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Research Article

Host Resistance and Morpho-Physiological Responses of Wheat Genotypes to Stripe Rust Caused by *Puccinia striiformis* f. sp. *tritici* at Seedling and Adult Stages

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ABSTRACT

Wheat (*Triticum aestivum* L.) is an important cereal crop and one of the main factors for food security in most countries, including Pakistan. Its production is seriously threatened by stripe rust, one of the most devastating diseases that causes significant yield losses. Therefore, this study evaluated the resistance of 21 wheat genotypes to stripe rust at seedling and adult stages by artificial inoculation. The results showed that LLR-9 and WC-19 were resistant to yellow rust at both stages, while Mexi-Pak, Sarsabz, and Sonora were highly susceptible to the disease, and severe infections occurred in the adult stage. Mexi-Pak was found to be susceptible to the disease at both stages, while Sarsabz and Sonora were only affected at maturity. Based on analysis of genetic variability and ANOVA, high genetic diversity was found for PHT, FLA, GY, TGW, and CC, out of which PHT, GY, and TGW were the crucial factors associated with resistance to the disease. Principal component analysis revealed that PHT, GY, and TGW were the most influential traits in distinguishing between resistant and susceptible genotypes. In contrast, CC and FLA also contributed to higher photosynthesis efficiency and supporting disease resistance. High PCV and GCV values were found for traits such as awn length, showing great potential for genetic improvement of these traits. NTP, SD, and AL had high heritability and showed good genetic control, whereas, in the case of NGS and CC, the heritability was low and thus involved a stronger environmental influence. This study demonstrates the need for wheat genotypes with disease resistance and high agronomic traits in breeding programs. It highlights LLR-9 and WC-19 as sources of resistance and provides helpful information about genetic factors responsible for resistance to the disease and productivity.

Keywords: Host resistance, Yellow rust, Genetic variation, Grain yield, Heritability, PC analysis

INTRODUCTION

Wheat (*Triticum aestivum* L.) is the most important cereal crop and is consumed as a major staple food in Pakistan. It is cultivated at 9.60 million hectares, producing 29.69 million tons (MNFSR 2024). Wheat faces precarious challenges due to biotic and abiotic factors (Jabran *et al.*, 2021). Under biotic stress, one of the worst threats is stripe rust caused by a biotrophic fungus (*Puccinia striiformis* f.sp. *tritici*).

It forms light yellow pustules, usually on the leaf in the form of stripes and sometimes also on the stem and ear. Later, these pustules turn into yellow-orange spores that release black spores when ripe. The infestation of stripe rust is favored by a mild and long winter (0-25°C) with high humidity (Maccaferri *et al.*, 2015). Stripe rust infestation has led to significant wheat losses worldwide as well as in Pakistan (Habib *et al.*, 2020). Due to this devastating pathogen, yield losses of up to 10 to 70% have been reported in susceptible varieties (Raza *et al.*, 2018). It is responsible for an annual yield decline of 5.5 million tonnes of wheat worldwide (Khanfri *et al.*, 2018; Kusunose *et al.*, 2023). Pakistan faced over 20% losses during rust epidemics in 1973, 1978, 1995, and 2003 (Ibrahim *et al.*, 2017) and annual losses were estimated at up to Rs. 1.5 billion. Stripe rust feeds on the host plant's nutrients, affecting the host plant's life cycle in many ways. These effects can include poor seed germination, reduced plant height, slow growth rate, poor forage quality, and increased leaf damage, ultimately reducing photosynthetic activity. These factors resulted in poor seed formation, shriveled grains, and reduced yields (Chen *et al.*, 2022).

Rust resistance in wheat is divided into two types (seedling resistance and adult resistance) depending on the stage of infestation. Genes controlling seedling resistance are expressed at both seedling and adult stages. However, the adult resistance genes only show their expression after the seedling stage. Previous studies indicate that genes controlling seedling resistance express different response types and do not directly affect yield (Lagudah *et al.*, 2006; Babu *et al.*, 2020). Prevention and control of this disease using fungicides is mostly ineffective, uneconomical, harmful to the environment, and poses health risks to farmers and consumers. Therefore, developing resistant varieties is the only alternative to producing wheat under disease-prone conditions (Luo *et al.*, 2023). By using high-yielding rust-resistant genotypes, wheat yield losses can be minimized. The spread of the disease can be controlled either by chemical control or breeding for rust resistance, as well as some cultural practices to reduce yield losses (Khan *et al.*, 2025). Aside from the effectiveness of cultural practices and chemical control, these are not widely used in most developing countries (Lodhi *et al.*, 2018). In contrast, the development and use of resistant varieties is feasible and environmentally friendly (Jabran *et al.*, 2024).

The present study represents a research gap by including old wheat varieties that serve as a historical benchmark that can appraise changes in stripe rust resistance. These older varieties have poorer agronomic performance; however, they may carry novel resistance mechanisms that are of value in elucidating the genetic basis of disease resistance. By comparing them with the advanced breeding lines, this research has estimated the improvement or divergence of the modern varieties for developing genotypes with more durable, long-lasting resistance. In this background, the present research has been focused on the measurement and testing of stripe rust and its influence on yield-promoting traits of some elite wheat genotypes of Pakistan.

MATERIALS AND METHODS

Experimental material

The seeds of twenty-one wheat genotypes, including commercial varieties, local landraces, and advanced lines, were collected from the Department of Plant Breeding and Genetics, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan (Table 1). The experiment was carried out under *In-vivo* and *In-vitro* conditions. The inoculum of the dominant virulent race *Puccinia striiformis* f. sp. *tritici* was collected from the Crop Disease Research Institute (CDRI), National Agriculture Research Centre, Islamabad, Pakistan.

In-vitro experiment

Six seeds per genotype were sown separately in disposable plastic cups (500 ml capacity) in three replicates according to a completely randomized design (CRD) for artificial inoculation. The cups were placed in a growth chamber with an optimal temperature of 23±2°C, relative humidity of 60-70%, and light for better germination. The seedlings were artificially inoculated two weeks after germination to monitor disease infestation.

Inoculation

Puccinia striiformis f. sp. *tritici* urediospores were weighed at 250 mg using an electric balance and placed in a petri dish to form a paste by adding a few drops of lukewarm water. The paste was diluted in 1000 ml of pure water, and 2-3 drops of Tween-20 were added to the solution to reduce surface tension. The freshly prepared inoculum was sprayed onto the seedlings as recommended by (Roelfs *et al.*, 1992).

Incubation

Inoculated seedlings were transferred to the growth chamber at 96% relative humidity and a temperature of 5–9°C under (suitable conditions for successfully establishing urediospores). After 48 hours, the inoculated seedlings were

transferred to the greenhouse with a temperature of 12–18°C and a photoperiod of 14 hours. Disease levels were measured two weeks later (Liu *et al.*, 2010).

Table 1. Wheat genotypes with their parentage used in the experiment.

Sr.No.	Variety	Category	Parentage
1	Bahawalpur-2000	Commercial variety	AU/UP301//GLL/Sx/3/PEW 'S'/4/MAI 'S'/MAY A 'S'//PEW'S'
2	Bahawalpur-94	Commercial variety	NA
3	Faisalabad-85	Commercial variety	MAYA-MONCHO 'S' X KVZ-TRM
4	Inqilab-91	Commercial variety	WL711/CROW 'S'
5	Kaghan-93	Commercial variety	TTR/JUN
6	Mexi-Pak	Commercial variety	PJ62 'S'-GB-55
7	Morocco	Commercial variety	NA
8	Nowsehra-96	Commercial variety	NA
9	Punjab-85	Commercial variety	KVZ/TRM//PTM/ANA
10	Rohtas-90	Commercial variety	INIA66/A.DISTT/INIA66/3/GEN.81
11	Sarsabz	Commercial variety	PI/FRND//MXP/3/PI/M20/70
12	Sonora	Commercial variety	NA
13	Shafaq-06	Commercial variety	LU26/HD2179//2*INQILAB91
14	Shalimar-94	Commercial variety	NA
15	Sehar-2006	Commercial variety	CHIL/2*STAR/4/BOW/CROW//BUC/PVN/3/2*VEE#10
16	Suleman-96	Commercial variety	F6.74/BUN//SIS/3/VEE#7
17	Sutluj-86	Commercial variety	CMT/YR//MON
18	Zarlashta	Commercial variety	URES/BOW 'S'
19	WC-19	Advance line	NA
20	WC-24	Advance line	NA
21	LLR-9	Land race	NA

Disease scaling

The seedling infestation was scored on the 0 to 9 disease rating scale reported by Line *et al.* (1974), known as the disease scale/infection type. The infection type (IT) scale consists of three sections. The IT ranged from 0-3 indicated resistance (no to few traces of spores), medium susceptible IT thresholds were 4-6 (mild to moderate spores), and susceptible IT scales were 7-9 (abundant spores).

In-vivo experiment

The field test was carried out in the 1st week of November 2016. The seeds were sown in rows using a dibbler machine. Each genotype was planted in two rows 3 m long and 30 cm apart in a randomized complete block design (RCBD) with three replicates. As a control, the universal stripe rust susceptible wheat variety Morocco (Afzal *et al.*, 2008; Lodhi *et al.*, 2018) was sown at the border and within the experimental units at regular intervals to promote natural rust spore formation.

Disease severity at the adult stage

The spores were collected manually from Morocco to find out the severity of stripe rust in wheat genotypes. The suspension was prepared by mixing 0.1 g of spores in 1000 ml of distilled water with 2-3 drops of Tween-20. The inoculum was sprayed in the evening during the booting phase. When 80% of the Morocco plants were found to be susceptible, data was collected by scaling all genotypes according to the modified Cobb scale (Peterson *et al.*, 1948), by recording the infection percentage on the flag leaf.

Observations and statistical analysis

Data from ten randomly selected plants per genotype from each replicate were recorded for plant height PHT (cm), flag leaf area FLA (cm²), number of tiller plants⁻¹ NTP, spike density SD, awn length AL (cm), number of grains spikelet⁻¹ NGS, peduncle length PL (cm), 1000-grain Weight TGW (g), chlorophyll content CC (%), grain yield GY (ton/ha), and analysis of variance and correlation analysis were performed using R package agricolae, Tukey's Honest Significant

Difference (HSD) test was applied on resulting ANOVA at $P < 0.05$ to find the pairwise comparison between the groups. Genetic variability was estimated by R package variability (version 0.1.0). Pearson's correlation was determined by corrplot (version 0.92) using R studio version: 2024.09.1+394 (R foundation for statistical computing, Vienna, Austria). Graphs were generated using GraphPad Prism version 9.0 for Windows.

Coefficient of Variability

Both phenotypic and genotypic coefficients of variability were calculated using the expression proposed by (Burton and De Vane, 1953):

$$\text{GCV (\%)} = \frac{\sigma_g}{\text{GM}} \times 100$$

$$\text{PCV (\%)} = \frac{\sigma_p}{\text{GM}} \times 100$$

Where GCV is the genotypic coefficient of variance, σ_g is the genotypic standard deviation, GM is the grand mean, PCV is the phenotypic coefficient of variance, and σ_p is the phenotypic standard deviation. GCV and PCV calculated values were characterized into three categories viz., low (0-10%), moderate (11-20%) and high (21% and above) as suggested by Sivasubramaniam and Madhava Menon (1973).

Heritability

Heritability in the broader sense was assessed using the method proposed by (Hanson *et al.*, 1956)

$$H^2 = \frac{\sigma_g^2}{\sigma_p^2} \times 100$$

Here, H^2 is the heritability (in the broadest sense, σ_g^2 is genotypic variance, σ_p^2 is phenotypic variance. The heritability was divided into three groups, namely: low (0–30%), moderate (31–60%), and high (61% and above), as described by (Robinson *et al.*, 1949).

Genetic advance

The genetic advance was estimated by following the equation of (Johnson *et al.*, 1955)

$$\text{GA} = H^2 \times \sigma_p \times K$$

Where H^2 is broad-sense heritability, σ_p is the standard deviation of phenotype, and K is the selection differential (1.755 at 10%). It is also categorized into three grades: low (0-10%), moderate (11-20%), and high (21% and more) (Johnson *et al.*, 1955).

Relative expected genetic advance

$$\text{EGA} = \frac{\text{GA}}{\text{GM}} \times 100$$

Where EGA is the expected genetic advance, GM is the general mean of the character. It is further categorized into three classes, i.e. low (0-10%), moderate (11-20%), and high (21% and above, as suggested by Johnson *et al.* (1955).

RESULTS AND DISCUSSION

Stripe rust disease assessment at the seedling stage

The data for infection type (IT) on wheat seedlings were recorded after 16 days of inoculation. The results showed resistance in eight genotypes where the IT value was between 0 and 3. Among the resistant genotypes, an IT value of 0 was observed in LLR-9 and WC-19, in which no visible yellow rust symptoms were observed, as shown in Table 2, Figure 1A. Necrotic/chlorotic spots were found in WC-24 and Sehar-2006, but no stripe rust spores were present; therefore, WC-24 and Sehar-2006 were categorized into IT type 1. For infection type 2 – 3, necrotic spots with trace spores were found in four genotypes viz., BWP-2000, Punjab-85, Shalimar-94, and Zarlasha. In FSD-85, Nowshera-96, Rohtas-90, Suleman-96, and Kaghan-93, moderate yellow rust with necrosis symptoms and mild to moderate spores with an infection type of 4 to 6 were detected (Figure: 1B). Eight genotypes were added to the yellow rust susceptible queue, including BWP-94 and Inqilab-91, which showed abundant spores along with streaks of chlorosis/necrosis, while Mexi-Pak and Morocco were found to be highly susceptible with symptoms of abundant spores without any signs of yellow rust was classified as necrosis or chlorosis (Figure 1C). It is concluded that varieties LLR-9 and WC-19 are resistant to yellow rust infection and could be considered for the yellow rust breeding program. Our results are consistent with those of (Bux *et al.*, 2011). They reported that varieties grown in Pakistan have a low genetic basis for significant biotic stresses. These varieties have few genes that control stripe rust at the seedling stage, making them susceptible to developing new pathotypes over time. These results were also supported by (Stepień *et al.*, 2003; McVey *et al.*, 2004; Najeeb *et al.*, 2005).

Table 2. Disease severity/ infection type (IT) of stripe rust on wheat seedlings.

Sr.No.	Variety	IT type*	Genotypes
1	Resistant	0	LLR-9, WC-19
		1	WC-24, Sehar-2006
		2-3	BWP-2000, Punjab-85, Shalimar-94, Zarlashtra
2	Intermediate susceptible	4-6	FSD-85, Nowsehra-96, Rohtas-90, Suleman-96
		6	Kaghan-93
3	Susceptible	7-8	BWP-94, Inqilab-91
		8	Sarsabz, Sonora, Sutluj-86
		8-9	Shafaq-06
		9	MexiPak, Morocco

*Whereas, the seedling infection type (IT) was scored on the 0 to 9 disease rating scale reported by Line *et al.* (1974).

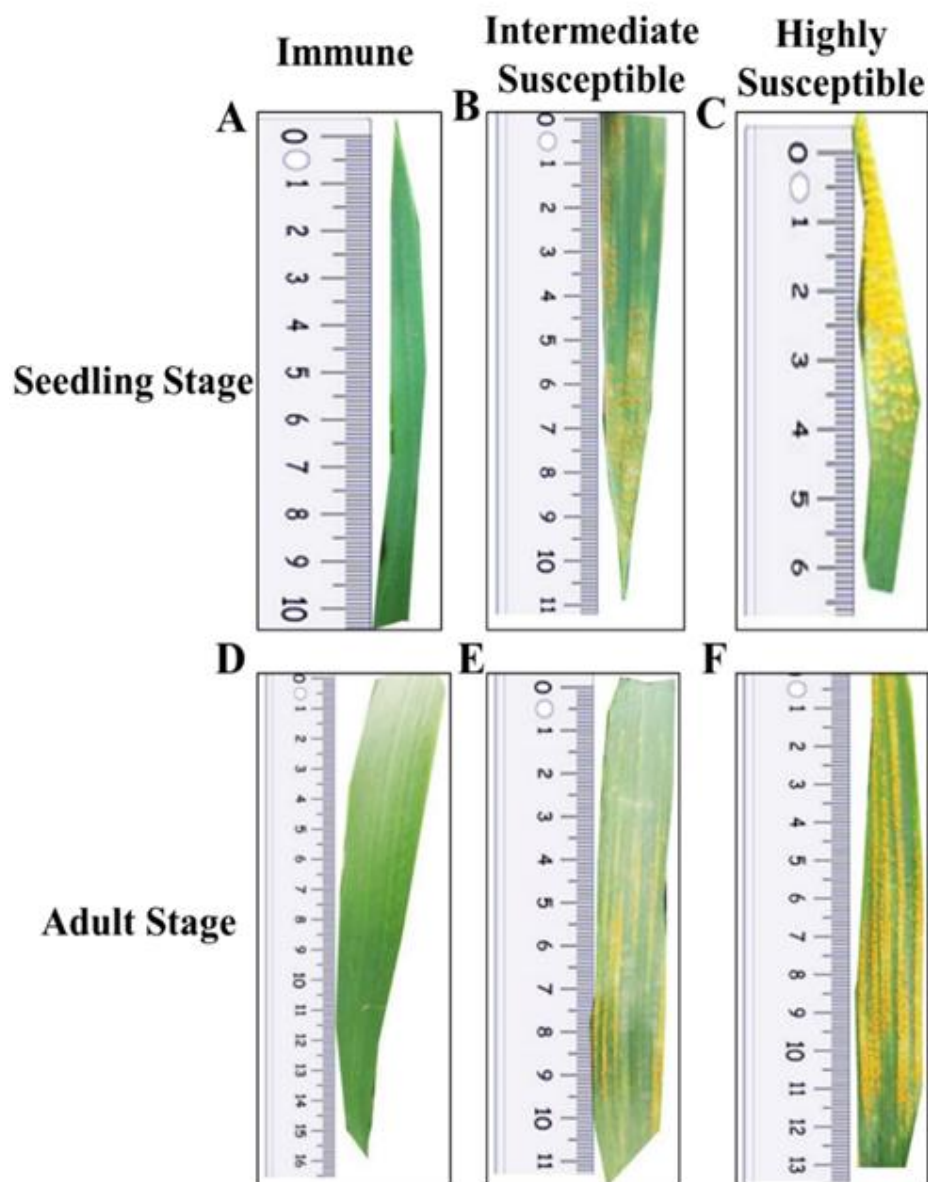


Figure 1. (A-C) Showing the effect of Disease severity/ infection type (IT) of stripe rust on wheat seedlings stage. (D-E) Showing the effect of Disease severity of stripe rust on wheat adult stage.

Stripe rust disease assessment at the adult stage

Disease severity was observed in the wheat genotypes in April when 80% of diseases occurred in the control genotype “Morocco”. According to the results, there are two genotypes viz. LLR-9 and WC-19 were found to be immune as no symptoms of yellow rust were observed, as shown in Table 3, Figure 1D. Two genotypes, WC-24 and Sehar-2006, were resistant to yellow rust as necrosis was observed without uredia. Three wheat genotypes including Nowsehra-96, Punjab-85 and Shalimar-94 were categorized as moderately resistant genotypes when small uredia surrounded by chlorosis were observed (Figure: 1E). Five genotypes consisting of BWP-2000, FSD-85, Rohtas-90, Suleman-96 and Zarlashtha (Table 3) were classified into the moderately resistant to the moderately susceptible group and showed symptoms of moderate uredia often surrounded by chlorosis. Five genotypes showed symptoms ranging from moderately susceptible to susceptible, including BWP-94, Inqilab-91, Kaghan-93, Shafaq-06, and Sutluj-86 (Table 3, Figure: 1F). In comparison, the remaining four genotypes from MexiPak, Morocco, Sarsabz and Sonora showed signs of high susceptibility with large uredia without necrosis and little or no chlorosis. Of twenty-one wheat genotypes, only seven (33%) fell within the immune to moderately resistant range, while fourteen (66%) ranged from moderately resistant to susceptible, as described in Table 3.

Table 3. Disease severity of stripe rust in wheat accessions assessed at the adult stage using Cobb's Scale.

S. No.	Disease reaction	Remarks	Genotypes
1	Immune	0	LLR-9, WC-19
2	Resistant	R	WC-24, Sehar-2006
3	Moderately resistant	MR	Nowsehra-96, Punjab-85, Shalimar-94
4	Moderately resistant to moderately susceptible	MRMS	BWP-2000, FSD-85, Rohtas-90, Suleman-96, Zarlashtha
5	Moderately susceptible to susceptible	MSS	BWP-94, Inqilab-91, Kaghan-93, Shafaq-06, Sutluj-86
6	Susceptible	S	MexiPak, Morocco, Sarsabz, Sonora

Once resistant to yellow rust, many wheat varieties become susceptible after being attacked by a particular breed of pathogen and can change resistance. A race 134E150 of the pathogen was able to attack the Yr-9 gene of wheat and ultimately break the resistance of FSD-85, which has two resistance genes, Yr-9 and Yr-4 (Ahmed, 2000; Basnet *et al.*, 2022), Kaghan-93 via Yr -9 gene along with other genes, Rohtas-90 protected by Yr-6 and Yr-7 genes, Sutluj-86 with Yr-9 gene, and other varieties with similar genes caused a loss of more than Rs. 270 billion (Afzal *et al.*, 2008). Inqilab-91 was one of the best-known wheat varieties in Pakistan and, along with other Yr genes, possesses the resistant Yr-27 gene. Its resistance was broken by *Attila virulence* (166E143A+) between 2002 and 2004, which changed the entire rust scenario (Yahyaoui and Rajaram, 2012; Gangwar *et al.*, 2019). Mexi-Pak was susceptible to yellow rust at both adult and seedling stages. Our results are consistent with the results of (Mirza *et al.*, 2012), as Mexi-Pak showed S response in 1999/2000 and 2002/2003. Sarsabz was also susceptible to yellow rust. Our results are related to the results of (Bux *et al.*, 2012; Gangwar *et al.*, 2019). It was observed that Sarsabz became susceptible to stripe rust only in the presence of the Yr-7 gene. Sehar-2006 possesses the unwanted susceptible Yr-9 gene (Iqbal *et al.*, 2016). WC-24 was found to resist yellow rust due to a few uncharacterized genes. Shafaq-06 with Yr-5 and Yr-10 (Marchal *et al.*, 2018) showed moderate susceptibility to susceptible reactions. Sonora proved highly vulnerable. Shalimar-94 was between resistant and moderately resistant. Suleman-96, Zarlashtha was MRMS. The above genotypes, i.e. LLR-9 and WC-19, were found to be resistant to yellow rust even in the adult stage, showing the varieties' strong genetic potential against rust. Our results are close to those of (Marchal *et al.*, 2018; Gangwar *et al.*, 2019; Basnet *et al.*, 2022; Luo *et al.*, 2023). Therefore, these varieties can be used in further breeding programs against *Puccinia striiformis*.

Morphological and Yield traits

Analysis of variance

Analysis of variance (ANOVA) was performed for all morpho-yield parameters. Highly significant ($p < 0.001$) variation in genotypes was observed for all the parameters except for NGS, which was found significant ($p < 0.05$). Table 4 presents the results of variance analysis, while Figure 2 presents the mean values of yield and agronomic parameters along with LSD values for a clear representation of significantly different groups. The CV value for all the parameters ranges from 2.10% in AL to 16.78% in FLA, highlighting the accuracy and reliability of the data and ensuring minimum

experimental error. Previous research on wheat genetics and breeding supports the results of the analysis, which show significant genetic variation for parameters such as PHT, FLA, NTP, SD and TGW. For example, (Upadhyay *et al.*, 2020) conducted a study to examine the genetic variation between the different agronomic parameters. As a result, heritability and coefficient of variation were found to exhibit a significant level of genetic diversity within the parameters studied (Upadhyay *et al.*, 2020). Similarly, numerous other studies have demonstrated strong genetic variation in awn length, chlorophyll content, and grain yield. Numerous genetic factors are responsible for this inconsistency (Upadhyay *et al.*, 2020; Prabha *et al.*, 2022). Our results indicate that all examined parameters show a significant genetic difference. In addition, a broader range of opportunities to improve wheat breeding programs to increase yield and other agricultural parameters have been highlighted.

Table 4: ANOVA means square values and coefficient of variation (CV) for wheat parameters

SOV	Dof	PHT	FLA	NTP	SD	AL	NGS	PL	TGW	CC	GY
REP	2	399.47	29.35	0.32	0.02	0.01	0.61	2.03	372.01	1.65	0.81
GEN	20	194.14**	137.5**	3.53**	0.88**	8.50**	0.82*	12.0**	74.9**	12.5**	4.53**
ERROR	40	24.94	25.25	0.18	0.04	0.01	0.31	0.47	14.15	2.89	0.78
CV %		4.64	16.78	4.58	10.30	2.10	15.29	3.39	8.89	3.77	15.95

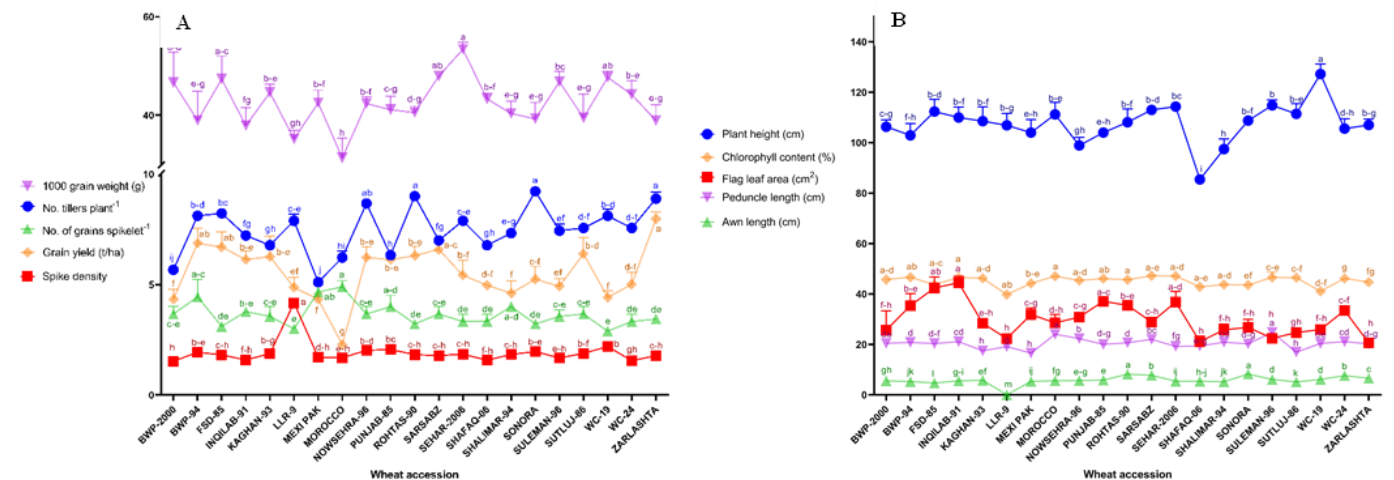


Figure 2. Mean performance of yield components (A) and morpho-physiological parameters (B) with grouping across wheat accessions.

Genetic variability

Genetic variation analysis of the main agronomic traits of wheat showed remarkable genetic variability, while moderate to high estimates of heritability were found for most of the studied traits. High heritability values for NTP, SD, and AL of 90.66%, 87.79%, and 99.48%, respectively, demonstrated substantial genetic control and the promise of improvement through selection. The heritability for PL and TGW is moderate and, therefore, can be used for improvement through genetic intervention. Low heritability was found for NGS and CC. Therefore, environmental factors play an essential role in these characteristics. These results are consistent with previous reports by (Santosh *et al.*, 2019; Khan *et al.*, 2025) and are further supported by (Fernandez *et al.*, 2022; Sewore and Abe, 2024), who demonstrated in their reports the paramount importance of genetic variability and heritability in wheat breeding. The results of the present study suggest that highly genetically controlled traits such as NTP, SD, and AL are recommended for breeding programs through selection. In contrast, environmentally dependent traits may require sophisticated breeding techniques. This is reflected in Table 5, which summarizes the genetic variability parameters. colour coding reflects the strength of the genetic contribution of each trait. Green represents a strong genetic contribution or variability (High); Orange represents a medium contribution, and yellow represents a low genetic influence.

Heritability and genetic advance

Estimates of heritability (h^2) and genetic advance in wheat are presented for various agronomic traits, as shown in Table 5, demonstrating that a significant genetic effect on some of the key traits should be improved using selection techniques. Traits including plant height, number of tillers plant⁻¹, spike density, awn length, peduncle length, and grain yield had high estimates of heritability, ranging from 61.73% to 99.48%, as shown in Table 5, indicating a strong genetic

influence on recorded phenotypic variation. Expected genetic advance was observed high for flag leaf area, number of tillers plant⁻¹, spike density, awn length and grain yield ranging from 32.52% to 59.95%, suggesting that the selection of these traits is likely to result in significant genetic improvements. Similarly, peduncle length (PL) and thousand-grain weight (TGW) showed moderate heritability and genetic progress, suggesting that they can be improved through breeding practices. This may also mean that traits such as NGS and CC, with a heritability of 35.5% and 52.42%, respectively, have a lower genetic gain of 13.93% and 5.91%. This is a clear indication that the impact of the environment on these features is quite significant; therefore, improving these traits requires more advanced breeding techniques.

Table 1. Genetic variability and heritability of wheat traits.

Parameters	PV	GV	PCV	GCV	H ²	GA	EGA
PHT	81.34	56.4	8.39	6.98	69.34	12.88	11.97
FLA	62.67	37.42	26.44	20.43	59.71	9.74	32.52
NTP	1.25	1.14	14.98	14.27	90.66	2.09	27.98
SD	0.32	0.28	29.47	27.61	87.79	1.02	53.29
AL	2.84	2.83	29.25	29.18	99.48	3.46	59.95
NGS	0.48	0.17	19.04	11.35	35.5	50.69	13.93
PL	4.32	3.85	10.21	9.63	88.99	3.81	18.71
TGW	34.41	20.25	13.85	10.63	58.87	7.11	16.8
CC	6.08	3.19	5.47	3.96	52.42	2.66	5.91
GY	2.03	1.25	25.78	20.26	61.73	1.81	32.78

Whereas yellow color shows low values, brown color medium, and green color shows higher values; PV; phenotypic variance, GV; genotypic variance, PCV; phenotypic coefficient of variance, GCV; genotypic coefficient of variance, H², heritability, GA; genetic advance, EGA; expected genetic advance, PHT; plant height, FLA; flag leaf area, NTP; numbers of tillers plant⁻¹, SD; spike density, AL; awn length, NGS; number of grains spikelet⁻¹, PL; peduncle length, TGW; thousand grain weight, CC; chlorophyll contents, GY; grain yield.

These results are consistent with some recent studies, such as by (Balkan, 2018), who estimated high heritability and genetic progress for yield-related traits, supporting the effectiveness of selection for these traits in wheat breeding programs. Furthermore, high heritability along with high genetic gain has been reported for certain key traits, such as the number of tillers plant⁻¹ and spike density, which is considered crucial in the case of high productivity in wheat under irrigated and drought conditions (Castellari *et al.*, 2023; Liu *et al.*, 2023). All these results confirm the importance of focusing on the traits of high heritability in the process of wheat improvement while paying attention to those with lower heritability due to environmental influences.

Principal component analysis (PCA) for agronomic and yield parameters

Relationships among the wheat's main agronomically essential traits, PHT, FLA, NTP, SD, NGS, PL, TGW, CC, and GY, and their response to disease were analyzed using Principal Component Analysis. Accordingly, the results were presented using three different types of PCA plots: PCA biplot, PCA individual plot, and PCA variables plot (Figure 3a, b and c). Finally, the PCA biplot presented a clear grouping of disease responses as highly susceptible, intermediate susceptible, and resistant genotypes based on their performance related to key traits. Highly susceptible genotypes were placed on the negative side of PC1, which portrayed poor performance concerning PHT, GY, and TGW traits important for plant disease resistance. In this context, resistant genotypes were positioned on the positive side of PC1, reflecting higher magnitudes concerning these traits and those related to CC and FLA, which are relevant to the photosynthetic efficiency and overall growth under disease stress. At positions closer to the origin, the intermediate susceptible genotypes showed average PHT, GY, and TGW values that can be considered to reflect partial resistance. Further supporting the results were seen in the individual plot of PCA, which clustered genotypes according to their responses to diseases since the resistant genotypes were separated from the highly Susceptible ones, and the intermediate susceptible genotypes fell between them. The variables plot of PCA described the distribution of the agronomic parameters of interest when the vectors corresponding to PHT, GY, and TGW were along the major axis of PC1, while the ones for CC and FLA were along PC2. Regarding selection in search of the development of disease-resistant wheat varieties, wheat breeders should pay more attention to PHT, GY, and TGW because these attributes

contribute most to resistance and productivity traits. This agrees with previously published literature that has established these as agronomic indices that determine the ability of wheat to resist diseases (Spanic *et al.*, 2023; Khan *et al.*, 2025). Breeding programs for these traits will raise the level of disease resistance in wheat varieties without compromising their yielding potential, ensuring food safety and sustainable agriculture (Jabran *et al.*, 2023).

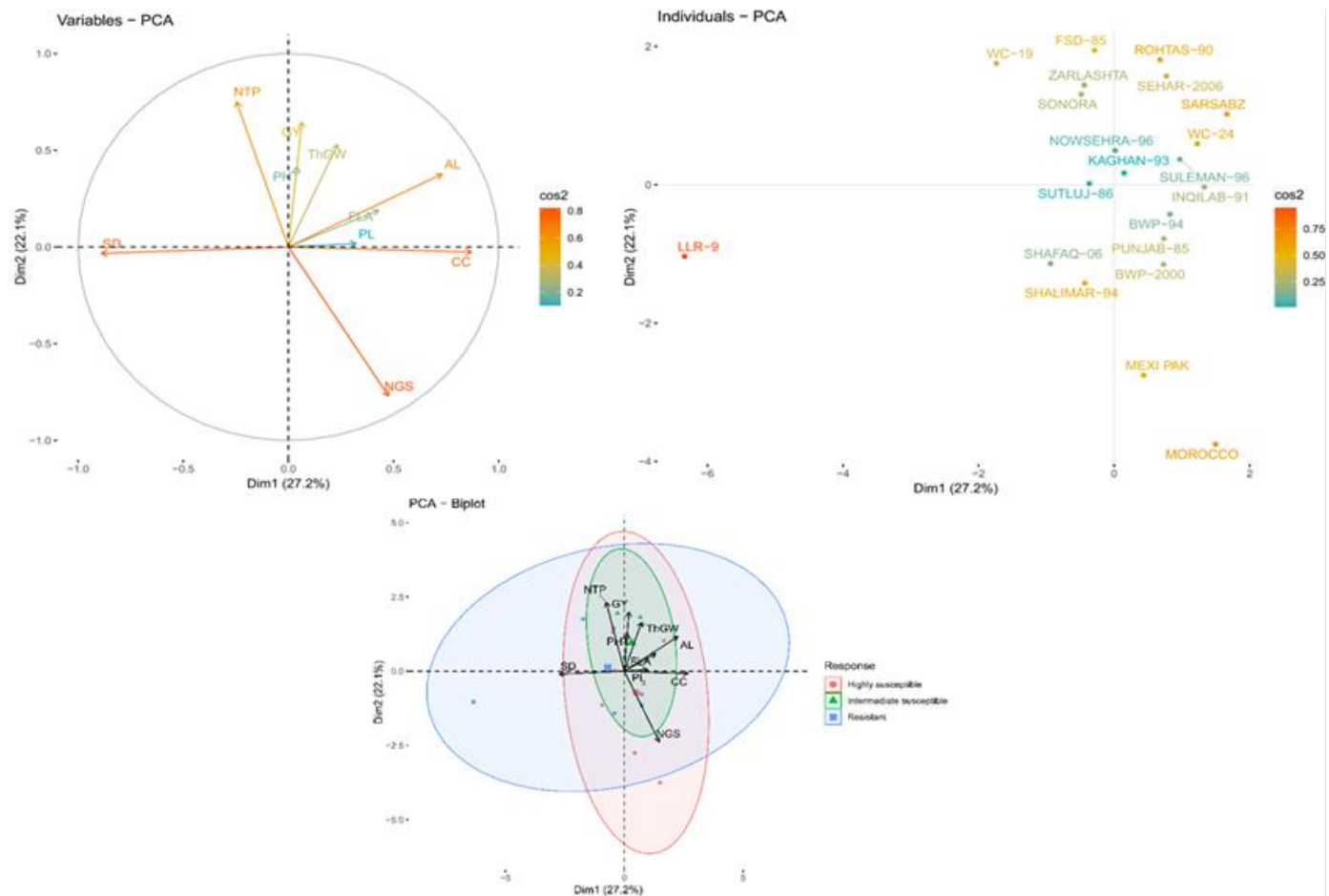


Figure 3. PCA visualization of wheat parameters and genotypes: biplot, individual plot, and variables plot.

Correlation analysis

The correlation among the wheat morpho-physiological and yield-related traits was determined, as shown in Figure 4. Using Pearson's correlation, several interesting correlations are revealed that explain the possible contribution of various factors towards grain yield and other related traits. A positive correlation between AL and CC at $p < 0.05$ may suggest that longer awns can support higher photosynthetic capacity. Chlorophyll content (CC) positively correlated with the number of grains per spikelet at $p < 0.05$, showing its supportive role in grain formation. Grain yield positively correlated with the number of tillers per plant at $p < 0.05$, hence underlining the importance of increasing arable production toward expanding yield potential. On the other hand, there was a significant negative correlation between NTP and NGS at $p < 0.001$, indicating the trade-off between production and the number of grains per spike, which is most probably due to resource competition. Higher values of seed density relate to lower photo vital tissue distribution, which is also evidenced by the high negative correlation of seed density with AL and CC at $p < 0.001$. No relationships between PHT and CC or between FLA and GY could be noticed. The above results show some significant dependencies among wheat traits. For the best yield and resource allocation, breeding programs consider trade-offs from the negative relationship between NTP and NGS and positive synergies represented by CC and GY. This supports the findings of (Duvnjak *et al.*, 2024), who reported a negative significant correlation between NTP and NGS in winter wheat under field conditions, proving that too high plant height can limit yield due to storage effects (Duvnjak *et al.*, 2024). Similarly, NTP also showed a negative association with grain yield in the present study, which is consistent with the findings of (Kumar *et al.*, 2023), who reported that although tillering is a crucial factor in grain number, excess

tillering competes for nutrients, ultimately reducing the number of grains per spike, and negatively impacting yield. On the other hand, chlorophyll content had a positive relationship with grain yield because chlorophyll is largely responsible for photosynthesis (Kumar *et al.*, 2023). Similarly, Elfanah *et al.* (2023) found that higher chlorophyll content increases plant photosynthetic efficiency and increases growth and yield performance, reinforcing the positive correlations between chlorophyll content and grain yield observed in this study. Such findings highlight the complexity of trait interactions in wheat. Optimizing one parameter may impact another, and the latter must be considered in breeding considerations (Elfanah *et al.*, 2023).

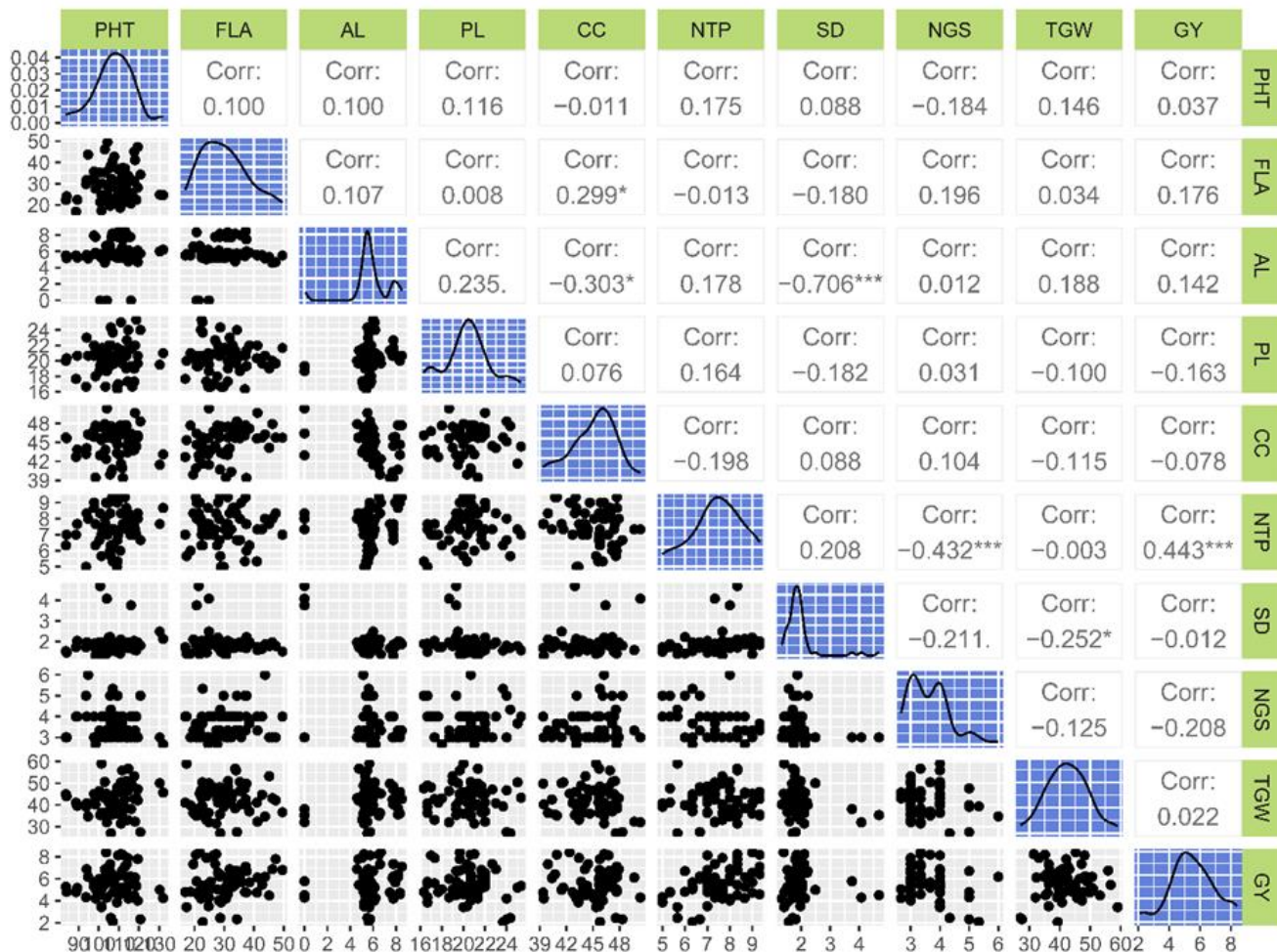


Figure 4. Correlation analysis of wheat morpho-physiological and yield-related traits. Whereas, *=Significant, **= Highly Significant, NS= Non-Significant, PHT; plant height, FLA; flag leaf area, NTP; numbers of tillers plant-1, SD; spike density, AL; awn length, NGS; number of grains spikelet-1, PL; peduncle length, TGW; thousand grain weight, CC; chlorophyll contents, GY; grain yield.

CONCLUSION

This study dealt with yellow rust resistance in wheat genotypes at the seedling and adult stages. LLR-9 and WC-19 exhibited resistance at seedling and adult stages and hence have the potential to be incorporated into disease-resistance breeding programs. The study also found considerable genetic variation among wheat genotypes concerning agronomic traits related to plant height, grain yield, flag leaf area, and 1000 grain weight, which is important in determining disease resistance and productivity. Principal component analysis identified the most prominent agronomic traits associated with disease resistance as PHT, GY, and TGW. A correlation analysis explained in more detail the complex trait associations of NTP and GY, with positive associations that could be useful for breeding purposes to improve disease resistance and productivity. Overall, the present study underlines the importance of genetic diversity in wheat improvement and indicates that disease resistance combined with a high yield potential will be a prerequisite for successfully breeding vigorous wheat cultivars in the pursuit of food security.

AUTHOR CONTRIBUTIONS

A.I., M.U.R., M.A. and T.M. conceived and designed the study. A.I., B., A., and M.I. collected and analyzed the data. A.I., S.G., F.K., N.A., and T.M. wrote and revised the manuscript. All authors reviewed and approved the final manuscript.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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