SEARCHING SEMIARID-ADAPTED COTTON CULTIVARS FOR SALT TOLERANCE USING GROWTH, MORPHOPHYSIOLOGICAL AND BIOCHEMICAL PARAMETERS

Teonis Batista da Silva[1], Gabriel Araujo Milarindo[2], Jenilton Gomes da Cunha[3], Maria Valnice de Souza Silveira1, Renato Oliveira de Sousa1, Rafael de Souza Miranda[4],

ABSTRACT: Cotton (*Gossypium hirsutum* L.) is a plant adapted to warm climates, often exposed to high temperatures and drought, which requires cultivars tolerant to adverse environmental conditions. This study aimed to evaluate the metabolic and agronomic performance of cotton cultivars under salt stress. The experiment was conducted in a greenhouse at the Federal University of Piauí, using a randomized block design in a 6 × 5 factorial scheme with five replications. Six cultivars (FM 911 GLTP, FM 912 GLTP RM, FM 970 GLTP RM, FM 974 GL, FM 978 GLTP RM, and FM 985 GLTP) were tested under five salinity levels (0, 4.0, 8.0, 12.0, and 16.0 dS m^{-1}). The electrolyte leakage, soil sodium and potassium content, pH, electrical conductivity, stomatal density, dry mass, root volume and morphology, leaf area, plant height, number of leaves, floral buds, protein content, and antioxidant enzyme activity were measured. The cultivars responded differently to salt stress, with FM 970 GLTP RM showing the highest tolerance, followed by FM 985 GLTP with moderate tolerance, while FM 911 GLTP was the most sensitive. Salinity levels of 12 and 16 dS m^{-1} caused the most severe effects, whereas 0 and 4 dS m^{-1} favored plant growth. A salinity level of 8 dS m^{-1} resulted in intermediate damage. The results highlight the importance of selecting salt-tolerant cultivars and adopting appropriate management strategies to maintain productivity in salinity-affected areas.

KEYWORDS: Saline water, agronomic indicators, *Gossypium hirsutum* L.

[1] Postgraduate Program in Agricultural Sciences, Federal University of Piauí, Bom Jesus, 64900-000, Piauí, Brazil; teonis.silva@ufpi.edu.br, maria.silveira@ufpi.edu.br, renato.sousa@ufpi.edu.br.
[2] Degree in Agricultural Engineering, Federal University of Piauí, Teresina, 64049-550, Piauí, Brazil; gabrielbjam@hotmail.com.
[3] Dr Professor, Campus Professora Cinobelina Elvas, Federal University of Piauí, Bom Jesus, 64900-000, Piauí, Brazil; jeniltongomes@ufpi.edu.br.
[4] PhD Professor, Plant Science Department, Federal University of Piauí, Teresina, 64049-550, Piauí, Brazil; rsmiranda@ufpi.edu.br.

SELEÇÃO DE CULTIVARES DE ALGODÃO TÍPICAS DO SEMIÁRIDO COM TOLERÂNCIA À SALINIDADE POR MEIO DE VARIÁVEIS DE CRESCIMENTO, MORFOFISIOLÓGICAS E BIOQUÍMICAS

RESUMO: O algodoeiro (*Gossypium hirsutum* L.) é uma planta adaptada ao clima quente, frequentemente exposta a altas temperaturas e estiagens, o que exige cultivares tolerantes a condições ambientais adversas. Este estudo teve como objetivo avaliar o desempenho agronômico e metabólico de cultivares de algodão sob estresse salino. O experimento foi conduzido em casa de vegetação na Universidade Federal do Piauí, utilizando delineamento em blocos casualizados, em esquema fatorial 6 × 5, com cinco repetições. Foram testadas seis cultivares (FM 911 GLTP, FM 912 GLTP RM, FM 970 GLTP RM, FM 974 GL, FM 978 GLTP RM e FM 985 GLTP) em cinco níveis de salinidade (0; 4,0; 8,0; 12,0 e 16,0 dS m^{-1}). Avaliaram-se variáveis fisiológicas e morfológicas, como vazamento de eletrólitos, teor de sódio e potássio no solo, pH, condutividade elétrica, densidade estomática, massa seca, volume e morfologia das raízes, área foliar, altura, número de folhas, botões florais, teor de proteínas e atividade enzimática antioxidante. As cultivares responderam de maneira distinta ao estresse salino, com destaque para FM 970 GLTP RM, que apresentou maior tolerância, seguida da FM 985 GLTP com tolerância moderada, enquanto FM 911 GLTP foi mais sensível. Os níveis de 12 e 16 dS m^{-1} causaram os efeitos mais severos, enquanto 0 e 4 dS m^{-1} favoreceram o crescimento. A condutividade de 8 dS m^{-1} provocou danos intermediários. Os resultados ressaltam a importância da seleção de cultivares tolerantes e do manejo adequado para garantir a produtividade em áreas afetadas pela salinidade.
PALAVRAS-CHAVE: Água salina, indicadores agronômicos, *Gossypium hirsutum* L.

INTRODUCTION

Cotton (*Gossypium hirsutum* L.) is a prominent crop in Brazil, ranked as fourth worldwide in production for the 2024/2025 harvest, with a projected growth of 4.7% compared to the previous season (ABRAPA, 2024). The crop species exhibits moderate salt tolerance, with an estimated threshold of 7.7 dS m^{-1} (ANWAR et al., 2024), although stages such as emergence and early flowering are more sensitive to salt stress (HAMANI et al., 2020).

Soil salinity is characterized by over accumulation of sodium (Na^{+}) ions, consisting one of the main abiotic factors negatively affecting plant development by altering physiological processes such as ionic homeostasis, photosynthesis and hormonal balance. In salt-sensitive plants, salinity decrease growth and productivity due to osmotic, ionic, and oxidative effects, which impair water uptake, nutrition and cellular integrity (WANI et al., 2020). To counteract salt damage, plants may activate physiological and biochemical mechanisms, including ion transport and compartmentalization, as well as antioxidant system activity, aiming to mitigate damage caused by reactive oxygen species (HAMANI et al., 2020).

In semi-arid regions, water scarcity and the use of saline water for irrigation represent significant challenges to agricultural production. Soil salinization is an increasing concern, resulting from inadequate water resource management and the increasing global food demand (ZHANG; DU, 2022). In this context, the cultivation of salt-tolerant species emerges as a strategic alternative to ensure agricultural sustainability in areas with ion toxic accumulation.

Our investigative study aimed to identify cotton cultivars with greater resilience and agronomic performance under saline stress conditions. The hypothesis was that cotton cultivars exhibit distinct responses to salt stress due to physiological adjustments that optimize plant growth.

MATERIAL AND METHODS

The experiment was conducted between October and December 2023 in a greenhouse from Federal University of Piauí (UFPI), Campus Professora Cinobelina Elvas, Bom Jesus/PI (9°04'46" S, 44°19'38" W). A randomized complete block design (RCBD), 6 × 5 factorial arrangement, with five replications was used, involving six cotton cultivars (FM 911 GLTP, FM 912 GLTP RM, FM 970 GLTP RM, FM 974 GL, FM 978 GLTP RM, and FM 985 GLTP) and five soil salinity levels (0; 4.0; 8.0; 12; and 16 dS m^{-1}).

During the experiment, temperature and relative humidity were monitored in the environment. Seeds were sown in 11 dm^3 plastic pots filled with soil, with thinning performed at 7 and 15 days after emergence, maintaining one plant per pot. Irrigation was conducted daily, maintaining 90% of field capacity, with salinity stress applied from the 27th day onward using saline solutions prepared by dissolving NaCl, $CaCl_2 \cdot 2H_2O$, and $MgCl_2 \cdot 6H_2O$ (MIRANDA et al., 2021). Pot weights were daily monitored to maintain adequate moisture levels.

Gas exchange parameters (photosynthesis, transpiration, internal CO_2 concentration, and stomatal conductance), stomatal density (adaxial and abaxial surfaces), biometric traits (height, stem diameter, number of leaves, flowers, buds, and nodes), photosynthetic pigments (chlorophylls and carotenoids), biomass production (dry weight of plant parts), electrolyte leakage in leaves and roots, root phenomics (length, volume, surface area, and diameter), leaf area, soil pH and electrical conductivity, and sodium and potassium content in the soil were evaluated. Antioxidant enzyme activities (APX, GPOD, SOD) and total soluble proteins in leaf and root extracts were also analyzed.

The data were subjected to statistical analysis by ANOVA, F-test (5%), and mean comparison using the Scott-Knott test (5%). A multivariate analysis (similarity dendrogram and principal component analysis) was also designed using the software R (v.4.2.3) and SigmaPlot (v.14.0).

RESULTS AND DISCUSSION

All cotton cultivars exhibited a progressive decline in performance parameters with increasing salinity, whereas the control plants consistently showed superior values across all cultivars when compared to those subjected to salt stress (Figure 1). A statistically significant variation in relative tolerance to salinity stress was detected at electrical conductivity (EC) levels ranging from 4 to 16 dS m^{-1}, with plant growth becoming progressively more impaired as salinity increased. The adverse effects of salinity were more pronounced in certain cultivars, particularly at EC levels of 12 and 16 dS m^{-1}. These results indicate that, up to a specific EC threshold, cotton plants may be classified as moderately tolerant to salinity.

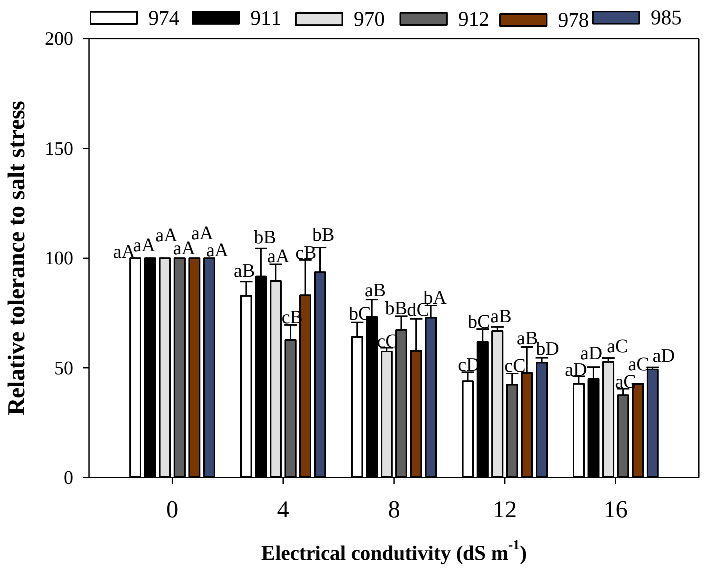

Figure 1. Relative tolerance to stress in cotton cultivars (FM 911 GLTP, FM 912 GLTP RM, FM 970 GLTP RM, FM 974 GL, FM 978 GLTP RM, FM 985 GLTP) subjected to five levels of soil electrical conductivity. Lowercase letters compare cultivars within the same EC level, whereas uppercase letters compare each cotton cultivar across different EC levels. Different letters indicate significant differences according to the Scott–Knott test ($p \leq 0.05$).

To perform a more detailed analysis of how irrigation water salinity affected the biochemical processes of cotton plants, the most contrasting treatments (EC of 0, 8, and 12 dS m⁻¹, and cultivars FM 970 GLTP RM and FM 911 GLTP) were selected to investigate plant defense-related variables, particularly the antioxidant pathway.

Salinity stress reduced protein contents at both EC levels evaluated (Figure 2). Under control conditions, cultivar FM 970 GLTP RM exhibited higher leaf protein contents compared to cultivar FM 911 GLTP (Figure 2a). At 8 dS m⁻¹ CE, no significant differences were observed between cultivars; however, at 12 dS m⁻¹ EC the FM 911 GLTP cultivar displayed protein contents lower than the control, whereas FM 970 GLTP RM showed a slight decrease. In roots, salinity stress did not alter protein contents in either cultivar. Nonetheless, FM 970 GLTP RM under 8 and 12 dS m⁻¹ salinity exhibited higher protein contents compared to FM 911 GLTP (Figure 2b).

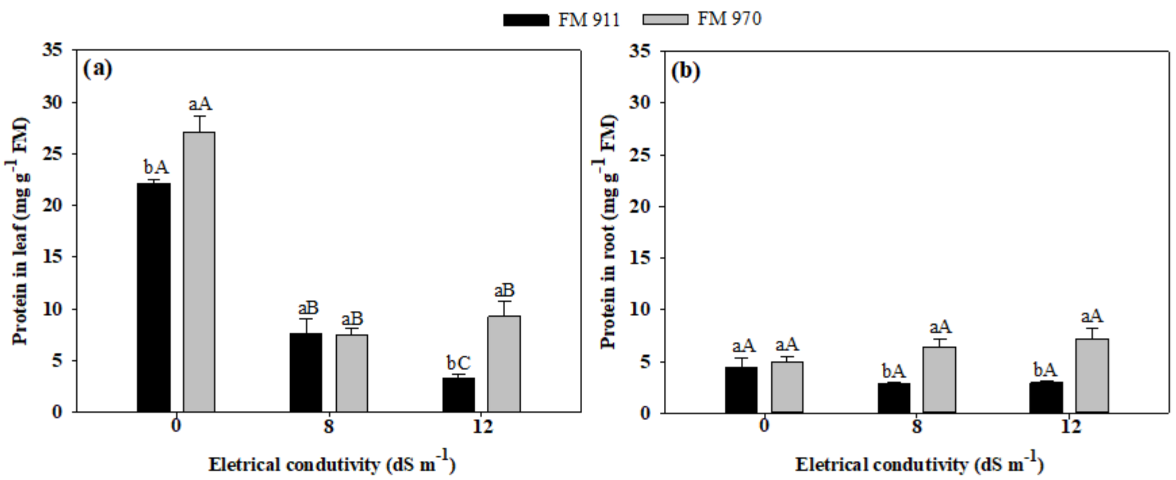

Figure 2. Total protein contents in leaves (a) and roots (b) of two cotton cultivars, FM 911 GLTP and FM 970 GLTP RM, subjected to two levels of electrical conductivity (8.0 and 12 dS m⁻¹) and the control. Lowercase letters compare cultivars within the same EC level, whereas uppercase letters compare each cotton cultivar across different EC levels. Different letters indicate significant differences according to the Scott–Knott test ($p \leq 0.05$).

Cotton plants of both cultivars exhibited progressive increases in leaf APX activity with increasing salinity stress (Figure 3a). In roots, increases were observed only in cultivar FM 911 GLTP, whereas FM 970 GLTP RM plants showed reductions compared to the control (Figure 3b). Under salinity stress, variations in leaf APX activity were recorded only at 12 dS m⁻¹, where cultivar FM 911 GLTP displayed higher APX activity values compared to FM 970 GLTP RM. In roots, cultivar FM 911 GLTP exhibited higher APX activity at all salinity stress levels.

Leaf GPOD activity increased significantly with increasing salinity stress in both cultivars, with a more pronounced response in FM 911 GLTP. In contrast, FM 970 GLTP RM exhibited lower antioxidant capacity compared to FM 911 GLTP, particularly at an EC of 12 dS m⁻¹. In roots, GPOD activity also increased with rising EC, with higher values recorded in FM 911 GLTP, especially at EC levels of 8 and 12 dS m⁻¹. Statistically significant differences between cultivars highlight the greater antioxidant efficiency of FM 911 GLTP.

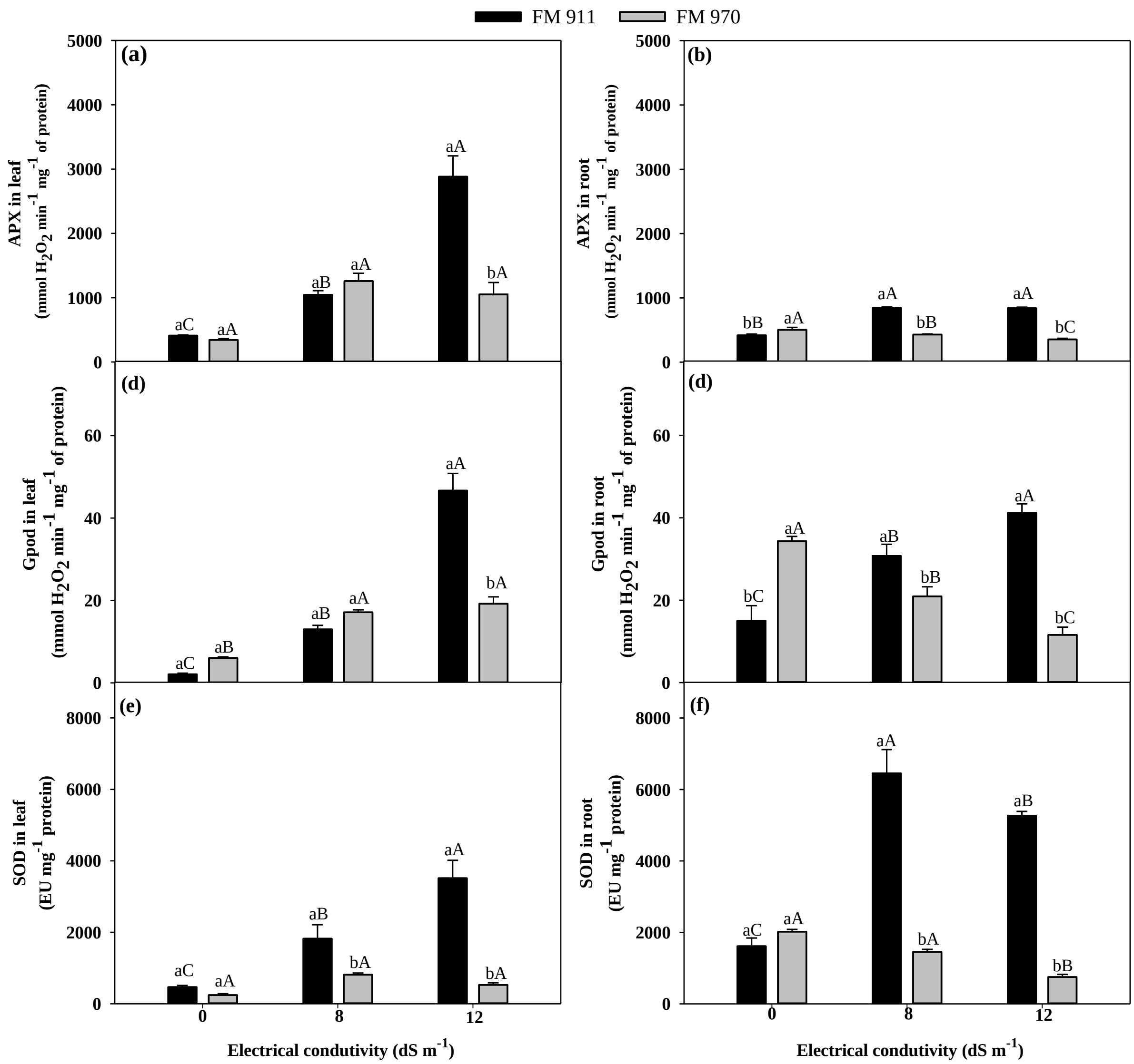

Figure 3. Activity of ascorbate peroxidase (APX), guaiacol peroxidase (G-POD) and superoxide dismutase (SOD) in leaves (a, c, e) in roots (b, d, f) of two cotton cultivars (FM 911 GLTP and FM 970 GLTP RM) subjected to two EC levels (8.0 and 12 dS m⁻¹) and the control. Lowercase letters compare cultivars within the same EC level, whereas uppercase letters compare each cotton cultivar across EC levels. Different letters indicate significant differences according to the Scott–Knott test ($p \leq 0.05$).

SOD activity in leaves showed an increasing trend with rising EC, reaching very high levels in FM 911 GLTP under an EC of 12 dS m⁻¹. In contrast, FM 970 GLTP RM exhibited lower antioxidant activity compared to FM 911 GLTP, particularly under conditions of higher salinity stress. In roots, the pattern was similar to that observed in leaves. Cultivar FM 911 GLTP displayed higher SOD activity under severe salinity stress, whereas FM 970 GLTP RM was less responsive, as indicated by the statistical results.

The PCA allowed the identification of four distinct groups among the cotton cultivars based on their responses to soil salinity (Figure 4). The principal component analysis (Figure 4) showed that the first two components explained 54.73% of the total variation (PC1 41.14%; PC2 13.59%). Group 1 consisted of FM 974 GL (12 dS m^{-1}) and FM 911 GLTP (8 dS m^{-1}) cultivars that showed a similar physiological pattern, suggesting tolerance to salt stress. Group 2 comprised cultivars under lower salinity levels (0 to 8 dS m^{-1}), such as FM 978 GLTP RM, FM 985 GLTP, and FM 912 GLTP RM, reflecting physiological stability under moderate conditions. Group 3 clustered cultivars exposed to different salinity levels, showing similar adaptive responses. Group 4 included treatments under high salinity conditions (12 and 16 dS m^{-1}), such as FM 978 GLTP RM, FM 911 GLTP, and FM 970 GLTP RM, indicating tolerance mechanisms under severe stress conditions.

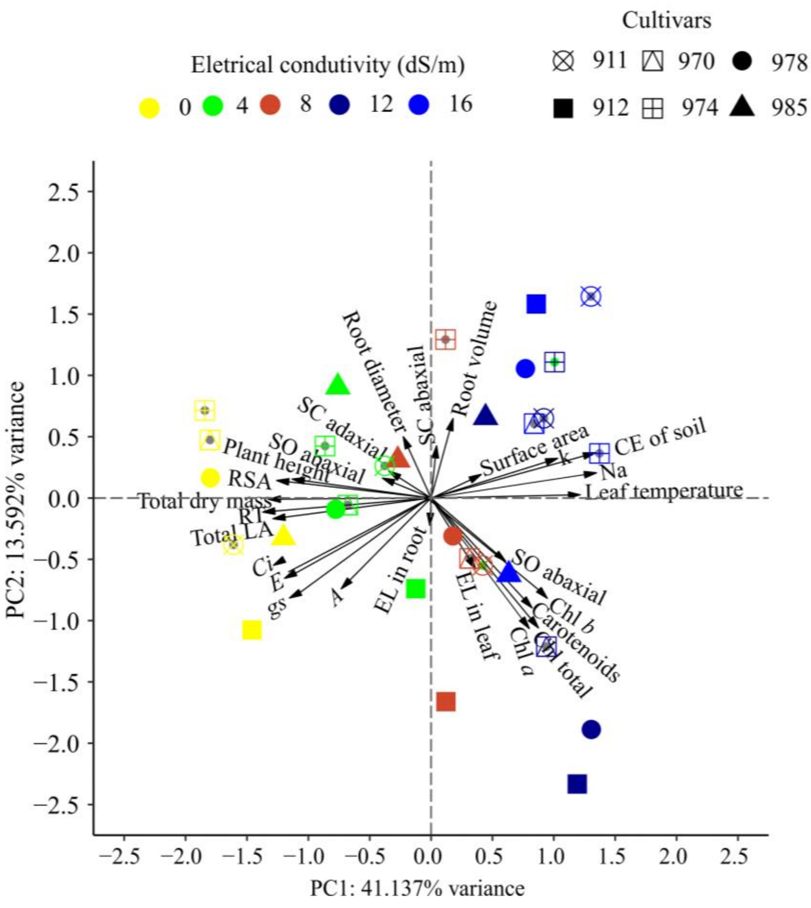

Figure 4. Principal component analysis (PCA) of cotton cultivars (FM 911 GLTP, FM 912 GLTP RM, FM 970 GLTP RM, FM 974 GL, FM 978 GLTP RM, FM 985 GLTP) subjected to five levels of electrical conductivity (control; 4.0; 8.0; 12.0; and 16.0 dS m-1. Variables: relative tolerance to salt stress; electrolyte leakage in leaves; electrolyte leakage in roots; soil sodium content; soil potassium content; soil electrical conductivity; soil pH; number of open and closed stomata on adaxial and abaxial surfaces; total dry mass of leaves; root volume, length, diameter, and dry mass; dry mass of apical, intermediate, and basal leaves; dry mass of flowers and stem; number of leaves; leaf area of apical, intermediate, and basal leaves; plant height; stem diameter; number of nodes; and number of floral buds.

Salinity negatively affected plant growth, with cultivars exposed to 0 and 4 dS m^{-1} showing better performance, associated with higher efficiency in gas exchange. Salinity at 8 dS m^{-1} caused intermediate effects, while levels of 12 and 16 dS m^{-1} induced severe stress. Some cultivars, such as FM 985 GLTP, FM 911 GLTP, FM 912 GLTP RM, and FM 978 GLTP RM, under high salinity, exhibited greater production of photosynthetic pigments, suggesting adaptive responses to salt stress.

A more concise second analysis of the similarity dendrogram (Figure 5) revealed the formation of three distinct groups among cultivars and salinity treatments. Group 1 consisted of the cultivar FM 911 GLTP subjected to a salinity level of 8 dS m^{-1}, indicating a differentiated physiological and morpho-agronomic behavior compared to the other treatments.

Group 2 included the cultivar FM 911 GLTP (under control conditions) and the cultivar FM 970 GLTP RM exposed to salinity levels of 8 and 12 dS m^{-1}, reflecting high similarity in responses to the analyzed variables, suggesting physiological consistency even under stress. Group 3 comprised FM 911 GLTP at 12 dS m^{-1} and FM 970 GLTP RM under control conditions, indicating similar response patterns between contrasting salinity conditions, which may demonstrate stability in certain cultivar attributes.

The study revealed differences in salt tolerance among cotton cultivars. FM 985 GLTP stood out for its higher resilience under moderate electrical conductivity (8 dS m^{-1}), whereas FM 912 GLTP RM exhibited greater sensitivity across all levels. Salt stress reduced leaf area, biomass, and physiological parameters such as photosynthesis and stomatal conductance, with varying intensity among cultivars.

The maintenance of photosynthetic pigments, such as chlorophyll and carotenoids, and stomatal regulation were strategies associated with tolerance. FM 970 GLTP RM exhibited protein stability and good physiological performance, while FM 911 GLTP responded with high activation of the antioxidant system, indicating a defense mechanism against oxidative stress.

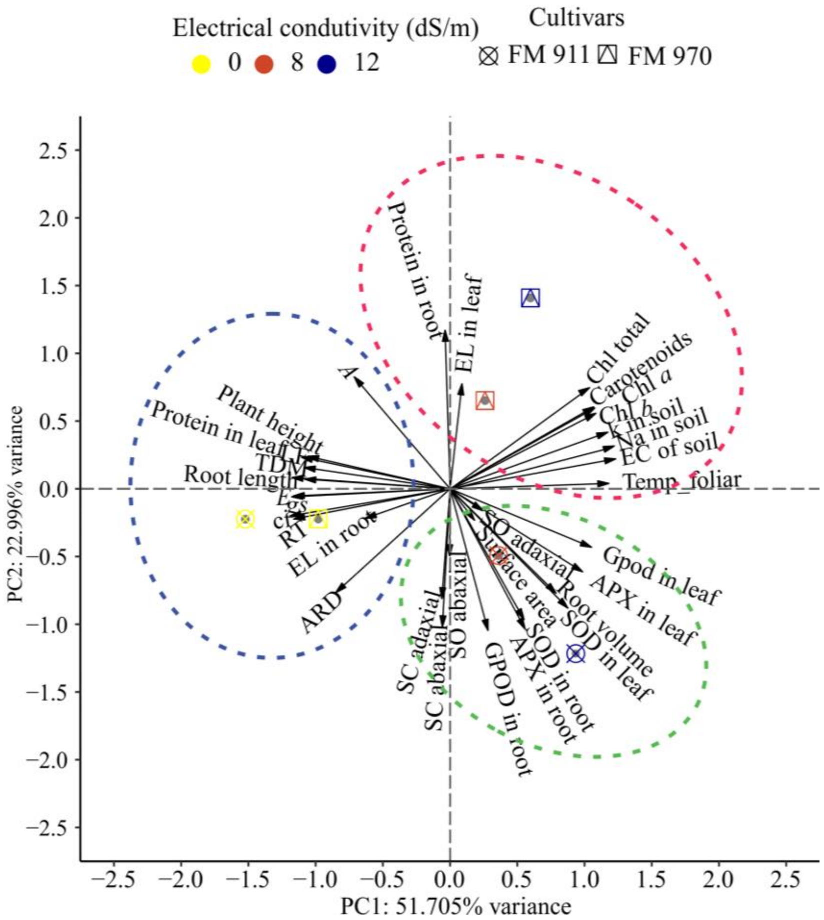

Figure 5. Principal component analysis (PCA) of cotton cultivars FM 911 GLTP and FM 970 GLTP RM under three electrical conductivity levels (0.0; 8.0; and 12.0 dS m⁻¹). Details from variables same as Figure 4.

Salinity tolerance varied among the cultivars studied and was lower at the highest salinity levels (EC of 12 and 16 dS m^{-1}; figure 01), suggesting that cotton plants may be moderately tolerant up to certain salinity thresholds. The assessment of tolerance involves the ability to survive and grow in saline soils (WANI et al., 2020). Sensitive plants exposed to irrigation with saline water experience reduced growth due to osmotic and ionic stress, which impairs water uptake and leads to nutrient imbalances and oxidative stress. In contrast, tolerant plants effectively regulate salt transport, thereby minimizing the harmful effects of salinity (OLIVEIRA et al., 2017).

Protein contents in leaves were more sensitive to salinity than those in roots. The cultivar FM 970 GLTP RM (Figure 02a), which showed the greatest tolerance to salt stress, also presented higher leaf protein contents, particularly under low to moderate salinity conditions. In contrast, protein contents in roots remained relatively stable across all salinity levels, suggesting a lesser impact of salt stress in this plant organ. Frukh et al. (2020) reported that, in rice cultivars, soluble protein contents were reduced by salinity stress, although the reduction was less pronounced in the tolerant cultivar.

The results indicated that cultivar FM 911 GLTP exhibited greater activation of the antioxidant system compared to FM 970 GLTP RM, especially under salt stress. Both cultivars showed increased activity of APX, GPOD, and SOD enzymes in leaves with increasing salinity; however, FM 911 GLTP displayed higher values at all stress levels in both leaves and roots.

These findings suggest that the sensitive cultivar FM 911 GLTP activated its defense system to eliminate excess reactive oxygen species (ROS), likely generated by the surplus energy resulting from impaired photosynthetic machinery. Although numerous studies have reported that oxidative stress tolerance is achieved through the removal of ROS by antioxidant enzymes (KERCHEV; VAN BREUSEGEM, 2021), the present data suggest that the greater antioxidant system activity in the sensitive cultivar represents an attempt to mitigate stress-induced damage (DVOŘÁK et al., 2020).

Therefore, the interaction between the maintenance of protein contents and the activation of the antioxidant system reflects the diversity of strategies employed by plants to cope with salinity stress. While FM 970 GLTP RM exhibits greater metabolic resilience under moderate conditions, FM 911 GLTP displays a specific defense mechanism against oxidative stress, particularly under high salinity levels. These results underscore the importance of selecting cultivars with specific traits for different salinity conditions, thereby contributing to the stability of agricultural production in environments prone to inducing salt stress in plants.

CONCLUSION

Salt stress promotes severe damage to physiological and biochemical pathways and growth of cotton crop. The cultivars FM 970 GLTP RM and FM 985 GLTP exhibited highest salt tolerance associated with physiological traits and antioxidant system to counteract salt damages at EC 8.0 dS m^{-1}. The FM 912 GLTP RM and FM 911 GLTP cultivars displayed elevated sensitivity to salinity. Our findings reinforce the importance of selecting cultivars with specific traits for different salinity levels, aiming at greater productive stability and sustainability in saline environments.

ACKNOWLEDGMENTS

The authors are grateful for the financial support granted by the Piauí State Research Support Foundation (FAPEPI), and the doctoral scholarship granted by the Coordination for the Improvement of Higher Education Personnel (CAPES) to T. B. Silva.

REFERENCES

ABRAPA - Associação Brasileira de Produtores de Algodão: **algodão no mundo**. 2024. Disponível em: https://www.abrapa.com.br/Paginas/default.aspx. Acesso em: 03 nov 2024.

ANWAR, M., SHAKEEL, A., SAEED, A., & SALEEM, M. F. Deciphering Salt Tolerance in Cotton: Unveiling Insights from Genetic Diversity Seedling Stage Growth Parameters. **Pakistan Journal of Agricultural Sciences**, v. 61, n. 1, 2024.

DVOŘÁK, P., KRASYLENKO, Y., OVEČKA, M., BASHEER, J., ZAPLETALOVÁ, V., ŠAMAJ, J., TAKÁČ, T. In vivo light-sheet microscopy resolves localization patterns of FSD1, a superoxide dismutase with function in root development and osmoprotection. **Plant, Cell & Environment**, v. 44, n. 1, p. 68-87, 2021.

FRUKH, A.; SIDDIQI, T. O.; KHAN, M. I. R.; AHMAD, A. Modulation in growth, biochemical attributes and proteome profile of rice cultivars under salt stress. Plant Physiology and Biochemistry, v. 146, p. 55-70, 2020.

HAMANI, A. K. M., WANG, G., SOOTHAR, M. K., SHEN, X., GAO, Y., QIU, R., MEHMOOD, F. Responses of leaf gas exchange attributes, photosynthetic pigments and antioxidant enzymes in NaCl-stressed cotton (*Gossypium hirsutum* L.) seedlings to exogenous glycine betaine and salicylic acid. **BMC Plant Biology**, v. 20, p. 1-14, 2020.

KERCHEV, P. I., VAN BREUSEGEM, F. Improving oxidative stress resilience in plants. **The Plant Journal**, v. 109, n. 2, p. 359-372, 2022.

MIRANDA, R. S.; SOUZA, F. I. L.; ALVES, A. F.; SOUZA, R. R.; MESQUITA, R. O.; RIBEIRO, M. I. D.; SANTANA-FILHO, J. A.; GOMES-FILHO, E. Salt-Acclimation physiological mechanisms at the vegetative stage of cowpea genotypes in soils from a semiarid region. **Journal of Soil Science and Plant Nutrition**, v. 21, p. 3530-3543, 2021.

OLIVEIRA, F. I. F.; MEDEIROS, W. J. F.; de LACERDA, C. F.; NEVES, A. L. R.; OLIVEIRA, D. R. Saline water irrigation managements on growth of ornamental plants. **Revista Brasileira de Engenharia Agrícola e Ambiental**, v. 21, n. 11, p. 739-745, 2017.

WANI, S. H., KUMAR, V., KHARE, T., GUDDIMALLI, R., PARVEDA, M., SOLYMOSI, K., KAVI KISHOR, P. B. Engineering salinity tolerance in plants: progress and prospects. **Planta**, v. 251, p. 1-29, 2020.

ZHANG, W.; DU, T. Fresh/brackish watering at growth period provided a trade-off between lettuce growth and resistance to NaCl-induced damage. **Scientia Horticulturae**, v. 304, p. 111283, 2022.