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THE EFFECT OF ALUMINUM TREATMENT ON METABOLISM IN OIL PALM SEEDLINGS

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Abstract

Due to rainfall and high temperatures, the Amazonian soil undergoes changes in its source material and leaching of base cations. This results in deep, infertile, and acidic soil. Aluminum present in acidic soil impairs plant growth and development by inhibiting root formation, enzymatic reactions, absorption, transport, and nutrient utilization. This study aimed to evaluate the effects of aluminum dosage on the metabolism of the oil palm *Elaeis guineensis* Jacq. The study was conducted in a greenhouse at the Federal Rural University of Amazonia. The experimental design was randomized, with five replications, in which dosages of 0, 10, 20, 30, and 40 mg L^{-1} aluminum chloride ($AlCl_3.6H_2O$) were administered. Electrolyte leakage, nitrate, nitrate reductase, free ammonium, soluble amino acids, proline content, and soluble proteins were analyzed in the leaves and roots of the oil palm. The highest concentration of aluminum was found in the roots. $AlCl_3$ treatment at 40 mg L^{-1} increased electrolyte leakage, nitrate, ammonium, and proline concentrations in the roots, and amino acid concentrations in both the leaves and roots. Furthermore, a decrease in nitrate reductase enzyme activity was observed in the roots. This study demonstrates that the oil palm has mechanisms of tolerance to aluminum toxicity.

Keywords: Acid soils. Arecaceae. Biochemistry. Toxicity.

1. Introduction

The African oil palm (*Elaeis guineensis* Jacq.) is grown in the largest cultivated area in the Amazon region of Brazil (Costa et al. 2018), where its development is boosted by the tropical climate (Green et al. 2019). The oil palm is a perennial, monocotyledonous, oleaginous plant that grows up to 15 m high and belongs to the family Arecaceae (Chagas et al. 2019).

At acidic pH, hydrogen ions (H^+) act on minerals in the soil and release aluminum ions (Al^{3+}). These Al^{3+} soil ions are maintained by negatively charged clay particles and are in equilibrium with Al^{3+} concentrations in the solution (Stefanello and Goergen 2019). Evidence suggests that the correction of soil acidity can benefit the oil palm (Costa et al. 2018). Aluminum can cause cytological changes and affect root development, as well as inhibit water and nutrient uptake and transport (Bojórquez-quintal et al. 2017).

In the meristematic region of the main and lateral roots, Al^{3+} can rapidly access the apoplast and compete for ligands expressed on the plasma membrane (Krzesłowska et al. 2019). This results in a disruption of calcium ion flux between cells, and consequently, the interruption of mitosis (Sharma et al. 2019). Aluminum also negatively influences cellular calcium homeostasis and membrane transport proteins (Krzesłowska et al. 2019). Aluminum and magnesium ions compete for membrane transporters since the binding sites of the enzymes do not distinguish well between targets and these ions due to similarities in hydrated rays (Stefanello and Goergen 2019).

Many plant species have developed mechanisms to improve survival in acidic soils (Ryan et al. 2011) that can induce aluminum toxicity. These mechanisms include aluminum exclusion from the roots (excluding or hindering the entry of Al^{3+} by the root) and aluminum tolerance (Al^{3+} enters root cells and is detoxified and stored in plant tissues). The response of various plants to aluminum toxicity has been studied (Krzesłowska et al. 2019; Stefanello and Goergen 2019). However, few studies have investigated the biochemical characterization of metabolic markers in plants treated with aluminum stress.

Due to the importance and increase of oil palm plantations in the Amazon region and the influence of aluminum toxicity, the present study aimed to evaluate the effect of aluminum chloride dosage on the metabolism of oil palm seedlings, *Elaeis guineensis* Jacq.

2. Material and Methods

The experiment was conducted in a greenhouse at the Institute of Agricultural Sciences of the Federal Rural University of Amazonia (UFRA), in Belém, Pará, Brazil between August 2015 and January 2016. Palm seedlings of the Deli × Lamé variety were grown at an average elevation of 10 m. According to Köppen and Geiger, the climate classification was Af, with an average temperature of 26.8°C and relative humidity of 81%. Seedlings were acclimatized to shady conditions, in hydroponic nutrient solution (Hoagland and Arnon 1950) with 1/2 ionic strength) for 45 days and were subsequently transplanted to 4.6 L Leonard pots. After 20 days, the nutrient solution was changed to total ionic strength, and aluminum treatment (aluminum chloride hexahydrate ($AlCl_3.6H_2O$)) began.

The experimental design included five randomized replicates, with one plant being an experimental unit that received a known concentration of aluminum. The experimental plants were treated with aluminum dosages of 0, 10, 20, 30, and 40 mg L^{-1} of Al^{3+} (added in the form of aluminum chloride hexahydrate 95% ($AlCl_3.6H_2O$)). Therefore a total of 25 plants were analyzed. The pH of the nutrient solution in the presence of aluminum was maintained at 4.8 by adjustment with hydrochloric acid (HCl (0.1 mol/L)) and sodium hydroxide (NaOH (1 M)).

Fresh leaf and root material were removed before sample collection. Shoots and roots were separated, wrapped in aluminum foil, and stored at 80°C. Samples were subsequently dried in a forced-air ventilation oven at 65°C for 48 hours. The dried sample was crushed in a mill until a fine powder was obtained and stored in falcon tubes for future biochemical analyses.

Biochemical analyses were performed at the Laboratory of Biodiversity Studies in Upper Plants (EBPS) (UFRA, Belém, Pará). Electrolyte leakage (Blum and Ebercon 1981), nitrates (NO_3^-) (Cataldo et al. 1975), nitrate reductase (RNO_3^-), enzyme activity (Hageman and Hucklesby 1971), free ammonium (NH_4^+) (Weatherburn et al. 1967), total soluble amino acids (TSA) (Yemm et al. 1955), proline (Bates et al. 1973), and total soluble proteins (Bradford et al. 1976) were analyzed.

Aluminum concentration in roots and leaves was analyzed at the Embrapa Amazônia Oriental Soils and Plants Laboratory using microwave plasma atomic emission spectroscopy.

Shapiro–Wilks (Shapiro and Wilks 1965), Levene (1960) and Box (1953) tests were used to check for normality and homoscedasticity, respectively. Bartlett's test was used to test for homogeneity. The F-test was conducted at a 5% probability. Regression analysis was conducted on the averages of the aluminum

levels and the most adequate equation was defined using the coefficient of determination (R2) as the criterion. Statistical analysis was conducted using the SISVAR software (Ferreira 2011) and graphs were generated in Excel (2013).

3. Results

At an aluminum dosage of 40 mg L^{-1}, the experimental plants had a 349.69% greater concentration of aluminum in the roots compared to the control plants (mean of 516,094 mg/kg and 102.15 mg/kg in treated and control plants, respectively) (Figure 1A). The quadratic regression equation model was used. Similarly, at an aluminum dosage of 40 mg L^{-1}, the experimental plants had a 422.69% greater concentration of aluminum in the leaves compared to the control plants (mean of 207.93 mg/kg and 40.16 mg/kg in treated and control plants, respectively) (Figure 1B). The data was adjusted through the cubic regression model.

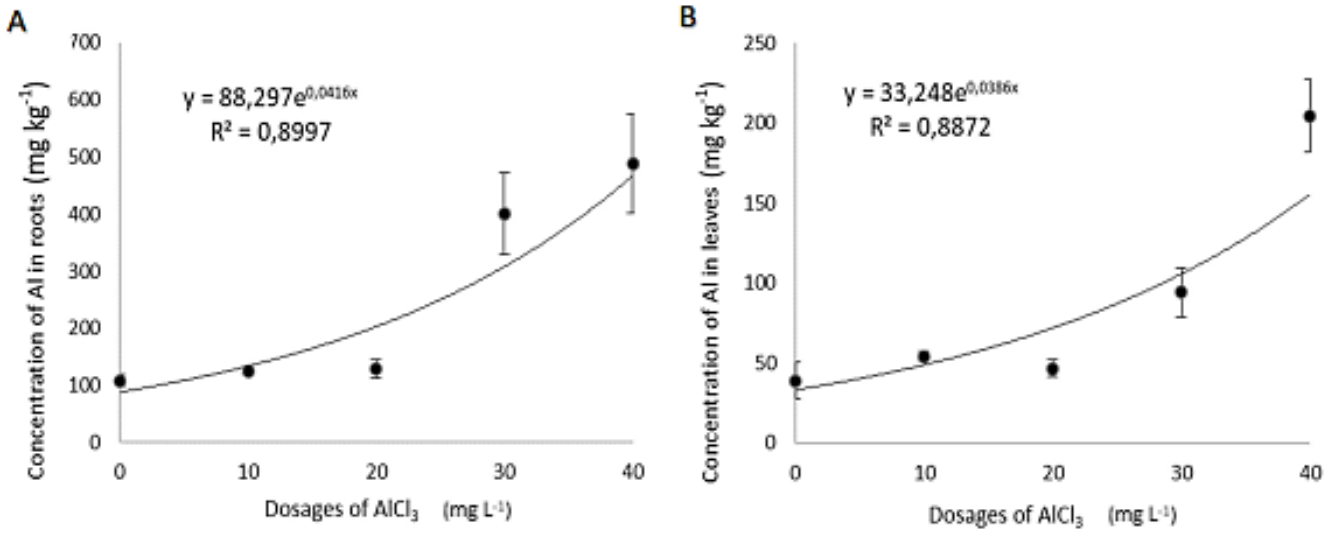

Figure 1. A - Concentration of aluminum in roots and B - leaves of oil palm seedlings submitted to aluminum dosages.

Electrolyte leakage was 47.32% higher in the roots of plants treated with 40 mg L^{-1} of aluminum, compared to those of plants receiving the control treatment (mean of 17.37 and 11.03%, respectively) (Figure 2). There was no significant effect on electrolyte leakage in leaves ($p>0.05$). The data was adjusted through the cubic regression model.

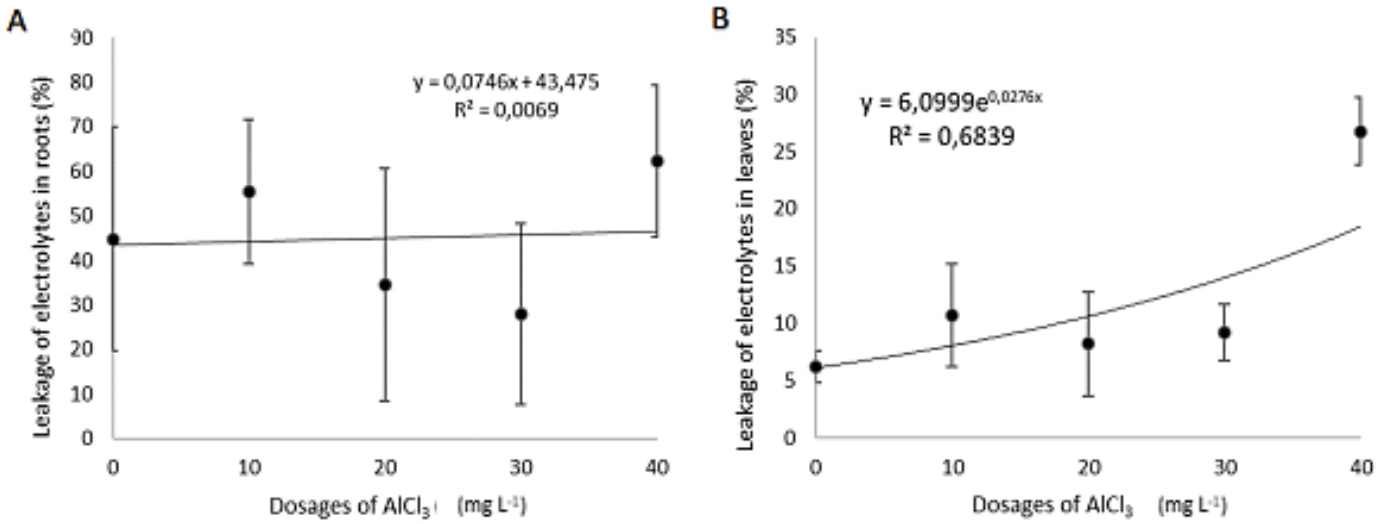

Figure 2. A - Electrolyte leakage in the roots and B - leaves of oil palm seedlings submitted to aluminum dosages.

The concentration of NO_3^- in the roots was 156.7% higher in plants treated with 40 mg L^{-1} of aluminum compared to those in plants receiving the control treatment (mean of 0.0242 NO_3^- kg^{-1}DM and 0.0882 NO_3^- kg^{-1} MS, respectively) (Figure 3). The data was adjusted through the positive linear regression model.

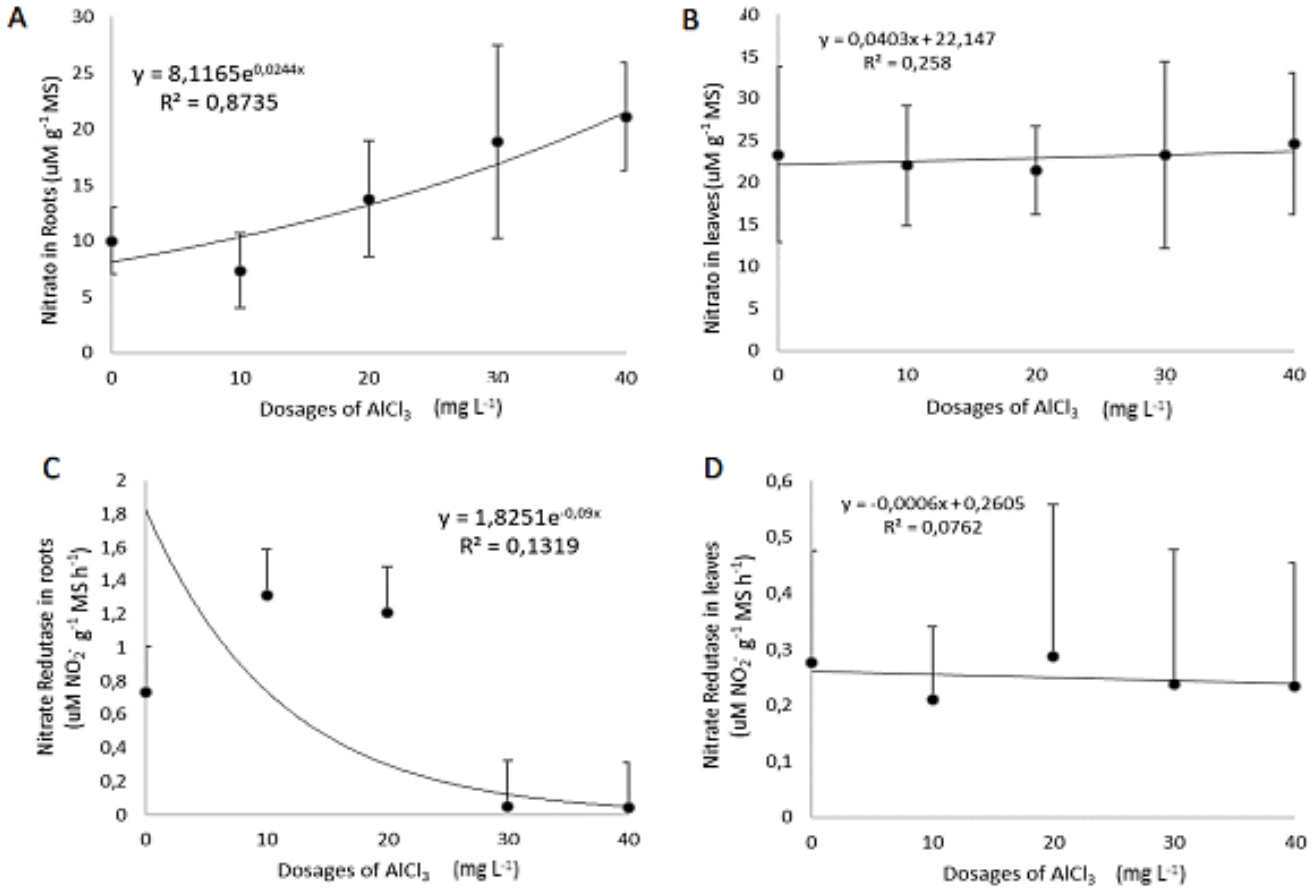

Figure 3. A - Nitrate activity in roots; B – leaves; C - the nitrate reductase enzyme in roots and D - leaves of oil palm seedlings submitted to aluminum dosages.

The RNO_3^- enzyme activity was 90.48% lower in the roots of plants treated with 40 mg L^{-1} $AlCl_3$ compared to those in plants receiving the control treatment (mean of 0.0202 and 1.0322 mmol of $NO_2^-g^{-1}$FM h^{-1}, respectively) (Figure 4). No significant effect was observed in the leaves. The data was adjusted through the cubic regression model.

Following treatment with 40 mg L^{-1} of $AlCl_3$, experimental plants had a 333.70% higher concentration of ammonium in the roots compared to control plants (mean of 48,807 µM g^{-1}DM and 11,251 µM g^{-1}DM, respectively) (Figure 5). There was no significant effect observed for ammonium concentrations in the leaves. The data was adjusted through the cubic regression model.

TSA were 271.89% higher in the roots of plants treated with 40 mg L^{-1} of $AlCl_3$ compared to those in plants receiving the control treatment (stocks of 127,67 mmol AA g^{-1} MS and 34,326 mmol AA g^{-1} DM, respectively) (Figure 6A). The data was adjusted through the cubic regression model. In the leaves, TSA were 592.20% higher in plants treated with 40 mg L^{-1} of $AlCl_3$ compared to those in plants receiving the control treatment (mean of 253,462 mmol of AA g^{-1}DM and 8,654 mmol of AA g^{-1}DM, respectively) (Figure 6B). The results were verified by adjusting the positive linear regression model.

Proline concentrations were 41.18% higher in the roots of plants treated with 40 mg L^{-1} of $AlCl_3$ compared to those in plants receiving the control treatment (a mean of 3.39 µM g^{-1} DM and 2.57 µM g^{-1} DM, respectively) (Figure 7A). The results were verified by adjusting the positive linear regression model. Similarly, in the leaves, proline concentrations were 66.66% higher in plants treated with 40 mg L^{-1} of $AlCl_3$ compared to those in plants receiving the control treatment (mean of 4.70 µM g^{-1} DM and 2.92 µM g^{-1} DM, respectively) (Figure 7B). The results were verified by adjusting the quadratic regression model.

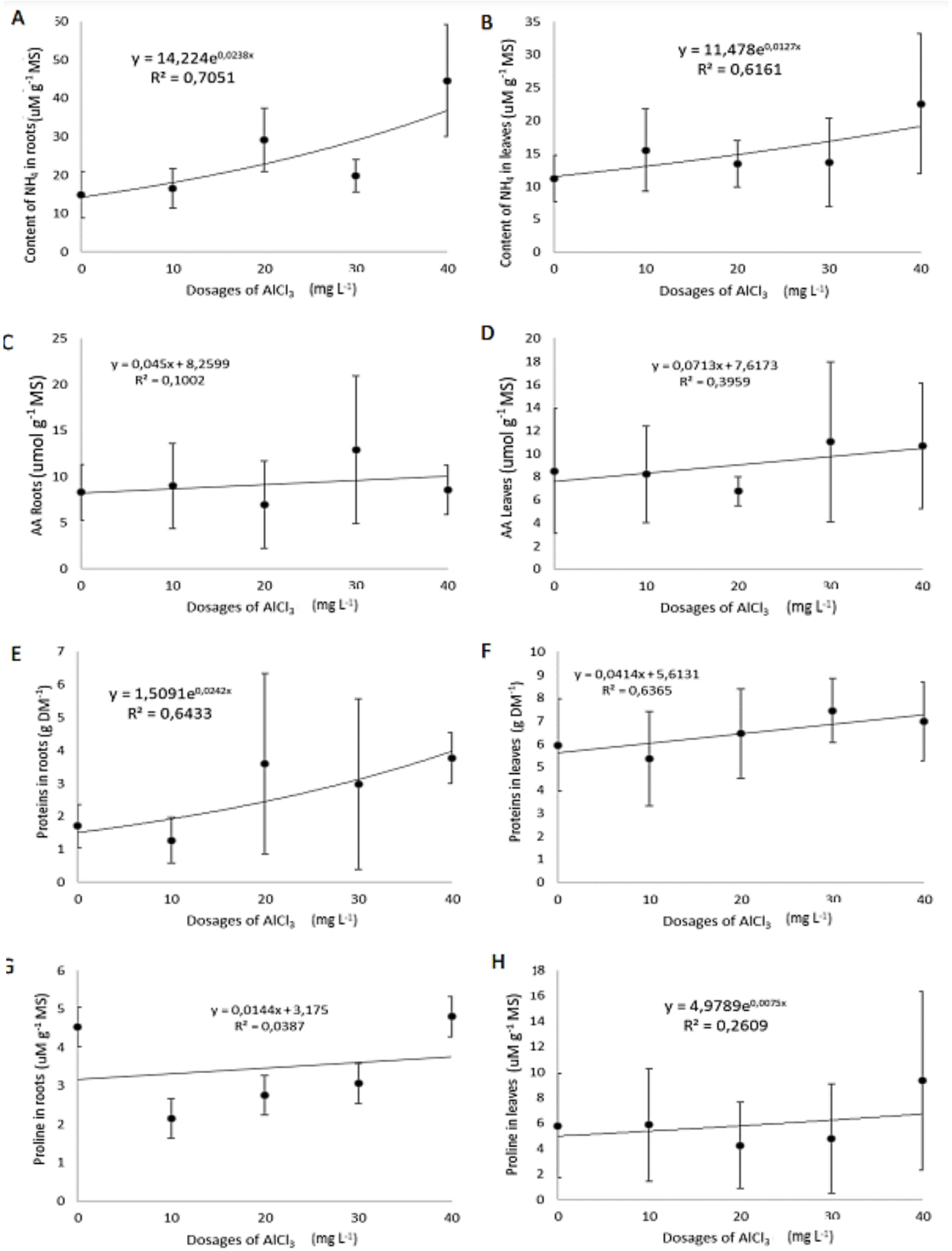

Figure 4. A - Ammonium concentrations in roots; B – leaves; C - amino acids in roots; D – leaves; E - proteins in roots; F – leaves; G - proline in roots and H - leaves of oil palm seedlings submitted to aluminum dosages.

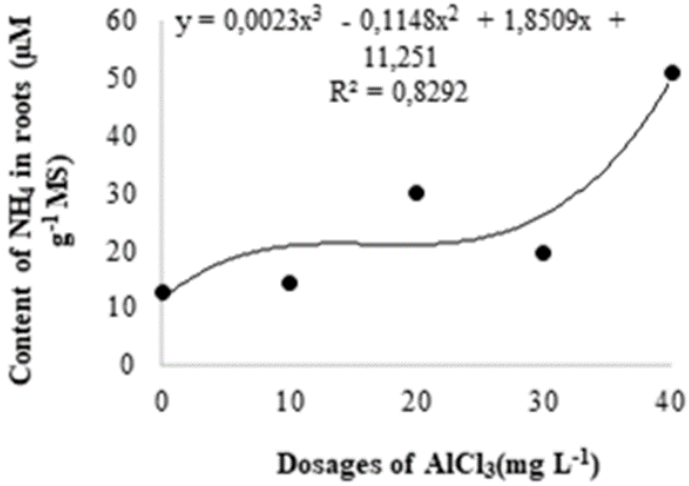

Figure 5. Free ammonium concentration in the roots of oil palm seedlings submitted to aluminum dosages.

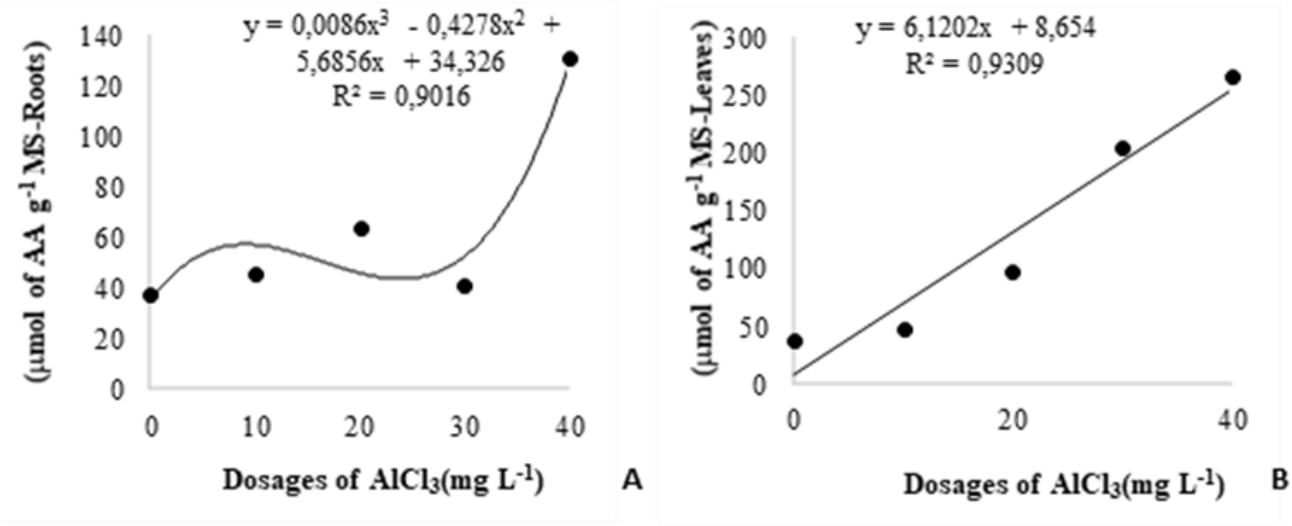

Figure 6. A - Amino acid concentration in roots and B - leaves of oil palm seedlings submitted to aluminum dosages.

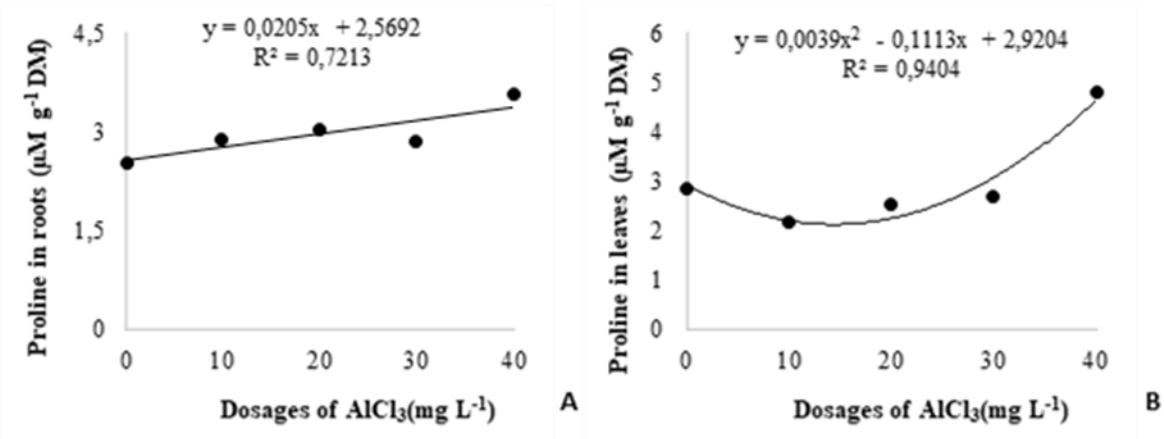

Figure 7. A - Proline concentrations in roots and B - leaves of oil palm seedlings submitted to aluminum dosages.

Total protein concentration was 70.90% higher in the roots of plants treated with 40 mg L^{-1} of $AlCl_3$ compared to those of plants receiving the control treatment (mean of 0.93 g^{-1} DM and 0.58 g^{-1} DM, respectively) (Figure 8A). The data were adjusted using the quadratic regression model.

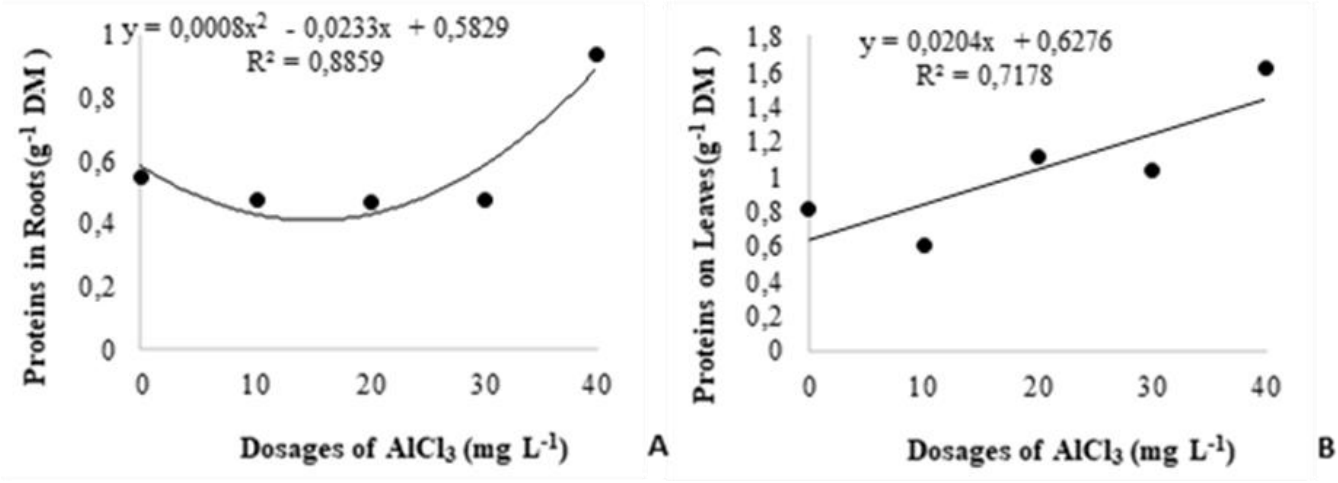

Figure 8. A - Proteins concentrations in roots and B - leaves of oil palm seedlings submitted to a aluminum dosages.

In the leaves, total protein concentration was 100% higher in plants treated with 40 mg L^{-1} of $AlCl_3$ compared to those in plants receiving the control treatment (mean of 1.44 mg protein g^{-1} DM and 0.63 mg protein g^{-1} DM, respectively) (Figure 8B). The results were verified by adjusting the positive linear regression model.

4. Discussion

The accumulation of aluminum in the leaves, relative to other parts of the plant, demonstrates that the transport of the element was approximately 9% for the genotypes, in the presence of aluminum in the nutrient solution. The results of this study suggest that the retention of aluminum in the roots, in the "apoplastic" and "simplistic" compartments is effective in reducing the discharge of aluminum in the xylem. These results also suggest that the oil palm uses the mechanism of aluminum tolerance to adapt to aluminum toxicity. This study showed that at a concentration of 40 mg L^{-1} of $AlCl_3$, aluminum is translocated to the aerial part of the plant (Singh et al. 2017).

The electrolyte leakage in the roots suggests that aluminum may have modified characteristics of the plasma membrane. This may represent a target sensitive to aluminum phytotoxicity (Singh et al. 2017).

Aluminum interacts with lipids and membrane proteins by modifying their molecular structure (Tokizawa et al. 2021). Aluminum increases the packaging density of lipids and, consequently, their rigidity (Tokizawa et al. 2021). Approximately 75% of all the aluminum absorbed into the cell wall is bound to the hemicellulose fraction (Sing et al. 2017). When aluminum binds to cell wall components it alters their cation exchange capacity (Sing et al. 2017).

The concentration of NO_3^- in the leaves was not significantly affected by aluminum treatment. The absorption of NO_3^- is an active process, brought about by the generation of energy from H^+ pumps (Maldaner et al. 2020). The absorption of NO_3^- is correlated with the pH level of the apoplasm, and an increase in pH is observed when NO_3^- is in abundance. This may be explained by the NO_3^- absorption process through $NO_3^-/2H^+$ (Vergara et al. 2018). This may also be explained by the inhibition of H^+ -ATPase activity, which may prevent the formation and maintenance of the proton gradient (Bojórquez-quintal et al. 2017). The decrease in RNO_3^- activity may be associated with the potential of aluminum to interfere with processes involved in absorption, transport, and nutrient use (Freitas et al. 2017).

The competition of aluminum with magnesium and the resulting lack of magnesium may also explain the decrease in RNO_3^- enzyme activity in the roots of oil palm seedlings. The lack of magnesium can stimulate the phosphorylation of serine residues, which interact with an inhibitory protein, resulting in reduced activity of the RNO_3^- enzyme (Kumar et al. 2017).

NH_4^+ accumulates in the cell through a passive absorption process via a single-carrier type transporter and an electrochemical potential gradient (Maldaner et al. 2020).

While the electric component of the proton motive force governs the anion absorption by the vacuole, the electrochemical potential gradient for H^+ is differentiated to govern the uptake of cations and sugars by the vacuole by secondary transport systems (Ibiang et al. 2017).

It is assumed that plants would be able to exclude absorbed aluminum and/or prevent it from entering and accumulating in the simplasma (Bojórquez-quintal et al. 2017).

The increase in the concentration of TSA may be associated with the accumulation of NH_4^+ in the roots. The accumulation of amino acids in this study may be a physiological response to aluminum toxicity since TSA have been shown to have an important role in plant aluminum tolerance (Tokizawa et al. 2021).

The higher proline concentration in aluminum-treated plants may also serve as a protective response to aluminum toxicity. Proline has an osmoprotective function and preserves the cellular integrity of proteins, enzymes, and membranes for the continuity of vital activities (Begum et al. 2019). Furthermore, in general, proline accumulation is considered an important parameter in the selection of stress-tolerant plants (Hussain et al. 2018).

The higher protein concentrations in the roots and leaves of plants treated with aluminum may be explained by the higher concentrations of amino acids observed in treated plants. The higher concentrations of amino acids may have promoted the synthesis of more proteins in aluminum-treated plants. During stress, plants are stimulated to synthesize new proteins that function in the synthesis and transport of organic acids (Bali et al. 2020). Concomitant to this, this result reflects an increase in the biosynthesis of proteins during aluminum stress (Bali et al. 2020).

5. Conclusions

The biochemical variables analyzed in this study indicate that the oil palm has mechanisms of tolerance to aluminum toxicity up to a concentration of 40 mg L^{-1} of $AlCl_3$.

The levels of free amino acids, proline, and proteins observed in this study can be used as an indicator of aluminum tolerance in plants and can guide breeding programs for this species.

Aluminum concentrations remained high in the root system, indicating a strategy for this plant to avoid toxicity.

With the increase in aluminum dosages, the enzyme activity of the plants was affected mainly in the roots, such as nitrate reductase, showing toxicity in this organ.

Authors' Contributions: BRITO, A.E.A.: conception and design, acquisition of data, analysis and interpretation of data, and drafting the article; CARDOSO, K.P.S.: analysis and interpretation of data, drafting the article, and critical review of important intellectual content; COSTA, T.C.: analysis and interpretation of data, drafting the article, and critical review of important intellectual content; MARTINS, J.T.S: analysis and interpretation of data, drafting the article, and critical review of important intellectual content; MACHADO, L.C.: analysis and interpretation of data, drafting the article, and critical review of important intellectual content; NOGUEIRA, G.A.S.: drafting the article and critical review of important intellectual content; CONCEIÇÃO, S.S.: conception and design, drafting the article and critical review of important intellectual content; DE OLIVEIRA, J.T.: conception and design, analysis and interpretation of data, drafting the article, and critical review of important intellectual content; SILVA, P.A.: conception and design, analysis and interpretation of data, drafting the article, and critical review of important intellectual content; DA SILVA, R.T.L.: conception and design, acquisition of data, analysis and interpretation of data, and drafting the article; CARDOSO, K.P.S.: analysis and interpretation of data, drafting the article, and critical review of important intellectual content; DE OLIVEIRA NETO, C.F.: conception and design, acquisition of data, analysis and interpretation of data, and drafting the article; CARDOSO, K.P.S.: analysis and interpretation of data, drafting the article, and critical review of important intellectual content. All authors have read and approved the final version of the manuscript.

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Ethics Approval: Not applicable.

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