

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

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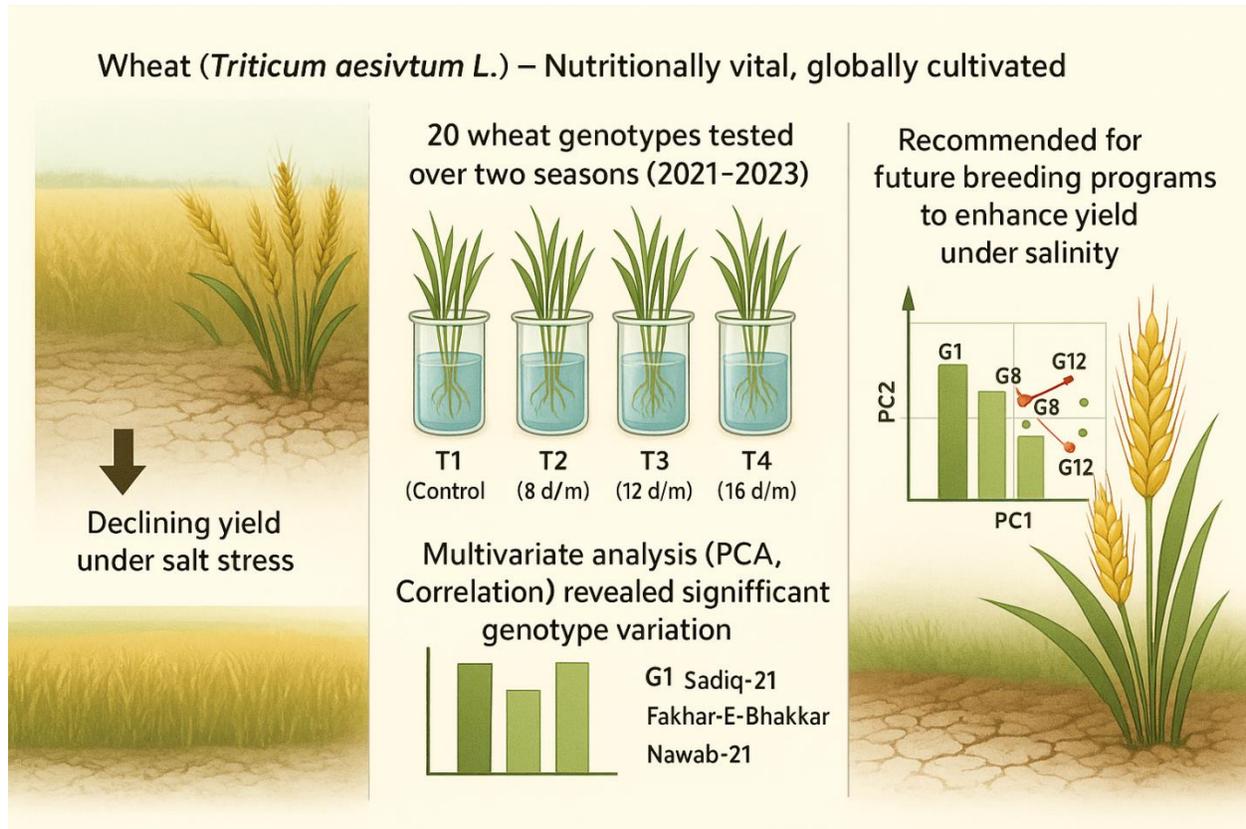
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16
17 **Abstract**

18 Wheat (*Triticum aestivum* L.) is a globally important cereal crop yet, its production is gradually
19 decreasing due to salinity stress while demand is increasing. Salt stress is an important abiotic
20 stressor affecting wheat from growth to harvest. The current study was conducted to evaluate 20
21 diverse wheat genotypes for two consecutive growing seasons in 2021-2022 and 2022-23, to
22 assess their salt tolerance potential against four salinity levels (control, 8dS/m, 12dS/m and 16
23 dS/m). Salinity tolerance among wheat genotypes was assessed on the basis of morpho-
24 physiological and yield related traits such as plant height (PH), peduncle length (PL), spike
25 length (SL), flag leaf area (FLA), chlorophyll content (CC), grain length (GL), grain width
26 (GW), grain area (GA), thousand grain weight (TGW) and yield per plant (YPP). A significant
27 genetic diversity was detected among the genotypes based on the evaluated traits. Correlation
28 analysis revealed positive association among all parameters across treatment conditions.
29 Principal component analysis (PCA) revealed that out of 10 components, only two had greater
30 than 1 eigenvalues and were significant across all treatments, explaining 70.49%, 60.67%,
31 60.62% and 51.562% of the total variation under T1 (control), T2 (8dS/m), T3 (12dS/m) and T4
32 (16dS/m) respectively. Additionally, the PCA biplot identified Sadiq-21 (G1), Fakhar-E-Bhkkar
33 (G8) and Nawab-21 (G12) as potential salt tolerant genotypes. In order to produce high yielding

34 and salt tolerant genotypes, future wheat breeding programs may utilize the tolerant genotypes
35 with salt tolerance potential.

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



37

38 Graphical abstract

39 1. INTRODUCTION

40 Spring wheat is an important cereal crop of semi-arid and arid regions of Pakistan. Wheat
41 is key crop for human nourishment due to its contribution in 30% world grain production and 45
42 % cereal nutrition's (Karimzadeh et al. 2023). Wheat contains Proteins (8-15%), moisture (12%),
43 Fats (1.5-2%) and Carbohydrate (60-80%) that are much needed for humans (Bakaaki et al.
44 2023). Wheat provides over half of the protein and more than half of the calories for about one-
45 third of the world's population thus considered to be the fundamental component of the human
46 diet due to its high nutritional value. It serves as a primary food source, providing essential
47 nutrients and energy for millions worldwide (Jamal et al. 2025). However, the demand for wheat

48 continues to rise, while its overall production faces significant challenges, one of the most
49 pressing being the impact of abiotic stressors such as salinity (Li et al., 2023).

50 Salt stress is a major environmental constraint that negatively affects wheat growth,
51 development, and yield, from seedling establishment to harvest. It poses a severe threat to global
52 wheat production, particularly in arid and semi-arid regions where soil salinization is prevalent (
53 Sewore and Abe, 2024) . Climate change is causing the rise in soil salinity due to higher
54 evaporation of irrigation water which also triggered by water scarcity and rising soil
55 temperatures (Eswar et al. 2021; Hamzah et al., 2024). Soil salinity affects total 831 million
56 hectares (mha) area all over the globe out of which 397 Million (ha) are classed as salt affected
57 while other 434 million (ha) as saline sodic (Phalke et al. 2020). In addition to the area already
58 impacted by salt, 1% to 2% more of the world's fertile lands are experiencing salt damage
59 each year (Yang et al. 2022). Reducing the risks of food insecurity brought on by population
60 growth and climate change is an important problem that has to be handled (Qiao et al. 2021).

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

79 valuable insights for breeding programs (Senapati et al., 2024, Qadeer et al. 2023, Jamal et al.
80 2025).

81 Physiological traits like flag leaf area and chlorophyll content are equally significant in
82 assessing a genotype's ability to tolerate salt stress (Nassima et al., 2024). The flag leaf is a
83 primary photosynthetic organ that contributes significantly to grain filling and overall plant
84 productivity. A larger flag leaf area under saline conditions indicates a plant's capability to
85 sustain photosynthetic efficiency, thereby supporting better growth and yield. Chlorophyll
86 content serves as an indicator of the plant's photosynthetic capacity, with salinity stress often
87 causing chlorophyll degradation, reducing the plant's energy production and overall health (Ali et
88 al. 2024, Saeed et al. 2024). Grain-related traits, including grain length, grain width, grain area,
89 and thousand grain weight, directly influence yield per plant and overall productivity. These
90 traits collectively determine the economic value of the crop and are essential for identifying
91 high-yielding genotypes that can withstand saline conditions (Ahmed et al. 2022b, Rashid et al.
92 2022, Ahmed et al. 2023). Understanding the genetic diversity associated with these traits
93 provides a foundation for selecting and breeding wheat varieties with enhanced tolerance to
94 salinity, ultimately contributing to food security in saline-affected regions.

95 Chlorophyll content is the key component for outstanding crop yield and negatively
96 impacted by salinity stress (Adil et al. 2022). This decrease in chlorophyll content is not simply
97 linked to decrease in cellular chlorophyll but can also non chlorophyll aspect like, photosynthesis
98 and yield per plant etc. (Mehta et al. 2010). So, the high salt causes distinct changes in the
99 physiological, morphological, and structural features of plant cells, tissues, and organs. Cell
100 replication and growth mechanisms are impacted by salt stress (Kumar et al. 2017). As a
101 consequence, meristem at the apex shrinks, which diminishes the cortex and the bundles of
102 vascular cells. Additional morphological alterations that happen in plants subjected to saline
103 stress are reduction in grain related attributes like grain length, width and area (Zeeshan et al.
104 2020). Many plant genotypes have altered physio-morphological attributes under salt stress,
105 however genetic diversity and evaluation of tolerance in commercial and old germplasm in
106 glasshouse conditions has not been done yet (Yang et al. 2022). This genotype screening lays the
107 basis for breeding efforts aimed at producing new wheat varieties with enhanced salt tolerance
108 (Ahmed et al. 2022a). So, genotypes resistant to salt can be found and used, ensuring more
109 sustainable and productive wheat production in the future.

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

116 The objectives of the current study were:

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

123

124 2. MATERIAL AND METHODS

125 Experimental Site and Design

126 Current study was executed over two consecutive wheat growing seasons (2023–2024 and 2024–
127 2025) at the experimental glasshouse facility of Department of Plant Breeding and Genetics, The
128 Islamia University of Bahawalpur, Pakistan (29.3544° N, 71.6911° E). Twenty wheat (*Triticum*
129 *aestivum* L.) genotypes including advanced breeding lines and conventional varieties were
130 sourced from Regional Agricultural Research Institute (RARI), Bahawalpur, Pakistan. The
131 objective was to assess genetic diversity among the genotypes, for salt stress tolerance based on
132 key morphological and physiological traits (Ilyas and Naz 2024).

133 The experiment was laid out in Completely Randomized Design (CRD) with three replications
134 per treatment each consisting of one pot per genotype per treatment. To ensure reliability, the
135 experiment was conducted across two consecutive seasons under controlled glasshouse
136 conditions, with replication minimizing environmental variation. Standard statistical procedures
137 (ANOVA, correlation, and PCA) further strengthened the consistency and reliability of the

138 results. As conditions were controlled and uniform so CRD was selected, which minimized
139 environmental variability (Gomez and Gomez, 1984).

140 **Glasshouse Conditions**

141 Glasshouse was maintained under semi-controlled conditions with an average temperature range
142 of 22–28°C, 60–70% relative humidity and a 12-hour photoperiod. Supplemental lighting and
143 ventilation were used to maintain consistent growth conditions.

144 **Pot Preparation and Salinity Treatments**

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

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

153 T2 (Moderate Stress): 8 dS/m NaCl

154 T3 (Severe Stress): 12 dS/m NaCl

155 T4 (Extreme Stress): 16 dS/m NaCl

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

158 **Trait Measurement**

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

162 Chlorophyll content was measured at the heading stage using a Hansatech CL-01 SPAD meter
163 with a wavelength range of 650–940 nm and a measurement range of 0–199.9 SPAD units
164 (Hansatech Instruments Ltd., UK). Three measurements were taken from different areas of the
165 flag leaf and averaged. Grain area (GA) was calculated following a modified version of the
166 method used by Ahmed et al. (2022), using the following equation:

$$167 \quad GA = \frac{13}{11} \times (GW + GH) \times GL$$

168 Where:

169 GW = Grain width (mm)

170 GH = Grain height (mm)

171 GL = Grain length (mm)

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

173 **Statistical Analysis**

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

$$178 \quad r_{xy} = \frac{\sum(x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum(x_i - \bar{x})^2 \sum(y_i - \bar{y})^2}}$$

179 where x_i and y_i represent trait values and \bar{x} , \bar{y} their respective means. Principal component
180 analysis (PCA) was performed using XLSTAT (Addinsoft, 2014) to assess multivariate
181 relationships and genotype clustering under salinity stress. Principal Component Analysis (PCA)
182 was conducted to reduce data dimensionality and identify key traits contributing to variation,
183 using eigenvalues > 1 as criteria for significant components. Data were also visualized using bar
184 graphs and biplots. To prepare the research data for future analysis, it was compiled using
185 XLSTAT 2014 and then submitted to principal component analysis (PCA) (Ahmed et al. 2019).
186 Graphs are used to depict the data for the investigated characteristics.

187

188 3. RESULTS

189 Estimation of variability under control and salinity stress conditions and association 190 analysis

191

192 Assessment of salinity tolerance among cultivars for salt tolerance wheat breeding is an integral
193 aspect. For this purpose, 20 wheat genotypes were evaluated. The selected genotypes were
194 screened for tolerance of salt stress using different morphological and physiological parameters.
195 The data for all studied traits under saline conditions among genotypes is presented in Table 2,
196 which showed a significant difference in all traits. Analysis of variance (ANOVA) (Table S1a,
197 S1b) showed highly significant differences among genotype, treatments and their interactions.
198 There is non-significant variation existent among the genotypes for seasons, therefore we used
199 mean data based on averaged over seasons (2021-2022 and 2022-2023) for further analysis. Over
200 all a decreasing trend was observed among parameters with increasing stress applications.
201 Strikingly the genotypes Sadiq-21 (G1), Fakhar-E-Bhkkar (G8) and Nawab-21 (G12) performed
202 quite well under all the salt treatment conditions for all the parameters. While Sutlej-86 (G10),
203 Blue Silver (G17) and Bahawalpur-79 (G18) performed poorly under all salt stress conditions for
204 all the studied attributes (Supplementary Tables S1-S11).

205 Simple linear correlation was performed to figure out the association of all studied indices with
206 yield under control and salt stress conditions based on averages over the seasons (2021-2022 and
207 2022–2023).. All the studied parameters showed positive and significant association with one
208 another under control and stress conditions. From this analysis, it was revealed that YPP was
209 most positively and significantly related to SL (0.966**, 0.960**, 0.974** and 0.962**)
210 followed by TGW (0.964**, 0.865**, 0.846** and 0.710**) and GA (0.923**, 0.733**, 0.848**
211 and 0.705**) under T1, T2, T3 and T4 conditions respectively (Table 3).

212 Principal Component Analysis (PCA)

213

214 There were a total of 10 Principal Components (PCs) in the analysis among which only two were
215 highly significant that cause the variability in data (Table 4). The first two PCs showed 70.49%,
216 60.67%, 60.62% and 51.562% total variation for T1, T2, T3 and T4 stress conditions

217 respectively. The first component had 60.01% variability in control (T1), 50.02% in T2, 40.49%
218 in T3 and 30.75% in T4 stress conditions. This PC had a major contribution from the YPP in T1
219 and T2 stressor while from GA and PH in T3 and T4 stress respectively. The second principal
220 component (PC2) has the 70.49% variability in T1, 60.67% in T2, 60.62% in T3 and 51.56%
221 variability in T4 (Figure 1). The second component has major contribution for variability has
222 from GL in treatment T1 and T2 while from PH and GL in T3 and T4 respectively as displayed
223 in Figure S1-S4. The data presented in Table 5 showed factor loading of all treatment. In the
224 given experiment traits like YPP in T1 and T2, GA in T3 and PH in T4 stress conditions showed
225 maximum variance present in the first PC (Figure 2). In second PC the maximum variance was
226 shown by GL in T1, T2 and T4 while PH in T3 in all treatments. In the first factor or PC, the
227 negative impact on the overall variance of the factor is CC among all treatments. In second factor
228 or PC the negative impact on overall variance was also given by CC. The division for every
229 variable for PC1 and PC2 exhibited the difference of variables for different character studied in
230 control and saline conditions. The biplot was generated between two main factors or PCs. The
231 first two components had majority of variability present in them (Figure 3). The biplot has four
232 main axes; the upper right axes has positive impact on PC1 and PC2 and the genotypes that are
233 situated on that block are best for selection as these have more variation among whole available
234 germplasm. The biplot was constructed between first two PCs and biplot result showed that
235 genotypes Sadiq-21 (G1), Fakhar-E-Bhkkar (G8) and Nawab-21 (G12) were present in positive
236 axes along with traits such as PL, FLA, CC, PH, GA, GL and TGW hence considered as salt
237 tolerant and genotypes Sutlej-86 (G10), Blue Silver (G17) and Bahawalpur-79 (G18) were
238 included in the negative axes hence can be considered as the salt susceptible genotypes while the
239 rest of the genotypes are considered neither tolerant nor susceptible (Figure 4).

240

241 **4. DISCUSSION**

242 Salt stress is one of the major environmental constraints to wheat productivity globally. Keeping
243 in view the highly complex nature of yield itself, identification and selection of yield associated
244 attributes could lead to development of high yielding cultivars under salt stress conditions. Plant
245 height is a crucial agronomic attribute for morphogenesis and crop production in wheat. An ideal
246 plant height is associated with lower rate of lodging, higher grain quantity and quality (Gudi et
247 al. 2023; Kradetskaya et al., 2024). Peduncle length (PL) is the measured length of the internode

248 beneath the spike of wheat, which is an essential attribute to influence photosynthesis
249 effectiveness and pollination success rate, determining grain production (Wang et al. 2023). The
250 growth of spikelet begins in the initial phase of spike formation, and salt stress can hinder this
251 procedure, resulting in less spikelet and eventually shorter spikes. Similar results are also
252 reported by (Dadshani et al. 2019). During periods of salt stress, leaves experience
253 decompression, which results in a decrease in the rate of photosynthesis and ultimately reduces
254 the potential for productivity. In the same way, the increase in salt concentrations plays a role in
255 the decrease in leaf water content, which subsequently leads to a decline in turgor pressure
256 within guard cells. This, in turn, eventually causes leaf area reduction (Masoudi et al. 2015).
257 Chlorophyll content (CC) serves as a crucial indicator for predicting and monitoring crop
258 growth. Its precise measurement on a large scale is vital for calculating crop productivity,
259 managing nutrition, pest control, and other agricultural applications (Gebregziabher et al. 2022;
260 Surendran and Krishnan, 2024,). The salt stress influenced grain length as salt stress increases
261 the grain length decreased. Similar results are also reported by (Zhang et al. 2022). The
262 photosynthetic activity and chlorophyll content reduced under saline stress conditions which
263 limit the availability of assimilates necessary for grain width development that resultantly
264 diminished the grain width (Zheng et al. 2023). Salt stress causes harmful ions like Na and Cl⁻ to
265 accumulate in plant tissues, which reduces grain area. Grain area is inhibited by these ions
266 because they interfere with cellular activities, such as nutrition intake and metabolism (Farooq et
267 al. 2017). Wheat crop under salinity stress spend more nutrients towards response to stress and
268 recovery functions like tissue regeneration and detoxification. This elevated level of metabolism
269 redirect energy from the process of grain filling which produce lighter grain and reduce the
270 thousand grain weight of crop (Mahdy et al. 2022). Additionally, the lowered rate of
271 photosynthesis and the negative effects of ion toxicity further impacts on crop output and
272 ultimately decrease the yield per plant (Adil et al. 2022).

273 The correlation analysis shows that as PH increase in control the CC, FLA, GA, GL, GW, PL,
274 SL, TGW and YPP was also increased. The similar result of correlation was also observed by
275 (Iqra et al. 2020) under normal condition. In saline condition these traits also correlate with shoot
276 length. Similar result in saline condition was also seen by (Gandahi et al. 2020). It showed that if
277 spike length is higher than all other attribute will increase or vice versa. Similar result for
278 association of spike length was presented by (Nezami et al., 2024). Flag leaf area contributed to

279 all the attributes under normal and salt stress. If plant has less leaf area than all the other
280 attributes like chlorophyll content, grain area and spike length well be lower. Likewise, if crop
281 has lower plant height than leaf area will also be reduced. Same results for correlation under salt
282 stress were also seen by (Blum, 2017; Alwahibi et al., 2024). Positive correlation among traits
283 indicates that good amount of chlorophyll content is necessary for the good performance of other
284 traits. If high level of chlorophyll content was present in the crop than good production is given
285 by the crops or vice versa. Same findings were given by (Enghiad et al. 2017; Amzeri et al.,
286 2024) for chlorophyll content. Higher grain length directly contributed to increased grain width
287 and grain area which ultimately increase thousand grain weight and yield per plant. If plant has a
288 good spike length, chlorophyll content and peduncle length than grain length is also affected.
289 Same correlation association was explained by (Omrani et al., 2022). The grain width has
290 positive correlation with grain attributes which increase with increasing grain width or vice
291 versa. The plant that has lager grain width has positive association with yield per plant. Same
292 association was observed by (Ahmed et al. 2021). The association showed that grain area directly
293 contributes to all their traits and increase the yield per plant or vice versa. Similar findings are
294 also given by (Akbarpour et al. 2015). Increase in grain length, area and width increase the
295 thousand grain weight or vice versa. It also showed that CC, FLA and YPP increases thousand
296 grain weight or vice versa. It coincides with the findings of (Moustafa et al. 2021). The all-other
297 trait contributes to the yield per plant. As yield per plant decrease all other parameters will also
298 decrease or vice versa. Same result was proposed by (Hasan et al. 2015). Similarly in another
299 study six wheat genotypes were characterized, measuring traits like plant height, flag leaf area,
300 and grain yield under salt stress. Significant differences were observed, with salt-tolerant
301 genotypes (Pasban-90, Bakhar-02) showing better performance in these parameters compared to
302 sensitive ones (Irshad et al. 2022).

303 With the PCA, large sets of complicated data are divided into smaller sets of simple factors that
304 can be correlated (Ahmed et al. 2019). The PCA was done to find out important traits in both salt
305 and normal conditions. (Ahmed et al. 2019) explained the statistical significance of eigenvalues,
306 which were then used to select the statistically significant principal components (PCs). All traits
307 have positive impacts on variability among first two components except CC. Similar result also
308 reported by (Ahmed et al. 2019). The reason of negative effect of chlorophyll content may be
309 due the deficiency of CC in the cell as salt in cell induces leakage of electrolyte and peroxidase

310 of lipids from thylakoid membrane of chloroplast which leads to loss of chlorophyll content that
311 has negative impact on all other traits. All the major traits have positive impact on second
312 principal component among all salt as well as normal conditions. Similar results are reported by
313 (Guellim et al. 2019). (Mahdy et al. 2022; Saihood et al., 2024) also reported that genotypes with
314 higher PCA-1 and smaller PCA-2 has more yield potential as compared to smaller PCA1 and
315 higher PCA-2. Furthermore, these findings emphasize importance of employing robust statistical
316 and computational methods for reliable crop performance evaluation (Reddy et al., 2023)

317 **5. CONCLUSION**

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

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339 **Authors Contribution**

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

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349 **Statements and Declarations**

350 **Competing interests**

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

352 **Data availability**

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

355

356

357 **Tables**

358 **Table 1** Genotypes name, their allocated code in experiment and pedigree with their major
359 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 | - |

360

361 **Table 2** Descriptive statistics of 20 wheat genotypes under control and salt conditions (8, 12 and
362 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 |

363 PH plant height, PL peduncle length, SL spike length, FLA flag leaf area, CC chlorophyll content, GL grain length,
364 GW grain width, GA grain area, TGW thousand grain weight, YPP yield per plant
365 T1= control, T2= 8dS/m, T3= 12dS/m, T4=16 dS/m

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

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 |

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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.

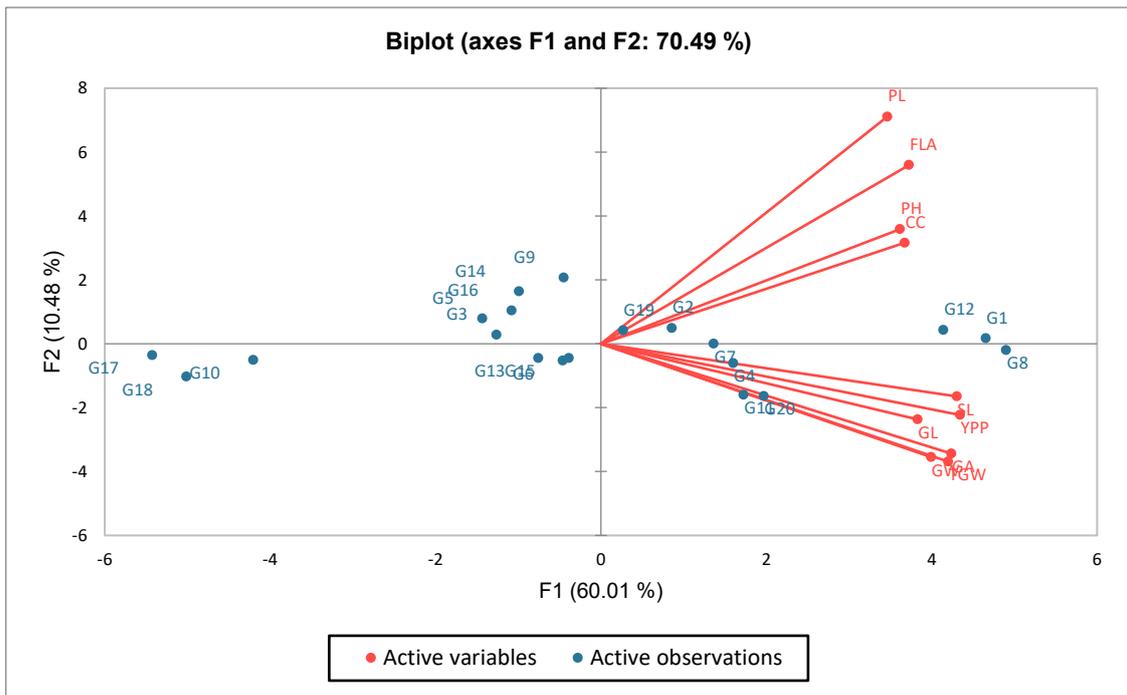


Figure 1: Biplot analysis graph for T1 (control) conditions

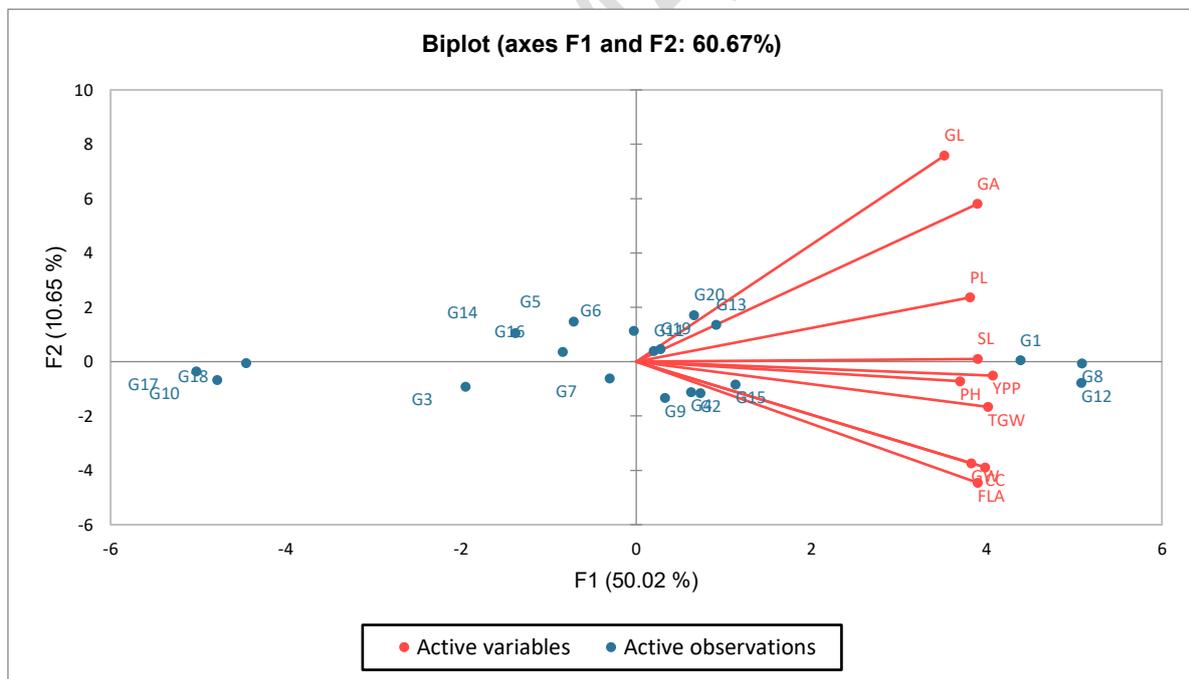


Figure 2: Biplot analysis graph for T2 (8dS/m) conditions

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