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RESEARCH ARTICLE

TOLERANCE OF CHIA (*SALVIA HISPANICA*) TO ALUMINUM

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ABSTRACT

The contamination of soil and water with toxic metals as aluminum (Al) is an obstacle to agricultural crops resulting in productivity losses of the affected plants. The objective of this work was to evaluate aluminum tolerance of chia during the germination process. Seeds were placed on paper embedded in an aqueous solution of aluminum chloride at concentrations of zero, 30, 60, 90 and 120 mg L^{-1}. The parameters evaluated were germination percentage, first count, germination speed index, and length and dry mass of seedlings. The results obtained indicate that concentrations of Al equal or less than 90 mg L^{-1} do not influence the germination and initial growth of chia. Based on the levels evaluated in this work, chia seeds are tolerant to the toxicity of Al in the substrate.

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INTRODUCTION

The presence of some toxic chemical elements in the soil, such as aluminum (Al), can damage plants and decrease their productivity (Freitas *et al*., 2012). Thus, there is great interest in studying the contamination of soil and water due the cumulative effect and toxicity of these pollutants (Mazzocato, 2009). Seed germination is a critical stage in the lifecycle of plants and tolerance to Al during germination is crucial for the establishment of plants that grow in acidic soils (Machado *et al*., 2015). The toxicity of aluminum can reduce germination by influencing the metabolism of seeds (Yamashita & Guimarães, 2011; Macedo *et al*., 2008). In addition, an increase in the concentration of toxic metals in plants causes various damaging effects, such as reduced growth that results in atrophy (Mazzocato, 2009). Exposure to high concentrations of Al causes cell membranes to change, leading to increased permeability and the loss of inner solutes. One of the initial effects on the phospholipid bilayer is lipid peroxidation. As a result, the electric potential of the wall changes and the Al degenerates the membrane protein channels (Macedo *et al*., 2008). In addition, Al can interfere with germination by modifying the cellular metabolism, in relation to water permeability, synthesis of proteins and cell walls, mobilization of lipids and mitotic cell division (Roy *et al*., 1988). Despite the importance, our understanding of the metabolic pathways associated with sensitivity, resistance and tolerance to Al by plants is still limited (Costa *et al*., 2014). For this reason, studies about the toxic effects of Al are important to help explain the geographic distribution of species and the role that Al plays in plant tissues. Some studies have used Al solutions at different concentrations on moistened substrates, where seeds are placed to germinate, in an attempt to simulate the toxic conditions of this element (Macedo *et al*., 2008; Yamashita & Guimarães, 2011; Lana *et al*., 2013; Nasr, 2013; Costa *et al*., 2014; Milane *et al*., 2014; Machado *et al*., 2015; Scheffer-Basso & Prior, 2015). However, no study was encountered that evaluated the toxic effect of Al on the germination of chia seeds. Chia (*Salvia hispanica* L. - Lamiaceae) is an annual herbaceous plant cultivated from seed that has high levels of fiber, proteins, oils and fatty acids (omega 3), making it a food with health benefits (Coelho & Salas-Mellado, 2014). When in contact with water the seeds release a transparent mucilage on their surface that protects the seedlings during initial stages of germination (Di Sapio *et al*., 2012). Chia seeds contain 5-6% mucilage that can be used as an emulsifier due to the high concentration of fibers (Reyes-Caudillo *et al*., 2008). The mucilage comprises mostly xylose, glucose and glucuronic acid, forming a ramified polysaccharide with a high molecular weight (Lin *et al*., 1994). Muñoz *et al*. (2012) studied the hydration capacity of chia mucilage and found that 100 mg of mucilage could absorb 2.7 g of water (27 times its own weight).

Chia seeds are subjected to conditions of stress by Al, which can limit development and chances of survival. Considering that the elevated concentrations of Al affect seed germination,

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and because understanding how species withstand determined conditions of stress helps provide adequate recommendations for planting and culture, the objective of this study was to evaluate how chia tolerates Al during germination and initial seedling growth.

MATERIALS AND METHODS

Chia seeds (crop 2015) were acquired from an company that produces and sells seeds, which is located in the municipality of Santa Cruz do Sul, RS (29º43’04”S, 52º25’33”W). To evaluate the toxic effect of the aluminum (Al) on the germination process, the chia seeds were sown on a paper substrate moistened with an aqueous solution of aluminum chloride ($AlCl_3$) at concentrations of zero (control) 30, 60, 90 and 120 mg L^{-1}. For the control (level zero), only distilled water was used. The toxic effect of Al on the seed germination process was evaluated using the tests listed below (Brasil, 2009):

Germination (%): conducted based on four repetitions of 100 seeds distributed in plastic boxes (gerbox), on germitest paper moistened with distilled water (2.5 times the weight of the paper). After sowing the seeds, the plastic boxes (gerbox) were maintained in BOD chambers at a constant temperature of 20 °C and 8 h of light and 16 hours of dark (Stefanello *et al*., 2015). Counts were made on days seven and 14 (when the test ended).

First count (%): conducted together with the germination test, where the percentage of normal seedlings was determined on day 7 of the test.

Germination speed index (GSI): counts of germinated seeds were made daily at the same hour. The germination criterion was the protrusion of the primary root. The germination speed index was calculated using the formula in Maguire (1962).

Seedlings length (cm): normal seedlings were obtained by sowing four repetitions of 20 seeds. Rolls of paper containing the seeds were kept in a germination chamber for six days, at a temperature of 20 °C. Total length, shoot length and root length of 10 seedlings were randomly evaluated for each repetition using a millimeter ruler. The average length of the seedlings was obtained by adding the number of measurements of each repetition and dividing this by the number of normal seedlings measured.

Dry mass of seedlings (mg): first, the fresh weight of 10 seedlings was measured (four repetitions), which were then placed in paper bags in an oven at 60 °C until mass constant (48 h). Subsequently, the seedlings were weighed again using a precision scale (0.001 g).

Data analysis: the experimental design was completely randomized, where treatments consisted of different concentrations of the solutions. The data was submitted to an analysis of variance using the F test and, when significant, a regression analysis was performed using the program Sisvar (Ferreira, 2011).

RESULTS AND DISCUSSION

The variance analysis indicated that the germination, first count and GSI variables were significantly different among the treatments (Table 1). In the absence of Al, the seeds had averages of 85% and 80% of normal seedlings for the germination and first count tests, respectively (Figure 1A) and these percentages were significantly lower for the highest concentration used (120 mg L^{-1}, 76% and 71%). Similarly, the germination speed (Figure 1B) was significantly reduced from 78.7 (control) to 64.1 (120 mg L^{-1}). According to Rampim & Lana (2013), species differ in the degree of tolerance to Al. Plant species such as *Fagopyrum esculentum* and *Camellia sinensis* have mechanisms of tolerance to Al by inactivating and storing the element in non-toxic forms in their leaves. Yamashita and Guimarães (2011) studied the toxic effect of Al on seeds of *Conyza* spp. and verified that the germination can be negatively influenced by the presence of Al. They found that the percentage of normal seedlings was reduced significantly starting at 135 mg L^{-1}, and that the germination speed was impaired starting at the lowest concentration of Al tested (45 mgL^{-1}). In addition, Nasr (2013) observed that when corn seeds were exposed to elevated concentrations of Al there was a decrease in the germination of seeds starting at 50 mg L^{-1}.

Although no significant differences were found for total length, shoot length and root length (Figure 2A), the seedlings showed typical symptoms of toxicity (thinner roots, darkened at the apices) in the presence of all Al concentrations. On the other hand, no significant effect was observed for the treatments in relation to dry mass of the chia seedlings (Figure 2B). It should be noted that not only measurements can express changes promoted by Al toxicity because damage caused by $AlCl_3$ was easily seen by the darkening of the growth points of the roots. This agrees with Meriño-Gergichevich *et al*. (2010), who observed that the roots of most plants become thinner and darker, causing them to be less efficient at absorbing water and nutrients. In the present study, the Al was applied to the substrate and, consequently, was closer to the roots. This supports the assertion by Foy *et al*. (1978) that the toxic effect of this element is more pronounced in seedlings compared to adult plants. Macedo *et al*. (2011) verified that only 3 mg L^{-1} of aluminum was sufficient to cause this darkening effect in the roots of *Jatropha curcas*. According to Gupta *et al*. (2013), the toxic effect of Al causes changes in root morphology,

Table 1. Summary of the analysis of variance for the variables germination (G), *first count (FC),* germination speed index (GSI), total length (TL), shoot length (SL), root length (RL) and dry mass (DM) of chia seedlings exposed to different concentrations of aluminum

Source of variation	Degrees of freedom	Mean square						
		G	FC	GSI	TL	SL	RL	DM
Treatment	4	56.950*	46.325*	144.528*	0.182	0.016	0.150	0.006
Residue	15	11.383	11.033	19.143	0.311	0.038	0.177	0.005
CV (%)		4.10	4.39	6.36	7.77	6.20	10.46	9.13

* Significant at 5% probability by F test. CV = Coefficient of variation

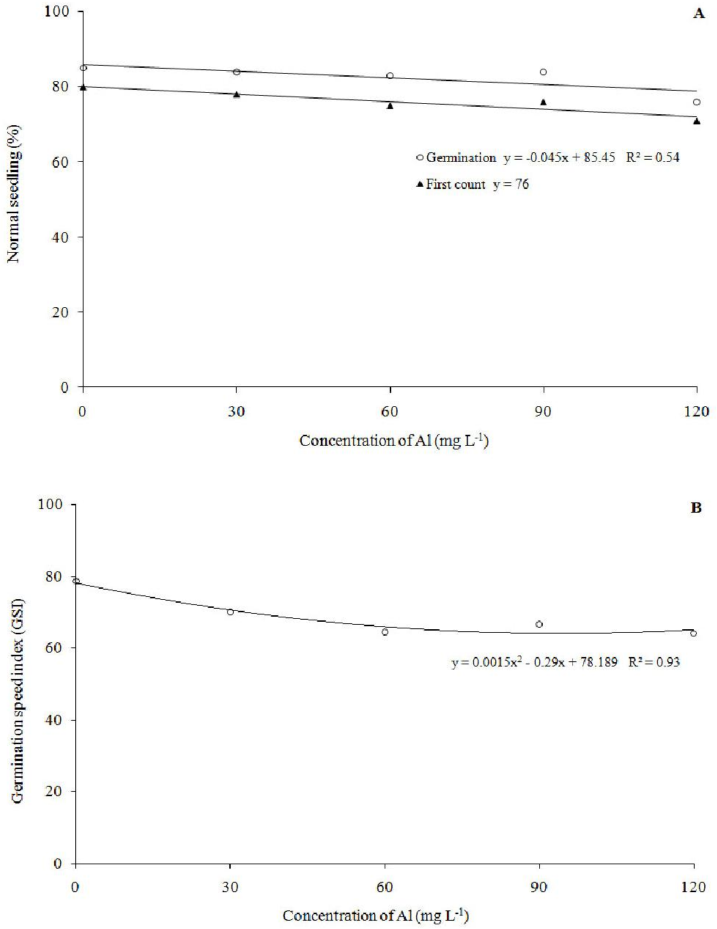

Figure 1. Percentage of normal seedlings (A) and germination speed index (B) of chia seeds exposed to different concentrations of aluminum

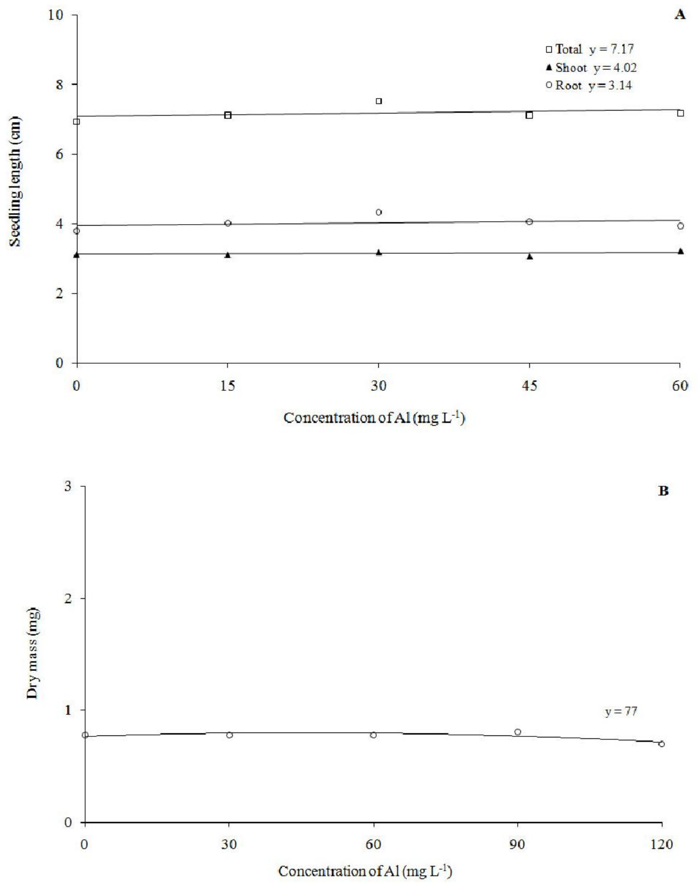

Figure 2. Length (A) and dry mass (B) of chia seedlings exposed to different concentrations of aluminum

including inhibiting the elongation of the radicle that results in short roots with thicker apices and few or no root hairs. The concentrations of Al used in this study are higher than normally found in the solution of acidic soils. According to Machado *et al*. (2015), in most acidic soils the concentration of soluble Al is less than 10 mg L^{-1}. The fact that the results were not significant for most of the variables evaluated, when the seeds were imbibed in a solution with the highest concentrations of Al, leads us to believe that the seeds have some tolerance. This is corroborated by Yang *et al*. (2010) who found that mucilage surrounding the seeds of *Artemisia sphaerocephala* acts as a kind of filter that prevents the harmful effects of toxic elements during germination. However, there is still no experimental evidence available about the physiological role of mucilage in protecting chia seeds against stress, and, therefore, studies about this topic would be useful.

Conclusion

Based on the results of this study, it can be concluded that chia seeds are tolerant to aluminum in the substrate and that it is possible to cultivate this species in agricultural areas at the concentrations proposed in this work.

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