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Research Article

EVALUATION OF ADVANCED BREAD WHEAT LINES FOR YIELD PERFORMANCE AND RESISTANCE TO STRIPE RUST

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The present study aimed to identify high-yielding wheat genotypes with resistance to yellow rust (Puccinia striiformis f.sp. tritici) through a field experiment conducted at Hazara University, Mansehra, during the 2019-20 crop season. Grain yield ranged from 2,013 kg/ha to 4,556 kg/ha, with a mean yield of 3,152 kg/ha. Several genotypes significantly outperformed the standard check varieties, demonstrating potential for both high yield and strong disease resistance. Yellow rust resistance was evaluated based on host reactions and coefficient of infection (CI) values, which revealed a broad spectrum of resistance levels, ranging from immune to highly susceptible. Several genotypes exhibited strong resistance (CI =0-9%), while others showed moderate resistance or varying degrees of susceptibility. Cluster analysis grouped the genotypes into six clusters based on their performance in terms of grain yield and yellow rust resistance. Genotypes combining high yield with strong resistance were identified as promising candidates for future breeding programs. Conversely, high-yielding but susceptible genotypes underscored the need for targeted improvement in disease resistance. This study highlights the importance of integrating high yield potential with durable disease resistance in wheat breeding efforts to ensure sustainable production, particularly in areas prone to yellow rust outbreaks. The findings contribute valuable understandings for breeders aiming to develop wheat varieties that are not only high yielding but also resilient to disease pressure.

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INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the most important staple crops worldwide, playing a vital role in global food security by providing a substantial portion of daily caloric and protein intake for millions of people. As the global population continues to grow and climate change intensifies, maintaining high yields and enhancing disease resistance in wheat has become increasingly critical (Beddow et al., 2015; Mukhtar et al., 2018; Mukhtar and Saeed, 2024). Among the various diseases that threaten wheat production, stripe rust, caused by the fungal pathogen *Puccinia striiformis* f.sp. *tritici*, is one of the most devastating. Stripe rust not only reduces grain yield but also affects grain quality, leading to significant economic

losses in wheat-growing regions around the world (Bux et al., 2012; Ahmad et al., 2024; Yaseen et al., 2025). Under favorable conditions, the disease can cause complete crop failure, with yield losses reaching up to 100% in susceptible cultivars (Reynolds and Borlaug, 2006).

Developing high-yielding and stripe rust-resistant wheat varieties is a top priority for breeders. Resistance breeding is regarded as a sustainable and long-term strategy, especially when compared to chemical control methods, which are often economically and environmentally unsustainable (Hyles et al., 2020). Genetic resistance to stripe rust can be broadly categorized into race-specific resistance and adult plant resistance (APR). Although race-specific resistance provides high levels of protection, it is frequently rendered ineffective by evolving pathogen populations. In contrast, APR offers durable, broad-spectrum resistance and is particularly valuable for long-term disease management (Roelfs et al., 1992).

Stripe rust thrives in cool, moist climates, making it a persistent threat in major wheat-growing regions such as South Asia, Europe, and North America. In countries like Pakistan, recurring stripe rust epidemics underscore the urgent need for resistant wheat varieties to ensure stable production (Bux et al., 2012). The integration of traditional breeding methods with modern molecular tools has greatly enhanced the identification and deployment of resistance genes in wheat. For example, molecular tagging of stripe rust resistance genes, such as *Yr10*, has facilitated the efficient introgression of these traits into elite wheat lines (Wang et al., 2002).

Yield potential and disease resistance in wheat are often influenced by genotype × environment ($G \times E$) interactions, making multi-environment evaluations essential for selecting stable and adaptable genotypes (Romagosa and Fox, 1993). Recent advancements in high-throughput phenotyping techniques, such as UAVbased imaging and hyperspectral analysis, have significantly enhanced the ability of breeders to evaluate wheat genotypes under diverse environmental conditions (Huang et al., 2020). These tools enable precise measurement of traits such as biomass, leaf area index, and disease severity, thereby accelerating the breeding process for high-yielding, diseaseresistant lines.

Historically, wheat improvement has relied on genetic diversity derived from wild relatives and landraces, which serve as valuable sources of resistance to both biotic and abiotic stresses (Feldman and Sears, 1981; Nevo, 2007). However, domestication and intensive selection have narrowed the genetic base of the crop, necessitating the continual exploration of diverse germplasm to identify new sources of resistance (Dubcovsky and Dvorak, 2007). This study aims to address this challenge by evaluating advanced wheat lines for their yield potential and resistance to stripe rust, thereby contributing to the development of resilient wheat varieties.

The primary objective of this study is to screen advanced bread wheat lines under field conditions to identify those with high yield potential and strong resistance to stripe rust. By integrating phenotypic assessments with insights into genetic resistance, this research seeks to support the development of durable wheat varieties capable of withstanding biotic stresses while maintaining high productivity. These findings are expected to enhance global food security by supporting the development of wheat varieties that ensure consistent and sustainable production amid climate variability and emerging pathogen pressures.

MATERIALS AND METHODS

A field experiment was conducted during the 2019-20 cropping season using 100 wheat genotypes, including three commercial varieties and Morocco (used as a susceptible check), arranged in an augmented block design. This design was chosen due to the large number of genotypes and limited seed availability, which made full replication impractical. To manage field variability and ensure reliable comparisons, the experiment was divided into blocks, each containing unreplicated test entries and repeated standard checks. The inclusion of replicated checks enabled the estimation and adjustment of environmental variation, thereby enhancing the accuracy of genotype performance evaluations. This approach is particularly suitable for preliminary screening in early-generation breeding trials where resources and seed quantities are limited.

Each experimental plot consisted of four rows; each 3 m long, with a row spacing of 30 cm. Standard agronomic practices were followed to ensure optimal growth and uniform management across all plots. Intercultural operations, including land preparation, seedbed formation, sowing, irrigation, weed control, and pesticide application, were carried out according to recommended guidelines to promote healthy plant development. Harvesting was done at the maturity stage for each plot, and grain yield (kg ha⁻¹) was calculated by converting the plot-specific grain weight using a standard formula.

Yellow rust disease severity was assessed for all 100 wheat genotypes at the peak disease stage. The selected field site in Mansehra is a well-established yellow rust hotspot, ensuring consistent natural infection. Disease scoring was performed twice: first, after heading, when initial symptoms began to appear, and second, at the peak disease stage to accurately assess disease intensity. Scoring was conducted under natural field conditions without artificial inoculation, relying on epiphytotic development favored by moderate temperatures (10-20°C) and high humidity. Disease severity was visually estimated as the percentage of infected leaf area on the flag leaf and penultimate leaf, using the modified Cobb scale (Peterson et al., 1948). The reaction of each genotype to infection was categorized as follows:

O (Immune): No visible signs of infection

R (Resistant): Necrotic areas, possibly with minor uredia MR (Moderately Resistant): Minor uredia surrounded by necrotic tissue

MS (Moderately Susceptible): Moderate uredia without necrosis, with varying degrees of chlorosis

S (Susceptible): Large uredia with little or no chlorosis

The coefficient of infection (CI) was calculated by multiplying the infection percentage by the response value (O = 0.0, R = 0.2, MR = 0.4, MS = 0.6, M = 0.8, S = 1.0).

Statistical analysis

Data on various traits were analyzed using analysis of variance (ANOVA) methods suitable for an augmented block design, implemented in R software (R Core Team, 2023). Cluster analysis was performed using the cluster package in R to classify the 100 wheat genotypes based on grain yield and yellow rust resistance. The data were standardized, and k-means clustering was applied to group genotypes with similar characteristics. The optimal number of clusters was determined using the elbow method by plotting the total within-cluster sum of squares (WSS) against a range of cluster numbers. The elbow point at k = 6 indicated the most suitable number of clusters, beyond which the marginal gain in explained variance was minimal. Moreover, the silhouette coefficient was computed to evaluate the consistency and compactness of the clustering results, further supporting the selection of six clusters. A dendrogram

was also generated using Ward's hierarchical agglomerative method to visualize genotype relationships and validate the clustering results (Maechler et al., 2023).

RESULTS

Grain yield

Grain yield data for the 100 wheat genotypes are presented in Table 1. The mean grain yield across all genotypes was 3,152 kg/ha, with the lowest yield recorded for HUM60 at 2,013 kg/ha and the highest for HUM07 at 4,556 kg/ha. The top 10 highest-yielding genotypes were: HUM07 (4,556 kg/ha), HUM22 (4,517 kg/ha), HUM05 (4,513 kg/ha), HUM72 (4,502 kg/ha), HUM17 (4,472 kg/ha), HUM12 (4,300 kg/ha), HUM62 (4,254 kg/ha), HUM15 (4,174 kg/ha), HUM13 (4,133 kg/ha), and HUM61 (4,133 kg/ha).

In contrast, the bottom 10 genotypes with the lowest grain yield were: HUM60 (2,013 kg/ha), HUM26 (2,041 kg/ha), HUM86 (2,056 kg/ha), HUM93 (2,139 kg/ha), HUM92 (2,204 kg/ha), HUM83 (2,224 kg/ha), HUM90 (2,239 kg/ha), HUM95 (2,397 kg/ha), HUM38 (2,435 kg/ha), and HUM01 (2,463 kg/ha).

Figure 1 compares the performance of the advanced bread wheat lines with four standard check varieties. The top-performing genotype, HUM07, yielded 4,556 kg/ha, significantly outperforming all the standard checks: Morocco (2,497 kg/ha), Kohat-2017 (3,559 kg/ha), Pakistan-2013 (3,439 kg/ha), and Pirsabak-2005 (3,165 kg/ha).

The susceptible check, Morocco, had the lowest yield among the standard varieties at 2,497 kg/ha, well below both the overall mean and the top-performing genotypes. Kohat-2017 and Pakistan-2013 performed relatively better, yielding 3,559 kg/ha and 3,439 kg/ha, respectively, both above the mean but still lower than HUM07. Pirsabak-2005 yielded 3,165 kg/ha, slightly above the mean, indicating average performance in comparison to the other genotypes.

Yellow rust

Yellow rust severity, based on host reactions and coefficient of infection (CI) values, revealed a consistent pattern of disease resistance and susceptibility among the genotypes. These were categorized into immune, resistant (R), moderately resistant (MR), moderately susceptible (MS), moderately resistant to moderately susceptible (M), and susceptible (S), as shown in Table 1 and Figure 2.

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Genotype	TR	CI	GY	Genotype	TR	CI	GY	Genotype	TR	CI	GY
HUM01	80S	80	2463	HUM35	30MS	24	2770	HUM69	5R	1	3500
HUM02	10MR	4	3896	HUM36	10MR	4	2933	HUM70	90S	90	3137
HUM03	0	0	2920	HUM37	5R	1	3065	HUM71	20MR	8	2676
HUM04	20MR	8	3543	HUM38	10MR	4	2435	HUM72	20MR	8	4502
HUM05	30M	18	4513	HUM39	30M	18	2711	HUM73	40M	24	2774
HUM06	30M	18	3078	HUM40	90S	90	3093	HUM74	20MR	8	3933
HUM07	30M	18	4556	HUM41	30M	18	3131	HUM75	10MR	4	3722
HUM08	5R	1	3354	HUM42	30MS	18	2687	HUM76	20MR	8	3217
HUM09	5R	1	3152	HUM43	30M	18	3200	HUM77	30MR	12	3002
HUM10	90S	90	2485	HUM44	20MR	8	3472	HUM78	30MR	12	3356
HUM11	10MR	4	3326	HUM45	30M	18	2696	HUM79	5R	1	3524
HUM12	0	0	4300	HUM46	20MR	8	2722	HUM80	90S	90	2530
HUM13	20MR	8	4133	HUM47	10MR	4	3489	HUM81	5R	1	3028
HUM14	5MR	2	3922	HUM48	30M	18	3178	HUM82	40M	24	2813
HUM15	10R	2	4174	HUM49	10MR	4	2846	HUM83	10MR	4	2224
HUM16	60S	60	2933	HUM50	90S	90	2593	HUM84	20MR	8	2680
HUM17	10MR	4	4472	HUM51	30MS	24	2591	HUM85	40M	24	2841
HUM18	20MR	8	3474	HUM52	20MR	8	3102	HUM86	40M	24	2056
HUM19	10MR	4	3409	HUM53	10MR	4	3700	HUM87	20MR	8	3354
HUM20	90S	90	2519	HUM54	30MS	24	3000	HUM88	5R	1	3419
HUM21	40M	24	2857	HUM55	5R	1	2848	HUM89	40M	24	2628
HUM22	30M	18	4517	HUM56	10MR	4	2587	HUM90	90S	90	2239
HUM23	30MS	24	2528	HUM57	0	0	3307	HUM91	40M	24	2497
HUM24	30MS	24	2600	HUM58	5R	1	3831	HUM92	60S	60	2204
HUM25	20MR	8	3967	HUM59	0	0	3639	HUM93	40M	24	2139
HUM26	30M	18	2041	HUM60	90S	90	2013	HUM94	40M	24	2559
HUM27	20MR	8	3974	HUM61	5R	1	4133	HUM95	40M	24	2397
HUM28	10MR	4	3628	HUM62	0	0	4254	HUM96	20MR	8	3464
HUM29	30M	18	3241	HUM63	30MS	24	3950	Morocco	90S	90	2497
HUM30	90S	90	2970	HUM64	30M	18	2546	Kohat-2017	40M	24	3559
HUM31	10MR	4	2937	HUM65	20MR	8	3469	Pakistan-2013	20MR	8	3439
HUM32	10MR	4	3278	HUM66	10MR	4	2956	Pirsabak-2005	30M	18	3165
HUM33	10MR	4	3435	HUM67	20MR	8	2876				
HUM34	10MR	4	2893	HUM68	80S	80	2800				

Table 1. Terminal reaction, coefficient of infection of yellow rust and grain yield (kg/ha) of 100 wheat genotypes

Note: TR = Terminal reaction, CI = Coefficient of infection, GY = Grain yield.

Among these, the immune and resistant genotypes exhibited strong resistance to the disease, making them promising candidates for breeding programs. The immune genotypes included HUM03, HUM12, HUM57, HUM59, and HUM62, while the resistant genotypes were HUM08, HUM09, HUM15, HUM37, HUM55, HUM58, HUM61, HUM69, HUM79, HUM81, and HUM88.

In contrast, the susceptible genotypes, HUM01, HUM10, HUM16, HUM20, HUM30, HUM40, HUM50, HUM60, HUM70, HUM80, HUM90, HUM92, Morocco, and Kohat-2017, exhibited pronounced disease symptoms.

Based on CI values, the genotypes were further classified into different resistance levels (Table 1 and

Figure 3). Genotypes classified as R, with CI values ranging from 0-9%, showed strong resistance to infection, totaling 56 genotypes. Eighteen genotypes were categorized as MR with CI values between 10-19%. The moderately resistant to moderately susceptible (MRMS) group comprised 22 genotypes with CI values between 20-39%.

No genotypes were found in the MS category. However, two genotypes, HUM16 and HUM92, were categorized as moderately susceptible to susceptible (MSS), with CI values ranging from 60-79%. Finally, 24 genotypes, including Morocco, were classified as highly susceptible, with CI values ranging from 80-100%.

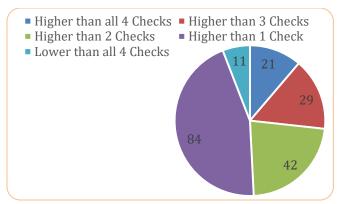


Figure 1. Distribution of genotypes based on grain yield compared to check cultivars.

- Immune (0)
- Resistant (R)
- Moderate Resistant (MR)
- Moderate Resistant-susceptible (M)
- Moderate susceptible (MS)
- Susceptible (S)

response to yellow rust.

Cluster analysis

Cluster analysis categorized the 100 wheat genotypes into six well-defined clusters based on their performance in grain yield and yellow rust resistance. The selection of six clusters was supported by the elbow method and further validated through silhouette analysis, indicating an optimal balance between intracluster similarity and inter-cluster distinction. Each cluster comprised genotypes with similar performance profiles, offering valuable understandings for potential candidates for breeding programs aimed at improving both yield and rust resistance (Table 2 and Figure 4).

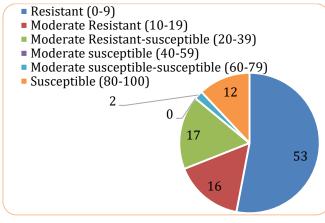


Figure 3. Distribution of genotypes based on the coefficient of infection to yellow rust.

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14

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Figure 2. Distribution of genotypes based on their

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Clusters	No. of Genotypes	Genotypes	Key Characteristics
Ι	07	HUM12, HUM13, HUM15, HUM17, HUM61, HUM62 and HUM72	High yielding and highly disease resistant
II	16	HUM02, HUM04, HUM14, HUM18, HUM25, HUM27, HUM28, HUM47, HUM53, HUM58, HUM59, HUM63, HUM69, HUM74, HUM75 and HUM79	Moderate yielding and highly disease resistant
III	08	HUM26, HUM60, HUM83, HUM86, HUM90, HUM92, HUM93 and Morroco	Low yielding and highly susceptible
IV	24	HUM01, HUM05, HUM07, HUM10, HUM20, HUM22, HUM23, HUM24, HUM38, HUM39, HUM42, HUM45, HUM46, HUM50, HUM51, HUM56, HUM64, HUM71, HUM80, HUM84, HUM89, HUM91, HUM94 and HUM95	Low yielding and moderately susceptible
V	20	HUM03, HUM16, HUM21, HUM30, HUM31, HUM34, HUM35, HUM36, HUM49, HUM54, HUM55, HUM66, HUM67, HUM68, HUM73, HUM77, HUM81, HUM82, HUM85 and Pirsabak-2005	Low yielding and moderately resistant
VI	25	HUM06, HUM08, HUM09, HUM11, HUM19, HUM29, HUM32, HUM33, HUM37, HUM40, HUM41, HUM43, HUM44, HUM48, HUM52, HUM57, HUM65, HUM70, HUM76, HUM78, HUM87, HUM88, HUM96, Kohat-2017 and Pakistan-13	Moderate yielding and moderate resistant

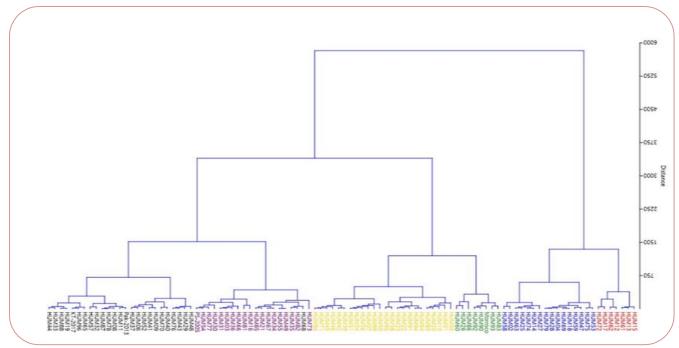


Figure 4. Dendrogram showing the distribution of 100 wheat genotypes into different clusters.

Cluster I emerged as the most promising group, consisting of seven high-performing genotypes, such as HUM12, HUM13, and HUM15, that demonstrated both high grain yield and strong resistance to yellow rust. Cluster II included 16 genotypes with moderate yield but strong rust resistance, including HUM02, HUM14, and HUM25, indicating potential for yield enhancement while maintaining durable resistance.

In contrast, Cluster III grouped eight highly susceptible and low-yielding genotypes, notably Morocco (the susceptible check), HUM26, and HUM60. Although these genotypes have limited breeding value, they are useful for studying host-pathogen interactions.

Cluster IV, comprising 24 genotypes such as HUM01, HUM05, and HUM39, was characterized by low yield and moderate susceptibility. Cluster V included 20 genotypes, including HUM03, HUM16, and Pirsabak-2005, which showed low yield but moderate resistance, suggesting their potential for introgressing disease resistance into high-yielding backgrounds.

Cluster VI, the largest with 25 genotypes, exhibited moderate yield and moderate resistance. It included widely adapted commercial varieties such as Kohat-2017 and Pakistan-13, along with experimental lines like HUM06 and HUM32, highlighting their balanced performance and potential utility as breeding parents for stabilizing desirable traits.

These genotypes represent excellent candidates for direct advancement and use in breeding programs targeting simultaneous improvement of yield and disease resistance.

This classification underscores the genetic diversity within the panel and facilitates the identification of genotypes with favorable trait combinations for targeted wheat improvement programs.

DISCUSSION

The present study provides valuable insights into the dual objectives of enhancing grain yield and improving resistance to yellow rust in wheat genotypes under the natural epidemic conditions of Hazara University, Mansehra. Particularly, immune and highly resistant genotypes such as HUM03, HUM12, and HUM57 were identified. These findings are consistent with those of Mujtaba et al. (2025), who also reported promising genotypes for yellow rust resistance in wheat across diverse environments.

Several other studies conducted in different regions, such as Central Asia (Sallam et al., 2022) and North America (Singh et al., 2011), have identified key resistance genes including *Yr5*, *Yr10*, *Yr15*, and *Yr24* as vital sources of resistance. Inamullah et al. (2021) further demonstrated the effectiveness of molecular markers in identifying resistance genes, underscoring

the utility of genetic tools in improving disease resistance. Among these, *Yr5* and *Yr15* have been widely incorporated into breeding programs worldwide due to their broad-spectrum effectiveness against multiple *Pst* races (McIntosh et al., 1995; Kaur et al., 2017). The resistance observed in this study aligns with global efforts to identify and deploy durable resistance genes (Sharma-Poudyal et al. 2013).

Furthermore, the moderate resistance observed in genotypes such as HUM08, HUM09, and HUM15 may be attributed to the presence of APR genes like *Yr18*, *Yr29*, and *Yr46*, which are known for their durability across diverse environments. The presence of APR genes in these genotypes reflects findings from regional studies in Pakistan (Hassan et al., 2016) and India (Prakash et al., 2017), where wheat cultivars carrying *Yr18* exhibited slow-rusting phenotypes under natural disease pressure. This suggests that regional breeding programs have successfully integrated *Yr18*-like resistance into their germplasm, contributing to the observed moderate resistance levels.

In contrast, genotypes such as HUM16, HUM60, and Morocco exhibited high levels of susceptibility, with CI values ranging between 80-100%. This susceptibility aligns with earlier studies that used Morocco as a susceptible check under similar conditions (Wellings, 2011). Such genotypes serve as essential tools for monitoring pathogen virulence and distinguishing resistant genotypes in breeding programs. The findings of our study are consistent with regional wheat breeding efforts in Pakistan, where Morocco has consistently been used as a susceptible check to evaluate resistance under field conditions. These results are in line with the regional efforts of Din et al. (2023) and Farooq et al. (2019), who characterized wheat lines for resistance to stripe rust and emphasized the importance of susceptible checks like Morocco.

Cluster analysis revealed high genetic and phenotypic diversity among the genotypes, with Cluster I containing high-yielding, disease-resistant lines such as HUM12, HUM15, and HUM17. These lines are promising candidates for wheat breeding programs focused on both yield and disease resistance. Similar results have been reported in regional studies, such as the work by Ahmed et al. (2018) in Pakistan, where cluster analysis of wheat genotypes identified elite lines with superior disease resistance and high yield potential. Our study aligns with their findings, confirming that combining both qualitative and quantitative resistance in highyielding genotypes can optimize wheat production in rust-prone regions.

The presence of high-yielding but susceptible genotypes, such as HUM05 and HUM22, underscores the challenge of enhancing disease resistance without compromising yield. These genotypes are ideal candidates for targeted improvement through marker-assisted selection (MAS), a technique that has shown considerable promise in regional breeding programs (Javed et al., 2020). MAS has been successfully employed in Pakistan and India to introgress *Yr5, Yr15,* and *Yr18* resistance genes into elite wheat lines, and it could serve as an effective approach to improve the resistance of these high-yielding genotypes.

The findings of this study also emphasize the significance of $G \times E$ interactions in determining wheat resistance to yellow rust. The field conditions at Hazara University, characterized by natural rust pressure, offer a realistic and effective environment for evaluating disease resistance, as suggested by Reynolds and Borlaug (2006) and validated in this study. These results are consistent with regional studies in Khyber Pakhtunkhwa (Zahid et al., 2020), which highlighted the importance of conducting field trials under natural disease pressure for accurate assessment of wheat genotypes' resistance levels.

Overall, the study underscores the importance of integrating classical breeding with modern genomic tools to enhance disease resistance in wheat. The findings align with broader regional efforts in wheat improvement, where durable resistance to yellow rust is prioritized alongside yield enhancement (Khan et al., 2021). In a separate study, Khan et al. (2025) emphasized the integration of genomic tools to complement traditional breeding approaches in improving yellow rust resistance. The immune and resistant genotypes identified, particularly those in Cluster I, represent valuable genetic resources for future wheat breeding programs, not only in Pakistan but also across similar agro-ecological zones in South Asia. Further molecular validation through QTL mapping and genomic-assisted selection will be essential for finetuning these resistance traits in elite wheat lines.

CONCLUSION

Genotypes HUM12 and HUM15 stand out as both highyielding and disease-resistant, making them prime candidates for breeding programs focused on improving yield and disease resilience. Genotypes such as HUM07, HUM17, and HUM72 offer a balanced combination of high yield and moderate resistance, providing versatile options for breeding. In contrast, high-yielding genotypes like HUM05 and HUM22, which are susceptible to yellow rust, require significant improvement in disease resistance to fully optimize their performance.

AUTHORS' CONTRIBUTIONS

IU and FUK designed the study; ZS prepared the materials, collected and analyzed the data; IUD helped in disease scoring. IU and FUK supervised the studies; ZS and FUK wrote the manuscript; All the authors proofread and approved the final manuscript.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

SUSTAINABLE DEVELOPMENT GOALS TARGETED

SDG 2: Zero Hunger SDG 12: Responsible Consumption and Production SDG 13: Climate Action SDG 15: Life on Land

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