BIOLOGIA PLANTARUM 55 (1): 178-182, 2011

BRIEF COMMUNICATION

Effect of aluminum on the *in vitro* activity of acid phosphatases of four potato clones grown in three growth systems

L.A. TABALDI[1], D. CARGNELUTTI[2], G.Y. CASTRO[1], J.F. GONÇALVES[1], R. RAUBER[1], D.A. BISOGNIN[3], M.R.C. SCHETINGER[2] and F.T. NICOLOSO[1]*

Departamento de Biologia, Centro de Ciências Naturais e Exatas (CCNE), Universidade Federal de Santa Maria (UFSM), Santa Maria - 97105-900, RS, Brasil[1]
Departamento de Química, CCNE, UFSM, Santa Maria - 97105-900, RS, Brasil[2]
Departamento de Fitotecnia, Centro de Ciências Rurais, UFSM, Santa Maria - 97105-900, RS, Brasil[3]

Abstract

The aim of this study was to assess the effect of aluminum on the *in vitro* activity of acid phosphatases (APases) of four potato clones, Macaca and Dakota Rose (Al-sensitive), and SMIC148-A and *Solanum microdontum* (Al-tolerant), grown *in vitro*, in hydroponics or in a greenhouse. The enzyme was assayed *in vitro* in the presence of 0, 1.85, 3.70, 5.55 and 7.40 mM Al. In plantlets grown *in vitro*, root APases were inhibited by Al in all clones, while shoot APases were inhibited by Al in *S. microdontum* and Dakota Rose and increased in Macaca at all Al concentrations. In plantlets grown in hydroponics, root APases increased in Macaca at 1.85 mM Al, whereas decreased at all Al levels in *S. microdontum*. In greenhouse plantlets, root APases decreased at 7.40 mM Al in *S. microdontum* and SMIC148-A, and at 3.70, 5.55 and 7.40 mM Al in Dakota Rose. Shoot APases decreased in Macaca and SMIC148-A. Conversely, in Dakota Rose, APases increased at 1.85 and 3.70 mM Al. These results show that the effect of Al toxicity on *in vitro* APase activity depends not only on Al availability but also on the plant organ, genetic background, and the growth conditions. Therefore, it suggests that acid phosphatases activity assessed *in vitro* might not be a good parameter to validate the screening for adaptation of potato clones to Al toxicity.

Additional key words: abiotic stress, phosphorus metabolism, *Solanum tuberosum*, *Solanum microdontum*, tolerance.

Toxic concentrations of aluminum (Al), generally found in acid soils (pH < 5.0), inhibit root growth (Dong *et al*. 2002, Tabaldi *et al.* 2007b, Liu *et al.* 2008), restricting water and nutrient uptake and leading to poor growth and yield (Marschner 1995, Kochian *et al.* 2004, Tabaldi *et al.* 2009a). Experimental data suggest that Al can be transported across the root plasma membrane and interact with components in the cell sap such as nuclear DNA, enzymes, calmodulin, tubulin and ATP (Delhaize and Ryan 1995). Aluminum causes oxidative stress (Tabaldi *et al*. 2007b, 2009b, Liu *et al*. 2008, Shamsi *et al.* 2008), probably because it affects enzyme-mediated reactions, especially those involving carbon, nitrogen and phosphorus metabolisms (Dong *et al*. 2002). In addition, Al can interfere with PO_4^{3-} binding (Rai *et al*. 1998). Therefore, it is important to study key enzymes involved in these processes, such as acid phosphatases (APases). Plant protein and enzyme responses to a variety of environmental factors may be useful in predicting the survival capacity of plant species or varieties under stress conditions.

Acid phosphatases (orthophosphoric-monoester phosphohydrolases, E.C.3.1.3.2) are a group of enzymes that catalyze the hydrolysis of a variety of phosphate esters releasing Pi from phosphorylated substrates in acidic environments (Yoneyama *et al*. 2007). They are widely distributed in plants (Luhová *et al*. 2006), and are present in the apoplast and in different cell compartments

Received 9 May 2009, *accepted* 15 December 2009.
Abbreviations: APase - phosphatase; EDTA - ethylenediaminetetraacetic acid; Pi - inorganic phosphate; TCA - trichloroacetic acid.
Acknowledgements: The authors thank the Coordenação e Aperfeiçoamento de Pessoal de Nível Superior, Conselho Nacional de Desenvolvimento Científico e Tecnológico and Fundação de Amparo à Pesquisa de Estado do Rio Grande do Sul for the research fellowship.
* Corresponding author; fax: (+55) 55 32208339, e-mail: ftnicoloso@yahoo.com

(Yoneyama *et al*. 2007), suggesting that these enzymes are involved at various metabolic pathways. They appear to be important in the production, transport and recycling of Pi (Tejera García *et al*. 2004). Phosphorus deficiency in higher plants has been shown to increase the activity of APases (Duff *et al*. 1994). Moreover, metals such as Hg and Zn can affect acid phosphatase activity (Tabaldi *et al*. 2007a).

Root growth has been used as a parameter for Al tolerance of different plant genotypes (Horst *et al*. 1999, Tabaldi *et al*. 2007b, 2009b). The induction of callose formation in root tips of Al-treated intact plants in nutrient solution led to a differentiation of cultivars in their sensitivity to Al (Eticha *et al*. 2005, Yu *et al*. 2009). In addition, it has been shown that in maize as well as in other species, exudation of organic anions from the root apex may play a key role in cultivar differences in Al tolerance (Kochian *et al*. 2004). Recently, using a hydroponic culture system with different concentrations of Al, we observed significant physiological responses including oxidative stress in potato clones (Tabaldi *et al*. 2007b, 2009b). Several factors have been shown to influence the response of plants to Al (Hairiah *et al.* 1995, Eticha *et al*. 2005, Shamsi *et al.* 2008, Yu *et al*. 2009) and then would limit substantially the screening for Al tolerance.

To improve and/or validate the screening for adaptation of potato genotypes to Al toxicity we hypothesized that acid phosphatase activity assessed *in vitro* might be a valid parameter. *In vitro* studies have been shown to be suitable approach for studying effect of metals on enzymes (Pereira *et al*. 2006, Tabaldi *et al*. 2007a). Therefore, the aim of this work was to assess the effect of Al on the *in vitro* activity of acid phosphatases of four potato clones grown in three growth systems.

Three adapted (2n=4x=48) potato (*Solanum tuberosum* L.) clones (Macaca, SMIC148-A and Dakota Rose) and one wild species (2n=2x=24) clone (PI595511-5/*Solanum microdontum* Bitter) were evaluated. These clones were obtained from the Potato Breeding and Genetics Program, Federal University of Santa Maria, RS, Brazil.

Nodal segments (1.0 cm long) were micropropagated in MS medium (Murashige and Skoog 1962), supplemented with 30 g dm^{-3} of sucrose, 0.1 g dm^{-3} of myo-inositol and 6 g dm^{-3} of agar. Plantlets were grown in a growth chamber at 25 ± 2 °C, 16-h photoperiod with photon flux density of 35 μmol m^{-2} s^{-1} for 30 d. At harvest, plantlets were collected, divided into roots and shoot and used for enzymatic assay (*in vitro* culture system).

Plantlets (20-d-old) from *in vitro* culture were transferred into plastic boxes (10 dm^3) filled with aerated full nutrient solution of low ionic strength. The nutrient solution had the following composition [mg dm^{-3}]: 85.31 of N; 3.77 of P; 23.68 of Mg; 104.75 of K; 97.74 of Ca; 11.54 of S; 181.08 of Cl; 0.03 of Cu; 0.11 of Mn; 0.13 of Zn; 0.01 of Ni; 0.27 of B; 0.05 of Mo; 2.68 of Fe ($FeSO_4$/Na-EDTA). These *ex vitro* cultured plantlets were grown in a growth chamber at the above mentioned conditions. After 10 d of culture, plantlets were collected, excised into roots and shoot and used for enzymatic assay (hydroponic system).

Ten tubers of each clone, with approximately 1.0 cm^3, were planted separately in 300 cm^3 plastic pots using sand as substrate and grown in greenhouse. The irrigation was made with the same nutrient solution used in the hydroponic culture. After 30 d of culture, all plantlets were collected and excised into shoot and roots for enzymatic analysis. Roots were rinsed with aliquots of distilled water to remove the substrate (greenhouse system).

The effect of the Al on APases was determined by assays in the presence of 0, 1.85, 3.70, 5.55 and 7.40 mM Al as $AlCl_3 \cdot 6\,H_2O$. These concentrations were chosen after preliminary tests (Tabaldi *et al*. 2007b, 2009b).

Enzyme extraction and assay were carried out at 4 ºC. For extraction, fresh potato samples of roots and shoot were ground with a mortar and pestle in three volumes of a buffer containing 100 mM Tris-HCl, pH 7.4, 0.1 mM ethylenediaminetetraacetic acid (EDTA) and 0.1 % albumin, centrifuged at 43 200 *g* for 30 min and the resulting supernatant was used for enzyme assay. Acid phosphatase activity was determined according to Tabaldi *et al*. (2007a) in a reaction medium consisting of 3.5 mM sodium azide, 2.5 mM calcium chloride, 100 mM citrate buffer, pH 5.5, and 0.02 cm^3 of Al, except in controls, at a final volume of 0.2 cm^3. A 0.02 cm^3 aliquot of the enzyme preparation (10 - 20 μg protein) was added to the reaction mixture, except in controls, and preincubated for 10 min at 35 ºC. The reaction was started by addition of substrate (PPi) and stopped by the addition of 0.2 cm^3 of 10 % trichloroacetic acid (TCA) to a final concentration of 5 %. Inorganic phosphate (Pi) was measured at 630 nm using malachite green as the colorimetric reagent and KH_2PO_4 as standard for the calibration curve. Controls were carried out to correct for nonenzymatic hydrolysis by adding enzyme preparation after TCA addition. All assays were performed in triplicate using PPi as substrate at a final concentration of 3.0 mM (according to Tabaldi *et al*. 2007a). In all the enzyme preparations, protein was determined by the method of Bradford (1976) using bovine serum albumin.

Each experiment was repeated at least five times. The data were subjected to analysis of variance (*ANOVA*), and means were compared by Tukey's range test when significance ($P < 0.05$) was detected.

Aluminum stress produced significant effects on APase activity in the four potato clones, but these effects depended on the growth system, genetic background, and plant organ analyzed. In the Macaca clone, Al-mediated APase inhibition was observed either in roots of *in vitro* grown plantlets (at all Al levels) (Fig. 1*A*) or in the shoot of plantlets grown in the greenhouse (at 7.40 mM Al) (Fig. 1*F*). In the *S. microdontum* clone, Al inhibited APase activity in roots of plantlets grown in all three systems used, but the degree of inhibition differed among them (Fig. 1*A,C,E*), as well as in the shoot of *in vitro* grown plantlets (at 7.40 mM Al) (Fig. 1*B*). In the

SMIC148-A clone, APase activity was inhibited in roots of *in vitro* grown plantlets (at 5.55 and 7.40 mM Al) (Fig. 1*A*) and in roots (at 7.40 mM Al) (Fig. 1*E*) and shoot (at 1.85, 3.70 and 7.40 mM Al) (Fig. 1*F*) of plantlets grown in the greenhouse. In the Dakota Rose clone, Al inhibited APase activity in roots (at 3.70, 5.55 and 7.40 mM Al) (Fig. 1*A*) and shoot (at 7.40 mM Al) (Fig. 1*B*) of *in vitro* grown plantlets and in roots of plantlets grown in the greenhouse (at 3.70, 5.55 and 7.40 mM Al) (Fig. 1*E*).

The mode of action of metals varies with enzymes and little is known about the exact mechanisms by which metals interact with many different enzymes. Enzyme reactions can be inhibited by metals, which can form a complex with the substrate, combine with protein-active groups of the enzymes, or react with the enzyme-substrate complex. Acid phosphatases (APases) represent a broad group of hydrolytic enzymes that catalyze the breakdown of P-monoesters with acid pH optima (Duff *et al*. 1994). These enzymes are expressed under a variety of conditions and in response to many stimuli. It is believed that APases play an important role during cell starvation for P scavenging and remobilization, and in other conditions that promote phosphate mobilization and/or oxidative stress (Del Pozo *et al*. 1999). Purified APase from leaves and nodules of common bean was strongly inhibited by Al (Tejera Garcia *et al*. 2004). Phosphatases are generally metalloenzymes dependent on Ca^{2+} or Mg^{2+}. One possible mechanism explaining Al-toxicity may be the replacement of Mg^{2+} by Al in the

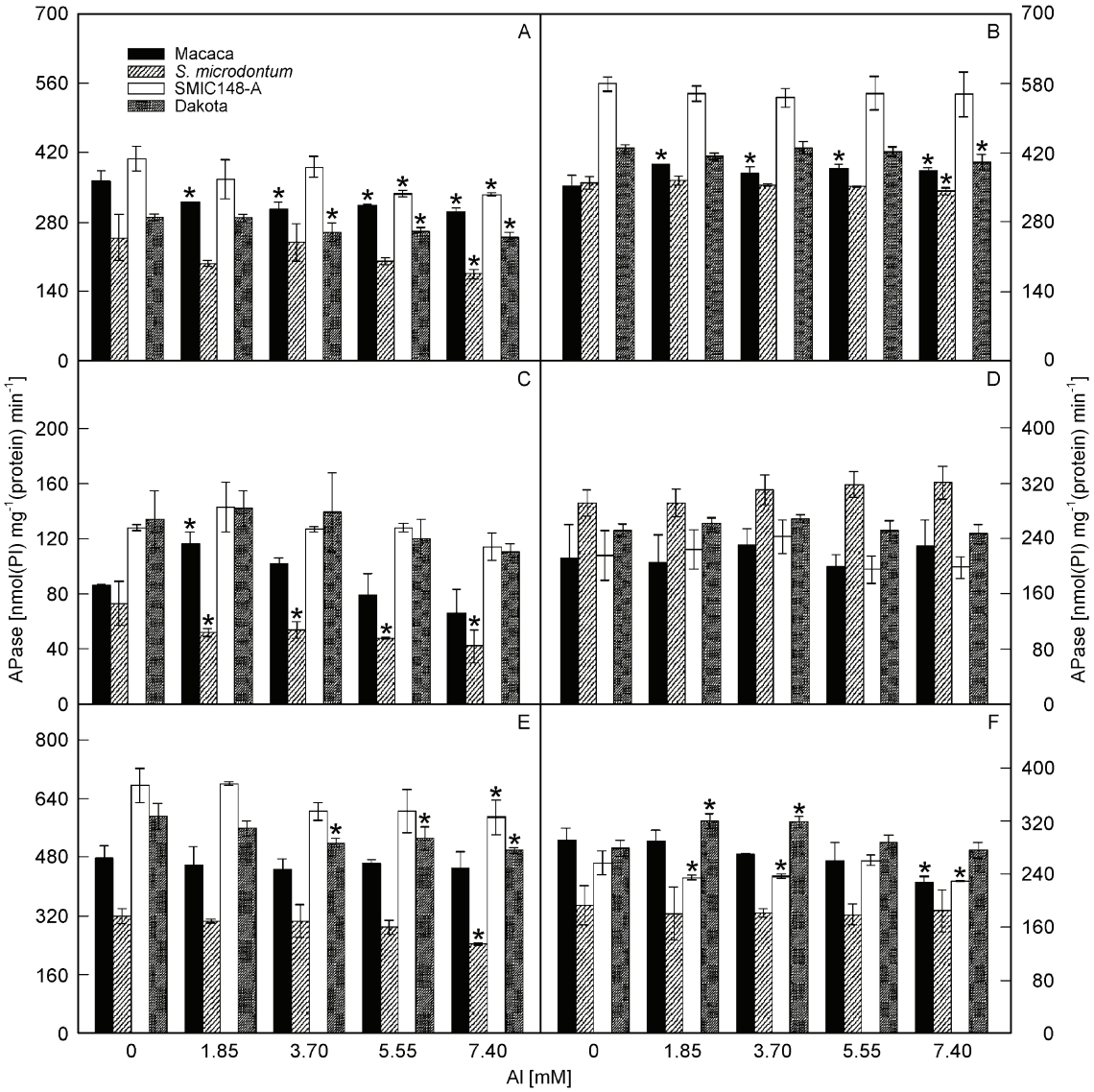

Fig. 1. Effect of increasing Al concentration on the *in vitro* acid phosphatase activity of roots (*A,C,E*) and shoot (*B,D,F*) of potato plantlets (clones Macaca, *S. microdontum*, SMIC148-A and Dakota Rose) grown *in vitro* (*A,B*), in hydroponics (*C,D*), and in the greenhouse (*E,F*). Data are means ± SD of five different replicates, * - different from control at $P < 0.05$.

active site of the enzyme. Besides, Al can influence the cytoplasmic Ca^{2+} homeostasis. It acts as a Ca^{2+}-channel blocker by binding to the verapamil-specific channel-receptor site and by interfering with the action of GTP-binding proteins (Zheng and Yang 2005). Another possibility, suggested by Rai *et al*. (1998), is that Al may interfere with the PO_4^{3-} binding sites. Other metals, such as Hg and Zn, also inhibited *in vitro* APase activity in cucumber seedlings (Tabaldi *et al*. 2007a). Therefore, it is feasible that Al-mediated inhibition of APase activity in potato may impair phosphate mobilization, since this enzyme is involved in the metabolism of P, an essential element for plant growth and development (Duff *et al*. 1994).

In the present study, APase activity was enhanced only in Al-sensitive potato clones. In the Macaca clone, APase activity increased in shoot of *in vitro* plantlets (at all levels of Al) (Fig. 1*B*), as well as in roots of hydroponic plantlets (at 1.85 mM Al) (Fig. 1*C*). Conversely, in shoot of the Dakota Rose clone, APase activity increased only in greenhouse plantlets (at 1.85 and 3.70 mM Al) (Fig. 1*F*). Intra- and/or extracellular APases of plants are induced under various environmental and developmental conditions (Duff *et al*. 1994), including exposure to cations, salt stress and in response to phosphate starvation (Gabbrielli *et al*. 1989, Yoneyama *et al*. 2007). In *Arabidopsis*, it was suggested that purple acid phosphatase had a bifunctional role, acting in phosphate mobilization and in the metabolism of reactive oxygen species (Del Pozo *et al*. 1999). SAP_1 and SAP_2, two secreted purple acid phosphatase isozymes from *Lycopersicum esculentum* may also be multifunctional proteins that operates as scavengers of Pi from extracellular phosphate-esters during Pi deprivation, or alkaline peroxidases that participate in the production of extracellular reactive oxygen species during the oxidative burst associated with the defense response of the plant to pathogen infection (Bozzo *et al*. 2002). Interestingly, using microarray technology, Goodwin and Sutter (2009) identified a total of 256 genes as Al-responsive in *Arabidopsis thaliana*, in which 94 of these genes were shown to be up-regulated and 162 were down-regulated. Moreover, within the up-regulated genes it was found a purple acid phosphatase with 2-fold change.

In all potato clones studied, the APase activity of *in vitro* and hydroponic plantlets was higher in shoot than in roots (Fig. 1*A,B,C,D*). On the contrary, in greenhouse plants, APase activity was higher in roots than in the shoot (Fig. 1*E,F*). This result might be related to different physiological features of plants grown in these three environments. Major differences exist between the environment of plants grown in tissue culture and that of a greenhouse. These include differences in medium mineral composition, growth regulators, irradiance (both quantity and quality), relative humidity, CO_2 and O_2 availability, *etc.* (Hazarika 2003). Differences between these two environments and their effect on plants have been recognized in numerous studies (Pospíšilová *et al*. 1999, Hazarika 2003). In the present study, greenhouse plants, grown under uncontrolled temperatures, had a higher transpiration rate than *in vitro* and hydroponic plants, where the temperature was maintained at 25 ºC. During acclimatization to *ex vitro* conditions, the transpiration rate usually decreases gradually because stomatal regulation of water loss becomes more effective and cuticle and epicuticular waxes develop (Pospíšilová *et al*. 1999). The net photosynthetic rate in *Solanum tuberosum* decreased in the first week after trans-plantation and increased thereafter (Baroja *et al*. 1995). An increase in the transpiration rate may enhance the uptake and translocation of mineral elements in the xylem (Marschner 1995). As APases are enzymes involved in the production, transport and recycling of Pi (Bozzo *et al*. 2002), it might be that Pi taken up by roots was not efficiently transported to the shoot in plants grown *in vitro* and in hydroponics due to the lower transpiration rate. Therefore, it can be suggested that APase activity in the shoot was higher than in the roots because Pi was more available in the latter. In addition, these data also suggest that the pool of acid phosphatases in roots and shoot is either different or that the mechanism of regulation of these enzymes is tissue specific. Zimmermann *et al*. (2004) reported a different expression of three purple acid phosphatases (PAP) from potato plants grown aeroponically either with or without Pi, where $StPAP_1$ was expressed more abundantly in root and stem than in young leaves, stolons and flowers. This gene was not responsive to P deprivation. $StPAP_2$, in contrast, responded strongly but locally to P deficiency stress and was also showed a higher expression in roots. Similar to $StPAP_2$, $StPAP_3$ was induced by P starvation, but showed a higher expression in the stem than in roots and leaves.

In our previous study (Tabaldi *et al*. 2007b), based on relative root growth of potato plants grown in a nutrient solution, SMIC148-A and *Solanum microdontum* were considered Al-tolerant clones, whereas Macaca and Dakota Rose were considered Al-sensitive. That study also showed that root H_2O_2 concentration in both Al-sensitive clones increased with increasing Al supply, whereas in Al-tolerant clones it either decreased (SMIC148-A) or demonstrated no alteration (*S. microdontum*). In addition, Al supply caused root lipid peroxidation only in the Al-sensitive clones. In the present study, it was shown that *in vitro* APase activity was affected by Al supply, but it depended on other factors such as the growth conditions, genetic background, and plant organ analyzed. Thus all of these factors must be considered in the development of protocols for the characterization of Al tolerant potato clones. On the other hand, the results of the present study also suggest that acid phosphatase activity assessed *in vitro* might not be a good parameter to validate the screening for adaptation of potato genotypes to Al toxicity.

References

Baroja, M.E., Aguirreolea, J., Sánchez-Díaz, M.: CO_2 exchange of *in vitro* and acclimatized potato plantlets. - In: Carre, F., Chagvardieff, P. (ed.): Ecophysiology and Photosynthetic *in vitro* Cultures. Pp. 187-188**.** Centre d'Études de Cadarache, Saint-Paul-lez-Durance 1995.

Bozzo, G.G., Raghothama, K.G., Plaxton, W.C.: Purification and characterization of two secreted purple acid phosphatase isozymes from phosphate-starved tomato (*Lycopersicon esculentum*) cell cultures. - Eur. J. Biochem. **269**: 6278-6286, 2002.

Bradford, M.M.: A rapid and sensitive method for the quantitation of microgram quantity of protein utilizing the principle of protein-dye binding. - Anal. Biochem. **72**: 248-254, 1976.

Delhaize, E., Ryan, P.R.: Aluminum toxicity and tolerance in plants. - Plant Physiol. **107**: 315-321, 1995.

Del Pozo, J.C., Allona, I., Rubio, V., Leyva, A., De la Peña, A., Aragoncillo, C., Paz-Ares, J.: A type 5 acid phosphatase gene from *Arabidopsis thaliana* is induced by phosphate starvation and by some other types of phosphate mobilizing/oxidative stress conditions. - Plant J. **19**: 579-589, 1999.

Dong, B., Sang, W.L., Jiang, X., Zhou, J.M., Kong, F.X., Hu, W., Wang, L.S.: Effects of aluminum on physiological metabolism and antioxidant system of wheat (*Triticum aestivum* L.). - Chemosphere **47**: 87-92, 2002.

Duff, S.M.G., Sarath, G., Plaxton, W.C.: The role of acid phosphatase in plant phosphorus metabolism. - Physiol. Plant. **90**: 791-800, 1994.

Eticha, D., Thé, C., Welcker, C., Narro, L., Staβ, A., Horst, W.J.: Aluminium-induced callose formation in root apices: inheritance and selection trait for adaptation of tropical maize to acid soils. - Field Crops Res. **93**: 252-263, 2005.

Gabbrielli, R., Grossi, L., Vergnano, O.: The effects of nickel, calcium and magnesium on the acid phosphatase activity of two *Alyssum* species. - New Phytol. **111**: 631-636, 1989.

Goodwin, S.B., Sutter, T.R.: Microarray analysis of *Arabidopsis* genome response to aluminum stress. - Biol. Plant. **53**: 85-99, 2009.

Hairiah, K., Noordwijk, J., Setijono, S.: Tolerance and avoidance of Al toxicity by *Mucuna pruriens* var. *utilis* at different levels of P supply. - Plant Soil **171**: 77-81, 1995.

Hazarika, B.N.: Acclimatization of tissue-cultured plants. - Curr. Sci. **85**: 1704-1712, 2003.

Horst, W.J., Schmohl, N., Kollmeier, M., Baluška, F., Sivaguru, M.: Does aluminium affect root growth of maize through interaction with the cell wall - plasma membrane - cytoskeleton continuum? - Plant Soil **215**: 163-174, 1999.

Kochian, L.V., Hoekenga, O.A., Piñeros, M.A.: How do crop plants tolerate acid soils? Mechanisms of aluminum tolerance and phosphorous efficiency. - Annu. Rev. Plant Biol. **55**: 459-493, 2004.

Liu, Q., Yang, J.L., He, L.S., Li, Y.Y., Zheng, S.J.: Effect of aluminum on cell wall, plasma membrane, antioxidants and root elongation in triticale. - Biol. Plant. **52**: 87-92, 2008.

Luhová, L., Lebeda, A., Kutrová, E., Hederervá, D., Peč, P.: Peroxidase, catalase, amine oxidase and acid phosphatase activities in *Pisum sativum* during infection with *Fusarium oxysporum* and *F. solani*. - Biol. Plant. **50**: 675-682, 2006.

Marschner, H. (ed.): Mineral Nutrition of Higher Plants. - Academic Press, London 1995.

Murashige, T., Skoog, F.: A revised medium for rapid growth and bioassays with tobacco tissue cultures. - Physiol. Plant. **15**: 473-497, 1962.

Pereira, L.B., Tabaldi, L.A., Gonçalves, J.F., Jucoski, G.O., Pauletto, M.M., Weis, S.N., Nicoloso, F.T., Borher, D., Rocha, J.B.T., Schetinger, M.R.C.: Effect of aluminum on δ-aminolevulinic acid dehydratase (ALA-D) and the development of cucumber (*Cucumis sativus*). - Environ. exp. Bot. **57**: 106-115, 2006.

Pospíšilová, J., Tichá, I., Kadleček, P., Haizel, D., Plzáková, Š.: Acclimatization of micropropagated plants to *ex vitro* conditions. - Biol. Plant. **42**: 481-497, 1999.

Rai, L.C., Husaini, Y., Mallick, N.: pH-altered interaction of aluminum and fluoride on nutrient uptake, photosynthesis and other variables of *Chlorella vulgaris*. - Aquat. Toxicol. **42**: 67-84, 1998.

Shamsi, I.H., Wei, K., Zhang, G.P., Jilani, G.H., Hassan, M.J.: Interactive effects of cadmium and aluminum on growth and antioxidative enzymes in soybean. - Biol. Plant. **52**: 165-169, 2008.

Tabaldi, L.A., Cargnelutti, D., Gonçalves, J.F., Pereira, L.B., Castro, G.Y., Maldaner, J., Rauber, R., Rossato, L.V., Bisognin, D.A., Schetinger, M.R.C., Nicoloso, F.T.: Oxidative stress is an early symptom triggered by aluminum in Al-sensitive potato plantlets. - Chemosphere **76**: 1402-1409, 2009b.

Tabaldi, L.A., Castro, G.Y, Cargnelutti, D., Skrebsky, E.C., Gonçalves, J.F., Rauber, R., Rossato, L.V., Schetinger, M.R.C., Bisognin, D.A., Nicoloso, F.T.: Micronutrient concentration in potato clones with distinct physiological sensitivity to Al stress. - Cienc. Rural **39**: 379-385, 2009a.

Tabaldi, L.A., Nicoloso, F.T., Castro, G.Y, Cargnelutti, D., Gonçalves, J.F., Rauber, R., Skrebsky, E.C., Schetinger, M.R.C., Morsch, V.M., Bisognin, D.A.: Physiological and oxidative stress responses of four potato clones to aluminum in nutrient solution. - Braz. J. Plant Physiol. **19**: 211-222, 2007b.

Tabaldi, L.A., Ruppenthal, R., Cargnelutti, D., Morsch, V.M., Pereira, L.B., Schetinger, M.R.C.: Effects of metal elements on acid phosphatase activity in cucumber (*Cucumis sativus* L.) seedlings. - Environ. exp. Bot. **59**: 43-48, 2007a.

Tejera Garcia, N.A., Oliveira, M., Iribarne, C., Lluch, C.: Partial purification and characterization of a non-specific acid phosphatase in leaves and root nodules of *Phaseolus vulgaris*. - Plant Physiol. Biochem. **42**: 585-591, 2004.

Yoneyama, T., Taira, M., Suzuki, T., Nakamura, M., Niwa, K., Watanabe, T., Ohyama, T.: Expression and characterization of a recombinant unique acid phosphatase from kidney bean hypocotyl exhibiting chloroperoxidase activity in the yeast *Pichia pastoris*. - Prot. Expression Purification **53**: 31-39, 2007.

Yu, M., Shen, R., Xiao, H., Xu, M., Wang, H., Wang, H, Zeng, Q., Bian, J.: Boron alleviates aluminum toxicity in pea (*Pisum sativum*). - Plant Soil **314**: 87-98, 2009.

Zheng, S.J., Yang, J.L.: Target sites of aluminum phytotoxicity. - Biol. Plant. **49**: 321-331, 2005**.**

Zimmermann, P., Regierer, B., Kossmann, J., Frossard, E., Amrhein, N., Bucher, M.: Differential expression of three purple acid phosphatases from potato. **-** Plant Biol. **6**: 519-528, 2004.