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Calcium Alleviation of Hydrogen and Aluminum Inhibition of Soybean Root Extension from Limed Soil into Acid Subsurface Solutions

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ABSTRACT

Alleviation by calcium (Ca) of inhibition of soybean [*Glycine max* (L.) Merr. cv. 'Ransom'] root elongation by hydrogen (H) and aluminum (Al) was evaluated in a vertical split-root system. Roots extending from a limed and fertilized soil compartment grew for 12 days into a subsurface compartment containing nutrient solution with treatments consisting of factorial combinations of either pH (4.0, 4.6, and 5.5) and Ca (0.2, 2.0, 10, and 20 mM), Al (7.5, 15, and 30 μM) and Ca (2.0, 10, and 20 mM) at pH 4.6, or Ca (2, 7, and 12 mM) levels and counter ions (SO_4 and Cl) at pH 4.6 and 15 μM Al. Length of tap roots and their laterals increased with solution Ca concentration and pH value, but decreased with increasing Al level. Length of both tap and lateral roots were greater when Ca was supplied as $CaSO_4$ than as $CaCl_2$, but increasing Ca concentration from 2 to 12 mM had a greater effect on alleviating Al toxicity than Ca source. In the absence of Al, relative root length (RRL) of tap and lateral roots among pH and Ca treatments was related to the Ca:H molar activity ratio of solutions ($R^2 \geq 0.82$). Tap and lateral RRL among solutions with variable concentrations of Al and Ca at pH 4.6 were related to both the sum of the predicted activities of monomeric Al ($R^2 \geq 0.92$) and a log-transformed and valence-weighted balance between activities of Ca and selected monomeric Al species ($R^2 \geq 0.95$). In solutions with 15 μM Al at pH 4.6, response of tap and lateral RRL to variable concentrations of $CaSO_4$ and $CaCl_2$ were related to predicted molar activity ratios of both Ca:Al^{3+} ($R^2 \geq 0.89$) and Ca:3 monomeric Al ($R^2 \geq 0.90$), provided that $AlSO_4$ and $Al(SO_4)_2$ species were excluded from the latter index. In all experiments H and Al inhibited length of lateral roots more than tap roots, and a greater Ca:H or Ca:Al concentration ratio was required in solutions to achieve similar RRL values as tap roots.

INTRODUCTION

Poor plant growth in acid soils is associated with Ca deficiency and toxicities of H, Al, and Mn (Kamprath, 1984; Marschner, 1991). Evidence continues to accumulate that Al constraints to root growth can be alleviated by Ca additions to soil and nutrient solutions (Alva et al., 1986; Noble et al., 1988; Horst, 1987; Wright and Wright, 1987). Within specific concentration ranges of Ca and Al, the effects of Ca additions on alleviation of Al rhizotoxicity cannot be directly attributed to a Ca deficiency or a reduction in Al activity through increased ionic strength. Increased solution Ca concentrations also can reduce root damage caused by excess H^+ (Lund, 1970; Moore, 1974; Runge and Rode, 1991).

We have previously investigated elongation of soybean roots exposed to varying solution concentrations of H and Al in a vertical split-root system in which roots extended from a limed and fertilized soil compartment into a subsurface solution compartment (Sanzonowicz et al., 1998). There was a greater inhibition in length of lateral roots than tap roots by H and Al. The objectives of the present investigation, using the same vertical split-root system with soybean, were to quantify the ameliorative effects of Ca on inhibition of tap and lateral root elongation by rhizotoxic H and Al levels, and to determine the effect of the counter anions from different Ca salts on Al toxicity to root growth.

MATERIALS AND METHODS

Experiments were conducted in greenhouse facilities at Raleigh, NC, using a vertical split-root system. Plastic cylinders, with a 10-cm inside diameter and 52-cm total length, were divided into two compartments separated by a root-permeable membrane. The 12-cm long surface compartment contained 1.3 kg of loamy sand taken from the Ap horizon of an Arenic Kandiudults. The soil was limed to pH 5.5 and received 30 mg K kg^{-1} before transfer to the surface compartment.

Treatments were imposed to 3.0 L of continuously aerated solution in the subsurface compartment. Three experiments were conducted to evaluate the effects

TABLE 1. Description of subsurface solution compartment treatments for each experiment.

	Treatment Variables			
Experiment	**pH**	**Al**	**Ca**	**Ca Source**
		μM	mM	
1	4.0	0	0.2, 2, 10, 20	$CaCl_2$
	4.6	0	0.2, 2, 10, 20	$CaCl_2$
	5.5	0	0.2, 2, 10, 20	$CaCl_2$
2	4.6	7.5	2, 10, 20	$CaCl_2$
	4.6	15	2, 10, 20	$CaCl_2$
	4.6	30	2, 10, 20	$CaCl_2$
3	4.6	15	2, 7, 12	$CaCl_2$
	4.6	15	2, 7, 12	$CaSO_4$

of Ca and Ca sources on alleviation of root growth inhibition by H and Al (Table 1). In Experiment 1 treatments consisted of a range of Ca concentrations at each of three pH values. Treatments in Experiment 2 consisted of a factorial combination of three levels each of Al and Ca in solutions maintained at pH 4.6. In Experiment 3 treatments consisted of three levels of Ca, supplied as either $CaCl_2$ or $CaSO_4$, in solutions with 15 μM Al maintained at pH 4.6. A randomized complete block design with three replications was used in all experiments. Solutions in all experiments contained 18.5 μM boron (B) as H_3BO_3 and 0.5 μM zinc (Zn) as ZnO, based on prior observations that soybean root elongation in the subsurface compartment was reduced when these nutrients were omitted (Sanzonowicz et al., 1998). Aluminum was supplied in Experiments 2 and 3 as metallic Al diluted in 5% HCl.

Changes in solution concentrations of Ca (Experiment 1) and Al (Experiments 2 and 3) were monitored in one treatment of each experiment. Treatments selected were those which had the lowest concentration of the element to be monitored and the most favorable conditions for root growth among remaining treatment variables. All solutions were replaced after eight days of root growth. At this time Ca in solutions for the treatment with 200 μM Ca at pH 5.5 (Experiment 1) had declined to 186 μM. In Experiment 2 Al reacting with ferron within 30 s had declined to 6.7 μM in the treatment with 7.5 μM Al and 20 mM Ca. In Experiment 3 ferron-reactive Al had declined to 14.4 μM in the treatment with 15 μM Al and 12 mM Ca as $CaSO_4$.

Seeds of soybean cv. "Ransom" were pre-germinated, and when their radicle lengths were 12±2mm seven were planted in each soil container. After two days each container was thinned to five plants. All experiments were conducted between

July and October. Natural light was supplemented with 150 microeinsteins m^{-2} s^{-2} of photosynthetically active radiation from metal halide lamps for 16 hours day^{-1}. Soil moisture was maintained at 80% of container capacity (Cassel and Nielsen, 1982) to avoid water movement from the soil to the solution compartment.

Tap roots began to cross the membrane between compartments on the third day after planting. At 15 days after planting plant shoots were harvested and dry weight was determined. Roots in the soil compartment were washed free of soil, and their lengths and dry weights were determined. Measurements in the solution compartment at harvest included tap root length, number and length of laterals on tap roots, and number and length of any other roots extending through the membrane from the soil compartment. Since we were unable to determine the specific class (basal, lateral or adventitious) for the latter category of roots, they were identified as "other" roots. Relative root length for a root class within each experiment was calculated as the ratio between a given treatment and the treatment with the greatest length.

Aluminum reacting in 30 s with buffered ferron was determined using a procedure adapted from Bersillion et al. (1980). Ionic activities of Al species in solution treatments were computed by the GEOCHEM-PC program (Parker et al., 1995). Additional details on soil chemical characteristics, root length measurements, ferron-reactive Al determinations and statistical analyses are reported elsewhere (Sanzonowicz et al., 1998).

RESULTS AND DISCUSSION

Most treatment means for dry weight of shoots, and total length and dry weight of roots in the soil compartment were not significantly different ($p<0.05$) within experiments (Table 2). The sole exception was significantly ($p<0.01$) less root length in the soil compartment in Experiment 2 with increasing Ca concentration in the solution compartment. Root length, averaged across Al treatments, decreased from 10.0 m $plant^{-1}$ with 0.2 mM Ca to 7.4 m $plant^{-1}$ with 20 mM Ca. Variations in means for measured plant shoot and soil compartment root parameters between experiments (Table 2) were attributed primarily to differences in ambient conditions in the greenhouse between growth periods for each experiment. Similarities in shoot biomass across treatments within experiments support the presumption that differences in root growth among subsurface compartment treatments were not confounded directly with differences in growth of plant tops.

Effects of Variable pH and Calcium in the Absence of Aluminum

Root Growth

Solution pH values and their maximum variation within any 24-h period for each pH treatment in Experiment 1 were 4.0±0.04, 4.6±0.07 and 5.5±0.12. There were

TABLE 2. Mean values and standard deviations across all treatments in each experiment for soybean shoot dry weight, dry weight, and total length of roots in the soil compartment at harvest.

	Shoot		Root			
Experiment	**Dry Weight**	**SD**	**Dry Weight**	**SD**	**Length**	**SD**
	------------g $plant^{-1}$------------				---m $plant^{-1}$---	
1	0.19	0.02	0.21	0.11	6.43	0.68
2	0.25	0.02	0.33	0.04	8.69	1.05
3	0.34	0.02	0.18	0.04	12.66	2.59

significant ($p<0.05$) main effects of solution pH and Ca treatments on the length of tap and lateral roots and on the number of lateral and other roots in the subsurface compartment. Interaction effects between pH and Ca treatments were significant only for length of tap and lateral roots.

Elongation of tap and lateral roots, in response to increasing solution Ca concentration, increased with increasing pH value (Figure 1). In solutions with 0.2 mM Ca and pH 4.0 tap root length was 1.1 cm $plant^{-1}$ and lateral roots failed to develop. When compared to the 0.2 mM Ca treatment, tap root length among solutions maintained at pH 4.0 increased by 2.1 cm $plant^{-1}$ with 2.0 mM Ca, 11.3 cm $plant^{-1}$ with 10 mM Ca, and 13 cm $plant^{-1}$ with 20 mM Ca. Among solutions maintained at pH 4.6, increases in tap root length relative to 0.2 mM Ca were 12, 13.5, and 15 cm $plant^{-1}$ for 2, 10, and 20 mM Ca, respectively. At pH 5.5 there were no significant differences in tap root length among treatments with Ca≥2 mM.

Our results are consistent with the comparisons of growth of soybean tap roots in a vertical split-root system by Lund (1970) who found elongation rates over a 48-h period for solutions with 1.25 µM Ca at pH 5.6 exceeded the rates obtained with either 12.5 µM Ca in solutions with pH 4.75 and 4.5 or 125 µM Ca in solutions with pH 4.0. Noble et al. (1988) reported no response in elongation of soybean tap roots to increasing Ca concentrations from 0.6 to 10 mM in solutions with pH values maintained in the range of 4.2 to 4.8. Other cations in their complete nutrient solutions may have contributed to alleviation of H injury to roots and thus minimized the response to added Ca.

Response in length of lateral roots to increasing Ca supply and solution pH was proportionately greater than for tap roots (Figure 1) and involved an increase in both the number and average length of lateral roots (Table 3). When averaged across Ca treatments, for each unit increase in solution pH the number of lateral roots increased by 51 $plant^{-1}$ ($r^2=0.99$) and their average length by 0.4 cm ($r^2=0.99$).

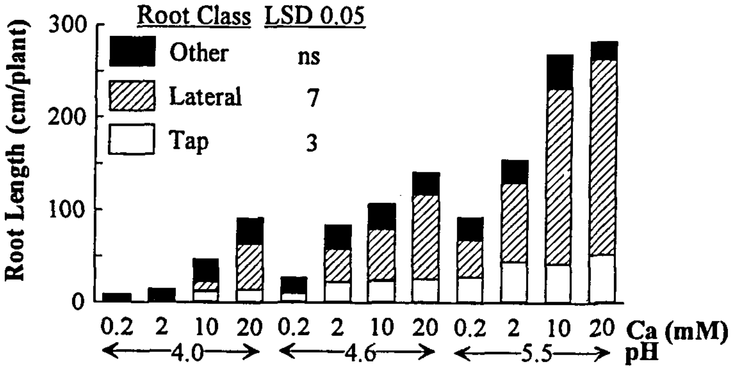


FIGURE 1. Total length as a function of soybean root classes in the subsurface compartment after 12 days of exposure to solution Ca and pH treatments. Least significant difference values are for interaction effects between Ca and pH treatments; ns indicates non significant at $p<0.05$.

For each mM increase in solution Ca, lateral root number increased by 2 plant^{-1} ($r^2=0.92$) and average length increased by 0.05 cm ($r^2=0.90$) when averaged across pH treatments. There were no consistent trends among treatments in average number of lateral roots per unit length of tap root (lateral root density) other than a decline from 2.5 cm^{-1} to 2.1 cm^{-1} with increasing pH levels. Since the average length of a lateral root did not remain constant across treatments, increased total length of lateral roots with increasing solution Ca concentration and pH value could not be solely attributed to increased elongation of tap roots.

Root Length Response Functions

Variations in relative length of both tap and lateral roots among subsurface compartment treatments were nonlinearly related to the Ca:H molar activity ratio (Ca/H) of the solutions, but the functions were different for tap and lateral roots (Figure 2). These relations indicated that maximum length of lateral roots required a greater (Ca/H) value than for tap roots, which is consistent with our observations of greater H inhibition of length in lateral roots than tap roots (Sanzonowicz et al., 1998). Relations between root length and (Ca/H) values also support the observation by Lund (1970) that minimum solution Ca concentrations required for elongation of soybean cv. 'Lee' tap roots depended on the pH of the solution in which roots were grown.

TABLE 3. Measured and calculated characteristics of lateral and other roots at harvest in the subsurface solution compartment as a function of pH and Ca treatments in Experiment 1.

Treatment		Root Number		Average Length[§]		Lateral Root[†]
pH	Ca	Lateral	Other	Lateral	Other	Density
	mM	-number plant^{-1}-		--------cm--------		number cm^{-1}
4.0	0.2	0	17	--	0.45	--
	2	9	14	0.12	0.76	2.7
	10	25	12	0.42	1.92	2.0
	20	39	10	1.28	2.84	2.7
	Mean	18	13	0.61	1.49	2.5
4.6	0.2	11	18	0.30	0.74	1.1
	2	59	11	0.61	2.44	2.7
	10	42	8	1.32	3.58	1.8
	20	68	5	1.35	4.83	2.7
	Mean	45	11	0.90	2.90	2.1
5.5	0.2	71	6	0.56	4.55	2.6
	2	79	6	1.08	4.35	1.8
	10	109	8	1.74	5.02	2.6
	20	121	4	1.74	5.34	2.3
	Mean	95	6	1.28	4.82	2.1
	----------Ca Means----------					
	0.2	27	14	0.29	1.91	1.2
	2	49	10	0.43	2.52	2.4
	10	59	9	1.16	3.51	2.1
	20	76	6	1.46	4.34	2.6
LSD 0.05:						
pH		2	1			
Ca		3	1			
pH x Ca		ns	ns			

[§]Derived from mean treatment values for root length and number.
[†]Number of lateral roots/length of tap root.

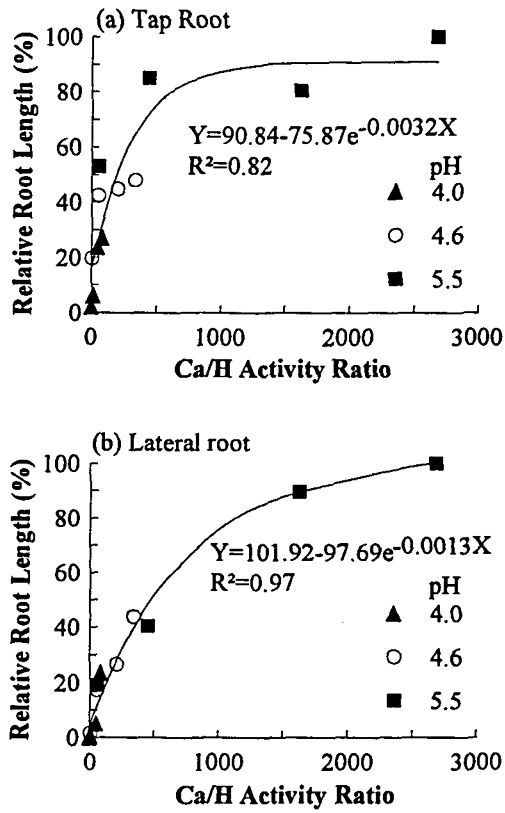


FIGURE 2. Observed (symbols) and predicted (lines) relative length of soybean (a) tap and (b) lateral roots in the subsurface solution compartment as a function of Ca:H molar activity ratios for treatments in Experiment 1.

Effects of Variable Calcium and Aluminum at pH 4.6

Root Growth

The relative contributions of specific root classes to total length of roots in the subsurface compartment were influenced by solution concentrations of both Ca and Al (Figure 3). Among treatments with 2 mM Ca, length of tap roots in solutions with 15 and 30 μM Al decreased by 84 and 88%, respectively, relative to the solution with 7.5 μM Al. Reductions in length of tap roots for similar comparisons among 10 mM Ca treatments was 25% for 15 μM Al and 78% for 30 μM Al. Among treatments with 20 mM Ca, length of tap roots declined only between solutions

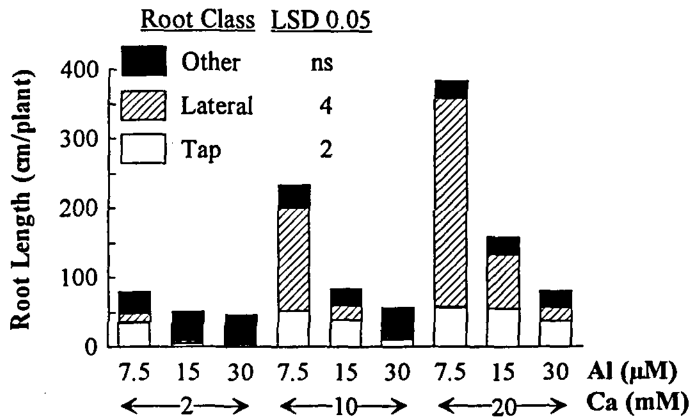


FIGURE 3. Total length as a function of soybean root classes in the subsurface compartment after 12 days of exposure to solution Al and Ca treatments at a fixed pH of 4.6. Least significant difference values are for interaction effects between Al and Ca treatments; ns indicates non significant at $p<0.05$.

with 15 and 30 μM Al. Length of lateral roots exhibited a greater response to Ca and Al treatments than tap roots. At 10 mM Ca, for example, tap root length decreased by a factor of 4.6 between solutions with 7.5 and 30 μM Al, whereas lateral root length decreased by a factor of 44. Increasing Ca concentration from 10 to 20 mM in solutions with 15 μM Al increased tap root length by 39% and lateral root length by 285%.

When averaged across Al treatments, each mM increase in Ca concentration increased lateral root number by 6.3 $plant^{-1}$ plant (r^2=0.98) and their average length by 0.04 cm (r^2=0.98; Table 4). Increasing solution Al concentration increased the number of other roots but decreased their average length in the subsurface compartment, whereas increasing Ca concentration had opposite effects. Consequently, the contribution of the other root class to total root length in the subsurface compartment was constant (32.3 cm $plant^{-1}$) across all treatments (Figure 3). These results are consistent with our previous observations that branching or elongation of existing roots in the surface soil compartment was stimulated with increasing severity of the constraint in root growth of solutions, and that more roots crossed the membrane into the subsurface compartment (Sanzonowicz et al., 1998).

Effects of Treatments on Solution Aluminum

Increasing Ca concentration in solution treatments increased ionic strength and decreased predicted activities of the sum of monomeric Al species (Table 5). The

TABLE 4. Measured and calculated characteristics of lateral and other roots at harvest in the subsurface solution compartment as a function Ca and Al treatments in Experiment 2.

Treatment		Root Number		Average Length[§]		Lateral Root[†]
pH	Ca	Lateral	Other	Lateral	Other	Density
	mM	-number plant^{-1}-		--------cm--------		number cm^{-1}
4.0	0.2	0	17	--	0.45	--
	2	9	14	0.12	0.76	2.7
	10	25	12	0.42	1.92	2.0
	20	39	10	1.28	2.84	2.7
	Mean	18	13	0.61	1.49	2.5
4.6	0.2	11	18	0.30	0.74	1.1
	2	59	11	0.61	2.44	2.7
	10	42	8	1.32	3.58	1.8
	20	68	5	1.35	4.83	2.7
	Mean	45	11	0.90	2.90	2.1
5.5	0.2	71	6	0.56	4.55	2.6
	2	79	6	1.08	4.35	1.8
	10	109	8	1.74	5.02	2.6
	20	121	4	1.74	5.34	2.3
	Mean	95	6	1.28	4.82	2.1
	----------Ca Means----------					
	0.2	27	14	0.29	1.91	1.2
	2	49	10	0.43	2.52	2.4
	10	59	9	1.16	3.51	2.1
	20	76	6	1.46	4.34	2.6
LSD 0.05:						
pH		2	1			
Ca		3	1			
pH x Ca		ns	ns			

[§]Derived from mean treatment values for root length and number.
[†]Number of lateral roots/length of tap root.

TABLE 5. GEOCHEM-predicted ionic strength and activities of Al species, and ferron-reactive Al as influenced by solution Ca and Al concentrations in Experiment 2.

Treatment		Ionic	Predicted Activities				Ferron
Ca	Al	Strength	Al^{3+}	$Al(OH)^{2+}$	$Al(OH)_2^+$	$\sum Al_{mono}$[§]	Al[‡]
mM	µM						
2	7.5	6023	2.7	1.1	0.3	4.1	5.6
	15	6052	5.4	3.0	0.7	9.1	11.7
	30	6109	10.6	4.3	1.3	16.3	23.9
10	7.5	30000	1.4	0.6	0.2	2.2	7.3
	15	30030	2.9	1.2	0.4	4.4	13.5
	30	30090	5.7	2.3	0.7	8.7	25.9
20	7.5	59760	1.0	0.4	0.1	1.5	7.5
	15	59790	2.0	0.8	0.2	3.0	14.0
	30	59860	3.9	1.5	0.5	5.9	27.6

[§] $\sum Al_{mono} = Al^{3+} + Al(OH)^{2+} + Al(OH)_2^{+} + Al(OH)_3^{0} + Al(OH)_4^{-}$.
[‡] Al reacting with ferron by 30s.

TABLE 6. Prediction equations for relative length of tap and lateral soybean roots in the subsurface solution compartment of Experiment 2, based on molar activities of different chemical parameters of the Ca and Al treatments.

Variables		Equation	R^2
Dependent	Independent		
Tap root length	$\sum Al_{mono}$[§]	$Y=13.3+149.5e^{-0.15X}$	0.92
	$Ca^{2+}/\sum Al_{mono}$	$Y=103.2-97.2e^{-0.0008X}$	0.84
	Ca^{2+}-Al Balance[¶]	$Y=48.9-18.2X+2.2X^2-0.06X^3$	0.97
Lateral root length	$\sum Al_{mono}$	$Y=1.5+450.0e^{-1.02X}$	0.99
	$Ca^{2+}/\sum Al_{mono}$	$Y=1.6e^{0.0009X}$	0.79
	Ca^{2+}-Al Balance	$Y=0.11e^{0.30X}$	0.95

[§] $\sum Al_{mono} = Al^{3+} + Al(OH)^{2+} + Al(OH)_2^{+} + Al(OH)_3^{0} + Al(OH)_4^{-}$.
[¶] Ca-Al activity balance $= \{2\log(Ca^{2+}) - [3\log(Al^{3+}) + 2\log(AlOH^{2+}) + \log(Al(OH)_2^{+})]\}$.

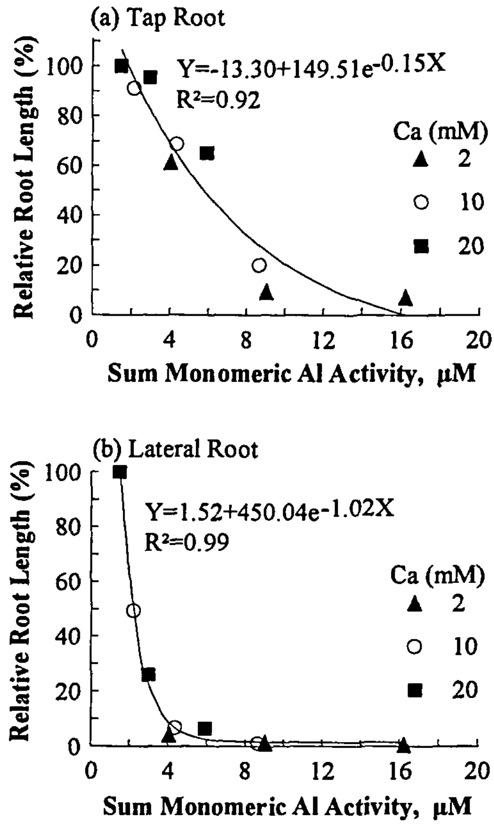


FIGURE 4. Observed (symbols) and predicted (lines) relative length of soybean (a) tap and (b) lateral roots in the subsurface solution compartment as a function of the sum of monomeric Al activities for treatments in Experiment 2.

monomeric species $Al(OH)_3^0$ and $Al(OH)_4^-$ were present at <0.02 μM activity in all treatments. Predominance of the Al^{3+} and $Al(OH)^{2+}$ species is consistent with the fixed solution pH value of 4.6. Although the proportion of monomeric Al in the Al^{+3} form decreased with increasing Ca, collinearity among species was high. All simple correlation coefficients between monomeric Al species were >0.98. Increased values for ferron-reactive Al with increasing Ca concentration suggested a matrix effect on this measurement of solution Al. Mean values of ferron Al among solution Ca levels differed by 20%. Similar cation interferences with Al measurements have been reported (Wright et al., 1987; Davenport, 1949).

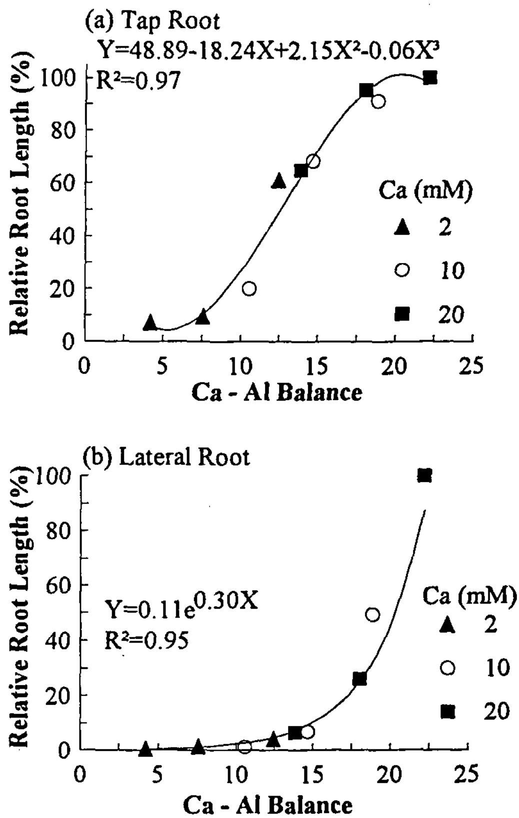


FIGURE 5. Observed (symbols) and predicted (lines) relative length of soybean (a) tap and (b) lateral roots in the subsurface solution compartment as a function of the Ca-Al activity balance for treatments in Experiment 2. Ca-Al activity balance = {2log(Ca) - [3log(Al^{3+}) + 2log($AlOH^{2+}$) + log($Al(OH)_2^{+}$)]}.

Root Length Response Functions

Relative lengths of tap and lateral roots among Ca and Al treatments in the subsurface solution compartment were related to the sum of monomeric Al activities ($\sum Al_{mono}$) (Table 6; Figure 4). The high collinearity among monomeric Al species resulted in similar relations between relative root length and individual monomeric Al species. Inclusion of the variation in Ca activities among treatments, as a Ca/ $\sum Al_{mono}$ molar activity ratio, did not improve prediction of relative root lengths. The Ca effect on root elongation was included in the relation with $\sum Al_{mono}$, since

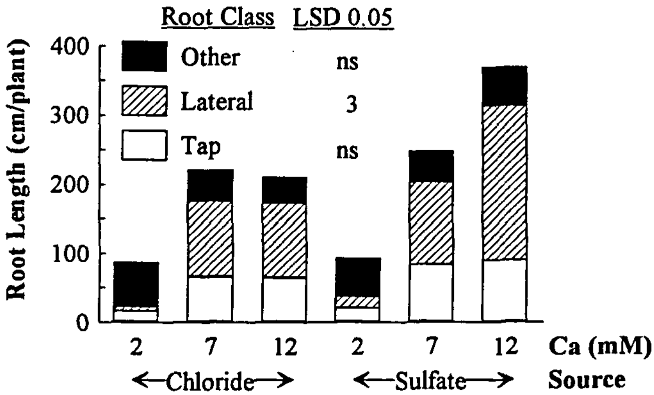


FIGURE 6. Total length as a function of soybean root classes in the subsurface compartment after 12 days of exposure to solutions with 15μM Al, a fixed pH of 4.6, and variable Ca levels supplied as either $CaCl_2$ or $CaSO_4$. Least significant difference values are for interaction effects between Ca level and Ca source treatments; ns indicates non significant at $p<0.05$.

the latter decreased with increasing Ca concentration (Table 5). The $\sum Al_{mono}$ corresponding to a 50% reduction in tap root elongation was 5.7 μM for all solution Ca concentrations. Alva et al. (1986) found that the $\sum Al_{mono}$ associated with a 50% reduction in elongation of soybean tap roots decreased from 12 to 17 μM as however, on 30 min reactions of solution aliquots with an aluminon buffer solution, whereas our estimates of $\sum Al_{mono}$ were calculated by GEOCHEM-PC based on pH values and ionic composition of solutions.

Noble et al. (1988) found that differences in the relative length of soybean tap roots among solutions with variable pH value and concentrations of Al and Ca were related to a log-transformed and valence-weighted balance between activities of Ca and selected monomeric Al species. The formulation of the index, which they defined as Ca-Al balance, was:

$$2\log(Ca) - [3\log(Al^{3+}) + 2\log(AlOH^{2+}) + \log(Al(OH)_2^{+})].$$

Application of this index to our data accounted for similar amounts of variation in relative lengths of tap and lateral roots as did $\sum Al_{mono}$ (Table 6 and Figure 5). The sigmoidal shape of the relation between length of tap roots and Ca-Al balance also was apparent in the data of Noble et al. (1988), but maximum root growth in their study occurred at index values >28.

Relative root length relations with both $\sum Al_{mono}$ (Figure 4) and the Ca-Al balance index (Figure 5) indicate a greater reduction in length of laterals than in tap roots to

TABLE 7. Measured and calculated characteristics of lateral and other roots at harvest in the subsurface solution compartment as a function of Ca levels and sources in Experiment 3.

Treatment		Root Number		Average Length[§]		Lateral Root[†]
Ca	Source	Lateral	Other	Lateral	Other	Density
mM		--number $plant^{-1}$--		--------cm--------		number cm^{-1}
2	Chloride	40	50	0.17	1.29	3.2
7		330	12	0.33	3.69	6.0
12		266	10	0.41	3.81	5.3
	Mean	212	24	0.30	2.93	4.8
2	Sulfate	42	27	0.40	2.04	2.9
7		347	10	0.35	4.35	5.1
12		401	9	0.56	6.09	5.3
	Mean	263	15	0.44	4.16	4.4
		Ca Means				
	2	41	38	0.28	1.66	3.0
	7	338	11	0.34	4.02	5.6
	12	334	10	0.48	4.95	5.3
LSD 0.05:						
Ca		4	2			
Source		ns	ns			
Ca x Source		ns	ns			

[§]Derived from mean treatment values for root length and number.
[†]Number of lateral roots/length of tap root.

a given level of solution Al or Ca:Al ratio. A 50% reduction in lateral root length occurred with 2.2 µM $\sum Al_{mono}$ and a Ca-Al balance index of 20.4. A similar reduction in tap root length required 5.7 µM $\sum Al_{mono}$ and a Ca-Al balance index of 14.0.

Effects of Calcium and Counter Ions on Aluminum Rhizotoxicity

Root Growth

Although elongation of tap and lateral roots with $CaSO_4$ was superior to $CaCl_2$ at 15 µM Al, concentration of Ca had a greater effect on alleviating inhibition of root growth than the Ca source (Figure 6). The combined length of tap and lateral roots averaged across Ca sources increased by 214 cm $plant^{-1}$ between 2 and 12 mM Ca, but averaged across Ca concentrations the difference in the sum of tap and lateral root length between Ca sources was 60 cm $plant^{-1}$. The greater response to Ca concentration than to Ca source in alleviating Al toxicity to root elongation supports previous reports that Al injury to elongation of soybean tap roots was reduced whether Ca was supplied as either $Ca(NO_3)_2$ (Alva et al., 1986) or $CaSO_4$ (Noble et al., 1988).

When averaged across Ca sources, elongation of tap roots increased significantly between 2 and 7 mM Ca, but length was not different between 7 and 12 mM Ca (data not shown). Likewise, number of lateral roots or lateral root density on tap roots did not increase above 7 mM Ca (Table 7). Each mM increase in Ca root by concentration to subsurface solutions increased the average length of a lateral 0.02 cm (r^2=0.92). When averaged across Ca levels, root length of treatments with $CaSO_4$ exceeded those with $CaCl_2$ by 32% for tap roots and 60% for lateral roots (data not shown). Solutions with $CaSO_4$ had 24% more lateral roots, and the average length of these laterals was 47% greater, than in $CaCl_2$ solutions (Table 7). The number of lateral roots per unit length of tap root, however, differed by only 9% between Ca sources. The number of other roots crossing the membrane decreased from 38/plant in solutions with 2 mM Ca to 10 $plant^{-1}$ in solutions with 12 mM Ca. The average length of other roots increased 0.34 cm (r^2=0.94) with each mM increase in Ca concentration in solution. These opposing trends in other root characteristics among solution Ca concentrations led to a constant total length by this root class (50 cm $plant^{-1}$) in all treatments (Figure 6).

Effects of Treatments on Solution Aluminum

Increasing concentrations of both Ca sources in solution increased ionic strength and decreased predicted activities of Al^{3+}, $AlOH^{2+}$ and $Al(OH)_2^+$ (Table 8). The predicted $\sum Al_{mono}$ activities also decreased with increasing $CaCl_2$. With $CaSO_4$ the proportion of monomeric Al in $AlSO_4^+$ and $Al(SO_4)_2^-$ forms increased with solution Ca concentration, but the predicted $\sum Al_{mono}$ activities remained relatively constant. Several investigators have presented evidence that $AlSO_4^+$ is either non-toxic (Pavan and Bingham, 1982; Cameron et al., 1986), or considerably less toxic to root elongation than Al^{3+} (Alva et al., 1991; Kinraide and Parker, 1987; Tanaka et al., 1987). Ferron reactive Al values ranged from 13.2 to 14.7 μM among treatments. Similarities in ferron Al values among $CaSO_4$ treatments are in agreement with previous observations (Kinraide and Parker, 1987; Alva et al., 1989) that Al complexed with SO_4 reacts with ferron to the same extent as other monomeric species.

Root Length Response Functions

Relative length of tap and lateral roots among Ca concentrations and sources were not closely related to activities of individual monomeric Al species or $\sum Al_{mono}$.

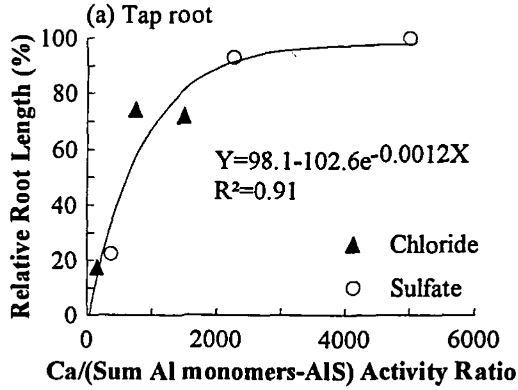
TABLE 8. GEOCHEM-predicted ionic strength and activities of Al species, and ferron-reactive Al as influenced by solution Al and Ca concentrations, and Ca source.

Treatment		Ionic	Predicted Activities¶						Ferron
Ca	Source	Strength	Al^{3+}	$AlOH^{2+}$	$Al(OH)_2^+$	$AlSO_4^+$	$Al(SO_4)_2^-$	$\sum Al_{mono}$§	Al‡
mM			-----	-----	-----	μM	-----	-----	-----
2	Chloride	6052	5.4	3.0	0.7	--	--	9.1	13.2
7		20960	3.4	1.3	0.4	--	--	5.1	13.5
12		36080	2.6	1.0	0.3	--	--	3.9	14.1
2	Sulfate	6842	2.0	0.8	0.3	7.7	0.7	11.5	14.5
7		20750	0.9	0.3	0.1	8.0	1.9	11.2	14.3
12		33800	0.6	0.2	0.1	7.6	2.5	11.0	14.7

¶Predicted activities of $Al(OH)_3^0$ and $Al(OH)_4^-$ were <0.02 μM in all treatments.

§$\sum Al_{mono}=Al^{3+}+Al(OH)^{2+}+Al(OH)_2^{+}+Al(OH)_3^{0}+Al(OH)_4^{-}+AlSO_4^{+}+Al(SO_4)_2^{+}$.

‡Al reacting with ferron by 30s.



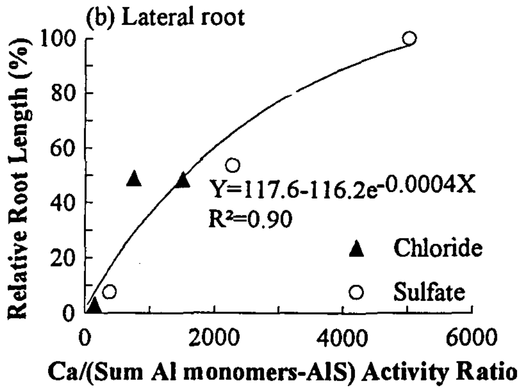


FIGURE 7. Observed (symbols) and predicted (lines) relative length of soybean (a) tap and (b) lateral roots in the subsurface solution compartment for Experiment 3 as a function of the molar activity ratio between Ca and $\sum Al_{mono}$ excluding Al ion pairs with SO_4 (AlS).

Relative lengths of both root classes were related to molar activity ratios between Ca and $\sum Al_{mono}$, provided monomeric Al complexed with SO_4 was subtracted from the latter (Figure 7). The greater sensitivity to Al inhibition by lateral roots than by tap roots is evident in Figure 7 by comparing the Ca-Al activity ratios required to achieve 50% maximum length in each root class. Since collinearity among Al^{3+} and mononuclear hydroxy-Al species was high, the Ca/Al^{3+} molar activity ratio also was closely related to relative lengths of tap and lateral roots among treatments.

Regression equations similar to those in Figure 7 for Ca/Al^{3+} activity ratios had R^2 values of 0.92 for tap roots and 0.89 for lateral roots.

CONCLUSIONS

Results from these experiments indicate that increasing Ca concentration in solutions alleviated the inhibitory effects of H and Al on soybean root elongation. Response in root length to Ca additions decreased with increasing severity of the H or Al constraint, indicating that Ca only alleviates H and Al injury within a given range of these toxic element concentrations. Ionic activity ratios between Ca and either H or monomeric Al were suitable indicators of conditions for a root growth response to added Ca. The need for a higher Ca level to increase length of lateral roots relative to tap roots, at similar levels of toxic H and Al, supports our previous observations that both H and Al affected elongation of lateral roots more by than elongation of tap roots (Sanzonowicz et al., 1998).

REFERENCES

Alva, A.K., D.G. Edwards, C.J. Asher, and F.P.C. Blamey. 1986. Effects of phosphorus/aluminum molar ratio and calcium concentration on plant response to aluminum toxicity. Soil Sci. Soc. Am. J. 50:133-137.

Alva, A.K., G.L. Kerven, D.G. Edwards, and C.J. Asher. 1991. Reduction in toxic aluminum to plants by sulfate complexation. Soil Sci. 152:351-359.

Alva, A.K., M.E. Sumner, Y.C. Li, and W.P. Miller. 1989. Evaluation of three aluminum assay techniques for excluding aluminum complexed with fluoride or sulfate. Soil Sci. Soc. Am. J. 53:38-44.

Bersillion, J.L., P.H. Hsu, and F. Fiessinger. 1980. Characterization of hydroxy aluminum solutions. Soil Sci. Soc. Am. J. 44:630-634.

Cameron, R.S., G.S.P. Richie, and A.D. Robson. 1986. Relative toxicities of inorganic aluminum complexes to barley. Soil Sci. Soc. Am. J. 50:1231-1236.

Cassel, D.K. and D.R. Nielsen. 1982. Field capacity and available water capacity. pp.901-924. In: A.L. Page, R.H. Miller, and D.R. Keeney (eds.), Methods of Soil Analysis. Part I. Agronomy 9. American Society of Agronomy, Madison, WI.

Davenport, W.H. 1949. Determination of aluminium in the presence of iron. J. Anal. Chem. 21:710-711.

Horst, W.J. 1987. Aluminium tolerance and calcium efficiency of cowpea genotypes. J. Plant Nutr. 10:1121-1129.

Kamprath, E.J. 1984. Crop response to lime on soils of the tropics. pp .349-368. In: F. Adams (ed.), Soil Acidity and Liming. Agronomy 12. American Society of Agronomy, Madison, WI.

Kinraide, T.B. and D.R. Parker. 1987. Non-phytotoxicity of aluminum sulfate ion, $AlSO_4^+$. Physiol. Plant. 71:207-212.

Lund, Z.F. 1970. The effect of calcium and its relation to several cations in soybean root growth. Soil Sci. Soc. Am. Proc. 34:456-459.

Marschner, H. 1991. Mechanisms of adaptation of plants to acid soils. Plant Soil 134:1-20.

Moore, D.P. 1974. Physiological effects of pH on roots. pp. 135-151. In: E.W. Carson (ed.), The Plant Root and its Environment. University Press of Virginia, Charlottesville, VA.

Noble, A.D., M.V. Fey, and M.E. Sumner. 1988. Calcium-aluminum balance and the growth of soybean roots in nutrient solutions. Soil Sci. Soc. Am. J. 52:1651-1656.

Parker, D.R., W.A. Norvell, and R.L. Chaney. 1995. GEOCHEM-PC: A chemical speciation program for IBM and compatible personal computers. pp. 253-269. In: R.H. Loeppert, A.P. Schwab, and S. Goldberg (eds.), Soil Chemical Equilibrium and Reaction Models. Soil Science Society of America, Madison, WI.

Pavan, M.A. and F.T. Bingham. 1982. Toxicity of aluminum to coffee seedlings grown in nutrient solution. Soil Sci. Soc. Am. J. 46:993-997.

Runge, M. and M.W. Rode. 1991. Effects of soil acidity on plant associations. pp. 183-202. In: B. Ulrich and M.E. Sumner (eds.), Soil Acidity. Springer-Verlag, New York, NY.

Sanzonowicz, C., T.J. Smyth, and D.W. Israel. 1998. Hydrogen and Al inhibition of soybean root extension from limed soil into acid subsurface solutions. J. Plant Nutr. 21:387-403.

Tanaka, A., T. Toshiaki, K. Yamamoto, and N. Kanamura. 1987. Comparison of toxicity to plants among Al^{3+}, $AlSO_4^+$, and Al-F complex ions. Soil Sci. Plant Nutr. 33:43-55.

Wright, R.J. and S.F. Wright. 1987. Effects of aluminum and calcium on the growth of subterranean clover in Appalachian soils. Soil Sci. 143:341-348.

Wright, R.J., V.C. Baligar, and S.F. Wright. 1987. Estimation of phytotoxic aluminum in soil solution using three spectrophotometric methods. Soil Sci. 144:225-232.