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Effects of organic acid fractions extracted from *Eucalyptus camaldulensis* leaves on root elongation of maize (*Zea mays*) in the presence and absence of aluminium

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Abstract

Complexes of aluminium (Al) with organic ligands are believed to represent an important detoxification mechanism in acid soils. However, relatively little is known about the particular ligands produced by decomposing vegetation or about their effects on plant growth in the presence or absence of toxic Al. This paper reports an experiment on the effects of decomposition products of *Eucalyptus camaldulensis* leaves on the root elongation of maize (*Zea mays*) cv. DK687 in the presence or absence of Al. The static solution culture experiment used fulvic acid (FA) and humic acid (HA), extracted from *E. camaldulensis* leaves, at three nominal concentrations, viz. 40, 120 and 360 mg C L^{-1}, replicated 4 times in the presence and absence of 30 μM Al. In the absence of Al, root elongation was increased by 30% by HA at 40 mg C L^{-1} and by 36% by FA at 120 mg C L^{-1}. In the presence of 30 μM Al, the effects of toxic Al on root elongation were negated by FA and HA at all concentrations. Aluminium was totally complexed in all treatments except FA at 40 mg C L^{-1} in which treatment only 2.7 μM Al was present in the monomeric form. The *E. camaldulensis* FA and HA at concentrations of 40 and 120 mg C L^{-1}, either in the presence or absence of Al, stimulated maize root elongation. Aluminium was strongly complexed by the *E. camaldulensis* FA and HA. The present results, in which FA and HA alleviated Al toxicity limitations on root elongation of maize, are relevant to the protection afforded to plant growth in acid soils amended with organic materials. They highlight the need to focus more on the role of FA and HA.

Introduction

The ability of both low and high molecular weight organic acids to complex aluminium (Al) is well documented. The high molecular weight fulvic acid (FA) and humic acid (HA) form much more stable complexes with Al than the low molecular weight organic acids, such as citrate and oxalate. Ritchie et al. (1988) showed that HA was superior to simple carboxylic acids in complexing Al, while Hargrove and Thomas (1981) showed that Al was so strongly bound to humic material (peat) that treatment with 2 M HCl was necessary to desorb it. Fulvic acid and HA are less susceptible to microbial degradation than low molecular weight acids and represent a potential means by which a more permanent amelioration of Al toxicity may be effected.

Various authors have used solution or sand culture to show that FA and HA detoxify Al (e.g. Suthipradit et al., 1990; Tan and Binger, 1986). In a pot study using an Al-toxic Ultisol, Hue and Amien (1989) showed that ground leafy amendments of cowpea (*Vigna unguiculata*), leucaena (*Leucaena leucocephala*) and guinea grass (*Panicum maximum*) at rates of application from 5 to 20 g kg^{-1} alleviated the toxic effects of Al. The detoxification of Al in that study was attributed to increased pH and the consequent reduction in monomeric Al concentration, plus the complexation of Al by organic molecules, notably low molecular weight aliphatic and aromatic acids. Bessho and Bell (1992) arrived at a similar conclusion from their pot trial with an Al-toxic Ultisol. Concentrations of FA and HA in the range of 25–250 mg C L^{-1} have been shown to stimulate root elongation (Linehan, 1976; Rauthan and Schnitzer, 1981; Schnitzer and Poapst, 1967; Vaughan and Linehan, 1976). However, Tadano et al. (1992) noted an inhibitory effect of canal waters drained from peat on rice (*Oryza sativa*) root elongation. They also showed that addition of a toxic Cu level to the toxic canal water resulted in no inhibition of rice root elongation, presumably because of the formation of a non-phytotoxic Cu-organic complex.

The present paper reports effects of FA and HA extracted from decomposing leaves of *E. camaldulensis* on root elongation of maize (*Zea mays*) in the presence and absence of Al.

Material and methods

Preparation and extraction of Eucalypt organic acids

Leaf material of *E. camaldulensis* was collected from Gympie State Forestry Research Station. One kg of the air dry material was aerated continuously in 15 L of water in each of five 25-L plastic trays for 1 month from 10 August 1992. The trays were maintained in a temperature controlled water bath at 30 °C. The solution was sampled daily for the first 10 days and subsequently every third day to monitor changes in the high molecular weight organic acids.

On 14 September 1992, a bulk extraction was conducted. Thirty L of extract was successively filtered through 800, 400, 200 and 45 μm nylon screens. The filtrate was then centrifuged at 20, 000 g for 20 min to remove microparticulate and microbial matter. The supernatant was decanted and acidified to pH 2.0 to precipitate the HA component. This suspension was then centrifuged at 10, 400 *g* for 15 min. The HA precipitate was redissolved in 0.1 *M* KOH, acidified to pH 2.0, and centrifuged to remove any FA present. The supernatant containing the non-HA components was passed through a 640 mL capacity glass column (300 × 52 mm) packed with Amberlite XAD-7 non-ionic polymeric resin conditioned to pH 2.0. The solution was pumped at a flow rate of 10 mL min^{-1} using a Masterflex pump (model 7015–20). The adsorbed FA was eluted with 0.1 *M* KOH.

The HA and FA extracts were dried in a vacuum oven at 55 °C and a pressure of 40 mm Hg. Industrial grade dry N_2 gas was bled into the oven.

Root elongation studies

Clean plastic buckets were near-filled with 1.9 L of deionised water, covered with plastic lids and placed in water baths at 27 °C. Stock nutrient solutions were added to achieve the following basal nutrient solution concentrations (μM): N 1350 (NO_3 1250 and NH_4 100), 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 (Kerven et al., 1991). Aliquots of HA or FA solution were added to appropriate pots to establish nominal organic carbon concentrations [OC] of 40, 120 and 360 mg L^{-1}. A control treatment with no organic acid addition was also set up. Eight pots of each treatment, including the control, were prepared. Four of each set of eight pots received $AlCl_3$ to give a total Al concentration of 30 μM. Thus, the experiment consisted of 14 treatments (6 organic acids plus 1 control × 2 Al concentrations) replicated 4 times for a total of 56 pots. Solution pH in all pots was adjusted to 4.5 by drop-wise addition of either 1 *M* KOH or HNO_3. Aeration tubes were placed in each pot and solutions were equilibrated for 1 day.

One thousand maize seeds were rinsed with tap water to remove fungicide and soaked in a 200 μM $CaSO_4$ plus 50 μM H_3BO_3 solution for 30 min. The seeds were rinsed three times with tap water and germinated at 28 °C using a rolled cloth towel moistened with tap water.

When the average radicle length was 30–50 mm, a seedling was placed in each of five plastic cups supported in the lid of each pot; the transplanted seedlings were covered with black polythene beads. After 4 days, all plants were harvested and the length of the longest root was determined. Prior to planting, a 10 mL solution sample was taken from each pot and analysed for monomeric Al concentration (Kerven et al., 1989); [OC] and total [Al] were measured using ICPAES. Root elongation was expressed relative to that in the Control-Al treatment.

Results and discussion

In the absence of Al, HA at 40 mg C L^{-1} and FA at 120 mg C L^{-1} both stimulated root elongation over that of the Control-Al plants by 30% and 36% respectively (Table 1). All other treatments had no significant effect in the absence of Al; in fact, HA at 360 mg C L^{-1} tended to reduce root elongation relative to the Control-Al treatment. The stimulation of root elongation by FA was significantly greater than that by HA in the presence of 120 and 360 mg C L^{-1}. Furthermore, there is evidence that the stimulation of root elongation by a low concentration of HA is superseded by a reduction in root elongation at the highest concentration (Table 1).

The mechanism by which FA and HA stimulated root elongation in the absence of Al was not investigated in this study; however, other workers have suggested possible mechanisms. Mato et al. (1972a,

Table 1. Relative root lengths of maize cv. DK687 plants grown in nutrient solutions containing fulvic or humic acid fractions in the presence and absence of 30 μM Al

Organic acid	Concn. (mg C L^{-1})	Relative root length[a] -Al	+Al
Control	0	1.00	0.63
FA	40	1.12	1.29
	120	1.36	1.46
	360	1.17	1.10
HA	40	1.30	1.34
	120	1.10	1.21
	360	0.90	0.94

[a]LSD (p = 0.05) = 0.21 for relative root length.

Table 2. Total and monomeric Al concentrations (μM) in nutrient solutions containing fulvic or humic acid fractions and Al

Organic acid	Concn. (mg C L^{-1})	Total [Al]	Monomeric [Al]
Control	0	27.3	18.6
FA	40	27.7	2.7
	120	30.2	ND[a]
	360	32.8	ND
HA	40	28.8	0.2
	120	31.5	ND
	360	33.1	ND

[a]ND = not detected.

b) have shown that FA and HA at concentrations as low as 5 mg C L^{-1} directly affect enzymatic functions in plants. They showed FA and HA at concentrations between 0 and 10 mg C L^{-1} increased the activity of indoleacetic acid oxidase (IAA oxidase), whilst at concentrations between 10 and 40 mg C L^{-1} the activity of IAA oxidase was decreased. Vaughan and Linehan (1976) suggested that FA and HA may have a direct biochemical or hormonal function, whilst Maggioni et al. (1987) have indicated that FA and HA can influence nutrient uptake via an enzymatic effect, viz. through the activity of K^+ and Mg^{2+} dependent ATPase.

The relative root length of plants grown in the Control+Al treatment was 0.63, the lowest for all treatments (Table 1). The measured monomeric Al concentration was 18.6 μM (Table 2). In all treatments which received both organic acid and 30 μM Al, relative root length was significantly greater than that in the Control+Al treatment (Table 1). At all FA and HA concentrations, with the exception of FA at 40 mg C L^{-1}, all of the added Al remained in solution, but in a totally complexed form (Table 2). There were no significant differences in relative root length between the + and - Al treatments in the presence of FA and HA at any concentrations. However, in the presence of Al, FA and HA at 40 and 120 mg C L^{-1} resulted in better root growth than that in the Control-Al alone. A comparison of the FA and HA at 40 mg C L^{-1} in the presence of Al suggested that HA was a better complexer of Al than was FA (Table 2). The FA and HA concentrations studied in this paper are considered realistic in relation to those likely to occur in the soil solutions of acid soils. Thus, Kerven et al. (1994) reported that the sum of FA and HA concentrations in soil solutions from acid soils amended with organic matter ranged from 30 to 110 mg C L^{-1}.

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