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Assessing Salt Stress Responses in Cotton Genotypes: Insights for Breeding Initiatives

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The growth and production of cotton on a global scale are significantly threatened by salinity stress. This study investigates the salt stress responses of eight cotton genotypes, focusing on their physiological and biochemical characteristics under varying salinity levels. Conducted at the Department of Plant Breeding and Genetics, University of Punjab, Lahore, the research employed a triple completely randomized design with two salinity levels and a control group. Genotypes were selected based on production potential and adaptability to Pakistan's cotton-growing regions. Physiological assessments included sodium (Na^+) and potassium (K^+) ion concentrations, while biochemical parameters encompassed antioxidant enzyme activities, soluble protein levels, and proline content. Statistical analysis revealed significant variations among genotypes and salinity levels, indicating genetic diversity and diverse reactions to salt stress. Genotypes displayed altered growth metrics, ion homeostasis, and K^+/Na^+ ratios under salt stress, highlighting their sensitivity and potential breeding value. However, it is crucial to thoroughly examine all associations between genotype and environment. The research listed above provide significant insights into the responses of cotton seedlings to salt stress.

Keywords: Salinity, Genotypes, Physiological Assessments, Cotton, Potassium.

Introduction:

Cotton, renowned for its delicate and soft fibers, originates from the protective boll near the plant seeds and is classified under the genus Gossypium, part of the family Malvaceae within the Malvales order, and belonging to the Gossypieae tribe. However, its production encounters numerous challenges, including biotic factors such as pest infestations and abiotic stresses. Among these, abiotic stress induced by environmental conditions significantly impacts plant growth, development, productivity, and seed quality [1].

Salinity stress, arising from elevated soil and water salt levels, severely limits global agricultural output, affecting around 20% of irrigated land. This has spurred increased efforts to develop salt-resistant agricultural varieties, especially for upland cotton, a major cash crop globally. Despite its widespread cultivation, upland cotton's yield diminishes significantly under salt stress, disrupting plant growth, photosynthesis, causing ion toxicity, and leading to oxidative damage [2]. Addressing salinity and sodicity issues in major irrigation projects is vital for improving productivity on salt-affected lands, transforming environmental challenges into economic opportunities. However, the lack of clear indicators for salinity tolerance hampers efforts in breeding salt-resistant upland cotton varieties [3].

Saline soils contain salt concentrations that inhibit plant growth, while non-saline soils have salt concentrations that do not disrupt soil structure. Salinity stress is particularly problematic in arid and semi-arid regions, especially affecting low-lying areas. Regions across Asia, the Pacific, and Australia are significantly impacted by salt, with approximately 6% of the total land area facing salinity issues. Salt stress triggers various plant responses—morphological, biochemical, and physiological—to cope with adverse effects [4]. In Pakistan, out of 22 million hectares of agricultural land, 6.28 million hectares are affected by salt. Among the salt-affected land, about 60.5% is salinesodic, while the remaining 39.5% suffers from salinity issues. Primary causes of salinity include improper agricultural practices, low rainfall, high evaporation rates, poor drainage, and the use of brackish water for irrigation [5]. With projections indicating that half of cultivated lands may face salt stress by 2050, addressing salinity during early plant stages, especially germination and seedling phases, is crucial due to the acute damage observed during these stages.

Salinity ranks as the second-most prevalent abiotic stress factor following drought, impacting not only plant growth but also steadily diminishing optimal crop yields globally [6]. Cotton's economic importance has propelled it to the designation of Pakistan's "white gold." As per researcher [7], cotton accounts for 0.6% of the country's gross domestic product (GDP) and constitutes 3.1% of the total value-added agricultural commodities in the nation. China currently has the prominent position as the foremost producer of Cotton, preceded by India, the United States of America, and Brazil. Pakistan holds the fifth position in the global ranking for cotton output. Cotton is an essential source of fiber and has a significant role in the production of edible oil in Pakistan. However, abiotic stresses, such as salinity, greatly impede the cultivation of cotton. Salinity stress has a significant effect on plant growth and presents a significant environmental problem, particularly in areas with little rainfall and semi-arid climates [8].

Globally, more than 800 million hectares face consistent salinity challenges. This affected area expands due to factors like climate change, rising sea levels, inadequate surface irrigation practices, and deficient drainage systems. Projections suggest that if current salinity trends persist, half of the cultivated land worldwide will grapple with salinity stress by 2050 [9]. In China alone, approximately 36 million hectares, accounting for 4.88% of available land, experience salinity stress. The global decline in cultivable land, coupled with escalating competition between cereal and fiber crops, is prompting a shift of cotton cultivation to saline and alkaline soils [10]. Salt resistance encompasses various complex mechanisms, with plants developing strategies at cellular, subcellular, and organ levels to combat this stress. The primary impact of salinity stress manifests as osmotic stress, initiated by heightened Na^+ and Cl^- concentrations in the root zone. This leads to ionic toxicity as salt accumulates in mature leaves due to transpiration. Ionic toxicity not only disrupts cellular metabolic processes but also hampers plant photosynthesis and triggers oxidative damage. Prolonged exposure to salt stress can result in biomass reduction and potential programmed cell death [11]. Additionally, excessive toxic ion accumulation in saline environments can disrupt K^+/Na^+ ion balance, elevate reactive oxygen species (ROS) generation, and accelerate lipid peroxidation. Plants respond by enhancing osmotic adjustment, regulating stomatal opening, balancing ion levels, boosting antioxidant defenses, and maintaining tissue water content [12][13]. However, the degree of salt tolerance varies depending on factors like growth stage, duration of exposure, salt concentration, plant species, and evaluation methods [14].

Objectives:

The objectives of the research article encompass a comprehensive investigation into the impact of salt stress on various aspects of cotton genotypes. Firstly, the study aims to evaluate the physiological and biochemical responses of different cotton genotypes when subjected to salt stress conditions, shedding light on their adaptive mechanisms. Through this evaluation, the research seeks to identify salt-tolerant cotton genotypes based on a holistic assessment of their morphological, physiological, and biochemical characteristics.

Literature review

Numerous studies have presented empirical evidence suggesting that the germination and seedling stages of cotton demonstrate a greater vulnerability to salt stress in comparison to the horizontal growth phases. Researcher [15] have projected that salt stress is anticipated to impact around 20% of cultivated land and nearly 50% of irrigated land worldwide. Elevated salinity has a negative effect on plant growth and production as it affects various physiological processes, including photosynthesis, osmotic balance, nutrient uptake, and other metabolic pathways. Although various approaches such as land reclamation techniques and the incorporation of organic manures into soil have been investigated, the creation of salt-tolerant cotton genotypes continues to be a viable and economical solution. Cotton has the ability to tolerate modest levels of salt; but, higher concentrations significantly affect its growth, development, fiber production, and overall quality. According to [16], the global cotton industry is encountering notable economic and environmental obstacles as a result of escalating salinization rates and the diminishing availability of arable land. Therefore, it is crucial to enhance cotton's ability to tolerate salt and explore methods to improve saline-alkali soil, thereby ensuring the consistent and sustainable development of the cotton industry. The genetic composition of plants influences their sensitivity to salt stress. According to its threshold level of 7.7 dSm-1, cotton is categorized as a crop that exhibits the ability to tolerate moderate levels of saline. [17] reported that the imposition of salt stress at concentrations of up to 15 dSm-1 can lead to a reduction of 50% in cotton yield per unit area. As per researcher [18] have reported that increased concentrations of salt in soil have adverse effects on a range of plant developmental and physiological processes. These mechanisms encompass the maintenance of cell water balance, improve the efficiency of nutrient absorption, regulate the rates of respiration and transpiration, stimulate development, and influence hormone regulation cc.

The emergence of cotton seedlings was delayed by 4-6 days when exposed to saline stress levels ranging from 10-15 dSm-1. Reported that salinity has a negative effect on the germination stage, possibly due to ion toxicity, uneven osmotic pressure, and the production of reactive oxygen species (ROS). The primary elements that determine plant growth restriction under salt stress are three important factors. To begin with, osmotic stress is a consequence of a reduction in water potential. Furthermore, the phenomenon of ion poisoning arises as a result of the increased uptake of sodium and chloride ions. Insufficient buildup or aberrant uptake of key plant nutrients from the soil can lead to nutritional shortages [20]. Plants have a proactive absorption of sodium and chloride, however in times of salt stress, they have a tendency to accumulate these elements to fatal levels. [21] reported that soils damaged by salt demonstrate a higher concentration of Na+, Mg++, Cl-, and SO- ions relative to essential elements such as K+, Ca++, H2PO-, and NO-. This disparity results in inadequate nutrition levels and abnormal movement of nutrients within plant tissues [22][23]. The occurrence of potassium and calcium ion absorption instability in plants has been documented in the presence of a significant sodium salt gradient. During instances of salt stress, calcium plays a crucial role in the maintenance of the sodium (Na+) gradient across the plasma membrane of root cells. Furthermore, it specifically promotes the uptake of potassium (K+), thereby assisting in the removal of salt from root cells [24]. The maintenance of the ideal Na+/K+ ratio in plant cells is more important for plant function under salt stress, rather than just concentrating on the removal of Na+ ions. The underlying cause of this phenomena can be attributed to the role of potassium (K+) as a regulator of stomatal aperture. According to [25], a decrease in potassium (K+) concentrations within the guard cells results in the closing of stomata, hence causing adverse impacts on plant physiology and growth.

This study facilitated the identification of salt-tolerant genotypes of cotton. The criteria for selection included important metrics like as biomass production, shoot length, and root length. Researchers [26][27] have reported that cotton plants with a high root/shoot ratio have decreased biomass due to the increased salt concentration in the soil. The observed augmentation in root growth in genotypes such as NIAB-545 and FH-490 under salt stress conditions can be attributed to osmotic compensation, a mechanism that facilitates the maintenance of root development even in the face of low water potential. Elevated salt stress adversely affects the growth and development of plants. As per the findings of there is a decrease in the shoot-to-root ratio when subjected to salt stress, indicating that shoot growth is more vulnerable to the negative effects of salt stress when compared to root growth. Furthermore, the variety of environmental factors that impact salt stress tolerance indices adds complexity to the selection process, making it intrinsically more challenging. The aim of this study is to evaluate the salt tolerance capabilities of several genotypes during the phase of seedling development. Considering the importance of the seedling stage in the comprehensive development of plants, it is crucial to investigate the modifications and adaptations that occur in reaction to salt stress conditions [28]. This study's findings will make a significant contribution to the discovery of salt-tolerant genotypes and salt-vulnerable genotypes. This approach would enhance the effective utilization of saline and dry regions in order to expand the cultivation of cotton. Furthermore, this study will offer valuable insights into effective breeding strategies aimed at enhancing the salt stress tolerance of cotton.

Methodology:

Figure 1 illustrates the flow of methodology utilized for this study.

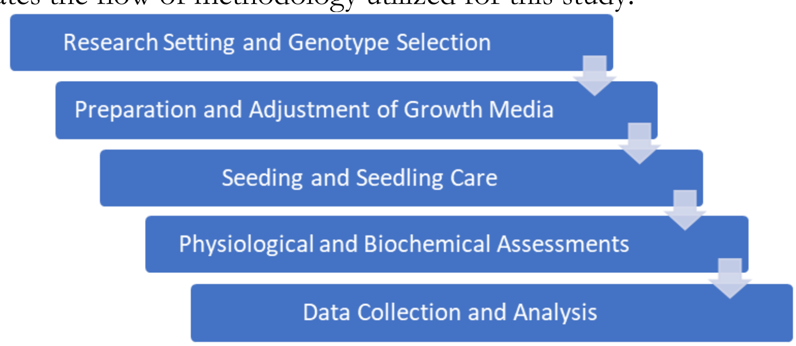

Figure 1: Flow of methodology

Research Setting and Genotype Selection:

The research was conducted at the Department of Plant Breeding and Genetics, University of Punjab, Lahore. Eight cotton genotypes were selected from various institutions including the Central Cotton Research Institute Multan, Nuclear Institute of Agriculture and Biology, Cotton Research Station Multan, and Ayub Agricultural Research Institute based on production potential and adaptability to different cotton-growing regions in Pakistan [29][30].

Table 1: Genotypes of Cotton seed

Genotype	Origin	Characteristics
NIAB - 545	Central Cotton Research Institute Multan	High salt tolerance, good yield potential
FH- Kehkshan	Ayub Agricultural Research Institute	Moderate salt tolerance, early maturity
FH-490	Central Cotton Research Institute Multan	High yield potential, medium salt tolerance
GH-Mubarak	Nuclear Institute of Agriculture and Biology	Good fiber quality, moderate salt tolerance
FH-303	Cotton Research Station Multan	Early maturity, low salt tolerance
FH-532	Ayub Agricultural Research Institute	High yield potential, moderate salt tolerance

FH-542	Central Cotton Research Institute Multan	Good fiber quality, low salt tolerance
SB-149	Cotton Research Station Multan	Moderate yield potential, high salt tolerance

Experimental Design:

A triple completely randomized design was employed, incorporating two salinity levels and a control group. Each genotype was grown in four plastic cups filled with a mud and sand mixture (1:2 ratio) serving as the growth medium.

Preparation and Adjustment of Growth Media:

Prior to seeding, the growth media's Electrical Conductivity (EC), saturation percentage, and pH were measured and adjusted to optimal levels: EC of 1.2 dS/m, saturation percentage of 60%, and pH range of 6.5-7.0. Regular monitoring and adjustments of EC and pH were conducted throughout the **e**xperiment.

Seeding and Seedling Care:

Initial seeding involved placing 3-5 seeds in each cup, followed by thinning to maintain one healthy seedling per cup. Seedlings were irrigated with a modified nutrient solution tailored specifically for salt stress during the initial 10-day period.

Physiological and Biochemical Assessments:

Physiological assessments focused on analyzing concentrations of sodium (Na^+) and potassium (K^+) ions using a flame photometer. Biochemical parameter analysis included extracting antioxidant enzymes, quantifying soluble protein levels, measuring superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX) activities, as well as estimating proline content and total soluble protein (TSP).

Data Collection and Analysis:

Data collection encompassed morphological measurements such as root length, shoot length, and seedling dry weight, in addition to biochemical data. The K^+/Na^+ ratio, an important indicator of salt tolerance, was calculated. Statistical analysis was performed using SPSS (Statistical Package for the Social Sciences) and analysis of variance (ANOVA) at a significance level of 5%.

Visualization and Dimension Reduction:

Genotypic performance was visualized using a heatmap generated in SPSS software. Principal Component Analysis (PCA) was utilized for dimension reduction and assessing factors influencing salt tolerance.

Results:

The statistical analysis of variance revealed statistically significant variations among different cotton genotypes across all seedling characteristics, irrespective of whether they were exposed to control or salt stress conditions. This observation indicates a significant degree of genetic diversity. The study unveiled significant mean square values associated with the effects of salinity, suggesting variations in the size and degree of tension induced by the two distinct salt concentrations (8 dSm-1 and 16 dSm-1). The need of considering specific salt levels when assessing their impact on the growth and physiochemical characteristics of Cotton seedlings is underscored. The statistical study demonstrates a significant disparity in the variance of genotype salinity interactions, suggesting that genotypes display diverse reactions to differing degrees of salinity.

In both normal and saline settings, the graphical presentation offers a visual depiction of the relative performance of various genotypes. Genetic variations that demonstrate resistance to salt are represented by darker shades on the positive end of the color spectrum, whereas lighter shades suggest genotypes that are susceptible to salt. This study considered the seedling and biochemical characteristics under three different conditions: normal, 8 dSm-1, and 16 dSm-

1. The findings suggest a significant correlation between salinity and genotype interaction, as well as the K+/Na+ ratio of potassium to sodium ions, and this association is statistically significant at a 5% level of significance. Figure 2 shows that the genotype NIAB - 545 exhibits distinct responses to varying salt treatments as observed in the provided data. In terms of root length, there is a consistent decrease with increasing salt levels, evident from 8.8 cm in the control group to 7.5 cm and 6.2 cm at 8 dSm-1 and 16 dSm-1, respectively. This trend is mirrored in shoot length, where the control group shows the longest shoots at 11.2 cm, followed by reductions to 10.3 cm and 9.1 cm at higher salinities. The seedling dry weight also reflects this pattern, with the control group having the highest biomass at 42.5 mg, decreasing to 35.2 mg and 28.7 mg under salt stress. Regarding ion concentrations, potassium (K+) levels decline from 10.9 mmol/L in the control to 8.5 mmol/L and 6.1 mmol/L at 8 dSm-1 and 16 dSm-1, respectively. Conversely, sodium (Na+) concentrations increase with salinity, rising from 9.2 mmol/L in the control to 14.1 mmol/L and 18.9 mmol/L at higher salt levels. This shift in ion balance is reflected in the K+/Na+ ratio, which decreases from 6.12 in the control to 4.54 and 4.24 under salt stress. These trends illustrate NIAB - 545's reduced growth and altered ion homeostasis in response to escalating salinity levels.

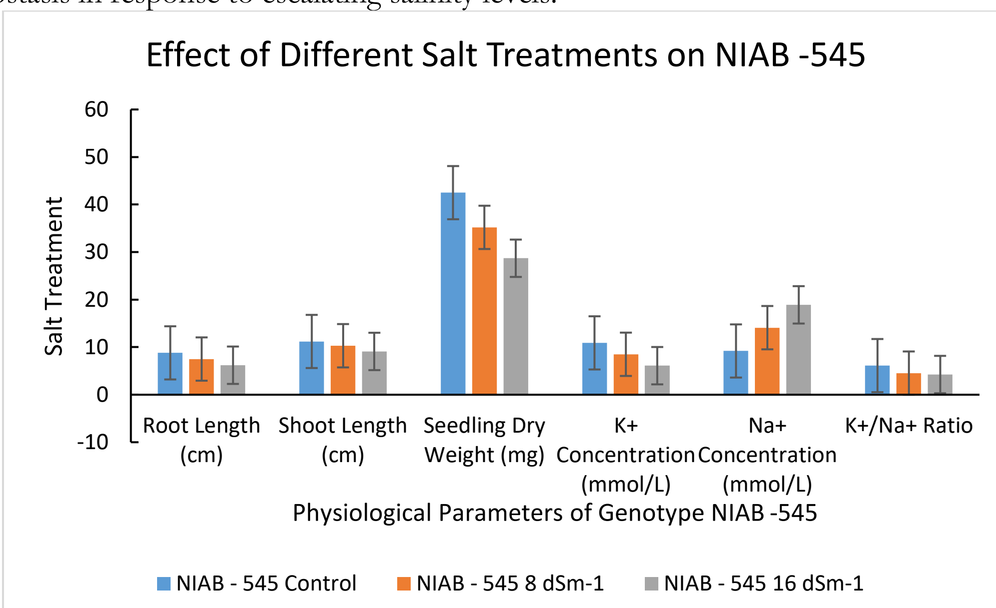

Figure 2: Effect of Different Salt Treatments on NIAB -54

FH-490 demonstrates notable variations in response to different salt treatments, as illustrated in figure 3. Depicting root length, there is a noticeable decline from 7.5 cm in the control group to 6.3 cm and further to 5 cm at 8 dSm-1 and 16 dSm-1, respectively. This trend is similarly reflected in shoot length, with the control group exhibiting the longest shoots at 10.8 cm, followed by reductions to 9.8 cm and 7.8 cm at higher salinities. The seedling dry weight also shows a decreasing pattern, with the control group having the highest biomass at 41.2 mg, decreasing to 36.8 mg and 30.5 mg under salt stress conditions. In terms of ion concentrations, potassium (K+) levels decline from 12.7 mmol/L in the control to 9.3 mmol/L and 9 mmol/L at 8 dSm-1 and 16 dSm-1, respectively. Conversely, sodium (Na+) concentrations increase with salinity, rising from 8 mmol/L in the control to 12.9 mmol/L and 17.5 mmol/L at higher salt levels. This shift in ion balance is reflected in the K+/Na+ ratio, which decreases from 6.18 in the control to 5.58 and 4.28 under salt stress. These findings highlight FH-490's reduced growth and altered ion homeostasis in response to escalating salinity levels, indicating its sensitivity to salt stress conditions.

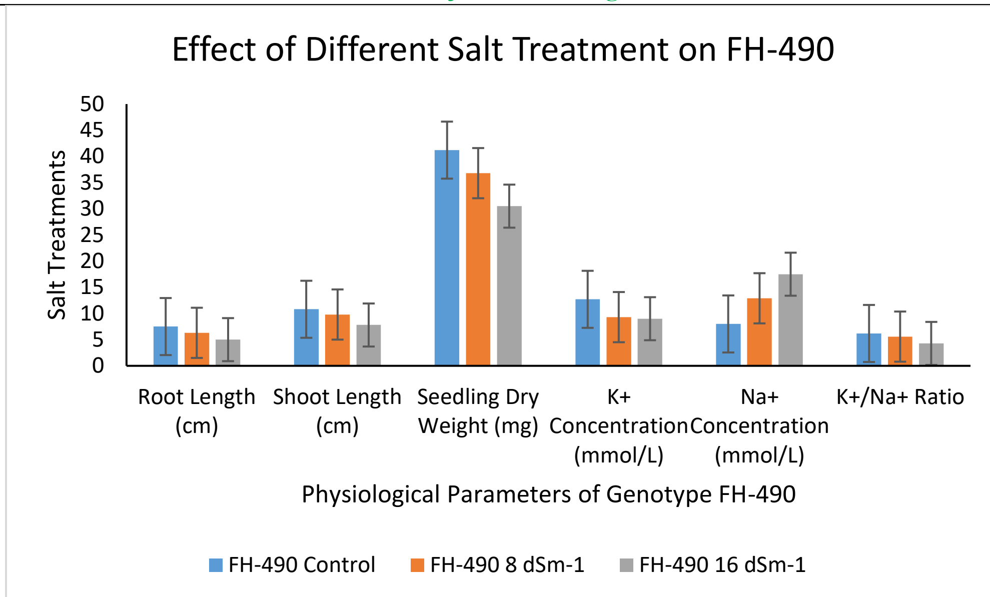

Figure 3: Effect of Different Salt Treatments on FH -490

GH-Mubarak, as depicted in the figure 4, showcases distinct responses across different salt treatments. Initially, in the control group, it exhibits a root length of 9.9 cm, which decreases to 8.6 cm and further to 5.3 cm at 8 dSm-1 and 16 dSm-1, respectively. A similar trend is observed in shoot length, with the control group recording the longest shoots at 8.1 cm, followed by reductions to 7.5 cm and 6.2 cm at higher salinity levels. The seedling dry weight also reflects this trend, starting at 43.2 mg in the control and decreasing to 37.9 mg and 31.6 mg under salt stress conditions. In terms of ion concentrations, potassium (K+) levels increase from 10.2 mmol/L in the control to 12.2 mmol/L at 8 dSm-1 and then decrease slightly to 8.5 mmol/L at 16 dSm-1. On the other hand, sodium (Na+) concentrations show an increasing pattern with salinity, rising from 9.3 mmol/L in the control to 13.8 mmol/L and 19.7 mmol/L at higher salt levels. Consequently, the K+/Na+ ratio decreases from 6.14 in the control to 4.61 and 3.29 under salt stress conditions. These results indicate GH-Mubarak's sensitivity to salt stress, with reduced growth and altered ion balance in response to increasing salinity levels.

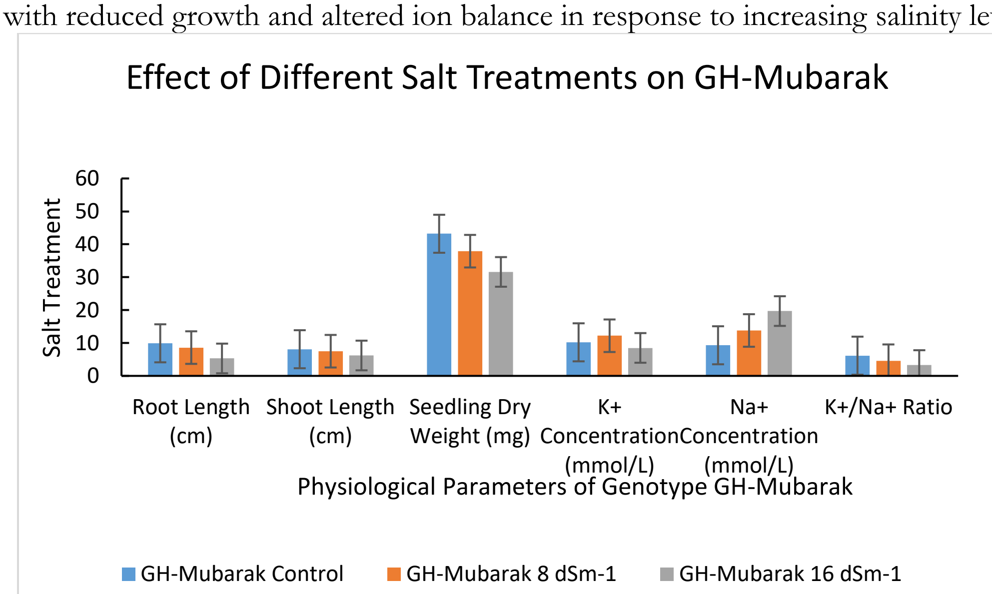

Figure 4: Effect of Different Salt Treatments on GH-Mubarak

FH-Kehkshan exhibits notable changes in response to varying salt treatments, as indicated by figure 5. In the control group, FH-Kehkshan demonstrates a root length of 9.2 cm, which reduces to 8.9 cm at 8 dSm-1 and further to 7.6 cm at 16 dSm-1. Similarly, shoot length follows a similar trend, starting at 12.5 cm in the control, decreasing to 10.8 cm at 8 dSm-1, and further to 9.4 cm at 16 dSm-1. The seedling dry weight also reflects this pattern, decreasing from 44.8 mg in the control to 38.5 mg and 32.1 mg under salt stress conditions.

Regarding ion concentrations, potassium (K+) levels start at 12.5 mmol/L in the control, decrease slightly to 9.8 mmol/L at 8 dSm-1, and further to 8.6 mmol/L at 16 dSm-1. In contrast, sodium (Na+) concentrations exhibit an increasing trend with salinity, rising from 8.5 mmol/L in the control to 13.5 mmol/L at 8 dSm-1 and 18.2 mmol/L at 16 dSm-1. Consequently, the K+/Na+ ratio decreases from 4.11 in the control to 2.68 and 2.37 under salt stress conditions. These results indicate FH-Kehkshan's response to salt stress, showing reduced growth parameters and altered ion concentrations with increasing salinity levels.

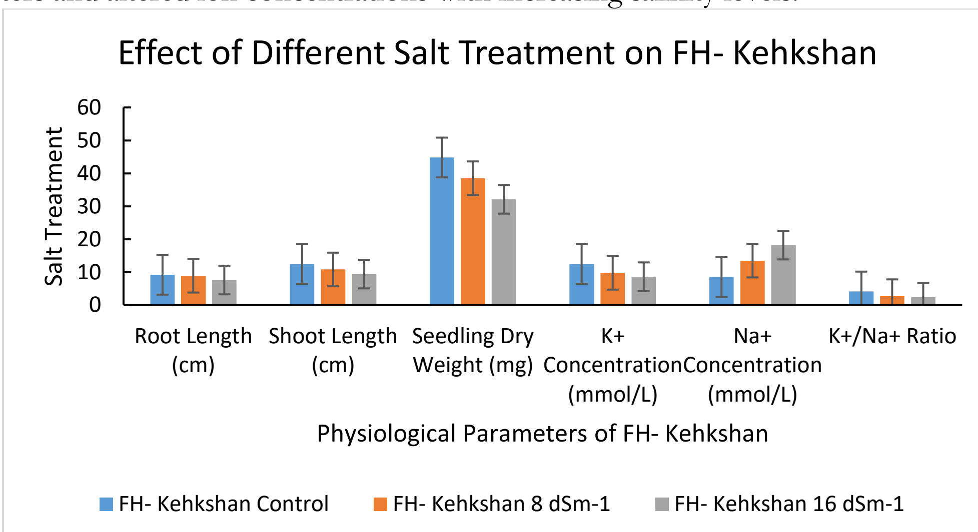

Figure 5: Effect of Different Salt Treatments on FH-Kehkshan

Figure 6 shows a consistent decrease in root length, shoot length, and seedling dry weight as salt treatment intensity increases. The K+ concentration decreases gradually, while Na+ concentration increases notably, resulting in a decreasing K+/Na+ ratio.

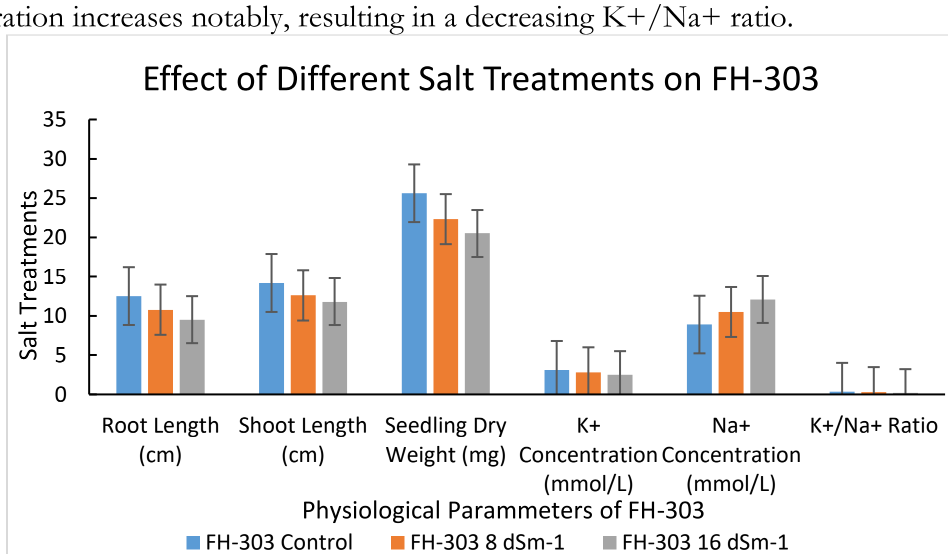

Figure 6: Effect of Different Salt Treatments on FH -303

Similar to FH-303, FH-532 exhibits reduced growth parameters under salt stress as shown in figure 7. Root length, shoot length, and seedling dry weight decrease with increasing salt treatment. K+ concentration decreases, Na+ concentration increases, and the K+/Na+ ratio declines as salinity levels rise.

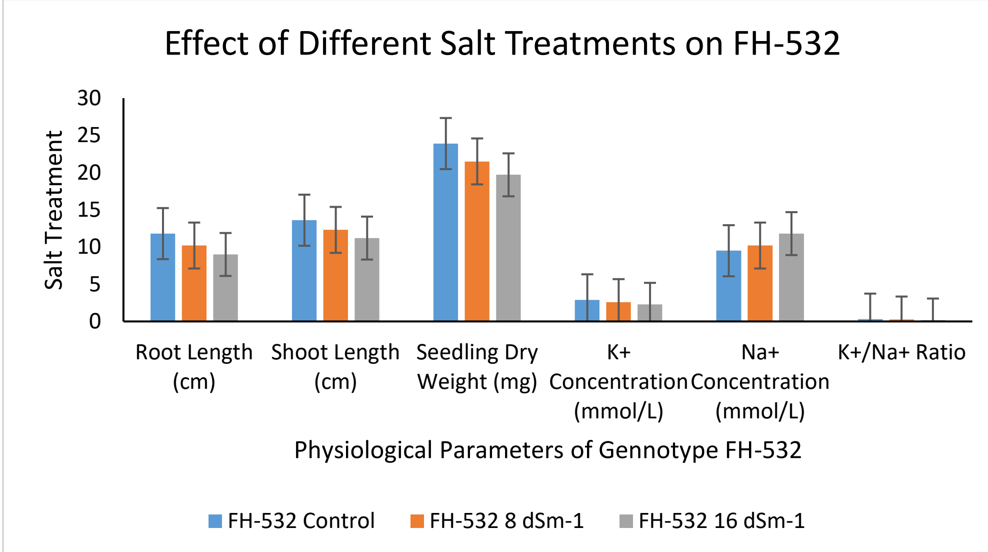

Figure 7: Effect of Different Salt Treatments on FH -532

FH-542 also demonstrates reduced growth metrics under salt stress conditions, as shown in figure 8. Root length, shoot length, and seedling dry weight decrease progressively with higher salt treatments. The K+ concentration decreases, Na+ concentration increases, and the K+/Na+ ratio decreases as salinity levels intensify.

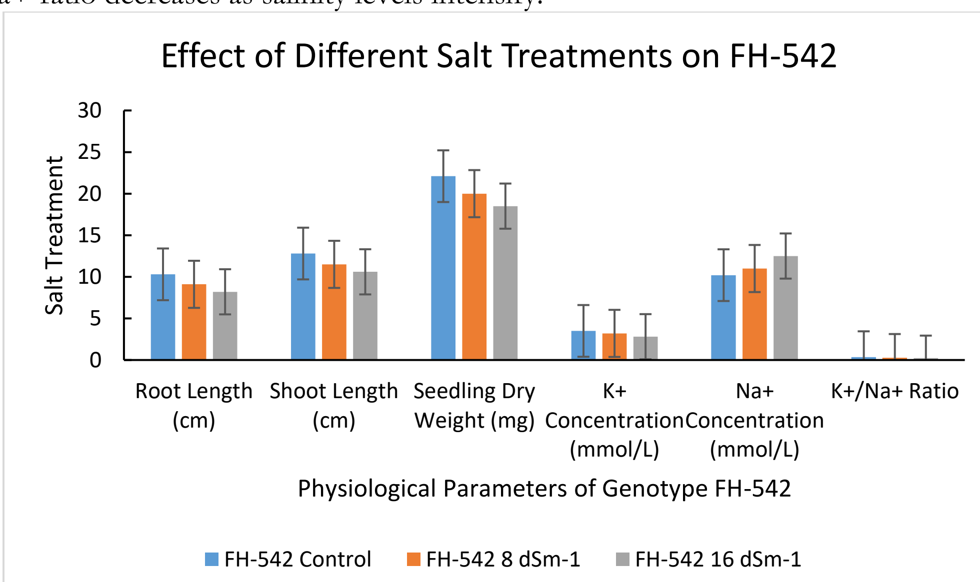

Figure 8: Effect of Different Salt Treatments on FH-542

In the case of SB-149 as shown in figure 9, there is a noticeable decrease in root length, shoot length, and seedling dry weight with increasing salt treatment levels. The K+ concentration decreases, Na+ concentration increases, and the K+/Na+ ratio shows a decreasing trend as salinity stress escalates.

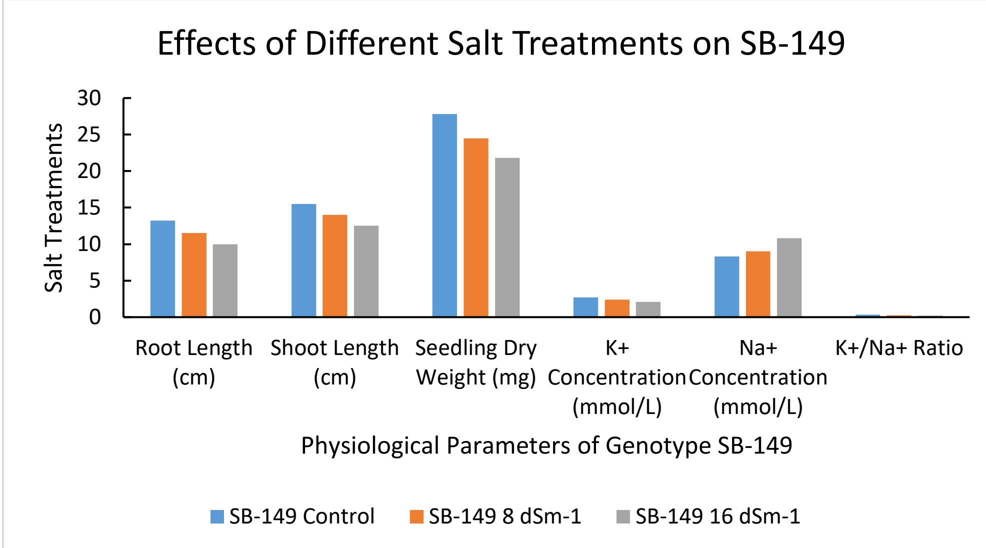

Figure 9: Effect of Different Salt Treatments on SB-149

Parameters encompassed in this study consist of sodium ion uptake (Na), root fresh weight, fresh weight of the entire plant , fresh weight of the shoot, potassium to sodium ions ratio (K.Na), root dry weight, shoot dry weight, shoot dry weight, shoot length, and potassium ion uptake (K).

Discussion:

Salinity exerts a considerable impact on growth-related characteristics, leading to reductions in shoot length, leaf numbers, and leaf area, collectively affecting the photosynthetic rate and overall biomass production. Leaf area, recognized as an immediate response to stress conditions, undergoes alterations under salt stress, altering leaf cellular structures and subsequently reducing the net photosynthetic rate. Biomass production is a critical indicator of stress severity, as emphasized in previous studies. In this study, all cotton genotypes experienced significant decreases in total biomass production under salt stress, identified as salt-sensitive. Existing literature suggests that salt-tolerant plants exhibit less biomass reduction and improved growth under salt stress compared to salt-sensitive counterparts, possibly due to functional disruptions in osmotic potential, ionic imbalances, and nutritional disparities. Other mechanisms contributing to lower biomass accumulation may involve energy redirection towards sodium ion exclusion, synthesis of compatible solutes, or direct effects on photosynthesis, aligning with similar growth inhibitions reported in various salt-stressed crops like tomato, wheat, melon, and maize.

The intricate relationship between photosynthesis and salt stress depends on several factors, including salt concentration, stress duration, and plant species. Stomatal conductance reduction can impede photosynthetic capacity in most glycophytes, including cotton. In our study, photosynthetic rates significantly declined with increasing salt concentration in the growth medium, although genotypes Z9807, Z0228, and Z7526 exhibited lower reductions in photosynthesis compared to the control, suggesting their ability to maintain higher photosynthetic rates by enhancing rubisco activity. Similar observations have been made in salt-tolerant cotton genotypes compared to salt-sensitive ones. Stomatal conductance also decreased under salt treatment, potentially due to increased abscisic acid levels or reduced stomatal aperture, as reported in salt-stressed cotton, pepper, date palm, melon, and sugarcane.

Pakistan faces a substantial obstacle in the form of salt stress, which has detrimental impacts on both food security and agricultural practices inside the country. This problem arises when there is an accumulation of high levels of salts in the soil due to various factors, including inefficient irrigation techniques, excessive extraction of groundwater, and changes in the hydrological cycle. According to [12], the application of salinity stress screening in pots during the seedling stage has been recognized as a feasible method with significant implications for the advancement of robust cotton cultivars. The evaluation of cotton plants' initial reactions to salt during the seedling stage is of utmost importance as it offers significant insights into their adaptability and capability for sustained growth under challenging conditions. Key parameters, such as shoot and root length, as well as biochemical indicators like the $K+/Na+$ ratio, have a major impact on the assessment of salinity tolerance. By conducting a systematic analysis of these parameters, researchers and breeders can identify cultivars that demonstrate enhanced salt tolerance, hence facilitating the development of resilient varieties through targeted breeding endeavors.

Genetic diversity enables the expression of different degrees of salt tolerance. The analysis of variance yields significant findings that offer vital insights into the intricate relationship between genetic factors and environmental pressures in cotton seedlings. The genetic diversity contained in genotypes is underscored by the observed variations in their properties, as emphasized by [10]. This research presents significant mean square values that are associated with the effects of salinity. These results represent alterations in the magnitude and degrees of stress induced by different quantities of salt. The presence of genetic variation among genotypes enables a broad spectrum of reactions in the presence of salt stress, spanning both phenotypes characterized by high tolerance and phenotypes characterized by high sensitivity. The importance of doing multi-environment screening is emphasized by the interaction effects between genotypes and environments, which give additional insight into the variable responses of different genotypes to different salinities.

The observation cited above underscores the need of considering different degrees of salinity when evaluating their impact on the growth and physiochemical traits of cotton seedlings. The findings pertaining to the variance of genotype salinity interactions indicate that different genotypes display distinct reactions to different levels of salt. The complex interplay between genetic factors and environmental pressures highlights the significance of acquiring a comprehensive understanding of how specific genotypes adapt and respond to varying salinity parameters. Consequently, the ability of plants to withstand salt stress is impacted by a range of morphological and physio-chemical characteristics. It is crucial to acknowledge that depending only on a solitary metric is not a reliable measure of salt tolerance. Furthermore, the variety of environmental factors that impact salt stress tolerance indices adds complexity to the selection process, making it intrinsically more challenging. It is imperative to employ selection indices that demonstrate higher heritability, alongside genetic advancement and screening across various salt stress levels, in order to identify genotypes that possess salt tolerance. To effectively assess salt tolerance, it is essential to employ a comprehensive approach that takes into account the challenges posed by environmental changes and the intricate interplay between genetic factors and salinity stress. The cotton genotypes were categorized into eleven distinct clusters, each indicating their distinct levels of resistance to salt stress. The evaluation of seedling performance in terms of morphological and biochemical parameters was carried out in this work, using the methodology outlined by [27]. Cluster 1 genotypes exhibited outstanding performance in salt stress conditions, as indicated by their higher levels of biomass output, shoot/root length, and shoot-root sodium-to-potassium ratio compared to the genotypes in the other two clusters. Moreover, these genotypes demonstrated reduced sodium levels, indicating a decrease in oxidative damage and serving as an indicator for salt-tolerant characteristics. The results indicated above highlight the significance of utilizing cluster analysis as a helpful analytical method for identifying salt-tolerant cotton genotypes, considering their morphological and physiological traits.

Salt has a substantial impact on growth indicators, including shoot length, root length, and root and shoot weight, resulting in a collective reduction in the overall biomass of the entire plant. [29] emphasize that the evaluation of salt stress tolerance is mostly based on the measurement of root and shoot length. The cotton genotypes NIAB-545 and FH-490 demonstrated the capacity to maintain average root lengths of 12.5 cm and shoot lengths of 9.8 cm under the influence of salt stress at a concentration of 17 dSm-1. Conversely, the genotype FH-303, which is prone to infection, saw a substantial decline in both root length, decreasing by 65%, and shoot length, decreasing by 45%. [29] reported that cotton genotypes commonly exhibit a reduction in root and shoot length when subjected to heightened salt stress conditions. Under conditions of salt stress, specific lines, notably FH-490, NIAB-545, GH-Mubarak, Kehkshan, and FH-326, had restricted impacts on soot and root length. According to [29], it may be inferred that these lines have the ability to produce a higher quantity of dry matter and effectively store salt within cells, therefore mitigating or minimizing the negative effects of salt stress. Conversely, genotypes that are prone to salt stress face challenges in attaining maximum performance because photosynthates are redirected and photosynthetic activity is restricted to mitigate the harmful effects of salt stress. Previous research on salt screening in various crops, including as barely, cotton, and wheat, have included comparable aspects connected to the roots and shoots. A decline in overall biomass output was seen in the genotypes FH-532, SB-149, FH-542, and FH-303, which were subsequently determined to be susceptible to salt stress.

The observation of reduced biomass decline and enhanced development in salt-tolerant plants has been documented in prior. [10] have demonstrated the influence of salt on the development and growth of maize and wheat. The behavior under observation can be ascribed to a decrease in osmotic potential, subsequently resulting in disruptions in ionic equilibrium, finally causing abnormalities in the nutritional balance inside plants. In their study, [10] have uncovered an additional mechanism that might potentially lead to a decrease in biomass production. This mechanism involves the redirection or use of the potential energy required for plant growth in order to exclude sodium ions.

Plants grown in seawater experience mostly osmotic stress, which causes anomalies in their cellular and metabolic functions. The presence of sodium in saline solutions is commonly acknowledged as a highly dangerous ion.

The high concentration of cytosolic components exerts an influence on environments. Therefore, a high level of salt has detrimental impacts on plants. The present investigation demonstrated that distinct cotton genotypes had varying levels of inorganic $Na+$ buildup. Delicate genotypes exhibited elevated amounts of sodium ions ($Na+$) in comparison to tolerant genotypes. [27] have reported that plants have adaptive mechanisms aimed at mitigating the adverse impacts of elevated sodium ion levels. The aforementioned mechanisms encompass the segregation of sodium ions within vacuoles and the efflux of salt via salt glands.

The importance of ion homeostasis resides in its influence on a crop's ability to efficiently regulate salt levels through the selective removal of sodium and the maintenance of an ideal equilibrium between potassium ions ($K+$) and sodium ions ($Na+$). The investigation showed significant differences in the concentrations of $Na+$ and $K+$ in the leaves, as well as the ratios of $K+$ to $Na+$. This suggests that the genotypes have different mechanisms for maintaining ion balance. The maintenance of ionic equilibrium is contingent upon the heightened expression of transporters, namely HKT and SOS1, which regulate the comprehensive uptake and transportation of $Na+$ ions. The observed increase in $K+/Na+$ ratios in genotypes with tolerance is likely attributable to this factor. The influence of salinity on cotton plants is apparent through several morphological and physiological alterations, such as changes in root and shoot traits, chlorophyll concentrations, and ion balance. The presence of salinity stress leads to a notable increase in the absorption of salt, as indicated by elevated levels of sodium ions (Na+), while also affecting the absorption of potassium ions (K+) and overall potassium levels. The importance of the potassium (K+) to sodium (Na+) ratio in regulating ion balance and assessing the tolerance of cotton plants to elevated salt levels has been established in previous studies. The significance of K+/Na+ ratios as potential biomarkers for evaluating the adaptive capacity of cotton genotypes in relation to varying salt concentrations is underscored by the analysis of variance. The interdependent relationship between Na+ and K+ concentrations serves as a diagnostic tool for assessing salinity-induced stress and also presents opportunities for targeted breeding strategies. The observed elevation in K+/Na+ ratios in both the root and shoot tissues of salt-tolerant plants can be attributed to the activation of genes related with salt tolerance, namely HKT1 and SOS1. The existence of these specific genes hinders the excessive uptake of Na+ ions into the xylem sap, leading to a reduction in the accumulation of detrimental Na+ ions and the preservation of optimal cellular K+ levels. [27] reported that the tolerant genotype demonstrated a higher K+/Na+ ratio in comparison to the sensitive genotypes.

Seedling screening plays a crucial role in expediting the selection process by enabling prompt assessment of salt responses and the identification of genotypes that exhibit tolerance and resource efficiency. Previous investigations stated that the screening of seedling stage can effectively predict salinity tolerance by taking into account the consistent variance observed across different cotton genotypes throughout control and salt treatments, even in the presence of potential interactions between genotypes and the environment. [10] provided evidence supporting the effectiveness of early screening by the validation of susceptible tests, such as FH-303, and established tolerant checks, such as NIAB-545. Nevertheless, to accommodate ontogenic variations, it remains imperative to carry out an evaluation throughout the adult phase. Taking into account several elements, the utilization of the seedling technique facilitates the commencement of breeding pipelines for cotton cultivars that exhibit expeditious salt tolerance. The examination of seedlings for research objectives holds the capacity to provide significant findings in physiological inquiries related to the dynamics of ion movement and buildup. The tolerant genotypes that have been found are important models for conducting thorough investigations into the mechanisms of adaptation. The present screening methodology demonstrates a high level of efficacy in evaluating the saline tolerance of various crops in their first stages of development.

The current investigation makes noteworthy advances to the understanding of the intricate dynamics of salt stress in various cotton genotypes. This study provides a comprehensive examination of the adaption processes and responses exhibited by these genotypes in relation to varying levels of salinity throughout the seedling phase. The salt-tolerant genotypes that have been found exhibit promise for future breeding endeavors focused on enhancing the cotton crop's capacity to endure salt stress.

Conclusion:

This study on assessing salt stress responses in cotton genotypes provides valuable insights into the genetic diversity and varying reactions of cotton genotypes to salt stress conditions. Through rigorous physiological and biochemical assessments, significant variations were observed among genotypes across different salinity levels, emphasizing their sensitivity and potential for breeding initiatives targeting salt tolerance.

The findings underscore the importance of considering specific salt levels when evaluating their impact on cotton seedling characteristics and biochemical parameters. The observed alterations in growth metrics, ion homeostasis, and K+/Na+ ratios highlight the genotypic differences in response to salt stress, indicating potential targets for further breeding efforts.

Furthermore, the cluster analysis identified genotypes with positive performance traits under salt stress, such as Mubarak, FH-490, NIAB-545, Kehkshan, and FH-326, showcasing their potential for salt tolerance enhancement. These genotypes could serve as valuable genetic resources for future breeding programs aimed at developing salt-tolerant cotton varieties.

Overall, this study contributes to the understanding of cotton genotypes' responses to salt stress, providing a foundation for targeted breeding initiatives to enhance salt tolerance and improve cotton productivity in salt-affected environments.

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