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## MORPHO-PHYSIOLOGICAL AND BIOCHEMICAL MARKERS FOR THE SELECTION OF SALT TOLERANT GENOTYPES IN *GOSSYPIUM HIRSUTUM*

M.N. KHALID<sup>1\*</sup>, A. SHAKEEL<sup>1\*</sup>, A. SAEED<sup>1</sup>, and G. MUSTAFA<sup>2</sup>

<sup>1</sup>Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad, Pakistan

<sup>2</sup>Center of Agricultural Biochemistry and Biotechnology, University of Agriculture, Faisalabad, Pakistan

Corresponding authors' emails: noumankhalidpbg@gmail.com, dramirpbg@gmail.com

Email of co-authors: drasifpbg@gmail.com, drmustafa8@gmail.com

### SUMMARY

The latest study investigated the salt tolerance of 55 cotton genotypes at the seedling stage, focusing on morphological, physiological, and biochemical traits at four salinity levels (1.8, 10, 15, and 20 dSm<sup>-1</sup>). Morphological parameters, including root and shoot lengths and weights, were adversely affected by increasing salinity levels. Chlorophyll contents decreased, indicating compromised photosynthetic efficiency. Sodium ion accumulation increased under salt stress, leading to altered ion balance. Biochemical assays highlighted increased activities of antioxidant enzymes, such as superoxide dismutase, peroxidase, elevated hydrogen peroxide levels, and proline content, indicating oxidative stress. The AA-933 showcased exceptional tolerance to salt stress across various levels for fresh and dry root and shoot lengths and weights. Also, NIAB-824 exhibited impressive performance for root-related traits. The resilience of SLH-33 was particularly noteworthy, excelling in morphological features, including fresh and dry root and shoot lengths, especially under the highest level of salinity stress (20 dSm<sup>-1</sup>). Likewise, CRIS-625, Hataf 3, and FH-498 demonstrated robust adaptability by maintaining elevated K<sup>+</sup>/Na<sup>+</sup> ratios. Hataf 3 stood out as a top performer across various physiological and biochemical traits, such as chlorophyll contents, K<sup>+</sup>, K<sup>+</sup>/Na<sup>+</sup>, superoxide dismutase, peroxidase, proline contents, and total antioxidant capacity, highlighting its remarkable salt tolerance. The biplot analysis further substantiated the distinct traits associated with different genotypes, aiding in identifying those with exceptional performance under varying salt-stress levels. This study highlights the importance of understanding salt-stress response in cotton, suggesting that breeding salt-tolerant varieties could improve crop resilience and productivity in challenging environments, promoting sustainable agriculture.

**Keywords:** Salinity, cotton germplasm, screening, oxidative stress, ionic imbalance, salt tolerance

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**Key findings:** Based on morphological markers like root and shoot-related traits, SLH-33 was the top performer under all salinity-stress levels. Hataf 3 was the option as a salt-tolerant genotype based on the physiological (chlorophyll contents) and biochemical markers ( $K^+/Na^+$ , superoxide dismutase, peroxidase, proline content, and total antioxidant capacity).

## INTRODUCTION

Soil salinization is a highly damaging event that can significantly impair the fertility and stability of land (Leogrande and Vitti, 2019). Based on data from 118 countries, which account for 85% of land surface worldwide, an estimation found salt affects over 424 million hectares of topsoil (0–30 cm) and 833 million hectares of subsoil (30–100 cm) (Negacz *et al.*, 2022). Most soils negatively impacted by salt prevail in arid and semiarid regions, which have a higher evaporation rate than precipitation. The deposition of salts within the root zone adversely affected the soil's biological, chemical, and physical properties (Sajid *et al.*, 2022; Shavkiev *et al.*, 2022; Zafar *et al.*, 2022a). Soil salt impairs the metabolic and biochemical processes of plants. When there is an excessive amount of sodium ion ( $Na^+$ ), cytosolic potassium ion ( $K^+$ ) efflux occurs (Abrar *et al.*, 2020). It results in altering the homeostasis of cells, deficiency of nutrients, oxidative stress, retarded growth, and cell death (Kumari *et al.*, 2021).

Previous research investigations have demonstrated that increased salinity significantly impairs plant photosynthesis. Hindering photosynthesis in plants can refer to various factors, including limitations in stomatal activity, such as stomatal closure, as well as non-stomatal factors, including chlorophyll malfunction, deficiency of enzyme proteins, and impairment of the chloroplast ultrastructure (Naik *et al.*, 2019). Salty soils have a higher  $Na^+$  to  $K^+$  ratio, leading to decreased  $K^+$  uptake, cell instability, and decreased enzymatic activity. Reactive oxygen species (ROS) can build up excessively in the cytoplasm, chloroplasts, and mitochondria, resulting from secondary stresses, i.e., oxidative and osmotic stresses and ionic toxicity (Arif *et al.*, 2020). Given their higher oxidative potential, ROS can induce harm to plant tissues, damage DNA, disturb cell

membranes, and degrade proteins, lipids, and photosynthetic pigments (Singh, 2022).

Plants undergo modifications to thrive in salty soil and adapt to their environment. Plants have developed numerous adaptations to flourish in saline soils (Arif *et al.*, 2020). The most significant of these processes are (i) the regulation of hormones (Belmecheri *et al.*, 2019), (ii) the biosynthesis of osmoprotectants and compatible solutes (Widodo *et al.*, 2009), (iii) the transport and uptake of ions (Koźmińska *et al.*, 2019), (iv) the activation of antioxidant (Wang *et al.*, 2020), (v) the synthesis of polyamines, and (vi) the production of nitric oxide (Sikder *et al.*, 2020a).

The cotton plant is a common source of both fiber and oil globally. Cotton can withstand considerable amounts of salt; its salinity threshold is  $7.7 \text{ dSm}^{-1}$ . High salinity levels notably inhibited its growth, and various salts diversely affected cotton's growth (Munawar *et al.*, 2021). High tissue  $K^+/Na^+$  is an essential selection criterion for salinity tolerance in cotton (Maryum *et al.*, 2022; Zafar *et al.*, 2023). Recent studies on the function of specific antioxidants in cotton salt tolerance indicate a correlation between more antioxidants and greater salt tolerance in cotton (Farooq *et al.*, 2022). Cotton's tolerance to salt can be increased through repeated selection cycles, as previously accomplished in alfalfa after nine cycles of recurrent selection (Afzal *et al.*, 2023).

Selection based on the seedling stage can help identify lines with better salt tolerance than the non-selected base population (Munawar *et al.*, 2021). Thus, early growth stage variability in cotton proved to be genetically based, and after only one cycle of selection, probing this variability resulted in enhanced salinity tolerance (Zafar *et al.*, 2022b). This investigation primarily sought to assess the salt tolerance of several genotypes during the seedling stage based on various

morphological, physiological, and biochemical markers.

## MATERIALS AND METHODS

### Collection of cotton germplasm

The current research obtained 55 genotypes of healthy seeds from two reputable institutions, namely, the Central Cotton Research Institute (CCRI) Multan and the Cotton Research Station, Ayub Agriculture Research Institute Faisalabad (CRS, AARI), Faisalabad (Table 1).

### Screening of cotton germplasm at the seedling stage

The cotton germplasm comprised 55 accessions assessed at four different salinity levels ( $T_0$ : 1.8 dSm<sup>-1</sup> as the control,  $T_1$ : 10 dSm<sup>-1</sup> as the lower level,  $T_2$ : 15 dSm<sup>-1</sup> as the

medium, and  $T_3$ : 20 dSm<sup>-1</sup> as the highest level). This experiment commenced in the research area of the Department of Plant Breeding and Genetics, University of Agriculture Faisalabad, Pakistan. For the seedling, sown experiment seeds in polystyrene plastic glass filled with sand transpired in May using a factorial completely randomized design (CRD). Each treatment had three replications maintained. The half-strength Hoagland solution (Malhotra *et al.*, 2014) earlier prepared kept the abovementioned levels of salinity sprinkled over the cups through showers to provide the needed nutrients and to maintain accurate salinity levels. Maintaining the salinity level occurred by continuously testing the electrical conductivity and pH of the half-strength Hoagland solution in the pots. Harvest of seedling experiment materialized after 40 days, with the following traits measured to find out the resistant genotypes:

**Table 1.** Coding 55 cotton genotypes used for screening under salt-stress conditions.

No.	Genotype	Codes	S.No.	Genotype	Codes
1	AA-933	G1	29	IR-901	G29
2	BH-200	G2	30	IUB-2015	G30
3	BH-224	G3	31	IUB-222	G31
4	BH-225	G4	32	MNH-1026	G32
5	BH-310	G5	33	MNH-1027	G33
6	BS-20	G6	34	MNH-1028	G34
7	BS-80	G7	35	MNH-1029	G35
8	CEMB-33	G8	36	MNH-1035	G36
9	CIM-620	G9	37	N-878	G37
10	CIM-622	G10	38	NIAB-1048	G38
11	CIM-625	G11	39	NIAB-820	G39
12	CIM-632	G12	40	NIAB-824	G40
13	CIM-663	G13	41	NIBGE-2	G41
14	CIM-785	G14	42	RH-Afnan-2	G42
15	CKC-03	G15	43	Saim-102	G43
16	CRIS-590	G16	44	SLH-33	G44
17	CRIS-601	G17	45	SLH-51	G45
18	CRIS-625	G18	46	SS-32	G46
19	CRIS-635	G19	47	Tarzan-5	G47
20	Cyto-515	G20	48	Tipo-01	G48
21	Cyto-608	G21	49	VH-330	G49
22	FBS-37	G22	50	VH-339	G50
23	FH-490	G23	51	VH-351	G51
24	FH-492	G24	52	VH-363	G52
25	FH-498	G25	53	VH-367	G53
26	FH-938	G26	54	VH-377	G54
27	Hataf 3	G27	55	VH-418	G55
28	IR-3701	G28			

### **Morphological parameters**

At the end of the experiment, the data recorded on the morphological traits included fresh root length - FRL (cm), fresh shoot length - FSL (cm), fresh root weight - FRW (g), fresh shoot weight - FSW (g), dry root weight - DRW (g), and dry shoot weight - DSW (g).

### **Physiological and biochemical parameters**

The chlorophyll concentration (CCI) measurement used a chlorophyll meter (SPAD 502 Plus) for the SPAD value. A procedure to assess  $K^+$  and  $Na^+$  concentrations in plant leaves followed the method explained by Farooq *et al.* (2018). Evaluating the quantification of  $H_2O_2$  concentration, estimation of proline content (PRO), the activity of catalase (CAT), peroxidase (POD), superoxide dismutase (SOD), and total antioxidant capacity of plant extracts (TAC) employed the procedure described by Munawar *et al.* (2021).

### **Statistical analysis**

The genetic variability of 55 different genotypes, evaluated concerning recorded attributes, used a factorial design analysis of variance with the help of STATISTIX 8.1. Applying the Dunnett's test, heat maps and biplot analysis further ran through the STATISTIX 8.1, the R software, and XLSTAT 2014, respectively.

## **RESULTS**

### **Assessment of genetic variability**

Results of ANOVA revealed all 50 genotypes and treatment showed highly significant outcomes for all the traits under study (Table 2). The treatment  $\times$  genotypes interaction effects were also substantial for all the traits, showing that different levels of salt stress affected the genotypes' performance.

### **Comparative analysis of traits of salt-treated and control seedlings**

Dunnett's test, performed on trait means, examined differences between the control and each of the salt levels (Table 3). Results showed that all parameters under study sustained adverse effects from salt stress compared with the control. Salt stress significantly reduced the FRL, FSL, DRL, DSL, FRW, FSW, DRW, and DSW versus the control. The reduction was severe at  $T_3$ . Drastic declines in the  $K^+$  ions and  $K^+/Na^+$  ratio concentrations were also evident due to salt stress. However, salt stress caused a significant rise in  $Na^+$ , SOD, POD, PRO,  $H_2O_2$ , CAT, and TAC. These differences are distinct in Figure 1.

### **Mean performance of genotypes under different levels of salt stress**

Mean performance of all the genotypes is available through heat maps under  $T_0$  (Figure 2),  $T_1$  (Figure 3),  $T_2$  (Figure 4), and  $T_3$  (Figure 5). For CCI, G1, G9, G14, G12, and G10 performed best at  $T_0$ , while G23, G41, G44, and G53 showed lowest values (Figure 2). For FSL, G34 provided maximum values at  $T_0$ , and G1, G10, G11, G27, G35, and G52 also displayed good performances. G47, G6, G4, and G31 gave minimum values for FSL at  $T_0$ . G1 also performed best for FSW and DSW at control ( $T_0$ ), whereas G47 performed poorly with minimum mean values for these traits. For root-related traits, G40 performed best for FRL, FRW, and DRW, and G18 provided minimum values. For biochemical traits like  $K^+/Na^+$ , POD, SOD, PRO, and CAT, G27 performed well at the control ( $T_0$ ), and it gave the lowest value for  $H_2O_2$ .

The G41 displayed the lowest mean performance for  $K^+/Na^+$ , POD, SOD, PRO, and CAT at  $T_0$ . At  $T_1$  and  $T_2$ , G1, G10, G11, and G27 showed better mean performance for CCI, FSL, FSW, and DSW, whereas G5, G7, and G18 showed the poorest performance for these traits (Figures 3 and 4). For root-related traits

**Table 2.** Mean squares for various morpho-physiological and biochemical traits under salinity stress conditions.

Source	DF	CCI	FSL	FSW	DSW	FRL	FRW	DRW	K <sup>+</sup>	Na <sup>+</sup>	K <sup>+</sup> /Na <sup>+</sup>	POD	SOD	PRO	TAC	H <sub>2</sub> O <sub>2</sub>	CAT
Gen	54	478.20**	86.14**	1.17**	0.08**	84.74**	0.21**	0.04**	2009**	175**	9.07**	46.5**	2.21**	0.19**	138.2**	0.02**	585**
Treatment	3	1580.19**	495.46**	5.82**	0.36**	393.38**	0.98**	0.11**	114525**	183775**	2980.96**	15706**	695.64**	15.9**	39593.4**	17.66**	104424**
Gen×treatment	162	3.33**	1.04**	0.01**	0.00**	0.51**	0.00**	0.00**	23**	14**	4.93**	1.6**	0.09**	0.01**	5.1**	0.00**	11**
Error	440	2.42	0.3	0.00	0.00	0.02	0.00	0.00	16	3	0.29	0.8	0.00	0.00	3.4	0.00	5

\*= significant at 95% probability level ( $\alpha=0.05\%$ ), \*\*= significant at 9% probability level ( $\alpha=0.01\%$ ).

**Table 3.** Comparative analysis of traits of salt-treated and control seedlings by using Dunnett's test.

Treatments	CCI	FSL	FSW	DSW	FRL	FRW	DRW	K <sup>+</sup>	Na <sup>+</sup>	K <sup>+</sup> /Na <sup>+</sup>	POD	SOD	PRO	TAC	H <sub>2</sub> O <sub>2</sub>	CAT
T <sub>0</sub> (Control)	38.49	19.38	1.98	0.49	13.82	0.69	0.23	164.14	17.19	10.07	10.19	1.44	0.31	12.56	0.15	23.60
T <sub>1</sub>	-1.96*	-1.30*	-0.13*	-0.03*	-1.18*	-0.05*	-0.019*	-28.84*	41.03*	-7.73*	4.30*	1.128*	0.358*	16.01*	0.402*	16.07*
T <sub>2</sub>	-4.02*	-2.41*	-0.26*	-0.06*	-2.14*	-0.10*	-0.035*	-47.15*	62.83*	-8.60*	9.01*	2.342*	0.536*	27.22*	0.616*	31.19*
T <sub>3</sub>	-7.24*	-4.08*	-0.44*	-0.11*	-3.65*	-0.18*	-0.060*	-60.96*	76.34*	-8.96*	22.51*	4.790*	0.729*	35.87*	0.748*	59.14*

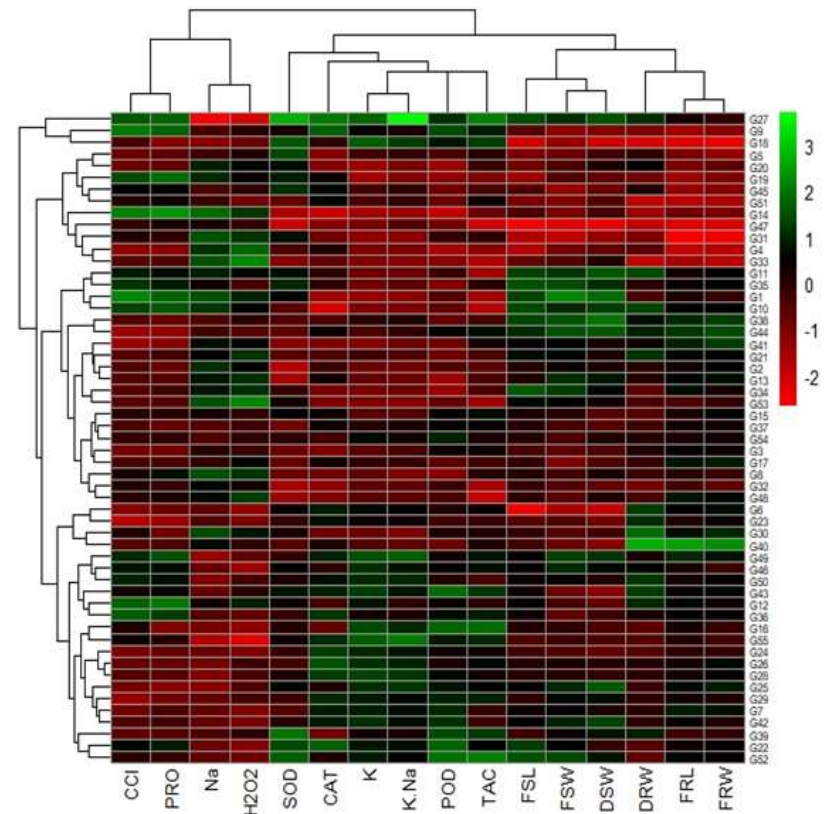
\*= significant at 95% probability level ( $\alpha=0.05\%$ ), \*\*= significant at 9% probability level ( $\alpha=0.01\%$ ).

**Table 4.** Principle components representing eigenvalues and variability of different levels of salt-stress conditions.

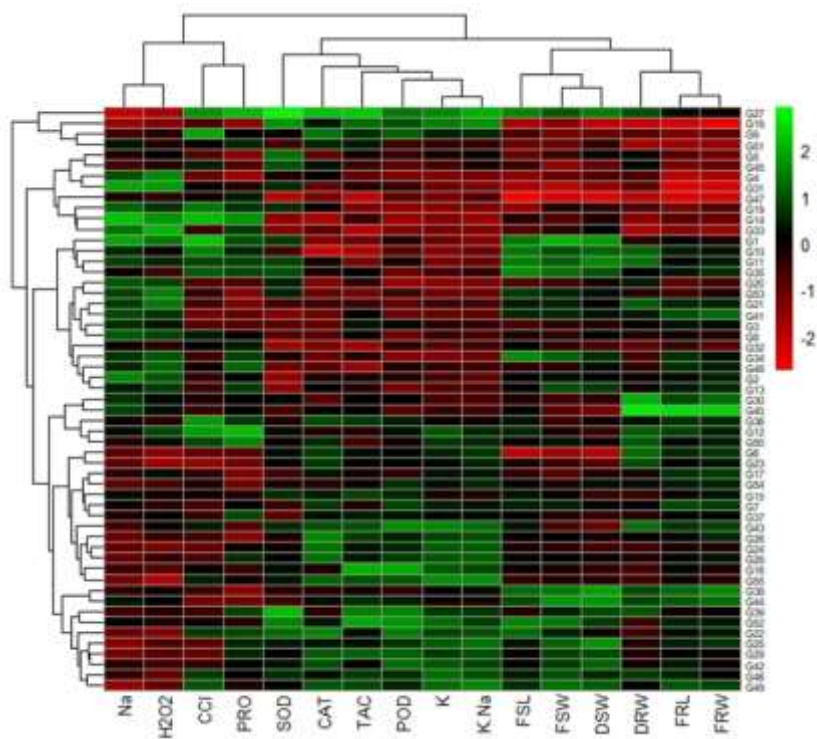
	Treatments	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11	PC12	PC13	PC14	PC15	PC16
Eigenvalue	T <sub>0</sub>	5.97	3.78	2.22	1.15	0.85	0.53	0.40	0.33	0.23	0.17	0.11	0.08	0.05	0.04	0.03	0.014
	T <sub>1</sub>	6.13	3.85	1.87	1.12	0.86	0.55	0.38	0.35	0.28	0.22	0.16	0.10	0.07	0.04	0.02	0.002
	T <sub>2</sub>	5.98	3.82	1.99	1.10	1.01	0.74	0.33	0.29	0.22	0.17	0.12	0.10	0.07	0.04	0.02	0.002
	T <sub>3</sub>	6.14	3.65	1.98	1.34	0.89	0.72	0.27	0.25	0.23	0.18	0.14	0.10	0.06	0.03	0.02	0.001
Variability (%)	T <sub>0</sub>	37.34	23.64	13.92	7.24	5.37	3.35	2.54	2.10	1.46	1.05	0.66	0.50	0.32	0.26	0.17	0.085
	T <sub>1</sub>	38.33	24.06	11.66	6.97	5.38	3.44	2.36	2.17	1.73	1.35	1.03	0.65	0.47	0.27	0.11	0.009
	T <sub>2</sub>	37.37	23.89	12.46	6.86	6.34	4.63	2.05	1.80	1.38	1.05	0.76	0.63	0.41	0.24	0.13	0.010
	T <sub>3</sub>	38.39	22.79	12.40	8.38	5.54	4.50	1.72	1.57	1.44	1.10	0.84	0.63	0.36	0.20	0.14	0.008
Cumulative %	T <sub>0</sub>	37.34	60.98	74.91	82.15	87.52	90.87	93.41	95.51	96.97	98.01	98.67	99.17	99.49	99.75	99.92	100
	T <sub>1</sub>	38.33	62.40	74.06	81.03	86.41	89.85	92.21	94.38	96.12	97.47	98.50	99.14	99.61	99.88	99.99	100
	T <sub>2</sub>	37.37	61.26	73.72	80.58	86.92	91.55	93.59	95.39	96.77	97.82	98.58	99.21	99.62	99.86	99.99	100
	T <sub>3</sub>	38.39	61.17	73.57	81.95	87.49	91.99	93.71	95.28	96.71	97.82	98.66	99.29	99.65	99.85	99.99	100



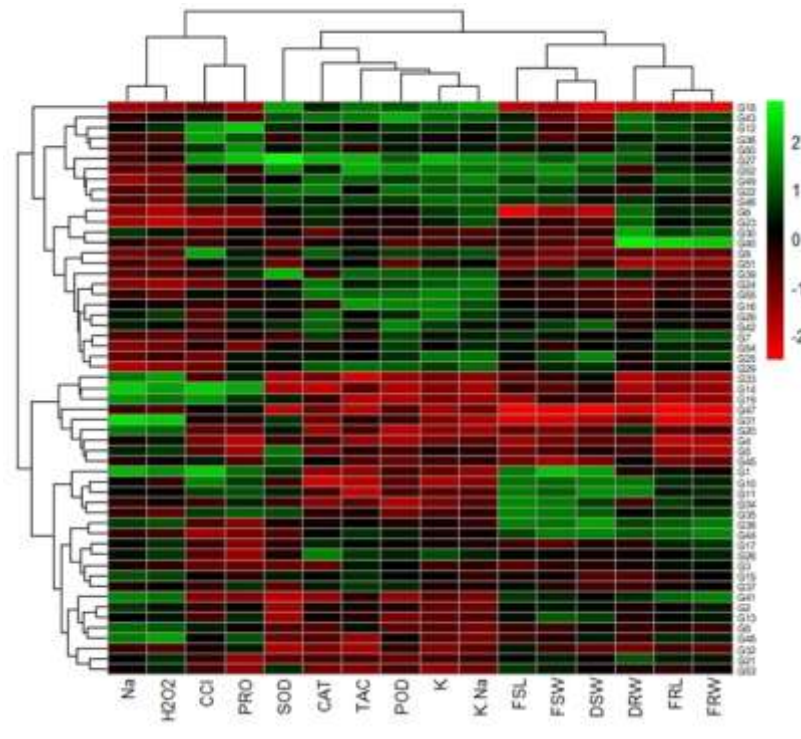
**Figure 1.** Box and whisker plots representing effects of various levels of salt stress on morpho-physiological and biochemical traits.



**Figure 2.** Heat map analysis of 55 cotton genotypes at T<sub>0</sub> (control).

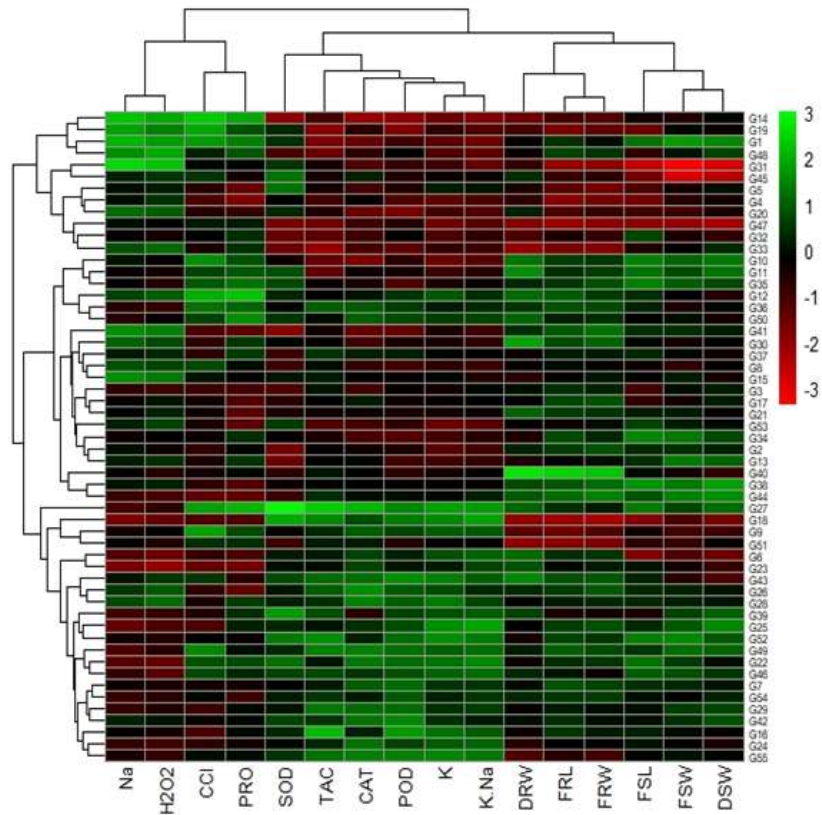


**Figure 3.** Heat map analysis of 55 cotton genotypes at  $T_1$  ( $10 \text{ dSm}^{-1}$ ).



**Figure 4.** Heat map analysis of 55 cotton genotypes at  $T_2$  ( $15 \text{ dSm}^{-1}$ ).





**Figure 5.** Heat map analysis of 55 cotton genotypes at  $T_3$  ( $20 \text{ dSm}^{-1}$ ).

like FRL, FRW, and DRW, G40, G30, and G38 showed maximum mean values at  $T_1$ . G48, G31, G18, G9, and G4 displayed the poorest mean performance for both shoot and root-related traits. For  $K^+/Na^+$ , G27 exhibited highest values, followed by G55 and G18, while G14, G31, G33, and G20 disclosed lowest values at  $T_2$ . G16 showed a high concentration of POD at  $T_1$  and  $T_2$ , followed by G52 and G44. For  $H_2O_2$ , G33 showed the highest concentration, followed by G53, G31, G14, and G21 at  $T_1$ . For CCI, genotype G14 showed a maximum mean value, followed by G12 and G19 at  $T_3$ , whereas minimum values were evident in G18, G23, and G44. G1 performed best among all genotypes for most traits, i.e., FSL, FSW, and DSW, for the control and all three levels of salinity stress.

The G40 showed maximum mean values for FRL, FRW, and DRW under all treatments. G44 also performed best for all the morphological traits under study, viz., FSL,

FSW, DSW, FRL, and FRW at  $T_3$  (Figure 5). For  $K^+/Na^+$  ratio, G18, G27, and G25 gave maximum mean values, while minimum ratios appeared in G14 and G31 under all levels of salinity stress. G14 also performed poorly for SOD and POD. Among all 55 genotypes, G27 showed maximum mean values for all biochemical traits under study, such as POD, SOD, PRO, and TAC, except for  $H_2O_2$  at  $T_3$ .

### Biplot analysis

A biplot analysis helped investigate the interrelationships between the attributes under study to identify any redundancies in the measured traits and visually represent the genotype profiles of these traits. At control ( $T_0$ ), PC1 explained 37.34% of the total variation, 23.63% with PC2, and 13.92% with PC3 (Table 4). Out of 16 PCs, four PCs showed Eigenvalues greater than one. At  $T_1$ , four PCs exhibited 81.03% cumulative variability,



having Eigenvalues greater than one, while five PCs showed 86.92% cumulative variability at T<sub>2</sub>. At T<sub>3</sub>, PC1 exhibited 38.38% total variation, followed by PC2 (22.78%) and PC3 (12.39%). PC1 had positive association with FSL, FSW, DSW, FRL, FRW, DRW, K<sup>+</sup>, K<sup>+</sup>/Na<sup>+</sup>, POD, SOD, TAC, and CAT, while negative with CCI, Na<sup>+</sup>, and H<sub>2</sub>O<sub>2</sub> under all levels of stress.

PC2's positive association comprised CCI, FSL, FSW, DSW, FRL, FRW, DRW, Na<sup>+</sup>, PRO, and H<sub>2</sub>O<sub>2</sub>, while negative with K<sup>+</sup>, K<sup>+</sup>/Na<sup>+</sup>, POD, SOD, TAC, and CAT at all salinity levels. The distance between the genotype and biplot origin represented the vector length of a genotype, and this distance measures the distinctive characteristics of the genotype. In this study, genotypes G1, G10, G11, G14, G18, G27, G33, and G47 had longer distances from the origin, which showed that these genotypes had high variability for all the traits under study at control and salinity-stress conditions. At control, G38 and G44 were closer to the DSW, FSW, FRW, FSL, FRL, and DRW, while G16, G43, G28, and G55 were closer to the SOD, POD, TAC, CAT, K<sup>+</sup>, and K<sup>+</sup>/Na<sup>+</sup> (Figure 6). At all three levels of stress, G12, G38, and G44 were nearer to the DSW, FSW, FRW, FSL, FRL, and DRW, and G16, G22, G26, G27, G28, and G43 were closer to the SOD, POD, TAC, CAT, K<sup>+</sup>, and K<sup>+</sup>/Na<sup>+</sup> (Figures 7, 8, and 9).

## DISCUSSION

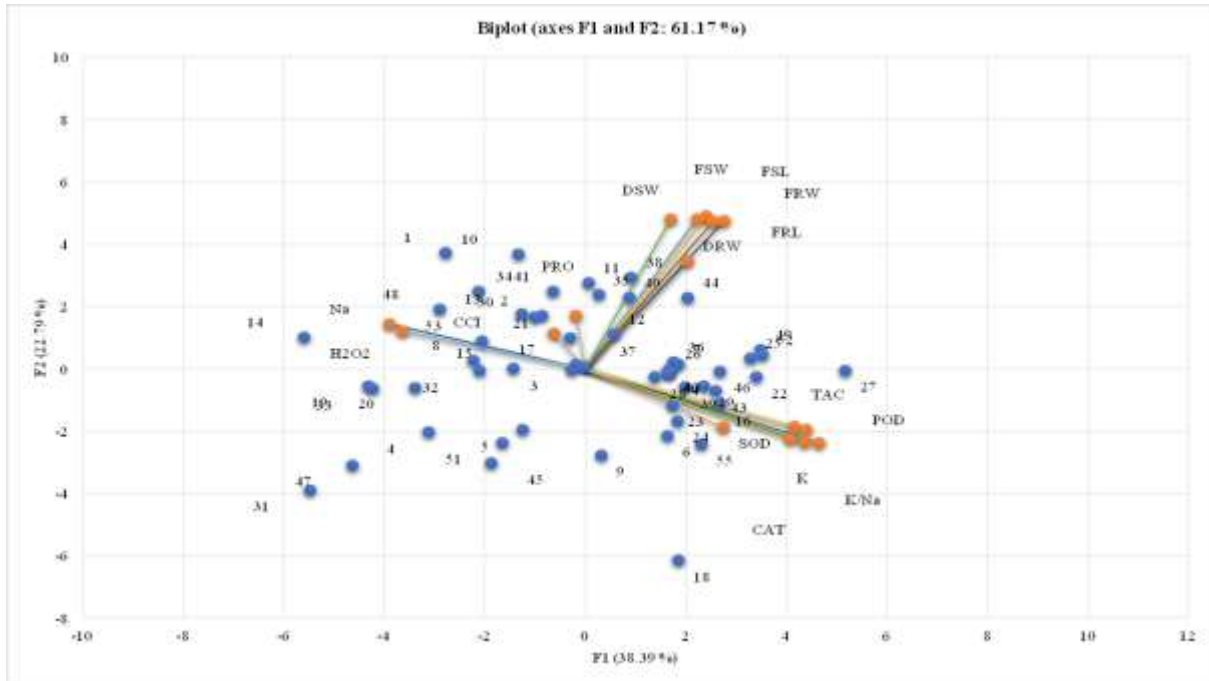
Salt stress is a major global food security challenge, affecting plant growth, development, and crop establishment. It can cause ionic toxicity, imbalance in nutrient uptake, and water stress, leading to reduced crop yield and compromised fiber quality (Zafar *et al.*, 2023). Screening methods for soil salinity tolerance are complex, but pot culture can efficiently evaluate cotton germplasm. Cultivating salt-tolerant cotton cultivars could mitigate salt stress effects in cotton farming, providing a sustainable and economically viable solution (Sharif *et al.*, 2019). The presented study screened out 55 cotton genotypes at three different salinity levels at the seedling stage. The presence of genetic variability is crucial in selecting genotypes under salinity.

Results of two-way ANOVA indicated variations among the various genotypes and revealed that salt stress had significantly affected all traits under observation.

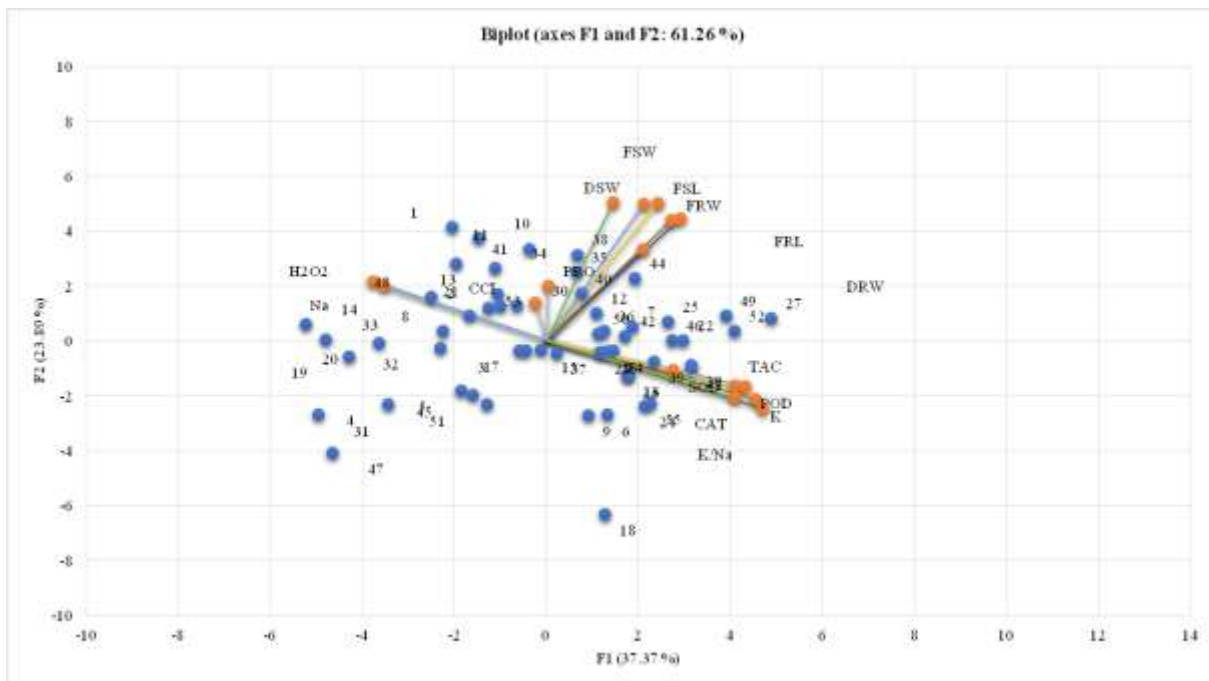
Various physiological traits, such as the chlorophyll concentration, have proven to be effective selection criteria due to their susceptibility to degradation under salt stress. This degradation ultimately results in a decrease in the rate of photosynthesis and overall plant growth. The concentration of chlorophyll has proven to have a positive correlation with the rate of photosynthesis, as well as with dry matter production and yield (Hussien *et al.*, 2019). This relationship suggests that higher levels of chlorophyll can contribute to increased photosynthetic activity, resulting in better plant growth and productivity. The genotypes that exhibited tolerance to salt stress demonstrated higher chlorophyll content levels than the lines that were susceptible to salt stress (Naik *et al.*, 2019). In the current experiment G9, G12, G14, G19 and G27 exhibited highest values for CCI under T<sub>3</sub> while G4, G18, G23, G25, G41 and G44 exhibited lowest values.

Assessing root and shoot-associated traits is critical in identifying salt-tolerant cotton genotypes (Sikder *et al.*, 2020b; Zafar *et al.*, 2022a). These traits have been successfully used as selection criteria in various field crops (Farooq *et al.*, 2022). In this experiment, salt stress significantly affected FRL and FSL. Genotype G38 showed maximum FSL, followed by G34, G35, G10, G27, and G11 at the highest level of salinity under study (T<sub>3</sub>). Genotypes G40, G44, G49, and G12 showed the highest FRL at T<sub>3</sub> among all genotypes.

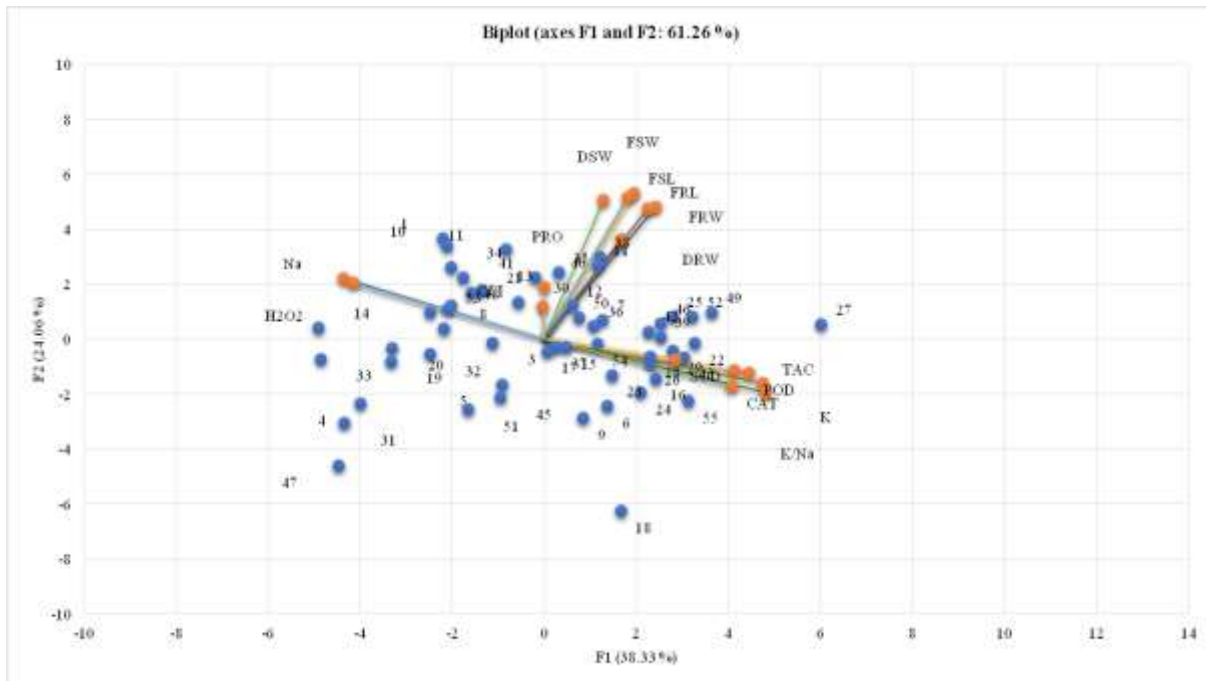
Our experiment also revealed a significant drop in both fresh and dry weights. Genotypes G3, G13, G17, G39, and G48 showed the lowest reduction in FSW and DSW at T<sub>3</sub>. Meanwhile, three genotypes, G36, G43, and G54, showed the lowest decrease for FRW and DRW. The presented study demonstrates that assessing plant biomass can be an applicable approach for gauging salt-stress tolerance in cotton. Compared with tolerant genotypes, the results showed that sensitive genotypes had a more profound biomass reduction (Munawar *et al.*, 2021). It implies



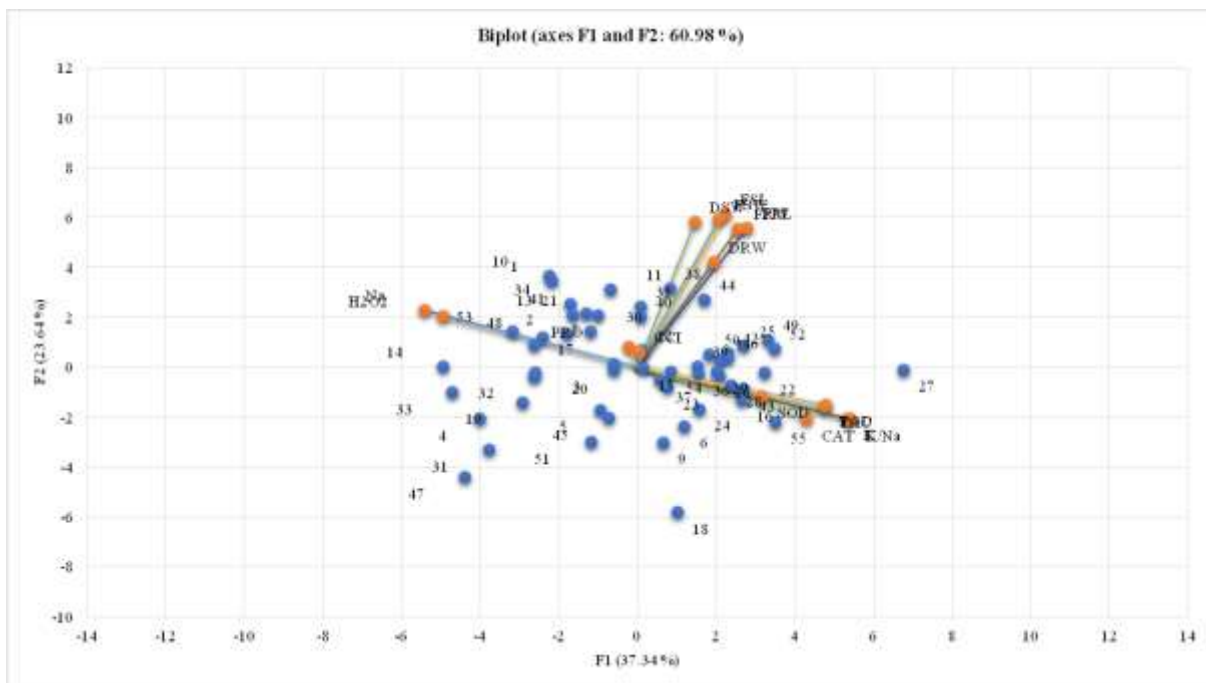
**Figure 6.** Biplot graph for T<sub>0</sub> (control) of 55 cotton genotypes for various morphological, physiological, and biochemical traits under study.



**Figure 7.** Biplot graph for T<sub>1</sub> (10 dSm<sup>-1</sup>) of 55 cotton genotypes for various morphological, physiological, and biochemical traits under study.



**Figure 8.** Biplot graph for  $T_2$  ( $15 \text{ dSm}^{-1}$ ) of 55 cotton genotypes for various morphological, physiological, and biochemical traits under study.



**Figure 9.** Biplot graph for  $T_3$  ( $20 \text{ dSm}^{-1}$ ) of 55 cotton genotypes for various morphological, physiological, and biochemical traits under study.

that measuring plant biomass can effectively differentiate between cotton genotypes with varying degrees of salt-stress tolerance.

The soil's high salt content significantly inhibits plant growth via three major mechanisms. Firstly, it generates osmotic stress, which limits plant water availability. Furthermore, high concentrations of Na<sup>+</sup> and Cl<sup>-</sup> ions in the soil can cause specific ion toxicity, which is detrimental to plants (Arif *et al.*, 2020). Lastly, elevating Na<sup>+</sup> and Cl<sup>-</sup> concentrations in soil can disturb nutrient ion balance and reduce the absorption of essential nutrients like potassium (K<sup>+</sup>), nitrate (NO<sup>-</sup>), and phosphate (PO<sub>4</sub><sup>3-</sup>). All these processes work together to prevent plant growth in salty soils. According to several studies, when plants experience salt stress, the concentrations of inorganic ions fluctuate (Choudhary *et al.*, 2023). Numerous studies have reported various responses, including increases in Na<sup>+</sup> and Cl<sup>-</sup> ion concentrations and decreases in Mg<sup>2+</sup>, K<sup>+</sup>, and Ca<sup>2+</sup> ion levels (Keya *et al.*, 2023). The pertinent investigation showed that the concentration of Na<sup>+</sup> ions rose significantly as the amount of salt stress increased. At highest levels of salt stress (T<sub>3</sub>), G1, G14, G19, and G31 showed maximum concentrations of Na<sup>+</sup> ions, whereas, G6, G18, G22, G23, and G25 showed lowest values.

A study found that Na<sup>+</sup> accumulation in plants harms various physiological processes, ultimately leading to lethal consequences (Abrar *et al.*, 2020). Additionally, this accumulation has linkages that decrease the available water in plants. A significant rise in the Na<sup>+</sup> and Cl<sup>-</sup> ions was apparent when the K<sup>+</sup>/Na<sup>+</sup> ratio decreased in cotton leaves. Salt-tolerant plants can regulate the entry of Na<sup>+</sup> ions through their root systems, ensuring survival in saline environments. Conversely, the plants lacking the capacity to uphold Na<sup>+</sup> homeostasis emerged as salt-susceptible. Numerous investigations have accentuated that maintaining an appropriate K<sup>+</sup>/Na<sup>+</sup> ratio, rather than excluding Na<sup>+</sup> ions, is critical in determining plant performance under salt-stress conditions (Basu *et al.*, 2021). Genotypes, G6, G18, G22, G24, G25, G27, G46, G49, G52, and G55 showed highest values for K<sup>+</sup>/Na<sup>+</sup> ions at T<sub>3</sub>.

Antioxidant activity is considerably a potential marker of plant salt tolerance. It is a fact that plants produce ROS in response to salt stress (Arif *et al.*, 2020). In this study, genotypes G1, G14, G31, G41, and G48 exhibited highest values of H<sub>2</sub>O<sub>2</sub>, whereas lowest values were evident in G6, G18, G22, G23, and G46 at 20 ds/m stress level. Under normal circumstances, intracellular antioxidants effectively neutralize ROS. However, when subjected to salt stress, an overabundance of ROS occurs, which leads to oxidative stress. This oxidative stress severely disrupts normal metabolic processes, breaking down proteins and the occurrence of mutations in nucleic acids (Singh, 2022).

Plants have two types of antioxidant systems: enzymatic and non-enzymatic, with enzymatic antioxidants like SOD, POD, and CAT regulating oxidative stress and maintaining cellular redox balance. SOD is the first line of defense against ROS by converting superoxide radicals into H<sub>2</sub>O<sub>2</sub>, which POD and CAT further detoxify (Wang *et al.*, 2020). This enzymatic network is necessary for keeping the equilibrium between oxygen radicals and H<sub>2</sub>O<sub>2</sub> within cells, making it an essential enzyme with potent antioxidant properties (Yu *et al.*, 2020). In the latest investigation, a significant increase in SOD concentrations was available in G2, G13, G32, G47, and G48 under the highest level of salt stress (20 dSm<sup>-1</sup>). A significant rise in POD concentration appeared in G4, G8, G13, G14, and G34 under 20 dSm<sup>-1</sup> salt stress. Noteworthy increases under 20 dSm<sup>-1</sup> of salt stress emerged in G1, G10, G14, G32, and G48 for CAT activity. A remarkable increase in proline activity was evident in G16, G25, G28, G30, and G37 at 20 dSm<sup>-1</sup> of salt stress compared with the control.

## CONCLUSIONS

Hence, the comprehensive assessment of genetic variability and trait responses under varying levels of salt stress in cotton genotypes has endowed valuable insights into the plant's ability to cope with salinity challenges. The substantial impact of salt stress on morphological, physiological, and biochemical

traits underscores the need to identify genotypes with enhanced salt tolerance. Among the genotypes studied, specific genotypes exhibited remarkable performance across different traits under salinity stress. The study identified genotypes with notable salt tolerance, such as G44, which excelled in multiple features, including root and shoot lengths. Genotype G27 demonstrated sizeable adaptability in maintaining a favorable  $K^+/Na^+$  ratio. These findings collectively contribute to understanding cotton's response to salt stress and can pave the way for future strategies to improve cotton productivity in saline environments. The selection of specific genotypes based on their performance in different traits under salinity stress holds potential for targeted breeding efforts to develop salt-tolerant cotton varieties, which can thrive in challenging soil conditions.

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