RESEARCH ARTICLE

ALUMINUM AFFECTS SEED GERMINATION AND INITIAL GROWTH OF SCHINUSMOLLE L. SEEDLINGS

1,3*Joseila Maldaner, 2Tamires Silveira Moro, 3Evandro Luiz Missio, 3Cleber Witt Saldanha, 4Gerusa Pauli Kist Steffen, 4Luciane Almeri Tabaldi

1Estudante de Pós-Doutorado na Universidade Federal de Santa Maria
2Estudante de Agronomia da Universidade Federal de Santa Maria, bolsista de iniciação científica
3Pesquisador da Fundação Estadual de Pesquisa Agropecuária (FEPAGRO), Centro de Pesquisa Fepagro Florestas – Santa Maria, RS
4Professora Adjunta da Universidade Federal de Santa Maria

ABSTRACT

Aluminum is the most abundant metal in the earth’s crust and its toxicity potentially limits the growth of plants grown in acid soils. The phytotoxic effects of aluminum on the seed germination and initial growth of Schinus molle seedlings were investigated in the present study. The treatments consisted of six aluminum concentrations (0, 25, 50, 100, 150 and 200 mg L\(^{-1}\)) in the form of chloride (AlCl\(_3\)) with four replicates of 12 seeds each. The majority of the evaluated variables responded in a negative quadratic form to Al concentrations. Our results indicate that intermediate Al concentrations tested were more harmful than the highest in this experiment for most of the evaluated variables, which shows that S. molle seeds have the potential to overcome the harmful effects of Al and to germinate in environments with high concentrations of this metal.

INTRODUCTION

Aluminum (Al\(^{3+}\)) is the third most abundant element in the lithosphere, surpassed only by oxygen and silicon, and makes up 8% of the constitution of the earth's crust (Rossiolo; JacobNeto, 2006), due to the fact that most primary and secondary minerals of rocks, formed by weathering, correspond to aluminosilicates (Miguel et al., 2010). This large amount of aluminum in soils can become toxic to living organisms. Its toxicity is widely recognized due to the inhibition of more than 200 important biological functions, which, in turn, causes serious adverse effects on plants, animals and humans (Exley and Mold, 2015). The degree of toxicity depends not only on the total concentration of Al, but also on the chemical form in which it is available, which is determined mainly by the pH of the medium (Kisnieriënë and Lapeikaitë, 2015). In plants Al toxicity is most significantly reflected in root development and activity (Yu et al., 2011), which occurs due to the interference of this metal in the mechanisms of acquisition and transport of water and nutrients, as well as to cytological changes (Sivaguru et al., 1999; Vasconcelos Filho, 2014).

As water and nutrient absorption is affected, plant growth can also be adversely affected (Vitorello et al., 2005). Thus, seed germination is a critical stage for plants and Al tolerance is a crucial factor for the establishment of plants that grow in acid soils (Machado et al., 2015). Metals are the soil contaminants posing the greatest environmental risk. The ecological costs attributed to soil problems are very high and the number of contaminated areas continues to increase. Among the alternatives for the recovery of contaminated soils, we highlight phytoremediation, by which plants and their associated microbiota remove, contain, transfer, stabilize or render harmless the metals present in the soil. At the end of the 20th century, the potential of plants to clean contaminated environments was identified, where Thlaspi caerulescens and Viola calaminaria were the first documented plant species to accumulate high levels of metals in the leaves (Lasat, 2000).

Plants that exhibit rapid growth, high biomass production, competitiveness, vigor and pollution tolerance are generally preferred in a phytoremediation process (Lamego and Vidal, 2007). Thus, forest species stand out for this purpose, due to their high biomass production, the wide root system and the high capacity to store metals compared to herbaceous species (Capuana, 2011). Schinus molle L., popularly known as Peruvian pepper, American pepper, or Peruvian peppertree,
Aluminum affects seed germination and initial growth of \textit{Schinus molle} L. seedlings

\section*{MATERIALS AND METHODS}

The experiment was carried out at the Laboratory of the FepagroFlorestas Research Center in Santa Maria, RS, Brazil and is part of the project entitled "Fitoremediação mediada por \textit{Schinus molle} e Trichoderma sp. em áreas contaminadas por aluminio". \textit{Schinus molle} seeds (lot 04/2014) belonging to the Renewable Forest Resources Bank, maintained at this research center, were used in this study. Amechanical scarification process was performed using sandpaper 80 mesh in a rotary drum for 150 seconds (as defined in previous tests); as well as a disinfection with commercial hypochlorite (2.5%; v/v) and water at a 1:1 ratio (v/v) plus two drops of liquid detergent per 100 mL of solution, for 5 minutes, followed by washing in distilled water. After that, the seeds were arranged on germinest paper in plastic boxes (gerbox - 11 x 11 x 3.5 cm).

The treatments consisted of six aluminum concentrations (0, 25, 50, 100, 150 and 200 mg L$^{-1}$) in the form of chloride (AlCl$_3$) with four replicates of 12 seeds each. Five milliliters of solution of the respective treatment were added to each replicate. The experiment was carried out in a growth room with a temperature of 25 $^\circ$C ± 2, artificial photoperiod (16 hours light) and light intensity of 35 μmol m$^{-2}$ s$^{-1}$ obtained by cold white lights. The treatment solutions were renewed every four days, at a volume of 3 mL per gerbox. To evaluate the germination potential, the germinated seeds were counted daily at the same time, during a period of 14 days, using root protrusion (2.0 mm) as the germination criterion. The mean data from the first count (Percentage of germinated plants at 7th day) and accumulated germination (percentage of plants sprouted on the 14th day) were analyzed according to the Rules of Analysis of Seeds (Brazili, 2009); and the Germination Speed Index (GSI) was calculated using the formula: $\text{GSI} = \frac{\text{IVG}}{\text{N1} + \text{G2} / \text{N2} + \ldots + \text{Gn} / \text{Nn}}$ (Maguire, 1962), where G1, G2, Gn = number of seeds germinated at the first count, second count until the last count and N1, N2, Nn = number of days from sowing, first, second, until the last count. At 28 days of sowing, the number of seedlings was counted, the mean height of seedlings was measured using a millimeter ruler and the number of leaves per plantlet was counted. Aerial parts and roots were separated and the biomass was measured in an analytical scale; to obtain the dry weighted data the material was placed in a drying oven at 60 ± 3 $^\circ$C until reaching a constant mass. The experimental data were submitted to analysis of variance and when significant ($P<0.05$), regression analysis was performed with the aid of the statistical program SISVAR 5.6 (Ferreira, 2011).

\section*{RESULTS AND DISCUSSION}

Most of the evaluated variables were affected by aluminum concentrations (Fig. 1, 2 and 3). Both the first count and germination speed index (GSI) presented a negative quadratic response to Al treatments (Fig. 1A and 1B). Only accumulated germination was not significantly influenced by Al supply (data not shown). Seed germination is a complex process which begins with water absorption to activate enzymes proteins (Rosiani et al., 2014). The whole germination process is regulated by the hormonal equilibrium of the species and environmental conditions (Atici et al., 2005). Heavy metal stress is a major abiotic stress which can affect seed germination (Casierra-Posada et al., 2009). Besides the effects on the reduction of water absorption, some studies have shown that heavy metals interfere with hormonal equilibrium balance, for example, decreasing endogenous cytokinins and GA$_3$ content in germinating seeds (Atici et al., 2005; Roshani et al., 2014). There are few studies that investigate the effects of Al on seed germination and results have been variable. Seed germination and initial growth of physic nut seedlings was drastically reduced by the addition of high concentrations of Al (Machado et al., 2015). Differently, some studies showed that concentrations up to 60 or 75 mg L$^{-1}$ do not affect germination, vigor, or initial growth on onion and corn seeds, respectively (Stefanello et al., 2016; Milane et al., 2014). In our study, intermediate concentrations tested (50 to 150 mg L$^{-1}$) were more harmful than the highest ones for the first count and GSI. Although the influence of Al on the germination of some species is recognized, Lima and Copeland (1990), while working with different germplasms of wheat, reported that seed germination was less sensitive to Al than seedling growth. The effects of Al on plant growth are observed primarily in the root system, particularly with interference in the elongation and division of meristematic cells (Rengel and Zhang, 2003; Kochian et al., 2004; Stivaguru et al., 2013). Consequently the absorption of water and nutrients is reduced, thereby reducing growth and plant biomass production (Inostroza-Blanchetseau et al., 2012). Similarly to those observed for the germination parameters, the variables of initial growth of \textit{S. molle} seedlings also presented a negative quadratic response to Al treatments (Fig. 2A and 2B). For example, height of seedlings and mean number of leaves were highly impaired by Al concentrations in a quadratic way. In relation to the height of the seedlings, the concentration of 100 mg L$^{-1}$ of Al caused a decrease of about 30%, which was greater than that caused by higher Al concentrations (200 mg L$^{-1}$). Among the effects of Al on plant shoot, the reduction in plant height, leaf area and dry matter yield are the most commonly reported (Lana et al., 2013). Evaluating the effects of aluminum concentrations on the initial growth of physic nut, Machado et al. (2015) observed lower shoot growth, especially at higher Al levels. Similarly Santos et al. (2010) reported significant reductions in root, shoot and total growth, number of leaves, and meandry weight of arugula plants at increasing concentrations of aluminum up to 60 mg L$^{-1}$. Moreover, dry weight both roots and shoot significantly decreased in response to Al concentrations in a quadratic way (Fig. 3A and 3B).
The biomass responses are a reflection of the effects of Al on germination and initial seedling growth. Most of wheat varieties analyzed by Alamgir and Akhter (2009) presented reduction of root and shoot dry mass under Al treatments, with the most severe damage in the roots. These authors agree that in addition to other growth variables, biomass can also be a criterion for classifying varieties with respect to Al tolerance. Toxicity of Al and other metals occurs at varying levels and depends on the concentration, plant species, stage of development, period of exposure and the organ and tissue of the plant (Benavides et al., 2005; Stefanello, 2016). Thus, the negative quadratic response observed for almost all variables evaluated in this experiment may indicate the possible activation of some mechanism of tolerance to this metal in *S. molle* plants.

Figure 1. First count (A) and Germination speed index (B) of *Schinus molle* seed at different aluminum concentrations

Figure 2. Height (A) and number of leaves (B) of *Schinus molle* seedlings at different aluminum concentrations

Figure 3. Dry weight of roots (A) and shoot (B) of *Schinus molle* seedlings at different aluminum concentrations

It is possible that at certain Al concentrations, some signaling route is triggered at this stage of growth, to relieve the negative effects of this metal. To prove this hypothesis, further studies are needed. It is important to remember that the Al concentrations used in this study are higher than those normally found in the solution of acid soils. High concentrations are important since this test brings preliminary results of a larger project that seeks to reach conclusions in regarding phytoremediation.

Conclusion

Under the conditions and for the variables evaluated in this experiment, the intermediate concentrations of Al were more damaging than the highest ones, showing that *S. molle* seeds have the potential to overcome the harmful effects of Al and to
germinate in environments with high concentrations of this metal.

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REFERENCES


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