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Influence of aluminum on root growth and of anatomy *Stenocalyx dysentericus* (DC.) O. Berg

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This study aimed to evaluate the effect of aluminum (Al) on root growth and root anatomical structure of *Stenocalyx dysentericus* seedlings. Newly emerged plants were grown in simple solution composed of 0.1 µM of $CaCl_2.2H_2O$ and five Al concentrations of 0, 150, 300, 600, and 1200 µM for 37 days in a hydroponic system. Subsequently, the seedlings were evaluated for root growth, relative root elongation, and anatomical studies using bright-field and fluorescence microscopy techniques. The results showed tolerance by *S. dysentericus*, with more root relative elongation in treatments with 150, 300, and 600 µM of Al. The anatomical studies revealed the presence of Al in root tissue, through the morin reagent, mainly in the 1200 µM treatment, characterizing some internal detoxification mechanism. *S. dysentericus* demonstrated tolerance in the tests with Al, principally at lower doses. These results may be entirely linked to its wide distribution in the cerrado domain, demonstrating to be a species adapted to soils with higher Al concentration. *S. dysentericus*, when subjected to treatment with Al, showed a stimulating effect on root growth; for this species, low concentrations of Al may be essential for better root growth.

Key words: Cerrado, acidic soils, plant toxicity, tolerance.

INTRODUCTION

Aluminum (Al) is the third most abundant chemical element in the earth's crust, with 8%; however, a small amount of this element occurs in a soluble, toxic form to plants. Its toxic form is observed when the pH is below 5, the Al^{3+} ion predominating, which gives way to the Al ions $(OH)^{2+}$, Al $(OH)_2^{+}$, and Al $(OH)_3$ as the soil pH value increases (Mossor-Pietraszewska, 2001; Frankowski et al., 2013). In acid soils, high Al levels and calcium deficiency are often considered the main limiting factors of plant growth. Under these conditions, the roots may have thickening and yellowing at the tips, degenerated and tortuous (Codognotto et al., 2002; Peixoto et al., 2007). On the other hand, some native cerrado species with distribution in acid soils show tolerance to the toxic

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effects of Al, being able to develop characteristics that make them resistant to higher concentrations of the metal and thus allowing their establishment in more acidified soils (Furley and Ratter, 1988; Andrade et al., 2011). As an example, there are *Qualea grandiflora, Vochysia thyrsoidea* and *Salvertia convallariaeodora* (Haridasan, 1982; Haridasan, 2008).

The study of plants tolerant to various concentrations of Al is considered the best alternative for increasing agricultural production in acid soils with high concentrations of this cation (Sanchez-Chacón et al., 2002; Echart and Cavalli-Molina, 2001). In addition, such plants provide important information on Al tolerance mechanisms that are necessary in breeding programs that aim to select the most productive plants with greater adaptability under stress conditions and can alleviate productivity problems in acid soils, caused by high Al levels (Freitas et al., 2006).

Among the diverse native cerrado species, cagaita (*Stenocalyx dysentericus* DC. O. BERG.), a representative of the Myrtaceae family, demonstrates high Al tolerance potential, has characteristics adaptive to sandy, acid and nutrient-poor soils. This species can be found in the extensive vegetation of the Cerrado area, mainly in the North, Southeast and Midwest (Vieira Neto et al., 2009). Therefore, the objective herein is to evaluate the effect of Al on root growth and root anatomical structure of *S. dysentericus* at the seedlings stage grown hydroponically in a simple nutrient solution.

MATERIALS AND METHODS

The fruits of *S. dysentericus* were collected on the Gameleira farm, located in the city of Montes Claros, Goiás, whose geographical coordinates are 16° 06'20 " S - 51° 17'11"W, at 592 m of altitude. The fruits were later pulped to obtain the seeds which were treated with fungicide, 30% Vitavax Tiran®, according to the manufacturer recommendations. Initially, the seeds were sown in beds containing washed sand as substrate. After 18 days emergence occurred and the standardization of size was at 40 days of cultivation, when the seedlings had an average of 14 cm in height. After the selection, the seedlings were transferred to hydroponic cultivation. Before immersing the roots solution, length of the principal root was measured, and the presence and visual stage of leaflets verified (cotyledonary and issued, up to this period).

The seedlings were then fixed in plastic caps with cotton support and placed in plastic pots containing 3 L of simple solution, consisting 0.1 µM de Ca L^{-1} in the form of $CaCl_2.2H_2O$, prepared according to the methodology proposed by Jacob Neto (1993). The pH of the solution was adjusted to 4.0 ± 0.2 with 1 M of HCl solution and the use of 0.1 M NaOH. The solution was changed every three days and constantly aerated using a compressor. To evaluate the effect of Al, concentrations of 0, 150, 300, 600, and 1200 µM of Al were adopted in the form of Al sulphate ($Al_2(SO_4)_3 \cdot 18H_2O$) in simple solution. From the start of the experiment, the seedling roots were maintained in solution containing the Al treatments and measured every 2 days for a period of 37 days, evaluating relative root elongation (RRE%), calculated according to the equation proposed by Vasconcelos et al. (2002), and shoot and root dry mass.

$$RRE = (LeAl_x - LiAl_x)/(Le\ Al_0 - LiAl_0) \times 100$$

where RRE is relative root elongation; iAlx is length initial root measured before exposure to the solution with “x” in Al; LeAlx is length end root measured before exposure of the solution with “x” in Al; $LiAl_0$ is length initial root before exposure to solution no Al; $LeAl_0$ is length end root measured after 37 days of exposure to the solution with Al.

The experimental design was completely randomized with 5 treatments with 4 repetitions each, with each replicate consisting of 4 seedlings per pot, totaling 20 experimental units. Data were subjected to analysis of variance by the F test and regression analysis.

Anatomy of root tips

After 37 days of hydroponic cultivation, samples with approximately 0.5 cm of root tips were collected with the help of disposable razor from one seedling per pot and fixed in Karnovsky solution (Karnovsky, 1965) for 24 h. After fixation, the samples were dehydrated in an ascending ethanol series, pre-infiltrated and infiltrated using historesin (Historesin, Leica) according to the manufacturer recommendations. The root tips were longitudinally sectioned to 5 mm with a rotary microtome (Model 1508R) and subsequently stained with toluidine blue-polychromatic staining, 0.05% in 0.1 M phosphate buffer, pH 6.8 (O’Brien et al., 1964), for structural analysis.

To evaluate Al location in the *S. dysentericus* seedling root tips, Morin fluorochrome was used (Eticha et al., 2005). 4',6 - Diamidino-2-phenylindole (DAPI), 1 µg ml^{-1} for 20 min, was also employed in order to evaluate the effect of Al on the DNA of meristematic cells. DAPI is a fluorochrome which binds strongly to DNA-rich regions. The anatomical images were obtained in an Olympus, BX61 bright-field and fluorescence microscope with a DP-72 camera. Fluorescence analysis was performed using a UV excitation cube (DAPI) 330-385.

RESULTS

Seedling growth under hydroponic cultivation with simple solution

The relative root elongation rate increased in treatments with 150, 300, and 600 µM of Al, providing evidence of Al tolerance for this species, since such concentrations stimulated root growth (Figure 1). Beyond the 600 µM dose, Al seedlings presented a root growth rate decrease, and phytotoxicity effects were observed from this dose onward. In the dry mass of root analysis, increase of 2.83 and 6.53% can be verified at the 300 and 600 µM Al dose and 26.98% reduction in the 1200 µM Al dose when compared with the control (Figure 2). For dry weight of shoot, the 300 µM Al dose promoted 6.02% increase as compared to the treatment without Al, while others had average lower than the control (Figure 2).

Visual analysis of seedlings at the end of the period of treatment showed greater root growth with Al treatments of 150, 300, and 600 µM (Figure 3). The apical region growth was stimulated up to the 600 µM Al dose. As for the Al dose of 1200 µM, a reduction was observed in root growth and the emergence of slight yellowing of the leaf edges, followed by premature leaf drop.

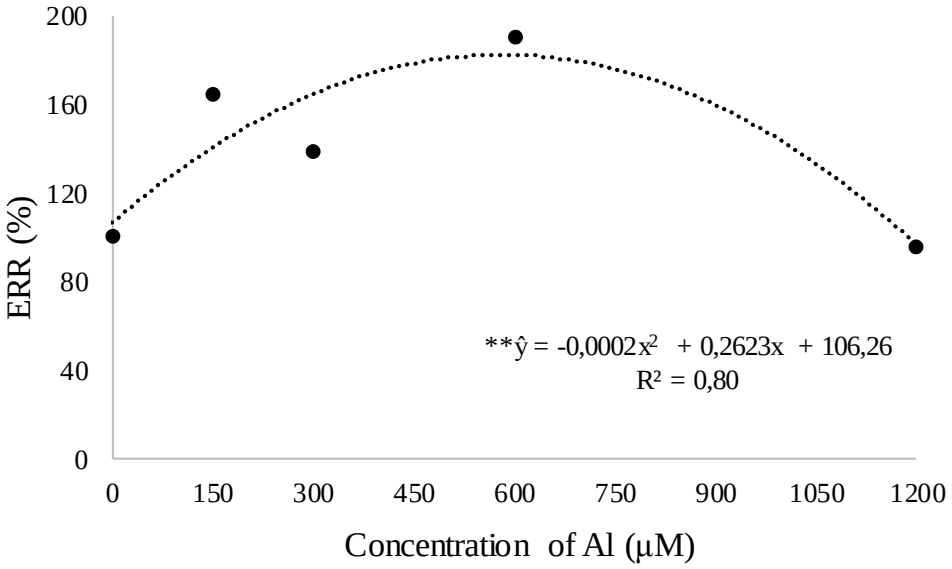

Figure 1. Relative root elongation (ERR%). ** Significant at the 5% level of probability. CV (%).

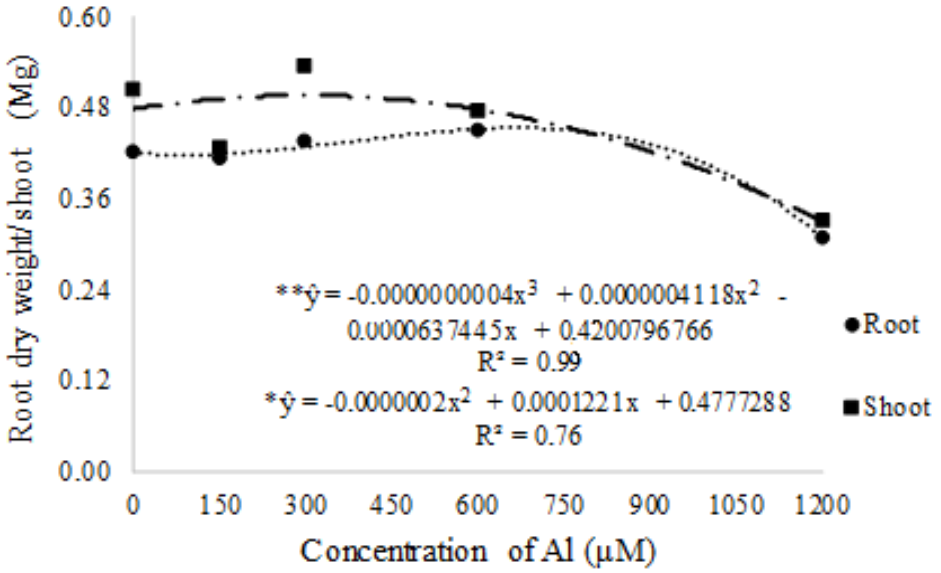

Figure 2. Root dry weight/shoot (Mg). ** Significant at the 1% level of probability and *Significant at 5% probability CV (%) = 13.55 (root) and 16.31 (shoot).

Root tip anatomy

Figure 4 shows *S. dysentericus* root tip sections exposed to different Al treatments, stained with toluidine blue. In analyzing Figure 4A and B, it was observed that the root apical meristems of the seedlings cultivated without Al consist of small juxtaposed cells with dense cytoplasm and an evident nucleus, tiny vacuoles also occur, the hood has uniform formation with apex cell integrity. In treatment with Al (Figure 4C, D, E, F, G and H), thicker roots were observed, consisting of increasingly larger cells and vacuoles in accordance to the Al dose increase. However, with 1200 µM of Al, the roots were thinner noting meristemic cells with walls of irregular outline, very large vacuoles with accumulation of content stained by toluidine blue, the presence of intercellular spaces and deformities in the epidermis; the promeristem is absent in this treatment, characterizing disorganization of the apical

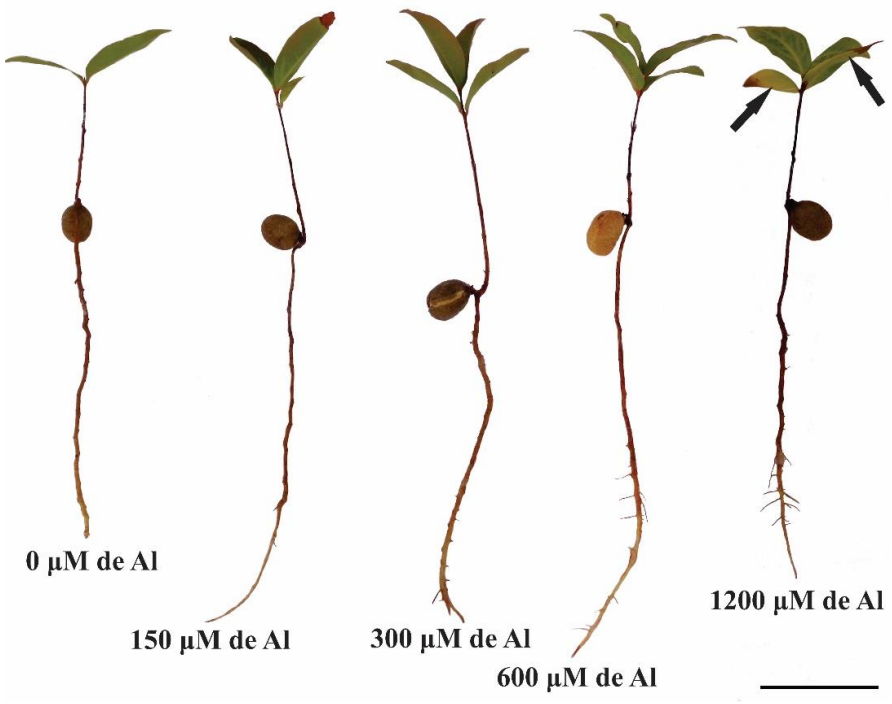

Figure 3. Visual appearance of seedlings at the end of the treatment period (*S. dysentericus*). Scale bar 5 cm.

meristem, which consequently leads to lower root growth (Figure 4I and J).

Tests with Morin show that for increasing Al doses promoted the highest intensity of green fluorescence (Figure 5A, C, E, G and I), especially from Figure 5I with a strong fluorescence signal in the cell wall, cytoplasm and nucleus demonstrating higher concentration of Al. The blue fluorescence with DAPI was with higher intensity to the treatments with 150, 300 and 600 µM of Al (Figure 5D, F and H), when compared with control (Figure 5B). For the treatment of 1200 µM Al, the blue fluorescence was lower, proving that for this does, Al adversely affects cell division inhibiting root growth (Figure 5J).

DISCUSSION

The central hypothesis of this study was that *S. dysentericus* presents Al tolerance, as do many native, usually woody, perennial species of the Cerrado that develop Al tolerance characteristics, being able to accumulate high concentrations in leaves, with levels above 1,000 mg Al kg^{-1} as a root Al detoxification method. These species are also called Al hyperaccumulators, frequent in families Euphorbiaceae, Myrtaceae, Rubiaceae, Melastomataceae, and Vochysiaceae (Cuenca et al., 1991; Jansen et al., 2002a, b).

S. dysentericus demonstrated tolerance and stimulated growth at Al doses up to 600 µM. As has been demonstrated by research works, Al can be beneficial when used in low concentration. Root growth inhibition tendency via Al application did not occur in some treatments, as reported for tea (Morita et al., 2008), corn (Comin et al., 1999) and apple (Stolf et al., 2008). Foy (1983) reported that in some species of plants, low doses of Al can be beneficial to growth. Silva (1992) found that the growth of rice plants was stimulated by the addition of up to 5 mg of Al^{3+} L^{-1} nutrient solution. For Silva (2007), in maize, sugar beet, and some species of tropical legumes, Al concentrations that result in stimulation of growth varied from 71.4 to 185 µM. However, the nature of the beneficial effects of Al is still unknown, but Huang and Bachelard (1993) postulated that this growth stimulation occurs under H^+ stress conditions, concluding that Al^{3+} minimizes the toxicity of H^+.

When a species shows sensitivity to Al, the first visible symptom is the inhibition of root elongation, although this root response has presented different behavior among plant species and even among cultivars (Matsumoto and Motoda, 2012). As an example, Matsumoto (2002) reported that the root growth elongation of Al-sensitive wheat was inhibited by a 3 h treatment with 5 µM of Al, while in the tolerant cultivar elongation was inhibited by a 10 fold higher concentration. The relative root elongation refers to a percentage assessment of the treatments effects on the root growth of seedlings in order to

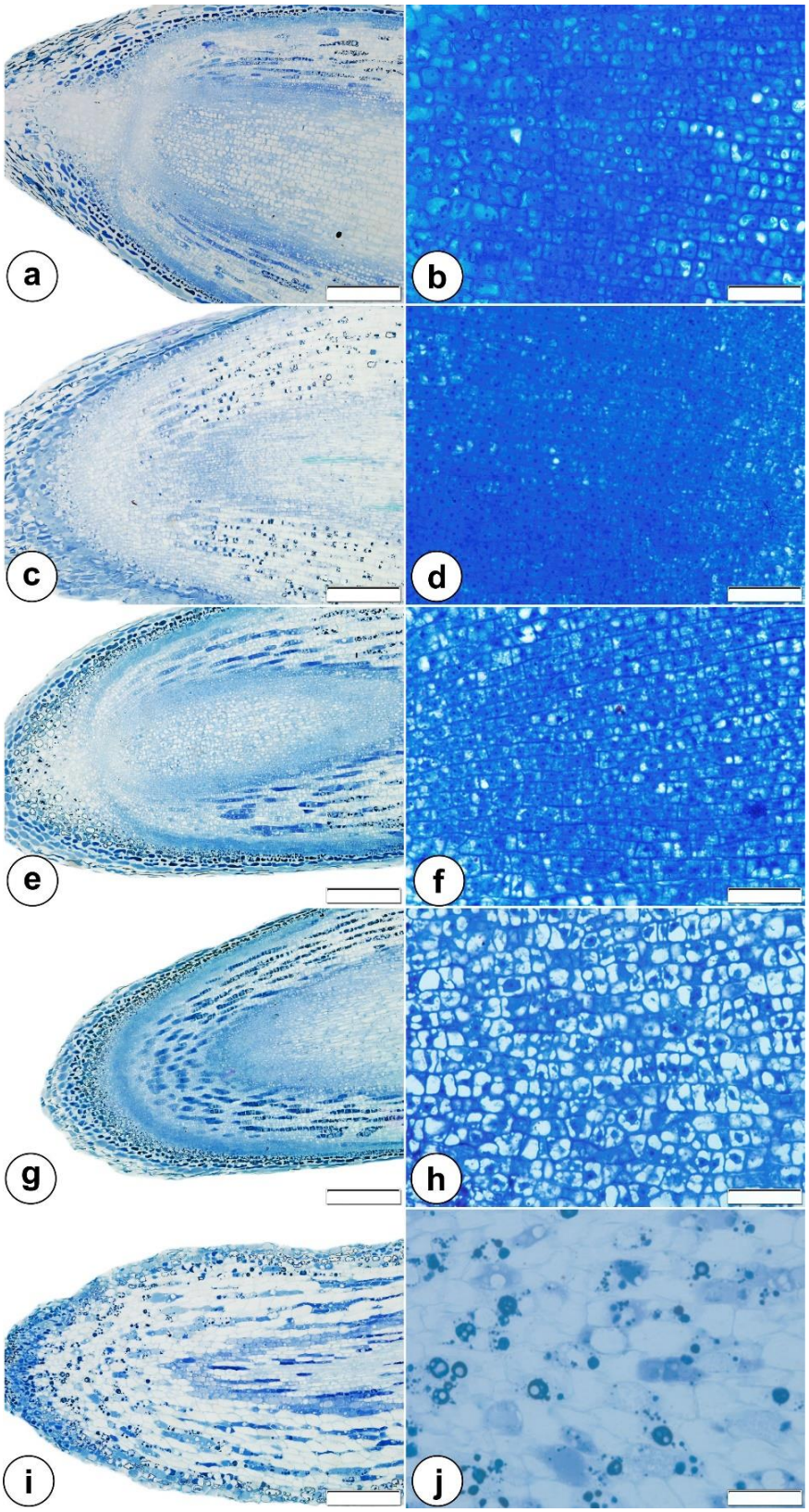

Figure 4. Optical micrographs of longitudinal sections of seedling roots *S. dysentericus*. Stained with toluidine blue. Where A - without Al; B - 150 µM of Al; C - 300 µM of Al; D - 600 µM of Al; and E - 1200 µM of Al A, C, E, G and I Bars = 200 µm; B, D, F, H and J Bar = 50 µm.

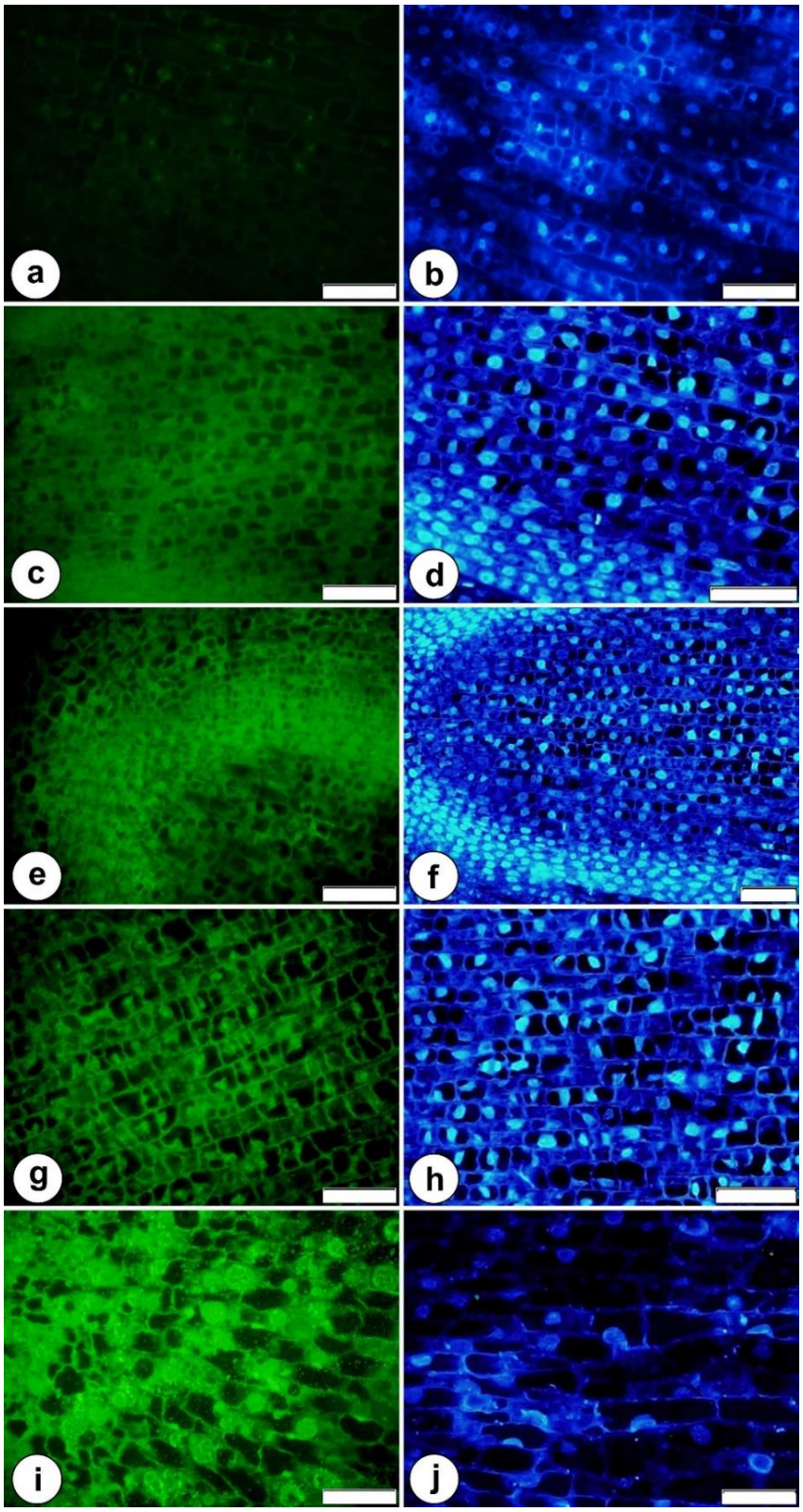

Figure 5. Fluorescence micrographs of cross sections of seedling roots *S. dysentericus*. Treated with morin fluorochrome (picture left) and fluorochrome DAPI (photo right). where: A and B - No Al; C and D - 150 µM dose of Al; E and F - 300 µM dose of Al; G and H - 600 µM dose of Al; I and J -. 1200 µM dose Al The green fluorescence indicates the presence of aluminum and blue presence of DNA. Bar 50 µm.

demonstrate the sensitivity or tolerance of species to Al. Vasconcelos et al. (2002), in a study using RRE% for evaluating the toxicity of Al in rice cultivars, concluded that this parameter was sufficient to identify differences in tolerance among cultivars, even at low concentrations.

The shoot dry mass at an Al dose of 300 µM presented the highest averages. Similar results were observed in castor beans by Lima et al. (2007), regarding the shoot dry matter, in which the increase was 6.3 times in treatments without Al and 15.8 times with a high degree of Al. Probably, the 300 µM Al concentration stimulated the best shoot development, demonstrating that, for this species, the solution with Al promoted greater growth of roots and shoots.

Root tip anatomy

The negative influence of Al in sensitive species alters the growth and cell expansion rate (Barceló et al., 1996). Thus, the cell volume increase at doses of 600 and 1200 µM of Al can be explained by the fact that the roots have a decreased pressure potential, which reduces the apparent hydraulic conductivity, thereby indicating that Al severely affects the proportion of water in the root (Echart and Cavalli-Molina, 2001).

Lima and Copeland (1994) indicated that the effects on the meristematic cells, that is, root growth reduction, become evident only after prolonged exposure to Al^{+3}. Thus, simultaneous alterations in cell elongation and thickness suggest that the effect of Al^{+3}, directly or indirectly, affect many cell expansion-related processes (Nichol et al., 1993). This information corroborates that of this present study, in which the effect of Al on increased cell size was observed at doses of 600 and 1200 µM (Figure 4H and I). In addition, other evidence indicates that cell elongation inhibition may be due to the result, at least in part, of changes in the cap cells, which act as environmental stress sensors (Marschner et al., 1991).

The toxicity of Al in the treatment with 1200 µM can be associated with the gross changes in the root morphology. Briefly, Al results in toxicity in root elongation, inhibiting root development, producing dark colored, thick apices and little secondary root formation due to the high saturation in such treatment for the seedling. Root damage results in a reduced root system, damaged, limiting water and mineral nutrient absorption (Delhaize et al., 1993; Maron et al., 2010). In characterizing the presence of Al in root tips, Garzon et al. (2011) reported that control plant root tips showed low fluorescence when stained with Morin and Al accumulation in the cell wall for treatments with higher doses of this element. Al internalization in the root and root growth stimulation may be associated with mechanisms of complexation by organic acids or internal detoxification mechanisms, impeding the genotoxic action of Al at doses of 150, 300, and 600 µM.

Achary and Panda (2010), working with *Allium cepa*, showed that at high concentrations, Al induces DNA damage, however, when in small concentrations, it can provide adaptive responses conferring genomic protection against genotoxic risk posed by the ion and promoting greater root system growth. When comparing the areas marked by Morin and DAPI, it was observed that increasing Morin fluorescence is related to the decrease of the DAPI fluorescence, that is, Al accumulation in cells causes cell death in the root apex, since such characteristics were demonstrated at higher Al doses at which root growth loss was observed.

Conclusion

S. dysentericus, when subjected to treatment with Al, showed that the Al may be essential for root growth, with smaller root tip diameter and nuclei division stimulus in the treatments with 150, 300 and 600 µM; however, the 1200 µM dose promoted a root growth decrease with cell expansion, showing that for this species, low concentrations of Al may be essential for better root growth.

Conflict of Interests

The authors have not declared any conflict of interests.

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