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Research Article

Genetic diversity and adaptation to aluminum toxicity in local upland rice of Kalimantan

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ABSTRACT

Acidic soils with high aluminum (Al) toxicity are a major constraint to upland rice productivity in many tropical regions, including Kalimantan. Local rice cultivars often possess adaptive traits that can be utilized for breeding tolerant varieties. This study aimed to investigate the genetic diversity of Kalimantan local upland rice cultivars using SSR markers and their adaptation to aluminum toxicity stress. A total of 93 rice accessions obtained from the Agricultural Gene Bank of IAARD were subjected to both genetic diversity and Al stress adaptation analyses. The genetic diversity analysis used 11 SSR markers, while the adaptation study employed a hydroponic method with 45 ppm Al in Yoshida nutrient solution. Root and shoot growth were observed after 21 days of stress treatment. The SSR markers were highly polymorphic, grouping the accessions into four clusters at a 35% similarity level. Most accessions showed tolerance to Al stress based on relative root and shoot growth parameters. Accessions P. Pulut Timai, Padai Cina, Raya, Parai Paliping, and P. Pulut Ayang/Pang were the most adaptive. Root length, shoot length, and shoot fresh weight exhibited moderate heritability. These findings provide valuable insights for breeding Al-tolerant upland rice adapted to acidic soils.

Keywords: adaptability; aluminum stress; molecular marker; SSR; upland rice

INTRODUCTION

The rice production in Indonesia fluctuates over time. In 2020, the production reached 54.64 million tons, an increase of 0.08% from the previous year (BPS, 2021). However, the continuously growing population demands an increase in food production. Among the factors faced in rice production in Indonesia is the availability of suitable land. Local upland rice, which is a type of rice that can grow in marginal land and can be used as an alternative to cope with this problem (Yullianida et al., 2019).

The utilisation of local Kalimantan upland rice genetic resources is essential because these genotypes have adapted to acidic soil conditions and high aluminum (Al) concentrations. Over generations, these local varieties have developed natural adaptation mechanisms that enable their growth and productivity in acidic soil conditions and nutrient-limited environments. This makes Kalimantan's local upland rice an ideal genetic resource for identifying and improving aluminum tolerance traits. In addition, the conservation and scientific research of local upland rice not only supports breeding programmes, but also supports the conservation of agricultural biodiversity and Indonesia's cultural heritage.

Aluminum (Al) is a significant factor inhibiting plant growth in acid soils. Aluminum toxicity can adversely affect plant growth and reduce crop yields (Rahman & Upadhyaya, 2021). Low Al concentrations stimulate nutrient absorption that increases the growth of rice roots (Sari et al., 2013), but high Al concentration causes roots to be ineffective in absorbing nutrients and water. Root growth is inhibited, becomes short, thick, and abnormal (Milatuzzahroh et al., 2019).

One of the ways to maintain rice production in acidic soil is by using varieties that are tolerant to Al toxicity. Such tolerant cultivars offer a practical solution to improve rice productivity on marginal lands, yet their availability remains limited. The selection method employed in this study offers an efficient strategy for identifying tolerant genotypes, as it integrates both phenotypic evaluation and molecular characterisation to capture adaptive traits associated with aluminum tolerance. Selection based on traits with high heritability ensures reliable genetic gains in breeding programs (Utami et al., 2016).

The development of rice varieties needs genetic resources as building blocks. The diversity of Indonesian rice may provide this substantial need. The development of genome-based technology currently makes it possible to exploit the genotypic diversity of rice germplasm to develop new tolerant varieties (Utami et al., 2016). Therefore, this study aimed to analyze the genetic diversity of local upland rice from Kalimantan and to evaluate its adaptation to Al stress.

MATERIALS AND METHODS

This research consisted of two main experiments, namely the molecular approach to observe genetic diversity and phenotype screening to observe rice response to Al stress. This research was conducted in Cikeumeuh Greenhouse and Molecular Biology Laboratory of Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development (ICABIOGRAD), Bogor, West Java, from September 2021 to April 2022. The genetic materials used in this study were 93 accessions of Kalimantan local upland rice from the Indonesian Agricultural Gene Bank collection. Kasalat and Dupa varieties were used as susceptible and tolerant checks, respectively.

Molecular analysis was conducted using SSR markers (Table 1). DNA extraction was performed using an extraction kit (GeneEx™ Plant Kit, GeneAll, Korea). The DNA amplification was conducted on a TI Thermalcycler PCR machine (Biometra, Germany). The PCR profiles were conducted according to Risliawati et al. (2021). The amplified DNA was then migrated in 6% polyacrylamide gel and then stained with ethidium bromide. The DNA was then visualized on a UV transilluminator (Biorad, USA).

The data scoring was conducted by using Gel Analyzer software (http://www.gelanalyzer.com). The data were analyzed using PowerMarker v3.25 software to obtain information on the number of alleles, gene diversity, polymorphic information content (PIC), and phylogenetic dendrogram. The clustering was performed according to Nei's genetic distance-based Unweighted Pair-Group Method with Arithmetic Mean (UPGMA) method.

The phenotypic study was conducted using a rapid test screening method in a hydroponic solution. The experimental design utilized a split-plot RCBD (randomized complete block design) with four replications. The main factor was Al stress, and the second factor was the rice accessions. Al treatment was administered by adding 45 ppm Al in Yoshida nutrient solution, while the control condition was set to 0 ppm Al.

The nutrient solution was maintained at a stable pH of 4.0±0.05 by daily adjustment using KOH or HCl. Observations were conducted weekly over a three-week period to monitor plant growth responses. During the observation period, the hydroponic solution was renewed weekly to ensure nutrient availability and maintain stable growing conditions. Observations had been made root length (RL), shoot length (SL), root fresh weight (RFW), shoot fresh weight (SFW), root dry weight (RDW), shoot dry weight (SDW), relative root length (RRL), relative shoot length (RSL), relative root fresh weight (RRFW), relative shoot fresh weight (RSFW), relative root dry weight (RRDW), and relative shoot dry weight (RSDW) had been followed the formula Hidayatun et al. (2017):

$$\mathrm{RRL, RSL, RRFW, RSFW, RRDW, RSDW} = \frac{\texttt{Stress (H21)}}{\texttt{Control (H21)}} \times\ 100\%$$

Data analysis was performed by using the SAS System software. F-test and DMRT (Duncan's Multiple Range Test) were conducted to determine the actual effect between the accessions. Adaptation grouping was carried out following Putri et al. (2020). Where ΔP = difference between groups for plant root length variable; b = highest root length; a = shortest root length; and n = number of adaptation groups (Table 2).

$$\Delta P = b – a/n$$

According to Solim & Nasution (2022), genetic variance, environmental variance, and phenotypic variance can be estimated using the following formulas:

$$\sigma^2_g = (MS - MSE)/r,\ \sigma^2_e = MSE/r, \text{ and } \sigma^2_p = \sigma^2_g + \sigma^2_e$$

Where: σ^2_g = genetic variance; σ^2_e = environmental variance; σ^2_p = phenotypic variance; MS = genotype total; MSE = environmental total; and r = replicates.

Furthermore, the expected heritability values and genetic variability are expressed as:

$$h^2_{bs} = \sigma^2_g / \sigma^2_p \text{ and } GCV = \sqrt{\sigma^2_g} / x \times 100\%$$

Where: h^2_{bs} = heritability; σ^2_g = genetic variance; σ^2_p = phenotypic variance, GCV = coefficient of genetic variability; σ^2_g = genetic variance; and x = mean.

Table 1. SSR markers used in the research

No	Marker	Chromosome	Repeat motif	Forward primer (5'-3')	Reverse primer (5'-3')
1.	RM 5	1	(GA)14	TGCAACTTCTAGCTGCTCGA	GCATCCGATCTTGATGGG
2.	RM 259	1	(CT)17	TTGAGTTTGAGAGGAGGG	CTTGTTGCATGGTGCCATGT
3.	RM 154	2	(GA)21	ACCCTCTCCGCCTCGCCTCCTC	CTCCTCCTCCTGCGACCGCTCC
4.	RM 514	3	(AC)12	AGATTGATCTCCCATTCCCC	CACGAGCATATTACTAGTGG
5.	RM 11	7	(GA)17	TGCCAATTACCTTCCCGTAC	TGCTCCGTATTGCTGCTATG
6.	RM 105	9	(CCT)6	GTCGTCGACCCATCGGAGCCAC	TGGTCGAGGTGGGGATCGGGTC
7.	RM 215	9	(CT)16	CAAAATGGAGCAGCAAGAGC	TGAGCACCTCCTTCTCTGTAG
8.	RM 474	10	(AT)13	AAGATGTACGGGTGGCATTC	TATGAGCTGGTGAGCAATGG
9.	RM 144	11	(ATT)11	TGCCCTGGCGCAAATTTGATCC	GCTAGAGGAGATCAGATGGTAGTGCATG
10.	RM 287	11	(GA)21	TTCCCTGTTAAGAGAGAAATC	GTGTATTTGGTGAAAGCAAC
11.	RM 19	12	(ATC)10	CAAAAACAGAGCAGATGAC	CTCAAGATGGACGCCAAGA

Table 2. Grouping of accessions through adaptation evaluation (Putri et al., 2020).

Score	Adaptation group	Group classification
1	Not adapted	$x < \Delta P + a$
2	Low adaptive	$\Delta P + a \le x < (\Delta P + a) + \Delta P$
3	Moderately adaptive	$(\Delta P + a) + \Delta P \le x < (\Delta P + a) + 2\,\Delta P$
4	Adaptive	$(\Delta P + a) + 2\,\Delta P \le x < (\Delta P + a) + 3\,\Delta P$
5	Highly adaptive	$x \ge (\Delta P + a) + 3\,\Delta P$

RESULTS AND DISCUSSION

Genetic diversity using SSR markers

All 11 SSR markers used in this study were polymorphic, producing a total of 75 alleles with 2 to 11 alleles per locus, with an average of 6.8 alleles per locus (Table 3). The major allele frequencies had an average value of 0.38. Genetic diversity showed an average of 0.72, indicating a high level of diversity among the genotypes. Polymorphic information content (PIC) ranged from 0.31 (RM105) to 0.83 (RM287), with an average of 0.68. These results indicate that the SSR markers used are highly informative and effective in detecting genetic variation among the tested upland rice genotypes.

According to Risliawati et al. (2021), markers with high PIC values are useful for assessing genetic diversity and germplasm characterisation. The PIC value indicates a marker's discriminatory ability to distinguish genotypes, and a value greater than 0.5 is categorised as highly informative. Therefore, the markers used in this study, especially RM287, RM5, and RM11, can be used effectively for population structure analysis and identification of genetic relationships between local upland rice genotypes.

The high average values of gene diversity (0.72) and PIC (0.68) indicate that local Kalimantan upland rice genotypes have a high level of genetic diversity. This diversity reflects a broad genetic base that can be utilised in rice breeding programmes. A broad genetic base is important for the development of new cultivars with superior traits such as drought tolerance, high yield potential, and good adaptability to marginal dryland environments. The relatively high allele richness indicates that this population has not experienced significant genetic bottlenecks, so that its genetic diversity is still preserved and can be effectively utilised in future genetic improvement programmes.

Compared to Nugroho et al. (2017), who reported 5.73 alleles per locus with a PIC of 0.65, and Sutoro et al. (2015), who reported 4.62 alleles per locus with a PIC of 0.49, this study found higher genetic diversity in the accessions tested. This indicates that local Kalimantan upland rice genotypes have allelic variation that has not been utilised in breeding programmes. The high genetic variation is also likely due to the diversity of ecotypes and genotype adaptation to the different microclimates and environments of Kalimantan.

These findings indicate that the SSR markers used in this study can provide a reliable molecular basis for genetic characterisation, cluster analysis, and selection of crossbreeding parents. This extensive genetic diversity supports the potential utilisation of local Kalimantan upland rice genotypes in breeding programmes to produce superior upland rice varieties that are tolerant to environmental stresses, have high yield stability, and are adaptable to climate change.

Table 3. Number of alleles, major allele frequency, gene diversity, and polymorphic information content (PIC)

Markers	Number of alleles	Major allele frequency	Gene diversity	PIC
RM5	9	0.28	0.82	0.80
RM11	10	0.27	0.81	0.78
RM19	4	0.41	0.69	0.63
RM105	2	0.74	0.38	0.31
RM144	4	0.41	0.70	0.64
RM154	7	0.28	0.81	0.78
RM215	8	0.32	0.77	0.73
RM259	11	0.31	0.80	0.77
RM287	10	0.28	0.85	0.83
RM474	3	0.55	0.56	0.47
RM514	7	0.37	0.74	0.70
Total	75			
Average	6.8	0.38	0.72	0.68

Cluster analysis showed high genetic diversity of local upland rice accessions from Kalimantan based on the eleven SSR markers (Figure 1). The phylogenetic tree grouped all accessions into four major clusters at a similarity coefficient of 35%, reflecting extensive genetic variation among genotypes. Table 4 shows that the detected diversity was mainly due to genetic differences at the molecular level, as reflected in the formation of four major clusters representing different genetic subpopulations among local upland rice accessions from Kalimantan.

Although the SSR markers used in this study were highly informative, with high mean PIC (0.68) and gene diversity (0.72) values, this analysis covered only eight of the twelve chromosomes in the rice genome (Table 3), thus limiting its ability to detect associations with specific adaptive traits such as tolerance to aluminum (Al) stress. The limited number of markers and their uneven distribution may explain the lack of clear relationships between genetic clusters and phenotypic adaptations. As explained by Powell et al. (1995) and Singh et al. (2012), comprehensive genetic diversity analysis generally requires 20–30 SSR or SNP markers evenly distributed throughout the genome to produce representative and reliable results. These findings are in line with the results of a study by Risliawati et al. (2021), which reported that SSR markers with high PIC values are effective for assessing genetic diversity in Indonesian rice germplasm, although their use alone is not sufficient to detect associations with specific traits.

Therefore, further research needs to use a larger number of markers and combine molecular data with phenotypic and environmental characteristics. Overall, these results confirm that Kalimantan upland rice has a broad genetic base and great potential as an important genetic resource for rice breeding programmes adapted to acidic soil conditions.

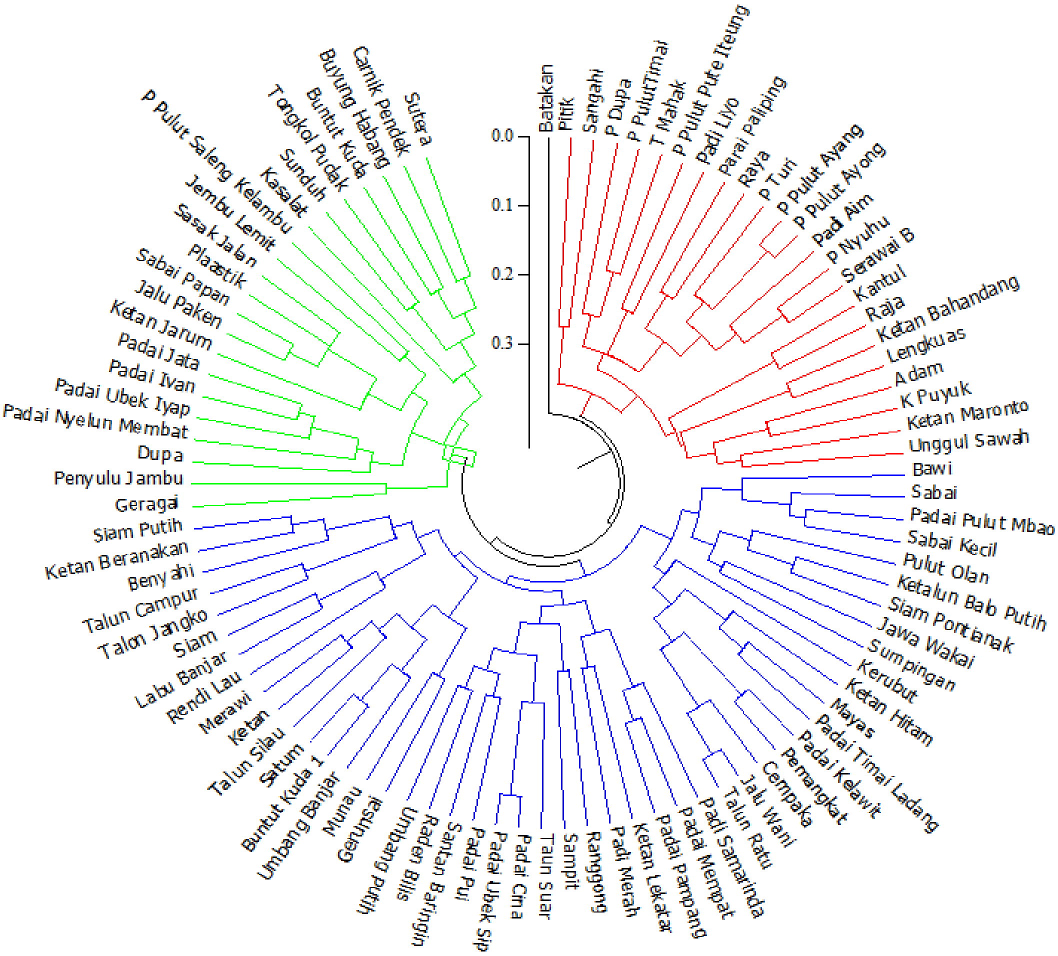

Figure 1. Phylogenetic dendrogram of 93 accessions based on 11 polymorphic SSR primers. The phylogenetic tree was constructed using 1,000 bootstrap replicates.

Apart from the limitation on the number, the markers used in this study were also not gene-specific markers. SSR markers are developed based on repetitive DNA sequences, which are generally found in the non-coding region. The repetitiveness of the sequence can occur throughout the genome, making it less specific. There are several SSR markers associated with rice tolerance to Al stress, but none of them were included in this study. This study needs to be continued by employing markers associated with tolerance to Al toxicity. The existing markers associated with rice tolerance to Al stress, such as RM 128, RM 164, and RM 570 in chromosome 3; RM 253 in chromosome 6; and RM 284 in chromosome 2, should be included (Famoso et al., 2011).

Table 4. Grouping of Kalimantan local upland rice based on cluster analysis.

Cluster	Total	Accession name
Cluster 1	1	Batakan.
Cluster 2	23	Pitik, Sangahi, P. Dupa, P. Pulut Timai, T. Mahak, P. Pulut Pute Iteung, Padi Liyo, Parai Paliping, Raya, P. Turi, P. Pulut Ayang, P. Pulut Ayong, Padi Aim, P. Nyuhu, Serawai B, Kantul, Raja, Ketan Bahandang, Lengkuas, Adam, K. Puyuk, Ketan Maronto, Unggul Sawah.
Cluster 3	48	Bawi, Sabai, Padai Pulut Mbao, Sabai Kecil, Pulut Olan, Ketalun Balo Putih, Siam Pontianak, Jawa Wakai, Sumpingan, Kerubut, Ketan Hitam, Mayas, Padai Timai Ladang, Padai Kewalit, Pemangkat, Cempaka, Jalu Wani, Talun Ratu, Padi Samarinda, Padai Mempat, Padai Pampang, Ketan/Lekatar, Padi Merah, Ranggong, Sampit, Taun Suar, Padai Cina, Padai Ubek Sip, Padai Pui, Santan Baringin, Raden Bilis, Umbang Putih, Gerunsai, Manau, Umbang Banjar, Buntut Kuda 1, Satum, Talun Silau, Ketan, Merawi, Rendi Lau, Labu Banjar, Siam, Talon Jangko, Talun Campur, Benyahi, Ketan Beranakan, Siam Putih.
Cluster 4	21	Gerangai, Penyulu Jambu, Dupa, Padai Nyelun Membat, Padai Ubek Iyap, Padai Ivan, Padai Jata, Ketan Jarum, Jalu Paken, Sabai Papan, Plaastik, Sasak Jalan, Jembu Lemit, P. Pulut Saleng Kalambu, Kasalat, Sunduh, Tongkol Pudak, Buntut Kuda, Buyung Habang, Camik Pendek, Sutera.

Adaptability of local upland rice to Al stress

Root and shoot performances were affected by the Al treatment, the accessions, and the interaction between these two factors. Al stress causes different impacts on the root and shoot growth of rice seedlings. The stress significantly affects the root growth but has no significant effects on the shoot length and shoot fresh weight. The accessions significantly affect all observation variables. The interaction between Al and accession significantly affects almost all root and shoot variables, except for shoot fresh weight and shoot dry weight (Table 5).

Previous studies also showed the same results as Novelia et al. (2019) and Putra et al. (2021), where the concentration of Al in the nutrient solution significantly decreased root growth, shoot length, fresh weight, and dry weight of plants.

Table 5. Variance analysis of Al, accession, and Al x accession interaction on the growth of Kalimantan local upland rice in Yoshida nutrient solution.

Source of variation	Mean square					
	Root length	Shoot length	Root fresh weight	Shoot fresh weight	Root dry weight	Shoot dry weight
Al	81.0414**	16.0983^{ns}	0.0399**	0.0112^{ns}	0.0008**	0.0016**
Accession	6.7220**	76.4253**	0.0019**	0.0154**	0.0004**	0.0005**
Al x Accession	3.7143**	12.8499*	0.0007*	0.0025^{ns}	0.0004**	0.0001^{ns}
CV (%)	24.54	18.37	56.51	32.02	189.49	30.789

Note: ** = significant at P<0.01; * = significant at P<0.05; ns = not significant; CV = coefficient of variation

In general, the stress of 45 ppm Al significantly affects RL, RFW, RDW, and SDW in all accessions. However, these variables can not be used directly to determine the tolerance of accessions. The accessions used in this study are natural populations, which means they are genetically different. Thus, those characters are not appropriate to be used as selection criteria. The relative value of each variable is more reliable and more accurately determines the response of all variables observed.

The relative values of root and shoot length, fresh weight, and dry weight show us the comparison of each variable under stress and control conditions. These relative values of root and shoot can differentiate the response of the two check varieties to Al stress. Dupa, the tolerant check, is among the five highest values of the RRFW, but not for the five other variables. Whereas Kasalat, the sensitive check, is among the five lowest values of the RRDW and RSDW, but not for the four other variables. Moreover, for RRL and RSL, the smallest value is not small. The majority of the other 91 rice accessions also showed a high value of the six variables. The five highest values are mostly higher than 1, except for the RRFW. On the other hand, the five smallest values are not small (Table 6; Table 7; Table 8). Considering this situation, the determination of the rice tolerance should be estimated by referring to the relative values of each variable and the check varieties as well.

The high values of the RRL, RRFW, RRDW, RSL, RSFW, and RSDW may reveal the high tolerance to Al stress. If we determine the tolerance only based on these values, the majority of the accessions will be categorized as tolerant. Even the sensitive check Kasalat will also be falsely categorized as tolerant because this variety has quite a high value of RRL and RSL, and did not show sensitive symptoms of the Al stress. However, when the sensitive variety did not show sensitive symptoms, this means that probably the concentration used in the treatment is less toxic for the rice accessions. This concentration of 45 ppm Al was rarely used in the screening of rice. Generally higher concentration is used (Hidayatun et al., 2017; Prasetiyono et al., 2012).

Al stress on the roots of rice plants can cause two opposite effects on root growth. Al can cause different effects, based on its concentration. Al can damage the roots and inhibit their growth or stimulate the roots to grow. Al toxicity has occurred when a certain amount of Al^{3+} exists surrounding the root plants. Conversely, when the concentration is below the ambient level, Al can give an opposite effect. Low Al concentrations can be a valuable element for several types of plants, including rice (Moreno-Alvarado et al., 2017). Al stimulation on rice growth was previously reported (Hidayatun et al., 2017). This phenomenon of growth stimulation has probably occurred in this study. Instead of growth inhibition, the accessions experienced growth stimulation.

Table 6. Relative root length and shoot length values of the 5 highest and 5 lowest.

Accession	0 ppm	45 ppm	RRL	Accession	0 ppm	45 ppm	RSL
Pulut Olan	6.50 a-h	11.21 a	1.73	P. Pulut Ayang/Pang	6.03 a-h	33.69 a-d	5.59
Benyahi	5.77 a-h	9.65 a-d	1.67	Ketan Beranakan	4.88 e-h	27.18 a-n	5.57
Jawa Wakai	5.54 c-h	9.01 a-g	1.63	Sampit	5.18 e-h	28.65 a-n	5.53
Padai Ivan	5.76 a-h	8.80 a-h	1.53	Benyahi	5.77 a-h	31.22 a-f	5.41
Buntut Kuda 1	6.38 a-h	9.36 a-e	1.47	Pitik	5.21 e-h	27.44 a-n	5.27
Mayas	6.47 a-h	5.14 k-r	0.79	Kantul	8.35 a	27.39 a-n	3.28
Padai Samarinda	5.55 c-h	3.90 q-r	0.70	Munau	7.11 a-f	23.14 e-p	3.26
Padi Timai Ladang	4.83 f-h	3.35 r	0.69	Ketan Jarum	6.83 a-h	21.98 g-p	3.22
Kantul	8.35 a	5.42 j-r	0.65	Batakan	6.83 a-h	20.97 j-p	3.07
Talun Ratu	6.91 a-g	4.35 o-r	0.63	Sasak Jalan	8.12 a-c	24.81 d-p	3.05

Note: Numbers followed by the same letter in the same column are not significantly different based on the DMRT test at the 5% level; RRL = relative root length; RSL = relative shoot length.

Table 7. Relative root fresh weight and shoot fresh weight values of the 5 highest and 5 lowest.

Accession	0 ppm	45 ppm	RRFW	Accession	0 ppm	45 ppm	RSFW
Padi Merah	0.042 d-h	0.156 a	3.69	Labu Banjar	0.179 d-m	0.252 a-h	1.41
Labu Banjar	0.044 d-h	0.105 a-c	2.37	Raya	0.207 b-m	0.286 a-c	1.38
Padi Aim	0.042 d-h	0.093 b-f	2.23	Parai Paliping	0.208 b-m	0.283 a-d	1.36
Parai Paliping	0.044 d-h	0.089 b-f	2.03	K. Puyuk	0.151 g-m	0.199 b-n	1.32
Dupa	0.018 h	0.035 d-g	1.99	Ketan Bahandang	0.164 f-m	0.212 b-n	1.29
P. Pulut Ayong	0.087 a-c	0.074 b-g	0.85	Padi Samarinda	0.188 b-m	0.130 k-r	0.69
Padi Samarinda	0.045 d-h	0.037 c-g	0.82	Padai Timai Ladang	0.158 f-m	0.107 m-r	0.68
Rendi Lau	0.072 a-e	0.059 b-g	0.81	Ketan Hitam	0.176 e-m	0.116 l-r	0.66
Carnik Pendek	0.028 g-h	0.020 g	0.71	Carnik Pendek	0.111 m-l	0.071 r	0.64
Batakan	0.051 c-h	0.036 d-g	0.70	P. Pulut Ayong	0.368 a	0.232 a-k	0.63

Note: Numbers followed by the same letter in the same column are not significantly different based on the DMRT test at the 5% level; RRFW = relative root fresh weight; RSFW = relative shoot fresh weight.

Table 8. Relative root dry weight and shoot dry weight values of the 5 highest and 5 lowest.

Accession	0 ppm	45 ppm	RRDW	Accession	0 ppm	45 ppm	RSDW
Labu Banjar	0.009b	0.014b	1.64	Labu Banjar	0.034 c-l	0.045 a-g	1.32
Ketan Bahandang	0.007b	0.011b	1.58	Raya	0.040 b-j	0.051 a-c	1.28
Padai Cina	0.012b	0.018b	1.57	Sabai Papan	0.024 h-l	0.030 h-s	1.28
Sabai Papan	0.005b	0.008b	1.52	Ketan Bahandang	0.031 d-l	0.039 b-m	1.25
P. Nyuhu	0.011b	0.017b	1.52	Parai Paliping	0.038 c-l	0.047 a-e	1.25
Ketan Maronto	0.009b	0.007b	0.79	Kasalat	0.024 h-l	0.016 p-s	0.67
Kasalat	0.007b	0.005b	0.73	P. Pulut Ayong	0.065 a	0.041 a-j	0.64
Padi Samarinda	0.011b	0.008b	0.71	Sabai Kecil	0.046 b-g	0.029 h-s	0.63
Sabai Kecil	0.020b	0.010b	0.49	Sasak Jalan	0.058 a-b	0.036 b-o	0.61
Padai Timai Ladang	0.050b	0.006b	0.13	Padai Timai Ladang	0.033 c-l	0.018 o-s	0.56

Note: Numbers followed by the same letter in the same column are not significantly different based on the DMRT test at the 5% level; RRDW = relative root dry weight; RSDW = relative shoot dry weight.

Despite the above-mentioned effects, the data can be used to estimate the tolerance of the accessions. Based on the RRL, the lowest value was found on Talun Ratu (0.63). The RRL of these accessions is below the RRL value of the sensitive check, Kasalat. However, only Talun Ratu showed a really small RRL value. On the other hand, the majority of accessions have high RRL value. Pulut Olan has the highest RRL value of 1.73. Dupa was not among the five highest RRL values, which means that many varieties might have higher tolerance levels than Dupa (Table 6). The RSL value shows that almost all accessions are tolerant to Al stress, with the values more than one. Padi Merah occupied the highest value of RRFW, whereas Labu banjar occupied the highest value of RSFW, but this accession Labu Banjar is also among the highest values of the RRDW and RSDW. Whereas, the lowest value of the RRDW and RSDW was found at Padai Timai Lading (Table 7; Table 8).

Al effects on root and shoot are quite different. The RRL, which is widely used as a primary parameter in determining rice response to Al stress, showed different impacts on rice accessions. Exposure to Al toxicity disrupts plant metabolism by reducing the absorption of water and mineral nutrients, thus inhibiting plant growth (Roy & Bhadra, 2014). This is because the plant needs to have the ability to adapt to high Al levels. High Al treatment can cause more severe root damage, resulting in a more significant reduction in root length and weight than in Al-tolerant plants (Hanum et al., 2009). Generally, moderate and tolerant accessions can prevent Al from entering the root vessels (Indrayani et al., 2017). Plants with long and good roots show better adaptability when exposed to Al stress (Anggraheni & Mulyaningsih, 2017). Rice genotypes have an adaptation mechanism to Al stress by using more energy for root growth than shoot growth (Utama, 2010).

Low Al concentrations can be a valuable element for several types of plants, including rice. But, when its concentration increases, Al will be toxic and inhibit plant growth (Moreno-Alvarado et al., 2017). Plants that are tolerant of Al toxicity can prevent the adverse effects of Al toxicity. Some criteria for tolerant plants, according to (Purnamaningsih & Mariska, 2008), are (1) roots can grow continuously and the root tips are not damaged, (2) able to change the pH in the root area, and (3) have a specific mechanism where Al cannot inhibit the uptake of Ca, Mg, and K so that plants can still meet their nutritional needs.

The provided Table 9 categorizes different adaptive response groups of rice accessions under 45 ppm aluminum (Al) stress based on various traits, including root length (RL), shoot length (SL), root fresh weight (RFW), shoot fresh weight (SFW), root dry weight (RDW), and shoot dry weight (SDW). The categorization of adaptive response groups provides valuable insights for future breeding programs. Breeding efforts can be tailored based on the level of adaptability observed in each group, with a focus on enhancing specific traits that contribute to aluminum stress tolerance in rice.

Table 9. Grouping of adaptation levels of Kalimantan local upland rice.

No.	Adaptation group	RL	SL	RFW	SFW	RDW	SDW
1	Not adapted	14	7	36	11	9	10
2	Low adaptive	21	16	38	33	40	28
3	Moderately adaptive	22	32	8	36	29	38
4	Adaptive	28	27	0	9	11	12
5	Highly adaptive	8	11	1	4	4	5

Note: RL = root length; SL = shoot length; RFW = root fresh weight; SFW = shoot fresh weight; RDW = root dry weight; SDW = shoot dry weight.

In this study, the rice shows adaptive responses to the treatment levels at 45 ppm Al stress. Among the total 93 rice accessions observed, 36 accessions are categorized as adaptive in the character of root length, 38 accessions in the shoot length, 1 accessions in the character of root fresh weight, 13 accessions in the shoot fresh weight, 15 accessions in the character of root dry weight, and 17 accessions in the character of shoot dry weight (Table 9). This shows that these accessions can adapt to 45 ppm Al stress. These adaptive responses provide insights into the genetic diversity within the rice accessions under 45 ppm aluminum stress. Future breeding programs can leverage this information to develop rice varieties with enhanced tolerance to aluminum stress, focusing on traits such as root and shoot length, fresh weight, and dry weight. Al stress in the early growth phase can be easily observed in the roots because the roots are the part of the plant directly affected by Al toxicity (Aini et al., 2019). According to Yartiwi et al. (2018), the root is the first part to come into contact with Al and gives a relatively fast reaction, so that it can be used to identify Al-tolerant plants.

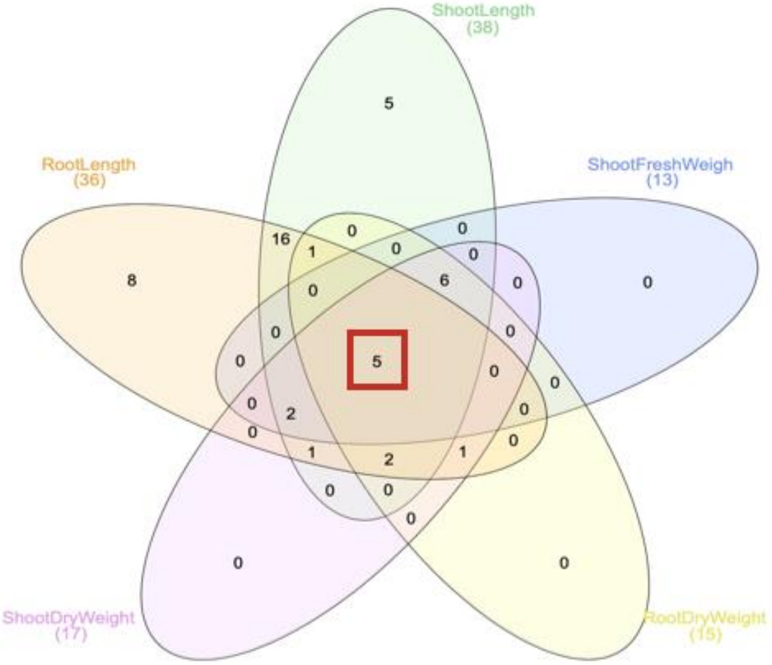

Figure 2. Venn diagram of the adaptive character of 45 ppm Al. The number in the red box showed adaptive accession based on oot length, shoot length, shoot fresh weight, root dry weight, and shoot dry weight.

Figure 2 shows that of the accessions included in the adaptive category, five accessions are adaptive to the parameters of root length, shoot length, shoot fresh weight, root dry weight, and shoot dry weight. These adaptive accessions are P. Pulut Timai, Padai Cina, Raya, Parai Paliping, and P. Pulut Ayang/Pang. The use of varieties or genotypes adaptive to abiotic stress is essential in rice cultivation.

Estimated heritability values of the rice genotypes on RL and SL belonged to high criteria, and SFW showed moderate criteria, which means these criteria provide opportunities for improvement through selection, while RFW, RDW, and SDW belonged to low criteria (Table 10). According to Marsinah et al. (2021), selection effectiveness can be improved by considering the expected value of moderate to high heritability. High heritability traits are those for which selection will be effective due to the small influence of the environment and the greater dominance of genetic variables in the development of plant genotypes. Selection will be less successful for traits with low heritability because environmental factors have a more significant influence on the phenotypic appearance of plants than genetic factors.

The coefficient of genetic diversity of Kalimantan local upland rice on the parameters of root length, shoot length, and shoot fresh weight showed the criteria for a moderate level of genetic diversity (Table 10). According to Marsinah et al. (2021), a low level of diversity indicates a tendency for individuals in the tested population to tend to be uniform, while the value of moderate to high diversity indicates that individuals in the tested population need to be improved at the next stage. The coefficient of genetic diversity is a matrix to assess the genetic diversity of an observed character. Traits with a significant coefficient of genetic diversity will be more likely to succeed if genetic manipulation is carried out. Conversely, traits with a slight genetic diversity coefficient will have a meager chance of success if the traits are improved.

Table 10. Broad sense heritability values (h^2_{bs}) and genetic coefficient of variation (GCV).

No.	Characters	σ^2_g	σ^2_p	h^2_{bs}	Criteria	GCV	Criteria
1	Root length (cm)	1.0280	1.6805	0.612	hight	11.681	medium
2	Shoot length (cm)	13.4106	19.1063	0.702	hight	11.151	medium
3	Root fresh weight (g)	0.0002	0.0021	0.112	low	0.000	NA
4	Shoot fresh weight (g)	0.0030	0.0068	0.435	medium	15.771	medium
5	Root dry weight (g)	0.0000	0.0005	0.016	low	0.000	NA
6	Shoot dry weight (g)	0.0000	0.0005	0.180	low	0.000	NA

Note: σ^2_g = genetic variance; σ^2_p = phenotypic variance; h^2_{bs} = heritability. Heritability (h^2_{bs}) criteria: low = <0.2; medium = >0.2->0.4; high = >0.5. GVC = coefficient of genetic diversity; GCV criteria, low: 0%-6.8%; medium: 6.9%-13.6%; moderately high: 13.7%-22%; high: > 22%. NA = data is not worth analyzing because the treatment mean square is smaller than the mean square error.

CONCLUSIONS

Upland rice accessions from Kalimantan Island have relatively high genetic diversity. The majority of the accessions are tolerant to 45 ppm Al stress, as revealed by the root and shoot responses. Among the parameters observed, root length, shoot length, and shoot fresh weight are considered the most suitable for studying rice adaptability to Al toxicity. Among the eleven SSR markers used, the SSR markers RM5, RM11, RM154, RM215, RM259, RM287, and RM514 are the most suitable for studying rice genetic diversity. P. Pulut Timai, Padai Cina, Raya, Parai Paliping, and P. Pulut Ayang/Pang have high adaptability to Al stress, and these accessions have the potential to be used as candidates for the development of Al-tolerant varieties. The information on the genetic diversity of the rice accessions, along with their adaptability to Al stress, can be used to support the breeding program, especially for the development of rice varieties targeting acidic soil conditions. However, to determine the most promising accessions, further studies are needed. In the genetic study, markers associated with Al tolerance should be used; while in the physiology study, a higher Al concentration should be used for treatment.

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