Tolerance Test of Upland Rice Promising Lines to Alumunium Stress in Water Culture Media

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Abstract. The use of aluminum-tolerant upland rice varieties is an effort that can be made to utilize ultisol land. The application of water culture techniques in greenhouses can be an alternative method in the testing of plant tolerances. This study aims to examine the tolerance level of F_8 upland rice lines to aluminum stress through low-pH water cultures. The method used is the Randomized Block Design with Split Plot pattern. The Concentration of Al as the main plot and genotypes of upland rice as subplot. The main plot treatment consists of 2 levels of treatment, i.e. 0 mg kg^{-1} and 60 mg kg^{-1} Al concentrations. The treatment of the subplot is consisting of 5 F_8 upland rice promising lines and 3 varieties as comparison. Concentration of Al 60 mg kg^{-1} is likely to inhibit the growth of upland rice lines. Lines 19I-06-09-23-03 and 23A-56-20-07-20 are moderate based on the sensitivity index of stress and anatomy of the roots, while 21B-57-21-21-23, 23F-04-10-18-18 and 23A-56-22-20-05 are sensitive to aluminum stress in water culture media. Line of 19I-06-09-23-03 is a promising line with the best growth in aluminum concentrartion of 60 mg kg^{-1}.

Keywords: Aluminum, Rice lines, Stress, Tolerance

1 Introduction

Most of Bangka area has ultisol soil type. Ultisol soils with a pH ranging from 3.7 to 6.4 have lower values of organic matter, available P, available K, and CEC [1]. The result of the research [2] reported that ultisol soils in Bangka Belitung have a low pH (3.5–6.5), low cation exchange capacity (CEC) of < 16 cmol kg^{-1}, high Al saturation (0% - 95%), and have a cation of 0.39-23.30 cmol+kg. [3] Added that the existing soil in Balunijuk Merawang Village, Bangka is ultisol soil with a pH range of 4.5, CEC 4.37 me 100 g^{-1}, Al-dd me 100 g^{-1}, and P_2O_5 Bray 1: 5.8 mg kg^{-1}.

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Ultisol has a high soil acidity and aluminum saturation [4]. Al elements have toxic properties that can inhibit other nutrients needed by plants. Al elements will be toxic at a pH of < 5.0 because they can absorb important nutrients such as P and Ca [5] . [6] report that high Al saturation limits root penetration to obtain nutrients.

Some of the ultisol lands spread across Indonesia are used as upland rice production land. According to [7], the limited adequate irrigated land makes upland rice cultivation an option for most people. The use of aluminum-tolerant upland rice varieties is an optimization effort that can be done to utilize ultisol land [8], [9].

One of the challenges in increasing upland rice production during cultivation is biotic and abiotic stresses that scale light to heavy so that they have the potential to reduce yields [10]. Knowing the ability of plants to adapt to the gripping environment such as the Al-scuffle is important information in the assembly of new high-yielding varieties. Efforts that can be used to obtain rice genotypes that have Al-tolerant properties include conventional pedigree crossing [11]; mutation induction, somaclonal diversity, and in vitro selection [12].

According to [13], the test method in acidic lands has limitations such as the time required (usually one growing season) and problems of soil variability. The application of water culture techniques (nutrient solution) is a solution to overcoming these problems. According to [14], test of the Al tolerance in nutrient solution screening have been found to correlate positively with those obtained under field condition.

One of the efforts to increase the productivity of rice plants is by breeding plants through the assembly of upland rice varieties with superior characters [15]. Currently, a cross has been carried out between mutant red rice, local accession of red rice, with commercial upland rice varieties, and has been obtained F_7 line of upland rice. The promising line used in this study was the F_8 generation line from the results of crossing varieties PBM UBB 1, Balok, Inpago 8, and Banyuasin. The brown rice upland rice line F_2 comes from seeds resulting from hybridization between MR1512 (PBM UBB 1 variety) × Inpago 8, MR1512 (PBM UBB 1 variety) × Banyuasin, Inpago 8 × Balok, Banyuasin × Balok, Inpago 8 × Balok [16].

The results of this study are expected to obtain lines of upland rice plants that are tolerant of aluminum (Al) through water culture. Al-tolerant promising lines are also expected to grow normally and obtain good results when planted on land that has a fairly high Al content.

2 Method

This research was conducted from December 30, 2021 to April 2022 at the greenhouse of the Experimental and Research Station and in Agrotechnology Laboratory of Universitas Bangka Belitung. The tools used in this study were styrofoam, TDS, pH meter, measuring cup, bucket, trash bag, seedling tub, hoe, drill, ruler, analytical scales, aerator, scissors, meter, name tag, RHS color chart, books, and stationery, microscope, preparation glass, razor blade, tweezers, pipette, 24 ml mineral cup, as well as a camera used as a documentation tool. The materials used in this study included rice seeds and as many as five lines of upland F_8 brown rice (19I-06-09-23-03, 21B-57-21-21-23, 23F-04-10-18-18, 23A-56-20-07-20, and 23A-56-22-20-05) and three varieties as comparisson (PBM UBB 1, Situ Pategang, and Danau Gaung), AB-Mix, $AlCl_3$, hematoxylin, pH Up and Down, aquades, FAA solution, water, topsoil, compost, sand, cotton wool, and duct tape.

The method used is a Randomized Block Design with Split Plot patterns. The main plot treatment consists of 2 levels of treatment, namely the concentration of Al 0 mg kg^{-1} and Al 60 mg kg^{-1}. The treatment of the subplot consisted of 5 lines of upland rice hope F_8 brown rice (19I-06-09-23-03, 21B-57-21-21-23, 23F-04-10-18-18, 23A-56-20-07-20 and 23A-56-22-20-05) and 3 comparison varieties (PBM UBB 1, Situ Pategang, and Danau Gaung). Each treatment combination consisted of 3 groups. All plants were observed without samples. Activities carried out consist of seed preparation, seed germination, preparation of water culture containers, preparation of water culture media, planting seedlings, giving treatment, maintenance, and harvesting. The characters observed were plant height, leaf length, number of leaves, number of saplings, root weight, root length, number of roots, header weight, root header ratio, percentage of damaged roots, and percentage of roots containing aluminum using histochemical analysis.

The quantitative data obtained were analyzed using Variance Analysis with a confidence level of 95%. If it shows that there is a significant, it is continued with the Duncan Multiple Range Test at a 95% confidence level. The measurement of the stress tolerance index is calculated using the formula proposed by Fischer and Maurer (1978) [17].

3 Result and Discussion

The promising line F_8 of upland rice showed differences in character to the treatment of Al and genotype. The interaction between the genotype of upland rice and aluminum chequered showed an unreal influence on all the characters observed. Some of the characteristics of rice growth did not differ markedly from the Al basin treatment including plant height, number of leaves, root length, root weight and root header ratio except for leaf length, number of saplings, number of roots, and heading weight. The genotype treatment of upland rice had a marked effect on plant height, leaf length, number of saplings, root length, heading weight and root header ratio while on number of leaves, number of roots, and root weight showed nonsifnificant influence (Table 1).

Table 1. Analysis of variance on upland rice genotypes, aluminum stress, and the interaction beetween the upland rice genotypes and aluminum stress on plant characters.

Characters	Aluminum stress		CV (%)	Genotypes		Interaction		CV (%)
	F-Hit	Pr > F		F-Hit	Pr > F	F-Hit	Pr > F	
Plant height	2.25	0.14^{ns}	20.18	3.10	0.015^{*}	1.04	0.43^{ns}	17.88
Leaf length	5.16	0.03^{*}	28.95	2.30	0.046^{*}	0.26	0.96^{ns}	20.31
Number of leaves (t)	0.50	0.49^{ns}	27.96	1.43	0.235^{ns}	0.64	0.72^{ns}	6.65
Number of saplings (t)	5.35	0.03^{*}	7.89	2.95	0.019^{*}	0.85	0.56^{ns}	7.55
Number of roots	4.87	0.04^{*}	7.22	2.07	0.082^{ns}	0.32	0.94^{ns}	19.44
Root length	1.30	0.26^{ns}	25.66	2.42	0.046^{*}	1.08	0.40^{ns}	23.71

Header weight (t)	8.19	0.008^{**}	8.35	2.68	0.029^{*}	0.78	0.61^{ns}	6.56
Root weight (t)	5.81	0.14^{ns}	5.88	1.37	0.256^{ns}	1.28	0.29^{ns}	6.24
Root header ratio (t)	1.79	0.20^{ns}	6.48	3.52	0.008^{**}	0.69	0.68^{ns}	5.96

Note: **, *: Significant at P≤0.01 and P≤0.05, respectively, ns: Nonsignificant, CV (Coefficient of variance), t (Data transformation '*Square Root'*=Sqrt (Original data + 0,5)).

The results of DMRT on the Al treatment showed marked differences in some of the observed characters (Table 2). The Al treatment 0 mg kg^{-1} is the best treatment on leaf length, number of saplings, number of roots, and heading weight. The Al basin treatment had nonsignificant on plant height, root length, number of leaves, root weight and root header ratio. The non-stress treatment showed the highest average yield than the 60 mg kg^{-1} Al basin treatment against plant height, root length, number of leaves, root weight and root header ratio (Table 2).

Table 2. The average character was observed in the treatment of aluminum stress.

Character	Al 0 mg kg^{-1} + pH 6	Al 60 mg kg^{-1} + pH 4
Plant height (cm)	96.58 a	84.47 a
Leaf length (cm)	65.29 a	57.60 b
Number of leaves (blade)	25.77 a	23.06 a
Number of saplings (stems)	4.05 a	2.76 b
Number of roots	71.51 a	63.10 b
Root length (cm)	53.16 a	50.16 a
Header weight (g)	15.76 a	10.60 b
Root weight (g)	8.22 a	6.48 b
Root header ratio	3.52 a	3.05 b

Note: The number followed by the same letter in the same column shows nonsignificant difference in the DMRT test with a 95% confidence level.

The results of further tests of DMRT with genotype treatment on plant height characters, leaf length, number of saplings, root length and root header ratio can be seen in Table 3. Danau Gaung variety obtained the highest yield on plant height followed by PBM UBB 1 and line 19I-06-09-23-03 and the one with the lowest plant height of 23A-56-22-20-05. The highest leaf length value is PBM UBB 1 followed by line 19I-06-09-23-03 and Danau Gaung. The lowest leaf length is 23A-56-22-20-05. The line 19I-06-09-23-03 obtained the highest number of saplings and root length than any other genotype. The highest heading weight is 19I-06-09-23-03 and the lowest is 23F-04-10-18-18. The highest root header ratio is Situ Pategang followed by 21B-57-21-21-23 and the lowest is 23F-04-10-18-18. All upland rice genotypes show unmarked differences in number of leaves, number of roots, and root weight. The average value of these characters can be seen in the histogram (Figure 1).

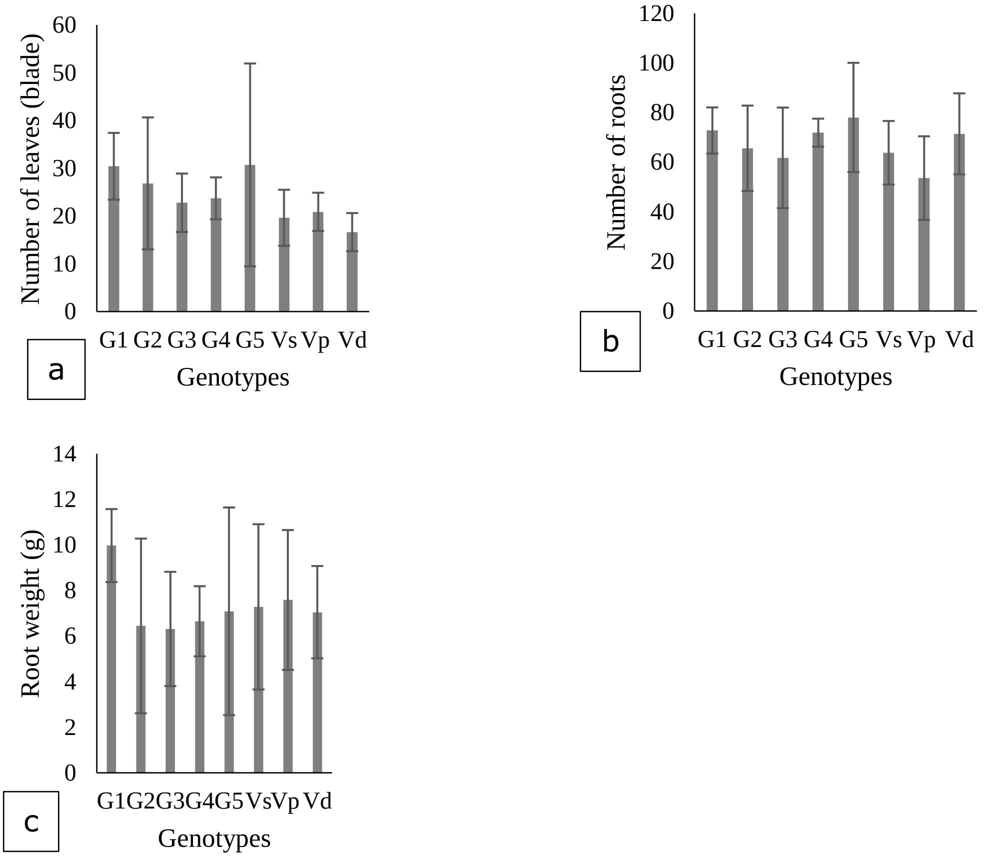

Figure 1. Averages at each genotype (data show average standard deviations); (a). the number of leaves, (b). the number of roots, (c). the weight of the roots; G1 (19I-06-09-23-03), G2 (21B-57-21-21-23), G3 (23F-04-10-18-18), G4 (23A-56-20-07-20), G5 (23A-56-22-20-05), Vs (Situ Pategang), Vp (PBM UBB 1), and Vd (Danau Gaung).

The highest average yield on Number of leaves in genotype treatment was found in line 23A-56-22-20-05, while the lowest was found in Danau Gaung variety (Figure 1a). The highest average yield of number of roots in genotype treatment was found in line 23A-56-22-20-05, while the lowest was found in PBM UBB 1 (Figure 1b). The highest mean root weight results in genotype treatment were found in the line 19I-06-09-23-03, while the lowest was in line 23F-04-10-18-18 (Figure 1c). The average yield of rice plant height in the aluminum treatment test showed nonsignificant difference between the line that was given the stress and the line without the firm. The highest average yield of plant height is found in the Danau Gaung variety. line 23A-56-22-20-05 shows the lowest average plant height compared to other lines (Table 3). PBM UBB 1 has the longest leaf length among other genotypes followed by the line 19I-06-09-23-03, and the lowest is indicated by the lines 23A-56-22-20-05. Line 19I-06-09-23-03 shows the most number of leaves and number of saplings compared to other genotypes. The average yield of the least number of leaves is Danau Gaung, while the average yield of the least number of saplings is the Situ Pategang (Table 3, Figure 1a).

Table 3. The average genotype in the character of plant height, leaf length, number of saplings, root length, header weight, and root header ratio.

Genotypes	Plant height (cm)	Leaf length (cm)	Number of saplings (stems)	Root length (cm)	Header weight (g)	Root header ratio
19I-06-09-23-03	99.85 ab	70.53 ab	5.32 a	68.28 a	21.25 a	3.18 bc
21B-57-21-21-23	81.03 bc	56.23 b	4.00 ab	46.80 b	13.75 ab	4.12 ab
23F-04-10-18-18	88.30 abc	55.07 b	2.40 b	43.83 b	9.65 b	2.45 c
23A-56-20-07-20	85.62 abc	56.04 b	4.03 ab	49.57 b	9.79 b	2.71 bc
23A-56-22-20-05	69.13 c	53.80 b	5.03 a	53.09 ab	11.75 ab	2.68 bc
Situ Pategang	95.77 ab	63.81 ab	1.73 b	50.17 b	12.82 ab	4.98 a
PBM UBB 1	100.39 ab	74.65 a	3.03 ab	56.96 ab	15.31 ab	3.37 bc
Danau Gaung	103.47 a	65.81 ab	1.90 b	43.61 b	11.12 ab	2.64 bc

Note: The number followed by the same letter in the same column shows nonsignificant difference in the DMRT test with a 95% confidence level

The determination of genotypes that are tolerant of aluminum stress was carried out through the calculation of the tolerance index or index of the sensitivity (IS) by Fischer and Maurer (1978) to the observed characters. The data used in the water flow test for optimum conditions were data on Al 0 mg kg^{-1} (without aluminum stress) with a pH of 6. The data on the choked condition uses Al 60 mg kg^{-1} (the addition of 60 mg kg^{-1} aluminum) which has a pH of 4. The sensitivity index results of each observed character were grouped in the category of tolerant when IS < 0.5, somewhat tolerant or moderate when 0.5 < IS < 1 and sensitive when IS >1. The results of the stress sensitivity index showed that the lines 19I-06-09-23-03, 23A-56-20-07-20, and the Danau Gaung variety were lines and varieties that were moderate of aluminum stress compared to the other three F_8 lines with comparative varieties of pategang situ and PBM UBB 1. The genotypes of upland rice that are sensitive to aluminum contamination are three F_8 lines, namely 21B-57-21-21-23, 23F-04-10-18-18 and 23A-56-22-20-05, as well as 2 comparison varieties, namely Situ Pategang and PBM UBB 1 (Table 4).

Table 4. The matrix of the tolerance level of the plant character is based on the value of the sensitivity index observed in each genotype.

G	PH	LL	NS	RL	RHR	NL	NR	HW	RW	IS	Category
G1	0.26	0.32	0.72	0.58	0.32	1.03	0.35	0.58	0.75	0.54	Moderate
G2	1.10	0.87	0.53	4.72	0.21	0.13	2.12	0.03	0.39	1.12	Sensitive
G3	0.38	0.48	1.46	3.22	0.86	1.83	0.64	1.03	1.35	1.25	Sensitive
G4	0.03	0.99	0.23	4.01	1.15	0.07	0.43	0.99	0.85	0.97	Moderate
G5	1.95	0.84	1.76	3.55	0.55	4.14	1.12	1.89	2.32	2.01	Sensitive
Vs	1.58	1.39	2.14	1.23	2.72	0.00	1.63	1.48	2.65	1.65	Sensitive
Vp	1.35	1.19	0.63	5.62	1.34	0.70	1.74	1.15	1.47	1.68	Sensitive
Vd	1.25	1.71	0.10	0.11	0.69	0.69	0.01	0.72	0.38	0.63	Moderate

Note: G (genotypes), PH (plant height), LL (leaf length); NS (number of saplings); RL (root length), RHR (root header ratio), NL (number of leaves), NR (number of roots), HW (header weight), and RW (root weight), SI (sensitivity index). G1 (19I-06-09-23-03), G2 (21B-57-21-21-23), G3 (23F-04-10-18-18), G4 (23A-56-20-07-20), G5 (23A-56-22-20-05), Vs (Situ Pategang), Vp (PBM UBB 1), and Vd (Danau Gaung).

The results of the observation of upland rice aluminum poisoning scoring were observed with reference to the assessment of IRRI (1996). The tolerance level of upland rice to aluminum stress has moderate criteria with a score of 3 in lines 19I-06-09-23-03, 23A-56-20-07-20, and the Danau Gaung, while lines 21B-57-21-21-23, 23F-04-10-18-18, 23A-56-22-20-05, Situ Pategang, and PBM UBB 1 have a score of 5 (slightly sensitive). Symptoms of Al poisoning for a score of 1 are normal growth and tillering. A score of 3 indicates normal growth and tillering, but there are spots of white or yellow color on the tips of older leaves. A score of 5 indicates stunted growth and tillering.

The highest percentage of damaged roots is found in PBM UBB 1, which means that the normal roots due to Al's stress are few. 19I-06-09-23-03 is the line that has the least percentage of damaged roots, which means that the roots are normally the most numerous than any other genotype. The line 19I-06-09-23-03 also has roots that contain the least Al among other lines and comparison varieties. The highest percentage of roots containing Al is PBM UBB 1. The qualitative character of the root color of the stress treatment of Al 60 mg kg^{-1} showed symptoms of root discoloration such as a change in root color which tended to be more pale white compared to normal root color visually in the non-stress treatment. The color of the roots of rice plants without stress indicates that there is no difference in color in each line and comparison variety (Table 5).

Table 5. Percentage of damaged roots and Al-containing roots with histochemical analysis.

Genotypes	Damaged roots (%)		Roots containing aluminum (%)	
	Al 0 mg kg^{-1}	Al 60 mg kg^{-1}	Al 0 mg kg^{-1}	Al 60 mg kg^{-1}
19I-06-09-23-03	0.00	17.18	0.00	13.00
21B-57-21-21-23	0.00	39.06	0.00	41.67
23F-04-10-18-18	0.00	43.75	0.00	46.35
23A-56-20-07-20	0.00	29.68	0.00	34.89
23A-56-22-20-05	0.00	51.56	0.00	48.44
Situ pategang	0.00	50.00	0.00	47.39
PBM UBB 1	0.00	56.25	0.00	51.04
Danau Gaung	0.00	34.37	0.00	37.50

The hematoxyline staining method by measuring the intensity of staining through histochemistry (microscopic preparations) to see the penetration of Al into the roots can be used to see the difference in plant tolerance to Al. The outermost layer of the root tip will be hematoxylin stained if there is Al at the root end. The normal root structure of genotype at the unstressed treatment can be seen in Figure 2. The addition of aluminum and pH treatment can cause injury to the roots of plants. Damage to the structure of the root part, namely in the epidermis and cortex, indicates a disfigurement in the root tip area (Figure 2).

Figure 2. Histochemical analysis of the roots; (a) normal roots, (b) damaged roots.

Detection of aluminum content is seen in hematoxylin color in plant root tissues after staining (Figure 3). A more intense hematoxylin color of the root tissue indicates the presence of aluminum content in plant tissues. The fading of hematoxyline staining of the root tissue indicates that there is no aluminum content in the root tissue of the plant.

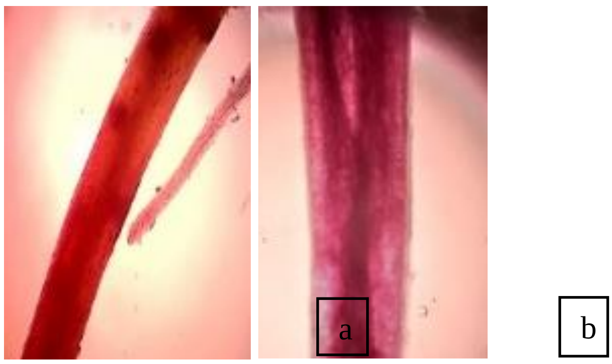

Figure 3. Root; (a) that do not contain aluminum, (b) contain aluminum

4 Conclusion

The promising line F_8 upland rice used in al tolerance testing is a line resulting from a pedigree selection which is the result of a cross between Bangka local accession and national varieties. Line 19I-06-09-23-03 is the result of crossing varieties of PBM UBB 1 × Inpago 8. The cross between the Inpago 8 varieties × the Balok accession produced a line 21B-57-21-21-23. Lines 23A-56-20-7-20 and 23A-56-22-20-5 are the result of crossing the accession of the Banyuasin × Balok. Line 23F-04-10-18-18 is the result of crossing the accession of the Inpago 8 × Balok. Screening test in nutrient solution can provides adequete Al stress for preliminary/basic evaluation of large number of genotypes in a small area and less expensive [18][19].

The growth of the promising line F_8 of upland rice grown through low-pH water cultures showed diversity in each character of the observation. This diversity occurs because each line and variety of comparisons have different genetic potentials in responding to the growing environment, especially aluminum stress [20]. The F_8 expectation line shows a marked effect on the growth of upland rice plants, namely on the character of plant height, leaf length, root length, number of saplings and root header ratio, as well as no real effect on the number of leaves, number of roots, heading weight and root weight based on genotype treatment factors (Table 3).

The stress factor of 60 mg kg^{-1} Al had a significant effect on the character of leaf length, number of saplings, number of roots, and header weight except plant height, number of leaves, root length, root weight and root header ratio (Table 2). Al poisoning can also inhibit the growth of the canopy by inhibiting the supply of nutrients, water and cytokinins from the roots due to poor penetration of the roots into the subsoil or low root hydraulic conditions [21]. According to [22] that the character of root growth is the main character that determines the level of tolerance of Al in plants, while the chlorophyll content of leaves is only a secondary symptom of plants in responding to Al-stress. The high number of roots in the Al-stress treatment is suspected to be due to the obstruction of the root lengthening process caused by damage to the root tip so that nutrient absorption is not optimal. The magnitude of the decrease in root length is influenced by the genotype, because each rice genotype has a different root system so that it provides a different response to nutrient treatment [23]. Similarly also conveyed by [24], the main consequences of Al exposure are a decrease in crop production and inhibition of root growth.

Plant height line 19I-06-09-23-03 is highest than the other four lines against aluminum stress. The lowest plant height is 23A-56-22-20-05. Plant height is not affected by Al-stress, there are differences due to genetic factors (Tables 1 and 2). This is in accordance with the results of research by [25], 23A-56-22-20-05 is a line of upland rice that has the shortest plant height than other lines. The character of plant height is also a determinant of plant yield which is closely related to the process of photosynthesis [26]. Stable lines have a good agronomic appearance, namely plant height, number of saplings, and moderate harvest age, and the total grain or panicle number is higher than the comparison variety [27]. The same thing was also stated by [28] that research that agronomically upland rice tolerant of Al's showed better agronomic character growth.

Line 19I-06-09-23-03 is the line that has the best average genotype character in aluminum stress treatment compared to the other four F_8 lines (Table 3 and Figure 1). The average character such as plant height, leaf length, root length, number of leaves, heading weight and root weight. This line of 19I-06-09-23-03- indicates a tolerance to Al poisoning. According to [29], tolerant plants have roots that are able to grow well and the tips of the roots are not damaged, can change the pH in the root, and have certain mechanisms where Al is unable to inhibit the uptake of Ca, Mg, and K so that plants can still meet their nutrient needs. The result of the stress sensitivity index (Table 4) showed that lines 19I-06-09-23-03, 23A-56-20-07-20, and Danau Gaung variety had moderate levels of tolerance.

According to Table 4, the genotypes of upland rice that are sensitive to aluminum stress are three F_8 lines, namely 21B-57-21-21-23, 23F-04-10-18-18 and 23A-56-22-20-05, as well as 2 comparison varieties, namely Situ Pategang and PBM UBB 1. In some of the characters observed, sensitive lines having stunted growth were indicated by the smallness of development and growth among other genotypes (Table 1, 3, and Figure 1). Aluminum stress has a negative impact on intolerant plants and can cause toxicity, resulting in inhibition of plant growth [30]. [31] stated that the accumulation of Al at the root turned out to be high in non-adaptive varieties, this caused the growth of the roots of the variety to be inhibited. [32] stated that due to aluminum poisoning, the roots become shortened, thicker and brownish and the leaves become necrotic due to chlorosis. Inhibition of root and bud growth is the earliest al-induced morphological change [33]. [34] report that aluminum deposits cause the roots to become swollen, curved, and discolored in rice seedlings. According to the results of the study that Al basin can decrease plant height and leaf area [35], as well as stem diameter [36].

Generally plants respond to Al stress by increasing the synthesis and exudation of organic acids as Al stress increases [37]. Citric organic acids are able to reduce Al toxicity in low concentrations thus turning Al into a form available to plant roots. The accumulation of Al in the root tissue occurs due to three possibilities, namely first, the absence of Al translocation to the leaves as is the case in plants [38], secondly, the ability of the roots to exude Al is low and thirdly the concentration of exuded organic acids is not able to glue all the Al present in the apoplas and the surface of the root [39].

[40] suggests that, plants that are able to adapt to high Al are caused by these plants that have a certain mechanism to suppress the adverse influence of Al so that it does not interfere with the absorption of nutrients and water, also able to streamline it. This efficiency can be in the process of absorption, reduction, translocation, and redistribution of nutrients. The tolerant genotype is thought to have the ability to prevent Al from crossing the plasma membrane and entering the simplas as well as other Al-sensitive places in the cytoplasm of the root [41]. According to [42], the mechanism that indicates the least accumulation of Al in the root tissue is called the external mechanism or the exclusion of Al i.e. the prevention of Al into the root tissue.

Based on the percentage of damaged roots and aluminum-containing roots in the upland rice genotype (Table 5) that 19I-06-09-23-03 is the best line compared to other lines. This shows that the line has a resistance mechanism to stress. According to [43], the intensity of the dark color formed as a result of the Alhematoxylin (Hematin) complex, is directly proportional to the number of Al accumulated in the root tissue. Al-tolerant genotypes are characterized by a small amount of damage to root tissue. [44] stated that the root is the most sensitive part of Al poisoning. At high Al saturation will inhibit the elongation of lateral roots. Genotypes that are sensitive to Al poisoning their root development will be impaired whereas tolerant varieties have no real influence [45]. [46] add that the root tips of sensitive plants accumulate Al more and have shorter roots than tolerant plants. The outermost layer of the root tip will be stained with hematoxylin if there is an Al at the root end, because Al acts as a hematein binding device which is an oxide component of the hematoxylin solution. Hematoxylin-stained roots show a distribution of absorption of Al. Staining is more intensive at the root tip, since the root tip is the area of cell elongation [47].

Aluminum has a beneficial and toxic effect on plants depending on the pH of the soil, the chemical species of Al, the genotype, and the growing conditions of the plant. Aluminum stress causes a series of morphological, physiological, biochemical, and molecular changes in growing plants that reduce plant growth, development, and yield. The results showed that with the presence of Al, the absorption of various nutrients, namely Fe, Ca, Mg, K, P, and N decreased at the roots and shoots of all cultivars [48]. [24] also points out that the main consequences of

Al exposure are a decrease in crop production and inhibition of root growth. Inhibition of root growth may be directly/indirectly responsible for the loss of crop production. [49] also added that the high Al stress greatly reduces the absorption of Ca and Mg so that nutrient absorption becomes poor which results in reduced root growth.

According to [50], the high Al^{3+} in the soil solution causes the acidity of the soil to increase and the dominant concentration so that the element Al^{3+} becomes toxic. The aluminum metal content in the roots is getting higher as the concentration of aluminum exposure treatment increases. Disruption of aluminum inhibits nutrient absorption occurs when dissolved aluminum ions will bind to nutrients contained in the growing environment, such as N, P, K, Ca, Mg, and Mo so that nutrients become unavailable and plants cannot use these nutrients [51].

The results of the research of [52] reported that the selection of Al tolerance in nutrient cultures and acidic fields with the same pH (4.0) experienced changes or shifts in the level of tolerance to Al. This is because not all lines that are tolerant in acidic fields are also tolerant in nutrient cultures which are thought to be largely determined by testing environmental factors. The effect of stress on testing in acidic fields is not necessarily due to dissolved Al levels but it is suspected that there are other factors such as microclimate, nutrient availability and varying soil fertility. While the test results in the greenhouse, the stress that occurs is uniform due to a single factor of dissolved Al.

Al 60 mg kg^{-1} is likely to inhibit the growth of upland rice lines. Lines 19I-06-09-23-03 and 23A-56-20-07-20 are moderate lines based on the sensitivity index and root anatomy, while lines 21B-57-21-21-23, 23F-04-10-18-18, and 23A-56-22-20-05 are promising lines that are sensitive to aluminum stress in water culture media. 19I-06-09-23-03 is the promising line that has the best growth against aluminum stress of 60 mg kg^{-1}.

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