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Uptake and Distribution of Aluminum in Root Apices of Two Rice Varieties under Aluminum Stress

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Aluminum (Al) toxicity is the major limiting factor of plant growth and production in acid soils. The target of Al toxicity is the root tip, which affects mainly on root growth inhibition. The aim of this research was to study the uptake and distribution of Al in root apices of two rice varieties IR64 (Al-sensitive) and Krowal (Al-tolerant), which were grown on nutrient solution containing 0, 15, 30, 45, and 60 ppm of Al. The root growth was significantly inhibited in both rice varieties at as low as 15 ppm Al concentration. The adventive roots of both varieties showed stunted growth in respons to Al stress. There was no difference in root growth inhibition between both rice varieties as well as among Al concentrations. Al uptake on root apices was qualitatively and quantitatively analyzed. Histochemical staining of roots using hematoxylin showed dark purple color on 1 mm region of Al-treated root apices. Rice var. IR 64 tended to take up more Al in root tip than Krowal did. However, there was no statistically significant difference (p = 0.176) in root Al content of both varieties in response to different concentration and period of Al treatments. Al distribution in root apices was found in the epidermal and subepidermal region in both rice varieties. Based on those results, rice var. Krowal that was previously grouped as Al-tolerant variety has similar root growth and physiological response to Al stress as compared to Al-sensitive variety IR64.

Key words: aluminum, uptake, distribution, root, rice

INTRODUCTION

Acid soils with low pH and high aluminum (Al) solubility widely distribute in Indonesia. Under pH 4 or less, most macronutrient such as nitrogen, phosphorus, potassium, calcium, and magnesium becomes limited to the plant and a toxic form of Al (Al^{3+}) increases its availability and can be a major limiting factor of plant growth and production in acid soils (Kochian 1995; Matsumoto 2000). Aluminum mainly inhibits root growth and causes short and stunted root (Taylor 1991; Delhaize & Ryan 1995). Subsequently it can affect water and nutrient uptake, which are essential for plant growth and development (Delhaize *et al*. 1993a). The main target of Al toxicity is a meristematic zone in root apex (Ryan *et al*. 1993; Delhaize *et al*. 1993a, b; Pellet *et al*. 1995; Delhaize & Ryan 1995). The presence of Al in root apex causes stunted seminal root and inhibit lateral root growth (Foy *et al*. 1978; Èiamporová 2002; Samac & Tesfaye 2003).

It has been known that there is a variation in Al tolerance among plant species or even among cultivars in a species. There are many Al tolerance levels among plant species or cultivars, ranging from Al-sensitive to tolerant. Plant adaptation to acid soil mostly depends on its tolerance to Al toxicity. Rye (*Secale cereale* L.) and rice (*Oryza sativa* L.) are the most Al-tolerant plant among grass species (Kim *et al*. 2001).

Al-tolerant and sensitive species accumulate Al at different level when they are grown in acid soil with high Al solubility (Samac & Tesfaye 2003). One of the Al tolerance criteria is that the Al-tolerant plant could reduce Al absorption and translocation to the shoot due to the most Al is stored in root cell vacuole (Rincón & Gonzales 1992; Matsumoto 2000). However, there are still conflicting results regarding the amount of Al being accumulated in the root cells between Al-tolerant and sensitive plant. For example, Al-sensitive wheat (*Triticum aestivum* L.) take up and accumulate Al in the root apices higher than Al-tolerant wheat (Delhaize *et al*. 1993a). It seems to be the relation between the level of Al accumulation in root cells and the level of Al-tolerance depends on the plant species. This means that Al uptake and accumulation in the root cell could be a specific Al tolerance criterion for certain plant species.

Rice is a major staple food for most Indonesian people. The problem of rice production in Indonesia is not merely because of culture practice, but also because of decreasing the land for rice cultivation due to the high land conversion for industry and rural development. One of the alternatives to produce rice is the use of marginal lands including acid soil. However, the major problem of the rice cultivation in acid soil is the limited Al-tolerant rice varieties that adapted to such soils. Attempts to develop such varieties had been initiated for many years ago but until today there are still many constraints in developing adapted rice varieties specific to acid soil with high Al toxicity. To develop Al-tolerant rice varieties requires good genetic resources and a selection tool. Plant selection based on physiological criteria to obtain parental lines could be the preliminary step in developing Al-tolerant rice varieties, and the uptake and distribution of Al in

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the root apices could be a choice as a selection tool. This paper presented the result in attempting to evaluate the uptake and distribution of Al in the root apices of two rice varieties that previously being classified by Jagau (2000) as Al-sensitive (IR64) and Al-tolerant (Krowal) varieties.

MATERIALS AND METHODS

Plant Materials. Rice var. IR64 and Krowal were used in this study. IR64 is a modern rice variety that sensitive to Al, whereas Krowal is a local Indonesian rice variety that previous study reported as an Al-tolerant variety (Jagau 2000).

Nutrient Culture and Aluminum Treatment. Seeds from both rice varieties were sterilized using 0.5% (v/v) NaOCl for 15 min, and then were rinsed with aquabidest three times. The seeds were then soaked in aquabidest for 24 h, followed by germinating it in petri dish for 48 h at 28-31 ^{o}C in dark conditions.

Nutrient culture and Al treatment were conducted in a growth chamber under controlled environment condition [29-31 ^{o}C, 12/12 h (light/dark), and 80% relative humidity]. Seedlings with similar root length from both varieties were grown on plastic screen floating on nutrient solution modified from Yoshida *et al*. (1976) [40 ppm N (NH_4NO_3), 10 ppm P (NaH_2PO_4), 40 ppm K (K_2SO_4), 40 ppm Ca ($CaCl_2$), 40 ppm Mg ($MgSO_4 \cdot 7H_2O$), 0.5 ppm Mn ($MnCl_2 \cdot H_2O$), 0.05 ppm Mo ($(NH_4)_6 \cdot Mo_7O_{24} \cdot H_2O$), 0.2 ppm B ($H_3BO_3$), 0.01 ppm Zn ($ZnSO_4 \cdot 7H_2O$), 0.01 ppm Cu ($CuSO_4 \cdot 5H_2O$), dan 2.0 ppm Fe (NaFeEDTA)] at pH 4.0 for 24 h before Al stress was applied. The solutions were then treated with 0, 15, 30, 45, and 60 ppm of Al in the form of $AlCl_3$. The Al treatments were administered for 24 and 72 h for Al uptake and distribution, and root growth analysis, respectively. Time course experiment was applied at 60 ppm of Al for 0, 6, 12, 24, and 48 h. The experiment was repeated three times. During the experiment, the nutrient solution was aerated and changed daily to maintain constant pH. At the end of the experiment, the length of seminal root of 10 seedlings was measured in all treatments of both varieties for root growth analysis. The root histochemical, Al uptake and distribution analyses were also conducted at the end of the experiment.

Hematoxylin Staining. The Al-treated rice root seedlings were rinsed with aquabidest for 30 min, and then soaked in 0.2% hematoxylin solution (in 0.02% $NaIO_3$) for 60 min. The roots were then rinsed one more time for 30 min before fixation in FAA solution (37% formaldehyde : glacial acetic acid : 70% ethanol) for 24 h. A purple color in root tip surface indicates Al presence in that area. Pictures were taken using Olympus FE-160 camera.

Aluminum Uptake. Analysis of Al uptake by root cell was conducted based on Al content of 15 mm-section of root tip. Approximately 100 Al-stressed root tips were analyzed. Roots were dried at 80 ^{o}C for 24 h, weighed using microbalance, and then ashed at 550 ^{o}C for 22 h. The root ash was then dissolved in 4 ml concentrated HNO_3 and 1 ml H_2O. The solution was heated at 200 ^{o}C for 2 h, cooled at room temperature and added 10 ml 6N HCl. The solution was then diluted with water to final volume 25 ml (Cunniff 1999) and the Al content was measured using *atomic absorption spectrophotometer* (Varian, Spectra AA-30).

Aluminum Distribution in Root Tissue. Distribution of Al in root tissue was analyzed based on the presence of Al in cross-section of root. Root seedlings were stained with hematoxylin as previously mentioned, and cross-sections of root division zone ($\pm$ 1 mm from root tip) were prepared manually, observed under microscope, and photographed using a photomicroscope Olympus CX-40. The purple color in root tissue indicated the presence of Al.

Data Analysis. Quantitative data was analyzed using univariate analysis of variance based on randomized block design of factorial experiment. The difference among treatment was tested using Duncan Multiple Range Test (DMRT) with $\alpha = 0.05$.

RESULTS

Root Morphology and Growth Analysis. Aluminum stress treatment on rice seedling caused morphological change and growth inhibition. Al-stressed seminal roots appeared shorter than that of normal roots (Figure 1). Al also caused inhibition of adventive root formation and growth. In general, the adventive roots only growth normally in non-stressed seedlings.

Seminal root growth of Al-stressed seedling was inhibited at as low as15 ppm Al. In both varieties, the inhibition occurred in all Al treatments. There was no difference in root elongation between both rice variety (IR64 and Krowal) under Al stress. In general, Al treatment significantly inhibited ($p < 10^{-3}$) rice root elongation. The root elongation decreased as the Al concentration increased (Table 1).

Periods of Al stress also significantly decreased ($p = 0.006$) root growth rate. The root growth rate of Al-stressed rice roots decreased significantly after being stressed for 6 h. The longer Al stress period, the slower root growth rate (Table 2), although the root growth rate was able to increased continuously in unstressed roots until 48 h.

Histochemical Analysis. Accumulation of Al in plant could be monitored using hematoxylin staining. The Al-stressed root showed dark purple color in root tip after hematoxylin staining, which indicated that Al was taken up and accumulated in this root zone. The higher concentration of Al applied, the darker purple color observed in the root tips. At any Al concentration, the root tip of rice var. IR64 showed darker color than that of Krowal (Figure 2). However, there was no difference in root color intensity among Al concentration in the same rice varieties. The result also showed that only the region of 1 mm root tip that intensely colored by hematoxylin.

Al Uptake and Distribution in Root Apices. Rice varieties IR64 and Krowal showed no significant difference ($p = 0.176$) in Al uptake (Table 3). Spectrophotometric analysis showed that only non-Al stressed root did not take up and accumulate Al, whereas all Al-treated roots took up and accumulated Al in the root tip area. There was no difference in Al uptake among Al-treated roots in both rice varieties (Table 3). However, at above 15 ppm Al stress level, rice variety IR64 tended to take up more Al than Krowal did (Figure 3).

The period of Al stress on rice root seedlings affected root Al uptake. The longer period of Al stress, the higher Al uptake by rice root. The highest Al uptake occurred in rice root that was stressed by Al for 48 h. There was no difference in Al uptake between both varieties under different periods of Al stress (Table 4).

Distribution of Al in root tissue was observed on microscope slide. The region of root apex that previously treated with Al was cross-sectioned every 1 mm interval from the root tip and observed using a microscope. The result showed that Al distributed only in epidermal and subepidermal region of rice root (Figure 4). There was no Al found in the deeper root cortex. The result also showed that there was no difference in Al distribution among rice root of both varieties that was treated with Al stress.

DISCUSSION

Aluminum toxicity has been known as a major problem in acid soil. One of the requirement in developing Al-tolerant rice varieties that can adapt to acid soil with high level of soluble Al is the availability of a good selection tool that is able to find parental resources based on Al-tolerant and sensitive plant characteristics. Al uptake and distribution in root apices could be used as a critetion to evaluate Al-tolerance of plant. This research was an attempt to evaluate Al-tolerance characteristic of two rice varieties based on their ability to take up and accumulate Al under Al stress condition.

Root morphological and growth analyses in this experiment showed that Al inhibited seminal root growth and blocked adventive root initiation. The root became short and stunted. The Al inhibition to the root growth occurred at as low as 15 ppm Al in both rice varieties. The higher Al-concentration, the slower root length elongation. The fenomenon that was observed in this experiment is because of the inhibition effect of Al that generally occurred in solution containing Al or acid soil. In general, the root inhibition occurred 24 h after Al stress (Kochian 1995). Although there is no clear explanation on how Al inhibit root growth, the fact showed that high Al solubility in acid soil could seriously inhibit root growth. The most likely explanation of root growth inhibition by Al is because Al ion binds on cell wall, plasma membrane, and nucleus. Binding Al ion on plasma membrane could disturb transport process through plasma membrane. Al ion could also inhibit cell division through its interference to DNA synthesis. Matsumoto (2000) found that mitosis activity rapidly decrease in meristematic zone of Al-stressed root.

Accumulation of Al in root tissue can be observed using hematoxylin staining, which is a simple and easy method to detect Al in plant tissue. This method was used by Polle *et al*. (1978) when detecting Al in wheat root, which is based on specific binding of hematoxylin to Al. The difference in root Al content was detected by difference in purple color intensity. The more Al accumulated in root tissue, the darker the purple color detected. Polle *et al*. (1978) reported that roots from all wheat cultivars showed increasing purple color intensity as the Al concentration increase in nutrient culture. However, the result of this experiment was different. There was no significant difference in rice root color intensity among different Al concentration (Figure 2). This qualitative observation suggested that Al uptake and accumulation in rice root apex did not increase significantly at Al stress level above15 ppm Al. Spectrosphotometric analysis supported this suggestion that rice root took up similar amount of Al at 15 ppm Al and above (Table 3).

Different with the level of Al stress, period of Al stress caused significant difference in Al uptake of rice root. The longer exposure to Al, the more Al being uptake by root. The highest uptake was achieved by roots that were exposed to Al for 48 h (Table 4). This result was in parallel with the root growth rate that decrease with longer period of Al stress (Table 2).

Hematoxylin staining also showed that rice var. IR64 had more intense color of root tip than Krowal at any level of Al stress, indicated that at any level of Al stress, root apex of IR64 took up and accumulated more Al than that of Krowal did. This result was partially in agreement with spectrophotometric analysis that showed rice var. IR64 tended to take up more Al than Krowal did at Al stress level above 15 ppm, although it was not statistically significant (Figure 3).

It has been known that accumulation of Al ion in the root tissue is correlated with different level of plant sensitivity to Al stress (Matsumoto 2000). It is predicted that, in general, the more sensitive to Al, the more Al accumulated in the root tissue. However, the result of this experiment showed that, in general, there was no significant difference in Al accumulation in the root tissue between rice var. IR64 and Krowal. This result indicated that rice var. Krowal that was previously classified as Al-tolerant rice has similar physiological response to Al stress compared to IR64 that was previously known as Al-sensitive.

Hematoxylin staining method can also be used to observe the distribution of Al ion among cells in root tissue. Cross section of Al-stressed roots that were previously stained using hematoxylin showed that dark purple color found in epidermal and subepidermal (outer layer of root cortex) cells of root apex (1 mm from root tips). This suggested that Al could only enter the root tissue not deeper beyond subepidermal layer of root tissue.

Al distribution in the rice root tissue was analyzed in different part of root ranging from 1 to 15 mm from the root tip with 1 mm interval. The result showed that there were no differences in Al distribution among sections. However, the clearest purple color was observed in the epidermal and subepidermal cell layers of 1 mm section from the root tip, which was indicated that the distribution of Al in the root tissue was mainly in the 1 mm root tip or cell division zone. The result of this experiment is in agreement with what Matsumoto (2000) stated that Al ion is accumulated in the root cap, root apical meristem, and root elongation zone. Yamamoto *et al*. (2001) also found that Al ion is highly accumulated in root apical meristem of *Pisum sativum*. The result of this experiment was also similar with the report of Piñeros *et al*. (2002) that found Al ion is accumulated in maize root in outer layer of cortex cells and it was not detected in endodermis and stele. Matsumoto (2000) also stated that accumulation of Al is occurred in root epidermis and cortex.

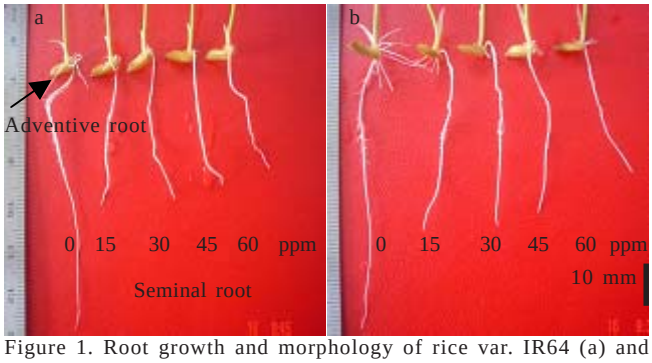

Figure 1. Root growth and morphology of rice var. IR64 (a) and Krowal (b) under different levels of Al stress.

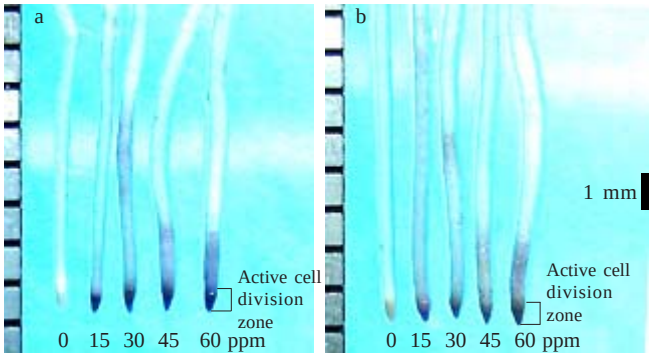

Figure 2. Hematoxylin staining of root apices of rice var. IR64 (a) and Krowal (b) after being Al-stressed for 24 h at 15, 30, 45, and 60 ppm.

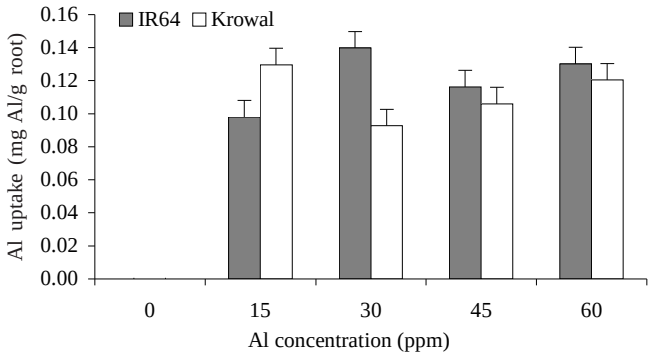

Figure 3. Al uptake of root apices of rice var. IR64 and Krowal at different levels of Al stress.

Table 1. Root elongation of rice var. IR64 and Krowal under different levels of Al stress

Factors	Root elongation (mm)
Varieties	
IR 64	4.88^{ns}
Krowal	4.75^{ns}
Al concentration (ppm)	
0	15.45^{a}
15	3.77^{b}
30	2.25^{c}
45	1.60^{cd}
60	1.00^{d}

^{ns}: not significant; number on the same column followed by the same letter was not significantly different based on DMRT (α = 0.05)

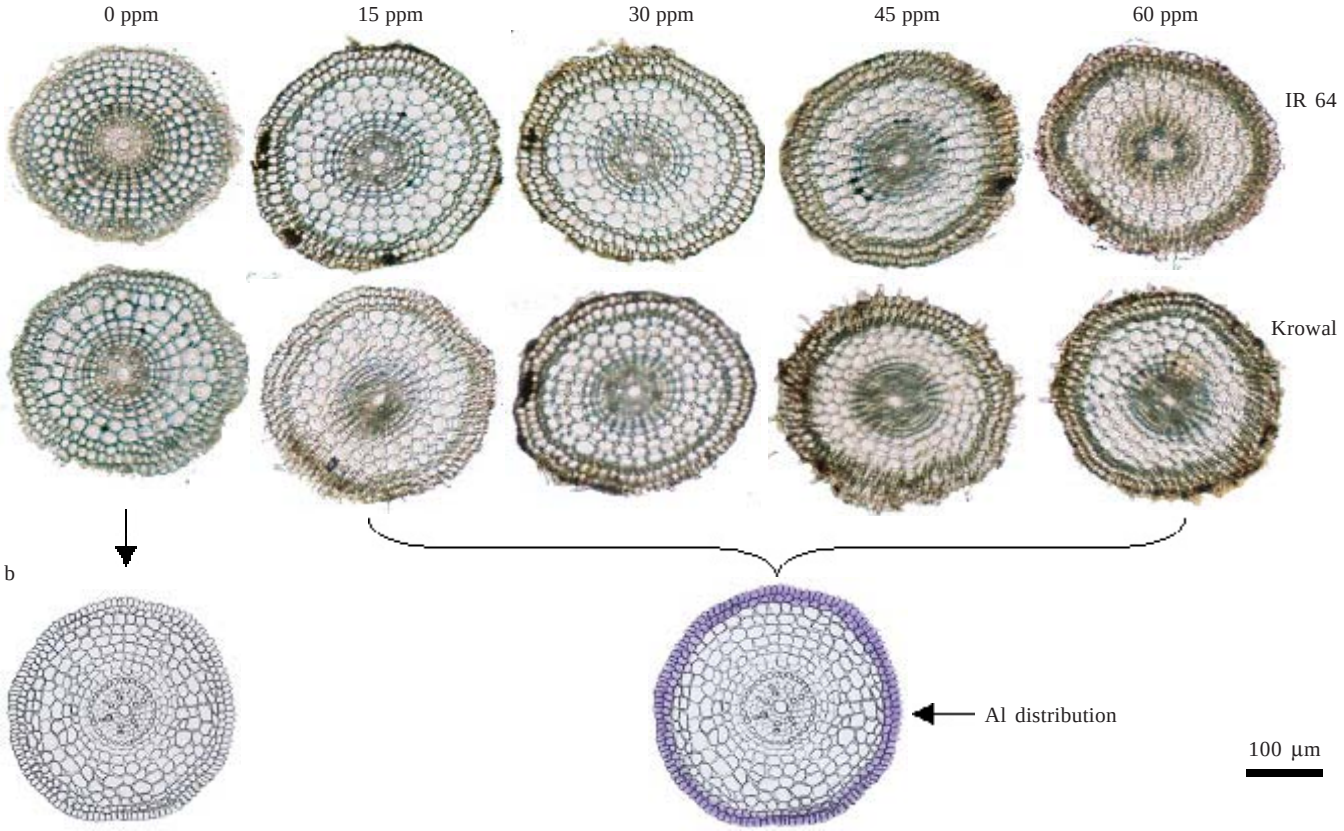

Figure 4. Distribution of Al ion in the root cells of rice var. IR64 and Krowal under different levels of Al stress. The purple color in root tissues indicated the presence of Al. (a) cross section of 1 mm root apex, (b) diagram of root cross section showing distribution of Al.

Table 2. Root growth rate of rice var. IR64 and Krowal under different periods of Al stress

Factors	Root growth rate (mm/h)	
	0 ppm	60 ppm
Varieties		
IR 64	0.41^{ns}	0.04
Krowal	0.43^{ns}	0.04
Period of Al stress (h)		
0	0.00^{d}	0.00^{b}
6	0.27^{c}	0.08^{a}
12	0.51^{b}	0.06^{a}
24	0.64^{ab}	0.04^{ab}
48	0.68^{a}	0.02^{b}

^{ns}: not significant; number on the same column followed by the same letter was not significantly difference based on DMRT (α = 0.05)

Table 3. Al uptake of rice root var. IR64 and Krowal under different levels of Al stress

Factors	Al uptake (mg Al/g root)
Varieties	
IR 64	0.12^{ns}
Krowal	0.10^{ns}
Al concentration (ppm)	
0	0.00^{a}
15	0.12^{b}
30	0.12^{b}
45	0.11^{b}
60	0.13^{b}

^{ns}: not significant; number on the same column followed by the same letter was not significantly difference based on DMRT (α = 0.05)

Table 4. Al uptake of rice root var. IR64 and Krowal under different periods of Al stress

Factors	Al uptake (mg Al/g root)
Varieties	
IR 64	0.09^{ns}
Krowal	0.08^{ns}
Period of Al stress (h)	
0	0.00^{a}
6	0.04^{b}
12	0.06^{b}
24	0.10^{c}
48	0.15^{d}

^{ns}: not significant; number on the same column followed by the same letter was not significantly difference based on DMRT (α = 0.05)

The Al uptake and distribution pattern were not different between both rice varieties at any different levels and periods of Al stress. These results were also supported by the data of root morphological and growth analyses that showed no significant difference in root morphology and growth of both rice varieties under Al stress treatment. These phenomena suggested that rice var. Krowal that was previously grouped as Al-tolerant cultivar based on Al stress level 45 ppm Al using the same nutrient solution (Jagau 2000) used in this experiment has similar characteristics in response to Al toxicity with IR64 and it may not be an Al-tolerant rice variety.

Carefull examination to this conclusion has been performed by repeating the analysis of root growth of both rice varieties under different Al stress level with different Al tolerance parameters, such as root re-growth, relative root length, and root elongation (data not included). The results showed that both rice varieties were not different in root growth response to Al stress. We also found two types of rice var. Krowal. One has long grain that used in this experiment and the other one with short grain. Both types have also been evaluated for the same Al tolerance parameters under Al stress. The result also indicated that both types of Krowal rice varieties has similar sensitivity to Al toxicity.

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