

Micronutrient concentration in potato clones with distinct physiological sensitivity to Al stress

Concentração de micronutrientes em clones de batata com distinta sensibilidade fisiológica ao estresse de alumínio

Luciane Almeri Tabaldi^I Gabriel Y Castro^I Denise Cargnelutti^{II} Etiane Caldeira Skrebsky^I
Jamile Fabbrin Gonçalves^I Renata Rauber^I Liana Rossato^I Maria Rosa Chitolina Schetinger^{II}
Dilson Antônio Bisognin^{III} Fernando Teixeira Nicoloso^{I*}

ABSTRACT

The objective of this study was to evaluate the effects of aluminum (Al) on the zinc (Zn), manganese (Mn), iron (Fe) and copper (Cu) concentrations in four potato clones (Macaca and Dakota Rose: both Al-sensitive clones; and SMIC148-A and *Solanum microdontum*: both Al-tolerant-clones), grown in a nutrient solution (pH 4.00) with 0, 50, 100, 150 and 200mg Al L⁻¹. Root Zn and Fe concentrations decreased linearly with the increase of Al levels in Macaca, SMIC148-A and Dakota Rose and increased linearly in *S. microdontum*. Shoot Zn concentration showed a quadratic relationship with Al in *S. microdontum* and SMIC148-A, but a curvilinear response in Dakota Rose. Shoot Fe concentration showed a quadratic relationship with Al in *S. microdontum*, SMIC148-A and Dakota Rose. Root Mn concentration decreased linearly in Macaca and SMIC148-A, and increased linearly in *S. microdontum* with Al levels. Mn concentration showed a quadratic relationship with Al in roots of Dakota Rose and in shoot of SMIC148-A, and increased curvilinearly with Al levels in shoot of Dakota Rose. In shoot, there was no alteration in Zn, Fe and Mn in Macaca and Mn concentration in *S. microdontum*. Roots and shoot Cu concentration increased linearly in Dakota Rose, and showed quadratic relationship with Al in Macaca. Roots Cu concentration showed a quadratic relationship with Al levels in *S. microdontum* and SMIC148-A. Shoot Cu concentration increased linearly in *S. microdontum*, and decreased linearly in SMIC148-A. Therefore, the excessive Al accumulation affected the uptake and distribution of Zn, Fe, Mn and Cu in roots and shoot of potato clones. The response of shoot Cu concentration to Al was less altered in the Al-tolerant clones than was in Al-sensitive clones. Aluminum tolerance in *S. microdontum* may be connected with greater levels of Zn, Fe and Mn in the roots.

Key words: *Solanum tuberosum*, *Solanum microdontum*, zinc, manganese, iron, copper.

RESUMO

O objetivo deste estudo foi caracterizar o efeito do alumínio (Al) na concentração de zinco (Zn), manganês (Mn), ferro (Fe) e cobre (Cu) em quatro clones de batata (Macaca e Dakota Rose: sensíveis ao Al; e SMIC148-A e *Solanum microdontum*: tolerantes ao Al) crescendo em solução nutritiva (pH 4,0) com 0, 50, 100, 150 e 200mg Al L⁻¹. A concentração de Zn e Fe em raízes diminuiu linearmente com o aumento dos níveis de Al nos clones Macaca, SMIC148-A e Dakota Rose e aumentou linearmente em *S. microdontum*. Na parte aérea, a concentração de Zn mostrou resposta quadrática ao Al em *S. microdontum* e SMIC148-A, enquanto no clone Dakota Rose houve uma resposta cúbica. Nos clones *S. microdontum*, SMIC148-A e Dakota Rose, a concentração de Fe mostrou resposta quadrática ao Al. A concentração de Mn em raízes diminuiu linearmente em relação ao Al nos clones Macaca e SMIC148-A e aumentou linearmente em *S. microdontum*. Para Dakota Rose e SMIC148-A, a concentração de Mn mostrou uma resposta quadrática em relação ao Al em raízes e parte aérea. A concentração de Mn na parte aérea aumentou de forma cúbica com os níveis de Al no clone Dakota Rose. Na parte aérea, não houve alteração na concentração de Zn e Fe na Macaca e de Mn nos clones Macaca e *S. microdontum*. Em raízes e na parte aérea, a concentração de Cu aumentou linearmente no clone Dakota Rose e mostrou resposta quadrática no clone Macaca. A concentração de Cu mostrou resposta quadrática com os níveis de Al em raízes dos clones *S. microdontum* e SMIC148-A. Na parte aérea, a concentração de Cu aumentou linearmente no clone *S. microdontum* e diminuiu linearmente no clone SMIC148-A com o aumento nos níveis de Al. Portanto, a acumulação excessiva de Al afetou negativamente a absorção e a distribuição de Zn, Fe, Mn e Cu nas raízes e na parte aérea dos clones de batata. A resposta da concentração de Cu na parte aérea ao Al foi menos alterada

^IDepartamento de Biologia, Centro de Ciências Naturais e Exatas (CCNE), Universidade Federal de Santa Maria (UFSM), 97105-900, Santa Maria, RS, Brasil. E-mail: fticoloso@yahoo.com. Autor para correspondência.

^{II}Departamento de Química, CCNE, UFSM, Santa Maria, RS, Brasil.

^{III}Departamento de Fitotecnia, Centro de Ciências Rurais (CCR), UFSM, Santa Maria, RS, Brasil.

nos clones Al-tolerantes que naqueles Al-sensíveis. A tolerância ao Al em *S. microdontum* pode estar conectada com os maiores níveis de Zn, Fe e Mn nas raízes.

Palavras-chave: *Solanum tuberosum*, *Solanum microdontum*, zinco, manganês, ferro, cobre.

INTRODUCTION

The mineral nutrient status of plants is directly related to their growth and productivity. Micronutrients are basic requirements for plant growth and development and their status is controlled by their genetically fixed nutrient uptake potential, the nutrient availability in the soil, and other environmental factors (MENGEL & KIRKBY, 2001). On the other hand, availability of most micronutrients depends, amongst other factors, on the pH of the soil solution, as well as the nature of binding sites on organic and inorganic particle surfaces (TUNA et al., 2008). Trace elements are adsorbed by inorganic constituents such as iron (Fe) and aluminum (Al) oxides and form complexes with organic matter (OMIL et al., 2007). Environmental variation of nutrient availability is expected to result in changes in plant physiology and morphology, and consequently in changes in the yield.

Acid soils are found throughout the world. It is estimated that about 40% of the world's arable soils and 12% of the land in crop production have a pH below 5.5 (VON UEXKÜLL & MUTERT, 1995). Moreover, soil acidification is increasing world-wide. These soils are often characterized by the reduced availability of several nutrients (FOY, 1992). In fact, soil acidification could bring about many other changes in the physical and chemical properties of the soil, which in turn affect plant growth and development.

In acid soil, some nutrients, such as P, Ca, and Mg, may be deficient, whereas others, such as Mn and B, could be toxic to plants. In addition, the continuing acidification of soils with the low buffering capacity leads to an increase of the Al mobilization in the environment and may be potentially hazardous to the all terrestrial and aquatic systems (MATÚS et al., 2006). These effects are further complicated by interactions of Al with other ions in different plant genotypes and under stress conditions (FOY, 1992). Although Al is the most abundant metal in the Earth's crust, it is nonessential for plants. Despite Al occurs in various chemical species, Al³⁺ is regarded the most toxic of the soluble forms of Al (PARKER et al., 1988). The Al cation Al³⁺ is toxic to many plants at micromolar concentrations, affecting primarily the normal functioning of roots. The rapid inhibition of Al-mediated

root growth (RYAN et al., 1993) results in poor nutrient and water acquisition and transport, consequently leads to nutrient deficiencies and decreased crop yields (KOCHIAN, 1995). Aluminum toxicity may be manifested as a deficiency of essential nutrients like Ca, Mg, Fe, Zn or Mo; decreased availability of P or as toxicity of Mn and H⁺ (SCHÖLL et al., 2005; GUO et al., 2007). Aluminum at high levels competes with cationic (mono or bivalents) ions for absorption sites in channels or transporters (KOCHIAN, 1995).

The interference of Al with uptake, transport and utilization efficiency of most of the mineral elements has been well documented (McCOLL et al., 1991; GUO et al., 2007). On the other hand, there is a lack of studies connecting the effects of Al on the uptake and transport of micronutrients in plants. In addition, GIANNAKOULA et al. (2008) suggested that higher levels of mineral nutrients may be connected with Al tolerance. In our previous research (TABALDI et al., 2007) was demonstrated that toxic concentrations of Al in nutrient solution caused oxidative stress in four potato clones, as evidenced by increased H₂O₂ formation, lipid peroxidation and oxidation of proteins in roots and shoots, mainly in Al-sensitive clones (Macaca and Dakota Rose). This study suggested that the reduced growth (length of roots and shoots) in Al-sensitive clones exposed to toxic levels of Al might be induced by an enhanced production of toxic oxygen species and subsequent lipid peroxidation. Moreover, it was possible to observe that Al-tolerant clones (SMIC148-A and *S. microdontum*) developed some defense mechanisms against oxidative stress. Utilizing the same plant biomass obtained by TABALDI et al. (2007) the present work aimed to analyze the influence of Al exposure in nutrient solution on the micronutrients concentration in roots and shoot of the same four potato clones.

MATERIAL AND METHODS

Three adapted (2n=4x=48) clones (Macaca, SMIC148-A and Dakota Rose) and one wild species (2n=2x=24) clone (PI595511-5 of *Solanum microdontum*) were evaluated. The *S. microdontum* was identified as highly resistant to *Phytophthora infestans* (BISOGNIN et al., 2005) and has been used in our breeding program. This clone will be referred to as *S. microdontum*. Tissue culture plantlets were obtained from the Potato Breeding and Genetics Program, Federal University of Santa Maria, Brazil. Nodal segments (1.0cm long) were micropropagated in MS medium (MURASHIGE & SKOOG, 1962), supplemented with 30g L⁻¹ of sucrose, 0.1g L⁻¹ of myo-inositol and 6g L⁻¹ of agar.

Twenty-day-old plantlets from *in vitro* culture were transferred into plastic boxes (10L) filled with aerated full nutrient solution of low ionic strength. The nutrient solution had the following composition (in μM): 6090.5 of N; 974.3 of Mg; 5229.5 of Cl; 2679.2 of K; 2436.2 of Ca; 359.9 of S; 0.47 of Cu; 2.00 of Mn; 1.99 of Zn; 0.17 of Ni; 24.97 of B; 0.52 of Mo; 47.99 of Fe ($\text{FeSO}_4/\text{Na-EDTA}$). Treatments consisted of the addition of 0, 50, 100, 150 or 200 mg Al L^{-1} as $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$. The solutions pH was adjusted daily to 4.0 ± 0.1 by titration with HCl or NaOH solutions of 0.1 M. Both *in vitro* and *ex vitro* cultured plants were grown in a growth chamber at $25 \pm 2^\circ\text{C}$ on a 16/8-h light/dark cycle with $35 \mu\text{mol m}^{-2} \text{s}^{-1}$ of irradiance. Aluminum-treated plantlets remained in each treatment for seven days. At harvest, the plantlets were divided into shoot and roots. Roots were rinsed twice with distilled water. Subsequently, micronutrients concentrations were determined.

After Al treatment, samples (roots and shoot) were dried at 60°C until reaching a constant weight. The dried tissue were weighed and ground into a fine powder before of the nitric-perchloric digestion. Micronutrients concentrations were determined by atomic absorption spectrometry.

All data were analyzed by ANOVA procedures. The effects of Al on micronutrients concentration in roots and shoot of potato plantlets were quantified using regression analysis with the SOC statistic package (Software Científico: NTIA/EMBRAPA). Coefficients were included in a regression equation when their values were significant ($P < 0.05$).

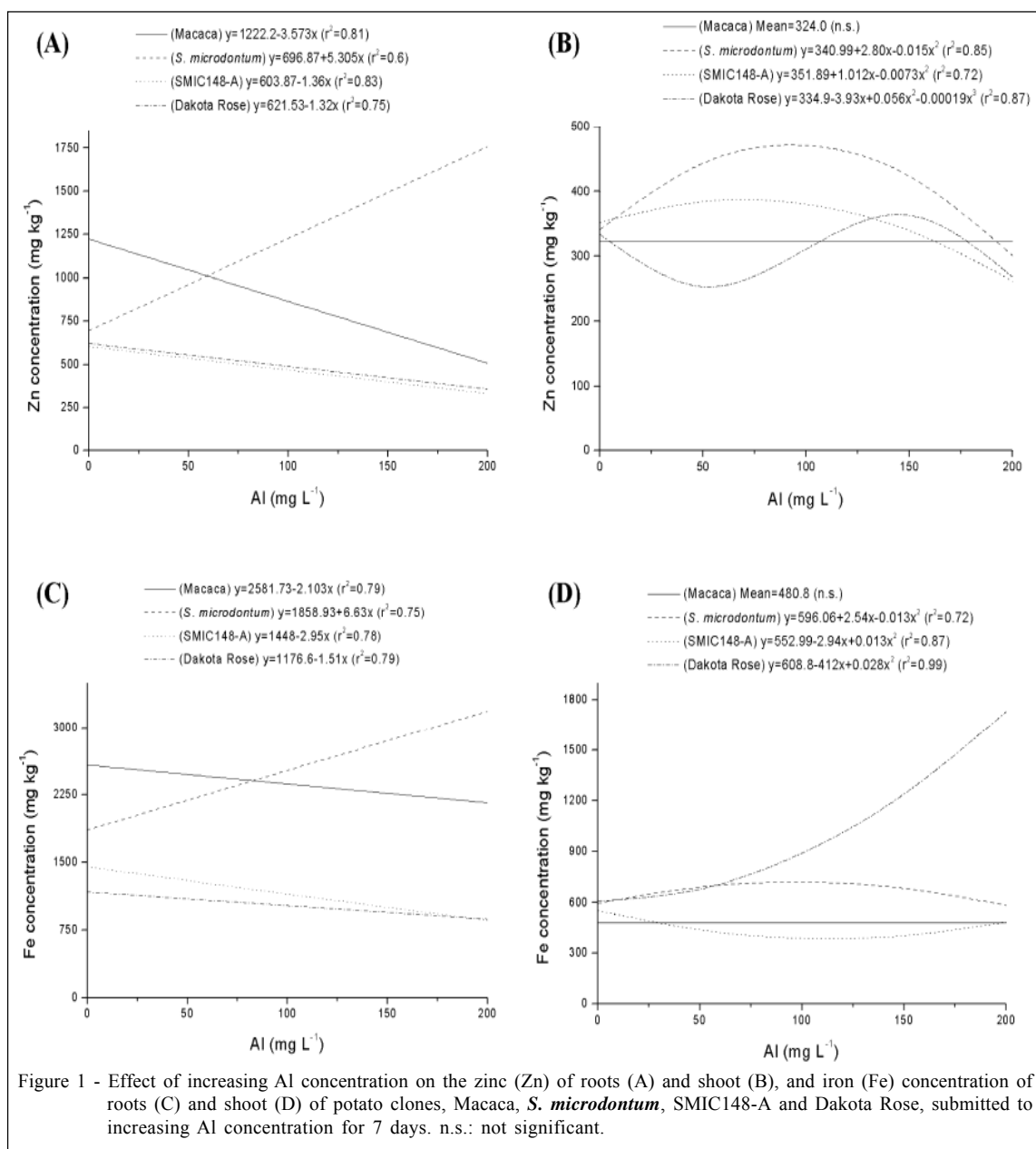
RESULTS AND DISCUSSION

Plants require an adequate supply of micronutrients for their normal physiological and biochemical functions. Deficiencies of essential micronutrients induce abnormal pigmentation, size, and shape of plant tissues, reduce leaf photosynthetic rates, and lead to various detrimental conditions (MASONI et al., 1996). In our previous study (TABALDI et al., 2007), based on relative root growth, it was demonstrated that *S. microdontum* and SMIC148-A are Al-tolerant clones, whereas Macaca and Dakota Rose are Al-sensitive clones. It was proposed that the reduced growth in Al-sensitive clones of potato exposed to toxic levels of Al might be induced by an enhanced production of toxic oxygen species and subsequent lipid peroxidation. In addition, regression analysis showed that the concentration of Al in both the roots and shoot of these clones increased linearly with the increase of Al levels, and the increase in tissue Al was

much steeper for Macaca and SMIC148-A clones. However, the maximum concentration of Al in roots and shoot was 49,300 and 17,900 mg kg^{-1} , as respectively found in Dakota Rose clone at 200 mg Al L^{-1} . Moreover, it was possible to observe that Al-tolerant plants developed some defense mechanisms against oxidative stress.

In this study, the concentration of some micronutrients in the tissue of roots and shoots of four potato clones was examined after seven days of Al exposure. A micronutrient- and organ-dependent response to Al toxicity was observed in all potato clones. Micronutrients concentration was higher in roots than in shoot of all potato clones tested, suggesting that more micronutrients were retained in the roots and lower amounts were transported to the shoot. Regression analysis showed that the concentration of zinc (Zn) decreased linearly with the increase of Al levels in roots of Macaca, SMIC148-A and Dakota Rose clones (Figure 1A). This result is similar to those reported by KOLAWOLE et al. (2000) and JEMO et al. (2007), who observed reduction in nutrient acquisition in cowpea genotypes exposed to Al. High concentrations of Al in the substrate decreased the uptake of Ca, K, P, Fe, and Zn in birch seedlings (*Betula pendula* Roth.), limiting the growth of roots and shoot (BOJARCZUK et al., 2002).

As it was observed for Zn concentration, root iron (Fe) concentration decreased linearly with the increase of Al levels in Macaca, SMIC148-A and Dakota Rose clones (Figure 1C). At 200 mg Al L^{-1} , root Fe concentration decreased by about 20%, 47% and 30%, in Macaca, SMIC148-A and Dakota Rose clones, respectively, when compared to the control. Metal-metal interactions may occur when cations compete for negatively charged binding sites at the cell surface (KINRAIDE et al., 1992). Since the cell wall is the major site of metal accumulation (KOCHIAN, 1995) and provides the bulk of charged surfaces in the apoplast, the metal-metal interactions should affect total metal accumulation. These data might indicate a direct competition between Al and essential nutrients for the same uptake site. Aluminum at high levels competes with cationics (mono or bivalents) ions for absorption sites in channels or transporters (KOCHIAN, 1995). This competition may reduce the ion absorption and utilization. In addition, Al ions may bind to the phospholipids heads of the plasma membrane, alter the lipid-protein interaction, and modify the activity of the transporters (SUHAYDA & HAUG, 1986). Another possibility is that Al binds directly to the transport proteins, thereby impairing their function (SCHROEDER, 1988).



Roots Zn and Fe concentrations increased linearly with the increase of Al levels in the *S. microdontum* clone (Figures 1A and 1C, respectively). In this study, *S. microdontum* clone had greater concentrations of most micronutrients analyzed. Therefore, as this potato clone is Al-tolerant (TABALDI et al., 2007) it seems that the Al levels tested was not enough to cause severe alteration in the metabolism. Thus, higher levels of mineral nutrients may be connected with Al tolerance, as suggested by GIANNAKOULA et al. (2008). Shoot Zn concentration showed a quadratic relationship in the *S. microdontum* and SMIC148-A clones, increasing in intermediary Al

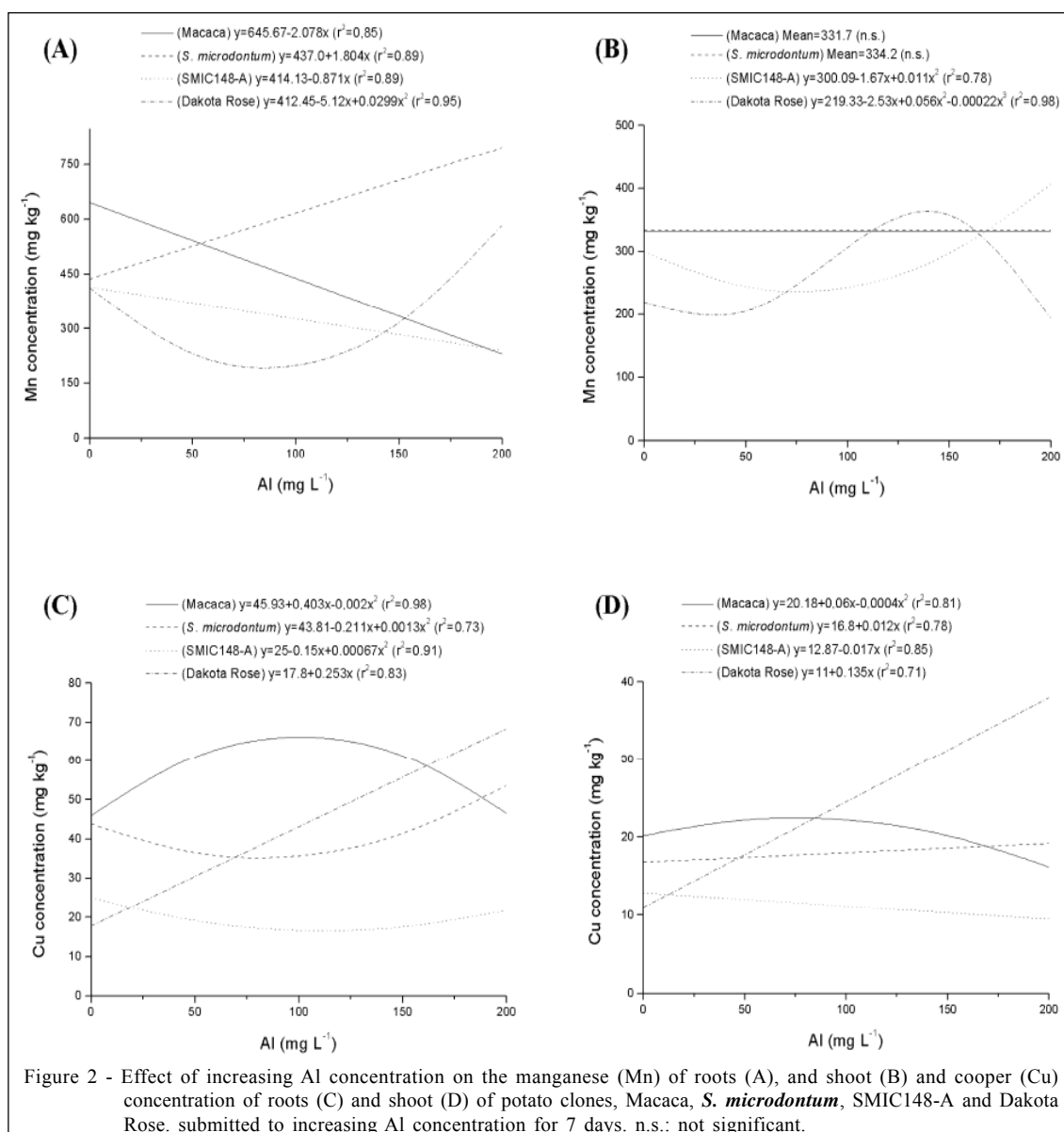
levels (Figure 1B). In Dakota Rose clone, shoot Zn concentration showed a curvilinear response, decreasing at approximately 50mg Al L⁻¹ and increasing at approximately 150mg Al L⁻¹ (Figure 1B). In SMIC148-A clone, shoot Fe concentration decreased between Al levels of approximately 50 and 150mg Al L⁻¹ and showed a quadratic increase in Dakota Rose clone. On the other hand, shoot Fe concentration slightly increased at Al levels between approximately 50 and 100mg Al L⁻¹ in the *S. microdontum* clone.

Roots manganese (Mn) concentration decreased linearly in both Macaca and SMIC148-A clones, and increased linearly with the increase of Al

levels in *S. microdontum* clone (Figure 2A). However, in Dakota Rose clone, Mn concentration showed a quadratic relationship to Al supply, decreasing at levels between approximately 50 and 100mg Al L⁻¹ and increasing at 200mg Al L⁻¹. Shoot Mn concentration showed a quadratic relationship with Al levels in the SMIC148-A clone (Figure 2B), decreasing at levels between approximately 50 and 100mg Al L⁻¹ and increasing at 200mg Al L⁻¹. For Dakota Rose clone, shoot Mn concentration showed a curvilinear response to Al supply, increasing at levels between approximately 100 and 150mg Al L⁻¹, while in the *S. microdontum* clone there was no alteration in Mn concentration.

There was no alteration in shoot Zn (Figure 1B), Fe (Figure 1D) and Mn (Figure 2B) concentration in Macaca clone. However, in the previous study (TABALDI et al., 2007), shoot growth in this clone decreased linearly with the increase of Al levels. Therefore, the interference of Al in the root growth and in the absorption and transport of water and other nutrients may have brought about lower shoot growth.

Copper (Cu) is an essential plant micronutrient playing an important role in both photosynthetic and respiratory electron transport, being a cofactor for many enzymes (OWEN, 1982). However, when present in elevated concentrations it



affects different parameters of plant metabolism, such as dry mass accumulation (ALI et al., 2002; ZHENG et al., 2004), chlorophyll (LOU et al., 2004), and water content (BURZYNSKI & KLOBUS, 2004) and the balance in macro- and micronutrient levels (ALI et al., 2002; BERNAL et al., 2007). Being a redox active metal, Cu generates reactive oxygen species (ROS) by Fenton reaction, which may result in oxidative stress leading to peroxidation of membrane lipids (STOHS & BAGCHI, 1995). The response of root Cu concentration in the Dakota Rose clone to Al levels was linear and positive (Figure 2C), whereas in Macaca it increased only at levels between approximately 50 and 150mg Al L⁻¹. By contrast, in both Al-tolerant clones, root Cu concentration showed in general an inverse relationship with the increase of Al levels, with exception of the *S. microdontum* clone that showed increased Cu concentration at 200mg Al L⁻¹ when compared to the control (Figure 2C). Shoot Cu concentration in the Dakota Rose clone increased linearly with the increase of Al levels (Figure 2D), while in the Macaca clone it slightly increased at Al levels between approximately 50 and 100mg Al L⁻¹. The response of shoot Cu concentration in the *S. microdontum* clone to Al levels was linear and positive (Figure 2D), while in SMIC148-A it was linear and negative (Figure 2D). However, for these two Al-tolerant clones shoot Cu concentration was less altered than that of Al-sensitive clones. Therefore, the increase in tissue Cu concentration observed in the potato clones exposed to Al might have caused disturbance in the metabolism, and hence to plant growth and development, mainly in the Al-sensitive clones.

Plants vary in sensitivity to toxic compounds in the soil. Many studies showed that the sensitivity depends on many factors, such as physicochemical properties of the soil, concentration of organic matter and nutrients, but primarily on soil pH (RENGEL, 1996). Aluminum is widespread in the Earth's crust and its availability to plants increases with decreasing pH of the soil (BOUDOT et al., 1994). The toxic Al ions present in the substrate can damage root cells, which become inefficient in absorption and translocation of both nutrients and water (MOSSOR-PIETRASZEWSKA et al., 1997), blocking their participation in important metabolic processes, such as photosynthesis and respiration (RENGEL, 1996).

Therefore, in the present study, the excessive Al accumulation observed could have affected the rate of uptake and distribution of certain micronutrients in roots and shoot of potato clones, and consequently would be responsible for mineral deficiencies/imbalance and depression of the plant

growth. Selection of plants tolerant to toxic ions contained in the soil can enable more effective management of degraded habitats.

CONCLUSIONS

The excessive Al accumulation affected the rate of uptake and distribution of Zn, Fe, Mn and Cu in roots and shoot of potato clones and the response of shoot Cu concentration to Al was less altered in the Al-tolerant clones than was in Al-sensitive clones. Aluminum tolerance in *S. microdontum* may be connected with greater levels of Zn, Fe and Mn in the roots.

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