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West Sumatera brown rice genotypes resistance to Aluminium in early growth phase

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Abstract. One of indicator of superior variety is resistant to abiotic stress. The research was conducted in Laboratory of Seed Technology and shade net house, Department of Agronomy, Faculty of Agriculture, Andalas University from March to June 2017. The research aimed to study the tolerance of 6 West Sumatera brown rice genotypes to aluminium stress in early phase. Factorial Design in Completely Randomized Design was used in this research. The first factor was West Sumatera brown rice genotypes, Padi Ladang, Sungai Abu, Duo Koto Putiah, Sibandung, Situjuah and Balingka. The second factor was AlCl3 dose with 5 levels of concentration, 0 ppm, 5 ppm, 10 ppm, 15 ppm and 20 ppm. The data was analysed by Duncan's Multiple Range Test in level 5%. The result showed that there was interaction between aluminium stress. Based on height and length of leaf, Balingka was the best genotype and resistant to Al stress and pH. Duo Koto Putiah was the best genotype based on length of relative root in 15 ppm. Based on level of tolerant, Situjuah was the susceptible genotype, Duo Koto Putiah was the tolerant genotype and Padi Ladang, Sungai Abu, Balingka, and Sibandung were the Moderate-Tolerant genotypes.

1. Introduction

Brown rice is one of rice that type consumed by half-people of the world [1]. In addition to be a source of protein, carbohydrates, vitamins and minerals, brown rice also contains beneficial nutrients for the human body and recently studies have reported rice as an abundant source of substances with biological effects, including y-oryzanol, tocopherol, tocotrienol, phenolic compounds, phytic acid and phytosterol [2]. Increasing of yield productivity of brown rice is main problem in Indonesia. One of strategy to expand the for obtaining that purpose is using the dry land which is widely available in Indonesia. Aluminium (Al) toxicity is main problem in rice cultivation [3]. Al harms the root hood and inhibit the root growth. It cause the plant can not absorb the water and nutrient optimally [4].

Al is rhizo-toxic ion that inhibit the growth and the productivity of plant in acid soil [5]. Even though, Al is harmful for the plant, but the tolerant plant can still tolerate the mineral in certain amount [4]. Several publications reported that inhibiting the plant root growth by Al is the basic to consider the tolerant and susceptible plants to Al stress [6]. It caused by inhabitation of root growth [6]. Beside that, the apoplast and simplast of root determine the cellular exclusion process or accumulation in sitoplasm. It determines the resistance of plant to Al. The response in this part is faster than the others and the different are significant [6].

Ref. [4] reported that 4 genotypes of brown rice that giving the 15 ppm of Al concentration showed that Al accumulation in tap or root. Generally, the rice that had tolerance to Al stress will be

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able to grow with certain mechanism to decrease the Al toxi by in its root zone. The tolerant plants to Al can tolerate or neutralizing it in sitoplasm of root cells [4]. The research aimed to study the resistance of several local brown rice to Al stress.

2. Materials and Methods

Research sites

The research was conducted in Laboratory of Seed Technology, Department of Agronomy and Shade Net House, Faculty of Agriculture, Andalas University, Padang, Indonesia from March to June 2017.

2. Procedures

Factorial design in Completely Randomized Design was used in this research. 2 factors and three replicates were used in this design. First factor was six brown rice genotypes, Padi Ladang, Sungai Abu, Duo Koto Putiah, Sibandung, Situjuh, Balingka. The second factor was AlCl₃ concentrations, AlCl₃ in 0 ppm, AlCl₃ in 5 ppm, AlCl₃ in 10 ppm, AlCl₃ in 15 ppm, AlCl₃ in 20 ppm. The seed were used in this research were the collection of Department of Agronomy, Faculty of Agriculture, Andalas University. 50 seeds in each genotype were soaked in NaOCl 0.5 % in 15 minutes for sterilization and then and rinsed by distillation water and soaked for 24 hours. The seeds then germinated in sandy medium for six days. During germination, the seeds were watered every day.

Stock solution was made by determining Hoagland Nutrient composition. By this result, 4 stock solution were obtained, A,B,C,D. the materials were dissolved with distillation water until homogenous by magnetic stirrer. The solution then poured to bottle and stored in refrigerator. 50 mg AlCl36H20 was dissolved with distillation water until volume 11. The solution was dissolved based on treatment until obtaining four AlCl36H20 solution. Nutrient solution was taken from stock solution. Making of nutrient culture media was conducted by taking 100 mL stock solution and moved to seed bed (size 35 x 45 cm). For Al medium, Al stock solution was added based on the treatment. Distillation water was added up to 10 mL/seedbed. The pH of media was measured by pH paper and pH was arranged in 4. If pH was below 4, NaOH was added to complete the pH up to 4.

15 seedlings per genotype was chosen to be grown in culture media that contained Hoagland nutrient in seedbed. 36 holes in Styrofoam with cotton was made for avoiding the seedlings sink. Nutrient solution was flowed by aerator for avoiding oxidation of solution. Planting the plants in culture media was conducted for two weeks. Plant maintenance was conducted from germination until the plant obtain the Al stress. The observation variable was height of plant and length of leaf, length of relative root and level of tolerance, weight of canopy and level of tolerance, weight of relative plant root and level of tolerance.

3. Result and Discussions

Height of plant and length of leaves

The result showed that there was interaction between brown rice genotypes and aluminium (Table 1). The height of plant of all genotypes were different respectively. The difference was caused by the tolerance of each genotypes were different [4]. The mechanism of tolerance to Al stress are different for each plant and variety in one species [6]. 4 genotypes (Padi Gogo, Sungai Abu, Sibandung Sungai Abu and Balingka) had best performance in height of plant than other genotypes (Duo Koto Putiah and Situjuah). The height of plant was influenced by genetic factor of each brown rice genotype and each genotype had different growth ability, depends on the genetic variance. The tolerant genotype to aluminium will govern the distribution of photosynthates to root zone to increase the root ability to absorb the nutrients in stress condition. It caused the plants can adapt in land with high level of Al. it is caused the plants had a certain mechanism to supress the influence of Al so that it doesn't interfere the minerals and water absorption and even they can be used [8].

The genetic tolerance diversity to Al has been reported in several species of agriculture plants specially cereals from Family Triticeae [9]. Rice plants that tolerant to Al stress tend multigenes and quantitative [10]. Tolerant genes to Al had similar function in governing tolerance characteristic of Al

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stress [11]. One of gene that can govern the tolerance of rice plant Al stress was ASR gene [12]. The result showed that there was interaction between brown rice genotypes and Al stress in length of leaves (Table 2). The difference of leaves length in each genotype had different growth ability depends on the genetic variance. Like height of plant, the poisonous leaves by Al undergo the growth inhabitation and cause the top of leave was yellowing, roll, and leaves will be shorter.

Table 1. Height of brown rice plant after giving Al stress in 21 days after planting

	Al concentration							
Brown rice genotypes	0 ppm	5 ppm	10 ppm	15 ppm	20 ppm			
Padi Gogo	30.27A	28.00AB	27.83B	25.93BC	24.63C			
	a	a	a	a	a			
Sungai Abu	31.27A	28.87B	28.00B	26.27BC	24.93C			
	a	a	a	a	a			
Ladang Duo Koto Putiah	26.10A	24.27AB	24.07 AB	22.33BC	19.07C			
	c	b	b	b	b			
Sibandung	29.07A	27.70AB	27.07AB	26.07B	24.10BC			
	bc	a	a	a	a			
Situjuah	26.40A	22.70B	20.13BC	18.03C	17.50C			
	c	bc	c	c	c			
Balingka	28.37A	27.83A	27.70A	25.83B	24.73B			
	c	a	a	a	a			

The numbers in the same row followed by the same uppercase letters and numbers in the same column followed by the same lower case are not significant according to DNMRT at the 5% level

The result also showed that the addition of Al in planting media caused the poisonous symptom in brown rice canopy. The top of leaves looked yellowing and later the leaves will die. This symptom was caused by lack of Phosphor [13]. In several plants, the toxicity of Al showed that the symptom was similar to Phosphorus deficiency, whole stunting, the smaller leaves, stem, leaves and root are reddish or purple, the leaves tip are yellowing and dies. The other plants showed that the Ca deficiency symptom [4]. Un-available of Phosphorus and Ca for plants was caused by Al binds them so that Phosphorus and Ca are not available for plants [14]. The result showed that the addition of Al in planting media caused the poisonous in brown rice plants. This symptom were visible in 15-20 ppm concentration (Figure 1). In these concentration, the leaves of plants were narrow and tip of leaves are yellowing because the plant could not tolerate the high dose of Al [12].

3.2. Length of root and level of tolerance based on length of relative root

The result showed that the length of root after giving the Al stress showed that the response of root length varied (Table 3). The result showed that there was no interaction between Al concentration and brown rice genotypes but the factor of brown rice genotypes influenced to the root growth of brown rice. The concentration of Al affected the growth of brown rice root. The poisonous plants by Al, the cell division were interfered particularly root cells. It was caused by Al binding with DNA and stopping the division of meristematic cells [4]. Root cap, meristematic, and elongation zone are most susceptible to Al. In these parts, Al will be accumulated more than another. The toxicity of Phosphorus caused P content decline so that the length of root that poisoned by Al will be shorter than un-poisonous plants [13].

The length of root of brown rice showed that the level of brown rice tolerance to Al based on length of relative roots (Table 4). Generally, all genotypes that examined showed the Moderate-Tolerant level (Table 4). This result showed that the plant could adapt to Al with the level of tolerance from

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Moderate to Tolerant. The plant that can adapt to Al was caused by a certain mechanism to supress the effect of Al so that the absorption of mineral and water are not interfered [15]. The tolerant plants to Al generally have a external and internal mechanism, neutralizing of Al. the absorption of nutrients or alkali of tolerant plant are higher than susceptible plants. Tillage of acid soil that poisoned by Al can be repaired by addition of organic matter [3].

Table 2. The length of brown rice leaves after giving Al stress in 21 days after planting

		Al concentration							
Brown rice genotypes	0 ppm	5 ppm	10 ppm	15 ppm	20 ppm				
Padi Gogo	24.37 A	22.43 A	21.90AB	20.00AB	19.50 C				
	ab	a	a	a	a				
Sungai Abu	23.90 A	23.53 A	22.23AB	21.90 A	20.90 AB				
	b	a	a	a	a				
Ladang Duo Koto Putiah	20.17 A	17.70AB b	16.47AB b	15.80AB b	11.27 C b				
Sibandung	26.37 A	22.30 B	21.20 B	19.47BC	18.90BC				
	a	a	a	a	a				
Situjuah	21.30 A	18.23 B	14.17 C	13.80 C	12.40 C				
	bc	b	b	c	b				
Balingka	22.00 A b	21.93 A a	21.43 A	20.00AB a	19.90 AB a				

The numbers in the same row followed by the same uppercase letters and numbers in the same column followed by the same lower case are not significant according to DNMRT at the 5% level

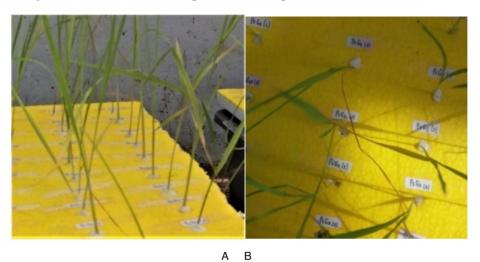


Figure 1. The symptom of Al toxicity (a) yellowing leaves, (b) the leaves roll

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Table 3. Length of brown rice root after giving the Al stress in 21 days after planting

	Al concentration						
Brown rice genotypes	2 0 ppm	5 ppm	10 ppm	15 ppm	20 ppm		
Padi Gogo	24.53 A b	23.77 A ab	22.33 A a	22.33 A a	20.20 B ab		
Sungai Abu	23.53 A b	22.87 A b	20.13 B b	19.27 B b	13.95 C d		
Ladang Duo Koto Putiah	18.37 A	12.30 B	12.73 B	11.50 B	10.03 B		
	27.53 A	24.43 B	22.57 B	22.73 B	22.40 B		
Sibandung	b 22.83 A	a 21.07 A	a 22.83 A	a 20.80 AB	а 12.70 В		
Situjuah	bc 27.47 A	b 25.40 AB	a 21.87 B	ь 21.00 В	d 18.00 C		
Balingka	a	a	ab	ab	С		

The numbers in the same row followed by the same uppercase letters and numbers in the same column followed by the same lower case are not significant according to DNMRT at the 5% level



Figure 2. The symptom of Al toxicity on brown rice roots.

3.3. Weight of canopy and level of tolerance based on canopy weight

The result of weight of canopy can be seen in Table 5. The result showed that the addition of Al decreased the relative dry weight of canopy of brown rice. In explained that addition of Al affected the dry weight of canopy. It was caused by the genotype was responsive to phosphorus even though in Al stress. The plants in Al stress but efficient in absorbing phosphorus will produce more biomass. High content of P can cause deficiency of P due to Al-P complex [16]. The Al toxicity influenced the phosphorus absorption in all rice varieties either susceptible or tolerant plants [17]. Correlation between P absorption that interfered indicates that the amount of absorbed nutrients will affect the formation of plant biomass [18]. The Ref. [19] reported that the genotype response in Al un-stressed showed that much better than another genotypes in dry weight of canopy. If Al toxicity was associated to phosphorus absorption by plant, evidently the symptom of phosphorus deficiency in upper parts of plants because phosphorus was deposited by Al in roots. It explained that there is interaction between Al and P. Al was deposited in cortex and placed in protoplasm and nucleus.

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Table 4. Level of tolerance of brown rice in Al stress based on length of relative root Genotype

_	Genotipe	Lev. of tol.	10 ppm	Lev. of tol.	15 ppm	Lev. of tol.	20 ppm	Lev. of tol.
	Padi Gogo	М-Т	90.65	М-Т	112.95	М-Т	93.59	M-T
	Sungai Abu	M-T	105.58	М-Т	115.69	М-Т	98.84	M-T
	Ladang Duo Koto Putiah	M-T	87.08	М-Т	161.80	Т	111.17	M-T
	Sibandung	М-Т	89.28	M-T	113.82	М-Т	93.37	M-T
	Situjuah	М-Т	78.54	M-T	101.61	М-Т	61.63	Sc
T -	Balingka	M-T	139.02	M-T	130.57	M-T	93.95	M-T

 $(T = Tolerant \ge 154.67\%), (M-T = Moderate-Tolerant\ 71.07\%-154.67\%), and\ (Sc=Susceptible \le 71.07\%)$

Table 5. Weight of canopy of brown rice after giving the Al stress in 21 days after planting Brown rice genotypes Al concentration

	Al concentration							
Brown rice genotypes	0 ppm	5 ppm	10 ppm	15 ppm	20 ppm			
Padi Gogo	0.05	0.04	0.04	0.04	0.03			
	0.05	0.04	0.04	0.04	0.04			
Sungai Abu Ladang Duo Koto	0.04	0.02	0.03	0.03	0.03			
Putiah	0.03	0.04	0.04	0.04	0.04			
Sibandung								
Situjuah	0.06	0.04	0.03	0.02	0.02			
, and the second	0.05	0.04	0.04	0.04	0.04			
Balingka	0.05	0.04	0.04	0.04				

The grouping of tolerance level based on dry weight can be seen in Table 6. All genotypes had Moderate-Tolerant (M-T) level. Based on this result the genotype that planted in un-stressed condition, the genotype had high dry weight. Otherwise, when 15 and 20 ppm of Al were given to the plant, the dry weight of canopy will be lower. The plant growth was characterized by the increasing of dry weight of plant. Optimal nutrients availability for the plants will be followed by the increasing of photosynthesis activity that producing assimilates in supporting the dry weight of plant [20].

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Table 6. Level of tolerance of brown rice in Al stress based on weight of canopy

Genotipe	5 ppm	Lev. of tol.	10 ppm	Lev. of tol.	15 ppm	Lev. of tol.	20 ppm	Lev. of tol.
Padi Gogo	108.81	M-T	90.65	М-Т	112.95	M-T	93.59	M-T
Sungai Abu	120.39	М-Т	105.58	М-Т	115.69	M-T	98.84	М-Т
Sungai Abu	120.39	IVI-1	105.56	IVI- I	113.09	IVI- I	90.04	IVI- I
Ladang Duo Koto Putiah	108.78	М-Т	87.08	М-Т	161.80	T	111.17	М-Т
Sibandung	92.50	M-T	89.28	М-Т	113.82	М-Т	93.37	М-Т
Situjuah	93.24	M-T	78.54	М-Т	101.61	М-Т	61.63	Sc
Balingka	109.87	М-Т	139.02	М-Т	130.57	M-T	93.95	М-Т

 $(T = Tolerant \ge 154.67\%)$. (M-T = Moderate-Tolerant 71.07%-154.67%). and $(Sc = Susceptible \le 71.07\%)$.

Conclusion

The result showed that there was interaction between aluminium stress. Based on height and length of leaf. Balingka was the best genotype and resistant to Al stress. Duo Koto Putiah was the best genotype based on length of relative root in 15 ppm. Based on level of tolerant. Situjuah was the susceptible genotype. Duo Koto Putiah was the tolerant genotype and Padi Ladang. Sungai Abu. Balingka. and Sibandung were the Moderate-Tolerant genotypes.

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