ASSESSMENT OF SALT STRESS ENVIRONMENT ADAPTATION OF BREAD WHEAT (*TRITICUM AESTIVUM* L.) GENOTYPES FOR SUSTAINABLE YIELD

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Abstract. Global wheat production faces a significant threat from salinity stress, influencing yield and quality traits. This study evaluated the salt tolerance of 102 wheat (*Triticum aestivum* L.) genotypes based on morpho-physiological traits at the seedling stage. Using a factorial complete randomized design (CRD), traits such as germination rate, vigor index, shoot and root length, root-to-shoot ratio, fresh and dry weights of shoots and roots, and salt tolerance index were measured under saline and control conditions. Analysis of variances revealed that all the studied parameters differed significantly among all genotypes, indicating the significance genetic variability existed. A strong association among most of the studied traits under both conditions underscores their importance for wheat breeding in salinity-stressed areas, where selecting one trait may enhance others. However, the coefficient of germination showed a negative, non-significant correlation with root and shoot length. Out of the 10 principal components (PCs), under normal conditions, five PCs had eigenvalues greater than one, accounting for 65% of the total variation. Under salinity stress, four PCs exceeded an eigenvalue of one, explaining 58% of the total variation. Based on the performance, Genotypes G7, G16, G24, G47, and G56 showed superior salt tolerance, making them ideal for improving wheat productivity and breeding resilient varieties, while G77, G84, G87, G94, and G99 were susceptible to salinity stress. The best performing germplasm under salinity stress might be a desirable genotype to be used as genetic resource for future breeding projects and early selection criteria for high yielding in wheat.
Keywords: *abiotic, germplasm, morphology, salinity, breeding*

Abbreviations: ROG: Rate of germination, COG: Coefficient of germination, GVI: Germination vigor index, RL: Root length, SL: Shoot length, RSR: Root to shoot length ratio, SFW: Shoot fresh weight, SDW: Shoot dry weight, RFW: Root fresh weight, RDW: Root dry weight, STI: Salt tolerance index, DF: Degree of freedom, SS: Sum of squares, MS: Mean Squares

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Introduction

Wheat (*Triticum aestivum* L.) is a major cereal crop in many parts of the world known as the "king of cereals." As a strategic crop, wheat is important for the global economy, production, food supply, and nutrition (Bashir et al., 2023; Sidra et al., 2024). It is one of the main species widely cultivated, providing food for over one-third of the world's population. However, saline soils in arid and semi-arid areas of the world restrict food production (Yassin et al., 2019; Kalsoom and Ahmed, 2023). Salinity is one of the main abiotic factors that slows down plant growth and productivity globally; it affects roughly 7% of the planet's land area. A high percentage of cultivated land is impacted by salt; 23% of cultivated land is saline, and 20% of irrigated land has secondary salinization (Singh et al., 2015; Sidra et al., 2024).

Certain agronomic techniques, such as using a lot of gypsum or leaching to remove salt from the soil, can lessen the detrimental effects of salinity on wheat production, but these methods are costly. However, cultivating genotypes tolerant to salt offers a long-term, practical, affordable solution that can be applied widely, making it the most efficient method (Al-Ashkar et al., 2019; Muhammad et al., 2023). The different salinity tolerance mechanisms are complex physiological and molecular processes that allow the wheat plants to survive due to high salt levels in the soils (Ijaz et al., 2023; Victoria et al., 2023). Included are ion homeostasis-a process whereby wheat cells maintain the balance of sodium and potassium ions, preventing the accumulation of toxic levels of sodium in tissues (Kalsoom and Ahmed, 2023; Ali, 2024). Equally important is osmotic adjustment, whereby wheat plants accumulate compatible solutes such as proline and sugars in maintaining cellular turgor under saline conditions. Antioxidant defense systems are reported to play a huge role in detoxifying oxidative stresses caused by excess salts (Afzal et al., 2024; Baloch et al., 2024; Chapagaee et al., 2024). Such an understanding of the mechanism with respect to the same is of paramount importance in developing salt-tolerant wheat varieties, hence informing the breeders where to source the respective traits for integration into new lines with improved performance in saline-prone ecosystems (Bibi et al., 2024; Zulfiqar et al., 2024).

Soil salinity is a serious ecological hazard for many countries across the globe. Agricultural lands degrade and turn into deserts due to the yearly expansion of saline soil areas brought on by secondary salinization processes; eventually, these lands vanish from circulation. Global agricultural productivity is declining as a result of these processes (Al-jughaif and Alobaidy, 2024). Because they violate ionic homeostasis and the osmotic state, high salt concentrations have a detrimental effect on cellular metabolism by expressing the toxic action of inorganic ions (Muhammad et al., 2023). Estimates indicate that 20% of croplands and 50% of agricultural fields globally are affected by soil salinity (Yokoi et al., 2002). The 102 wheat genotypes selected for this study were highly relevant for the salt tolerance evaluation. These genotypes obtained from diverse environmental conditions across various regions, including areas with known saline soils. The selection was performed both on naturally occurring and induced salt tolerance traits (Ahmed et al., 2022). Primary emphasis given to varieties that have already shown resistance to salinity stress in previous works and plant breeding programs. It would aim at capturing the widest genetic diversity to study the response of different genotypes under salt-stressed conditions. Such genotypes have been derived from various geographical locations; hence, the mechanism of salt tolerance derived would most likely serve useful applications in agriculture in these saline-prone areas (Pangaribuan et al., 2023; Khan et al., 2024).

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Therefore, it is now more important than ever to develop wheat genotypes that are both salt-tolerant and high-yielding, especially in light of the ongoing global population growth and climate changes (Mansour et al., 2020). However, there are barriers to improving salt-tolerant genotypes, including a lack of genetic diversity, a limited ability to select under salinity stress, and a lack of understanding of the intricate mechanisms underlying salt tolerance (El-Hendawy et al., 2017). To address the limitations on agricultural output, improving breeding for salt tolerance is an important global concern.

In contrast to genotypes that are sensitive to salt, those that are salt-tolerant are able to finish their growth cycle and yield the proper amount of grain when exposed to salinity stress (Oyiga et al., 2016). Different wheat genotypes are more or less effective at producing a grain yield that is acceptable under salinity stress. To determine which wheat genotypes are sensitive to salt and which are salt-tolerant, it is crucial to address salinity conditions (El-Hendawy et al., 2017). Since the plants are screened under realistic and natural conditions like soil heterogeneity, drought stress, and fluctuations in air temperature at the same time as salinity stress, evaluation of wheat genotypes under natural salinization is an important assessment approach (Dadshani et al., 2019). Therefore, genotypic evaluation in naturally salinized fields helps identify appropriate genotypes that could be cultivated in salinity and possible parents that could be included in salt-tolerance breeding programs (Moustafa et al., 2021; Ali et al., 2024).

The main goal of this experiment was to determine the salinity tolerance of 102 different wheat genotypes using characteristics of the seedlings, in order to establish the relationship between the seedling traits under study and to choose appropriate selection requirements for both salinity and normal conditions.

Material and methods

Experimental location and site

The experiment aimed to evaluate the response of various 102 wheat genotypes (*Table 1*) to salt stress at the seedling stage. The seeds of the genotypes were collected from the seed store of the Department of Plant Breeding and Genetics (PBG) at the Islamia University of Bahawalpur (IUB). The current experiment was conducted in the Department of PBG laboratory at the IUB, Punjab, Pakistan. Two treatments, salinity (S) at 6 ds/m level and normal (N), were applied in a completely randomized design (CRD), with each treatment replicated three times. In petri dishes filled with sand, five seeds of each genotype were sown. Saline solution which was made by adding NaCl in sterilized water was applied in petri dishes as per treatment plan (Sparks et al., 2020). Each treatment received 100 ml of solution (6 ds/m). Same amount (100 ml) of sterilized water was added in control treatment petri dishes. In order to keep three plants per replication, thinning was done after germination. The petri dishes were placed in a growth chamber at 25°C temperature and 50% relative humidity under 16 h light and 8 h dark photoperiod for 20 days to completion of the experiment (Gholizadeh et al., 2021).

Traits to be measured

During the experiment, the following characteristics were examined: salt tolerance index (STI), root fresh weight (g), root dry weight (g), shoot fresh weight (g), shoot dry

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weight (g), root to shoot length ratio, rate of germination (%), coefficient of germination (%) etc. For a maximum of seven days, germination data were recorded at 120-h intervals. The confirmation of germination occurred when the radical and plumule sizes exceeded 2 mm. Precision scales and fine scales were used to measure the length of the shoot and root, respectively. Electronic balances were used to measure the fresh weights, and oven drying at 70°C for 72 h was used to determine the dry weights. The ratio of plant values treated with NaCl to control values was used to compute the salt tolerance index (Ashraf et al., 2008; Gholizadeh et al., 2021). The following formulas (*Eqs. 1-4*) were used to determine the Rate of germination, coefficient of germination, germination vigor index and Reduction percentage (Krishnasamy and Seshu, 1990).

$$Rate\ of\ germination\ (\%) = \frac{No.of\ seeds\ germinated\ at\ 120h}{No.of\ seeds\ germinated\ at\ 168h} \times 100 \qquad \text{(Eq.1)}$$

$$Co - efficient\ of\ germination\ (\%) = \frac{100(A1+A2+\cdots An}{A1T1+A2T2+\ldots AnTn} \times 100 \qquad \text{(Eq.2)}$$

$$Germination\ Vogor\ Index\ (\%) = \left(\frac{A1}{T1} + \frac{A2}{T2} + \frac{An}{Tn}\right) \times 100 \qquad \text{(Eq.3)}$$

where A = number of seeds germinated, T = time (hours) corresponding to A, n = number of days to final count.

$$Reduction\ Percentage = \frac{Mean\ for\ Normal\ conditions - mean\ for\ stress\ conditions}{Mean\ for\ Normal\ conditions} \times 100 \qquad \text{(Eq.4)}$$

Statistical data analysis

Analysis of variance (ANOVA) was carried out with Statistic 8.1. Mini-tab was used for Principal Component Analysis (PCA). R Studio was utilized for the analysis of genotypic phenotypic correlations. Reduction Percentage was employed to find out best and worst genotypes under both normal and stress condition.

Results

Analysis of variance

The ANOVA results revealed significant variability among genotypes (GEN), treatments (TRT), and their interaction (GEN × TRT) across all studied traits. For Rate of Germination (RG), significant effects were observed for both genotypes and treatments, indicating that both genetic variability and treatment application influenced germination rates. Similarly, the Coefficient of Germination (CG) showed significant variation due to genotypes and treatments, but the GEN × TRT interaction was non-significant, suggesting a consistent response to treatments across genotypes for this trait. For Germination Vigor Index (GVI), highly significant effects of genotypes were detected, reflecting substantial genetic variability. Treatments also significantly influenced GVI, while the GEN × TRT interaction showed significant effects, indicating that genotypes responded differently to treatments regarding germination vigor. Root Length (RL) and Shoot Length (SL) were significantly influenced by genotypes and treatments, with significant GEN × TRT interactions suggesting that genetic diversity affected root and shoot growth differently under treatment conditions. In terms of biomass traits, Shoot Fresh Weight (SFW) was significantly affected by genotypes and

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GEN × TRT interactions, while treatments showed no significant effects, indicating that treatment application did not directly influence fresh shoot weight. Conversely, Shoot Dry Weight (SDW) exhibited highly significant effects for genotypes and treatments, as well as a highly significant GEN × TRT interaction, highlighting the combined influence of genetic and treatment variability on this trait. Root Fresh Weight (RFW) showed significant effects for genotypes and treatments, but its GEN × TRT interaction was non-significant, indicating stable responses across genotypes to treatment. Lastly, Root Dry Weight (RDW) was significantly influenced by genotypes, treatments, and their interaction, reflecting the combined effects of genetic diversity and treatment application on root dry matter production (*Table 2*).

Table 1. *Code and genotypes name of 102 studied wheat genotypes*

Code	Genotypes name	Code	Genotypes name	Code	Genotypes name
G1	IHSAN16	G35	BATHUR	G69	ASS-15-STRN
G2	LALMA-13	G36	HASHIM	G70	PIUB-5523
G3	MH-97	G37	NN GANDAM I	G71	SILVER-BLUE
G4	PASBAN 90	G38	GOMAL 7	G72	TD-01
G5	NARC-2009	G39	PIRSABAK 08	G73	FSD-08
G6	PIRSABAK-2013	G40	BARANI 05	G74	NISHAN-E-BAKHAR
G7	SHAHKAR-2013	G41	IMDAD 2005	G75	SEHER-6
G8	BENAZIR	G42	KHIRMAN	G76	SHAHFAQ 2006
G9	HAMAL-FAQIR	G43	SASSUI	G77	UGALA 16
G10	NIFA-LALMA	G44	SKD-1	G78	BALHAR-STAR
G11	NIA SAARANG	G45	KOHSAR 95	G79	MH-21
G12	MILLAT-2011	G46	KIRAN 95	G80	AKBAR
G13	DHARABI-2011	G47	NOWSHERA 96	G81	SHALKOT-14
G14	PUNJAB-2011	G48	SINDHU16	G82	GALAXY-13
G15	JAUHAR16	G49	ABADGAR 93	G83	IUB-2120
G16	AARI-2011	G50	BWP-97	G84	BOURLAG
G17	NIFA-BARSAT-10	G51	Pakistan 20	G85	AAS-2011
G18	JANBAZ-10	G52	204088	G86	DILKASH
G19	SIRAN-2007	G53	Akbar-19	G87	GHAZI-19
G20	ATA HABIB 2010	G54	ANAJ-17	G88	SUBHANI-19
G21	KT 2009	G55	166	G89	FAKHR-e-BAKHAR
G22	NIA AMBER	G56	AS-021	G90	PBG-4596
G23	NIA-SUNEHRI	G57	PAK-13	G91	SUBHANI-21
G24	AMIN-2008	G58	Aruj	G92	AZRC-1
G25	KT-2010	G59	ZINCOL 2016	G93	NAWAB
G26	NIFA AMAN	G60	15-STRN-NAWAB	G94	ASS-02
G27	S-24	G61	178	G95	SUPER
G28	BARSAT-10	G62	204171	G96	MARKAR
G29	ATTA-HABIB-10	G63	204164	G97	PBG-4492
G30	TIJABAN-2010	G64	SADIQ-15-STRN	G98	IUB-2122
G31	LASANI 2008	G65	214313	G99	PIUB-5521
G32	CHAKWAL 50	G66	10-SATY	G100	V-16164
G33	MAIRAJ 2008	G67	HTYT-9	G101	BWP-2000
G34	GOMAL 08	G68	GOLD-16	G102	NARC-11

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Table 2. *Mean sum of squares (MS) for studied traits obtained from analysis of variances (ANOVA)*

SOV	DF	RG	CG	GVI	RL	SL	MS	SFW	SDW	RFW	RDW
GEN	101	125.61^{*}	225.48^{*}	1225.65^{**}	325.66^{*}	325.61^{*}	1253.29^{**}	112.21^{*}	1613.18^{**}	114.26^{*}	625.21^{*}
TRT	1	1342.24^{**}	214.97^{*}	885.39^{*}	910.71^{*}	225.7^{*}	1342.2^{**}	63.14^{ns}	1258.62^{**}	115.52^{*}	622.24^{*}
GEN*TRT	101	122.26^{*}	75.01^{ns}	118.82^{*}	425.25^{*}	119.23^{*}	1199.01^{**}	115.03^{*}	1666.03^{**}	31.27^{ns}	416.28^{*}
Error	406	12.35	35.12	95.98	75.72	15.42	21.32	1.25.83	17.3.28	13.78	11.24
Total	611	1602.46	550.58	2325.84	1737.34	685.96	3815.82	290.38	4537.83	274.83	1674.97

SOV: Sources of Variations, GEN: Genotypes, TRT: Treatment, DF: Degree of freedom, SS: Sum of squares, MS: Mean Squares, ROG: Rate of germination, COG: Coefficient of germination, GVI: Germination vigor index, RL: Root length, SL: Shoot length, RSR: Root to shoot length ratio, SFW: Shoot fresh weight, SDW: Shoot dry weight, RFW: Root fresh weight, RDW: Root dry weight. **: highly significant ($p < 0.01$), *: significant ($p < 0.05$), ns: non-significant

Performance of genotypes based on their reduction percentage

The rate of germination (ROG) for genotypes G7 (8.31), G16 (8.35), G24 (8.32), G47 (8.36), and G56 (8.31) showed a low reduction percentage, signifying their better performance. In contrast, genotypes G84 (9.00), G99 (9.29), G87 (9.23), G94 (9.29), and G77 (9.34) exhibited high reduction percentages, correlating with their poor performance, as illustrated in *Figure 1A*. The COG was found to be highly significant. The genotypes G16 (6.13), G24 (6.44), G7 (6.57), G56 (6.58), and G47 (6.75) demonstrated good performance, as evidenced by their low reduction percentage values. The high reduction percentage values of genotypes G84 (9.25), G77 (9.43), G94 (9.52), G87 (9.57), and G99 (10.09) indicate that these varieties did not perform well (*Fig. 1A*).

Genotypes G7 (15.18), G24 (15.34), G16 (15.50), G47 (15.58), and G56 (15.77) demonstrated superior performance in the germination vigor index (GVI) due to their low reduction percentage values. Conversely, genotypes G99 (19.02), G84 (19.11), G94 (19.12), G87 (19.17), and G77 (19.68) exhibited high reduction percentages, reflecting their poor performance (*Fig. 1A*). Similarly, the low reduction percentage values in root length (RL) for genotypes G24 (37.84), G7 (38.67), G47 (39.54), G56 (39.72), and G16 (41.02) were indicative of their strong performance. In contrast, genotypes G99 (61.87), G84 (65.08), G87 (66.57), G77 (67.65), and G94 (72.04) displayed high reduction percentages, which contributed to their poor performance, as illustrated in *Figure 1A.*

Current results showed that the genotypes G56 (37.84), G24 (38.63), G7 (39.61), G16 (39.44), and G47 (40.82) demonstrated low reduction percentages, indicating superior performance. In contrast, genotypes G84 (59.57), G94 (63.19), G87 (63.62), G99 (64.49), and G77 (68.72) exhibited the highest reduction percentages and performed poorly (*Fig. 1A*). In terms of root-to-shoot length ratio, the genotypes G47 (-0.17), G7 (-0.01), G24 (0.07), G16 (0.08), and G56 (0.26) performed well due to their low reduction percentages. Conversely, genotypes G99 (5.45), G84 (5.70), G87 (8.10), G77 (11.89), and G94 (12.12) exhibited high reduction percentages and lower performance (*Fig. 1B*). Genotypes with the greatest root-to-shoot length ratios under salinity stress were classified as salinity-tolerant, while those with the lowest performance levels were identified as salinity-susceptible. These findings provide critical insights into genotype responses to salinity stress and can inform breeding strategies for enhanced stress tolerance.

Genotypes G7 (37.04), G56 (39.22), G24 (39.62), G47 (40.00), and G16 (40.38) exhibited low reduction percentage values for shoot fresh weight (SFW), reflecting their superior performance. Conversely, genotypes G84 (57.14), G94 (60.61), G99 (60.61),

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G87 (63.64), and G77 (66.67) displayed high reduction percentages, indicating their weakest performance (*Fig. 1B*). For shoot dry weight (SDW), genotypes G7 (41.94), G24 (41.94), G56 (43.33), G16 (43.33), and G47 (44.83) demonstrated strong performance due to their low reduction percentages (*Fig. 1B*). In contrast, genotypes G84 (65.00), G87 (68.42), G99 (68.42), G77 (70.59), and G94 (73.68) showed high reduction percentages, resulting in poor performance (*Fig. 1B*). Similarly, root fresh weight (RFW) performance was superior in genotypes G7 (41.94), G24 (41.94), G56 (43.33), G16 (43.33), and G47 (44.83), which had low reduction percentages. Genotypes G84 (65.00), G87 (68.42), G99 (68.42), G77 (70.59), and G94 (73.68) exhibited high reduction percentages, correlating with their poorest performance (*Fig. 1B*). These results emphasize the critical role of reduction percentages in assessing genotype performance under salinity stress.

In this study, the genotypes G7 (41.38), G24 (42.86), G47 (44.44), G56 (44.44), and G16 (46.43) exhibited low reduction percentages in shoot dry weight (SDW), reflecting superior performance. In contrast, genotypes G99 (66.67), G87 (68.75), G94 (70.59), G84 (70.59), and G77 (73.33) showed high reduction percentages, indicating poor performance, as shown in *Figure 1B*. Under both normal and salinity stress conditions, based on their reduction percentage values and overall performance, genotypes G7, G16, G24, G47, and G56 demonstrated superior resilience and were classified as salinity-tolerant. Conversely, genotypes G77, G84, G87, G94, and G99 exhibited poor performance across most studied traits and were categorized as salinity-susceptible wheat genotypes (*Table 3*).

Table 3. *Best and worst performer genotypes with their reduction percentage*

Trait	Best performer wheat genotypes with reduction %	Worst performer wheat genotypes with reduction %
ROG	G7 (8.31), G56 (8.31), G24 (8.32), G16 (8.35), G47 (8.36)	G84 (9.00), G99 (9.29), G87 (9.23), G94 (9.29), G77 (9.34)
COG	G16 (6.13), G24 (6.44), G7 (6.57), G56 (6.58), G47 (6.75)	G84 (9.25), G77 (9.43), G94 (9.52), G87 (9.57), G99 (10.09)
GVI	G7 (15.18), G24 (15.34), G16 (15.50), G47 (15.58), G56 (15.77)	G99 (19.02), G84 (19.11), G94 (19.12), G87 (19.17), G77 (19.68)
RL	G24 (37.84), G7 (38.67), G47 (39.54), G56 (39.72), G16 (41.02)	G99 (61.87), G84 (65.08), G87 (66.57), G77 (67.65), G94 (72.04)
SL	G7 (37.04), G56 (39.22), G24 (39.62), G47 (40.00), G16 (40.38)	G84 (57.14), G94 (60.61), G99 (60.61), G87 (63.64), G77 (66.67)
RSR	G47 (-0.17), G7 (-0.01), G24 (0.07), G16 (0.08), G56 (0.26)	G99 (5.45), G84 (5.70), G87 (8.10), G77 (11.89), G94 (12.12)
SFW	G7 (37.04), G56 (39.22), G24 (39.62), G47 (40.00), G16 (40.38)	G84 (57.14), G94 (60.61), G99 (60.61), G87 (63.64), G77 (66.67)
SDW	G7 (41.94), G24 (41.94), G56 (43.33), G16 (43.33), G47 (44.83)	G84 (65.00), G87 (68.42), G99 (68.42), G77 (70.59), G94 (73.68)
RFW	G7 (38.78), G24 (39.58), G16 (40.43), G47 (42.22), G56 (42.55)	G84 (60.00), G87 (64.29), G94 (65.52), G99 (65.52), G77 (69.23)
RDW	G7 (41.38), G24 (42.86), G47 (44.44), G56 (44.44), G16 (46.43)	G99 (66.67), G87 (68.75), G94 (70.59), G84 (70.59), G77 (73.33)

ROG: Rate of germination, COG: Coefficient of germination, GVI: Germination vigor index, RL: Root length, SL: Shoot length, RSR: Root to shoot length ratio, SFW: Shoot fresh weight, SDW: Shoot dry weight, RFW: Root fresh weight, RDW: Root dry weight

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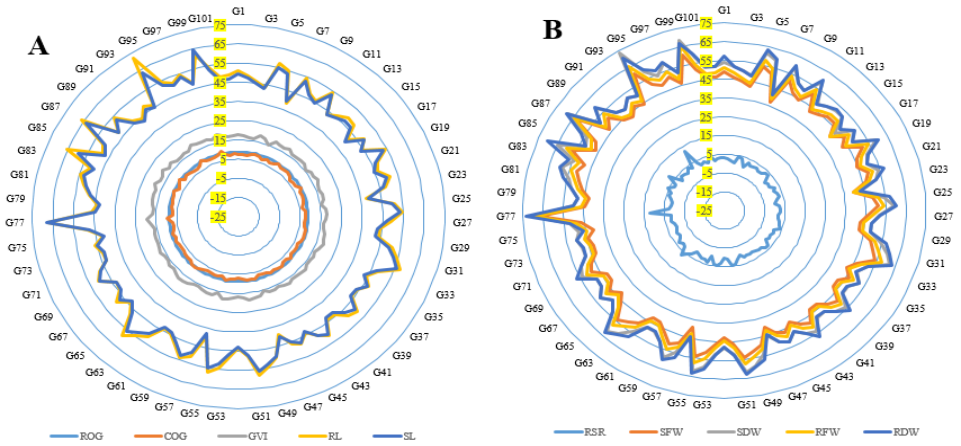

Figure 1. *Radar graph for reduction percentage under observed environments in 102 wheat genotypes for studied attributes like (A) ROG: Rate of germination, COG: Coefficient of germination, GVI: Germination vigor index, RL: Root length, SL: Shoot length. (B) RSR: Root to shoot length ratio, SFW: Shoot fresh weight, SDW: Shoot dry weight, RFW: Root fresh weight, RDW: Root dry weight*

Correlation analysis under normal and salinity stress conditions

Correlation coefficients quantify the strength and direction of relationships between two variables or factors. Under normal conditions, genotypic correlations revealed a strong positive relationship between root length and both shoot and root fresh weights (0.99**). Similarly, a significant positive correlation was observed between root length and the germination rate (0.99**) within the phenotypic association. As presented in *Table 4*, the germination rate exhibited a positive correlation (0.97**) with the germination vigor index under both genotypic and phenotypic correlations. Furthermore, a genotypic correlation of 0.98** was identified between the Salt Tolerance Index (STI) and root length, while a phenotypic correlation of 0.97** was noted for the same traits. Salt Tolerance Index and root length, emphasizing its relevance for wheat breeding programs.

Interestingly, the coefficient of germination demonstrated a negative correlation with shoot length (-0.16** genotypically and -0.30** phenotypically) under normal conditions. A negative correlation was also observed between root dry weight (-0.42** genotypically and -0.54** phenotypically) and the coefficient of germination under normal conditions. In both genotypic and phenotypic contexts, root length negatively correlated with the coefficient of germination (-0.55** and -0.42**, respectively), although it positively correlated with other traits under normal conditions.

Under salinity stress conditions, both the genotypic and phenotypic correlations showed a positive relationship between the germination vigor index (0.99**) & (0.96**) and root length. Additionally, a positive correlation between root length and the rates of germination (0.99** & 0.98**) under genotypic and phenotypic correlation, respectively. Furthermore, there was a phenotypic correlation of Root fresh weight (0.97**) and a positive genotypic correlation of Germination vigor index (0.70**) with shoot length. According to both genotypic and phenotypic correlation, there was a strong and positive correlation between the root fresh weight and the shoot fresh weight (0.99**). As indicated in *Table 4*, the root-to-shoot length ratios (-0.33^{ns} & -0.51**) under both genotypic correlations had a negative correlation with SL. Shoot length and

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COG had a negative correlation (-0.30^{ns} & -0.16^{ns}). Likewise, there was a negative correlation between ROG (-0.43 ^{ns}) and (-0.36^{ns}) and RSR, but a positive correlation with the other traits.

Table 4. *Genotypic and phenotypic correlation under normal conditions*

Traits	ROG	COG	GVI	RL	SL	RSR	SFW	SDW	RFW	RDW	STI
ROG	1**	0.42*	0.97**	0.99**	0.60**	0.85**	0.99**	0.99**	0.99**	0.99**	0.97**
COG	0.54**	1**	0.42*	-0.42 ^{ns}	-0.16 ^{ns}	0.44*	0.42*	0.42*	0.43*	0.42*	0.45*
GVI	0.99**	0.56**	1**	0.96**	0.58**	0.84**	0.96**	0.96**	0.96**	0.96**	0.95**
RL	0.99**	-0.55**	0.99**	1**	0.60**	0.85**	0.99**	0.99**	0.99**	0.99**	0.97**
SL	0.70**	-0.30^{ns}	0.70**	0.70**	1**	0.54**	0.61**	0.60**	0.61**	0.60**	0.62**
RSR	0.85**	0.57**	0.87**	0.86**	0.63**	1`**	0.85**	0.85**	0.86**	0.86**	0.89**
SFW	0.99**	0.54**	0.99**	0.99**	0.71**	0.86**	1**	0.99**	0.99**	0.99**	0.98**
SDW	0.99**	0.54**	0.99**	0.99**	0.69**	0.85**	0.99**	1**	0.99**	0.98**	0.97**
RFW	0.99**	0.55**	0.99**	0.99**	0.71**	0.87**	0.99**	0.99**	1**	0.99**	0.98**
RDW	0.99**	0.54**	0.99**	0.99**	0.70**	0.86**	0.99**	0.98**	0.99**	1**	0.97**
STI	0.97**	0.58**	0.98**	0.98**	0.70**	0.89**	0.98**	0.97**	0.98**	0.97**	1**

ROG: Rate of germination, COG: Coefficient of germination, GVI: Germination vigor index, RL: Root length, SL: Shoot length, RSR: Root to shoot length ratio, SFW: Shoot fresh weight, SDW: Shoot dry weight, RFW: Root fresh weight, RDW: Root dry weight, **: highly significant ($p < 0.01$), *: significant ($p < 0.05$), ns: non-significant

Under both normal and salinity-stressed conditions, root length (RL) exhibited strong positive correlations with shoot fresh weight (SFW), root fresh weight (RFW), and the rate of germination (ROG), emphasizing its central role in plant vigor (*Table 5*). The germination vigor index (GVI) was positively linked to ROG, while RL's positive correlation with the salt tolerance index (STI) highlights its importance for salinity resilience. In contrast, the coefficient of germination (COG) and root-to-shoot length ratio showed negative correlations with traits like shoot length and root dry weight (RDW), indicating trade-offs. These findings underline the complex trait interdependencies and offer crucial insights for breeding salt-tolerant, vigorous wheat genotypes.

Principal component analysis

A statistical technique called principal component analysis (PCA) is applied in multivariate data analysis to find connections, trends, and differences within datasets, especially when it comes to salinity tolerance characteristics. Under normal conditions, the first five (under normal) and first four (salinity) of the eleven main components (*Table 6*) displayed eigenvalues of more than one, indicating significance. Because the other six PCs' eigenvalues were less than 1, they were deemed non-significant and unusable for additional analysis. The first five PCs under investigation showed 65% total variation under normal circumstances, while the first four PCs showed 58% under stressed conditions. The first PC typically explained 16% of the variation, followed by the second PC at 15%, the third PC at 13%, the fourth PC at 12%, and the fifth PC at 9% under normal conditions. While in the salinity stressed conditions, the first PC contributed 17% of the total variation during drought conditions, the second contributed 15%, the third contributed 14%, and the fourth contributed 11% as mentioned in

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Figure 2A, B. The total eleven principal components studied for the salinity stress in wheat genotype based on their yield related traits, five PCs (normal) and Four PCs (salinity) among eleven PCs had eigenvalue greater than 1 and having variations in study traits among 102 wheat genotypes.

Table 5. *Genotypic and phenotypic correlation under salinity stress condition*

Traits	ROG	COG	GVI	RL	SL	RSR	SFW	SDW	RFW	RDW
ROG	1**	0.44**	0.95**	0.98**	0.58**	0.36**	0.98**	0.98**	0.98**	0.98**
COG	0.56**	1**	0.42**	-0.42 ns	-0.16 ns	0.28**	0.43**	0.41**	0.42*	0.4**
GVI	0.99**	0.56**	1**	0.96**	0.58**	0.35**	0.96**	0.96**	0.97**	0.96**
RL	0.99**	0.55**	0.99**	1**	0.60**	0.36**	0.99**	0.99**	0.99**	0.98**
SL	0.70**	-0.30 ns	0.70**	0.70**	1**	-0.51	0.61**	0.59**	0.61**	0.59**
RSR	0.43**	0.37**	0.43**	0.43**	-0.33 ns	1**	0.35**	0.36**	0.35**	0.36**
SFW	0.99**	0.55**	0.99**	0.99**	0.71**	0.42*	1**	0.99**	0.99**	0.99**
SDW	0.99**	0.53**	0.99**	0.99**	0.69**	0.43*	0.99**	1**	0.99**	0.98**
RFW	0.99**	0.55**	0.43**	0.99**	0.70**	0.42**	0.99**	0.99**	1**	0.99**
RDW	0.99**	0.54**	0.99**	0.99**	0.69**	0.43**	0.99**	0.98**	0.99**	1**

ROG: Rate of germination, COG: Coefficient of germination, GVI: Germination vigor index, RL: Root length, SL: Shoot length, RSR: Root to shoot length ratio, SFW: Shoot fresh weight, SDW: Shoot dry weight, RFW: Root fresh weight, RDW: Root dry weight, **: highly significant ($p < 0.01$), *: significant ($p < 0.05$), ns: non-significant

Table 6. *Variability under normal and salinity conditions*

	Environment	PC1	PC2	PC3	PC4	PC5
Eigenvalue	Normal	1.74	1.65	1.41	1.31	1.02
	Salinity	1.74	1.47	1.43	1.11	0.95
Proportion (%)	Normal	16	15	13	12	9
	Salinity	17	15	14	11	1
Cumulative (%)	Normal	16	31	44	56	65
	Salinity	17	2	47	58	67

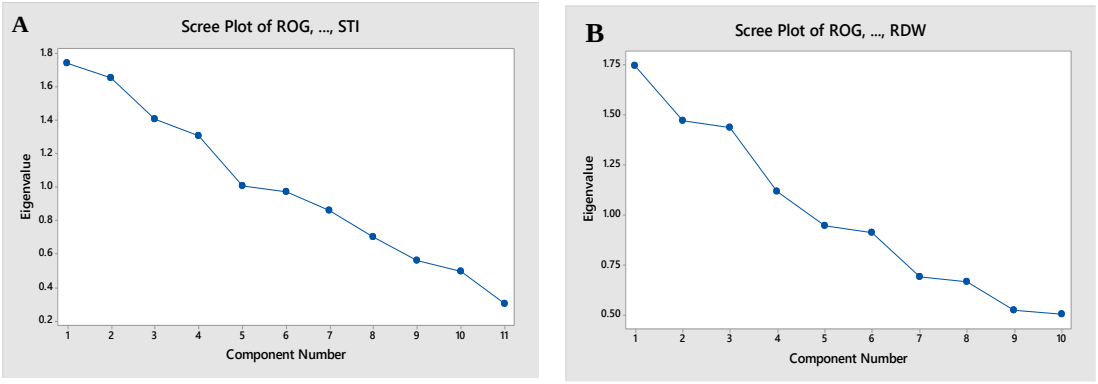

Figure 2. *The Scree plot under normal condition (A) and under salinity stressed conditions (B) illustrates the eigenvalues of principal components in studied traits of 102 bread wheat genotypes, showing their relative contributions to the total variance in the dataset. A clear inflection point indicates the optimal number of principal components to retain for effective dimensionality reduction without significant loss of information*

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The graph has two plots: one showing the data under salinity conditions and the other showing the data under normal conditions. The two axes present in every plot are known as the first and second main components, or PC1 and PC2. SFW, SDW, RFW, RDW, germination vigor index, coefficient of germination, RL, SL, and root to shoot length ratio all exhibited a strong positive correlation with each other. When two lines meet at an acute angle, it indicates a positive correlation between the variables; when two lines meet at an obtuse angle, it indicates a negative correlation. Under conditions of salt stress, all characteristics exhibited positive correlation, with the exception of shoot length, which had a strong negative association with a significant variable (*Fig. 3A, B*).

In *Figure 4*, the genotypes that appeared in the same square box performed the same, but the genotypes that appeared in different squared boxes performed differently. Principle component analysis also helpful in selecting diverse parents for hybridization and other plant breeding techniques. Under normal conditions, G37, G72, G40, and G74 were opposite to G25, G31, G32 and G26. Genotypes G39, G77, G84 and G50 were opposite to G27, G24, G47 and G56 as shown in *Figure 4A*. There was a clear difference between salinity-tolerant and Salinity-susceptible genotypes. Under Salinity stress condition, G2, G3, G4 and G9 were opposite to G99, G100, G82 and G102. Genotypes G5, G77, G32 and G94 were opposite to G75, G79, G74 and G78 showed diversity as in *Figure 4B*.

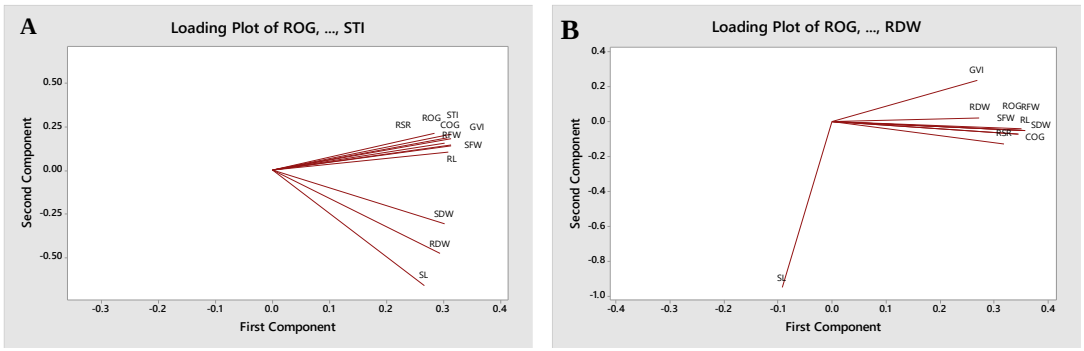

Figure 3. *The Loading plot under normal condition (A) and under salinity stressed conditions (B) depicts the contribution of individual variables to the principal components. Variables with higher loadings are more influential in defining the principal components, reflecting key traits under unstressed conditions*

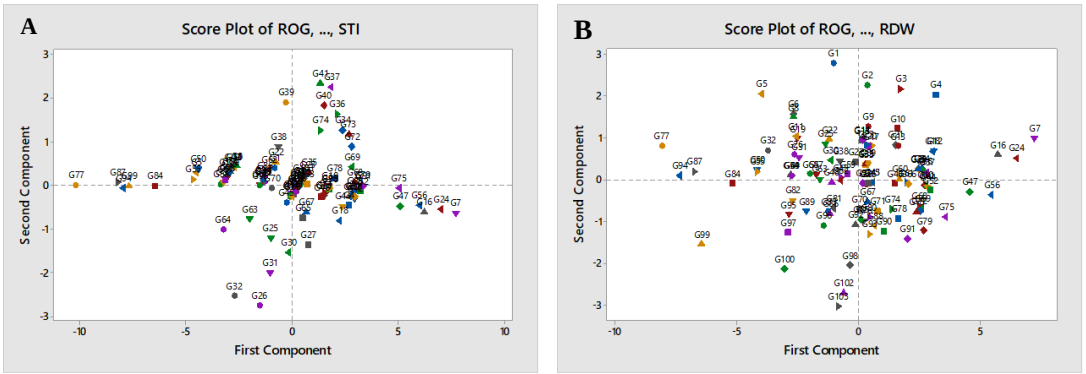

Figure 4. *The Score plot under normal conditions (A) and under salinity stressed conditions (B) showed the distribution of samples in the reduced-dimensional space defined by the principal components. It shows clustering patterns, reflecting similarities or differences among samples under unstressed conditions*

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Biplot analysis was performed using the PCA for parental selection in breeding programs (*Fig. 5A, B*). In the PCA Biplot, the angle between the inverses of the trait vectors is used to roughly represent the correlation between the traits. A positive correlation is indicated by an angle larger than 90 degrees, whereas a less than 90-degree angle denotes independence between the features. However, our results clearly showed that the correlations between a trait pair and the trait pair's contributions to the Biplot for PCA were well coordinated with the approximate vector angles.

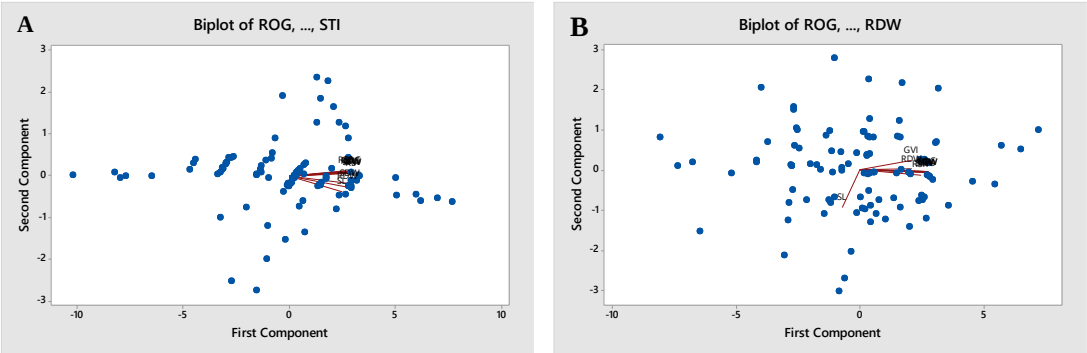

Figure 5. *The Biplot under normal conditions (A) and under salinity stress conditions (B) combines the score and loading plots, showing both sample distribution and variable contributions in the principal component space. It highlights relationships between samples and variables under unstressed conditions, revealing key traits influencing sample differentiation*

Discussion

The ANOVA results in this study highlight the significant role of genetic diversity and treatments in influencing traits like Germination Vigor Index (GVI), Shoot Dry Weight (SDW), and Root Dry Weight (RDW). Highly significant genotype × treatment (GEN × TRT) interactions for SDW and RDW indicate genotype-specific responses to treatments. In contrast, non-significant GEN × TRT interactions for Coefficient of Germination (CG) and Root Fresh Weight (RFW) suggest consistent responses across genotypes, while the non-significant treatment effect for Shoot Fresh Weight (SFW) underscores its greater reliance on genetic factors. These findings demonstrate that genetic variation is a key determinant of the studied traits, with treatment effects and GEN × TRT interactions being particularly critical for traits like GVI, SDW, and RDW. Previously findings align with the current findings (Alghabari and Shah, 2024; Chapagaee et al., 2024). Therefore, both genotype selection and targeted treatments can effectively optimize these traits. Wheat scientists reported similar findings about salinity stress in wheat crop (Khan et al., 2024; Murtaza et al., 2024). Accuracy in this research is ensured by using a CRD with a controlled saline environment; however, this does constrain the external validity. While CRD is effective in reducing experimental error, it cannot capture the spatial variability and associated environmental complexities that typify field conditions. Besides, the controlled environment isolates salinity as the only major stress factor, which can hardly be real in the field, since it is often combined with other abiotic stresses such as drought and nutrient deficiencies, and pests. These controlled conditions overestimate or underestimate the resilience of genotypes against multiple stressors. While the study gives valuable information on mechanisms related to salt tolerance, the field validation step is crucial in ensuring applicability across diverse

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agro-ecological environments and under more realistic conditions of stress (Alom et al., 2016; Dadshani et al., 2019; Ahmed et al., 2022; Khan et al., 2024).

The study revealed substantial genotypic variation in wheat's response to salinity stress, showing that salt tolerance is influenced by both genetic and environmental factors (Mansour et al., 2020; Uzair et al., 2022). Genotypes G7, G16, G24, G47, and G56 exhibited high salt tolerance, making them promising candidates for breeding programs, whereas G77, G84, G87, G94, and G99, which performed poorly, could serve as baselines for identifying salt-sensitive traits. Salinity stress significantly reduced the rate of germination (ROG), consistent with the findings of previously studies (Alom et al., 2016; Chapagaee et al., 2024), they reported that tolerant genotypes maintained higher germination percentages. Some scientists (Atak et al., 2006; Khan et al., 2024) further explained that high salinity concentration prevents water absorption by seeds, leading to germination failure and reduced plant density.

Genotypes G7, G16, G24, G47, and G56 also showed superior performance for Coefficient of Germination (COG) and GVI, Furthermore, GVI and coefficient of germination (COG) were significantly higher in these genotypes, corroborating reports by wheat scientists (Oyiga et al., 2016; Ahmed et al., 2022), which noted that enhanced germination indices are critical indicators of salt tolerance. In another study, Fercha and Gherroucha (2014) similarly observed declines in germination index and seedling vigor under increasing salinity. Root length (RL) emerged as a crucial trait for salinity tolerance, with tolerant genotypes G7, G16, G24, G47, and G56 showing minimal reductions in RL, their minimal reductions in RL under salinity stress validate findings by wheat breeders (Gholizadeh et al., 2021; Al-jughaif and Alobaidy, 2024), they emphasized the importance of root development for water uptake under high salinity. Longer roots of these genotypes probably reflect efficient ion exclusion and osmotic adjustment strategies mitigating the damage under salt stress. The susceptible genotype G77 and G99 showed a significant reduction in RL, most probably because of salt accumulation on the root surfaces thereby impairing water absorption. Their superior shoot length and root-to-shoot ratios further support observations by wheat scientists (Sharma, 2015; Kalsoom and Ahmed, 2023), indicating adaptive strategies such as optimized resource allocation and maintenance of growth under stress.

Shoot length (SL) was also negatively affected by salinity stress, with tolerant genotypes maintaining better shoot growth, in agreement with Akbarimoghaddam et al. (2011), who found a gradual decline in shoot length with increasing NaCl concentrations. The root-to-shoot ratio, an indicator of salinity adaptation, was higher in tolerant genotypes, further validating previous findings by Sharma (2015), Kalsoom and Ahmed (2023). Fresh and dry weights of roots and shoots were significantly reduced under salt stress, with G7, G24, G56, G47, and G16 showing better weight ratios compared to salt-sensitive genotypes. Ahmed et al. (2022) noted a similar reduction in shoot fresh weight under salinity stress, attributing it to osmotic imbalances and ion toxicity. Additionally, their better shoot and root dry weights echo findings by plant scientists, which linked biomass retention under salinity to improved salt tolerance. These results underscore the potential of these genotypes for breeding programs targeting enhanced resilience to saline environments.

Among the quantitative traits, root length showed a high positive genotypic and phenotypic correlation with shoot fresh weight, root fresh weight, and root dry weight, especially under salinity stress conditions. These results confirm those of Ahmed et al. (2022) in wheat crop. GVI also exhibited strong positive correlations with ROG,

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highlighting its role in enhancing seedling vigor. Additionally, a positive correlation between Salt Tolerance Index (STI) and RL (0.98**) reinforces the importance of root development for salt tolerance (Choudhary et al., 2021; El Sabagh et al., 2021). In contrast, COG showed a negative correlation with SL and Root Dry Weight (RDW), indicating trade-offs between these traits. In contrast, the root-to-shoot ratio was negatively correlated with SL, while positively related to other studied traits, reflecting a complex dependency possibly utilized in breeding for salt tolerance and improvement of seedling vigor. Fresh shoot weight and plant biomass have already been reported by previous studies as applicable criteria of selection for salt tolerance at the seedling stage, among others, by Bahrani and Hagh Joo (2012) and Muhammad et al. (2023).

Principal Component Analysis (PCA) provided critical insights into trait variability and associations under normal and salinity-stressed conditions. Eigenvalues greater than 1 led to the selection of five PCs under normal conditions and four under salinity stress, explaining 65% and 58% of the total variance, respectively. PC1 contributed the highest proportion of variance (16%) under normal conditions and 17% under salinity stress, indicating shifts in trait contributions due to stress. Previously scientists conducted PCA in wheat crop and their findings aligns with current study (Ahmed et al., 2022, 2024a; Khan et al., 2023). Traits like SFW, RFW, and GVI exhibited strong positive correlations across conditions, reflecting their critical role in salinity tolerance.

The PCA biplot revealed positive correlations through acute trait vector angles and negative associations through obtuse angles (Ahmed et al., 2024b; Alghabari and Shah, 2024). Salinity stress altered these associations, with SL showing a strong negative correlation, aligning with findings by Khan et al. (2024). The distinct clustering of salinity-tolerant genotypes (G7, G16, G24, G47, and G56) and susceptible genotypes (G77, G84, G87, G94, and G99) in the PCA plots underscores the effectiveness of PCA in distinguishing genotypic performance and identifying adaptive strategies. Previous research has indicated similar findings about salinity stress in wheat crop (Kalsoom and Ahmed, 2023; Muhammad et al., 2023; Zulfiqar et al., 2024). This highlights PCA's utility for selecting diverse parents in hybridization programs aimed at improving salinity tolerance. Overall, the study underscores the importance of selecting genotypes with multiple stress-tolerant traits. Genotypes G7, G16, G24, G47, and G56 emerged as promising candidates for future wheat breeding programs, offering resilience to salinity stress and enhancing yield stability under adverse conditions.

Conclusion

Developing salinity-resistant wheat varieties is essential for sustainable crop production in saline-prone regions. This study evaluated the morphological responses of 102 wheat genotypes (*T. aestivum* L.) to salinity stress at the seedling stage. Using a factorial randomized design, key traits such as germination rate, vigor index, shoot and root length, root-to-shoot ratio, fresh and dry weights, and salt tolerance index were analyzed under saline and control conditions. Strong associations among most traits under both conditions emphasized their significance for breeding programs, suggesting that selecting one trait could enhance others. Principal component analysis revealed that under normal conditions, five components explained 65% of the total variation, while under salinity stress, four components accounted for 58%. Superior salt tolerance was observed in genotypes G7, G16, G24, G47, and G56, reflecting their genetic potential to withstand salinity and improve productivity. Conversely, genotypes G77, G84, G87,

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G94, and G99 performed poorly under stress, indicating their susceptibility. These findings provide a foundation for identifying salt-tolerance genes, exploring genetic mechanisms, and utilizing QTL mapping and molecular markers to enhance breeding strategies. By developing resilient wheat varieties, sustainable yields can be ensured in regions affected by soil salinization, addressing global food security challenges.

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