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## Tolerance of Traditional Maize Varieties (*Zea mays* L.) to Salt Stress

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**ABSTRACT:** Managing salinity in irrigation water is essential to ensure water supply for plants, especially in semi-arid regions, where the most readily available water often has high salt content. In this context, the tolerance of traditional maize varieties to salt stress was evaluated using morphological and biochemical parameters at the initial developmental stage. The experiment was conducted in a greenhouse, using polyethylene trays with 200 cells filled with inert coconut fiber-based substrate. The experimental design was completely randomized in a  $15 \times 2$  factorial scheme, with four replicates and 50 seeds per plot, for a total of 120 experimental plots. The factors corresponded to 15 traditional maize varieties and two levels of irrigation water salinity ( $S1 = 0.5 \text{ dS m}^{-1}$  and  $S2 = 7.5 \text{ dS m}^{-1}$ ). Emergence was evaluated from the first to the eighth day after sowing, while growth, phytomass, and biochemical analyses were performed on the eighth day. The results showed that irrigation with saline water affected the development of the native varieties, with growth and biomass accumulation of the aerial part and rootlets being the most impacted variables. The varieties V3—Grão do Sertão, V5—Alho Pipoca, V8—Jaboatão Areia, V6—Pontinha, and V14—Logradouro stood out for their greater tolerance to saline stress, showing reductions of less than 4% in biomass accumulation, maintaining high root dry mass, and high salinity tolerance indices ( $ITS > 97\%$ ). In contrast, V10—Sol da Manhã, V11—Índio, and V12—Jaboatão Remígio were the most sensitive, with significant reductions in SDM and TDM and ITS values close to 80%, in addition to greater impairment of initial growth.

**KEYWORDS:** Native varieties; morphology; biochemistry; salinity; semi-arid region

### 1 Introduction

Maize (*Zea mays* L.), an annual grass belonging to the Poaceae family, is among the main grain crops produced and consumed worldwide. It is a versatile and multifunctional crop with significant socioeconomic relevance, due to its wide range of uses, from human and animal food to industrial applications. In addition to its high productive potential and nutritional value, maize is widely used as a raw material in the manufacture of food, beverages, biofuels, and other derivatives, consolidating itself as a strategic crop in different sectors of the global economy [1–3]. It is grown on a large scale under various soil and climatic conditions, but it is sensitive to salt stress, especially during the seedling stage [4].

In the global agricultural context, Brazil stands out as one of the leading maize producers, driven by its vast cultivated areas and high production volume. Recent data from the National Supply Company [5] indicate that, for the 2024/2025 harvest, national maize production is estimated at approximately 137 million tons, consolidating itself as the highest ever recorded in the institution's historical series. The area devoted to cereal cultivation covers approximately 21.14 million hectares, with an estimated average yield of 5806 kg per hectare, representing an improvement over the previous cycle. The Brazilian Cerrado, especially in the Midwest, remains the country's main maize-producing region. Recent data indicate that this region accounts for approximately 54% of national maize production, reaffirming its historical and leadership role and reinforcing the importance of this state in national productivity. The Brazilian Institute of Geography and Statistics (IBGE) estimated that Brazil harvested 128.2 million tons of maize in 2025, reflecting an increase of 11.8% over 2024. Average national productivity also increased, reaching 5834 kg ha<sup>-1</sup> in the first harvest [6].

Creole seeds are traditional varieties cultivated and preserved by family farmers over generations. They are adapted to local conditions, having been selected over generations for their agronomic characteristics and their ability to adapt to diverse climates, soils, and habitats [7–9]. In addition to their agronomic value, traditional varieties play a key role in preserving the genetic and cultural heritage of rural communities, as they maintain broad biological diversity, which is essential for the resilience of agricultural systems in the face of diseases, pests, and climate change, while perpetuating knowledge, practices, and collective identities associated with seed cultivation and exchange [10,11]. In this context, seed guardians play a decisive role in maintaining, selecting, multiplying, and circulating these varieties, ensuring the continuity of genetic and cultural heritage by preserving ancestral practices and strengthening community exchange networks [12]. Seed houses serve as community spaces for the storage, conservation, and collective management of native seeds, functioning as living banks of biodiversity and centers for the preservation of traditional knowledge associated with their use, especially in rural and indigenous territories [11]. Based on this set of social, cultural, and ecological practices that ensure the conservation of native varieties, it is essential to investigate their agronomic performance in the face of adverse environmental challenges to which they are exposed, such as water stress, salt stress, and climate change, reinforcing the need to evaluate them under different environmental contexts to guide conservation and sustainable use strategies [13]. Thus, evaluating the response of these varieties to salinity, especially during germination and early growth stages, is a fundamental step in identifying genetic materials with greater adaptive potential, ensuring both their conservation and strategic use in environments subject to salt stress.

Despite being well adapted to the climatic conditions of the Brazilian semi-arid region, maize is considered moderately sensitive to salinity, particularly during the early growth stages, with negative impacts on essential processes such as photosynthesis, respiration, and germination [12]. The cultivation of this grass in semi-arid regions often depends on irrigation with brackish water as a viable alternative to mitigate the effects of water scarcity [14]. However, continuous use of this type of water can lead to the accumulation of soil ions, such as sodium (Na<sup>+</sup>) and chlorine (Cl<sup>-</sup>), resulting in deleterious effects on crops [15,16]. Maize production is affected by several abiotic and biotic factors, with abiotic stress being the most critical for global agriculture [2,4]. Among these, saline stress is one of the main constraints to crop development and productivity. Its negative effects manifest throughout the plant's cycle, from germination and seedling establishment to the vegetative and reproductive stages, directly interfering with physiology, morphology, and final yield [3,17,18].

Salinity causes two main effects on plants: primary effects are related to reduced soil water potential, leading to cell dehydration and ionic cytotoxicity, caused by excessive accumulation of ions such as

sodium ( $\text{Na}^+$ ) and chlorine ( $\text{Cl}^-$ ) in the root environment. Secondary effects include physiological and metabolic changes such as reduced cell and leaf expansion, reduced cellular and metabolic activities, stomatal closure compromising gas exchange, photosynthetic inhibition, leaf abscission, increased ROS production, destabilization of membranes and proteins, and cell death [4,19,20]. The response of plants to salinity involves morphological, physiological, and biochemical changes [21]. A reduction in the osmotic potential of the soil solution, which hinders the absorption of water and nutrients by the roots and promotes ionic toxicity [22]. As a result, several vital processes are affected, including photosynthesis, stomatal conductance, transpiration, germination, growth, cellular metabolism, cell expansion, biomass, productivity, and translocation of photoassimilates [23,24].

For maize and several other crops, sodium ions ( $\text{Na}^+$ ) are considered highly toxic. Their excess in the environment disrupts ionic homeostasis in plant cells, negatively affecting cellular metabolism. In addition, the accumulation of  $\text{Na}^+$  stimulates the generation of reactive oxygen species (ROS), such as hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), which can cause significant oxidative damage to proteins, membrane lipids, and nucleic acids. These effects compromise essential cellular functions, impairing plant development and survival under saline stress conditions [4]. During germination and the early stages of development, seedlings are highly susceptible to salt stress, as their physiological mechanisms are not yet fully established to mitigate the osmotic and ionic effects resulting from salinity [18], which directly affects their phenotypic characteristics, physiological functions, and biochemical indicators [3]. Seed germination in saline environments is a decisive stage for the initial establishment of plants. Due to the proximity of the seed to the soil surface, this phase is particularly vulnerable to salt stress, which can compromise water absorption and the metabolic processes essential for germination. Therefore, seedling germination and growth characteristics are fundamental criteria for selecting salt-tolerant cultivars [2,25].

When faced with salt stress, maize plants activate a series of complex physiological, biochemical, and metabolic mechanisms, such as stomatal opening regulation, photosynthesis modulation, respiration, and transpiration, as well as hormonal adjustment, antioxidant enzyme activity, and ion transport, to minimize the damage caused by this type of stress and ensure the survival and development of the plant [2,17,26]. Although most studies on salt tolerance in maize focus on commercial and hybrid strains. Traditional maize varieties represent a promising alternative for cultivation in salinized environments, thanks to their hardiness and the potential to select more tolerant genotypes. Evaluating the response of these varieties to salinity, especially during germination and early growth stages, is essential for identifying genetic materials with greater adaptive potential [8].

We hypothesized that traditional maize varieties exhibit greater tolerance to salt stress, as evidenced by superior initial seedling growth compared to commercial genotypes, making them strategic alternatives for cultivation in environments with high salinity.

Therefore, we aimed to assess how traditional maize varieties respond to salt stress during germination and early seedling development. Our goal was to identify the most tolerant genotypes by integrating morphological and biochemical parameters, thereby supporting the selection of genetic materials with greater adaptive potential under saline conditions.

## 2 Materials and Methods

### 2.1 Location of the Experiment

The experiment was conducted in a greenhouse belonging to the Department of Agronomic and Forest Sciences (DCAF) of the Federal Rural University of the Semi-Arid Region (UFERSA), located in Mossoró, Rio Grande do Norte, in June 2024. The experimental area is located at the geographical coordinates  $5^{\circ}11'31''$  S

and 37°20'40'' W, at an altitude of 18 m. The region's climate is classified as semi-arid (BSH) according to the Köppen climate classification, characterized by irregular rainfall and a high temperature range [27].

During the experiment, the maximum and minimum temperatures were 40.4°C and 22.8°C, respectively. Relative humidity ranged from 93% (maximum) to 28% (minimum), as measured with a Minipa® MTH1300 Digital thermo-hygrometer.

## **2.2 Experimental Design**

The experiment was conducted using a completely randomized design in a 15 × 2 factorial scheme, with four replicates and 50 seeds per replicate, for a total of 120 experimental plots. The treatments consisted of a combination of 15 traditional maize varieties and two levels of irrigation water salinity. The varieties used were: V1—Santana do Mato, V2—Brejinho, V3—Grão do Sertão, V4—BR 106 (Commercial genotype), V5—Alho Pipoca, V6—Pontinha, V7—Zé de Julho, V8—Jaboatão Areia, V9—Peba, V10—Sol da Manhã, V11—Índio, V12—Jaboatão Remígio, V13—Pontinha Remígio, V14—Logradouro, V15—Ideal, which were subjected to two levels of electrical conductivity of irrigation water: S1 = 0.5 dS m<sup>-1</sup> (control condition) and S2 = 7.5 dS m<sup>-1</sup> (saline condition).

## **2.3 Water Sources and Plant Material**

The water salinity levels were obtained from two sources. The first level, corresponding to the control treatment (S1), used water from the local supply system, with electrical conductivity (ECa) of 0.5 dS m<sup>-1</sup>. We obtained the second water salinity (S2) from the saline reject from the reverse osmosis desalinator that supplies the Jurema settlement, located on the banks of the RN-013 highway, between the cities of Mossoró and Tibau, in the state of Rio Grande do Norte.

To achieve the desired electrical conductivity of 7.5 dS m<sup>-1</sup>, the saline tailings were diluted with the supply water. The salinity level of 7.5 dS m<sup>-1</sup> was selected to assess the performance of the varieties under high-salinity stress conditions. The water was stored in 150 L plastic containers, with one reservoir for each salinity level. The electrical conductivity of the water was monitored regularly using a portable conductivity meter (Lutron, model CD-4301), with readings adjusted to a reference temperature of 25°C, as recommended by the manufacturer.

Traditional maize varieties were obtained directly from rural producers in the states of Rio Grande do Norte and Paraíba, who belong to communities that preserve the use of traditional seeds. The seeds used were from the 2023 harvest, acquired from collections maintained by native seed guardians. To preserve their physiological quality, the seeds were stored in sealed PET bottles to maintain their original moisture content. Storage was carried out in dry, well-ventilated environments free of preservatives, thereby preserving the material's physiological quality.

## **2.4 Sowing and Irrigation Management**

Sowing was carried out on 04 June 2024, in polyethylene trays with 200 cells, filled with coconut fiber-based substrate. One seed per cell was used, positioned at a depth of 1.0 cm. After sowing, the trays were placed in a greenhouse, where they remained for eight days, a period intended for germination and initial seedling development.

Irrigation management consisted of two daily irrigations using a floating irrigation system, in which the trays were placed in direct contact with a 3.0 cm deep layer of water. This method allowed water to reach the substrate via capillary rise, promoting humidification of the growth medium from the base.

This strategy prevented direct contact between the aerial parts of the seedlings and the salts in the saline solution, thereby avoiding the deleterious effects on the leaves.

To enable the formation of the water mirror, two reservoirs measuring 1.0 m<sup>2</sup> and 0.05 m deep were built, both lined with waterproof canvas. Each reservoir was assigned to one of the irrigation water salinity levels (0.5 and 7.5 dS m<sup>-1</sup>), ensuring separate and efficient management of saline water.

### 2.5 Emergence Test

From the third day after sowing (DAS), the number of emerged seedlings was counted, considering emergence to be the presence of cotyledons above the surface of the substrate, as established by the Rules for Seed Analysis [28]. Counts were performed daily until the eighth day after sowing, without discarding seedlings that had already emerged. The number of seedlings emerging each day was obtained by subtracting the cumulative number of seedlings that had emerged on the previous day from the number that had emerged on the current day. This provided a temporal distribution of emergence, enabling further analysis, such as the calculation of the emergence speed index (ESI) and the emergence percentage. The emergence percentage (%) was calculated using Eq. (1).

$$E (\%) = \frac{Pn}{N} 100 \quad (1)$$

where:

$E$  = Seedling emergence percentage (%);

$Pn$  = Normal seedlings;

$N$  = Total number of seeds placed for germination.

The number of seedlings that emerged each day was used to calculate the Emergence Speed Index (ESI) according to the method proposed by [29] with assessments performed daily until seedling emergence stabilized. The calculation was performed according to Eq. (2).

$$ESI = \frac{E1}{N1} + \frac{E2}{N2} + \dots + \frac{Ei}{Ni} \quad (2)$$

where:

$ESI$  = Emergence velocity index;

$E1$ ,  $E2$ , and  $Ei$  = number of normal seedlings counted in the first, second, and last counts;

$N1$ ,  $N2$ , and  $Ni$  = number of days from sowing in the first, second, and last counts.

### 2.6 Morphological Evaluations

At the end of the emergence test, conducted 8 days after sowing (DAS), the lengths of the aerial part and the root were measured in each experimental plot. Measurements were taken from the tip of the main root to the leaf apex using a ruler graduating in millimeters, with results expressed in centimeters (cm). This assessment allowed us to verify the initial development of seedlings under different salinity conditions, contributing to the analysis of native varieties' tolerance to salt stress during the initial establishment phase. After collecting, the plants were separated into morphological parts (leaves, stem, and root). The samples were placed in Kraft paper bags and dried in an oven with forced-air circulation at 65°C for 72 h. Once the drying process was complete, the samples were weighed on a precision analytical balance (0.0001 g). The data obtained were used to calculate the shoot dry mass (SDM), the root dry mass (RDM), and the total dry mass (TDM = SDM + RDM), all expressed in mg.

## 2.7 Salinity Tolerance Assessment

Based on biomass data, the salinity tolerance index (STI, %) was calculated using Eq. (3). The salinity tolerance index (STI) values were classified into four levels, according to the methodology proposed by [30], as follows: Tolerant (T) for STI between 80.1–100.0%, Moderately Tolerant (MT) with STI between 60.1–80.0%, Moderately Susceptible (MS) between 41.1–60%, and Susceptible (S) for values below 40% (STI < 40%). For this index, the total dry mass of each variety was used as the primary parameter to determine the materials' tolerance to salt stress.

$$STI (\%) = \frac{TDM \text{ in salt stress}}{TDM \text{ in controle}} \times 100 \quad (3)$$

where:

STI = salinity tolerance index;

TDM = total dry mass.

## 2.8 Biochemical Evaluations

The remaining fresh material from each replication was frozen in liquid nitrogen (−196°C) and stored in an ultra-freezer (−80°C). Samples were then manually macerated to obtain crude extract, which was divided into triplicates containing approximately 0.2 g of macerated material. Each sample was placed in a hermetically sealed plastic tube containing 3 mL of 60% ethanol (v/v), heated in a water bath at 60°C for 20 min, and centrifuged at 10,000 rpm at 4°C for 10 min. The supernatant was collected for the determination of the following variables:

1. Total soluble sugars (TSS): determined by the anthrone method [18], using glucose as the standard, with results expressed in  $\mu\text{mol GLU g}^{-1}$  fresh mass;
2. Total free amino acids (TFAA): determined by the acid ninhydrin method [19], using a glycine standard curve, with results expressed in  $\mu\text{mol GLY g}^{-1}$  fresh mass;
3. Free proline (PRO): determined according to Bates; Waldren; Teare [20], with results expressed in  $\mu\text{mol PRO g}^{-1}$  fresh mass.

## 2.9 Statistical Analysis

The data obtained were analyzed using the F test for analysis of variance to identify significant effects. For the test of means, the Scott-Knott grouping test ( $p \leq 0.05$ ) was applied for the variety factor and the Student's *t*-test ( $p \leq 0.05$ ) for the salinity level factor. The statistical analyses were performed using SISVAR<sup>®</sup> software, version 5.8 [31]. Hierarchical cluster analysis using dendrograms was performed in PAST4.

## 3 Results

### 3.1 Emergence and Growth Parameters

According to the F test, a significant interaction was observed between the factors salinity and traditional maize varieties for most of the variables evaluated: emergence (E,  $p \leq 0.001$ ), emergence speed index (ESI,  $p \leq 0.05$ ), shoot length (SL,  $p \leq 0.001$ ), root length (RL,  $p \leq 0.001$ ), shoot dry mass (SDM,  $p \leq 0.05$ ), total dry mass (TDM,  $p \leq 0.05$ ), total soluble sugars (TSS,  $p \leq 0.001$ ), total free amino acids (TFAA,  $p \leq 0.001$ ), free proline (PRO,  $p \leq 0.001$ ), and salinity tolerance index (ITS,  $p \leq 0.001$ ), except for root dry mass (RDM), whose interaction was not significant ( $p > 0.05$ ), indicating that the effect of salinity on this variable was similar among varieties. These results show that the response to salt stress is genotype-dependent, i.e., some



varieties are more tolerant to salt than others, reflecting differences in their physiological and biochemical adaptation mechanisms. The significant interaction between salinity and variety across most variables underscores the importance of selecting more tolerant varieties, given their distinct physiological and biochemical adaptations.

Table 1 shows the mean emergence values (E%) and Emergence Speed Index (ESI) of traditional maize varieties subjected to two salinity levels (0.5 and 7.5 dS m<sup>-1</sup>) during the initial development phase. Salinity did not significantly compromise seed emergence, which remained above (>90%) even under high salinity conditions, except for the V6 variety—Pontinha, which showed a significant reduction of 24% in emergence (63% to 39%) under salinity of 7.5 dS m<sup>-1</sup>, indicating a lower degree of tolerance of this variety at the emergence stage (Table 1).

**Table 1:** Test of means for emergence (E) and Emergence Speed Index (ESI) of traditional maize varieties subjected to salt stress in the early stages of development.

| Maize Varieties     | E (%)                  |       |                        |       | ESI                    |       |                        |       |
|---------------------|------------------------|-------|------------------------|-------|------------------------|-------|------------------------|-------|
|                     | 0.5 dS m <sup>-1</sup> |       | 7.5 dS m <sup>-1</sup> |       | 0.5 dS m <sup>-1</sup> |       | 7.5 dS m <sup>-1</sup> |       |
| 1—Santana do Mato   | 99 <sup>aA</sup>       | ±1.00 | 99 <sup>aA</sup>       | ±1.00 | 3.37 <sup>aC</sup>     | ±0.04 | 3.44 <sup>aC</sup>     | ±0.10 |
| 2—Brejinho          | 95 <sup>aA</sup>       | ±1.91 | 97 <sup>aA</sup>       | ±3.00 | 3.72 <sup>aB</sup>     | ±0.03 | 3.81 <sup>aB</sup>     | ±0.10 |
| 3—Grão do Sertão    | 99 <sup>aA</sup>       | ±1.00 | 98 <sup>aA</sup>       | ±2.00 | 3.21 <sup>aD</sup>     | ±0.06 | 3.38 <sup>aC</sup>     | ±0.06 |
| 4—BR 106            | 99 <sup>aA</sup>       | ±1.00 | 92 <sup>bA</sup>       | ±2.83 | 3.41 <sup>aC</sup>     | ±0.08 | 3.49 <sup>aC</sup>     | ±0.05 |
| 5—Alho Pipoca       | 91 <sup>aB</sup>       | ±1.00 | 83 <sup>bA</sup>       | ±5.74 | 4.29 <sup>bA</sup>     | ±0.23 | 4.00 <sup>aA</sup>     | ±0.18 |
| 6—Pontinha          | 63 <sup>aC</sup>       | ±5.26 | 39 <sup>bB</sup>       | ±3.42 | 4.14 <sup>aA</sup>     | ±0.25 | 4.07 <sup>aA</sup>     | ±0.03 |
| 7—Zé de Julho       | 100 <sup>aA</sup>      | ±0.00 | 99 <sup>aA</sup>       | ±1.00 | 3.29 <sup>aD</sup>     | ±0.06 | 3.42 <sup>aC</sup>     | ±0.07 |
| 8—Jaboatão Areia    | 98 <sup>aA</sup>       | ±1.15 | 97 <sup>aA</sup>       | ±1.91 | 3.49 <sup>aC</sup>     | ±0.05 | 3.76 <sup>bB</sup>     | ±0.08 |
| 9—Peba              | 97 <sup>aA</sup>       | ±1.00 | 98 <sup>aA</sup>       | ±1.15 | 3.56 <sup>aC</sup>     | ±0.06 | 3.55 <sup>aC</sup>     | ±0.07 |
| 10—Sol da Manhã     | 95 <sup>aA</sup>       | ±1.91 | 96 <sup>aA</sup>       | ±1.63 | 3.24 <sup>aD</sup>     | ±0.08 | 3.21 <sup>aC</sup>     | ±0.06 |
| 11—Índio            | 98 <sup>aA</sup>       | ±1.15 | 95 <sup>aA</sup>       | ±2.52 | 3.99 <sup>bA</sup>     | ±0.07 | 3.54 <sup>aC</sup>     | ±0.10 |
| 12—Jaboatão Remigio | 98 <sup>aA</sup>       | ±1.15 | 96 <sup>aA</sup>       | ±0.00 | 3.19 <sup>aD</sup>     | ±0.02 | 3.23 <sup>aC</sup>     | ±0.04 |
| 13—Pontinha Remigio | 92 <sup>aA</sup>       | ±4.62 | 97 <sup>aA</sup>       | ±1.91 | 3.49 <sup>aC</sup>     | ±0.08 | 3.41 <sup>aC</sup>     | ±0.07 |
| 14—Logradouro       | 96 <sup>aA</sup>       | ±1.63 | 100 <sup>aA</sup>      | ±0.00 | 3.78 <sup>aB</sup>     | ±0.07 | 3.78 <sup>aB</sup>     | ±0.03 |
| 15—Ideal            | 97 <sup>aA</sup>       | ±1.00 | 98 <sup>aA</sup>       | ±2.00 | 3.28 <sup>aD</sup>     | ±0.06 | 3.41 <sup>aC</sup>     | ±0.03 |

Note: Capital letters compare traditional varieties within salinity using the Scott-Knott test at a 5% probability level. Lowercase letters compare salinity within traditional varieties using Student's *t*-test at a 5% probability level. Standard error, *n* = 4.

The emergence speed index (ESI) showed less pronounced variations than the emergence index, suggesting greater stability with respect to salinity. Varieties such as V5—Alho Pipoca (ESR = 4.00) and V6—Pontinha (ESR = 4.07) showed the highest emergence speed rates even under saline stress. Under high salinity conditions, varieties such as V3—Grão do Sertão, V10—Sol d da Manhã, V12—Jaboatão Remigio, and V15—Ideal showed the lowest ESI values, despite maintaining high germination rates (Table 1).

Table 2 shows the average values for root length (RL) and shoot length (SL) of traditional maize varieties subjected to two levels of salinity (0.5 and 7.5 dS m<sup>-1</sup>) during the initial development phase. It can be observed that salt stress significantly reduced seedling growth in both the root and aerial parts, highlighting the deleterious effects of salinity on morphological development. The greatest reductions in RL were observed in varieties V5—Alho Pipoca and V6—Pontinha, with decreases of 30.15% and 44.20%, respectively, indicating high salinity sensitivity. SL proved to be even more sensitive to salinity, with significant reductions in almost all varieties below 7.5 dS m<sup>-1</sup>, notably V12—Jaboatão remigio, V2—Brejinho, and V14—Logradouro. In the SL, varieties such as V12—Jaboatão Remigio, V13—Pontinha Remigio, and V14—Logradouro maintained relatively high values even under salinity. The increase in RL observed in some varieties such as V9—Peba (21.85%) and V12—Jaboatão Remigio (16.21%) under saline stress.

**Table 2:** Test of means for root length (RL) and shoot length (SL) of traditional maize varieties subjected to salt stress in the early stages of development.

| Maize Varieties     | RL (cm)                |       |                        |       | SL (cm)                |       |                        |       |
|---------------------|------------------------|-------|------------------------|-------|------------------------|-------|------------------------|-------|
|                     | 0.5 dS m <sup>-1</sup> |       | 7.5 dS m <sup>-1</sup> |       | 0.5 dS m <sup>-1</sup> |       | 7.5 dS m <sup>-1</sup> |       |
| 1—Santana do Mato   | 16.80 <sup>aB</sup>    | ±0.40 | 15.30 <sup>aA</sup>    | ±0.59 | 28.77 <sup>aB</sup>    | ±0.86 | 25.44 <sup>bA</sup>    | ±0.85 |
| 2—Brejinho          | 17.23 <sup>aB</sup>    | ±0.46 | 13.75 <sup>bB</sup>    | ±0.96 | 30.59 <sup>aA</sup>    | ±0.35 | 25.39 <sup>bA</sup>    | ±0.48 |
| 3—Grão do Sertão    | 17.90 <sup>aB</sup>    | ±0.70 | 17.10 <sup>aA</sup>    | ±0.24 | 27.28 <sup>aB</sup>    | ±1.26 | 24.56 <sup>bA</sup>    | ±0.35 |
| 4—BR 106            | 18.61 <sup>aA</sup>    | ±0.93 | 15.59 <sup>bA</sup>    |       | 24.27 <sup>aC</sup>    | ±0.88 | 23.82 <sup>aB</sup>    | ±0.46 |
| 5—Alho Pipoca       | 14.66 <sup>aC</sup>    | ±0.63 | 10.24 <sup>bC</sup>    | ±0.35 | 21.25 <sup>bD</sup>    | ±0.11 | 23.60 <sup>aB</sup>    | ±0.63 |
| 6—Pontinha          | 18.80 <sup>aA</sup>    | ±1.85 | 10.49 <sup>bC</sup>    | ±0.40 | 21.04 <sup>aD</sup>    | ±1.80 | 21.61 <sup>aB</sup>    | ±0.30 |
| 7—Zé de Julho       | 20.29 <sup>aA</sup>    | ±0.58 | 15.86 <sup>bA</sup>    | ±0.72 | 29.01 <sup>aB</sup>    | ±0.37 | 25.95 <sup>bA</sup>    | ±0.29 |
| 8—Jaboatão Areia    | 20.31 <sup>aA</sup>    | ±0.25 | 15.62 <sup>bA</sup>    | ±1.12 | 30.01 <sup>aA</sup>    | ±0.17 | 26.19 <sup>bA</sup>    | ±0.74 |
| 9—Peba              | 13.00 <sup>bC</sup>    | ±0.36 | 15.84 <sup>aA</sup>    | ±0.80 | 26.58 <sup>aC</sup>    | ±0.44 | 25.03 <sup>aA</sup>    | ±0.29 |
| 10—Sol da Manhã     | 14.04 <sup>aC</sup>    | ±0.84 | 12.96 <sup>aB</sup>    | ±0.53 | 26.35 <sup>aC</sup>    | ±0.70 | ±0.23                  | ±0.46 |
| 11—Índio            | 16.70 <sup>aB</sup>    | ±1.36 | 17.56 <sup>aA</sup>    | ±1.39 | 25.33 <sup>aC</sup>    | ±0.63 | 22.61 <sup>bB</sup>    | ±0.64 |
| 12—Jaboatão Remígio | 11.35 <sup>aC</sup>    | ±0.47 | 13.19 <sup>aB</sup>    | ±0.72 | 31.58 <sup>aA</sup>    | ±1.04 | 25.03 <sup>bA</sup>    | ±0.77 |
| 13—Pontinha Remígio | 12.73 <sup>aC</sup>    | ±0.81 | 14.04 <sup>aB</sup>    | ±0.58 | 31.71 <sup>aA</sup>    | ±1.10 | 26.80 <sup>bA</sup>    | ±0.74 |
| 14—Logradouro       | 18.69 <sup>aA</sup>    | ±0.80 | 17.36 <sup>aA</sup>    | ±1.00 | 31.42 <sup>aA</sup>    | ±0.32 | 26.25 <sup>bA</sup>    | ±0.49 |
| 15—Ideal            | 17.80 <sup>aB</sup>    | ±1.39 | 15.67 <sup>aA</sup>    | ±0.85 | 27.79 <sup>aB</sup>    | ±1.16 | 24.89 <sup>bA</sup>    | ±0.33 |

Note: Capital letters compare Traditional varieties within salinity using the Scott-Knott test at a 5% probability level. Lowercase letters compare salinity within Traditional varieties using Student's *t*-test at a 5% probability level. Standard error, *n* = 4.

### 3.2 Phytomass Parameters

The root dry mass (RDM) showed significant variation both among salinity levels and among the traditional maize varieties evaluated. It was observed that salinity significantly reduced DRW, with an average of 95.02 mg in the control (0.5 dS m<sup>-1</sup>) and 89.12 mg under stress (7.5 dS m<sup>-1</sup>), representing an approximate drop of 5.9%. This result confirms the negative impact of salinity on root growth, even in the early stages (Table 3). The V4—BR 106 and V5—Alho Pipoca varieties stood out for having the lowest RDM values, indicating greater sensitivity to salt stress, with reductions of 34.14% and 47.52%, respectively, compared to the control. The varieties V8—Jaboatão Areia (133.25 mg), V9—Peba (107.50 mg), V11—Índio (107.25 mg), V13—Pontinha Remígio (113.00 mg), and V14—Logradouro (109.00 mg) stood out for having the highest root biomass accumulations, all with values above 100 mg.

The accumulation of shoot dry mass (SDM) and total dry mass (TDM) was affected by salt stress (7.5 dS m<sup>-1</sup>), causing significant reductions in the masses of most of the traditional maize varieties evaluated, evidencing the negative impact of salinity on plant growth (Table 4). The greatest reductions in SDM and TDM were observed in varieties V10—Sol da Manhã (17.37% SDM; 13.40% TDM), V11—Índio (18.78% SDM; 20.12% TDM), and V12—Jaboatão Remígio (23.97% SDM; 20.16% TDM), the latter being the most affected by salt stress. On the other hand, some varieties showed minimal reductions, suggesting greater tolerance to salt stress. Noteworthy are V3—Grão do sertão (2.92% SDM; 1.42% TDM), V6—Pontinha (0.27% SDM; 1.66% TDM), and V8—Jaboatão Areia (3.99% SDM; 2.33% TDM), with reductions of less than 4%. The V5—Alho Pipoca variety showed unique behavior. Despite having the lowest absolute biomass values, it was the only one to show increases in SDM (10.40%) and TDM (9.79%) under salt stress.



**Table 3:** Test of means for root dry mass (RDM) of traditional maize varieties subjected to salt stress in the early development stage.

| Salinity               | RDM (mg)           |       |
|------------------------|--------------------|-------|
| 0.5 dS m <sup>-1</sup> | 95.02 <sup>a</sup> | ±3.02 |
| 7.5 dS m <sup>-1</sup> | 89.12 <sup>b</sup> | ±2.85 |

| Maize Varieties     | RDM (mg)            |       |
|---------------------|---------------------|-------|
| 1—Santana do Mato   | 76.63 <sup>D</sup>  | ±2.71 |
| 2—Brejinho          | 93.75 <sup>C</sup>  | ±3.09 |
| 3—Grão do Sertão    | 91.50 <sup>C</sup>  | ±3.94 |
| 4—BR 106            | 60.88 <sup>E</sup>  | ±1.74 |
| 5—Alho Pipoca       | 47.50 <sup>F</sup>  | ±1.10 |
| 6—Pontinha          | 73.00 <sup>D</sup>  | ±3.09 |
| 7—Zé de Julho       | 88.38 <sup>C</sup>  | ±1.68 |
| 8—Jaboatão Areia    | 133.25 <sup>A</sup> | ±3.14 |
| 9—Peba              | 107.50 <sup>B</sup> | ±5.63 |
| 10—Sol da Manhã     | 92.38 <sup>C</sup>  | ±4.16 |
| 11—Índio            | 107.25 <sup>B</sup> | ±5.89 |
| 12—Jaboatão Remigio | 96.88 <sup>C</sup>  | ±3.40 |
| 13—Pontinha Remigio | 113.00 <sup>B</sup> | ±3.76 |
| 14—Logradouro       | 109.00 <sup>B</sup> | ±2.41 |
| 15—Ideal            | 90.13 <sup>C</sup>  | ±2.42 |

Note: Capital letters compare traditional varieties within salinity using the Scott-Knott test at the 5% probability level. Lowercase letters compare salinity within traditional varieties using tuk at the 5% probability level. Standard Error of Salinity, n = 60. Standard Error of Variety, n = 8.

**Table 4:** Test of means for shoot dry mass (SDM), total dry mass (TDM) of traditional maize varieties subjected to salt stress in the early development stage.

| Maize Varieties     | SDM (mg)               |        |                        |       | TDM (mg)               |        |                        |        |
|---------------------|------------------------|--------|------------------------|-------|------------------------|--------|------------------------|--------|
|                     | 0.5 dS m <sup>-1</sup> |        | 7.5 dS m <sup>-1</sup> |       | 0.5 dS m <sup>-1</sup> |        | 7.5 dS m <sup>-1</sup> |        |
| 1—Santana do Mato   | 119.75 <sup>aB</sup>   | ±8.61  | 112.75 <sup>aC</sup>   | ±4.85 | 199.50 <sup>aC</sup>   | ±10.52 | 186.25 <sup>aC</sup>   | ±7.31  |
| 2—Brejinho          | 129.00 <sup>aB</sup>   | ±1.87  | 114.50 <sup>aC</sup>   | ±1.76 | 226.75 <sup>aC</sup>   | ±4.71  | 204.25 <sup>aC</sup>   | ±4.89  |
| 3—Grão do Sertão    | 120.00 <sup>aB</sup>   | ±9.47  | 116.50 <sup>aC</sup>   | ±2.22 | 211.25 <sup>aC</sup>   | ±12.53 | 208.25 <sup>aC</sup>   | ±8.41  |
| 4—BR 106            | 91.00 <sup>aC</sup>    | ±1.78  | 84.00 <sup>aD</sup>    | ±0.91 | 153.75 <sup>aD</sup>   | ±3.25  | 143.00 <sup>aD</sup>   | ±1.91  |
| 5—Alho Pipoca       | 74.50 <sup>aD</sup>    | ±0.87  | 82.25 <sup>aD</sup>    | ±3.28 | 120.00 <sup>aE</sup>   | ±0.00  | 131.75 <sup>aD</sup>   | ±4.40  |
| 6—Pontinha          | 91.00 <sup>aC</sup>    | ±10.96 | 90.75 <sup>aD</sup>    | ±6.54 | 165.25 <sup>aD</sup>   | ±15.15 | 162.50 <sup>aD</sup>   | ±10.88 |
| 7—Zé de Julho       | 134.50 <sup>aB</sup>   | ±2.53  | 121.75 <sup>aC</sup>   | ±3.17 | 223.75 <sup>aC</sup>   | ±5.57  | 209.25 <sup>aC</sup>   | ±4.71  |
| 8—Jaboatão Areia    | 156.75 <sup>aA</sup>   | ±2.81  | 150.50 <sup>aA</sup>   | ±3.07 | 290.25 <sup>aA</sup>   | ±8.07  | 283.50 <sup>aA</sup>   | ±5.61  |
| 9—Peba              | 141.75 <sup>aA</sup>   | ±6.34  | 124.50 <sup>bC</sup>   | ±3.30 | 250.50 <sup>aB</sup>   | ±14.06 | 230.75 <sup>aB</sup>   | ±11.76 |
| 10—Sol da Manhã     | 129.50 <sup>aB</sup>   | ±8.03  | 107.00 <sup>bC</sup>   | ±5.12 | 225.75 <sup>aC</sup>   | ±10.55 | 195.50 <sup>bC</sup>   | ±12.78 |
| 11—Índio            | 135.75 <sup>aB</sup>   | ±7.35  | 110.25 <sup>bC</sup>   | ±6.21 | 256.00 <sup>aB</sup>   | ±5.52  | 204.50 <sup>bC</sup>   | ±11.77 |
| 12—Jaboatão Remigio | 152.25 <sup>aA</sup>   | ±4.99  | 115.75 <sup>bC</sup>   | ±5.15 | 256.75 <sup>aB</sup>   | ±4.68  | 205.00 <sup>bC</sup>   | ±8.57  |
| 13—Pontinha Remigio | 154.75 <sup>aA</sup>   | ±8.32  | 131.75 <sup>bB</sup>   | ±4.59 | 270.00 <sup>aB</sup>   | ±12.50 | 242.50 <sup>bB</sup>   | ±7.92  |
| 14—Logradouro       | 150.75 <sup>aA</sup>   | ±6.61  | 132.00 <sup>bB</sup>   | ±4.04 | 263.75 <sup>aB</sup>   | ±10.02 | 237.00 <sup>bB</sup>   | ±5.61  |
| 15—Ideal            | 132.50 <sup>aB</sup>   | ±8.41  | 117.00 <sup>aC</sup>   | ±5.60 | 225.75 <sup>aC</sup>   | ±8.81  | 204.00 <sup>aC</sup>   | ±9.86  |

Note: Capital letters compare Traditional varieties within salinity using the Scott-Knott test at a 5% probability level. Lowercase letters compare salinity within Traditional varieties using Student's *t*-test at a 5% probability level. Standard error, n = 4.

### 3.3 Biochemical and Salinity Tolerance Parameters

The increase in salinity caused significant changes in total soluble sugars (TSS) and total free amino acids (TFAA) sugars and amino acids, revealing different physiological strategies in response to two salinity levels (0.5 and 7.5 dS m<sup>-1</sup>) during the initial development phase (Table 5). The increase in total soluble sugars

(TSS) was significant in varieties such as V1—Santana do Mato, V2—Brejinho, V3—Grão do Sertão, and V4—BR 106, with increases of up to 28.88 units at  $7.5 \text{ dS m}^{-1}$ . For total free amino acids (TFAA), the behavior was more variable. Varieties such as V4—BR 106, V10—Sol da Manhã, V5—Alho Pipoca, and V9—Peba showed accumulations greater than 4 units under saline stress, suggesting adaptive physiological responses with multiple functions. On the other hand, varieties such as V3—Grão do Sertão and V12—Jaboatão Remígio showed reduced amino acid levels, suggesting a lower capacity for metabolic adjustment to salinity, which may reflect greater stress sensitivity.

**Table 5:** Test of means for total soluble sugars (TSS,  $\mu\text{mol GLU g}^{-1}$  fresh mass) and total free amino acids (TFAA,  $\mu\text{mol GLY g}^{-1}$  fresh mass) in the aerial parts of traditional maize varieties subjected to salt stress in the initial development phase.

| Maize Varieties     | TSS                     |       |                         |       | TFAA                    |       |                         |       |
|---------------------|-------------------------|-------|-------------------------|-------|-------------------------|-------|-------------------------|-------|
|                     | $0.5 \text{ dS m}^{-1}$ |       | $7.5 \text{ dS m}^{-1}$ |       | $0.5 \text{ dS m}^{-1}$ |       | $7.5 \text{ dS m}^{-1}$ |       |
| 1—Santana do Mato   | 27.42 <sup>bc</sup>     | ±2.83 | 56.30 <sup>aA</sup>     | ±3.53 | 10.50 <sup>aC</sup>     | ±0.56 | 10.90 <sup>aB</sup>     | ±1.03 |
| 2—Brejinho          | 32.81 <sup>bb</sup>     | ±2.58 | 52.04 <sup>aA</sup>     | ±2.98 | 12.64 <sup>aB</sup>     | ±1.79 | 10.81 <sup>aB</sup>     | ±0.93 |
| 3—Grão do Sertão    | 28.32 <sup>bc</sup>     | ±1.26 | 46.26 <sup>aB</sup>     | ±2.76 | 16.05 <sup>aA</sup>     | ±1.26 | 11.77 <sup>bb</sup>     | ±0.81 |
| 4—BR 106            | 28.31 <sup>bc</sup>     | ±2.40 | 43.41 <sup>aB</sup>     | ±3.06 | 9.51 <sup>bc</sup>      | ±0.70 | 15.32 <sup>aA</sup>     | ±1.16 |
| 5—Alho Pipoca       | 43.58 <sup>aA</sup>     | ±2.43 | 44.24 <sup>aB</sup>     | ±1.95 | 10.87 <sup>bc</sup>     | ±1.30 | 15.81 <sup>aA</sup>     | ±0.74 |
| 6—Pontinha          | 42.92 <sup>aA</sup>     | ±3.47 | 42.55 <sup>aB</sup>     | ±2.53 | 15.40 <sup>aA</sup>     | ±1.48 | 14.93 <sup>aA</sup>     | ±0.69 |
| 7—Zé de Julho       | 29.21 <sup>bc</sup>     | ±2.84 | 42.99 <sup>aB</sup>     | ±3.62 | 13.51 <sup>aB</sup>     | ±0.79 | 14.91 <sup>aA</sup>     | ±0.41 |
| 8—Jaboatão Areia    | 35.67 <sup>bb</sup>     | ±2.57 | 44.98 <sup>aB</sup>     | ±3.79 | 13.68 <sup>aB</sup>     | ±1.38 | 15.58 <sup>aA</sup>     | ±1.31 |
| 9—Peba              | 36.08 <sup>aB</sup>     | ±2.37 | 40.18 <sup>aB</sup>     | ±3.49 | 11.17 <sup>bc</sup>     | ±1.63 | 15.71 <sup>aA</sup>     | ±0.25 |
| 10—Sol da Manhã     | 26.08 <sup>bc</sup>     | ±0.30 | 37.54 <sup>aC</sup>     | ±4.07 | 9.90 <sup>bc</sup>      | ±0.78 | 14.88 <sup>aA</sup>     | ±0.53 |
| 11—Índio            | 40.94 <sup>aA</sup>     | ±1.83 | 38.38 <sup>aC</sup>     | ±0.75 | 10.60 <sup>aC</sup>     | ±0.94 | 11.59 <sup>aB</sup>     | ±1.10 |
| 12—Jaboatão Remígio | 48.87 <sup>aA</sup>     | ±1.09 | 39.90 <sup>bb</sup>     | ±1.42 | 12.74 <sup>aB</sup>     | ±0.65 | 9.82 <sup>aB</sup>      | ±1.00 |
| 13—Pontinha Remígio | 45.76 <sup>aA</sup>     | ±3.18 | 33.08 <sup>bc</sup>     | ±0.82 | 11.36 <sup>aC</sup>     | ±0.89 | 11.72 <sup>aB</sup>     | ±1.51 |
| 14—Logradouro       | 46.99 <sup>aA</sup>     | ±4.47 | 42.01 <sup>aB</sup>     | ±0.97 | 15.85 <sup>aA</sup>     | ±1.01 | 16.24 <sup>aA</sup>     | ±1.40 |
| 15—Ideal            | 35.05 <sup>aB</sup>     | ±1.55 | 33.11 <sup>aC</sup>     | ±3.15 | 13.01 <sup>aB</sup>     | ±0.87 | 13.41 <sup>aA</sup>     | ±0.38 |

Note: Capital letters compare Traditional varieties within salinity using the Scott-Knott test at a 5% probability level. Lowercase letters compare salinity within Traditional varieties using Student's *t*-test at a 5% probability level.

Traditional maize varieties showed distinct average free proline contents (PRO) in the aerial part when subjected to two salinity levels ( $0.5$  and  $7.5 \text{ dS m}^{-1}$ ) during the initial stage of development. The treatments and varieties differed significantly, revealing specific responses to salt stress (Table 6). In general, salt stress significantly increased proline accumulation in most of the varieties analyzed, confirming its importance as an adaptive mechanism. The varieties V7—Zé de Julho, V10—Sol da Manhã, and V11—Índio stood out with the highest contents below  $7.5 \text{ dS m}^{-1}$  (48.24, 48.60, and 48.89  $\text{mg g}^{-1}$ , respectively), representing increases of more than 200% compared to the control condition. Varieties such as V5—Alho Pipoca and V6—Pontinha also showed significant increases (40.45 and 33.73  $\text{mg g}^{-1}$ ), reinforcing their adaptive potential. In contrast, genotypes such as V1—Santana do Mato, V12—Jaboatão Remígio, and V15—Ideal exhibited low proline levels under salinity.

Analysis of the Salinity Tolerance Index (STI) revealed significant differences among traditional maize varieties in their response to salt stress during the initial stage of development (Table 6). Under control conditions ( $0.5 \text{ dS m}^{-1}$ ), all varieties presented an ITS equal to 100%, as expected, since this salinity level is used as a reference for calculating the index. However, under high salinity ( $7.5 \text{ dS m}^{-1}$ ), significant reductions were observed in several varieties. Noteworthy are varieties V11—Índio and V12—Jaboatão Remígio, which presented the lowest ITS values (79.88% and 79.84%, respectively), indicating greater sensitivity to salt stress.

On the other hand, the varieties V3—Grão do Sertão, V5—Alho Pipoca, and V8—Jaboatão Areia maintained ITS values above 96% (Table 6).

**Table 6:** Test of means for free proline content (PRO,  $\mu\text{mol PRO g}^{-1}$  fresh mass) and salinity tolerance index (STI, %) of traditional maize varieties subjected to salt stress in the early stages of development.

| Maize Varieties     | PRO                    |       |                        |       | STI                    |    |                        |       |
|---------------------|------------------------|-------|------------------------|-------|------------------------|----|------------------------|-------|
|                     | 0.5 dS m <sup>-1</sup> |       | 7.5 dS m <sup>-1</sup> |       | 0.5 dS m <sup>-1</sup> |    | 7.5 dS m <sup>-1</sup> |       |
| 1—Santana do Mato   | 26.99 <sup>aA</sup>    | ±5.80 | 13.41 <sup>bD</sup>    | ±2.41 | 100 <sup>aA</sup>      | ±0 | 92.54 <sup>bB</sup>    | ±2.95 |
| 2—Brejinho          | 17.36 <sup>aA</sup>    | ±1.64 | 23.28 <sup>aC</sup>    | ±2.91 | 100 <sup>aA</sup>      | ±0 | 90.08 <sup>bB</sup>    | ±2.16 |
| 3—Grão do Sertão    | 26.05 <sup>aA</sup>    | ±4.41 | 30.46 <sup>aC</sup>    | ±4.19 | 100 <sup>aA</sup>      | ±0 | 96.63 <sup>aA</sup>    | ±3.18 |
| 4—BR 106            | 20.30 <sup>aA</sup>    | ±3.82 | 27.88 <sup>aC</sup>    | ±2.76 | 100 <sup>aA</sup>      | ±0 | 93.01 <sup>bB</sup>    | ±1.25 |
| 5—Alho Pipoca       | 18.73 <sup>bA</sup>    | ±3.01 | 40.45 <sup>aB</sup>    | ±3.63 | 100 <sup>aA</sup>      | ±0 | 100.00 <sup>aA</sup>   | ±0.00 |
| 6—Pontinha          | 18.76 <sup>bA</sup>    | ±0.19 | 33.73 <sup>aB</sup>    | ±5.61 | 100 <sup>aA</sup>      | ±0 | 93.57 <sup>aB</sup>    | ±3.72 |
| 7—Zé de Julho       | 15.62 <sup>bB</sup>    | ±3.59 | 48.24 <sup>aA</sup>    | ±5.13 | 100 <sup>aA</sup>      | ±0 | 93.52 <sup>aA</sup>    | ±2.11 |
| 8—Jaboatão Areia    | 21.03 <sup>aA</sup>    | ±0.82 | 21.59 <sup>aC</sup>    | ±1.54 | 100 <sup>aA</sup>      | ±0 | 97.52 <sup>aA</sup>    | ±1.86 |
| 9—Peba              | 16.28 <sup>aB</sup>    | ±3.48 | 20.54 <sup>aC</sup>    | ±4.22 | 100 <sup>aA</sup>      | ±0 | 91.07 <sup>bB</sup>    | ±3.84 |
| 10—Sol da Manhã     | 7.16 <sup>bB</sup>     | ±0.89 | 48.60 <sup>aA</sup>    | ±4.66 | 100 <sup>aA</sup>      | ±0 | 86.60 <sup>bC</sup>    | ±5.66 |
| 11—Índio            | 15.13 <sup>bB</sup>    | ±3.72 | 48.89 <sup>aA</sup>    | ±4.37 | 100 <sup>aA</sup>      | ±0 | 79.88 <sup>bD</sup>    | ±4.60 |
| 12—Jaboatão Remigio | 8.85 <sup>aB</sup>     | ±1.74 | 11.08 <sup>aD</sup>    | ±3.43 | 100 <sup>aA</sup>      | ±0 | 79.84 <sup>bD</sup>    | ±3.34 |
| 13—Pontinha Remigio | 13.87 <sup>aB</sup>    | ±2.55 | 15.58 <sup>aD</sup>    | ±0.96 | 100 <sup>aA</sup>      | ±0 | 89.81 <sup>bB</sup>    | ±2.93 |
| 14—Logradouro       | 20.79 <sup>aA</sup>    | ±4.50 | 27.96 <sup>aC</sup>    | ±2.97 | 100 <sup>aA</sup>      | ±0 | 89.86 <sup>bB</sup>    | ±2.13 |
| 15—Ideal            | 7.92 <sup>aB</sup>     | ±1.44 | 12.35 <sup>aD</sup>    | ±3.66 | 100 <sup>aA</sup>      | ±0 | 90.37 <sup>bB</sup>    | ±4.37 |

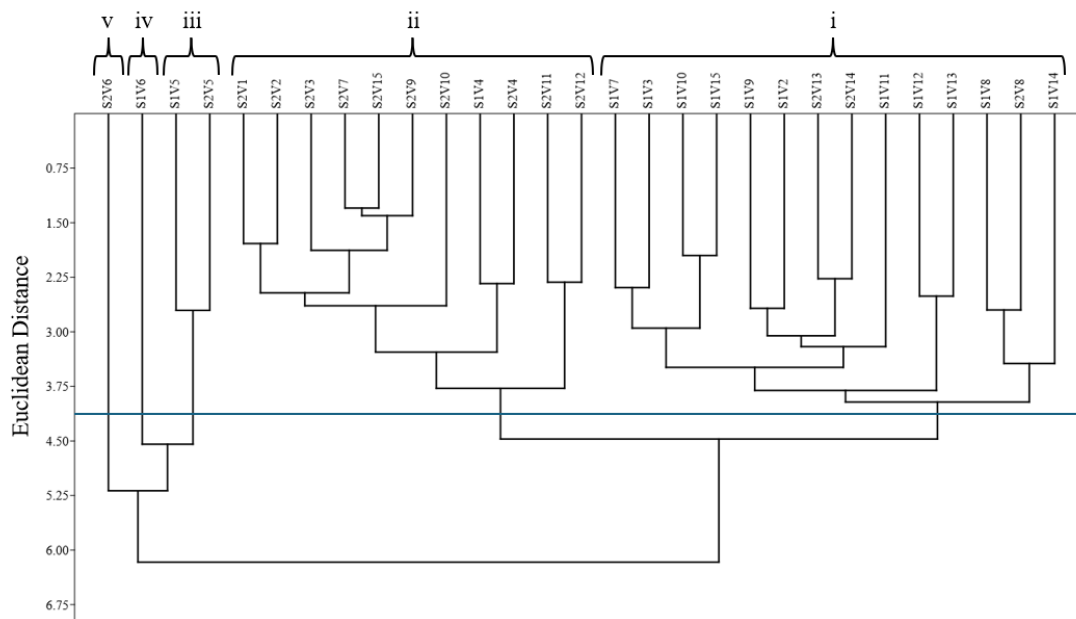
Note: Capital letters compare traditional varieties within salinity using the Scott-Knott test at a 5% probability level. Lowercase letters compare salinity with the s of traditional varieties using Student's *t*-test at a 5% probability level. Standard error, *n* = 4.

Based on the classification proposed by Fageria [30], most varieties were considered tolerant (ITS  $\geq 80.1\%$ ), demonstrating good performance even under moderate saline conditions. Only two varieties, V11—Índio and V12—Jaboatão Remigio, were classified as moderately tolerant ( $60.1\% \leq \text{ITS} < 80.0\%$ ), showing a slight reduction in performance but still able to adapt. No variety was classified as susceptible (ITS  $< 40\%$ ). During the early growth stage, all varieties were tolerant to salt stress at 7.5 dS m<sup>-1</sup>, except for V11 and V12, which showed only moderate tolerance. This result suggests that most traditional maize varieties possess sufficient physiological and biochemical mechanisms to cope with high salinity during the initial phases of development. However, tolerance expressed at early stages does not necessarily persist throughout the plant's life cycle. Future studies should therefore evaluate the effects of this salinity level across subsequent developmental stages, including vegetative growth, flowering, and grain production, to determine whether these varieties maintain their performance under prolonged salt stress.

The dendrogram shows the formation of five distinct groups of samples, defined by the cut-off point on the Euclidean distance line ( $\approx 4.1$ ). Samples that cluster at lower levels show greater similarity to each other, while those that cluster only at higher levels show greater dissimilarity (Fig. 1).

The results of the hierarchical cluster analysis, represented in the dendrogram, reveal a clear differentiation between the traditional maize genotypes subjected to the two salinity levels (S1 = 0.5 dS m<sup>-1</sup> and S2 = 7.5 dS m<sup>-1</sup>). Under the lower salinity condition (S1), Group I included varieties V7 (Zé de Julho), V3 (Grão do Sertão), V15 (Ideal), V9 (Peba), V2 (Brejinho), V11 (Índio), V12 (Jaboatão Remigio), V13 (Pontinha Remigio), V8 (Jaboatão Areia), and V14 (Logradouro). In S2, this same group was reduced to only three varieties: V13, V14, and V8, suggesting that more intense salinity promoted greater divergence among genotypes; however, the tolerance of these three varieties is similar to that of the others in condition S1. Group II presented, in S1, only variety V4 (BR 106), while in S2, it added a broad set of genotypes (V1, V2, V3, V7, V15, V9, V10, V4, V11, and V12), showing that, under higher salinity, these materials share physiological

responses similar to V4 under S1. Group III maintained the V5 variety (Alho Pipoca) in both conditions, demonstrating consistent and differentiated behavior in relation to the others. Group IV included only V6 (Pontinha) in S1, while in S2, this same variety migrated to Group V, reinforcing its unique response to salt stress.



**Figure 1:** The dendrogram was constructed based on Euclidean distance, grouping the 15 native varieties (V1 to V15) into five main groups (i to v), defined by the cut on the blue line ( $\approx 4.1$  distance) V1—Santana do Mato; V2—Brejinho; V3—Grão do Sertão; V4—BR 106; V5—Alho Pipoca; V6—Pontinha; V7—Zé de Julho; V8—Jaboatão Areia; V9—Peba; V10—Sol da Manhã; V11—Índio; V12—Jaboatão Remígio; V13—Pontinha Remígio; V14—Logradouro; V15—Ideal. S1 =  $0.5 \text{ dS m}^{-1}$  and S2 =  $7.5 \text{ dS m}^{-1}$ .

In summary, the results show that the distribution of genotypes across groups varied with salinity level, with greater dispersion and reorganization in S2. This dynamic shows that some varieties maintain stable response patterns (such as V5 and V6). In contrast, others substantially shift their positions within the groupings, reflecting different adaptive strategies in response to salt stress. This differential behavior across groups highlights the genetic diversity within native varieties. It suggests potential sources of tolerance that can be explored in breeding programs aimed at cultivation in saline environments.

#### 4 Discussion

Saline stress significantly reduces crop yields in arid and semi-arid regions. In these regions, maize serves as a staple crop, primarily within smallholder family farming. Consequently, identifying salt-tolerant maize cultivars is crucial to ensure reliable yields in saline soils. To address this issue, we conducted a screening experiment to identify salt-stress-tolerant genotypes. The results show that maize's response to salt stress depends heavily on its genotype. Specifically, traditional maize varieties use different physiological and biochemical strategies to adapt. Given the strong link between salinity and genotype across most variables, it is important to identify and select more tolerant genetic materials; this selection becomes increasingly vital as soil salinization becomes more common. In salty soils, lower osmotic potential makes it harder for seeds to take up water, which affects early germination [2]. In addition, excess  $\text{Na}^+$  and  $\text{Cl}^-$  are toxic, impairing enzyme activity and slowing germination and early seedling growth [15]. Notably, these

effects match earlier findings [32], which reported that salinity lowers soil osmotic potential, reducing water absorption and delaying germination. Even with these challenges, some varieties still had high emergence rates under high salinity, suggesting that they activate efficient physiological mechanisms. These include osmotic adjustment, increased antioxidant activity, and maintenance of cell integrity [19]. Furthermore, according to [33], salt-tolerant maize usually accumulates more proline, reduces reactive oxygen species (ROS), and boosts antioxidant enzymes such as SOD, POD, and GPX. Collectively, these responses protect cells and help seedlings stay healthy.

The emergence speed index (ESI) remained mostly stable across genotypes, suggesting that salinity affects germination speed less than the total number of seeds that emerge. However, differences in ESI between genotypes indicate that metabolic efficiency varies during early growth. Fast germination under saline conditions is usually linked to the production of compatible osmolytes and the activation of antioxidant defenses [23,26]. Conversely, slow emergence may reflect lower metabolic efficiency or slower activation of enzymes like amylases, which help break down starch for energy [20]. These results are consistent with those of Praxedes et al. [8], who reported that salinity delays emergence in sensitive genotypes but does not fully prevent germination. Salty conditions lead to shorter roots and shoots due to osmotic stress. Increased soil salt means less water is available, which lowers turgor pressure, may cause plasmolysis, and halts cell expansion [34]. In addition to these osmotic effects, toxic ions and nutrient imbalances caused by  $\text{Na}^+$  and  $\text{Cl}^-$  build-up disrupt important processes, including nutrient transport, enzyme activity, and energy metabolism [2,35]. Structural changes, such as increased suberization in roots and earlier Casparian strip formation, also limit water and nutrient flow to the plant's vascular system [15]. Finally, Liu et al. found that salinity damages root structure by reducing root length and dry mass, making primary roots thicker and lateral roots fewer. These changes help limit root exposure to salt.

Some genotypes maintained or even increased root growth under stress. This indicates they have mechanisms to compensate for it. Longer roots in some varieties may help them reach more soil, water, and nutrients in tough conditions [36]. Hormones such as ABA and auxins control these responses. Certain genes for ion transport and osmolyte production must also be active [37]. Important tolerance systems include ion exclusion or compartmentalization, balancing  $\text{Na}^+$  and  $\text{K}^+$ , and producing osmolytes like proline and glycine betaine. Antioxidant enzymes (SOD, CAT, APX) are also involved [20,38,39]. Thicker root tissues and more barriers in the root help block ions and reduce oxidative damage [34]. Lower root dry mass (RDM) under salt stress indicates that roots are sensitive to it, as others have found [4]. Salinity reduces water uptake and builds up toxic ions. These harm membranes and slow cell division. Li et al. [40] say roots are the first to sense and respond to salt stress. The root system's ability to change shape and function is very important for stress tolerance. Most genotypes had less shoot dry mass (SDM) and total dry mass (TDM). This shows the effects of both osmotic stress and toxic ions. The buildup of  $\text{Na}^+$  and  $\text{Cl}^-$  reduces water uptake. This causes a form of drought that slows cell growth and lowers biomass. Competition between  $\text{Na}^+$  and nutrients, such as  $\text{K}^+$  and  $\text{Ca}^{2+}$ , also disrupts key processes. These include photosynthesis, respiration, protein synthesis, and nutrient transport [16,17,39,41]. These issues reduce the production and use of photoassimilates. This results in lower biomass accumulation.

Some genotypes lost little or even gained biomass under salt stress. This shows strong tolerance. They may store ions in vacuoles to protect cellular functions and have higher antioxidant activity, which is enhanced by proline. Higher total soluble sugars (TSS) during salt stress show that these sugars help plants adjust. Sugars like sucrose, glucose, and fructose help maintain cell water balance and do not disrupt metabolism. They also act as antioxidants, stabilize membranes and proteins, and protect against ROS

damage [42–44]. SWEET family genes, such as ZMsweet7, help transport sugars. These genes improve photosynthesis and enhance stress tolerance [45,46].

Total free amino acids (TFAA) have several roles during stress. Amino acids such as proline, glutamine, alanine, and glycine betaine help with osmotic adjustment, neutralize ROS, and stabilize cellular structures [47,48]. Genotypes differ in the flexibility of their metabolism and their ability to adjust to salinity, affecting their salt stress tolerance [13]. Proline build-up is a well-known sign of salt stress tolerance. A big increase in proline under salt stress shows its role as a multifunctional osmolyte. It helps keep osmotic balance, keeps cells firm, removes ROS, and stabilizes proteins and membranes [2,40,43]. High proline in some genotypes means strong defense. Low levels in others suggest weaker adaptation.

The Salinity Tolerance Index (STI) is a useful tool for assessing how well genotypes perform under stress. STI values make it easy to quickly sort genotypes by their tolerance. High STI in most varieties indicates they are generally tolerant of salinity at early stages. This tolerance may not persist later. The hierarchical cluster analysis showed clear genetic differences among genotypes. It also highlighted how their responses to salinity can change. Genotypes changed groupings at higher salinity, revealing different adaptive strategies. This points to strong genetic diversity in traditional maize varieties. Such diversity is valuable to breeding programs developing crops for saline environments.

## 5 Conclusions

Salinity at 7.5 dS m<sup>-1</sup> affected post-emergence growth and biomass accumulation more severely than germination, indicating that seedling establishment is the most sensitive stage for identifying salt tolerance in traditional maize varieties. Although most varieties maintained stable emergence, significant reductions in root and shoot growth highlighted substantial genotypic variability in morphophysiological responses to salt stress. The varieties V5—Alho Pipoca, V8—Jaboatão Areia, and V3—Grão do Sertão exhibited the highest tolerance indices (>97%) and should therefore be given priority in breeding programs targeting saline environments. Additionally, V8—Jaboatão Areia and V9—Peba sustained strong root development under salinity, suggesting adaptive functions that improve water and nutrient acquisition. In contrast, V10—Sol da Manhã, V11—Índio, and V12—Jaboatão Remígio showed the greatest biomass reductions and the lowest tolerance indices, indicating greater sensitivity. Biochemical responses, particularly the accumulation of soluble sugars and proline, demonstrated activation of osmotic adjustment and cellular protection mechanisms, reinforcing their potential as complementary markers for early screening of salt tolerance.

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