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Research Article

Utilization of amelioration and bioremediation to reduce Al stress in upland rice “Inpago 12”

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ABSTRACT

The contribution of rice production in drylands (upland rice) to national rice production is still low, due to several limiting factors including limited water, low pH, and low soil fertility. This study aimed to obtain improved recommendations for amelioration and bioremediation in drylands with Al stress to enhance rice production. The upland rice variety of Inpago 12 was planted at Taman Bogo Experimental Farm, East Lampung in the first planting season of 2020. The experiment was arranged in a split-plot design with three replications. The main plot was the application of amelioration materials consisting of control (without amelioration), $CaMg(CO_3)_2$*,* $CaCO_3$*, and rock phosphate. The subplot was the application of bioremediation materials consisting of control (without bioremediation), biofertilizer, endophytic microbes, and citric acid. Results showed that amelioration and bioremediation on dry land with low and high Al stress did not significantly increase the growth of rice plants. However, bioremediation in the form of citric acid on land with low Al stress significantly increased grain production by 6.21% than without bioremediation.*

Keywords: Aluminium, dryland, limited water, rice production, low soil fertility, low pH

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INTRODUCTION

The conversion of irrigated paddy fields of lowland into non-agricultural areas is a challenge to maintan rice production in Indonesia (Paiman et al., 2020). To coop with such problem, rice extensification to sub-optimal land is one of solutions (Lakitan et al., 2018). One of the potential sub-optimal lands to be developed as agricultural land is dryland (Rawung et al., 2021), or *tegalan* and *ladang* in Indonesian. The area of dryland in Indonesia is around 17 million ha suitable for agriculture production (Mulyani & Sarwani, 2013).

Most of the drylands in Indonesia are classified as acidic (Kuswantoro, 2014). From 17 million ha about 44% are acid drylands, and only 2.2% are non-acid drylands (Mulyani & Sarwani, 2013). Acidic drylands have pH < 5 and rainfall > 2.000 mm per year (Berek, 2019). It is has been known that process of acid soil formation in dryland relates to rainfall intensity, where the higher the rainfall the higher the level of soil weathering meaning soil acidity. In acid soil of dryland, nutrients leaching is very intensive resulted in low base saturation and high aluminum saturation (Mahanani et al., 2020; Fendiyanto et al., 2021).

Acid soils can affect the replacement of exchangeable base cations such as calcium (Ca^{2+}), magnesium (Mg^{2+}), and potassium (K^+) by H^+ and Al^{3+}, and the dissolution of Al and Mn minerals, as well as the dissolution of Fe-containing minerals, are significant aspects of soil acidification (Goulding, 2016). This can result in toxicity by certain metals namely, Mn, Fe, and Al as well as nutrient imbalances especially P in acid soils, where Al toxicity is the most significant threat to plant survival in acid soils (Bojórquez-Quintal et al., 2017).

One solution to tackle the issue of dryland agriculture is by using Al-tolerant varieties, such as Inpago 12 (Hairmansis, 2019). This approach is considered effective and efficient in increasing rice productivity in low-pH soils. The yield loss due to aluminum toxicity varies depending on the soil aluminum saturation, plant species, and specific varieties used (Kochian et al., 2015). Rice crops still experience a yield loss of up to 30% when using tolerant varieties on land with certain element toxicity (de Freitas et al., 2017; Mahender et al., 2019). Therefore, efforts are needed to modify the growing environment to maximize the potential yield of tolerant varieties and support growth and yield for vulnerable varieties when planted in acidic soil with aluminum toxicity.

Several cultivation strategies can be implemented to overcome the problem of Al toxicity in drylands. These strategies include the use of P fertilizer and amelioration by liming with $CaCO_3$ or $CaMg(CO_3)_2$ (dolomite) to reduce Al accumulation in the soil (Chen & Liao, 2016). Liming, by adding $CaCO_3$ or $CaMg(CO_3)_2$, increases soil pH and converts soluble Al into less soluble forms that are not toxic to plants (Husain et al., 2021; Mahmud & Chong, 2022). However, it should also be noted that liming and the use of P fertilizer in the long term can have an impact on high production costs and environmental health (Holland et al., 2019).

Bioremediation refers to the technique of using living organisms, typically microbes, to metabolize and thereby decrease or neutralize harmful environmental contaminants (Indu, 2020). Bioremediation serves as a potential alternative due to its relative cost-effectiveness, eco-friendly nature, and significant removal efficiency (Purwanti et al., 2019). Certain microorganisms are capable of adapting to and resisting contaminants. Biofertilizers are substances that contain living microorganisms, such as bacteria, fungi, or algae, which can improve soil fertility by increasing the availability of essential nutrients (Vessey, 2003). In some cases, the organisms in biofertilizers can also help to degrade or detoxify pollutants in the soil, making them a form of bioremediation (Raklami et al., 2022; Fouad et al., 2023). Endophytic microbes, a group of fungi that form a mutualistic relationship with many plant species, could play a role in bioremediation as well (Spagnoletti & Giacometti, 2020).

The use of organic acids, one of which is citric acid, is expected to be an alternative in dealing with Al toxicity in agricultural land (Kaur et al., 2018; Tahjib-Ul-Arif et al., 2021). Citric acid is one organic chelating agent that is harmless to the environment (Shinta et al. 2021). It can bind to metal ions, such as Al, forming a complex that can be more easily mobilized or extracted from the environment (Yang et al., 2020; Lee & Khor, 2023). At 2 mM Al stress, citric acid increased epicotyl length, sprouts, and roots by 11–26%. At 5 mM Al stress, citric acid increased the length of epicotyls, sprouts, and roots by 19–60%. In addition, citric acid also increased fresh weight up to 12%, but decreased chlorophyll content. Under normal conditions (no Al stress) citric acid did not affect the growth of soybean sprouts (Abdullahi et al., 2004).

Previous research conducted on a greenhouse scale showed that amelioration and bioremediation aimed at reducing the exchangeable-Al content in soil for rice plants appeared to work well. Compared to the control, the exchangeable-Al content in all treatments appeared to decrease. Amelioration using agricultural lime, in the form of $CaMg(CO_3)_2$ and $CaSiO_3$, respectively, can reduce exchangeable-Al by 20.22% and 17.77%. It is also evident that adding organic acid to the $CaMg(CO_3)_2$ treatment and using endophytic nutrient solubilizing microbes in the $CaSiO_3$ treatment increase the effectiveness of reducing Al stress (Margaret et al., 2019). This study aimed to obtain improved recommendations for amelioration and bioremediation in drylands with Al stress to enhance rice production.

MATERIALS AND METHODS

The research was carried out during the initial planting season of the region between February and May 2020. The research was conducted in two locations of the Taman Bogo Experimental Farm under the jurisdiction of the Indonesian Soil Research Institute, located in Purbolinggo District, East Lampung, Lampung Province. The research was funded by the Indonesian Agency for Agricultural Research and Development in 2019.

Soil is categorized as Ultisol. The first location had a moderate saturation of Al (low Al-stress) and second location had high saturation of aluminum (high Al-stress). The experiment was arranged in a split-plot design with three replications. The main plot treatment was ameliorant material, while the subplot treatment was bioremediant material (Table 1).

Table 1. Treatments arrangement of farming technique for field-scale aluminum stress reduction in 2020.

Code	Treatment
Main plot (Amelioration)	
A1	Control (without amelioration)
A2	$CaMg(CO_3)_2$ 2 tons ha^{-1}
A3	$CaCO_3$ 2 tons ha^{-1}
A4	Rock phosphate 1 tons ha^{-1}
Subplot (Bioremediation)	
B1	Control (without bioremediation)
B2	Biofertilizer
B3	Endophytic microbes
B4	Citric acid

Tillage was done manually by hoeing in two steps, namely: 1) at the beginning of the rainy season or after the first rain, 2) before planting was done with minimum tillage. The new superior variety of upland rice Inpago 12 was planted with a distance 2:1 in “jajar legowo” method. Fertilizer application followed the recommendations of the upland soil test kit, known as the "perangkat uji tanah kering" (PUTK test), i.e., Urea 200 kg ha^{-1}, SP-36 200 kg ha^{-1}, and KCl 100 kg ha^{-1}. Weed control was done mechanically, whereas pest and disease was controlled by utilizing light traps.

Observations were conducted on soil characteristics, growth (height, number of tillers per m^2, root volume), yield components, and yield (number of panicles per m^2, number of grains per panicle, percent of filled grains, 1,000-grain weight and dry grain yield) as well as scoring of scale, symptoms and criteria against Al toxicity based on the standard evaluation system (IRRI, 2013). The results were converted from the sampling area of the tiller to the ha area with 14% moisture content. The data collected were analyzed by variance analysis and if there were significant effects, it would be followed by the Duncan Multiple Range Test (DMRT) at a 5% level.

RESULTS AND DISCUSSION

Soil chemical and physical properties

The research was conducted in two locations with moderate to high levels of Al saturation. The chemical and physical properties of soil samples taken compositely are presented in Table 2. The soil samples collected from the land with moderate Al content had a clayey texture, with a higher proportion of clay fraction (54%) than dust and sand fractions. The soil C-organic content, N-total, and cation exchange capacity (CEC) were low, with values of 1.41%, 0.12%, and 9.78 cmolc kg^{-1}, respectively. On the other hand, the soil samples from land with high Al content showed a sandy clay texture, dominated by the sand fraction (51%), followed by clay (27%) and dust (22%). These results suggest that the soil is infertile, low in organic matter, and has an acidic pH (Peverill et al., 1999).

The degree of soil acidity, as indicated by soil pH, was categorized as very acidic with a value of 4.3 at both locations. This acidity is caused by the exchange complex on the surface of colloids and soil solutions, which is dominated by Al^{3+} cations. The high Al saturation parameter (27.06) further supports this observation. Additionally, the contents of C-organic, N-total, Na, Ca, Mg, and CEC were very low. The low CEC value is attributed to the predominance of sand fraction particles, which have a small colloidal surface area (Meimaroglou & Mouzakis, 2019). Base saturation is an important indicator of soil fertility. As shown in Table 2, the base saturation values for both locations were low to moderate, with a value of 30-45. A soil is considered very fertile if its base saturation is ≥80%, moderately fertile if it is between 50-80%, and infertile if it is ≤50% (Bünemann et al., 2018).

In sandy soils with low pH, Al becomes more soluble and can be toxic. It can bind to organic matter and clay, but in soils dominated by sand with limited clay or organic matter, the toxic Al^{3+} ions are more likely to be freely available to harm plant roots. Al binding can lead to P deficiency by forming insoluble complexes with it, further reducing the already low nutrient-holding capacity of sandy soils (Saentho et al., 2022). While Al's impact on soil structure might be less pronounced in sandy soils, its interactions with nutrients and plant roots still affect overall soil fertility. The addition of organic matter can sometimes mitigate Al toxicity, but this may be limited in soils already dominated by sand and low in organic matter.

Table 2. Soil properties of two sites in Taman Bogo Experimental Farms, Lampung, 2020.

No	Parameter	Site 1 (Low Al-stress)		Site 2 (High Al-stress)	
		Value	Criteria	Value	Criteria
1	Texture (%)				
	Sand	19.00	Clay	51.00	Sandy clay
	Dust	27.00		22.00	
	Clay	54.00		27.00	
2	pH				
	Actual	4.90		4.30	Very acid
	Potential	4.20	Very acid	3.80	Very acid
3	C-org (%)	1.41	Low	0.76	Very low
4	N-total (%)	0.12	Low	0.07	Very low
5	Available-P (ppm)	13.70	High	9.90	Moderate
6	Available-K (ppm)	27.00	Moderate	17.00	Low
7	Cation Exchange Capacity ($cmol_c\ kg^{-1}$)	9.78	Low	4.73	Very low
8	Na ($cmol_c\ kg^{-1}$)	0.02	Very low	0.06	Very low
9	Ca ($cmol_c\ kg^{-1}$)	3.59	Low	1.10	Very low
10	Mg($cmol_c\ kg^{-1}$)	0.73	Low	0.22	Very low
11	Base saturation	45.00	Moderate	30.00	Low
12	Al^{3+}	0.00		1.28	
13	Al saturation	0.00	Very low	27.06	High

Source: Indonesian Soil Research Institute (ISRI) Laboratory, 2020.

Plant growth

The study on plant growth, considering factors like plant height, number of tillers, and root volume, found no significant impact from amelioration and bioremediation treatments in varying Al stress conditions (Table 3). According to the coefficient of variance (CV), plant height had the least variability, making it relatively stable across treatments. Number of tillers showed moderate variability, more so under high Al saturation. Root volume exhibited the most variability, especially in high Al conditions, making it the most sensitive parameter. This sensitivity can be attributed to Al toxicity's primary impact on the root system, given that roots are the first plant organs to encounter Al^{3+} ions in the soil, as they are in direct contact with the rhizosphere. The effects of this exposure can be wide-ranging, affecting nutrient uptake, growth, and overall plant health.

This suggests root volume could be a key focus for assessing the efficacy of different treatments.

Plant height in the amelioration treatment ranged from 119.41-123.41 cm, while in the bioremediation ranged from 120.74-122.01 cm. Similarly, in land with high Al stress soil characteristics, the analysis of variance showed that application of ameliorant did not significantly increase plant height posture. The range of plant height at harvest was 121.70-123.16 cm, and the growth of plant height at harvest age did not show significant differences. It is worth noting that while plant height is used as a growth parameter, it does not always guarantee higher yields (Abdulrachman et al., 2014; Brito et al., 2018). In high Al-stress soil, endophytic microbes showed a promising potential to increase rice plant height. Endophytic microbes have shown the ability to adapt to high soil acidity, high Fe and Al, and dissolve P from Fe-P and Al-P forms, as well as spur plant growth under these abiotic stress conditions (Yuniarti & Susilowati, 2021).

Table 3. Plant height, number of tillers, and root volume in fields with low to moderate Al stress soil characteristics and high in 2020 first planting season.

Treatment	Low Al-stress			High Al-stress		
	Plant height (cm)	Number of tillers per m^2	Root volume (mL)	Plant height (cm)	Number of tillers per m^2	Root volume (mL)
Amelioration						
Without amelioration	123.41	140.00	500.00	121.86	152.00	466.67
$CaMg(CO_3)_2$ 2 tons ha^{-1}	119.41	139.00	466.67	121.70	159.00	455.56
$CaCO_3$ 2 tons ha^{-1}	121.11	147.00	455.56	123.16	151.00	422.22
Rock phosphate 1 tons ha^{-1}	122.48	146.00	477.78	121.90	152.00	433.33
Bioremediation						
Without bioremediation	121.73	140.00	466.67	122.84	153.00	411.11
Biofertilizer	122.01	141.00	433.33	120.37	147.00	444.44
Endophytic microbes	120.74	144.00	488.89	125.13	154.00	422.22
Citric acid	121.93	147.00	511.11	120.27	161.00	500.00
Average	121.60	143.00	474.99	122.15	154.00	444.44
CV (%)	3.14	9.58	32.66	3.53	11.30	40.92

Note: Values followed by the same letter in the same column are not significantly different based on DMRT at 0.05 level.

Specific treatments, such as $CaCO_3$ 2 tons ha^{-1} in low Al soil and $CaMg(CO_3)_2$ 2 tons ha^{-1} in high Al soil, slightly increased number of tillers, with citric acid appears to be the most effective under both conditions. There was more variability in high Al soil (11.30%) compared to low Al soil (9.58%), but neither amelioration nor bioremediation significantly impacted tiller growth. This lack of effect might be due to other influencing factors or possibly insufficient doses or materials used in the treatments.

Root volume remained generally unaltered with amelioration treatments, except for a notable response to rock phosphate. This might be attributed to its specific properties or synergistic effects, as evidenced in studies where it increased nutrient uptake while decreasing metal absorption in specific plants (Fayiga & Ma, 2006). Bioremediation with citric acid emerged as a promising enhancer of root volume under high Al stress, possibly due to multifaceted mechanisms like aluminum chelation, soil pH modification, nutrient enrichment, microbial stimulation, and direct cellular impacts on roots (Tahjib-Ul-Arif et al., 2021). However, the inconsistency in high Al scenarios, indicated by a greater coefficient of variance, suggests a complex interaction that might require further investigation.

Al-toxicity score

Figures 1A and 1B provide insights into the score of aluminum (Al) toxicity in the shoot based on the standard evaluation system (IRRI, 2013), recorded at two-week intervals throughout the growth period. In the land with medium-low stress, the highest toxicity score of 7 (categorized as susceptible) was observed in the amelioration treatment using $CaMg(CO_3)_2$ and the bioremediation treatment using citric acid (Figure 1). These high toxicity scores indicate that these treatments were less effective in mitigating Al toxicity in the plants.

However, as the growth period progressed and approached harvest, the toxicity scores decreased in all treatments, resulting in a shift toward the resistant category. Despite the reduction in toxicity, the treatment with the highest aluminum toxicity score remained the amelioration treatment using $CaMg(CO_3)_2$ or known also as dolomite. This suggests that while the overall toxicity decreased, the $CaMg(CO_3)_2$ treatment still exhibited some residual toxicity effects.

In the bioremediation treatment, the biofertilizer showed the highest toxicity score, with a value of 5, categorizing it as somewhat vulnerable. This indicates that the bioremediation approach using the biofertilizer may have had a moderate impact on reducing Al toxicity but was not as effective as desired. These findings highlight the challenges in mitigating Al toxicity in the shoot and suggest that the amelioration treatment with $CaMg(CO_3)_2$ and the bioremediation treatment with citric acid or the biofertilizer did not fully eliminate the negative effects of Al toxicity throughout the growth period.

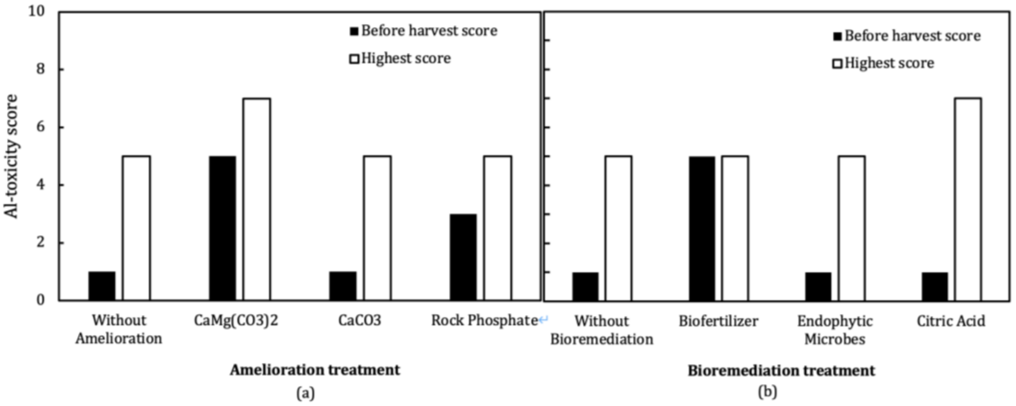

Figure 1. Effect of amelioration (a) and (b) bioremediation to Al-toxicity score on moderate to low Al-stress soil at Taman Bogo, 2020.

Yield component

The statistical analysis conducted on lands with low to high Al stress soil characteristics revealed interesting findings regarding the effects of amelioration and bioremediation treatments on various yield parameters (Table 4). In the case of the land with low Al stress, the results indicated that the amelioration treatment did not yield significant effects on the number of panicles per m^2, the percentage of filled grains, and the weight of 1,000 grains. The bioremediation treatment only showed a significant difference in the number of grains per panicle parameter. The lack of significant effects on certain yield parameters suggests that the plants may not have fully optimized their absorption of the materials used in the treatments. Consequently, this may have led to lower productivity compared to the control plots. Despite this, it is important to note that the average values for each parameter were recorded. In this case, the average values for the land with moderate Al stress were 97 panicles per m^2, with 139 grains per panicle, a percentage of filled grains at 68.21%, and a 1,000-grain weight of 19.67 g. These values provide a baseline understanding of the yield potential under the given conditions.

In the lands with high Al stress soil characteristics, neither the amelioration nor the bioremediation treatments exhibited significant effects on any of the yield component parameters. The average values for each parameter, in this case, were as follows: 92 panicles per m^2, with 127 grains per panicle, a percentage of filled grains at 69.18%, and a 1,000-grain weight of 20.77 g. These findings suggest that the amelioration and bioremediation treatments employed in this study did not result in significant improvements in yield parameters under the given soil conditions with moderate to high

Al stress. Further research and exploration of alternative treatment methods may be necessary to identify more effective strategies for enhancing crop productivity in these challenging soil conditions.

Table 4. Number of panicles per m^2, number of grains per panicle, percentage of filled grain, and 1,000 grain weight in the first planting season 2020 in low to high Al-saturated soil.

Treatment	Low Al-stress				High Al-stress			
	Number of panicle per m^2	Number of grain per panicle	Filled grain (%)	1,000 grain weight (g)	Number of panicle per m^2	Number of grain per panicle	Filled grain (%)	1,000 grain weight (g)
Amelioration								
Without amelioration	106a	134a	69.25a	20.33a	90a	126a	65.75a	20.65a
$CaMg(CO_3)_2$ 2 tons ha^{-1}	96a	146a	67.12a	19.80a	96a	134a	67.09a	20.68a
$CaCO_3$ 2 tons ha^{-1}	90a	133a	71.49a	18.72a	95a	125a	72.37a	20.79a
Rock phosphate 1 tons ha^{-1}	95a	142a	65.18a	19.77a	89a	124a	71.51a	20.81a
Bioremediation								
Without bioremediation	103a	150a	65.34a	19.83a	88a	126a	69.70a	20.73a
Biofertilizer	101a	129a	70.51a	20.40a	89a	138a	69.57a	20.77a
Endophytic microbes	94a	139ab	66.95a	18.56a	94a	123a	69.63a	20.85a
Citric acid	90a	137ab	70.41a	19.98a	98a	122a	67.82a	20.59a
Average	97.00	139	68.21	19.67	92	127.00	69.18	20.73
CV (%)	20.65	10.75	12.61	14.41	18.24	13.51	7.14	231.00

Note: Values followed by the same letter in the same column are not significantly different based on DMRT at 0.05 level.

Harvest yield

The statistical analysis results presented in Table 5 suggest that the application of amelioration materials in all tested treatments did not elicit a significant response, with a yield range of 4.94–5.23 dry milled rice (DMR) tons ha^{-1}. However, the application of $CaMg(CO_3)_2$ resulted in a higher yield compared to both control plots and other treatments. These findings suggest that on land with very low aluminum stress, using tolerant varieties may be sufficient without the need for amelioration. In contrast, the bioremediation treatment showed a significant response when citric acid was applied to the soil, resulting in the highest yield of 5.30 DMR tons ha^{-1} compared to the control plot and other treatments. This response could be attributed to citric acid's ability to reduce the solubility of Al in the soil while increasing the soil pH (Gondal et al., 2021). A decrease in soil Al solubility can improve plant rooting conditions, leading to increased productivity (Muktamar et al., 2016).

Table 5. Harvest yield of DMR (rice after drying, GKG in Indonesian) in low to moderate and high Al-saturation soil of dry land, 2020.

Treatment	DMR yield (tons ha^{-1})	
	Low Al-stress	High Al-stress
Main Plot		
Without ameliorant	5.19a	4.18a
$CaMg(CO_3)_2$ 2 tons ha^{-1}	5.23a	4.21a
$CaCO_3$ 2 tons ha^{-1}	5.06a	4.34a
Rock phosphate 1 tons ha^{-1}	4.94a	4.06a
Subplot		
Without bioremediant	4.99b	4.40a
Biofertilizer	5.13ab	4.16a
Endophytic microbes	4.99b	4.11a
Citric acid	5.30a	4.12a
Average	5.10	4.20
CV (%)	4.70	8.15

Note: Numbers followed by the same letter in the same column are not significantly different based on DMRT at 0.05 level.

Citric acid might not have been able to alleviate Al stress during the vegetative phase due to the timing and severity of Al stress. This stress affects root growth and nutrient uptake, which in turn impairs vegetative growth (Malkanthi et al., 1995). If Al stress is severe during this period, citric acid might not have been able to fully mitigate the damage. On the other hand, the yield component of the plant might have been positively influenced by citric acid. This influence could be due to improved nutrient uptake and soil structure facilitated by citric acid (Duarte et al., 2007). Moreover, the phenomenon of stress priming might play a role; citric acid treatment during the vegetative phase might have induced physiological changes that aided the plants during their reproductive stage, leading to improved yield (Hilker & Schmülling, 2019; Tahjib-Ul-Arif et al., 2021).

Application of both amelioration and bioremediation materials did not increase grain yields significantly, with an average yield of 4.20 DMR tons ha^{-1}. This lack of response is likely due to the fact that the soil used had not received any soil amendments, as evidenced by the initial soil analysis, which showed very low soil fertility criteria. Therefore, intensive and continuous application of soil conditioners is necessary. Recently, it is difficult to find a type of soil conditioner capable of repairing and improving all soil functions (physical, chemical, and biological) with a single application (Dariah et al., 2015). Furthermore, it is suspected that the recommended doses of materials used in the 2019 greenhouse-scale research, when applied to field conditions, were insufficient to increase plant productivity due to various environmental factors such as weathering of minerals and rocks, and leaching.

CONCLUSIONS

The use of tolerant varieties on low Al stress land without amelioration has been successful in reducing the negative impact of aluminum stress. Additionally, providing bioremediation materials in the form of citric acid has proved to be effective only under low aluminum stress. This selective approach emphasizes the importance of tailoring interventions to specific soil conditions.

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