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The effect of Ca and Al concentration on alleviation of Al toxicity on non-symbiotic growth of groundnut

(Kesan kepekatan Ca dan Al untuk mengurangkan keracunan Al terhadap pertumbuhan bukan simbiotik kacang tanah)

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Key words: Al toxicity, *Arachis hypogaea* L., Ca, Mg, solution culture

Abstrak

Kajian kultur larutan selama 21 hari telah dijalankan untuk menentukan kepekatan kalsium (Ca) luaran yang diperlukan untuk mengurangkan keracunan aluminium (Al) terhadap pertumbuhan bukan simbiotik kacang tanah (*Arachis hypogaea* L.) cv. Matjam pada pH 4.3. Kepekatan larutan Ca dikekalkan pada 500, 1 000, 2 500, 5 000 dan 10 000 µM manakala jumlah aktiviti spesies Al monomerik (Σa_{Almono}) pada 0, 15, 30 dan 60 µM. Hasil relatif bahagian atas pokok didapati menurun dengan 15 µM Σa_{Almono} di dalam larutan kecuali pada 2 500 µM Ca. Hasil relatif semakin menurun dengan 30 µM Σa_{Almono} dan lebih menurun lagi dengan 60 µM Σa_{Almono}. Kesannya lebih ketara pada kepekatan Ca yang rendah. Kepekatan Ca luaran sebanyak 2 500 µM diperlukan untuk mengurangkan keracunan Al pada 24 µM Σa_{Almono}. Kepekatan Ca di dalam daun termuda yang telah berkembang (YEL) didapati menurun dengan rawatan Al tetapi meningkat dengan peningkatan Ca di dalam larutan. Walau bagaimanapun, kepekatan magnesium di dalam YEL menurun dengan peningkatan Ca dan Al.

Abstract

A 21-day solution culture study was conducted to determine the external calcium (Ca) concentration required to alleviate aluminium (Al) toxicity on non-symbiotic growth of groundnut (*Arachis hypogaea* L.) cv. Matjam at pH 4.3. The Ca concentrations were maintained at 500, 1 000, 2 500, 5 000 and 10 000 µM, while the sum activities of monomeric Al species (Σa_{Almono}) were 0, 15, 30 and 60 µM. The relative yield of plant tops was decreased with Σa_{Almono} of 15 µM in solution except at 2 500 µM Ca. Relative yields declined further with Σa_{Almono} of 30 µM, and even further with 60 µM Σa_{Almono}. The effect was more marked at low Ca concentration. A 2 500 µM external Ca concentration was required to alleviate Al toxicity at 24 µM Σa_{Almono}. The Ca concentration in the youngest expanded leaf (YEL) decreased with Al treatment but increased with increased Ca concentration in solution. However, magnesium (Mg) concentration in YEL was decreased with increased Ca and Al.

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Introduction

Aluminium (Al) toxicity is probably one of the growth limiting factors for many plants, particularly in acid soils of pH below 5.0 but it may occur in kaolinitic soils of pH as high as 5.5 (Foy 1974, 1984; Alam and Adams 1979; Carvalho et al. 1980; Friesen et al. 1980; Kamprath and Foy 1985). It has been recognized that the activities of the monomeric Al species [particularly Al^{3+}, $Al(OH)^{2+}$ and $Al(OH)_2^{+}$] give the best measure of phytotoxicity of Al (Adams and Lund 1966; Blamey et al. 1983; Alva, Blamey et al. 1986). In recent years, there have been considerable advances regarding the role of Ca in alleviating Al toxicity. An ameliorative Al toxicity due to an increase in solution Ca concentration on *Zea mays*, *Medicago sativa* L. and *Pennisetum clandestinum* Chiov. cv. Whittet has been demonstrated (Munns 1965; Rhue and Grogan 1977; Huett and Menary 1980).

Alva, Edwards et al. (1986) showed that a Ca concentration of 500–5 000 µM substantially decreased Al toxicity in *Glycine max* (L.) Merr., *Trifolium subterraneum* L., *M. sativa* L. and *Helianthus annus* L. when the sum of activities of monomeric Al species (Σa_{Almono}) was used to characterize the solution Al concentration. They also reported that 15 000 µM Ca would alleviate Al toxicity at 18 µM Σa_{Almono} in soybean, and this threshold toxic Σa_{Almono} may vary with legume species. However, little information is available regarding the external Ca concentration required to alleviate Al toxicity in groundnut, based on the threshold concentration of the Σa_{Almono}. A solution culture experiment was conducted to determine the external Ca concentration required to alleviate Al toxicity on non-symbiotic growth of groundnut using Σa_{Almono} to characterize the toxic concentration of Al.

Materials and methods

A solution culture experiment was conducted in a glasshouse at Universiti Pertanian Malaysia, Serdang, Malaysia, using *Arachis hypogaea* cv. Matjam. The daily temperature of the nutrient solution ranged from 28 ^{o}C to 32 ^{o}C. The mean minimum and maximum air temperatures during the experimental period were 23.1 ^{o}C and 31.6 ^{o}C, respectively; mean relative humidity 70–97% and mean daily evaporation 3.5 mm.

Seeds were surface sterilized with 95% ethanol for 1 min, followed by 0.1% $HgCl_2$ for 2 min, and rinsed five times with sterile deionized water for 1 min before sowing in sterilized sand. The 4-day-old seedlings were then transferred to 30 L containers, 4 plants/pot, after the Al and Ca treatments were imposed. The plants were grown for 21 days. The solutions were aerated by continuous bubbling using an aquarium pump with four outlets, one to each container.

Four Al levels were obtained by the addition of appropriate volumes of $Al_2(SO_4).16H_2O$ stock solution providing the following sum activities of monomeric Al species (Σa_{Almono}): 0, 15, 30 and 60 µM (Blamey et al. 1983). Five Ca concentrations (500, 1 000, 2 500, 5 000 and 10 000 µM Ca as $CaSO_4.2H_20$) were also included. The treatments were arranged factorially in a randomized complete block design with three replicates. Solution pH was measured daily and adjusted with 0.005 M HCl or 0.005 M NaOH to 4.3 if necessary. Phosphorus concentrations were maintained at 5 µM (measured every 3 days using the molybdenum blue method of Jackson 1957) to limit the formation of non-phytotoxic Al polymers. Concentrations of Ca and Al were monitored every 4 days and adjusted by adding appropriate volumes of stock solutions when necessary. Calcium concentration in solution was determined using atomic absorption spectrometry (AAS). The concentration of monomeric Al in solution was determined using the modified aluminon technique (Blamey et al. 1983), and Σa_{Almono} calculated using solution electrical conductivity as an estimate of ionic strength (Griffin and Jurinak 1973; Gillman and Bell 1978). The solution compositions are shown in *Table 1*. All other nutrients were supplied daily for 21 days using the programmed nutrient addition technique (Asher and Blamey 1987) to give a total nutrient solution concentrations (µM) of N 381, K 132, Mg 64, S 19.24, Fe 1.13, Mn 0.56, Cl 30.5, Cu 0.13, Zn 0.05, B 1.14 and Mo 0.01. The incremental additions of stock solution are shown in *Table 2*.

Plants were harvested after 21 days of growth in nutrient solution by separating into tops and roots, and taking the youngest expanded leaf (YEL) from each plant. The root length was measured; plant tops, YEL and roots were oven dried at 65 °C for 4 days and weighed. The YEL were ground using a Wiley mill with stainless steel blades and lining. Subsamples were taken for determination of Ca and Mg concentrations by AAS after dry ashing (Anon. 1980).

Results and discussion

Dry matter yield of tops

The result clearly showed that increasing the Ca concentration up to 2 500 µM had a beneficial effect on the growth of groundnut under Al stress. Though 1 000 µM Ca concentration seemed to be inadequate to alleviate Al toxicity at 15 µM Σa_{Almono}, 2 500 µM Ca was sufficient to alleviate Al toxicity up to 24 µM Σa_{Almono} and produced 90% relative yield of tops, but not at 30 µM and 60 µM Σa_{Almono} *(Figure 1)*. These results suggested that the addition of Ca up to 2 500 µM Ca, in the presence of Al, reduced the phytotoxic effect of Al, which could be attributed to a reduction in the activity of phytotoxic Al species through an increase in the ionic strength of the solution (Noble and Sumner 1988) and/or a direct physiological effect of the added Ca (Alva, Asher et al. 1986; Kinraide and Parker 1987). At 5 000 µM Ca concentration, it failed to alleviate the toxic effect of >11 µM Σa_{Almono}. Alva, Edwards et al. (1986) found that the beneficial effect of 15 000 µM Ca on growth of soybean was only in the range of 5–18 µM Σa_{Almono}.

Table 1. Ca and Al treatments and the calculated activities of Al monomers in nutrient solution, total Al, monomeric Al and electrical conductivity at pH 4.3 before planting

Treatment		Solution composition							
Ca conc. (µM)	Σa_{Almono} (µM)	Total Al (µM)	Mono Al (µM)	EC (mS/cm)	Al^{3+} (µM)	$Al(OH)^{2+}$ (µM)	$Al(OH)_2^+$ (µM)	$Al(OH)_3$ (µM)	$AlSO_4^+$ (µM)
500	15	25	21	0.520	5.01	0.98	3.98	0.03	4.92
	30	44	42	0.547	9.93	1.94	7.89	0.05	9.79
	60	87	85	0.569	19.98	3.90	15.87	0.10	19.67
1 000	15	25	21	0.609	4.31	0.84	3.42	0.02	6.49
	30	44	42	0.634	8.56	1.67	6.80	0.04	12.93
	60	87	85	0.660	17.26	3.37	13.72	0.09	25.82
2 500	15	25	21	0.841	3.10	0.61	2.45	0.02	9.24
	30	43	41	0.867	6.04	1.18	4.80	0.03	17.93
	60	84	82	0.883	12.07	2.35	9.59	0.06	35.66
5 000	15	23	20	1.205	2.07	0.40	1.65	0.01	10.75
	30	42	40	1.244	4.14	0.81	3.29	0.02	21.35
	60	83	81	1.250	8.39	1.64	6.66	0.04	43.20
10 000	15	23	20	1.768	1.35	0.26	1.07	0.01	12.39
	30	42	40	1.786	2.70	0.53	2.15	0.01	24.74
	60	83	81	1.812	5.47	1.07	4.35	0.03	49.94

Table 2. Incremental addition of stock solution until 21 days after planting

Nutrient conc.	Addition of stock solution (mL/pot)										
	Day 1	2	3	4	5	6	7	8	9	10	11–21
0.1 M NH_4NO_3	0.65	0.74	0.84	0.95	1.08	1.22	1.39	1.57	1.78	2.02	2.29
0.1 M KNO_3	0.69	0.78	0.88	1.00	1.14	1.29	1.46	1.66	1.88	2.13	2.42
0.1 M $MgSO_4$	0.10	0.11	0.12	0.14	0.16	0.18	0.21	0.23	0.26	0.30	0.34
0.1 M $MgCl_2$	0.08	0.09	0.10	0.12	0.13	0.15	0.17	0.19	0.22	0.25	0.28
1.0 mM FeEDTA	0.59	0.67	0.76	0.86	0.97	1.10	1.25	1.42	1.61	1.82	2.07
1.0 mM $MnSO_4$	0.29	0.33	0.37	0.42	0.49	0.54	0.62	0.70	0.79	0.90	1.02
1.0 mM $CuSO_4$	0.07	0.08	0.09	0.10	0.11	0.13	0.14	0.16	0.19	0.21	0.24
1.0 mM $ZnSO_4$	0.03	0.03	0.03	0.04	0.04	0.05	0.05	0.06	0.07	0.08	0.09
1.0 mM H_3BO_3	0.60	0.68	0.77	0.87	0.99	1.12	1.27	1.44	1.63	1.85	2.10
0.1 mM MoO_3	0.06	0.06	0.07	0.08	0.09	0.11	0.12	0.14	0.15	0.17	0.20

Phosphorus concentration was maintained at 5 µM (measured every 3 days) by addition of $NaH_2PO_4.2H_20$

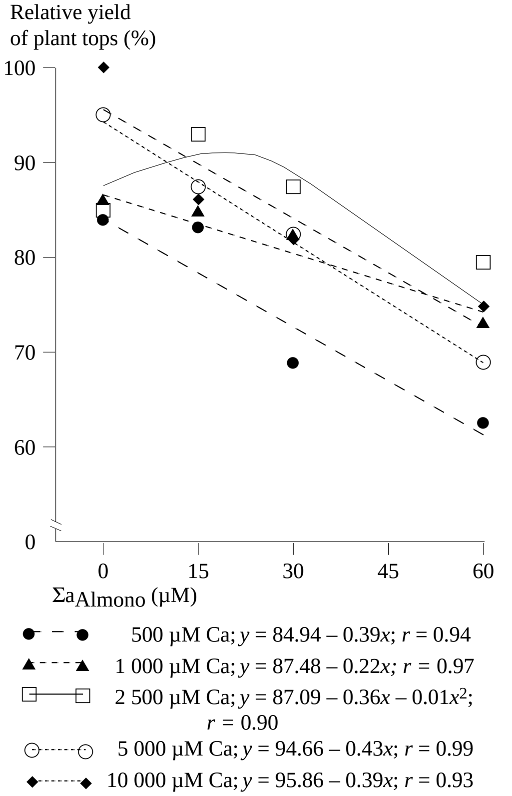

Figure 1. Relative yield of plant tops of groundnut grown in mineral N at different concentrations of Σa_{Almono} and Ca

Root elongation

In all Ca treatments, except the 10 000 µM Ca, a stimulation in relative root length was observed at 15 µM followed by a decline with further increase in Σa_{Almono} *(Figure 2)*. At 15 µM Σa_{Almono}, plants supplied with 1 000 µM Ca produced the greatest root growth (100% relative root length). The toxic effects of Al at 30 µM and 60 µM Σa_{Almono} on root length were more deleterious on plants provided with 500 µM and 1 000 µM Ca than those with ≥ 2 500 µM. A soil solution concentration of 1 000–5 000 µM Ca was generally required to protect plant roots against deleterious effects of low pH, toxic ions and other nutritional imbalances (Raju et al. 1972; Foy 1974; Marschner 1974; Moore 1974).

At 60 µM Σa_{Almono}, plants supplied with low levels of Ca (500 µM and 1 000 µM) produced roots which were corralloid with many stubby lateral roots but few branches. Wagatsuma (1983) indicated that Al was bound to pectic substances in the cell walls of root but a small proportion of Al entered the protoplast and combined with nucleic acids and acid soluble phosphates; the destruction of roots and the high concentration of Al in the medium (1 852 µM) increased the passive movement of Al into the protoplast. An increase in the Ca supply from 2 500 µM to 10 000 µM

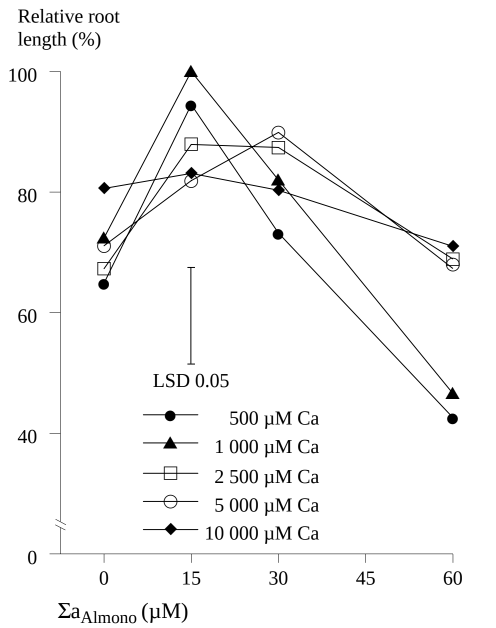

Figure 2. Relative root length of groundnut grown in mineral N at different concentrations of Σa_{Almono} and Ca

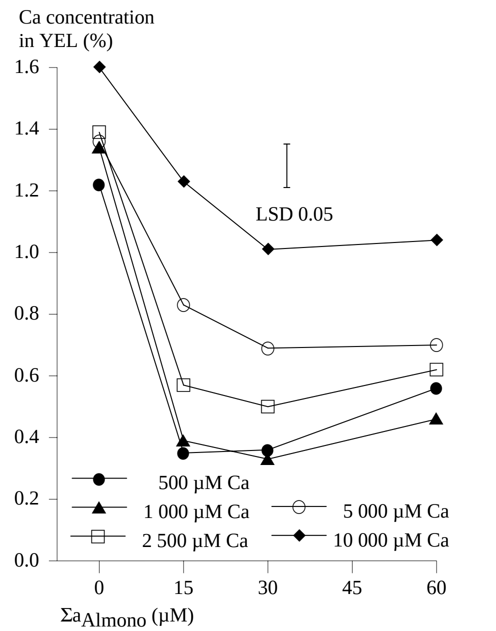

Figure 3. Ca concentration in YEL of groundnut grown in mineral N at different concentrations of Σa_{Almono} and Ca

improved root morphology and promoted elongation of primary roots, but inhibited the growth of lateral roots. Thus, increasing the Ca concentration to >2 500 µM increased root growth and overcame the toxic effect of Al. Wagatsuma (1983) also reported that Ca in the medium containing Al could suppress Al toxicity when the concentration of Ca was considerably higher than that of Al; a Ca concentration of 50 000 µM reduced the Al content of roots from 111 µM to 0 µM.

Ca and Mg concentrations in YEL

In the absence of Al, the Ca concentration in YEL was increased from 1.22% to 1.60%, corresponding to an increase in Ca concentration from 500 µM to 10 000 µM *(Figure 3)*. Increased Al concentration decreased Ca concentration in YEL. The effect was more deleterious in the lower Ca treatments. At 15, 30 and 60 µM Σa_{Almono}, all plants had low Ca concentration (<1.25%) in YEL inadequate for optimum growth requirement of 1.47% (Kasran 1989). Similar suppressing effects of Al on the uptake of cations, especially Ca, have been previously reported (Foy and Brown 1964; Mac Leod and Jackson 1965, 1967; Munns 1965). The inhibitory effects of Al^{3+} on Ca^{2+} uptake have been attributed to Al^{3+} replacement of Ca^{2+} on binding or exchange sites of cell walls (Foy et al. 1972) which partly accounted for Al toxicity in plants grown on acid soils (Noble and Sumner 1988). Therefore, Al stress could increase the plant requirement for Ca. Lance and Pearson (1969) reported that the inhibition of Ca uptake by roots of cotton seedlings previously treated with 11 µM of Al could not be avoided until the Ca concentration in the medium was increased to 15 000 µM. Johnson and Jackson (1964) indicated that the reduction in Ca concentration in wheat due to Al toxicity could not be overcome by supplying additional Ca. They further suggested that a portion of the Ca-accumulating mechanism was inactivated completely by 5.56 µM Al concentration or less.

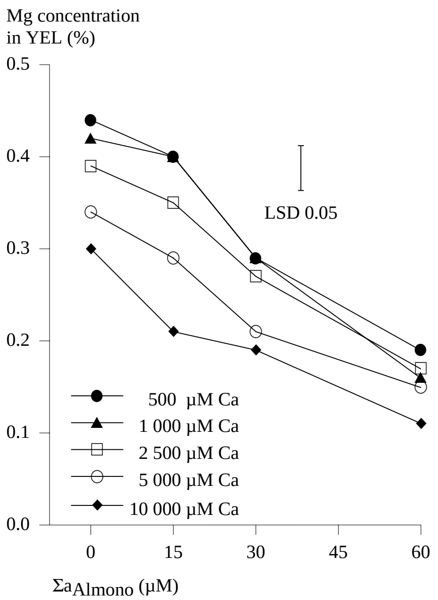

Figure 4. Mg concentration in YEL of groundnut grown in mineral N at different concentrations of Σa_{Almono} and Ca

Generally, the increase in Ca supply lowered the Mg concentrations in YEL, as shown in the Al control treatment *(Figure 4)*. Further increase in Al concentrations lowered the Mg concentrations in YEL. The decrease was directly related to the increase in Ca concentration. At 15 µM Σa_{Almono}, plants supplied with 5 000 µM and 10 000 µM Ca had low Mg concentrations in YEL, <0.30%, inadequate for optimum growth (Kasran 1989). An increase in the Al concentration to 30–60 µM Σa_{Almono} markedly decreased the Mg concentrations in plants supplied with <2 500 µM Ca to <0.30%. This seems to indicate that a high level of Ca with a high Al concentration collectively produced a more severe inhibition in the translocation of Mg. Similar results were also observed by Shamsuddin et al. (1992). Kasran et al. (1992) found that the presence of Al (1.5–80 µM Σa_{Almono}) lowered the Mg concentrations in YEL but not in the roots, indicating that translocation of Mg from the roots to the plant tops was inhibited by Al.

Conclusions

Data obtained from the study indicated that 2 500 µM external solution Ca concentration was required to alleviate Al toxicity at 24 µM Σa_{Almono} and produce 90% relative yield of groundnut tops. The results also indicated that Ca could not alleviate Al toxicity effects at 30 µM and 60 µM Σa_{Almono} on growth of top, root, and root length of groundnut. Apparently, Ca concentration >5 000 µM depressed top dry weight and the Mg concentration in YEL to 0.19% at 60 µM Σa_{Almono}.

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