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Article Revision Letter for Authors - (AJAB-2020-05-295)

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Your manuscript entitled \"Assessment of Aluminum Tolerant of Double Haploid Lines for Developing New Type of Upland Rice\" (Ms.Nr. AJAB-2020-05-295) was reviewed by editorial board members of the Asian Journal of Agriculture and Biology. As initial decision, your manuscript was found interesting but some revisions have to be made before it can reach a publishable value.
Please answer all the comments below point-by-point in an accompanying response letter to your revised submission.

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COMMENTS for Authors:

=> Reviewer # 1

1. Authors need to fix all mistakes that higlighted in the manuscript.
2. When author stated some lines or varieties, it should be clearly stated which lines are tolerant and sensitive (at the first time are in abstract).
3. Make sure that author wrote the complete phare of an abbreviation when state it at the first time, and it should be consistent to using the abbreviation for further writing.
4. There is formula of SSR and RRL in the methods. But in discussion the SSR was changed to become RSR, so which is the right one.
5. Authors need to provide the measurment unit of the variables.
6. References: 20 out of 36 are not up to date or older than 2014. Probably these need to be changed with up to date literatures.
9. Some corrections as highligted in References are needed to be fixed.

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Assessment of Aluminum Tolerant of Double Haploid Lines for Developing New Type of Upland Rice

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ABSTRACT

Aluminum (Al) has a direct or indirect adverse effect on plant growth which is not the same for all plants, even in the same species. The roots of plants are the most sensitive to Al toxicity. The initial symptoms of Al toxicity are inhibition of cell extension and the retarded development of root systems. This study was aimed to evaluate doubled haploid line (DHL) of upland rice ~~lines~~ derived from anther culture to Al stress and to study the genetic diversity and population distribution of DHL due to Al stress. Al tolerant testing was carried out in a greenhouse arranged in a factorial randomized complete block design (RCBD) with three replicates. The first factor was concentration of Yoshida nutrient included Al of 0 and 45 ppm was the first factor. The second factor was upland rice lines obtained from previous experiments (DHL), four parents (SGJT36, SGJT28, Fatmawati, and Way Rarem), while Dupa, and ITA131, respectively as an Al tolerant and susceptible checks. The results showed that root length, shoot length, and shoot dry weight had high heritability values and correlated well with the observed characters. Al tolerant doubled haploid upland rice lines derived from anther culture varied widely. Based on relative root length (RRL), of the 58 lines tested, 19, 29, and 10 genotypes were highly tolerant, tolerant, and moderate tolerant, respectively. DH1 rice derived from P3 showed highly tolerant, tolerant, and moderate tolerant, while from P6 showed highly tolerant and tolerant.

Keywords: Aluminum (Al) tolerance, Doubled Haploid (DH), Upland rice lines.

INTRODUCTION

The transition of land functions into residential areas, construction of social facilities and infrastructure has led to a reduction of land for agriculture. It resulted in the expansion of agricultural land directed to areas of marginal land (dry land), especially on ultisol soils that reacted acid. It was often lack of Ca, Mg, P, K, and N as well as Al toxicity. The high of Al in acid soils has been shown to inhibit plant growth (Silva et al., 2010; Brunner and Sperisen, 2013). Utilization of acidic land faced with various obstacles, including low pH, which will reduce the availability of nutrients for plant growth. On the other hand, Al toxicity is increasing. In very acid soils (pH <4.5), Al solubility can increase Al saturation. Aluminum has detrimental

effects on plant, not only inhibit the growth of rice roots but also cause damage to rice root systems which can both lead to significant reductions in rice yields (Ismail et al., 2007; Liu et al., 2012). The effect of Al stress is not the same in all plants, even in the same species. The roots is the most sensitive to Al toxicity.

The mechanism of Al toxicity in plants are inhibition of cell extension and the retarded development of root systems. The availability of Al in soil solution depends on the acidity. In very acidic soil ~~reaction~~ conditions (pH <4.5), Al becomes very soluble, especially in the form of Al^{3+}, which is toxic~~ity~~ to plants. Aluminum also interferes ~~with~~ uptake, transport, and the utilization of nutrients, and inhibits enzyme activity and hormonal balance (Lupwayi et al., 2014; Wan et al., 2019; Yamamoto, 2019). The presence of high soluble Al causes stunted root growth and ultimately decreases the ability of roots to absorb mineral and water nutrients (Silva et al., 2012; Ma et al., 2014; Kochian et al., 2015). Inhibition of root growth occurs due to cell division and elongation in the root meristem by Al stress.

Al accumulation in root tissue will determine the level tolerance of plant genotypes and correlated with the level of root damage. Al accumulation in the root tissue is lower than in the sensitive genotype (Ma 2000; Zang et al., 2019). The small number of negative charges on the cell wall in tolerant genotype caused the lower interaction. (Watanabe and Okada 2005; Kochian et al. 2015). This phenomenon has also been reported by some previous researchers that stated tolerant rice had a mechanism by reducing the interaction of Al on the root cell walls (Nursyamsi 2000; Awasthi et al., 2017; Qian et al., 2018).

Until now, not many of rice varieties have tolerated acid soils, and some lines are still in the testing process. High genetic diversity is one of the main factors in improving plant traits, both by conventional and biotechnological methods. Previous genetic diversity studied on DH1 had produced 58 double haploid of upland rice lines that are ready for further evaluated (Herawati et al. 2009). Therefore, the selection of available genotypes needs to be done to obtain genotypes that are tolerant to aluminum stress. Identification of differences in root growth characteristics is one indicator that can be used in the tolerance selection of Al stress because roots are the main target of damage by Al. In rice, a quick method for evaluating tolerant genotypes to Al stress can be done by observing the root length in the vegetative phase (Bakhtiaret al., 2007; Belachew et al., 2017; Awasthi et al., 2017; Qian et al., 2018). This study was aimed to evaluate the DH1 of upland rice derived from anther culture, and to study genetic diversity and population distribution due to aluminum stress.

MATERIALS AND METHODS

The experiment was carried out in the greenhouse of the Indonesian Center for Research and Development on Biotechnology and Agricultural Genetic Resources, Cimanggu, Bogor. The materials used were 58 DH1 rice lines, four elders (SGJT36, SGJT28, Fatmawati, and Way Rarem), and two varieties checked, namely Dupa and ITA131 respectively as tolerant and sensitive aluminium (Al) (Prasetyono, 2003; Bakhtiar et al., 2007). The nutrient solution used was Yoshida nutrient solution (Yoshida et al., 1976).

The experiment organized in a factorial randomized complete block design (RCBD) which repeated three times. Yoshida nutrient solution (Yoshida et al. 1976) was added with Al-solution at 0 ppm and 45 ppm as the first factor, while the second factor was 64 of upland rice lines/varieties.

The seeds were roasted at 45 ° C for 3 x 24 hours, and then the seeds were sown in a nursery on husk media. Seeds ~~germination~~ were germinated in the dark room for five days. Rice seeds that were healthy, uniform, and have a height of ± 5 cm were selected for planting. The nutrient solution used was Yoshida method with the final composition as follows: 40 ppm N, ten ppm P, 40 ppm K, 40 ppm Ca, 40 ppm Mg, 0.5 ppm Mn, 0.05 ppm Mo, 0.2 ppm B, 0.01 ppm Zn, 0.01 ppm Cu and two ppm Fe (Yoshida et al. 1976). To reduce the formation of Al polymer, the pH of the nutrient solution was adjusted by using 0.1 N NaHCO3 to pH 4.5 before the addition of Al. The addition of Al by adding 0 and 2 ml of Al stock solution that had been made for 1000 ml of Al (source AlCl3.5H2O) to get the treatment concentration of 45 ppm Al. The pH of the nutrient solution was adjusted to 4.0 ± 0.1 using 0.1 N NaHCO3 or 0.1 N HCl.

Five-day-old healthy sprouts ~~on~~ with uniform root length were transplanted to the media. Sprout stems wrapped in soft foam and then put into styrofoam holes that had been prepared and floated on a nutrient solution in a pot. Each pot was planted with five sprouts and maintained for 14 days in a greenhouse. A growth period of 14 days was used because the composition of the Yoshida nutrient solution was designed for 14 days (Yoshida et al. 1976). During this period, the addition of water and pH adjustment was carried out with 0.1 N NaHCO3 or 0.1 N HCl every two days. Data were collected on plants at 14 days after planting by measuring root length, plant height, root dry weight, shoot dry weight. The formula estimated shoot root weight ratio (SRR):

$$SRR = \frac{root\ dry\ weight}{shoot\ dry\ weight}$$

The formula measures variable relative root length (RRL):

$$RRL = \frac{root\ length\ under\ Al\ stress}{root\ length\ without\ Al}$$

Data analysis was performed using the Least Significant Difference Test (LSD). The level of Al tolerance of rice was grouped into a susceptible = RRL<0.5, rather tolerant = 0.5 <RRL <0.70, tolerant = 0.70 <RRL <0.85, and highly tolerant = RRL> 0.85. Analysis of variance and correlation between variables using Pearson were performed using SAS software version 9.1. Genetic parameters were calculated based on the method used by Singh and Chaudhary (1979) as follows:

Source of variance	df	Means Square	expectation value
Genotipe	(g-1)	M2	$\sigma_e^2 + 3\sigma_g^2$
Error	(r-1)(g-1)	M1	σ_e^2

σ_e^2 = enviroment variance; σ_g^2 = genetic variance

$$\sigma_g^2 = \frac{M2 - M1}{r}\sigma_e^2 = M1\sigma_p^2 = \sigma_g^2 + \sigma_e^2$$

The standard deviation of genetic variance using the formula:

$$\sigma_{\sigma_g^2} = \sqrt{\left(\frac{2}{r}\right)\left[\left(\frac{M2_g^2}{df_g} + 2\right) + \left(\frac{M1_e^2}{df_e} + 2\right)\right]}$$

M2 = Means squared genotype
M1 = Means squared error
r = replication
dfg = degree of freedom genotype
dfe = degree of freedom error

Genetic diversity could be estimated from genetic variance (σ2g) and standard deviation of genetic variance (σσ2g). A character has a broad genetic diversity if σ2g>2σσ^{2}g. The estimation of Coefficient Genotype Diversity (CGD) using the formula:

$$CGD = \frac{\sqrt{\sigma_g^2}}{\bar{x}} \text{ x } 100\%\bar{x} = \text{average population observed}$$

if 0 <CGD ≤10.94 (narrow); 0 <CGD≤21.88 (rather narrow); 0 <CGD≤32.83 (rather broad); 0 <CGD≤43.77 (broad); 43.77 <CDG (very broad).

The formula estimatedof coefficient phenotype diversity (CPD) as follows:

$$CPD = \frac{\sqrt{\sigma_p^2}}{\bar{x}} x100\%$$

if 0 <CPD≤24.94 (narrow); 0 <CPD ≤49.71 (rather narrow); 0 <CPD ≤74.71 (rather broad); 0 <CPD≤99.65 (broad); 99.65 <CPD (very broad).

Heritability in a broad sense (h^2_{bs}) was calculated according to the formula:

$$h_{bs}^2 = \frac{\sigma_g^2}{\sigma_p^2}$$

Heritability values (h^2_{bs}) are grouped according to Stanfield (1983) as follows:
0.50 <h^2_{bs}<1.00 = height; 0.20 <h^2_{bs}<0.50 = moderate; h^2_{bs}<0.20 = low.

Genotypic correlations can be calculated using the formula:

$$r_{g(xiji)} = \frac{cov.g(xixj)}{\sqrt{\left(\sigma^2_{g(xi)}.\sigma^2_{g(xj)}\right)}}$$

cov.g(xixj) = genotypic variation between properties i and j
$\sigma^2_{g(xi)}$ = genetic variability i
$\sigma^2_{g(xj)}$= genetic variability j

RESULTS AND DISCUSSION

Analysis of genetic diversity

Analysis of variance of DH1 lines for Al stress in nutrient culture showed significant differences on all observed variables (Table 1). The response of each variable was different from Al stress. Al stress reduced root length by 21.95 percent and shoots dry weight by 22.14 percent, while decreased shoot length and root dry weight by only 6 percent (Figure 1).

Table 1. Analysis of variance of DH1 lines of new type upland rice under Al stress in nutrient solution

Variable	Sum Square	Mean Square	F value
Root length	1159.4	20.3	4.80**
Shoot length	0.35	0.006	2.92**
Root dry weight	0.089	0.0016	1.10*
Shoot dry weight	0.11	0.002	4.46**
Soot root weight ratio (SRR)	0.35	0.0062	2.92**

*Significant different at level 0.05; ** Significant different at level 0.01

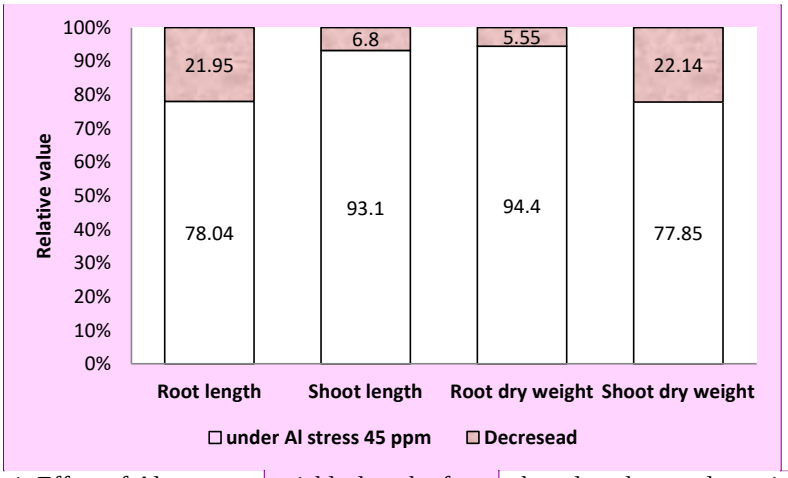

Figure 1. Effect of Al stress on ~~variableslength of root~~, shoot length, root dry weight, and shoot dry weight of DH1 lines

Decreasing in root length was caused by obstruction of the elongation of the primary and lateral roots. Field and laboratory experiments showed that there were mixed responses to Al toxicity in rice (Watanabe and Okada 2005; Bakhtiaret al., 2007;Qian et al., 2018). Reduction in shoot dry weight was due to nutrients available for suboptimal growth because of impaired nutrient absorption and transport in roots (Kochian et al. 2015;Qian et al., 2018). The decreased in root dry weight was only 5.55 percent, not as much as in dry shoot weight (22.14 percent) (Figure 1) although the root length decreased, the roots were shorter, and adventitious roots grew more. It showed that under Al stress conditions, more carbohydrates were directed to root growth. Bakhtiar et al. (2007) and Belachew et al. (2017) also found that shoot dry weight was more sensitive than root dry weight to Al toxicity. Inhibition of shoot growth is a secondary effect due to nutrient deficiency, especially Mg, Ca, and P. The inhibition of water absorption

Comment [MS1]: It is better if the decreased variables drown under 0 (zero), so the values are negative !!!!, such as --21.95; - 6.8; -5.5; and - 22,14. Please write the number in the right form such as 21.91 not 21,91 and soon..... !!!

Comment [MS2]: root length

caused dwarf rice growth (Ma et al., 2014). Wang et al.(2015) demonstrated that the application of NH4 decreased the Al content in rice roots by reducing ~~the~~ pectin content in rice roots. Freitas et al. (2019) revealed that aluminum chloride was more useful in producing aluminum toxicity in the upland rice plants grown in the nutrient solution.

Table 2. Genetic diversity of root length, shoot length, root dry weight,shoot dry weight, and root shoot weight ratio under stress conditions Al

Variable	Mean	GV*	PV	2xSD GV	GVC	PVC	h^2bs
Root length	15.75	5.37	9.61	5.43	14.71	19.68	0.56
Shoot length	42.14	30.74	38.41	21.41	13.61	14.70	0.80
Root dry weight	0.037	0.00007	0.0015	3.25	22.12	100.0	0.05
Shoot dry weight	0.114	0.00053	0.0009	3.25	20.19	26.75	0.57
Shoot root weight ratio (SRR)	0.29	0.0014	0.0035	3.25	12.92	20.40	0.40

*GV =Genotipe Variability, PV=PhenotipeVariability, PVC=Phenotipe Variability Coefficient, GVC= Genotipe Variability Coefficient, SDGV=standar deviate genetic variability, h^2_{bs}= heritability in a broad sense

The estimates of genetic parameters are shown in Table 11. Root length characters had a narrow diversity of genotypes but had a broad coefficient of the diversity of genotypes, respectively, 5.37 and 14.71 percent. Shoot length had a broad genotype diversity that was 30.74 percent but had a narrow coefficient of genotype diversity by 13.61 percent. Root dry weights both had a broad of the coefficient of genotypic diversity and coefficient of phenotype diversity (Table 2). The estimated heritability values for dry weight and shoot length were 0.05 and 0.8, respectively (Table 2). Heritability value of root length, shoot length, and shoot dry weight were classified as high. Characters that have high heritability values indicate that genetic factors are more dominant than the environment so that the selection of these characters can be made in the first generations (Akinwaleet al., 2011; Herawati et al., 2019).

Correlation and Relative Root Length (RRL)

Correlation analysis of all observed characters were positive, except for shoot length and RSR, while shoot dry weight and RSR were negatively (Table 3). Characters that have significantly different and positive correlations can be used as selection criteria. Root length, shoot length, and shoot dry weight can be selected as one of the criteria for Al tolerance for DH1 line. These characters had high genetic diversity and heritability values and have positively correlated with other characters.

Table 3. Correlation of root length, shoot length, root dry weight,shoot dry weight, and shoot root weight ratio (SRR) under Al stress condition

Characters	Shoot length	Root dry weight	Shoot dry weight	Shoot root weight ratio (SRR)
Root length	0.42**	0.28**	0.53**	0.12*
Shoot length		0.25*	0.65**	-0.25*

Root dry weight			0.43**	0.11^{ns}
Shoot dry weight				-0.14*

*= significant at level 005; **= very significant at level 001, ns=no significant

Among these characters, root length was more easily and quickly observed, so the researchers used relative root length (RRL) to distinguish tolerant and Al-susceptible genotypes. Previous research indicated that the main target of Al toxicity was the root tissue of the plant. Root damage occurs in sensitive genotypes due to Al toxicity, characterized by a decrease in protein content in the cytoplasm and increased membrane damage to cell walls, which results in cell membrane leakage (Zhu et al., 2018). Qian et al. (2018) reported that the fresh and dry weights of the rice seedlings were significantly positively correlated with chlorophyll content. This result indicates that a low Al concentration increases the fresh and dry weights of rice seedlings by increasing leaf chlorophyll content and promoting photosynthesis.

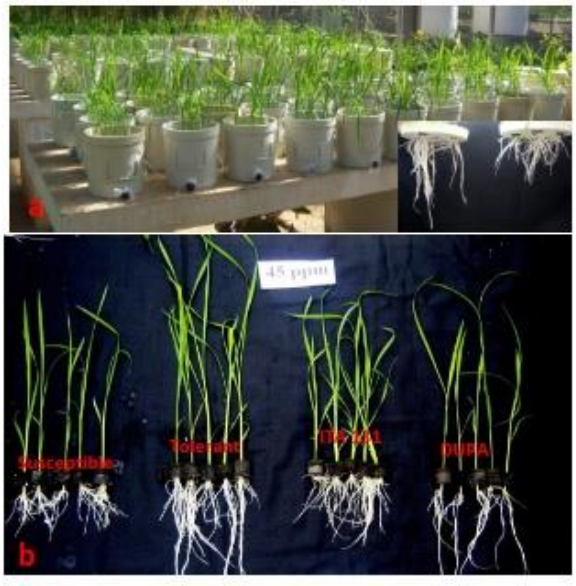

Figure 2. The experiment of Al stress on Yoshida nutrient solution (a); the appearance of root of susceptible lines, tolerant lines, ITA 131 (susceptible check), DUPA (tolerant check) under 45 ppm Al (b, from left to right)

Root shortening is one of the consequences of Al inhibition of root length. The morphology of secondary roots appeared shorter, fat, and reduced branching, while adventitious roots grew more on the root neck (Figure 2a). The roots have hardy penetrating the soil layer so that the absorption of nutrients and water will be inhibited. The level of Al toxicity depends on the activity of Al^{+3} in the soil media. The root activity of the seedlings at the concentrations also significantly decreased. Al decreases the fresh weight by inhibiting the absorption of water and mineral substances (Qian et al., 2018).

RRL values in the DH1 lines varied between 0.53-1.03 (Table 4). The RRL value of the Dupa (tolerant check) was 0.74, while ITA131 (susceptible check) was 0.53 (Figure 2b). The 5%

LSD test showed no significant difference between the RRL values for the rather tolerant genotypes and the RRL values for susceptible checks (Table 4). It is consistent with previous experiments carried out by Prasetiyono (2003), Bakhtiaret al. (2007) that Dupa was tolerant at RRL value 0.7, however, for ITA131 (0.53) was an increase from the previous experiment of 0.41 (Bakhtiaret al., 2007). For this reason, it is necessary to review using ITA varieties as susceptible checks (Figure 2b). The 5% LSD test on DH1-lines resulted in 8 lines having significantly different and higher RRL values than the Dupa check varieties (RRL = 0.74), namely lines P6-274, P6-314, P3-196, P6-273, P6- 311, P6-250, P6-267, and P6-278 (Table 4).

Table 4. Root lengths in treatments of 0 and 45 ppm Al and the relative value of the root length (RRL) of DH1 lines at 14 days after planting.

Lines	Al_0	Al_{45}[1]	RRL	Criteria[2]	Lines	Al_0	Al_{45}	RRL	Criteria
	(cm)					(cm)			
P6-274	16.2	16.7	1.03*	HT	P6-319	20.4	16.0	0.78	T
P6-314	20.3	20.3	1.01*	HT	P6-275	20.3	15.6	0.78	T
P3-196	17.1	16.8	0.98*	HT	P6-297	25.1	19.3	0.77	T
P6-273	19.9	19.5	0.97*	HT	P3-210	20.6	15.8	0.76	T
P6-311	15.3	14.9	0.96*	HT	P3-161	20.2	15.8	0.76	T
P3-250	16.8	15.9	0.95*	HT	P3-135	23.1	17.2	0.76	T
P6-267	10.6	10.1	0.95*	HT	P3-175	21.8	16.6	0.76	T
P6-278	19.4	18.3	0.94*	HT	P3-221	23.8	18.1	0.76	T
P6-286	23.4	21.6	0.93	HT	P3-190	20.2	15.3	0.75	T
P6-266	12.5	11.7	0.93	HT	P6-320	19.9	15.2	0.75	T
P3-191	21.5	19.6	0.90	HT	P3-162	20.9	15.4	0.74	T
P6-264	14.0	12.6	0.90	HT	P1-108	20.2	15.0	0.74	T
P3-238	17.9	15.1	0.88	HT	P6-317	16.3	12.2	0.73	T
P3-204	17.2	15.1	0.88	HT	P3-131	21.3	15.2	0.72	T
P6-291	14.9	13.1	0.87	HT	P3-248	18.7	13.5	0.72	T
P6-265	12.4	10.9	0.87	HT	P6-103	20.6	14.7	0.70	RT
P6-261	17.1	14.8	0.87	HT	P3-160	24.2	16.8	0.70	RT
P6-257	20.6	17.8	0.86	HT	P3-31	22.4	13.8	0.63	RT
P6-255	21.0	17.9	0.85	HT	P3-26	23.7	14.6	0.61	RT
P6-276	20.1	16.9	0.85	T	P4-45	22.1	13.3	0.60	RT
P6-271	21.7	17.8	0.84	T	P5-50	22.1	12.9	0.59	RT
P3-148	20.9	17.3	0.83	T	P2-1	18.5	11.1	0.59	RT
P3-120	23.2	19.6	0.83	T	P3-27	25.7	14.0	0.54*	RT
P6-272	20.5	16.6	0.83	T	P2-2	18.5	10.1	0.54*	RT
P6-62	20.6	16.8	0.83	T	P3-28	23.9	12.7	0.53*	RT
P6-105	16.6	13.7	0.83	T	Dupa	24.7	18.2	0.74	T
P6-295	21.8	17.8	0.83	T	ITA131	21.1	11.3	0.53	RT
P3-159	24.5	19.9	0.81	T	SGJT-28			0.89	HT
P3-134	19.3	15.6	0.80	T	SGJT-36			0.86	HT

Lines	Al_0	Al_{45}[1]	RRL	Criteria[2]	Lines	Al_0	Al_{45}	RRL	Criteria
	(cm)					(cm)			
P3-150	21.9	17.6	0.80	T	W.Rarem			0.52	RT
P6-302	20.3	15.5	0.79	T	Fatmawati			0.76	T
P3-158	24.1	19.2	0.79	T	BNT 0.05			0.2	
P3-249	20.6	16.3	0.78	T	KK (%)			15.69	

*Significantly different from Dupa based on LSD 0.05 test; [1]Al_0= 0 $AlCl_3$, Al_{45}= 45 ppm $AlCl_3$; [2]HT = Highly tolerant, T=tolerant, AT=Rather tolerant

In tolerance genotypes, Al is prevented from passing through the plasma membrane and entering the symplast and sites that are sensitive to Al in the cytoplasm of the root tip. The ability of the root cell wall to absorb low Al and the permeability of the cell membrane is thought to be involved in the mechanism of external tolerance. Zhu et al. (2018) that Hydrogen sulfide (H2S) plays an essential role in Al stress resistance in plants. HS reduces Al toxicity by reducing the Al content in the apoplast and symplast rice root. Wang et al.(2017) revealed that the activity of cytosolic glucose-6-phosphate dehydrogenase is also involved in resistance to Al through mediating ROS levels in soybean. Reports by Qian et al. (2018) indicated that H2O2 accumulation is also a key factor contributing to the decrease in root activity.

In Al tolerance, plants will be able to raise the pH around the root area (Kochian et al., 2004; Ma, 2007). Increasing pH around the roots occurs due to the influx of H^+ at the root tip. It resulted in the deposition of Al and a decreasing Al^{3+} activity so that it becomes a less toxic form to plants (Samac and Tasfaye, 2003; Zhao et al., 2014). Plants avoid from Al toxicity trough absorb NO^{3-} in large amounts. It caused the release of hydroxyl ions (OH^-) or bicarbonate ions (HCO^{3-}) into the rhizosphere,increased pH, and suppressed the solubility of Al (Justino et al., 2006; Zhao et al., 2018).

Table 5. The results of the DH1 lines selection for a new type of upland rice under Al stress

Criteria	Genotype	Number of lines
Highly tolerant	P6: 274, 314, 273, 311, 267, 278, 286, 266, 264, 291, 265, 261, 257, 255, dan P3: 196, 191, 238, 204, 250	19
Tolerant	P6: 276, 271, 272, 62, 105, 295, 302, 319, 275, 297, 320, 108, 317, dan P3: 148, 120, 159, 134, 150, 158, 249, 210, 161, 135, 175, 221, 190, 162, 131, 248	29
Rather tolerant	P2: 1, 2; P3:160, 31, 26, 27, 28; P4-45, P5-50, P6-103	10

The RRL values of the genotype P3-27, P2-2, P3-28 were lower than tolerant checks, classified as moderately tolerant genotypes (low) by 0.53-0.54, almost the same as the RRL values of theITA as susceptible checks by 0.53 (Table 4).The grouping was based on RRL values in 58 DH1 lines tested on nutrient cultures at 0 and 45 ppm Al, that is susceptible = RRL <0.5, rather tolerant = 0.5 < RRL <0.70, tolerant = 0.70 < RRL <0.85, and highly tolerant = RRL > 0.85, so 19 genotypes were highly tolerant, 29 tolerant genotypes, and 10 genotypes rather tolerant (Table 5).

Distribution of Population from Cross of P3 (Fatmawati x SGJT-36) and P6 (SGJT-36 x Fatmawati)

Aluminum tolerance based on the relative root length (RRL) and root shoot weight ratio (RSR) in DH1 populations from the crossing of P3 (Fatmawati x SGJT-36) and P6 (SGJT-36 x Fatmawati) and the two parents are presented in Table 6. The relative root lengths (RRL) in the P3 population ranges from 0.53 - 0.98, while the P6 population ranges from 0.70 - 1.03. The Fatmawati elders had an RRL of 0.77, while the SGJT-36 elders were 0.87. There was diversity in all observed characters. The root shoot weight ratio (RSR) of the P3 population ranged from 0.20 to 0.32, while the P6 population ranged from 0.22 to 0.39. The Fatmawati elders had an RSR value of 0.30, while the SGJT-36 elders had an RRL value of 0.32 (Table 6).

Table 6. Relative root length (RRL) and root shootweight (RSR) ratio of DH1 lines in populations of crossing P3 (Fatmawati x SGJT-36)and P6 (SGJT-36 x Fatmawati)

Characters	X ± SD DH1*	Range of DH1 population		Mean value of parent **	
		P3	P6***	Fatmawati	SGJT-36
Relative Root Length	0.8 ± 0.11	0.53 – 0.98	0.70 – 1.03	0.77	0.87
Root shoot weight ratio (RSR)	0.29 ± 0.04	0.20 – 0.32	0.22 – 0.39	0.30	0.32

*X ± SD DH1 is mean ± standard deviate, **Fatmawati and SGJT-36 5 plants each,***P3 were 26 lines, and P6 were 27 lines

RRL and RSR values observed in DH1 populations varied greatly, some of which were similar to their parents, intermediates, and exceed both of their parents. The frequency distribution of P3 and P6 populations based on RRL values is presented in Table 7.Based on aluminum tolerance criteria, the frequency distribution of the two elders did not overlap. Fatmawati had tolerant criteria, while SGJT-36 had highly tolerant. The frequency distribution of DH1 populations of P3 derivatives was highly tolerant, tolerant, and rather tolerant, while the frequency distribution of P6 populations was highly tolerant to tolerant (SGJT-36 elders) (Table 7).

Table 7. Distribution of DH1 lines in each population of crossing P3 (Fatmawati x SGJT-36) and P6 (SGJT-36 x Fatmawati) based on aluminum tolerance

Criteria	Parent*		DH1**	
	Fatmawati	SGJT-36	P3	P6
Highly tolerant	0	√	5	14
Tolerant	√	0	16	12
Rather tolerant	0	0	5	1
Susceptible	0	0	0	0

*The Fatmawati elders and SGJT-36 each with five plants, ** P3 were 26 lines, and P6 were 27 lines, √ Al tolerance criteria on elders

It was due to the presence of transgressive segregation in the combination of an anther, which produced lines with different tolerance levels. Many genes control Al tolerance levels in rice, so not all genotypes will have this gene. Zang et al. (2019) were found that there were significant differences between the gene expression patterns of Indica Al-tolerant and Japonica

Al-tolerant varieties, the gene expression patterns of the Al-tolerant varieties in the mixed subgroup, which was inclined to Japonica, were similar to the Al-tolerant varieties in Japonica. Each gene or combination will have a role in regulating the mechanism of Al tolerance in rice that will be expressed in each phase of plant growth (Wu et al. 2000). Thus the elders used in this study produced lines that were tolerant to aluminum stress. The next step will be an evaluation of the leaf blast disease in the greenhouse.

CONCLUSION

Evaluation of Al tolerance based on RRL in nutrient culture showed that 19, 29, and 10 genotypes were highly tolerant, tolerant, and rather tolerant, respectively. The tolerance level of Al in the DH1 lines of upland rice produced by anther culture varied greatly. Root length, shoot length, and shoot dry weight had high coefficient of diversity and heritability and correlated with each other. The distribution of DH1 populations of P3 derivatives produced highly tolerant, tolerant, and rather tolerant criteria, while the populations of P6 derivatives produced highly tolerant to tolerant criteria.

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Assessment of Aluminum Tolerant of Double Haploid Lines for Developing New Type of Upland Rice

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ABSTRACT

Aluminum ~~has a~~ can possibly have direct direct or indirect adverse effect on plant growth~~. The~~ , however, this effect ~~of Al stress~~ is not the same for all plants, even in the same species. The roots of plants are most sensitive to Al ~~toxicity. The~~ toxicity accompanied to initial symptoms ~~of Al toxicity in plants are~~ such as the inhibition of cell extension and ~~the~~ retarded development of root systems~~. .~~ . This study ~~aimed~~ aims to evaluate doubled haploid (DH1) upland rice lines derived from anther ~~culture to~~ aluminum ~~stress~~ culture, and ~~studying~~ also examine the genetic diversity and ~~population~~ the distribution of doubled haploid lines due to aluminum stress. Al tolerant ~~testing~~ test was carried out in a greenhouse using factorial ~~randomized complete block design~~ Randomized Complete Block Design (RCBD) with three replicates. Yoshida nutrient solution containing Al of 0 and 45 ppm was the first factor~~. The~~ , while the second ~~factor~~ was the lines obtained from previous experiments (DH1), the four parents (SGJT36, SGJT28, Fatmawati, and Way Rarem), Dupa, and ~~ITA131, respectively as Al tolerant and~~ the ITA131 susceptible checks. The results showed that the shoot and root length, ~~shoot length, and shoot~~ with their dry weight values had high ~~heritability values~~ coefficient of diversity, heritability, and significantly correlated ~~well~~ with ~~the observed characters~~each other. The tolerance level of Al ~~tolerant doubled haploid~~ in DH1- lines of upland rice ~~lines derived from anther~~ produced by another culture varied ~~widely~~significantly. Based on ~~relative root length~~ the Relative Root Length (RRL), out of 58 lines tested, 19 genotypes were highly tolerant, 29 lines ~~tolerant~~were moderate, ~~and ten moderate tolerant~~while 10 were low. The DH1 rice derived from P3 showed ~~highly tolerant~~high, ~~tolerant~~moderate, and ~~moderate tolerant~~low tolerance, while those from P6 showed ~~highly tolerant~~ high and ~~tolerant.~~ moderate tolerance only.

Keywords: Aluminum tolerance, Doubled haploid, Upland rice

INTRODUCTION

The transition of land ~~functions~~ into residential areas, the construction of social facilities and infrastructure has led to a reduction in the field of ~~land for agriculture~~agricultural land. It also resulted in the ~~expansion~~ shifting of agricultural land ~~directed~~ to ~~areas of~~ a marginal ~~land~~ (dry land~~)~~) area, especially on ultisol soils that reacted ~~sourly. It was often found~~ sourly to plant cultivation as a result of some symptoms ~~of~~ such as lack of Ca, Mg, P, K, and N as well as the presence of Al toxicity. ~~Al content~~ The high content of Al in ~~acid soils~~ acidic soil has ~~been~~ shown to inhibit plant growth (Silva et al., 2010; Brunner and Sperisen, 2013). The utilization of acidic land is faced with various obstacles, ~~including~~ such as low ~~pH,~~ pH which ~~will reduce~~ reduces the availability of nutrients for plant growth. On the other hand, Al toxicity ~~is increasing. In~~ increases in very ~~acid soils~~ acidic soil (pH <4.5), with increasing Al ~~solubility can increase Al saturation. Aluminum~~ solubility, which has detrimental effects on ~~plant, not~~ plants. Not only is the growth of rice roots inhibited, but ~~rice root systems can~~ also ~~be~~ damaged by high concentrations of Al in the soil, which ~~can both~~ lead to significant reductions in rice yields (Ismail et al., 2007; Liu et al., 2012). The impact of Al ~~stress~~ is not the same on all plants, even in the same species~~. The root is the part of the plant, which is most sensitive to Al toxicity.~~ .

The initial symptoms of Al toxicity in plants are inhibition of cell extension and the retarded development of root systems. ~~The~~ Its availability ~~of Al~~ in ~~soil~~ land solution depends on the level of soil acidity. In very acidic ~~soil reaction~~ conditions (pH <4.5), Al becomes very soluble, especially in the form of Al^{3+} ion, which is ~~toxicity~~ highly toxic to plants. ~~Aluminum~~ It also interferes with the uptake, transport, and the utilization of nutrients, and also inhibits enzyme activity and hormonal balance (Lupwayi et al., 2014; Wan et al., 2019; Yamamoto, 2019). The ~~presence of~~ high content of Al ~~solubility~~ solution in the soil causes stunted root growth and ~~ultimately~~ decreases the ability of roots to absorb mineral and water nutrients (Silva et al., 2012; Ma et al., 2014; Kochian et al., 2015). ~~Inhibition~~ The inhibition of root growth by Al ~~stress occurs~~ occurs, due to cell division and elongation in the root meristem.

~~Al~~ The accumulation of Al in root tissue ~~will determine~~ determines the ~~level~~ tolerance rate of plant ~~genotypes and correlated~~ genotypes, which correlate with the level of root damage. ~~In genotypes tolerant~~In tolerant genotype, the Al ~~accumulation in the~~ aggregation root ~~tissue~~ was ~~generally~~ lower than the sensitive genotype (Ma 2000; Zang et al., 2019). The small number of negative charges on the cell wall ~~in genotype~~ in tolerant ~~caused~~ genotype reduces the interaction of Al with the ~~lower interaction.~~ root layer (Watanabe and Okada 2005; Kochian et al. 2015). This phenomenon has also been reported ~~by some~~ in previous ~~researchers~~ studies (Nursyamsi 2000; Awasthi et al., 2017; Qian et al., 2018) that ~~tolerant~~ rice ~~had~~ tolerance has a mechanism ~~by~~ of reducing the interaction of Al on the root cell walls.

~~Until now~~Currently, ~~not~~ many rice varieties have not tolerated ~~acid~~ acidic soils, and some ~~lines~~ are still ~~in the testing stage.~~ being tested. High genetic diversity is one of the main factors used in improving plant traits, both ~~conventionally~~ by conventional and ~~biotechnology methods.~~ biotechnological method. Previous study of genetic diversity ~~studied~~ on DH1 had produced 58 double haploid upland rice lines that ~~are~~ were ready to be further evaluated (Herawati et al. 2009). Therefore, ~~the~~ proper selection ~~of available genotypes~~ needs to be done to obtain genotypes that ~~are tolerant to~~ tolerate aluminum stress~~. Identification of~~ . The differences in root growth character is one indicator that can be used in the tolerance ~~selection of Al stress because~~ selection, since roots are the main target of damage by Al. In upland rice, a quick method for evaluating genotypes that tolerate Al stress can be done by observing the root length in the vegetative phase (Bakhtiar et al., 2007; Belachew et al., 2017; Awasthi et al., 2017; Qian et al., 2018). This study ~~aimed~~ aims to ~~evaluate the~~ examine DH1 of upland rice ~~from~~ derived ~~anther~~

from another culture, and also study genetic diversity, ~~and~~ as well as the population distribution due to aluminum stress.

MATERIALS AND METHODS

The experiment was carried out in the greenhouse of the Indonesian Center for Research and Development on Biotechnology and Agricultural Genetic Resources, Cimanggu, Bogor. The materials used were 58 DH1 rice lines, the four elders (SGJT36, SGJT28, Fatmawati, and Way Rarem), ~~and two varieties checked~~Dupa, ~~namely Dupa~~ and ITA131 ~~respectively as tolerant and sensitive Al~~ susceptible check (Prasetyono, 2003; Bakhtiar et al., 2007)~~. The nutrient solution used was Yoshida nutrient solution (Yoshida et al., 1976)~~.

Experiments using factorial ~~randomized complete block design~~ Randomized Complete Block Design (RCBD) were repeated three times~~. Experiments using~~, with the Yoshida nutrient solution (Yoshida et al. 1976~~) were given a~~). A solution of ~~Al as much as~~ aluminum at the concentrations of 0 ~~ppm~~ and 45 ppm were given as the first factor, while the second ~~factor~~ was 64 rice ~~lines/~~line varieties.

The rice seeds were roasted for 3 x 24 hours at 45 ° ~~C,~~ C and ~~then the seeds were~~ sown ~~in a nursery~~ on husk media. ~~Seed germination~~ They were allowed to germinate in the dark for five days. ~~Rice seeds~~ After which those that were ~~healthy, uniform,~~ healthy and ~~have~~ uniform with a height of ± 5 cm were selected for planting. The nutrient ~~solution~~ used was Yoshida ~~method~~ solution with the final composition as follows: 40 ppm N, ~~ten~~ 10 ppm P, 40 ppm K, 40 ppm Ca, 40 ppm Mg, 0.5 ppm Mn, 0.05 ppm Mo, 0.2 ppm B, 0.01 ppm Zn, 0.01 ppm ~~Cu~~ Cu, and ~~two~~ 2 ppm Fe (Yoshida et al. 1976). In the Al ~~treatment,~~ treatment to reduce the formation of ~~Al~~ polymer, the pH of the nutrient solution was adjusted to 4.5 by using 0.1 N ~~NaHCO3 to pH 4.5 before the addition of Al~~NaHCO3. ~~The addition of Al by adding 0 and~~ After this, 2 ml of Al ~~stock~~ solution ~~that had been~~ made ~~for 1000 ml~~ from 1000ml of ~~Al (source~~ AlCl3.~~5H2O)~~ 5H2O was added to get ~~the~~ a treatment concentration of 45 ~~ppm Al~~ppm. The pH of the nutrient solution was adjusted to ~~pH~~ 4.0 ± 0.1 with 0.1 N NaHCO3 or 0.1 N HCl.

Five-day-old healthy sprouts ~~on~~ from a uniform root ~~length~~ were transferred to the media. Sprout stems were then wrapped in soft foam and ~~then put into styrofoam holes that had been prepared and floated~~ placed on a nutrient solution in ~~a pot~~styrofoam holes. Each ~~pot~~ pothole was planted with five sprouts and maintained for 14 days in a greenhouse. A growth period of 14 days was used ~~because~~ due to the composition of ~~the~~ Yoshida nutrient solution ~~was designed for 14 days~~ (Yoshida et al. 1976). During this ~~period~~phase, ~~the~~ water addition ~~of water~~ and pH adjustment ~~was~~ were carried out with 0.1 N NaHCO3 or 0.1 N HCl every two days. Observations were made on plants aged 14 days after ~~planting~~ planting, by measuring root length, plant height, root ~~dry weight,~~ and shoot dry weight. The formula ~~estimated shoot root~~ used to estimate the Shoot Root weight ~~ratio~~ Ratio (SRR~~)~~) was as follows:

$$SRR = \frac{root\ dry\ weight}{shoot\ dry\ weight} SRR = \frac{root\ dry\ weight}{shoot\ dry\ weight}$$

The formula ~~measures~~ used to measure the variable ~~relative root length~~ Relative Root Length (RRL~~)~~) was as follows:

$$RRL = \frac{root\ length\ under\ Al\ stress}{root\ length\ without\ Al} RRL = \frac{root\ length\ under\ Al\ stress}{root\ length\ without\ Al}$$

Data analysis was performed using the Least Significant Difference Test (LSD). Tolerance of rice lines to Al stress ~~was~~ were grouped into a susceptible = RRL <0.5, ~~rather tolerant~~ low = 0.5 <RRL <0.70, ~~tolerant~~ moderate = 0.70 <RRL <0.85, and ~~highly tolerant~~ high tolerance = RRL> 0.85. Analysis of variance and the correlation between variables were performed using Pearson analysis ~~of~~ and SAS software version 9.1. Genetic parameters were calculated based on the ~~method used by~~ Singh and Chaudhary (1979) method as follows:

Source of variance	df	Means Square	expectation value
~~Genotipe~~Genotype	(g-1)	M2	σ_e^2 σ_e^2 + $3\sigma_g^2$ σ_g^2
Error	(r-1)(g-1)	M1	σ_e^2 σ_e^2

σ_e^2 σ_e^2 = enviroment variance; σ_g^2 σ_g^2 = genetic variance

$$\sigma_g^2 = \frac{M2-M1}{r} \sigma_g^2 = \frac{M2-M1}{r} \qquad \sigma_e^2 = M1 \sigma_e^2 = M1 \qquad \sigma_p^2 = \sigma_g^2 + \sigma_e^2 \sigma_p^2 = \sigma_g^2 + \sigma_e^2$$

The standard deviation ~~of~~ formula for genetic ~~variance using the formula~~variance:

$$\sigma_{\sigma_g^2} = \sqrt{\left(\frac{2}{r}\right)[(\frac{M2_g^2}{df_g} + 2) + (\frac{M1_e^2}{df_e} + 2)]} \quad \sigma_{\sigma_g^2} = \sqrt{\left(\frac{2}{r}\right)[(\frac{M2_g^2}{df_g} + 2) + (\frac{M1_e^2}{df_e} + 2)]}$$

M2 = Means squared genotype
M1 = Means squared error
r = replication
dfg = degree of freedom genotype
dfe = degree of freedom error

Genetic diversity could be estimated from the genetic variance (σ2g) and the standard deviation of genetic variance (σσ2g). A character ~~has~~ had a broad genetic diversity ~~if σ2g>~~when σ2g > 2σσ^2_g. The ~~estimates of~~ Coefficient Genotype Diversity (CGD) was estimated using the ~~formula~~formula as follows:

$$CGD = \frac{\sqrt{\sigma_g^2}}{\bar{x}} \text{x } 100\% CGD = \frac{\sqrt{\sigma_g^2}}{\bar{x}} \text{x } 100\%$$

x̄ = average population observedx̄ = average population observed

~~if~~ When 0 ~~<~~< CGD ~~=~~< 10.94 (narrow); 0 ~~<CGD=~~< CGD < 21.88 (~~rather narrow~~narrower); 0 < CGD ~~=~~< 32.83 (~~rather broad~~broader); 0 < ~~CGD=~~CGD < 43.77 (broad); 43.77 ~~<~~< CDG (~~very broad~~broadest).

The ~~formula estimated of coefficient phenotype diversity~~ Coefficient Phenotype Diversity (CPD) was estimated using the formula as follows:

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$$CPD = \frac{\sqrt{\sigma_p^2}}{\bar{x}} x100\% CPD = \frac{\sqrt{\sigma_p^2}}{\bar{x}} x100\%$$

if When 0 < CPD ≤ 24.94 (narrow); 0 < CPD ≤49.71 (rather narrownarrower); 0 < CPD ≤ 74.71 (rather broadbroader); 0 < CPD ≤ 99.65 (broad); 99.65 < CPD (very broadbroadest).

Heritability in a broad sense (h^2_{bs}) was calculated according to the formula:

$$h_{bs}^2 = \frac{\sigma_g^2}{\sigma_p^2} h_{bs}^2 = \frac{\sigma_g^2}{\sigma_p^2}$$

Heritability The heritability values (h^2_{bs}) are were grouped according to Stanfield (1983) as follows:

0.50 < h^2_{bs} << 1.00 = heighthigh; 0.20 < h^2_{bs} << 0.50 = moderate; h^2_{bs} << 0.20 = low.

Genotypic correlations can be were calculated using the formula:

$$r_{g(xiji)} = \frac{cov.g(xixj)}{\sqrt{\left(\sigma^2_{g(xi)} . \sigma^2_{g(xj)}\right)}} r_{g(xiji)} = \frac{cov.g(xixj)}{\sqrt{\left(\sigma^2_{g(xi)} . \sigma^2_{g(xj)}\right)}}$$

cov.g(xixj) = genotypic variation between properties i and j

$\sigma^2_{g(xi)}\sigma^2_{g(xi)}$ = genetic variability i

$\sigma^2_{g(xj)}\sigma^2_{g(xj)}$= genetic variability j

RESULTS AND DISCUSSION

Analysis of of genetic diversity

Analysis of variance of DH1 lines in of rice with Al stress on in nutrient culture showed significant differences in all observed variables (Table 1). The response of each variable was different from Al stress. Al stress reduced root length by 21.95 percent and shoots dry weight by 22.14 percent, while it decreased shoot length and root dry weight by only 6 percent (Figure 1).

Table 1. Analysis of variance of DH1 lines of new type upland rice under Al stress in nutrient solution

Variable	Sum Sum Square	Mean Square	F value
Root length	1159.4	20.3	4.80**
Shoot length	0.35	0.006	2.92**
Root dry weight	0.089	0.0016	1.10*
Shoot dry weight	0.11	0.002	4.46**
Root shoot weight Ratio (RSR)	0.35	0.0062	2.92**

*Significant different at level 0.05; ** Significant different at level 0.01

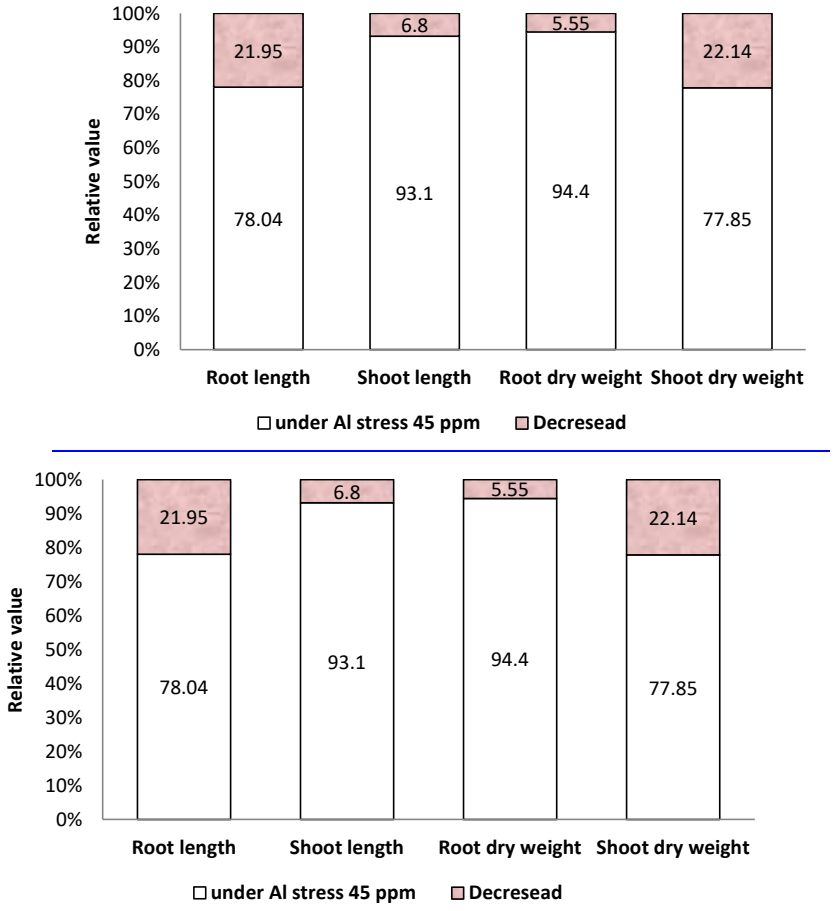

Figure 1. Effect of Al stress on variables of the length and dry weight of ~~root, shoot length, the~~ root ~~dry weight, and~~ shoot ~~dry weight~~ of DH1 lines.

~~Decreasing~~ The decrease in root length ~~is~~ was caused by ~~obstruction of the elongation~~ obstruction of ~~the~~ primary and lateral ~~roots~~roots elongation. ~~Field~~ The field and laboratory experiments showed ~~that there were~~ the mixed responses to Al toxicity in rice (Watanabe and Okada 2005; Bakhtiar et al., 2007; Qian et al., 2018). Reduction in shoot dry weight was due to the unavailable nutrients ~~available~~ for suboptimal ~~growth because~~ growth, as a result of the impaired ~~nutrient~~ mineral absorption and transport in roots (Kochian et al. 2015; Qian et al., 2018). The ~~decreased~~ decrease in root dry weight was only 5.55 percent, ~~not as much as in~~ compared to the dry shoot weight (22.14 percent) (Figure 1~~) because although~~). Since the root length ~~decreased, the roots were~~ decreased and became shorter, ~~and~~ therefore the adventitious roots grew the more. ~~It~~ These showed that under ~~stress~~ Al conditions, more carbohydrates were directed to root growth. Bakhtiar et al. (2007) and Belachew et al. (2017~~)~~). It was also ~~found~~ observed that shoot dry weight was more sensitive to Al toxicity than root dry weight~~. Inhibition~~

. The inhibition of shoot growth ~~is~~ was a secondary effect due to nutrient deficiency, especially Mg, Ca, ~~and~~ P, and the ~~inhibition~~ restriction of water absorption ~~causes~~ which caused dwarf rice growth (Ma et al., 2014). Wang et al. (2015) demonstrated that the application of NH4 decreased the Al content in rice roots by reducing the pectin content in ~~rice~~ their roots. Freitas et al. (2019) ~~reveal~~ showed that aluminum chloride was more ~~useful~~ important in producing ~~aluminum~~ Al toxicity in the upland rice ~~plants~~ plants, grown in the nutrient solution.

Table 2. Genetic diversity of root ~~length,~~ and shoot length, root ~~dry weight,~~ and shoot dry weight, and root shoot weight ratio under Al stress ~~conditions Al~~conditions

Variable	Mean	GV*	PV	2xSD GV	GVC	PVC	h^2bs
Root length	15.75	5.37	9.61	5.43	14.71	19.68	0.56
Shoot length	42.14	30.74	38.41	21.41	13.61	14.70	0.80
Root dry weight	0.037	0.00007	0.0015	3.25	22.12	100.0	0.05
Shoot dry weight	0.114	0.00053	0.0009	3.25	20.19	26.75	0.57
Root shoot weight Ratio (RSR)	0.29	0.0014	0.0035	3.25	12.92	20.40	0.40

*GV =Genotipe Variability~~,~~ , PV=PhenotipeVariability, PVC=Phenotipe Variability Coefficient, GVC= Genotipe Variability Coefficient, SDRG=standar deviate genetic variability, h^2_{bs}= heritability in a broad sense

The ~~estimates of~~ estimated genetic parameters ~~are~~ were shown in Table 11. Root length ~~characters~~ had a narrow diversity of genotypes ~~but had~~ with a broad coefficient of ~~the diversity of genotypes, respectively,~~ 5.37 and 14.71 percent. Shoot length had a broad ~~genotype~~ genetic diversity that was 30.74 percent but had a narrow coefficient of ~~genotype diversity by~~ 13.61 ~~percent. Root dry weights both had a broad of the coefficient of genotypic diversity and coefficient of phenotype diversity~~ percent (Table 2). The estimated heritability values ~~for dry weight~~ of root and shoot ~~length~~ dry weight were 0.05 and 0.8, respectively (Table 2). ~~Heritability value of root length, shoot length, and shoot dry weight~~ The estimate for their lengths were ~~classified as~~ considerably high. Characters that ~~have~~ had high heritability values ~~indicate~~ indicated that these genetic factors ~~are~~ were more dominant than ~~the environment so that the selection of these characters can be~~ others, therefore, their selections were made in the first ~~generations~~ generation (Akinwale et al., 2011; Herawati et al., 2019).

Correlation and Relative Root Length (RRL)

~~Correlation analysis of all~~ Positive correlations were observed ~~characters showed a positive~~for all characters, except for shoot length and RSR, ~~while shoot dry weight and RSR were negatively~~ which showed negative (Table 3). ~~Characters~~ Features that ~~have significantly different~~ had significant differences and positive ~~correlations can be~~ relationships were used as selection criteria. Root ~~length,~~ and shoot length, and the shoot dry weight ~~can be~~ were selected as one of the ~~criteria for~~ requirements of Al tolerance for DH1 line. These characters had high genetic ~~diversity and~~ diversity, heritability ~~values~~ values, and ~~have~~ were positively correlated with other ~~characters~~features.

Table 3. Correlation of root ~~length,~~ and shoot length, ~~root dry weight, shoot~~ their dry ~~weight~~weights, and ~~root shoot~~ the Root Shoot weight ~~ratio~~ Ratio (RSR) under Al stress condition

Characters	Shoot length	Root dry weight	Shoot dry weight	Root shoot weight ratio (RSR)
Root length	0.42**	0.28**	0.53**	0.12*
Shoot length		0.25*	0.65**	-0.25*
Root dry weight			0.43**	0.11^{ns}
Shoot dry weight				-0.14*

*= significant at ~~level~~ level 005; **= very significant at level 001, ns=no significant

Among these characters, root length was more easily ~~and quickly~~ observed, ~~so~~ therefore, the researchers used relative root length (RRL) to distinguish tolerant and Al-susceptible genotypes. Previous research indicated that the main target of Al ~~toxicity~~ toxicity was the root tissue of the plant. Root damage ~~occurs in sensitive genotypes due to Al toxicity,~~ was characterized by ~~a decrease in~~ decreased protein content in the cytoplasm and increased membrane damage to cell walls, which ~~results in cell membrane~~ resulted to leakage (Zhu et al., 2018). Qian et al. (2018) reported ~~that~~ that the fresh and dry weights of the rice seedlings were ~~significantly positively correlated~~ in significant correlation with chlorophyll content. This result ~~indicates~~ indicated that a low Al concentration ~~increases~~ increased the seedlings' fresh and dry weights ~~of rice seedlings~~ by increasing the leaf chlorophyll content and promoting photosynthesis.

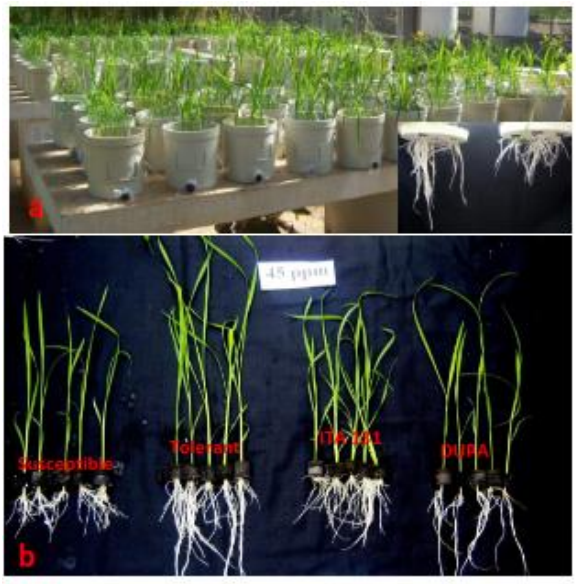

Figure 2. The experiment of Al stress on Yoshida nutrient solution ~~(a);~~ showed the ~~appearance of~~ root lengths of ~~susceptible line, tolerant lines,~~ ITA 131 (susceptible check), and DUPA (tolerant check) under 45 ~~ppm Al (b)~~ppm.

Root shortening is one of the consequences of Al ~~inhibition of root length. The morphology of secondary roots~~ inhibition, therefore, its structure appeared to be shorter, fat, and reduced branching, while its adventitious roots grew ~~more on~~ the ~~root neck~~ more (Figure 2a). The penetration of roots ~~have hardy penetrating the~~ into hard soil ~~layer so that the absorption of~~ layers also inhibit nutrients and water ~~will be inhibited~~absorption. The ~~level of Al~~ toxicity level depends on the ~~activity~~ concentration of Al^{+3} ions in the soil ~~media~~solution. ~~The root activity of the seedlings at the concentrations also significantly decreased.~~ Al ~~decreases~~ decreased the fresh weight by inhibiting the absorption of water and mineral substances (Qian et al., 2018).

~~RRL~~ The Relative Root Length (RRL) values ~~in the~~ for DH1 lines ~~tested~~ varied between 0.53-1.03 (Table 4). The RRL value of the Dupa (tolerant check) was 0.74, while ITA131 (susceptible check) was 0.53 (Figure 2b). The 5% LSD test ~~results~~ showed no significant difference between the PAR values for ~~the rather~~ more tolerant genotypes and ~~the PAR values~~ for susceptible checks (Table 4)~~. It is consistent~~. This test corresponded with the previous experiments carried out by Prasetiyono (2003), Bakhtiar et al. (2007) that Dupa ~~was tolerant~~ had tolerance at RRL value of 0.7, however, for ~~ITA131 (~~ITA131, it was 0.53~~)~~, which was ~~an~~ found to increase from the previous ~~experiment~~ test of 0.41 (Bakhtiar et al., 2007). For this reason, it ~~is~~ was necessary to review using ITA varieties as susceptible checks (Figure 2b). The 5% LSD test on DH1-lines resulted in 8 lines having significantly different ~~and~~ higher RRL values than the Dupa check varieties (PAR = 0.74), ~~namely lines~~ such as line P6-274, P6-314, P3-196, P6-273, P6- 311, P6-250, P6-267, and P6-278 (Table 4).

Table 4. Root lengths in the treatments of 0 ~~Al~~ and 45 ppm Al ~~and the relative value of~~ with the ~~root length~~ Relative Root Length (RRL) value of ~~DH1~~ DH1-lines at 14 days after planting

Lines	Al_0	Al_{45}[1]	RRL	Criteria[2]	Lines	Al_0	Al_{45}	RRL	Criteria
	(cm)					(cm)			
P6-274	16.2	16.7	1.03*	HT	P6-319	20.4	16.0	0.78	T
P6-314	20.3	20.3	1.01*	HT	P6-275	20.3	15.6	0.78	T
P3-196	17.1	16.8	0.98*	HT	P6-297	25.1	19.3	0.77	T
P6-273	19.9	19.5	0.97*	HT	P3-210	20.6	15.8	0.76	T
P6-311	15.3	14.9	0.96*	HT	P3-161	20.2	15.8	0.76	T
P3-250	16.8	15.9	0.95*	HT	P3-135	23.1	17.2	0.76	T
P6-267	10.6	10.1	0.95*	HT	P3-175	21.8	16.6	0.76	T
P6-278	19.4	18.3	0.94*	HT	P3-221	23.8	18.1	0.76	T
P6-286	23.4	21.6	0.93	HT	P3-190	20.2	15.3	0.75	T
P6-266	12.5	11.7	0.93	HT	P6-320	19.9	15.2	0.75	T
P3-191	21.5	19.6	0.90	HT	P3-162	20.9	15.4	0.74	T
P6-264	14.0	12.6	0.90	HT	P1-108	20.2	15.0	0.74	T
P3-238	17.9	15.1	0.88	HT	P6-317	16.3	12.2	0.73	T
P3-204	17.2	15.1	0.88	HT	P3-131	21.3	15.2	0.72	T
P6-291	14.9	13.1	0.87	HT	P3-248	18.7	13.5	0.72	T
P6-265	12.4	10.9	0.87	HT	P6-103	20.6	14.7	0.70	RT
P6-261	17.1	14.8	0.87	HT	P3-160	24.2	16.8	0.70	RT
P6-257	20.6	17.8	0.86	HT	P3-31	22.4	13.8	0.63	RT

Lines	Al_0	Al_{45}[1]	RRL	Criteria[2]	Lines	Al_0	Al_{45}	RRL	Criteria
	(cm)					(cm)			
P6-255	21.0	17.9	0.85	HT	P3-26	23.7	14.6	0.61	RT
P6-276	20.1	16.9	0.85	T	P4-45	22.1	13.3	0.60	RT
P6-271	21.7	17.8	0.84	T	P5-50	22.1	12.9	0.59	RT
P3-148	20.9	17.3	0.83	T	P2-1	18.5	11.1	0.59	RT
P3-120	23.2	19.6	0.83	T	P3-27	25.7	14.0	0.54*	RT
P6-272	20.5	16.6	0.83	T	P2-2	18.5	10.1	0.54*	RT
P6-62	20.6	16.8	0.83	T	P3-28	23.9	12.7	0.53*	RT
P6-105	16.6	13.7	0.83	T	Dupa	24.7	18.2	0.74	T
P6-295	21.8	17.8	0.83	T	ITA131	21.1	11.3	0.53	RT
P3-159	24.5	19.9	0.81	T	SGJT-28			0.89	HT
P3-134	19.3	15.6	0.80	T	SGJT-36			0.86	HT
P3-150	21.9	17.6	0.80	T	W.Rarem			0.52	RT
P6-302	20.3	15.5	0.79	T	Fatmawati			0.76	T
P3-158	24.1	19.2	0.79	T	BNT 0.05			0.2	
P3-249	20.6	16.3	0.78	T	KK (%)			15.69	

*Significantly different from Dupa based on LSD 0.05 test; [1]Al_0= 0 $AlCl_3$, Al_{45}= 45 ppm $AlCl_3$; [2]HT = Highly tolerant, T=tolerant, AT=Rather tolerant

In tolerance genotypes, Al ~~is~~ was prevented from passing through the plasma membrane and entering the symplast and sites that ~~are~~ were sensitive ~~to Al~~ in the cytoplasm ~~of the~~ root tip. The ability of the root cell wall to absorb low Al and the permeability of ~~the cell~~ its membrane ~~is thought to be~~ were involved in the mechanism of external tolerance. Zhu et al. (2018) explained that Hydrogen sulfide (H2S) ~~plays~~ played an essential role ~~in~~ on Al stress resistance in plants. ~~HS reduces~~ H2S lowered Al toxicity by reducing ~~the Al~~ its content in the apoplast and symplast rice root. Wang et al. (2017) ~~revealed~~ showed that the activity of cytosolic glucose-6-phosphate dehydrogenase ~~is~~ was also involved in resistance to Al ~~through mediating~~ with the intervention of ROS levels in soybean. ~~Result~~ The result by Qian et al. (2018) indicated that H2O2 accumulation ~~is~~ was also a key factor contributing to the ~~decrease in~~ decreased root activity.

In Al tolerance, ~~plants will be able to raise the~~ plant pH ~~around~~ was raised at the root ~~area~~ tip (Kochian et al., 2004; Ma, 2007). ~~Increasing pH around the roots occurs~~ This was due to the influx of H^+ ~~at the root tip. It~~ around this area, which resulted in the deposition of Al and a decreasing Al^{3+} ion activity ~~so that it becomes a less toxic form to plants~~ (Samac and Tasfaye, 2003; Zhao et al., 2014). ~~Plants avoid from Al toxicity trough absorb~~ High NO^{3-} content in ~~large amounts.~~ plants tend to reduce Al toxicity. It also caused the release of hydroxyl ~~ions~~ (OH^-) or bicarbonate ions (HCO^{3-}) into the rhizosphere, increased pH, and suppressed the solubility of ~~Al~~ Al (Justino et al., 2006; Zhao et al., 2018).

Table 5. The results of the ~~DH1 lines selection~~ DH1-line selections for a new type of upland rice under Al stress

Criteria	Genotype	Number of lines
Highly	P6: 274, 314, 273, 311, 267, 278, 286, 266, 264, 291,	19

tolerant	265, 261, 257, 255, dan P3: 196, 191, 238, 204, 250	
Tolerant	P6: 276, 271, 272, 62, 105, 295, 302, 319, 275, 297, 320, 108, 317, dan P3: 148, 120, 159, 134, 150, 158, 249, 210, 161, 135, 175, 221, 190, 162, 131, 248	29
Rather tolerant	P2: 1, 2; P3:160, 31, 26, 27, 28; P4-45, P5-50, P6-103	10

The RRL values of ~~the genotype~~ P3-27, P2-2, P3-28 were lower than the tolerant checks, and classified as ~~moderately~~ the moderate tolerant genotypes (~~low) by~~ 0.53-0.54), which was almost the same as the ~~RRL values of the~~ ITA ~~as~~ susceptible checks ~~by 0.53~~ (0.53) (Table 4). The grouping was based on the RRL values in 58 ~~DH1 lines~~ DH1-lines, tested on nutrient cultures at 0 and 45 ppm Al, ~~that is~~ and produced susceptible = PAR ~~<~~<< 0.5, ~~rather tolerant~~ with low tolerance = 0.5 ~~<~~<< PAR ~~<~~<< 0.70, ~~tolerant~~ moderate = 0.70 ~~<~~<< PAR ~~<~~<< 0.85, and ~~highly tolerant~~ high = ~~PAR~~PAR > 0.85, ~~so 19 genotypes were highly tolerant~~therefore, 19 high, 29 ~~tolerant genotypes~~moderate, and 10 ~~genotypes rather~~ low tolerant genotype were produced (Table 5).

Distribution of Population from Cross of P3 (Fatmawati x SGJT-36) and P6 (SGJT-36 x Fatmawati)

Aluminum tolerance was based on the ~~relative root length~~ Relative Root Length (RRL) and ~~root shoot~~ the Root Shoot weight ~~ratio~~ Ratio (RSR) in DH1 ~~populations from the~~ populations. The crossing of P3 (Fatmawati x SGJT-36) and P6 (SGJT-36 x Fatmawati) ~~and~~ with the two parents ~~are~~ were presented in Table 6. The ~~relative root lengths~~ Relative Root Lengths (RRL) in the P3 population ~~ranges~~ ranged from 0.53 - 0.98, while the P6 population ~~ranges~~ ranged from 0.70 - 1.03. The Fatmawati elders had an RRL value of 0.77, while ~~the~~ that of SGJT-36 ~~elders were~~ was 0.87~~.~~. There ~~was diversity~~ were diversities in all observed characters~~.~~ ~~The root shoot weight ratio (RSR)~~ , with the RSR of the P3 population that ranged from 0.20 to 0.32, while ~~the~~ that of P6 ~~population ranged~~ graded from 0.22 to 0.39. The Fatmawati elders had ~~an~~ RSR ~~value~~ values of 0.30, while ~~the~~ those of SGJT-36 ~~elders had an RRL value of~~ were 0.32 (Table 6).

Table 6. The Relative ~~root length~~ Root Length (RRL) and ~~root shoot~~ the Root Shoot weight Ratio (RSR) ~~ratio~~ of ~~DH1~~ DH1-lines in populations of crossing P3 (Fatmawati x SGJT-36) and P6 (SGJT-36 x Fatmawati)

Characters	X ± SD DH1*	Range of DH1 population		Mean value of parent **	
		P3	P6***	Fatmawati	SGJT-36
Relative Root Length	0.8 ± 0.11	0.53 – 0.98	0.70 – 1.03	0.77	0.87
Root shoot weight ratio (RSR)	0.29 ± 0.04	0.20 – 0.32	0.22 – 0.39	0.30	0.32

*X ± SD DH1 is mean ± standard deviate, **Fatmawati and SGJT-36 5 plants each,*** P3 were 26 lines, and P6 were 27 lines

RRL and RSR values observed in DH1 populations varied ~~greatly, some of which were similar to their parents, intermediates, and exceed both of their parents~~significantly. The frequency distribution of P3 and P6 populations based on RRL values ~~is~~ were presented in Table 7. Based on aluminum tolerance criteria, the frequency distribution of the two elders did not

overlap. Fatmawati had ~~tolerant criteria~~moderate, while SGJT-36 had ~~highly tolerant~~high tolerance. The frequency distribution of DH1 populations of P3 derivatives ~~was highly tolerant~~had extreme, ~~tolerant~~moderate, and ~~rather tolerant~~low tolerance, while ~~the frequency distribution~~ those of P6 ~~populations was highly tolerant to tolerant~~ had high and moderate tolerance only (SGJT-36 elders~~) (Table 7).~~

~~).~~ Table 7. Distribution of ~~DH1~~ DH1-lines in each population of crossing P3 ~~(Fatmawati x SGJT-36)~~ and P6 (SGJT-36 x Fatmawati) based on aluminum tolerance.

Criteria	Parent*		DH1**	
	Fatmawati	SGJT-36	P3	P6
~~Highly~~ High tolerant	0	√	5	14
~~Tolerant~~Moderate tolerant	√	0	16	12
~~Rather~~ Low tolerant	0	0	5	1
Susceptible	0	0	0	0

*The Fatmawati elders and SGJT-36 each with five plants, ** P3 were 26 lines, and P6 were 27 lines, √ Al tolerance criteria on elders

~~It was due to the presence of~~ The frequent transgressive segregation in the ~~combination~~ anther of ~~an anther, which~~ a plant produced lines with different tolerance levels. ~~Many~~ Few genes were observed to control Al ~~tolerance~~ acceptance levels in rice, therefore, ~~so~~ not all genotypes ~~will have~~ possessed this gene. Zang et al. (2019) ~~were~~ found that there were significant differences between the gene expression patterns of Indica ~~Al-tolerant~~ and Japonica Al-tolerant varieties. Therefore, the gene ~~expression patterns of the Al-tolerant varieties~~ arrangement in the ~~mixed subgroup, which was inclined to Japonica~~subgroups, were similar to ~~the Al-tolerant varieties~~ those in ~~Japonica~~Japonica species. Each ~~gene~~ gene, or their combination ~~will have~~ played a role in regulating the mechanism of ~~Al~~ Al-tolerance in ~~rice that will be~~ rice, and expressed in each phase of plant growth (Wu et al. 2000). ~~Thus~~ Thus, the ~~elders~~ aged species used in this study produced lines that were tolerant to aluminum stress. ~~The next step will be an~~ Therefore, further research was needed for the evaluation of ~~the~~ leaf blast disease in ~~the~~ greenhouse.

CONCLUSION

The results of the evaluation of Al tolerance based on RRL in nutrient culture produced ~~19 genotypes~~ 19, 29, and 10 genotypic tolerance that were ~~highly tolerant~~high, ~~29 genotypes tolerant~~moderate, and ~~ten genotypes rather tolerant~~low, respectively. The tolerance level of Al in the ~~DH1~~ DH1-lines of upland rice produced by anther culture varied ~~greatly~~significantly. ~~Root length, shoot length,~~ The root and shoot length with the shoot dry weight had a high coefficient of ~~diversity~~ diversity, heritability, and ~~heritability and~~ significantly correlated with each other. The distribution of DH1 populations of P3 derivatives produced ~~highly tolerant~~high, ~~tolerant~~moderate, and ~~rather tolerant~~ low tolerance criteria, while ~~the population~~ those of P6 ~~derivatives produced highly tolerant to tolerant criteria.~~yielded high and moderate only.

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Assessment of Aluminum Tolerant of Double Haploid Lines for Developing New Type of Upland Rice

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ABSTRACT

Aluminum can possibly have direct or indirect adverse effects on plant growth; however, this effect is not the same for all plants, even in the same species. The roots of plants are most sensitive to Al toxicity accompanied to initial symptoms such as the inhibition of cell extension and retarded development of root systems. This study was aimed to evaluate doubled-haploid (DH1) upland rice derived from anther culture to Al stress and to study the genetic diversity and population distribution of DH lines due to Al stress. Al tolerant test was carried out in a greenhouse using factorial Randomized Complete Block Design (RCBD) with three replicates. Yoshida nutrient solution containing Al of 0 and 45 ppm was the first factor, while the second was the lines obtained from previous experiments (DH1), the four parents (SGJT36, SGJT28, Fatmawati, and Way Rarem), while Dupa, and ITA131, respectively as an Al tolerant and susceptible checks.. The results showed that root length, shoot length, and shoot dry weight had high heritability values and correlated well with the observed characters. Al tolerant doubled haploid upland rice lines derived from anther culture varied widely. Based on the Relative Root Length (RRL), out of 58 lines tested, 19 genotypes were highly tolerant, 29 lines were moderate, while 10 were low. The DH1 rice derived from P3 showed high, moderate, and low tolerance, while those from P6 showed high and moderate tolerance only.

Keywords: Aluminum (Al) tolerance, Doubled Haploid (DH), Upland rice lines.

INTRODUCTION

The transition of land into residential areas, the construction of social facilities and infrastructure has led to a reduction in the field of agricultural land. It also resulted in the shifting of agricultural land to a marginal (dry land) area, especially on ultisol soils that reacted sourly to plant cultivation as a result of some symptoms such as lack of Ca, Mg, P, K, and N as well as the presence of Al toxicity. The high content of Al in acidic soil has shown to inhibit plant growth (Silva et al., 2010; Brunner and Sperisen, 2013). The utilization of acidic land is faced with various obstacles, such as low pH, which reduces the availability of nutrients for plant growth. On the other hand, Al toxicity increases in very acidic soil (pH <4.5), with increasing Al

solubility, which has detrimental effects on plants. Not only is the growth of rice roots inhibited, but also damaged by high concentrations of Al in the soil, which leads to significant reductions in rice yields (Ismail et al., 2007; Liu et al., 2012). The impact of Al is not the same on all plants, even in the same species.

The initial symptoms of Al toxicity in plants are inhibition of cell extension and the retarded development of root systems. Its availability in land solution depends on the level of soil acidity. In very acidic conditions (pH <4.5), Al becomes very soluble, especially in the form of Al3+ ion, which is highly toxic to plants. It also interferes with the uptake, transport, and the utilization of nutrients, and also inhibits enzyme activity and hormonal balance (Lupwayi et al., 2014; Wan et al., 2019; Yamamoto, 2019). The high content of Al solution in the soil causes stunted root growth and decreases the ability of roots to absorb mineral and water nutrients (Silva et al., 2012; Ma et al., 2014; Kochian et al., 2015). The inhibition of root growth by Al occurs, due to cell division and elongation in the root meristem.

The accumulation of Al in root tissue determines the tolerance rate of plant genotypes, which correlate with the level of root damage. In tolerant genotype, the Al aggregation root was lower than the sensitive genotype (Ma 2000; Zang et al., 2019). The small number of negative charges on the cell wall in tolerant genotype reduces the interaction of Al with the root layer (Watanabe and Okada 2005; Kochian et al. 2015). This phenomenon has also been reported in previous studies (Nursyamsi 2000; Awasthi et al., 2017; Qian et al., 2018) that rice tolerance has a mechanism of reducing the interaction of Al on the root cell walls.

Currently, many rice varieties have not tolerated acidic soils, and some are still being tested. High genetic diversity is one of the main factors used in improving plant traits, both by conventional and biotechnological methods. The previous study of genetic diversity on DH1 had produced 58 double haploid upland rice lines that were ready to be further evaluated (Herawati et al. 2009). Therefore, the proper selection needs to be done to obtain genotypes that tolerate aluminum stress. The differences in root growth character are one indicator that can be used in the tolerance selection, since roots are the main target of damage by Al. In upland rice, a quick method for evaluating genotypes that tolerate Al stress can be done by observing the root length in the vegetative phase (Bakhtiar et al., 2007; Belachew et al., 2017; Awasthi et al., 2017; Qian et al., 2018). This study aims to examine DH1 of upland rice derived from anther culture, and also study genetic diversity, as well as the population distribution due to aluminum stress.

MATERIALS AND METHODS

The experiment was carried out in the greenhouse of the Indonesian Center for Research and Development on Biotechnology and Agricultural Genetic Resources, Cimanggu, Bogor. The materials used were 58 DH1 rice lines, the four elders (SGJT36, SGJT28, Fatmawati, and Way Rarem), Dupa, and ITA131 susceptible check (Prasetyono, 2003; Bakhtiar et al., 2007).

Experiments using factorial Randomized Complete Block Design (RCBD) were repeated three times, with the Yoshida nutrient solution (Yoshida et al. 1976). A solution of aluminum at the concentrations of 0 and 45 ppm were given as the first factor, while the second was 64 rice line varieties.

The rice seeds were roasted for 3 x 24 hours at 45 °C and sown on husk media. They were allowed to germinate in the dark for five days. After which those that were healthy and uniform with a height of ± 5 cm were selected for planting. The nutrient used was Yoshida

solution with the final composition as follows: 40 ppm N, ten ppm P, 40 ppm K, 40 ppm Ca, 40 ppm Mg, 0.5 ppm Mn, 0.05 ppm Mo, 0.2 ppm B, 0.01 ppm Zn, 0.01 ppm Cu, and two ppm Fe (Yoshida et al. 1976). In the Al treatment to reduce the formation of the polymer, the pH of the nutrient solution was adjusted to 4.5 by using 0.1 N NaHCO3. After this, 2 ml of Al solution made from 1000 ml of AlCl3.5H2O was added to get a treatment concentration of 45 ppm. The pH of the nutrient solution was adjusted to 4.0 ± 0.1 with 0.1 N NaHCO3 or 0.1 N HCl.

Five-day-old healthy sprouts from a uniform root were transferred to the media. Sprout stems were then wrapped in soft foam and placed on a nutrient solution in styrofoam holes. Each pothole was planted with five sprouts and maintained for 14 days in a greenhouse. A growth period of 14 days was used due to the composition of the Yoshida nutrient solution (Yoshida et al. 1976). During this phase, water addition and pH adjustment were carried out with 0.1 N NaHCO3 or 0.1 N HCl every two days. Observations were made on plants aged 14 days after planting, by measuring root length, plant height, root and shoot dry weight. The formula used to estimate the Shoot Root weight Ratio (SRR) was as follows:

$$SRR = \frac{root\ dry\ weight}{shoot\ dry\ weight}$$

The formula used to measure the variable Relative Root Length (RRL) was as follows:

$$RRL = \frac{root\ length\ under\ Al\ stress}{root\ length\ without\ Al}$$

Data analysis was performed using the Least Significant Difference Test (LSD). Tolerance of rice lines to Al stress were grouped into a susceptible= RRL<0.5, rather tolerance= 0.5<RRL<0.70, tolerance=0.70<RRL<0.85, and highly tolerance=RRL>0.85. Analysis of variance and the correlation between variables were performed using Pearson analysis and SAS software version 9.1. Genetic parameters were calculated based on the Singh and Chaudhary (1979) method as follows:

Source of variance	df	Means Square	expectation value
Genotipe	(g-1)	M2	$\sigma_e^2 + 3\sigma_g^2$
Error	(r-1)(g-1)	M1	σ_e^2

σ_e^2 = enviroment variance; σ_g^2 = genetic variance

$$\sigma_g^2 = \frac{M2 - M1}{r}\sigma_e^2 = M1\sigma_p^2 = \sigma_g^2 + \sigma_e^2$$

The standard deviation formula for genetic variance:

$$\sigma_{\sigma_g^2} = \sqrt{\left(\frac{2}{r}\right)[(\frac{M2_g^2}{df_g} + 2) + (\frac{M1_e^2}{df_e} + 2)]}$$

M2 = Means squared genotype
M1 = Means squared error

r = replication
dfg = degree of freedom genotype
dfe = degree of freedom error

Genetic diversity could be estimated from the genetic variance (σ2g) and the standard deviation of genetic variance (σσ2g). A character had a broad genetic diversity when σ2g > 2σσ2g. The Coefficient Genotype Diversity (CGD) was estimated using the formula as follows:

$$CGD = \frac{\sqrt{\sigma_g^2}}{\bar{x}} \text{ x } 100\% \bar{\text{x}} = \text{average population observed}$$

When 0 < CGD ≤ 10.94 (narrow); 0 < CGD ≤ 21.88 (narrower); 0 < CGD ≤ 32.83 (broader); 0 < CGD ≤ 43.77 (broad); 43.77 < CDG (broadest).

The Coefficient Phenotype Diversity (CPD) was estimated using the formula as follows:

$$CPD = \frac{\sqrt{\sigma_p^2}}{\bar{x}} x100\%$$

When 0 < CPD ≤ 24.94 (narrow); 0 < CPD ≤49.71 (narrower); 0 < CPD ≤ 74.71 (broader); 0 < CPD ≤ 99.65 (broad); 99.65 < CPD (broadest).

Heritability in a broad sense (h^2_{bs}) was calculated according to the formula:

$$h_{bs}^2 = \frac{\sigma_g^2}{\sigma_p^2}$$

The heritability values (h2bs) were grouped according to Stanfield (1983) as follows:
0.50 < h2bs < 1.00 = high; 0.20 < h2bs < 0.50 = moderate; h2bs < 0.20 = low.

Genotypic correlations were calculated using the formula:

$$r_{g(xiji)} = \frac{cov.g(xixj)}{\sqrt{\left(\sigma_{g(xi)}^2 . \sigma_{g(xj)}^2\right)}}$$

cov.g(xixj) = genotypic variation between properties i and j
$\sigma_{g(xi)}^2$ = genetic variability i
$\sigma_{g(xj)}^2$= genetic variability j

RESULTS AND DISCUSSION

Analysis of genetic diversity

Analysis of variance of DH1 lines of rice with Al stress in nutrient culture showed significant differences in all observed variables (Table 1). Al stress reduced root length by 21.95 percent and shoots dry weight by 22.14 percent, while it decreased shoot length and root dry weight by only 6 percent (Figure 1).

Table 1. Analysis of variance of DH1 lines of new type upland rice under Al stress in nutrient solution

Variable	Sum Square	Mean Square	F value
Root length	1159.4	20.3	4.80**
Shoot length	0.35	0.006	2.92**
Root dry weight	0.089	0.0016	1.10*
Shoot dry weight	0.11	0.002	4.46**
Shoot root weight ratio (SRR)	0.35	0.0062	2.92**

*Significant different at level 0.05; ** Significant different at level 0.01

Figure 1. Effect of Al stress on variables of the length and dry weight of the root and shoot of DH1 lines.

The decrease in root length was caused by the obstruction of primary and lateral roots elongation. The field and laboratory experiments showed mixed responses to Al toxicity in rice (Watanabe and Okada, 2005; Bakhtiar et al., 2007; Qian et al., 2018). Reduction in shoot dry weight was due to the unavailable nutrients for suboptimal growth, as a result of the impaired mineral absorption and transport in roots (Kochian et al. 2015; Qian et al., 2018). The decrease in root dry weight was only 5.55 percent, compared to the dry shoot weight (22.14 percent) (Figure 1). Since the root length decreased and became shorter, therefore the adventitious roots grew the more. These showed that under Al conditions, more carbohydrates were directed to root growth.

Bakhtiar et al. (2007) and Belachew et al. (2017) also observed that shoot dry weight was more sensitive to Al toxicity than root dry weight. The inhibition of shoot growth was a secondary effect due to nutrient deficiency, especially Mg, Ca, P, and the restriction of water absorption, which caused dwarf rice growth (Ma et al., 2014). Wang et al. (2015) demonstrated that the application of NH4 decreased the Al content in rice roots by reducing the pectin content in their roots. Freitas et al. (2019) showed that aluminum chloride was more important in producing Al toxicity in the upland rice plants, grown in the nutrient solution.

Table 2. Genetic diversity of root and shoot length, root and shoot dry weight, and root shoot weight ratio under Al stress conditions

Variable	Mean	GV*	PV	2xSD GV	GVC	PVC	h^2bs
Root length	15.75	5.37	9.61	5.43	14.71	19.68	0.56
Shoot length	42.14	30.74	38.41	21.41	13.61	14.70	0.80
Root dry weight	0.037	0.00007	0.0015	3.25	22.12	100.0	0.05
Shoot dry weight	0.114	0.00053	0.0009	3.25	20.19	26.75	0.57
Shoot root weight ratio (SRR)	0.29	0.0014	0.0035	3.25	12.92	20.40	0.40

*GV =Genotipe Variability, PV=PhenotipeVariability, PVC=Phenotipe Variability Coefficient, GVC= Genotipe Variability Coefficient, SDGV=standar deviate genetic variability, h^2_{bs}= heritability in a broad sense

The estimated genetic parameters were shown in Table 11. Root length had a narrow diversity of genotypes with a broad coefficient of 5.37 and 14.71 percent. Shoot length had a broad genetic diversity that was 30.74 percent but had a narrow coefficient of 13.61 percent. Root dry weights both had a broad of the coefficient of genotypic diversity and coefficient of phenotype diversity (Table 2). The estimated heritability values of root and shoot dry weight were 0.05 and 0.8, respectively (Table 2). The estimate for root length, shoot length, and shoot dry weight were considerably high. Characters that had high heritability values indicated that these genetic factors were more dominant than the environment; therefore, their selections were made in the first generation (Akinwale et al., 2011; Herawati et al., 2019).

Correlation and Relative Root Length (RRL)

Positive correlations were observed for all characters, except for shoot length and SRR, which showed negative (Table 3). Features that had significant differences and positive relationships were used as selection criteria. Root length, shoot length, and the shoot dry weight were selected as one of the requirements of Al tolerance for DH1 line. These characters had high genetic diversity, heritability values, and were positively correlated with other features.

Table 3. Correlation of root length, shoot length, root dry weight,shoot dry weight, and shoot root weight ratio (SRR) under Al stress condition

Characters	Shoot length	Root dry weight	Shoot dry weight	Shoot root weight ratio (SRR)
Root length	0.42**	0.28**	0.53**	0.12*
Shoot length		0.25*	0.65**	-0.25*
Root dry weight			0.43**	0.11^{ns}

Shoot dry weight				-0.14*

*= significant at level 005; **= very significant at level 001, ns=no significant

Among these characters, root length was more easily observed; therefore, the researchers used relative root length (RRL) to distinguish tolerant and Al-susceptible genotypes. Previous research indicated that the main target of Al toxicity was the root tissue of the plant. Root damage was characterized by decreased protein content in the cytoplasm and increased membrane damage to cell walls, which resulted in leakage (Zhu et al., 2018). Qian et al. (2018) reported that the fresh and dry weights of the rice seedlings were in significant correlation with chlorophyll content. This result indicated that a low Al concentration increased the seedlings' fresh and dry weights by increasing the leaf chlorophyll content and promoting photosynthesis.

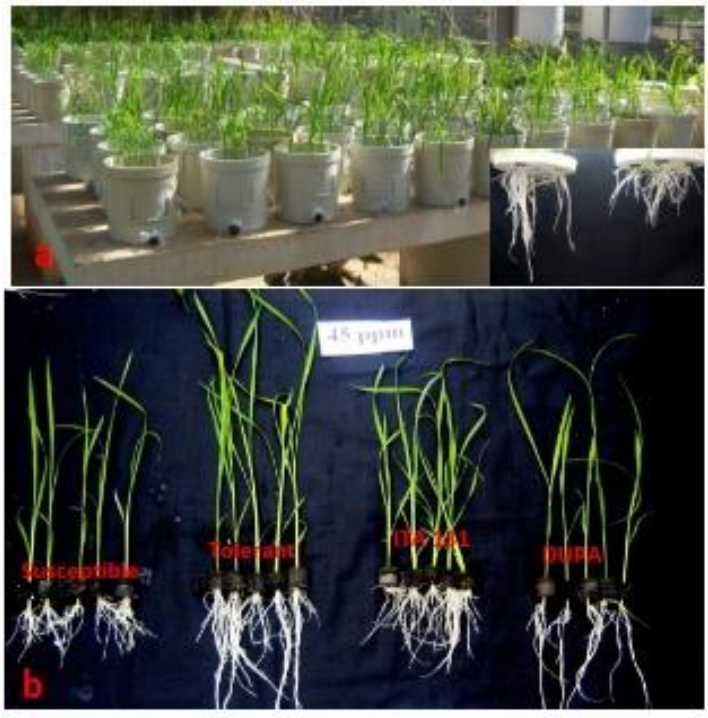

Figure 2. The experiment of Al stress on Yoshida nutrient solution (a); the root lengths of ITA 131 (susceptible check), and DUPA (tolerant check) under 45 ppm (b)

Root shortening is one of the consequences of Al inhibition; therefore, its structure appeared to be shorter, fat, and reduced branching, while its adventitious roots grew the more (Figure 2a). The roots have hardy penetrating the soil layer also inhibit nutrients and water absorption. The toxicity level depends on the concentration of Al^{+3} ions in the soil solution. Al decreased the fresh weight by inhibiting the absorption of water and mineral substances (Qian et al., 2018).

The Relative Root Length (RRL) values for DH1 lines varied between 0.53-1.03 (Table 4). The RRL value of the Dupa (tolerant check) was 0.74, while ITA131 (susceptible check) was 0.53 (Figure 2b). The 5% LSD test showed no significant difference between the RRL values for more tolerant genotypes and for susceptible checks (Table 4). This test corresponded with the previous experiments carried out by Prasetiyono (2003), Bakhtiar et al. (2007) that Dupa had tolerance at RRL value of 0.7, however, for ITA131, it was 0.53, which was found to increase

from the previous test of 0.41 (Bakhtiar et al., 2007). For this reason, it was necessary to review using ITA varieties as susceptible checks (Figure 2b). The 5% LSD test on DH1-lines resulted in 8 lines having significantly different higher RRL values than the Dupa check varieties (RRL = 0.74), such as line P6-274, P6-314, P3-196, P6-273, P6- 311, P6-250, P6-267, and P6-278 (Table 4).

Table 4. Root lengths in the treatments of 0 and 45 ppm Al with the Relative Root Length (RRL) value of DH1-lines at 14 days after planting

Lines	Al_0	Al_{45}[1]	RRL	Criteria[2]	Lines	Al_0	Al_{45}	RRL	Criteria
	(cm)					(cm)			
P6-274	16.2	16.7	1.03*	HT	P6-319	20.4	16.0	0.78	T
P6-314	20.3	20.3	1.01*	HT	P6-275	20.3	15.6	0.78	T
P3-196	17.1	16.8	0.98*	HT	P6-297	25.1	19.3	0.77	T
P6-273	19.9	19.5	0.97*	HT	P3-210	20.6	15.8	0.76	T
P6-311	15.3	14.9	0.96*	HT	P3-161	20.2	15.8	0.76	T
P3-250	16.8	15.9	0.95*	HT	P3-135	23.1	17.2	0.76	T
P6-267	10.6	10.1	0.95*	HT	P3-175	21.8	16.6	0.76	T
P6-278	19.4	18.3	0.94*	HT	P3-221	23.8	18.1	0.76	T
P6-286	23.4	21.6	0.93	HT	P3-190	20.2	15.3	0.75	T
P6-266	12.5	11.7	0.93	HT	P6-320	19.9	15.2	0.75	T
P3-191	21.5	19.6	0.90	HT	P3-162	20.9	15.4	0.74	T
P6-264	14.0	12.6	0.90	HT	P1-108	20.2	15.0	0.74	T
P3-238	17.9	15.1	0.88	HT	P6-317	16.3	12.2	0.73	T
P3-204	17.2	15.1	0.88	HT	P3-131	21.3	15.2	0.72	T
P6-291	14.9	13.1	0.87	HT	P3-248	18.7	13.5	0.72	T
P6-265	12.4	10.9	0.87	HT	P6-103	20.6	14.7	0.70	RT
P6-261	17.1	14.8	0.87	HT	P3-160	24.2	16.8	0.70	RT
P6-257	20.6	17.8	0.86	HT	P3-31	22.4	13.8	0.63	RT
P6-255	21.0	17.9	0.85	HT	P3-26	23.7	14.6	0.61	RT
P6-276	20.1	16.9	0.85	T	P4-45	22.1	13.3	0.60	RT
P6-271	21.7	17.8	0.84	T	P5-50	22.1	12.9	0.59	RT
P3-148	20.9	17.3	0.83	T	P2-1	18.5	11.1	0.59	RT
P3-120	23.2	19.6	0.83	T	P3-27	25.7	14.0	0.54*	RT
P6-272	20.5	16.6	0.83	T	P2-2	18.5	10.1	0.54*	RT
P6-62	20.6	16.8	0.83	T	P3-28	23.9	12.7	0.53*	RT
P6-105	16.6	13.7	0.83	T	Dupa	24.7	18.2	0.74	T
P6-295	21.8	17.8	0.83	T	ITA131	21.1	11.3	0.53	RT
P3-159	24.5	19.9	0.81	T	SGJT-28			0.89	HT
P3-134	19.3	15.6	0.80	T	SGJT-36			0.86	HT
P3-150	21.9	17.6	0.80	T	W.Rarem			0.52	RT
P6-302	20.3	15.5	0.79	T	Fatmawati			0.76	T

Lines	Al_0	Al_{45}[1]	RRL	Criteria[2]	Lines	Al_0	Al_{45}	RRL	Criteria
	(cm)					(cm)			
P3-158	24.1	19.2	0.79	T	BNT 0.05			0.2	
P3-249	20.6	16.3	0.78	T	KK (%)			15.69	

*Significantly different from Dupa based on LSD 0.05 test; [1]Al_0= 0 $AlCl_3$, Al_{45}= 45 ppm $AlCl_3$;
[2]HT = Highly tolerant, T=tolerant, RT=Rather tolerant

In tolerance genotypes, Al was prevented from passing through the plasma membrane and entering the symplast and sites that were sensitive in the cytoplasm root tip. The ability of the root cell wall to absorb low Al and the permeability of its membrane were involved in the mechanism of external tolerance. Zhu et al. (2018) explained that Hydrogen sulfide (H_2S) played an essential role in Al stress resistance in plants. H_2S lowered Al toxicity by reducing its content in the apoplast and symplast rice root. Wang et al. (2017) showed that the activity of cytosolic glucose-6-phosphate dehydrogenase was also involved in resistance to Al with the intervention of ROS levels in soybean. The result by Qian et al. (2018) indicated that H_2O_2 accumulation was also a key factor contributing to the decreased root activity.

In Al tolerance, plant pH was raised at the root tip (Kochian et al., 2004; Ma, 2007). This was due to the influx of H+ around this area, which resulted in the deposition of Al and a decreasing Al3+ ion activity (Samac and Tasfaye, 2003; Zhao et al., 2014). High NO3- content in plants tend to reduce Al toxicity. It also caused the release of hydroxyl (OH-) or bicarbonate ions (HCO^{3-}) into the rhizosphere, increased pH, and suppressed the solubility of Al (Justino et al., 2006; Zhao et al., 2018).

Table 5. The results of the DH1 lines selection for a new type of upland rice under Al stress

Criteria	Genotype	Number of lines
Highly tolerant	P6: 274, 314, 273, 311, 267, 278, 286, 266, 264, 291, 265, 261, 257, 255, dan P3: 196, 191, 238, 204, 250	19
Tolerant	P6: 276, 271, 272, 62, 105, 295, 302, 319, 275, 297, 320, 108, 317, dan P3: 148, 120, 159, 134, 150, 158, 249, 210, 161, 135, 175, 221, 190, 162, 131, 248	29
Rather tolerant	P2: 1, 2; P3:160, 31, 26, 27, 28; P4-45, P5-50, P6-103	10

The RRL values of P3-27, P2-2, P3-28 were lower than the tolerant checks, and classified as the moderate tolerant genotypes (0.53-0.54), which was almost the same as the ITA susceptible checks (0.53) (Table 4). The grouping was based on the RRL values in 58 DH1-lines, tested on nutrient cultures at 0 and 45 ppm Al, and produced susceptible = RRL <0.5, rather tolerant = 0.5 <RRL <0.70, tolerant = 0.70 <RRL <0.85, and highly tolerant = RRL> 0.85, therefore, 19 highly, 29 tolerant, and 10 rather tolerant genotype were produced (Table 5).

Distribution of Population from Cross of P3 (Fatmawati x SGJT-36) and P6 (SGJT-36 x Fatmawati)

Aluminum tolerance was based on the Relative Root Length (RRL) and the Root Shoot weight Ratio (SRR) in DH1 populations from the crossing of P3 (Fatmawati x SGJT-36) and P6 (SGJT-36 x Fatmawati) with the two parents were presented in Table 6. The Relative Root

Lengths (RRL) in the P3 population ranged from 0.53 - 0.98, while the P6 population ranged from 0.70 - 1.03. The Fatmawati elders had an RRL value of 0.77, while that of SGJT-36 was 0.87. There were diversities in all observed characters, with the SRR of the P3 population that ranged from 0.20 to 0.32, while that of P6 graded from 0.22 to 0.39. The Fatmawati elders had SRR values of 0.30, while those of SGJT-36 was 0.32 (Table 6).

Table 6. The Relative Root Length (RRL) and the Root Shoot weight Ratio (RSR) of DH1-lines in populations of crossing P3 (Fatmawati x SGJT-36) and P6 (SGJT-36 x Fatmawati)

Characters	X ± SD DH1*	Range of DH1 population		Mean value of parent **	
		P3	P6***	Fatmawati	SGJT-36
Relative Root Length (RRL)	0.8 ± 0.11	0.53 – 0.98	0.70 – 1.03	0.77	0.87
Shoot Root weight ratio (SRR)	0.29 ± 0.04	0.20 – 0.32	0.22 – 0.39	0.30	0.32

*X ± SD DH1 is mean ± standard deviate, **Fatmawati and SGJT-36 5 plants each,***P3 were 26 lines, and P6 were 27 lines

RRL and RSR values observed in DH1 populations varied greatly, some of which were similar to their parents, intermediates, and exceed both of their parents. The frequency distribution of P3 and P6 populations based on RRL values is presented in Table 7. Based on aluminum tolerance criteria, the frequency distribution of the two elders did not overlap. Fatmawati had tolerant criteria, while SGJT-36 had highly tolerant. The frequency distribution of DH1 populations of P3 derivatives was highly tolerant, tolerant, and rather tolerant, while the frequency distribution of P6 populations was highly tolerant to tolerant (SGJT-36 elders) (Table 7).

Table 7. Distribution of DH1 lines in each population of crossing P3 (Fatmawati x SGJT-36) and P6 (SGJT-36 x Fatmawati) based on aluminum tolerance

Criteria	Parent*		DH1**	
	Fatmawati	SGJT-36	P3	P6
Highly tolerant	0	√	5	14
Tolerant	√	0	16	12
Rather tolerant	0	0	5	1
Susceptible	0	0	0	0

*The Fatmawati elders and SGJT-36 each with five plants, ** P3 were 26 lines, and P6 were 27 lines, √ Al tolerance criteria on elders

The frequent transgressive segregation in the anther of a plant produced lines with different tolerance levels. Few genes were observed to control Al acceptance levels in rice; therefore, not all genotypes possessed this gene. Zang et al. (2019) found that there were significant differences between the gene expression patterns of Indica and Japonica Al-tolerant varieties. Therefore, the gene arrangement in the subgroups was similar to those in Japonica species. Each gene, or their combination, played a role in regulating the mechanism of Al-tolerance in rice and expressed in each phase of plant growth (Wu et al. 2000). Thus, the parent used in this study produced lines that were tolerant to aluminum stress. Therefore, further

research was needed for the evaluation of leaf blast disease in the greenhouse to obtain the superior upland rice line.

CONCLUSION

The results of the evaluation of Al tolerance based on RRL in nutrient culture produced 19, 29, and 10 genotypic that was highly tolerance, tolerance, and rather tolerance, respectively. The tolerance level of Al in the DH1-lines of upland rice produced by anther culture varied significantly. The root length, shoot length, and the shoot dry weight had a high coefficient of diversity, heritability, and significantly correlated with each other. The distribution of DH1 populations of P3 derivatives produced highly tolerant, tolerant, and rather tolerant criteria, while those of P6 derivatives produced highly tolerant to tolerant only.

Acknowledgment

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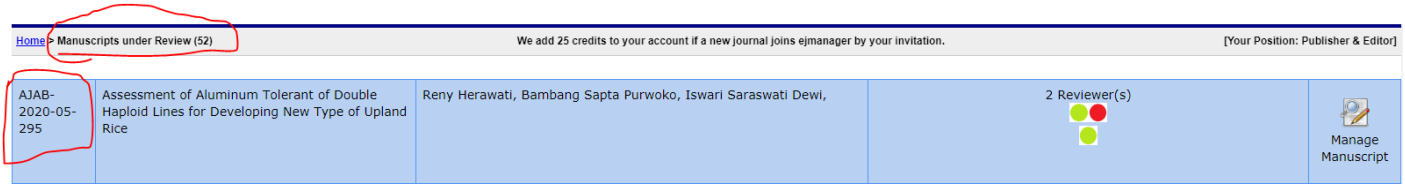

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Assessment of Aluminum Tolerant of Double Haploid Lines for Developing New Type of Upland Rice

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ABSTRACT

Aluminum has a can possibly have direct direct or indirect adverse effect on plant growth. The , however, this effect of Al stress is not the same for all plants, even in the same species. The roots of plants are most sensitive to Al toxicity. The toxicity accompanied to initial symptoms of Al toxicity in plants are such as the inhibition of cell extension and the retarded development of root systems. . This study aimed aims to evaluate doubled haploid (DH1) upland rice lines derived from anther culture to aluminum stress culture, and studying also examine the genetic diversity and population the distribution of doubled haploid lines due to aluminum stress. Al tolerant testing test was carried out in a greenhouse using factorial randomized complete block design Randomized Complete Block Design (RCBD) with three replicates. Yoshida nutrient solution containing Al of 0 and 45 ppm was the first factor. The , while the second factor was the lines obtained from previous experiments (DH1), the four parents (SGJT36, SGJT28, Fatmawati, and Way Rarem), Dupa, and ITA131, respectively as Al tolerant and the ITA131 susceptible checks. The results showed that the shoot and root length, shoot length, and shoot with their dry weight values had high heritability values coefficient of diversity, heritability, and significantly correlated well with the observed characterseach other. The tolerance level of Al tolerant doubled haploid in DH1- lines of upland rice lines derived from anther produced by another culture varied widelysignificantly. Based on relative root length the Relative Root Length (RRL), out of 58 lines tested, 19 genotypes were highly tolerant, 29 lines tolerantwere moderate, and ten moderate tolerantwhile 10 were low. The DH1 rice derived from P3 showed highly toleranthigh, tolerantmoderate, and moderate tolerantlow tolerance, while those from P6 showed highly tolerant high and tolerant. moderate tolerance only.

Keywords: Aluminum tolerance, Doubled haploid, Upland rice

INTRODUCTION

The transition of land ~~functions~~ into residential areas, the construction of social facilities and infrastructure has led to a reduction in the field of ~~land for agriculture~~agricultural land. It also resulted in the ~~expansion~~ shifting of agricultural land ~~directed~~ to ~~areas of~~ a marginal ~~land~~ (dry land~~)~~) area, especially on ultisol soils that reacted ~~sourly. It was often found~~ sourly to plant cultivation as a result of some symptoms ~~of~~ such as lack of Ca, Mg, P, K, and N as well as the presence of Al toxicity. ~~Al content~~ The high content of Al in ~~acid soils~~ acidic soil has ~~been~~ shown to inhibit plant growth (Silva et al., 2010; Brunner and Sperisen, 2013). The utilization of acidic land is faced with various obstacles, ~~including~~ such as low ~~pH,~~ pH which ~~will reduce~~ reduces the availability of nutrients for plant growth. On the other hand, Al toxicity ~~is increasing. In~~ increases in very ~~acid soils~~ acidic soil (pH <4.5), with increasing Al ~~solubility can increase Al saturation. Aluminum~~ solubility, which has detrimental effects on ~~plant, not~~ plants. Not only is the growth of rice roots inhibited, but ~~rice root systems can~~ also ~~be~~ damaged by high concentrations of Al in the soil, which ~~can both~~ lead to significant reductions in rice yields (Ismail et al., 2007; Liu et al., 2012). The impact of Al ~~stress~~ is not the same on all plants, even in the same species~~. The root is the part of the plant, which is most sensitive to Al toxicity.~~ .

The initial symptoms of Al toxicity in plants are inhibition of cell extension and the retarded development of root systems. ~~The~~ Its availability ~~of Al~~ in ~~soil~~ land solution depends on the level of soil acidity. In very acidic ~~soil reaction~~ conditions (pH <4.5), Al becomes very soluble, especially in the form of Al^{3+} ion, which is ~~toxicity~~ highly toxic to plants. ~~Aluminum~~ It also interferes with the uptake, transport, and the utilization of nutrients, and also inhibits enzyme activity and hormonal balance (Lupwayi et al., 2014; Wan et al., 2019; Yamamoto, 2019). The ~~presence of~~ high content of Al ~~solubility~~ solution in the soil causes stunted root growth and ~~ultimately~~ decreases the ability of roots to absorb mineral and water nutrients (Silva et al., 2012; Ma et al., 2014; Kochian et al., 2015). ~~Inhibition~~ The inhibition of root growth by Al ~~stress occurs~~ occurs, due to cell division and elongation in the root meristem.

~~Al~~ The accumulation of Al in root tissue ~~will determine~~ determines the ~~level~~ tolerance rate of plant ~~genotypes and correlated~~ genotypes, which correlate with the level of root damage. ~~In genotypes tolerant~~In tolerant genotype, the Al ~~accumulation in the~~ aggregation root ~~tissue~~ was ~~generally~~ lower than the sensitive genotype (Ma 2000; Zang et al., 2019). The small number of negative charges on the cell wall ~~in genotype~~ in tolerant ~~caused~~ genotype reduces the interaction of Al with the ~~lower interaction.~~ root layer (Watanabe and Okada 2005; Kochian et al. 2015). This phenomenon has also been reported ~~by some~~ in previous ~~researchers~~ studies (Nursyamsi 2000; Awasthi et al., 2017; Qian et al., 2018) that ~~tolerant~~ rice ~~had~~ tolerance has a mechanism ~~by~~ of reducing the interaction of Al on the root cell walls.

~~Until now~~Currently, ~~not~~ many rice varieties have not tolerated ~~acid~~ acidic soils, and some ~~lines~~ are still ~~in the testing stage.~~ being tested. High genetic diversity is one of the main factors used in improving plant traits, both ~~conventionally~~ by conventional and ~~biotechnology methods.~~ biotechnological method. Previous study of genetic diversity ~~studied~~ on DH1 had produced 58 double haploid upland rice lines that ~~are~~ were ready to be further evaluated (Herawati et al. 2009). Therefore, ~~the~~ proper selection ~~of available genotypes~~ needs to be done to obtain genotypes that ~~are tolerant to~~ tolerate aluminum stress~~. Identification of~~ . The differences in root growth character is one indicator that can be used in the tolerance ~~selection of Al stress because~~ selection, since roots are the main target of damage by Al. In upland rice, a quick method for evaluating genotypes that tolerate Al stress can be done by observing the root length in the vegetative phase (Bakhtiar et al., 2007; Belachew et al., 2017; Awasthi et al., 2017; Qian et al., 2018). This study ~~aimed~~ aims to ~~evaluate the~~ examine DH1 of upland rice ~~from~~ derived ~~anther~~

from another culture, and also study genetic diversity, ~~and~~ as well as the population distribution due to aluminum stress.

MATERIALS AND METHODS

The experiment was carried out in the greenhouse of the Indonesian Center for Research and Development on Biotechnology and Agricultural Genetic Resources, Cimanggu, Bogor. The materials used were 58 DH1 rice lines, the four elders (SGJT36, SGJT28, Fatmawati, and Way Rarem), ~~and two varieties checked~~Dupa, ~~namely Dupa~~ and ITA131 ~~respectively as tolerant and sensitive Al~~ susceptible check (Prasetyono, 2003; Bakhtiar et al., 2007)~~. The nutrient solution used was Yoshida nutrient solution (Yoshida et al., 1976)~~.

Experiments using factorial ~~randomized complete block design~~ Randomized Complete Block Design (RCBD) were repeated three times~~. Experiments using~~, with the Yoshida nutrient solution (Yoshida et al. 1976~~) were given a~~). A solution of ~~Al as much as~~ aluminum at the concentrations of 0 ~~ppm~~ and 45 ppm were given as the first factor, while the second ~~factor~~ was 64 rice ~~lines/~~line varieties.

The rice seeds were roasted for 3 x 24 hours at 45 ° ~~C,~~ C and ~~then the seeds were~~ sown ~~in a nursery~~ on husk media. ~~Seed germination~~ They were allowed to germinate in the dark for five days. ~~Rice seeds~~ After which those that were ~~healthy, uniform,~~ healthy and ~~have~~ uniform with a height of ± 5 cm were selected for planting. The nutrient ~~solution~~ used was Yoshida ~~method~~ solution with the final composition as follows: 40 ppm N, ~~ten~~ 10 ppm P, 40 ppm K, 40 ppm Ca, 40 ppm Mg, 0.5 ppm Mn, 0.05 ppm Mo, 0.2 ppm B, 0.01 ppm Zn, 0.01 ppm ~~Cu~~ Cu, and ~~two~~ 2 ppm Fe (Yoshida et al. 1976). In the Al ~~treatment,~~ treatment to reduce the formation of ~~Al~~ polymer, the pH of the nutrient solution was adjusted to 4.5 by using 0.1 N ~~NaHCO3 to pH 4.5 before the addition of Al~~NaHCO3. ~~The addition of Al by adding 0 and~~ After this, 2 ml of Al ~~stock~~ solution ~~that had been~~ made ~~for 1000 ml~~ from 1000ml of ~~Al (source~~ AlCl3.~~5H2O)~~ 5H2O was added to get ~~the~~ a treatment concentration of 45 ~~ppm Al~~ppm. The pH of the nutrient solution was adjusted to ~~pH~~ 4.0 ± 0.1 with 0.1 N NaHCO3 or 0.1 N HCl.

Five-day-old healthy sprouts ~~on~~ from a uniform root ~~length~~ were transferred to the media. Sprout stems were then wrapped in soft foam and ~~then put into styrofoam holes that had been prepared and floated~~ placed on a nutrient solution in ~~a pot~~styrofoam holes. Each ~~pot~~ pothole was planted with five sprouts and maintained for 14 days in a greenhouse. A growth period of 14 days was used ~~because~~ due to the composition of ~~the~~ Yoshida nutrient solution ~~was designed for 14 days~~ (Yoshida et al. 1976). During this ~~period~~phase, ~~the~~ water addition ~~of water~~ and pH adjustment ~~was~~ were carried out with 0.1 N NaHCO3 or 0.1 N HCl every two days. Observations were made on plants aged 14 days after ~~planting~~ planting, by measuring root length, plant height, root ~~dry weight,~~ and shoot dry weight. The formula ~~estimated shoot root~~ used to estimate the Shoot Root weight ~~ratio~~ Ratio (SRR~~)~~) was as follows:

$$SRR = \frac{root\ dry\ weight}{shoot\ dry\ weight} SRR = \frac{root\ dry\ weight}{shoot\ dry\ weight}$$

The formula ~~measures~~ used to measure the variable ~~relative root length~~ Relative Root Length (RRL~~)~~) was as follows:

$$RRL = \frac{root\ length\ under\ Al\ stress}{root\ length\ without\ Al} RRL = \frac{root\ length\ under\ Al\ stress}{root\ length\ without\ Al}$$

Data analysis was performed using the Least Significant Difference Test (LSD). Tolerance of rice lines to Al stress ~~was~~ were grouped into a susceptible = RRL <0.5, ~~rather tolerant~~ low = 0.5 <RRL <0.70, ~~tolerant~~ moderate = 0.70 <RRL <0.85, and ~~highly tolerant~~ high tolerance = RRL> 0.85. Analysis of variance and the correlation between variables were performed using Pearson analysis ~~of~~ and SAS software version 9.1. Genetic parameters were calculated based on the ~~method used by~~ Singh and Chaudhary (1979) method as follows:

Source of variance	df	Means Square	expectation value
~~Genotipe~~Genotype	(g-1)	M2	$\sigma_e^2 \sigma_e^2 + 3\sigma_g^2 \sigma_g^2$
Error	(r-1)(g-1)	M1	$\sigma_e^2 \sigma_e^2$

$\sigma_e^2 \sigma_e^2$ = enviroment variance; $\sigma_g^2 \sigma_g^2$ = genetic variance

$$\sigma_g^2 = \frac{M2-M1}{r} \sigma_g^2 = \frac{M2-M1}{r} \qquad \sigma_e^2 = M1 \sigma_e^2 = M1 \qquad \sigma_p^2 = \sigma_g^2 + \sigma_e^2 \sigma_p^2 = \sigma_g^2 + \sigma_e^2$$

The standard deviation ~~of~~ formula for genetic ~~variance using the formula~~variance:

$$\sigma_{\sigma_g^2} = \sqrt{\left(\frac{2}{r}\right)[(\frac{M2_g^2}{df_g} + 2) + (\frac{M1_e^2}{df_e} + 2)]} \quad \sigma_{\sigma_g^2} = \sqrt{\left(\frac{2}{r}\right)[(\frac{M2_g^2}{df_g} + 2) + (\frac{M1_e^2}{df_e} + 2)]}$$

M2 = Means squared genotype
M1 = Means squared error
r = replication
dfg = degree of freedom genotype
dfe = degree of freedom error

Genetic diversity could be estimated from the genetic variance (σ2g) and the standard deviation of genetic variance (σσ2g). A character ~~has~~ had a broad genetic diversity ~~if σ2g>~~when σ2g > 2σσ^2_g. The ~~estimates of~~ Coefficient Genotype Diversity (CGD) was estimated using the ~~formula~~formula as follows:

$$CGD = \frac{\sqrt{\sigma_g^2}}{\bar{x}} \text{x } 100\% CGD = \frac{\sqrt{\sigma_g^2}}{\bar{x}} \text{x } 100\%$$

x̄ = average population observedx̄ = average population observed

~~if~~ When 0 ~~<~~< CGD ~~=~~< 10.94 (narrow); 0 ~~<CGD=~~< CGD < 21.88 (~~rather narrow~~narrower); 0 < CGD ~~=~~< 32.83 (~~rather broad~~broader); 0 < ~~CGD=~~CGD < 43.77 (broad); 43.77 ~~<~~< CDG (~~very broad~~broadest).

The ~~formula estimated of coefficient phenotype diversity~~ Coefficient Phenotype Diversity (CPD) was estimated using the formula as follows:

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$$CPD = \frac{\sqrt{\sigma_p^2}}{\bar{x}} x100\% CPD = \frac{\sqrt{\sigma_p^2}}{\bar{x}} x100\%$$

~~if~~ When 0 < CPD ~~<~~≤ 24.94 (narrow); 0 < CPD ≤49.71 (~~rather narrow~~narrower); 0 < CPD ~~<~~≤ 74.71 (~~rather broad~~broader); 0 < CPD ~~<~~≤ 99.65 (broad); 99.65 < CPD (~~very broad~~broadest).

Heritability in a broad sense (h^2_{bs}) was calculated according to the formula:

$$h_{bs}^2 = \frac{\sigma_g^2}{\sigma_p^2} h_{bs}^2 = \frac{\sigma_g^2}{\sigma_p^2}$$

~~Heritability~~ The heritability values (h^2_{bs}) ~~are~~ were grouped according to Stanfield (1983) as follows:

0.50 < h^2_{bs} ~~<~~≤ 1.00 = ~~height~~high; 0.20 < h^2_{bs} ~~<~~≤ 0.50 = moderate; h^2_{bs} ~~<~~≤ 0.20 = low.

Genotypic correlations ~~can be~~ were calculated using the formula:

$$r_{g(xiji)} = \frac{cov.g(xixj)}{\sqrt{\left(\sigma^2_{g(xi)} . \sigma^2_{g(xj)}\right)}} r_{g(xiji)} = \frac{cov.g(xixj)}{\sqrt{\left(\sigma^2_{g(xi)} . \sigma^2_{g(xj)}\right)}}$$

cov.g(xixj) = genotypic variation between properties i and j

$\sigma^2_{g(xi)}\sigma^2_{g(xi)}$ = genetic variability i

$\sigma^2_{g(xj)}\sigma^2_{g(xj)}$= genetic variability j

RESULTS AND DISCUSSION

Analysis ~~of~~ of genetic diversity

Analysis of variance of DH1 lines ~~in~~ of rice with Al stress ~~on~~ in nutrient culture showed significant differences in all observed variables (Table 1). ~~The response of each variable was different from~~ Al ~~stress. Al~~ stress reduced root length by 21.95 percent and shoots dry weight by 22.14 percent, while it decreased shoot length and root dry weight by only 6 percent (Figure 1).

Table 1. Analysis of variance of DH1 lines of new type upland rice under Al stress in nutrient solution

Variable	~~Sum~~ Sum Square	Mean Square	F value
Root length	1159.4	20.3	4.80**
Shoot length	0.35	0.006	2.92**
Root dry weight	0.089	0.0016	1.10*
Shoot dry weight	0.11	0.002	4.46**
Root shoot weight Ratio (RSR)	0.35	0.0062	2.92**

*Significant different at level 0.05; ** Significant different at level 0.01

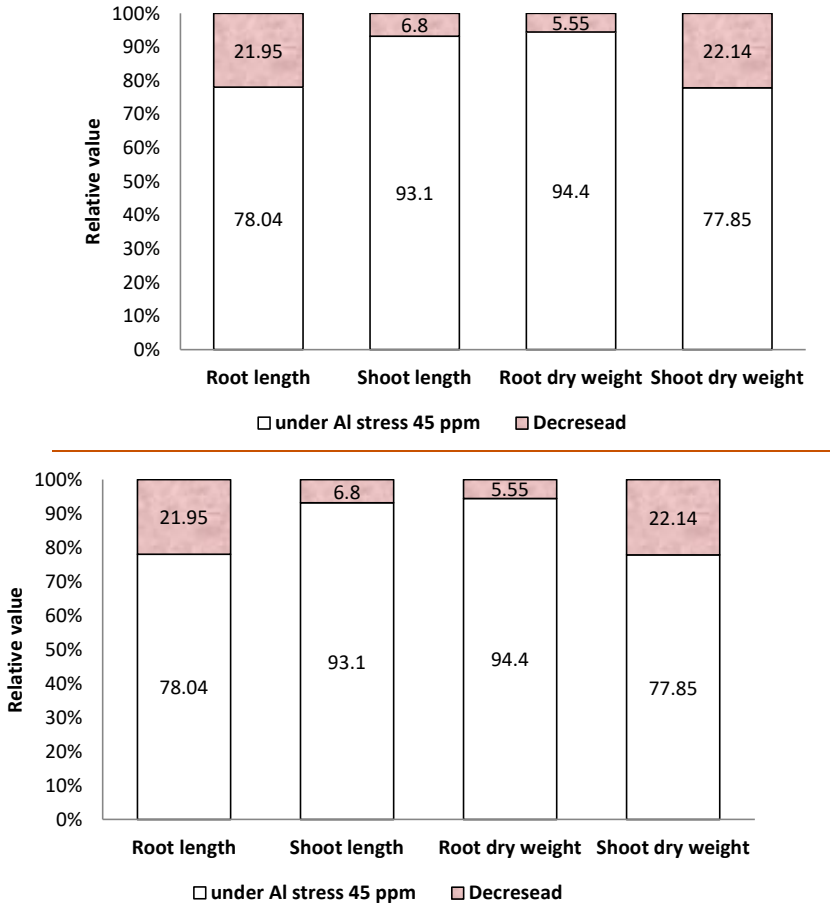

Figure 1. Effect of Al stress on variables of the length and dry weight of ~~root, shoot length, the~~ root ~~dry weight, and~~ shoot ~~dry weight~~ of DH1 lines.

~~Decreasing~~ The decrease in root length ~~is~~ was caused by ~~obstruction of the elongation~~ obstruction of ~~the~~ primary and lateral ~~roots~~roots elongation. ~~Field~~ The field and laboratory experiments showed ~~that there were~~ the mixed responses to Al toxicity in rice (Watanabe and Okada 2005; Bakhtiar et al., 2007; Qian et al., 2018). Reduction in shoot dry weight was due to the unavailable nutrients ~~available~~ for suboptimal ~~growth because~~ growth, as a result of the impaired ~~nutrient~~ mineral absorption and transport in roots (Kochian et al. 2015; Qian et al., 2018). The ~~decreased~~ decrease in root dry weight was only 5.55 percent, ~~not as much as in~~ compared to the dry shoot weight (22.14 percent) (Figure 1~~) because although~~). Since the root length ~~decreased, the roots were~~ decreased and became shorter, ~~and~~ therefore the adventitious roots grew the more. ~~It~~ These showed that under ~~stress~~ Al conditions, more carbohydrates were directed to root growth. Bakhtiar et al. (2007) and Belachew et al. (2017~~)~~). It was also ~~found~~ observed that shoot dry weight was more sensitive to Al toxicity than root dry weight~~. Inhibition~~

. The inhibition of shoot growth ~~is~~ was a secondary effect due to nutrient deficiency, especially Mg, Ca, ~~and~~ P, and the ~~inhibition~~ restriction of water absorption ~~causes~~ which caused dwarf rice growth (Ma et al., 2014). Wang et al. (2015) demonstrated that the application of NH4 decreased the Al content in rice roots by reducing the pectin content in ~~rice~~ their roots. Freitas et al. (2019) ~~reveal~~ showed that aluminum chloride was more ~~useful~~ important in producing ~~aluminum~~ Al toxicity in the upland rice ~~plants~~ plants, grown in the nutrient solution.

Table 2. Genetic diversity of root ~~length,~~ and shoot length, root ~~dry weight,~~ and shoot dry weight, and root shoot weight ratio under Al stress ~~conditions Al~~conditions

Variable	Mean	GV*	PV	2xSD GV	GVC	PVC	h^2bs
Root length	15.75	5.37	9.61	5.43	14.71	19.68	0.56
Shoot length	42.14	30.74	38.41	21.41	13.61	14.70	0.80
Root dry weight	0.037	0.00007	0.0015	3.25	22.12	100.0	0.05
Shoot dry weight	0.114	0.00053	0.0009	3.25	20.19	26.75	0.57
Root shoot weight Ratio (RSR)	0.29	0.0014	0.0035	3.25	12.92	20.40	0.40

*GV =Genotipe Variability~~, ~~, PV=PhenotipeVariability, PVC=Phenotipe Variability Coefficient, GVC= Genotipe Variability Coefficient, SDRG=standar deviate genetic variability, h^2_{bs}= heritability in a broad sense

The ~~estimates of~~ estimated genetic parameters ~~are~~ were shown in Table 11. Root length ~~characters~~ had a narrow diversity of genotypes ~~but had~~ with a broad coefficient of ~~the diversity of genotypes, respectively,~~ 5.37 and 14.71 percent. Shoot length had a broad ~~genotype~~ genetic diversity that was 30.74 percent but had a narrow coefficient of ~~genotype diversity by~~ 13.61 ~~percent. Root dry weights both had a broad of the coefficient of genotypic diversity and coefficient of phenotype diversity~~ percent (Table 2). The estimated heritability values ~~for dry weight~~ of root and shoot ~~length~~ dry weight were 0.05 and 0.8, respectively (Table 2). ~~Heritability value of root length, shoot length, and shoot dry weight~~ The estimate for their lengths were ~~classified as~~ considerably high. Characters that ~~have~~ had high heritability values ~~indicate~~ indicated that these genetic factors ~~are~~ were more dominant than ~~the environment so that the selection of these characters can be~~ others, therefore, their selections were made in the first ~~generations~~ generation (Akinwale et al., 2011; Herawati et al., 2019).

Correlation and Relative Root Length (RRL)

~~Correlation analysis of all~~ Positive correlations were observed ~~characters showed a positive~~for all characters, except for shoot length and RSR, ~~while shoot dry weight and RSR were negatively~~ which showed negative (Table 3). ~~Characters~~ Features that ~~have significantly different~~ had significant differences and positive ~~correlations can be~~ relationships were used as selection criteria. Root ~~length,~~ and shoot length, and the shoot dry weight ~~can be~~ were selected as one of the ~~criteria for~~ requirements of Al tolerance for DH1 line. These characters had high genetic ~~diversity and~~ diversity, heritability ~~values~~ values, and ~~have~~ were positively correlated with other ~~characters~~features.

Table 3. Correlation of root ~~length,~~ and shoot length, ~~root dry weight, shoot~~ their dry ~~weight~~weights, and ~~root shoot~~ the Root Shoot weight ~~ratio~~ Ratio (RSR) under Al stress condition

Characters	Shoot length	Root dry weight	Shoot dry weight	Root shoot weight ratio (RSR)
Root length	0.42**	0.28**	0.53**	0.12*
Shoot length		0.25*	0.65**	-0.25*
Root dry weight			0.43**	0.11^{ns}
Shoot dry weight				-0.14*

*= significant at ~~level~~ level 005; **= very significant at level 001, ns=no significant

Among these characters, root length was more easily ~~and quickly~~ observed, ~~so~~ therefore, the researchers used relative root length (RRL) to distinguish tolerant and Al-susceptible genotypes. Previous research indicated that the main target of Al ~~toxicity~~ toxicity was the root tissue of the plant. Root damage ~~occurs in sensitive genotypes due to Al toxicity,~~ was characterized by ~~a decrease in~~ decreased protein content in the cytoplasm and increased membrane damage to cell walls, which ~~results in cell membrane~~ resulted to leakage (Zhu et al., 2018). Qian et al. (2018) reported ~~that~~ that the fresh and dry weights of the rice seedlings were ~~significantly positively correlated~~ in significant correlation with chlorophyll content. This result ~~indicates~~ indicated that a low Al concentration ~~increases~~ increased the seedlings' fresh and dry weights ~~of rice seedlings~~ by increasing the leaf chlorophyll content and promoting photosynthesis.

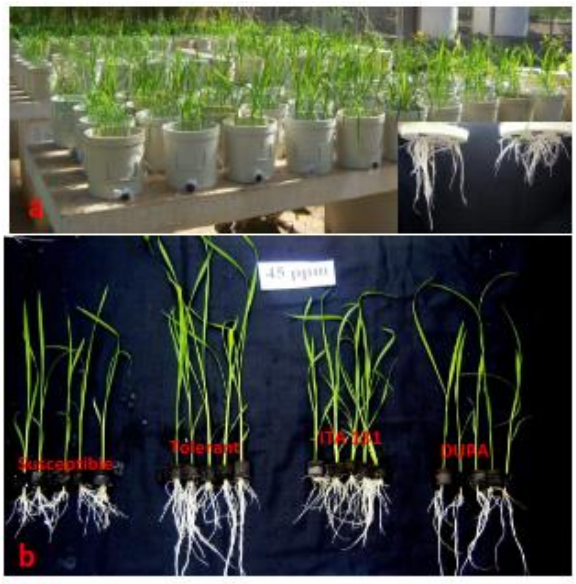

Figure 2. The experiment of Al stress on Yoshida nutrient solution ~~(a);~~ showed the ~~appearance of~~ root lengths of ~~susceptible line, tolerant lines,~~ ITA 131 (susceptible check), and DUPA (tolerant check) under 45 ~~ppm Al (b)~~ppm.

Root shortening is one of the consequences of Al ~~inhibition of root length. The morphology of secondary roots~~ inhibition, therefore, its structure appeared to be shorter, fat, and reduced branching, while its adventitious roots grew ~~more on~~ the ~~root neck~~ more (Figure 2a). The penetration of roots ~~have hardy penetrating the~~ into hard soil ~~layer so that the absorption of~~ layers also inhibit nutrients and water ~~will be inhibited~~absorption. The ~~level of Al~~ toxicity level depends on the ~~activity~~ concentration of Al^{+3} ions in the soil ~~media~~solution. ~~The root activity of the seedlings at the concentrations also significantly decreased.~~ Al ~~decreases~~ decreased the fresh weight by inhibiting the absorption of water and mineral substances (Qian et al., 2018).

~~RRL~~ The Relative Root Length (RRL) values ~~in the~~ for DH1 lines ~~tested~~ varied between 0.53-1.03 (Table 4). The RRL value of the Dupa (tolerant check) was 0.74, while ITA131 (susceptible check) was 0.53 (Figure 2b). The 5% LSD test ~~results~~ showed no significant difference between the PAR values for ~~the rather~~ more tolerant genotypes and ~~the PAR values~~ for susceptible checks (Table 4)~~. It is consistent~~. This test corresponded with the previous experiments carried out by Prasetiyono (2003), Bakhtiar et al. (2007) that Dupa ~~was tolerant~~ had tolerance at RRL value of 0.7, however, for ~~ITA131 (~~ITA131, it was 0.53~~)~~, which was ~~an~~ found to increase from the previous ~~experiment~~ test of 0.41 (Bakhtiar et al., 2007). For this reason, it ~~is~~ was necessary to review using ITA varieties as susceptible checks (Figure 2b). The 5% LSD test on DH1-lines resulted in 8 lines having significantly different ~~and~~ higher RRL values than the Dupa check varieties (PAR = 0.74), ~~namely lines~~ such as line P6-274, P6-314, P3-196, P6-273, P6- 311, P6-250, P6-267, and P6-278 (Table 4).

Table 4. Root lengths in the treatments of 0 ~~Al~~ and 45 ppm Al ~~and the relative value of~~ with the ~~root length~~ Relative Root Length (RRL) value of ~~DH1~~ DH1-lines at 14 days after planting

Lines	Al_0 (cm)	Al_{45}[1] (cm)	RRL	Criteria[2]	Lines	Al_0 (cm)	Al_{45} (cm)	RRL	Criteria
P6-274	16.2	16.7	1.03*	HT	P6-319	20.4	16.0	0.78	T
P6-314	20.3	20.3	1.01*	HT	P6-275	20.3	15.6	0.78	T
P3-196	17.1	16.8	0.98*	HT	P6-297	25.1	19.3	0.77	T
P6-273	19.9	19.5	0.97*	HT	P3-210	20.6	15.8	0.76	T
P6-311	15.3	14.9	0.96*	HT	P3-161	20.2	15.8	0.76	T
P3-250	16.8	15.9	0.95*	HT	P3-135	23.1	17.2	0.76	T
P6-267	10.6	10.1	0.95*	HT	P3-175	21.8	16.6	0.76	T
P6-278	19.4	18.3	0.94*	HT	P3-221	23.8	18.1	0.76	T
P6-286	23.4	21.6	0.93	HT	P3-190	20.2	15.3	0.75	T
P6-266	12.5	11.7	0.93	HT	P6-320	19.9	15.2	0.75	T
P3-191	21.5	19.6	0.90	HT	P3-162	20.9	15.4	0.74	T
P6-264	14.0	12.6	0.90	HT	P1-108	20.2	15.0	0.74	T
P3-238	17.9	15.1	0.88	HT	P6-317	16.3	12.2	0.73	T
P3-204	17.2	15.1	0.88	HT	P3-131	21.3	15.2	0.72	T
P6-291	14.9	13.1	0.87	HT	P3-248	18.7	13.5	0.72	T
P6-265	12.4	10.9	0.87	HT	P6-103	20.6	14.7	0.70	RT
P6-261	17.1	14.8	0.87	HT	P3-160	24.2	16.8	0.70	RT
P6-257	20.6	17.8	0.86	HT	P3-31	22.4	13.8	0.63	RT

Lines	Al_0	Al_{45}[1]	RRL	Criteria[2]	Lines	Al_0	Al_{45}	RRL	Criteria
	(cm)					(cm)			
P6-255	21.0	17.9	0.85	HT	P3-26	23.7	14.6	0.61	RT
P6-276	20.1	16.9	0.85	T	P4-45	22.1	13.3	0.60	RT
P6-271	21.7	17.8	0.84	T	P5-50	22.1	12.9	0.59	RT
P3-148	20.9	17.3	0.83	T	P2-1	18.5	11.1	0.59	RT
P3-120	23.2	19.6	0.83	T	P3-27	25.7	14.0	0.54*	RT
P6-272	20.5	16.6	0.83	T	P2-2	18.5	10.1	0.54*	RT
P6-62	20.6	16.8	0.83	T	P3-28	23.9	12.7	0.53*	RT
P6-105	16.6	13.7	0.83	T	Dupa	24.7	18.2	0.74	T
P6-295	21.8	17.8	0.83	T	ITA131	21.1	11.3	0.53	RT
P3-159	24.5	19.9	0.81	T	SGJT-28			0.89	HT
P3-134	19.3	15.6	0.80	T	SGJT-36			0.86	HT
P3-150	21.9	17.6	0.80	T	W.Rarem			0.52	RT
P6-302	20.3	15.5	0.79	T	Fatmawati			0.76	T
P3-158	24.1	19.2	0.79	T	BNT 0.05			0.2	
P3-249	20.6	16.3	0.78	T	KK (%)			15.69	

*Significantly different from Dupa based on LSD 0.05 test; [1]Al_0= 0 $AlCl_3$, Al_{45}= 45 ppm $AlCl_3$; [2]HT = Highly tolerant, T=tolerant, AT=Rather tolerant

In tolerance genotypes, Al ~~is~~ was prevented from passing through the plasma membrane and entering the symplast and sites that ~~are~~ were sensitive ~~to Al~~ in the cytoplasm ~~of the~~ root tip. The ability of the root cell wall to absorb low Al and the permeability of ~~the cell~~ its membrane ~~is thought to be~~ were involved in the mechanism of external tolerance. Zhu et al. (2018) explained that Hydrogen sulfide (H2S) ~~plays~~ played an essential role ~~in~~ on Al stress resistance in plants. ~~HS reduces~~ H2S lowered Al toxicity by reducing ~~the Al~~ its content in the apoplast and symplast rice root. Wang et al. (2017) ~~revealed~~ showed that the activity of cytosolic glucose-6-phosphate dehydrogenase ~~is~~ was also involved in resistance to Al ~~through mediating~~ with the intervention of ROS levels in soybean. ~~Result~~ The result by Qian et al. (2018) indicated that H2O2 accumulation ~~is~~ was also a key factor contributing to the ~~decrease in~~ decreased root activity.

In Al tolerance, ~~plants will be able to raise the~~ plant pH ~~around~~ was raised at the root ~~area~~ tip (Kochian et al., 2004; Ma, 2007). ~~Increasing pH around the roots occurs~~ This was due to the influx of H^+ ~~at the root tip. It~~ around this area, which resulted in the deposition of Al and a decreasing Al^{3+} ion activity ~~so that it becomes a less toxic form to plants~~ (Samac and Tasfaye, 2003; Zhao et al., 2014). ~~Plants avoid from Al toxicity trough absorb~~ High NO^{3-} content in ~~large amounts.~~ plants tend to reduce Al toxicity. It also caused the release of hydroxyl ~~ions~~ (OH^-) or bicarbonate ions (HCO^{3-}) into the rhizosphere, increased pH, and suppressed the solubility of ~~Al~~ Al (Justino et al., 2006; Zhao et al., 2018).

Table 5. The results of the ~~DH1 lines selection~~ DH1-line selections for a new type of upland rice under Al stress

Criteria	Genotype	Number of lines
Highly	P6: 274, 314, 273, 311, 267, 278, 286, 266, 264, 291,	19

tolerant	265, 261, 257, 255, dan P3: 196, 191, 238, 204, 250	
Tolerant	P6: 276, 271, 272, 62, 105, 295, 302, 319, 275, 297, 320, 108, 317, dan P3: 148, 120, 159, 134, 150, 158, 249, 210, 161, 135, 175, 221, 190, 162, 131, 248	29
Rather tolerant	P2: 1, 2; P3:160, 31, 26, 27, 28; P4-45, P5-50, P6-103	10

The RRL values of ~~the genotype~~ P3-27, P2-2, P3-28 were lower than the tolerant checks, and classified as ~~moderately~~ the moderate tolerant genotypes (~~low) by~~ 0.53-0.54), which was almost the same as the ~~RRL values of the~~ ITA ~~as~~ susceptible checks ~~by 0.53~~ (0.53) (Table 4). The grouping was based on the RRL values in 58 ~~DH1 lines~~ DH1-lines, tested on nutrient cultures at 0 and 45 ppm Al, ~~that is~~ and produced susceptible = PAR ~~<~~<< 0.5, ~~rather tolerant~~ with low tolerance = 0.5 ~~<~~<< PAR ~~<~~<< 0.70, ~~tolerant~~ moderate = 0.70 ~~<~~<< PAR ~~<~~<< 0.85, and ~~highly tolerant~~ high = ~~PAR~~PAR > 0.85, ~~so 19 genotypes were highly tolerant~~therefore, 19 high, 29 ~~tolerant genotypes~~moderate, and 10 ~~genotypes rather~~ low tolerant genotype were produced (Table 5).

Distribution of Population from Cross of P3 (Fatmawati x SGJT-36) and P6 (SGJT-36 x Fatmawati)

Aluminum tolerance was based on the ~~relative root length~~ Relative Root Length (RRL) and ~~root shoot~~ the Root Shoot weight ~~ratio~~ Ratio (RSR) in DH1 ~~populations from the~~ populations. The crossing of P3 (Fatmawati x SGJT-36) and P6 (SGJT-36 x Fatmawati) ~~and~~ with the two parents ~~are~~ were presented in Table 6. The ~~relative root lengths~~ Relative Root Lengths (RRL) in the P3 population ~~ranges~~ ranged from 0.53 - 0.98, while the P6 population ~~ranges~~ ranged from 0.70 - 1.03. The Fatmawati elders had an RRL value of 0.77, while ~~the~~ that of SGJT-36 ~~elders were~~ was 0.87~~.~~. There ~~was diversity~~ were diversities in all observed characters~~. The root shoot weight ratio (RSR)~~ , with the RSR of the P3 population that ranged from 0.20 to 0.32, while ~~the~~ that of P6 ~~population ranged~~ graded from 0.22 to 0.39. The Fatmawati elders had ~~an~~ RSR ~~value~~ values of 0.30, while ~~the~~ those of SGJT-36 ~~elders had an RRL value of~~ were 0.32 (Table 6).

Table 6. The Relative ~~root length~~ Root Length (RRL) and ~~root shoot~~ the Root Shoot weight Ratio (RSR) ~~ratio~~ of ~~DH1~~ DH1-lines in populations of crossing P3 (Fatmawati x SGJT-36) and P6 (SGJT-36 x Fatmawati)

Characters	X ± SD DH1*	Range of DH1 population		Mean value of parent **	
		P3	P6***	Fatmawati	SGJT-36
Relative Root Length	0.8 ± 0.11	0.53 – 0.98	0.70 – 1.03	0.77	0.87
Root shoot weight ratio (RSR)	0.29 ± 0.04	0.20 – 0.32	0.22 – 0.39	0.30	0.32

*X ± SD DH1 is mean ± standard deviate, **Fatmawati and SGJT-36 5 plants each,*** P3 were 26 lines, and P6 were 27 lines

RRL and RSR values observed in DH1 populations varied ~~greatly, some of which were similar to their parents, intermediates, and exceed both of their parents~~significantly. The frequency distribution of P3 and P6 populations based on RRL values ~~is~~ were presented in Table 7. Based on aluminum tolerance criteria, the frequency distribution of the two elders did not

overlap. Fatmawati had ~~tolerant criteria~~moderate, while SGJT-36 had ~~highly tolerant~~high tolerance. The frequency distribution of DH1 populations of P3 derivatives ~~was highly tolerant~~had extreme, ~~tolerant~~moderate, and ~~rather tolerant~~low tolerance, while ~~the frequency distribution~~ those of P6 ~~populations was highly tolerant to tolerant~~ had high and moderate tolerance only (SGJT-36 elders~~) (Table 7).~~

~~).~~ Table 7. Distribution of ~~DH1~~ DH1-lines in each population of crossing P3 ~~(Fatmawati x SGJT-36)~~ and P6 (SGJT-36 x Fatmawati) based on aluminum tolerance.

Criteria	Parent*		DH1**	
	Fatmawati	SGJT-36	P3	P6
~~Highly~~ High tolerant	0	√	5	14
~~Tolerant~~Moderate tolerant	√	0	16	12
~~Rather~~ Low tolerant	0	0	5	1
Susceptible	0	0	0	0

*The Fatmawati elders and SGJT-36 each with five plants, ** P3 were 26 lines, and P6 were 27 lines, √ Al tolerance criteria on elders

~~It was due to the presence of~~ The frequent transgressive segregation in the ~~combination~~ anther of ~~an anther, which~~ a plant produced lines with different tolerance levels. ~~Many~~ Few genes were observed to control Al ~~tolerance~~ acceptance levels in rice, therefore, ~~so~~ not all genotypes ~~will have~~ possessed this gene. Zang et al. (2019) ~~were~~ found that there were significant differences between the gene expression patterns of Indica ~~Al-tolerant~~ and Japonica Al-tolerant varieties. Therefore, the gene ~~expression patterns of the Al-tolerant varieties~~ arrangement in the ~~mixed subgroup, which was inclined to Japonica~~subgroups, were similar to ~~the Al-tolerant varieties~~ those in ~~Japonica~~Japonica species. Each ~~gene~~ gene, or their combination ~~will have~~ played a role in regulating the mechanism of ~~Al~~ Al-tolerance in ~~rice that will be~~ rice, and expressed in each phase of plant growth (Wu et al. 2000). ~~Thus~~ Thus, the ~~elders~~ aged species used in this study produced lines that were tolerant to aluminum stress. ~~The next step will be an~~ Therefore, further research was needed for the evaluation of ~~the~~ leaf blast disease in ~~the~~ greenhouse.

CONCLUSION

The results of the evaluation of Al tolerance based on RRL in nutrient culture produced ~~19 genotypes~~ 19, 29, and 10 genotypic tolerance that were ~~highly tolerant~~high, ~~29 genotypes tolerant~~moderate, and ~~ten genotypes rather tolerant~~low, respectively. The tolerance level of Al in the ~~DH1~~ DH1-lines of upland rice produced by anther culture varied ~~greatly~~significantly. ~~Root length, shoot length,~~ The root and shoot length with the shoot dry weight had a high coefficient of ~~diversity~~ diversity, heritability, and ~~heritability and~~ significantly correlated with each other. The distribution of DH1 populations of P3 derivatives produced ~~highly tolerant~~high, ~~tolerant~~moderate, and ~~rather tolerant~~ low tolerance criteria, while ~~the population~~ those of P6 ~~derivatives produced highly tolerant to tolerant criteria.~~yielded high and moderate only.

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Assessment of Aluminum Tolerant of Double Haploid Lines for Developing New Type of Upland Rice

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ABSTRACT

Aluminum can possibly have direct or indirect adverse effects on plant growth; however, this effect is not the same for all plants, even in the same species. The roots of plants are most sensitive to Al toxicity accompanied to initial symptoms such as the inhibition of cell extension and retarded development of root systems. This study aims to evaluate doubled haploid (DH1) upland rice lines derived from anther aluminum culture, and also examine the genetic diversity and the distribution of doubled haploid lines due to aluminum stress. Al tolerant test was carried out in a greenhouse using factorial Randomized Complete Block Design (RCBD) with three replicates. Yoshida nutrient solution containing Al of 0 and 45 ppm was the first factor, while the second was the lines obtained from previous experiments (DH1), the four parents (SGJT36, SGJT28, Fatmawati, and Way Rarem), Dupa, and the ITA131 susceptible checks. The results showed that the shoot and root length, with their dry weight values had a high coefficient of diversity, heritability, and significantly correlated with each other. The tolerance level of Al in DH1- lines of upland rice produced by another culture varied significantly. Based on the Relative Root Length (RRL), out of 58 lines tested, 19 genotypes were highly tolerant, 29 lines were moderate, while 10 were low. The DH1 rice derived from P3 showed high, moderate, and low tolerance, while those from P6 showed high and moderate tolerance only.

Keywords: Aluminum tolerance, Doubled haploid, Upland rice

INTRODUCTION

The transition of land into residential areas, the construction of social facilities and infrastructure has led to a reduction in the field of agricultural land. It also resulted in the shifting of agricultural land to a marginal (dry land) area, especially on ultisol soils that reacted sourly to plant cultivation as a result of some symptoms such as lack of Ca, Mg, P, K, and N as well as the presence of Al toxicity. The high content of Al in acidic soil has shown to inhibit plant growth (Silva et al., 2010; Brunner and Sperisen, 2013). The utilization of acidic land is faced with

various obstacles, such as low pH, which reduces the availability of nutrients for plant growth. On the other hand, Al toxicity increases in very acidic soil (pH <4.5), with increasing Al solubility, which has detrimental effects on plants. Not only is the growth of rice roots inhibited, but also damaged by high concentrations of Al in the soil, which leads to significant reductions in rice yields (Ismail et al., 2007; Liu et al., 2012). The impact of Al is not the same on all plants, even in the same species.

The initial symptoms of Al toxicity in plants are inhibition of cell extension and the retarded development of root systems. Its availability in land solution depends on the level of soil acidity. In very acidic conditions (pH <4.5), Al becomes very soluble, especially in the form of Al^{3+} ion, which is highly toxic to plants. It also interferes with the uptake, transport, and the utilization of nutrients, and also inhibits enzyme activity and hormonal balance (Lupwayi et al., 2014; Wan et al., 2019; Yamamoto, 2019). The high content of Al solution in the soil causes stunted root growth and decreases the ability of roots to absorb mineral and water nutrients (Silva et al., 2012; Ma et al., 2014; Kochian et al., 2015). The inhibition of root growth by Al occurs due to cell division and elongation in the root meristem.

The accumulation of Al in root tissue determines the tolerance rate of plant genotypes, which correlate with the level of root damage. In tolerant genotype, the Al aggregation root was lower than the sensitive genotype (Ma 2000; Zang et al., 2019). The small number of negative charges on the cell wall in tolerant genotype reduces the interaction of Al with the root layer (Watanabe and Okada 2005; Kochian et al. 2015). This phenomenon has also been reported in previous studies (Nursyamsi 2000; Awasthi et al., 2017; Qian et al., 2018) that rice tolerance has a mechanism of reducing the interaction of Al on the root cell walls.

Currently, many rice varieties have not tolerated acidic soils, and some are still being tested. High genetic diversity is one of the main factors used in improving plant traits, both by conventional and biotechnological methods. The previous study of genetic diversity on DH1 had produced 58 double haploid upland rice lines that were ready to be further evaluated (Herawati et al. 2009). Therefore, the proper selection needs to be done to obtain genotypes that tolerate aluminum stress. The differences in root growth character are one indicator that can be used in the tolerance selection since roots are the main target of damage by Al. In upland rice, a quick method for evaluating genotypes that tolerate Al stress can be done by observing the root length in the vegetative phase (Bakhtiar et al., 2007; Belachew et al., 2017; Awasthi et al., 2017; Qian et al., 2018). This study aims to examine DH1 of upland rice derived from another culture, and also study genetic diversity, as well as the population distribution due to aluminum stress.

MATERIALS AND METHODS

The experiment was carried out in the greenhouse of the Indonesian Center for Research and Development on Biotechnology and Agricultural Genetic Resources, Cimanggu, Bogor. The materials used were 58 DH1 rice lines, the four elders (SGJT36, SGJT28, Fatmawati, and Way Rarem), Dupa, and ITA131 susceptible check (Prasetyono, 2003; Bakhtiar et al., 2007).

Experiments using factorial Randomized Complete Block Design (RCBD) were repeated three times, with the Yoshida nutrient solution (Yoshida et al. 1976). A solution of aluminum at the concentrations of 0 and 45 ppm were given as the first factor, while the second was 64 rice line varieties.

The rice seeds were roasted for 3 x 24 hours at 45 ° C and sown on husk media. They were allowed to germinate in the dark for five days. After which those that were healthy and uniform with a height of ± 5 cm were selected for planting. The nutrient used was Yoshida solution with the final composition as follows: 40 ppm N, ten ppm P, 40 ppm K, 40 ppm Ca, 40 ppm Mg, 0.5 ppm Mn, 0.05 ppm Mo, 0.2 ppm B, 0.01 ppm Zn, 0.01 ppm Cu, and two ppm Fe (Yoshida et al. 1976). In the Al treatment to reduce the formation of the polymer, the pH of the nutrient solution was adjusted to 4.5 by using 0.1 N NaHCO3. After this, 2 ml of Al solution made from 1000ml of AlCl3.5H2O was added to get a treatment concentration of 45 ppm. The pH of the nutrient solution was adjusted to 4.0 ± 0.1 with 0.1 N NaHCO3 or 0.1 N HCl.

Five-day-old healthy sprouts from a uniform root were transferred to the media. Sprout stems were then wrapped in soft foam and placed on a nutrient solution in styrofoam holes. Each pothole was planted with five sprouts and maintained for 14 days in a greenhouse. A growth period of 14 days was used due to the composition of the Yoshida nutrient solution (Yoshida et al. 1976). During this phase, water addition and pH adjustment were carried out with 0.1 N NaHCO3 or 0.1 N HCl every two days. Observations were made on plants aged 14 days after planting, by measuring root length, plant height, root and shoot dry weight. The formula used to estimate the Shoot Root weight Ratio (SRR) was as follows:

$$SRR = \frac{root\ dry\ weight}{shoot\ dry\ weight}$$

The formula used to measure the variable Relative Root Length (RRL) was as follows:

$$RRL = \frac{root\ length\ under\ Al\ stress}{root\ length\ without\ Al}$$

Data analysis was performed using the Least Significant Difference Test (LSD). Tolerance of rice lines to Al stress were grouped into a susceptible = RRL <0.5, low = 0.5 <RRL <0.70, moderate = 0.70 <RRL <0.85, and high tolerance = RRL> 0.85. Analysis of variance and the correlation between variables were performed using Pearson analysis and SAS software version 9.1. Genetic parameters were calculated based on the Singh and Chaudhary (1979) method as follows:

Source of variance	df	Means Square	expectation value
Genotype	(g-1)	M2	$\sigma_e^2 + 3\sigma_g^2$
Error	(r-1)(g-1)	M1	σ_e^2

σ_e^2 = enviroment variance; σ_g^2 = genetic variance

$$\sigma_g^2 = \frac{M2-M1}{r} \qquad \sigma_e^2 = M1 \qquad \sigma_p^2 = \sigma_g^2 + \sigma_e^2$$

The standard deviation formula for genetic variance:

$$\sigma_{\sigma_g^2} = \sqrt{\left(\frac{2}{r}\right)\left[\left(\frac{M2_g^2}{df_g} + 2\right) + \left(\frac{M1_e^2}{df_e} + 2\right)\right]}$$

M2 = Means squared genotype
M1 = Means squared error
r = replication
dfg = degree of freedom genotype
dfe = degree of freedom error

Genetic diversity could be estimated from the genetic variance (σ2g) and the standard deviation of genetic variance (σσ2g). A character had a broad genetic diversity when σ2g > $2\sigma\sigma^2_g$. The Coefficient Genotype Diversity (CGD) was estimated using the formula as follows:

$$CGD = \frac{\sqrt{\sigma_g^2}}{\bar{x}} \text{ x } 100\% \qquad \bar{x} = \text{average population observed}$$

When 0 < CGD ≤ 10.94 (narrow); 0 < CGD ≤ 21.88 (narrower); 0 < CGD ≤ 32.83 (broader); 0 < CGD ≤ 43.77 (broad); 43.77 < CDG (broadest).

The Coefficient Phenotype Diversity (CPD) was estimated using the formula as follows:

$$CPD = \frac{\sqrt{\sigma_p^2}}{\bar{x}} x100\%$$

When 0 < CPD ≤ 24.94 (narrow); 0 < CPD ≤49.71 (narrower); 0 < CPD ≤ 74.71 (broader); 0 < CPD ≤ 99.65 (broad); 99.65 < CPD (broadest).

Heritability in a broad sense (h^2_{bs}) was calculated according to the formula:

$$h_{bs}^2 = \frac{\sigma_g^2}{\sigma_p^2}$$

The heritability values (h^2_{bs}) were grouped according to Stanfield (1983) as follows:
0.50 < h^2_{bs} < 1.00 = high; 0.20 < h^2_{bs} < 0.50 = moderate; h^2_{bs} < 0.20 = low.

Genotypic correlations were calculated using the formula:

$$r_{g(xiji)} = \frac{cov.g(xixj)}{\sqrt{\left(\sigma^2_{g(xi)}.\sigma^2_{g(xj)}\right)}}$$

cov.g(xixj) = genotypic variation between properties i and j
$\sigma^2_{g(xi)}$ = genetic variability i
$\sigma^2_{g(xj)}$ = genetic variability j

RESULTS AND DISCUSSION

Analysis of genetic diversity

Analysis of variance of DH1 lines of rice with Al stress in nutrient culture showed significant differences in all observed variables (Table 1). Al stress reduced root length by 21.95 percent and shoots dry weight by 22.14 percent, while it decreased shoot length and root dry weight by only 6 percent (Figure 1).

Table 1. Analysis of variance of DH1 lines of new type upland rice under Al stress in nutrient solution

Variable	Sum Square	Mean Square	F value
Root length	1159.4	20.3	4.80**
Shoot length	0.35	0.006	2.92**
Root dry weight	0.089	0.0016	1.10*
Shoot dry weight	0.11	0.002	4.46**
Root shoot weight Ratio (RSR)	0.35	0.0062	2.92**

*Significant different at level 0.05; ** Significant different at level 0.01

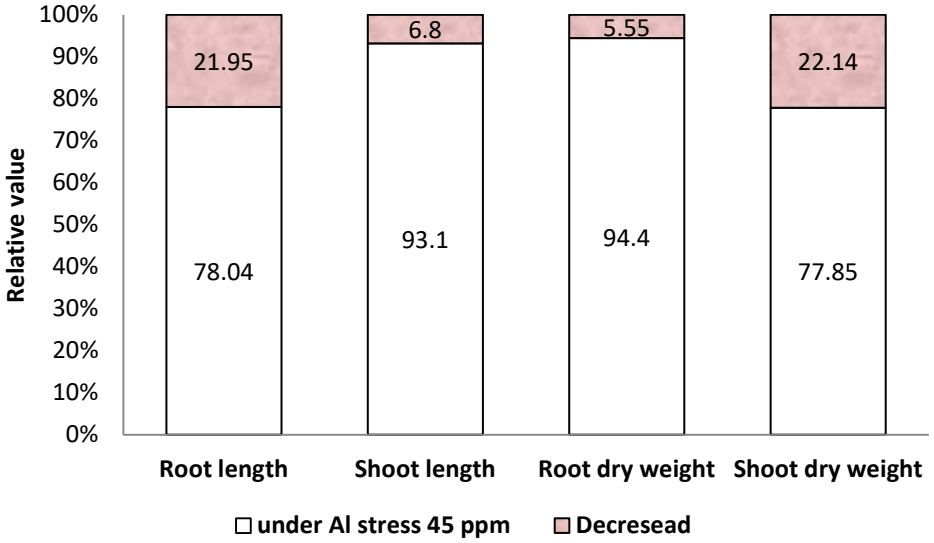

Figure 1. Effect of Al stress on variables of the length and dry weight of the root and shoot of DH1 lines.

The decrease in root length was caused by the obstruction of primary and lateral roots elongation. The field and laboratory experiments showed mixed responses to Al toxicity in rice (Watanabe and Okada, 2005; Bakhtiar et al., 2007; Qian et al., 2018). Reduction in shoot dry weight was due to the unavailable nutrients for suboptimal growth, as a result of the impaired mineral absorption and transport in roots (Kochian et al. 2015; Qian et al., 2018). The decrease in root dry weight was only 5.55 percent, compared to the dry shoot weight (22.14 percent) (Figure 1). Since the root length decreased and became shorter, therefore the adventitious roots grew the more. These showed that under Al conditions, more carbohydrates were directed to root growth. Bakhtiar et al. (2007) and Belachew et al. (2017). It was also observed that shoot dry weight was more sensitive to Al toxicity than root dry weight. The inhibition of shoot growth was a secondary effect due to nutrient deficiency, especially Mg, Ca, P, and the restriction of water absorption, which caused dwarf rice growth (Ma et al., 2014). Wang et al. (2015) demonstrated that the application of NH4 decreased the Al content in rice roots by reducing the pectin content in their roots. Freitas et al. (2019) showed that aluminum chloride was more important in producing Al toxicity in the upland rice plants, grown in the nutrient solution.

Table 2. Genetic diversity of root and shoot length, root and shoot dry weight, and root shoot weight ratio under Al stress conditions

Variable	Mean	GV*	PV	2xSD GV	GVC	PVC	h^2bs
Root length	15.75	5.37	9.61	5.43	14.71	19.68	0.56
Shoot length	42.14	30.74	38.41	21.41	13.61	14.70	0.80
Root dry weight	0.037	0.00007	0.0015	3.25	22.12	100.0	0.05
Shoot dry weight	0.114	0.00053	0.0009	3.25	20.19	26.75	0.57
Root shoot weight Ratio (RSR)	0.29	0.0014	0.0035	3.25	12.92	20.40	0.40

*GV =Genotipe Variability, PV=PhenotipeVariability, PVC=Phenotipe Variability Coefficient, GVC= Genotipe Variability Coefficient, SDRG=standar deviate genetic variability, h^2_{bs}= heritability in a broad sense

The estimated genetic parameters were shown in Table 11. Root length had a narrow diversity of genotypes with a broad coefficient of 5.37 and 14.71 percent. Shoot length had a broad genetic diversity that was 30.74 percent but had a narrow coefficient of 13.61 percent (Table 2). The estimated heritability values of root and shoot dry weight were 0.05 and 0.8, respectively (Table 2). The estimate for their lengths was considerably high. Characters that had high heritability values indicated that these genetic factors were more dominant than others; therefore, their selections were made in the first generation (Akinwale et al., 2011; Herawati et al., 2019).

Correlation and Relative Root Length (RRL)

Positive correlations were observed for all characters, except for shoot length and RSR, which showed negative (Table 3). Features that had significant differences and positive relationships were used as selection criteria. Root and shoot length and the shoot dry weight were selected as one of the requirements of Al tolerance for DH1 line. These characters had high genetic diversity, heritability values, and were positively correlated with other features.

Table 3. Correlation of root and shoot length, their dry weights, and the Root Shoot weight Ratio (RSR) under Al stress condition

Characters	Shoot length	Root dry weight	Shoot dry weight	Root shoot weight ratio (RSR)
Root length	0.42**	0.28**	0.53**	0.12*
Shoot length		0.25*	0.65**	-0.25*
Root dry weight			0.43**	0.11^{ns}
Shoot dry weight				-0.14*

*= significant at level 005; **= very significant at level 001, ns=no significant

Among these characters, root length was more easily observed; therefore, the researchers used relative root length (RRL) to distinguish tolerant and Al-susceptible genotypes. Previous research indicated that the main target of Al toxicity was the root tissue of the plant. Root damage was characterized by decreased protein content in the cytoplasm and increased

membrane damage to cell walls, which resulted in leakage (Zhu et al., 2018). Qian et al. (2018) reported that the fresh and dry weights of the rice seedlings were in significant correlation with chlorophyll content. This result indicated that a low Al concentration increased the seedlings' fresh and dry weights by increasing the leaf chlorophyll content and promoting photosynthesis.

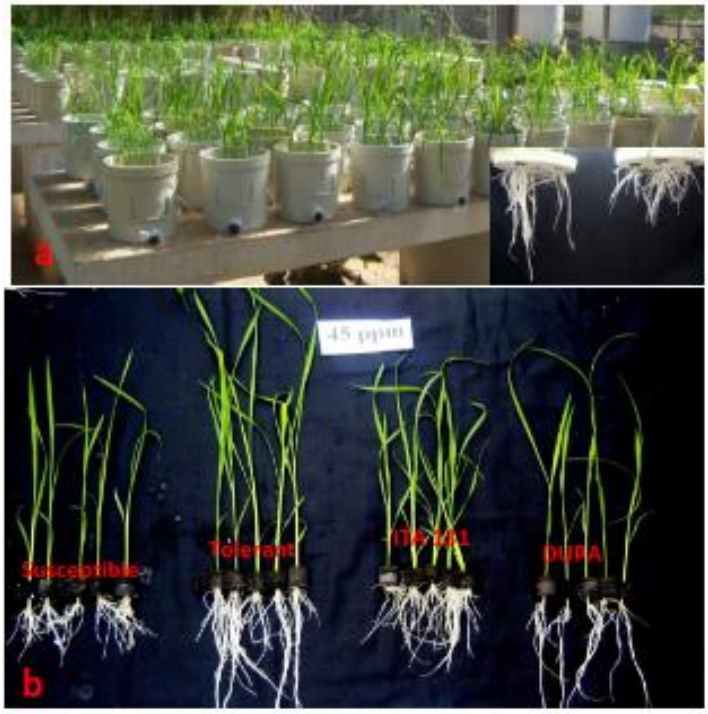

Figure 2. The experiment of Al stress on Yoshida nutrient solution showed the root lengths of ITA 131 (susceptible check), and DUPA (tolerant check) under 45 ppm.

Root shortening is one of the consequences of Al inhibition; therefore, its structure appeared to be shorter, fat, and reduced branching, while its adventitious roots grew the more (Figure 2a). The penetration of roots into hard soil layers also inhibit nutrients and water absorption. The toxicity level depends on the concentration of Al^{+3} ions in the soil solution. Al decreased the fresh weight by inhibiting the absorption of water and mineral substances (Qian et al., 2018).

The Relative Root Length (RRL) values for DH1 lines varied between 0.53-1.03 (Table 4). The RRL value of the Dupa (tolerant check) was 0.74, while ITA131 (susceptible check) was 0.53 (Figure 2b). The 5% LSD test showed no significant difference between the PAR values for more tolerant genotypes and for susceptible checks (Table 4). This test corresponded with the previous experiments carried out by Prasetiyono (2003), Bakhtiar et al. (2007) that Dupa had tolerance at RRL value of 0.7, however, for ITA131, it was 0.53, which was found to increase from the previous test of 0.41 (Bakhtiar et al., 2007). For this reason, it was necessary to review using ITA varieties as susceptible checks (Figure 2b). The 5% LSD test on DH1-lines resulted in 8 lines having significantly different higher RRL values than the Dupa check varieties (PAR = 0.74), such as line P6-274, P6-314, P3-196, P6-273, P6- 311, P6-250, P6-267, and P6-278 (Table 4).

Table 4. Root lengths in the treatments of 0 and 45 ppm Al with the Relative Root Length (RRL) value of DH1-lines at 14 days after planting

Lines	Al_0	Al_{45}[1]	RRL	Criteria[2]	Lines	Al_0	Al_{45}	RRL	Criteria
	(cm)					(cm)			
P6-274	16.2	16.7	1.03*	HT	P6-319	20.4	16.0	0.78	T
P6-314	20.3	20.3	1.01*	HT	P6-275	20.3	15.6	0.78	T
P3-196	17.1	16.8	0.98*	HT	P6-297	25.1	19.3	0.77	T
P6-273	19.9	19.5	0.97*	HT	P3-210	20.6	15.8	0.76	T
P6-311	15.3	14.9	0.96*	HT	P3-161	20.2	15.8	0.76	T
P3-250	16.8	15.9	0.95*	HT	P3-135	23.1	17.2	0.76	T
P6-267	10.6	10.1	0.95*	HT	P3-175	21.8	16.6	0.76	T
P6-278	19.4	18.3	0.94*	HT	P3-221	23.8	18.1	0.76	T
P6-286	23.4	21.6	0.93	HT	P3-190	20.2	15.3	0.75	T
P6-266	12.5	11.7	0.93	HT	P6-320	19.9	15.2	0.75	T
P3-191	21.5	19.6	0.90	HT	P3-162	20.9	15.4	0.74	T
P6-264	14.0	12.6	0.90	HT	P1-108	20.2	15.0	0.74	T
P3-238	17.9	15.1	0.88	HT	P6-317	16.3	12.2	0.73	T
P3-204	17.2	15.1	0.88	HT	P3-131	21.3	15.2	0.72	T
P6-291	14.9	13.1	0.87	HT	P3-248	18.7	13.5	0.72	T
P6-265	12.4	10.9	0.87	HT	P6-103	20.6	14.7	0.70	RT
P6-261	17.1	14.8	0.87	HT	P3-160	24.2	16.8	0.70	RT
P6-257	20.6	17.8	0.86	HT	P3-31	22.4	13.8	0.63	RT
P6-255	21.0	17.9	0.85	HT	P3-26	23.7	14.6	0.61	RT
P6-276	20.1	16.9	0.85	T	P4-45	22.1	13.3	0.60	RT
P6-271	21.7	17.8	0.84	T	P5-50	22.1	12.9	0.59	RT
P3-148	20.9	17.3	0.83	T	P2-1	18.5	11.1	0.59	RT
P3-120	23.2	19.6	0.83	T	P3-27	25.7	14.0	0.54*	RT
P6-272	20.5	16.6	0.83	T	P2-2	18.5	10.1	0.54*	RT
P6-62	20.6	16.8	0.83	T	P3-28	23.9	12.7	0.53*	RT
P6-105	16.6	13.7	0.83	T	Dupa	24.7	18.2	0.74	T
P6-295	21.8	17.8	0.83	T	ITA131	21.1	11.3	0.53	RT
P3-159	24.5	19.9	0.81	T	SGJT-28			0.89	HT
P3-134	19.3	15.6	0.80	T	SGJT-36			0.86	HT
P3-150	21.9	17.6	0.80	T	W.Rarem			0.52	RT
P6-302	20.3	15.5	0.79	T	Fatmawati			0.76	T
P3-158	24.1	19.2	0.79	T	BNT 0.05			0.2	
P3-249	20.6	16.3	0.78	T	KK (%)			15.69	

*Significantly different from Dupa based on LSD 0.05 test; [1]Al_0= 0 $AlCl_3$, Al_{45}= 45 ppm $AlCl_3$; [2]HT = Highly tolerant, T=tolerant, AT=Rather tolerant

In tolerance genotypes, Al was prevented from passing through the plasma membrane and entering the symplast and sites that were sensitive in the cytoplasm root tip. The ability of the root cell wall to absorb low Al and the permeability of its membrane were involved in the

mechanism of external tolerance. Zhu et al. (2018) explained that Hydrogen sulfide (H2S) played an essential role in Al stress resistance in plants. H2S lowered Al toxicity by reducing its content in the apoplast and symplast rice root. Wang et al. (2017) showed that the activity of cytosolic glucose-6-phosphate dehydrogenase was also involved in resistance to Al with the intervention of ROS levels in soybean. The result by Qian et al. (2018) indicated that H2O2 accumulation was also a key factor contributing to the decreased root activity.

In Al tolerance, plant pH was raised at the root tip (Kochian et al., 2004; Ma, 2007). This was due to the influx of H^{+} around this area, which resulted in the deposition of Al and a decreasing Al^{3+} ion activity (Samac and Tasfaye, 2003; Zhao et al., 2014). High NO^{3-} content in plants tend to reduce Al toxicity. It also caused the release of hydroxyl (OH^{-}) or bicarbonate ions (HCO^{3-}) into the rhizosphere, increased pH, and suppressed the solubility of Al (Justino et al., 2006; Zhao et al., 2018).

Table 5. The results of the DH1-line selections for a new type of upland rice under Al stress

Criteria	Genotype	Number of lines
Highly tolerant	P6: 274, 314, 273, 311, 267, 278, 286, 266, 264, 291, 265, 261, 257, 255, dan P3: 196, 191, 238, 204, 250	19
Tolerant	P6: 276, 271, 272, 62, 105, 295, 302, 319, 275, 297, 320, 108, 317, dan P3: 148, 120, 159, 134, 150, 158, 249, 210, 161, 135, 175, 221, 190, 162, 131, 248	29
Rather tolerant	P2: 1, 2; P3:160, 31, 26, 27, 28; P4-45, P5-50, P6-103	10

The RRL values of P3-27, P2-2, P3-28 were lower than the tolerant checks, and classified as the moderate tolerant genotypes (0.53-0.54), which was almost the same as the ITA susceptible checks (0.53) (Table 4). The grouping was based on the RRL values in 58 DH1-lines, tested on nutrient cultures at 0 and 45 ppm Al, and produced susceptible = PAR < 0.5, with low tolerance = 0.5 < PAR < 0.70, moderate = 0.70 < PAR < 0.85, and high = PAR > 0.85, therefore, 19 high, 29 moderate, and 10 low tolerant genotype were produced (Table 5).

Distribution of Population from Cross of P3 (Fatmawati x SGJT-36) and P6 (SGJT-36 x Fatmawati)

Aluminum tolerance was based on the Relative Root Length (RRL) and the Root Shoot weight Ratio (RSR) in DH1 populations. The crossing of P3 (Fatmawati x SGJT-36) and P6 (SGJT-36 x Fatmawati) with the two parents were presented in Table 6. The Relative Root Lengths (RRL) in the P3 population ranged from 0.53 - 0.98, while the P6 population ranged from 0.70 - 1.03. The Fatmawati elders had an RRL value of 0.77, while that of SGJT-36 was 0.87. There were diversities in all observed characters, with the RSR of the P3 population that ranged from 0.20 to 0.32, while that of P6 graded from 0.22 to 0.39. The Fatmawati elders had RSR values of 0.30, while those of SGJT-36 was 0.32 (Table 6).

Table 6. The Relative Root Length (RRL) and the Root Shoot weight Ratio (RSR) of DH1-lines in populations of crossing P3 (Fatmawati x SGJT-36) and P6 (SGJT-36 x Fatmawati)

Characters	X ± SD DH1*	Range of DH1 population		Mean value of parent **	
		P3	P6***	Fatmawati	SGJT-36
Relative Root Length	0.8 ± 0.11	0.53 – 0.98	0.70 – 1.03	0.77	0.87
Root shoot weight ratio (RSR)	0.29 ± 0.04	0.20 – 0.32	0.22 – 0.39	0.30	0.32

*X ± SD DH1 is mean ± standard deviate, **Fatmawati and SGJT-36 5 plants each,*** P3 were 26 lines, and P6 were 27 lines

RRL and RSR values observed in DH1 populations varied significantly. The frequency distribution of P3 and P6 populations based on RRL values were presented in Table 7. Based on aluminum tolerance criteria, the frequency distribution of the two elders did not overlap. Fatmawati had moderate, while SGJT-36 had a high tolerance. The frequency distribution of DH1 populations of P3 derivatives had extreme, moderate, and low tolerance, while those of P6 had high and moderate tolerance only (SGJT-36 elders). Table 7. Distribution of DH1-lines in each population of crossing P3 and P6 (SGJT-36 x Fatmawati) based on aluminum tolerance.

Criteria	Parent*		DH1**	
	Fatmawati	SGJT-36	P3	P6
High tolerant	0	√	5	14
Moderate tolerant	√	0	16	12
Low tolerant	0	0	5	1
Susceptible	0	0	0	0

*The Fatmawati elders and SGJT-36 each with five plants, ** P3 were 26 lines, and P6 were 27 lines, √ Al tolerance criteria on elders

The frequent transgressive segregation in the anther of a plant produced lines with different tolerance levels. Few genes were observed to control Al acceptance levels in rice; therefore, not all genotypes possessed this gene. Zang et al. (2019) found that there were significant differences between the gene expression patterns of Indica and Japonica Al-tolerant varieties. Therefore, the gene arrangement in the subgroups was similar to those in Japonica species. Each gene, or their combination, played a role in regulating the mechanism of Al-tolerance in rice and expressed in each phase of plant growth (Wu et al. 2000). Thus, the aged species used in this study produced lines that were tolerant to aluminum stress. Therefore, further research was needed for the evaluation of leaf blast disease in the greenhouse.

CONCLUSION

The results of the evaluation of Al tolerance based on RRL in nutrient culture produced 19, 29, and 10 genotypic tolerance that was high, moderate, and low, respectively. The tolerance level of Al in the DH1-lines of upland rice produced by anther culture varied significantly. The root and shoot length with the shoot dry weight had a high coefficient of diversity, heritability, and significantly correlated with each other. The distribution of DH1 populations of P3 derivatives produced high, moderate, and low tolerance criteria, while those of P6 yielded high and moderate only.

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