

Characterization of spring wheat genotypes reveals differential salt tolerance potential based on quantitative attributes

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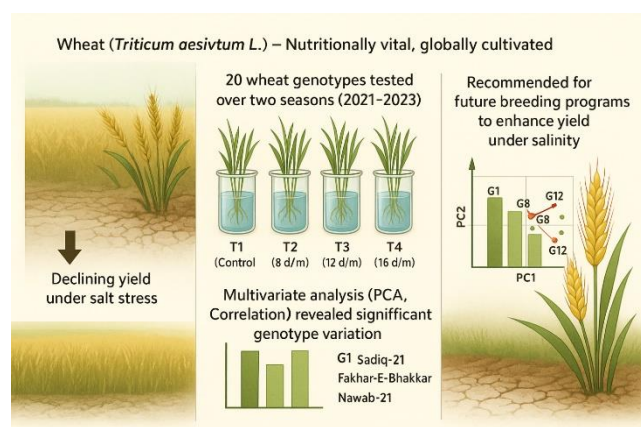
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Graphical abstract



Abstract

Wheat (*Triticum aestivum* L.) is a globally important cereal crop yet, its production is gradually decreasing due to salinity stress while demand is increasing. Salt stress is an important abiotic stressor affecting wheat from growth to harvest. The current study was conducted to evaluate 20 diverse wheat genotypes for two consecutive growing seasons in 2021–2022 and 2022–23, to assess their salt tolerance potential against four salinity levels (control, 8dS/m, 12dS/m and 16 dS/m). Salinity tolerance among wheat genotypes was assessed on the basis of morpho-physiological and yield related traits such as plant height (PH), peduncle length (PL), spike length (SL), flag leaf area (FLA), chlorophyll content (CC), grain length (GL), grain width (GW), grain area (GA), thousand grain weight (TGW) and yield per plant (YPP). A significant genetic diversity was detected among the genotypes based on the evaluated traits. Correlation analysis revealed positive association among all parameters across treatment conditions. Principal component analysis (PCA) revealed

that out of 10 components, only two had greater than 1 eigenvalues and were significant across all treatments, explaining 70.49%, 60.67%, 60.62% and 51.562% of the total variation under T1 (control), T2 (8dS/m), T3 (12dS/m) and T4 (16dS/m) respectively. Additionally, the PCA biplot identified Sadiq-21 (G1), Fakhar-E-Bhakar (G8) and Nawab-21 (G12) as potential salt tolerant genotypes. In order to produce high yielding and salt tolerant genotypes, future wheat breeding programs may utilize the tolerant genotypes with salt tolerance potential.

Keywords: Wheat; Salt stress, Yield, Genotypes, Correlation, PCA

1. Introduction

Spring wheat is an important cereal crop of semi-arid and arid regions of Pakistan. Wheat is key crop for human nourishment due to its contribution in 30% world grain production and 45 % cereal nutrition's (Karimzadeh *et al.* 2023). Wheat contains Proteins (8–15%), moisture (12%), Fats (1.5–2%) and Carbohydrate (60–80%) that are much needed for humans (Bakaaki *et al.* 2023). Wheat provides over half of the protein and more than half of the calories for about one-third of the world's population thus considered to be the fundamental component of the human diet due to its high nutritional value. It serves as a primary food source, providing essential nutrients and energy for millions worldwide (Jamal *et al.* 2025). However, the demand for wheat continues to rise, while its overall production faces significant challenges, one of the most pressing being the impact of abiotic stressors such as salinity (Li *et al.* 2023).

Salt stress is a major environmental constraint that negatively affects wheat growth, development, and yield, from seedling establishment to harvest. It poses a severe threat to global wheat production, particularly in arid and

semi-arid regions where soil salinization is prevalent (Sewore and Abe 2024). Climate change is causing the rise in soil salinity due to higher evaporation of irrigation water which also triggered by water scarcity and rising soil temperatures (Eswar *et al.* 2021; Hamzah *et al.* 2024). Soil salinity affects total 831 million hectares (mha) area all over the globe out of which 397 Million (ha) are classed as salt affected while other 434 million (ha) as saline sodic (Phalke *et al.* 2020). In addition to the area already impacted by salt, 1% to 2% more of the world's fertile lands are experiencing salt damage each year (Yang *et al.* 2022). Reducing the risks of food insecurity brought on by population growth and climate change is an important problem that has to be handled (Qiao *et al.* 2021).

Salinity affects wheat plants by disrupting physiological and morphological processes. Key traits such as plant height, spike development, and chlorophyll content are particularly sensitive to saline conditions, which lead to impaired photosynthesis and reduced biomass accumulation (Senapati *et al.* 2024, Jamal *et al.* 2025). Understanding the genetic basis of salt tolerance and the underlying physiological mechanisms is crucial for developing salt-tolerant wheat varieties. Traditional breeding strategies have made some progress, but integrating knowledge of genetic diversity and physio-morphological indices offers a promising avenue for advancing salt tolerance in wheat (Chaouch *et al.* 2024). The salinity is a serious problem for soil and is present in more than 100 countries worldwide. Salinity stress triggers a range of adverse physiological and morphological changes in wheat plants, making the evaluation of specific traits crucial for understanding and enhancing salt tolerance. Traits such as plant height and peduncle length are critical indicators of overall plant vigor and developmental stability under stressful conditions (Ur *et al.* 2024; Li *et al.* 2023). Reduced plant height and peduncle length under high salinity levels often reflect osmotic and ionic imbalances, leading to stunted growth and reduced biomass. Additionally, spike length plays a pivotal role in determining the potential reproductive output of the plant, as shorter spikes under salinity stress correlate with lower grain production. By examining these morphological indices, researchers can identify genotypes that exhibit resilience in maintaining these traits even under saline environments, thereby offering valuable insights for breeding programs (Senapati *et al.* 2024, Qadeer *et al.* 2023, Jamal *et al.* 2025).

Physiological traits like flag leaf area and chlorophyll content are equally significant in assessing a genotype's ability to tolerate salt stress (Shoukat *et al.* 2025). The flag leaf is a primary photosynthetic organ that contributes significantly to grain filling and overall plant productivity. A larger flag leaf area under saline conditions indicates a plant's capability to sustain photosynthetic efficiency, thereby supporting better growth and yield. Chlorophyll content serves as an indicator of the plant's photosynthetic capacity, with salinity stress often causing chlorophyll degradation, reducing the plant's energy production and overall health (Ali *et al.* 2024, Saeed *et al.*

2024). Grain-related traits, including grain length, grain width, grain area, and thousand grain weight, directly influence yield per plant and overall productivity. These traits collectively determine the economic value of the crop and are essential for identifying high-yielding genotypes that can withstand saline conditions (Ahmed *et al.* 2022b, Rashid *et al.* 2022, Ahmed *et al.* 2023). Understanding the genetic diversity associated with these traits provides a foundation for selecting and breeding wheat varieties with enhanced tolerance to salinity, ultimately contributing to food security in saline-affected regions.

Chlorophyll content is the key component for outstanding crop yield and negatively impacted by salinity stress (Adil *et al.* 2022). This decrease in chlorophyll content is not simply linked to decrease in cellular chlorophyll but can also non chlorophyll aspect like, photosynthesis and yield per plant etc. (Mehta *et al.* 2010). So, the high salt causes distinct changes in the physiological, morphological, and structural features of plant cells, tissues, and organs. Cell replication and growth mechanisms are impacted by salt stress (Kumar *et al.* 2017). As a consequence, meristem at the apex shrinks, which diminishes the cortex and the bundles of vascular cells. Additional morphological alterations that happen in plants subjected to saline stress are reduction in grain related attributes like grain length, width and area (Zeeshan *et al.* 2020). Many plant genotypes have altered physio-morphological attributes under salt stress, however genetic diversity and evaluation of tolerance in commercial and old germplasm in glasshouse conditions has not been done yet (Yang *et al.* 2022). This genotype screening lays the basis for breeding efforts aimed at producing new wheat varieties with enhanced salt tolerance (Ahmed *et al.* 2022a). So, genotypes resistant to salt can be found and used, ensuring more sustainable and productive wheat production in the future.

Therefore, it would be beneficial for breeders to examine these variations to understand the adaptive structural changes that wheat undergoes in response to salt stress. Hence, to increase the tolerance in various wheat genotypes, it is crucial to identify characteristics linked to salinity stress tolerance (Shahid *et al.* 2020). Currently, computational strategies have also highlighted the significance of accuracy in agricultural prediction models, where the Naive Bayes algorithm achieved 88% precision compared to 83% for RNN (Reddy *et al.* 2024).

The objectives of the current study were:

- Evaluation of genetic diversity in a range of local and promising wheat varieties with regard to salinity stress.
- Study the quantitative attributes of the promising wheat genotypes against salt stress.
- Determine the association between physio-morphological, and grain yield related traits under both control and salt stress environments.
- Identification of salt tolerant and salt susceptible genotypes.

2. Material and methods

2.1. Experimental site and design

Current study was executed over two consecutive wheat growing seasons (2023–2024 and 2024–2025) at the experimental glasshouse facility of Department of Plant Breeding and Genetics, The Islamia University of Bahawalpur, Pakistan (29.3544°N, 71.6911°E). Twenty wheat (*Triticum aestivum* L.) genotypes including advanced breeding lines and conventional varieties were sourced from Regional Agricultural Research Institute (RARI), Bahawalpur, Pakistan. The objective was to assess genetic diversity among the genotypes, for salt stress tolerance based on key morphological and physiological traits (Ilyas and Naz 2024).

The experiment was laid out in Completely Randomized Design (CRD) with three replications per treatment each consisting of one pot per genotype per treatment. To ensure reliability, the experiment was conducted across two consecutive seasons under controlled glasshouse conditions, with replication minimizing environmental variation. Standard statistical procedures (ANOVA, correlation, and PCA) further strengthened the consistency and reliability of the results. As conditions were controlled and uniform so CRD was selected, which minimized environmental variability (Gomez and Gomez 1984).

2.2. Glasshouse conditions

Glasshouse was maintained under semi-controlled conditions with an average temperature range of 22–

28°C, 60–70% relative humidity and a 12-hour photoperiod. Supplemental lighting and ventilation were used to maintain consistent growth conditions (Table 1).

2.3. Pot Preparation and Salinity Treatments

Seed sowing was done in plastic pots (12" × 12") filled with 4 kg of air dried loamy soil. Soil electrical conductivity (ECe) was initially measured using a portable EC meter (HANNA HI98331) and all baseline EC readings were maintained below 1.5 dS/m. After 15 days interval of seed sowing, for salinity induction various concentrations of sodium chloride (NaCl) were used, with solutions prepared to EC levels of 8, 12, and 16 dS/m using distilled water, following the protocol of Nassar *et al.* (2020). Salinity treatments were applied after every 15 days interval to maintain consistent stress conditions:

T1 (Control): 0 dS/m (no salt)

T2 (Moderate Stress): 8 dS/m NaCl

T3 (Severe Stress): 12 dS/m NaCl

T4 (Extreme Stress): 16 dS/m NaCl

Each pot received 500 mL of respective salt solution per application. Leaching and water loss were controlled to avoid EC drift.

2.4. Trait Measurement

The following morpho-physiological attributes were recorded: Plant height (PH), peduncle length (PL), spike length (SL), chlorophyll content (CC), flag leaf area (FLA), grain length (GL), grain area (GA), grain width (GW), thousand grain weight (TGW) and yield per plant (YPP).

Table 1. Genotypes name, their allocated code in experiment and pedigree with their major characteristics

Code	Name	Pedigree	Notable Trait
G1	Sadiq-21		Rust resistant
G2	Fareed-06	PT'S'/3/TOB/LFN//BB/4/BB/HD-832-5//ON/5/G-V/ALD'S'/HPO	-
G3	Bahawalpur-2000	AU/UP301//GLL/Sx/3/PEW S/4/MAI S/MAY A S//PEWS	Loose Smut Resistance
G4	Jauhar-16	KAUZ/PASTOR//V.3009	High yielding and rust resistant
G5	Ghazi-19	N/A	-
G6	Mairaj-08	SPARROW/INIA//V.7394/WL711/13/BAUS	Smut, leaf and Rust resistant
G7	Manthar-03	KAUZ, MEX//ALTAR-84/(AOS)AWNED-ONAS	Lodging, rust, smut, bunt and blight resistant
G8	Fakhar-E-Bhkkar	93T347 and Auqab-2000	Temperature and Rust resistance
G9	Bahawalpur-97	SUSONOKOMUGI/NORIN/(SIB)BOBWHITE	Smut and leaf rust resistant
G10	Sutlej-86	CLEMENT/YECORA-70//((SIB)MONCHO	Susceptible to smut
G11	Gold-16	PR-32(BAU)/INQ-91	-
G12	Nawab-21	N/A	-
G13	Subhani-21	N/A	-
G14	Aas-11	PRL/PASTOR//2236(V6550/SUTLEH-86)	-
G15	Punjnad-01	PUNJAB-85/NEELKANT	Leaf, yellow rust and Salt resistant
G16	Derawar-97	ORE F1 158/FUNDULEA//KAL/BB/3/NAC	Leaf rust resistant, Susceptible to Smut
G17	Blue Silver	II-54-388/AN/3/YT54/N10B//LR64	Leaf rust resistant, Susceptible to Smut
G18	Bahawalpur-79	CIANO-67(SIB)/2*LERMA-ROJO-64//2*SONORA-64	Leaf rust, Brown rust resistant
G19	Nishan-21	N/A	-
G20	Akbar-21	N/A	-

Chlorophyll content was measured at the heading stage using a Hansatech CL-01 SPAD meter with a wavelength

range of 650–940 nm and a measurement range of 0–199.9 SPAD units (Hansatech Instruments Ltd., UK). Three

measurements were taken from different areas of the flag leaf and averaged. Grain area (GA) was calculated following a modified version of the method used by Ahmed *et al.* (2022), using the following equation:

$$GA = \frac{13}{11}(GW+GH) \times GL \quad (1)$$

Where: GW = Grain width (mm), GH = Grain height (mm), GL = Grain length (mm)

All dimensions were measured using a digital caliper with 0.01 mm precision.

Table 2. Descriptive statistics of 20 wheat genotypes under control and salt conditions (8, 12 and 16 dS/m).

Traits	Conditions	Minimum	Maximum	Mean	SD(n-1)
PH (cm)	T1	60.66	83.00	74.782	4.9134
	T2	59.00	75.00	66.65	4.5988
	T3	49.67	61.33	54.582	3.789
	T4	38.67	51.33	44.833	3.2553
PL (cm)	T1	7.00	18.17	13.017	3.4914
	T2	5.33	13.5	9.183	2.6014
	T3	3.00	12.83	6.975	3.0627
	T4	2.80	5.83	4.043	1.0559
SL (cm)	T1	6.52	7.87	7.377	0.3385
	T2	6.12	7.64	7.0355	0.4742
	T3	5.88	7.59	6.6015	0.4819
	T4	5.16	6.52	5.673	0.379
FLA	T1	32.4	53.37	44.091	6.2117
	T2	25.37	44.2	32.755	5.652
	T3	21.0	32.57	25.915	3.6125
	T4	19.13	26.87	21.951	2.5753
CC	T1	19.4	38.05	29.189	4.6381
	T2	16.72	30.82	22.858	3.4932
	T3	14.28	19.01	17.275	1.3131
	T4	9.27	14.71	11.785	1.4501
GL	T1	4.13	4.96	4.5385	0.2403
	T2	3.73	4.70	4.2575	0.2959
	T3	3.26	4.32	3.7575	0.3111
	T4	2.85	3.73	3.3985	0.2237
GW	T1	2.11	2.84	2.414	0.2691
	T2	1.51	2.14	1.7635	0.1498
	T3	1.29	1.74	1.5345	0.1145
	T4	0.77	1.39	1.0465	0.1535
GA	T1	30.71	40.27	35.207	2.9885
	T2	24.74	33.71	29.654	2.5037
	T3	17.09	24.62	20.813	2.0606
	T4	12.78	18.64	16.041	1.462
TGW	T1	39.35	47.63	43.715	2.7443
	T2	32.97	38.9	36.273	1.7312
	T3	30.38	34.14	32.061	1.1266
	T4	26.3	30.56	27.68	1.0163
YPP	T1	4.95	13.01	8.796	2.0428
	T2	3.7	11.01	6.86	1.9331
	T3	3.07	8.67	4.969	1.5075
	T4	0.98	4.65	2.455	0.9428

PH plant height, PL peduncle length, SL spike length, FLA flag leaf area, CC chlorophyll content, GL grain length, GW grain width, GA grain area, TGW thousand grain weight, YPP yield per plant.

T1= control, T2= 8dS/m, T3= 12dS/m, T4=16 dS/m.

2.5. Statistical analysis

Data were analyzed using analysis of variance (ANOVA) to test for significance among treatments and genotypes, following Heinisch (1962). For traits with significant differences, Pearson's correlation coefficients were computed to determine trait interrelationships using the formula:

$$r_{xy} = \frac{\sum (x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum (x_i - \bar{x})^2 \sum (y_i - \bar{y})^2}} \quad (1)$$

where x_i and y_i represent trait values and $\bar{x}$, $\bar{y}$ their respective means. Principal component analysis (PCA) was

Table 3. Correlation analysis for all studies traits in control and salt stress conditions.

Traits		CC	FLA	GA	GL	GW	PH	PL	SL	TGW
FLA	T1	0.734**								
	T2	0.885**								
	T3	0.686**								
	T4	0.601**								
GA	T1	0.689**	0.689**							
	T2	0.654**	0.641**							
	T3	0.745**	0.681**							
	T4	0.762**	0.628**							
GL	T1	0.666**	0.643**	0.926**						
	T2	0.562**	0.527*	0.971**						
	T3	0.653**	0.574**	0.973**						
	T4	0.799**	0.522**	0.915**						
GW	T1	0.642**	0.624**	0.886**	0.679**					
	T2	0.820**	0.853**	0.690**	0.538*					
	T3	0.831**	0.704**	0.816**	0.692**					
	T4	0.626**	0.845**	0.790**	0.583**					
PH	T1	0.635**	0.697**	0.677**	0.594**	0.669**				
	T2	0.735**	0.778**	0.710**	0.649**	0.728**				
	T3	0.638**	0.712**	0.670**	0.568**	0.701**				
	T4	0.691**	0.817**	0.691**	0.668**	0.825**				
PL	T1	0.666**	0.810**	0.601**	0.569**	0.559**	0.698**			
	T2	0.720**	0.697**	0.765**	0.715**	0.605**	0.656**			
	T3	0.639**	0.617**	0.787**	0.727**	0.832**	0.429*			
	T4	0.601**	0.614**	0.609**	0.664**	0.555**	0.776**			
SL	T1	0.753**	0.741**	0.896**	0.792**	0.852**	0.705**	0.683**		
	T2	0.706**	0.655**	0.713**	0.617**	0.702**	0.565**	0.744**		
	T3	0.704**	0.620**	0.844**	0.768**	0.728**	0.578**	0.661**		
	T4	0.535*	0.672**	0.608**	0.464*	0.835**	0.736**	0.591**		
TGW	T1	0.671**	0.669**	0.916**	0.770**	0.909**	0.663**	0.590**	0.955**	
	T2	0.818**	0.771**	0.685**	0.612**	0.701**	0.706**	0.726**	0.856**	
	T3	0.629**	0.708**	0.854**	0.820**	0.662**	0.479*	0.705**	0.866**	
	T4	0.652**	0.750**	0.673**	0.662**	0.697**	0.703**	0.706**	0.721**	
YPP	T1	0.737**	0.713**	0.923**	0.808**	0.887**	0.752**	0.656**	0.966**	0.964**
	T2	0.778**	0.718**	0.733**	0.647**	0.753**	0.643**	0.749**	0.960**	0.865**
	T3	0.663**	0.560*	0.848**	0.781**	0.730**	0.518*	0.699**	0.974**	0.846**
	T4	0.584**	0.698**	0.705**	0.539*	0.876**	0.768**	0.582**	0.962**	0.710**

** Highly significant (0.01); * significant (0.05); ns non-significant.

3. Results

3.1. Estimation of variability under control and salinity stress conditions and association analysis

Assessment of salinity tolerance among cultivars for salt tolerance wheat breeding is an integral aspect. For this purpose, 20 wheat genotypes were evaluated. The selected genotypes

performed using XLSTAT (Addinsoft 2014) to assess multivariate relationships and genotype clustering under salinity stress. Principal Component Analysis (PCA) was conducted to reduce data dimensionality and identify key traits contributing to variation, using eigenvalues > 1 as criteria for significant components. Data were also visualized using bar graphs and biplots. To prepare the research data for future analysis, it was compiled using XLSTAT 2014 and then submitted to principal component analysis (PCA) (Ahmed *et al.* 2019). Graphs are used to depict the data for the investigated characteristics.

were screened for tolerance of salt stress using different morphological and physiological parameters. The data for all studied traits under saline conditions among genotypes is presented in **Table 2**, which showed a significant difference in all traits. Analysis of variance (ANOVA) (**Table S1a, S1b**) showed highly significant differences among genotype, treatments and their interactions. There is non-significant variation existent

among the genotypes for seasons, therefore we used mean data based on averaged over seasons (2021-2022 and 2022-2023) for further analysis. Over all a decreasing trend was observed among parameters with increasing stress applications. Strikingly the genotypes Sadiq-21 (G1), Fakhar-E-Bhkkar (G8) and Nawab-21 (G12) performed quite well under all the salt treatment conditions for all the parameters. While Sutlej-86 (G10), Blue Silver (G17) and Bahawalpur-79 (G18) performed poorly under all salt stress conditions for all the studied attributes (Supplementary **Tables S1-S11**).

Simple linear correlation was performed to figure out the association of all studied indices with yield under control and salt stress conditions based on averages over the seasons (2021-2022 and 2022-2023). All the studied parameters showed positive and significant association with one another under control and stress conditions. From this analysis, it was revealed that YPP was most positively and significantly related to SL (0.966**, 0.960**, 0.974** and 0.962**) followed by TGW (0.964**, 0.865**, 0.846** and 0.710**) and GA (0.923**, 0.733**, 0.848** and 0.705**) under T1, T2, T3 and T4 conditions respectively (**Table 3**).

3.2. Principal Component Analysis (PCA)

There were a total of 10 Principal Components (PCs) in the analysis among which only two were highly significant that cause the variability in data (**Table 4**). The first two PCs showed 70.49%, 60.67%, 60.62% and 51.562% total variation for T1, T2, T3 and T4 stress conditions respectively. The first component had 60.01% variability in control (T1), 50.02% in T2, 40.49% in T3 and 30.75% in T4 stress conditions. This PC had a major contribution from the YPP in T1 and T2 stressor while from GA and PH in T3 and T4 stress respectively. The second principal component (PC2) has the 70.49% variability in T1, 60.67% in T2, 60.62% in T3 and 51.56% variability in T4 (**Figure 1**). The second component has major contribution for variability has from GL in treatment T1 and T2 while from PH and GL in T3 and T4 respectively as displayed in **Figure S1-S4**. The data presented in **Table 5** showed factor loading of all treatment. In the given experiment traits like YPP in T1 and T2, GA in T3 and PH in T4 stress conditions showed maximum variance present in the first PC (**Figure 2**). In second PC the maximum variance was shown by GL in T1, T2 and T4 while PH in T3 in all treatments. In the first factor or PC, the negative impact on the overall variance of the factor is CC among all treatments. In second factor or PC the negative impact on overall variance was also given by CC. The division for every variable for PC1 and PC2 exhibited the difference of variables for different character studied in control and saline conditions. The biplot was generated between two main factors or PCs. The first two components had majority of variability present in them (**Figure 3**). The biplot has four main axes; the upper right axes has positive impact on PC1 and PC2 and the genotypes that are situated on that block are best for selection as these have more variation among whole available germplasm. The biplot was constructed between first two PCs and biplot result showed that genotypes Sadiq-21 (G1),

Fakhar-E-Bhkkar (G8) and Nawab-21 (G12) were present in positive axes along with traits such as PL, FLA, CC, PH, GA, GL and TGW hence considered as salt tolerant and genotypes Sutlej-86 (G10), Blue Silver (G17) and Bahawalpur-79 (G18) were included in the negative axes hence can be considered as the salt susceptible genotypes while the rest of the genotypes are considered neither tolerant nor susceptible (**Figure 4**).

Table 4. Eigenvalues, Variability % and Cumulative % of Traits in Control and saline condition.

		F1	F2
Eigenvalue	T1	6.013	1.483
	T2	5.021	1.656
	T3	4.492	2.132
	T4	3.705	2.857
Variability (%)	T1	60.013	10.483
	T2	50.021	10.656
	T3	40.492	20.132
	T4	30.705	20.857
Cumulative %	T1	60.013	70.496
	T2	50.021	60.677
	T3	40.492	60.624
	T4	30.705	51.562

Table 5. Factors loading of PCA for control and saline conditions

		F1	F2	F3	F4	F5
PH	T1	0.828	0.055	-0.394	0.078	0.337
	T2	0.828	0.055	-0.394	0.078	0.337
	T3	0.722	0.548	-0.297	-0.118	0.191
	T4	0.906	0.065	0.188	0.129	-0.261
PL	T1	0.853	0.180	0.069	0.402	-0.210
	T2	0.853	0.180	0.069	0.402	-0.210
	T3	0.828	0.100	0.494	0.112	0.044
	T4	0.784	0.165	0.512	-0.209	-0.163
SL	T1	0.873	0.008	0.453	-0.090	0.033
	T2	0.873	0.008	0.453	-0.090	0.033
	T3	0.906	0.233	-0.222	-0.114	-0.215
	T4	0.844	0.430	-0.091	-0.272	0.016
FLA	T1	0.873	0.340	-0.218	0.063	-0.106
	T2	0.873	0.340	-0.218	0.063	-0.106
	T3	0.790	0.383	-0.070	0.437	-0.132
	T4	0.845	0.205	0.148	0.436	0.082
CC	T1	-0.892	-0.296	-0.088	0.082	-0.104
	T2	-0.892	-0.296	-0.088	0.082	-0.104
	T3	-0.833	-0.261	0.126	-0.227	-0.199
	T4	-0.803	-0.387	-0.107	0.073	0.059
GL	T1	0.787	0.578	-0.147	-0.076	-0.004
	T2	0.787	0.578	-0.147	-0.076	-0.004
	T3	0.884	0.222	-0.030	-0.006	0.377
	T4	0.802	0.548	-0.116	-0.071	-0.013
GW	T1	0.856	0.285	-0.164	-0.314	-0.165
	T2	0.856	0.285	-0.164	-0.314	-0.165
	T3	0.891	0.229	0.278	-0.153	-0.058
	T4	0.904	0.260	-0.205	0.178	-0.080
GA	T1	0.871	0.442	-0.132	-0.136	-0.055
	T2	0.871	0.442	-0.132	-0.136	-0.055
	T3	0.959	0.109	-0.018	-0.028	0.241
	T4	0.869	0.312	-0.304	-0.012	-0.042
TGW	T1	0.899	0.127	0.216	0.092	0.231

	T2	0.899	0.127	0.216	0.092	0.231
	T3	0.886	0.276	-0.132	0.265	-0.073
	T4	0.857	0.008	0.200	-0.069	0.452
YPP	T1	0.911	0.039	0.348	-0.102	0.045
	T2	0.911	0.039	0.348	-0.102	0.045
	T3	0.892	0.315	-0.145	-0.143	-0.176
	T4	0.878	0.358	-0.187	-0.211	-0.038

Please see Table 2 for trait abbreviations.

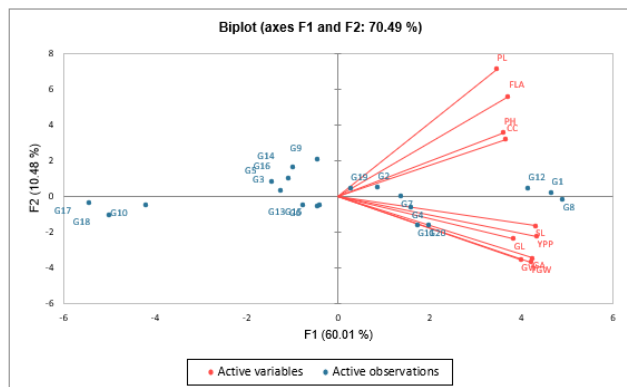


Figure 1. Biplot analysis graph for T1 (control) conditions

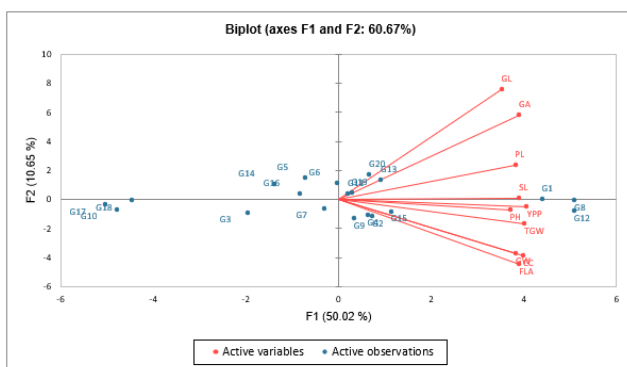


Figure 2. Biplot analysis graph for T2 (8dS/m) conditions

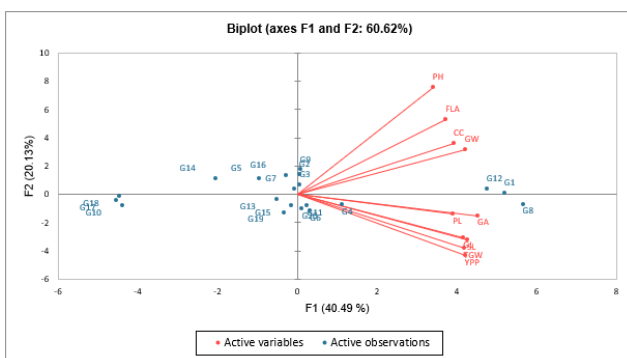


Figure 3. Biplot analysis graph for T3 (12dS/m) conditions

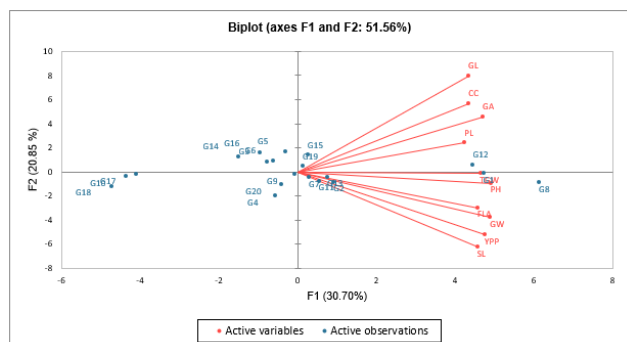


Figure 4. Biplot analysis graph for T4 conditions (16 dS/m).

4. Discussion

Salt stress is one of the major environmental constraints to wheat productivity globally. Keeping in view the highly complex nature of yield itself, identification and selection of yield associated attributes could lead to development of high yielding cultivars under salt stress conditions. Plant height is a crucial agronomic attribute for morphogenesis and crop production in wheat. An ideal plant height is associated with lower rate of lodging, higher grain quantity and quality (Gudi *et al.* 2023; Kradetskaya *et al.* 2024). Peduncle length (PL) is the measured length of the internode beneath the spike of wheat, which is an essential attribute to influence photosynthesis effectiveness and pollination success rate, determining grain production (Wang *et al.* 2023). The growth of spikelet begins in the initial phase of spike formation, and salt stress can hinder this procedure, resulting in less spikelet and eventually shorter spikes. Similar results are also reported by (Dadshani *et al.* 2019). During periods of salt stress, leaves experience decompression, which results in a decrease in the rate of photosynthesis and ultimately reduces the potential for productivity. In the same way, the increase in salt concentrations plays a role in the decrease in leaf water content, which subsequently leads to a decline in turgor pressure within guard cells. This, in turn, eventually causes leaf area reduction (Masoudi *et al.* 2015). Chlorophyll content (CC) serves as a crucial indicator for predicting and monitoring crop growth. Its precise measurement on a large scale is vital for calculating crop productivity, managing nutrition, pest control, and other agricultural applications (Gebregziabher *et al.* 2022; Surendran and Krishnan 2024,). The salt stress influenced grain length as salt stress increases the grain length decreased. Similar results are also reported by (Zhang *et al.* 2022). The photosynthetic activity and chlorophyll content reduced under saline stress conditions which limit the availability of assimilates necessary for grain width development that resultantly diminished the grain width (Zheng *et al.* 2023). Salt stress causes harmful ions like Na and Cl⁻ to accumulate in plant tissues, which reduces grain area. Grain area is inhibited by these ions because they interfere with cellular activities, such as nutrition intake and metabolism (Farooq *et al.* 2017). Wheat crop under salinity stress spend more nutrients towards response to stress and recovery functions like tissue regeneration and detoxification. This elevated level of metabolism redirect energy from the process of grain filling which produce lighter grain and reduce the thousand grain weight of crop (Mahdy *et al.* 2022). Additionally, the lowered rate of photosynthesis and the negative effects of ion toxicity further impacts on crop output and ultimately decrease the yield per plant (Adil *et al.* 2022).

The correlation analysis shows that as PH increase in control the CC, FLA, GA, GL, GW, PL, SL, TGW and YPP was also increased. The similar result of correlation was also observed by (Iqra *et al.* 2020) under normal condition. In saline condition these traits also correlate with shoot length. Similar result in saline condition was also seen by

(Gandahi *et al.* 2020). It showed that if spike length is higher than all other attribute will increase or vice versa. Similar result for association of spike length was presented by (Nezami *et al.* 2024). Flag leaf area contributed to all the attributes under normal and salt stress. If plant has less leaf area than all the other attributes like chlorophyll content, grain area and spike length will be lower. Likewise, if crop has lower plant height than leaf area will also be reduced. Same results for correlation under salt stress were also seen by (Blum 2017; Alwahibi *et al.* 2024). Positive correlation among traits indicates that good amount of chlorophyll content is necessary for the good performance of other traits. If high level of chlorophyll content was present in the crop than good production is given by the crops or vice versa. Same findings were given by (Enghiad *et al.* 2017; Amzeri *et al.* 2024) for chlorophyll content. Higher grain length directly contributed to increased grain width and grain area which ultimately increase thousand grain weight and yield per plant. If plant has a good spike length, chlorophyll content and peduncle length than grain length is also affected. Same correlation association was explained by (Omran *et al.* 2022). The grain width has positive correlation with grain attributes which increase with increasing grain width or vice versa. The plant that has larger grain width has positive association with yield per plant. Same association was observed by (Ahmed *et al.* 2021). The association showed that grain area directly contributes to all their traits and increase the yield per plant or vice versa. Similar findings are also given by (Akbarpour *et al.* 2015). Increase in grain length, area and width increase the thousand grain weight or vice versa. It also showed that CC, FLA and YPP increases thousand grain weight or vice versa. It coincides with the findings of (Moustafa *et al.* 2021). The all-other trait contributes to the yield per plant. As yield per plant decrease all other parameters will also decrease or vice versa. Same result was proposed by (Hasan *et al.* 2015). Similarly in another study six wheat genotypes were characterized, measuring traits like plant height, flag leaf area, and grain yield under salt stress. Significant differences were observed, with salt-tolerant genotypes (Pasban-90, Bakhar-02) showing better performance in these parameters compared to sensitive ones (Irshad *et al.* 2022).

With the PCA, large sets of complicated data are divided into smaller sets of simple factors that can be correlated (Ahmed *et al.* 2019). The PCA was done to find out important traits in both salt and normal conditions. (Ahmed *et al.* 2019) explained the statistical significance of eigenvalues, which were then used to select the statistically significant principal components (PCs). All traits have positive impacts on variability among first two components except CC. Similar result also reported by (Ahmed *et al.* 2019). The reason of negative effect of chlorophyll content may be due the deficiency of CC in the cell as salt in cell induces leakage of electrolyte and peroxidase of lipids from thylakoid membrane of chloroplast which leads to loss of chlorophyll content that has negative impact on all other traits. All the major traits have positive impact on second principal component

among all salt as well as normal conditions. Similar results are reported by (Guellim *et al.* 2019). (Naz *et al.* 2025) also reported that genotypes with higher PCA-1 and smaller PCA-2 has more yield potential as compared to smaller PCA1 and higher PCA-2. Furthermore, these findings emphasize importance of employing robust statistical and computational methods for reliable crop performance evaluation (Reddy *et al.* 2023)

5. Conclusion

Salinity in the soil is an abiotic stressor that seriously compromises agricultural productivity. In this study total 20 wheat genotypes were studied for their quantitative traits against saline conditions. The analysis of variance (ANOVA) revealed that there was a distinct and notable variation present among genotypes for the parameters under consideration. Based on averages over the seasons (2021–2022 and 2022–2023) the genotypes G1, G8 and G12 performed well under salt stress conditions showing that they have salt tolerance potential than others. This study emphasizes on importance of these tolerant genotypes to provide enough yields in salinity-affected areas. Conversely the genotypes G10, G17 and G18 did not perform well under salt stress conditions and regarded as salt susceptible genotypes. The correlation analysis showed highly significant and positive association among all traits under saline and control conditions. The PCA also revealed similar findings indicating that these G1, G8 and G12 are present on positive axes of biplot and have association with PL, FLA, CC, PH, GL, and TGW. While G10, G17 and G18 are present on negative axes of biplot considered susceptible genotypes. Future research should emphasize on authenticating these genotypes under multi-location field trials to confirm tolerance against salinity stress. Furthermore, incorporating molecular markers and genomic tools with morphological screening will speed up breeding of salt-tolerant wheat varieties

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Authors Contribution

Adel A. Rezk and Mueen Alam Khan designed the study and supervised the research. Mueen Alam Khan and Hafiz Ghulam Muhu Din Ahmed and conducted the experiments and collected data. Ishtiaq Ahmad and Maryam performed data analysis. Othman Al-Dossary, Hossam S. El-Beltagi, Mohamed I. Aldaej, and Jameel M. Al-Khayri contributed to manuscript revision and scientific input. All authors reviewed and approved the final manuscript.

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Competing interests

The authors showed no relevant financial or non-financial interests to disclose.

Data availability

All data generated or analysed during this study are included in this published article [and its supplementary information files].

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