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RESEARCH ARTICLE

Evaluation of salt tolerance in cotton (*Gossypium hirsutum* L.) seedlings using the multitrait genotype-ideotype distance index (MGIDI)

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Abstract

Cotton is a vital agricultural crop and a primary source of natural fibres, linters and oil. However, soil salinity poses a major abiotic threat to crop productivity worldwide, particularly in arid and semi-arid regions. Although cotton is considered moderately salt-tolerant, its early growth stages are highly sensitive to stress. This study evaluated the morphological response of 28 cotton cultivars to controlled salt stress conditions. Results revealed a clear, dose-dependent reduction in seedling growth under increasing salinity levels. Analysis of variance (ANOVA) confirmed a significant cultivar × treatment interaction (PC × PT), highlighting substantial genetic variability and contrasting responses of cultivars to salinity. Pearson correlation analysis indicated stronger relationships between linear growth traits and biomass accumulation under saline conditions, suggesting a coordinated adaptive modulation of growth mechanisms. Principal component analysis (PCA) confirmed the dominant effect of salinity on phenotypic variability and distinguished distinct adaptive growth strategies among cultivars. The multitrait genotype-ideotype distance index (MGIDI) effectively identified the cultivars Bukhoro-14 (C5), C-4727 (C7), Kelajak (C14) and Nasaf (C19) as genotypes with enhanced salt tolerance at specific levels of induced salt stress. These genotypes represent valuable genetic resources for breeding programs aimed at developing salt-tolerant cotton cultivars suited to arid environments.

Keywords: cotton; *Gossypium hirsutum* L.; MGIDI; salt stress; tolerance

Introduction

Cotton (*Gossypium hirsutum* L.) is one of the world's most important textile crops, constituting nearly 35 % of global natural fiber production and serving as a crucial raw material for the textile industry (1). *G. hirsutum* accounts for approximately 90 % of global cotton production and occupies nearly 95 % of the total cultivated area (2). Currently, Uzbekistan ranks among the top ten cotton producers in the world, producing about 700 thousand metric tons annually (1). Most modern medium-fiber cotton cultivars are the result of long-term breeding programs focused on improving the primary agronomic trait of fiber quality (2, 3). However, prolonged reliance on traditional breeding methods, particularly backcrossing and inbreeding, has progressively narrowed the genetic base, leading to reduced variability for essential traits such as disease resistance and tolerance to environmental stresses (2).

Soil salinization, caused by the accumulation of elevated concentrations of sodium, chloride, sulfate and other ions, affects approximately 8.7 % of agricultural land and is a major cause of declining crop yields worldwide, particularly in arid and semi-arid regions (4-6). In Uzbekistan, soil salinization driven by global climate change, geographical factors and anthropogenic activities poses a significant challenge to agriculture (4, 5). Although cotton is generally considered a moderately salt-tolerant crop, it exhibits substantial reductions in yield and growth under high salinity, especially during the critical seedling stage. Salt stress significantly limits plant growth and development during early ontogenesis, reducing shoot and root length, as well as the fresh and dry weight of seedlings (2, 6-9). These morphological traits can serve as reliable criteria for screening cotton cultivars for salt tolerance at the seedling stage (6, 10).

Notably, despite the wide diversity of Uzbek cotton cultivars, a comprehensive screening of salt stress resistance has not been undertaken. Such screening is essential for a more systematic and efficient approach to selecting donors and recipients in breeding programs. In addition, the application of multivariate statistical analysis tools, such as principal component analysis (PCA) and the multitrait genotype-ideotype distance index (MGIDI), allows for the extraction of robust and integrative insights when assessing crop responses to stress (11). PCA summarizes the variability and relationships between different traits, identifying the most informative variables (11). Similarly, MGIDI combines multiple traits into a single index, thereby reducing multicollinearity and facilitating the identification of superior genotypes for a given stress intensity (11). Accordingly, the present study aims to identify salt-tolerant cotton genotypes based on morpho-physiological traits evaluated at the seedling stage.

Materials and Methods

Plant materials

The study involved 28 medium-fiber cotton (*G. hirsutum*) cultivars (Table 1). The standard cultivar TM-1 was used as a control. Experiments were conducted under controlled laboratory conditions.

Substrate and growth conditions

Pre-soaked seeds of each cultivar were sown in 2 L plastic pots filled with a substrate mixture of sterilized fine river sand and field soil in a 3:1 ratio. The experiment was conducted using three replications to ensure statistical validity, with ten seeds per pot to account for germination variability and provide a sufficient sample size for analysis (9). This sand-dominant substrate composition provided a light, highly permeable medium suitable for salinity experiments, ensuring uniform salt distribution and preventing localized salt accumulation. The loose substrate also facilitated the intact extraction of seedling roots for morphometric measurements on day 21. To prevent water stagnation and root decay, a 2-3 cm layer of small stones was placed at the bottom of the pots to serve as a drainage system.

Plants were grown in a phytotron for 21 days under controlled environmental conditions, including a 16 h light / 8 h dark photoperiod, a light intensity of 1000 lux and a temperature range maintained between 25-30 °C.

Salinity treatments

To assess salt tolerance, a modified method was applied (12). The electrical conductivity (EC) of the irrigation solutions was estimated based on NaCl concentration, using the common approximation that 10 mM NaCl corresponds to approximately 1.0 dS/m. Accordingly, the theoretical EC values for the treatments were calculated as follows: 0 mM (control, ~0 dS/m), 50 mM (5.0 dS/m), 100 mM (10.0 dS/m), 150 mM (15.0 dS/m) and 200 mM (20.0 dS/m). Plants were irrigated every two days with 100 mL of water or the corresponding NaCl solution to field capacity. Because of the adequate drainage, the soil solution EC (ECe) was assumed to remain in equilibrium with the EC of the irrigation water.

Determination of morphometric traits

On the 21st day after sowing, morphometric traits were assessed by analyzing digital photographs captured against a millimeter-scale background using the ImageJ v.1.53e software (13). The shoot length (SL), root length (RL) and total seedling length (TL) were measured. The fresh root weight (FRW) was measured using a CAS MWP-300 precision balance.

Statistical analysis

Preliminary data processing and descriptive statistical analyses were performed using Microsoft Excel. Subsequent statistical analyses were conducted using the Python 3.13.4 programming language within the VS Code environment (v.1.104.1). Specifically, one-way analysis of variance (ANOVA) was performed using the Stats models library, Pearson’s correlation coefficients were calculated using SciPy. Stats and PCA were carried out using Scikit-Learn. To identify the superior cotton cultivars, the MGIDI with a selection intensity of 20 % was applied, calculated using the Metan package in RStudio v. 4.5.1 (11, 14).

Table 1. List of *G. hirsutum* L. cultivars used in the experiment to assess salt tolerance at the seedling stage

Codes	Cultivar	Species name	Origin	Codes	Cultivar	Specie name	Origin
C1	Afsona	*G. hirsutum*	CGB	C15	Kupaysin	*G. hirsutum*	CGB
C2	Baraka	*G. hirsutum*	CGB	C16	Namangan-102	*G. hirsutum*	CGB
C3	Buhoro-10	*G. hirsutum*	CGB	C17	Namangan-34	*G. hirsutum*	CGB
C4	Buhoro-102	*G. hirsutum*	CGB	C18	Namangan-77	*G. hirsutum*	CGB
C5	Buhoro-14	*G. hirsutum*	CGB	C19	Nasaf	*G. hirsutum*	CGB
C6	Buhoro-6	*G. hirsutum*	CGB	C20	Novbahor-2	*G. hirsutum*	CGB
C7	C-4727	*G. hirsutum*	CGB	C21	Omad	*G. hirsutum*	CGB
C8	Chimboy	*G. hirsutum*	CGB	C22	Porloq-1	*G. hirsutum*	CGB
C9	Chuntay - 2	*G. hirsutum*	PSC	C23	Ravnaq-1	*G. hirsutum*	CGB
C10	Gulbahor-2	*G. hirsutum*	CGB	C24	Sulton	*G. hirsutum*	CGB
C11	Ishonch	*G. hirsutum*	CGB	C25	TM-1	*G. hirsutum*	CGB
C12	Jinken-1402	*G. hirsutum*	PSC	C26	Turkan	*G. hirsutum*	PSC
C13	Junjen	*G. hirsutum*	PSC	C27	Xin Lu Zhong-87	*G. hirsutum*	PSC
C14	Kelajak	*G. hirsutum*	CGB	C28	Xin Lu Zoa-78	*G. hirsutum*	PSC

*CGB - Center of Genomics and Bioinformatics (Uzbekistan), PSC - private seed company.

Results

Descriptive statistics

A comparative assessment of the phenotypic traits of 28 cotton cultivars under control (0 mM) and salt stress conditions (50-200 mM) was conducted using descriptive statistical analysis of morphometric traits including shoot length (SL), root length (RL), total seedling length (TL) and fresh root weight (FRW) (Table 2, Fig. 1).

The results of the experiment demonstrate a pronounced decrease in the growth parameters of the cotton cultivars studied depending on the salt concentration. Under control conditions (0 mM), the mean SL was 13.98 ± 2.37 cm, however, significant growth suppression was observed, under salt stress. Mean SL progressively decreased to 9.00 ± 1.21 cm at 50 mM and further to 5.88 ± 1.27 cm at the maximum concentration 200 mM, which is more than three times lower than the control (Table 2, Fig. 1).

A similar trend was observed for RL, which decreased from 27.93 ± 3.19 cm in the control to 19.61 ± 2.57 cm at 200 mM (Table 2, Fig. 1). Total seedling length also declined with increasing salt stress, decreasing from 41.91 ± 5.32 cm in the control group to 32.96 ± 3.08 cm at 50 mM and reaching a minimum of 25.48 ± 3.64 cm at 200 mM. FRW showed a comparable pattern: values dropped from 2.44 ± 0.38 g in the control to 2.04 ± 0.40 g at 50 mM and continued to decline to 1.05 ± 0.29 g at 200 mM.

ANOVA revealed that both the cultivar effect (PC) and the treatment effect (PT) were highly significant ($p < 0.001$) for all studied traits. Moreover, a highly significant cultivar and treatment interaction (PC × PT) was observed, indicating that the cultivars responded differentially to increasing salinity levels (Table 2).

Pearson's correlation coefficient

Pearson's correlation analysis revealed statistically significant positive correlations ($p < 0.001$) between all studied growth parameters and root fresh weight. Under control conditions (0 mM), the correlation between SL and RL was r = 0.828 ($p < 0.05$); however, it decreased to r = 0.621 ($p < 0.001$) at 150 mM and increased again to r = 0.776 ($p < 0.001$) at 200 mM. The correlation between RL and TL remained consistently high both in the control and under salt stress (r = 0.968–0.976, $p < 0.001$). The correlation between RL and FRW under control conditions was r = 0.422 ($p < 0.05$), while correlations between SL and TL with FRW were statistically non-significant. Under salt stress conditions (starting from 50 mM), correlations between all linear parameters (SL, RL, TL and FRW) became statistically significant (Fig. 2).

Principal component analysis (PCA)

To simplify the interpretation of multidimensional morphometric data, PCA was performed. The analysis identified two principal components - the first component (Dim 1) was dominant, explaining 90.8 1% of the total variance, while the second component (Dim 2) accounted for a minor proportion of 5.98 %. Together, these two components captured 96.79 % of the total variation, providing a robust representation of the dataset structure on the combined biplot (Fig. 3).

The Dim 1 axis distinctly separated the treatment groups: the control group was positioned in the positive range, whereas increasing salt stress levels (from 50 to 200 mM) caused a progressive shift of the cultivar group centroids toward the negative range, indicating a strong dose-dependent effect. All vectors of the studied traits (SL, RL, TL and FRW) were oriented to the right and closely aligned with the Dim 1 axis. The acute angles between the vectors indicated a strong positive correlation among all four traits. Conversely, the vertical Dim 2 axis, which explained a relatively small proportion of the remaining variation, reflected more subtle differences in growth strategies: the pronounced positive slope of the FRW vector along this axis, in contrast to the negative slope of the length-related vectors (SL, RL and TL) suggested distinct adaptive strategies. Specifically, some cultivars under salt stress maintain higher rates of linear elongation with moderate biomass accumulation, while others prioritize biomass accumulation over linear dimensions.

Multitrait genotype-ideotype distance index (MGIDI)

MGIDI was employed to identify cotton cultivars with enhanced resistance to salt stress. The analysis was performed separately for each of the five treatment levels using a 20 % selection intensity, which facilitated the identification of the most promising cultivars.

Under control conditions, four cultivars - Namangan-102 (C16), Baraka (C2), Gulbahor-2 (C10) and Chimboy (C8) exhibited the most favorable MGIDI values, indicating their optimal productivity in the absence of stress (Fig. 4a). Under mild salt stress (50 mM NaCl), the leading group included cultivars Namangan-102 (C16), C-4727 (C7), TM-1 (C25) and Chimboy (C8), reflecting a shift in the composition of the top-performing cultivars under stress conditions (Fig. 4b). A further increase in salinity to 100 mM, cultivars Baraka (C2), C-4727 (C7), Ishonch (C11) and Kelajak (C14) were selected, demonstrating high potential for tolerance to this level of salt stress (Fig. 5a). At 150 mM, cultivars Nasaf (C19), Porloq-1 (C22), Sulton (C24) and Buhoro-10 (C3) ranked highest, characterizing them as the most adapted to higher stress levels (Fig. 5b).

Table 2. Descriptive statistics and results of analysis of variance (ANOVA) for seedling traits of 28 cotton cultivars under control (0 mM) and salt stress (50-200 mM) conditions

Traits	0 mM	mM	mM	mM	mM	ANOVA	Cultivar × Treatment
	$\frac{\text{Mean} \pm \text{SD}}{min - max}$	$\frac{\text{Mean} \pm \text{SD}}{min - max}$	$\frac{\text{Mean} \pm \text{SD}}{min - max}$	$\frac{\text{Mean} \pm \text{SD}}{min - max}$	$\frac{\text{Mean} \pm \text{SD}}{min - max}$		
SL (cm)	13.98 ± 2.37 9.75 - 19.12	9.00 ± 1.21 4.66 - 10.67	7.72 ± 1.26 5.52 - 10.27	6.87 ± 0.94 4.95 - 8.85	5.88 ± 1.27 3.80 - 8.11	PC = *** PT = ***	PC × PT = ***
RL (cm)	27.93 ± 3.19 22.48 - 34.48	23.97 ± 2.08 18.17 - 27.23	22.17 ± 3.02 13.36 - 27.40	21.07 ± 2.87 15.14 - 26.84	19.61 ± 2.57 14.49 - 23.97	PC = *** PT = ***	PC × PT = ***
TL (cm)	41.91 ± 5.32 32.23 - 52.43	32.96 ± 3.08 22.83 - 37.72	29.89 ± 3.99 19.20 - 35.42	27.94 ± 3.53 20.10 - 35.69	25.48 ± 3.64 19.31 - 31.94	PC = *** PT = ***	PC × PT = ***
FRW (g)	2.44 ± 0.38 1.80 - 3.47	2.04 ± 0.40 0.55 - 2.69	1.57 ± 0.49 0.77 - 2.59	1.25 ± 0.39 0.64 - 2.05	1.05 ± 0.29 0.58 - 1.72	PC = *** PT = ***	PC × PT = ***

The analyzed traits include shoot length up to the epicotyl (SL), root length (RL), total seedling length (TL) and fresh root weight (FRW); PC - *p*-value of cultivar effect, PT - *p*-value of treatment effect and PC × PT - *p*-value of cultivar and treatment interaction; (*$p < 0.05$), (**$p < 0.01$) and (***$p < 0.001$) indicate significant differences at the corresponding levels of significance in the studied parameters.

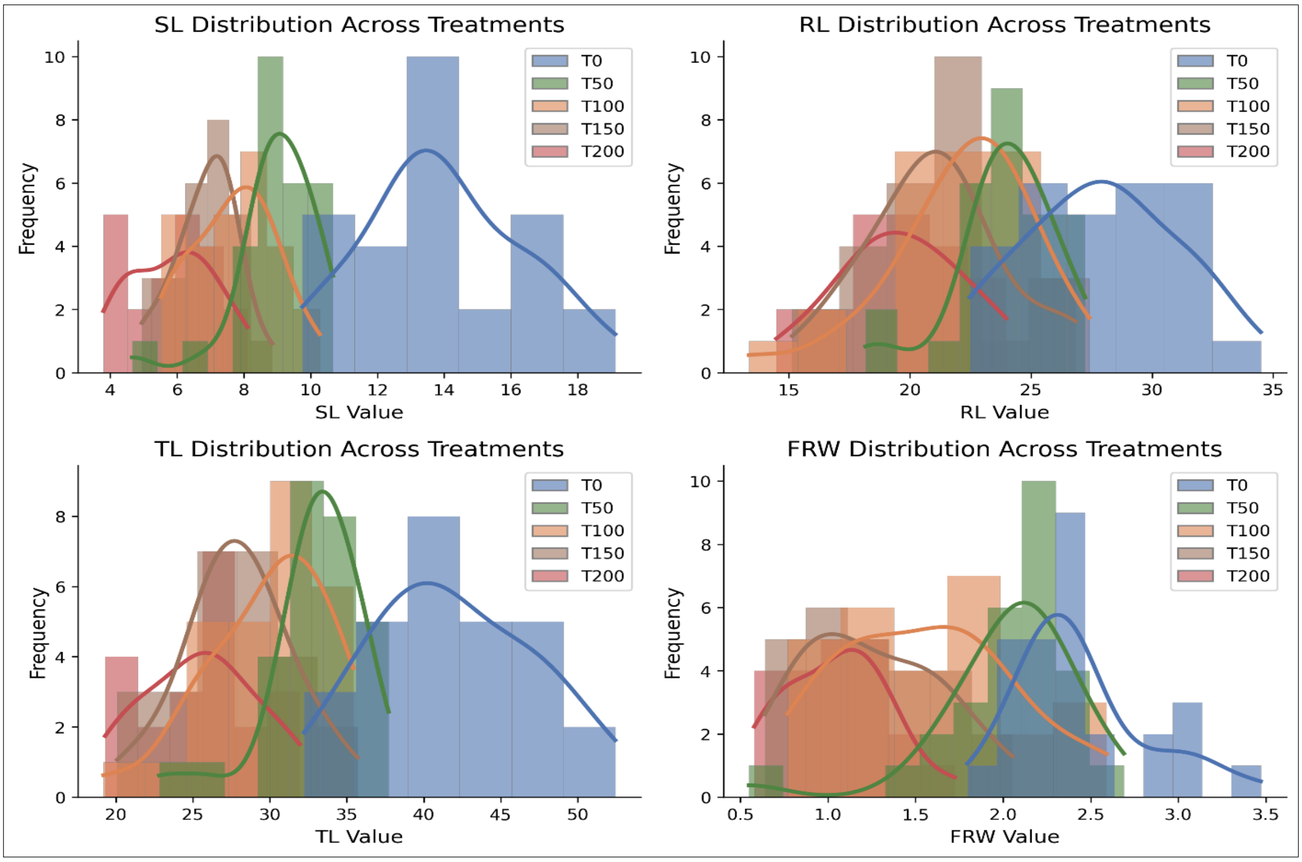

Fig. 1. Frequency distribution of seedling phenotypic traits under different NaCl concentrations: T0 – control (0 mM), T50 - 50 mM NaCl, T100 - 100 mM NaCl, T150 - 150 mM NaCl, T200 - 200 mM NaCl.

Fig. 2. Pairwise correlation matrix of the studied traits in cotton cultivars, illustrating changes in interrelationships depending on the salinity level: SL - shoot length, RL - root length, TL - total seedling length, FRW - fresh root weight; T0 – control (0 mM), T50 - 50 mM NaCl, T100 - 100 mM NaCl, T150 - 150 mM NaCl, T200 - 200 mM NaCl; *p<0.05, **p<0.01 and ***p<0.001 indicate significant differences at the corresponding levels of significance in the studied parameters.

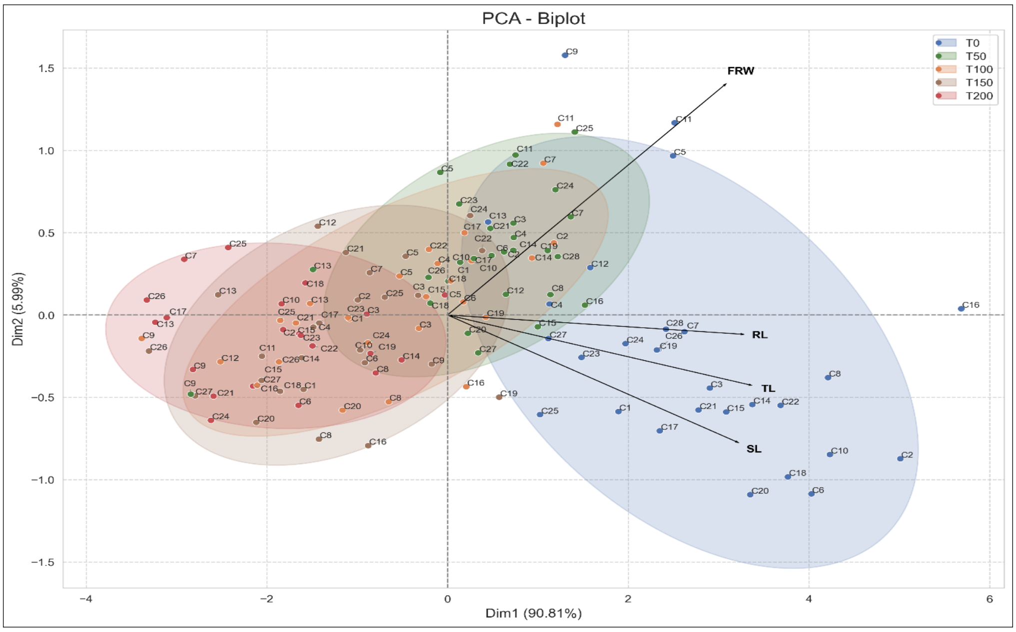

Fig. 3. Combined principal component (PCA) plot illustrating the distribution of cotton cultivars (C) and the contribution of the studied traits: SL - shoot length, RL - root length, TL - total seedling length, FRW - fresh root weight; T0 – control (0 mM), T50 - 50 mM NaCl, T100 - 100 mM NaCl, T150 - 150 mM NaCl, T200 - 200 mM NaCl.

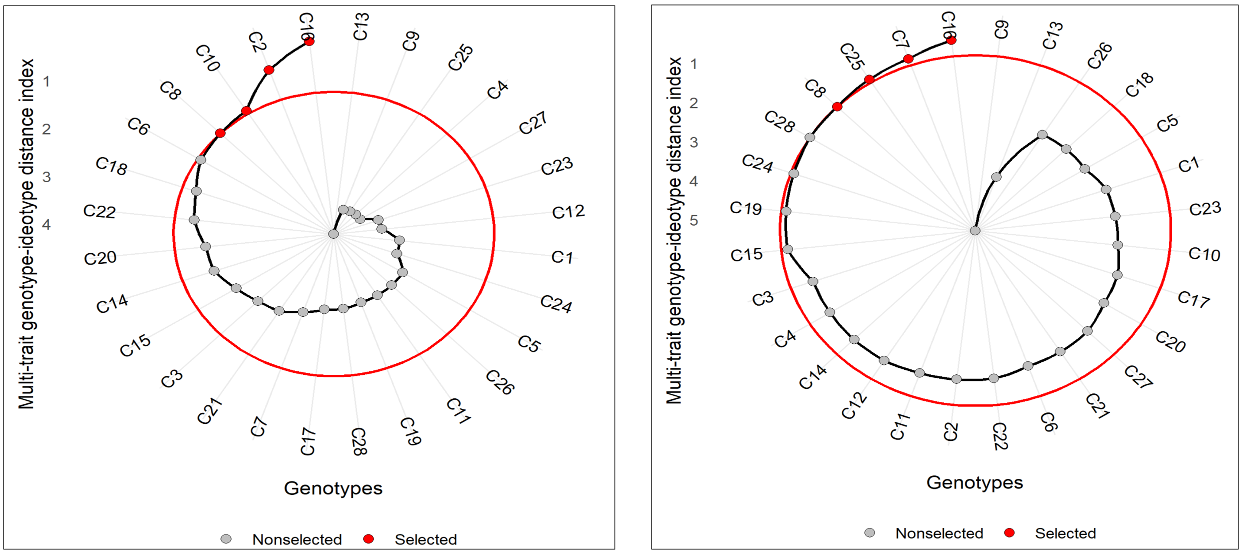

Fig. 4. Ranking of cotton cultivars based on the MGIDI index: (a) under control conditions (0 mM NaCl); (b) under salt stress conditions (50 mM NaCl).

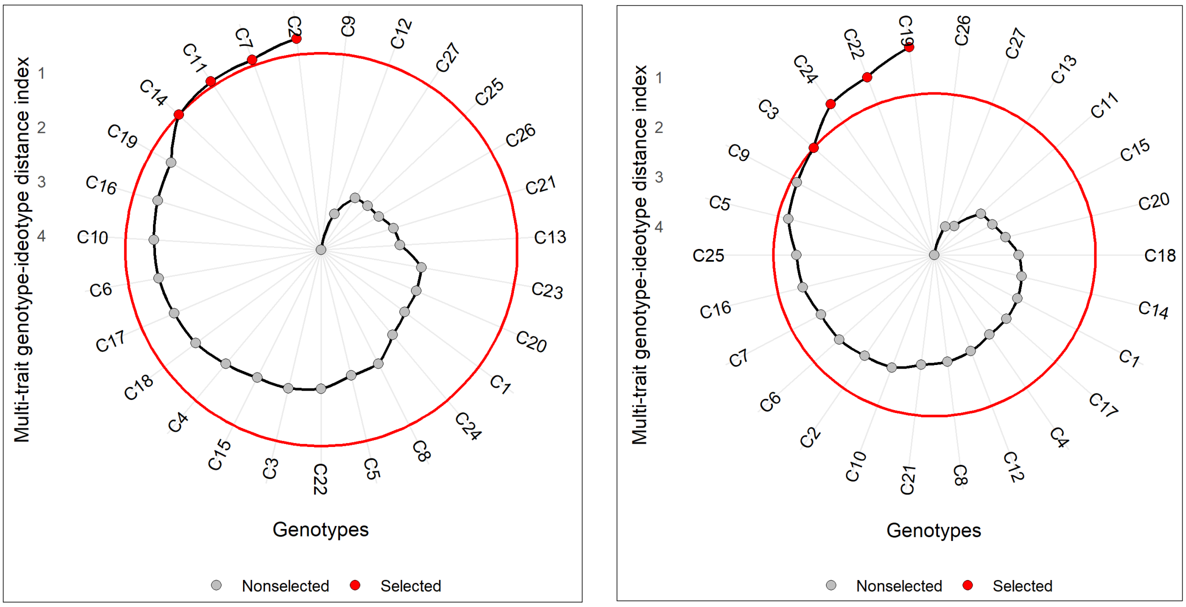

Fig. 5. Ranking of cotton cultivars based on the MGIDI index: (a) under salt stress conditions (100 mM NaCl); (b) under salt stress conditions (150 mM NaCl).

The maximum NaCl concentration of 200 mM, most cultivars were excluded by the selection index and only a few highly resistant cultivars remained. According to the MGIDI analysis, cultivars Buhoro -14 (C5), Kelajak (C14) and Nasaf (C19) showed the best performance at this treatment level (Fig. 6). Thus, the MGIDI analysis identified a set of cotton cultivars with increased salt stress tolerance. Collectively, across different concentrations (from 50 mM to 200 mM), the following cultivars were selected as superior: Baraka (C2), Buhoro-10 (C3), Buhoro-14 (C5), C-4727 (C7), Chimboy (C8), Gulbahor-2 (C10), Ishonch (C11), Kelajak (C14), Namangan-102 (C16), Nasaf (C19), Porloq-1 (C22), Sulton (C24) and TM-1 (C25).

Discussion

The results of this study confirm that salt stress exerts a statistically significant dose-dependent inhibitory effect on all morphometric traits of cotton seedlings, while the identified genotype × treatment interaction highlights genetic differences in adaptive potential among the studied cultivars. Correlation analysis showed that the relationship between shoot and root development weakens under moderate stress but recovers at high concentrations, suggesting the ability of resistant cultivars to coordinate growth processes for survival. PCA confirmed the dominant influence of the stress factor on phenotypic variability and revealed differences in resource allocation strategies between linear growth and biomass accumulation. Based on the MGIDI index, cultivars with different sensitivity thresholds were identified namely, Chimboy (C8) and Namangan-102 (C16) are effective under low salt stress (50 mM), while Baraka (C2), Buhoro -14 (C5), C-4727 (C7), Kelajak (C14) and Nasaf (C19) demonstrate stable high resistance under moderate (100-150 mM) and severe (200 mM) salinity conditions.

Growth responses to salinity

The observed dose-dependent inhibitory effect of salt stress on all morphometric traits of cotton seedlings aligns with findings reported in previous studies (15, 16). This growth suppression is attributed to a dual mechanism of osmotic stress, which leads to a rapid reduction in turgor pressure and restricts water uptake, followed by specific ion toxicity (Na^+ and Cl^-) combined with oxidative stress (6, 7). A crucial finding is the identification of a significant genotype × treatment interaction. This result provides direct evidence of significant genetic diversity among the studied cultivars in terms of salt tolerance, which confirms the feasibility of targeted breeding programs (7-9, 11).

Correlation and adaptive mechanisms

The results of the correlation analysis regarding the coordination of growth processes are particularly significant (9, 11). The weakening of the correlation between shoot and root development under moderate stress (100–150 mM) likely reflects stress-induced plasticity and an imbalance in resource allocation (17, 18). Under moderate stress, a plant may redistribute resources, prioritizing specific organs as an adaptive response (11, 17). This can manifest both as predominant root development to enhance water absorption efficiency and as the relative maintenance of shoot growth to preserve photosynthetic capacity (18). This divergence leads to asymmetrical growth, altering the root-to-shoot biomass ratio, often favoring below-ground expansion depending on the genetic strategy of the cultivar (17, 18).

In contrast, the restoration and strengthening of the correlation between above- and below-ground traits at maximum stress (200 mM) indicate tighter physiological coordination in the most resistant cultivars. This suggests that maintaining a precise balance between shoot and root development is essential for survival under conditions of extreme salinity. Such efficient resource allocation is a key feature of high adaptive potential and aligns with survival strategies under resource-limited conditions (19).

Principal component analysis (PCA)

The results of the PCA presented in this study align well with established physiological patterns of cotton adaptation to salt stress (11). The first principal component (Dim 1) serves as the productivity/tolerance axis, where the distinct separation of treatments-shifting from the control group (positive direction associated with high SL, RL, TL and FRW values) to the salt-stressed groups (negative region) illustrates a strong dose-dependent inhibitory effect. Furthermore, the second component (Dim 2) captures an alternative growth strategy, contrasting biomass accumulation (FRW) with linear elongation

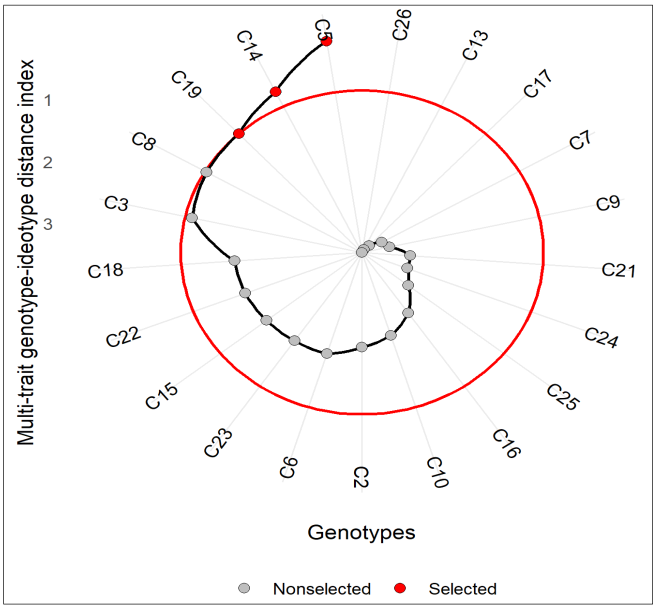

Fig. 6. Ranking of cotton cultivars based on the MGIDI index under salt stress conditions (200 mM NaCl).

(SL, RL and TL), demonstrating more subtle differences in the adaptive potential of cultivars. The observed phenotypic plasticity is corroborated by the physiological data reported by (11). Those studies indicated that under optimal growth conditions, the plant is characterised by high metabolic activity (high TSP and K^+) and biomass accumulation, whereas under increasing stress, an energy-intensive metabolic shift occurs towards the synthesis of protective osmolytes and antioxidants (proline, SOD, CAT and POD), which inevitably leads to a trade-off that limits vegetative growth (11).

MGIDI results for specific cultivars and breeding implications

For a comprehensive and balanced assessment of cultivars, the MGIDI was employed. This index has proven to be an effective breeding tool that circumvents the issues of multicollinearity and biased estimates characteristic of traditional indices (20). The application of the MGIDI index enabled the identification of the specific adaptations of the studied cotton cultivars to varying levels of salt stress. These findings directly correlate with the established dose-dependent inhibitory effect on morphometric traits and confirms the significant interaction between genotype × treatments.

The results showed that the composition of the top-performing cultivars shifted markedly with increasing treatment levels. Cultivars Namangan-102 (C16) and Chimboy (C8) demonstrated high potential under optimal and mildly saline conditions (0-50 mM), while others gained an advantage under moderate and high stress levels. This reflects their specific adaptation to distinct salinity regimes and emphasizes the need for targeted selection based on these adaptive characteristics.

Cultivars that demonstrated resistance at various stress levels of treatment are particularly valuable for breeding. Specifically, cultivar C-4727 (C7) exhibited resistance at low to moderate salt stress (50-100 mM), aligns with the differential shoot and root growth observed at these concentrations. Cultivar Baraka (C2) showed dynamic adaptation, despite a performance decline at 50 mM, it reached maximum MGIDI values at 100 mM. This suggests a threshold-dependent activation of defence systems, triggered only when specific salinity level is reached. Its strong response to moderate stress, contrasted with sensitivity to high salt levels, makes this cultivar a valuable model for studying adaptation mechanisms of adaptation and stress response regulation.

Cultivar Nasaf (C19) displayed exceptional stability under moderate and high salt stress (150–200 mM), while Kelajak (C14) demonstrated broad adaptation across the 100–200 mM range. This stability under maximum stress mirrors the restoration and strengthening of inter-organ correlation at 200 mM, pointing to precise physiological coordination as a key feature of high adaptive potential. Finally, Buhoro-14 (C5) proved to be highly specialized, exhibiting maximum resistance only under severe salt stress (200 mM). Thus, the application of the multivariate MGIDI index effectively identified Buhoro-14 (C5), C-4727 (C7), Baraka (C2), Kelajak (C14) and Nasaf (C19) as genotypes with enhanced salt tolerance at specific levels of induced salt stress.

Conclusion

In conclusion, this study confirms the pronounced inhibitory effect of salt stress on the morphometric traits of cotton seedlings and highlights significant genotypic diversity in response to salinity. The application of the multivariate MGIDI analysis effectively identified cultivars Buhoro-14 (C5), C-4727 (C7), Kelajak (C14) and Nasaf (C19) as valuable genetic resources with enhanced salt tolerance under varying treatment levels. Future research should focus on investigating biochemical markers to provide deeper insights into physiological adaptation mechanisms and facilitate the targeted use of these cultivars in breeding programs.

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Authors' contributions

IBS, DAM, VSK, FSR and DEU carried out the experiments and wrote and revised the manuscript, performed statistical analysis. DKB, ASI, NRR, KAU, SES, SOK, SSA, BMS, AAA, AKM, VVU, AAB, ZZY, RMA, BAA, MRZ and BKR participated in the experiments, collected the data and prepared the manuscript. ZTB edited and approved the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: The authors declare that they have no competing interests.

Ethical issues: None

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