Evaluation of salt tolerance in cotton genotypes (*G.hirsutum* L.) at seedling stage using multiple salt tolerance indices

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Abstract

Salt stress is one of the most detrimental factors adversely affecting cotton, and cotton plants display considerable diversity in salt tolerance. Developing cultivars that can withstand salt stress is imperative to meet the growing cotton demand for the textile industry. High-yielding and salt-tolerant cotton cultivars could be developed with the help of efficient salt-tolerance screening methods. The primary objective of the current study was to efficiently screen cotton genotypes for salt tolerance using salt tolerance indices such as tolerance index, mean productivity, geometric mean productivity, yield index, stress susceptibility index, stress tolerance index, and modified stress tolerance index under control and three salt stress conditions (10 dS/m, 15 dS/m, and 20 dS/m). The effects of genotype, environment, and their interaction on seedling dry weight were highly significant. As the salt stress level increased, there was a consistent decrease in the dry weight of the seedlings with different genotypes exhibiting varying levels of performance, suggesting the presence of genetic variability. Salt tolerance indices and combined ranking criteria indicated that the genotypes Hataf-1, FH-158, CIM-446, and VH-305 exhibited the highest level of salt tolerance. Conversely, the genotypes Cyto-178, VH-259, MNH-996, MNH-992, NS-131, and AA-703 had the highest sensitivity to salt stress. The identified salt-tolerant genotypes could be cultivated in saline conditions and should be considered for future breeding programs. The findings also proved that the salt tolerance indices employed in this study are valuable for efficiently screening cotton germplasm to enhance salinity tolerance in breeding programs.

Keywords: cotton, seedling, salinity, tolerance indices, salt tolerance, dry weight.

Introduction

Soil salinity is a growing global concern and poses a major threat to agricultural productivity, particularly in regions reliant on irrigation due to the high sodium chloride content of the water (Qureshi and Perry, 2021). According to reports from the United Nations, 20% of agricultural land and 50% of global cropland are salt affected (Zaman *et al.*, 2018). Soil salinity leads to soil degradation, which in turn reduces agricultural output in various places worldwide (Zaman *et al.*, 2016). Soil salinization affects around 800 million hectares of land worldwide (Singh, 2022). In Pakistan, soil salinity ranks high among the most pressing issues, reducing agricultural productivity. An annual decline in cultivated lands directly results from salinity and waterlogging; every five minutes, an acre of farmland is rendered unusable for agricultural production (Syed *et al.*, 2020). Future increases in soil salinity may be caused by climatic changes, low annual precipitation, rising sea levels, water scarcity for irrigation, excessive fertilizer use, and the absence of an effective drainage system (Corwin, 2021). According to published reports, salinity diminishes the amount of arable land that can be used for agricultural purposes (Mukhopadhyay *et al.*, 2021). In Pakistan, roughly 18% of Sindh province's land, 2% of Khyber Pakhtun Khawa province's land, and 3% of Punjab province's land are affected by salinity (Qureshi *et al.*, 2008). These provinces comprise both primary as well as secondary cotton cultivation regions. Salinity has a detrimental impact on the quality and productivity of plants, including cotton, globally (Wu *et al.*, 2023). The seedling, vegetative, and reproductive growth phases are adversely affected by high salinity. Compared to fully developed plants, seedlings exhibit a greater susceptibility to salinity (Munawar *et al.*, 2021). Salinity primarily induces hyper-osmotic stress and hyper-ionic toxic effects, resulting in the reduction of germination and seedling growth (Sikder *et al.*, 2020b). Elevated levels of salinity hinder the germination of seeds and the emergence of roots as a result of the osmotic effect (Muhammad *et al.*, 2023). This is detrimental to the plant as it hinders its ability to maintain the essential nutritional requirements required for the healthy development of cotton plants (Munawar *et al.*, 2021). Furthermore, elevated salinity levels are responsible for ion toxicity, osmotic stress, and mineral deficits, all of which have a negative impact on photosynthetic, physiological, and biochemical processes, which in turn affect the overall biomass accumulation (Saleh, 2012). The total dry weight is a crucial factor in assessing the salinity tolerance of cotton seedlings (Basal, 2010). It comprehensively measures the plant's growth and biomass accumulation in saline environments (Chaudhary *et al.*, 2020). Researchers can evaluate the extent of growth restriction caused by salinity stress while identifying genotypes with improved salt resistance by measuring the total dry weight of cotton seedlings subjected to various degrees of salinity. Cotton plant species exhibit varying degrees of salt tolerance, which is determined by their genetic composition and response to the concentration of salts present in the soil (Morton *et al.*, 2019; Maryum *et al.*, 2022). Cotton plants possess an innate ability to tolerate the detrimental impacts of salt in either their leaves or root zone (Abdelraheem *et al.*, 2019). Stress tolerance indices (STI) have been acknowledged as valuable measurements in assessing genotypes' capacity to tolerate stress and produce high yields (Mahmood *et al.*, 2022). These different salt tolerance indices can be employed to evaluate plant germplasm's ability to tolerate salt stress. Multiple studies in the literature have examined the effectiveness of these indices in finding genotypes that exhibit greater yield stability under situations with minimal moisture (Moussouraki *et al.*, 2019; Ullah *et al.*, 2019; Sikder *et al.*, 2020a).

Keeping in view the increasing soil salinity and its impact on cotton's growth, researchers must develop distinctive cotton genotypes that can withstand stress and maintain high fibre quality to fulfil the growing textile requirements of the global population, particularly in the face of anticipated climate changes such as salinity stress. The current investigation was initiated with the purpose of accomplishing the subsequent goals: evaluate the impact of soil salinity on cotton seedlings and identify salt-tolerant cotton genotypes by utilizing salt tolerance indices (STI).

Materials and Methods

The experimental material of twenty-five genotypes was sown in pots using a two-factor, completely randomized design arrangement to assess their response to NaCl stress in a controlled environment as well as under three different levels of salt stress: 10 dS/m, 15 dS/m, and 20 dS/m. Concentrated NaCl solutions were formulated and subsequently administered to the specified treatments. The NaCl concentration was consistently monitored and controlled by periodically measuring the electrical conductivity of the sand using an EC meter (Hardie and Doyle, 2012). The Hoagland solution was used to ensure precise measurement and adequate nutrient provision to the plants (Hoagland and Arnon, 1950). Water with an electrical conductivity of 1.18 dS/m was used to irrigate the control group. Seedlings were harvested 40 days after the date of sowing. To determine the dry weight of the roots and shoots, a drying technique similar to that described by Zhang *et al.* (2014) was followed, following which the dry weight was measured using an electronic scale.

Statistical Analysis

Analysis of variance was performed on the mean seedling dry weight data (Steel *et al.*, 1997). Eight salt tolerance indicators were calculated based on the total dry weight of the seedlings, which was determined by combining the dry weight of the root and shoot. Salt tolerance indices, namely the Tolerance Index (TOL) (Rosielle and Hamblin, 1981), Stress Susceptibility Index (SSI) (Mousavi *et al.*, 2008), Stress Susceptibility Percentage Index (SSPI)(Fischer and Maurer, 1978), Stress Tolerance Index (STI) (Fernandez George, 1992), Yield Index (YI)(Gavuzzi *et al.*, 1997), Mean Productivity (MP) (Rosielle and Hamblin, 1981), Geometric Mean Productivity (GMP) (Fernandez George, 1992) and Modified Stress Tolerance Index (MSTI) (Bouslama and Schapaugh Jr, 1984) were computed using their formulae (Table 1).

Table 1. The name and formula used for all salt tolerance indices.

Name	Formula
Genotypes with low values of these indices show higher stability in saline conditions.	
Tolerance Index (TOL)	$Y_p - Y_s$
Stress Susceptibility Index (SSI)	$[1-(Y_s/Y_p)]/[1-(\bar{Y}_s / \bar{Y}_p)]$
Stress Susceptibility Percentage Index (SSPI)	$TOL\times100/(2\times\bar{Y}_p)$
Genotypes with high values of these indices show higher stability in saline conditions.	
Stress Tolerance Index (STI)	$(Y_p\times Y_s)/(\bar{Y}_p)^2$
Yield Index (YI)	$Y_s/\bar{Y}_s$
Mean Productivity (MP)	$(Y_p+Y_s)/2$
Geometric Mean Productivity (GMP)	$\sqrt{(Y_p\times Y_s)}$
Modified Stress Tolerance Index (MSTI)	$(YI)^2\times STI$

Y_s and Y_p are the genotype-specific measures of total dry matter under stress and control conditions, respectively, while $\bar{Y}_s$ and $\bar{Y}_p$ represent the mean total dry matter under stress and non-stress conditions for all genotypes, respectively.

Results

Two factorial ANOVA was used to evaluate the importance of the total dry matter, and the results showed statistically significant differences ($p < 0.01$) between the genotypes, treatments, and their interaction (Table 2). The increase in salt stress led to a decrease in total dry weight by 12%, 29%, and 41% under 10dS/m, 15dS/m, and 20 dS/m of salt stress compared to control conditions. The results of the tolerance index (TOL) under 10 dS/m of salt stress level indicated that NS-121 exhibited the lowest value (0.042), followed by FH-158 (0.049) and Tarzan-1 (0.048), with MNH-992 recording the highest value (0.164), followed by IUB-65 (0.16) and FH-172 (0.13). The findings regarding the tolerance index (TOL) under 15 dS/m of salt stress indicated that CIM-599 obtained the lowest value (0.10), followed by Tarzan-1 (0.14) and VH-259 (0.15). Conversely, AA-703 obtained the highest value (0.40), which was further followed by CIM-602 (0.33) and MNH-992 (0.31).

Table 2. Analysis of variance for total dry weight

Source	DF	SS	MS	F-Value
Gen	24	0.88	0.03	20.16**
Treatment	3	4.87	1.62	886.97**
Gen × Treatment	72	0.34	0.0047	2.59**
Error	200	0.36	0.0018	

** = Highly Significant

CIM-599 had the lowest value (0.21) under 20 dS/m of salt stress, followed by Tarzan-1 (0.22) and IUB-63 (0.23). On the contrary, AA-703 attained the highest value of 0.49, which was succeeded by CIM-446 (0.46) and MNH-992 (0.42) (Figure 1A). The stress susceptibility index (SSI) metrics under 10 dS/m of salt stress revealed that NS-121 demonstrated the lowest value (0.45), followed by FH-158 (0.47) and Tarzan-1 (0.56). In contrast, MNH-992 obtained the highest value (1.611), with IUB-65 (1.45%) and MNH-996 (1.32) following closely. CIM-599 had the lowest stress susceptibility index (SSI) under 15 dS/m salt stress (0.44), followed by FH-158 (0.56) and Tarzan-1 (0.61). On the other hand, CIM-602 (1.50) and MNH-992 (1.39), in that order, succeeded AA-703 (0.40), which had the lowest value. FH-158 exhibited the most minimal value (0.60) when subjected to salt stress of 20 dS/m, with Tarzan-1 (0.63) and CIM-599 (0.67) following closely behind.

On the contrary, AA-703 had the highest value of 1.70, followed by CIM-446 (1.49) and MNH-992 (1.44) (Figure 1B). The stress susceptibility percentage index (SSPI) results showed that NS-121 had the lowest value (2.63), followed by Tarzan-1 (3.01), while MNH-992 had the highest value (10.31), followed by IUB-65 (10.11), under the salt stress level of 10 dS/m. The results of the SSPI under 15 dS/m salt stress revealed that CIM-599 achieved the minimum value of 6.7, with Tarzan-1 followed closely at 9.2. On the other hand, AA-703 achieved the highest value of 25.59, while CIM-602 had a value of 21.32. CIM-599 exhibited the minimum value (13.61) when subjected to a salt stress level of 20 dS/m, with Tarzan-1 following closely behind at 13.90. In contrast, AA-703 achieved the highest value of 31.21, followed by CIM-446, with a value of 28.15 (Figure 1C). According to the salt tolerance index (STI), under 10 dS/m of salt stress, Hataf-1 had the highest value (1.25), followed by CIM-446 (1.12), while IUB-63 had the lowest value (0.60), followed by CIM-599 (0.62). The salt tolerance index, assessed under 15 dS/m of salt stress, revealed that Hataf-1 had the highest value (1.02), followed by FH-158 (0.88). In contrast, IUB-63 had the lowest value (0.50), followed by Cyto-178 (0.78). When subjected to salt stress of 20 dS/m, FH-158 exhibited the highest value of 0.77, with Hataf-1 following closely at 0.75. In contrast, Cyto-178 achieved the lowest value of 0.42, followed by IUB-63, with a value of 0.43 (Figure 2A). Under 10 dS/m of salt stress, Hataf-1 had the highest yield index (YI) (1.22), followed by CIM-446 (1.13), while IUB-63 had the lowest (0.83), followed by VH-259 (0.84). When subjected to salt stress of 15 dS/m, Hataf-1 exhibited the highest yield index of 1.25, followed by FH-158 (1.20). On the other hand, Cyto-178 had the lowest yield

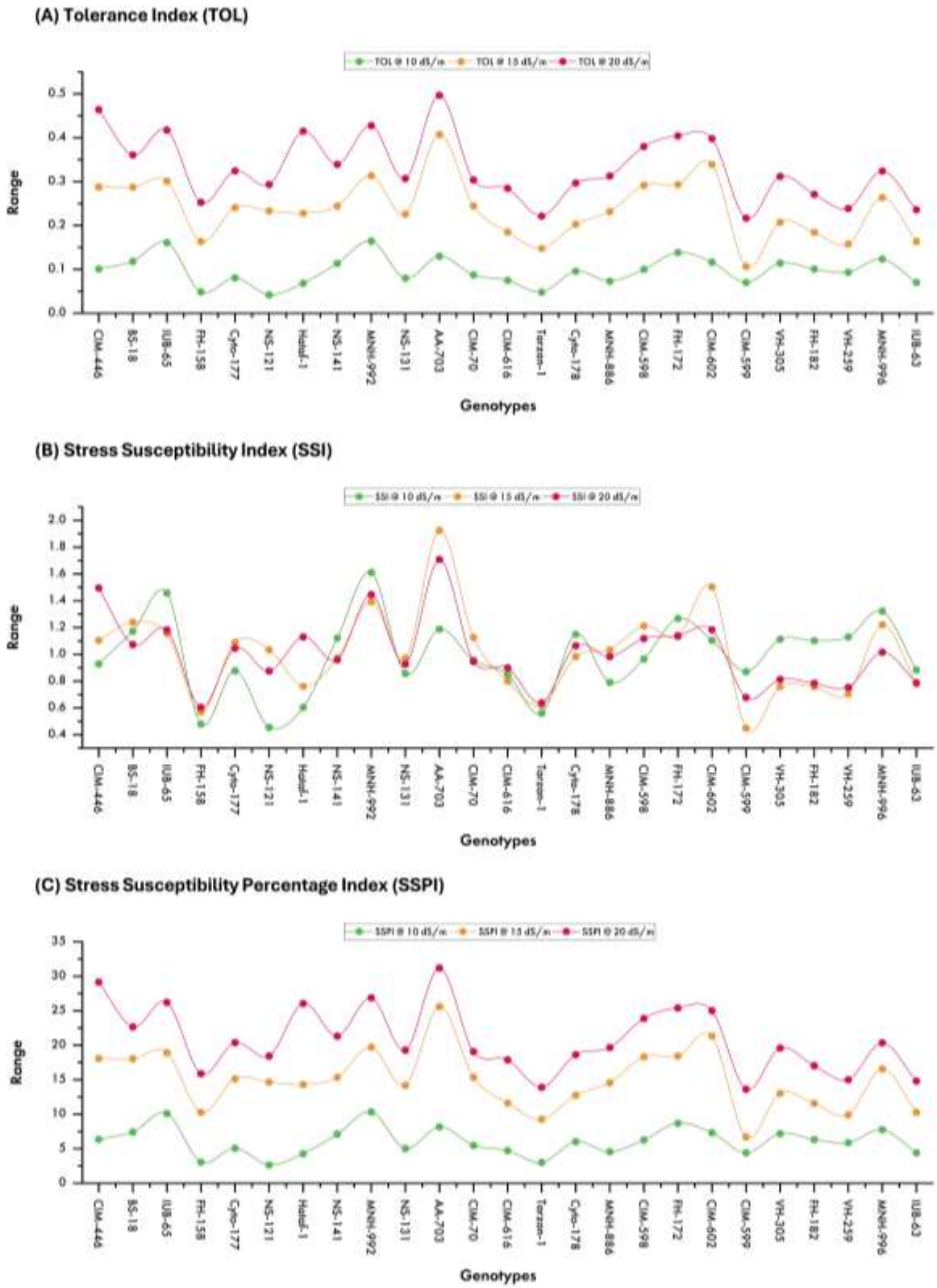

Figure 1. Tolerance index, stress susceptibility percentage index and stress susceptibility index for cotton genotypes under three different salt stress regimes.

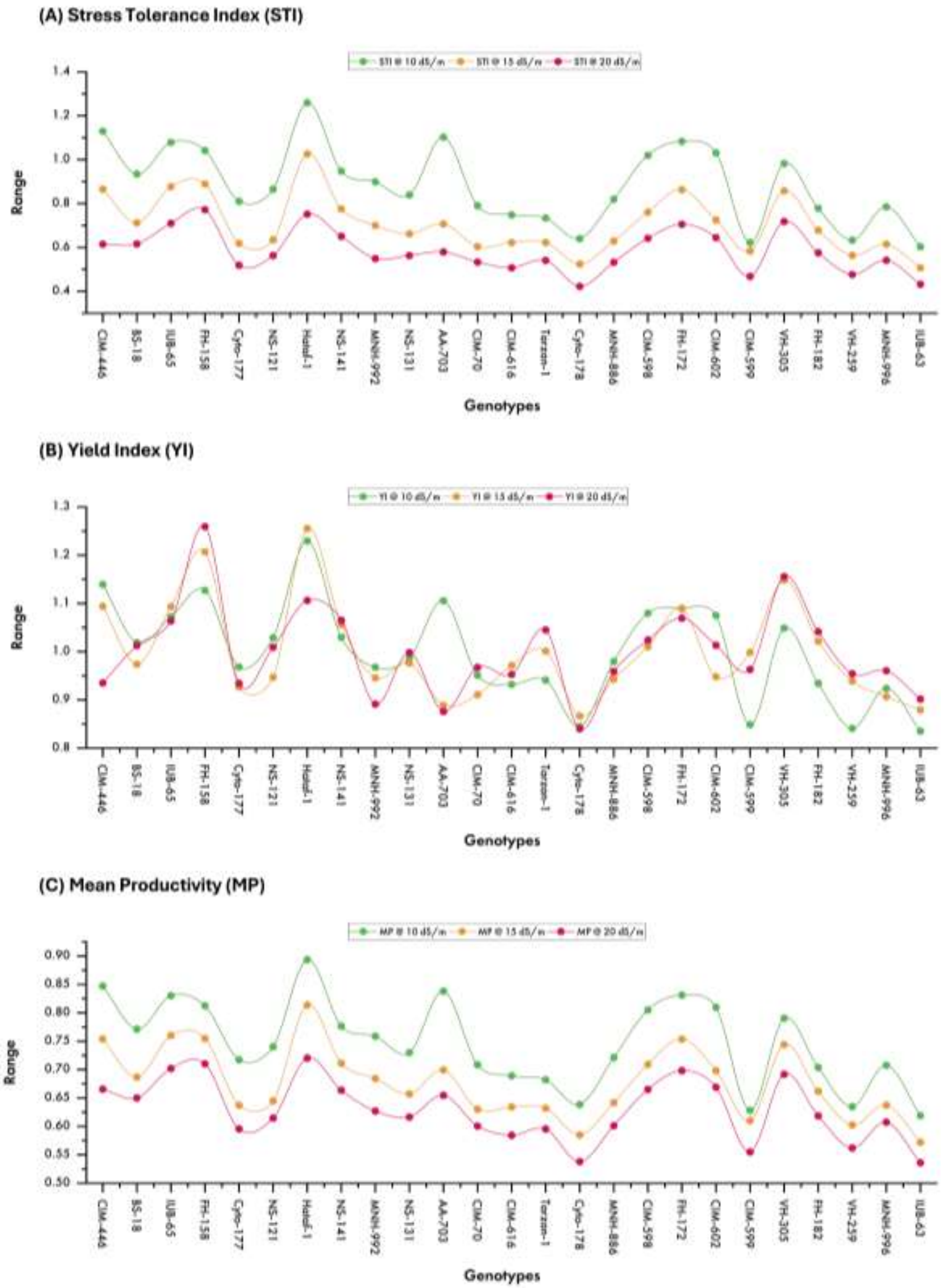

Figure 2. Stress tolerance index, yield index, and mean productivity for cotton genotypes under three different salt stress regimes.

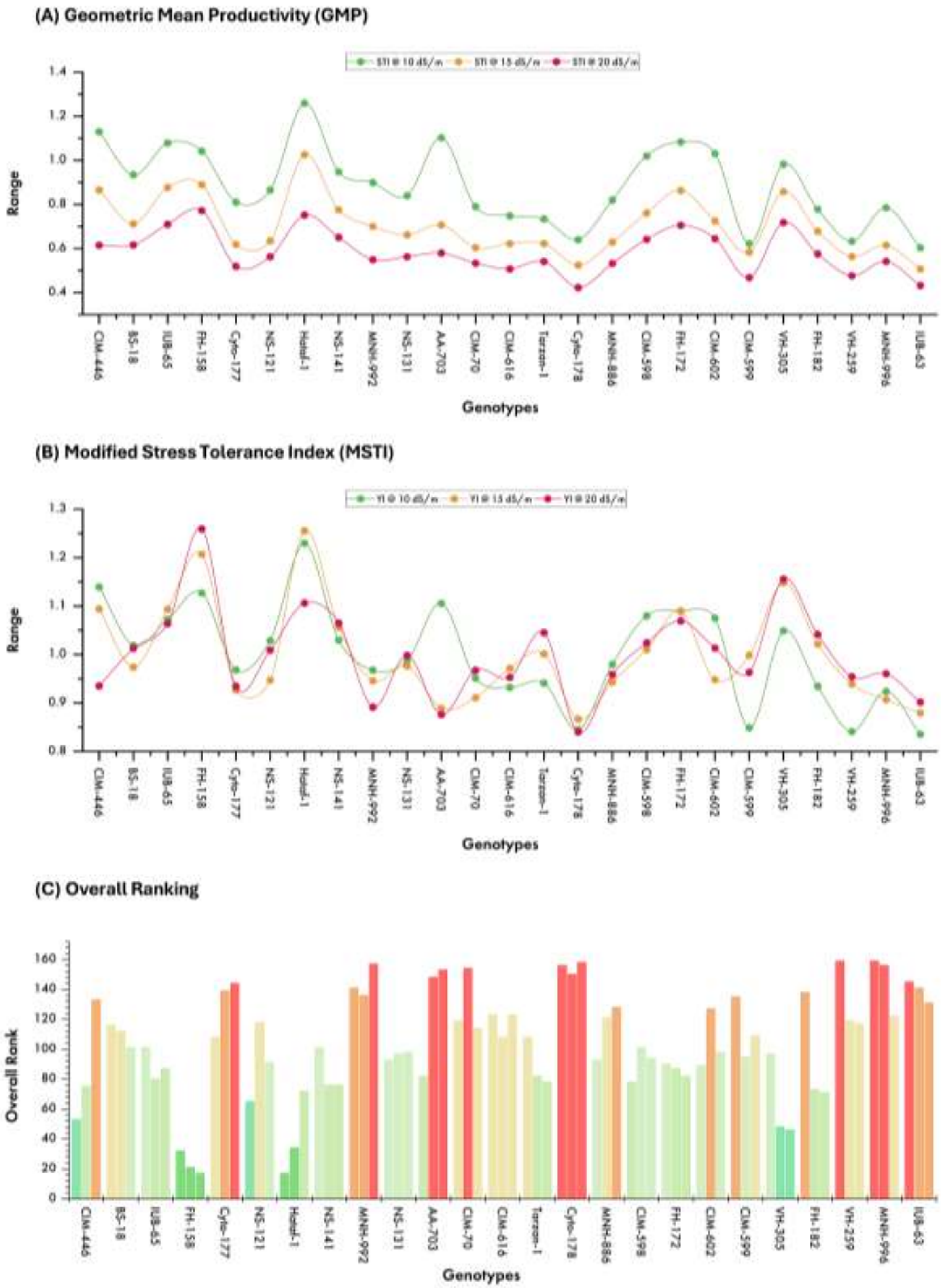

Figure 3. Geometric mean productivity, modified stress tolerance index, and overall ranking for cotton genotypes under three different salt stress regimes.

index of 0.86, followed by IUB-63 with a value of 0.87. Under 20 dS/m of salt stress, FH-158 had the highest yield index value of 1.25, followed by VH-305 (1.15). In contrast, Cyto-178 achieved a minimum score of 0.84, followed by AA-703, with a value of 0.87 (Figure 2B). The mean productivity (MP) results indicated that Hataf-1 exhibited the highest value (0.89), followed by CIM-446 (0.84), while IUB-63 displayed the lowest value (0.61), followed by CIM-599 (0.62), under the salt stress level of 10 dS/m. When exposed to salt stress of 15 dS/m, Hataf-1 exhibited the highest value of 0.81, with Tarzan-1 closely behind at 0.76. Conversely, IUB-63 had the lowest recorded value of 0.67, closely followed by Cyto-178, with a value of 0.58. Under a salt stress level of 20 dS/m, Hataf-1 had the highest value of 0.72, with FH-158 closely following at 0.71. In contrast, IUB-63 had the lowest value of 0.536, followed by Cyto-178, with a value of 0.538 (Figure 2C). Under 10 and 15 dS/m of salt stress, Hataf-1 had the highest geometric mean productivity (MP) values, 0.89 and 0.80, respectively, while IUB-63 had the lowest values in both salt regimes (0.61 and 0.56). When exposed to a salt stress level of 20 dS/m, FH-158 exhibited the highest value of 0.69, followed closely by Hataf-1 at 0.68. On the contrary, Cyto-178 acquired the minimum value of 0.51, followed by IUB-63 (0.52) (Figure 3A). The results of the modified stress tolerance index (MSTI) indicated that Hataf-1 had the highest value (1.35), followed by CIM-446 (1.09), while MNH-992 had the lowest value (0.43), followed by VH-259 (0.44), at a salt stress level of 10 dS/m. Under 15 dS/m salt stress, the MSTI results showed that Hataf-1 had the highest value of 1.28, closely followed by FH-158 at 1.09. Conversely, Cyto-178 and IUB-63 had the lowest values, at 0.43 and 0.44, respectively. FH-158 demonstrated the highest value (1.12) at salt stress conditions of 20 dS/m, with VH-305 closely trailing at 0.92. On the other hand, Cyto-178 demonstrated the lowest value of 0.37, while IUB-63 attained a value of 0.43 (Figure 3B).

Discussion

Cotton is generally considered to have a moderate level of salt tolerance, although there are significant variations in salt tolerance among different species and cultivars (Maryum *et al.*, 2022; Muhammad *et al.*, 2023). It is widely recognized that the level of tolerance exhibited by adult plants indicates the level of tolerance exhibited by seedlings (Rehman and Iqbal, 2022). Due to the variable abilities of different cultivars to adapt to environmental traits, it is crucial to consider their interactions. This emphasizes the importance of evaluating genotypes in various contexts to identify the most suitable ones. The current study revealed considerable variations in the total dry weight of seedlings among different genotypes (Table 2), indicating a wide range of variability that allows us to identify salt-tolerant genotypes (Tiwari *et al.*, 2013). The considerable variance in the total dry weight of seedlings under control and salt stress circumstances suggests the presence of genetic diversity and the potential for selecting advantageous genotypes in both conditions (Farooq *et al.*, 2018). Consistent with previous research, which found that salinity reduced photosynthetic capacity and, in turn, biomass buildup in plants, the present data shows that total dry weight has decreased considerably with an increase in salt stress (Basel, 2011; Shaheen *et al.*, 2012; Ma *et al.*, 2021). The reduction in the overall dry mass of cotton seedlings under salt stress can be linked to a convergence of physiological and biochemical variables (Basel, 2011). The imbalance in salt concentrations, known as osmotic stress, prevents roots from taking in water, which causes cellular drought and stunts growth (Munawar *et al.*, 2021). In addition, the accumulation of sodium and chloride ions due to salt stress can lead to ion toxicity, which disrupts the key metabolic processes and the intake of nutrients (Guo *et al.*, 2020). So, one plausible explanation for the decrease in dry matter could be the augmented inhibition of mineral nutrient absorption and utilization by plants experiencing salt stress. According to Pessarakli (2021), as salinity increased, cotton plants exhibited a decline in dry matter, predominantly attributable to a decrease in nitrogen uptake.

Researchers have employed diverse techniques to assess genetic variations in salt tolerance and to find the most salt-tolerant cotton varieties. Researchers have turned to stress tolerance indices, generally utilized to measure salt tolerance (Dong *et al.*, 2020; Sikder *et al.*, 2020a). The yield differential between stress and non-stress conditions is the stress tolerance (TOL) metric (Rosielle and Hamblin, 1981). At the same time, an SSI of more than 1 indicates a higher-than-average vulnerability to stress, while an SSI of less than 1 indicates a lower-than-average vulnerability (Abro *et al.*, 2015). A smaller TOL, SSI, and SSPI value is preferred among the stress tolerance indicators since bigger values indicate a comparatively higher sensitivity to stress (Devendra *et al.*, 2006). Genotypes with limited yield potential under non-stress settings and high yield under stress conditions are preferred in selection based on these parameters. In our present study, the tolerance and stress susceptibility indexes indicated that genotypes, particularly NS-121, Tarzan-1, and CIM-599, obtained the lowest score under salt stress conditions besides scoring less than 1 for the stress susceptibility index (Figure 1). It was interesting to observe that the stress susceptibility percentage index (SSPI) also identified these genotypes with minimal ranking variation. Similar results have been reported by Taghizadeh *et al.* (2018) and Mahdy *et al.* (2021). Breeders interested in relative performance frequently utilize geometric mean productivity (GMP) and means productivity (MP) since the degree of salinity stress might fluctuate over the years in the field (Mohammadi, 2016). Furthermore, in addition to these two factors, the yield index is an effective measurement metric that can be utilized to evaluate the relative performance under salt stress conditions (Anwaar *et al.*, 2020). In our present study, CIM-446 and Hataf-1 showed the higher relative performance for these three metrics, namely geometric mean productivity and means productivity and yield index. In contrast, IUB-63 and VH-259 recorded the lowest relative performance (Figure 2). The salt tolerance index and its modified version, the modified salt tolerance index (MSTI) developed to improve STI efficiency, indicated that CIM-446, Hataf-1, and VH-305 were salt tolerant as they obtained the highest values in both these indices while IUB-63 and Cyto-178 (Figure 3). However, it was interesting that both indexes showed similar genotypes, demonstrating a clear classification and variability under stress and non-stress conditions. Similar findings have been reported by Singh *et al.* (2015) and Bakhshi *et al.* (2021).

The computed salt tolerance indices revealed that identifying salt-tolerant genotypes based on a single criterion was conflicting. Various indices have identified distinct genotypes that exhibited salt tolerance. So, in order to determine the most desirable and tolerant genotype, the total rank of all salt tolerance indicators was calculated (Figure 3C). The genotype that scored the lowest based on this criterion was determined to be the most desirable salt-tolerant genotype. After evaluating all indices, the genotypes Hataf-1, FH-158, CIM-446, and VH-305 were the most salt tolerant. On the other hand, the genotypes Cyto-178, VH-259, MNH-996, MNH-992, NS-131, and AA-703 were found to be the most sensitive to salt. Researchers can effectively determine genotypes with higher salt tolerance degrees by evaluating the salt tolerance indices of different genotypes (Kumawat *et al.*, 2017). Breeders can use the genetic diversity in these populations to their advantage by selecting salt-tolerant cultivars from the most promising individuals (Hanin *et al.*, 2016). The current work essentially reaffirms that stress tolerance indices can identify cultivars with high tolerance to salt stress.

Conclusion

Our thorough evaluation in this study revealed that salt stress significantly and negatively impacts the total biomass of cotton plants during their earliest growth stages. It was observed that plant dry weight decreased as salt stress increased, with notable variance observed between cultivars. Since genetic variations serve as the foundation for improvement in cotton, breeders can improve desired characteristics in the succeeding generations. The results of this study definitively showed that salt tolerance indices can be efficiently and swiftly used to screen cotton germplasm for salt tolerance. They also offer valuable insights into salinity stress tolerance, which can help cotton breeders identify and improve genotypes that are resistant to salt. Based on the findings of this study, the genotypes Hataf-1, FH-158, CIM-446, and VH-305 exhibited the highest salt stress tolerance. Consequently, it can be advised to cultivate these genotypes in saline conditions and utilize them in subsequent breeding programs to develop cotton cultivars that can withstand high levels of salt stress.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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