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Towards Resilient Cotton: Morphological and growth responses of three INIFAP cotton cultivars under salt stress in hydroponics

García-Campusano, Florencia[1*]; García-Pérez, José L.[1]; Vallejo-Reyna, Miguel A.[2]; Félix-Fuentes, José L.[3]; Cruz-Estrada, Ángel E.[2]

[1] Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias. Campo Experimental La Campana. Cd. Aldama, Chihuahua, México. C. P. 32910.

[2] Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias. Centro Nacional de Investigación Disciplinaria en Conservación y Mejoramiento de Ecosistemas Forestales. Coyoacán, Ciudad de México, México. C. P. 04010.

[3] Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias. Campo Experimental Norman E. Borlaug. Valle del Yaqui, Cd. Obregón Sonora, México. C. P. 85000.

* Correspondence: garcia.florencia@inifap.gob.mx

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ABSTRACT

Objective: To evaluate the aerial and root morphological response in seedlings from three cotton cultivars under moderate and severe sodicity conditions.
Design/methodology/approach: Nineteen growth traits were analyzed in seedlings of INIFAP cotton cultivars Cian 95, Cian Precoz, and Juárez 91, grown for 21 days in a hydroponic system with nutrient solutions supplemented with NaCl at two electrical conductivity levels: 12 and 19.6 dS/m.
Results: Sodicity significantly affected all evaluated traits, with more pronounced effects on aerial growth than on root development. The only significant genotype × sodicity level interaction was observed in leaf area, with Juárez 91 being the most sensitive. Root growth displayed a bimodal pattern: it was maintained under moderate sodicity but was significantly inhibited under severe conditions. The multivariate functional value (MFV) index effectively integrated the morphological data into a single metric, allowing for the ranking of relative tolerance among cultivars.
Limitations on study/implications: This study provides a morphological basis for understanding adaptive strategies in early developmental stages. The MFV index proved to be a valuable tool for characterizing the overall response to sodicity and may be incorporated into cotton breeding programs focused on salinity tolerance.
Findings/conclusions: Although the cultivars showed similar responses, Cian Precoz exhibited greater stability under both sodicity levels and was classified as tolerant. Cian 95 and Juárez 91 were moderately tolerant, with Cian 95 standing out due to its lower variation under high sodicity conditions.

Keywords: Aerial biomass, *Gossypium hirsutum*, root growth, sodicity, tolerance.

INTRODUCTION

Soil salinization represents one of the greatest challenges for global agriculture, as it directly affects the growth, development, and productivity of most agri-food crops

(Munns, 2002). It is characterized by the accumulation of soluble salts, primarily sodium chloride (NaCl) and sodium sulfate $\left(Na_2SO_4\right)$, though it can also result from the buildup of ions such as calcium $\left(Ca^{2+}\right)$, magnesium $\left(Mg^{2+}\right)$, and nitrate $\left(NO_3^-\right)$, among others. Salinization arises from both natural processes such as parent materials rich in salts, proximity to the sea, low precipitation, and high evaporation, as well as from practices, including inadequate irrigation and the excessive use of fertilizers (Eswar *et al.*, 2021). The impact of salinity on plants manifests through osmotic stress, ionic toxicity, and nutritional imbalances, all of which inhibit water and nutrient uptake and directly or indirectly restrict photosynthesis, ultimately reducing plant growth and crop yield (Munns & Tester, 2008; Kader & Lindberg, 2010). Salinity affects more than 20% of irrigated agricultural soils and contributes to the annual loss of between 1-2% of cultivable land worldwide (Arzani, 2008). This problem is expected to intensify in the coming decades due to climate change, particularly in arid and semi-arid regions, where its effects are exacerbated by high evapotranspiration rates and low rainfall (Hassani *et al.*, 2021), further increasing pressure on agricultural systems already facing water and productivity stress. In this context, the development of salt-tolerant cultivars has become a strategic priority in genetic improvement programs for various crop species (Arzani, 2008). Cotton (*Gossypium hirsutum* L.) is the leading source of natural fiber used in the textile industry and is also a valuable resource for the food and energy sectors, owing to its oil- and protein-rich seeds. It is considered moderately tolerant to salinity, with a threshold of 7.7 dS/m (EC: electrical conductivity) without yield reduction (Chaudhary *et al.*, 2024). However, increasing salt concentrations (>12 dS/m) hinder or delay germination, seedling establishment, biomass accumulation, and the quality of fiber and seeds, although significant variations are observed between cultivars and developmental stages (Gul *et al.*, 2025). This inherent tolerance makes cotton suitable for soil restoration programs in degraded areas and coastal dunes, as well as in regions where salinity has rendered the cultivation of other crops such as maize or wheat (4 dS/m) unviable (Sharif *et al.*, 2019). The National Institute of Forestry, Agriculture, and Livestock Research (INIFAP) has a long-standing history in the development of plant varieties in Mexico and conserves seeds from five conventional cotton cultivars developed between 1960 and 1995 CIAN Precoz (CP), CIAN 95 (C95), Nazas 87 (N87), Laguna 89 (L89), and Juárez 91 (J91) which exhibit potential as base germplasm due to their fiber quality and yield (Pérez *et al.*, 2011; Bonilla-Barrientos *et al.*, 2020; Hernández-Leal *et al.*, 2025). However, their potential resilience to adverse environmental conditions, particularly their response to stress factors such as salinity, has not yet been explored. Under unfavorable developmental conditions, plants adjust their morphology to minimize stress and optimize the use of key resources such as water, light, and nutrients. A detailed characterization of structural changes, growth patterns, and phenology in different genetic backgrounds can provide valuable insights into adaptive strategies and guide future germplasm selection efforts with targeted breeding objectives. In cotton, germination, establishment, and early development are considered key stages in the plant's response to salinity (Munis *et al.*, 2010), as they largely determine the plant's capacity to cope with adverse conditions from its earliest stages and to develop an architecture that supports future productivity (Pan *et al.*, 2023). Exploring these early phases allows for the identification of cotton genotypes with more efficient and resilient responses, which are fundamental for developing cultivars adapted to changing environments. With the aim of identifying critical tolerance thresholds and potential differences among cultivars, the present study aimed to characterize the aerial and root morphological growth responses of juvenile plants from three INIFAP cotton cultivars CP, C95, and J91 under NaCl-induced salt stress in hydroponic conditions. Exposure to moderate and severe sodicity levels (12 and 19.6 dS/m) revealed that the cultivars demonstrated notable tolerance at this developmental stage. These findings may help prioritize promising genetic materials for breeding programs aimed at the recovery of saline soils and agricultural sustainability in vulnerable regions, thereby strengthening national cotton production with conventional varieties adapted to saline conditions.

MATERIALS AND METHODS

Biological material

Seeds from the cultivars Cian Precoz (CP), Cian 95 (C95), and Juárez 91 (J91) were used. These cultivars had previously exhibited contrasting responses during the germination stage, with CP and C95 showing greater resistance than J91 (Carrillo-Cruz *et al.*, 2023). For germination, seeds were sown in polystyrene trays with 46 cm^3 microcells, using a substrate mixture of peat:perlite:vermiculite in a 2:1:1 ratio, and watered with tap water every third day. The experiment was conducted during July and August 2024 at the La Campana Experimental Station of the National Institute of Forestry, Agriculture, and Livestock Research (INIFAP) in Aldama, Chihuahua (28° 51’ 4.29” N and 105° 52’ 0.38” W), at an elevation of 1,252 meters above sea level. Temperature and relative humidity were recorded throughout the experiment (Table 1) at five-minute intervals using a HOBO U12 data logger (Onset Computer Corporation, Inc., Southern MA, USA).

Experimental Setup and Design

The trial aimed to evaluate the vegetative development of plants from the three cultivars under two sodium chloride (NaCl) concentrations, simulating moderate (12 dS/m) and severe (19.6 dS/m) sodicity conditions (Shahid *et al.*, 2018; Gul *et al.*, 2025), along with a control treatment (no NaCl). A total of 54 seedlings across the three cultivars that had reached the two-leaf stage (approximately two weeks) were selected. Seedlings were randomly assigned to treatments in a completely randomized factorial design. Each combination of factors (Cultivar×Sodicity) consisted of six seedlings.

Transplanting was carried out in PVC tubes adapted as pots, measuring 60 cm in height and 10 cm in diameter, with a volume of 4.71 L. These were filled with a 1:1 mixture of perlite and vermiculite and irrigated with 50% strength Hoagland nutrient

Table 1. Average maximum and minimum relative temperature and humidity recorded during the study.

Temperature (°C)			Relative humidity (%)		
Maximum	**Minimum**	**Average**	**Maximum**	**Minimum**	**Average**
37.88±0.26	19.69±0.21	28.05±0.23	74.19±1.66	23.51±0.77	47.12±1.38

solution (Hoagland & Arnold, 1950). To prepare the solution, various commercial fertilizers were used: $Ca(NO_3)_2$2 (YaraTera® Calcinit), KNO_3 (NKS Plus Ultrasol®), $MgSO_4$ (Sal Epson Peñoles®), MAP (Ultrasol®), and a commercial micronutrient mix (Kelatex® Multi) (Table 2).

Subsequently, the tubes were placed in plastic trays measuring 72.4 cm in length, 49.3 cm in height, and 38.6 cm in width, which were filled with nutrient solution to ensure that the substrate in the containers remained moist. Plants were maintained under these conditions for one week to ensure post-transplant establishment before initiating the sodicity stress treatments. The salinity concentration was gradually adjusted by dissolving NaCl into the nutrient solution over the course of one week, to prevent osmotic shock in the seedlings, until the desired electrical conductivity (EC) was reached, measured with a portable pH/EC/TDS meter HI98130 Hanna®. The control nutrient solution had an EC of 1.2 dS/m. The experiment lasted for three weeks and was conducted under semi-controlled conditions in a shade house with 65% light transmissivity. During this period, each container's substrate was saturated daily with its corresponding nutrient solution. To maintain sodicity levels in each treatment, EC was monitored every third day and adjusted as needed to compensate for changes caused by factors such as evaporation.

Evaluated variables

At 21 days, the following aerial morphological variables were measured: plant height and stem diameter at the base, number of nodes, leaf area, dry biomass of leaves and stems, and the presence and number of squares. Additionally, the vigor index (height/number of nodes), slenderness index (height/stem diameter), and specific leaf area (leaf area/dry mass) were calculated. Leaf area was determined using the ImageJ software (Schneider *et al.*, 2012) based on digitized images from a CanonScan LiDE 700F scanner (900 dpi resolution).

To evaluate root growth, roots were separated from each plant and washed with water to remove residual substrate. The root systems of four plants per cultivar and treatment were digitized using the previously mentioned scanner and analyzed with WinRhizo version 3.0 (Regent Instruments, Quebec, Canada). The following parameters were obtained: root length (cm), absorption surface area (cm^2), average diameter (mm), and root volume (cm^3).

Table 2. Fertilizer dose and elemental contribution for the preparation of the nutrient solution.

Fertilizer	Dose (g/L)	Input	meq/L
$Ca(NO_3)_2$2(15.5% N, 26% CaO→18.56% Ca)	0.216	Ca^{2+}, NO_3^-	2.0 Ca^{2+}, 4.0 NO_3^-
KNO_3 (12% N, 46% K_2O→38.2% K)	0.307	K^+, NO_3^-	3.0 K^+, 3.0 NO_3^-
MAP (12% N, 61% P_2O_5→26.6% P)	0.058	NH_4^+, $H_2PO_4^-$	~0.5 NH_{22}, 0.5 $H_2PO_4^-$ 2
$MgSO_4$ (16.18% Mg, 32.1% S)	0.297	Mg^{2+}, SO_4^{2-}	2.0 Mg^{2+}, 2.0 SO_4^{2-} 2
Micronutrient mix	0.05	Fe, Mn, Zn, B, Cu, Mo	Traces

After processing, all roots were collected and oven-dried at 52 °C for 72 hours to determine root dry biomass, which was then used to calculate specific root length.

A two-way analysis of variance was conducted using mixed models, where the fixed factors were cultivar and NaCl levels, including their interaction. Tray was introduced as a random factor. When statistical significance was observed, Tukey's test ($p<0.05$) was used for mean comparisons. Prior to this, assumptions of normality and homogeneity of variance were verified using the Shapiro-Wilk and Levene tests, respectively. All analyses were performed in R version 4.4.1 (R Core Team, 2024), using the lmer function from the *lme4* package (Bates *et al.*, 2015) for mixed model fitting, and the glm function from the *stats* package. For mean comparisons, the *emmeans* package (Lenth, 2016) was used.

To assess salinity tolerance, all variables generated in the study were included: plant height (cm), stem diameter (mm), leaf area (cm^2), specific leaf area (cm^2/g), stem dry weight (mg), leaf dry weight (mg), aerial biomass (mg), root biomass (g), shoot-to-root ratio, root length (m), absorption surface (cm^2), mean diameter (mm), root volume (cm^3), specific root length (m/g), and root tissue density (g/cm^3). These variables are widely recognized as sensitive indicators for inferring physiological mechanisms of tolerance or susceptibility to salt stress, due to their relevance in water transport and balance, as well as in nutrient absorption and distribution (Chen *et al.*, 2012; Wu *et al.*, 2019). Relative performance under stress was compared using the Salt Tolerance Index (STI), calculated independently for each trait and treatment as the ratio of the average value under salinity to the average value under control conditions.

$$STIi = X_i \;/\; X_0$$

Where $X_i{}^2$ is the value of the variable under saline treatment (12.0 or 19.6 dS/m) and $X_0{}^2$ is the corresponding value under the control treatment (1.2 dS/m). To enable comparison across traits with different scales, the *STI* values were normalized using a min-max membership function, generating a relative reference index (MFV, Membership Function Value) ranging from 0 to 1 for each variable and treatment.:

$$MFVi = \left(STIi - \min(STI) \,/\, \left(\max(STI) - \min[\![STI]\!]\right)\right)$$

MFV values were averaged by treatment for each cultivar, and the average under the most severe treatment (19.6 dS/m) was used to classify relative tolerance. The classification was based on the mean MFV value relative to the overall mean (μ) and standard deviation (σ), allowing for categorization into five tolerance levels: highly tolerant ($\geq\mu+1.64\sigma$), tolerant ($\geq\mu+1.0_2$ and $<\mu+1.64\sigma$), moderately tolerant ($\mu\pm1.0\sigma$), susceptible ($\leq\mu-1.0\sigma$ and $>\mu-1.64\sigma$), and highly susceptible ($\leq\mu-1.64\sigma$), following criteria adapted from Wu *et al.* (2019). Statistical and graphical analyses were conducted using custom scripts in Python 3.12.5, employing the *pandas* library for structured data manipulation (McKinney, 2010), *matplotlib* for graph generation (Hunter, 2007), and *seaborn* for high-level statistical visualizations (Waskom, 2021). Heatmaps were generated using the heatmap() function from seaborn, using the Membership Function Value (MFV) calculated for the 19 morphophysiological variables as input. These values were normalized between 0 and 1 using a min-max function, allowing for a comparative analysis of each cultivar's relative performance under saline stress conditions.

RESULTS AND DISCUSSION

During the experimental period, 100% plant survival was observed despite the high concentrations of NaCl used. For most of the evaluated traits, including both shoot and root growth, NaCl levels had a significant effect ($\alpha < 0.05$). However, no interaction was detected between cultivar and sodicity level, except for leaf area. These findings confirm cotton's capacity to withstand moderate-to-severe NaCl levels during early vegetative growth, although detrimental effects on performance were evident. Furthermore, the results suggest that the three cultivars exhibit similar morphological response mechanisms, which may reflect a conserved strategy to cope with osmotic and/or ionic stress at this developmental stage. Vegetative shoot growth. Changes in plant height and stem diameter are considered general or integrated adaptive responses, reflecting trade-offs in growth and resource redistribution to favor tolerance mechanisms that support survival under osmotic and ionic stress induced by salinity (Gul *et al.*, 2025). All three cultivars exhibited a similar growth pattern in response to increasing sodicity (Figure 1). An average reduction of 25% in stem diameter was observed compared to the control, at both 12 and 19.6 dS/m. This reduction in radial growth of the stem (and roots) is a common consequence of osmotic stress induced by salinity (Castillo-Campohermoso *et al.*, 2020; Kokebie *et al.*, 2024), as excess salts decrease water mobility (Munns & Tester, 2008). Evidence from other species suggests that changes in radial growth are due to reduced cell expansion in the vascular cambium (Castillo-Campohermoso *et al.*, 2020). Although a reduced vascular diameter limits the overall transport of water within the plant, under osmotic stress it helps maintain hydraulic pressure (and thus water conduction), while restricting the upward movement of Na^+ and Cl^-, which tend to accumulate in shoots and are particularly harmful to aerial tissues (Munns & Tester, 2008; Kokebie *et al.*, 2024). Plant height reduction was likely the most pronounced morphological response to sodicity and was similar across all cultivars, with a decrease ranging from 26% to 56% under 12 and 19.6 dS/m, respectively (Figure 1). These results are consistent with studies on 50 cotton genotypes exposed to comparable NaCl levels, which also reported adverse effects on vertical growth, even under moderate salinity (Gul *et al.*, 2025). Overall, the reductions in both stem diameter and height resulted in more compact plants, as reflected by a lower slenderness index (Figure 1). The number of nodes at 12 dS/m was comparable to the control, whereas a significant reduction was observed at 19.6 dS/m (Figure 1). This indicates that at moderate NaCl levels, shorter plant height may be attributed to a decreased internodal elongation rate (Barrick *et al.*, 2015), while under severe sodicity, both internode elongation and apical meristem activity are affected. Evidence from Arabidopsis shows that salt stress reduces the number of mitotically active cells in the shoot apical meristem, impairing its maintenance, proliferation, and capacity for tissue differentiation (Jun *et al.*, 2019). These outcomes are reflected in the vigor index as a disruption in vegetative development progression, and also as a diminished

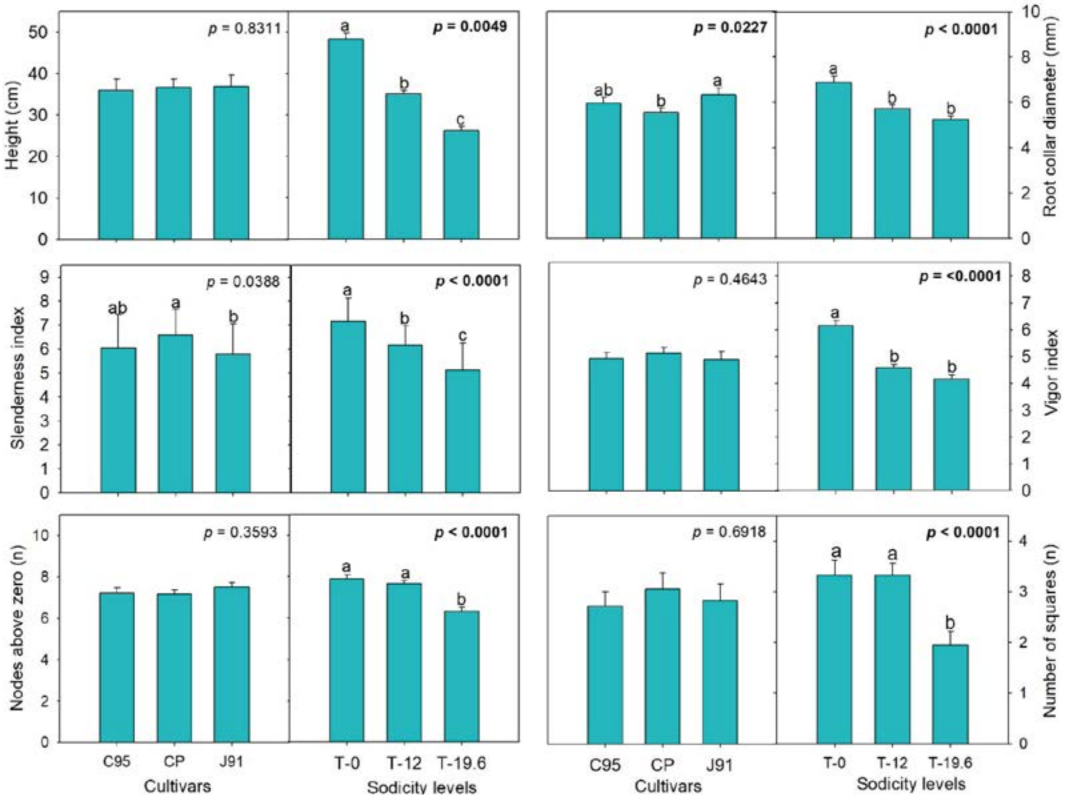

Figure 1. Aerial development of juvenile cotton plants under salt stress. Means ± Standard error. P-values are indicated in each graph for the corresponding variable. Cultivars: C95- Cian 95, CP- Cian Precoz, J91- Juárez 91. Different letters within each factor denote significant differences (Tukey, α=0.05).

potential for producing new leaves and branches an effect that is expected to negatively impact yield over the crop cycle (Yousaf *et al.*, 2023).

Leaf formation and growth. The number of leaves was not affected by moderate NaCl concentrations; however, it significantly decreased under the severe sodicity level of 19.6 dS/m (Figure 2). No leaf abscission was observed during the evaluation period, suggesting that the reduction may be attributed to delayed initiation of new leaves, which aligns with the decrease in the number of nodes formed at this level. In addition, a significant reduction in leaf size was recorded even at 12 dS/m though the severity increased further at 19.6 dS/m. For this trait, a significant interaction was detected between sodicity level and cultivar; although all cultivars were affected, J91 exhibited the greatest reduction compared to the control.

Leaf area reduction is a characteristic response of cotton to salt stress and is considered a key selection criterion for salinity tolerance in this crop (Munis *et al.*, 2010; Saleh, 2012). This phenomenon is typically attributed to the inhibition of cell expansion and division caused by osmotic stress and potential ionic toxicity induced by excess NaCl (Gul *et al.*, 2025). Nonetheless, it also serves as a strategy for reducing transpiration and conserving water under saline conditions or for conserving and reallocating resources to other organs and physiological functions (Munns, 2002).

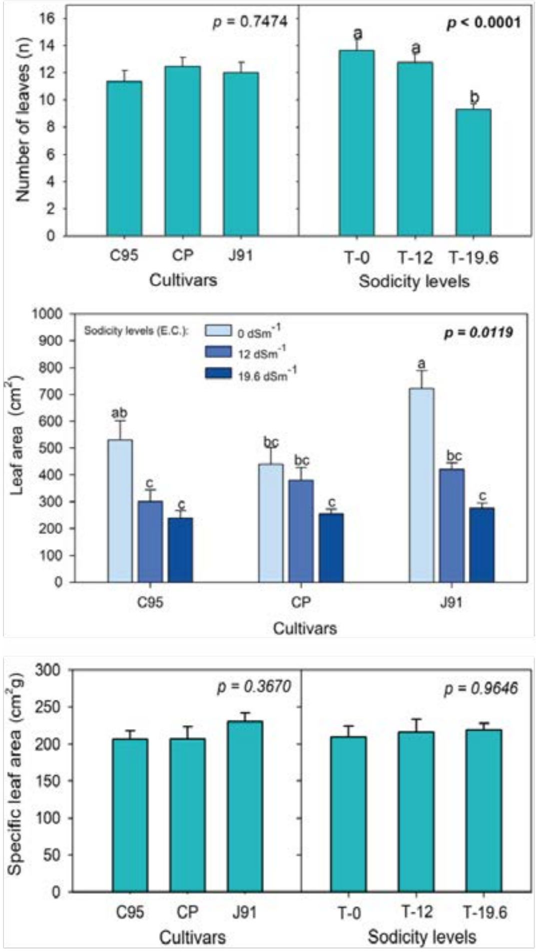

Figure 2. Effect of sodicity stress on leaf number, leaf area, and specific leaf area (SLA) in juvenile cotton plants. Means ± Standard Error. P-values are indicated in each graph for each variable. Cultivars: C95- Cian 95, CP- Cian Precoz, J91- Juárez 91. Different letters within each factor denote significant differences (Tukey, $\alpha=0.05$).

Since specific leaf area remained consistent across cultivars and NaCl levels throughout the exposure period, the limitation in leaf growth appears to act as a tolerance mechanism in which newly formed leaves retain their structural density and morphological characteristics despite being smaller, thereby preserving photosynthetic efficiency per unit biomass (Zhang *et al.*, 2014). However, the long-term impact of reduced photosynthetic surface area should not be overlooked, as it may negatively affect biomass accumulation, the transition to reproductive stages, seed production, and ultimately the quantity and quality of harvested fiber (Hu *et al.*, 2012; Zhang *et al.*, 2012).

Square formation

Approximately 90% of the plants reached the square formation stage, beginning from the fourth or fifth node. No differences were observed between cultivars under the control and 12 dS/m treatments. However, at 19.6 dS/m, reproductive transition was significantly impaired, with square formation reduced by up to 50% (Figure 2). This reduction may be the result of delayed flowering (timing) or a consequence of fewer floral initiation sites (fruiting positions) (Park *et al.*, 2013), potentially linked to the decreased number of formed nodes. No square abortion was observed during the evaluation period. Although the study did not continue into the reproductive phase, this variable suggests a likely impact on cotton yield. Research on other cultivars indicates that this is a recurrent phenomenon beyond 140 mol m^{-3}, making it a relevant trait for identifying levels of tolerance (Ashraf & Ahmad, 2000).

Root growth

To identify potential differences in root architecture adjustments under different sodicity levels, seven traits were evaluated in the cultivars, describing both root growth and biomass distribution. No cultivar effect was detected on any root attributes during the evaluated development stage ($\alpha < 0.05$). However, distinct responses were evident regarding sodicity level: at 12 dS/m, root development was similar to the control, with a non-significant upward trend observed across all traits; at 19.6 dS/m, significant changes were detected, indicating reduced growth, particularly when compared to the moderate sodicity level (Figure 3). This suggests that root growth rates were maintained under moderate-to-high salinity conditions but declined under severe stress. This pattern has been previously reported for the species (Gul *et al.*, 2025). In studies measuring cortical cell behavior in the

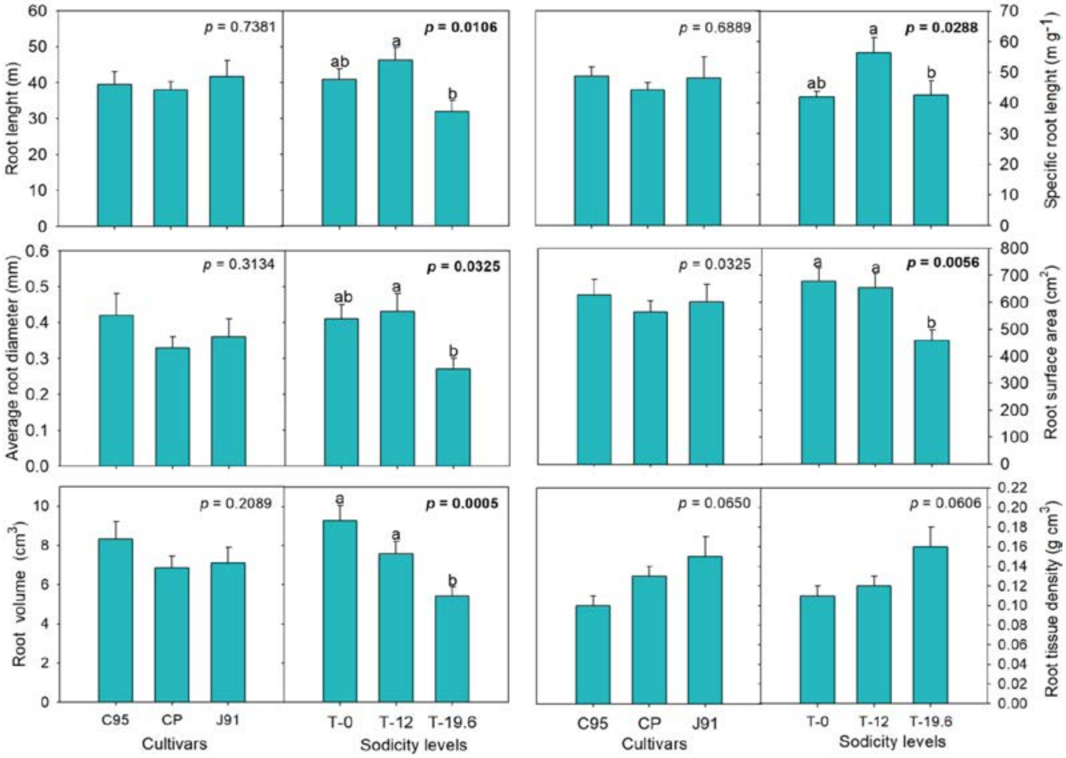

Figure 3. Root morphological traits in three cotton cultivars subjected to three sodicity levels during establishment. Means ± Standard Error. P-values are indicated in each graph for each variable. Cultivars: C95-Cian 95, CP- Cian Precoz, J91- Juárez 91. Different letters within each factor denote significant differences (Tukey, $\alpha=0.05$).

elongation zone of cotton plants exposed to NaCl concentrations between 25 and 100 mM (approximately 2 to 10 dS/m), cell growth rates increased, especially when the growing medium was supplemented with calcium (Kurth *et al.*, 1986; Zhong & Lauchli, 1993; Husain *et al.*, 2004) or boron (Lu *et al.*, 2023). The contrast between moderate and severe sodicity was evident in traits related to the root's ability to explore the substrate, such as root length and specific root length, as well as in traits associated with water and nutrient absorption and transport, including absorption surface area, mean diameter, and root volume (Figure 3). Root tissue density showed a marginal, non-significant increase in J91, as well as under the 19.6 dS/m treatment compared to the other conditions. These results suggest that under severe sodicity, root growth is reduced, and there is a tendency toward increased tissue thickness. This trait is considered a key adaptive root response associated with stress tolerance (Kramer-Walter *et al.*, 2016), as it enhances cell resistance and protects against dehydration-induced collapse, preserving the root's conductive function albeit at the cost of rapid growth and absorption efficiency.

Aerial and root biomass accumulation

In most crop species, the inhibitory effect of salinity on growth is more pronounced in the shoot than in the root system (Gul *et al.*, 2025). Overall, aerial biomass production (stem and leaves without petiole) was similar across cultivars; however, it decreased by approximately 25% and 60% compared to the control under 12 and 19.6 dS/m, respectively (p=0.0001) (Figure 4). This reduction may be associated with inhibited vegetative growth and reduced leaf area, ultimately affecting photosynthetic capacity and leading to lower sugar production and, therefore, reduced biomass (Zhang *et al.*, 2014; Peng *et al.*, 2016). Additionally, decreased biomass may result from the inhibition of photosynthesis due to the accumulation of toxic levels of Cl_2 in plant tissues. Regarding root biomass accumulation, similar values were recorded between the control and 12 dS/m, although a downward trend was observed. At 19.6 dS/m, a significant 20%

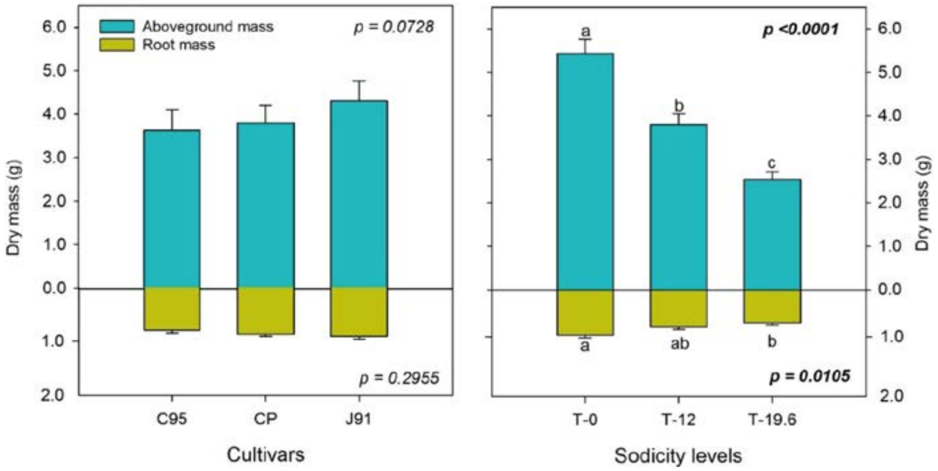

Figure 4. Shoot-to-root biomass ratio. Means ± Standard Error. P-values are indicated in each graph for each variable. Cultivars: C95- Cian 95, CP- Cian Precoz, J91- Juárez 91. Different letters within each factor denote significant differences (Tukey, α=0.05).

reduction was recorded ($p=0.01$), consistent with the observed decreases in root length and volume (Figure 4).

This was reflected in a significant decrease in the shoot-to-root biomass ratio with increasing sodicity ($p<0.0001$). While biomass accumulation under control conditions was skewed toward the shoot, this part of the plant was the most affected starting at moderate sodicity levels, whereas the root system exhibited greater initial resilience and was only significantly compromised under severe conditions. This shift in growth allocation suggests an adaptive strategy based on moderating shoot growth possibly to reduce water loss via transpiration and redirecting photosynthates toward the root system to support essential functions such as nutrient and water absorption, as well as other stress defense mechanisms (Shelden & Munns, 2023).

Tolerance level characterization

Although the three cultivars exhibited similar responses to the evaluated sodicity levels, the use of the Membership Function Value (MFV) index allowed the integration of 19 morphological variables into a single composite metric, facilitating the ranking of their tolerance to salt stress under both conditions (12 and 19.6 dS/m). Additionally, this tool enabled the identification of specific strengths and weaknesses of each cultivar in relation to individual traits. Based on the average MFV values during the juvenile stage, CP was classified as tolerant, while C95 and J91 were categorized as moderately tolerant. It is worth noting that, overall, the negative impact on growth at 19.6 dS/m was more pronounced in J91 than in CP or C95 (Figure 5).

At 12 dS/m, CP stood out for its high values in most traits related to shoot growth and displayed intermediate performance in traits associated with the root system. In contrast, J91 exhibited the opposite pattern, showing a stronger negative impact on shoot development and a milder effect on root growth, suggesting distinct physiological strategies for coping

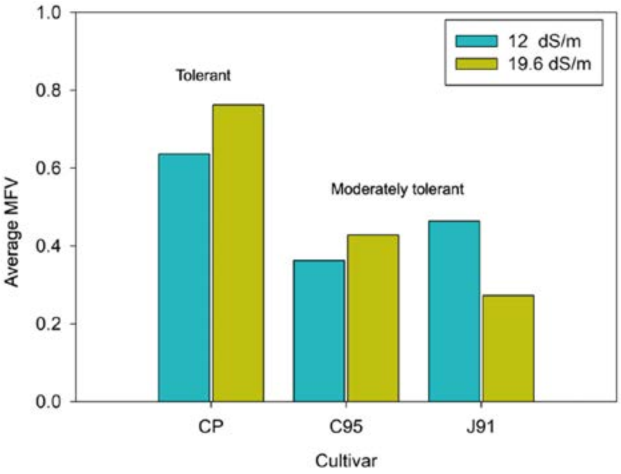

Figure 5. Average values of the membership function index (MFV) per cotton cultivar at two sodicity levels (12 and 19.6 dS/m). The classification is indicated above each bar and is based on the average MFV values compared to the population mean and standard deviation, according to the method proposed by Wu *et al.* (2019). Cultivars: C95- Cian 95, CP- Cian Precoz, J91- Juárez 91.

with salt stress at this sodicity level. Meanwhile, C95 showed intermediate performance in shoot growth but was the most affected cultivar in traits related to root architecture (Figure 6). CP maintained its classification as tolerant at 19.6 dS/m, preserving relatively stable growth in both shoot and root systems. In contrast, J91 and C95 remained classified as moderately tolerant, though their response profiles shifted: J91 showed a decline in root performance and continued to be the most affected in terms of shoot growth, whereas C95, although impacted, exhibited a more balanced performance compared to the other two cultivars (Figure 6).

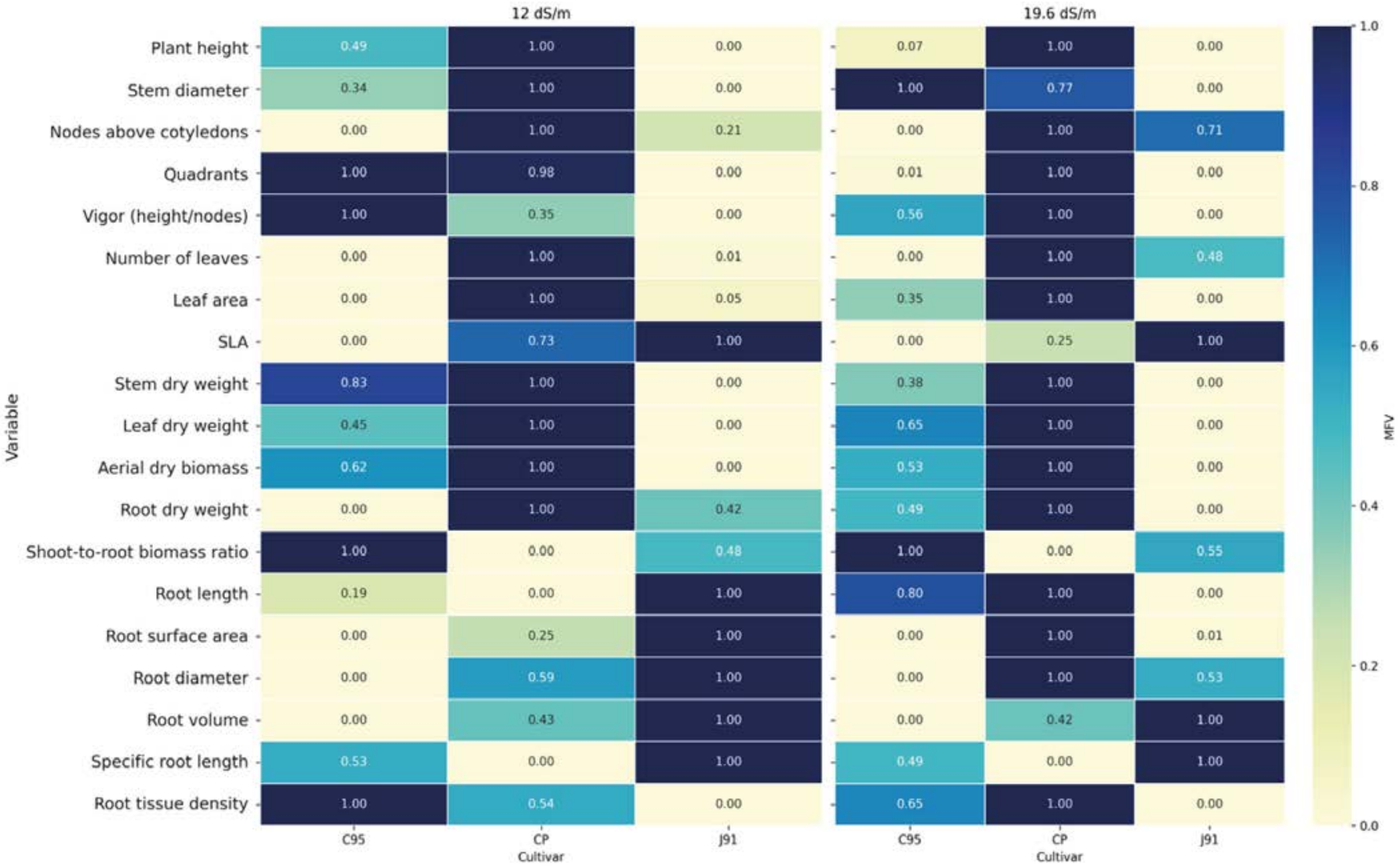

Figure 6. Heat map of the Membership Function Value (MFV) by morphological variable for three cotton cultivars under two sodicity levels: 12 dS/m (left) and 19.6 dS/m (right). Each value represents the individual MFV per variable, where 1.00 corresponds to the best relative performance among cultivars. Variation across treatments suggests differential responses by trait and genotype, and allows for the identification of specific strengths to guide the selection of tolerant materials. SLA=Specific Leaf Area. Cultivars: C95- Cian 95, CP- Cian Precoz, J91- Juárez 91.

CONCLUSIONS

In this study, three cotton cultivars developed by INIFAP Cian 95, Cian Precoz, and Juárez 91 were compared for their responses to intermediate (12 dS/m) and severe (19.6 dS/m) sodicity levels during the establishment and vegetative growth stages. Nineteen aerial and root growth traits were analyzed, and the Membership Function Value (MFV) index was incorporated as an integrative metric to visualize each cultivar's strengths and weaknesses across traits. All cultivars demonstrated the capacity to survive and grow, although a detrimental effect was observed across all traits with increasing sodicity, with aerial growth being the most affected. The root system showed a bimodal response: it maintained growth under moderate sodicity but declined under severe conditions, indicating an inhibitory effect. Cian Precoz exhibited the most robust and consistent performance under both sodicity levels, emerging as the most tolerant cultivar. Cian 95 and Juárez 91, while classified as moderately tolerant, displayed specific weaknesses and strengths that may inform future breeding programs. The multivariate approach employed underscores its utility in comprehensively assessing tolerance to abiotic stress in early developmental stages of cotton.

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