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The critical period of aluminum stress on soybean root growth

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ABSTRACT

Aluminum is prevalent in soils of tidal swamps. Soybean is known to be very sensitive to aluminum stress and so when tidal swamps are converted to soybean cropland, considerable effort and expense are required to overcome Al toxicity in soybean roots. It is therefore necessary to determine at what time in early development soybeans can best endure aluminum stress and identify aluminum-tolerant cultivars. This study was conducted by testing the impact of aluminum exposure on three soybean cultivars (Tanggamus, Karasumame, and M652) (relative to no-exposure controls) at four time periods at 10, 20, and 30 days after planting. No significant effect of aluminum on root growth in the first five days after exposure was observed, but the toxic effects became evident after soybeans had been exposed to aluminum for 10 days. Soybean seedlings that experienced aluminum stress earliest (at 10 days after planting) were more negatively impacted by Al exposure than seedlings exposed later (e.g., 30 days after planting). Root growths of the three cultivars we tested in this study were all detrimentally impacted by aluminum exposure. However, the M652 cultivar was the most sensitive to aluminum exposure. We conclude that the critical threshold period for soybean root growth to succumb to aluminum stress is within the first 30 days after planting, whereas the tolerance to aluminum stress occurs only during the first 10 days of exposure.

INTRODUCTION

Since 2009, soybean research in Indonesia has been conducted on how saturated-soil-culture technology can be used to increase productivity on cropland converted from tidal swamps. Those studies provided much-needed information on tidal land management. Soil-culture technology is not complicated and it can produce a high yield of soybean. Soybean seed productivity using this technology can attain 4.5 tons ha^{-1} (Ghulamahdi, Melati, & Sagala, 2009) and an average of 2.5 tons ha^{-1}. These yield estimates are much higher than the national (Indonesia) average of 1.4 tons ha^{-1} of soybean on non-tidal lands (Statistics, 2018) and of 0.8 tons ha^{-1} on tidal lands without using saturated-soil-culture technologies (Djayusman, Suastika, & Soelaeman, 2001). These yield comparisons are significant because tidal land is marginal for crop production due to its low pH and high concentrations of pyrite, Fe, and Al; however, this land is being converted to cropland in Indonesia.

Aluminum (Al) limits plant growth. According to Zheng (2010) and Sopandie (2014), Al dissolves in solution (in ionic form) when soil is acidic. Also, phosphorus (P) binds to soil minerals and becomes unavailable to plants under acidic conditions (Dermawan, 2011; Liao et al., 2006). Kataoka, Nakanishi, Verlag, Kataoka, and

Nakanishi (2001) concluded that Al accumulates about 1 mm from root tips when it is being absorbed by roots. Al interferes with root growth by binding to plant matter, such as cell walls, plasma membranes, and nuclei. According to Duressa, Soliman, & Chen (2010) and Duressa, Soliman, Taylor, & Senwo (2011), the destruction of cells and tissues in roots likely inhibits plant growth and thus reduces the yield of Al-sensitive soybeans. In contrast, Al-tolerant soybeans have both physiological and molecular mechanisms enabling them to survive under acidic conditions and even produce seeds under what would normally be considered an Al-stressed environment.

Saturated-soil-culture technology has been proven to prevent pyrite oxidation in saturated soils in former tidal swamp cropland (Ghulamahdi et al., 2013) and this technology also seems to increase concentrations of P, K, and Ca, while decreasing Al and Fe concentrations (Noya, Ghulamahdi, Sopandie, Sutandi, & Melati, 2014). Saturated-soil-culture technology uses about 2.5 tons ha^{-1} of lime to increase soil pH and reduce soil Al solubility (Ghulamahdi, Melati, Sagala, & Sahuri, 2011; Ghulamahdi, Welly, & Sagala, 2018; Noya et al., 2014; Sagala, 2010).

Information is still very limited regarding the timing of Al stress during root growth and the stage at which soybean roots are most susceptible to Al stress. Therefore, in this study, we wanted to determine: (1) the critical period at which Al negatively inhibits the growth of soybean roots, (2) how many days Al impacts root growth, (3) which life stage of soybean is most sensitive to Al toxicity, and (4) whether any soybean cultivars are Al-tolerant. The amount of lime used on field could probably be reduced if the critical period of Al stress on soybean roots is known or if an optimal planting approach can be devised based on a critical period of growth inhibition.

MATERIAL AND METHODS

Experimental design

Our experiment was arranged in a factorial, completely-randomized design with three replications. The first factor was soybean cultivar, consisting of the cultivars 'Tanggamus', 'Karasumame', and 'M652'. The second factor was period relative to Al application [i.e., no Al application (control) (T0), Al application 10 days after planting (DAP) (T1), Al application 20 DAP (T2), and Al application 30 DAP (T3)].

The three cultivars of soybean were selected based on our previous study (Sagala & Suzanna, 2016). 'Tanggamus' is an Indonesian cultivar and 'Karasumame' is a Taiwan cultivar, while 'M652' is an Indian cultivar. Both 'Karasumame' and 'M652' were obtained from the gene bank of the National Institute of Agrobiological Sciences (NIAS, Genetic Resources Center) of National Agriculture and Food Research Organization (NARO), Japan.

Preparation of Nutrient Solution and Experimental Procedure

The experiment was conducted in a greenhouse in nutrient cultures. Each treatment pot contained two liters of nutrient solution, consisting of 1.5 nM of $Ca(NO_3)_2.4H_2O$, 1.0 mM of NH_4NO_3, 1.0 mM of KCl, 0.4 mM of $MgSO_4.7H_2O$, 1 mM of KH_2PO_4, 0.50 ppm of $MnSO_4.4H_2O$, 0.02 ppm of $CuSO_4.5H_2O$, 0.05 ppm of $ZnSO_4.7H_2O$, 0.5 ppm of H_3BO_3, 0.01 ppm of $(NH_4)2MO_7O_{24}.4H_2O$, and 0.068 mM of $FeSO_4.7H_2O$. This is the same chemical recipe used by Sopandie (1990). Aluminum was provided to soybeans as $AlCl_3.6H_2O$ at a concentration of 0.7 mM (169 ppm) (Noya et al., 2014). According to Noya et al. (2014), soybean should be poisoned at this dosage.

Figure 1. Experimental set-up arrangement with attached aerators.

Soybean seeds of the three selected cultivars were seeded into a sand medium and grown there until five days after sprouting. The 5-day-old sprouts were then transplanted into pots (five seedlings per pot) containing the culture solution. Each seedling was held in place with styrofoam material (Fig. 1). An aerator was installed on each pot to create oxic conditions in the culture medium.

Measurement and Data analysis

Measurements of root length and root dry weight were made immediately before the first application of Al (i.e., just prior to time T1) and at 5 and 10 days after Al applications for treatments T1, T2, and T3. Measurements prior to T1 were performed on all pots. Measurements on the fifth and tenth days after T1 were only performed on plants in pots T0 and T1 because soybeans in pots T2 and T3 had not yet been exposed to Al (they provided control-like conditions). Measurements on the fifth and tenth days after T2 were only made for plants in pot T0 and T2 (for the same reason as stated above). Measurements on the fifth day after T3 were made only on T0 and T3 pots, while measurements on the tenth day after T3 (40 DAP) were performed on all pots.

We created a sensitivity index (SI) for roots subjected to Al stress, calculated using the Fischer and Maurer (1978) formula: SI=(1-(y/p))/(1-(x/xp)) where y is the mean of stressed cultivars, yp is the mean of control cultivars, x is the mean of all stressed cultivars, and xp is the mean of all control cultivars. These SI data provided us with three tolerance threshold categories: tolerant ($SI < 0.5$), moderately tolerant ($0.5 < IS \leq 1$), and sensitive ($SI \geq 1$).

All statistical analyzes were performed using SPSS version 22. Data were first analyzed with analysis of variance. This was followed by a Duncan Multiple Range Test, which is a post-hoc multiple (pairwise) comparison test. We applied this test to two sets of data: (1) on root length measurements relative to cultivar type and Al effects for the periods prior to the T1 application of Al and 10 days after the T3 application and (2) on measurements of root dry weight relative to the influence of cultivars and Al applications.

The Duncan multiple comparison test was conducted to evaluate the impacts of Al treatment on root growth, but there were only three treatments that could be compared with controls (i.e., T0 vs. T1, T0 vs T2, and T0 vs T3). These comparisons were made to establish a tolerance threshold for soybean roots relative to the time of Al exposure.

RESULTS AND DISCUSSION

Tolerance threshold of soybean roots relative to the time of aluminum exposure

Although Al-exposed soybeans tended to have shorter roots than controls (non-Al-treated soybeans), root length was not statistically shorter after five days of growth following Al application (Fig. 2). However, inhibition of root growth was significantly expressed by Day 10 after Al exposure. In contrast, the soybeans exposed to aluminum at 30 DAP showed no significant growth inhibition after the fifth nor tenth day after exposure (Figs 3–6).

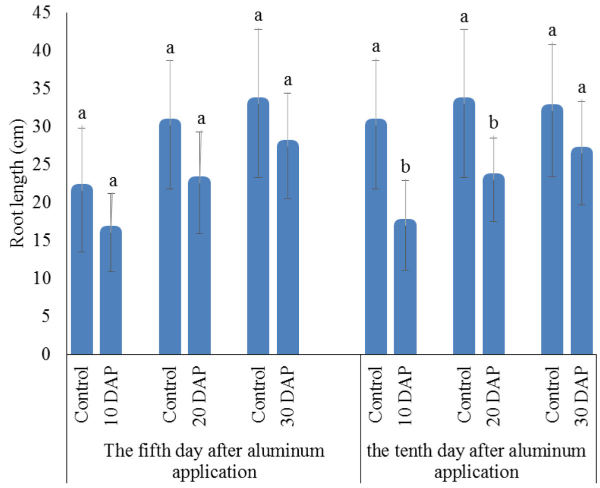

Figure 2. Mean root length relative to aluminum treatment effects on the fifth and tenth days after Al application. Error bars represent one standard deviation. Identical letters in adjacent bars (i.e., between control and DAP) indicate no significant difference in length based on a Duncan Multiple Range Test ($P < 0.05$).

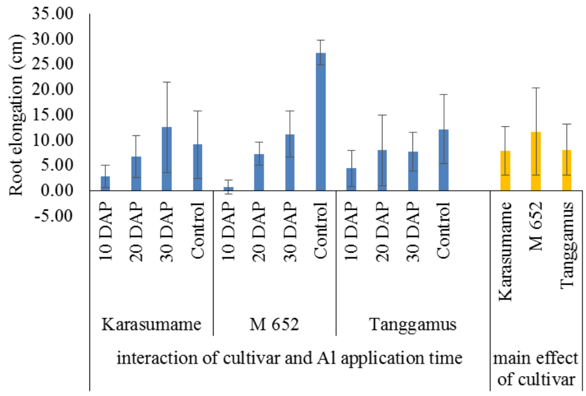

Figure 3. Differences in mean root elongation (growth) relative to the timing of Al applications. Root growth was determined from differences in root lengths based on the last measurement (the tenth day after Al

application at 30 DAP) minus the first measurement (just before Al application at 10 DAP). Error bars represent one standard deviation.

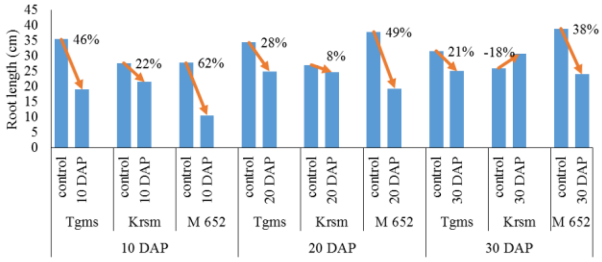

Figure 4. Root length and percentage of its decrease of 3 soybean cultivars with Al treatment at age 10, 20, 30 days after planting (DAP). Tgms is Tanggamus cultivar. Krsm is Karasumame cultivar

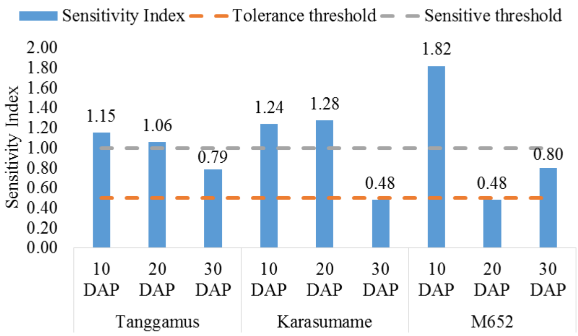

Figure 5. Sensitivity index (SI) values of three soybean cultivars exposed to Al. SI thresholds: Al-tolerant (SI < 0.5), moderately Al-tolerant (0.5 < SI ≤ 1), and Al-sensitive (SI ≥ 1).

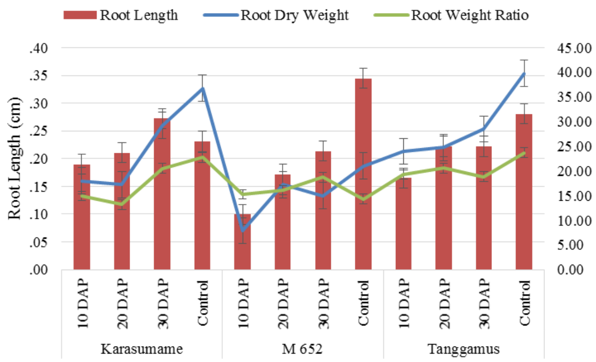

Figure 6. Root length, root dry weight, and root length-to-weight ratio of three cultivars of soybean following Al exposure at three time periods. Error bars represent one standard deviation.

Kataoka et al. (2001) found that accumulation of Al at root tips and disturbance of cellular growth typically occurs 15 minutes after roots absorb Al, with Al entering the cytoplasm within 15 minutes after Al exposure. Furthermore, they found that within 30 minutes of exposure to Al, Al had entered into the nuclei of cells located 1 mm from the root tip. This 1 mm location is most sensitive to gravitational stimulation. That is why roots are more sensitive to Al than shoots and why roots become stunted when they are poisoned by Al. The shortening of root length (relative to controls) in soybeans exposed to Al agrees with the results of previous studies. However, based on our staggered application of Al to various soybean cultivars, we found that the accumulation of Al and cellular disturbance in roots is delayed for about 10 days, which can perhaps be attributable to various physiological adaptation mechanisms (Duressa et al., 2010, 2011; Liao et al., 2006; Zheng, 2010). After 10 days of exposure to Al in our study, soybeans became significantly stressed. Therefore, it appears that soybean roots may tolerate Al stress for no more than 10 days.

Root length increments

Fig. 3 shows differences in root length (growth) between initial measurements and last measurements for each treatment combination of cultivar, Al application period, and the main effects of cultivars. The three cultivars differed in their responses to Al stress at specific periods (days) after planting (DAP), but they showed the same general pattern in that root growth in the controls were longer than those in Al-stressed plants. This confirms that aluminum exposure inhibits the growth of soybean roots, as also shown in other research (Liao et al., 2006; Milivojević & Stojanović, 2003; Mustafa, Sakata, & Komatsu, 2015; Noya et al., 2014). However, our study also found that all three cultivars we tested showed inhibited root growth when they were exposed to high Al concentrations in early growth (by 10 DAP), whereas soybeans exposed to Al at 30 DAP mostly resisted Al toxicity. It appeared that the 'Karasumame' cultivar did not respond negatively to Al added after 30 days of growth (i.e., its root growth did not differ from controls). When root growth is examined as percent inhibition of root growth (i.e., percent difference in growth), we found that for all cultivars, percent growth inhibition at 30 DAP was less than at 20 DAP, which was in turn less than at 10 DAP (Fig. 4). For example, root growth inhibition of 'Tanggamus' at 10, 20, and 30 DAP were 46%, 28%, and 21%, respectively. The sensitivity index (SI) also indicates that 10- and 20-day-old soybean roots are more sensitive to Al exposure than are 30-day-old roots (Fig. 5).

Root growth in 10 DAP ‘M652’ plants became extremely inhibited after the first 10 days of Al exposure. The roots grew only 0.73 cm over 40 days. However, for control ‘M652’ plants, root elongation was much longer relative to its three Al treatments than were the other cultivars to their treatments. In other words, although the growth of ‘Karasumame’ and ‘Tanggamus’ were also inhibited by exposure to Al, they did not respond as drastically to Al exposure as did the ‘M652’ cultivar. Therefore, we speculate that the ‘M652’ cultivar could be more responsive (or sensitive) to Al toxicity than the other two cultivars. In addition, although both the ‘Karasumame’ and ‘Tanggamus’ cultivars exhibited better root growth at 10 DAP than did the ‘M652’ cultivar, the ‘Tanggamus’ cultivar was the least responsive to Al exposure because its root growth did not differ from controls as much as the other cultivars did (i.e., its magnitude of root inhibition was less).

The effect of aluminum exposure on soybean growth

Aluminum stress affects plant growth, as shown by differences in the dry weight of shoots in the Al-stressed plants relative to controls; that is, the mean shoot dry weight of control plants was higher than it was in plants for all three Al treatment periods (10, 20, and 30 DAP) and for all cultivars (data not shown). Roots are crucial to plant survival. Plant life starts from the roots. Root health affects the overall health of a plant. The raw material of photosynthesis is transported to the leaf through the vascular tissue after it is first absorbed by the roots.

Similar effects of Al toxicity on growth are also represented in the weight of dried rooted berries (Fig. 6). Root elongation (growth) impacted the dry weight of shoots (data not shown), dry weight of roots, and the length-to-weight ratio of roots.

CONCLUSION AND RECOMMENDATIONS

The presence of aluminum in cropland of former tidal swamps disrupts growth and thus decreases yields of soybeans. Al primarily interferes with soybean root growth in that root growth is disrupted when Al reaches toxic concentrations. We determined that soybeans can deal with aluminum toxicity for only about 10 days of exposure. Although some prior studies have shown that Al quickly enters root cytoplasm and nuclei, root-growth inhibition does not manifest during the first five days of exposure to Al, but that indicators of stress (e.g., root-growth rate) appear after about 10 days of exposure, particularly in the youngest seedlings. Our study also found that young seedlings exposed to aluminum are more sensitive to Al toxicity than older seedlings. Root growth in all three cultivars we tested was negatively affected by Al toxicity. However, the ‘M652’ cultivar appeared to be the most sensitive to Al than the other two cultivars we tested. We conclude that the critical Al-tolerance threshold for soybeans (relative to root growth) occurs within about 30 days after planting; i.e., soybeans are most tolerant toward Al toxicity effects after that. Thirty days after planting is about when the vegetative (non-seedling) stage begins. However, tolerance to Al toxicity only lasts for about 10 days after exposure to Al no matter when soybean seedlings are first exposed.

Further research is required to identify the most efficient type of intervention needed to minimize the negative effects of Al toxicity at the beginning stages of soybean growth. Additional research is warranted to examine the best approach for transplanting >20-day-old seedlings in cropland converted from tidal swamps.

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