PROTECTIVE EFFECT OF DIVALENT CATIONS AGAINST ALUMINUM TOXICITY IN SOYBEAN[1]

Ivo Ribeiro da Silva[2], Tarcísio Fernando Côrtes Corrêa[3], Roberto Ferreira Novais[2], Fabrício de Oliveira Gebrim[4], Flancer Novais Nunes[4], Eulene Francisco da Silva[4] & Thomas Jot Smyth[5]

SUMMARY

A large proportion of soybean fields in Brazil are currently cultivated in the Cerrado region, where the area planted with this crop is growing considerably every year. Soybean cultivation in acid soils is also increasing worldwide. Since the levels of toxic aluminum (Al) in these acid soils is usually high it is important to understand how cations can reduce Al rhizotoxicity in soybean. In the present study we evaluated the ameliorative effect of nine divalent cations (Ca, Mg, Mn, Sr, Sn, Cu, Zn, Co and Ba) in solution culture on Al rhizotoxicity in soybean. The growth benefit of Ca and Mg to plants in an acid Inceptisol was also evaluated. In this experiment soil exchangeable Ca:Mg ratios were adjusted to reach 10 and 60 % base saturation, controlled by different amounts of $CaCl_2$ or $MgCl_2$ (at proportions from 100:0 up to 0:100), without altering the soil pH level. The low (10 %) and adequate (60 %) base saturation were used to examine how plant roots respond to Al at distinct (Ca + Mg)/Al ratios, as if they were growing in soils with distinct acidity levels. Negative and positive control treatments consisted of absence (under native soil *or* undisturbed conditions) or presence of lime ($CaCO_3$) to reach 10 and 60 % base saturation, respectively. It was observed that in the absence of Aluminum, Cu, Zn, Co and Sn were toxic even at a low concentration (25 µmol L^{-1}), while the effect of Mn, Ba, Sr and Mg was positive or absent on soybean root elongation when used in concentrations up to 100 µmol L^{-1}. At a level of 10 µmol L^{-1} Al, root growth was only reverted to the level of control plants by the Mg treatment. Higher Tin doses led to a small alleviation of Al rhizotoxicity, while the other cations reduced root growth or had no effect. This is an indication that the Mg effect is ion-specific and not associated to an electrostatic protection mechanism only, since all ions were divalent and used at low concentrations. An increased exchangeable Ca:Mg

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(2) Professor do Departamento de Solos, Universidade Federal de Viçosa – UFV. Av. PH Rolfs s/n, CEP 36570-000 Viçosa (MG). E-mails: ivosilva@ufv.br

(3) Graduando em Agronomia, Departamento de Solos, UFV. Bolsista do CNPq.

(4) Doutorando em Solos e Nutrição de Plantas, Departamento de Solos, UFV.

(5) Soil Science Department, North Carolina State University. Raleigh, NC, USA.

ratio (at constant soil pH) in the acid soil almost doubled the soybean shoot and root dry matter even though treatments did not modify soil pH and exchangeable Al^{3+}. This indicates a more efficient alleviation of Al toxicity by Mg^{2+} than by Ca^{2+}. The reason for the positive response to Mg^{2+} was not the supply of a deficient nutrient because $CaCO_3$ increased soybean growth by increasing soil pH without inducing Mg^{2+} deficiency. Both in hydroponics and acid soil, the reduction in Al toxicity was accompanied by a lower Al accumulation in plant tissue, suggesting a competitive cation absorption and/or exclusion of Al from plant tissue stimulated by an Mg-induced physiological mechanism.

Index terms: heavy metals, cation amelioration, soil acidity, Ca:Mg ratio

RESUMO: *EFEITO PROTETOR DE CÁTIONS DIVALENTES CONTRA A TOXIDEZ DE ALUMÍNIO EM SOJA*

Uma grande parte da soja no Brazil é cultivada atualmente na região do Cerrado, e é nessa região que ocorre a maior expansão da cultura. Nessa região, a maioria dos solos é ácida e, geralmente, apresenta níveis elevados de alumínio (Al) tóxico. Portanto, é importante compreender como determinados cátions reduzem a rizotoxidez de Al em plantas de soja. Neste estudo foram avaliados, em solução nutritiva, o efeito protetor de nove cátions divalentes (Ca, Mg, Mn, Sr, Sn, Cu, Zn, Co and Ba) contra a rizotoxidez de Al e o efeito positivo do Ca e Mg no crescimento das plantas de soja num solo com elevada saturação por Al. A relação entre Ca e Mg trocáveis foi variada por meio de adição de diferentes quantidades de $CaCl_2$ ou $MgCl_2$ (com as proporções variando entre 100:0 até 0:100), sem que ocorresse alteração no pH do solo. Tratamentos testemunhas consistiram de solo sem aplicação ou com aplicação de calcário ($CaCO_3$). Foi observado que, na ausência do Al, os cátions Cu, Zn, Co e Sn foram tóxicos mesmo em baixas concentrações (25 $\mu mol\ L^{-1}$), enquanto Mn, Ba, Sr e Mg em concentrações até 100 $\mu mol\ L^{-1}$ apresentaram nenhum efeito ou efeito positivo no elongamento radicular da soja. Com 10 $\mu mol\ L^{-1}$ de Al, apenas no tratamento com Mg as raízes apresentaram recuperação no elongamento para o mesmo patamar que o tratamento controle sem Al. O Sn em doses elevadas proporcionou uma pequena melhora na rizotoxidez de Al, enquanto outros cátions divalentes tiveram nenhum efeito ou reduziram o crescimento radicular. Isso é uma indicação de que o efeito do Mg é específico e não está apenas associado a uma proteção por meio de efeito eletrostático, porque todos os outros cátions usados eram divalentes e foram fornecidos em baixas concentrações. No experimento com solo ácido, observou-se que a redução da relação Ca:Mg trocáveis praticamente dobrou o acúmulo de matéria seca da parte aérea e das raízes, embora os tratamentos não tenham modificados o pH do solo e o Al^{3+} trocável, suportando o maior efeito protetor do Mg contra o Al^{3+} em relação ao Ca. A resposta positiva ao Mg não foi devida ao suprimento do nutriente que se encontrava deficiente, pois a aplicação de $CaCO_3$ melhorou o crescimento radicular por meio do incremento do pH (e precipitação do Al^{3+}) sem induzir deficiência de Mg. Tanto em condições hidropônicas quanto no solo ácido, a redução na toxidez do Al^{3+} foi acompanhada por menor acúmulo de Al no tecido vegetal, sugerindo a ocorrência de inibição competitiva na absorção, e, ou , exclusão do Al na rizosfera em resposta a um mecanismo fisiológico promovido pelo Mg.

Termos de indexação: metais pesados, efeito protetor de cátions, acidez do solo, relação Ca:Mg.

INTRODUCTION

Soybean is one of the most important export crops grown in Brazil. A vast area of soybean fields is currently cultivated in the Cerrado region, and increases considerably every year. Acid soils with variable charge minerals (kaolinite and oxides) have low bases saturation (Silva et al., 2008). In this region, acidity levels of more than 50 % of the soils are high and aluminum (Al) levels in the surface and subsurface layers are toxic (Eswaran, 1997; Lopes, 1983). Grain yield of several crops was shown to be reduced by base saturation lower than 60 % and enchageable Al^{3+} greater than 0,3 $cmol_c\ dm^{-3}$ (Nicolodi et al., 2008). This makes liming a key practice of soil fertility management (Lopes, 1996). A better understanding of related issues and enhanced management will benefit this as well as other regions, since soybean cultivation in acid soils is quickly expanding worldwide.

Although there is significant variation for Al tolerance among soybean genotypes (Sartain & Kamprath, 1978; Spehar, 1994; Menosso et al., 2000; Silva et al., 2000), the species is relatively sensitive to soil acidity in comparison with other crop and tree species (Moyer-Henry et al., 2003). Several studies have reported substantial gains in growth and yield in acid soils treated with lime (Oliveira & Pavan, 1996; Martins et al., 1998; Fageria, 2001). The positive effects of liming on acid soils are numerous, and the reduction of Al toxicity is certainly one of the most beneficial. Knowledge on the amelioration of Al toxicity due to its precipitation as low solubility Al-hydroxides under higher soil pH is not new, but the effects of basic cations (mainly Ca and Mg) on the reduction of the deleterious effect of Al have almost always been overlooked. Experiments in hydroponics have clearly shown that basic cations are able to alleviate Al toxicity in soybean (Alva et al., 1986; Ferrufino et al., 2000; Silva et al., 2001a). In fact, soybean responses to liming in high Al soils with sufficient Ca and Mg availability are usually low or insignificant (Caires et al., 1998, 2003). Conversely, substantial responses to liming have been observed in soils with high Al and lower cation availability (Martins et al., 1998; Fageria, 2001).

Previous research has shown that the ability of cations to reduce Al^{3+} toxicity, except for the small cation H^+, is directly related to their valences in the order: $H^+ > C^{3+} > C^{2+} > C^{1+}$ (Kinraide & Parker, 1987; Grauer & Horst, 1992; Kinraide et al., 1992, 1994a; Kinraide, 1998). However, it seems that the amelioration of Al toxicity by cations is not only a matter of a competitive effect with Al by absorption sites at the root surface because cations of a same valence may have a distinct, species-dependent ameliorative effect. For example, it was observed that Ca and Mg confer a similar protection against Al toxicity in wheat (Kinraide, 1998), but there are results in hydroponics indicating that Mg is more efficient than Ca in soybean (Silva et al., 2001a,b). Under field conditions it was found that Mg has a fundamental function against Al toxicity because the response of soybean yield to liming increases as a consequence of greater availability and Mg uptake (Caires et al., 2001). It is currently not known whether other divalent cations have a similar ameliorative effect as Mg^{2+} and whether Mg^{2+} is equally protective in soil as in hydroponic conditions. This study investigates the protective role of physiologically relevant concentrations of several divalent cations against Al^{3+} rhizotoxicity in soybean and evaluates whether the beneficial effects of Mg^{2+} can be extended from hydroponics to soil conditions.

MATERIAL AND METHODS

Experiment 1: Protective effect of divalent cations against Al rhizotoxicity in solution culture

Soybean seeds of the cultivars UFV-16 (Al-tolerant) and Confiança (Al-sensitive) were germinated on 0.01 mmol L^{-1} $CaSO_4$ - soaked germination paper at 25 °C for 72 h. Six to eight uniform seedlings of each cultivar were transferred to plastic trays containing 10 L of aerated solution culture with 0.5 mmol L^{-1} $CaCl_2$. The solution pH had previously been adjusted to 4.5 with 0.1 mmol L^{-1} H_2SO_4, to avoid Al precipitation.

Treatments were analyzed in a 8 x 2 factorial design of eight divalent cations (Mg^{2+}, Zn^{2+}, Cu^{2+}, Mn^{2+}, Sr^{2+}, Sn^{2+}, Co^{2+} and Ba^{2+}), either in the absence or presence of 10 μmol L^{-1} Al. Due to the large number of experimental units, the experiment was run independently for the two soybean cultivars. The divalent cations were applied at 0, 25, 50 and 100 μmol L^{-1}. Background Ca levels were varied from 0.4 to 0.5 mmol L^{-1} depending on the concentration of other divalent cations added, in order to maintain a constant ionic strength. The solution pH was adjusted daily to 4.5 by slowly adding 0.1 mol L^{-1} KOH or HCl as required, under continuous stirring. The plants were grown in a greenhouse at 27 ± 5 °C. After 16–18 h acclimation to the basal $CaCl_2$ solution, the primary root length was measured and treatments initiated. Root length was measured again 90 h after treatment initiation. The experiments were arranged in a randomized block design, with three replicates of six seedlings each.

At harvest, roots and shoots were separated, dried for 72 h (70 °C) in a forced draft oven, weighed, ground in a Wiley mill with a 1 mm stainless steel sieve and wet-digested with a nitro-perchloric mixture (3:1) according to Sarruge & Haag (1974). After appropriate dilutions the element concentration was determined by ICP-AES.

Parallel experiments were run in hydroponics to evaluate the effect of Ca and Mg on root growth in more detail, under similar conditions as described above. Additional information on treatments is presented in the section Results and Discussion.

Experiment 2: Effect of varying Ca:Mg ratio on soybean growth in an acid soil

In order to test the effectiveness of Ca and Mg as Al toxicity barrier in soil, soybean growth was evaluated in soil samples of the 0-20 cm layer of an Al-toxic sandy-loam Inceptisol with varying Ca and Mg ratios. After air-drying, sub-samples were taken for chemical and physical soil analysis. The soil consists of 18 % clay, 11 % silt, 36 % fine sand and 35 % coarse sand. Under native conditions the soil presented ($cmol_c$ kg^{-1}): 0.10 Ca, 0.04 Mg, and 1.00 Al. Except for the nutrients involved in the treatments, all macro and micronutrients were supplied in amounts to ensure optimal growth. Fourteen treatments were arranged in a 7 x 2 factorial design of seven Ca and, or Mg amendments, at two soil base saturations (10 and 60 %). The seven Ca and Mg treatments consisted of: (1) control with all nutrients (without Ca and Mg). All nutrients except Ca and Mg were supplied in doses and sources according to Novais et al. (1991); (2) soil limed with $CaCO_3$ to reach 60 % base saturation (3.8 t ha^{-1}); (3) Ca + Mg equivalent to Ca present in the $CaCO_3$ applied in treatment 1, at 100:0 Ca:Mg; (4) Ca + Mg equivalent to Ca present in the $CaCO_3$ applied in treatment 1, at 75:25 Ca:Mg; (5) Ca + Mg equivalent to Ca present in the $CaCO_3$ applied in treatment 1, at 50:50 Ca:Mg; (6) Ca + Mg equivalent to Ca present in the $CaCO_3$ applied in treatment 1, at 25:75 Ca:Mg, and (7) Ca + Mg equivalent to Ca present in the $CaCO_3$ applied in treatment 1, at 00:100 Ca:Mg. In treatments 3 to 7 and 8 to 14 Ca and Mg were supplied as chloride salts to avoid changes in soil pH, Al precipitation and Al complexation, thus minimizing the chance for confounding effects. Treatments 8 to 14 were the same as described above, except that a lower lime dose (10 % base saturation) was used. The low (10 %) and adequate (60 %) base saturation were created to examine how plant roots would respond to Al at distinct (Ca + Mg)/Al ratios, as if they were growing in soils with distinct acidity limitations. After treatment application, the soil moisture was adjusted to field capacity and incubated for two weeks in a greenhouse. Thereafter, it was air-dried, weighed (0.8 kg) and transferred to 1 L plastic pots. Six seeds of soybean cv. UFVS-2001 were sown in each pot and three days after germination, seedlings were thinned to the three most regular in size. Pots were watered twice a day with deionized water in order to maintain moisture at around 80 % of field capacity. Two weeks days after germination the plants (vegetative stage between V_2 and V_3) were removed from pots, separated in shoot and roots, dried at 70 °C and then weighed to the fourth decimal place. The material was wet-digested in a nitro-perchloric acid mixture and the Ca, Mg and Al concentrations were measured in the extracts as described above.

RESULTS AND DISCUSSION

Protective effect of Mg and other divalent cations against Al rhizotoxicity

In the first part of this study the effect of several divalent cations on the elongation of soybean roots was evaluated in the absence and presence of Al^{3+} in solution. The differences among divalent cations regarding their effects on root elongation were marked, statistically significant ($p < 0.05$) (Figures 1 and 2). In control solutions without Al^{3+}, cations such as Cu^{2+},

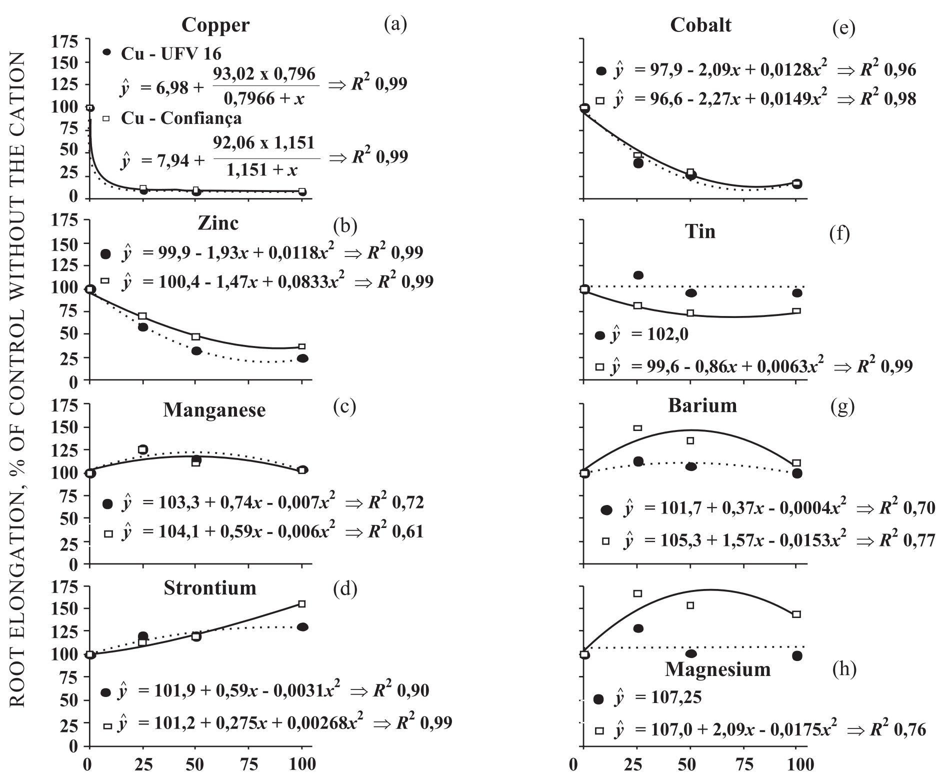

Figure 1. Relative root elongation of soybean cultivars UFV-16 and Confiança as a function of increasing doses of divalent cations in a $CaCl_2$ 500 mmol L^{-1} (pH 4.5) basal solution, in the absence of Al. Error bars represent standard error (n = 3).

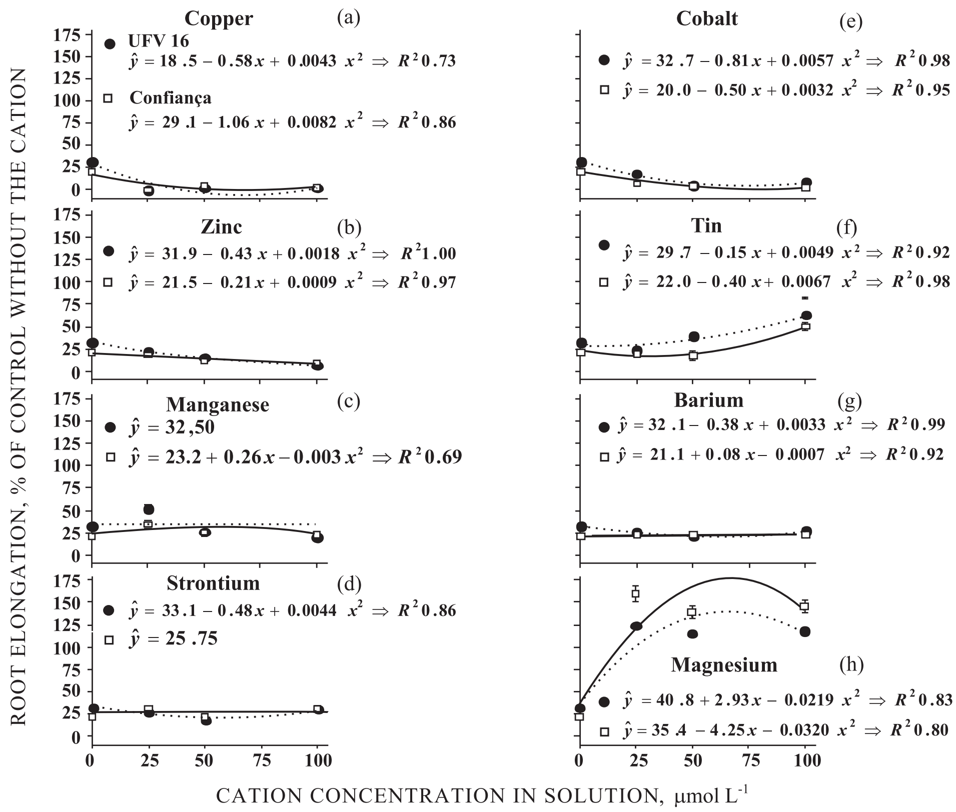

Figure 2. Relative root elongation of soybean cultivars UFV-16 and Confiança as a function of increasing doses of divalent cations in a $CaCl_2$ 500 mmol L^{-1} (pH 4.5) basal solution, in the presence of 10 µmol L^{-1} Al. Error bars represent standard error (n = 3).

Zn^{2+} and Co^{2+} were highly toxic to both soybean cultivars, drastically reducing root elongation even at the lowest concentration (Figure 1). Previous studies have also shown that Cu^{2+} and Zn^{2+} at low doses were highly toxic to plant roots (Kinraide et al., 2004; Pedler et al., 2004). Tin was slightly toxic to cv. Confiança only. Although Cu^{2+} and Zn^{2+} are essential elements for plant growth, they are required at low concentrations (less than 10 µmol L^{-1}) in solution, while excess supply leads to toxicity, with a consequent reduction of plant growth. Cobalt is only considered a beneficial (non-essential) element for plants, but is essential for N_2-fixing bacteria (Marschner, 1995). The effect on root elongation of the other cations (Mn^{2+}, Ba^{2+}, Sr^{2+} and Mg^{2+}), mainly the last three, was positive ($p < 0.05$). The positive effect of these cations is possibly related to alleviation of H^+ toxicity (Kinraide, 1991) at low pH levels, as used here, rather than a response to nutrient deficiency in the growth medium. The beneficial effects of Ba^{2+} and Sr^{2+}, which are non-essential elements for plants, seem to support this hypothesis.

The two genotypes differed ($p < 0.05$) in response to solution Al. In solutions containing 10 µmol L^{-1} Al (in 500 µmol L^{-1} $CaCl_2$ background) and with addition of no other divalent cation, root growth of cv. Confiança was reduced by approximately 75 %, and around 55 % in cv. UFV-16. This differential Al tolerance of soybean genotypes has been reported previously (Sartain & Kamprath, 1978; Spehar, 1994; Menosso et al., 2000; Silva et al., 2000) and is related to the ability of Al-tolerant plants to produce and secrete organic acids, particularly citrate, in the presence of Al (Yang et al., 2000; Menosso et al., 2001; Silva et al., 2001a).

The addition of increasing amounts of Mn^{2+}, Sr^{2+} and Ba^{2+} led to no substantial improvement in root elongation of both soybean cultivars. Cobalt, Zn^{2+}, Cu^{2+} and Sn^{2+} even aggravated the Al rhizotoxicity (Figure 2), probably as a result of their intrinsic toxicity in the absence of Al^{3+} (Figure 1). In contrast, the toxic effect of Al on root growth of both soybean cvs. was eliminated with the addition of as little as 25 µmol L^{-1} Mg^{2+} (Figure 2). The improvement in root elongation by Mg was paralleled by a reduced Al concentration in root tissue (Figure 3h). This was not restricted to Mg since increasing doses of all cations led to a reduced Al concentration in roots, with exception of Sr^{2+} and Ca^{2+}. The former reduced Al accumulation up to a dose of 50 µmol L^{-1} and the latter was not very effective in reducing Al accumulation in the root (Figure 3).

The high Ca^{2+} demand in the growth medium to maintain normal root elongation (Kinraide, 1998;

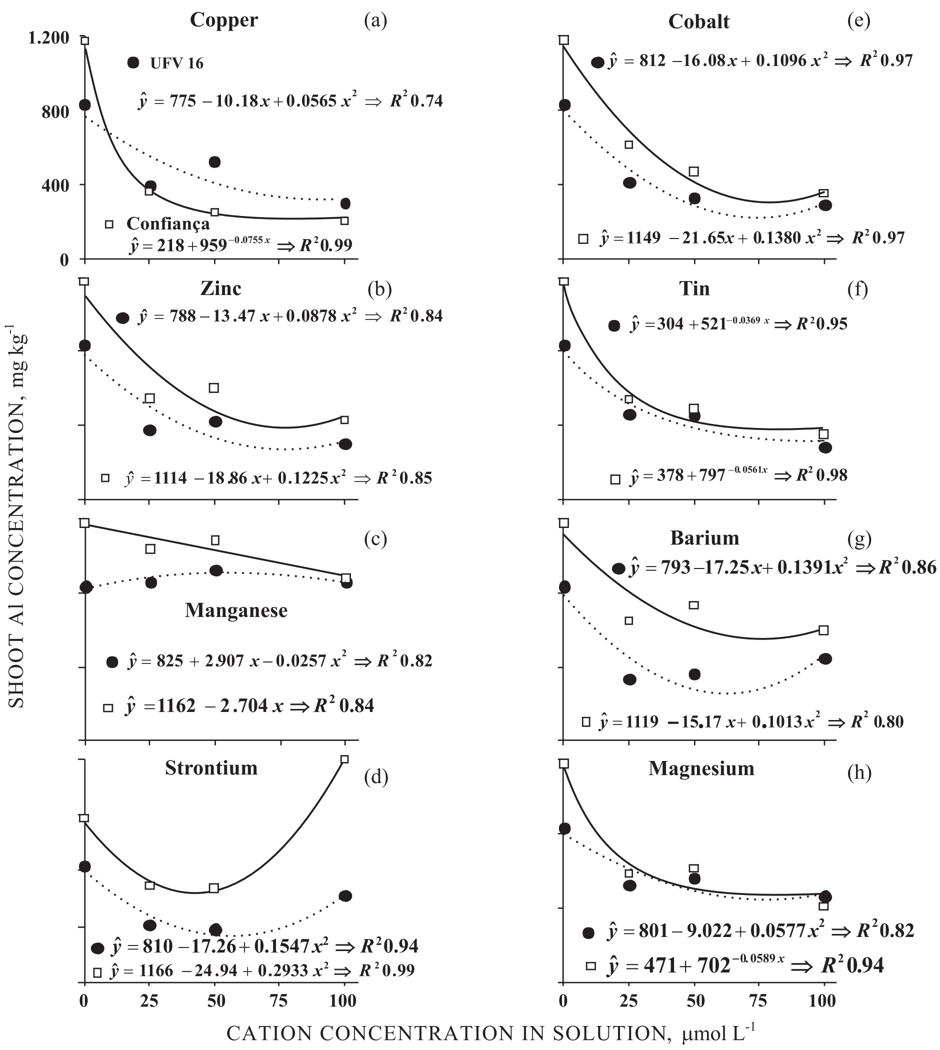

Figure 3. Aluminum concentration in roots of soybean cvs. UFV-16 and Confiança as a function of increasing doses of divalent cations in a $CaCl_2$ 500 mmol L^{-1} (pH 4.5) basal solution, in the presence of 10 µmol L^{-1} Al. Error bars represent standard error (n = 3).

Sanzonowicz et al., 1998; Silva et al., 2001b) negates a separate analysis of the beneficial effects of supplying a deficient nutrient from others related to the alleviation of Al damage. In the solution without Al, the absence of Ca^{2+} (deionized water, pH 4.5) completely inhibited root elongation, which reached normal growth only when at least 250 µmol L^{-1} Ca^{2+} was present in the solution (Figure 4a). When Ca^{2+} was supplied at a non-limiting concentration (> 250 µmol L^{-1}; pH 4.5), the presence of 10 µmol L^{-1} Al limited root elongation by more than 50 % (Figure 4a), which confirms the lower protective effect of Ca^{2+} (Figure 4a) in comparison to Mg^{2+} (Figure 5). In fact, only 500 µmol L^{-1} Ca^{2+} was sufficient to completely alleviate the deleterious effect of 10 µmol L^{-1} Al on soybean root growth with 25 µmol L^{-1} Mg^{2+} in the solution.

It has been shown that strontium is able to substitute for Ca in the dimerization of pectic compounds of the cell wall *in vitro* (O´Neill et al., 1996). The possibility of using Sr^{2+} in substitution of Ca^{2+} in solution would allow a more accurate quantification of the ameliorative effect of Ca on Al rhizotoxicity. However, Sr^{2+} alone was unable to substitute Ca^{2+}, leading to restricted root elongation even in Al-free solution (Figure 4b). The protective effect of cations at low concentrations (micromolar range) against Al rhizotoxicity in soybean therefore seems to be somewhat ion-specific. The mitigating effect of Mg^{2+} is not due do an increase in the ionic strength, which was maintained constant in all treatments. Moreover, since all cations evaluated were divalent, it is unlikely that the ameliorative effect of Mg^{2+} was associated to Al displacement from the root cell surface. The low cation concentrations employed do not support the explanation of a protective action based on the increase of the electrical potential of root cell plasma membrane either (Kinraide et al.,

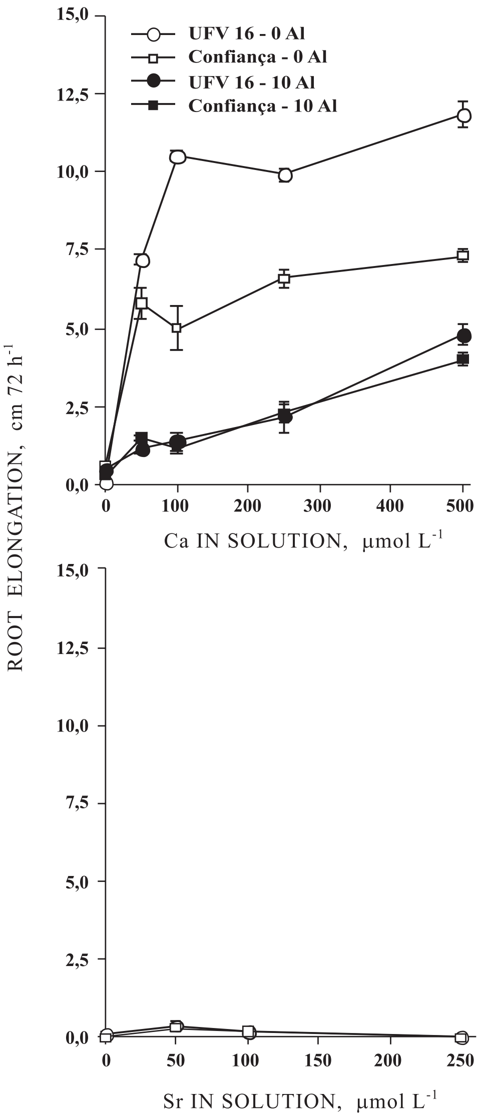

Figure 4. Root elongation of soybean cultivars UFV-16 and Confiança as a function of increasing Ca (a) or Sr (b) in solution (pH 4.5), in the absence or presence of 10 µmol L^{-1} Al. Error bars represent standard error (n = 3).

1992; Kinraide, 1994b, 1998). In wheat, Pedler et al. (2004) found that low Mg^{2+} doses were able to completely alleviate Zn^{2+} rhizotoxicity, while Ca amelioration, even at much higher doses, was much weaker. The nature of this protective role of Mg^{2+} is not quite clear at the moment, but the high effectiveness of low Mg^{2+} concentrations indicates the involvement of a biochemical/physiological mechanism. One possibility could be the stimulatory function by Mg^{2+} on the biosynthesis and citric acid secretion by soybean root tips in the presence of Al (Silva et al., 2001c). Since citric acid is a potent Al chelator, it could well be involved in Al detoxification in the rhizosphere or within root tip cells (Ma et al., 2001; Ryan et al., 2001). Due to the ionic radii similarity, an Al uptake blockage due to competitive transport (Macdiarmid & Gardner, 1996) can not be completely ruled out.

Calcium and magnesium effects on soybean growth in acid soil

We further tested the protective effect of Ca and Mg by growing soybean on an acid, Al-toxic soil under variable Ca^{2+} and Mg^{2+} availability. For the purpose of comparison, the treatments liming (plus all nutrients but Ca^{2+} and Mg^{2+}) and control (no liming plus all nutrients, except Ca^{2+} and Mg^{2+}) were established (Table 1). A decreasing exchangeable Ca:Mg ratio in the soil from 26 to 0.17 (Table 1) almost doubled soybean shoot and root dry matter f (Figure 6). A similar trend was observed for the treatment with smaller amounts of Ca and Mg application (10 % base saturation). Interestingly, these treatments did not substantially modify the soil pH and exchangeable Al^{3+}, thus indicating a more efficient alleviation of Al toxicity by Mg^{2+} than by Ca^{2+}. These results also show that the existing exchangeable Ca^{2+} in the soil was not limiting, otherwise no response to Mg^{2+} would have been observed. The positive response to Mg^{2+} was not due to a supply of a deficient nutrient because $CaCO_3$ increased soybean growth by increasing soil pH and precipitating toxic Al without inducing Mg^{2+} deficiency, as observed for other acid, Mg-deficient soils (Tan et al., 1992).

A low lime dose (10 % saturation) was sufficient to raise soil pH from 4.4 to 5.1 and improved shoot growth. Nevertheless, root growth was still limited under such conditions (Figure 6a). It appears that the remaining 0.6 $cmol_c$ dm^{-3} Al^{3+} in the soil was

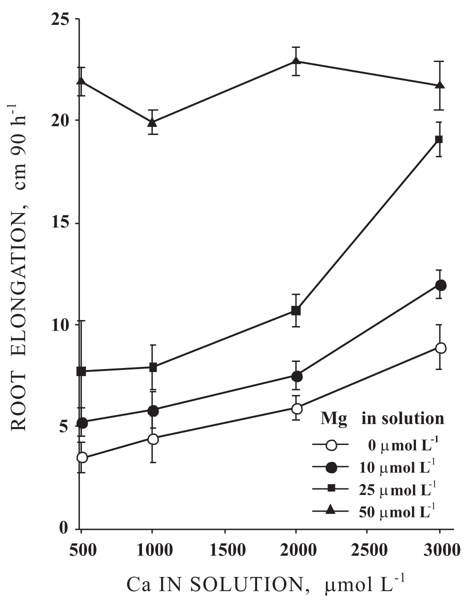

Figure 5. Relative root elongation of soybean cultivar Confiança as a function of increasing Ca and Mg in solution (pH 4.5), in the presence of 10 µmol L^{-1} Al. Error bars represent standard error (n = 3).

Table 1. Selected chemical characteristics of the soil after establishing the treatments

Treatment			pH	Ca^{2+}	Mg^{2+}	Al^{3+}	$Ca^{2+}:Mg^{2+}$ ratio
				$cmol_c\ dm^{-3}$			
				Low lime dose (10 % base saturation)			
1		100:0	4.6	0.40	0.05	1.0	8.00
2		75:25	4.8	0.30	0.10	1.0	3.00
3	Ca:Mg ratio	50:50	4.5	0.30	0.20	0.9	1.50
4		25:75	4.7	0.20	0.20	0.9	1.00
5		0:100	4.7	0.10	0.30	0.9	0.33
6	$CaCO_3$		5.1	0.50	0.05	0.6	10.00
				Recommended lime dose (60 % base saturation)			
		100:0	4.2	1.30	0.05	0.9	26.00
7		75:25	4.3	1.10	0.40	0.9	2.75
8	Ca:Mg ratio	50:50	4.4	0.90	0.80	1.0	1.13
9		25:75	4.4	0.50	1.10	0.9	0.45
10		0:100	4.3	0.20	1.20	0.9	0.17
11	$CaCO_3$		5.7	2.60	0.05	0.0	52.00
12	Control (no liming)		4.4	0.1 0	0.05	0.9	2.0 0

pH in H_2O (2.5:1). Al, Ca and Mg in the 1 mol L^{-1} KCl extract.

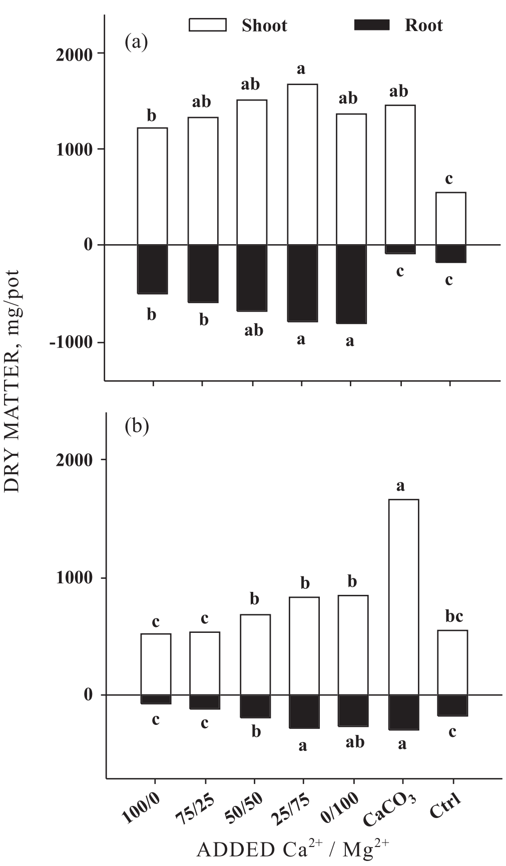

Figure 6. Dry matter production by soybean cv. UFVS-2001 grown in an acid soil with variable Ca:Mg ratios. (a) 10 % base saturation; (b) 60 % base saturation. Lime treatment received $CaCO_3$ and all other nutrients, except Mg. Control (Ctrl) treatment received no liming, but the supply of all other nutrients, except Ca and Mg, was ensured. Treatment means followed by the same letter are not significantly different by Tukey´s test (α = 0.05).

enough to restrict root growth. In fact, Al^{3+} concentration above 0,3 $cmol_c\ dm^{-3}$ results in lower grain yield for several crops, including soybean (Nicolodi et al., 2008). Contrastingly, root elongation was increased by several orders of magnitude when Mg was added (Figure 6A), even though 0.9 $cmol_c\ dm^{-3}$ Al^{3+} was still present (Table 1). The better root development at 10 % than that at 60 % base saturation in treatments with chloride salts may be due to chloride toxicity since the soil was a sandy loam and soybean is known to be chloride-sensitive (Pantalone et al., 1997).

The results also show that soybean plants grow well in a wide range of Ca:Mg ratios, as long as Al^{3+} is absent. This is supported by the fact that plant growth was maximized in limed soil (60 % base saturation) at a Ca:Mg ratio of 52 (Figure 6), whereas at a lower Ca:Mg ratio of 8 or 26 in soils of treatments 1 and 7 in which Al^{3+} was high, both shoot and root growth was reduced. It should be noted that even in the treatment with highest Ca:Mg ratio shoot growth was lower than in limed soil (Figure 6), indicating that alleviation of Al toxicity was not complete, and greater amounts of the cation may be required for full protection. Although the application of greater Ca and Mg amounts (60 % base saturation) led to higher levels of tissue Ca and Mg (compare Figure 7b,c with Figure 7e,f), the responses in shoot and root growth were not as positive as one would expect (compare Figure 6A to Figure 6B). Perhaps, growth was more limited as a result of a higher Al^{3+} concentration in the soil solution due to Ca and Mg displacement of exchangeable Al^{3+}, which in turn led to a higher Al uptake (Figure 7). Additionally, the possibility of Cl^- toxicity can not be completely ruled out.

The chemical characteristics of the soil used here parallel those of acid subsoils, namely low exchangeable Ca^{2+} and Mg^{2+} and high Al^{3+}. Hence, in acid subsoils as those in the Cerrado region of

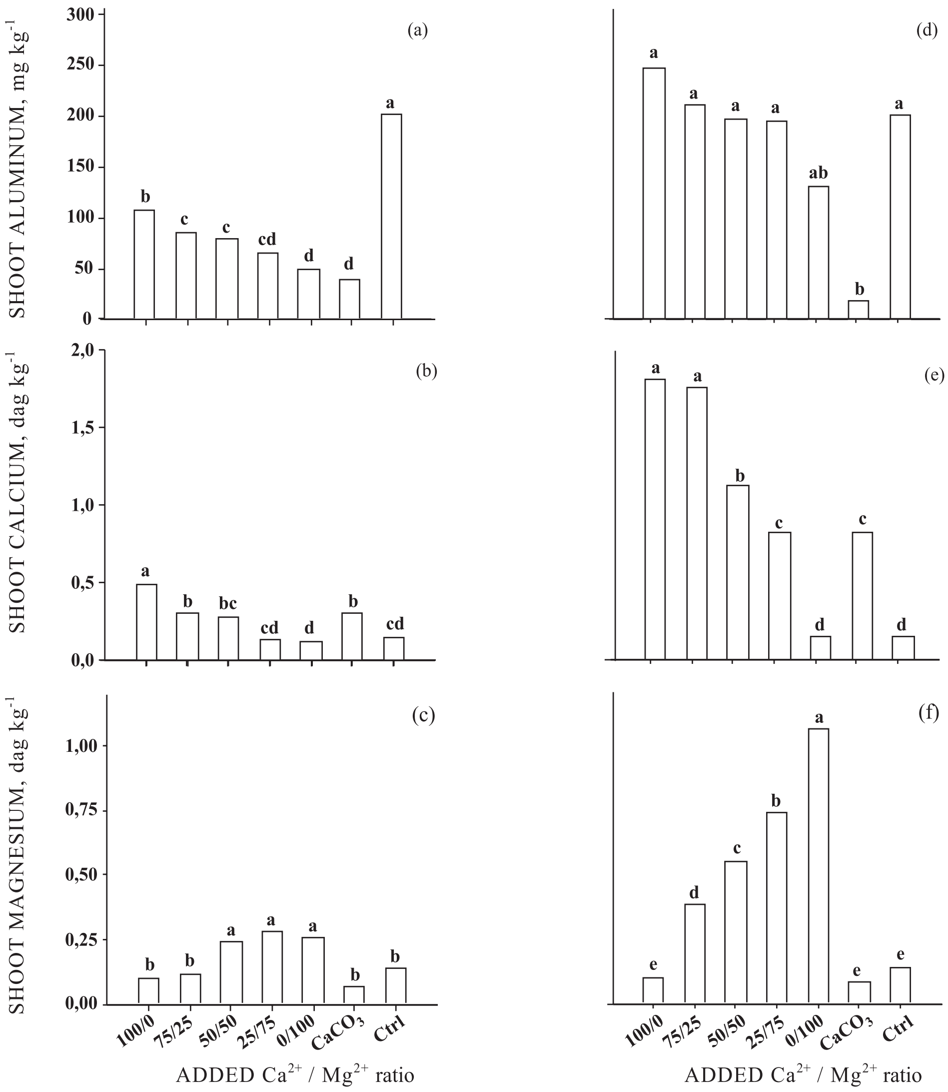

Figure 7. Shoot concentration of Al, Ca and Mg of soybean cv. UFVS-2001 grown in an acid soil with variable Ca:Mg ratios. A-C 10 % base saturation; D-F 60 % base saturation. Lime treatment received $CaCO_3$ and all other nutrients, except Mg. Control (Ctrl) treatment received no liming, but the supply of all other nutrients, except Ca and Mg, was ensured. Treatment means followed by the same letter are not significantly different by Tukey´s test (α = 0.05).

Brazil, soybean rooting depth and drought resistance could be maximized by supplying not only a mobile source of Ca^{2+} (Ritchey et al., 1980; Lopes, 1996), but probably also Mg^{2+}. Earlier studies have suggested that a similar soybean growth in acid soil could be obtained with lower lime rates if the amendment contained Mg^{2+} in addition to Ca^{2+} (Muchovej et al., 1986). Furthermore, it has been pointed out that responses to liming in high-Al^{3+}, acid soils seem to be substantial under conditions with low Mg^{2+}, even if Ca^{2+} is not limiting (Caires et al., 2003). Therefore, the importance of a Mg^{2+} source in areas where soybean is frequently used in crop rotations must not be overlooked. The observations that Ca^{2+} and Mg^{2+} from low solubility sources such as carbonate move downward in the soil profile under no-till conditions over time (Caires et al., 2000) further emphasizes the need to use dolomitic limestone, at least until Mg^{2+} levels in the (sub)soils are adequately raised.

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