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Full Length Article

Effect of Organic Acids Amendment on the Growth and Yield of Soybean (*Glycine max*) in Ultisol

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ABSTRACT

Aluminum toxicity and low soil P are the major fertility constraints in Ultisols. The mechanism of resistance to aluminum toxicity has been attributed to the Al-dependent release of organic anions from roots. The present study examined ameliorative effect of adding free citrate, malate and lactate into the soil to the growth and grain yield of soybean. The organic acids were added at equivalent weight ratios of 1:2, 1:1 and 2:1 to exchangeable Al. Significantly longer root lengths, higher root biomass and higher shoot biomass were recorded in soil amended with malate and lactate at equivalent weight ratio of 1:2 to exchangeable Al. However, addition of organic acids at equivalent weight ratio of 2:1 produce significantly shorter root lengths, lower root biomass and lower shoot biomass. Severe growth retardations were observed in lactate (2:1) amended soil. While showing ameliorative effect on soybean growth in Ultisol, addition of lactate at equivalent weight ratio of 1:2 resulted in higher aluminum concentration in plant organs. Amendments using lactate at equivalent weight ratio of 1:2 also increase grain yield of soybean. © 2010 Friends Science Publishers

Key words: Ultisol; Soybean; Aluminum toxicity; Citrate; Malate; Lactate

INTRODUCTION

Soybean is an important source of nutritious foods that are a valuable part of the human diet in Indonesia. However, soybean production is relatively stagnant on a low level and even decreasing during the period of 2005 until 2007 (BPS, 2009). Naturally, it could not fulfill domestic consumption. To meet the demand, increases of productivity through intensive agriculture as well as expansion of harvested area are necessary. Efforts to increase harvested area, however, have to deal with red soils (Siradz, 2008).

Highly weathered, leached Ultisols is often used for the expansion of agricultural production in Indonesia (Donner, 1987). Soil acidity and low soil P are the major fertility constraints in these soils (Siradz, 2008). Soil acidity constraints are a complex interaction of several growth-limiting factors for plants including H, Al, or Mn toxicities, as well as deficiencies of Ca, Mg, P, or Mo (Kamprath, 1984; Foy, 1992). However, the major limitation to plant productivity on most acid soils is aluminum (Al) toxicity (Taylor, 1988). Aluminum toxicity affects the plant roots growing in the acid soil by disrupting the metabolically active cells at the root apex (Ryan *et al*., 1993; Sivaguru & Horst, 1998), resulting in the inhibition of root elongation (Ciamporová, 2002).

The mechanism of resistance has been attributed to the Al-dependent release of organic anions from roots (Delhaize *et al*., 1993; Ryan *et al*., 1995). This model proposes that organic acids released from roots protects the sensitive growing apices by binding with and detoxifying the harmful Al^{3+} cations in the apoplast (Delhaize *et al*., 1993; Delhaize & Ryan, 1995; Ryan *et al*., 2001). The release of organic anions is an important mechanism for Al resistance in cereal and non-cereal species (Zheng *et al*., 1998b; Ma *et al*., 2001; Ryan *et al*., 2001). The nature of released organic anions from roots differs between species. The release of malate was reported in wheat (Delhaize *et al*., 1993; Ryan *et al*., 1995), oxalate was reported in buckwheat (*Fagopyrum esculentum*) and taro (*Colocasia esculenta*) (Ma & Miyasaka, 1998; Zheng *et al*., 1998a), while citrate was reported from maize (*Zea mays*), snapbean (*Phaseolus vulgaris*) and *Cassia tora* (Miyasaka *et al*., 1991; Pellet *et al*., 1995; Ma *et al*., 1997). Moreover, Meriga *et al*. (2003) were able to alleviate toxic effects of aluminum toward rice seedling by adding free citrate into the culture medium.

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The objective of this study was to elucidate the effects of different organic acids addition in alleviating aluminum toxicity toward soybean grown in Ultisol.

MATERIALS AND METHODS

Soil properties: Soil used for the greenhouse experiment was a surface soil (0–20 cm) of Ultisol (sub ordo Typic Tropudult) collected from Jasinga, Bogor Regency, West Java, Indonesia (106.99 E, 6.15 S). Soil sample was air-dried, passed through 2-mm sieve and analyzed for its water holding capacity, pH, exchangeable Al, Al saturation, exchangeable Na, K, Ca and Mg. Water holding capacity of soil was determined according to Wilde *et al*. (1972). Soil pH was determined potentiometrically in distilled water (1:2.5, wt/vol) (Tan, 1996). Exchangeable Al was extracted in 1.0 mol L^{-1} KCl (1:10 soil/solution & shaken for 30 min) and determined by titration with 0.02 mol L^{-1} NaOH solution (Mc Lean, 1965). Exchangeable Na, K. Ca and Mg were extracted in 1 mol L^{-1} NH_4OAc at pH 7.0 (Chapman, 1965), prior to analysis by atomic absorption spectroscopy (Tan, 1996). Aluminum saturation was calculated as a measure of Al toxicity using Eq. [1] (Johnson *et al*., 1997).

$$\text{Al saturation (\%)} = (Al_{KCl}/ECEC) \times 100 \quad [1]$$

Where Al_{KCl} is exchangeable Al, expressed in $cmol_c\ kg^{-1}$ and ECEC (effective cation exchange capacity) is the sum of exchangeable Na, K, Ca and Mg, expressed in $cmol_c\ kg^{-1}$, plus the concentration of Al_{KCl}. Water holding capacity of the soil was determined according to Tan (1996). Triplicate analyses were conducted in the measurement of soil properties.

Soil treatments: Each pot (25 cm diameter & 35 cm deep) contained 6 kg of soil. The soil was amended with ammonium nitrate 87 mg kg^{-1}, potassium phosphate monobasic 133.3 mg kg^{-1}, potassium chloride 33.3 mg kg^{-1}, magnesium sulfate heptahydrate 40 mg kg^{-1}, ammonium molybdate tetrahydrate 0.17 mg kg^{-1} and sodium tetraborate decahydrate 0.17 mg kg^{-1}. Organic acids were added separately following the treatment. Citrate, malate and lactate, were added as their acid forms. Their addition rates were calculated to be at equivalent weight ratios of 1:2, 1:1 and 2:1 to exchangeable Al. Citrate was added at 5.11 g, 10.22 g and 20.44 g kg^{-1} soil to obtain equivalent weight ratios of 1:2, 1:1 and 2:1 to exchangeable Al, respectively. Malate was added at 5.35 g, 10.70 g and 21.40 g kg^{-1} soil to obtain equivalent weight ratios of 1:2, 1:1 and 2:1 to exchangeable Al, respectively. On the other hand, liquid lactate (90%) was added at 6.59 mL, 13.19 mL and 26.38 mL kg^{-1} soil to obtain equivalent weight ratios of 1:2, 1:1 and 2:1 to exchangeable Al, respectively. No addition of organic acid was utilized as a control. Chemical amendments and organic acids were thoroughly mixed with the soil and tap water was added to reach 85% of water holding capacity. Pots were weighed daily and brought back to initial weight to maintain moisture. Soil moisture was kept at 85% of water holding capacity throughout the development period of the soybean grown.

Soybean growth: Soil was incubated for 7 d before seed sowing. Following the incubation, 3 soybean (*Glycine max* (L.) Merr.) seeds from local variety of Wilis were sown per pot. Three replicates of each treatment were planted in a completely randomized design. Pots were thinned to one plant per pot at 7 d. Plants were grown in a green house, where temperatures ranged between 26 and 32°C. Plants were harvested at 21 days after sowing (DAS) and at their maximum vegetative growth (indicated by a single open flower at any node on the main stem). At each harvest roots were carefully separated from soil and thoroughly washed under tap water. Total root length was determined using the modified line intersect method (Tennant, 1975). Roots and shoot materials were separated and dried in oven at 75°C to constant weights. Powder of the root and shoot materials harvested at their maximum vegetative growth were dissolved in dilute acid mixture ($HClO_4$:HNO_3; 1:1 v/v) and their Al content was determined using atomic absorption spectroscopy. Absorbed Al in plant parts were calculated from the dry weights and their Al of content. At their maximum generative growth (indicated by full mature color of 95% of the pods), data regarding pod number and grain yield were also recorded. The data of root length, root and shoot dry weight at 21 DAS and at their maximum vegetative growth, number of pod, grain yield, Al concentration at root and shoot, as well as total accumulated Al in soybean root and shoot, were analyzed by applying Duncan's Multiple Range (DMR) Test (Gomez & Gomez, 1984).

RESULTS AND DISCUSSION

Soil characterization: The soil used in this study shows characteristics of Ultisol (Table I). Soil pH was low (pH = 4.3). Exchangeable Na, K, Ca and Mg were 0.50, 0.28, 3.37 and 1.17 cmol kg^{-1}, respectively. Exchangeable Al was 15.97 $cmol_c\ kg^{-1}$ and Al saturation was high (Al sat = 75%). Al saturation and exchangeable Al were well correlated to the reductions of plant growth (Shuman *et al*., 1990). Dry weight of tops increased as the Al saturation was decreased.

Effect of organic acids amendment on root and shoot growth: The most obvious symptom of soil toxicity is inhibition of root growth (Haynes & Mokolobate, 2001), which then followed by inhibition of plant growth (Adams & Moore, 1983). Observation of root length, root biomass and shoot biomass were done at 21 DAS and at maximum vegetative growth. The maximum vegetative growth was reached at 42 DAS. At 21 DAS observation, significantly longer root lengths, higher root biomass and higher shoot biomass were recorded in soil amended with malate and lactate at equivalent weight ratio of 1:2 to exchangeable Al (Table I). No significant responses were recorded on amendments with citrate at equivalent weight ratios of 1:2

Table I: Effect of organic acids amendment on soybean growth

Amendments	At 21 DAS			At 42 DAS		
	Root length (cm)	Root dry wt (g)	Shoot dry wt (g)	Root length (cm)	Root dry wt (g)	Shoot dry wt (g)
Citrate: Exchangeable Al at equivalent weight ratios of						
1:2	347.7^{cd}	0.11^{ij}	0.41^{pq}	1205.7^{B}	0.26^{I}	1.53^{Q}
1:1	453.0^{ab}	0.14^{gh}	0.44^{op}	1314.9^{B}	0.28^{I}	2.11^{P}
2:1	232.0^{e}	0.08^{k}	0.21^{t}	766.4^{D}	0.16^{K}	0.95^{S}
Malate: Exchangeable Al at equivalent weight ratios of						
1:2	484.4^{a}	0.15^{g}	0.45^{o}	1564.4^{A}	0.33^{H}	2.19^{P}
1:1	389.5^{bc}	0.12^{hi}	0.32^{r}	1298.9^{B}	0.28^{I}	1.21^{RS}
2:1	295.2^{de}	0.09^{jk}	0.26^{s}	938.9^{C}	0.20^{J}	1.04^{S}
Lactate: Exchangeable Al at equivalent weight ratios of						
1:2	506.6^{a}	0.16^{g}	0.44^{op}	1705.7^{A}	0.36^{G}	2.47^{O}
1:1	295.9^{de}	0.09^{jk}	0.33^{r}	547.7^{C}	0.12^{L}	1.43^{QR}
2:1	32.0^{f}	0.01^{l}	0.05^{u}	299.9^{F}	0.02^{M}	0.12^{T}
Control	379.8^{bc}	0.12^{hi}	0.39^{q}	1314.5^{B}	0.28^{I}	1.45^{QR}
S.E.	2.66	0.014	0.027	7.68	0.039	0.037
P_{acid}	<0.0001	0.0009	<0.0001	<0.0001	<0.0001	<0.0001
$P_{equiv\ wt\ rt}$	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
$P_{acid\ x\ equiv\ wt\ rt}$	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Mean values (n=3), standard errors (S.E.), and probabilities (P) of treatment effects for the ANOVA. Means followed by the same letter are not significantly different (P>0.05)

and 1:1 and with malate at equivalent weight ratio of 1:1. Moreover, addition of citrate, malate and lactate at equivalent weight ratio of 2:1 produce significantly shorter root lengths, lower root biomass and lower shoot biomass. Severe growth retardations were observed in lactate (2:1) amended soil. Similar growth responses were observed at 42 DAS.

Longer root lengths, higher root biomass and higher shoot biomass in soil amended with malate and lactate at equivalent weight ratio of 1:2 to exchangeable Al were in disagreement with those reported by Hue *et al*. (1986) and Ginting *et al*. (1998). Using cotton taproot as bioindicator Hue *et al*. (1986) observed that citrate was a strong detoxifier in nutrient solutions containing aluminum, while malate and lactate were moderate and weak detoxifiers, respectively. Using soybean root as bioindicator, Ginting *et al*. (1998) also reported that the ability of organic acids in alleviating Al phytotoxicity was in the order of citrate ≈ malate >> lactate.

Additions of malate and lactate at equivalent weight ratio of 1:2 to exchangeable Al were equal to 3.99 and 7.98 cmol kg^{-1}, respectively. The concentrations of added malate and lactate were a quarter and a half, respectively to the concentration of exchangeable Al. Additions of citrate, malate and lactate at equivalent weight ratio of 2:1 to exchangeable Al which were equal to 10.65, 15.96 and 31.92 cmol kg^{-1}, respectively resulted in significant shorter root lengths, lower root biomass and lower shoot biomass. The result was in disagreement with that of Ginting *et al*. (1998), who suggested that the concentration of the organic anion must be at least that of the Al to reduce phytotoxicity significantly. Ginting *et al.* (1998) also suggested that twice as much oxalate as citrate was required to achieve the same reduction in phytotoxicity. The differences between our results and that of Hue *et al*. (1986) and Ginting *et al*. (1998) may be caused by the difference of the growth media. While Hue *et al*. (1986) and Ginting *et al*. (1998) used nutrient solution amended with Al for their experiments; Ultisol was used in this experiment. Interaction with complex physical and chemical components in soil may cause the differences.

Effect of organic acids amendment on yield parameters of soybean: Observation on yield parameters at maximum generative growth (42 DAS) recorded a sustained effect of organic acid amendments to Ultisol. Significant increases of pod numbers were also recorded following the addition of malate and lactate at equivalent weight ratio to exchangeable Al of 1:2 (Table II). Citrate addition at equivalent weight ratio of 1:1 also significantly increased pod number. However, only amendments using malate and lactate at equivalent weight ratio of 1:2 increase grain yield of soybean grown in Ultisol. Severe growth retardations observed in lactate (2:1) amended soil was followed by severe decrease of grain yield. As in the effect of organic acids amendment on root and shoot growth, increases concentration of added organic acid resulted in decreases of plants grain yield.

Effect of organic acids amendment on the concentration of aluminum in soybean: Observation of aluminum concentrations in soybean root at 42 DAS recorded significant increases of aluminum concentration following the additions of citrate and lactate at equivalent weight ratio of 1:2 to exchangeable Al (Table III). On the other hand, significant decreases of aluminum concentration in soybean root were recorded following the additions of malate and higher equivalent weight ratios of citrate and lactate. However, a tendency of Al concentration decrease in root compared to that of control was observed from the overall data.

At 42 DAS, significant decreases of aluminum concentration in soybean shoot was recorded following the additions of citrate and malate at equivalent weight ratio of

Table II: Effect of organic acids amendment on different yield parameters of soybean at maturity

Amendments	Number of Pod	Grain yield (g)
Citrate : Exchangeable Al at equivalent weight ratios of		
1:2	19.33^{cd}	2.26^{rs}
1:1	21.00^{b}	3.76^{o}
2:1	18.33^{cd}	2.42^{q}
Malate : Exchangeable Al at equivalent weight ratios of		
1:2	21.33^{b}	4.30^{n}
1:1	18.00^{d}	3.27^{p}
2:1	14.00^{e}	1.89^{s}
Lactate : Exchangeable at equivalent weight ratios of		
1:2	38.67^{a}	5.72^{m}
1:1	19.67^{c}	2.81^{q}
2:1	2.00^{f}	0.28^{t}
Control	19.33^{cd}	3.52^{op}
S.E.	0.62	0.085
P_{acid}	<.0001	<.0001
$P_{equiv\ wt\ rt}$	<.0001	<.0001
$P_{acid\ x\ equiv\ wt\ rt}$	<.0001	<.0001

Mean values (n=3), standard errors (S.E.), and probabilities (P) of treatment effects for the ANOVA. Means followed by the same letter are not significantly different (P>0.05)

Table III: Effect of organic acids amendment on the concentration of aluminum in soybean root and shoot

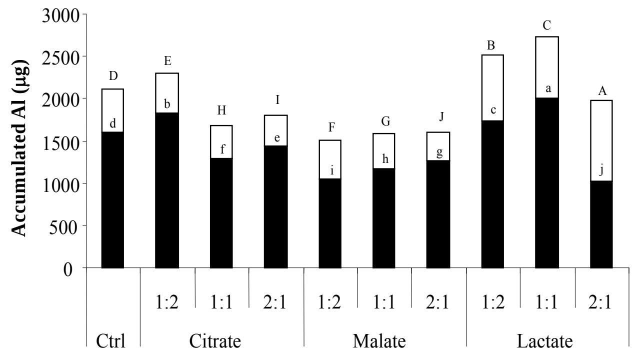
Amendments	Aluminum concentration (μg g^{-1} dry weight)	
	Root	Shoot
Citrate : Exchangeable Al at equivalent weight ratios of		
1:2	7346.5^{a}	336.6^{mn}
1:1	5589.8^{d}	284.5^{opq}
2:1	6234.2^{c}	260.2^{pq}
Malate : Exchangeable Al at equivalent weight ratios of		
1:2	4552.7^{f}	323.7^{mno}
1:1	5100.6^{e}	294.9^{nop}
2:1	5484.2^{d}	239.2^{q}
Lactate : Exchangeable at equivalent weight ratios of		
1:2	7523.9^{a}	561.6^{l}
1:1	6699.2^{b}	518.0^{l}
2:1	4727.5^{ef}	677.6^{k}
Control	6941.2^{b}	363.3^{m}
S.E.	4.36	3.57
P_{acid}	<.0001	<.0001
$P_{equiv\ wt\ rt}$	<.0001	<.0001
$P_{acid\ x\ equiv\ wt\ rt}$	<.0001	<.0001

Mean values (n=3) standard errors (S.E.) and probabilities (P) of treatment effects for the ANOVA. Means followed by the same letter are not significantly different (P>0.05)

1:1 and 2:1. Alike to the tendency of aluminum accumulation in soybean root, decreasing aluminum concentrations in soybean shoot was observed following addition of citrate and malate at increasing equivalent weight ratios. On the contrary, increase aluminum concentrations in soybean shoot were observed following increase concentration of added lactate.

Significant longer root lengths, higher root biomass and higher shoot biomass of soybean grown in Ultisol amended with malate and lactate at equivalent weight ratio of 1:2 to exchangeable Al (Table I) were not well associated with aluminum concentrations in root and shoot. Alleviation of aluminum toxicity to plants by organic acids were related to their ability to form stable chelate complexes with

Fig. 1: Accumulated Al in soybean root (■) and shoot (□) after amendment with organic acids. Mean values (n=3) presented; data bars carrying same alphabet differ non-significantly (P>0.05). Standard errors are too small to be presented



monomeric Al in soil solution thus greatly reduce its availability (Haynes & Mokolobate, 2001). Longer root lengths, higher root biomass and higher shoot biomass of soybean grown with malate amendment Al were in agreement with this mechanism. Accumulated Al in plant organs indicate availability of Al to plant. Accumulated Al in root was increased significantly following lactate addition, except at equivalent weight ratio of 2:1. On the other hand, citrate and malate additions generally resulted in significant decreases of Al accumulation in root (Fig. 1). Accumulated Al in shoot was also increased significantly following lactate addition, while citrate and malate additions resulted in significant decreases of Al accumulation in shoot. Significant increase of Al availability following addition of lactate may be caused by high solubility of aluminum lactate (ATSDR, 1999). Highly soluble aluminum lactate was presumably well diffused into the soybean and transported to shoot. Wang *et al*. (2007) reported similar mechanism on the enhancing effect of organic acids on lead uptake by wheat root.

Higher aluminum content, compared to control, in the root and shoot of soybean following the addition of lactate at equivalent weight ratio of 1:2 to exchangeable Al coincide with higher root and shoot growth as well as higher grain yield. It raises a question on the toxicity of aluminum to plant cells and organs. We hypothesized that toxicity of aluminum to plant root was caused by precipitation of Al, which is soluble in acid condition of soil, inside or around the root as a result of its scarce solubility in the physiological pH range (Marschner, 1995). Precipitated Al inhibits nutrient uptake into root. Lactate chelated aluminum, which is soluble in physiological pH range, was not precipitated and, therefore, was not inhibit root functions. However, additions of lactate at higher concentrations may cause a higher adsorption of aluminum, which then caused phytotoxicity to the plant. Moreover, higher concentrations of organic acids may disturb physiological process in the plants.

The organic acids at the equivalent weight ratio of 1:2 to exchangeable Al could amend the problem of aluminum toxicity in Ultisol and the mechanism for detoxification may differ among organic acids.

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