



Available Online at EScience Press

International Journal of Phytopathology

ISSN: 2312-9344 (Online), 2313-1241 (Print)
<https://esciencepress.net/journals/phytopath>

EVALUATION OF WHEAT GERMPLASM FOR MORPHOLOGICAL AND MOLECULAR RESISTANCE TO POWDERY MILDEW

^aNasr Ullah Khan*, ^bIhteram Ullah, ^bMaham Jamshed, ^bMarium Haroon, ^cAbdul Majid, ^dAdnan Shehzad, ^eRubina Naz Qaisrani, ^fDouglas Law, ^gDaniel K.Y. Tan, ^hWael Elmenofy, ^aMuhammad Yasin*

^a Gomal Center of Biochemistry and Biotechnology, Gomal University, Dera Ismail Khan, Khyber Pakhtunkhwa, Pakistan.

^b Department of Plant Breeding and Genetics, Faculty of Agriculture, Gomal University, Dera Ismail Khan, Khyber Pakhtunkhwa, Pakistan.

^c Department of Agricultural Chemistry and Biochemistry, The University of Agriculture Peshawar, Khyber Pakhtunkhwa, Pakistan.

^d Department of Biotechnology, COMSATS University Islamabad, Abbottabad Campus, Abbottabad, Pakistan.

^e Institute of Chemical Sciences, Gomal University, Dera Ismail Khan, Khyber Pakhtunkhwa, Pakistan.

^f Faculty of Health and Life Sciences, INTI International University Nilai, Negeri Sembilan, Malaysia.

^g Plant Breeding Institute, Sydney Institute of Agriculture, School of Life and Environmental Sciences, Faculty of Science, The University of Sydney, Sydney, NSW 2006, Australia.

^h Department of Arid Land Agriculture, College of Agricultural and Food Sciences, King Faisal University, Al-Ahsa, Saudi Arabia.

ARTICLE INFO

Article History

Received: January 15, 2026

Revised: March 17, 2026

Accepted: April 23, 2026

Keywords

Powdery mildew

Bgt

Wheat landraces

Molecular markers

1000 GW

Food Production

Food security

ABSTRACT

Powdery mildew, a devastating wheat foliar disease caused by fungus *Blumeria graminis* f. sp. *tritici* (Bgt), causes global wheat yield losses in cool and humid environments. The current study aimed to characterize ninety-eight Pakistani wheat landraces along with two check cultivars for yield potential and to unravel their potential for resistance against wheat powdery mildew. Wheat was sown in Randomized Complete Block (RCB) Design at Hazara University, Mansehra, Khyber Pakhtunkhwa, Pakistan in the 2021-2022 and 2022-2023 wheat growing seasons. The study utilized STATISTIX 8.1 for statistical analysis of powdery mildew resistance and significant agronomic traits. The data revealed that 56 out of 98 landraces and check variety NARC-2009 were susceptible while 42 wheat landraces and check variety SH-2002 showed moderate to high level resistance to powdery mildew. Three promising wheat landraces with high powdery mildew resistance, short height, good seed length and high 1000 KW (thousand kernel weight) identified in this study were acc.#11126, acc.#11212 and acc.#11392. Weak but highly significant negative correlation ($r = -0.159^{***}$) of powdery mildew with thousand grain weight might suggest a probable trade-off where higher powdery mildew levels are associated with a reduction in 1000 KW. Molecular markers analysis confirmed the presence of *Pm1*, *Pm2*, *Pm3*, *Pm4* and *Pm8* in different PM-resistant wheat landraces. Based on the molecular marker analysis of 100 wheat experimental panel, the most abundant gene possessed by landraces was *Pm3* (23%), followed by *Pm2* (10%), *Pm1* (8%), *Pm4* (4%) and *Pm8* (1%). Only three wheat landraces, namely, acc.#11126, acc.#11212 and acc.#11392, simultaneously possessed two PM resistance genes. The three promising candidate wheat landraces identified in this study could be potential breeding materials for developing powdery resistant wheat varieties for Pakistan's cool and humid environments with high prevalence of powdery mildew.

*Corresponding Authors: Nasr Ullah Khan and Muhammad Yasin

Email: nasrkhanpbg@gu.edu.pk; drmywazir-biotech@gu.edu.pk

© The Author(s) 2026.

INTRODUCTION

The yield of common wheat, one of the most widely grown crops, is often challenged by biotic and abiotic stresses (Alotaibi *et al.*, 2023; Liang *et al.*, 2016). Powdery mildew (PM), caused by *Blumeria graminis* f. sp. *tritici* (Bgt) is a devastating wheat foliar disease, which results in severe yield losses globally and locally (Hao *et al.*, 2015). Powdery mildew is prevalent in regions with cool and humid climate, especially in Hazara division and northern areas of Pakistan. The deployment of powdery mildew resistant wheat cultivars is the most economical and environmentally safe strategy to curb this devastating disease (Mohler *et al.*, 2013; Zou *et al.*, 2023; Tony *et al.*, 2025; Hao *et al.*, 2026). However, the resistant cultivars are soon overcome by novel virulent strains of Bgt owing to evolutionary pressure posed by host resistance and current global climate change. Thus, there is constant need to characterize local germplasm and discover novel sources of powdery mildew resistance.

Screening of wheat genetic resources and identification of genetic variation is pre-requisite for identification, mapping, cloning and deployment of novel PM resistance genes in wheat breeding programs. The emergence of novel virulent Bgt strains caused by rapid drift in climate change and the loss of resistance of the major PM resistance loci such as *Pm1* and *Pm3* in Asia and Europe further necessitate exploration of novel wheat genetic resources for powdery mildew resistance. In general, only a few PM resistance genes have shown broad spectrum and durable resistance globally such as *Pm21* (encoding CC-NBS-LRR protein) identified in wheat wild relative *Haynaldia villosa* and introgressed into bread wheat (He *et al.*, 2018). Thus, large scale screening of different wheat genetic resources complemented by molecular markers is pivotal for identification of novel, broad spectrum and durable PM resistance genes and the deployment of these genes in developing PM resistant wheat cultivars. Molecular markers are important tools to perform genetic mapping, tagging, cloning and marker-assisted selection (MAS) in wheat breeding programs aimed at developing PM resistant wheat cultivars. Different types of molecular markers have been extensively used to identify more than 200 formally and temporarily designed PM resistance loci (genes, alleles and Quantitative Trait Loci-QTLs) in the past few decades (Xue *et al.*, 2024).

The bottleneck to using modern wheat cultivars in PM resistance wheat breeding programs is their low level of genetic diversity. The majority of modern wheat cultivars

exhibit a low level of resistance to powdery mildew. For instance, screening of 100 Pakistani wheat varieties for powdery mildew resistance produced only two varieties that were resistant to local Bgt isolates in the field (Asad *et al.*, 2014). On the contrary, wheat landraces have a diverse gene pool owing to their intraspecific genetic diversity. Wheat landraces are better adapted to local agro-climatic conditions and act as repositories of genes and elite alleles useful for biotic and abiotic stress improvement (Ullah *et al.*, 2018; Li *et al.*, 2019). However, Asian wheat landraces, including those of Pakistan, are rarely used in modern wheat breeding programs (Balfourier *et al.*, 2019). A recent report evaluated a global panel of 755 wheat landraces for powdery mildew resistance and revealed rich genetic diversity in Pakistani, Turkish and Iranian wheat landraces, and novel resistance loci to diverse powdery mildew isolates via subsetting approaches in association studies (Leber *et al.*, 2024). This study further suggested screening local wheat landraces to unlock their unexplored potential for powdery mildew resistance. Previously, more than a dozen PM resistance genes have been identified in wheat landraces such as *Pm2c*, *Pm3b*, *Pm4e*, *Pm5d*, *Pm5e*, *Pm24*, *Pm47*, *Pm59*, *Pm61*, *Pm63* and *PmXNM* (Xu *et al.*, 2015; Xue *et al.*, 2024). However, the majority of wheat landraces are still locked in local and global germplasm banks, including those in Pakistan, and have not yet been fully characterized for powdery mildew resistance. Pakistani wheat landraces constitute an untapped reservoir of powdery mildew resistance, and phenotypically resistant accessions are expected to carry detectable Pm resistance genes associated with improved agronomic performance under disease pressure. The current study aimed to characterize ninety-eight Pakistani wheat landraces along with two check cultivars for yield-related traits and to unravel their potential for resistance against wheat powdery mildew. The findings of this study are important for local wheat breeders to develop elite wheat cultivars with powdery mildew resistance and better yield-related traits for powdery mildew prevalent regions of Pakistan with cool and humid environments.

MATERIALS AND METHODS

The current research was conducted in experimental area of Hazara University, Mansehra (powdery mildew prevalent region), Pakistan in the wheat growing season of November 2021 - June 2022. All germplasm was re-evaluated at the

same single location for powdery mildew resistance in wheat growing season of November 2022 - June 2023.

Research Design

Research design layout for this study was standardized as randomized complete block design (RCBD) with three replications. The RCBD experimental design included the randomization procedure and single row plot type, resulting in 300 experimental units/plots (100 genotypes $\times$ 3 replications). Experimental area per plot was kept at 0.675 m² with 98 wheat landraces and two check (control) varieties SH-2002 and NARC-2009, grown on the grass planted area (300 plots) of 202.5 m². Seeds were manually dibbled at a plant-to-plant spacing of 15 cm, with 15 seeds sown per row. Row length and row-row spacing were 2.25 m and 30 cm, respectively. Standard agronomic practices such as fertilizer application of recommended doses of nitrogen and phosphorus, irrigation and hoeing were regularly performed. Morphological and agronomic observations were recorded from all healthy plants within each accession, while border plants, where applicable, were excluded from data collection to

minimize edge effects. Consequently, each accession was represented once in each replication, providing three independent observations for statistical analysis.

Germplasm Collection

In this study, ninety-eight wheat landraces and two check varieties (SH-2002 and NARC-2009) were acquired from National Agricultural Research Centre (NARC), Islamabad Pakistan. Detailed enlistment is provided in Table 1. Accessions with “unknown origin” is a limitation of the germplasm documentation process.

Morphological Characterization for Powdery Mildew Resistance and Yield Related Traits

Scoring powdery mildew resistance

Powdery mildew was scored as 0, 1, 2, 3, and 4 according to modified protocol of Si *et al.*, (1992). The 0, 1, 2, 3 and 4 in this scale represent no visible symptoms, visible necrosis without sporulation, sparse sporulation, abundant sporulation, and abundant sporulation with mycelia covering more than 80% of the leaf area, respectively. A score of 0–2 was considered as resistant and 3–4 as susceptible (Figure 1).

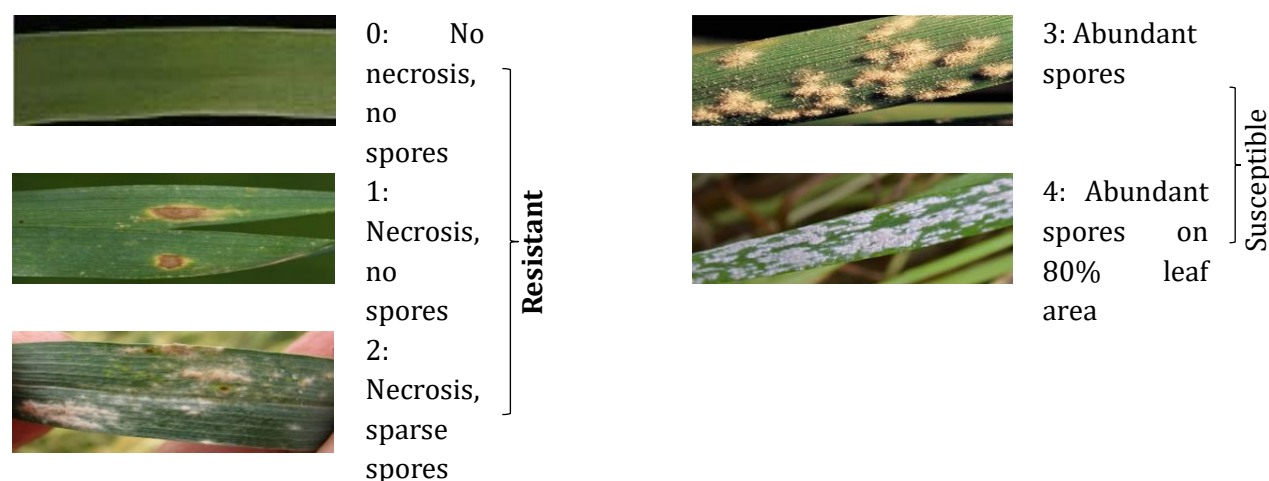


Figure 1. Powdery mildew scoring at Hazara University wheat field experiment.

Plant height (cm)

Plant height data was taken after all the wheat plants matured. Individual selected plants were subjected to measurement from soil surface to the top of ear exclusive of the awns and average was estimated.

Spike length (cm)

Spike length of the randomly chosen plants was calculated after measuring the spike from base to the pinnacle of spike exclusive of awns and further analyzed on their average calculated.

Seed length (mm)

Seed length was estimated with the help of digital Vernier caliper of three randomly selected seeds and further analyzed on their average calculated.

1000-kernel weight (g)

At the stage of physiological maturity, 1000 kernel weight (1000 KW) were calculated from the randomly selected plants, and their weight was taken in grams.

Table 1. Detail of wheat germplasm used.

Sr.	Acc.#	Remarks	Geographic Origin	Sr.	Acc.#	Remarks	Geographic Origin
1	010725	LU-26-S	Pakistan	51	011181	000216(01)	Pakistan, Kharan, Balochistan
2	010726	S-1406	Pakistan	52	011183	000218(01)	Pakistan, Kharan, Balochistan
3	010730	SOFTYXTOB'S'	Pakistan	53	011185	000220(01)	Pakistan, Kharan, Balochistan
4	010731	C-273	Pakistan	54	011189	000223(02)	Pakistan, Chagai, Balochistan
5	010810	SVP-75	Pakistan	55	011190	000224(01)	Pakistan, Chagai, Balochistan
6	010811	SVP-75	Pakistan	56	011199	000226(06)	Pakistan, Ziarat, Balochistan
7	010865	SVP-117	Pakistan	57	011209	000234(01)	Pakistan, Ziarat, Balochistan
8	010866	SVP-116	Pakistan	58	011212	000237(02)	Pakistan, Ziarat, Balochistan
9	010871	SVP-141	Pakistan	59	011214	000239(03)	Pakistan, Loralai, Balochistan
10	010890	SVP-150	Pakistan	60	011219	000242(09)	Pakistan, Loralai, Balochistan
11	010891	SVP-150	Pakistan	61	011234	000250(07)	Pakistan, Sibi, Balochistan
12	010895	SVP-153	Pakistan	62	011236	000253(01)	Pakistan, Sibi, Balochistan
13	010940	SVP-18	Pakistan	63	011238	000254(06)	Pakistan, Sibi, Balochistan
14	010941	SVP-19	Pakistan	64	011239	000254(07)	Pakistan, Sibi, Balochistan
15	010945	SVP-26	Pakistan	65	011242	000257(13)	Pakistan, Sibi, Balochistan
16	010951	SVP-29	Pakistan	66	011249	000261(05)	Pakistan, Quetta, Balochistan
17	010957	SVP-88	Pakistan	67	011250	000262(02)	Pakistan, Quetta, Balochistan
18	011016	WP124,37P8 N-634	Pakistan	68	011255	000265(02)	Pakistan, Quetta, Balochistan
19	011021	WP317,PV2P6	Pakistan	69	011256	000265(05)	Pakistan, Quetta, Balochistan
20	011022	WP242,11P10 N-627	Pakistan	70	011260	000267(05)	Pakistan, Quetta, Balochistan
21	011027	WP557,211 10	Pakistan	71	011262	000350(03)	Pakistan, Qila Abdullah, Balochistan
22	011041	WP571,AK-4	Pakistan	72	011274	000362(01)	Pakistan, Pishin, Balochistan
23	011042	WP572,AK-5	Pakistan	73	011280	000365(04)	Pakistan, Pishin, Balochistan
24	011046	WP576,AK-10	Pakistan	74	011286	000373(03)	Pakistan, Pishin, Balochistan
25	011061	WP591,AK-26	Pakistan	75	011296	000384(01)	Pakistan, Chagai, Balochistan
26	011062	WP592,AK-29	Pakistan	76	011306	000392(03)	Pakistan, Chagai, Balochistan
27	011063	WP594,AK-31	Pakistan	77	011311	000394(04)	Pakistan, Chagai, Balochistan
28	011066	WP597,AK-34	Pakistan	78	011324	000406(04)	Pakistan, Quetta, Balochistan
29	011067	WP598,AK-35	Pakistan	79	011332	000426(03)	Pakistan, Ziarat, Balochistan
30	011071	WP602,AK-39	Pakistan	80	011351	Punjab Type-9	Pakistan, Faisalabad, Punjab
31	011072	WP603,AK-40	Pakistan	81	011383	000487(03)	Pakistan, NaushahroFiroz, Sindh
32	011076	WP607,AK-49	Pakistan	82	011387	000490(09)	Pakistan, Larkana, Sindh
33	011077	WP608,AK-50	Pakistan	83	011388	000490(10)	Pakistan, Larkana, Sindh
34	011078	WP609,AK-51	Pakistan	84	011389	000599(05)	Pakistan, Bannu, KP
35	011079	WP610,AK-53	Pakistan	85	011392	000594(04)	Pakistan, D.I.Khan, KP
36	011081	WP612,AK-56	Pakistan	86	011395	000507(03)	Pakistan, Larkana, Sindh

37	011091	WP623,AK-72	Pakistan	87	011397	000592(02)	Pakistan, D.I.Khan, KP
38	011101	WP636,NPE-3	Pakistan	88	011398	000585(05)	Pakistan, Bhakkar, Punjab
39	011116	WP649,NPE-27	Pakistan	89	011407	000523(07)	Pakistan, Jacobabad, Sindh
40	011121	WP657,N-15E(1) 28P7	Pakistan	90	011452	000478(04)	Pakistan, Hyderabad, Sindh
41	011126	WP666,N1010E(4) 28P4	Pakistan	91	011491	000890(03)	Pakistan, Okara, Punjab
42	011136	WP679,N-691(D32)15P6	Pakistan	92	011513	000980(02)	Pakistan, Parachinar, KP
43	011145	000177(02)	Pakistan, Pishin, Balochistan	93	011523	000890(03)	Pakistan, Okara, Punjab
44	011154	000196(02)	Pakistan, Quetta, Balochistan	94	011524	001055(02)	Pakistan, Nawabshah, Sindh
45	011166	000207(01)	Pakistan, Kalat, Balochistan	95	011612	001362(04)	Pakistan, Swat, KP
46	011169	000210(05)	Pakistan, Kalat, Balochistan	96	011613	001364(06)	Pakistan, Swat, KP
47	011174	000212(01)	Pakistan, Kalat, Balochistan	97	011705	82/1	Pakistan, Faisalabad, Punjab
48	011175	000212(02)	Pakistan, Kalat, Balochistan	98	011706	82/3	Pakistan, Faisalbad, Paunjab
49	011179	000215(03)	Pakistan, Kharan, Balochistan	99	SH-2002	INQ91/FINK'S'	Pakistan
50	011180	000215(04)	Pakistan, Kharan, Balochistan	100	NARC-2009	INQALAB 91*2/TUKURU	Pakistan

DNA Extraction and PCR Amplification

DNA extraction from all 100 wheat germplasm using the leaves of young seedlings and their PCR amplification was performed with the reported procedures (Ma *et al.*, 1994; Ullah *et al.*, 2018). Polymerase chain reaction of markers was performed in PE9600 thermal cycler (Perkin Elmer), in a volume of 10 µL containing 10–20 ng of template, 2 pmol of each of the primers, 2 nmol of each of the deoxynucleoside triphosphates, 15 nmol of MgCl₂, 0.1 U Taq DNA polymerase, and 1 × PCR buffer. The PCR profile was set as one cycle of 94 °C for 3 min, followed by 36 cycles of 94 °C for 30 s, 50–60 °C (depending on the specific primers) for 40 s, and 72 °C for 50 s, and a final extension at 72 °C for 5 min. PCR products from amplification with publicly available candidate markers of most prevalent powdery mildew resistance genes *Pm1*, *Pm2*, *Pm3*, *P4* and *Pm8* (Table 2) were separated on a 1% agarose gel, and viewed under ultra-violet (UV) light after staining with ethidium bromide.

Statistical analysis

All statistical analyses were performed using Statistix version 8.1 (Analytical Software, Tallahassee, FL, USA). Data recorded for powdery mildew resistance and agronomic traits were subjected to analysis of variance (ANOVA) according to the Randomized Complete Block Design (RCBD) model:

$$Y_{ij} = \mu + G_i + R_j + \epsilon_{ij}$$

where Y_{ij} is the observed value, μ is the overall mean, G_i is the effect of the i th genotype, R_j is the effect of the j th replication, and ϵ_{ij} is the experimental error. When significant differences among genotypes were detected, means were separated using the Least Significant Difference (LSD) test at the 5% probability level. Pearson's correlation coefficients were calculated to determine associations among powdery mildew resistance and agronomic traits. Statistical significance was evaluated at $P \leq 0.05$ and $P \leq 0.01$.

RESULTS

Phenotypic Characterization for Powdery Mildew Resistance

Powdery mildew resistance of ninety-eight wheat landraces and two varieties was scored at seedling and adult stages for two consecutive years at Hazara University, Mansehra as shown in Figure 1. Different landraces and varieties responded differently to Bgt isolates in the cool, humid field of Hazara University. All five types of resistant (highly resistant, resistant, moderately resistant) and susceptible (moderately susceptible, highly susceptible) phenotypes were observed in the field (Figure 1).

As shown by Figure 2, among all germplasm tested, check variety NARC-2009 was highly susceptible with score 4 while 56 landraces were moderately to highly susceptible (36 moderately susceptible with score 3 and 20 highly susceptible with score 4).

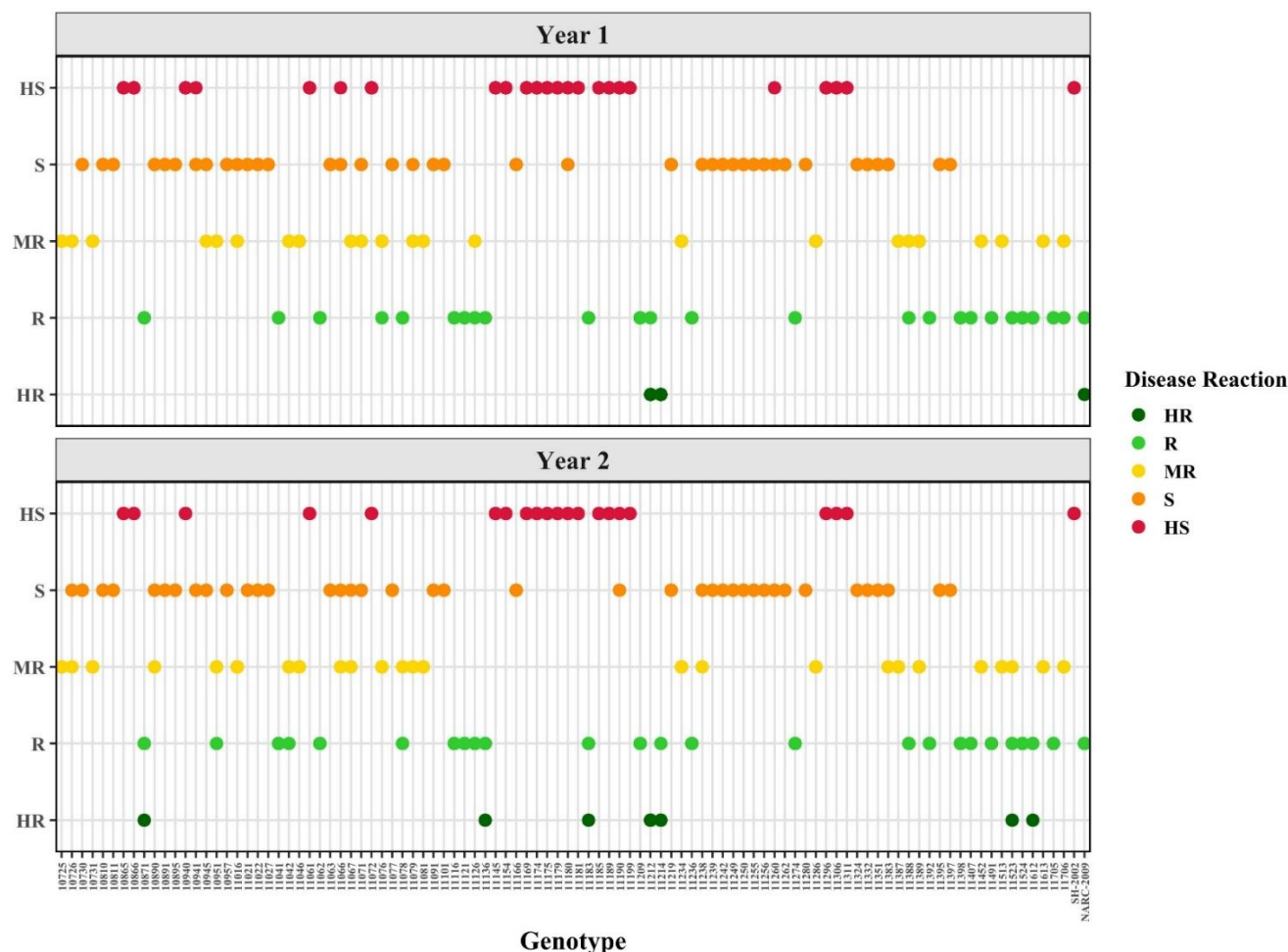


Figure 2 Powdery mildew status of 100 wheat germplasm in the field. Powdery mildew score 0-2: Resistant, 3-4: Susceptible. Year 1 (2021-2022) and year 2 (2022-2023).

Similarly, 42 out of 98 landraces were resistant to powdery mildew in which 19 wheat landraces were moderately resistant (score: 2), 21 resistant (score: 1) and 2 were highly resistant (score: 0). Check variety SH-2002 was resistant to powdery mildew with score 1. Two of the immune landraces from Ziarat (11212) and Loralai (11214) were extremely resistant without having spores or necrosis (Supplementary Table 1). Consistency of powdery mildew resistance versus susceptibility prevailed among all landraces across three replications at Hazara University research station. All tested genotypes showed consistency in powdery mildew resistance versus susceptibility for both years (Supplementary table 1).

Agronomic Potential

Powdery mildew resistance cultivars were further screened for traits with better yield characters such as plant height, spike length, seed length and 1000 kernel

weight. A two-year combined analysis of variance (ANOVA) revealed highly significant differences ($p < 0.001$) among all wheat genotypes for powdery mildew, plant height, spike length, seed length and 1000 KW. The non-significant year x genotype interaction for all traits, except spike length, indicates substantial genetic variability and stable expression of traits across two years, thereby supporting their potential for effective selection in the breeding program (Table 3). Landrace 11189 had minimum mean seed length of 5.58 mm and 5.66 mm, and landrace 11212 had maximum of 8.34 mm and 8.65 mm for 2021/22 and 2022/23, respectively (Figure 3). Landrace 11091 had a minimum mean 1000 KW of 30.03 g and 31.8 g, and Landrace 11212 had maximum mean 1000 KW of 59.43 g and 61.23 g for 2021/22 and 2022/23, respectively (Figure 4). Landrace 10810 had a minimum mean spike length of 7.07 cm and

7.34 cm, and Landrace 11214 had maximum of 16.15 cm and 16.84 cm for 2021/22 and 2022/23, respectively (Figure 5). Similarly, landrace 11260 had a minimum of 62.76 cm and 62.2 cm mean plant height, and landrace 11311 had maximum of 114.53 cm and 112.02 cm mean plant height for 2021/22 and 2022/23, respectively (Figure 6). Based on the above-mentioned traits scored

for two years, it is evident that landrace 11212 from Ziarat Balochistan with highly resistant response to powdery mildew, semi-dwarf height (78.1 cm), maximum spike length (16.4 cm), seed length (8.49 mm) and 1000 KW (60.33 g) suggests it is a promising candidate among all germplasm tested in this study.

Table 3. Analysis of Variance (ANOVA) for powdery mildew and yield related traits.

Source	PM	PH	SPL	SDL	1000 KW
Genotype	7.36***	760.24***	641.88***	2.00***	207.18***
Year	0.13ns	103.48ns	13.15***	9.96***	289.39***
Replication	0.02ns	0.957ns	0.10ns	0.05ns	0.78ns
Genotype*Year	0.051ns	5.03ns	1.27***	0.03ns	1.19ns
Error	0.053	4.316	0.109	0.029	1.18
F-Value	133.14	181.26	5640.7	75.79	186.16
P-Value	<0.001	<0.001	<0.001	<0.001	<0.001
LSD (5%)	0.370680053	3.345045266	0.531587218	0.274195473	1.749050241

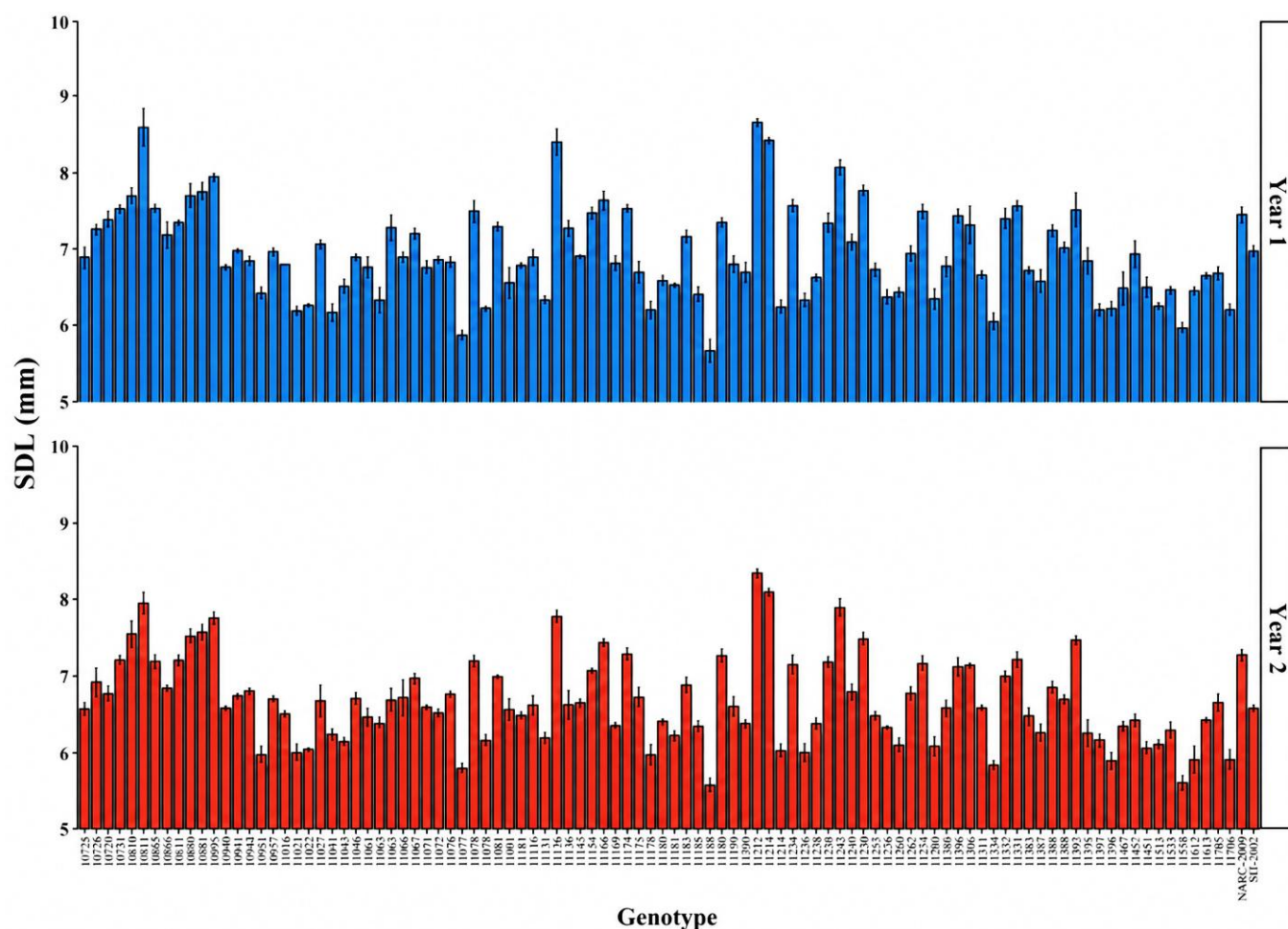


Figure 3. Comparative performance of 100 wheat genotypes for seed length. Values on Y-axis show seed length (mm) and genotypes are on X-axis. Year 1 (2021-2022) and year 2 (2022-2023).

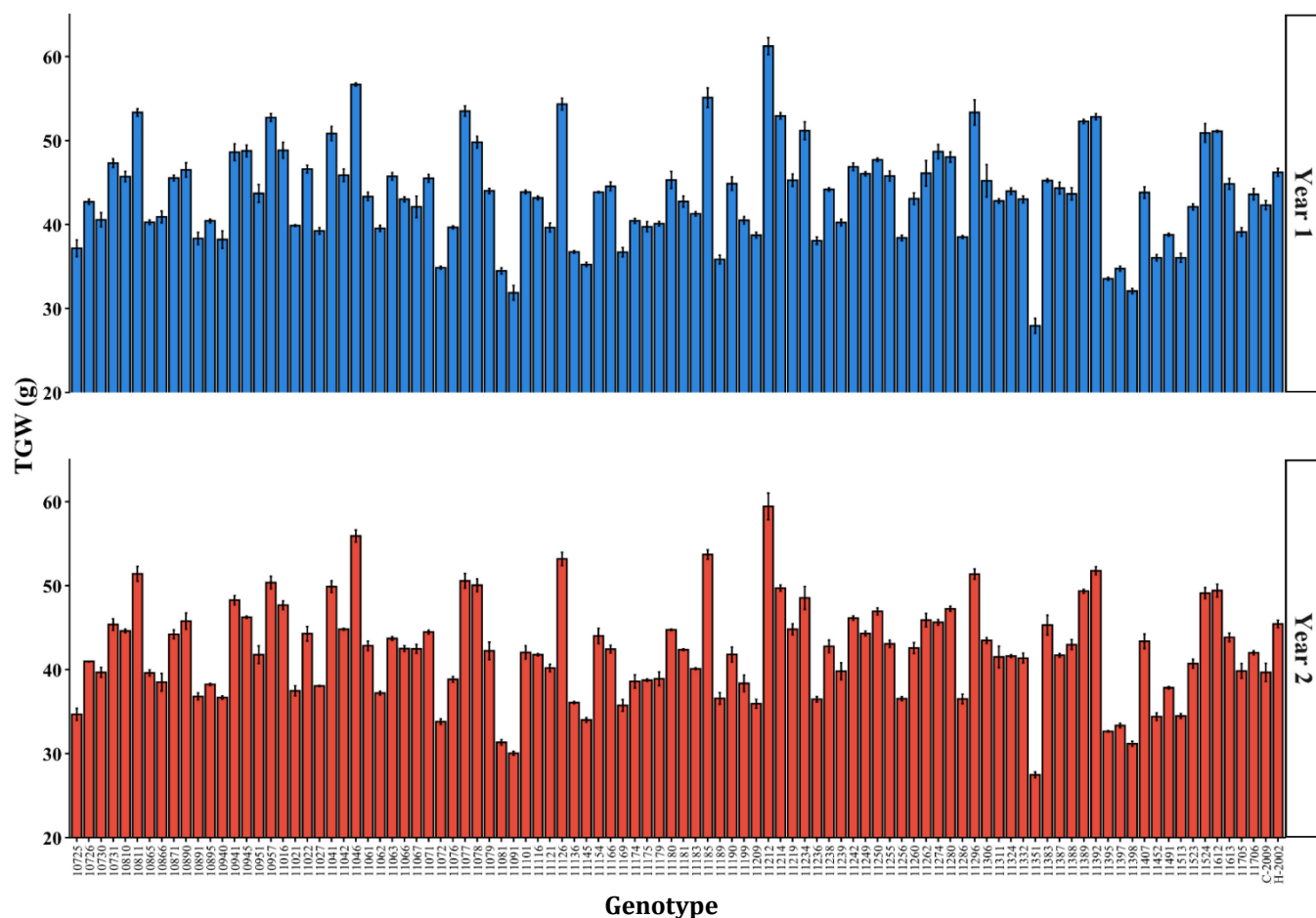


Figure 4. Comparative performance of 100 wheat genotypes 1000 kernel weight (1000 KW). Values on Y-axis show 1000 KW (g) and genotypes are on X-axis. Year 1 (2021-2022) and year 2 (2022-2023).

Molecular Characterization for Powdery Mildew Resistance Genes

Five Pm genes including *Pm1*, *Pm2*, *Pm3*, *Pm4* and *Pm8* were chosen as candidates and the primer sequences of their functional markers were used to screen 100 germplasm with the help of PCR. These five genes were chosen as candidates owing to their widespread

application in local and global breeding programs for developing powdery mildew resistant cultivars. Another reason to choose these genes is their successful cloning in the past decades and availability of their functional markers (Kang *et al.*, 2020). The markers names, their primer sequences and expected amplicon sizes are given in Table 2.

Table 2. Marker information for Pm1, Pm2, Pm3, Pm4 and Pm8.

Marker	Type	Pm gene	F- and R-primers	Band size	Reference
<i>Pm1aSTS1</i>	STS	<i>Pm1</i>	CAATATAAACTTCAGATGTTCT AT CTACATTGGCTATGCGTGTAG TC	333bp	Hewitt <i>et al.</i> , 2021
<i>TaPm2a</i>	Functional	<i>Pm2</i>	GGTTTGAATCCAAGAGATGATGCATATTTGAT TAAGTGGAATAGCTCAATCTGAGAACCTT	212bp	Chen <i>et al.</i> , 2019
<i>STSPm3</i>	Functional	<i>Pm3</i>	TGGTTGCACAGACAATCC GTAGCTAAGCTTGAGTATTGGCA	946bp	Srichumpa <i>et al.</i> , 2005
<i>TaPm4_V1</i>	Functional	<i>Pm4</i>	TAGGTTGGAGAGATCACAACGA CTGAGGTAGAGGAGGCAACTT	179bp	Sánchez-Martín <i>et al.</i> , 2021
<i>sfr43</i>	Functional	<i>Pm8</i>	TGGCTTCCAACAGCCCTAGC AGGCTTTTGCACCTTCTCTC	662bp	Hurni <i>et al.</i> , 2013

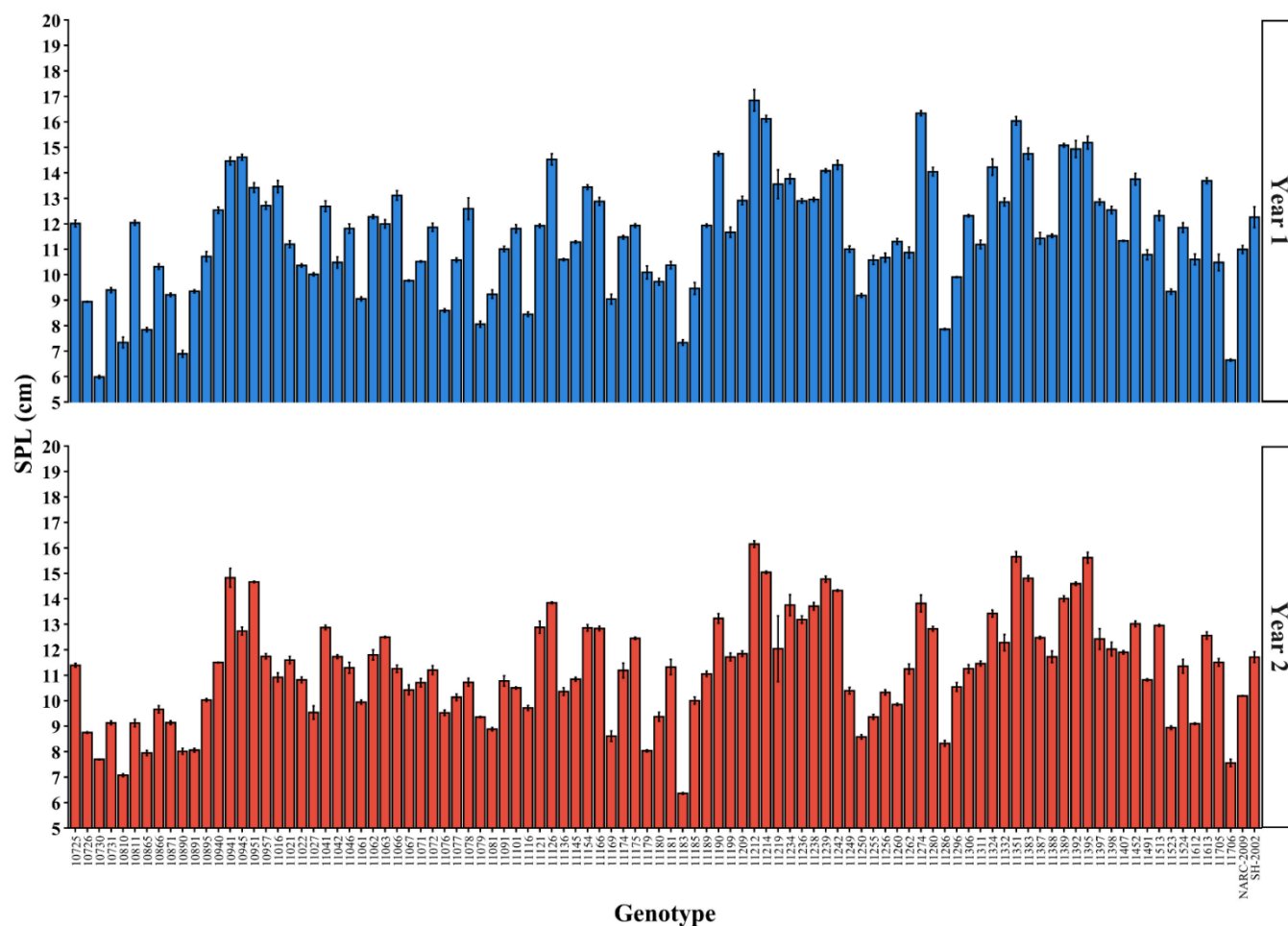


Figure 5. Comparative performance of 100 wheat genotypes for spike length. Values on Y-axis show spike length (cm) and genotypes are on X-axis. Year 1 (2021-2022) and year 2 (2022-2023).

All five Pm genes were identified in different landraces and check varieties used in this study. The most abundant gene possessed by landraces was Pm3 (23%), followed by Pm2 (10%), Pm1 (8%), Pm4 (4%) and Pm8 (1%). The Pm gene frequency data set represents the frequency of the respective Pm genes in the entire tested wheat panel (N = 100) (Table 4, Figure 7). Some of the germplasm possessed a single PM resistance gene while some possessed two at a time. None of the landraces or varieties possessed more than two Pm genes together. None of the susceptible lines possessed Pm genes except landraces 11169 and 11174 which despite possessing Pm1 were highly susceptible (Table 4).

Correlation of powdery mildew and agronomic traits

As shown by Figure 8, several traits exhibited highly significant positive associations ($p < 0.001$). For instance,

the highly significant positive correlation was observed between SDL and TGW ($r = 0.294^{***}$), suggesting that increases in seedling length or related developmental traits positively influence 1000 KW. In contrast, significant negative correlations were identified between specific trait pairs ($p < 0.001$). Pm was highly significantly negatively correlated with 1000 KW ($r = -0.159^{***}$). Although the magnitude of this association was very small, accounting for only approximately 2.5% of the variation, it might confer a probable trade-off where higher Pm levels are associated with a reduction in 1000 KW. Plant height exhibited the same trend (Figure 8). Overall, all five traits demonstrated consistency in correlation across both seasons.

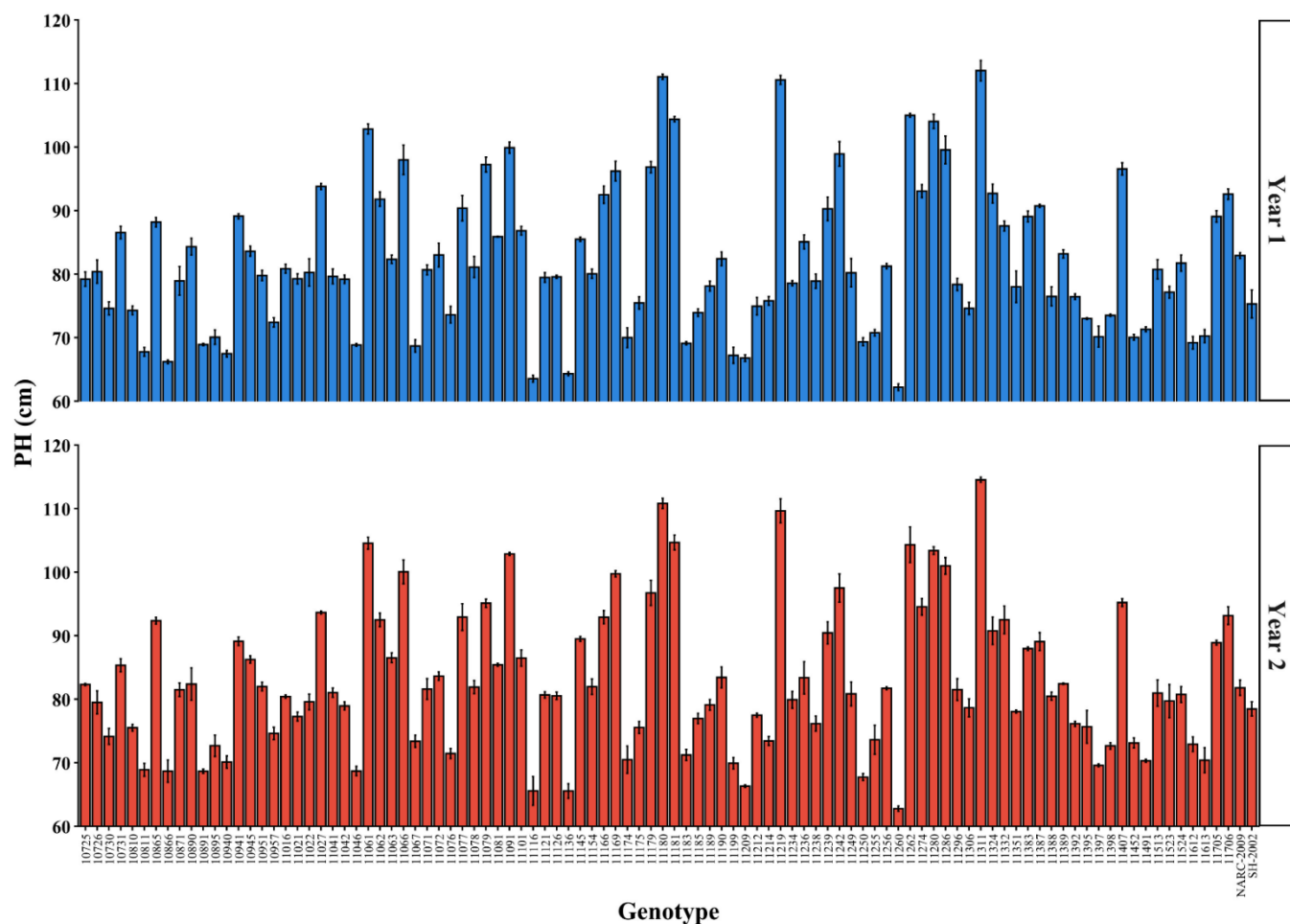


Figure 6 Comparative performance of 100 wheat genotypes for plant height (cm). Values on Y-axis show plant height (cm) and genotypes are on X-axis. Year 1 (2021-2022) and year 2 (2022-2023).

Table 4. Distribution of Pm genes in Pakistani wheat landraces.

Sr.	Acc.#	PM score	PM status	Pm1	Pm2	Pm3	Pm4	Pm8	Sr.	Acc.#	PM score	PM status	Pm1	Pm2	Pm3	Pm4	Pm8
1	10725	2	MR	-	+	-	-	-	51	11181	4	HS	-	-	-	-	-
2	10726	2	MR	-	+	-	-	-	52	11183	1	R	-	-	+	-	-
3	10730	3	MS	-	-	-	-	-	53	11185	4	HS	-	-	-	-	-
4	10731	2	MR	-	-	+	-	-	54	11189	4	HS	-	-	-	-	-
5	10810	3	MS	-	-	-	-	-	55	11190	4	HS	-	-	-	-	-
6	10811	3	MS	-	-	-	-	-	56	11199	4	HS	-	-	-	-	-
7	10865	4	HS	-	-	-	-	-	57	11209	1	R	-	+	-	-	-
8	10866	4	HS	-	-	-	-	-	58	11212	0	HR	-	+	+	-	-
9	10871	1	R	-	+	-	-	-	59	11214	0	HR	-	-	+	-	-
10	10890	3	MS	-	-	-	-	-	60	11219	3	MS	-	-	-	-	-
11	10891	3	MS	-	-	-	-	-	61	11234	2	MR	-	-	+	-	-
12	10895	3	MS	-	-	-	-	-	62	11236	1	R	-	-	+	-	-
13	10940	4	HS	-	-	-	-	-	63	11238	3	MS	-	-	-	-	-
14	10941	3	MS	-	-	-	-	-	64	11239	3	MS	-	-	-	-	-
15	10945	3	MS	-	-	-	-	-	65	11242	3	MS	-	-	-	-	-
16	10951	2	MR	-	-	+	-	-	66	11249	3	MS	-	-	-	-	-
17	10957	3	MS	-	-	-	-	-	67	11250	3	MS	-	-	-	-	-
18	11016	2	MR	-	+	-	+	-	68	11255	3	MS	-	-	-	-	-

19	11021	3	MS	-	-	-	-	-	69	11256	3	MS	-	-	-	-	-
20	11022	3	MS	-	-	-	-	-	70	11260	3	MS	-	-	-	-	-
21	11027	3	MS	-	-	-	-	-	71	11262	3	MS	-	-	-	-	-
22	11041	1	R	-	-	+	-	-	72	11274	1	R	-	-	+	-	-
23	11042	2	MR	-	-	-	+	-	73	11280	3	MS	-	-	-	-	-
24	11046	2	MR	+	-	-	-	-	74	11286	2	MR	-	-	+	-	-
25	11061	4	HS	-	-	-	-	-	75	11296	4	HS	-	-	-	-	-
26	11062	1	R	-	-	+	-	-	76	11306	4	HS	-	-	-	-	-
27	11063	3	MS	-	-	-	-	-	77	11311	4	HS	-	-	-	-	-
28	11066	3	MS	-	-	-	-	-	78	11324	3	MS	-	-	-	-	-
29	11067	2	MR	-	-	+	-	-	79	11332	3	MS	-	-	-	-	-
30	11071	3	MS	-	-	-	-	-	80	11351	3	MS	-	-	-	-	-
31	11072	4	HS	-	-	-	-	-	81	11383	3	MS	-	-	-	-	-
32	11076	2	MR	-	+	-	-	-	82	11387	2	MR	-	+	-	-	-
33	11077	3	MS	-	-	-	-	-	83	11388	1	R	-	-	+	-	-
34	11078	1	R	-	-	+	-	-	84	11389	2	MR	+	-	-	-	-
35	11079	2	MR	-	+	-	-	-	85	11392	1	R	+	+	-	-	-
36	11081	2	MR	-	-	+	-	-	86	11395	3	MS	-	-	-	-	-
37	11091	3	MS	-	-	-	-	-	87	11397	3	MS	-	-	-	-	-
38	11101	3	MS	-	-	-	-	-	88	11398	1	R	-	-	+	-	-
39	11116	1	R	-	-	-	+	-	89	11407	1	R	-	-	+	-	-
40	11121	1	R	-	-	-	-	-	90	11452	2	MR	-	-	-	-	-
41	11126	1	R	-	-	+	+	-	91	11491	1	R	+	-	-	-	-
42	11136	1	R	-	-	+	-	-	92	11513	2	MR	+	-	-	-	-
43	11145	4	HS	-	-	-	-	-	93	11523	1	R	-	-	+	-	-
44	11154	4	HS	-	-	-	-	-	94	11524	1	R	-	-	+	-	-
45	11166	3	MS	-	-	-	-	-	95	11612	1	R	+	-	-	-	-
46	11169	4	HS	+	-	-	-	-	96	11613	2	MR	-	-	-	-	-
47	11174	4	HS	+	-	-	-	-	97	11705	1	R	-	-	+	-	-
48	11175	4	HS	-	-	-	-	-	98	11706	2	MR	-	-	+	-	-
49	11179	4	HS	-	-	-	-	-	99	SH- 2002	1	R	-	-	-	-	+
50	11180	4	HS	-	-	-	-	-	100	NARC- 2009	4	HS	-	-	-	-	-

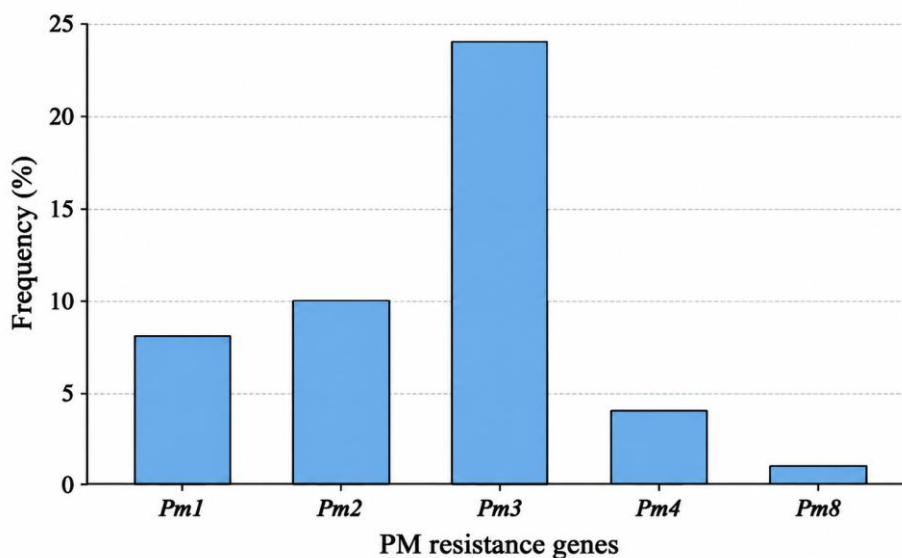


Figure 7. Relative distribution of powdery resistance genes in Pakistani wheat germplasm. Pm gene frequencies are based on the 100 wheat genotypes/accessions evaluated in the complete experimental panel (N = 100), including the check cultivars SH-2002 and NARC-2009.

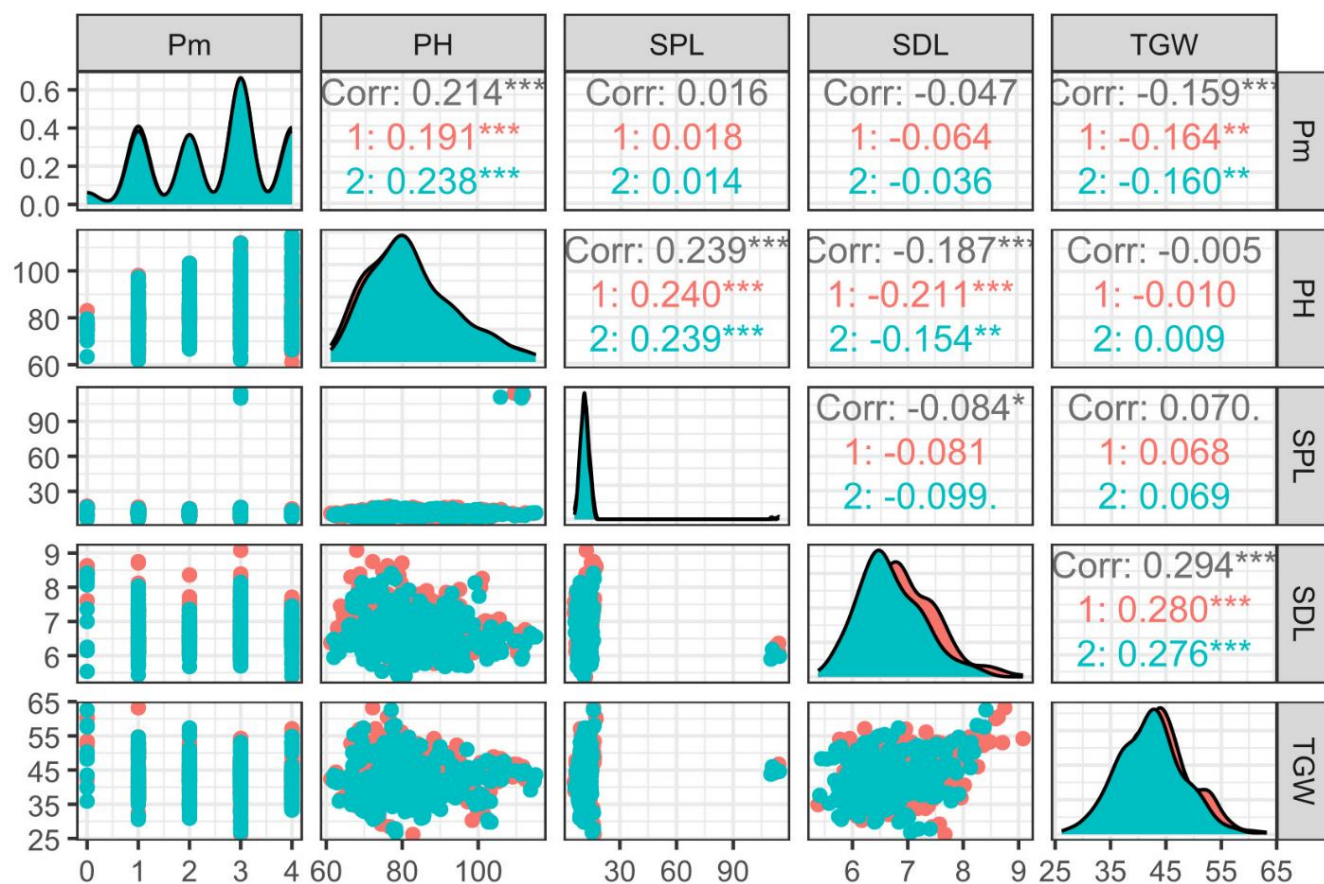


Figure 8. Correlation of powdery mildew resistance and agronomic traits. PH: Plant height, SDL: Seed length, SPL: Spike length, PM: Powdery mildew resistance, 1000 KW: 1000 kernel weight, NS: Non significant, **,***: significant at p-value < 0.01 and 0.001. 1: 2021-2022, 2: 2022-2023.

DISCUSSION

Wheat landraces are important wheat genetic resources and act globally as repositories of genes conferring resistance to various wheat diseases such as rust and powdery mildew (Lodhi *et al.*, 2020; Nadeem *et al.*, 2021). Globally, phenotypic screening of wheat landraces in the field (against mixture of Bgt strains) and disease nurseries (against controlled single Bgt isolates) revealed a diverse spectrum of resistance and susceptibility. The phenotypic resistance complemented by molecular resistance analysis revealed the identification of several pm-resistance loci such as *Pm2*, *Pm3*, *Pm5* and *Pm24* (Bapela *et al.*, 2023). Nearly half (42%) of the wheat landraces identified as powdery mildew resistant in the current study necessitates the large scale phenotypic and molecular analysis for powdery mildew resistance of the wheat landraces locked in global seed banks. Such large-scale screening will broaden the genetic base of wheat

germplasm against newly emerging virulent Bgt strains in the era of constantly changing climate. Since the resistance was evaluated only against the naturally occurring local Bgt population, the results should not be generalized to all geographical regions. Controlled inoculation using characterized isolates will be required for validation.

Wheat landraces, cultivated for thousands of years in extreme environments, possess stable yield and contribute genes/alleles of agronomic importance and resistance against abiotic and biotic stresses (Ullah *et al.*, 2018). Besides stable yield and yield related attributes, wheat landraces contributed more than dozens of genes and alleles conferring race-specific powdery mildew resistance. For instance, the identification of *Pm47* in Chinese wheat landrace Hongyanglazi (Xiao *et al.*, 2013) and *Pm24* in landrace Baihulu (Xue *et al.*, 2012) justify the utilization of wheat landraces in wheat breeding

programs aimed at developing powdery mildew resistance. Both these *Pm* genes were later transferred to elite Chinese cultivar Yangmai158 with the help of molecular markers. One wheat landrace identified in our study, 11212, conferred high phenotypic resistance and harbored *Pm2* and *Pm3*. This landrace exhibited comparatively promising yield related attributes such as semi-dwarf height (78.1 cm), spike length (16.4 cm), seed length (8.49 mm) and 1000 KW (60.33 g). This landrace can be utilized as a promising candidate breeding parent. Very few studies have investigated phenotypic and molecular resistance of Pakistan's indigenous wheat germplasm against powdery mildew. For instance, investigation of the phenotypic resistance of historic Pakistani cultivars against powdery mildew in an open field experiment at Kaghan Valley, a powdery mildew hot spot in Pakistan, showed that all historic wheat cultivars, except SH-2002 and Parwaz-94, revealed moderate to high susceptibility to the local Bgt isolates in open field condition (Asad *et al.*, 2014). Similarly, investigation of the phenotypic and molecular resistance of European wheat cultivars against powdery mildew in 38 different open field environments of northern region (powdery mildew prevalent region) of Pakistan identified several *Pm* genes. Phenotypic resistance followed by molecular markers analysis revealed the presence of powdery mildew resistant genes *Pm8*, *Pm38* and *Pm39* in the European wheat cultivars (Iqbal *et al.*, 2023). Our study is the first report on phenotypic and molecular screening of Pakistan's indigenous wheat landraces against powdery mildew and offer opportunities to utilize the powdery mildew resistant candidate wheat landraces in breeding programs. The race-specific characterization was beyond the scope of this study.

Race-specific powdery mildew resistance confers resistance against specific strain of Bgt. In the current study, majority of the powdery mildew resistant landraces possessed single *pm* gene while few possessed two genes simultaneously. None of the landraces or varieties possessed more than two *Pm* genes together. None of the susceptible lines possessed *Pm* genes except landraces 11169 and 11174 which despite possessing *Pm1* were highly susceptible. The *Pm1* gene encodes a classic race-specific, major immune receptor (NLR protein) that works on a "gene-for-gene" basis. It only triggers a hypersensitive defense response if it recognizes a specific matching avirulence effector (*AvrPm1*) secreted by the fungus. There could be several possible

explanations to this phenotype-genotype mismatch of *Pm1* in 11169 and 11174, including genetic background effects (Gallois *et al.*, 2018), marker-gene recombination (Tailor and Bhatla, 2024), quantitative resistance (Corwin and Kliebenstein, 2017), modifier genes (Yin *et al.*, 2019) and heterogeneous pathogen populations (Clin *et al.*, 2022). Previous reports demonstrated that wheat genotypes carry a non-functional or mutated allele of R-gene (a pseudogene or silent allele) that contains a premature stop codon, frameshift mutation, or transposon insertion blocking proper protein transcription or translation. The molecular marker may still bind to a conserved flank or region of the DNA, resulting in a "positive" band or signal, even though the plant fails to produce a functional, active receptor protein (Kang *et al.*, 2020). In addition to disease resistance, studies have shown that mutated alleles produced and amplified band on agarose gel, yet contributing less to the phenotypic trait. For instance, the wild type NAM-B1 allele causes higher accumulation of Zn and Fe in wheat grain. The mutant NAM-B1 allele, though with amplify specific band on agarose gel, failed to accumulate higher Zn and Fe contents in wheat kernel (Uauy *et al.*, 2006; Shoormij *et al.*, 2023). Besides, non-specific amplification, recombination between the marker locus and the functional resistance gene, or sequence variation affecting marker-gene association could potentially produce a marker-positive but phenotypically susceptible genotype. In the *Pm1* case of this study, we suggest that marker detection should be interpreted as evidence of the presence of the marker-associated genomic region rather than definitive proof of a functional resistance allele. Race-specific characterization followed by molecular markers analysis along with amplified locus sequencing is recommended to resolve the phenotype-genotype mismatch in these two landraces.

The abundantly present *Pm3* gene coupled by stable resistance in Pakistani landraces suggests its exploitation in local breeding programs. Previous studies have identified the presence of *Pm4* gene alone or in combination in several landraces, cultivars and synthetic hexaploid wheat (Hysing *et al.*, 2007; Rafique *et al.*, 2017). Both these studies support the presence of *Pm4* alleles identified in several Pakistani wheat landraces in this study. Similarly, *Pm3* alleles (*Pm3CS*) have been identified in three landraces, namely IG41606, IG41554 and AUS4856 belonging to Baluchistan province of Pakistan *Pm3CS*. The presence of *Pm3CS* in the latter two landraces

was found to be the ancestral *Pm3* gene which later on diverged into a multi-allelic locus containing 17 different alleles of *Pm3* distributed in global wheat landrace pool (Bhullar *et al.*, 2009). The presence of *Pm8* in variety SH-2002 in this study might be attributed to the fact that *Pm8* originated from rye as wheat rye 1BL.1RS line. This wheat rye translocation has widely been applied in wheat breeding programs in Pakistan and India (Khan *et al.*, 2013). The presence of *Pm8* in SH-2002 and not in landraces supports the hypothesis that the *Pm8* in SH-2002 might be the consequence of 1BL.1RS breeding. Previously, *Pm8* has been demonstrated to be a homoeologous gene of *Pm3* (Hurni *et al.*, 2013). The reason for screening these five R-genes *Pm1*, *Pm2*, *Pm3*, *Pm4* and *Pm8* is that they are widely distributed in wheat germplasm, being major powdery mildew resistance loci, cloned and functionally validated (Yahiaoui *et al.*, 2004; Hurni *et al.*, 2013; Sánchez-Martín *et al.*, 2016; Hewitt *et al.*, 2021; Sánchez-Martín *et al.*, 2021). Different studies identified novel variants of these cloned genes (Jin *et al.*, 2018; Chen *et al.*, 2019; Manser *et al.*, 2021; Jin *et al.*, 2022; Kunz *et al.*, 2026; Saqlain *et al.*, 2026), offering possibility to broaden the narrow base of wheat powdery mildew resistance. Since only five major resistance genes were screened in this study, resistant accessions lacking these markers may possess novel resistance loci or additional *Pm* genes requiring future investigation. This hypothesis is supported by the fact that some landraces lacking *Pm1*, *Pm2*, *Pm3*, *Pm4* and *Pm8* alleles in this study still conferred resistance to powdery mildew, such as 11121, 11452 and 11613.

Since the study relies on natural infection, a morphological or molecular confirmation of *Blumeria graminis* f. sp. *tritici* is further recommended to confirm these findings. A more rigorous investigation is needed to screen Pakistani wheat landraces in mixed Bgt environment the field and in the presence of single isolates in the greenhouse. Such resistance spectrum analysis might uncover novel *Pm* alleles in Pakistani wheat landraces. Besides resistance spectrum analysis, Genome-Wide Association Study (GWAS) and multi-site experiments may also reveal a new spectrum of alleles in Pakistani wheat landrace pool.

Thousand kernel weight is a critical yield contributing trait in wheat and is highly affected by disease incidence. The highly significant negative correlation of powdery mildew with 1000 KW suggests that yield contributing traits such as TGW are adversely affected by powdery

mildew, especially in heavy and prolonged rainy spells and high humidity, which are in agreement with previous findings that incidence of powdery mildew cool and humid environment lead to heavy yield losses while the presence of PM resistance genes in wheat landraces or cultivars contribute to better yield-related traits in powdery mildew-prone regions (Ullah *et al.*, 2018). Similarly, also investigation of the correlation of wheat powdery mildew and yield losses of Egyptian wheat germplasm for consecutive two years attributed to incidence of wheat powdery mildew (Ashmawy *et al.*, 2014). They found that cultivars resistant to powdery mildew performed better with stable yield-related traits while those susceptible to powdery mildew observed yield losses from 20% to 22.52%, especially during prolonged rainy spells. Additionally, PH and SDL shared a significant negative relationship ($r = -0.156$), revealing that individuals with greater plant height under field conditions may display reduced seedling length traits, or vice-versa. Tall plants are more prone to lodging especially in flood irrigation or heavy rain followed by winds, thereby leading to low yield, low 1000 KW and shriveled seeds (Gao *et al.*, 2020; Jamil *et al.*, 2026).

CONCLUSION

The study concluded that more than half of the germplasm tested were susceptible to Bgt mix powdery mildew fungal strains in the field. The resistant germplasm comprised both moderate and highly resistant wheat landraces. Molecular characterization identified the presence of most prevalent PM resistance genes in different landraces. Based on wheat experimental panel (N = 100 tested with molecular markers, the most abundant *Pm* gene possessed by landraces was *Pm3* (23%), followed by *Pm2* (10%), *Pm1* (8%), *Pm4* (4%) and *Pm8* (1%). Powdery mildew resistance showed weak but highly significant correlation with 1000 KW and seed length. Based on powdery mildew resistance, molecular marker analysis and yield related attributes, three wheat landraces, especially wheat landrace acc.# 11212 from Ziarat Baluchistan province were the most superior breeding material for developing powdery mildew resistant and high yielding wheat cultivars for powdery mildew-prone regions of Pakistan.

DECLARATIONS

Funding: All authors extend their gratitude to Deanship

of Scientific Research, Vice Presidency for Graduate Studies and Scientific Research, King Faisal University, Al-Ahsa, Saudi Arabia for funding this research work (Grant Number: KF264924). This work was funded by Higher Education Commission (HEC) Islamabad, Pakistan under SRGP grant (455/IPFP-II (Batch-I)/SRGP/NAHE/HEC/2020/62) awarded to Nasr Ullah Khan.

Conflict of interest/Competing interests: The authors have no relevant financial or non-financial interests to disclose.

Availability of data and material: All data generated or analyzed during this study are included in this manuscript.

Consent to participate: All authors consent to participate in the manuscript publication

Consent for publication: All authors approved the manuscript to be published

AUTHOR CONTRIBUTIONS

NUK and IU conceptualized the study; NUK and MJ assisted with methodology and project administration; MH and AS helped with software, resources and writing—original draft preparation; AM and RNQ performed validation; MY carried out formal analysis; WE conducted investigation; DL curated the data; DKYT contributed to writing—reviewing and editing; MJ took part in visualization; IU and MY participated in supervision and funding acquisition. All authors have read and agreed to the published version of the manuscript.

ACKNOWLEDGEMENT

Appreciation to the Deanship of Scientific Research, Vice Presidency for Graduate Studies and Scientific Research, King Faisal University, Al-Ahsa, Saudi Arabia (Grant Number: KF264924). We are also thankful to the National Academy of Higher Education-NAHE (HEC) of Pakistan and Hazara University Mansehra for providing all necessary services.

REFERENCES

- Alotaibi, M. O., N. M. Alotaibi, A. M. Ghoneim, N. ul Ain, M. A. Irshad, R. Nawaz, T. Abbas, A. Abbas, M. Rizwan and S. Ali. 2023. Effect of green synthesized cerium oxide nanoparticles on fungal disease of wheat plants: a field study. *Chemosphere*, 339: 139731.
- Asad, S., M. Fayyaz and A. Munir. 2014. Screening of wheat commercial varieties for resistance against powdery mildew (*Blumeria graminis* f. sp. *tritici*) at Kaghan valley, Pakistan. *Pakistan Journal of Phytopathology*, 26: 07-13.
- Ashmawy, M., W. El-Orabey, A. E. A. Abu Aly and A. Shahin. 2014. Losses in grain yield of some wheat cultivars infected with powdery mildew. *Egyptian Journal of Phytopathology*, 42: 71-82.
- Balfourier, F., S. Bouchet, S. Robert, R. De Oliveira, H. Rimbart, J. Kitt and E. Paux. 2019. Worldwide phylogeography and history of wheat genetic diversity. *Science Advances*, 5: eaav0536.
- Bapela, T., H. Shimelis, T. Terefe, S. Bourras, J. Sanchez-Martin, D. Douchkov, F. Desiderio and T. J. Tsilo. 2023. Breeding wheat for powdery mildew resistance: genetic resources and methodologies-a review. *Agronomy*, 13: 1173.
- Bhullar, N. K., K. Street, M. Mackay, N. Yahiaoui and B. Keller. 2009. Unlocking wheat genetic resources for the molecular identification of previously undescribed functional alleles at the *Pm3* resistance locus. *Proceedings of the National Academy of Sciences*, 106: 9519-9524.
- Chen, F., H. Jia, X. Zhang, L. Qiao, X. Li, J. Zheng and Z. Chang. 2019. Positional cloning of *PmCH1357* reveals the origin and allelic variation of the *Pm2* gene for powdery mildew resistance in wheat. *The Crop Journal*, 7: 771-783.
- Clin, P., F. Grognaud, D. Andrivon, L. Mailleret and F. M. Hamelin. 2022. Host mixtures for plant disease control: Benefits from pathogen selection and immune priming. *Evolutionary Applications*, 15: 967-975.
- Corwin, J. A. and D. J. Kliebenstein. 2017. Quantitative resistance: more than just perception of a pathogen. *The Plant Cell*, 29: 655-665.
- Gallois, J. L., B. Moury and S. German-Retana. 2018. Role of the genetic background in resistance to plant viruses. *International Journal of Molecular Sciences*, 19: 2856.
- Gao, Y., Y. Li, W. Xia, M. Dai, Y. Dai, Y. Wang, H. Ma and H. Ma. 2023. The regulation of grain weight in wheat. *Seed Biology*, 2(1): 1-13.
- Gao, Z., Y. Wang, G. Tian, Y. Zhao, C. Li, Q. Cao, R. Han, Z. Shi and M. He. 2020. Plant height and its relationship with yield in wheat under different irrigation regime. *Irrigation Science*, 38: 365-371.
- Hao, C., H. Mao, Y. Zong, P. Lu, X. Wang, J. Liu and X. Zhang. 2026. A decade of excellent development in sciences

- and technologies drives more efficient wheat breeding and production in China. *Journal of Integrative Agriculture*,
- Hao, Y., R. Parks, C. Cowger, Z. Chen, Y. Wang, D. Bland and J. Johnson. 2015. Molecular characterization of a new powdery mildew resistance gene *Pm54* in soft red winter wheat. *Theoretical and Applied Genetics*, 128: 465-476.
- He, H., S. Zhu, R. Zhao, Z. Jiang, Y. Ji, J. Ji and T. Bie. 2018. *Pm21*, encoding a typical CC-NBS-LRR protein, confers broad-spectrum resistance to wheat powdery mildew disease. *Molecular Plant*, 11: 879-882.
- Hewitt, T., M. C. Müller, I. Molnár, M. Mascher, K. Holušová, H. Šimková and P. Zhang. 2021. A highly differentiated region of wheat chromosome 7AL encodes a *Pm1a* immune receptor that recognizes its corresponding *AvrPm1a* effector from *Blumeria graminis*. *New Phytologist*, 229: 2812-2826.
- Hurni, S., S. Brunner, G. Buchmann, G. Herren, T. Jordan, P. Krukowski and B. Keller. 2013. Rye *Pm8* and wheat *Pm3* are orthologous genes and show evolutionary conservation of resistance function against powdery mildew. *The Plant Journal*, 76: 957-969.
- Hysing, S. C., A. Merker, E. Liljeroth, R. M. Koebner, F. J. Zeller and S. L. Hsam. 2007. Powdery mildew resistance in 155 Nordic bread wheat cultivars and landraces. *Hereditas*, 144: 102-119.
- Jamil, M., W. Ahmad, R. Shafqat, H. Nawaz and A. Ameer. 2026. Digital morphometry identifies significant association between seed dimensions and thousand kernel weight under water-limited conditions. *Cereal Research Communications*, 54: 1071-1082.
- Jin, Y., T. Gu, X. Li, H. Liu, G. Han, Z. Shi and D. An. 2022. Characterization of a new splicing variant of powdery mildew resistance gene *Pm4* in synthetic hexaploid wheat YAV249. *Frontiers in Plant Science*, 13: 1048252.
- Jin, Y., H. Xu, P. Ma, X. Fu, L. Song, Y. Xu and D. An. 2018. Characterization of a new *Pm2* allele associated with broad-spectrum powdery mildew resistance in wheat line Subtil. *Scientific Reports*, 8: 475.
- Kang, Y., M. Zhou, A. Merry and K. Barry. 2020. Mechanisms of powdery mildew resistance of wheat—a review of molecular breeding. *Plant Pathology*, 69: 601-617.
- Khan, N., J. Hussain, A. Hassan and M. M. Shah. 2013. PCR-based identification of Rye translocations in Pakistani wheat germplasm. *Minerva Biotechnologica*, 25: 87-94.
- Kunz, L., Z. Bernasconi, M. Heuberger, J. Isaksson, A. G. Sotiropoulos, U. Stirnemann and B. Keller. 2026. Dual recognition of structurally unrelated mildew effectors underlies the broad-spectrum resistance of *Pm3e* in wheat. *Nature Communications*, 17: 1-12.
- Leber, R., M. Heuberger, V. Widrig, E. Jung, E. Paux, B. Keller and J. Sánchez-Martín. 2024. A diverse panel of 755 bread wheat accessions harbors untapped genetic diversity in landraces and reveals novel genetic regions conferring powdery mildew resistance. *Theoretical and Applied Genetics*, 137: 88.
- Li, G., J. Zhou, H. Jia, Z. Gao, M. Fan, Y. Luo and Z. Ma. 2019. Mutation of a histidine-rich calcium-binding-protein gene in wheat confers resistance to Fusarium head blight. *Nature Genetics*, 51: 1106-1112.
- Liang, J., B. Fu, W. Tang, N. U. Khan, N. Li and Z. Ma. 2016. Fine mapping of two wheat powdery mildew resistance genes located at the *Pm1* cluster. *The Plant Genome*, 9: 1-9.
- Lodhi, S. S., S. Maryam, K. Rafique, A. Shafique, Z. A. Yousaf, A. M. Talha, A. Gul and R. Amir. 2020. Overview of the prospective strategies for conservation of genomic diversity in wheat landraces, In: Climate change and food security with emphasis on wheat. Academic Press, Cambridge, MA, USA. p. 293-309.
- Ma, Z. Q., M. E. Sorrells and S. D. Tanksley. 1994. RFLP markers linked to powdery mildew resistance genes *Pm1*, *Pm2*, *Pm3*, and *Pm4* in wheat. *Genome*, 37: 871-875.
- Manser, B., T. Koller, C. R. Praz, A. C. Roulin, H. Zbinden, S. Arora and J. Sánchez-Martín. 2021. Identification of specificity-defining amino acids of the wheat immune receptor *Pm2* and powdery mildew effector *AvrPm2*. *The Plant Journal*, 106: 993-1007.
- Mohler, V., C. Bauer, G. Schweizer, H. Kempf and L. Hartl. 2013. *Pm50*: a new powdery mildew resistance gene in common wheat derived from cultivated emmer. *Journal of Applied Genetics*, 54: 259-263.
- Nadeem, M. A., M. Z. Yeken, M. Tekin, Z. Mustafa, R. Hatipoğlu, H. Aktaş, A. Alsaleh, E. Cabi, E. Habyarimana, N. Zencirci and T. Karaköy. 2021. Contribution of landraces in wheat breeding, In: Wheat Landraces. Springer, Cham, Switzerland. p. 215-258.
- Rafique, K., C. A. Rauf, A. Gul, H. Bux, R. A. Memon, A. Ali and S. Farrakh. 2017. Evaluation of D-genome synthetic hexaploidy wheats and advanced derivatives for

- powdery mildew resistance. *Pakistan Journal of Botany*, 49: 735-743.
- Sánchez-Martín, J., B. Steuernagel, S. Ghosh, G. Herren, S. Hurni, N. Adamski and B. B. Wulff. 2016. Rapid gene isolation in barley and wheat by mutant chromosome sequencing. *Genome Biology*, 17: 221.
- Sánchez-Martín, J., V. Widrig, G. Herren, T. Wicker, H. Zbinden, J. Gronnier and B. Keller. 2021. Wheat *Pm4* resistance to powdery mildew is controlled by alternative splice variants encoding chimeric proteins. *Nature Plants*, 7: 327-341.
- Saqlain, M., T. Chen, J. Ma, M. Noshervan, Z. Yang, D. Wu and Y. Li. 2026. The diversity of cloned wheat powdery mildew resistance genes and the resistance mechanisms. *WheatOmics*, 2: 4.
- Shoormij, F., A. Mirlohi, D. Chan-Rodriguez, H. Bolibok-Brągoszewska and G. Saeidi. 2023. Characterization of 14 *Triticum* species for the *NAM-B1* gene and its associated traits. *PLoS ONE*, 18: e0287798.
- Si, Q. M., X. X. Zhang, X. Y. Duan, B. Q. Sheng and Y. L. Zhou. 1992. On gene analysis and classification of powdery mildew (*Erysiphe graminis* f. sp. *tritici*) resistant wheat varieties. *Acta Phytopathologica Sinica*, 22: 349-355.
- Srichumpa, P., S. Brunner, B. Keller and N. Yahiaoui. 2005. Allelic series of four powdery mildew resistance genes at the *Pm3* locus in hexaploid bread wheat. *Plant Physiology*, 139: 885-895.
- Tailor, A. and S. C. Bhatla. 2024. *R* gene-mediated resistance in the management of plant diseases. *Journal of Plant Biochemistry and Biotechnology*, 33: 5-23.
- Tony, A., D. Mullan, H. T. Phan, F. J. Lopez-Ruiz, K. N. Dodhia, B. J. Saunders and K. C. Tan. 2025. Evidence of a distinct *Blumeria graminis* f. sp. *tritici* pathotype structure in Australian wheat powdery mildew: implications for resistance breeding. *bioRxiv*,
- Uauy, C., A. Distelfeld, T. Fahima, A. Blechl and J. Dubcovsky. 2006. A *NAC* gene regulating senescence improves grain protein, zinc, and iron content in wheat. *Science*, 314: 1298-1301.
- Ullah, K. N., N. Li, T. Shen, P. Wang, W. Tang, S. Ma and Z. Ma. 2018. Fine mapping of powdery mildew resistance gene *Pm4e* in bread wheat (*Triticum aestivum* L.). *Planta*, 248: 1319-1328.
- Xiao, M., F. Song, J. Jiao, X. Wang, H. Xu and H. Li. 2013. Identification of the gene *Pm47* on chromosome 7BS conferring resistance to powdery mildew in the Chinese wheat landrace Hongyanglazi. *Theoretical and Applied Genetics*, 126: 1397-1403.
- Xu, H., Y. Yi, P. Ma, Y. Qie, X. Fu, Y. Xu and D. An. 2015. Molecular tagging of a new broad-spectrum powdery mildew resistance allele *Pm2c* in Chinese wheat landrace Niaomai. *Theoretical and Applied Genetics*, 128: 2077-2084.
- Xue, F., C. Wang, C. Li, X. Duan, Y. Zhou, N. Zhao and W. Ji. 2012. Molecular mapping of a powdery mildew resistance gene in common wheat landrace Baihulu and its allelism with *Pm24*. *Theoretical and Applied Genetics*, 125: 1425-1432.
- Yahiaoui, N., P. Srichumpa, R. Dudler and B. Keller. 2004. Genome analysis at different ploidy levels allows cloning of the powdery mildew resistance gene *Pm3b* from hexaploid wheat. *The Plant Journal*, 37: 528-538.
- Yin, J., H. Yi, X. Chen and J. Wang. 2019. Post-translational modifications of proteins have versatile roles in regulating plant immune responses. *International Journal of Molecular Sciences*, 20: 2807.
- Zou, S., Y. Xu, Q. Li, Y. Wei, Y. Zhang and D. Tang. 2023. Wheat powdery mildew resistance: from gene identification to immunity deployment. *Frontiers in Plant Science*, 14: 1269498.

Publisher's note: EScience Press remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license and indicate if changes

were made. The images or other third-party material in this article are included in the article's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit <http://creativecommons.org/licenses/by/4.0/>.