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 as 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 $NaHCO_3$. After this, 2 ml of Al solution made from 1000ml of $AlCl_3.5H_2O$ 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 $NaHCO_3$ 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 $NaHCO_3$ 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:

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

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 ($\sigma 2g$) and the standard deviation of genetic variance ($\sigma\sigma 2g$). A character had a broad genetic diversity when $\sigma 2g > 2\sigma\sigma^2_g$. 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 $0 < CGD \leq 10.94$ (narrow); $0 < CGD \leq 21.88$ (narrower); $0 < CGD \leq 32.83$ (broader); $0 < CGD \leq 43.77$ (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 $0 < CPD \leq 24.94$ (narrow); $0 < CPD \leq 49.71$ (narrower); $0 < CPD \leq 74.71$ (broader); $0 < CPD \leq 99.65$ (broad); $99.65 < CPD$ (broadest).

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

$$h^2_{bs} = \frac{\sigma_g^2}{\sigma_p^2}$$

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:

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

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

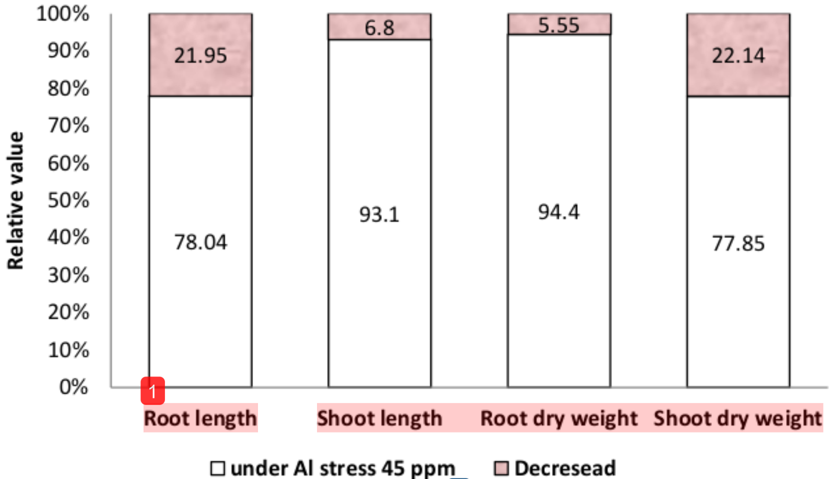

Figure 1. Effect of Al stress on variables of the length and dry weight of the root and shoot of DH1 lines.

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.

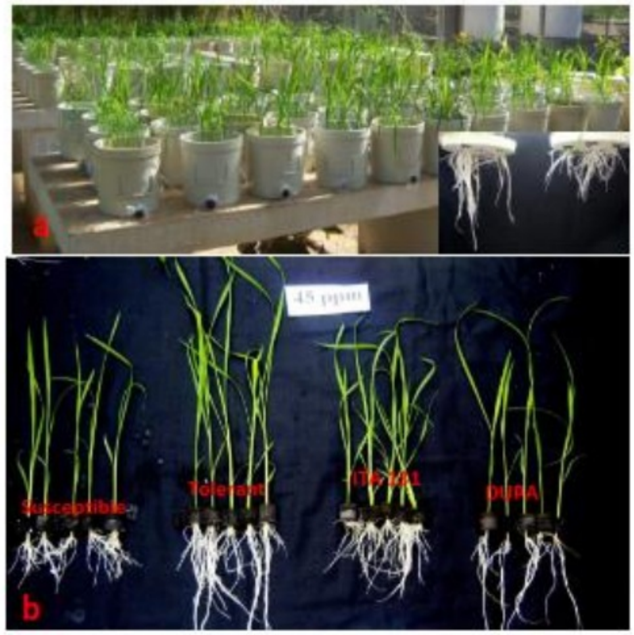

Figure 2. The experiment of Al stress on Yoshida nutrient solution showed the root lengths of ITA 131 (susceptible check), and DUPA (tolerant check) under 45 ppm.

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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