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Australian Journal of Soil Research

Volume 36, 1998

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Australian Academy of Science

Aust. J. Soil Res., 1998, **36**, 913–19

Effects of humic and fulvic acids on the rhizotoxicity of lanthanum and aluminium to corn

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Abstract

Effects of varying lanthanum (La) or aluminium (Al) concentrations (0–30 μM) on corn (*Zea mays* L.) root elongation were examined in the presence and absence of (*i*) humic acid (HA) at 35 mg carbon (C)/L, or (*ii*) fulvic acid (FA) at 15 mg C/L, using dilute nutrient solutions. The organic acids were extracted from a mixture of decomposed grass (*Sorghum halepense*) and lucerne (*Medicago sativa*) hay. In the absence of added HA or FA, the addition of La at $\geq$5 μM and Al at 30 μM was toxic to the root growth of corn. The rhizotoxic effects of La at 5 and 10 μM were negated by HA. The ability of FA to overcome La rhizotoxicity was much less, significantly ameliorating the toxic effects of 5 μM La but not those of 10 or 30 μM La. HA and FA did not precipitate La from solution. Both organic acids ameliorated Al toxicity by complexing Al and reducing monomeric Al in solution.

It is concluded that concentrations of HA and FA, commonly present in soil solutions, are capable of forming non-rhizotoxic complexes with La, hence plant tolerance to La in the soil solution may be appreciably higher than would be indicated by results of solution culture experiments in which these ligands are not present.

Additional keywords: Al, La, root length, root growth, trivalent cation, toxicity.

Introduction

Aluminium (Al) and the rare earth element (REE) lanthanum (La), both trivalent cations, have been shown to be highly toxic to plants. Brady *et al.* (1990) showed that 2 μM Al in solution caused significant reductions in the root growth of soybean (*Glycine max*). Diatloff *et al.* (1995*a*) found that 3·1 μM La caused a 50% reduction in the root growth of mungbean (*Vigna radiata*), and that 4·8 μM La caused a similar reduction in the root growth of corn (*Zea mays* L.). La at 5 μM has been reported to reduce the growth of wheat (*Triticum aestivum* L.) roots by 50% (Delhaize *et al.* 1993). In flowing solution culture studies, Diatloff *et al.* (1995*b*) found that as little as 0·4 μM La could cause a significant reduction in whole plant yields of mungbean.

Increases in Al solubility are associated with decreases in soil pH and often lead to Al toxicity, a serious yield-limiting factor in many acidic soils (Wright 1989). Computer modelling studies indicate that the solubility of La also increases with decreasing pH (Diatloff *et al.* 1993), and La concentrations measured in the

10.1071/SR98018 0004-9573/98/060913$05.00

solutions extracted from some acidic Australian soils exceeded the 0·4 μM threshold for toxicity to mungbean (Diatloff *et al.* 1996). However, it is well established that the phytotoxicity of Al can be alleviated by the formation of complexes with humic acid (HA) and fulvic acid (FA) (Bartlett and Riego 1972; Hue *et al.* 1986; Suthipradit *et al.* 1990; Harper 1994; Harper *et al.* 1995). There is evidence that REEs also form strong complexes with HA and FA (Torres and Choppin 1984; Ephraim *et al.* 1989; Bidoglio *et al.* 1991*a*, 1991*b*), but it is not known whether the formation of such complexes can overcome the phytotoxic effects of La.

This paper reports the results of experiments in which effects of HA and FA on the toxicity of La and Al to corn roots were examined.

Materials and methods

Two experiments were conducted in 2-L plastic buckets immersed in a constant temperature waterbath (27°C) located in a glasshouse in Brisbane, Queensland (27°28′ S, 152°55′ E). The clean, 2-L polythene buckets were filled with 1·9 L of deionised water and covered with plastic lids, and aeration tubes were inserted. Stock solutions were then added to achieve the following basal nutrient solution concentrations (μM): N 1350 (nitrate 1250 plus ammonium 100), Ca 500, K 250, Mg 200, S 200, Fe 10, B 3, P 1, Zn 1, Mn 0·5, Cu 0·1, Co 0·04, and Mo 0·02. HA and FA were extracted from a mixture of decomposed grass (*Sorghum halepense*) and lucerne (*Medicago sativa*) hay and concentrated, and stock solutions were prepared (Harper 1994). To remove adsorbed cations, FA was passed through a column packed with Sigma Dowex macroporous cation exchange resin (hydrogen form), and HA was twice dialysed against triple-deionised water. The dried FA and HA contained <50 μg/g of Mg, Ca, Fe, Cu, and Mn, <20 μg/g of Al, and <1 μg/g of La as measured by inductively coupled plasma-mass spectrometry (ICP-MS). Aliquots of HA (Expt 1) and FA (Expt 2) stock solution were added to appropriate buckets to establish organic carbon (C) concentrations of 35 mg C/L HA and 15 mg C/L FA. Solution pH was adjusted to 4·5 by drop-wise addition of either 1 M KOH (HA, Expt 1) or HNO_3 (FA, Expt 2). Appropriate volumes of 2 mM $La(NO_3)_3$ or 30 mM $AlCl_3$ stock solutions were added in a drop-wise manner to impose treatments of 0, 5, 10, and 30 μM La, and 0, 10, and 30 μM Al. The treatments were replicated 3 times. The nutrient solutions were equilibrated for 24 h prior to sampling for chemical analysis and planting of seedlings.

Solutions were sampled at a depth of 50 mm and analysed for total La by ICP-MS, total Al by inductively coupled plasma-atomic emission spectrometry (ICP-AES), and monomeric Al by the colorimetric procedure of Kerven *et al.* (1989). Samples analysed for La were ultra-filtered to 0·025 μm according to Menzies *et al.* (1991) prior to analysis. C was measured in the nutrient solutions by ICP-AES after the samples had been acidified and purged for 20 min with nitrogen gas to remove dissolved CO_2 (Oweczkin *et al.* 1995).

Corn seeds (*Zea mays* L. cv. DK687) were rinsed with tap water to remove fungicide and soaked for 30 min in a solution containing 200 μM $CaSO_4$ and 50 μM H_3BO_3. The seeds were placed in rolled cloth towels moistened with $CaSO_4/H_3BO_3$ solution and germinated at 28°C. One corn seedling with roots 40 mm long was transplanted into each of 5 plastic cups supported in the lid of each bucket. The transplanted seedlings were covered with black polythene beads and allowed to grow for 4 days before the length of the longest root was measured.

Results

Effects of HA and FA on La and Al concentrations measured in solution

The concentrations of C in the nutrient solutions prior to planting the corn seedlings were 35±4 mg C/L in the HA experiment and 15±3 mg C/L in the FA experiment. The addition of HA or FA had no effect on the total concentrations of La or Al measured in solution (Table 1). This indicates that the addition of HA and FA did not precipitate La or Al from solution. The addition of HA and

FA decreased monomeric Al concentrations in solution. HA was more effective than FA in decreasing the concentration of monomeric Al solution.

Table 1. Effects of humic acid (HA) and fulvic acid (FA) on the measured concentrations (±s.e.) of total lanthanum (La), total aluminium (Al), and monomeric Al in nutrient solutions to which 0, 5, 10, or 30 μM of either La or Al had been added

	Total La (μM)				Al (μM)					
	0	5	10	30	Total			Monomeric		
					0	10	30	0	10	30
Experiment 1										
Control	<0·01	5·2	12·0	32·1	<0·1	12·0	29·4	<0·1	7·9	26·9
		±0·3	±1·3	±3·0		±1·2	±2·5		±0·5	±2·1
+HA	<0·01	5·0	11·0	29·2	<0·1	11·0	31·4	<0·1	2·8	3·3
		±0·2	±1·4	±3·1		±1·0	±3·0		±0·1	±0·1
Experiment 2										
Control	<0·01	5·1	11·2	33·8	<0·1	10·5	30·0	<0·1	7·9	24·0
		±0·3	±1·5	±3·5		±1·0	±2·0		±0·4	±2·0
+FA	<0·01	5·5	11·0	32·6	<0·1	10·3	30·2	<0·1	4·8	8·4
		±0·4	±1·3	±2·9		±0·9	±2·6		±0·2	±0·6

Effects of HA, La, and Al on corn root length

In the absence of HA, the addition of La at 5, 10, or 30 μM significantly reduced corn root length compared with control plants, 30 μM La being significantly more rhizotoxic than lower concentrations (Fig. 1*a*). Without HA addition, 30 μM Al significantly reduced corn root length, whereas 10 μM Al significantly enhanced root growth compared with control plants. La was more rhizotoxic than Al at equivalent nominal concentrations. In the presence of La, the addition of HA increased corn root growth above that of plants grown in the presence of La alone. This ameliorative effect of HA was greater at low solution concentrations of La. The reduction in root growth when the concentration of Al was increased from 10 to 30 μM was overcome by the addition of HA. In the absence of Al or La, the addition of HA significantly enhanced corn root length.

Effects of FA, La, and Al on corn root length

In the absence of FA, the rhizotoxic effects of La and Al were similar to those observed in the absence of HA (Fig. 1*b*). Thus, 5, 10, and 30 μM La, and 30 μM Al, significantly reduced corn root length relative to the control treatment. As shown in the HA experiment, La was more toxic than Al at equivalent nominal concentrations. In contrast to HA, FA addition significantly reduced corn root length in the absence of added La or Al. The addition of FA to La-treated plants ameliorated rhizotoxicity, the greatest effect being observed in the 5 μM La treatment. The addition of FA and 30 μM Al resulted in the length of corn roots exceeding that of control plants.

Discussion

Effects of HA and FA on La and Al concentrations measured in solution

The addition of HA at 35 mg C/L and FA at 15 mg C/L reduced the concentration of monomeric Al measured in solution (Table 1). This reduction in

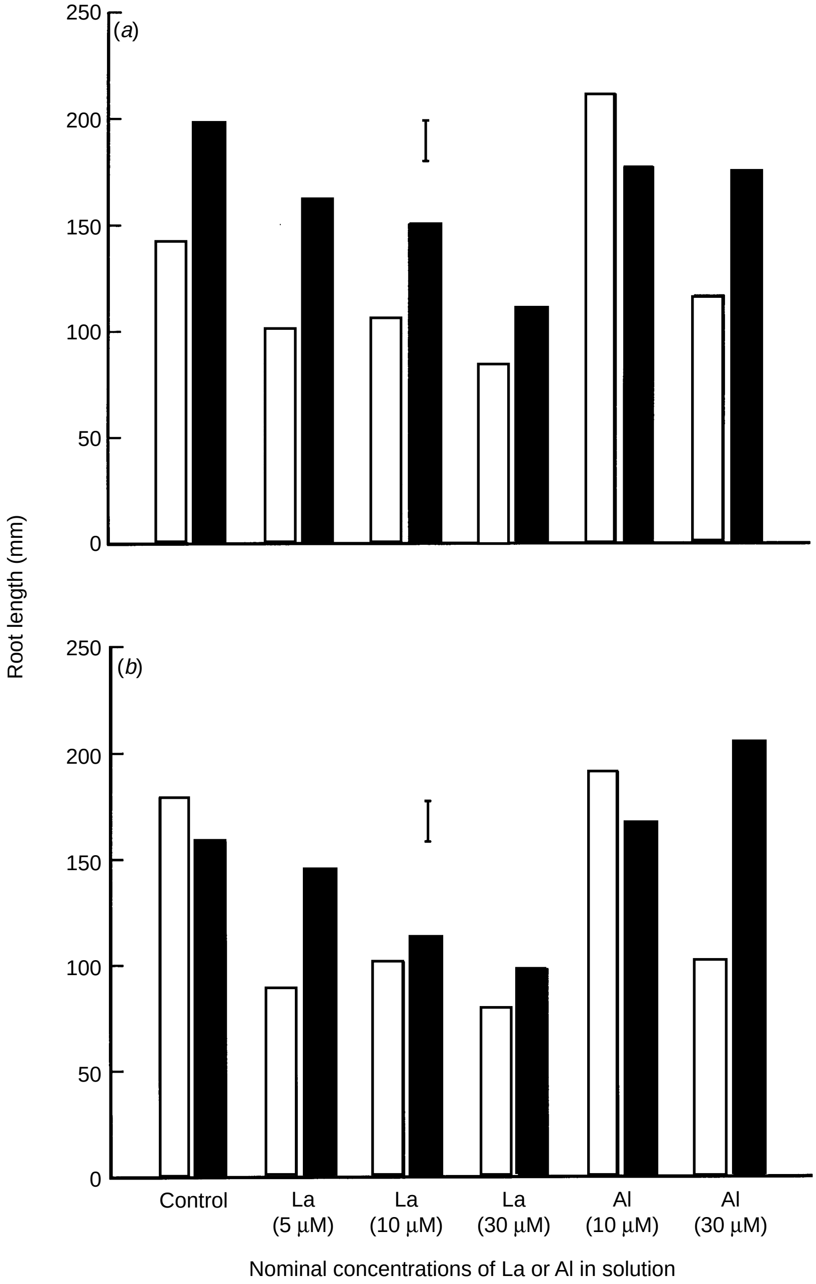

Fig. 1. Effects of the addition or omission of (*a*) humic acid (35 mg carbon/L) or (*b*) fulvic acid (15 mg carbon/L) to nutrient solutions on the root length of corn seedlings grown for 4 days in nutrient solutions containing varying initial concentrations of lanthanum (La) and aluminium (Al). Controls (□); humic or fulvic acid (■); capped lines are l.s.d. at $P = 0 \cdot 05$.

monomeric Al by HA and FA has been reported previously and is attributed to the ability of the organic acids to complex Al (Bartlett and Riego 1972; Hue *et al*. 1986; Suthipradit *et al*. 1990; Harper 1994; Harper *et al*. 1995). Rhizotoxic monomeric Al species can be discriminated from other less toxic Al species, in solution, using a short-term pyrocatechol violet colorimetric method (Kerven *et al*. 1989).

Although analytical methods designed for measuring monomeric Al in solution are available, similar colorimetric methods for monomeric La determinations in solution have not been developed. Computer simulations using GEOCHEM-PC predicted that in dilute complete nutrient solutions the major soluble forms of La would be La^{3+} and $LaSO_4^+$ (Diatloff *et al*. 1993). In the present study, the total concentration of La in solution as measured by ICP-MS did not alter with the addition of HA or FA to the nutrient solutions (Table 1), showing that all of the added La remained in solution.

Effects of HA and FA on La and Al rhizotoxicity

La concentrations of 5, 10, and 30 μM in solution were rhizotoxic to corn, in the absence of HA or FA (Fig. 1). Toxic effects of similar concentrations of La in solution have been observed on the root elongation of corn, mungbean, and wheat (Diatloff *et al*. 1995*a* and references within). The present experiments demonstrated that HA and FA can ameliorate the rhizotoxic effects of La on corn. The addition of HA to the nutrient solution containing 5 or 10 μM La restored root elongation of corn to that seen in controls (Fig. 1). The addition of FA also ameliorated the rhizotoxic effects of La. The ability of FA to detoxify La was much less than that of HA (Fig. 1) and may simply reflect the lower concentrations of FA (15 mg C/L) added to the nutrient solutions than HA (35 mg C/L). Notwithstanding, Harper (1994), using the same FA and HA, further demonstrated that HA at the same concentration as FA was more effective at complexing Al.

The ameliorative effects of both organic acids on La toxicity were not due to the precipitation of La from solutions, the addition of HA and FA having no effect on the La concentrations measured in solution (Table 1). The ability of both organic acids to detoxify La is most likely due to their capacity to complex La. For HA, it is possible, but less likely that the ameliorative effect may be independent of La toxicity as the stimulatory effect on root growth was similar at 0, 5, and 10 μM La. Notwithstanding, both HA and FA have been shown to form strong complexes with REE such as europium and terbium (Torres and Choppin 1984; Ephraim *et al*. 1989; Bidoglio *et al*. 1991*a*, 1991*b*). The present experiments showed such HA and FA complexes with La to be non-phytotoxic or at least less toxic that free uncomplexed La.

Both HA and FA ameliorated the toxic effects of Al on corn root length (Fig. 1). These results are in agreement with other studies also showing the reduced toxicity of organically complexed Al relative to inorganic monomeric Al ions (Bartlett and Riego 1972; Hue *et al*. 1986; Suthipradit *et al*. 1990; Harper 1994; Harper *et al*. 1995). The beneficial effects of 10 μM Al and those of HA in the absence or presence of Al are consistent with those recorded and discussed in more detail by Harper (1994) and Harper *et al*. (1995).

Extracted soil solutions often contain substantial concentrations of organic C. Menzies (1991) surveyed over 40 acid soils and found that total soluble C in soil solutions ultra-filtered to 0·025 μm varied from <40 to >600 mg/L. This range encompasses the 35 and 15 mg C/L HA and FA, respectively, used in the present study. The concentrations of REEs in soil solutions are much less studied. A survey of 19 unamended soils indicated that the concentrations of La in ultra-filtered (0·025 μm) soil solutions ranged from <0·007 to 0·13 μM (Diatloff *et al.* 1996). However, the addition of $CaSO_4$, increased the concentrations of La in soil solution, the highest concentration measured being 1·4 μM. If the soluble C commonly present in soil solutions forms complexes with La, it is possible that a large proportion of the low concentrations of La present in soil solution would be organically complexed. Our results suggest that such complexes have lower phytotoxicity than uncomplexed La. Hence, plants may be tolerant of higher concentrations of REEs in soil solution than would be indicated by results of solution culture experiments in which these ligands were not present.

Acknowledgments

The authors thank Hans Simons for assistance with ICP-MS analyses.

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Manuscript received 26 February 1998, accepted 6 July 1998