

Study of the plantain banana plants (*Musa paradisiaca* L.) adaptation to soil salinity: synthesis of the biochemical compounds, total soluble sugars, proline, glycine betaine and foliar pigments

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Abstract

Plantain bananas are consumed food in tropical countries. In Côte d'Ivoire, yields remain very low in coastal strip due to soil salinity. This study was conducted to evaluate acclimatization of banana plants to salinity through synthesis of photosynthetic pigments, total soluble sugars, proline, and glycine betaine. Plants aged 30 and 60 days were treated with 5, 10 and 15 g/L of NaCl. Results showed that synthesis of photosynthetic pigments increased with plant age. With 5 g/L of NaCl, chlorophyll b content was 0.44 (30-day-old plants) and 1.06 $\mu\text{g/gFW}$ (60-day-old plants). It remained elevated in 30-day control plants (0.63 $\mu\text{g/g WF}$). At 10 g/L of NaCl, chlorophyll a and carotenoids reached values of 1.51 and 2.78 $\mu\text{g/gFW}$ and 0.78 and 0.97 $\mu\text{g/gFW}$, respectively, in 30- and 60-day plants. Total soluble sugars and proline were more abundant in leaves. Sugars showed high values at 10 g/L of NaCl ($17.68 \times 10^{-2} \mu\text{g/gFW}$ in 30-day plants and $21.76 \times 10^{-2} \mu\text{g/gFW}$ in 60-day plants), and proline at 15 g/L of NaCl ($10.29 \times 10^{-2} \mu\text{g/gFW}$ in 30-day plants and $8 \times 10^{-2} \mu\text{g/gFW}$ in 60-day plants). Glycine betaine was elevated in both leaves and roots. At 5 g/L of NaCl, values were high in 30-day leaves ($11.82 \times 10^{-2} \mu\text{g/gFW}$) and 60-day roots ($10.89 \times 10^{-2} \mu\text{g/gFW}$). At 15 g/L of NaCl, values in 30-day roots and 60-day leaves were 13.92×10^{-2} and $12.81 \times 10^{-2} \mu\text{g/gFW}$, respectively. In conclusion, banana plant achieves acclimatization through synthesis of biochemical compounds

Keywords: *Musa paradisiaca*; Soil Salinity; Biochemical Compounds; Acclimatization

1. Introduction

Soil salinity is becoming an increasingly major problem for the global agricultural sector because it limits crop growth and productivity. According to FAO reports [1], approximately 10.7% of the Earth's land surface is affected by salinity, representing about 1.381 million hectares. Arid and semi-arid climates are the most impacted. It is primarily linked to an excess of Na^+ , Ca^{2+} , and Mg^{2+} cations and Cl^- , SO_4^{2-} , and HCO_3^- anions in the growing medium [2]. However, Na^+ and Cl^- are the most toxic to plants when they accumulate in large quantities [3]. Natural salinization is the most widespread. It results from the decomposition of minerals or when a saline aquifer is located close to the Earth's surface [4]. In arid and semi-arid ecosystems, it results from high evaporation of water from the soil [5]. Oceanic or coastal salts also contribute to soil salinization. These so-called "cyclic" salts are transported by wind and deposited on land following rainfall. Studies on plants under salt stress reveal that salt stress affects plant morphology, physiological and metabolic functions, leading to reduced growth and decreased yield [6]. In bananas (*Musa paradisiaca* L.), high salinity levels lead to delayed flowering and reduced yield [7]. However, in Côte d'Ivoire, this plant ranks third after yam (*Dioscorea alata* L.) and cassava (*Manihot esculenta* Crantz), with an estimated annual production of over 1.7 million tons [8]. It contributes to food security and provides income for producers [9]. Despite its nutritional and economic importance, plantain cultivation is largely confined to the eastern, central-western, southwestern, and western regions [10]. In the

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northern and southeastern coastal areas, plantain cultivation is almost nonexistent. In northern Côte d'Ivoire, the scarcity of rainfall over extended periods makes growing this plant difficult.

As for the southeastern coastal region, in addition to biotic factors common to all plants [11], soil salinity significantly reduces yields. This study was conducted in the context of climate change and investigated the impact of salinity in the agricultural environment on the synthesis of leaf pigments, total soluble sugars, proline and glycine betaine.

2. Materials and methods

2.1. Plant material

The plantain banana plants used in this study were 30 and 60 days old. These plants were obtained using the PIF technique in a germination chamber and transferred to a nursery in pots containing a sawdust substrate.

2.2. Experimental setup and plant treatment

The experimental setup consisted of 40 plantain banana plants, each placed in a tray containing sawdust, arranged in completely randomized blocks. The 40 plants were divided into two groups: 20 plants aged 30 days and 20 plants aged 60 days. Each group was further subdivided into four subgroups of five plants each, according to the following treatment types: plants in the subgroup treated with 5 g/L of NaCl solution; plants in the subgroup treated with 10 g/L of NaCl solution; plants in the subgroup treated with 15 g/L of NaCl solution; and untreated plants in the subgroup, referred to as control plants. The treatment was carried out by watering the substrate containing the plants with 100 mL of NaCl solution. Banana plants were placed in a greenhouse, and the leaves and roots were harvested after 24 hours of processing and stored at 4°C in a melting ice bath.

2.3. Quantification of chlorophyll and carotenoid pigments in leaves

The extraction and quantification of leaf pigments were performed according to the method of Ferus & Arkosiova [12]. A quantity of 0.5 g of fresh plantain banana leaves was ground in the presence of 25 mL of 80% (v/v) acetone and a pinch of sodium bicarbonate. The ground material was centrifuged at 5000 rpm for 10 min at 4°C. The supernatant was collected and stored at 4°C in tubes lined with aluminum foil. For the determination of leaf pigments, 3 mL of extract were transferred into a test tube and the absorbance was measured at 645 and 663 nm by spectrophotometer against a control made of 80% acetone. The chlorophyll and carotenoid content of the leaves was evaluated according to the formulas of Lichtenthaler [13] and values were expressed in micrograms per gram of fresh weight (µg/gFW).

- Chlorophyll a (mg/l) = $12.7 A_{663} - 2.69 A_{645}$
- Chlorophyll b (mg/l) = $22.9 A_{645} - 4.68 A_{663}$
- Total chlorophyll (mg/l) = $8.02 A_{663} + 20.20 A_{645}$
- Carotenoids = $[(1000 \times A_{470}) - (2.13 \text{ chlorophyll a}) - (97.64 \text{ chlorophyll b})] / 198$

2.4. Quantification of total soluble sugars in the leaves and roots of banana plants

The method of Das et al. [14] modified and adapted to our equipment, was used for the extraction of total soluble sugars. A 1 g mass of fresh leaves or roots was ground in 10 mL of 80% (v/v) methanol. The ground material was transferred to a test tube and incubated at 80°C for 10 min in a water bath. After cooling in an ice bath, the ground material was centrifuged at 10000 rpm for 10 min. The supernatant was collected and stored at 2°C. The total sugars were determined in a reaction medium containing 1 mL of supernatant, 1 mL of 5% (v/v) phenol, and 5 mL of concentrated sulfuric acid solution. When a yellow-orange color appeared on the surface, the mixture was vortexed to homogenize the solution. After a 10-minute rest period, the tubes were heated in a water bath at 30°C for 15 minutes. After cooling, the absorbance was determined at 585 nm using a spectrophotometer against a blank or a sample where the extract was replaced with distilled water. Soluble sugar concentrations were determined using a standard curve prepared from a glucose solution with concentrations of 0 to 1000 µg/mL. The soluble sugar content of the samples was expressed in micrograms per gram of fresh weight (µg/gFW).

2.5. Estimation of the amount of proline in the leaves and roots of plantain banana plants

Proline was extracted and quantified according to the method proposed by Monneveux & Nemmar [15]. For the extraction, 1 g of fresh roots or leaves was placed in 2 mL of 40% (v/v) methanol, hermetically sealed in a test tube, and heated to 80 °C for 60 min in a water bath. After cooling, 1 mL of the solution was taken and added to 1 mL of acetic acid and 1 mL of a mixture (120 mL distilled water + 300 mL acetic acid + 25 mg ninhydrin + 80 mL orthophosphoric acid).

The mixture was boiled at 100 °C for 30 min until it turned red. After cooling, 5 mL of toluene was added to the extract, and the mixture was shaken for 10 to 20 seconds. The upper organic phase containing proline was recovered and dehydrated by adding 5 mg of sodium sulfate (Na_2SO_4) using a spatula, and was used for the assay. The absorbance was determined at 528 nm. The spectrophotometer zero was set using a blank composed of 1 mL of 40% (v/v) methanol, 1 mL of acetic acid, 1 mL of the mixture, and 25 mg of ninhydrin. Proline concentrations in the extract were determined using a standard curve prepared from increasing proline concentrations from 0 to 1000 $\mu\text{g/mL}$. Proline contents are expressed in micrograms per gram of fresh weight ($\mu\text{g/gFW}$).

2.6. Estimation of glycine betaine content in plantain banana leaves and roots

The glycine betaine content was determined according to the method of Grieve & Grattan [16]. A 0.5 g mass of finely ground, dry plant material was mixed with 20 mL of distilled water and kept under mechanical stirring for 48 h at 25 °C. The mixture was centrifuged at 10000 rpm for 10 min. The supernatant was collected and stored in a freezer until analysis. The samples were thawed and diluted (1:1, v/v) with 2 N sulfuric acid (H_2SO_4). A 0.5 mL aliquot was taken and incubated for 1 h on ice. Then, 0.2 mL of cold potassium iodide-iodine (KI-I_2) was added and gently vortexed. The samples were stored between 0 and 4 °C for 16 h. The mixture was centrifuged at 10000 rpm for 15 min at 0 °C. The pellet was dissolved in 9 mL of 1,2-dichloroethane, thoroughly homogenized, and allowed to stand for 2 h. Absorbance was determined at 365 nm using a spectrophotometer. The amount of glycine betaine in the extract was determined from a standard curve (50 to 200 $\mu\text{g/mL}$). The stock solution was prepared by diluting 1 mg/mL in 1 N sulfuric acid.

2.7. Data analysis

Statistical analyses of the data were performed using Excel 2013 and STATISTICA 7.1 software. A one-way analysis of variance (ANOVA) was performed for each parameter studied at the 5% significance level. When $p < 0.05$, the difference between the means is considered significant. Homogeneous groups were determined using the Newman-Keuls test.

3. Results

3.1. Variation in the leaf pigment content of plantain banana plants after treatment of the culture medium with NaCl solution.

Table 1 shows the amount of chlorophyll and carotenoids extracted from the leaves of plantain banana plants treated with the NaCl solution. In general, the amount of these receptor pigments varied according to the age of the plants and the NaCl concentration. In 30-day-old plants, the amount of chlorophyll a (Chla), b (Chlb), and total (Chlt), as well as that of carotenoids (Car), was 0.86 (Chla), 0.63 (Chlb), 1.49 (Chlt), and 0.10 $\mu\text{g/gFW}$ (Car), respectively, in the control group. Initially at 0.86 $\mu\text{g/gFW}$ for Chla, the quantity remained almost identical in the presence of 5 g/L NaCl (0.82 $\mu\text{g/gFW}$). However, with 10 g/L NaCl, it reached a value of 1.51 $\mu\text{g/gFW}$. At a concentration of 15 g/L NaCl, the quantity was 0.82 $\mu\text{g/gFW}$. In these plants, the quantity of Chlb decreased from 0.63 (control) to 0.44 (5 g/L NaCl) and to 0.22 $\mu\text{g/gFW}$ (10 g/L NaCl). At a concentration of 15 g/L NaCl (0.43 $\mu\text{g/gFW}$), the content remained identical to that of 5 g/L NaCl. As for Chlt, only treatment of the plants with 10 g/L NaCl resulted in an increase, reaching a value of 1.73 $\mu\text{g/gFW}$. The carotenoid content of the leaves was also influenced by the NaCl treatment. The highest quantity of carotenoids was observed in the plants treated with 10 g/L NaCl (0.78 $\mu\text{g/gFW}$).

In 60-day-old plants, the leaf content of chlorophyll pigments and carotenoids was higher in the control group than in the 30-day-old control group, except for Chla. When the plants were treated with the NaCl solution, the quantity of these pigments increased regardless of the concentration. However, the quantity of Chlb and Chlt decreased with increasing concentration. decreasing from 1.06 to 0.84 $\mu\text{g/gFW}$ for Chlb and from 3.80 to 3.39 $\mu\text{g/gFW}$ for Chlt. That of Chla (2.78 $\mu\text{g/gFW}$) and of Car (0.97 $\mu\text{g/gFW}$) was maximal at NaCl 10 g/L.

Table 1 Chlorophyll and carotenoid content in plantain banana plant leaves after treatment of the culture medium with NaCl solution

Treatments	Leaf pigments (µg/gFW)							
	Chlorophyll a		Chlorophyll b		Total Chlorophyll		Carotenoids	
	30 j	60 j	30 j	60 j	30 j	60 j	30 j	60 j
NaCl 5g/L	0.82 ±0.10 ^a	2.74 ±0.49 ^a	0.44 ±0.12 ^a	1.06 ±0.20 ^b	1.26 ±0.02 ^a	3.80 ±0.68 ^b	0.24 ±0.08 ^b	0.77 ±0.11 ^b
NaCl 10g/L	1.51 ±0.11 ^a	2.78 ±0.08 ^a	0.22 ±0.08 ^a	0.87 ±0.31 ^b	1.73 ±0.16 ^a	3.66 ±0.22 ^b	0.78 ±0.05 ^b	0.97 ±0.08 ^c
NaCl 15g/L	0.82 ±0.11 ^a	2.55 ±0.31 ^a	0.43 ±0.05 ^a	0.84 ±0.19 ^b	1.26 ±0.15 ^a	3.39 ±0.17 ^b	0.29 ±0.04 ^b	0.58 ±0.07 ^a
Control	0.86 ±0.73 ^a	2.01 ±0.64 ^a	0.63 ±0.38 ^a	0.20 ±0.12 ^a	1.49 ±0.44 ^a	2.21 ±0.56 ^a	0.10 ±0.26 ^a	0.50 ±0.08 ^a
<i>p-values</i>	<i>0.14</i>	<i>0.21</i>	<i>0.13</i>	<i>0.002</i>	<i>0.19</i>	<i>0.006</i>	<i>0.011</i>	<i>0.001</i>

In each column, values with the same letter are statistically identical at the 5% threshold (Newman-Keuls test).

3.2. Variation in total soluble sugar content in the leaves and roots of plantain banana plants after treatment of the culture medium with NaCl solution.

The total soluble sugar content extracted from the leaves and roots of plantain banana plants treated with NaCl solution is recorded in Table 2 below. Generally, the amount of total soluble sugars in these organs was higher in the leaves. When 30-day-old plants were treated with NaCl solution, the amount of total soluble sugars increased from 8.10×10^{-2} (control) to 14.01×10^{-2} µg/gFW (5 g/L of NaCl). With increasing NaCl concentration in the culture medium, the total sugar content also increased, reaching 17.88×10^{-2} µg/gFW at a NaCl concentration of 10 g/L before decreasing to 12.77×10^{-2} µg/gFW at 15 g/L of NaCl. In the roots, the highest levels were recorded at NaCl concentrations of 5 g/L (6.24×10^{-2} µg/gFW) and 10 g/L (6.26×10^{-2} µg/gFW). In 60-day-old plants, the total soluble sugar content (3.03×10^{-2} µg/gFW) was slightly low in the roots of untreated (control) plants. After treatment, it increased significantly in both the leaves and roots. In the leaves, the values exceeded 15×10^{-2} µg/gFW. It was maximal with NaCl 10 g/L (21.77×10^{-2} µg/gFW). Similarly, in the roots, it reached a value of 13.42×10^{-2} µg/gFW at the same concentration.

Table 2 Total soluble sugar content of leaves and roots of plantain banana plants after treatment of the culture medium with NaCl solution

Treatments	Total sugar content of organs ($\times 10^{-2}$ µg/gFW)			
	30-day-old plants		60-day-old plants	
	Leaves	Roots	Leaves	Roots
NaCl 5g/L	14.02±0.64 ^b	6.24±0.53 ^b	18.38±0.27 ^b	10.50±0.52 ^c
NaCl 10g/L	17.68±1.61 ^c	6.26±0.50 ^b	21.77±0.79 ^c	13.41±0.44 ^d
NaCl 15g/L	12.76±2.31 ^b	5.56±0.27 ^b	16.21±0.13 ^b	5.31±0.16 ^b
Control	8.10±0.10 ^a	4.93±0.45 ^a	8.01±2.29 ^a	3.03±0.15 ^a
<i>p-values</i>	0.001	0.018	0.001	0.001

In each column, values bearing the same letter of the alphabet are statistically identical at the 5% threshold (Newman-Keuls test)

3.3. Variation in proline content in the leaves and roots of plantain banana plants after treatment of the culture medium with NaCl solution.

Table 3 shows the amount of proline extracted from the leaves and roots of plantain banana plants treated with NaCl solution. In 30-day-old plants, the proline content in the leaves and roots increased with the NaCl concentration in the culture medium. Initially 5.98×10^{-2} µg/gFW in the leaves, it increased to 7.71 µg/gFW (5 g/L of NaCl), 8.28 µg/gFW

(10 g/L of NaCl), and 10.29×10^{-2} $\mu\text{g/gFW}$ (15 g/L of NaCl) when the culture medium was treated with NaCl. Similarly, in the roots, the amount of proline increased from 5.75 (control) to 8.8×10^{-2} $\mu\text{g/gFW}$ (15 g/L of NaCl). However, in 60-day-old plants, the proline content in the leaves and roots was slightly higher than in 30-day-old plants in the control group. When the plants were treated with the NaCl solution, a small increase in proline content was observed at all concentrations used. It ranged from 7.61 to 8×10^{-2} $\mu\text{g/gFW}$ in the leaves and from 6.21 to 6.33×10^{-2} $\mu\text{g/gFW}$ in the roots.

Table 3 Proline content in the leaves and roots of plantain banana plants after treatment of the culture medium with NaCl solution

Treatments	Proline content of organs ($\times 10^{-2}$ $\mu\text{g/gFW}$)			
	30-day-old plants		60-day-old plants	
	Leaves	Roots	Leaves	Roots
NaCl 5g/L	7.71 $\pm$ 0.01 ^b	6.83 $\pm$ 0.00 ^b	7.61 $\pm$ 0.00 ^b	6.21 $\pm$ 0.00 ^b
NaCl 10g/L	8.25 $\pm$ 0.00 ^b	8.23 $\pm$ 0.01 ^c	7.72 $\pm$ 0.00 ^b	6.23 $\pm$ 0.00 ^b
NaCl 15g/L	10.29 $\pm$ 0.00 ^c	8.80 $\pm$ 0.01 ^c	8.00 $\pm$ 0.00 ^b	6.33 $\pm$ 0.00 ^b
Control	5.98 $\pm$ 0.00 ^a	5.75 $\pm$ 0.00 ^a	6.73 $\pm$ 0.01 ^a	5.67 $\pm$ 0.00 ^a
<i>p-values</i>	0.000	0.000	0.019	0.006

In each column, values bearing the same letter of the alphabet are statistically identical at the 5% threshold (Newman-Keuls test)

3.4. Variation in glycine betaine content in the leaves and roots of plantain banana plants after treatment of the culture medium with NaCl solution.

Table 4 shows the glycine betaine content extracted from the leaves and roots of plantain banana plants treated with NaCl solution. For 30-day-old plants, treatment with NaCl solution resulted in an increase in glycine betaine content in the roots and a reduction in this amino acid in the leaves when the elicitor concentration in the culture medium was increased. Initially, the amount was 6×10^{-2} $\mu\text{g/gFW}$ in the leaves of untreated plants (control), increasing to 8×10^{-2} $\mu\text{g/gFW}$ with 5 g/L of NaCl. It then increased with increasing salt concentration, reaching a value of 14×10^{-2} $\mu\text{g/gFW}$ (NaCl 15 g/L) in the roots. In contrast, the amount of 7×10^{-2} $\mu\text{g/gFW}$ in the control plants increased to 12×10^{-2} $\mu\text{g/gFW}$ when these plants were exposed to NaCl 5 g/L. From this initial amount of glycine betaine, the content decreased with increasing NaCl concentration. However, in 60-day-old plants, the effects of NaCl were the opposite of those observed in 30-day-old plants. Increasing the NaCl concentration led to an increase in glycine betaine content in the leaves and a decrease in the roots. In the control plants, the values were 5 and 7×10^{-2} $\mu\text{g/gFW}$ in the roots and leaves, respectively. At a NaCl concentration of 5 g/L, the concentration increased to 7×10^{-2} $\mu\text{g/gFW}$ in the leaves and 11×10^{-2} $\mu\text{g/gFW}$ in the roots. From these values, the quantity increased in the leaves to reach 13×10^{-2} $\mu\text{g/gFW}$ and decreased in the roots to 6×10^{-2} $\mu\text{g/gFW}$ at a NaCl concentration of 15 g/L.

Table 4 Glycine betaine content in the leaves and roots of plantain banana plants after treatment of the culture medium with NaCl solution

Treatments	Glycine betaine content of organs ($\times 10^{-2}$ $\mu\text{g/gFW}$)			
	30-day-old plants		60-day-old plants	
	Leaves	Roots	Leaves	Roots
NaCl 5g/L	11.82 $\pm$ 0.01 ^b	8.04 $\pm$ 0.00 ^b	7.20 $\pm$ 0.01 ^a	10.89 $\pm$ 0.00 ^d
NaCl 10g/L	7.00 $\pm$ 0.00 ^a	8.62 $\pm$ 0.00 ^{bc}	9.35 $\pm$ 0.00 ^b	7.48 $\pm$ 0.00 ^c
NaCl 15g/L	6.78 $\pm$ 0.01 ^a	13.92 $\pm$ 0.00 ^c	12.81 $\pm$ 0.02 ^c	5.61 $\pm$ 0.00 ^b
Control	6.77 $\pm$ 0.01 ^a	6.23 $\pm$ 0.00 ^a	7.41 $\pm$ 0.00 ^a	5.39 $\pm$ 0.00 ^a
<i>p-values</i>	0.001	0.001	0.001	0.001

In each column, values bearing the same letter of the alphabet are statistically identical at the 5% threshold (Newman-Keuls test)

4. Discussion

The quantity of biochemical compounds extracted from the leaves and roots of plantain banana was strongly influenced by sodium chloride (NaCl) solution. The chlorophyll (Chl) content of leaves from 60-day-old plants was higher than that of 30-day-old plants. When plants were treated with 10 g/L of NaCl, a significant increase in chlorophyll content was observed in the 30-day-old plants. This increase in chlorophyll content in young plants could be explained by the fact that their cells are not yet physiologically viable and are therefore seeking to mature. This maturation would be linked to the synthesis of photosynthetic pigments. However, in general, in 60-day-old plants, the amount of chlorophyll decreased with increasing NaCl concentration. Nguyen et al. [17] explained that the decrease in chlorophyll precursors and a reduction in the expression of the ChlD, ChlH and ChlI genes, which encode the Mg-chelatase subunits, as well as a reduction in the expression of protochlorophyllide oxidoreductase B, are observed. Furthermore, in these older glycophytic plants, salt stress triggers the synthesis of osmoprotectants. These osmoprotectants share glutamic acid. Glutamate can be redirected to the proline biosynthesis pathway, reducing its availability for the chlorophyll biosynthesis pathway. In maize (*Zea mays*), exposure to 100-200 mM of NaCl induced a significant decrease in chlorophyll a and b [18]. Levitt [19] explained that the degradation of leaf chlorophyll under salt stress is due to the fact that excess salt in the soil induces osmotic stress and ionic toxicity, leading to a decrease in leaf size and quality, as well as the degradation of pigments necessary for photosynthesis. Indeed, NaCl promotes the production of reactive oxygen species (ROS), which causes the oxidation and degradation of chlorophyll pigments. The increase in carotenoid content in leaves under stress has been reported by some authors as a protective response of the plant against oxidative stress [20; 21]. In fact, carotenoids play a role in protecting chlorophylls against oxidation by ROS.

Furthermore, our study showed that proline content increased preferentially in leaves when the concentration of the medium increased with the NaCl solution. This finding was also reported by Kinsou et al. [22] on tomato (*Solanum lycopersicum* Mill.) and Djerroudi et al. [23] on saltbush (*Atriplex halimus* L.). These authors explain that the increase in proline synthesis under salt stress can determine the plant's salinity tolerance levels. According to EL-Iklil et al. [24], the variation in proline accumulation according to NaCl concentrations in the medium is due to compartmentalization of the amino acid, hence the expression of sites of resistance in the plant to salt stress. In a saline environment, proline accumulation protects the cell membrane and participates in osmotic adjustment [25]. Maggio et al. [26] add that enhanced proline accumulation can regulate several processes necessary for plant survival under salt stress. Proline accumulation has been demonstrated in numerous species and under various stress conditions, whether water-related [27] or saline [28]. Our study showed that proline content is higher in older leaves than in younger leaves. This was observed by De-Lacerda et al. [29] in sorghum (*Sorghum bicolor* L. Moench), who showed that 100 mM of NaCl causes an increase in proline in all parts of the plant, but particularly in the oldest leaves. According to the work of Ould El Hadj [30], the oldest basal leaves participate in the sequestration of excess Na⁺ ions, benefiting the growing young leaves. Generally, the role attributed to proline in plant stress responses is multifaceted. According to Qian et al. [31], proline accumulation contributes to the acquisition of resistance through osmotic adjustment. It could also play a role in regulating cytoplasmic pH or constitute a reserve of reduced carbon and nitrogen, used by the plant after the stress period [32]. Our study showed an increasing correlation between proline content and applied stress. Renzetti et al. [33] report that proline is the most characteristic amino acid in plants subjected to salt stress; the importance of proline as an indicator of stress appears to play a role in maintaining vacuole pressures, as well as in protecting membranes and enzymatic systems.

The accumulation of soluble sugars in the leaves of plantain bananas under the influence of salinity has also been demonstrated in numerous species such as barley (*Hordeum vulgare* L.) [25] and sunflower (*Helianthus annuus* L.) [34]. However, in this study, salinity reduced the sugar content in the roots and leaves of the banana plants studied. These same results were observed by Ottow et al. [35] in olive (*Olea europaea* L.) leaves. These findings could suggest the sensitivity of bananas to salinity. In contrast to our results, numerous studies have shown that salinity causes an increase in soluble sugar content in most plants subjected to salt stress [25; 36]. The decrease in soluble sugars in roots can be explained by their consumption [37]. Indeed, under stress conditions, plants increase their respiration. This respiration contributes to the consumption of soluble sugars to produce ATP. Furthermore, the sugar produced can be used in other metabolic pathways or stored as insoluble sugar (starch), which reduces its availability during measurement. Thus, Udomchalothorn et al. [38] observed a decrease in the activity of fructose 2,6-bisphosphate (F26BP) in rice (*Oryza sativa* L.) plants subjected to stress, leading to an accumulation of sucrose and thereby contributing to increased salt tolerance in certain varieties by increasing the internal osmolarity of cells and available carbon reserves.

Glycine betaine levels increased in the roots and decreased in the leaves of plants 30 days after the application of increasing concentrations of NaCl. The increase in glycine betaine levels in the roots could be explained by maintaining root cellular osmotic pressure to allow water absorption. It also limits the influx of Na⁺ ions into the root via the

activation of plasma membrane ATPases [39]. Initially high in leaves at 5 g/L of NaCl, its levels decreased with increasing concentration. Numerous studies have shown an increase in the levels of this compound in leaves under moderate stress [40;41]. Glycine betaine appears to act as an osmoregulator, helping to stabilize osmotic pressure within plant cells.

5. Conclusion

The banana plant's tolerance to environmental salinity is associated with the plant's physiological and biochemical reactions. It is reflected in its capacity to produce metabolites such as leaf pigments, total sugars, proline and glycine betaine in the plant's various organs. The accumulation of these compounds in plantain bananas is thought to be involved in osmotic adjustment mechanisms and also to act as osmoprotectants. These metabolic elements can be considered "biochemical markers" of the degree of tolerance to salt stress and, consequently, can be used for the early selection of salt-tolerant varieties in the studied species.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interest.

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