

MULTIVARIATE PHENOTYPIC PROFILING REVEALS TRAITS ASSOCIATED WITH STRIPE RUST RESISTANCE AND YIELD STABILITY IN BREAD WHEAT GENOTYPES

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Abstract

Wheat productivity is seriously threatened by prevalence of stripe rust, caused by *Puccinia striiformis* f. sp. *tritici*, across the globe. The current study evaluated 53 wheat genotypes under artificial stripe rust pathogen inoculation to investigate disease severity Cobb's scale and its impact on agronomic traits. Disease intensity was quantified using the modified, while adult-plant resistance (APR) was assessed by coefficient of infection (CI) values. Eighteen genotypes exhibited high APR with low CI scores, signifying notable resistance diversity. Agronomic traits including grain-filling period (GFP), plant height (PH), peduncle length (PL), spike length (SL), and 1000-grain weight (TGW) were significantly influenced by rust stress. Positive correlations between GFP and traits such as PH, TGW, and grain weight/spike (GW/S) suggest that extended grain filling contributes to better yield resilience under disease pressure. Conversely, flag leaf area (FLA) showed negative correlations with several yield related traits, indicating potential susceptibility to pathogen infiltration. Principal component analysis (PCA) reduced trait variability to 5 major components explaining 77.42% of the total variance; with PC1 being most influential for GW/S. Biplot analysis identified genetically divergent genotypes (e.g., G7, G14, G20) and traits contributing most to phenotypic variation under rust stress. Cluster and profile analyses further categorized genotypes into 6 groups, revealing distinct trait associations with disease tolerance. Integration of disease assessment with multivariate statistical analyses provides valuable insights for wheat breeding programs, paving a way for effective selection of genotypes with strong APR, high yield and physiological robustness against stripe rust.

Key words: Plant resistance; Disease severity; diversity; *Triticum aestivum* L.

Introduction

Globally, wheat (*Triticum aestivum* L.) is considered as a major staple food crop, essential for food security and human nutrition (Fu *et al.*, 2026). Wheat is being cultivated in diverse agro-climatic zones across the globe with production of approximately 760 million metric tons annually (Anon., 2022). Wheat is a widely cultivated crop and being used in numerous forms including bread, pasta, and various other forms of bakery items fulfilling nutrient requirements for billions of humans (Rezk *et al.*, 2025). However, its production faces different biotic and abiotic stresses, among those stress stripe rust is one of the most threatening biotic factors caused by fungal pathogen (*Puccinia striiformis* f. sp. *tritici*), leading severe damage to wheat production (Khan *et al.*, 2025; Sun *et al.*, 2025). The causal fungus is adapted to wide range of

environmental conditions in the regions of wheat cultivation. Previously reported studies revealed that stripe rust caused yield losses in wheat approximately up to 70%. The invasion of this disease is highly influenced by environmental factors like temperature, humidity, and rainfall, which varies its occurrence in different regions of the globe. Rainfall along with prevailing low temperatures creates ideal conditions for occurrence of wheat stripe rust, making the disease more prevalent during the growing season (Singh *et al.*, 2014; Zulkiffal *et al.*, 2024).

The severity of stripe rust depends on a combination of factors, including the virulence of the pathogen, the genetic composition of the wheat plant, and the environmental conditions during the growing season. Environmental stressors such as high humidity and moderate temperatures favor the spread of stripe rust, while other factors like soil fertility and water availability can

influence plant resistance and susceptibility (Zhang *et al.*, 2022). The pathogen is capable of rapidly evolving, leading to the emergence of new rust races that can overcome earlier effective genetic resistance (Singh *et al.*, 2014; Tabouktirt *et al.*, 2026). As a result, wheat breeders are continually faced with the challenge of developing cultivars with durable resistance to this disease.

Genetic makeup of genotypes is crucial for stripe rust resistance in wheat which can be categorized into two categories including race-specific resistance and APR. Genotype specific stripe rust resistance is ruled by single genes that recognize specific strains of the fungus which generally exhibit for short period due to mutation in pathogen leading to overcome the resistance. In contrast, APR offers a sustainable solution by conferring resistance across a broad range of pathogen races (McIntosh *et al.*, 2021). Shahin (2017) identified stripe rust resistance genes *Yr2+*, *Yr5*, *Yr10*, *Yr15* and *YrND* as effective, while *Yr18* and *Yr29* were found to be partially effective during study periods with moderate susceptibility. In another study by Shahin *et al.*, (2024), identified rust-resistant genes *Yr5*, *Yr9*, *Yr10*, *Yr15*, *Yr17*, *Yr18*, *Yr26*, *Yr29*, *Yr30*, and *Yr36*. While, genes *Yr26*, *Yr30*, and *Yr36* were described in all studied genotypes.

Outbreak of stripe rust can be controlled through application of fungicides, but it is not sustainable and effective strategy. Furthermore, it is also a source of environmental pollution, leading to the development of fungicide-resistant strains of the pathogen (Oliver, 2014; Almusallam *et al.*, 2025). Moreover, relying fully on fungicides can cause contamination of soil and water resources, negatively impacting ecosystems and human health. While, breeding for resistant wheat varieties is more sustainable and environmentally friendly approach to mitigate stripe rust (Mukhtar & Mohamad, 2022). The continued occurrence of the stripe rust in wheat highlights the need for improved wheat cultivars through integrated approaches i.e., conventional breeding, screening of germplasm, marker-assisted selection and field-oriented trials (Aliyeva *et al.*, 2026).

Stripe rust is one of the most destructive diseases of wheat worldwide and a constant threat to food security. Short term control is possible through application of conventional fungicides but sustainable solution lies in breeding resistant wheat varieties. Through integration of APR genes and yield traits highly protective and resistant to stripe rust, cultivars could be evolved for sustainable wheat production. Continued efforts are crucial for genetic improvement and the development of sustainable agricultural practices to mitigating the impact of stripe rust on global wheat production. The objective of the research was therefore to assess the efficiency of multivariate analyses tools in identifying potential high-yielding genotypes and indicators under stripe rust pressure.

Materials and Methods

Research was conducted to determine how stripe rust affects the agronomic characteristics of wheat (*Triticum aestivum* L). The experiment was carried out on November 2024-2025, in the Department of Plant Breeding and Genetics (PBG) field research area at the Islamia

University of Bahawalpur (IUB), Pakistan, which is situated at latitude 29° 24' and longitude 71° 47'. In the early stages of crop growth, the temperature typically ranges from 21°C to 11°C, with an average humidity of 57%. Fifty-three genotypes were obtained from the Regional Agriculture Research Institute, Bahawalpur, Pakistan and grown in an augmented design. A similar augmented design strategy has previously been used by Khan *et al.*, (2025) for the evaluation of wheat genotypes, demonstrating its suitability and reliability for large-scale germplasm assessment and minimizing the effects of uncontrolled environmental variations. Each entry consisted of two rows, of two meters long each. A highly susceptible wheat genotype (Morocco) was sown after every 10 entries as a check and spreader to ensure uniform pathogen dissemination across the field. Split doses of the recommended amount of urea were applied in the field. When the plants were at the tillering stage, artificial inoculation was performed using a knapsack throughout the field. Spores (300 mg) were added to 1 liter of double-distilled water to create the suspension, inoculum was applied using a knapsack sprayer as a fine mist to the plant and leaf undersides to maintain moisture. Artificial moisture was maintained in the field with the aid of an atomizer. The plants were graded using the Modified Cobb's scale (Peterson *et al.*, 1948) every 15 days from the time the first symptoms appeared until the disease stopped progressing. Calculations were performed to determine disease severity as a percentage (Roelf *et al.*, 1992). Data on agronomic traits were collected from 10 plants in each line at various growth stages.

Disease monitoring: A modified version of Cobb's scale, with 0 representing no sickness and 100 representing severe infection, was used to evaluate disease severity. There were different categories for severity: resistant (R), moderately resistant (MR), resistant to moderately resistant (RMR), moderately susceptible (MS), moderately resistant-moderately susceptible (M), moderately susceptible to susceptible (MSS), and susceptible (S), all distinguished by large compatible uredinia without necrosis or chlorosis, and severity quantified as a percentage from 0 to 100.

Measurement of growth, physiological and grain-related attributes: Chlorophyll content (C.CNT) was measured at three points on both healthy and diseased leaves early in the morning using Han's Tech Instruments Model CL-01. Flag leaf area (FLA) was determined early in the morning (when transpiration was minimal and leaves were turgid), placing them on blank paper, measuring length and width at three points, and multiplying the result by a constant factor of 0.75 (Aldesuquy *et al.*, 2014). Using a meter rod, the plant height (PH) was measured in centimeters, starting from the base of the plant and ending at the top of the spike. Spike length (SL) was measured using a ruler in centimeters from base to tip (without including the awn length). The number of spikelet/spike (S/S) was counted from the base of the spike to its tip. The peduncle length (PL) was measured in centimeters, beginning at the first node below the spike and ending at the base of the spike. Grain-filling period (GFP) was calculated as the number of days between anthesis and physiological maturity. Anthesis was recorded when

approximately 50% of the spikes exhibited visible anthers, while physiological maturity was determined by the loss of green color from the peduncle, indicating completion of grain development. At maturity, the main spike was harvested and threshed to determine the number of grains/spike (G/S). Utilizing electronic scales, grain weight/spike and 1000-grain weight (TGW) was measured.

Statistical analysis

The CI was calculated following Pathan & Park (2006). Correlation coefficient formulas were used to assess how closely the two sets of data were related. The formulas output a number between -1 and 1. Correlation analysis of agronomic data was performed using Statistix 8.1. Principal component analysis (PCA) was performed to generate PC₁ and PC₂, which were then used to create a biplot. Two dendrograms were created using IBM SPSS 26 software. In this analysis, tree diagram was constructed using Ward's method and the Euclidean distance.

Results

This study employed artificial inoculation of stripe rust pathogen to examine its effects on the agronomic characteristics of several wheat genotypes. Disease severity was assessed for each genotype using the modified Cobb's scale. Correlation analysis was performed to determine the relationship between different studied traits, such as grain-filling period (GFP), chlorophyll content (C.CNT), flag leaf area (FLA), plant height (PH), peduncle

length (PL), spike length (SL), spikelet/spike (S/S), grains/spike (G/S), 1000-grain weight (TGW), grain weight/spike (GW/S). PCA analysis with a biplot was used to further support the findings of the correlation analysis. Dendrograms and biplots were generated to demonstrate the diversity of the genotypes studied in relation to observed parameters.

Disease severity score and association among studied traits:

The studied germplasm exhibited substantial amount of variation in stripe rust response. Based on CI, 18 genotypes showed high APR; 15 genotypes showed moderate APR; and 20 genotypes indicated low APR (Table 1). GFP correlated positively with PH, PL, GW/S, and TGW. FLA was negatively correlated with GFP, PH, S/S, G/S, TGW, and GW/S (Fig. 1). PH showed a positive correlation with GFP, PL, SL, S/S, G/S, TGW, and GW/S. SL had a negative correlation with GFP, C.CNT, G/S, TGW, and GW/S (Fig. 1).

Principal component analysis: Five of the 10 principal components (PCs) had eigenvalues larger than one, suggesting that they were statistically significant and accounted for 77.42% of the variance according to the data (Table 2, Fig. 2). The first two PCs explained 44.41% of the total variability and capturing a significant amount of the observed phenotypic diversity. PC₁ stands out for the GW/S because of its exceptionally high factorial coefficient value of 36.00. PC₂ for PL, whereas PC₃ for GFP because of exceptionally high factorial coefficient values (Table 3).

Table 1. Categorization of 53 wheat genotypes based on stripe rust severity, coefficient of infection (CI), and adult-plant resistance (APR).

Genotype code	Severity %	CI	APR	Genotype code	Severity %	CI	APR
G1	5MR	2	High	G28	60S	60	Low
G2	40MSS	36	Moderate	G29	20MR	8	High
G3	60S	60	Low	G30	60S	60	Low
G4	40MSS	36	Moderate	G31	20MR	8	High
G5	5MR	2	High	G32	20MR	8	High
G6	40MSS	30	Moderate	G33	20MR	8	High
G7	20MR	8	High	G34	5MR	2	High
G8	40MSS	36	Moderate	G35	60S	60	Low
G9	20MR	8	High	G36	40MSS	36	Moderate
G10	60S	60	Low	G37	40MSS	36	Moderate
G11	5MR	2	High	G38	60S	36	Low
G12	40MSS	36	Moderate	G39	5MR	2	High
G13	40MSS	36	Moderate	G40	5MR	2	High
G14	40MSS	36	Moderate	G41	5MR	2	High
G15	5MR	2	High	G42	20MR	8	High
G16	60S	60	Low	G43	40MSS	36	Moderate
G17	60S	60	Low	G44	40MSS	36	Moderate
G18	60S	60	Low	G45	60S	60	Low
G19	60S	60	Low	G46	40MSS	36	Moderate
G20	60S	60	Low	G47	40MSS	36	Moderate
G21	40MSS	36	Moderate	G48	60S	60	Low
G22	60S	60	Low	G49	60S	60	Low
G23	60S	60	Low	G50	20MR	8	High
G24	60S	60	Low	G51	40MSS	36	Moderate
G25	5MR	2	High	G52	5MR	2	High
G26	60S	60	Low	G53	60S	60	Low
G27	60S	60	Low				

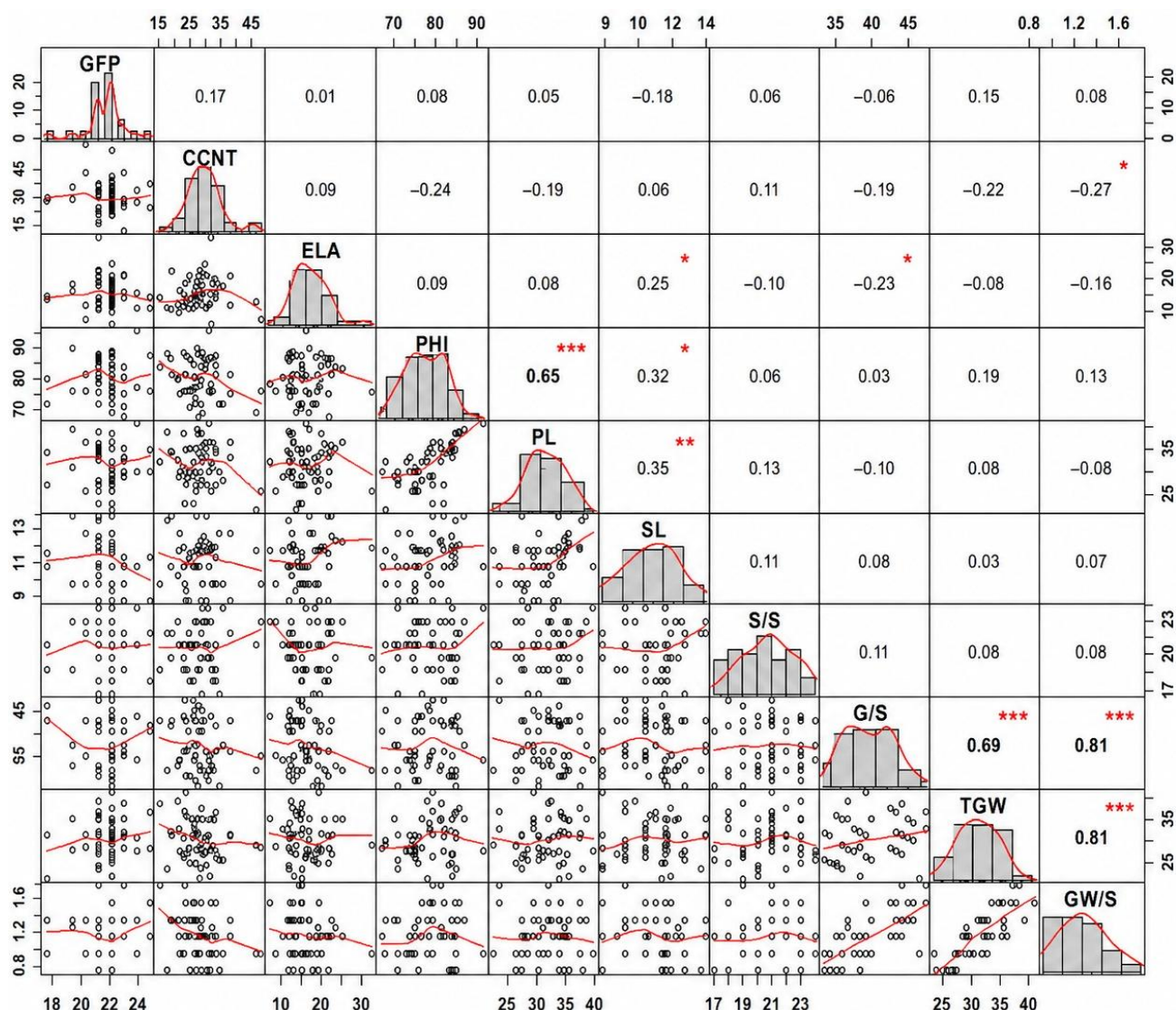


Fig. 1. Correlation matrix showing the relationships between yield-related traits and stripe rust resistance in 53 wheat genotypes.

Table 2. Eigenvalues and explained variance of the first ten principal components from PCA of yield-related traits in 53 wheat genotypes under stripe rust stress.

Variability	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
Eigenvalue	2.41	2.03	1.15	1.12	1.04	0.69	0.66	0.56	0.33	0.02
Variability (%)	24.12	20.29	11.47	11.19	10.35	6.92	6.59	5.62	3.26	0.2
Cumulative %	24.12	44.41	55.88	67.06	77.42	84.33	90.92	96.54	99.8	100

Biplot: Genetic diversity among genotypes for the analyzed attributes is displayed in the biplot. Genotypes that were nearby showed a high degree of genetic similarity, while those that were far exhibited an extensive amount of genetic diversity. Biplot indicated that G7, G14, G20, G22, G29, G17, G43, and G53 were genetically diverse as those were positioned farther from the origin. In addition, the length of the vectors showed the relative fraction of variation among genotypes; in other words, a longer vector indicated more genetic diversity for that trait, and a shorter vector indicated less genetic diversity. The vector lengths for the three traits (SL, GFP, PH, and PL) were greater than those for SL, S/S, C.CNT, GW/S, G/S, and PLA. The angle between two vectors, independent of their length, depicted the relationship between two traits; for instance, a negative correlation is indicated an angle larger than 90, a positive correlation by an angle smaller

than 90, and a right angle (90) by no correlation or independent behavior. TGW, PH, PL, and TGS were favorably connected with GFP, whereas S/S and SL were negatively correlated (Fig. 3).

Cluster analysis: Cluster analysis further categorized 53 genotypes into 6 primary groups with a Euclidean distance of 0.950. The first cluster consisted of only G17. While G24, G3, G13, G18, G22, G27, and G28 were in the second cluster, accounting for 12.97% of all genotypes. There were 17 genotypes in the third cluster comprising of 31.48% of the total genotypes. The fourth cluster contained 11 genotypes, accounting for 20.37% of the total genotypes analyzed. Similarly, there were only four genotypes (G20, G42, G9, G11) in fifth cluster. G35, G44, G43, G51, G6, G12, G15, G31, G2, G14, G25, and G34 were among the 12 genotypes found in the sixth cluster (Fig. 4).

Profile plot: The profile plot revealed significant differences among clusters (Fig. 5). It was observed that highest C.CNT was found in cluster 6, similarly PL and PH was higher in cluster 4. However, results displayed that cluster 3 had intermediate values for most of studied traits. Interestingly, cluster 1 and 2 showed maximum G/S and cluster 5 displayed maximum TGW. Among the clusters moderate variation was recorded in the traits like GW/S, SL, S/S indicating limited variability exhibited among the traits. However, overall considerable amount of variability was observed in profile plot.

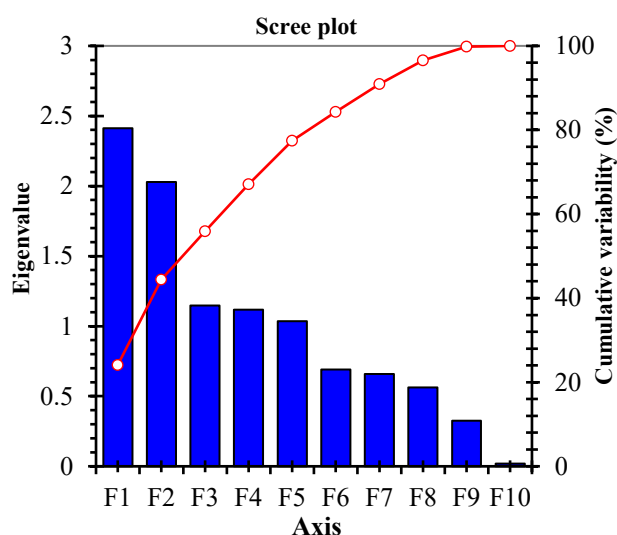


Fig. 2. Scree plot illustrating the eigenvalues and cumulative variability explained by the first ten PCs.

The blue bars represent the eigenvalues of each principal component, while the red line with circles shows the cumulative percentage of variance explained by these components.

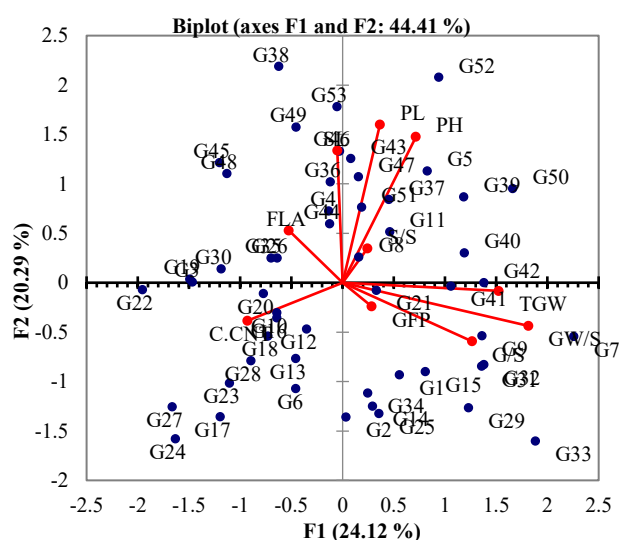


Fig. 3. PCA biplot of the first two principal components for yield-related traits and stripe rust resistance in 53 wheat genotypes.

The blue dots represent the genotypes (G1-G53), and the red vectors represent the traits: grain-filling period (GFP), chlorophyll content (C.CNT), flag leaf area (FLA), plant height (PH), peduncle length (PL), spike length (SL),

spikelets/spike (S/S), grains/spike (G/S), 1000-grain weight (TGW), and grain weight/spike (GW/S). The percentage of variance explained by each principal component is indicated on the respective axes.

Table 3. Percentage contribution (trait loading) of yield-related traits to the first five principal components in 53 wheat genotypes under stripe rust stress.

Traits	F1	F2	F3	F4	F5
GFP	0.88	0.75	44.68	16.13	7.26
C.CNT	9.47	1.93	12.24	6.13	12.12
FLA	3.03	3.65	0.97	28.03	33.71
PH	5.62	28.35	0.8	1.3	2.08
PL	1.45	33.31	0.86	2.9	2.58
SL	0.03	23.29	7.57	5.82	2.25
S/S	0.65	1.58	21.59	35.8	9.97
G/S	17.47	4.58	9.72	2.05	7.00
TGW	25.41	0.09	0.56	0.08	20.48
GW/S	36.00	2.48	1.02	1.76	2.55

Abbreviation: grain-filling period (GFP), chlorophyll content (C.CNT), flag leaf area (FLA), plant height (PH), peduncle length (PL), spike length (SL), spikelets/spike (S/S), grains/spike (G/S), 1000-grain weight (TGW), grain weight/spike (GW/S)

The diagonal displays the distribution of each trait. Scatter plots in the lower triangle illustrate the pairwise relationships, with a red line indicating the trend. Correlation coefficients are shown in the upper triangle, with asterisks indicating the significance level (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

Abbreviation: grain-filling period (GFP), chlorophyll content (C.CNT), flag leaf area (FLA), plant height (PH), peduncle length (PL), spike length (SL), spikelets/spike (S/S), grains/spike (G/S), 1000-grain weight (TGW), grain weight/spike (GW/S)

Discussion

Stripe rust is arguably one of the most destructive diseases of wheat crop rapidly evolving and overcoming the race specific resistance. Therefore, identifying genetically diverse wheat genotypes with durable APR is essential for the development of climate smart wheat cultivars. Wheat genotypes were evaluated against artificially inoculated stripe rust pressure focusing on yield-related traits. The wheat genotypes showed a great deal of divergence to resistance against stripe rust reflecting available genotypes of having rich gene pool valuable for future breeding programs and sustainable wheat production to improve food security (Jan *et al.*, 2026). Results clearly displayed that approximately 18 genotypes exhibited low CI values. This is in line with findings of several researchers (Afzal *et al.*, 2015; Shahin *et al.*, 2024). Wheat germplasm has mainly been evaluated mostly for yield and against abiotic stress. However, in the present study, we evaluated germplasm not only against stripe rust but also to identify yield-related indices as stripe rust interferes with photosynthesis indirectly causing severe yield losses (Chen *et al.*, 2026). All morphological, physiological and yield traits were affected due to stripe rust (Atsbeha *et al.*, 2026). GFP and major agronomic traits like PH, PL, GW/S and TGW suggested that prolonged GFP might enhance grain development and yield stability even under disease pressure (Iftikhar *et al.*, 2002; Amjad *et al.*, 2023; Dukamo *et al.*, 2023). FLA, which is typically associated with photosynthetic capacity, interestingly

showed negative correlations with most yield-related traits in this study under disease pressure as greater leaf area may provide greater space for pathogen development leading to decreased photosynthetic efficiency.

PCA identified 5 major components and having eigenvalue greater than 1 which cumulatively explained 77.42% of the total variability. Distinct position of genotypes such as G7, G14, G20, and G43 in PCA biplot indicated high genetic divergence for agronomic and disease resistance. Traits like SL, GFP, PH, and PL contributed most significantly to genotypic variability thus could well serve as potential selection indicators for identification of disease resistant germplasm. The finding of the current investigation did not agree with previous studies where yield-related traits often dominated variation, thus emphasizing the distinct physiological responses of current germplasm set under stripe rust infection (Jevtić *et al.*, 2026).

Genotypes with longer GFPs may tolerate the stripe rust thus maintaining grain yield (Mullualem *et al.*, 2024). Wide range of diversity on the basis of agronomic and yield traits was demonstrated as cluster analysis revealed that 53 genotypes of wheat were distributed in 6 distinct groups indicating that each group possessed distinct adaptive mechanism of handling the disease pressure. Our results are in accordance with the findings of (Liu *et al.*, 2019; Khan & Iqbal, 2025; Panthi & Poudel, 2026). Normally it was observed that stripe rust in wheat affect the photosynthesis causing the yield losses but genotypes having resistance against stripe rust can mitigate the adverse effects of stripe rust (Khan *et al.*, 2025; Sivhada *et al.*, 2025).

This study advances the methodological approach to wheat genotype evaluation by combining multivariate analyses with artificial disease screening. The integration of PCA, biplots, and cluster profiling under biotic stress is still rare in the literature, particularly in relation to stripe rust. These findings provide actionable insights for breeders aiming to select diverse, resilient genotypes with superior agronomic traits under pathogen pressure. Future research should further explore the molecular underpinnings of these trait associations to enhance marker-assisted selection strategies.

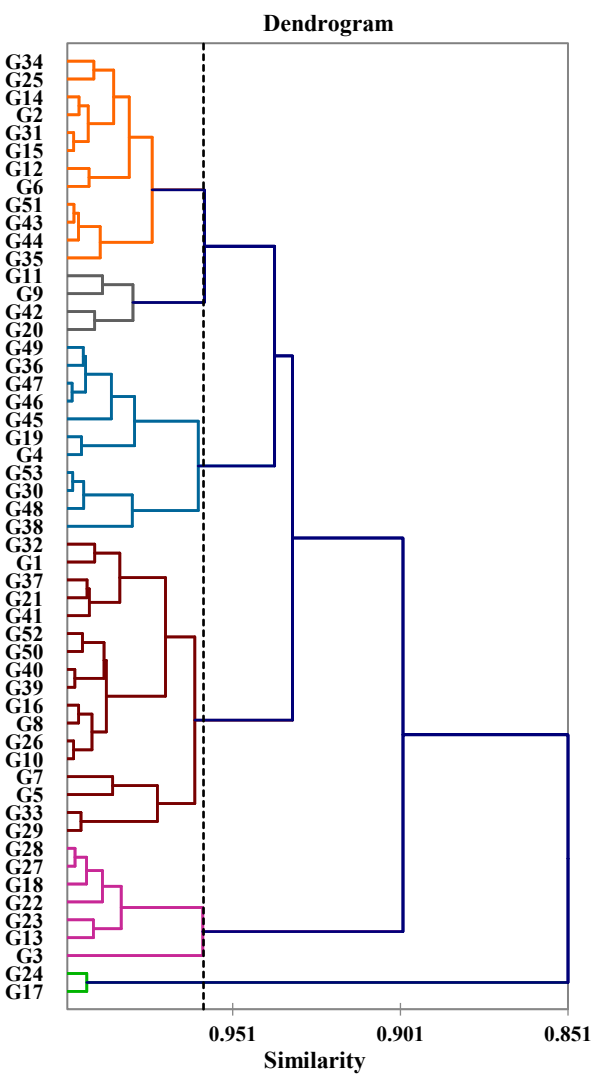


Fig. 4. Dendrogram illustrating the hierarchical clustering of 53 wheat genotypes based on their yield-related traits and stripe rust resistance. The x-axis represents the similarity coefficient, and the vertical lines connect genotypes or groups of genotypes based on their similarity. Different colored branches indicate distinct clusters of genotypes.

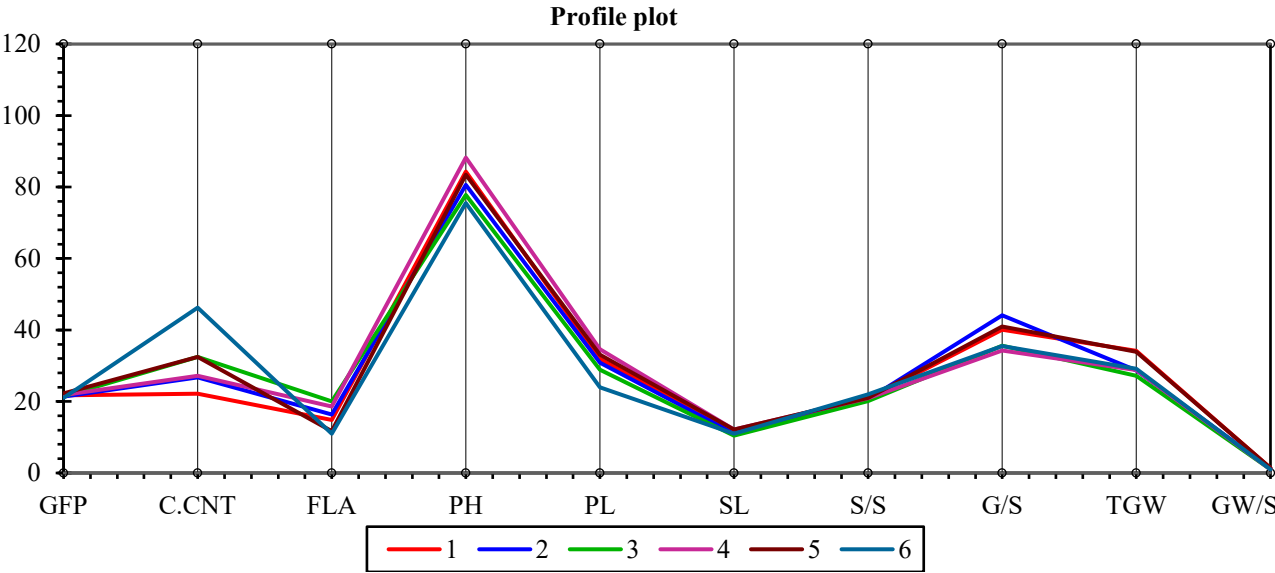


Fig. 5. Profile plot showing the mean performance of six distinct clusters of wheat genotypes for different yield-related traits and potential stripe rust resistance indicators.

The x-axis represents the traits: grain-filling period (GFP), chlorophyll content (C.CNT), flag leaf area (FLA), plant height (PH), peduncle length (PL), spike length (SL), spikelets/spike (S/S), grains/spike (G/S), 1000-grain weight (TGW), and grain weight/spike (GW/S). The y-axis represents the relative values of these traits for each cluster.

Conclusion

The present study evaluated 53 wheat genotypes grown under artificially induced stripe rust inoculation; consequently, resistant genotypes were identified on the basis of low CI values. Multivariate statistical tools effectively identified major traits contributing to phenotypic variation leading to discrimination of potentially divergent genotypes. Results revealed that GFP, PH, and PL were closely linked with improved disease tolerance, but in contradiction to these traits larger FLA was strongly associated with susceptibility to stripe rust. The identified genotypes may serve as promising donors for developing high yielding and disease resistant cultivars. Furthermore, integrating field based phenotypic screening with multivariate analyses offers an effective strategy for efficient selection of sustainable and resilient wheat genotypes under the changing environmental conditions.

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