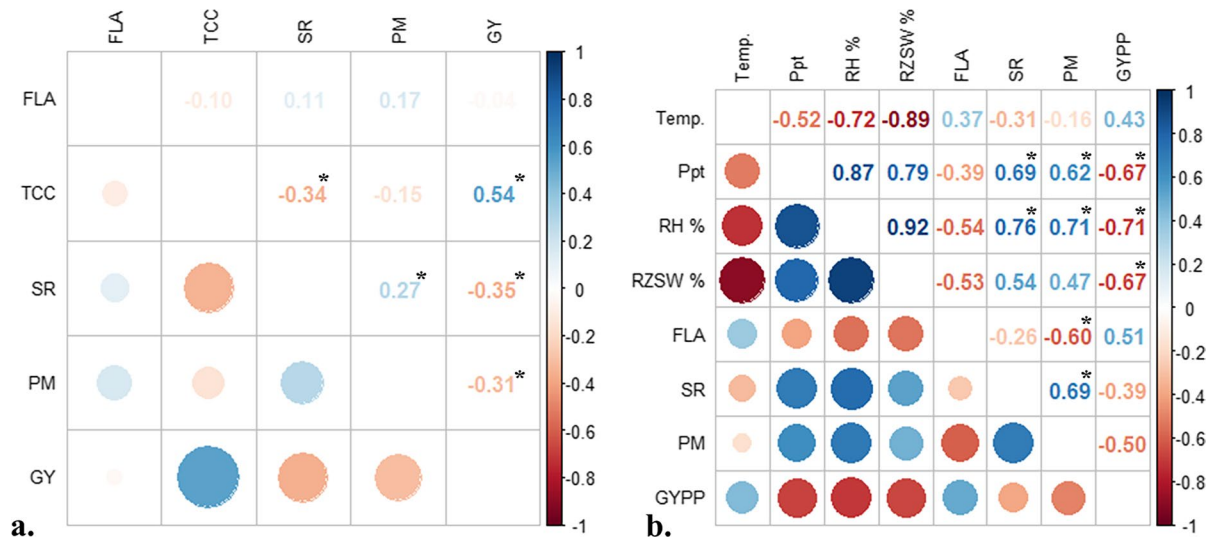

Fig. 4 Heatmap displaying Pearson's correlation coefficient pooled across locations among **a** studied traits with disease scores, and **b** studied traits and disease scores with agrometeorological data (* $P < 0.05$) (Temp-Temperature(ºC), Ppt- Precipitation, RH%- Relative Humidity, RZSW- Root zone soil wetness)

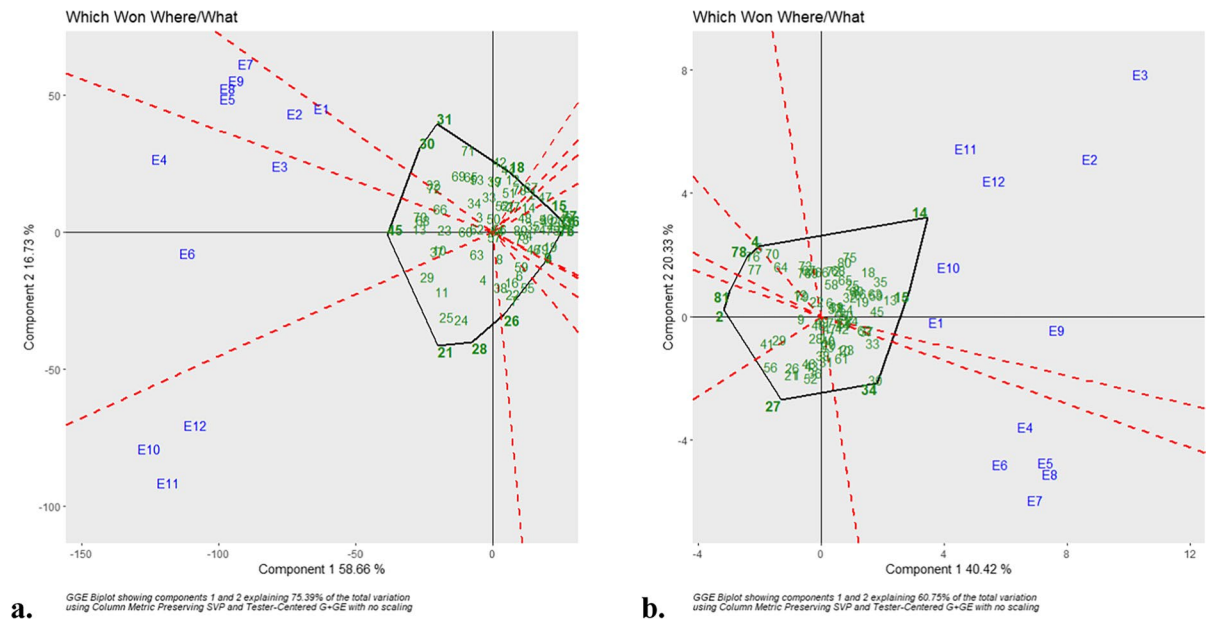

Fig. 5 Which-won-where view of GGE biplot based on **a** SR, and **b** PM disease scores on 81 genotypes of wheat under 12 environments Green numbers correspond to genotypes as listed in (Supplementary Table S1)

(CIMMYT Entry no.107), G-37 (CIMMYT Entry no.164), G-45 (CIMMYT line 60-24) and G-59 (CIMMYT line 30-7) consistently showed high level of disease score and were located outermost to the right side of the origin of the biplot (Fig. 5b).

The polygon view partitioned the biplot into several sectors, aiding in the clustering of environments into mega-environments (ME). All the environments were categorized into three MEs for SR and two MEs for PM. Mega environments I (ME-I) for SR includes

environments E-1 (Palampur 2019–20), E-2 (Palampur 2021–22), E-5 (Sundernagar 2021–22), E-7 (Bajaura 2019–20), E-8 (Bajaura 2021–22) and E-9 (Bajaura 2022–23), ME-II comprised environments E-3 (Palampur 2022–23), E-4 (Sundernagar 2019–20) and E-6 (Sundernagar 2022–23) and ME-III comprised E-10 Kukumseri 2019), E-11 (Kukumseri 2021) and, E-12 (Kukumseri 2022) (Fig. 5a). While, for PM, ME-I include E-1 (Palampur 2019–20), E-2 (Palampur 2021–22), E-3 (Palampur 2022–23), E-9 (Bajaura 2022–23), E-10 (Kukumseri 2019), E-11 (Kukumseri 2021) and E-12 (Kukumseri 2022) and ME-II comprised environments E-4 (Sundernagar 2019–20), E-5 (Sundernagar 2021–22), E-6 (Sundernagar 2022–23), E-7 (Bajaura 2019–20) and E-8 (Bajaura 2021–22) (Fig. 5b).

Mean vs. Stability

The GGE biplot of "Mean vs. Stability" view ranked genotypes based on their average performance across 12 environments for SR and PM. The single arrowed line indicated the AEC (average environment coordinate) abscissa, pointing towards higher disease intensity (Yan and Tinker 2006) (Figs. 7). The GGE biplot revealed that, in terms of the least disease occurrence for SR, the overall best performing genotypes with wider adaptability were G-9 (DH 202), G-15 (DH 208), G-19 (DH 216), G-20 (DH 217), G-36 (CIMMYT Entry no.133), G-43 (CIMMYT Entry no.278), G-49 (CIMMYT line 60-44), G-58 (CIMMYT line 30-10), G-75 (HPW 360), G-76 (HPW 368), G-77 (HPW 373), G-79 (VL892) and G-81 (DH 114) (Fig. 6a). In case of PM, i.e., G-2 (DH 195), G-4 (DH 197), G-5 (DH 198), G-76 (HPW 368), G-77 (HPW 373), G-78 (VL 829) and G-81 (DH 114) were the overall best genotypes (Fig. 6b).

Evaluating test environments: discrimination ability and representativeness

The 'discrimitiveness vs. representativeness' view of GGE biplot for test environments explained that E-10 (Kukumseri 2019) and E-11 (Kukumseri 2021) had greater vector length for SR, while E-3 (Palampur 2022–23) exhibited a greater vector length for PM compared to other environments, suggesting the higher capability of these environments for discriminating and distinguishing genotypes based on the respective disease (Fig. 7).

A smaller (acute) angle of test environment vector with AEC signifies stronger representativeness of

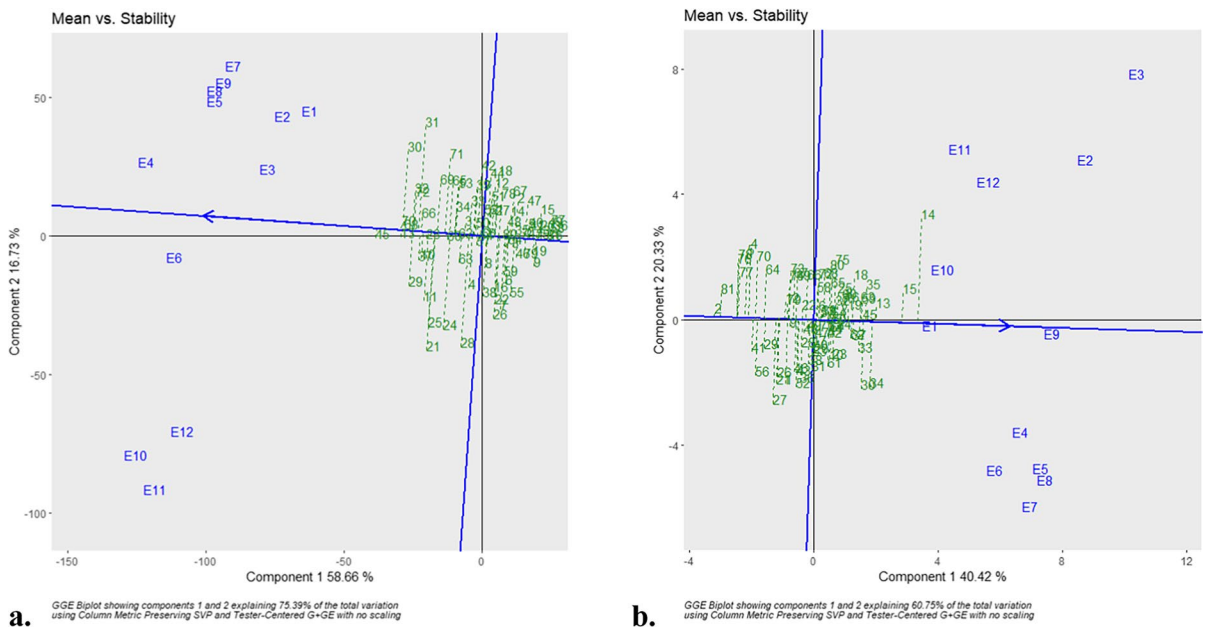

Fig. 6 Mean vs stability view of GGE biplot based on, **a** SR, and **b** PM disease scores on 81 genotypes of wheat under 12 environments

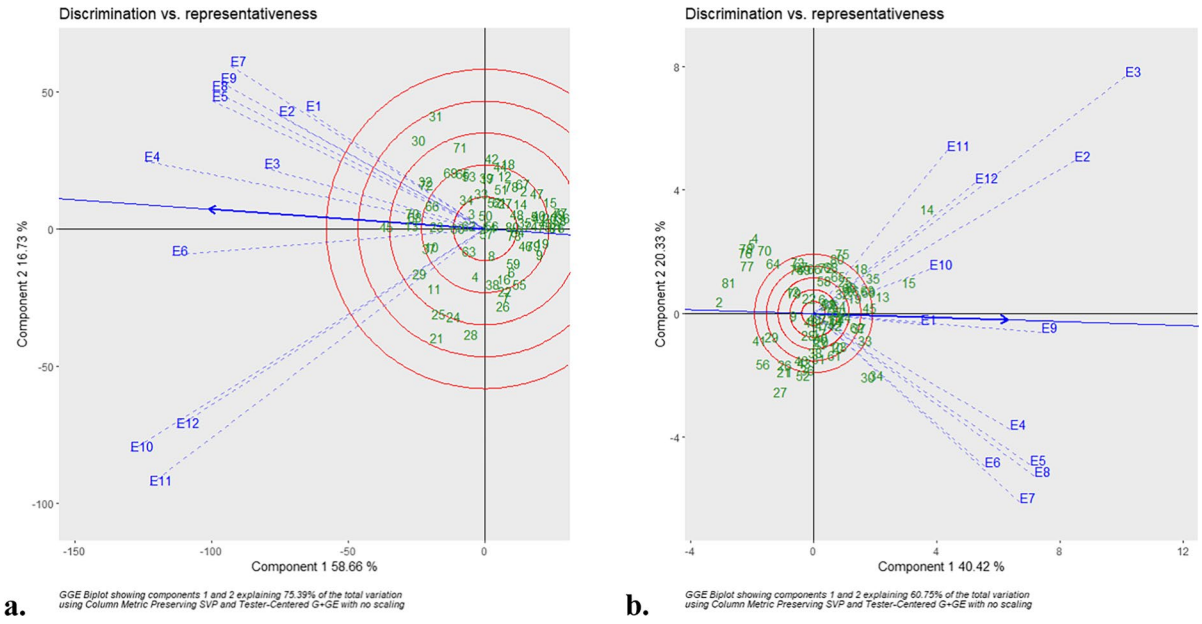

Fig. 7 Discriminativeness vs. representativeness view of GGE biplot based on, **a** SR, and **b** PM disease scores on 81 genotypes of wheat under 12 environments

the environment (Mehta et al. 2022; Das et al. 2019). Small angles of E-3 (Palampur 2022–23), E-4 (Sundernagar 2019–20) and E-6 (Sundernagar 2022–23) with AEC for SR; and E-1 (Palampur 2019–20), E-9 (Bajaura 2022–23) and E-10 (Kukumseri 2019) for PM, were indicative of stronger representativeness (Fig. 7 a and b).

Discussion

Developing elite wheat cultivars through resistant breeding programs represents a financially and environmentally sustainable strategy for disease management. However, despite developing resistant varieties against SR and PM, there remains a potential risk of resistance loss, owing to pathogen evolution and emergence of new strains. This highlights the need for regular disease screenings and the identification of diverse resistance genes.

Periodic screening of wheat germplasm over seasons and locations, against prevalent pathogen races is crucial to develop high-yielding varieties with durable resistance to multiple diseases (Singh et al. 2015). The present study was such a kind of periodic screening, conducted at four different locations in diverse agroclimatic zones of the north-western Himalayan region, well-recognized as a hotspot for SR and PM diseases. These locations provide varied habitats for both the crop and pathogens, enabling effective natural screening of resistant germplasm against SR and PM. Phenotypic evaluation of the tested genotypes for various agro-morphological traits showed significant differences across all the four locations. Five genotypes, namely DH 202, DH 210, DH 217, CIMMYT Entry no. 23 and Chamba landrace 3, demonstrated significantly higher yields compared to their respective check varieties across various locations. Sharma et al. (2022) and Jee et al. (2019) also observed significant differences among wheat genotypes they evaluated. High heritability coupled with moderate genetic advance for FLA, indicating additive and non-additive gene action in the inheritance, providing scope of improvement through selection for this trait. Conversely, moderate heritability combined with low genetic advance was observed for GY, indicating non-additive gene action. Therefore, selections based solely on GY would not be effective. The findings of this study align with those of Adhikari et al. (2018) and Singh et al. (2018). Field screening of the germplasm revealed that 12 and 28 genotypes were highly

resistant to SR and PM, respectively (Table 5). Among these, four genotypes, namely DH 202, HPW 368, HPW 373 and DH 114, demonstrated resistance to both SR and PM. Notably, DH 202, DH 208 and DH 217 exhibited high yields along with resistance to SR, while DH 202, CIMMYT Entry no. 23 and VL 829 showed high yields coupled with resistance to PM. Overall, a high-yielding genotype DH 202 exhibited resistance to both SR and PM. In the study conducted by, Mishra et al. (2015) it was observed that out of 616 accessions, 197 were found to be resistant to SR. Kumar et al. (2016) screened 19,460 accessions for SR at a hotspot, Gurdaspur (Punjab) and identified 498 potential resistant accessions to multiple rusts. Similar set of wheat germplasm (19,460 accessions) were screened by Vikas et al (2020) at Wellington, a hotspot for PM, for two consecutive seasons, results indicated that 7,271 accessions were resistant.

The primary goal of plant breeders is to develop varieties that consistently resist multiple diseases and produce higher yields. This is achieved by identifying and combining diverse disease-specific resistance genes. Molecular analysis showing distinct amplification patterns in resistant and susceptible genotypes confirmed the phenotypic evaluation. Among the twelve genotypes demonstrating the presence of both SR resistance genes (*Yr5* and *Yr10*), eleven exhibited resistance in the field across multiple locations.

The results of molecular screening for SR resistance are consistent with those reported by Rani et al. (2019), who observed the presence of STS7/8 (*Yr5*) in 23 genotypes out of 68 wheat genotypes and by Haider et al. (2023), where Xp3000 (*Yr10*) marker was amplified in ten out of 45 tested wheat accessions. Cheng et al. (2022) while assessing 332 wheat germplasms for PM resistance noted that, 16 accessions amplified *Pm24* gene (Xgwm337). The resistant disease reactions observed in certain genotypes, despite lacking the studied genes associated with SR and PM resistance, may be attributed to the presence of other race-specific genes not included in the study (Kokhmetova et al. 2021; Brar and Kutcher 2016). Likewise, some genotypes amplifying the genes under consideration were found susceptible in field conditions, potentially attributed to environmental factors, the new emerging pathogen races or gene-environment interactions (Ali et al 2017; Wang and Chen 2017).

Correlation analysis indicated an inverse relationship between disease incidences with GY. These findings align with those of Sharma-Poudyal and Chen (2011) and Murray et al. (1994) for SR and Draz et al. (2019) and Cerón and Martel (2003) for PM, who concluded that the proportion of leaf area, affected by SR/PM significantly contributes to yield losses in wheat. Further, the negative correlation of disease scores with TCC is indicative of cellular damage caused by the fungal pathogen to the infected plants. These findings align with Mishra et al. (2015) and Cao et al. (2009) regarding SR and PM incidence, respectively. Correlation studies between agro-morphological traits, disease responses and weather parameters are pivotal for understanding the epidemiology of the pathogen and its impact on crop productivity (Ali et al 2023; Liu et al. 2015). The findings of correlation analysis among all the traits under consideration indicate a significant contribution of the weather parameters towards disease development, consistent with previous research by EI Jarroudi et al. 2020 in SR; Mehta et al. (2018), Singh and Pannu (2014) in PM and Mehta et al. (2022), Basandrai and Basandrai 2018 and Kumar et al. 2016 in both diseases. These studies underscore the role of low temperatures and high relative humidity in promoting PM and YR incidence.

For effective resistance breeding programs, understanding the significance of the GEI is crucial for ensuring the stability of host genotype across different locations (Das et al. 2019; Sankar et al. 2021). The 'which-won-where' view of the GGE biplot aids in interpreting GEI in multi-environment trial data. The genotypes, positioned at distinct vertices of the polygon (convex hull), exhibit the lowest and highest SR and PM scores, thus can be categorized into best and worst performers, among the environments in that sector (Yan and Tinker 2006; Yan et al. 2007). Overall, the biplot revealed that five genotypes (DH 198, CIMMYT Entry No. 242, HPW 368, HPW 373 and DH 114) exhibited low levels of SR and PM intensities across various locations. As observed in current research, resistance sources for SR have been documented worldwide (Mehta et al. 2022; Sood et al. 2020; El-Orabey et al. 2020). Furthermore, sources of resistance to PM have been identified by various researchers (Mehta et al. 2022; Vikas et al. 2020; Draz et al. 2019; Gupta et al. 2016; HaiRong et al.

2011). After partitioning of the biplot, all the environments were categorized into three and two MEs for SR and PM, respectively. These results were in concise with Mehta et al. (2022) where the total environments were classified into three MEs for SR and PM. "Mean vs. Stability" view helps in inferring the most stable genotypes across different environments. The research findings were in alignment with the biplot studies conducted by earlier workers (Mehta et al. 2022; Das et al. 2019). In multi-environmental trials, the longer vector length indicates the greater discriminatory capability of the environments, whereas smaller angle formed by each vector with the abscissa signifies stronger representativeness of a mega-environment (Yan et al. 2007). In the study, Kukumseri exhibited a longer vector length for SR, whereas Palampur displayed a longer vector length for PM, indicating their enhanced capacity to discriminate between genotypes based on the respective diseases. Similar findings were reported in the research conducted by Mehta et al. (2022). The variation in disease intensity across different locations may be attributed to spatial evolution of pathogen pathotypes in the north-western Himalayan region or/and genetic variability among the genotypes (Aggarwal et al. 2018; Vikas et al. 2020).

The findings of our current investigation are quite promising and consistent with previous studies (Mehta et al. 2022; Vikas et al. 2020; Sood et al. 2020; Yang et al. 2017), demonstrating the development of wheat varieties showing dual resistant to SR and PM, while maintaining higher yields. In our research, we have identified wheat genotypes resistant to both diseases. This phenotypic screening was further confirmed by molecular studies using PCR amplification with a disease-specific gene-linked markers. Using GGE biplot analysis, stable genotypes with low levels of SR and PM intensities across various locations were identified. Kukumseri and Palampur have shown potential as optimal test sites for screening wheat germplasm against SR and PM, respectively. The high-yielding genotypes, possessing resistance to both diseases, can be released as varieties following multi-location trials. Furthermore, these genotypes can serve as valuable donors in future sustainable wheat improvement and can be used in molecular mapping studies and association analyses to identify and characterize new sources of resistance.

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Author contributions HKC conceptualized and planned the experiments; SV executed the experiments, drafted the manuscript and analyzed the data & HKC, SV, AB, KS, KSD, AP and MS provided suggestions and polished the manuscript.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Conflict of interest The authors declare that they have no conflict of interests regarding the publication of this paper.

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