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Aluminum toxicity assessment in *Coffea arabica* cv. Catiguá MG2 under hydroponic conditions

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ABSTRACT

Aluminum is an element commonly found in acid soils, notably known by their pH values ranging around 5. At soil pH values at or below pH 5, aluminum may drastically interfere with phosphorus uptake by plants, inhibit root growth, and induce cell death. This study aimed to assess the tolerance of *Coffea arabica* cv. Catiguá MG2 seedlings in a solution containing Al under hydroponic conditions using a simple, relatively fast protocol. Seedlings at 6 months of age, established *in vitro*, were cultivated in Hoagland solution (¼ strength and pH 4.0) supplemented with different concentrations of Al (0; 0.888; 1.666; and 2.499 mM) provided from the source $AlK(SO_4)_2.12H_2O$ for 30 days. The higher Al^{3+} concentrations caused more evident symptoms of toxicity, unlike the 0.888 mM that caused little damage to the roots. The control seedlings did not exhibit any symptoms of nutritional deficiencies. Although the protocol has been used only for a specific coffee cultivar, it is expected to be useful in the assessment of Al-caused toxicity in other coffee materials when exposing seedlings to Al in the hydroponic system with a dilution of Hoagland solution, and could be useful for the quick identification of coffee genotypes with certain Al tolerance.

Key words: Acid soils; Aluminum tolerance; Root elongation inhibition; Cell death; Hoagland solution.

1 INTRODUCTION

Coffee production is one of the most common and financially agricultural activities in several regions of Brazil since coffee is a commodity widely consumed and sold in a global socioeconomic context. The entire coffee production chain reaches billions of dollars annually worldwide, and Brazil is the largest producer and exporter, besides being a great consumer (Companhia Nacional de Abastecimento - CONAB, 2018; Filho; Ramalho; Andrade, 2018). Nevertheless, even with all the technological advances obtained in agriculture, environmental factors and low fertility of Brazilian Cerrado soils, characterized by their high acidity level (Peixoto et al., 2010; Vendrame et al., 2010), still affect the optimal cultivation and yield of coffee plants.

Available aluminum (Al) in the rhizosphere is a crucial limiting factor for the yield of species grown in regions with acid soils below pH 5 (Liu; Piñeros; Kochian, 2014; Rao et al. 2016; Sade et al., 2016), the most used soil for plant cultivation on Earth. Al effects on plant metabolism are rapidly evident and become more pronounced in highly acidic soils, which trigger the formation of toxic cations for plants like Al^{3+} and $AlOH^{2+}$ (Bojórquez-Quintal et al., 2017; Sade et al., 2016; Yamamoto, 2019). Two prominent symptoms observed in plants exposed to Al include inhibition of root growth, problems in nutrient uptake (Liu; Piñeros; Kochian, 2014), and cell death due to the loss of plasma membrane integrity (Yamamoto, 2019).

Different genotypes can exhibit a certain tolerance to Al in the environment based on internal mechanisms evolutionary developed to deal with this metal. Strategies adopted by plants enclose the exclusion of organic acids to the rhizosphere, changes in the cell wall composition and plasma membrane properties, the elimination of chelating molecules, and mucilage secretion (Bojórquez-Quintal et al., 2017; Yamamoto, 2019). Therefore, assessment of plant tolerance to Al-induced stress requires the design of experiments to identify susceptibility levels of specific genotypes and, mainly, to understand the Al effects on the root system of target species.

The hydroponic system is a viable method widely used to grow plants under controlled conditions for studying the allocation and response mechanisms of plants concerning the availability of nutrients and exposure to toxic elements (Braccini et al., 1998; Giannakoula et al., 2010; Liang et al., 2013; Nguyen; Mcinturf; Mendoza-Cózatl, 2016). Considering the physical characteristics of the hydroponic system, generally established on liquid nutrient solutions, it is possible to prevent mechanical damages commonly observed in experiments with soil such as those generated by handling roots and loss

of nutrients that bind to soil particulates (Nguyen; Mcinturf; Mendoza-Cózatl, 2016).

Macedo et al. (2011) evaluated the Al toxicity with nutrient solutions in four *Coffea arabica* cultivars (Catuaí Amarelo IAC62, Iapar 59, Obatã IAC-1669/20, and Oeiras MG6851) using only 0.83 mM Al during the initial development stages of seedlings. Braccini et al. (1998) also evaluated the Al tolerance of *C. arabica* genotypes in nutrient solution using an even lower Al concentration (0.296 mM). These authors showed that plants exposed to Al have differences in uptake and translocation of P and Ca. Nonetheless, it isn't clear if tolerance attributed to some genotypes is due to individual genetic factors from tolerant genotypes or the low Al concentration added into the solution since acidic soils contain higher amounts of Al (Cunha et al., 2015).

According to the context, our purpose was to develop a quick and practical protocol to evaluate toxicity caused by relatively high concentrations of Al in the root system of *C. arabica* cv. Catiguá MG2 seedlings under hydroponic conditions. The perspective is to apply this methodology at the initial tolerance evaluation stages of coffee cultivars that experience harmful effects from Al available in the field.

2 MATERIAL AND METHODS

2.1 Plant material and growth conditions

Seeds of *C. arabica* cv. Catiguá MG2 (CG) were rinsed under running water for 1 hour to remove parchment, immersed in 1.6% formaldehyde solution for 30 minutes, and washed with autoclaved distilled water. Then, the seeds were kept into a sterile 0.5% (v/v) boric acid (H_3BO_3) solution for 72 hours under shaking. The embryos were excised from the seeds under aseptic conditions and inoculated in GER medium (Etienne, 2005) composed of MS (Murashige; Skoog, 1962) medium salts, specific vitamins for coffee (10 mg L^{-1} of thiamine, 1.0 mg L^{-1} of nicotinic acid, 1.0 mg L^{-1} of pyridoxine, 2.0 mg L^{-1} of glycine), 40 g L^{-1} of sucrose, 10 mg L^{-1} de cysteine, and 2.5 g L^{-1} of Phytagel (Sigma Chemical Co., USA). The embryos were maintained in the dark at 22 °C for 10 days and then transferred to lighted conditions with a 16-hour photoperiod for an additional 20 days.

2.2 Hydroponic experiment

Coffee seedlings from embryos established *in vitro* at 30 days of age were grown in GER medium supplemented with 0.5 µM indole butyric acid (IBA) and 20 g L^{-1} sucrose for five months, with subculturing every 15 days. Seedlings of the same age were removed from the culture medium, washed with distilled water, and transferred to Hoagland and Arnon (1950) nutrient solution diluted to ¼ strength for 15 days, with later exchange to a ½ strength solution. The solution pH was kept around 5.5 throughout the adaptation of the plant material into a hydroponic solution.

The experiment took place in dark opaque trays (7 cm h x 23 cm w x 36 cm l) with a 5.0-liter capacity, with ten seedlings per tray, without aeration, replacing the nutrient solution every three days. The seedlings stayed under these conditions for 30 days before beginning the Al toxicity experiments.

2.3 Assessment of Al toxicity in coffee seedlings

The trials of plant tolerance to Al were carried out in a greenhouse in Lavras, MG (21º14'45'' S latitude, 44º59'59'' W longitude and 920 m altitude), Brazil, with a daytime temperature of 27±3 °C, the nighttime temperature of 20±3 °C, and 12-hour photoperiod, using Hoagland nutrient solution diluted to ¼ strength and modified regarding phosphorus concentration (10x less). The Al supply occurred at concentrations of 0.888 mM, 1.666 mM, and 2.499 mM through adding aluminum potassium sulfate dodecahydrate ($AlK(SO_4)_2.12H_2O$) into the solutions. The control was the absence of its compound (0.0 mM Al). The solutions' pHs were kept at 4.0 ± 0.2 through daily adjustments with 0.1 M HCl for 30 days. The nutrient solutions had no aeration and were replaced every three days. The phosphorus concentration (25 µM) and the solution pH were conserved at low levels to reduce the possibility of aluminum precipitations in the solution.

The experiment design had four treatments (three Al concentrations and the control) set up in a completely randomized design with six replications and one plant per plot. The variables analyzed were length of the main root and number of lateral roots (from the main root) measured previously to the maintenance of seedlings in Al-containing nutrient solutions. These measurements were performed once more after 30 days of plants exposure to the Al-containing solutions.

2.4 Statistical analysis

Statistical analysis was performed in two different periods: before (day 0) and after (30 days) exposure of seedlings to nutrient solutions containing Al, according to the statistical model $Yij = \mu + t_i + e_{ij,}$ wherein: Y_{ij} is the observation referring to the plot that received the i^{th} treatment in the j^{th} block; μ is the constant associated with the observations; t_i:= is the effect of the i^{th} treatment; e_{ij} is the experimental error associated with observation of plot *ij*, in which $e \sim N(0, I\sigma^2)$. Percentage of reduction in root length of the seedlings was performed through the mean of the six seedlings of the four treatments for the two variables under analysis: number of lateral roots and length of the main root.

For statistical analyses, Dunnett's test was performed using the package DescTools (Signorell et al., 2021) available in the software R v.4.1 (R Core Team, 2021) and Tukey's test through software Sisvar (Ferreira, 2014), both at 5% significance for qualitative factors. Polynomial regression analysis was applied for quantitative factors.

3 RESULTS

The cultivar Catiguá MG2 showed sensitivity to all Al levels supplied into the nutrient solution. Seedlings at day 30 (Figure 1, I-L) exhibited thicker roots, lower root growth, a decrease in the number of absorbent hairs, and root tip necrosis compared to those at day 0 (Figure 1, A-D). A reduction in emission of absorbent hairs and tip necrosis of already existing roots were some of the apparent symptoms in seedlings exposed to Al after 15 days (Figure 1, E-H). On day 7th, roots in contact with 2.499 mM of Al^{3+} began to exhibit light brown coloring and initial apical necrosis. Seedlings exposed to concentrations lower than 2.499 mM responded similarly to the control, i.e., the roots still were with conventional color, thickness, and branching. On day 15th, seedlings exposed to 1.666 mM started showing development inhibition of lateral roots (Figure 1G), while those exposed to 0.888 mM presented thickened roots with the onset of apical necrosis (Figure 1F). After 30 days of exposure, roots and root tips became thicker (Figure 2) in all the seedlings in contact with any level of Al^{3+}. Meanwhile, coffee seedlings grown in the nutrient solution without Al^{3+} (control) did not show any of those symptoms (Figure 1I).

The main root length of seedlings kept in solutions containing 1.666 and 2.499 mM Al^{3+} was statistically different between 0 and 30 days (Table 1). A considerable reduction in root length was registered in seedlings exposed for 30 days to the higher Al concentrations, unlike those exposed to 0.888 and 0.0 mM Al^{3+}, which exhibited a slight reduction or even an increase in root length, respectively.

The number of lateral roots at day 0 did not differ statistically among the treatments. However, after 30 days of exposure to Al solutions, the number of roots declined as the Al^{3+} concentrations in solutions increased (Figure 3). The Al^{3+} concentrations of 0.888, 1.666, and 2.499 mM led to reductions of 22.72%, 28.55%, and 63.85% in the number of roots, respectively. Nonetheless, the control exhibited the opposite behavior since the number of lateral roots raised 39.72% at the end of the experiment (Table 2).

Even at the lowest Al concentration was possible to observe the beginning of chlorosis in some leaves (Figure 4). Chlorosis was more severe in mature leaves of seedlings exposed to the highest Al concentration with a start point at the leaf edge that progressed toward the center of the foliar blade (Figure 4D). The control did not show any deficiency symptoms (Figure 4A).

4 DISCUSSION

The use of nutrient solution at ¼ strength proved to be effective in assessing Al toxicity in coffee since visual morphological differences were evident in the roots and shoots of Al exposed seedlings. Dilution of nutrient solution is often necessary because the excess of ions can interfere with chemical activity and uptake of certain nutrients by plants (Shri; Pillay, 2017), which would underestimate the Al^{3+} effects. In the present study, dilution of the Hoagland nutrient solution to ¼ the original concentration allowed the assessment of Al effects on coffee seedlings grown in a hydroponic system. The control showed no symptoms of nutritional deficiency over 30 days.

The most common morphological characteristics at the end of 30 days were the formation of short, thick roots of brownish color that were brittle and with fine and scarce branching. Other studies related to Al-caused toxicity in solution for different coffee genotypes also report similar symptoms and morphological characteristics (Londoño; Valencia, 1983; Pavan; Bingham, 1982; Macedo et al., 2011). Furthermore, other crops like maize (Bennet; Breen; Fey, 1986) and sorghum (Baligar et al., 1993) also presented these morphological abnormalities, which highlight the existence of a standard toxicity profile in the radicular zone of plants exposed to some Al concentrations, regardless of species and genotypes.

Several studies have shown that Al toxicity can affect root growth (Giannakoula et al., 2010; Liang et al., 2013; Sade et al., 2016; Yamamoto, 2019; Muhammad; Zbobgo; Guo-Ping, 2019). The necrotic spots may appear likely from tissue darkening caused by Al-induced oxidative stress (Ma et al., 2012; Sun et al., 2014). The reduction in capacity for root elongation is likely a response to the Al toxicity, which first acts in the plant root system through retarding the root growth and development, increasing their diameter, and leading to a lower number of lateral roots. This reduction in root growth is likely due to Al ions interaction with negatively charged cell membrane components and with the phosphate group belonging to deoxyribonucleic acid within the nucleus, which would reduce the membrane permeability to other nutrients, and the replication and transcription cellular activity, respectively (Liu; Piñeros; Kochian, 2014; Pavan; Bingham, 1982; Sade et al., 2016; Yamamoto, 2019; Wagatsuma, 2017).

The decreased number of lateral roots is another common symptom in seedlings exposed to toxic Al concentrations. A hypothesis is that Al can bind to the cell wall and triggers oxidative stress into the root system through producing reactive oxygen species (ROS), which causes the degradation of cell compounds and consequently may lead to cell death (Kopittke et al., 2016).

Figure 1: Visual aspect of the coffee seedling root system before the exposition to ¼ strength Hoagland nutritive solution with different Al^{3+} concentrations for 30 days. Seedlings cultivated for 0 day (A-D); 15 days (E-H); and 30 days (I-L) into Hoagland solution containing 0.0 mM (control) (A,E,I); 0.888 mM (B,F,J); 1.666 mM (C,G,K); and 2.499 mM (D,H,L) of Al^{3+}.

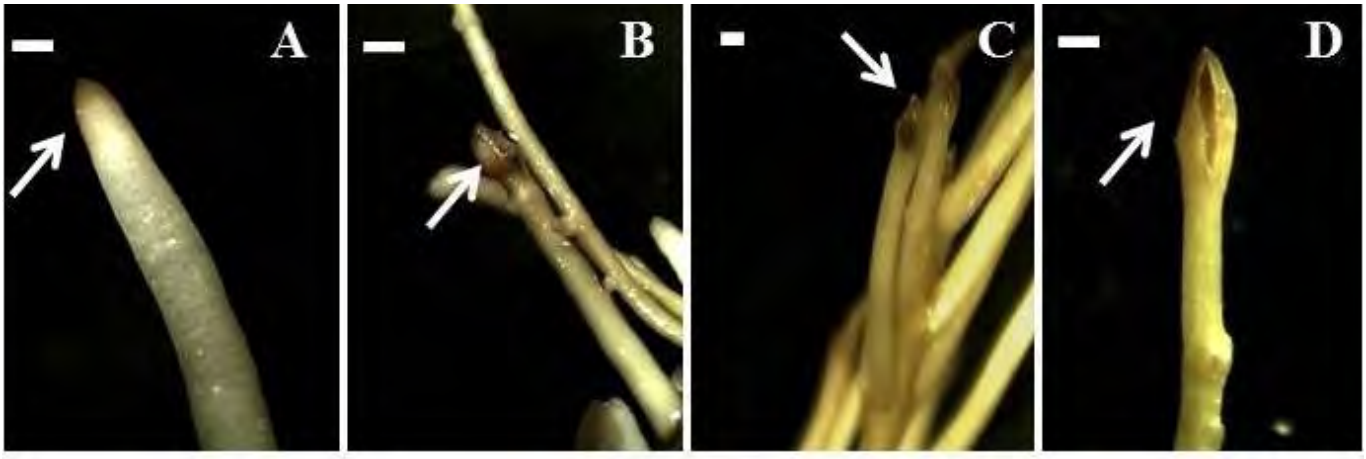

Figure 2: Root apices (white arrows) of coffee seedlings exposed to ¼ strength Hoagland nutritive solution containing Al^{3+} after 30 days. Seedlings were exposed to (A) 0.0 mM (control); (B) 0.888 mM; (C) 1.666 mM; and (D) 2.499 mM of Al^{3+}. Bar = 1.0 mm.

Table 1: The main root length (cm) and number of lateral roots of *C. arabica* cv. Catiguá seedlings grown in ¼ strength Hoagland nutritive solution in hydroponic system supplied with different Al concentrations.

$[Al^{3+}]$ (mM)	Main root length (cm)		Number of lateral roots	
	day 0	day 30	day 0	day 30
0	4.08 Aa	4.25 Aa	11.33 Aa	15.83 Aa
0.888	5.00 Aa	3.50 Aa	11.00 Aa	08.50 Aa
1.666	6.25 Ba	3.92 Ab	8.16 Aa	05.83 Ba
2.499	6.42 Ba	3.17 Ab	13.83 Aa	05.00 Bb

Mean values followed by the same uppercase letter on columns or lowercase letters on lines, for each variable, do not differ statistically at 5% significance ($p<0.05$) and refer to data analyzed by Dunnett's (columns) or Tukey's (lines) test.

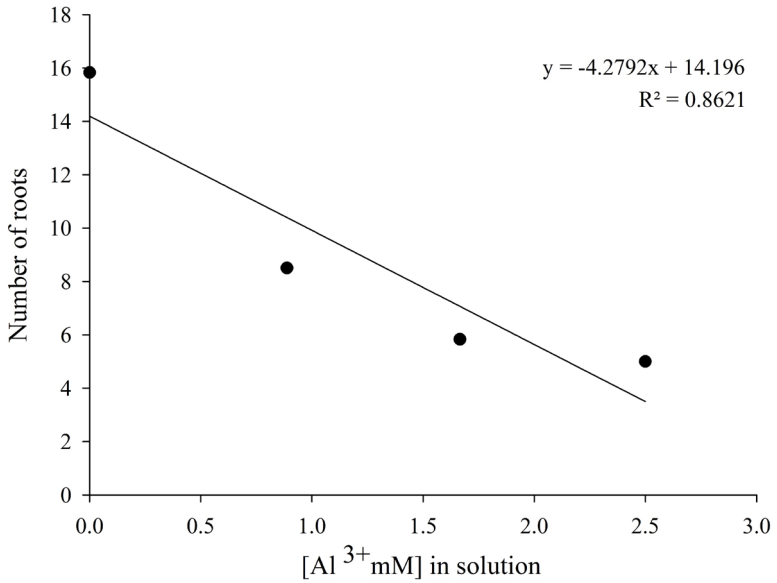

Figure 3: The number of lateral roots of the main root of the coffee seedlings exposed to the ¼ strength Hoagland nutritive solution after 30 days. Seedlings were exposed to different Al^{3+} concentrations (0.888, 1.666, and 2.499 mM) and to Al-free solution (control).

Table 2: Relative growth rate of the main root and number of lateral roots of *C. arabica* cv. Catiguá seedlings cultured in the ¼ strength Hoagland nutritive solution in hydroponic system supplied with different Al concentrations after 30 days.

[Al^{3+}] (mM)	Main root length (%)	Number of lateral roots (%)
0	(+) 04.17	(+) 39.72
0.888	(-) 30.00	(-) 22.72
1.666	(-) 37.28	(-) 28.55
2.499	(-) 50.62	(-) 63.85

(+) Positive or (-) negative relative rate of the variable under analysis for the 30 days of assessment indicating an increase or decrease in root growth or number of lateral roots, respectively;

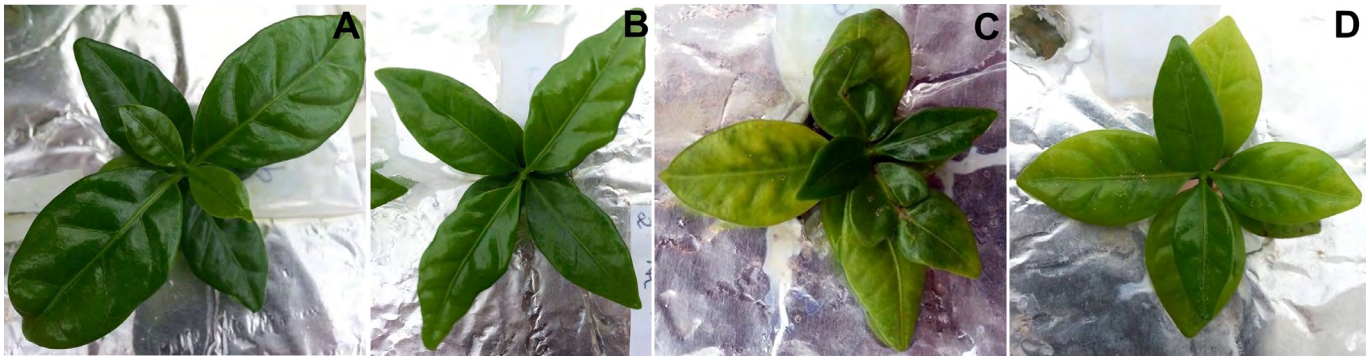

Figure 4: Al toxicity symptoms in coffee seedlings exposed to ¼ strength Hoagland nutritive solution containing Al^{3+} after 30 days in a hydroponic system. Seedlings were exposed to (A) 0.0 mM (control); (B) 0.888 mM; (C) 1.666 mM; and (D) 2.499 mM of Al^{3+}.

Pavan and Bingham (1982) reported that Al-caused toxicity in coffee shoots is characterized by a rise of tissue chlorosis, with necrotic points at the edge of leaves and curling of young leaves. In our study, some of these symptoms appeared in seedlings exposed to 1.666 mM and 2.499 mM Al after 30 days (Figure 4).

The Al available to plants can lead to other toxic effects that exceed the damage observed in the plant's root system. Given the potential of Al to interfere in the uptake and movement of some nutrients as calcium, phosphorus, and magnesium (Baligar et al., 1993; Bennet; Breen; Fey, 1986; Braccini et al., 1998), such symptoms may arise from deficiencies of these nutrients in the leaves. Magnesium can be directly associated with chlorosis appearance as it plays a crucial role in photosynthesis as an atom belonging to chlorophyll (Chen et al., 2018). Accordingly, Mg deficiencies might lead to leaf chlorosis based on the low chlorophyll content (Kobayashi et al., 2018).

5 CONCLUSIONS

The 0.888 mM Al^{3+} in the nutrient solution under suitable conditions is enough to cause damage to the coffee root tips even though the higher concentrations lead to more evident morphological damage to the plant root system and shoots. Furthermore, dilution of the Hoagland nutrient solution to ¼ strength does not affect the seedlings from control, which shows the viability of its application in experiments of Al toxicity evaluation without interfering with the maintenance of nutritional quality and plant growth. We believe that the simplicity of this protocol is enough to induce symptoms of aluminum toxicity in coffee seedlings in practice and quickly, which would assist the identification, at least from the morphological point of view, the more tolerant cultivars in a short time. Additionally, more studies at the molecular and physiological levels are undoubtedly needed to get the knowledge deeper on a better understanding of how Al influences other characteristics inside and outside of coffee plants. It will bring light on mechanisms involved with the tolerance and susceptibility of coffee plants exposed to Al in the field.

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7 AUTHORS' CONTRIBUTION

MHC contributed to the study conception and design, performed the experiments, and wrote the manuscript; WPFM

co-wrote, reviewed and edited the manuscript, and approved the final version of the work; KOGD conducted all statistical analyses; ASA and JCA performed the experiments; EGM contributed to the study conception and design, and supervised the experiments; LVP got funding acquisition for the financial support from funding agencies, supervised the experiments, and approved the final version of the work.

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