Bul. Plasma Nutfah 23(2):81–90

Exploring Aluminum Tolerance at Seedling Stage in Rice (*Oryza sativa*, Linn) by Using Modified Magnavaca Nutrient Solution

(Eksplorasi Toleransi Keracunan Aluminium pada Bibit Padi (*Oryza sativa*, Linn) menggunakan Larutan Hara Magnavaca yang Dimodifikasi)

Nurul Hidayatun[1]*, Maria Genaleen Q. Diaz[2], and Abdelbagi M. Ismail[3]

[1]Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development, Jl. Tentara Pelajar 3A, Bogor 16111, Indonesia
Phone (+62251) 8337975; Fax. (+62251) 8338820
[2]University of the Philippines, Los Banos, Philippines
[3]International Rice Research Institute, Los Banos, Philippines
*E-mail: nurulhi23@yahoo.com

Submitted: 24 Mei 2017; Revised: 1 Agustus 2017; Accepted: 29 September 2017

ABSTRAK

Keracunan aluminium (Al) merupakan salah satu hambatan utama dalam produksi tanaman pangan di lahan masam. Pengembangan varietas toleran Al memerlukan serangkaian tahapan, salah satunya ialah seleksi terhadap ketahanan terhadap cekaman Al. Penelitian ini bertujuan mengetahui respons pertumbuhan akar dan tunas terhadap keracunan Al pada delapan varietas padi yang dipelihara pada larutan hidroponik. Larutan nutrisi yang merupakan modifikasi dari larutan Magnavaca digunakan untuk membandingkan pengaruh Al pada beberapa variabel pengamatan, yaitu perpanjangan akar relatif (PAR), panjang tunas relatif (PTR), dan bobot akar relatif (BAR). Penelitian dilakukan dalam rancangan split plot. Delapan varietas padi diskrining pada empat tingkat kejenuhan Al (0 µM, 540 µM, 750 µM, dan 1.300 µM). Panjang akar, panjang tunas, dan bobot kering akar diukur setelah 7 hari perlakuan cekaman Al, kemudian nilai PAR, PTR, dan BAR dihitung. Hasil penelitian menunjukkan bahwa cekaman Al secara nyata menurunkan PAR dan PTR tetapi menaikkan BAR. PTR menurun seiring dengan peningkatan konsentrasi Al, sementara penurunan PAR mulai terjadi pada konsentrasi Al 750 µM. Sementara itu, bobot kering akar menunjukkan peningkatan pada konsentrasi Al 540 dan 750 µM, tetapi tidak ada perbedaan nyata pada konsentrasi 1.300 µM. Peningkatan bobot akar disebabkan oleh penebalan dinding akar, tetapi efek ini tertutupi oleh penghambatan pertumbuhan akar pada konsentrasi 1.300 µM. Dari ketiga variabel yang diamati, panjang akar dan panjang tunas merupakan variabel yang lebih baik untuk mengukur ketahanan terhadap Al, dibanding bobot kering akar. Namun, kedua variabel ini tidak cukup mewakili penghambatan pertumbuhan akar dan tunas, sehingga kurang memadai untuk digunakan sebagai satu-satunya variabel dalam kegiatan skrining Al.

Keywords: padi, toleransi aluminium, skrining fenotipik, larutan Magnavaca

ABSTRACT

Aluminum (Al) toxicity is considered as one of the main constraints for crop production in acidic soil. This study was subjected to observe the response of root and shoot growth of eight rice varieties under Al stress in hydrophonic solution. A modified Magnavaca's solution was used to compare the effect of Al stress using different variables which were relative root elongation (RRE), relative shoot length (RSL) and relative root weight (RRW). The experiment was conducted in split-plot experimental design. Eight rice varieties were screened in four Al levels (0 µM, 540 µM, 750 µM, and 1,300 µM). Root length, shoot length, and root dry weight were measured after 7 days of treatment, then the RRE, RSL, and RRW were calculated. The results showed that Al significantly reduced RRE and RSL but increased RRW. RSL was reduced as the Al concentration increased while RRE reduction started only at 750 µM Al concentration. It was observed that RRW was significantly higher under 540 and 750 µM Al concentration. However, no significant difference was observed in 1,300 µM Al concentration. The increase in root weight is partly attributed by the thickening of the root wall, but this effect was diminished due to root hair inhibition under 1,300 µM Al concentration. Among these three variables observed, root and shoot lengths indicated better variables in determining Al tolerance in rice, compared to root weight. However, these variables were not sufficient to represent root and shoot growth inhibition, and not sufficient to be used solely for Al toxicity screening

Kata kunci: rice, aluminum tolerance, phenotypic screening, Magnavaca solution.

INTRODUCTION

Al toxicity is the single most important factor and a major constraint for crop production on 67% of the total acid soils worldwide (Hede et al. 2001). It is considered one of the primary causes of low rice productivity on upland and lowland rice growing areas (Dobermann & Fairhurst 2000). As tropical country, the high rainfall in most areas in Indonesia leads to leaching of nutrients and soil bases, and left only H ion in the clay complexes, which provides acidic soil with high aluminum saturation. Acidic dry-lands, generally called Red Yellow Podzolic soil is sensitive to erosion and poor in nutrient elements (Adiningsih & Sudjadi 1993). The soil become infertile and with low productivity (Suwarno et al. 2005). However, since the more suitable lands were already used, these marginal areas now become the hope of future agriculture.

The major and most easily recognized symptom of Al toxicity is the inhibition of root growth, which will lead to reduce plant vigor and yield. Delhaize & Ryan (1995) found that the apical region of the root and the root meristem are the primary site of Al-toxicity. Exposure to high Al in this region resulted in growth inhibition, while Al exposure in other regions of the root can cause root damage but no significant growth inhibition. Thus, the root growth inhibition has become a widely accepted to measure high Al stress tolerance in plants. Al accumulates preferentially in the root tips at sites of cell division and cell elongation. Dobermann & Fairhurst (2000) reported that long-term exposure of plants to Al also inhibits shoot growth by inducing nutrients (Mg, Ca, P) deficiencies, drought stress, and phytohormone imbalances.

Upland rice has enormous potential to support national rice production, however the utilization of these marginal lands for agriculture production is still facing various technical obstacles. Traditionally, farmers mitigate the effects of aluminum toxicity by liming and application of P fertilizer to increase the bioavailability of P in acid soils. However, this practice is not economically and physically feasible. P application does not always alleviate Al toxicity, and could be effective only after Al stress is overcome, especially for Al-sensitive species (Chen et al. 2012). The improvement of crop production in acidic lands by using tolerant cultivars is therefore considered as a more effective strategy and is more affordable to farmers. Breeding programs that develop rice genotype with ability to cope with aluminum toxicity and phosphorus deficiency will support low input agricultural systems that can sustain agricultural productivity. Plant species, including varieties within a species varies in their response to Al, some are more tolerant to others. Those plants can be used as genetic resources for crop improvement. Thus, development or screening of genotypes with higher Al tolerance will support sustenance of agriculture in acidic soils.

A screening method was developed to measure the symptoms caused by Al in the target areas that suffer the most. Screening can be performed hidroponic by using nutrition media, bioassay with soil media, or evaluation in the field (Howeler & Cadavid 1976). whose comparing nutrient and field screening for Al-toxicity on rice found that the values of relative root length of eight cultivars that were used as standard are correspond to field observations of their relative tolerance to acid soils. Various screening methods have been employed for Al toxicity tolerances, including field screening, soil, and nutrient solution culture. However, most of the screening have been conducted using hydroponic nutrient solutions.

Screening with hydroponics is considered easier and more practical than field screening, so many of the Al toxicity screening activities of Al are conducted in this way. The hydroponic method facilitates the preparation, maintains the homogeneity of the pH, and the availability of the nutrient, as well as the ease with which the system of observation and scoring (Wang et al. 2006). With this method, the development of rooting can be monitored at any time. In addition, the sample of the plant can be easily retrieved, even then it can be returned back into the media after scoring, allowing for observation of a variable over different time periods. These conveniences also allow for faster and larger scale of screening.

Diverse media and nutrient solutions are being used for germination and subsequent seedling growth. Various seedling ages, Al concentrations and stress durations was used in the screening. Ma et al. (1997) used four levels of Al (5, 10, 20, and 40 µM Al) in 1 mM $CaCl_2$ solution at pH 4.5 for a rapid hydroponic screening for Al toxicity tolerance in barley. In rice, one of the most Al tolerant crops, higher Al concentrations were used. Wu et al. (2000) used 7 days old seedlings and exposed them to 1 mM Al for 3 weeks. Nguyen et al. (2001) used seedlings with 4 days increment in age, and exposed them to 30 mM Al for 10 days, and mention that this level of stress was optimal for differentiating tolerant and sensitive rice genotypes.

Magnavaca solution is generally used for screening in maize and sorghum (Magnavaca et al. 1987). In 2010, Kochian et al. (2004) developed and optimized a nutrient solution and a high-throughput Al tolerance screening method for rice by modifying this nutrient solution. Modifications were made to ensure a sufficient supply of essential nutrients and to minimize the chemical interactions between Al and other mineral species in the nutrient solution at the high Al concentrations needed for rice. The modified nutrient solution was optimized and compared with Yoshida solution that is commonly used for Al tolerance studies in rice. Modified Magnavaca solution has significantly reduced precipitation of P, Fe, and Al in the Al treatment solutions compared with the Yoshida solution. The modified Magnavaca solution provide higher Al^{3+}, the active Al species that inhibit root growth, the variable for Al tolerance.

The degree of tolerance to Al is determined by comparing the root growth under stress versus control condition. The comparison of root length under stress versus root length under control condition, designed as a relative root growth (RRG) of the longest root, relative root length (RRL), or the root tolerance index (RTI) (Kochian et al. 2004; Nguyen et al. 2001; Wu et al. 2000). are the most commonly used parameters for estimating Al tolerance in cereals.

The objective of the study was to observe the response of shoot and root growth to varoius level of Al-toxicity in Magnavaca solution in order to find the best parameter to be used for Al toxicity screening.

MATERIALS AND METHODS

The experiment was conducted at IRRI greenhouse during December 2011–January 2012. Eight rice varieties obtained from IRRI GRC collection were used (Table 1). Azucena and Chadungda are known as Al-tolerant varieties, while IR20 is considered as susceptible and IR64 and IR74 are considered as intermediate. Pokkali is considered tolerant to acid sulfate soils (Tuan & Nghia 1982) and Fe toxicity (Wu et al. 2014).

Seed preparation and establishment of seedlings were conducted following the procedure below: The seeds (100 seeds per genotype) were placed in an oven at 50°C for 5 days to break the dormancy. Seeds were sterilized with 15% bleach, rinsed thoroughly and soaked in distilled water and kept at 32ºC for 24 h. Seeds were germinated on moist paper towel in petri disc for 48 h and evenly germinated seeds were incubated in rolled paper towel for another 48 h. Afterwards, healthy seedlings with similar root lengths were selected and sown in holes on styrofoam sheets floating in trays containing 7 l of nutrient solution, either with or without Al. Modified Magnavaca nutrient solution has been used as the medium for the screening (Table 2).

The experiment was laid in a split plot design with 2 replications. The Al concentration was assigned as the main factor. Ten seedlings per variety were used as the experimental unit. Four different Al concentrations were applied; 0, 540, 750, and 1,300 µM. The solution was maintained for five days and the pH adjusted daily to 4±0.05 by KOH or HCl. A one day optimization of nutrient solution (pH 4) was conducted prior to the 5 days treatments. The root length was measured manually with a ruler, before treatment (initial root length) and after 5 days of the treatment (the longest root length). After treatment, the root was oven dried in 50°C for 5 days and weighed.

Table 1. The genetic material used for the experiment.

Variety name	IRGC No.
IR20	14503
IR64	66970
IR74	-
Azucena	47125
Azucena	52992
Azucena	112854
Chadungda	96244
Pokkali	108921

Table 2. Elements and the concentrations in the Magnavaca's nutrient solution*.

Compound	Concentration
KCl	1 mM
NH_4NO_3	1.5 mM
$CaCl_2.2H_2O$	1 mM
KH_2PO_4	45 µM
$MgSO_4\ 7H_2O$	200 µM
$Mg(NO_3)_2.6H_2O$	500 µM
$MgCl_2.6H_2O$	155 µM
$MnCl_2.4H_2O$	11.8 µM
H_3BO_3	33 µM
$ZnSO_4.7H_2O$	3.06 µM
$CuSO_4.5H_2O$	0.8 µM
$Na_2MoO.4H_2O$	1.07 µM
Fe-HEDTA	77 µM

*Adopted from Famoso et al. (2010).

The degree of tolerance to Al was determined by comparing the root growth under stress versus under control (no Al) conditions (Kochian et al. 2004; Nguyen et al. 2001; Wu et al. 2000). The comparison of root length under stress versus root length under control conditions was defined as relative root elongation (RRE), relative root growth of the longest root length (RRG), relative shoot elongation (RSE) and relative root dry-weight (RRW). The RRE, RRG, RSE, and RRW were determined using the formula:

$$RRE = \frac{\text{Stress (longest root length after treatment-initial root length)}}{\text{Control (longest root length after treatment-initial root length)}} \times 100\%$$

$$RSE = \frac{\text{Stress (shoot length after treatment-initial shoot length)}}{\text{Control (shoot length after treatment-initial shoot length)}} \times 100\%$$

$$RRG = \frac{\text{Stress (total root length after treatment)}}{\text{Control (total root length after treatment)}} \times 100\%$$

$$RRW = \frac{\text{Root dry-weight after 5 days on Al stress}}{\text{Root dry-weight after 5 days in control condition}} \times 100\%$$

RESULTS AND DISCUSSION

Al inhibits plant growth via various ways, either directly by damaging root system, which in turn inhibit nutrient uptake, or indirectly by disrupting metabolic and biological systems. Al stress reduced plant biomass and uptake of various nutrient elements. Al targets multiple parts of the plant cell. Plants have to detoxify Al for survival. Multiple Al resistance mechanisms exist in plants. Two major Al resistance mechanisms that exist in plants are an external mechanism (Al exclusion) and an internal mechanism (Al tolerance). The external Al exclusion mechanism takes place outside the roots and prevents the entry of Al into the cell. These mechanisms include rhizosphere pH barrier formation, Al-binding by mucilage secreted from the roots, cell wall Al immobilization, increasing selective permeability of the plasma membrane, quelling by exudation of chelating compound (such as organic acids and phenolic compounds), and Al efflux from the root apex (Kochian et al. 2004). This study showed that shoot and root growth were reduced by Al stress. However, the effect of Al toxicity was more pronounced on root growth than on shoot growth.

Effect of Al Toxicity on the Inhibition of Root Growth

The rooth growth performance was significantly affected by both Al concentration ($P = 0.0008$) and rice genotype ($P<0.0000$). There was interaction between Al concentration and rice genotype ($P<0.0001$) revealing the severity of the stresses is different in each genotype (Table 3).

Root growth was significantly reduced by Al stress. The effect of Al toxicity was seen starting from the second day of exposure and gradually become more severe with the duration of exposure. This effect is clearly visible after 3−4 days of exposure. Root showed various symptoms of injury. They became hairless, thick, stunted and rigid. Whereas, under the control condition, the root system showed normal growth. The initial primary root growed long with lateral root-branching and soft hair. No toxicity symptoms found (Figure 1 & Figure 2).

The growth inhibition was more severe on the lateral root/root branches and root hair than on the primary root. Some plants showed severe reduction of the root branch and root hair while maintaining the primary root length (Figure 2).

Root is considered as the primary target site of Al toxicity and the severity of the effect of Al toxicity is commonly measured by the inhibition of root growth. Inhibition of root growth is typically calculated by comparing the root elongation in Al stress relative to control conditions. However, the root response to Al toxicity was affected (but not determined) by the initial root length. Especially in the case of short-term duration of treatment: the better vigor of initial root length, the better it will cope up with stress. Data of initial root length can be used as correction factor in the calculation of root growth inhibition. Thus, the parameter of relative root elongation (RRE) is more appropriate and independent of the bias caused by differences in the inital root length.

This study showed that Al significantly affected RRE ($P = 0.0008$; $R^2 = 0.54$; $n = 598$). The effect varied depending on the genotype ($P<0.0001$) and the interaction ($P<0.0001$). These eight genotypes showed different level and pattern of RRE as a response to Al concentration (Table 3 and 4, Figure 3).

The eight rice genotypes showed different response to Al stress. IR20, IR64, and IR74 showed higher RRE than Azucena, Chadungda, and Pokkali. In IR20, IR64, and IR74, the RRE were increased under low Al concentration (540 µM) and then decreased at higher level of Al (750

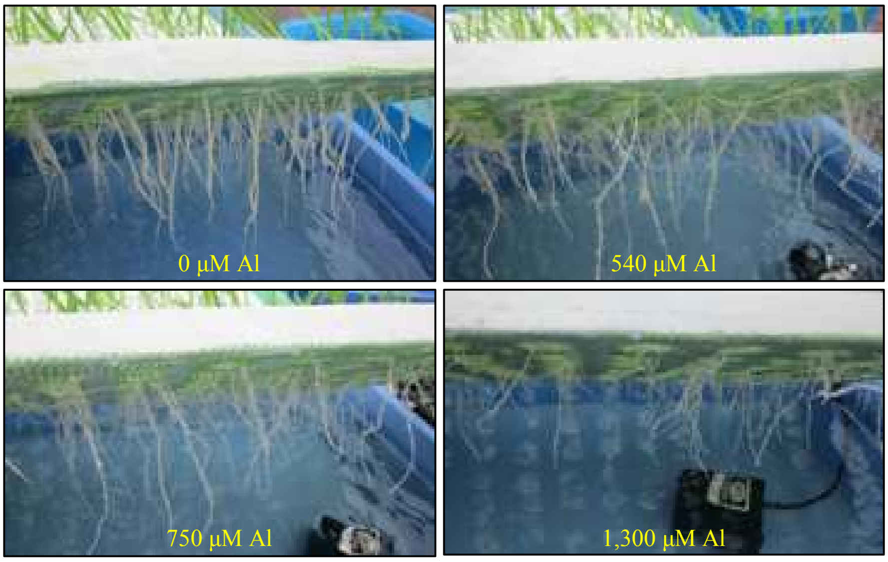

Figure 1. Root growth at four days after exposure to different Al concentrations.

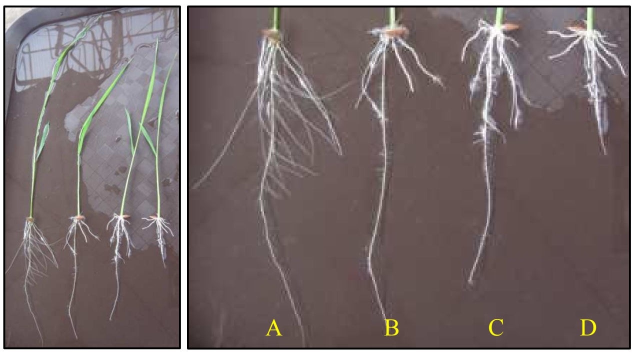

Figure 2. Root and shoot performance after 5 days of treatment with four different Al concentrations. A = 0 µM Al, B = 540 µM Al, C = 750 µM, D = 1,300 µM.

and 1,300 µM). This indicating that in low concentration, instead of inhibiting root growth, Al probably stimulated the absorption of nutrients resulting in enhanced growth.This phenomena of stimulating effect was reported previously by Hai et al. (1989), where the low Al concentration stimulate plant growth. The stimulating effect occurs in the low Al concentration until the treshold that Al will exerts toxicity effect.

Azucena, Chadungda, and Pokkali showed lower RRE. The RRE was gradually decreased as the Al concentration increased, and no stimulating effect found in these varieties. Thus, there is a threshold for Al concentration to be toxic as was also previously reported by Hai et al. (1989). Threshold concentrations determine when toxicity begins. At concentrations below the threshold, Al probably acts as a substrate or cofactor for other processes, while at concentrations above the threshold Al acts as an inhibitor.

Al toxicity threshold is typically depends on the age of seedlings. Previous experiments showed that in the older age of the seed, concentration of 540 µM Al stimulated root growth in six varieties of rice (data not shown). However, in this study, by using the same seedling stage, the response of rice plants were different. It is clear that the threshold of toxicity not only depends on the age of sprouts, but also is influenced by the genotype.

Irrigated rice, represented by IR20, IR64, and IR74 in this study, had higher RRE than the upland rice (Azucena, Chadungda) and the landrace Pokkali. RRE inhibition began at concentrations of 540 µM on upland rice, whereas in the irrigated varieties it stimulated root elongation. RRE inhibition started at the 750 µM Al concentration in irrigated rice. This seems contrary to the fact that upland rice is considered more tolerant to Al toxicity. Upland rice roots growth might have been affected by the lower aeration in hydroponic solution. In the other hand, there might be showed the drawback of the use of the longest root length as variable to determine the RRE to represent the tolerance to Al toxicity. A more reliable variable is probably need to determine the plant response to Al toxicity.

Famoso et al. (2010) showed that RRG of the longest root is not the best indicator of Al tolerance because a genotype may appear tolerant based on longest root measurements when, in fact, total root growth is inhibited. A comparison based on the relationship between RRG of the longest root and RRG of the total root system showed an R^2 of 0.172. Thus, RRG of the longest root is not a good proxy for RRG of the total root system. Despite of the drawback, the use of the longest root length was widely performed in the evaluation of

Table 3. P value of the effect of Al concentration, genotype, and the interaction on the variation of relative root elongation (RRE).

Effect	Pr>F
Aluminum concentration	0.0008
Variety	<0.0001
Aluminum concentration variety	<0.0001

Table 4. Relative root elongation of eight rice varieties under four different Al concentrations.

Variety	Initial root length	RRE under four different Al concentration			
		0 µM Al	540 µM Al	750 µM Al	1,300 µM Al
Azucena112854	19.68	1	1.063611	0.837222	0.42544
Azucena47125	5.59	1	1.124939	0.950616	0.793048
Azucena52992	17.55	1	0.927253	0.806673	0.661381
Chadungda	28.38	1	0.68804	0.585208	0.265972
IR20	22.95	1	1.446609	1.084674	0.731121
IR64	7.42	1	1.246676	0.992105	0.475789
IR74	11.49	1	1.38645	1.154141	0.553828
Pokkali	15.60	1	0.839691	0.62732	0.299794

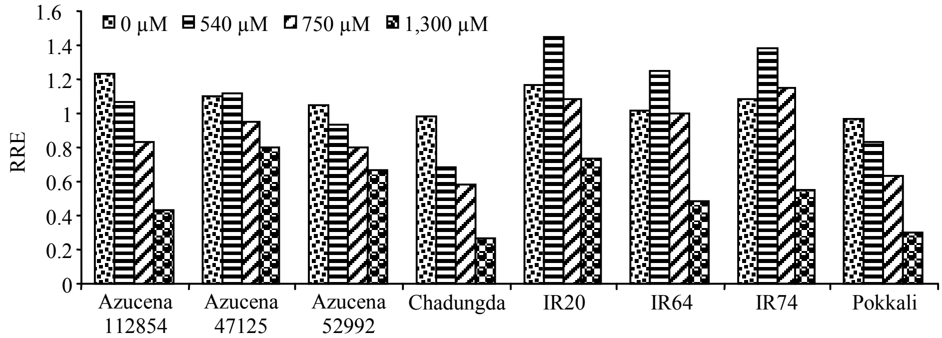

Figure 3. Effect of aluminum on the relative root elongation (RRE).

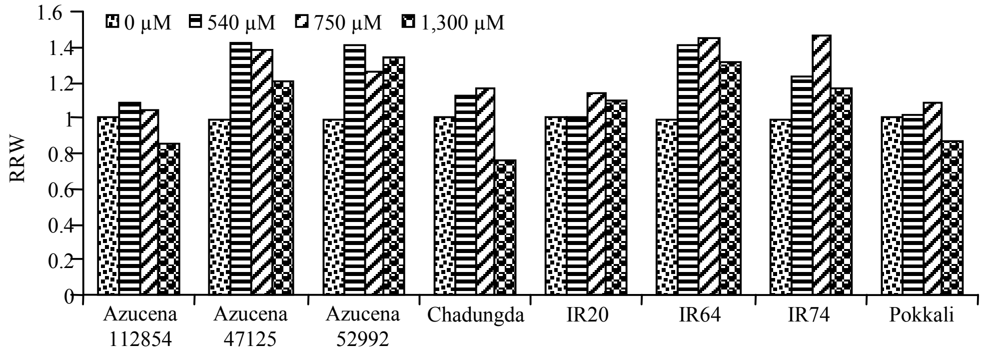

Figure 4. Effect of Al on relative root dry weight (RRW).

tolerance to Al toxicity. In rice, this method were used in various study (Nguyen et al. 2002, 2003; Wu et al. 2000).

In order to obtain accurate estimations of total root growth, Famoso et al. (2010) developed a custom root digital imaging system to quantify root length parameters for root systems of rice. The system was based on digital photography and semiautomatic measurements of individual primary, secondary, and tertiary roots using RootReader2D software. In this system, the length of the total root system can be reliably measured and a high quality digital images of each root system can be captured. This measurement system will more suitable for root growth measurement, especially for cerealia with fibrous root architecture. Lateral roots develop as branches of seminal root and these branches are also profusely re-branched forming complex ramification.

Effect of Al Concentration on Root Dry Weight

The root dry weight was not significantly affected by Al concentration (0.1098). Pearson correlation analysis was also showed a weak association (R^2 = 0.1117) between the Al concentration and the RRW. The RRW was more significantly affected by rice genotypes ($P<0.0001$) and its interaction ($P<0.0001$). This revealing that the differences on the responses are determined by rice genotypes. In all varieties tested, the 540 µM and 750 µM Al increased root dry weight. In the highest stess level tested (1,300 µM), the root dry weight were reduced (except for Azucena 52992). In some varieties RRW under 1,300 µM is higher than that of under control condition (Figure 6). Azucena, IR64 and IR74 showed higher RRW under Al stress than in the control condition.

Among the symptoms of Al toxicity are root wall thickening. Root dry weight, which is typically used to represent root mass, is determined by various components such as the number of root branches, length, size and volume. Apparently, the root wall thickening increased root mass, as reflected in root dry weight. The wall thickening, which actually is among the symptoms of Al toxicity, indirectly contribute to the increase of root dry weight. Thus, aside of the root mass, root dry weight also influenced by root thickness. The root wall thickening lead to the root rigidity. This type of root can not absorb nutrient efficiently. Thus, the contribution of the root wall thickening can not be accounted positively in the root growth, instead, this make an ambiguous on the use of root dry weight as variable for screening for tolerance to Al toxicity, especially in the seedling age. Due to these opposing effects, variation in root biomass was not significant and, consequently, this variable is considered not appropriate for determining tolerance to Al toxicity.

The rigidity of the root represent damage of the cell membrane and the loss of the plasma membrane integrity. Yamamoto et al. (2001) stated that mebrane damage is typical of the peroxidation of lipids, as a typical symptom under oxidative stress. Histochemical observation and quantification of the loss of plasma membrane integrity suggest that membrane damage induced by aluminum is due to mechanical disruption of cells at the periphery of cracks in the root at the elongation zone after aluminum exposure. The cracks in the root formed by differential cell expansion due to the inhibition of root elongation: there is an inhibition of surface cell expansion, whereas the expansion of internal cells occurs normally.

The root stunning suggesting the disruption of cell wall division. It was reported that aluminum also disrupts the cytoskeletal dynamics, either indirectly via alteration of signaling cascades that are involved in cytoskeletal stabilization or via a direct interaction with cytoskeletal elements. The disruption of the cytoskeletal elements (microtubules, microfilaments, and cortical

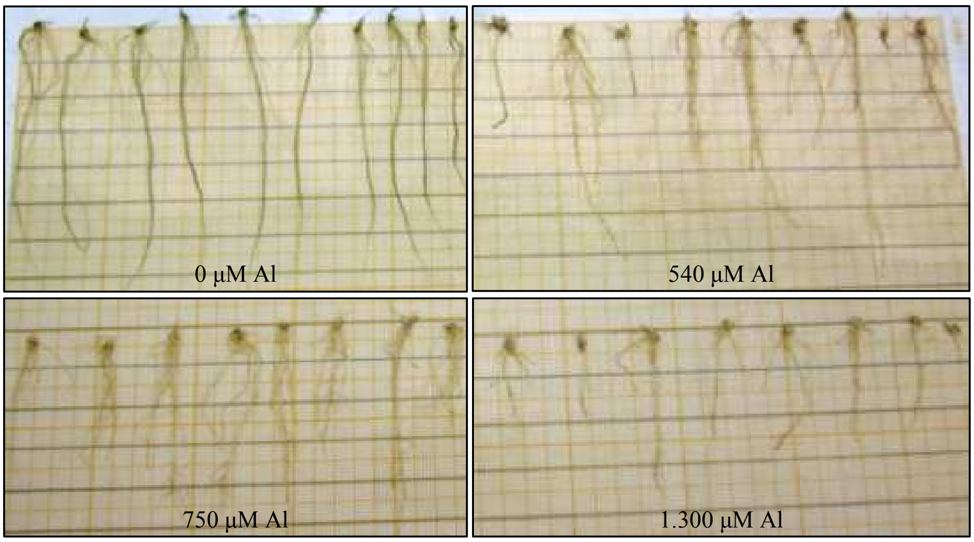

Figure 5. Root growth of rice variety Chadungda under four different levels of Al concentrations.

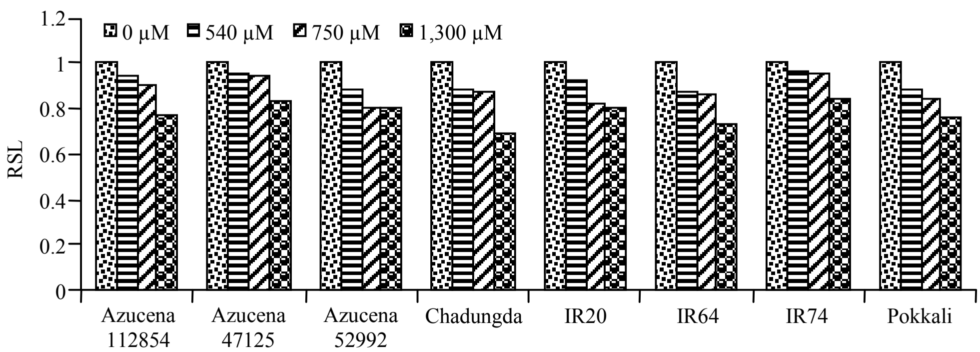

Figure 6. Effect of Al on relative shoot length (RSL).

microtubules) leads to the disruption of the cell wall division (Horst et al. 1999).

Effect of Al Toxicity on the Inhibition of Shoot Growth

Shoot growth was significantly affected by Al concentration (P = 0.0180) and rice genotypes (0.0042), but not by the interaction (0.6074). Al negatively correlated with RSL (R^2 = 0.47; n = 598) in all eight varieties. Al toxicity has multi target sites and multi symptoms. In the shoot, Al toxicity symptoms appear as the inhibition of shoots growth. Al caused necrotic, inhibit shoot expansion, and reduced leaf number (Thornton et al. 1986). In this study those symptoms were not strikingly clear.

The toxicity effect on shoot was less severe than those on the root. However, there was no stimulating effect found in the shoot part. The shoot growth was inhibited by the presence of Al stress and the growth gradually decreased as the toxicity level increased.

The common responses of shoots to Al, include: cellular and ultrastructural changes in leaves, increased rates of diffusion resistance, reduction of stomatal aperture, decreased photosynthetic activity leading to chlorosis and necrosis of leaves, total decrease in leaf number and size, and a decrease in shoot biomass (Thornton et al. 1986).

Typically, the effect of Al toxicity was more pronounced on root growth than on shoot growth. Early symptoms in roots are rapid, while Al translocation to the upper parts of plants is slow (Ma et al. 1997). Consequently, its effect was not immediately seen. The slow transport of Al was also reported previously by Hai et al. (1989). It was assumed that Al does not directly inhibit accumulation of plant biomass, but indirectly inhibit nutrient uptake and other biochemical processes. Thus, apparently shoot is not directly affected by Al toxicity. Thornton et al. (1986) found that the Al toxicity symptom on shoot growth inhibition of honeylocust was detected only after 3 weeks exposure with 1,500 μM Al. The slow response of the shoot made it not reliable to use this part as variable for determining tolerance to Al toxicity.

Conclusion and Future Work

Root length, root dry weight, and shoot length were affected by Al toxicity. Among these three variables, root length was considered better parameters for screening. However, there were some drawbacks of using the only longest root length for assessing Al tolerance on rice. Other parameters such as root hairs, total root length, and root surface area could better express total root growth and might provide better estimates to quantify the magnitude of the effect of Al toxicity in rice.

The optimum concentration of Al in the hydroponic using modified Magnavaca solution is 750 μM. This concentration can be used to conduct screening for tolerance to Al toxicity in the 5 days seedling age.

REFERENCES

Adiningsih, J.S. & Sudjadi, M. (1993) *Peranan sistem bertanam lorong (Alley cropping) dalam meningkatkan kesuburan tanah pada lahan kering masam*. *Risalah Seminar Hasil Penelitian Tanah dan Agroklimat*. Bogor, Pusat Penelitian Tanah dan Agroklimat.

Chen, Z.C., Yamaji, N., Motoyama, R., Nagamura, Y. & Ma, J.F. (2012) Up-regulation of a magnesium transporter gene *OsMGT1* is required for conferring aluminum tolerance in rice. *Plant Physiology*, 159, 1624–1633.

Delhaize, E. & Ryan, P.R. (1995) Aluminum toxicity and tolerance in plants. *Plant Physiology*, 107, 315−321.

Dobermann, A. & Fairhurst, T. (2000) *Rice. Nutrient disorders & nutrient management*. *Handbook series.* Potash & Phosphate Institute (PPI), Potash & Phosphate Institute of Canada (PPIC), and International Rice Research Institute (IRRI).

Famoso, A.N., Clark, R.T., Shaff, J.E., Craft, E., McCouch, S.R. & Kochian, L.V. (2010) Development of a novel aluminum tolerance phenotyping platform used for comparisons of cereal Al tolerance and investigations into rice Al tolerance mechanisms. *Plant Physiology*, 153 (4), 1678−1691.

Hai, T.V., Hai, T.V. & Loud, H.L. (1989) Effect of aluminium on the mineral nutrition of rice. *Plant and Soil*, 114, 173−185 .

Hede, A.R., Skovmand, B. & López-Cesati, J. (2001) Acid soils and aluminum toxicity. In: Reynolds, M.P., Ortiz-Monasterio, J.I. & McNab, A. (eds.) *Application of physiology in wheat breeding*. Mexico, CIMMYT. pp. 172−182.

Howeler, R.H. & Cadavid, L.F. (1976) Screening of rice cultivars for tolerance to Al-toxicity in nutrient solution as compared with a field screening method. *Agronomy Journal*, 68 (4), 551−555.

Horst, W.J., Schmohl, N., Kollmeier, M., Baluska, F. & Sivaguru, M. (1999) Does aluminium affect root growth of maize through interaction with the cell wall–plasma membrane–cytoskeleton continuum? *Plant and Soil*, 215, 163–174.

Kochian, L.V., Hoekenga, O.A. & Pineros, M.A. (2004) How do crop plants tolerate acid soils? Mechanism of aluminum tolerance and phosphorus eficiency. *Annual Review of Plant Biology*, 55, 459–93.

Ma, J.F., Zheng, S.J., Li, X.F., Takeda, K. & Matsumoto, H. (1997) A rapid hydroponic screening for aluminium tolerance in barley. *Plant and Soil*, 191, 133–137.

Magnavaca, R., Gardner, C.O. & Clark, R.B. (1987) Evaluation of inbred lines for aluminum tolerance in nutrient solution. In: Gubelman & Loughman (eds.) *General aspect of plant mineral nutition*. Dordrecht-Boston-Lancaster, Martinus Nijhoff Publisher. pp. 255–265.

Nguyen, V.T., Burow, M.D., Nguyen, H.T., Le, B.T., Le, T.D. & Paterson, A.H. (2001) Molecular mapping of genes conferring aluminum tolerance in rice (*Oryza sativa* L.) *Theoretical and Applied Genetics*, 102, 1002–1010.

Nguyen, V.T., Nguyen, B.D., Sarkarung, S., Martinez, C., Paterson, A.H. & Nguyen, H.T. (2002) Mapping of genes controlling aluminum tolerance in rice: Comparison of different genetic backgrounds. *Molecular Genetics and Genomics*, 267 (6), 772−780.

Nguyen, B.D., Brar, D.S., Bui, B.C., Nguyen, T.V., Pham, L.N. & Nguyen, H.T. (2003) Identification and mapping of the QTL for aluminum tolerance introgressed from the new source, *Oryza rufipogon* Griff., into indica rice (*Oryza sativa* L.). *Theoretical and Applied Genetics*, 106, 583–593.

Suwarno, Toha, H.M. & Ismail, B.P. (2005) Ketersediaan teknologi dan peluang pengembangan padi gogo. *Inovasi Teknologi Padi*. Bogor, Pusat Penelitian dan Pengembangan Tanaman Pangan. hlm.129−143.

Thornton, F.C., Schaedle, M. & Raynal, D.J. (1986) Effects of aluminum on growth, development, and nutrient composition of honeylocust (*Gleditsia triacanthos* L.) seedlings. *Tree Physiology*, 2, 307−316.

Tuan, D.T. & Nghia, N.H. (1982) Varietal tolerant for acid sulfate soil in North Vietnam. *International Rice Research Newsletter*, 7 (4), 12–13.

Wang, J.P., Raman, H., Zang, G.P., Mendham, N. & Zhou, M. (2006) Aluminium tolerance in barley (*Hordeum vulgare* L.): Physiological mechanisms, genetics, and screening methods. *Journal of Zhejiang University Science B*, 7 (10), 769−787.

Wu, P., Liao, C.Y., Hu, B., Yi, K.K., Jin, W.Z., Ni, J.J. & He, C. (2000) QTLs and epistasis for aluminum tolerance in rice (*Oryza sativa* L.) at different seedling stages. *Theoretical and Applied Genetics*, 100, 1295–1303.

Wu, L.B., Shhadi, M.Y., Gregorio, G., Matthus, E., Becker, M. & Frei, M. (2014) Genetic and physiological analysis of tolerance to acute iron toxicity in rice. *Rice*, 7 (8), 1–12.

Yamamoto, Y., Kobayashi, Y. & Matsumoto, H. (2001) Lipid peroxidation is an early symptom triggered by. Aluminum, but not the primary cause of elongation inhibition in pea roots. *Plant Physiology*, 125, 199–208.