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Effect of Toxic Concentrations of H^+ and Al on Nonsymbiotic Growth of Groundnut in Solution Culture

ROSMIN KASRAN *+, ZULKIFLI H. SHAMSUDDIN * and D.G. EDWARDS**

** Department of Soil Science, Universiti Pertanian Malaysia, 43400 UPM Serdang, Selangor, Malaysia.*

*** Department of Agriculture, The University of Queensland, Queensland, Australia 4072.*

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ABSTRAK

Dua percubaan kultura nutrien telah dijalankan untuk mengkaji kesan kepekatan toksik H^+ dan aluminium terhadap pertumbuhan bukan-simbiotik kacang tanah (Arachis hypogaea L.) *cv. Matjam. Lima paras pH yang digunakan ialah 3.5, 4.0, 4.8, 5.5 dan 6.5 manakala jumlah aktiviti spesies Al monomerik (Σa_{Almono}) ialah 0, 1.5, 3.0, 15, 35 dan 80 μM pada paras pH 4.3. Kepekatan ion H^+ pada pH 3.5 tidak menjejaskan berat kering bahagian atas pokok tetapi menjejaskan pertumbuhan akar dan pemanjangannya. Peningkatan pH kepada 4.0 menambahkan lagi pertumbuhan akar. Paras pH yang disyorkan untuk tumbesaran kacang tanah ialah $\geq$ 4.4. Kepekatan aluminium yang tidak toksik untuk pertumbuhan bahagian atas dan pemanjangan akar ialah $\leq$ 12.2 μM Σa_{Almono} ($\equiv$ 17.1μM Al-monomeric atau 20.3 μM monomerik Al-jumlah). Peningkatan kepekatan Σa_{Almono} kepada 80 μM merendahkan kepekatan magnesium pada daun termuda berkembang penuh (YEL) kepada paras tidak mencukupi, 0.18%. Kepekatan magnesium di dalam YEL lebih sensitif terhadap ketoksikan aluminium daripada kepekatan magnesium di dalam akar. Keputusan juga mendapati kacang tanah memberi respon berat kering akar dan pemanjangan akar yang positif pada kepekatan aluminium yang rendah (1.5 μM Σa_{Almono}); kesan ini tidak ditunjukkan pada berat kering bahagian atas pokok.*

ABSTRACT

Two solution culture experiments were conducted to study the effects of toxic concentrations of H^+ and aluminium on nonsymbiotic growth of groundnut (Arachis hypogaea L.) *cv. Matjam. The five pH levels used were 3.5, 4.0, 4.8, 5.5 and 6.5, and the sum of activities of monomeric Al species (Σa_{Almono}) were 0, 1.5, 3.0, 15, 35 and 80 μM at pH 4.3. The H^+ ion concentration at pH 3.5 did not affect top dry weight but markedly decreased root dry weight and length. Raising the solution pH to 4.0 improved the growth of roots. The pH level recommended for growth of groundnut was $\geq$ 4.4. The non-toxic concentration of aluminium for growth of groundnut and root elongation was $\leq$ 12.2 μM Σa_{Almono} ($\equiv$ 17.1 μM monomeric-Al or 20.3 μM monomeric total-Al). Increasing the Σa_{Almono} to 80 μM decreased the magnesium concentration in the youngest expanded leaf (YEL) to 0.18%, a value known to be deficient for groundnut. Magnesium concentration in the YEL was more sensitive to aluminium toxicity than magnesium concentration in the roots. The results also showed that root dry weight and root length responded positively to low concentrations of aluminium (1.5 μM Σa_{Almono}); this effect was not observed for dry weight of tops.*

INTRODUCTION

Many soils of the humid tropics have pH values of less than 5.0 (1:5 soil : water) and low levels of exchangeable calcium (Pearson 1975). Low pH *per se* seems unlikely to inhibit growth of legumes severely, even in acid-sensitive species, such as *Medicago sativa* and *Phaseolus vulgaris*. These plants have been shown to tolerate acid conditions (pH 4.5) in solution culture with a low concentration of aluminium ($\leq$ 10 μM), provided there was an adequate supply of calcium and combined nitrogen (Asher and Edwards 1978; Franco and Munns 1982).

+ Present address:
Cocoa and Coconut Research Division, MARDI Hilir Perak, P.O. Box 25, 36307, Sungai Sumun, Perak, D.R., Malaysia

Aluminium toxicity is a major problem for the growth of many plant species in acid soils of pH (1:5 soil : water) below 5.0, or even below pH 5.5 in kaolinitic soils (Foy 1984; Kamprath and Foy 1985). Excess available aluminium in subsoils can reduce plant access to subsoil water and nutrients due to limited root proliferation into subsoil horizons (Foy 1988). The visual symptoms of aluminium toxicity in legumes include marked stunting and thickening of roots (Munns 1965 a,b,c; Blamey *et al.* 1983; Haug 1983). There is often a close relationship between aluminium status in soils and soil pH; increases in exchangeable aluminium are often associated with an increase in soil acidity (Blamey *et al.* 1987). Reports on the effects of H^+ and aluminium on non-symbiotic growth of groundnut are limited. In relation to this, two solution culture experiments were undertaken to determine the toxic concentrations of H^+ and aluminium for non-symbiotic growth of groundnut. Solution culture experiments have been used to overcome the difficulty in studying the effects of soil pH *per se* on plant growth.

MATERIALS AND METHODS

Two solution culture experiments were conducted at Universiti Pertanian Malaysia, Serdang, Malaysia where daily temperatures of the nutrient solution ranged from 28° to 32°C. The minimum and maximum air temperatures during the experimental period were 21.5° and 33.8°C, respectively. Solutions in the 5l pots were aerated by continuous bubbling using an aquarium pump with four outlets, one to each pot. Groundnut cv. Matjam seeds were surface-sterilised with 95% alcohol for one minute, followed by 0.1% $HgCl_2$ for two minutes and five rinses with sterile deionised water for one minute. Four-day-old seedlings, germinated on sterilised sand, were transferred by wrapping a sponge around each seed and suspending the roots in the 5l nutrient solution, three plants per pot.

pH Experiment

The experiment was conducted using the depleting nutrient method (Hewitt 1966; Asher and Edwards 1983). Solution pH was measured daily and adjusted with 0.05 M HCl and 0.05 M KOH to the desired pH, if necessary. The five pH values used were 3.5, 4.0, 4.8, 5.5 and 6.5. The treatments were arranged in a randomized complete block design with four replicates and a total of 20 pots.

The initial nutrient solution contained (µM): N 1000, P 100, K 500, Ca 400, Mg 100, S 677, Na 114, Cl 100, Fe 14, Zn 0.38, Mo 0.05, Mn 2.2, B 23, Co 0.02, and Cu 0.16. These concentrations were achieved by calculating the total amount of the respective element required in 5l volumes of solution. No additional nutrient was given during the experiments. Plants were grown for 21 days in the absence of aluminium.

Aluminium Experiment

The experiment was conducted using the Programmed Nutrient Addition Technique (Asher and Blamey 1987). The treatments were arranged in a randomized complete block design with four replicates and a total of 24 pots. Six concentrations of aluminium, as $Al_2(SO_4)_3 \cdot 16H_2O$, were applied, viz. 0, 4, 8, 25, 50, and 110 µM total-Al, equivalent to 0, 2, 4, 21, 47 and 108 µM monomeric-Al or 0, 1.5, 3, 15, 35 and 80 µM (a_{Almono} (sum of activities of monomeric-Al).

$$\text{Al monomeric} = Al^{3+} + Al(OH)^{2+} + Al(OH)_2^{+} + Al(OH)^0_3 + Al(SO_4)^{+} \quad \text{(Equation 1)}$$

The culture solution was continuously aerated for seven days to allow for equilibration of nutrients and treatments imposed (equilibration period). On the seventh day, the solution was agitated and allowed to stand for 1h before solution samples were taken at a depth of 4cm from the 17 cm-high pots. Solution pH and electrical conductivity (mS cm^{-1}) were measured. Molar ionic strength was estimated as 0.013 3 electrical conductivity (Griffin and Jurinak 1973; Gillman and Bell 1978). Phosphate (5 µM) and pH (4.3) levels were kept low to maintain the levels of Al-treatments as described by Kim (1985), to avoid formation of soluble, non-phytotoxic aluminium-polymers and to prevent precipitation of amorphous aluminium phosphate. Solution pH was measured daily and adjusted to pH 4.3 with 0.05 M HCl or 0.05 M NaOH, if necessary. Phosphorus concentrations in the solution were measured daily by the molybdenum-blue method (Jackson 1957) and maintained at 5 µM by addition of $NaH_2PO_4.2H_2O$. All other nutrients were supplied daily for 28 days using the Programmed Nutrient Addition Technique (Asher and Blamey 1987) to give total nutrient solution concentrations as follows (µM) : N 582, K522, Ca 400, Mg 154, S 96, Fe 4.5, B 4.5, Mn 2.2, Cl 3.05, Cu 0.5, Zn 0.2 and Mo 0.05.

Total-Al in solution was determined colorimetrically, using the aluminon method of Hsu (1963). The concentration of monomeric-Al in solution was determined by the modified aluminon method (Blamey *et al.* 1983). This method employed the same procedure as that described by Hsu (1963) for total-Al, but without the acid digestion (addition of HCl and heating for 30 min in a waterbath at 80°C). Colour intensity was read 30 min after the addition of aluminon buffer solution. The Σa_{Almono} was calculated from solution parameters as described by Blamey *et al.* (1983), which assumed the presence of sulphate in solution, and pH range to be between 4.0 and 6.0.

The sum of activities of the five monomeric-Al species (Σa_{Almono}) (Equation 1) was calculated using solution pH, electrical conductivity, total sulphate and the measured concentrations of total and monomeric-Al in solution by the Almono Computer Programme, the Debye-Huckel equation

$$-\log f = (Az^2\sqrt{\mu}) / (1 + Ba\sqrt{\mu}) \quad \text{(Equation 2)}$$

where f = activity coefficient of the ion; μ = ionic strength of the solution ; z = valence of the ion; a = ionic diameter; A = 0.5115; and B = 0.3291 (Kielland 1937), and the following equations (Marion *et al.* 1976):

$$Al(OH)^{2+} \rightleftharpoons Al^{3+} + OH^- \quad pK\ 8.99$$

$$Al(OH)_2^+ \rightleftharpoons Al^{3+} + 2OH^- \quad pK\ 19.3$$

$$Al(OH)_3^0 \rightleftharpoons Al^{3+} + 3OH^- \quad pK\ 26.8$$

$$Al(SO_4)^+ \rightleftharpoons Al^{3+} + SO_4^{2-} \quad pK\ 3.2$$

The ionic diameters used were as follows: Al^{3+} δ 0.9 nm; $Al(OH)^{2+}$ 0.7 nm; $Al(OH)_2^+$ δ 0.6 nm; $Al(SO_4)^+$ 0.43 nm; and SO_4^{2-} 0.4 nm (Kielland 1937; Nair and Prenzel 1978). The solution compositions are shown in Table 1.

Leaf tissues and roots required for chemical analyses were dried in an oven at 65°C for 4 days and later ground using a Wiley mill with stainless steel blades and lining. After dry ashing (Anon 1980), phosphorus and potassium concentrations were determined using an autoanalyzer, and calcium and magnesium were determined using atomic absorption spectrophotometer. After wet ashing (Anon 1980), nitrogen was determined with an autoanalyzer.

The following data were recorded: top and root dry weight, root length, and nitrogen, phosphorus, potassium, calcium and magnesium concentrations in the YEL and roots.

RESULTS AND DISCUSSION

1. *Effects of pH* per se *on Growth of Groundnut*
   a. *Dry Matter Yield of Tops and Roots, and Root Length*

The H^+ ion concentration at pH 3.5 did not affect the top dry weight of groundnut and the plants produced 90% of maximum total dry matter yeild (Table 2). There was no significant difference between pH values from 3.5 to 6.5 on dry matter yield of tops. In contrast, Blamey *et al.* (1982) found that a solution pH of 3.5 was lethal for plant top growth of sunflower (*Helianthus annuus*). In that study, the critical solution pH corresponding to 90% of maximum total dry matter yield for four sunflower cultivars ranged from 3.9 to 4.5. In the present experiment, the root dry weight and root length were reduced at the lower pH (3.5) compared to the higher pH treatments (4.0-6.5) (Table 2). At pH 3.5, the

TABLE 1

Calculated activities of aluminium monomers in nutrient solution, total-Al, monomeric-Al and electrical conductivity at pH 4.3 prior to planting

Total-Al (µM)	Mono-Al (µM)	EC (mS/cm)	Σa_{Almono} (µM)	Al^{3+} (µM)	$Al(OH)^{2+}$ (µM)	$Al(OH)_2^+$ (µM)	$Al(OH)_3^0$ (µM)	$Al(SO_4)^+$ (µM)
0	0	0.29	0	0	0	0	0	0
4	2	0.30	1.5	0.59	0.09	0.29	0.002	0.52
8	4	0.30	3.0	1.17	0.19	0.59	0.003	1.05
25	21	0.31	15.0	5.94	0.84	2.93	0.02	5.41
50	47	0.33	35.0	13.42	2.05	6.72	0.03	12.83
110	108	0.35	80.0	29.64	4.65	14.81	0.07	30.82

TABLE 2
Relative yield of tops, top and root dry weights and root length of groundnut grown at different solution pH

Solution pH	Rel. Yield of tops (%)	Top dry weight (g plant^{-1})	Root dry weight (g plant^{-1})	Root length (cm)
3.5	90[a]	3.45[a]	0.59[a]	30.3[a]
4.0	94[a]	3.58[a]	0.79[b]	39.0[b]
4.8	100[a]	3.83[a]	0.77[b]	45.2[c]
5.5	95[a]	3.63[a]	0.75[b]	45.8[c]
6.5	94[a]	3.59[a]	0.72[b]	45.2[c]

Values followed by different letters within a column are significantly different at 5% level by Duncan's Multiple Range Test

roots were short, thickened, and brown. Islam *et al.* (1980) showed that lateral root development of *Manihot esculenta* (cassava), *Phaseolus vulgaris* (French bean), *Zingiber officinale* (ginger), *Zea mays* (maize), *Lycopersicon esculentum* (tomato) and *Triticum aestivum* (wheat) was suppressed and in some cases the root tips were killed at pH 3.3. A solution pH < 4.2 has been shown to greatly reduce the growth rate of the primary roots of cotton (*Gossypium* spp.) (Howard and Adams 1965). Suthpradit (1988) found that groundnut cv. Red Spanish grew best in flowing culture with adequate inorganic nitrogen at pH 3.5 compared to other higher pH treatments (maximum pH 5.5). Groundnut was the most tolerant crop legume studied and reached maximal growth of shoots and roots after 25 days at pH 3.5.

In contrast to roots of plants grown at low pH, roots of plants grown at pH ≥ 4.8 were light-coloured and finely branched. Relative root length increased with increase in solution pH from 3.5 to 4.8 (*Fig. 1*); interpolation indicates that 90% of maximum relative root length was achieved at pH 4.4. Thus, raising the solution pH was more beneficial to growth of roots than tops.

b. Nutrient Concentrations in Youngest Expanded Leaves

Cation uptake is suppressed in the presence of high H^+ concentrations (Islam *et al.* 1980). This was evident in the present experiment where pH 3.5 markedly reduced the calcium concentration in the YEL compared to pH ≥ 4.0 (Table 3). In contrast to the strong effects of H^+ ions in inhibiting calcium absorption at pH 3.5, effects of H^+ ions at pH 3.5 in inhibiting magnesium absorption were not apparent. Soybean tap roots grown in the nutrient solution portion of a split medium also showed a lower calcium concentration at pH 4.5 than at 5.6 (Lund 1970). Several nutrient solution studies have shown that a high H^+ ion concentration decreases the uptake of calcium, magnesium and potassium (Islam *et al.* 1980), manganese (Robson and Loneragan 1970),

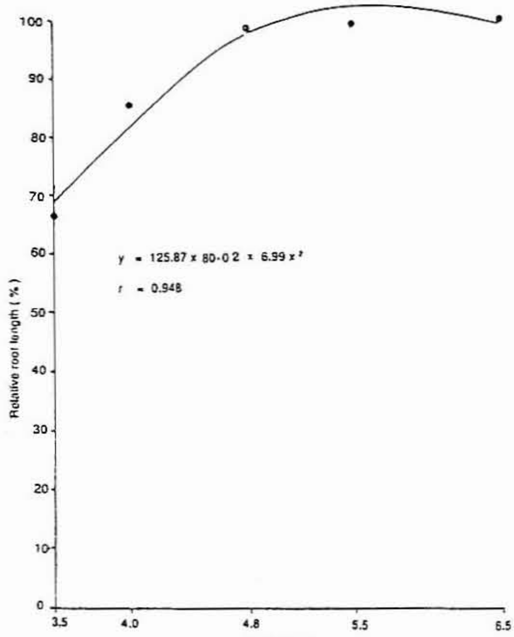

Fig. 1: Relative root length of groundnut at different solution pH

zinc (Rashid *et al.* 1976) and copper (Bowen 1969). Fawzy *et al.* (1954) showed that pretreatment of barley roots for 3 hours at pH 3 in the presence of Al^{3+} and Ca^{2+} considerably reduced the loss of endogenous potassium. The presence of these ions in the pretreatment phase had a protective effect on the subsequent ability of barley roots to absorb potassium at pH 5.0. However, in the present solution culture study, nitrogen, phosphorus, potassium and magnesium concentration in the YEL were not limited by excess H^+ even at a pH as low as 3.5 (Table 3). This ability of groundnut to better maintain adequate concentrations of macronutrients in its tissues than other plant species when grown at low pH makes it one of the more acid-tolerant legume species, in keeping with the findings of Adams and Pearson (1970).

2. Effects of Aluminium on Groundnut

a. Dry Matter Yield of Tops and Roots, and Root Length

The relative yield of groundnut tops was independent of Σa_{Almono} from 0 to 15 µM, but declined strongly with further increase in Σa_{Almono} to 35 µM and 80 µM (Table 4). The critical Σa_{Almono} corresponding to 90% relative yield of tops was 12.2µM (*Fig. 2*). Root dry weight was lower in the zero aluminium control treatment and in the highest aluminium treatment (Σa_{Almono} of 80 µM) than in all other treatments (Table 4).

Symptoms of aluminium toxicity were not apparent on the leaves but a thickening of the roots and inhibition in root growth were obvious within 3 days of applying the higher concentrations of aluminium. The root systems in the

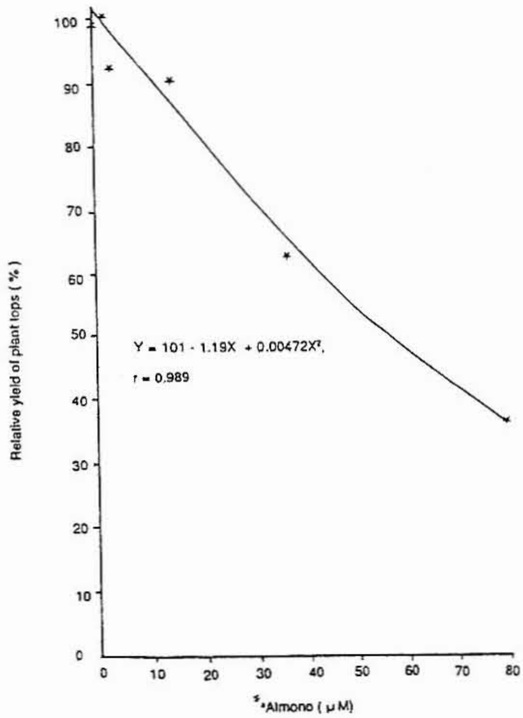

Fig. 2: Relative yield of groundnut tops at different values of Σa_{Almono}

Σa_{Almono} of 35 and 80 µM treatments were markedly inhited in length, coralloid shaped, and brown, and the lateral roots were stubby with no fine branching, symptoms characteristic of aluminium toxicity (Foy 1984). Bouma *et al.* (1981) found that in Al-injured plants, aluminium typically accumulates in or on roots but not in tops.

A stimulation in root length was observed at the lower (Σa_{Almono} of 1.5 µM) treatment while at the higher ($\Sigma a_{Almono} \geq 5$ µM) treatments a marked

TABLE 3
Nitrogen, phosphorus, potassium, calcium and magnesium concentrations in YEL of groundnut grown at different solution pH

Solution pH	Nutrient concentrations in YEL (%)				
	Nitrogen	Phosphorus	Potassium	Calcium	Magnesium
3.5	2.33[a]	0.15[a]	2.34[a]	0.29[a]	0.46[b]
4.0	2.29[a]	0.15[a]	2.51[a]	0.76[b]	0.41[ab]
4.8	2.60[a]	0.15[a]	2.64[a]	0.76[b]	0.38[a]
5.5	2.65[a]	0.14[a]	2.87[a]	0.87[bc]	0.36[a]
6.5	2.77[a]	0.15[a]	3.35[b]	0.91[c]	0.40[a]

Values followed by different letters within a column are significantly different at 5% level by Duncan's Multiple Range Test

TABLE 4
Relative yield of tops, top and root dry weights, and
root length of groundnut grown at different values of $(a_{Almono}$

Total-Al in soln. (μM)	Mono-Al (μM)	$(a_{Almono}$ (μM)	Rel. yield of tops (%)	Top dry weight (g plant^{1})	Root dry weight (g plant $^{-1}$)	Root length (cm)
0	0	0	98^{C}	2.79^{C}	0.33^{a}	38.9^{C}
4	2	1.5	100^{C}	2.85^{C}	0.40^{b}	43.1^{d}
8	4	3.0	92^{C}	2.61^{C}	0.40^{b}	41.6^{cd}
25	21	15.0	91^{C}	2.59^{C}	0.44^{b}	42.4^{d}
50	47	35.0	61^{b}	1.75^{b}	0.40^{b}	22.0^{b}
110	108	80.0	37^{a}	1.05^{a}	0.33^{a}	14.1^{a}

Values followed by different letters within a column are significantly different at 5% level by Duncan's Multiple Range Test

reduction in root length (Table 4) was observed. Other studies have also shown a positive growth response of tropical legumes at low concentrations of aluminium, although aluminium is not regarded as an essential plant nutrient (Andrew *et al.* 1973; Foy *et al.* 1978; Foy and Fleming 1978).

b. Nutrient Concentrations in YEL

In general, an increase in $(a_{Almono}$ resulted in an increase in the nitrogen, phosphorus, potassium and calcium concentrations in the YEL; a reverse trend was observed for magnesium (Table 5). Truman *et al.* (1986) found that aluminium was antagonistic to the uptake of calcium and magnesium but increased the uptake of potassium by *Pinus radiata.* Similar observations on aluminium increasing the uptake of phosphorus have been reported (Andrew and Vanden Berg 1973; Andrew *et al.* 1973; Murphy *et al.* 1984). Murphy *et al.* (1984) showed that the phosphorus concentration in the tops of four tropical pasture legumes increased with increase in solution aluminium concentration to 25 or 50 μM, and then decreased with further increase in solution aluminium concentration to 100 or 125 μM. Thus, the toxic effect of aluminium on the uptake of several nutrient elements varies with plant species and genotype.

An increase in the activity of monomeric aluminium species to 80 μM depressed the

TABLE 5
Nitrogen, phosphorus, potassium, calcium and magnesium concentrations in YEL and magnesium
concentrations in roots of groundnut grown at different values of $(a_{Almono}$

Total-Al in soln.	Mono-Al	$(a_{Almono}$	Nutrient concentrations (%) in: YEL					Root
(μM)	(μM)	(μM)	N	P	K	Ca	Mg	Mg
0	0	0	3.05^{a}	0.28^{a}	1.77^{a}	0.06^{a}	0.29^{c}	0.05^{a}
4	2	1.5	3.37^{ab}	0.33^{b}	1.89^{a}	0.19^{b}	0.29^{c}	0.07^{b}
8	4	3.0	3.70^{b}	0.34^{b}	1.93^{a}	0.20^{b}	0.30^{c}	0.08^{c}
25	21	15.0	3.69^{b}	0.36^{b}	1.93^{a}	0.25^{b}	0.28^{c}	0.08^{c}
50	47	35.0	5.49^{c}	0.52^{d}	2.50^{b}	0.36^{c}	0.24^{b}	0.09^{d}
110	108	80.0	5.80^{c}	0.44^{c}	3.08^{c}	0.42^{c}	0.18^{a}	0.07^{b}

Values followed by different letters within a column are significantly different at 5% level by Duncan's Multiple Range Test magnesium concentration in the YEL to the deficient value of 0.18% (Reuter and Robinson 1986). In this study, magnesium concentration in the YEL and plant growth were significantly reduced at $\Sigma a_{Almono} \geq 35$ µM; however, magnesium deficiency may have attenuated the growth response only at Σa_{Almono} of 80 µM. The visual magnesium deficiency symptoms appeared as greyish-green areas on the leaf, first at the tip and then along the leaf margin. Gradually this discoloration changed to yellow and eventually brown with the chlorosis extending interveinally. The decrease in magnesium concentration in the YEL at Σa_{Almono} of 35 µM was not accompanied by a corresponding decrease in magnesium concentration in the root (Table 5). This indicated that the translocation of magnesium to the plant tops, and not the uptake of magnesium by roots, was reduced at high ($\geq$ 35 µM Σa_{Almono}) aluminium concentrations. Thus, in agreement with Foy (1988), we concluded that for plants grown under aluminium stress, the accumulation of nutrients in plant tops probably reflects prior root damage resulting in differential transport of elements.

The experiment showed that aluminium was not toxic up to Σa_{Almono} of 12.2 µM, which corresponds to 90% of maximum growth of groundnut. The results also indicated that magnesium concentration in the YEL was more sensitive to aluminium toxicity than magnesium concentration in the roots.

CONCLUSION

The pH level recommended for growth of groundnut was $\geq$4.4. The H^+ ion concentration at pH 3.5 did not affect top dry weight of groundnut but markedly decreased root dry weight and length and reduced the uptake of calcium. Raising the solution pH to 4.0 was more beneficial to growth of roots than tops.

The non-toxic level of aluminium for growth of groundnut tops and root elongation was $\Sigma a_{Almono} \leq 12.2$ µM, which corresponds to a 10% depression of growth of tops from the maximum achieved in the absence of aluminium while a low concentration of 4 µM total-Al ($\equiv$ 1.5 µM Σa_{Almono}) produced a positive growth response. Magnesium concentration in the YEL was more sensitive to aluminium toxicity than magnesium concentration in the roots.

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