Effect of aluminum stress in early-stage growth of soybean

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Abstract. The sensitivity of soybean to aluminum stress is well known. One of the abiotic stresses in tidal swamps is the aluminum toxicity. Therefore, it is necessary to find the appropriate management and cropping pattern to obtain a high yield of soybean in the tidal land. We supposed that it would be related to the development stage of soybean. This study was aimed to find the most sensitive of soybean vegetative growth stage to aluminum. Three cultivars of soybean (tanggamus, Karasumame, and M652) and four aluminum treatments (control, 10 days after planting/DAP, 20 DAP, 30 DAP) were arranged in a completely randomized design with three replications. Measurements have been made on root length, root/shoot ratio, biomass dry weight, leaves Aluminum content, leaves the area, and sensitivity index. Overall, observation results indicate that the earlier soybean experience aluminum stress, the more its vegetative growth is disturbed.

1. Introduction

Fertile soil has been converting to either non-agriculture or non-crop agriculture use for years in Indonesia. It is about 110000 ha year⁻¹[1]. Extensification has to be done to meet the soybean national need in addition to intensification program. Developing the tidal swamps for soybean cultivation should be one of the extensification programs. Indonesia has a large of tidal swamps. According to reference [2], there is about 9.53 million ha of tidal swamps suitable for agriculture activity. However, there are some obstacles faced in the development of the tidal swamps.

Aluminum (Al) is one of the chemical element that can be found in tidal swamps soil and inhibit the soybean root growth[3–6]. Reference [7] concluded in his review that Al toxicity initially disrupted root growth. Al is absorbed by root and enters to plant tissue through apoplast and symplast system[8]. Soybean Al-sensitive cultivar will experience the enlargement of plasma membrane and plasmolysis in the root[9].

There are many reports about the danger of Al to soybean root growth. However, it is lack of information about which stage of the soybean development is the most sensitive to Al stress. The damage to the soybean root caused by Al should affect the entire soybean growth. Therefore, this recent work will show the soybean vegetative growth at Al stress and the most affected soybean growth stage. This information will be important to the development of tidal swamp for soybean cultivation in Indonesia. It is expected to be useful, especially, for saturated soil culture studies of soybean done by references[10–12].

2. Materials and Method

The experiment tested 4 levels of Al application time treatment for 3 soybean cultivars. The Al application time treatments were control (No Al treatment), 10, 20, and 30 days after planting (DAP).
The three soybean cultivars were Tanggamus (Indonesian cultivar), Karasumame (Nepali cultivar), and M652 (Indian cultivar). They were arranged in a completely randomized design. The experiment was replicated three times.

The experiment was conducted in a greenhouse. A nutrient solution was used as growing medium of the soybean. The use of nutrient solution as a growing medium was intended to control the Al concentration and to ensure the homogeneity of all environmental factors other than the treatment. The end of the experiment was at age of 40 DAP i.e. 10 days after 30 DAP treatment.

2.1. Nutrient Solution Composition
A nutrient solution composition of reference[13] was referred in this experiment. Some chemical compounds consisted of 1.5 mM Ca(NO_3)_2 \cdot 4H_2O; 1.0 mM NH_4NO_3; 1.0 mM KCl; 0.4 mM MgSO_4 \cdot 7H_2O; 1 mM KH_2PO_4; 0.50 ppm MnSO_4 \cdot 5H_2O; 0.02 ppm CuSO_4 \cdot 5H_2O; 0.05 ppm ZnSO_4 \cdot 7H_2O; 0.5 ppm H_3BO_3; 0.01 ppm (NH_4)_2MoO_4 \cdot 24H_2O and 0.068 mM FeSO_4 \cdot 7H_2O were dissolved in water so that the volume of the nutrient solution was 2 l each pot. The concentration and form of Al were referred from reference[14] study. They found that soybean started to be stressed at 0.7 nM AlCl_3 \cdot 6H_2O (169 ppm).

Soybean was sowed in sand medium until the age of 5 days. The experimental pots were filled with the 2 l nutrient solution. The 5-days seedling was transferred to the pot. Each pot was connected to installed aerator for oxidation need (Figure 1).

![Figure 1. Arranged pots of the experiment](image)

2.2. Data Collecting and Analysis
Data were collected on root length, root/shoot ratio, biomass dry weight, leaves Aluminum content, leaves area, and sensitivity index. They were measured at the end of the experiment except for leaves area. Leaf area was observed 1 week after each Al treatment. Sensitivity index formula was referred from reference[15] as follows:

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SI = \frac{1 - \frac{y}{yp}}{1 - \frac{x}{xp}}
\]

SI = Sensitivity Index
y = mean value of cultivar treated by Al
yp = mean value of cultivar not treated by Al (control)
x = mean of all cultivars treated by Al
xp = mean of all cultivars not treated by Al (control)
Criteria: SI < 0.5 = tolerant; 0.5 < SI ≤ 1 = moderate; SI ≥ 1 = sensitive

The variants of the data were analyzed. The Duncan Multiple Range Test probability 5% was used to see the mean comparison. This statistical analysis was done by using SPSS 22.
3. Result

Root length was affected by the presence of Al. Figure 2 (a) shows that the data pattern of root length follows the pattern of the Al treatment. Control is insignificantly different from 30 DAP but significantly different from 20 DAP, and so on up to 10 DAP. The root of soybean control is longer than those treated by Al. The shortest root is found in 10 DAP treatment and significantly different from control.

Figure 2 (b) shows the root/shoot ratio of all soybean cultivar according to the time of Al application. These root/shoot ratio values show that the root dry is lighter than shoot dry weight for all of the values are less than 1. The time of Al application insignificantly affects the root/shoot ratio although the root/shoot ratio pattern tends to be the same as the pattern of root length. This implies that the root growth affects the shoot growth. The development of root in soybean control tends to be faster than the other.

Interaction of cultivar and time of Al application affected total dry weight. Figure 3 (a) shows the total dry weight of different Al application time in each cultivar. The pattern of dry weight value also tends to be the same to root length and root/shoot ratio pattern. The dry weight of Tanggamus treated Al at 10 and 20 DAP is significantly lighter than those treated Al at 30 DAP and control. The dry weight of Karasumame treated by Al earlier is lighter than those treated later. The dry weight of M652 treated Al at 10 DAP is significantly different from the other. The dry weight of M652 treated Al at 20 DAP is insignificantly different from 30 DAP but they are significantly different from 10 DAP and control.

Figure 3 (b) shows that cultivar M652 is significantly the lightest in 10 and 30 DAP treatment. Cultivar Tanggamus and Karasumame are statistically similar at all of the Al treatment although insignificantly different from Tanggamus and Karasumame at 20 DAP and control.

Interaction of cultivar and time of Al application affected the Al content. Al content was derived from soybean leaves. Figure 4 (a) shows the Al content of different Al application time in each cultivar. The Al content of Tanggamus at 10 and 20 DAP are significantly different from 30 DAP and control. The Al content of Karasumame at 10 DAP, 20 DAP, and control are statistically different from 30 DAP. The Al content of M652 at 30 DAP is the highest but insignificantly different from 10 and 20 DAP.

Figure 4 (b) shows that the highest Al content is found in M652 leaves at 30 DAP. The Al content of Tanggamus, Karasumame, and M652 are statistically similar at 10 DAP, 20 DAP, and control. The
high Al content of M652 at 30 DAP influenced the dry weight of M652 at 30 DAP (Figure 3). The dry weight of M652 at 30 DAP is low while its Al content is high.

![Figure 3](image_url)

**Figure 3.** Interaction of cultivar and Al treatment on the total dry weight of soybean biomass. (a) Mean comparison of Al treatments in each cultivar. (b) Mean comparison of cultivars in each Al treatment. DAP is the day after planting. Within each cultivar (a) or each Al treatment (b), values followed by a different letter are significant at P<0.05.

![Figure 4](image_url)

**Figure 4.** Interaction of cultivar and Al treatment on Al content of soybean. (a) Mean comparison of Al treatments in each cultivar. (b) Mean comparison of cultivars in each Al treatment. DAP is the day after planting. Within each cultivar (a) or each Al treatment (b), values followed by a different letter are significant at P<0.05.

Figure 5 shows the difference of leaves area of control and Al treatment at 10 DAP (a), 20 DAP (b), and 30 DAP (c). All cultivar leaves are larger in control than those at Al treatment. The differences leave area of Tanggamus between control and Al treatment 10 DAP, 20 DAP and 30 DAP respectively are 46%, 15%, and 6%. The differences of leaves area of Karasumame between control and Al treatment 10 DAP, 20 DAP and 30 DAP respectively are 59%, 15%, and 14%. The differences of leaves area of M652 between control and Al treatment 10 DAP, 20 DAP and 30 DAP respectively are 73%, 65%, and 24%. The higher the difference means the more disrupted the soybean. The difference of leaves area is higher in 10 DAP treatment and lowest in 30 DAP treatment.
Figure 5. The difference of soybean leaf area between control and Al treatment in each cultivar. (a) leaf area of soybean for 10 DAP treatment. (b) leaf area of soybean for 20 DAP treatment. (c) leaf area of soybean for 30 DAP treatment. The observation was done at 5 days after each Al treatment, for example, the leaf area of (a) was measured at 15 DAP (5 days after 10 DAP treatment). DAP is the day after planting.

Figure 6 shows that sensitivity index of the three cultivars studied at 10 DAP is more than 1. It means that soybean at the age 10 DAP is sensitive to Al toxicity. The sensitivity index of Tanggamus tends to decrease with the time of Al application. Sensitivity index of Karasumame is more than 1 at 10 and 20 DAP but decline sharply at 30 DAP. The sensitivity index of M652 is more than 1 at 10 DAP and decline at 20 and 30 DAP.

4. Discussion
Soybean growth was disrupted due to Al toxicity. Al affected soybean growth since Al was still inside the roots. Root growth was disrupted by cellular damage occurring within the plant tissue. Physiological processes within the roots were disrupted due to cellular damage. Al also affected the physiology process directly due to plasmolysis that occurs in the presence of Al [9].

Sensitive Soybean cultivars experience growth disturbance to all plant organs. We found a relationship between inhibitions of root and shoot growth in this study. The root growth pattern over the time of the al application tends to be same as the root/shoot ratio pattern. This suggests that impaired root growth also results in a disruption to shoot growth [14].

The three cultivars studied in this experiment absorbed Al to leaf. This indicates that there is no Al-detention mechanism at the root for not getting into the leaf[16]. The higher the concentration of Al in
the leaves the greater the growth disturbance experienced by the Tanggamus. This does not occur in the two other cultivars. It was not found a regular pattern in both varieties.

It was found that the Al treatment at age 10 DAP made the worst growth of soybean compared to other treatments all parameters observed. Therefore, this study has found that early toxicity of Al is more dangerous than in older age.

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\begin{array}{|c|c|c|c|c|}
\hline
\text{Age (DAP)} & \text{Tanggamus} & \text{Karasuame} & \text{M652} \\
\hline
10 & 1.08 & 0.99 & 0.74 \\
20 & 1.37 & 1.41 & 0.53 \\
30 & 1.65 & 0.44 & 0.73 \\
\hline
\end{array}
\]

Figure 6. Sensitivity index of soybean on all Al treatment. SI < 0.5 = tolerant; 0.5 <SI ≤ 1 = moderate; SI ≥ 1 = sensitive. SI was counted using the formula of [15].

5. Conclusion
Al affected all of the soybean vegetative organs observed. Cultivar M652 was the most vulnerable cultivar to Al toxicity. All cultivar were sensitive at 10 DAP. So, we conclude that the earlier all soybean cultivar experienced Al, the more sensitive all of the soybean cultivar to Al

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