

SEÇÃO IV - FERTILIDADE DO SOLO E NUTRIÇÃO DE PLANTAS

TIMING, LOCATION AND CROP SPECIES INFLUENCE THE MAGNITUDE OF AMELIORATION OF ALUMINUM TOXICITY BY MAGNESIUM⁽¹⁾

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SUMMARY

The protective effect of cations, especially Ca and Mg, against aluminum (Al) rhizotoxicity has been extensively investigated in the last decades. The mechanisms by which the process occurs are however only beginning to be elucidated. Six experiments were carried out here to characterize the protective effect of Mg application in relation to timing, location and crop specificity: Experiment 1 – Protective effect of Mg compared to Ca; Experiment 2 – Protective effect of Mg on distinct root classes of 15 soybean genotypes; Experiment 3 – Effect of timing of Mg supply on the response of soybean cvs. to Al; Experiment 4 – Investigating whether the Mg protective effect is apoplastic or simplastic using a split-root system; Experiment 5 – Protective effect of Mg supplied in solution or foliar spraying, and Experiment 6 – Protective effect of Mg on Al rhizotoxicity in other crops. It was found that the addition of 50 $\mu\text{mol L}^{-1}$ Mg to solutions containing toxic Al increased Al tolerance in 15 soybean cultivars. This caused soybean cultivars known as Al-sensitive to behave as if they were tolerant. The protective action of Mg seems to require constant Mg supply in the external medium. Supplying Mg up to 6 h after root exposition to Al was sufficient to maintain normal soybean root growth, but root growth was not recovered by Mg addition 12 h after Al treatments. Mg application to half of the root system not exposed to Al was not sufficient to prevent Al toxicity on the other half exposed to Al without Mg in rooting medium, indicating the existence of an external protection mechanism of Mg. Foliar spraying with Mg also failed to decrease Al toxicity, indicating a possible apoplastic role of Mg. The protective effect of Mg appeared to be soybean-specific since Mg supply did not substantially improve root elongation in sorghum, wheat, corn, cotton, rice, or snap bean when grown in the presence of toxic Al concentrations.

Index terms: soybean, cations, calcium, rhizotoxicity, soil acidity.

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RESUMO: ÉPOCA, LOCALIZAÇÃO E ESPÉCIE VEGETAL NA MAGNITUDE DO EFEITO PROTETOR DO MAGNÉSIO CONTRA O ALUMÍNIO TÓXICO

O efeito protetor de alguns cátions, especialmente o do Ca e do Mg, contra a rizotoxidez do alumínio (Al) tem sido investigado extensivamente nas últimas décadas. No entanto, os mecanismos envolvidos apenas começaram a ser elucidados. No presente estudo foram conduzidos seis experimentos, visando caracterizar o efeito protetor do Mg em relação à sua época de aplicação, localização, além da especificidade da cultura: Experimento 1 – efeito protetor do Mg em comparação ao Ca; Experimento 2 – efeito protetor do Mg em distintas classes de raízes de 15 genótipos de soja; Experimento 3 – efeito da época da aplicação do Mg na resposta de cultivares de soja ao Al; Experimento 4 – determinando se o efeito protetor do Mg é apoplástico ou simplástico; Experimento 5 – efeito protetor do Mg suprido via solução nutritiva ou via foliar, e; Experimento 6 – efeito protetor do Mg contra a rizotoxidez em outras culturas. Observou-se que a adição de 50 $\mu\text{mol L}^{-1}$ Mg às soluções nutritivas contendo Al aumentou a tolerância ao metal por 15 quinze cultivares de soja. Isso fez com que cultivares de soja conhecidas por serem sensíveis ao Al se comportassem como tolerantes. A ação protetora do Mg parece requerer o suprimento constante de Mg no meio de crescimento. O suprimento de Mg até seis horas após a exposição das raízes ao Al foi suficiente para manter o crescimento radicular normal da soja, porém adições de Mg depois de 12 h da aplicação do Al não foram capazes de recuperar o crescimento radicular. A suplementação do Mg à metade do sistema radicular não exposta ao Al não foi suficiente para prevenir a toxidez do Al à outra metade do sistema radicular exposta ao metal na ausência de Mg no meio de crescimento, confirmando a existência de um mecanismo externo de proteção pelo Mg. Aplicações foliares de Mg também não conseguiram reduzir a toxidez por Al, indicando uma possível função apoplástica de proteção pelo Mg. O efeito protetor do Mg pareceu ser específico para a soja porque o suprimento de Mg não resultou em melhoria substancial no crescimento radicular de plantas de sorgo, trigo, milho, algodão, arroz e feijão quando estes foram cultivados na presença de concentrações tóxicas de Al.

Termos de indexação: soja, cátions, rizotoxidez, acidez do solo.

INTRODUCTION

Several ions are known as ameliorators of Al rhizotoxicity in plants, especially Ca and Mg. Because of their abundance or reduced availability in acid soils, the protective role of Ca and Mg against Al rhizotoxicity has been extensively investigated (Gonzalez-Erico et al., 1979; Alva et al., 1986; Kinraide & Parker, 1987; Keltjens & Dijkstra, 1991; Rengel, 1992; Brady et al., 1993; Keltjens & Tan, 1993; MacDiarmid & Gardner, 1996; Ryan et al., 1997; Kinraide, 1998; Sanzonowicz et al., 1998; Ferruffino et al., 2000; Silva et al., 2001a).

Nevertheless, the mechanisms of protective action of Ca and Mg against Al rhizotoxicity are not well understood. Alleviating effects beyond the solution ionic strength have been attributed to: (a) restoration of Ca at the root cell surface above the deficient levels induced by Al^{3+} ; (b) electrostatic displacement of Al^{3+} from the root surface due to an increase in the root electrical potential (making it less negative) or direct competition, and; (c) protection of Al-sensitive organelles or intracellular reactions (Kinraide & Parker, 1987; Kinraide, 1998; Edmeades et al., 1991; Grauer & Horst, 1992; Rengel, 1992). Consistent with the above roles, Al root uptake was reduced and root

growth improved under conditions of Ca or Mg supplementation (Ryan et al., 1997; Silva et al., 2001bc).

The effectiveness of Ca and Mg to alleviate Al^{3+} rhizotoxicity in long-term experiments (2–3 weeks) seems to be plant species-dependent; Ca seems to have a greater protective effect in dicots and Mg be more efficient in monocots (Keltjens & Tan, 1993). Extensive research by Kinraide and co-authors has shown that the effect of Ca on wheat root protection against Al^{3+} damage was similar or even slightly superior to Mg (Kinraide et al., 1985; Kinraide & Parker, 1987; Kinraide et al., 1992, 1994; Kinraide, 1998; Ryan et al., 1997). However, there are other situations where Mg was reported to be more efficient than Ca (Edmeades et al., 1991; Keltjens & Dijkstra, 1991), but there may differential responses in Al-sensitive and Al-tolerant genotypes (Hecht-Buchholz & Schuster, 1987).

More recently, it was found that low concentrations of Mg (200 $\mu\text{mol L}^{-1}$) improved soybean root elongation in the presence of toxic Al^{3+} concentrations, but this protective effect was not observed for wheat roots (Silva et al., 2001a). In another study with soybean, it was observed that Mg was more efficient than Ca in reducing Al accumulation

at the root tip and improving root elongation when supplemented at micromolar concentrations. However, the two cations were equally effective in alleviating Al rhizotoxicity at millimolar concentrations (Silva et al., 2001bc). MacDiarmid & Gardner (1996) also reported that the toxic effect of Al^{3+} on *Sacharomyces cerevisiae* was mitigated by Mg supplementation, unlike similar additions of Ca to the growth medium. Furthermore, it was recently reported that the overexpression of an Arabidopsis magnesium transport gene in *Nicotiana benthamiana* increased Mg uptake in the presence of Al and conferred Al tolerance (Deng et al., 2006).

The protective effect of low Mg concentrations ($50 \mu\text{mol L}^{-1}$) against Al toxicity was shown to have some ion specificity because it could not be mimicked by equal concentrations of Mn. Additionally, this beneficial effect, unlike Ca, could not be explained based on its effects on root surface electric potential (Silva et al., 2001b). These results suggest that the protective effect of low Mg concentrations against Al is not simply due to a charge effect, but involves a more physiological mechanism. Induction of citrate exudation by roots is an important mechanism contributing to Al tolerance in soybean (Silva et al., 2001b; Shen et al., 2005). The detoxification of Al^{3+} via stimulation by Mg of biosynthesis and exudation of citrate by root tips is one possibility (Silva et al., 2001c). Whether the Mg effect is simplastic or apoplastic is still unclear. Support for a simplastic role comes from a study where Mg prior to Al supplementation to the culture solution anticipated root citrate exudation when exposed to Al (Silva et al., 2001b). However, it was not determined if this would result in additional Al plant tolerance. This study further investigated the protective role of physiologically relevant Mg concentrations against Al^{3+} rhizotoxicity in soybean and evaluated whether the beneficial effects of Mg could be extended to other important crops.

MATERIAL AND METHODS

The experiments described below were carried out under greenhouse conditions at the Soil Science Department of the Viçosa Federal University during spring and summer months. In the spring the minimum and maximum temperatures averaged, respectively, 15 and 26 °C, while in the summer the average minimum and maximum temperatures were 19 and 34 °C.

Experiment 1 – Protective effect of Mg compared to Ca

Soybean seeds of cv. Confiança (Al-sensitive) were germinated in $0.01 \text{ mmol L}^{-1} \text{ CaSO}_4$ soaked germination paper at 25 °C for 72 h. For each replication, six to eight uniform seedlings were

transferred to plastic trays containing 10 L of aerated $0.5 \text{ mmol L}^{-1} \text{ CaCl}_2$. Solution pH had been previously adjusted to 4.5 with $0.1 \text{ mmol L}^{-1} \text{ HCl}$. Treatments consisted of a combination of solutions containing 0 or $10 \mu\text{mol L}^{-1} \text{ Al}$, either in the absence or presence of $25 \mu\text{mol L}^{-1} \text{ Mg}$, combined with five Ca concentrations (0, 250, 500, 1000 and $3000 \mu\text{mol L}^{-1}$). In solutions containing Mg, Ca concentrations were $25 \mu\text{mol L}^{-1}$ lower. Solution pH was daily adjusted to 4.5 throughout the experiment by slowly adding $0.1 \text{ mol L}^{-1} \text{ KOH}$ or $0.1 \text{ mol L}^{-1} \text{ HCl}$ as needed, under continuous stirring. After 16–18 h acclimation to the basal CaCl_2 solution, six uniform seedlings were selected and maintained in each tray. The primary root length of these seedlings was measured and treatments initiated. Root length was measured again 90 h after beginning treatments. The experiment was arranged in a completely randomized block design, with three replications. The experimental unit consisted of a tray with six regular, selected seedlings.

Experiment 2 – Protective effect of magnesium on distinct root classes of 15 soybean genotypes

Seeds of 15 soybean cultivars grown together on commercial farms in the Brazilian cerrado were germinated and transferred to a $0.5 \text{ mmol L}^{-1} \text{ CaCl}_2$ basal solution (pH 4.5). The soybean cultivars used were: Monsoy 8001, Monsoy 8400, Monsoy 8411, Monsoy 9001, UFV 16, Confiança, Emgopa 315, Jataí, Emgopa 316, IAC 21, Segurança, Vencedora, Liderança, Conquista and Garantia. After the acclimation period in solution, the primary root length was measured and seedlings were transferred to treatment solutions consisting of four Al concentrations (0, 2.5, 5, 10 and $20 \mu\text{mol L}^{-1} \text{ Al}^{3+}$, either in the absence or presence of $50 \mu\text{mol L}^{-1} \text{ Mg}$. Primary root length was measured again 90 h after solution treatments were initiated. At harvest, the length of the two longest lateral roots of each plant was also measured. Experimental design and conditions were similar to experiment one.

Experiment 3 – Timing effect of Mg supply on response of soybean cvs. to Al

The experiment consisted of a $2 \times 4 \times 2$ factorial (two soybean genotypes, four Mg treatment periods, applied before and/or after Al addition (pre + post) or only before Al addition to the solution. Soybean seedlings of cvs. UFV-16 (Al-tolerant) and Confiança (Al-sensitive) were germinated and acclimated as already described. Treatments were established in $0.5 \text{ mmol L}^{-1} \text{ CaCl}_2$ basal solution (pH 4.5) and consisted of Mg supply for four different periods: 24, 12, 6, 3 and 0 h before (x axis with negative values in Figure 3) or 24, 12, 6, 3 and 0 h after (x axis with positive values in Figure 3) Al addition to the solution. Only at time “0” Mg and Al were added simultaneously. Thus, Al was present only after time “0”, but Mg remained in solution throughout the experiment. The

other treatments consisted of applying and maintaining Mg in solution only in the pre-treatment period (from 24 h to 0 h). That is, plants were pre-treated with Mg for different periods before adding Al (x axis labeled with negative values in Figure 2). On the day Al was going to be added (time "0"), the solutions were replaced by similar ones, but without Mg. That is, Mg was only present in solution as a pretreatment before Al application. It was hypothesized that plants pre-treated with Mg would have a higher Mg concentration in the tissue than those pre-cultured without Mg in solution, and this would provide information on the effect of previously taken up (internal) Mg on the Al³⁺ rhizotoxicity. The treatments in figure 2a therefore differ from those in figure 2b only in that Mg added as pre-treatment before Al application (-24 to -1) remained in solution after Al addition in 2a, while Mg added as pre-treatment (-24 to -1) was not maintained in solution after Al addition in 2b. In treatments for periods 0–24 Mg was added after Al application and thus remained in solution with Al until the end of the experiment. All treatments were established in a variable (0.45 to 0.5 mmol L⁻¹) CaCl₂ basal solution (pH 4.5). Tap root length was measured at time "0" and again 90 h later. Root elongation for the 90 h period is reported. The experiment was conducted in a greenhouse in a completely randomized block design, with three replications. Each experimental unit consisted of a tray with six uniform seedlings. Because of operational and logistic limitations, root growth evaluation and plant tissue analysis were not possible. The same experiment was therefore run in the following week to measure Al and Mg shoot and root concentrations, with the difference that the Mg pre- and post-treatment lasted 48 h instead of 24 h.

Experiment 4 – Investigating whether the Mg protective effect is apoplastic or symplastic using a split-root system

Seeds of soybean cv. UFV-16 were germinated for 72 h and uniform seedlings were cultivated for one week in Clark's nutrient solution (Clark, 1975), reduced to half-strength, then had their primary root

cut approximately one cm below the root-shoot transition to stimulate growth of lateral roots. Seedlings remained in this solution for an additional week, and were then transferred to split-pots (5 L each), placing half of the root system in each compartment. A small cotton roll wetted with deionized water was kept between the two pots just below the severed primary root in order to prevent root dehydration. Both compartments had nutrient solutions, containing the treatments (Table 1). Magnesium concentration in solution in compartment 2 was varied (0, 50, 100, 250, 500 µmol L⁻¹), either in the absence or presence of 10 µmol L⁻¹ Al in compartment 1. All other nutrients were supplied in compartment 2, where roots were grown in Clark's solution. In order to avoid undesirable elemental interaction with Al (i.e. Al-phosphate precipitation), in compartment 1 only 500 µmol L⁻¹ Ca and 0.1 mg L⁻¹ B was added. As a control treatment, Mg was added to both compartments, in the absence and presence of Al in compartment 1. Since Mg is phloem-mobile (Marschner, 1995) this experimental setting allowed an evaluation of the Mg effect supplied either separately or together with Al, so inferences on an apoplastic or symplastic protective role of Mg would be possible. The experiment was arranged in a completely randomized block design, with three replications. Each "twin-pot" contained one plant with the root system split in two halves, which represented one experimental unit.

Fifteen days after exposing plants to solution treatments, root length was measured for each compartment. Thereafter, they were rinsed three times with deionized water. Roots and shoots were separated and dried at 70 °C for 72 h, ground, wet digested with a nitro-perchloric mixture and analysed for Al, Ca and Mg by ICP-AES.

Experiment 5 – Protective effect of Mg supplied in solution or foliar spraying

In order to further test if internal Mg would result in protection against Al, Mg was supplied to plants either via solution or foliar spray. The three

Table 1. Composition of solution treatments in the split-root system set-up

Treatment	Compartment 1	Compartment 2
T1	500 µmol L ⁻¹ Ca, 0.1 mg L ⁻¹ B, 0 µmol L ⁻¹ Al	Clark's (0 µmol L ⁻¹ Mg)
T2	500 µmol L ⁻¹ Ca, 0.1 mg L ⁻¹ B, 0 µmol L ⁻¹ Al	Clark's (50 µmol L ⁻¹ Mg)
T3	500 µmol L ⁻¹ Ca, 0.1 mg L ⁻¹ B, 0 µmol L ⁻¹ Al	Clark's (100 µmol L ⁻¹ Mg)
T4	500 µmol L ⁻¹ Ca, 0.1 mg L ⁻¹ B, 0 µmol L ⁻¹ Al	Clark's (250 µmol L ⁻¹ Mg)
T5	500 µmol L ⁻¹ Ca, 0.1 mg L ⁻¹ B, 0 µmol L ⁻¹ Al	Clark's (500 µmol L ⁻¹ Mg)
T6	500 µmol L ⁻¹ Ca, 0.1 mg L ⁻¹ B, 0 µmol L ⁻¹ Al, 50 µmol L ⁻¹ Mg	Clark's (500 µmol L ⁻¹ Mg)
T7	500 µmol L ⁻¹ Ca, 0.1 mg L ⁻¹ B, 10 µmol L ⁻¹ Al	Clark's (0 µmol L ⁻¹ Mg)
T8	500 µmol L ⁻¹ Ca, 0.1 mg L ⁻¹ B, 10 µmol L ⁻¹ Al	Clark's (50 µmol L ⁻¹ Mg)
T9	500 µmol L ⁻¹ Ca, 0.1 mg L ⁻¹ B, 10 µmol L ⁻¹ Al	Clark's (100 µmol L ⁻¹ Mg)
T10	500 µmol L ⁻¹ Ca, 0.1 mg L ⁻¹ B, 10 µmol L ⁻¹ Al	Clark's (250 µmol L ⁻¹ Mg)
T11	500 µmol L ⁻¹ Ca, 0.1 mg L ⁻¹ B, 10 µmol L ⁻¹ Al	Clark's (500 µmol L ⁻¹ Mg)
T12	500 µmol L ⁻¹ Ca, 0.1 mg L ⁻¹ B, 10 µmol L ⁻¹ Al, 50 µmol L ⁻¹ Mg	Clark's (500 µmol L ⁻¹ Mg)

treatments tested were: Mg supplied via solution together with Al; leaf supplied Mg and a control that received no Mg. Plants were germinated and acclimated to nutrient solution as described above. Plants of the foliar treatment were then sprayed twice daily with a $50 \mu\text{mol L}^{-1}$ MgCl_2 solution. This leaf application of Mg began after the acclimation period and lasted for three days until the addition of Al to the solution. On the first day of Al treatments, primary root length was measured and Al added to the solution. The second group of plants was treated with $50 \mu\text{mol L}^{-1}$ Mg in the nutrient solution. Control plants were grown in a 0.5 mmol L^{-1} CaCl_2 basal solution (pH 4.5). All plants were grown in the presence of $10 \mu\text{mol L}^{-1}$ Al. The experiment was conducted in a greenhouse in a completely randomized block design, with three replications. Each experimental unit consisted of a tray with six uniform seedlings.

Experiment 6 – Protective effect of Mg on Al rhizotoxicity in other crop plants

Seeds of soybean, wheat, sorghum, cotton, rice, and common bean were germinated and acclimated to the 0.5 mmol L^{-1} CaCl_2 basal solution (pH 4.5). Crops were treated with solution treatments of six Al rates (0, 5, 10, 25, 50 and $100 \mu\text{mol L}^{-1}$), except sorghum. For this plant species the maximum Al dose was $50 \mu\text{mol L}^{-1}$. Plants were exposed to the variable Al concentrations either in the absence ($500 \mu\text{mol L}^{-1}$ Ca basal solution) or presence of $50 \mu\text{mol L}^{-1}$ Mg ($450 \mu\text{mol L}^{-1}$ Ca basal solution). The experiment was conducted in a greenhouse in a completely randomized block design, with three replications. Each experimental unit consisted of a tray with six regular seedlings.

RESULTS AND DISCUSSION

Protective effect of magnesium and calcium against Al rhizotoxicity

When Ca was supplied at a non-limiting concentration ($> 250 \mu\text{mol L}^{-1}$; pH 4.5), the presence of $10 \mu\text{mol L}^{-1}$ Al limited root elongation by more than 50 % in comparison to plants grown in the absence of Al. Not even $3.000 \mu\text{mol L}^{-1}$ Ca was sufficient to completely alleviate the toxic effect of Al. On the other hand, root growth in the presence of Al was fully recovered when $25 \mu\text{mol L}^{-1}$ Mg was present in solution also, as long as solution Ca was not limiting (Figure 1). These results confirm that the degree of Al toxicity to plants depends strongly on the type and concentration of other ions in solution, mainly Ca and Mg (Kinraide et al., 1985; Alva et al., 1986; Kinraide & Parker, 1987; Rengel, 1992; Ryan et al., 1997; Kinraide, 1998; Ferrufino et al., 2000; Silva et al., 2001a), and that in soybean the protective effect of Ca is lower than of Mg (Silva et al., 2001c).

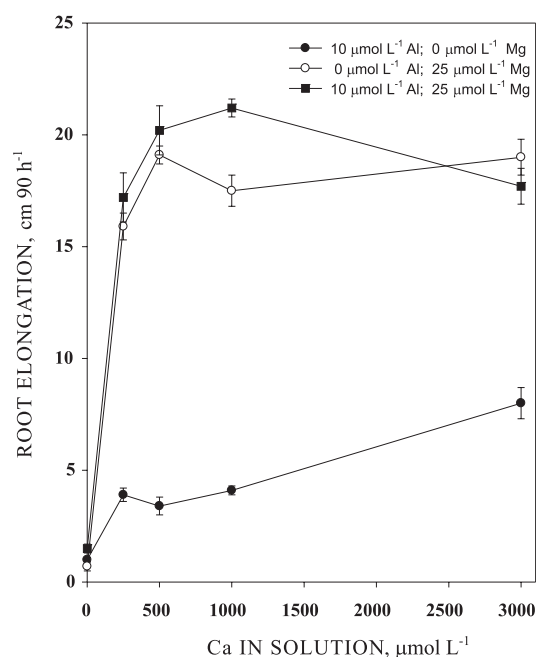


Figure 1. Primary root elongation of soybean cultivar Confiança (Al-sensitive) in the absence and presence of Al and Mg, as a function of increasing Ca in solution (pH 4.5).

Protective effect of magnesium on distinct root classes of 15 soybean genotypes

Additional studies were carried out in order to evaluate whether the beneficial effect of Mg against Al rhizotoxicity would be consistent when studies involved a larger number of soybean genotypes. Because soybean root classes differ in Al sensitivity (Sanzonowicz et al., 1998; Ferrufino et al., 2000; Silva et al., 2001b), the Mg effect on both primary and lateral root growth was monitored under conditions of variable Al rates in solution (Table 2). When 15 soybean cvs. were cultivated in the presence of increasing Al concentrations in a $500 \mu\text{mol L}^{-1}$ CaCl_2 solution (pH 4.5) without Mg addition, a substantial reduction in primary root elongation was observed, reaching up to 80 % at the highest Al dose ($20 \mu\text{mol L}^{-1}$ Al). At this relatively high Al concentration the variation in primary root elongation among soybean genotypes was only small (Table 2). However, the differences among the genotypes in relation to their ability to tolerate low to intermediate Al concentrations in solution were greater. The greater tolerance of cvs. UFV-16, Conquista, Garantia and M-Soy 8001 is noteworthy, with a respective primary root elongation of 50, 58, 58 and 50 % in a $10 \mu\text{mol L}^{-1}$ Al solution, compared to control plants growing in the absence of Al. In the cultivars IAC 21, Confiança, Liderança, and Vencedora the degree of sensitivity to Al toxicity was highest, with root elongation reduced to 17, 23, 24 and 25 % of control plants, respectively (Table 2). This differential Al tolerance could be due to the distinct abilities of roots to detoxify Al through

citrate production and secretion in response to Al (Silva et al., 1999, 2001a; Yang et al., 2000). Confirming the previously observed protective effect of Mg, when 50 mol L⁻¹ Mg were added to the basal 450 µmol L⁻¹ CaCl₂, Al inhibition of root elongation was substantially reduced for all cultivars (Table 2). The relative primary root elongation of the cvs. considered Al-tolerant (UFV-16, Conquista, Garantia and M-Soy 8001), increased, respectively, from 50, 58, 58 and 50 % in the presence of 10 µmol L⁻¹ to 93, 104, 103, and 97 % of control plants growing without Al addition. On other hand, primary root elongation of the cvs. known as Al-sensitive (IAC 21, Confiança, Liderança, and Vencedora) increased from 17, 23, 24 and 25 % to 101, 104, 113 e 109 %, respectively, in comparison with control plants (Table 2). The average

relative root elongation across all cultivars and Al concentrations was 63 % when Mg was absent. Primary root elongation virtually doubled, reaching 99 % of control plants when 50 µmol L⁻¹ Mg was added to the solution.

Lateral roots were also Al sensitive, but the differences among genotypes were less evident than for primary roots (Table 2). The addition of 50 µmol L⁻¹ Mg restored lateral root growth from an average of 54 to 94 %. Therefore, Mg was also important to improve growth of branched roots of all soybean genotypes under Al stress conditions and supports the idea that the alleviating effect of low Mg concentrations on Al rhizotoxicity in soybean does not seem to be specific for a root class or is restricted to a limited number of cultivars.

Table 2. Root elongation (relative to control without Al) of the primary and lateral roots of 15 soybean cultivars exposed to increasing Al concentration in solution for 90 h, in the absence of Mg (500 µmol L⁻¹ Ca; pH 4.5) or presence of 50 µmol L⁻¹ Mg (450 µmol L⁻¹ Ca; pH 4.5)

	Al in solution, µmol L ⁻¹					Mean	Al in solution, µmol L ⁻¹					Mean
	0	2.5	5	10	20		0	2.5	5	10	20	
	0 µmol L ⁻¹ Mg						50 µmol L ⁻¹ Mg					
	Primary root											
M-SOY 8400	100	99	75	48	13	59	100	98	100	92	78	94
UFV16	100	105	91	50	15	65	100	104	105	93	81	97
Confiança	100	59	27	23	9	29	100	101	110	104	90	101
M-Soy 9001	100	84	81	32	6	51	100	109	108	106	95	104
Engopa315	100	96	96	48	5	61	100	114	105	104	81	101
Jataí	100	74	67	32	12	46	100	109	100	110	88	101
M-SOY 8411	100	84	71	37	13	51	100	105	103	95	84	97
Engopa 316	100	108	77	46	16	62	100	101	100	103	88	98
IAC 21	100	86	77	17	8	47	100	105	100	101	82	97
Segurança	100	107	88	29	12	59	100	111	107	94	73	97
Vencedora	100	112	81	25	13	58	100	117	106	109	98	106
Liderança	100	68	49	24	8	37	100	118	105	113	98	107
Conquista	100	110	99	58	12	70	100	99	92	104	82	96
Garantia	100	106	71	58	11	62	100	107	98	103	73	96
M-Soy 8001	100	88	52	50	10	50	100	115	100	97	65	95
Mean	100	92	73	38	11		100	108	103	102	84	
	Lateral roots											
M-SOY 8400	100	96	49	35	9	58	100	99	101	89	82	94
UFV16	100	88	33	29	8	52	100	159	70	74	45	90
Confiança	100	76	19	29	9	47	100	100	90	90	84	93
M-Soy 9001	100	78	29	36	7	50	100	108	88	76	45	83
Engopa3 15	100	77	28	37	7	50	100	148	119	105	72	109
Jataí	100	73	30	26	5	47	100	156	78	74	45	91
M-SOY 8411	100	79	24	37	8	50	100	159	89	82	74	101
Engopa 316	100	64	34	31	7	47	100	122	94	77	86	96
IAC 21	100	79	56	38	9	56	100	132	87	78	69	93
Segurança	100	87	62	54	9	62	100	91	94	68	51	81
Vencedora	100	73	45	43	6	53	100	105	94	89	58	89
Liderança	100	51	19	22	6	39	100	133	115	84	64	99
Conquista	100	50	42	33	6	46	100	100	92	72	67	86
Garantia	100	81	35	35	6	51	100	112	121	89	90	102
M-Soy 8001	100	74	33	30	6	48	100	134	124	106	91	111
Mean	100	75	36	34	7		100	124	97	84	68	

Is Mg protection against Al an apoplastic or symplastic effect?

The mechanism by which Mg ameliorates the deleterious effects of Al is not well understood. It is not known whether the effect occurs in the apoplast or in the symplast of root cells. It is also still unclear whether Mg must be present together with Al in the growth medium to have a mitigating effect, or if an adequate tissue concentration would be sufficient. The ameliorative effect of Mg is maximized when is supplied to plants in pre-treatment solution (before Al addition), together with Al (time zero "0"), or up to 6 h after exposing roots to toxic Al, as long as Mg remains in solution with Al³⁺ (Figure 2a). After 6 h of root exposure to Al in the absence of Mg, the toxicity effects becomes irreversible, and Mg additions afterwards can not completely restore root elongation (Figure 2a). The response pattern of Al-sensitive (cv. Confiança) and Al-tolerant (UFV-16) soybean is similar, but in the latter Al damage appeared to be less pronounced (Figure 2).

Plants that were pre-treated for up to 24 h in the presence of Mg and exposed to Al in solution without Mg (beginning at time zero), showed only a small improvement in root elongation in comparison to plants pre-treated with Mg for periods varying from 0 to 12 h (Figure 2b). However, this beneficial effect was much smaller when compared to plants exposed to Al in the presence of Mg in solution (Figure 2b). These results suggest that a higher tissue Mg concentration generated by the pretreatments does not ensure protection against Al damage and reinforces the idea that Mg must be present together with Al in solution so that its alleviating function is fully expressed. Alternatively, it can be speculated that even though internal Mg reserves are satisfactory for normal soybean growth in the absence of Al, the Mg delivery to the rapidly dividing and expanding root tip cells is not adequate under Al stress conditions if Mg is not present in the external solution. This internal Mg deficiency status induced by Al is alleviated by the additional Mg taken up when Mg is added to the growth solution. In fact, aluminum is chemically more similar to Mg than to Ca (Martin, 1990) and competitively inhibits Mg uptake by plant roots (Rengel & Robinson, 1989). This Al-induced Mg deficiency has been suggested as a primary cause of Al toxicity (MacDiarmid & Gardner, 1996). Support for this hypothesis comes from research where overexpression of yeast and bacterial Mg transporters in yeast increased Mg (Co²⁺) uptake and improved Al tolerance significantly (Fowler et al., 1999) and the more recent findings that overexpression of an *Arabidopsis* magnesium transport gene in *Nicotiana benthamiana* increased Mg uptake in the presence of Al and conferred Al tolerance (Deng et al., 2006).

Exposure of roots to Al reduced Mg concentration in roots and shoots only when Mg was added in pre-treatment and was absent from the solution after Al

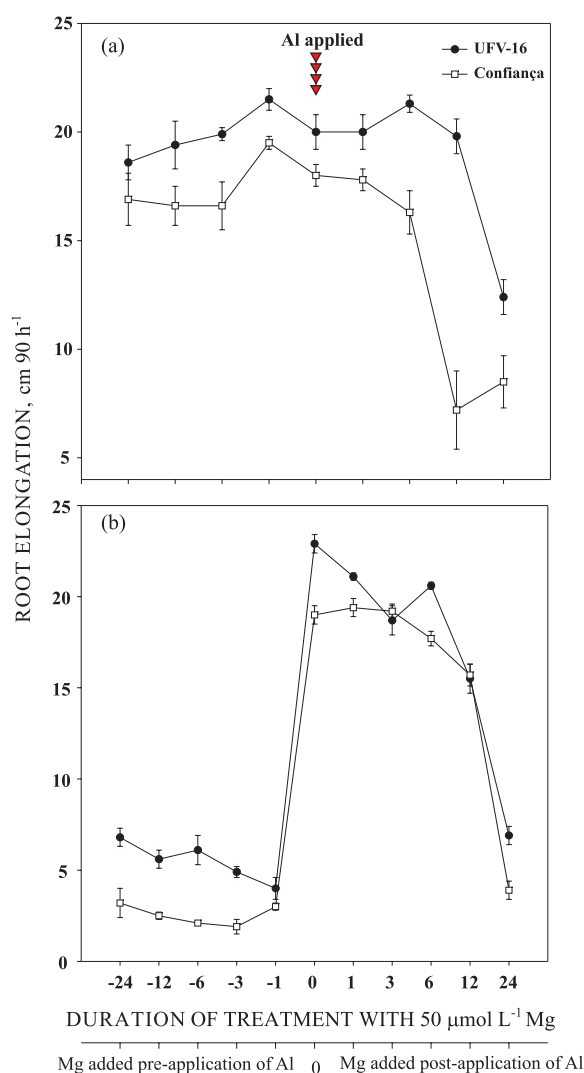


Figure 2. Primary root elongation of soybean cvs. Confiança and UFV-16 as affected by different treatment periods with 50 μmol L⁻¹ Mg in pre (-24 to 0) and post (0 to 24) addition of 10 μmol L⁻¹ Al. Aluminum was always added at time "0", just after initial root length measurement. (a) After adding Mg to the solution (before or after Al addition), Mg remained in solution with Al during the 90 h-Al treatment period. (b) plants were pre-conditioned for distinct periods in the presence of Mg, but in the absence of Al (-24 to -1 h) and then beginning at time 0 they were grown in the presence of Al without Mg. The plants of the treatments 0-24 h received timed applications of Mg in the same manner as plants of group 0-24 h of figure 2a. Thus, treatments in figure 2a differ from those in figure 2b mainly because Mg added as pre-treatment before Al application (-24 to -1) remained in solution after Al addition in 2a, while Mg added as pre-treatment (-24 to -1) was not maintained in solution after Al addition in 2b. All treatments were established in a variable (0.45 to 0.5 mmol L⁻¹) CaCl₂ basal solution (pH 4.5).

addition (Figure 3c). It also occurred when Mg was added 6–12 h after initiating Al exposure (Figure 3d). Moreover, the Al content in roots of these plants was higher when Mg was only supplied in the pre-treatment (no Mg present in solution after Al addition at time “0”; Figure 3a; -48 to 0 h) in comparison with plants that received Mg in pre-treatment and also in post-treatment with Al (-48 to 0 h Figure 3a vs 48 to 0 h Figure 3b). Delaying the supply of Mg by more than 12 h after adding Al to solution led to an increment in the Al root content (Figure 3a,b), which is directly related with the increase in Al toxicity and inhibition of root growth (Figure 2). The reduction in Al uptake induced by Mg was more than 20 % in comparison to control plants treated with Al in the absence of Mg (Figure 3a,b; +Al-Mg). This effect would probably be much greater if root apices, instead of whole roots, had been analyzed (Silva et al., 2001d). Compared to roots, the Al shoot contents were more than 10 times lower and no substantial effect of Mg was detected.

To investigate whether the protective effect is dependent on the presence of Mg together with Al in the solution (an indication of existence of an apoplastic mechanism) an experiment was carried out using a split-root approach. Solution treatments are shown in table 1. There were no substantial differences on root growth in the two compartments among treatments T1 to T6 (absence of Al in compartment 1). Root growth in compartment 2 (without Al) also showed no variation among treatments T7 to T12 (Al present in compartment 1). However, when Al was added to compartment 1, the root system was reduced by more than 50 % in treatments T7 to T11 as compared to equivalent treatments without Al (T1 to T5). There was an adequate root growth only when $50 \mu\text{mol L}^{-1}$ Mg was present in solution together with Al (Figure 4). The increasing Mg levels in compartment 2, which led to greater translocation of Mg to roots of compartment 1 (Figure 5) was not sufficient to counter the deleterious effect of Al. In fact, results of a study with an Al-tolerant soybean

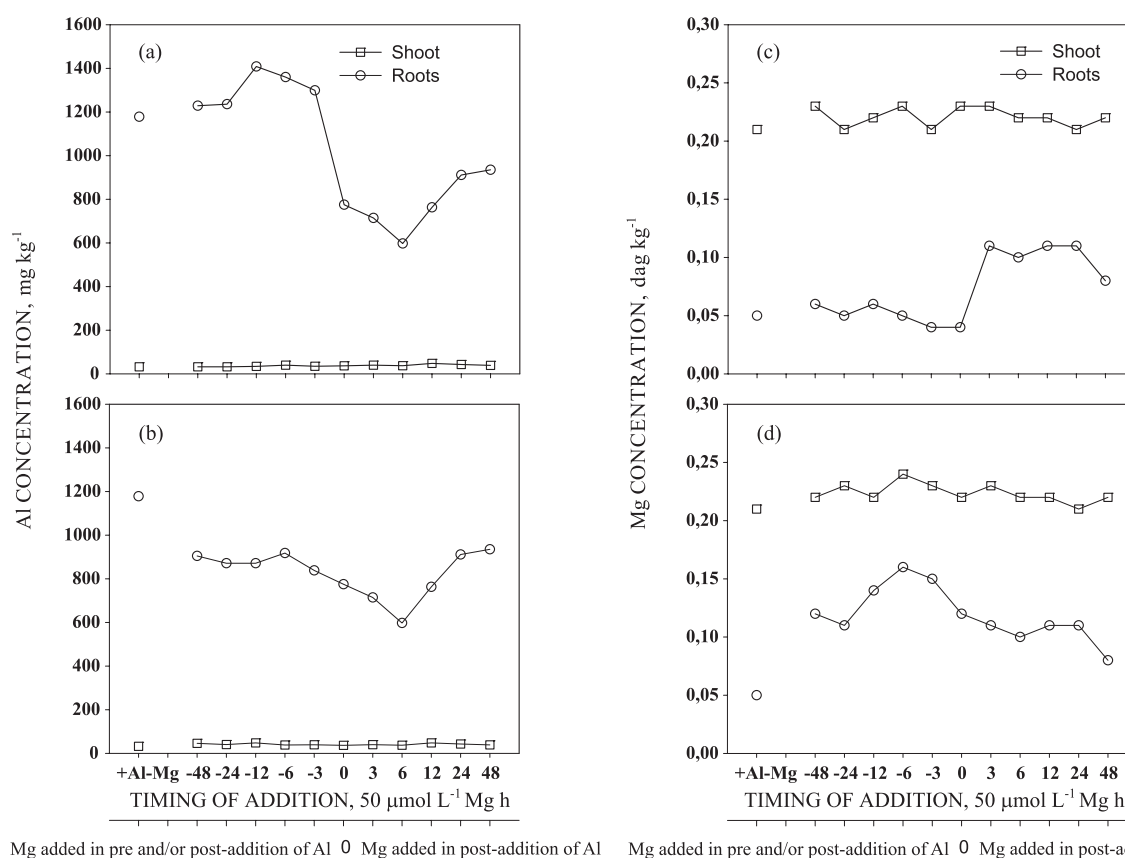


Figure 3. Aluminum (a-b) and magnesium (c-d) concentration in roots and shoots of soybean cv. UFV-16 as a function of different periods of supply of $50 \mu\text{mol L}^{-1}$ Mg in pre- (-48 to 0) and post-(0 to 48) addition of $10 \mu\text{mol L}^{-1}$ Al. Aluminum was always added at time “0”, just after initial root length measurement. Figure a-c: Plants were pre-conditioned for distinct periods in the presence of Mg, but in the absence of Al (-48 to -1 h) and then beginning at time 0 they were grown in the presence of Al without Mg. The plants of the treatments 0–48 received timed applications of Mg in the same manner as plants of group 0–48 h of figure a-c. All treatments were established in a variable (0.45 to $0.5 \mu\text{mol L}^{-1}$) CaCl_2 basal solution (pH 4.5). Figure b-d: After adding Mg to the solution (before or after Al addition), Mg remained in solution with Al during the 90 h-Al treatment period.

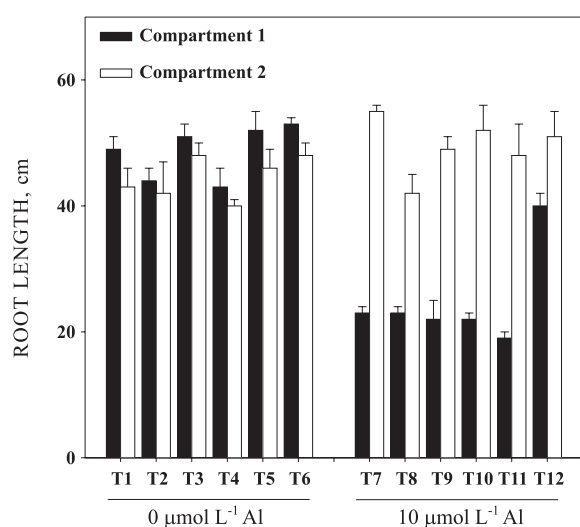


Figure 4. Primary root length of soybean cv. UFV-16 as related to solution treatments in the split-root experiment. Treatments 1-5 and 7-11 received increasing Mg rates in solution in compartment 1 (where roots were not in direct contact with Al), in the absence and presence of $10 \mu\text{mol L}^{-1}$ Al in compartment 2, respectively. Treatments six and twelve received Mg in compartment 2, in the same compartment where roots were exposed to Al (treatment 12).

genotype using a split root system indicate that exposure of one half of the root system to Al did not induce organic acid secretion by the other part of the root system, suggesting that no signals are involved in the citrate secretion in soybean (Nian et al., 2004). Thus, the presence of Mg, in the same way as Al, would be required in the external medium in order to induce organic acid secretion and/or activate other tolerance mechanism. In another study where Mg was supplied by foliar spraying or nutrient solution, a similar behavior was observed since Mg protection was only effective against Al when supplied together with Al via nutrient solution (Figure 6). Thus, these results once again support the idea that the Mg effect is based on a physiological mechanism in the apoplast and appears to go beyond an alleviation of an Mg deficiency via competitive inhibition of Mg uptake by Al. Alternatively, the Mg role is internal, but Al blocks the Mg transport via phloem from one part of the root system (compartment 2) to the other (compartment 1). Additionally, based on the decreasing trend of Mg root contents grown in compartment 1 in the presence of Al (T7 to T11) in comparison to control plants grown in the absence of Al (T1 to T5; Figure 5b), it can not be completely ruled out that the main toxic Al effect is due to an Al-induced Mg-deficiency (Rengel & Robinson, 1989; MacDiarmid & Gardner, 1996; Fowler et al., 1999). In fact, in yeast there are indications that Al toxicity occurs as a result of reduced Mg^{2+} influx via the Alr proteins and overexpression of the

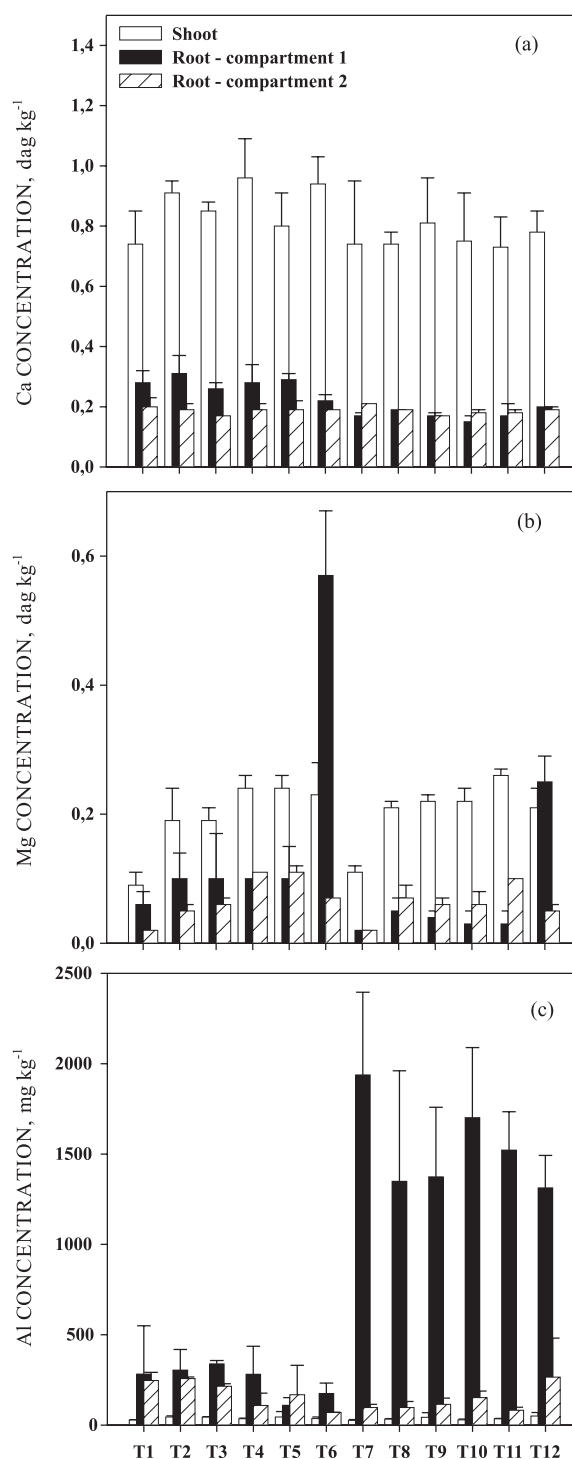


Figure 5. Shoot and root (in the two compartments) contents of Ca (a), Mg (b) and Al (c) of soybean cv. UFV-16 as related to solution treatments (Table 1) in the split-root experiment.

Mg transport system led to a greater Al tolerance (MacDiarmid & Gardner, 1998). The observed trend for lower Ca concentration in roots grown in the presence of Al (Figure 5a) is unlikely to be the main cause of Al toxicity, as shown by Ryan et al. (1997).

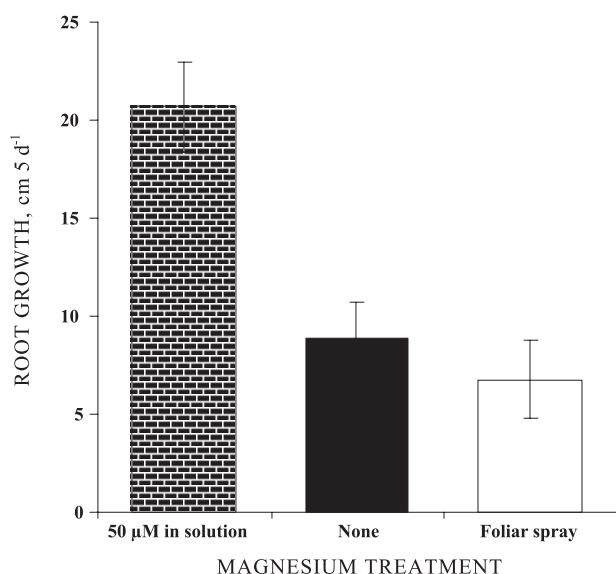


Figure 6. Primary root growth of soybean cv. Confiança as influenced by no Mg supply, Mg supplied via solution or foliar spray, in the presence of 10 $\mu\text{mol L}^{-1}$ Al.

Mg protection against Al in other crops

The ameliorative effect of Mg is interesting because it seems to differ from other divalent cations in relation to plant species specificity (Keltjens & Tan, 1993; Silva et al., 2001a). In a study involving seven important annual crops, it was observed that rice (Figure 7a), corn (Figure 7c) and wheat (Figure 7f) were most Al tolerant, whereas cotton (Figure 7e), sorghum (Figure 7g), snap bean (Figure 7b), and soybean (Figure 7g) were most sensitive to Al rhizotoxicity in the absence of Mg. In the presence of 50 $\mu\text{mol L}^{-1}$ Mg root elongation of rice, snap bean, corn and wheat was slightly improved, but soybean root elongation was consistently greater compared to control plants growing without Mg at low to intermediate Al concentrations (Figure 7g). These results confirm data from previous studies where Mg increased Al tolerance in soybean, but not in wheat (Silva et al., 2001b), and increase the number of species for which the alleviating effect of Mg is not substantial. Our results also corroborate previous studies where Ca and Mg were found to be equally effective against Al rhizotoxicity in wheat (Kinraide et al., 1985, 1992, 1994; Grauer & Horst, 1992; Kinraide, 1998). We observed no trend suggesting that Mg is more efficient ameliorating Al toxicity in monocots than in dicots, as reported by Keltjens & Tan (1993).

At the moment it is unclear whether the protective effect of Mg occurred exclusively due to an apoplastic (possibly competitive) action. Additional research with the cloned Arabidopsis AtMGT1 and AtMGT2 genes, which have been shown to encode plasma membrane located Mg transporter proteins and are sensitive to Al blockage (Legong et al., 2001) may throw some light on this issue.

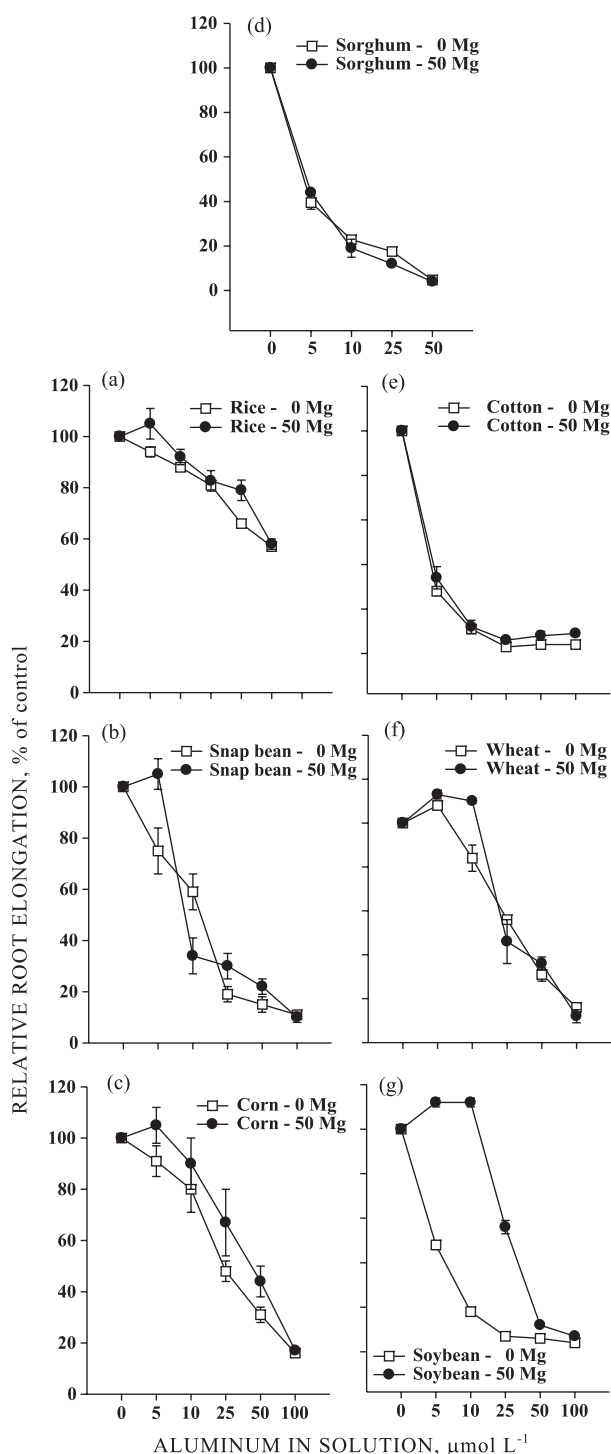


Figure 7. Root elongation of soybean, wheat, corn, cotton, sorghum and snap bean as a function of increasing Al doses, in the absence or presence of 50 $\mu\text{mol Mg L}^{-1}$ in a 0.45 mmol L^{-1} CaCl_2 .

CONCLUSIONS

1. Magnesium at a low concentration is an efficient alleviator of Al rhizotoxicity; this effect is significant in soybean only.

2. Soybean cultivars differ in Al-tolerance, and the presence of Mg in the growth medium causes Al-sensitive cultivars to behave as somewhat Al-tolerant.

3. To be more effective against Al toxicity Mg must be present together with Al in the growth media, which is in line with an apoplastic role.

4. Adding Mg after Al inflicting damage does not recover root growth.

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