

## Influence of soil solution aluminum on root elongation of wheat seedlings

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### Abstract

Wheat (*Triticum aestivum* L.) seedlings were grown for 4 days in an acid soil horizon treated with 10 levels each of  $\text{Ca}(\text{OH})_2$ ,  $\text{CaSO}_4$  and  $\text{CaCl}_2$ . The treatments resulted in a wide range of Al levels and Al speciation in soil solution. Seedling root length in the  $\text{Ca}(\text{OH})_2$  treatments was significantly related ( $p < 0.01$ ) to calculated  $\text{Al}^{3+}$  activity in soil solution. The Al- $\text{SO}_4$  complex in soil solution had a negligible effect on the root growth of Hart wheat, thus confirming the previously reached conclusion concerning the non-phytotoxicity of Al- $\text{SO}_4$ . The short-term seedling root growth technique used in this investigation allowed for separation of Al effects on root elongation from those on plant nutrition and should be useful for studying Al toxicity relationships in soil.

### Introduction

Aluminum in aqueous systems exists in a variety of forms, some of which have biological toxicity. Solution culture techniques have been employed to study the basic relationships between Al speciation and root elongation (Alva *et al.*, 1986; Cameron *et al.*, 1986; Kinraide and Parker, 1987a). Recent work using solutions containing only mononuclear Al suggest that while  $\text{Al}^{3+}$  is not necessarily the sole toxic species, its activity is the best single indicator of Al stress (Parker *et al.*, 1988).

Several investigators (Adams and Hathcock, 1984; Pavan *et al.*, 1982; Wright *et al.*, 1987) have attempted to relate activities of Al species in soil solution to root growth limitations. These results have generally been less satisfactory than those obtained under carefully controlled solution culture conditions. Acid soil limitations to root growth may involve both nutritional effects and direct effects on root elongation. Short-term (2–4 day) bioassay techniques using root development of seedlings in soil have been used to identify soils with Al toxicity problems (Karr *et al.*, 1984; Ritchey *et al.*, 1983). In these methods the seed supplies

the nutrients and only Al toxicity and Ca and B deficiencies limit seedling root elongation. These short-term methods may be useful for studying Al toxicity relationships in soil solution. The current investigation was undertaken to determine the feasibility of using these methods in Al phytotoxicity studies by evaluating the root growth response of wheat seedlings (*Triticum aestivum* L.) to changes in Al speciation in soil solution.

### Materials and methods

The soil used in the investigation was the BA horizon of the Lily (fine-loamy, siliceous, mesic Typic Hapludult) soil series. After collection, the soil was air-dried and passed through a 2-mm screen. The soil had the following chemical properties: pH (1:1  $\text{H}_2\text{O}$ ), 4.6; exchangeable Ca, 0.08  $\text{cmol}(+) \text{kg}^{-1}$ ; and exchangeable Al, 2.88  $\text{cmol}(+) \text{kg}^{-1}$ . The cation exchange capacity, determined by summation of exchangeable bases (Thomas, 1982), plus exchangeable acidity (Yuan, 1959) was 3.29  $\text{cmol}(+) \text{kg}^{-1}$ .

### Experiment 1

Experiment 1 was designed to evaluate the influence of soil solution Al level and speciation on the root elongation of three wheat cultivars of differing Al tolerance. Calcium hydroxide was added to the soil to give Ca at rates of 0, 0.025, 0.05, 0.1, 0.2, 0.4, 0.8, 1.6, 3.2, and 6.4 cmol(+) kg<sup>-1</sup>. The Ca(OH)<sub>2</sub> was mixed with the soil, water was added to bring the moisture content to a level corresponding to 33 kPa tension, and the soil treatments were taken through two wetting and drying cycles over a 2 week period. One day prior to planting, the soil treatments were remoistened to a level corresponding to 33 kPa tension.

Two hard red spring wheats ('Yecora Rojo' CI 17414 - U. of Cal. Foundation Seed and 'Wampum' CI 17691 - Washington State Crop Imp. Association) and one soft red winter wheat ('Hart' - Devine Seed Co., Yellow Springs, OH) were used in the investigation. Details of the method have been reported previously (Ritchey *et al.*, 1988). Briefly, the method consisted of planting five pregerminated seeds in 200 ml plastic cups containing moist soil. There were three replications for each lime level-cultivar combination in a randomized complete block design. The cups were placed on trays containing moist paper towels and covered with a plastic dome so that watering would not be required during the 4 day growth period. The containers were kept in a growth chamber at the following conditions: 21°C, 12 hour day length, and

illumination of 115 μmol m<sup>-2</sup> s<sup>-1</sup>. At harvest the longest root and the total root system length of each seedling were measured.

### Experiment 2

Experiment 2 was designed to test the phytotoxicity of the AlSO<sub>4</sub> complex in soil solution. Treatments consisted of 10 levels each of CaCl<sub>2</sub> and CaSO<sub>4</sub> at rates which gave amounts of Ca comparable to those added with Ca(OH)<sub>2</sub> in Experiment 1. Experimental conditions and measurements were as described for Experiment 1 except that only Hart wheat was planted.

### Chemical analysis

At harvest soil was shaken from the wheat seedling roots and soil solutions were removed by centrifugation (Elkhabit *et al.*, 1987). Total soil solution concentrations of K, Ca, Mg, Na, Al, Mn, Fe and P were determined by ICP emission spectroscopy. Gradient elution ion chromatography was used to determine SO<sub>4</sub>, NO<sub>3</sub>, F, Cl, citrate and oxalate concentrations in soil solution. Reactive Al in soil solution was estimated by a 15 second reaction with 8-hydroxyquinoline (James *et al.*, 1983). A modified version (Parker *et al.*, 1987) of the GEOCHEM computer program (Sposito and Mattigod, 1980) containing equilibrium constants from Lindsay

Table 1. Length (cm) of longest root and total root system length<sup>a</sup> of Hart, Wampum, and Yecorra Rojo wheat cultivars grown for 4 days in Lily BA soil treated with 10 levels of Ca(OH)<sub>2</sub>

Ca(OH) <sub>2</sub> Treatment (cmol(+)kg <sup>-1</sup> )	Hart		Wampum		Yecorra Rojo	
	Longest root	Total root length	Longest root	Total root length	Longest root	Total root length
0	8.6 <sup>f</sup>	27.1 <sup>f</sup>	10.5 <sup>c</sup>	35.8 <sup>f</sup>	11.1 <sup>fg</sup>	34.9 <sup>d</sup>
0.025	9.3 <sup>c</sup>	28.6 <sup>ef</sup>	11.1 <sup>c</sup>	39.4 <sup>def</sup>	10.9 <sup>g</sup>	34.6 <sup>d</sup>
0.05	9.6 <sup>de</sup>	30.6 <sup>de</sup>	10.3 <sup>c</sup>	37.6 <sup>ef</sup>	11.7 <sup>de</sup>	36.6 <sup>cd</sup>
0.1	9.9 <sup>d</sup>	32.0 <sup>d</sup>	10.8 <sup>c</sup>	37.4 <sup>ef</sup>	11.7 <sup>cde</sup>	38.5 <sup>bc</sup>
0.2	10.1 <sup>d</sup>	33.3 <sup>d</sup>	12.4 <sup>b</sup>	43.6 <sup>cd</sup>	11.5 <sup>ef</sup>	38.1 <sup>bc</sup>
0.4	12.1 <sup>c</sup>	37.4 <sup>c</sup>	12.3 <sup>b</sup>	44.4 <sup>bcd</sup>	11.6 <sup>ef</sup>	38.2 <sup>bc</sup>
0.8	13.2 <sup>ab</sup>	42.7 <sup>a</sup>	13.2 <sup>ab</sup>	43.1 <sup>cde</sup>	12.2 <sup>bcd</sup>	39.0 <sup>bc</sup>
1.6	13.5 <sup>a</sup>	41.9 <sup>ab</sup>	13.9 <sup>a</sup>	50.3 <sup>a</sup>	12.8 <sup>a</sup>	42.0 <sup>a</sup>
3.2	13.6 <sup>a</sup>	41.3 <sup>ab</sup>	13.1 <sup>ab</sup>	45.9 <sup>abc</sup>	12.4 <sup>ab</sup>	38.6 <sup>bc</sup>
6.4	12.7 <sup>b</sup>	38.9 <sup>bc</sup>	13.7 <sup>a</sup>	49.7 <sup>ab</sup>	12.3 <sup>abc</sup>	40.1 <sup>ab</sup>
LSD (0.05)	0.5	3.1	1.0	5.8	0.6	2.9

<sup>a</sup> Values in the table represent a mean from 15 plants. Means in a column followed by the same letter are not significantly different at the *P* = 0.05 level by the LSD test.

(1979) was used to calculate concentrations and activities of free ions and complexes in soil solution.

## Results and discussion

### Experiment 1

Mean values for length of the longest root and total root system length of the three wheat cultivars are shown in Table 1. A significant ( $p < 0.01$ ) linear relationship existed between longest root and total root system length for a given cultivar (Hart longest root =  $-0.14 + 0.32$  total root length,  $r^2 = 0.98$ ). Length of the longest root will be referred to as root length and will be used as a measure of plant response to  $\text{Ca}(\text{OH})_2$  treatments. Hart was the most sensitive and Yecorra Rojo the least sensitive to  $\text{Ca}(\text{OH})_2$  additions. Maximum root length increases relative to the 0  $\text{Ca}(\text{OH})_2$  treatment were 58.1, 32.4 and 15.3% for Hart, Wampum and Yecorra Rojo, respectively. These results are consistent with the reported relative soil acidity tolerance of the three cultivars (Ritchey *et al.*, 1988). A slight drop in root length was observed at the highest  $\text{Ca}(\text{OH})_2$  rate, but was only significant for Hart.

Soil solution Al reacting in 15 seconds with 8-hydroxyquinoline (reactive Al) ranged from 9 to  $67 \mu\text{M}$  (Table 2) and represented from 41 to 75% of total soil solution Al. Reactive Al is operationally defined and generally thought to represent most of the mononuclear inorganic Al, some weakly coor-

inated organic Al and some of the Al associated with dispersed colloidal particles (James *et al.*, 1983; Bartlett *et al.*, 1987). Activities of Al species in soil solution were estimated from the GEOCHEM program using reactive Al, pH and soil solution composition as inputs. Calculated  $\text{Al}^{3+}$  activity ( $\{\text{Al}^{3+}\}$ ) decreased from 12 to  $0.004 \mu\text{M}$  with  $\text{Ca}(\text{OH})_2$  additions (Table 2).

A plot of relative root length (RRL) of the three cultivars as a function of calculated  $\{\text{Al}^{3+}\}$  is shown in Fig. 1, where  $\text{RRL} = (\text{root length of a given treatment}/\text{longest root length of any treatment}) \times 100$ . Yecorra Rojo root length was not greatly influenced by the range of  $\{\text{Al}^{3+}\}$  values found in this experiment. Hart RRL (Fig. 1) was significantly related ( $p < 0.01$ ) to  $\{\text{Al}^{3+}\}$  by an equation of the form:  $\text{RRL} = \text{Ae}^{\text{B}\{\text{Al}^{3+}\}}$ . While  $\{\text{Al}^{3+}\}$  alone satisfactorily explained the variation in RRL of Hart, additional factors such as cation amelioration (Kinraide and Parker, 1978a), pH (Kinraide *et al.*, 1985) and potential toxicity of Al polymers (Parker *et al.*, 1988) have also been shown to influence the expression of Al toxicity.

The  $\{\text{Al}^{3+}\}$  values in Fig. 1 are higher than those associated with toxicity to 'Al-sensitive' Tyler wheat in carefully controlled solution experiments (Kinraide and Parker, 1987a,b). The  $\{\text{Al}^{3+}\}$  values in Fig. 1 are approximations and are limited by the inability of the 8-hydroxyquinoline method to unequivocally separate forms of Al. Additional work needs to be done to develop techniques that will allow fractionation of soil solution Al so that forms of Al can be related to plant response. The short-

Table 2. Composition, reactive<sup>a</sup> Al, and calculated  $\text{Al}^{3+}$  activity<sup>b</sup> in soil solutions removed from Lily BA soil treated with 10 levels of  $\text{Ca}(\text{OH})_2$

Ca(OH) <sub>2</sub> Treatment (cmol(+)kg <sup>-1</sup> )	pH	Soil solution composition <sup>c</sup> (μM)							Reactive <sup>a</sup> Al (μM)	Al <sup>3+</sup> activity <sup>b</sup> (μM)
		Ca	Mg	Mn	Al	SO <sub>4</sub>	F	P		
0	4.44	110	145	123	89	85	38	2	67	12
0.025	4.54	134	140	123	89	118	35	3	67	11
0.05	4.51	157	136	124	92	98	45	3	64	8.2
0.1	4.53	225	149	123	93	82	34	2	59	8.9
0.2	4.55	328	133	108	83	115	37	3	49	4.8
0.4	4.58	502	116	96	72	133	35	3	43	3.6
0.8	4.76	885	117	87	64	171	36	4	35	1.4
1.6	4.85	1273	93	53	49	296	33	4	27	0.6
3.2	5.14	2193	90	35	41	637	39	8	19	0.1
6.4	5.79	3154	60	10	22	1384	62	10	9	0.0

<sup>a</sup> Al reacting with 8-hydroxyquinoline in 15 seconds.

<sup>b</sup> Calculated using the GEOCHEM program.

<sup>c</sup> Trace levels of oxalate ( $\leq 5.7 \mu\text{M}$ ) and citrate ( $\leq 2.6 \mu\text{M}$ ) were found in these soil solutions, but are not listed in the table.

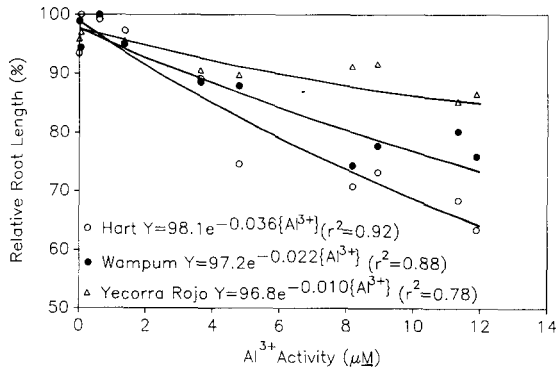


Fig. 1. Relative root length of Hart, Wampum and Yecorra Rojo wheat as a function of  $Al^{3+}$  activity in soil solutions removed from a soil treated with 10 levels of  $Ca(OH)_2$ .

term growth technique should be useful in carrying out these studies since it allows separation of the influence of Al on root elongation from the longer term influence of Al on plant nutrition.

### Experiment 2

Root length of Hart wheat in the  $CaSO_4$  and  $CaCl_2$  treatments is plotted (Fig. 2) as a function of summation of Al activities ( $\Sigma\{Al\}$ ) calculated from the GEOCHEM program. Activities included in  $\Sigma\{Al\}$  were  $Al^{3+}$  and Al complexed with  $OH$ ,  $SO_4$ ,  $F$ , oxalate and citrate. Total soil solution Al and  $\Sigma\{Al\}$  increased with  $CaCl_2$  and  $CaSO_4$  additions.

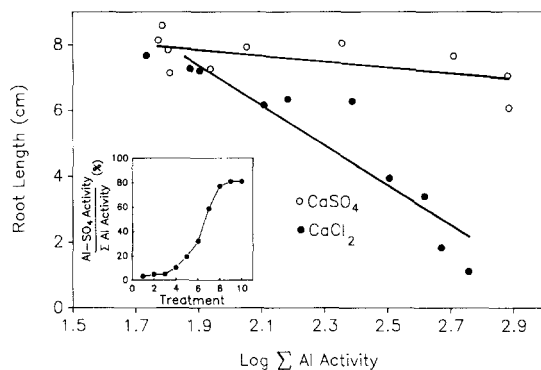


Fig. 2. Root length of 4 day old Hart wheat seedlings as a function of  $\log_{10} \Sigma Al$  activities ( $\mu M$ ) in soil solutions removed from a soil treated with 10 levels of  $CaSO_4$  and 10 levels of  $CaCl_2$ . **Insert:** Percentage of soil solution Al activity due to  $Al-SO_4$  in a soil treated with 10 levels of  $CaSO_4$ .

Hart root length decreased significantly with increasing  $\Sigma\{Al\}$  in the  $CaCl_2$  treatments but only showed a slight decrease in the  $CaSO_4$  treatments. The insert in Fig. 2 shows a plot of  $\{Al-SO_4\}$  as a percentage of  $\Sigma\{Al\}$  for the ten  $CaSO_4$  treatments. The  $\{Al-SO_4\}$  increased from 2 to 623  $\mu M$  across the ten  $CaSO_4$  treatments and constituted an increasingly larger percentage of  $\Sigma\{Al\}$ . These levels of  $\{Al-SO_4\}$  had a negligible effect on the root growth of Hart wheat. These findings confirm the conclusion reached by Cameron *et al.* (1986) and Kinraide and Parker (1986b) concerning the non-phytotoxicity of the  $Al-SO_4$  complex. In the  $CaCl_2$  treatments  $\{Al-SO_4\}$  was  $\leq 3\%$  of  $\Sigma\{Al\}$  in all the treatments and  $\{Al^{3+}\}$  increased with  $CaCl_2$  additions. Hart root length was related to  $\{Al^{3+}\}$  in the  $CaCl_2$  treatments by an equation of the form: Root length =  $7.77 - 0.017 \{Al^{3+}\}$  ( $r^2 = 0.94$ ). Soil solution pH dropped with  $CaCl_2$  additions, and along with  $\{Al^{3+}\}$ , could have contributed to inhibition of root growth.

In Experiments 1 and 2, Hart root growth was very sensitive to changes in Al levels and speciation in soil solution. Root growth limitations were significantly related to  $\{Al^{3+}\}$  and the nonphytotoxicity of  $AlSO_4$  was confirmed. This short-term seedling root growth technique allows separation of Al effects on root elongation from those on nutrition and should be useful for studying Al toxicity relationships in soil.

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