

Nutrient uptake of soybean genotypes under aluminum toxicity

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Abstract

The objective of this research was to study the nutrient uptake of soybean exposed to aluminium (Al) toxicity. The factorial design consisted of two treatments arranged in a randomized block design with three replications. Liming was the first factor which consisted of four levels, *i.e.* i) without liming; ii) liming with $0.5 \times \text{Al}(\text{exchangeable}/\text{ec})$; iii) liming with $1 \times \text{Al}(\text{ec})$; and iv) liming with $1.5 \times \text{Al}(\text{ec})$. Five genotypes were used as second factor, *i.e.* three tolerant genotypes (W3898-14-3, Willis, and Kawi), and two sensitive genotypes (MLG 3209 and MLG 3083). It was found that two tolerant genotypes, W3898-14-3 and Kawi, had a higher potassium (K) and sodium (Na) uptake than susceptible genotypes. Liming affected significantly the ratio of $\text{Al}/[\text{calcium}(\text{Ca}) + \text{magnesium}(\text{Mg})]$ in roots and leaves, the content of Ca and Mg in the roots and the content of Mg in the leaves. The K content in the roots and the content of Ca, K, and Na in the leaves were unresponsive to the alteration of pH and Al saturation.

Introduction

Among the earth's crust minerals, aluminum (Al) is the third most abundant after oxygen and silicon (Bhalerao and Prabu, 2013). It can have

a toxic effect on plants under specific soil conditions, especially when the soil is acid. The root is the plant organ most affected by Al toxicity, before the upper part organs (Meriño-Gergichevich *et al.*, 2010). However, some plants can tolerate Al toxicity, whereas other cannot tolerate it at all. This suggests that there is a specific mechanism associated with Al toxicity tolerance. Al-tolerant plants can be classified in three groups according to the plant tissues that accumulate Al (Foy, 1984). The first group includes plants in which the Al concentration in the leaves is not always different from that of sensitive plants and remains at lower levels. The second group includes plants with no Al in the leaves and/or Al trapped in the roots. The third group includes plants in which Al tolerance is directly related to Al accumulation in the upper part of the plant. These plants have therefore a high internal tolerance towards Al. In principle, the mechanisms of Al tolerance facilitate Al exclusion from the root and/or confer the plant the ability to tolerate it in its organs (Vardar and Unal, 2007). Watanabe and Osaki (2002) proposed other Al toxicity tolerance mechanisms by classifying plant into two groups. The first group includes the Al excluders, *i.e.* plants that exclude Al from their organs. The second group includes the Al accumulators, *i.e.* the plants that inactivate Al in their organs. Al accumulator plants present specific mechanisms involved in tolerance, such as the immobilization of Al in the cell wall, cytoplasmic Al complexation by organic acids, vacuolar isolation of Al tolerant enzymes, and isolation of Al in the vacuoles (Sopandje *et al.*, 2000). Inorganic nutrients are also involved in this mechanism due to the interaction of Al with other nutrients to form inactive molecules. When Al concentration increases, the concentrations of magnesium (Mg), calcium (Ca), potassium (K), and sodium (Na) decrease in the foliage and the roots (Bojarczuk *et al.*, 2006). However liming can increase Ca, Mg, K, and Na in both the soil and the plant; because it increases the soil pH, thus increasing the availability of Ca, Mg, K, and Na in the soil itself. Some studies reported that the use of Ca and Mg led to a decrease of Al in the soil and the plants (Wang and Kao, 2004; Loide, 2004), because the presence of Ca and Mg can precipitate Al. On the contrary, the binding of Al^{3+} to negative charges and the precipitation of Al in the apoplast may decrease the loading of Mg^{2+} ions (Bose *et al.*, 2011). The effect of Mg is ion-specific and is not only associated to an electrostatic protection mechanism (da Silva *et al.*, 2008). Being a cation, K can also potentially decrease Al toxicity. Na was also reported among nutrients that can decrease Al toxicity (Maas *et al.*, 2000). The tissue concentration of these nutrients may vary among soybean genotypes. The tolerant group shows higher concentrations of Mg and Ca than the sensitive group (Yan *et al.*, 2009). Changes in the content of these nutrients in roots (Bojarczuk *et al.*, 2006) and leaves (Poolpipatana and Hue, 1994) indicate that these traits can be useful tolerance or sensitive indicators (Bojarczuk *et al.*, 2006; Poolpipatana and Hue, 1994).

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Materials and methods

Soil preparation

Before planting, the soil was limed with dolomite. Soil and dolomite (39% CaO and 21% Mg) were mixed to obtain a homogenous soil medium. The mixture was then incubated for 30 d to enable the reaction

Table 1. Nutrient content of the soil with liming at 0.0×Al(ec), 0.5×Al(ec), 1×Al(ec), and 1.5×Al(ec).

Soil properties	Liming			
	0×Al(ec)	0.5×Al(ec)	1×Al(ec)	1.5×Al(ec)
pH H ₂ O	4.95	5.65	6.2	6.6
pH KCl	4.1	4.6	5.25	5.8
K (Cmol.kg ⁻¹)	0.16	0.22	0.28	0.29
Na (Cmol.kg ⁻¹)	0.46	0.58	0.62	0.79
Ca (Cmol.kg ⁻¹)	1.81	2.97	9.88	16.86
Mg (Cmol.kg ⁻¹)	0.37	1.14	3.23	7.84
CEC (Cmol.kg ⁻¹)	4.4	8.8	17.6	22
CEC _{effective} (Cmol.kg ⁻¹)	4.57	6.36	15.01	26.11
Al _{saturation} (%)	27.13	15.88	4.59	0.19

Al(ec), aluminium exchangeable; K, potassium, Na, sodium, Ca, calcium, Mg, magnesium.

between soil and lime to take place. During the incubation period, every four days the soil was watered to maintain soil moisture under field capacity conditions. Planting was performed in a polybag with a capacity of 10 kg of soil with two plants per polybag.

Greenhouse experiment

The greenhouse experiment was conducted with Ultisol soils in the province of Lampung-Indonesia. The design was factorial, included two factors, and was arranged in a randomised complete block with three replications. The first factor was liming which consisted of four levels, *i.e.* i) without liming; ii) liming with 0.5×Al(exchangeable/ec); iii) liming with 1×Al(ec); and iv) liming with 1.5×Al(ec). The second factor was the genotype which consisted of five genotypes: three tolerant genotypes (W 3898-14-3, Wilis, and Kawi) and two sensitive genotypes (MLG MLG 3209 and 3083).

Plant nutrient analysis

Plant nutrient contents were measured during the flowering stage. Measurements were carried out on the physiological characters, such as content of nutrients like Al, K, Na, Ca, and Mg in roots and leaves. Before plant nutrients were analysed, shoots and roots were separated, and then roots were washed under running water to eliminate the soil. The clean shoots and roots were dried under the sunshine. The five nutrients were analysed by grinding the roots and the leaves with a grinding mill. Then they were subsequently put in an acid solution for further destruction. The materials were then diluted and observed under an atomic absorption/flame emission spectrophotometer (AA-Shimidzu 630-12) to measure the content of Al, Ca, and Mg, and under a flame photometer (CORNING Flamephotometer 410) to measure the content of K and Na.

Results and discussion

Liming increased the pH from 4.95 up to 5.65, 6.20 and 6.60 at 0.5×Al(ec), 1×Al(ec), and 1.5×Al(ec) respectively. Consequently, the macronutrients increased and the micronutrients decreased (Table 1). The highest increase was shown by Ca after applying liming at 1.5 Al(ec). Mg and Na also increased significantly after Ca. In general, the nutrients increased significantly at 1×Al(ec) and tended to flatten out at 1.5×Al(ec). Similar results were also reported by other authors (Pengshouvana *et al.*, 2009) showing that liming increased the concentration of Ca and Mg, but decreased Al in the soil. This effect is due to

Table 2. Effect of liming on calcium (Ca) and magnesium (Mg) uptake and aluminium/(Ca+Mg) ratio in root of soybean genotypes.

Liming	Ca (ppm)	Mg (ppm)	Al/(Ca+Mg)
0×Al(ec)	306.98 ^c	1551.25 ^c	80.30 ^a
0.5×Al(ec)	349.62 ^b	2383.83 ^b	53.27 ^b
1×Al(ec)	406.30 ^a	2566.44 ^{ab}	48.22 ^b
1.5×Al(ec)	370.70 ^b	2692.18 ^a	42.43 ^b

Ca, calcium; Mg, magnesium; Al(ec), aluminium exchangeable. ^{a,b,c}Values in the same column followed by the same letter were not significantly different with Duncan's multiple range test of 5%.

the ability of lime to increase the soil pH, thus increasing macronutrient solubility, yet decreasing micronutrient solubility, including Al solubility.

The variance analysis of the nutrient contents in soybean roots showed a significant difference in the contents of Ca, Mg, K and Al/(Ca+Mg) ratio, whereas Al and Na were not significantly different (data not shown). Unlike other nutrients, the content of Mg in the leaves of soybean was significantly different. The genotype factor also showed a significant difference in relation to the content of K, Ca, and Na, thus explaining the difference in the uptake of the three nutrients in each genotype. A similar result was also reported by Lee (1989).

The increase in lime led to an increased uptake of Ca and Mg, but the Al/(Ca+Mg) ratio decreased (Table 2). Ca uptake increased from 0.5×Al(ec) to 1×Al(ec), but it decreased at 1.5×Al(ec). On the contrary, Mg uptake increased up to 1.5×Al(ec). A similar result was also reported by another study (Keltjens, 1990) which showed that an increasing concentration of (Ca+Mg) in the medium also increased concentrations of Ca and Mg in roots and shoots. The highest Al/(Ca+Mg) ratio was reached with no liming and decreased under liming conditions. There was no statistically significant difference in Al uptake in roots and leaves under liming conditions, probably due to the interaction of Al with Ca and Mg. This can be explained by the Al/(Ca+Mg) ratio in roots and leaves, which was significantly different under liming condition (Tables 2 and 3). This occurred because Al can interact with other nutrients, which can therefore neutralise its detrimental effect.

Root Ca uptake increased, as liming increased with the highest uptake occurring at 1×Al(ec) (Table 2). This showed that root Ca uptake was more influenced by the environmental factors rather than the genotype factors. Ca plays a fundamental role in Al-Ca interactions in the improvement of Al toxicity (Meriño-Gergichevich *et al.*, 2010). The increase in Ca uptake was caused by the neutralization due to liming and not by the addition of Ca in Ca-deficient plants (Sunarto,

1985). In this experiment, the increase in soil Ca availability was affected by both neutralisation and Ca addition. This can be explained by the sharp increase in the Ca content, although the soil pH did not increase significantly. The low root Ca uptake at $1.5\times\text{Al}(\text{ec})$ occurred because this liming level had already influenced significantly the availability of soil Ca. Furthermore, at certain concentrations, Al may enhance the absorption of Ca in the roots. Nursyamsi *et al.* (2000) reported that a concentration of 5 ppm of Al can stimulate Ca uptake. Increasing Ca uptake by the roots was reported by Ferufino *et al.* (2000), indicating that Ca uptake was higher in lateral roots than in the taproot. Ca uptake in cells led to favorable conditions for cell enlargement and root cell extension (Blamey, 2003; Okada *et al.*, 2003). Ca application decreased the Al content in the root tips (Watanabe and Okada, 2005).

Mg uptake in roots and leaves increased with the increase in lime. However, when liming exceeded $0.5\times\text{Al}(\text{ec})$, Mg uptake in the leaves did not increase (Table 3), but it kept increasing in the roots (Table 2). Increasing Mg uptake in the leaves with increasing Al(ec) was also reported by Pan *et al.* (1989). Increasing Mg uptake in the roots and leaves was triggered by increasing Mg in the soil due to liming. Increasing Mg in the soil was affected by increasing soil pH that lead to better soil conditions for Mg availability, and increasing Mg as liming materials (dolomite). In addition, the plant Mg uptake was strongly influenced by Mg(ec) and soil pH. A similar result was reported by Basri *et al.* (1991) who stated that Mg absorption decreased by liming with a low Mg content material.

An interaction was identified between the genotype and the environment in relation to K uptake in the roots (Table 4). Genotypes W3898-14-3 and Wilis showed the highest root K uptake at $1.5\times\text{Al}(\text{ec})$. The Kawi genotype showed the highest K uptake with no liming. Sensitive genotype MLG 3209 also showed the highest K uptake at $1.5\times\text{Al}(\text{ec})$, while MLG 3083 showed the highest K uptake at $1\times\text{Al}(\text{ec})$. The interaction between the genotypes and liming indicated that both the genotype and the environment equally influenced K uptake. However, Al can also inhibit K^+ uptake by blocking K inward (Liu and Luan, 2001), thus leading to a decreasing K uptake.

The effect of liming on the uptake of Mg and $\text{Al}/(\text{Ca}+\text{Mg})$ ratio in the leaves showed that Mg uptake in leaves increased when liming increased, whereas the $\text{Al}/(\text{Ca}+\text{Mg})$ ratio decreased (Table 3). A significant difference was identified between control and liming treatments, but not among liming treatments. This indicates that liming at $0.5\times\text{Al}(\text{ec})$ could increase Mg uptake, whereas there was no increasing Mg uptake with liming doses higher than at $0.5\times\text{Al}(\text{ec})$. Andric *et al.* (2012) also reported that liming treatment significantly decrease Mg concentrations in leaves. Like in Mg uptake, a significant difference was seen between control and liming treatments, but not among liming treatments. A decreasing $\text{Al}/(\text{Ca}+\text{Mg})$ ratio is in line with the increasing of Mg uptake in the leaves, since Mg is the component of the ratio.

Beside, Ca uptake in leaves was low lead the contribution to the $\text{Al}/(\text{Ca}+\text{Mg})$ ratio was not significant.

The interaction between the genotype and the environment indicated by Ca uptake in the leaves is reported in Table 5. W3898-14-3 achieved the highest uptake at $0.5\times\text{Al}(\text{ec})$, whereas Willis and Kawi achieved it at $1.5\times\text{Al}(\text{ec})$ and $1\times\text{Al}(\text{ec})$ respectively. MLG 3209 reached the highest Ca uptake at $1\times\text{Al}(\text{ec})$ and the lowest at $1.5\times\text{Al}(\text{ec})$. The highest Ca uptake in the leaves in MLG 3083 occurred at $0.5\times\text{Al}(\text{ec})$, while the lowest at $1\times\text{Al}(\text{ec})$ although no statistically significant difference was identified between $0\times\text{Al}(\text{ec})$ and $1.5\times\text{Al}(\text{ec})$. Ca uptake in the leaves showed an interaction between the genotype and liming, thus proving that both genotype and environmental factors had an influence on Ca uptake in the leaves. The interaction between the genotypes and the environment lead to a different response of the genotypes to the environmental changes (Table 5). Nursyamsi *et al.* (2000) also showed an increase in Ca uptake in the leaves, when Al concentration decrease.

Genotypes W3898-14-3, MLG 3209 and Kawi showed a higher K and Na uptake in the leaves than Willis and MLG 3083 (Table 6). As to Na uptake, MLG 3209 was not significantly different from MLG 3083. The relationship of K and Na uptake with plant tolerance to acidic soils has not been widely studied. In general K and Na uptake also determine Al saturation in the soil. In this experiment root K uptake was not significantly different in the liming treatments, whereas leaf K uptake was significantly different among the genotypes (Table 6). Acid soil tolerant genotypes and Kawi W3898-14-3 showed a high uptake of K in the leaves and were significantly different from susceptible genotype MLG 3083. Basri *et al.* (1991) reported a decreased K uptake with increasing liming. The decreasing K uptake is due to the Ca^{2+} competitive effect on K, because increased liming lead to the dominance of Ca^{2+} in the absorption site.

Uptake of Na in the leaves was also significantly different among the genotypes (Table 6). Similar to the K uptake in the leaves, the leaf Na uptake of acid-tolerant genotypes W3898-14-3 and Kawi showed high

Table 3. Effect of liming on magnesium (Mg) uptake and aluminium/(calcium+Mg) ratio in soybean leaves.

Liming	Mg (ppm)	Al/(Ca+Mg)
$0\times\text{Al}(\text{ec})$	3354.74 ^b	11.29 ^a
$0.5\times\text{Al}(\text{ec})$	4424.26 ^a	7.53 ^b
$1\times\text{Al}(\text{ec})$	4331.95 ^a	8.62 ^b
$1.5\times\text{Al}(\text{ec})$	4296.78 ^a	8.27 ^b

Mg, magnesium; Ca, calcium; Al(ec), aluminium exchangeable. ^{a,b}Values in the same column followed by the same letter were not significantly different at Duncan's multiple range test 5%.

Table 4. Effect of liming on potassium uptake in roots of soybean genotypes.

Genotypes	K (ppm)			
	$0\times\text{Al}(\text{ec})$	$0.5\times\text{Al}(\text{ec})$	$1\times\text{Al}(\text{ec})$	$1.5\times\text{Al}(\text{ec})$
W 3898-14-3	10,149.92 ^{b-e}	11,249.15 ^{ab}	11,552.11 ^{ab}	13,147.80 ^a
Wilis	10,549.64 ^{b-d}	12,148.51 ^{ab}	11449.01 ^{ab}	13,047.87 ^a
Kawi	11,648.86 ^{ab}	5853.67 ^h	7451.83 ^{f-h}	7152.04 ^{gh}
MLG 3209	8750.91 ^{c-g}	8351.62 ^{d-g}	6652.40 ^{gh}	9750.21 ^{b-f}
MLG 3083	8251.27 ^{d-g}	8752.33 ^{c-g}	10849.43 ^{a-c}	7951.48 ^{e-h}

K, potassium; Al(ec), aluminium exchangeable. ^{a,b,c,d,e,f,g,h}Values followed by the same letter were not significantly different at Duncan's multiple range test 5%.

Table 5. Effect of liming on calcium uptake in the leaves of soybean genotypes.

Genotypes	Ca (ppm)			
	0×Al(ec)	0.5×Al(ec)	1×Al(ec)	1.5×Al(ec)
W 3898-14-3	1059.61 ^{a-d}	1270.93 ^a	952.53 ^{b-e}	915.90 ^{b-f}
Wilis	842.64 ^{c-f}	680.59 ^f	1006.07 ^{b-e}	1107.51 ^{ab}
Kawi	827.11 ^{d-f}	1039.88 ^{b-e}	1124.41 ^{ab}	1011.70 ^{b-e}
MLG 3209	883.47 ^{b-f}	960.99 ^{b-e}	991.98 ^{b-e}	798.94 ^{ef}
MLG 3083	821.48 ^{d-f}	1082.15 ^{a-c}	798.94 ^{ef}	884.91 ^{b-f}

Ca, calcium; Al(ec), aluminium exchangeable. ^{ab,c,d,e,f}Values numbers followed by the same letter were not significantly different at Duncan's multiple range test 5%.

Table 6. Effect of liming on potassium and sodium uptake in leaves of soybean genotypes.

Genotypes	K (ppm)	Na (ppm)
W 3898-14-3	23,040.69 ^a	256.71 ^a
Wilis	18,966.93 ^b	222.11 ^b
Kawi	23,604.36 ^a	256.71 ^a
MLG 3209	21,862.12 ^a	235.95 ^{ab}
MLG 3083	18,864.45 ^b	215.19 ^b

K, potassium; Na, sodium. ^{ab}Values in the same column followed by the same letter were not significantly different at Duncan's multiple range test 5%.

uptake of Na in the leaves and was significantly different from susceptible genotype MLG 3083. Na influx into plant cells occurred by active transport (Taiz and Zeiger, 1991), which had no impact on the uptake of Na, despite it increased in the soil due to liming. In other words, increasing Na in the plant did not affect soil Na availability, but the increase of Na in the plant was caused by plant active absorption. This indicates that plants need Na in the physiological process and not as an influxing excessive nutrient due to the high Na availability. A similar result was reported by Maas *et al.* (2000) that studied the improvement of soil acidity by using sea water and reported that the application of higher sea water levels led to a higher suppression of Al.

Conclusions

Different genotypes had different different nutrient uptake. At different level of liming, the contents of K in the roots and Ca in the leaves were also different among the genotypes. Acid-tolerant genotypes and acid-susceptible genotypes did not show any difference in terms of uptake of Ca, Mg, and Al/(Ca+Mg) ratio in the roots, and Mg and Al/(Ca+Mg) ratio in the leaves. The differences in the uptake of these nutrients were more influenced by liming levels rather than by the genotypes. K and Na uptake were not affected by the liming levels, but they were influenced by the genotypes. Acid-tolerant genotypes W 3898-14-3 and Kawi had a higher K and Na uptake than susceptible genotypes.

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