INTERACTIONS BETWEEN MAGNESIUM, CALCIUM, AND ALUMINUM ON SOYBEAN ROOT ELONGATION(1)

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ABSTRACT

Alleviation of Al rhizotoxicity by Ca and Mg can differ among species and genotypes. Root elongation of soybean [*Glycine max* (L.) Merr.] line N93-S-179 and cvs. Young and Ransom exposed to varying concentrations of Al, Ca and Mg were compared in two experiments using a vertically split root system. Roots extending from a surface compartment with limed soil grew for 12 days into a subsurface compartment with nutrient solution treatments maintained at pH 4.6 with either 0 or 15 µmol L^{-1} Al. Calcium and Mg concentrations in treatments ranging from 0 to 20 mmol L^{-1}. Although an adequate supply of Mg was provided in the surface soil compartment for soybean top growth, an inclusion of Mg was necessary in the subsurface solutions to promote root elongation in both the presence and absence of Al. In the absence of Al in the subsurface solution, tap root length increased by 74 % and lateral root length tripled when Mg in the solutions was increased from 0 to either 2 or 10 mmol L^{-1}. In the presence of 15 µmol L^{-1} Al, additions of 2 or 10 mmol L^{-1} Mg increased tap root length fourfold and lateral root length by a factor of 65. This high efficacy of Mg may have masked differences in Al tolerance between genotypes N93 and Young. Magnesium was more effective than Ca in alleviating Al rhizotoxicity, and its ameliorative properties could not be accounted for by estimated electrostatic changes in root membrane potential and Al^{3+} activity at the root surface. The physiological mechanisms of Mg alleviation of Al injury in roots, however, are not known.

Index terms: cation amelioration, aluminum toxicity, root stress, soil acidity.

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RESUMO: *INTERAÇÃO ENTRE MAGNÉSIO, CÁLCIO E ALUMÍNIO NA ELONGAÇÃO RADICULAR DA SOJA*

A redução na rizotoxidez de Al por Ca e Mg difere entre espécies e genótipos. A elongação radicular da linhagem N93-S-179 e cvs. Young e Ransom de soja [Glycine max *(L.) Merr.] exposta a concentrações variáveis de Al, Ca e Mg foi comparada em dois experimentos, usando um sistema de raízes subdivididas verticalmente. As raízes cresceram por 12 dias a partir de um compartimento superficial com solo corrigido para um compartimento subsuperficial com soluções nutritivas que continha os tratamentos e mantidas a pH 4,6. As concentrações de Ca e Mg nos tratamentos variaram de 0 a 20 mmol* L^{-1}*. Embora tenha sido providenciado um suprimento adequado de Mg para o crescimento da parte aérea da soja no compartimento superficial com solo, a adição de Mg nas soluções subsuperficiais foi necessária para promover a elongação radicular tanto na presença como na ausência de Al. Na ausência de Al na solução em subsuperfície, o comprimento de raízes aumentou 74 % e o comprimento das raízes laterais triplicou quando Mg da solução foi aumentado de 0 para 2 ou 10 mmol* L^{-1}*. Na presença de 15 µmol* L^{-1} *de Al, adições de 2 ou 10 mmol* L^{-1} *de Mg aumentaram o crescimento da raiz principal em quatro vezes e das raízes laterais por uma fator de 65. Esta elevada eficiência do Mg pode ter mascarado as diferenças na tolerância ao Al entre os genótipos N93 e Young. O Mg foi mais eficiente que o Ca para reduzir a rizotoxidez do Al, e sua propriedade protetora não pode ser explicada pelas mudanças eletrostáticas estimadas para o potencial da membrana e atividade do* Al^{3+} *na superfície da raiz. No entanto, os mecanismos fisiológicos envolvidos no efeito protetor do Mg contra a toxidez do Al às raízes ainda não são conhecidos.*

Termos de inndexação: proteção por cátions; toxidez por alumínio; estresse radicular; acidez do solo.

INTRODUCTION

Proposed mechanisms for alleviation of aluminum (Al) rhizotoxicity by calcium (Ca) and magnesium (Mg) additions to soil and nutrient solutions involve a reduction of Al activity at the plasma membrane or competition for binding sites in the root apoplasm (Alva et al., 1986; Kinraide & Parker, 1987; Grauer & Horst, 1992; Kinraide, 1994; 1998). Some investigators suggest that Mg is more effective than Ca in competing with Al for binding sites (Keltjens & Dijkstra, 1991), but others postulate that it is less effective than Ca in competing with Al for exchange sites at the plasma membrane (Kinraide & Parker, 1987; Grauer & Horst, 1992). These discrepancies may be partially explained by the differential effectiveness of these cations according to the plant species evaluated. Keltjens & Tan (1993) found that Mg was more efficient in alleviating Al toxicity in monocots, while Ca was more effective in the dicots. More specifically in soybean, low concentrations of Mg (micromolar range) are as effective as higher concentrations of Ca (milimolar range), but in wheat no such difference between cations is evident (Silva et al., 2001).

Aluminum can interfere with physiological processes involving Mg because their similar ionic radii is more important than their charge differences in biological reactions (Martin, 1992). Aluminum is more effective than Ca in displacing Mg ions from binding sites in the root apoplasm (van Praag et al., 1997) and can substitute Mg at critical enzymatic and regulatory sites in the symplasm (Macdonald & Martin, 1988). Magnesium is a cofactor of enzymes involved in phosphate transfer (Macdonald & Martin, 1988) and ATP-synthesis (Bush & Ninnemann, 1997), and contributes to the structural stability of nucleic acids, ribosomes (Cammarano et al., 1972), and membranes (Clarkson & Hanson, 1980). Calcium is involved in cell wall and membrane stability and plays an important role mediating enzyme activity in the symplasm (Clarkson & Hanson, 1980; Hanson, 1984; Marschner, 1995; Carpita & McCann, 2000; White & Broadley, 2003). Calcium is also a key second messenger involved in cell signaling cascades, which are not only important components of the whole cell metabolism but also offer plants the possibility to adapt to changing environments (Bush, 1995; Trewawas & Malhó, 1997; Sanders et al., 1999; White & Broadley, 2003).

The objectives of this study were to compare root elongation of two soybean genotypes to variable levels of Mg and Al supply in a subsurface solution compartment and to evaluate interactions between Mg and Ca in alleviating Al rhizotoxicity.

MATERIALS AND METHODS

A vertical split-root system (Sanzonowicz et al., 1998b) was used in two experiments to evaluate elongation of soybean roots extending from a limed surface soil layer into a subsurface compartment containing 3.0 L of continuously aerated solutions with various combinations of Al, Ca, and Mg. The soil compartment contained 1.1 kg of the Ap layer from a Wagram soil (loamy, siliceous, thermic, Arenic Kandiudults) that was limed to pH 5.5 with $CaCO_3$ and that received 30 mg kg^{-1} of K as sulfate salt. Soil exchangeable Mg was 0.08 $cmol_c$ kg^{-1}. The soil compartment was separated from the subsurface solution compartment by a root-permeable membrane made of cheesecloth impregnated with a mixture of paraffin and petrolatum. Solutions in all experiments contained 18.5 µmol L^{-1} B as H_3BO_3 and 0.5 µmol L^{-1} Zn as $ZnCl_2$, to avoid decreased soybean root elongation in the subsurface compartment when these nutrients are omitted (Sanzonowicz et al., 1998b). Aluminum was supplied to solutions as $AlCl_3$ from a 0.037 mol L^{-1} acidified stock solution, Ca as $CaCl_2$ and Mg as $MgCl_2$. Solution pH in the subsurface compartment was adjusted daily by titration with 0.05 mol L^{-1} solutions of either HCl or NaOH.

Experiment 1 was conducted in a walk-in chamber of the phytotron at North Carolina State University, Raleigh, NC (Thomas & Downs, 1991). Subsurface solution treatments consisted of nine combinations of Al (0, 15 µmol L^{-1}), Ca (2, 10 mmol L^{-1}) and Mg (0, 2, 10 mmol L^{-1}) concentrations maintained at pH 4.6. Treatments in the absence of Al consisted of three Mg concentrations with Ca maintained at 2 mmol L^{-1}. In the presence of 15 µmol L^{-1} Al, there were six treatments comprised of the factorial combination of Ca and Mg concentrations (Table 1). Root elongation of soybean breeding line N93-S-179 (N93) and cv. Young were compared during 12 days of exposure to the subsurface solution treatments. Each pot contained four plants. Solutions in the subsurface compartment were replaced every four days. Treatments were arranged in a randomized complete block design with three replications. Statistical analysis of variables measured over time was performed with a split-plot model with the factorial combination of genotypes, Ca and Mg nested within Al levels as main plots, and with time as the subplot variable (Steel et al., 1996).

Plants were harvested 13 days after planting, which coincided with natural abscission of cotyledons and emergence of the second trifoliates. Details of seed preparation, planting, watering the soil compartment, harvesting, above-ground plant tissue analysis, and ambient conditions in the phytotron chamber are reported elsewhere (Ferrufino et al., 2000).

Tap root elongation in the solution compartment was measured every two days. Microbial contamination was minimized by disinfecting all tools with 20.6 mol L^{-1} ethanol. Tap roots, lateral roots, and roots extending into the solution compartment from the soil layer were separated at harvest and counted. Roots extending through the membrane from the soil compartment could not be distinguished in lateral and basal classes (Zobel,

Table 1. GEOCHEM-predicted ionic strength, activities of Ca^{2+}, Mg^{2+}, and Al^{3+} in the subsurface compartment solutions for Experiments 1 and 2, and the potential and Al^{3+} activity at the root plasma membrane surface as estimated by a modified Gouy-Chapman-Stern model

Treatment			Ionic strenght	Predicted activities in solution			Root plasma membrane	
Ca	Mg	Al		Ca^{2+}	Mg^{2+}	Al^{3+}	Al^{3+}	Potential
— mmol L^{-1} —		µmol L^{-1}		—— mmol L^{-1} ——		—— µmol L^{-1} ——		mV
				Experiment 1				
2	0	0	0.005	1.5	0.0	0.0	0.0	-21.8
	2	0	0.010	1.3	1.3	0.0	0.0	-15.7
	10	0	0.030	1.0	5.2	0.0	0.0	-6.1
	0	15	0.005	1.5	0.0	5.6	8.3	-3.8
	2	15	0.010	1.3	1.3	4.6	5.8	-2.6
	10	15	0.030	1.0	5.2	2.9	2.5	0.4
10	0	15	0.025	5.4	0.0	3.1	2.9	-0.2
	2	15	0.030	5.2	1.0	2.8	2.5	0.4
	10	15	0.050	4.5	4.5	2.2	1.5	2.4
				Experiment 2				
2	0	15	0.005	1.5	0.0	5.6	8.3	-3.9
	2	15	0.010	1.3	1.3	4.6	5.8	-2.6
	10	15	0.030	1.0	5.2	2.9	2.5	0.4
	20	15	0.055	0.9	8.8	2.1	1.3	2.8
0	20	15	0.050	0	9.0	2.2	1.5	2.4
20	0	15	0.050	9.1	0.0	2.2	1.5	2.4

1991) and were therefore combined into a group labeled “other” roots. Roots were refrigerated in a 4.3 mol L^{-1} ethanol solution prior to measuring length by edge discrimination (Pan & Bolton, 1991) with a desktop scanner preset to a resolution of 98 dots cm^{-1}. Soil was washed off the roots from the soil compartment and their combined total length was measured using the same procedure described for the solution compartment.

Experiment 2 was conducted in a greenhouse at North Carolina State University. Natural light was supplemented by 150 mol m^{-2} s^{-1} of photosynthetically active radiation from metal halide lamps for 16 h day^{-1}. There were six treatments in the subsurface solution compartment, all with 15 µmol L^{-1} Al: 0, 2, 10 and 20 mmol L^{-1} Mg with 2 mmol L^{-1} Ca; 20 mmol L^{-1} Mg without Ca; and 20 mmol L^{-1} Ca without Mg. The pH of the solutions was adjusted daily to 4.6 and they were replaced once after eight days of root growth. Treatments were arranged in a randomized complete block design with three replications.

Six seedlings of soybean cv. Ransom were planted in the soil compartment of each container and thinned to five plants after two days. After 12 days of exposure to solution treatments, tap roots, their lateral roots and “other” roots in the subsurface compartment were separated and the length measured by the line intercept method (Tennant, 1975).

Activities of Al species in all solution treatments of both experiments were calculated by the GEOCHEM-PC program (Parker et al., 1995). Free concentrations of ions in solution, as predicted by GEOCHEM-PC, were used as input to a modified Gouy-Chapman-Stern model (Kinraide et al., 1998) to estimate the negative potential and ionic activities at the surface of root plasma membrane.

RESULTS AND DISCUSSION

Subsurface solution

Additions of Ca and Mg increased the predicted ionic strength of solutions in both experiments and reduced predicted molar activities for Al^{3+} (Table 1). Increased ionic strength upon the addition of Mg also reduced predicted molar activities for Ca^{2+} among solutions containing either 2 or 10 mmol L^{-1} total Ca. Since solution pH was maintained at 4.6 in all treatments, collinearity between predicted activities of monomeric Al species was high.

Experiment 1

Magnesium Effects on Root Length in the Absence of Al

During the 12 days of root exposure to subsurface solutions there were no significant differences ($p > 0.05$) between genotypes in tap root elongation among the Mg treatments (data not shown). Tap root length averaged across genotypes was similar among Mg levels during the first seven days of exposure to solution treatments (Figure 1). During subsequent periods, the elongation rate of tap roots in solutions without Mg was lower than with either 2 or 10 mmol L^{-1} Mg.

There were no differences between genotypes in the length of tap, lateral or “other” root classes in the solution compartment at harvest time (data not shown). When averaged across genotypes, however, the addition of 2 mmol L^{-1} Mg increased tap root length 1.5 times and tripled the lateral root length (Figure 2). Lateral roots accounted for 60 % of the total root length in the solution compartment when Mg was absent and for an average of 81 % in solutions with 2 and 10 mmol L^{-1} Mg. This larger contribution of lateral roots to total root length in the presence of Mg (Figure 2) was not only due to an increase in the number of lateral roots that emerged from the tap root, but also to their greater length relative to treatments without Mg (Table 2). The number of lateral roots, averaged across genotypes, doubled when solution Mg increased from 0 to 2 mmol L^{-1}, but then decreased by 20 % with Mg concentrations between 2 and 10 mmol L^{-1}. The average length of lateral roots was 1.6 times greater in treatments with 2 or 10 mmol L^{-1} Mg relative to the treatment without Mg. Since the number of lateral roots per unit length of tap root remained constant among Mg treatments, differences in average lateral root length resulted primarily from changes in root elongation rather than lateral root initiation.

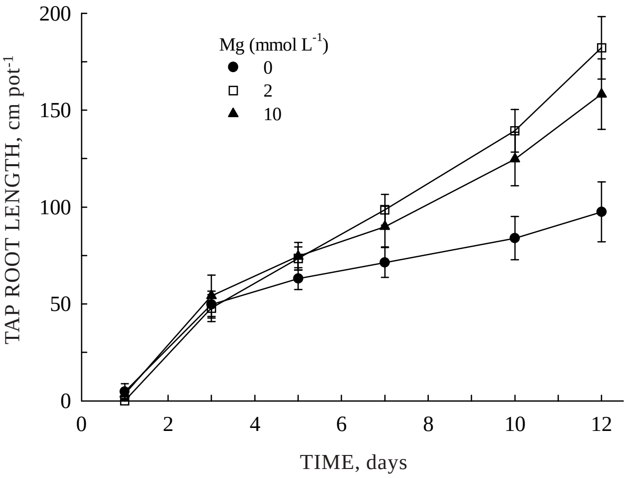

Figure 1. Mean tap root elongation of soybean genotypes N93-S-179 (N93) and Young with time of exposure to solutions in Experiment 1 without Al, at pH 4.6, and variable Mg concentrations. Vertical bars denote standard errors.

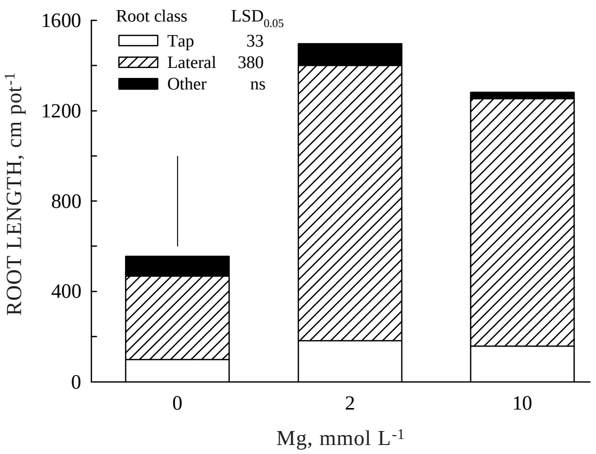

Figure 2. Mean total length in the subsurface compartment as a function of root classes for soybean genotypes N93-S-179 (N93) and Young after 12 days of exposure to solution Mg treatments in Experiment 1 containing no Al, 2 mmol L^{-1} Ca and maintained at pH 4.6. Least significant difference values are for the main effects of Mg on individual root classes and total root length (vertical line); ns stands for non significant at $p < 0.05$.

The observed improvement in root elongation with Mg additions may be linked to alleviation of H^+ toxicity by Mg. In experiments with a similar vertical split-root system, Sanzonowicz et al. (1998a) reported that increasing Ca concentrations from 2 to10 mmol L^{-1} alleviated H^+ toxicity for roots of soybean cv. Ransom grown in subsurface solutions maintained at pH 4.6 with no Al. The reductions we observed in tap root length and lateral root number upon increasing Mg supply from 2 to 10 mmol L^{-1} may also be related to competition between Ca and Mg. Lund (1970) noted reductions in soybean root length for solutions without Al when the Ca/(Ca + Mg) concentration ratios were less than 0.2. In our experiment the Ca/(Ca + Mg) ratio was 0. 17.

Root growth in soil, shoot dry weight and Magnesium concentration

Total root length in the surface soil compartment and soybean top dry weight did not differ significantly ($p > 0.05$) among genotypes or subsurface solution treatments (data not shown). Mean values were 25 m/pot for total root length and 0.5 g/pot for top dry weight. Magnesium concentration in plant tops averaged across genotypes increased linearly by 0.04 % for each milimolar increase of Mg concentration in solution (data not shown). However, soil Mg was apparently sufficient for top growth because concentrations in tops (mean of 0.34 %) in treatments without Mg supply in the subsurface solution were within the sufficiency range for soybean plants in the vegetative stage of development (Henderson & Kamprath, 1970; Jiménez et al., 1996).

Magnesium and Calcium interactions in the presence of 15 µmol L^{-1} Aluminum

Length of tap, lateral and "other" root classes in the solution compartment at harvest were not affected by solution Ca concentration and differences among genotypes were not significant (data not shown). The addition of 2 or 10 mmol L^{-1} Mg increased length of tap, lateral, and total root length relative to the treatment without Mg, when averaged across genotypes and solution Ca treatments (Figure 3). In the presence of 15 µmol L^{-1} Al, tap root length increased fourfold and lateral root length by a factor of 65 upon additions of 2 or 10 mmol L^{-1} Mg. Thus, most of the response in root elongation to Mg additions to solutions occurred in lateral roots. The relative contribution of lateral roots to the total root length was 12 % in the absence of Mg and an average of 76 % in treatments with 2 and 10 mmol L^{-1} Mg.

Comparisons of total root length among solution Mg treatments with and without 15 µM Al (Figure 3 vs. Figure 2) indicates that Al-inhibition of root elongation was greater in the absence of Mg. Relative to treatments without Al, total root length

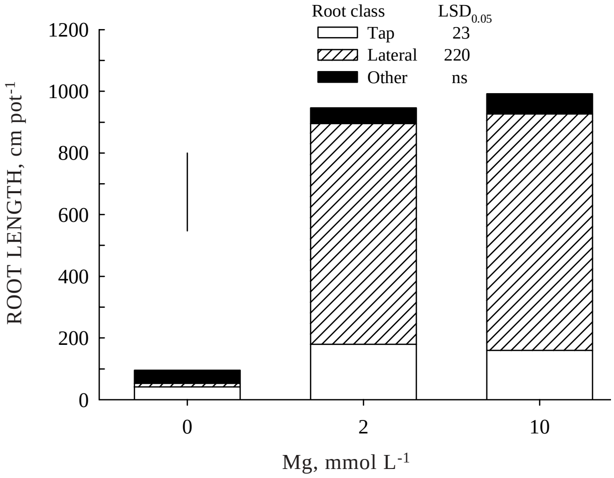

Figure 3. Total soybean root length and length of individual root classes at harvest in subsurface solution Mg treatments which received 15 µmol L^{-1} Al in Experiment 1. Root length is averaged across solution Ca levels and genotypes N93-S-179 (N93) and Young. Least significant difference values are for the main effects of Mg on individual root classes and total root length (vertical line); ns stands for non significant at $p < 0.05$.

in the presence of 15 µmol L^{-1} Al decreased by 83 % in solutions with 0 mmol L^{-1} Mg and an average of 30 % in solutions with 2 and 10 mmol L^{-1} Mg. Furthermore, there was no response in root elongation to 2 or 10 mmol L^{-1} Mg in either the presence or absence of Al.

Experiment 2

Calcium and Mg supply to subsurface solutions with 15 µmol L^{-1} Al influenced the length of all root classes for soybean cv. Ransom (Figure 4). Responses in root elongation to solution Mg treatments with 2 mmol L^{-1} Ca are similar to those observed with soybean line N93 and cv. Young in Experiment 1 (Figure 3); lateral roots accounted for most of the increase in total root length and there was no response to Mg concentrations above 2 mmol L^{-1}. Increasing solution Ca concentration from 2 to 20 mmol L^{-1} in the absence of Mg tripled total root length. In contrast to the Ca response, the addition of 2 mmol L^{-1} Mg to solutions with 2 mmol L^{-1} Ca increased total root length tenfold. Total root length in solutions with 20 mmol L^{-1} Mg without Ca was smaller than in the treatment with 2 mmol L^{-1} Ca and no Mg. Tap root elongation stopped after 1 day of exposure to the solution without Ca, and no lateral roots were detected on tap roots. Our results for root growth in solutions without Ca are consistent with prior reports that an external Ca supply is essential for normal root development (Clarkson, 1984; Kinraide, 1998; Sanzonowicz et al., 1998b).

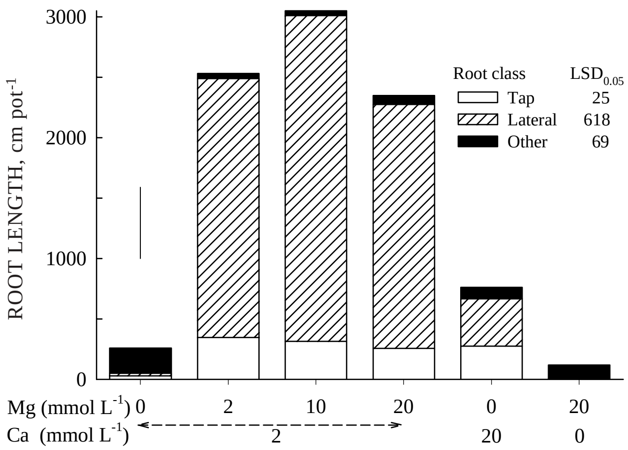

Figure 4. Total root length at harvest as a function of root classes and solution Ca and Mg treatments in the subsurface compartment for soybean cv. Ransom in Experiment 2. All solution treatments were maintained at pH 4.6 and contained 15 µmol L^{-1} Al. Vertical bar denotes least significant difference ($p < 0.05$) among treatments for total root length.

Solution Calcium and Magnesium effects on predicted free Al^{3+} activities at the root surface

Amelioration of Al rhizotoxicity in wheat by additions of cations to hydroponic cultures was closely related to changes in electrical potentials of the root plasma membrane and Al^{3+} activity at the membrane surface as estimated by a modified Gouy-Chapman-Stern model (Kinraide et al., 1992; Kinraide, 1994, 1998). Additions of cations increase the predicted ionic strength of solutions, increase the predicted electrical potential of root surface membranes (E_0), and decrease the predicted Al^{3+} activity at the membrane surface $\{Al^{3+}\}_m$ (Table 2). These electrostatic changes lead to less Al accumulation in both the root apoplast and symplast. Experiments comparing the amelioration of Al rhizotoxicity for wheat by Ca and Mg have consistently shown that either both cations have similar protective effects or that Ca is marginally superior to Mg (Kinraide et al., 1985, 1992; Kinraide & Parker, 1987; Kinraide, 1994, 1998).

Estimates by the modified Gouy-Chapman-Stern model of E_0 and $\{Al^{3+}\}_m$ for soybean roots in each of the solution treatments for Experiments 1 and 2 are provided in table 1. Additions of Ca and Mg increased ionic strength of solutions and E_0 in both experiments. In solutions with Al, additions of Ca and Mg also decreased $\{Al^{3+}\}_m$. Variations in total soybean root length among treatments containing Al and different amounts of Ca and Mg (Figures 3 and 4) were however not consistently related to predicted $\{Al^{3+}\}_m$ values. In Experiment 2, for example, there were no differences in total root length among solutions with 2 mmol L^{-1} Ca and supplemented with 2, 10 or 20 mmol L^{-1} Mg, but predicted values for $\{Al^{3+}\}_m$ decreased by a factor of 4.3 across this range of Mg concentrations. Likewise, there were no increases in total root length among Al treatments in Experiment 2 for solution Mg concentrations > 2 mmol L^{-1}, but the predicted $\{Al^{3+}\}_m$ values averaged across Ca treatments decreased from 5.59 to 1.97 as Mg in solution increased from 2 to 10 mmol L^{-1}.

The alleviation of Al rhizotoxicity for soybean at lower concentrations of Mg than Ca suggests that mechanisms other than electrostatic action are involved. The observed response in root elongation to Mg in the absence of Al lends further support to the involvement of physiological processes. The reasons for the substantial differences between wheat and soybean root elongation response to amelioration of Al rhizotoxicity by Mg are the subject of ongoing investigations in our lab.

Table 2. Characteristics of lateral roots at harvest in the subsurface solution compartment of Experiment 1 for soybean line N93-S-179 (N93) and cv. Young as a function of Mg concentrations in solution maintained at pH 4.6 without Al

Treatment		Number	Density(1)	Average length(2)
Mg	Genotype			
mmol L^{-1}		roots/pot	number/cm	cm/root
0	N93	461	4.6	0.6
	Young	413	4.0	1.1
	Mean	437	4.3	0.8
2	N93	1007	6.1	1.1
	Young	938	5.2	1.3
	Mean	973	5.6	1.2
10	N93	862	5.7	1.3
	Young	671	4.7	1.5
	Mean	767	5.2	1.4
Mean	N93	777	5.5	1.0
	Young	674	4.6	1.3
LSD 0.05:				
Mg		165	NS	0.4
Genotype		NS	NS	NS
Mg x Genotype		NS	NS	NS

(1) Number of lateral roots/length of tap root. (2) Total length/number of roots.
NS: Non-significant ($p > 0.05$) F test.

CONCLUSIONS

1. Despite adequate supply of Mg for soybean top growth in an upper soil compartment, Mg added to a subsurface solution compartment promotes root elongation in both the presence and absence of Al.

2. The ameliorative action of Mg at relatively low concentrations against Al injury is more efficient than that of Ca and is not directly associated with predicted changes in electrostatic properties of the root plasma membrane.

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