



Research Article

Evaluation of Aluminium Tolerance in Pasaman Local Genotypes of Brown Rice During the Early Growth Phase

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Abstract

Background and Objective: One of brown rice resistance is tolerant to Aluminium stress. The research was conducted in Seed Technology Laboratory and shade net house of Faculty of Agriculture, Andalas University from March to June 2017. The research aimed to study the tolerance of 6 Pasaman brown rice genotypes to Al stress. **Materials and Methods:** Factorial design in Completely Randomized Design was used in this research. The first factor was brown rice genotypes, Sigambiri, Ladang Talamau, Sikarujuk, Silomlom Pulen and Perbatasan. The second factor was AlCl₃ doses, 0 ppm, 5 ppm, 10 ppm, 15 ppm and 20 ppm. The data was analysed by F test and extended by Duncan's New Multiple Range Test in 5%. **Results:** The result showed that there was interaction between Al concentration and 6 brown rice genotypes for height of plant, length of leaves. Based on tolerance level, all genotypes were grouped to Moderate-Tolerant level.

Keywords: Aluminium, Brown rice, Genotypes

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Introduction

Brown rice (*Oryza nivara*) is a type of rice that contains many nutrients. Brown rice also contains soluble dietary fibers (β -glucan, pectin, and gum) and ferulic acid from nonlignified cell walls. (Ryan 2011). Recent studies have shown that brown rice has a wide range of biological activities, including antioxidant, anti-carcinogenic, antiallergenic activities, anti-atherosclerosis, and amelioration of iron deficiency anemia in the body (Mir et al., 2015). In addition to this, brown rice has the ability to fight heart diseases and cancer. For people living with celiac disease, it is one way that they can consume bread and other bakery goods due to the absence of gluten-forming proteins in brown rice (Morita 2007).

The increasing of brown rice productivity is the main problem in Indonesia. One way that should be conducted for solving the problem is by using the dry land that widely available in Indonesia. The Aluminium (Al) toxicity is the main problem in cultivating brown rice. The Al harms the root hairs and inhibiting the growth of root hairs and causing the plant undergoes the nutrients deficiency (Wang et al., 2015).

Al is rhizo toxic ion that inhibiting the growth and productivity of plant in acid soil (Zheng 2010). Even though Al inhibits the metabolism process and plant growth, it can be tolerated by the plant in certain threshold (Abate et al., 2013). The several publications reported that the inhibiting of root growth is the basis for determining the tolerant or susceptible plant to Al stress (Zhao and Shen 2013). It is caused by the inhibiting of root apex that consisted of hood and root meristem that as the main target of Al toxicity. Meanwhile, the growth of the extended root zone is not influenced by Al. Besides that, the symplast and apoplast of the root are the part that can determine the cellular exclusion process or accumulation in the cytoplasm. The accumulation of Al in this part as the basis for determining the resistance of a plant to Al. the response in this part are faster than the other parts and the difference are significant (Zhao and Shen 2013).

The result of Elvariza's research(year of publishing?) reported that four rice genotypes that were examined to Al stress showed that 15 ppm concentration showed the accumulation of Al occurred in the end of roots. The color of root was lighter than un-poisoned root. The strong color was shown in Ciherang variety and followed by IR 42, Situbagendit and HawaraBunar varieties. The response to 15 ppm in RRG (Root Re-Growth) characteristic of rice plant root was influenced by variety of differences. In 0 ppm, the length of root was influenced by the difference of rice plant, but in 15 ppm, that character was not influenced by rice plant varieties.

Generally, the tolerant plant to Al stress will be able to grow by a certain mechanism for decreasing Al toxicity in the root zone. Awasthi et al., (2017) reported that the tolerant plant can tolerate Al in the cytoplasm, decreasing Al absorption or neutralizing it if it is in root cells. The research aimed to study the interaction of brown rice genotypes and several Al concentration doses.

Materials and Methods

Plant Material and Culture Conditions

The research was conducted in experimental design and Completely Randomized Design (CRD) in Factorial with 2 factors was used in this research. The first factor was

brown rice genotypes and the second factor was Al concentration and the trial was replicated in 3 times. The genotypes were Sigambiri, LadangTalamau, Sikarujuk, SilolomPulen, Pasaman Timur and Perbatasan. The concentration Al was AlCl₃ 30 ppm, 5 ppm, 10 ppm, 15 ppm, and 20 ppm.

The seedlings of rice were the collection of Department of Agronomy, Faculty of Agriculture, Andalas University. 50 seedlings of each genotype were soaked in NaOCl 0,5 % for 15 minutes for seedling sterilization. The seedlings then were washed in distillation water and soaked in distillation water for 24 hours. The seedlings then were sowed in sandy media by using the seedbed for 6 days. During the germination process, the media was watered every day. The solution was made by weighing the chemical materials based on Hoagland solution so that 4 groups of stock solution were obtained which were A, B, C, D. The materials were dissolved in distillation water and stirred until homogenous by a magnetic stirrer and entered into a bottle and stored in the refrigerator. For Al solution stock by weighing 50 mg of AlCl₃6H₂O and dissolved by distillation water until 11 level of volume and dissolved based on treatment so that 4 groups of AlCl₃6H₂O were obtained.

Nutrient solutions were taken from stock solutions that were made before. The making of nutrient culture media was conducted by taking 100 ml of each stock solution and entered to seedbed size 35 x 45 cm. For Al stress media, a stock solution of Al was added based on treatment. The distillation water was added until 10 L/seedbed. pH of nutrient culture was measured by pH paper and pH was arranged in 4.15 seedlings/genotypes were chosen for growing in nutrient culture that filled by Hoagland in the seedbed. Before planting, the seedlings were washed first by distillation water.

In order for the planted seeds not to sink into the solution, the seedbed is covered with styrofoam which has 36 holes and cotton was put in there. In the middle of the cotton, seedlings were inserted until the roots reach the solution that was made. Then in each seedbed that was filled with a nutrient solution, it was flowed by an aerator to prevent oxidation from the solution. Planting on nutrient media was carried out for 2 weeks. The maintenance of brown rice was conducted from germination of seeds until after being transferred to nutrient solution and obtaining the aluminum stress treatment in 2 MST. When maintenance germination was carried out, which was watering the plants once a day every afternoon, maintenance was carried out when the medium of nutrient culture was conducted to control the aerator always alive and the hose of aerator was not folded. And once a day, plants were watered using spray bottles every afternoon. Every maintenance and observation, the documentation was conducted during the growth of the brown rice.

Data Analysis

The observation variables were the height of the plant, length of leaves, length of plant root and tolerant level, canopy dry weight and tolerant level. The data was analyzed by the F test in 5% and continued by Duncan's New Multiple Range Test in 5%.

Results

Height and length of leaves of the plant

The result showed that there was an interaction between brown rice genotypes and Al concentration for the height of the plant (Table 1). The difference in plant height was caused by the tolerance of each brown rice genotype to Al were different (Awasthi et al.,

2017). Zhao et al., (2013) added that the mechanism of tolerance to Al was different among the varieties in one species. The genotype Sikarojuik showed the shorter plant height than others genotypes. It was caused by the genetic factor of each genotype and each genotype has different growth potency. Kang et al., (2011) stated that the tolerant genotype to Al will lead the distribution of photosynthate more to root zone for increasing the root ability to absorb the minerals in stress condition and can adapt in a high level of Al concentration. There was a mechanism in root for suppressing the bad effect of Al so that it can't disturb the nutrients absorption and water even can make them efficient.

Table 1. Height of plant of brown rice after giving the several Aluminium doses in 21 days after planting (dap).

Brown rice genotypes	Height of plant (cm) in each aluminium concentration (ppm)				
	0	5	10	15	20
					19,33
Sigambiri	28,53 ab	26,87 b	26,83 b	22,73 d	cd
	A	A	A	B	C
LadangTalamau	31,57 a	29,83 a	27,67 ab	25,63 c	22,80 d
	A	AB	BC	CD	D
Sikarojuk	19,03 a	18,50 c	17,90 c	16,80 e	15,20 e
	A	A	AB	AB	B
SilomlomPulen	29,10 ab	28,73 a	26,77 b	26,73 b	24,90 c
	A	AB	B	B	BC
Pasaman Timur	30,80 a	29,80 a	29,40 a	27,43 ab	27,23 a
	A	A	A	B	B
Perbatasan	29,50 ab	29,20 a	28,00 a	28,00 a	28,17 a
	A	A	A	A	A

The numbers in the same row followed by the same uppercase letters and the numbers in the same column followed by the same lowercase letters differ not significantly according to the DNMRT at the level of 5%

The biodiversity of plant tolerance genetic to Al has been reported by in several species of cultivation plants especially cereals from Triticeae (Magalhaes et al., 2004). In the rice plant, the characteristic of tolerant is multigene and quantitative (Famoso et al., 2011). Arenhart et al., (2014) stated that the tolerant genes to Al probably has a similar function for controlling tolerant characteristic to Al. One gene that can control the tolerance of rice plant to Al was ASR gene (Arenhart et al., 2013).

The result showed that there was interaction among the genotypes of brown rice and Al doses (Table 2). The difference in leaves length for each genotype was caused by the genetic variation of each plant. Each genotype has different growth potency depends on the genetic variety. Similar to the height of the plant, the length of leaves generally will undergo the inhibition of growth and cause the end of leaves are yellowing, rolling and the length of leaves are shorter than usual. The result also explains that the addition of Al in planting media caused the symptom of toxicity in the canopy. In a tip of leaves, they were yellowing and finally die. This symptom was caused by the inhibition of P absorption (Aluwiharee et al., 2016).

Table 2. Length of leaves of brown rice after giving several Al doses in 21 days after planting (dap).

Brown rice genotypes	Length of leaf (cm) in each alumunium concentration (ppm)				
	0	5	10	15	20
	23,57 b	22,53 a	20,83 a	19,00 cd	15,00 c
Sigambiri (kr)	A	A	AB	B	C
	26,37a	23,93 a	22,57 a	17,03 b	17,53 b
LadangTalamau	A	B	B	C	C
Sikarojuk	14,80 c	13,17 b	13,13 b	13,13 d	12,97 d
	A	A	A	A	A
	22,87 bc	21,10 bc	20,60 a	19,50 a	16,53 bc
SilomlomPulen	A	A	AB	B	C
	23,47 b	22,83 a	21,17 a	22,07 a	22,97 a
Pasaman Timur	A	A	A	A	A
Perbatasan	23,27 b	22,77 a	22,73 a	21,47 a	21,53 a
	A	A	A	A	A

The numbers in the same row followed by the same uppercase letters and the numbers in the same column followed by the same lowercase letters differ not significantly according to the DNMRT at the level of 5%

In several plants, the toxicity of Al showed the symptom is similar to P deficiency, whole stunting, the leaves shrink, the color dark green. The stem, leaves, and the color of leaves veins was purple, leaves tip was yellowing and died. In other plants, they showed the induced Ca deficiency symptom or the transportation of Ca was depressed in plant and caused the young leaves were curling or rolling and the growth points died (Awasthi et al., 2017). Un-available of P and Ca for the plant were caused by Al binds both elements so that P and Ca are not available for the plant (Arredondo et al., 2014). This result showed that the addition of Al in culture media caused the toxicity in brown rice plant leaves. These symptoms are visible in 15-200 ppm (Figure 1). In that concentration, the leaves were tighter and the tip of leaves was yellowing because they could not tolerate the Al stress (Arehart et al., 2014).

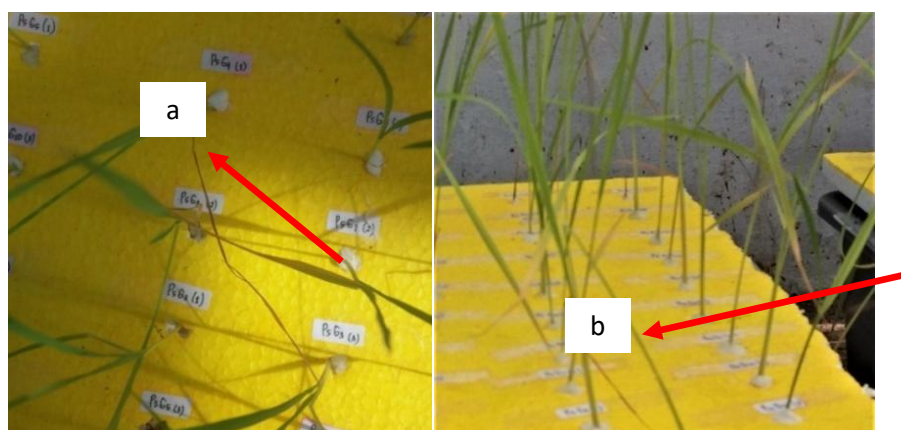


Figure 1. The symptom of Al toxicity. (a) the leaves are yellowing, (b) the leaves are rolling

Length of plant root and level of tolerance based on the length of the root

Based on the result of the length of root, the response of brown rice genotypes to Al concentration varied (Table 3). This result showed that the Al concentration did not affect the brown rice growth but brown rice genotype factor affected the root growth. As well as the concentration factor, it affected the root growth.

Table 3. Length of the root of brown rice after giving several Al doses in 21 days after planting (dap)

Brown rice genotypes	Length of roots (cm) in each Al concentration					Average of genotypes
	0 ppm	5 ppm	10 ppm	15 ppm	20 ppm	
Sigambiru	24,00	22,67	20,27	18,57	16,00	20,32 a
LadangTalamau	25,33	20,47	20,00	18,77	17,93	20,50 a
Sikarojuk	20,97	16,47	15,07	13,70	13,43	15,93 b
SilomlomPulenu	27,13	21,00	20,67	20,40	18,57	21,55 a
Pasaman Timur	21,80	20,57	19,77	18,70	17,93	19,75 a
Perbatasan	24,20	21,80	20,47	19,40	16,43	20,46 a
Average of concentration	23,90 A	20,50 B	19,37 B	18,26 BC	16,71 C	

The numbers in the same row followed by the same uppercase letters and the numbers in the same column followed by the same lowercase letters differ not significantly according to the DNMRT at the level of 5%

The division of cells will be inhibited in the poisonous plants especially in root cells. It was caused by Al binding with DNA and ending the cells division process of apical meristem (Awasthi et al., 2017). Root cap, meristem, and root elongation zone are the most susceptible to Al. In these parts, the accumulation of Al is more than others. The toxicity of Al caused the P level in root decreased and it caused the root will be shorter (Aluwihareet al., 2016). The poisoned root can be seen in Figure 2.



Figure 1. the poisoned roots by Al. (1) the shorter root that poisoned by Al than un-poisoned root by Al

The length of roots showed that the tolerance level of brown rice to Al. the observation of tolerance level to Al-based length of root was shown in Table 4.

Table 4. Level of tolerance of brown rice genotypes to Al stress based on the length of the root

Genotypes	Length of roots (cm) in each Al concentrations (Level of tolerance)							
	5 ppm	10 ppm	15 ppm	20 ppm	5 ppm	10 ppm	15 ppm	20 ppm
Sigambiri (kr)	105.78 (M-T)	89.96 (M-T)	82.16 (M-T)	70.73 (Sc)				
Ladang Talamau								
Sikarujok								
Silomlom Pulen								
Pasaman Timur								
Perbatasan								
Genotype	5 ppm	Level of tolerance	10 ppm	Level of tolerance	15 ppm	Level of tolerance	20 ppm	Level of tolerance
Sigambiri (kr)	105,78	M-T	89,96	M-T	82,16	M-T	70,73	Sc
LadangTalamau	127,70	M-T	99,00	M-T	91,93	M-T	86,96	M-T
Sikarujuk	139,16	M-T	109,78	M-T	91,06	M-T	86,98	M-T
SilomlomPulen	147,76	M-T	112,91	M-T	112,01	M-T	109,37	M-T
Pasaman Timur	103,06	M-T	96,45	M-T	90,78	M-T	87,84	M-T
Perbatasan	152,00	M-T	126,08	M-T	122,39	M-T	117,26	M-T

The numbers in the same row followed by the same uppercase letters and the numbers in the same column followed by the same lowercase letters differ not significantly according to the DNMRT at the level of 5%

Generally, all genotypes showed the level of tolerance was Moderate-Tolerant (M-T). This result showed that the plants can be adapted to Al stress. The plant could adapt in Al stress was caused by there was a mechanism for suppressing the Al effect and it didn't disturb the nutrients and water absorption (Fagiera 2012). Tolerant plants generally had a mechanism both external and internal that is neutralizing Al. the absorption of nutrients in tolerant nutrient is higher than susceptible plants. Land acid management can be repaired by adding organic materials (Wang et al., 2016).

The dry weight of canopy and level of tolerance

The result of the dry weight of canopy was presented in Table 5. The result showed that the addition of Al decreased canopy dry weight. It explained that the Al concentration affected brown rice weight. It was caused by the genotype responded to P even though in stress condition by Al. Syafruddin (2006) stated that the plant in Al stress but could use P produced more biomass. Awasthi et al., (2017) stated that 11 rice genotypes showed the decreasing of biomass in Al stress.

High level of P can produce Al-P binding and cause P stress deficiency. Swastiet al., (2004) reported that Al toxicity could suppress P absorption rate in all genotypes of rice both tolerant and susceptible genotypes. The inhabitation of P absorption caused the inhabitation of root extending and finally, it affected the plant biomass.

Table 5. The dry weight of brown rice after giving several Al concentrations in 21 days after planting (dap)(gram)

Brown rice genotypes	Dry weight (g) in each Al concentration					Average of genotypes
	0 ppm	5 ppm	10 ppm	15 ppm	20 ppm	
Sigambiri (kr)	0,04	0,03	0,03	0,03	0,03	0,034 e
LadangTalamau	0,06	0,05	0,05	0,04	0,03	0,046 c
Sikarujuk	0,04	0,02	0,03	0,03	0,02	0,026 f
SilomlomPulen	0,05	0,04	0,04	0,05	0,04	0,043d
Pasaman Timur	0,06	0,05	0,05	0,05	0,05	0,052 a
Perbatasan	0,06	0,05	0,04	0,04	0,04	0,046 b
Average of Al concentration	0,052	0,039	0,038	0,040	0,037	
	A	C	CD	B	D	

The numbers in the same row followed by the same uppercase letters and the numbers in the same column followed by the same lowercase letters differ not significantly according to the DNMRT at the level of 5%

Takehisa et al., (2013) reported that genotypes response in un-stressed condition by Al showed a better response than the stressed condition for dry weight of canopy. If Al toxicity was correlated to P absorption, the deficiency of P symptom occurred in upper parts because P was deposited by Al in roots. Al was in cortex and placed in protoplasm and nucleus.

The grouping of tolerance level based on dry weight presented in Table 6. All genotypes had Moderate-Tolerant level. Based on this result, the genotypes that planted in un-stress condition showed the weight of canopy was heavier that stress condition. Doniet al., (2014) stated that the growing plant can be measured by dry weight. The availability of nutrients for plant followed by photosynthesis activity that produced assimilate for supporting the dry weight of the plant.

Table 6. Level of tolerance of brown rice in Al stress based on dry weight of canopy in 21 days after planting (dap)

Brown rice genotypes	Al concentration							
	B1	Level of tolerance	B2	Level of tolerance	B3	Level of tolerance	B4	Level of tolerance
Sigambiri (kr)	87,96	M-T	77,27	M-T	64,84	M-T	61,81	M-T
LadangTalamau	78,30	M-T	74,36	M-T	59,62	M-T	56,51	M-T
Sikarojuk	73,22	M-T	72,52	M-T	53,44	M-T	49,85	M-T
SilomlomPulen	116,31	M-T	90,63	M-T	76,90	M-T	77,30	M-T
Pasaman Timur	87,08	M-T	81,27	M-T	80,74	M-T	76,42	M-T
Perbatasan	78,77	M-T	74,22	M-T	74,48	M-T	73,75	M-T

The numbers in the same row followed by the same uppercase letters and the numbers in the same column followed by the same lowercase letters differ not significantly according to the DNMRT at the level of 5%

Conclusions

The result showed that there was an interaction between brown rice genotypes and Al concentration and affected the plant height, length of leaves, length of root and dry weight canopy.

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