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REGRESSION AND CORRELATION ANALYSIS OF MORPHOLOGICAL TRAITS IN COTTON (*GOSSYPIUM HIRSUTUM* L.) UNDER DIFFERENT SALINITY LEVELS

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SUMMARY

Salinity is one of the major abiotic stresses that considerably and adversely affects cotton growth, development, and seed cotton yield. The relevant study aimed to analyze regression and correlation among the morphological traits of 12 cotton (*Gossypium hirsutum* L.) cultivars exposed to different concentrations of sodium chloride (NaCl). Four levels of NaCl stress (0, 100, 150, and 200 mM) were successful in their application under controlled conditions. The results revealed different levels of salt stress conditions negatively influence cotton traits, particularly the fresh plant weight and shoot length, which showed a decrease of about 50% and 29%, respectively. The dry root weight and root length demonstrated a high degree of stability under salt stress conditions. According to the correlation analysis, plant length and root length appeared as positively correlated with other traits under different mM of NaCl stress conditions. These findings provide useful insights for identifying salt-tolerant cotton genotypes through marker-assisted selection and physiological screening.

Keywords: Correlation, salt stress, ions, SNP markers, SSR, cotton cultivar

Key findings: The regression value of plant length against other cotton (*G. hirsutum* L.) traits, as calculated, enabled the probabilistic estimation of those traits. The considerable positive correlations were evident among the assessed phenotypic traits under different salt stress conditions. The study identified the most susceptible and tolerant cotton traits.

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INTRODUCTION

Soil salinization, high temperatures, and water deficit conditions lead to extensive functional disorders and deterioration of photosynthesis in crop plants (Shavkiev *et al.,* 2020; Khusenov *et al.,* 2025). Soil salinization is one of the harmful abiotic stress factors; it adversely affects the growth and development of different crops for harvest globally. Saline degradation is steadily increasing and causing critical environmental problems (Ahammed *et al.,* 2018; Panta *et al.,* 2014). Past reports indicated more than 6% of the world's total arable area, or approximately 800 million hectares, suffers from significant salt stress conditions (Setia *et al.,* 2013).

The steady rise in population is driving an increased demand for cotton production. *Gossypium hirsutum* L. is one of the most widely cultivated cotton species worldwide and serves as an important source of both fiber and oilseed. At present, the species *G. hirsutum* L. (90%) (Campbell *et al.,* 2010) and *G. barbadense* (8%) (Wang *et al*., 2015) dominate the global cotton production. The early germination stage of cotton is particularly vulnerable to soil salinity, and the young seedling exhibited more sensitivity than mature plants (Gilland*,* 2002; Shaheen *et al.,* 2012; Tiwari *et al.,* 2013; Normamatov *et al.,* 2023).

With soil salinity levels of 15–20 dS/m^{-1} (10 mM NaCl solution per 1.0 dS/m^{-1}), these delay seed germination and growth of young seedlings relative to control plants (Saqib *et al.,* 2002; Zhang *et al.,* 2012), which considerably declined plant yield (Saranga *et al.,* 2004). In general, salinity stress negatively affects all stages of plant growth and development, from seed germination to crop maturity. Past findings revealed how salt stress inhibited the vegetative growth and development and, eventually, the fiber yield in cotton (Dong, 2012). Increased NaCl stress during the cotton's early phase caused a decline of Ca^{2+}, K^{+}, Mg^{2+}, P_3, and Mn^{4+} ions in roots and young leaves, resulting in a disruption of the plant's nutritional balance (Wang *et al.,* 2011).

For assessing salt tolerance in cotton plants at the germination stage, it is possible to evaluate the seed traits by studying germination potential, germination rate, germination index, and fresh biomass (Guo-Wei *et al.,* 2011). Soil salinity negatively alters cotton phenotypic traits, including plant length, fresh and dry plant weights, root length, leaf area, chlorophyll content, photosynthesis, and transpiration rate (Loka *et al.,* 2011). Although cotton is a moderately salt-tolerant crop as compared to other agricultural crops, the crop's tolerance is relatively low and varies based on plant growth and development stages and environmental conditions. Several studies have reported the effects of salt stress on the germination, vegetative development, and seed cotton yield (Guo *et al.,* 2012; Ahmads *et al.,* 2002; Umedova *et al.,* 2024).

Correlation analysis revealed the relationship between various traits, and multivariate analysis allows for precise measurement of similarities and differences between the traits (Kakar *et al.,* 2019). The following study aimed to determine the most susceptible and tolerant cotton traits to salt stress conditions. Moreover, this research sought to study the relationship of plant length with other growth traits. These are shoot length, root length, fresh plant weight, fresh shoot weight, fresh root weight, dry plant weight, dry shoot weight, and dry root weight. Likewise, it partitioned the observed phenotypic correlations under different NaCl stress conditions.

MATERIALS AND METHODS

Plant material and experimental design

The presented experiment on cotton (*G. hirsutum* L.) commenced during 2024–2025 in laboratory conditions at the Center of Genomics and Bioinformatics, Academy of Sciences, Uzbekistan. Twelve upland cotton cultivars, including Ravnaq-1, Ravnaq-2, L-2022, GenBio-4, Zangi Ota, Baraka, Guliston, Hapicala-19, Ishonch, An-Boyovut-2, C-8290, and C-8296, received evaluation for phenotypic traits under different NaCl stress conditions.

The research took place under controlled greenhouse conditions, with a day/night temperature of 30 °C±5 °C/25 °C±5 °C and a photoperiod of light 14 h/dark 10 h. The cotton cultivars' seeds entailed planting in the sterilized soil mixture (soil 60% and sand 40%) in plastic pots (Normamatov *et al.,* 2023).

Salinity treatments

One control and three NaCl stress levels were treatments applied, namely, 0 mM NaCl (control), 100, 150, and 200 mM NaCl. Salt stress treatment began at the young cotton seedling stage on the 21st day of the growth. Plant irrigation employed the respective NaCl solutions every three days, maintaining soil moisture close to 70% of the field capacity.

Measured traits

After 35 days of salinity treatment, the harvested plants underwent evaluation for the following morphological traits: plant length (PL, cm), shoot length (SL, cm), root length (RL, cm), fresh plant weight (FPW, g), fresh shoot weight (FSW, g), fresh root weight (FRW, g), dry plant weight (DPW, g), dry shoot weight (DSW, g), and dry root weight (DRW, g). Fresh plant weight measurement used the digital analytical balance (FA-2204), while plant dry weight determination proceeded after oven-drying samples at 80 °C for 48 h (Shaheen *et al.,* 2012).

Statistical analysis

Data analysis performed engaged STATA17 and OriginPro-2024 software, applying the analysis of variance (ANOVA) to assess the effects of NaCl stress levels. Meanwhile, correlation matrix visualization used a scatter matrix plot to identify relationships among the morphological parameters under different NaCl stress conditions. The correlation strength assessment was by the r value, dividing into three categories, namely, a strong positive relationship ($r > 0.7$), a moderate relationship ($r = 0.3–0.7$), and a weak relationship ($r < 0.3$).

RESULTS AND DISCUSSION

The soil's highest salt concentration critically affects agricultural crops, viz., a delay in seed germination rate, crop growth, development, and reproduction; an inhibition of enzymatic activities; and a considerable decrease in the process of photosynthesis. Therefore, it is crucial to determine the morphological and physiological responses of specific crops to soil salinity stress before attempting to introduce genetic and environmental factors to alleviate salt stress conditions. In general, salt stress induces an imbalance of cellular ions, resulting in ion toxicity and osmotic stress in plant cells. Plant growth-regulating compounds, including nitric oxide and melatonin, have attracted considerable attention due to their potential to reduce salinity stress in cotton cultivars (Akmal *et al.,* 2023). Hence, reduced photosynthetic efficiency adversely impacts cellular metabolic processes, leading to abnormal plant development (Zhang *et al.*, 2016).

In the presented study, using the bootstrap method helped analyze each cotton (*G. hirsutum* L.) trait with an observed mean value and standard error (Table 1). The nine cotton plant traits included plant length, shoot length, root length, fresh plant weight, fresh shoot weight, fresh root weight, dry plant weight, dry shoot weight, and dry root weight. These sustained analyses were under four different environmental conditions comprising optimal and three salinity levels. Based on the results, the salinity levels significantly influence most phenotypic traits of cotton at the early growing stage. The cotton traits fresh shoot weight and fresh plant weight showed the highest variability under 200 mM NaCl conditions with considerable sensitivity to salinity, with more than 50% decrease in mean values (51.1% and 49.5%, respectively). These viable cotton traits can be useful as biological stress indicators in cotton crops. Despite the highest salt stress, the trait dry root weight did not undergo significant fluctuations and showed a 0% reduction in mean value, maintaining its absolute value at 0.02 at the NaCl stress levels. This indicates the highest level of genetic stability and tolerance to salinity in this trait. Shader *et al.*

Table 1. Analysis of mean values based on the bootstrap method for cotton traits assessed under various NaCl-stress conditions.

Traits	Optimal		100 mM NaCl			150 mM NaCl			200 mM NaCl		
	Observed mean	Bootstrap std. err.	Observed mean (g)	Decreasing (%)	Bootstrap std. err	Observed mean (g)	Decreasing (%)	Bootstrap std. err.	Observed mean (g)	Decreasing (%)	Bootstrap std. err.
PL	26.49	0.32	23.76	10.2	0.36	22.40	17.6	0.32	21.38	19.3	0.30
SL	16.01	0.23	13.49	15.6	0.25	12.34	23.1	0.20	11.33	29.2	0.18
RL	10.48	0.13	10.26	2.1	0.15	10.05	4.1	0.15	10.05	1.2	0.16
FPW	2.12	0.03	1.63	23.1	0.03	1.40	33.0	0.03	1.07	49.5	0.02
FSW	1.82	0.02	1.36	25.2	0.02	1.16	36.20	0.02	0.89	51.1	0.02
FRW	0.29	0.01	0.27	6.9	0.01	0.24	17.20	0.01	0.18	37.9	0.00
DPW	0.23	0.01	0.19	17.4	0.01	0.17	26.10	0.01	0.15	34.8	0.00
DSW	0.21	0.00	0.17	19.0	0.00	0.15	28.60	0.00	0.13	38.1	0.00
DRW	0.02	0.00	0.02	-	0.00	0.02	-	0.00	0.02	-	0.00

PL - plant length (cm), SL - shoot length (cm), RL - root length (cm), FPW - fresh plant weight (g), FSW - fresh shoot weight (g), FRW - fresh root weight (g), DPW - dry plant weight (g), DSW - dry shoot weight (g), and DRW - dry root weight (g).

(2023) reported that 100 and 200 mM NaCl stress negatively influence the morphological traits of cotton, resulting in reduced seed cotton yield.

The results revealed cotton phenotypic traits responded differently to salinity stress conditions. In particular, a 200 mM NaCl concentration caused the maximum negative effect on phenotypic traits of almost all cotton cultivars studied, especially the traits fresh plant weight (with a 50% decrease) and shoot length (with a 29% decrease). However, the cotton traits, such as dry root weight and root length, demonstrated the highest degree of stability relative to the various NaCl stress conditions. The results suggested these traits could play a crucial role in the cotton plant’s mechanisms for maintaining vital functions under salt stress conditions (Table 1). Treatment with 150 mM NaCl concentration resulted in statistically significant differences among cotton genotypes at the early seedling phase (Du *et al.,* 2018). Assessing diversity within cotton cultivars is essential for cotton breeding by marker-assisted selection (MAS) programs and for the effective conservation of plant genetic resources (Mirzakamol *et al.,* 2023).

The multivariate approach allows better analysis of the studied genetic diversity traits in different crop plants (Rezai and Frey, 1990; Malaquias *et al.,* 2017; Ullah *et al*., 2024). An analysis of variance of the regression model of plant length on the other eight traits in cotton cultivars under optimal and NaCl stress conditions appears in Table 2. The analysis evaluates how well the regression model fits. In this case, the F value (3.66123E30) and p-value ($P < 0.0001$) under optimal conditions revealed the selected variables emerged as almost closely related to plant length.

Table 2 shows the results of the ANOVA regression model applied to the trait of plant length under 100 mM NaCl (mild salt stress condition). The regression model value (SS = 14 825.54003) was slightly larger than the optimal (SS = 13 174), indicating some variation occurred among the traits under such salt stress conditions. According to the regression analysis results under 150 mM NaCl stress, the regression model value (SS = 12 487.11838) was slightly lower than the optimal, and 100 mM NaCl, which enunciated a moderate salt stress, limits the growth of plant length. The traits’ coefficient of variation under the optimal environment was higher, which was the same at 100 mM NaCl. However, it decreased at 150 mM NaCl, indicating that the traits’ growth and development lowered as the salt stress level increased, but the correlations among the traits were high. The observed traits’ reduction could refer to osmotic damage or toxicity caused by excessive ion uptake (Meloni *et al*., 2001).

Table 2. ANOVA results for the regression model of cotton traits under optimal and salt-stress conditions.

Salt-stress Conditions	Trait	Source of variation	d.f.	Sum of square	Mean square	F Value	Prob>F
Control	TPL	Regression model	8	13174.61515	1646.82689	3.66123E30	<0.0001
		Residual error	351	1.5788E-25	4.49801E-28		
		Total	359	13174.61515			
100 mM NaCl		Regression model	8	14825.54003	1853.1925	2.69955E30	<0.0001
		Residual error	351	2.40955E-25	6.86481E-28		
		Total	359	14825.54003			
150 mM NaCl		Regression model	8	12487.11838	1560.8898	2.8792E31	<0.0001
		Residual error	351	1.90286E-26	5.42125E-29		
		Total	359	12487.11838			
200 mM NaCl		Regression model	8	11838.07193	1604.75899	6.64084E30	<0.0001
		Residual error	351	8.48192E-26	2.4165E-28		
		Total	359	12838.07193			

Following the analysis of variance, the statistical indicators attained an explanation for the regression model of cotton traits under 200 mM NaCl stress conditions. The regression model constructed for the trait of plant length measured under 200 mM NaCl stress was statistically very well fitted (residual SS was almost zero). The model has a statistical p-value of <0.0001, the predictors in the model explained the variation in plant length trait very effectively, and the variations detected for this trait under salt stress conditions were considerably reliable (Table 2). In total, the identification of the most appropriate salt-tolerance traits was successful through a regression approach using multiple traits on cotton plants (Sikder *et al.,* 2020).

The correlation analysis of morpho-biological traits studied during the germination stage of cotton plants allows further in-depth analysis of the relationship between plant resistance levels to various stresses and the primary traits that boost productivity. This approach also serves as an effective tool for identifying salt-tolerant genotypes at the early vegetative stage, in combination with molecular markers (SSR—simple sequence repeat and SNP—single nucleotide polymorphism) in the selection process. Phenotypic correlation revealed the visual observations, while the genotypic correlation allows assessing the heritability of cotton traits (Desalegn *et al.,* 2009; Bobokhujaev *et al.,* 2021).

The correlation analysis of plant morphological traits under optimal conditions revealed that plant length exhibited a considerable positive association with numerous traits. These are shoot length (r = 0.94, P < 0.001), root length (r = 0.77, P < 0.001), fresh root weight (r = 0.46, P < 0.001), dry plant weight (r = 0.51, P < 0.001), dry plant weight and dry shoot weight (r = 0.51, P < 0.001), and dry root weight (r = 0.51, P < 0.001). Meanwhile, it displayed weak associations (P > 0.05) with fresh plant weight and fresh shoot weight (r = 0.24 and r = 0.16, respectively) (Figure 1). Furthermore, shoot length showed the highest (P < 0.001) positive correlation with root length, dry plant weight, and dry shoot weight (r = 0.51, r = 0.51, and r = 0.50, respectively), and a moderate (P < 0.001) positive association with the traits of fresh plant weight, fresh shoot weight, fresh root weight, and dry root weight. The root length demonstrated considerable positive correlations with fresh root weight (r = 0.49, P < 0.001) and dry root weight (r = 0.40, P < 0.001), and a highly significant (P < 0.001) positive association existed between the fresh plant weight and fresh shoot weight (r = 0.98). No negative correlation occurred between the traits under the optimal environment. According to past findings, the analysis of variance revealed significant differences among the cotton genotypes, indicating significant associations of leaf chlorophyll content, seed

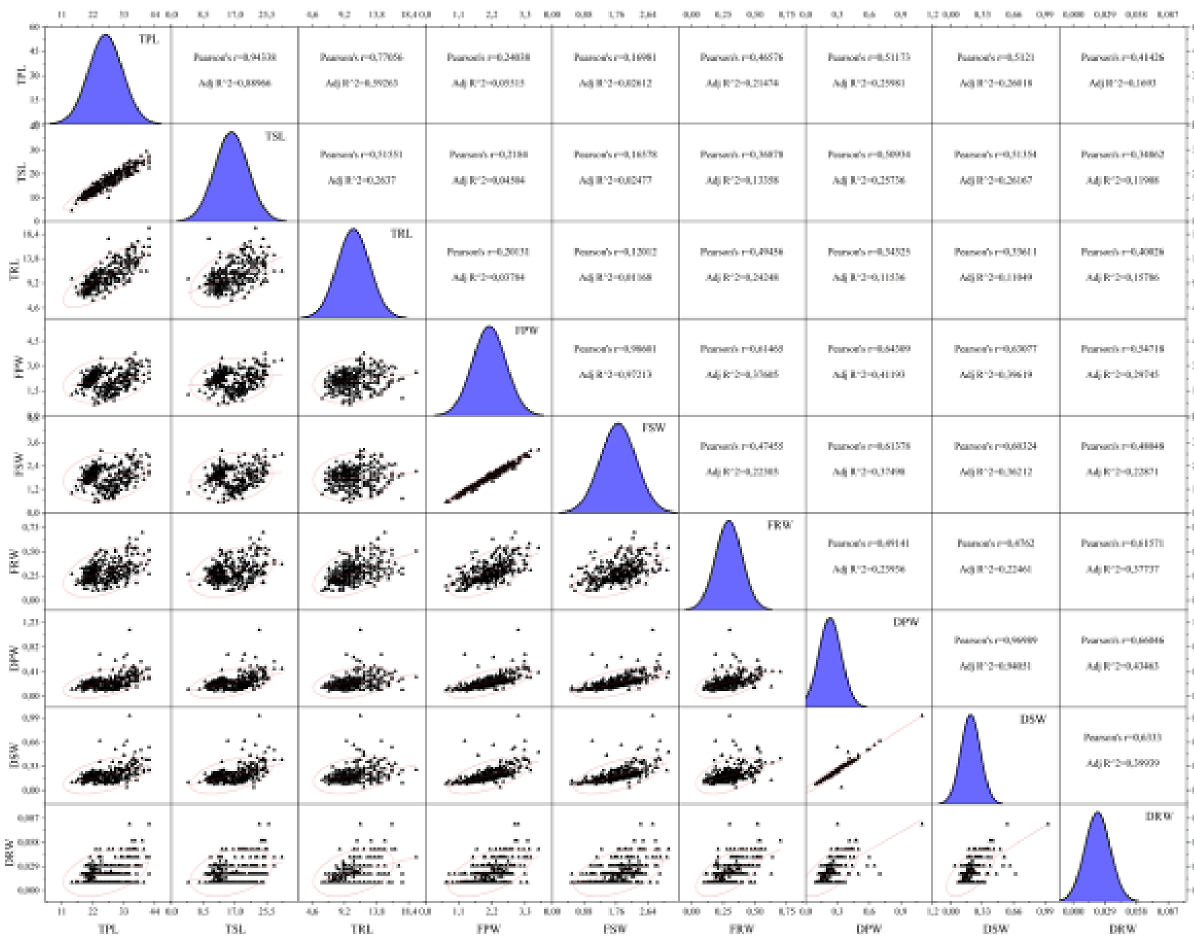

Figure 1. Correlation analysis among nine morphological traits at the early growth stage of cotton under optimal condition. Traits included plant length - PL (cm), shoot length - SL (cm), root length - RL (cm), fresh plant weight - FPW (g), fresh shoot weight - FSW (g), fresh root weight - FRW (g), dry plant weight - DPW (g), dry shoot weight - DSW (g), and dry root weight - DRW (g). Denoting correlation matrix was with $P < 0.05$ (*), $P < 0.01$ (**), and $P < 0.0001$ (***).

cotton weight per boll, seed weight, and plant height (Cetin *et al*., 2009).

Under 100 mM NaCl stress conditions, the correlation analysis of cotton phenotypic traits revealed that plant length showed a significant ($P < 0.001$) positive correlation with traits of shoot length, root length, fresh plant weight, fresh root weight, and dry plant weight. However, dry shoot weight gave a moderate ($P < 0.001$) positive correlation with dry root weight (Figure 2). The trait of shoot length also showed the highest positive correlation with all the cotton traits, except for dry root weight. The root length was one of the most important traits under salt stress environments. It showed a moderate ($P < 0.001$) positive correlation with other traits, such as fresh plant weight, fresh shoot weight, fresh root weight, dry plant weight, dry shoot weight, and dry root weight. All the cotton phenotypic traits evaluated under 100 mM NaCl stress conditions were nonsignificantly different from those studied under optimal conditions, and the 100 mM NaCl stress displayed a nonsignificant effect on cotton growth and development. Identifying the genetic mechanisms governing these traits is key to generating cotton genotypes capable of

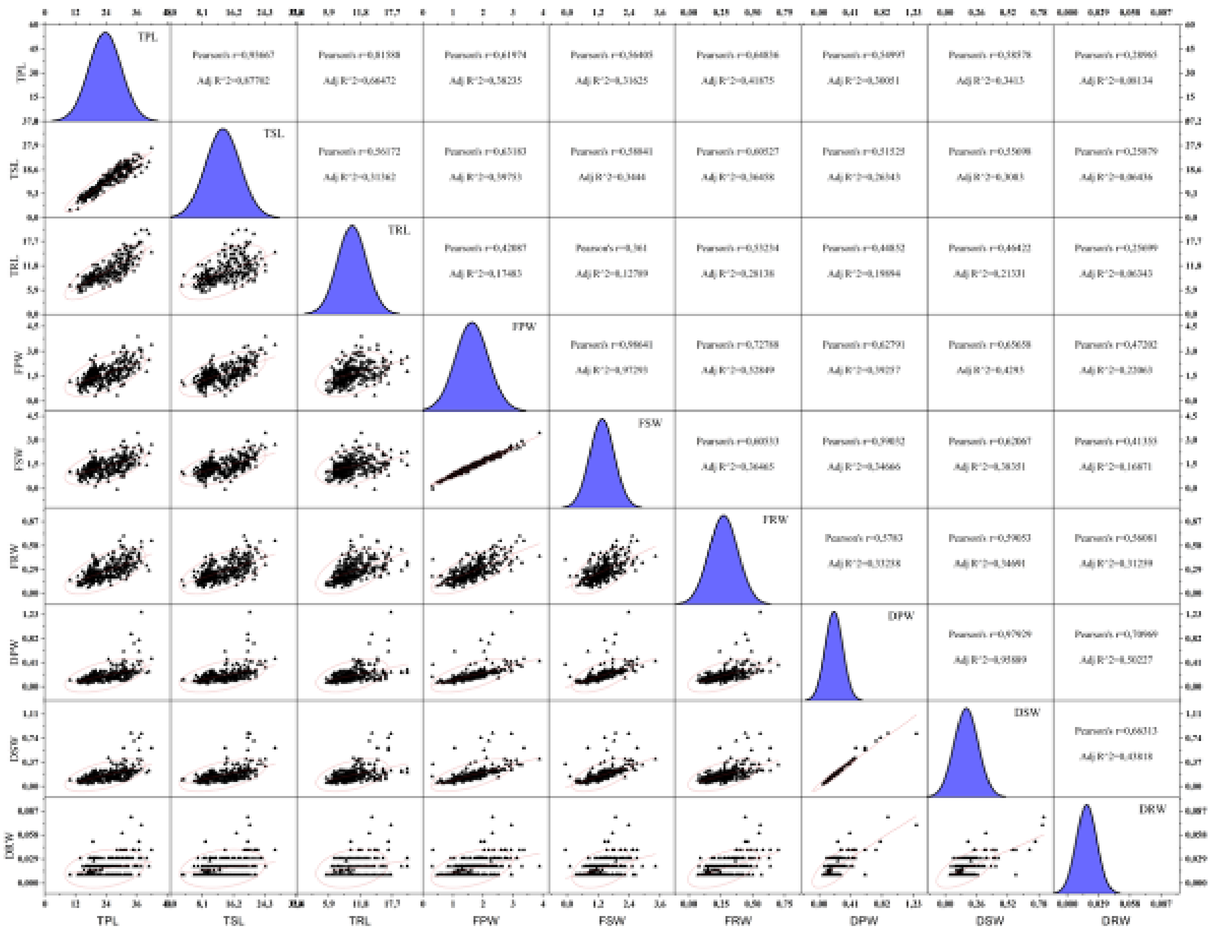

Figure 2. Correlation analysis among nine morphological traits at the early growth stage of cotton under 100 mM-NaCl-stress condition. Traits included plant length - PL (cm), shoot length - SL (cm), root length - RL (cm), fresh plant weight - FPW (g), fresh shoot weight - FSW (g), fresh root weight - FRW (g), dry plant weight - DPW (g), dry shoot weight - DSW (g), and dry root weight - DRW (g). Denoting correlation matrix was with $P < 0.05$ (*), $P < 0.01$ (**), and $P < 0.0001$ (***).

withstanding salinity stress, notably during the early seedling growth (Gul *et al.,* 2025).

The correlation analysis of plant morphological traits under 150 mM NaCl salt stress conditions disclosed plant length exhibiting a significant positive association with other growth traits like shoot length (r = 0.93, P < 0.001), root length (r = 0.84, P < 0.001), and fresh root weight (r = 0.75, P < 0.001). However, it had an average positive association with fresh plant weight (r = 0.62, P < 0.001), fresh shoot weight (r = 0.53, P < 0.001), dry plant weight (r = 0.45, P < 0.001), and dry shoot weight (r = 0.44, P < 0.001), while a weaker positive relationship with dry root weight (r = 0.29, P < 0.001) (Figure 3). The root length, identified as an important trait under salt stress environments, proved to be highly and positively correlated with the fresh root weight (r = 0.58, P < 0.001) trait. Although it showed a slightly weaker positive correlation with other traits, such as fresh plant weight, fresh shoot weight, dry plant weight, dry shoot weight, and dry root weight. Their values range between r = 19 and r = 38. In numerous research, a highly significant positive correlation was evident between fresh root weight and dry root weight, indicating a maintained yet salt stress-affected growth (Gul *et al.,* 2025).

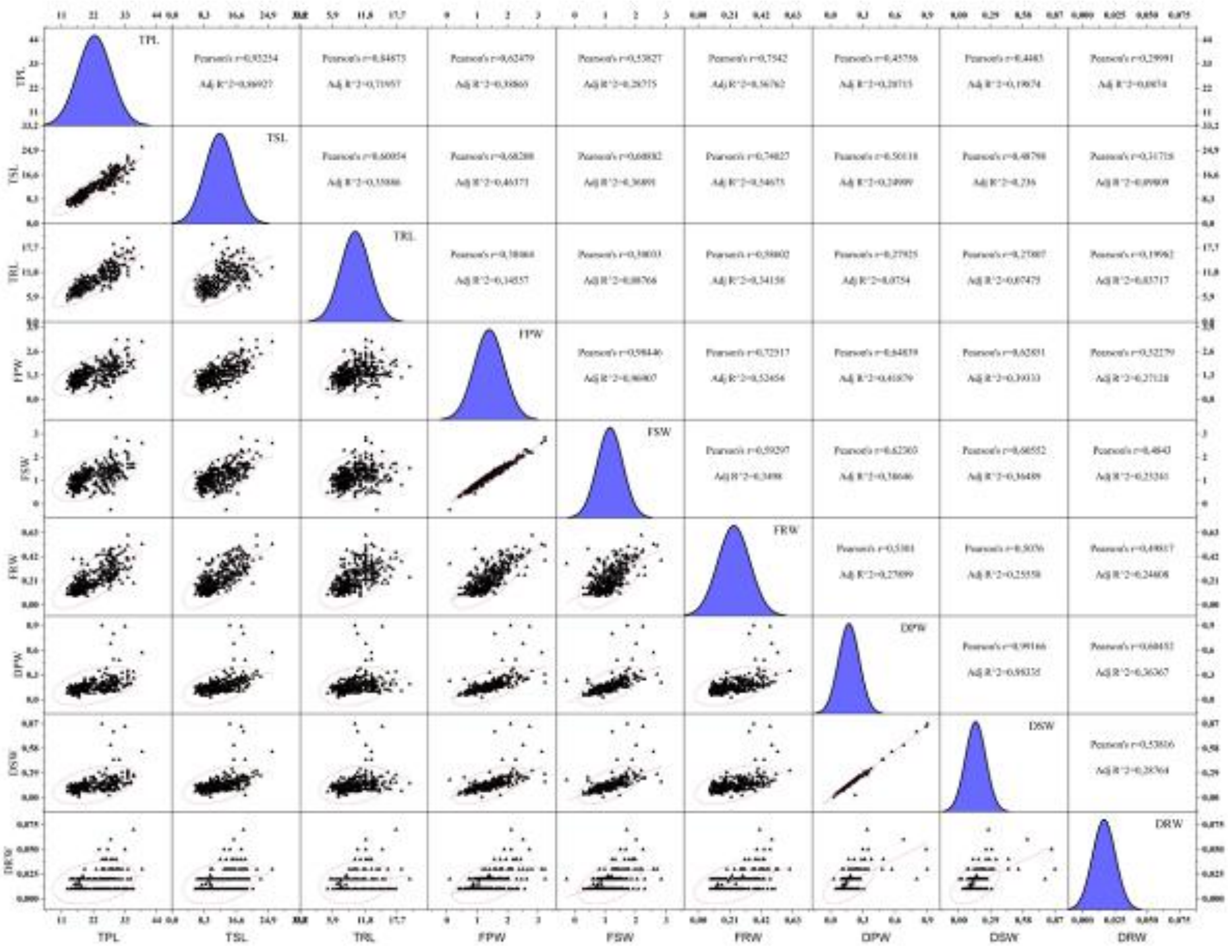

Figure 3. Correlation analysis among nine morphological traits at the early growth stage of cotton under 150-mM-NaCl stress condition. Traits included plant length - PL (cm), shoot length - SL (cm), root length - RL (cm), fresh plant weight - FPW (g), fresh shoot weight - FSW (g), fresh root weight - FRW (g), dry plant weight - DPW (g), dry shoot weight - DSW (g), and dry root weight - DRW (g). Denoting correlation matrix was with $P < 0.05$ (*), $P < 0.01$ (**), and $P < 0.0001$ (***).

The scatter plots and histograms, based on the correlation coefficient, showed the relationship between various phenotypic traits of the cotton cultivars under 200 mM NaCl stress (Figure 4). In this case, the plant length expressed a significant positive correlation with shoot length (r = 0.90, P < 0.001), root length (r = 0.82, P < 0.001), and fresh root weight (r = 0.73, P < 0.001). Meanwhile, it had a moderate correlation with traits of fresh plant weight, fresh shoot weight, dry plant weight, dry shoot weight, and dry root weight. Under 200 mM of NaCl stress, the root length trait displayed a high correlation only with the TPL trait and has an average positive correlation with other phenotypic traits. The trait of fresh plant weight showed a considerable positive correlation with dry plant weight (r = 0.72, P < 0.001) and dry shoot weight (r = 0.70, P < 0.001). The trait of dry root weight revealed a moderate (P < 0.001) positive correlation with other traits, ranging from r = 27 to r = 61. Correlation analysis indicated that the balance of K, Ca, Mg, and Na in cotton stems and leaves plays a key role in determining salt tolerance (Li *et al.,* 2025).

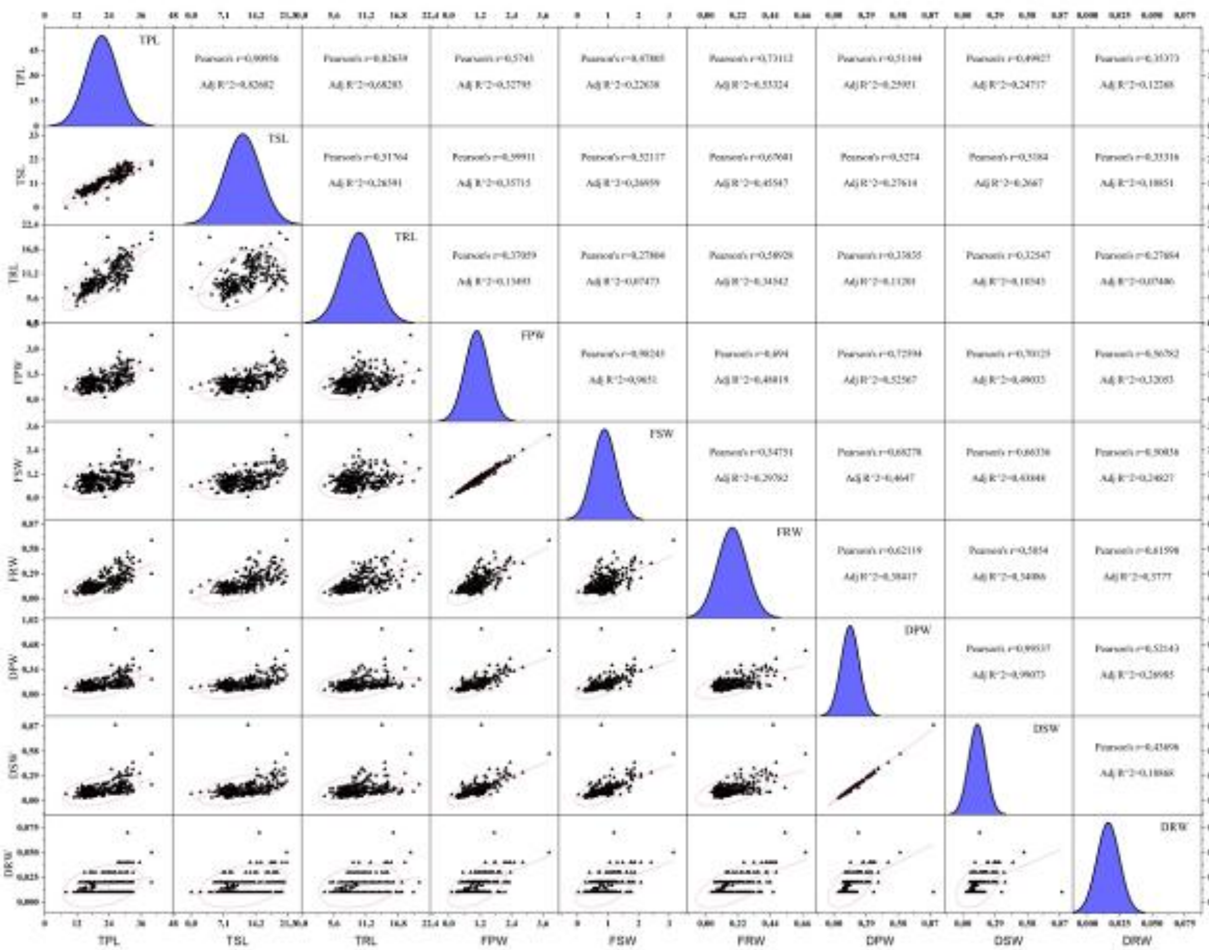

Figure 4. Correlation analysis among nine morphological traits at the early growth stage of cotton under 200-mM-NaCl stress condition. Traits included plant length - PL (cm), shoot length - SL (cm), root length - RL (cm), fresh plant weight - FPW (g), fresh shoot weight - FSW (g), fresh root weight - FRW (g), dry plant weight - DPW (g), dry shoot weight - DSW (g), and dry root weight - DRW (g). Denoting correlation matrix was with $P < 0.05$ (*), $P < 0.01$ (**), and $P < 0.0001$ (***).

CONCLUSIONS

The cotton (*G. hirsutum* L.) phenotypic traits revealed differential responses to salinity stress conditions. The results indicated that 200 mM NaCl caused the highest negative effect in almost all cotton traits, including FPW and TSL, which showed a decrease of about 50% and 29%, respectively. The traits DRW and TRL showed the highest degree of stability under salt stress conditions and proved to have a crucial role in the plant's mechanisms for maintaining vital functions under salt stress conditions. The correlation analysis provided the traits TPL and TRL as vital under salt stress environments, proving positively correlated with other traits under different NaCl stress conditions. Additionally, the obtained backcross lines from salt-tolerant and susceptible cotton genotypes, based on molecular analysis, will continue in future studies. These findings will serve as a foundation for identifying and selecting the salt-tolerant and high-yielding cotton genotypes in plant breeding programs.

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