Buletin Peternakan 47 (4): 242-247, November 2023

ISSTAP
Indonesian Society for Sustainable Tropical Animal Production

Bulletin of Animal Science

ISSN-0126-4400/E-ISSN-2407-876X Accredited: 230/E/KPT/2022
http://buletinpeternakan.fapet.ugm.ac.id/

Doi: 10.21059/buletinpeternak.v47i4.84290

Tolerance Level of Butterfly Pea (*Clitoria ternatea* L.) to Stress Acidity Through Tissue Culture Technique

Kennaya Putri Aura Jannah*, Panca Dewi Manu Hara Karti, and Iwan Prihantoro

Department of Nutrition and Feed Technology, Faculty of Animal Science, Institut Pertanian Bogor, Bogor, 16689, Indonesia

Article history
Submitted: 8 May 2023
Accepted: 20 September 2023

* Corresponding author:
E-mail:
kennayaputrikennaya@gmail.com

ABSTRACT

Butterfly pea (*Clitoria ternatea* L.) a high-quality legume that is rich in protein and grows on various soil types with a pH range of 5.5-8.9. This experiment was conducted to get the level of tolerance of butterfly pea plants to stress acidity at different levels through tissue culture technique. The study was designed using a complete randomized design with 6 treatments with the different levels of $AlCl_3$ addition using Murashige Skoog (MS) media with 20 replications (P0 (0 ppm $AlCl_3$), P1 (100 ppm $AlCl_3$), P2 (200 ppm $AlCl_3$), P3 (300 ppm $AlCl_3$), P4 (400 ppm $AlCl_3$), and P5 (500 ppm $AlCl_3$)). Data were analyzed using analysis of variance (ANOVA), and if there was a significant difference, data were further analyzed using Duncan's multiple range test. The variables observed were acidity media changes, plant height gain, number of leaves, number of branches, number of tillers, percentage of leaves withering, and leaf color. The results showed that the butterfly pea plant has mechanism of adaptation to acid stress on the parameters of plant height gain and number of leaves at the end of the observation. However, the level of plant tolerance on the parameters of the number of branches and the number of tillers was ≤ 300 ppm (pH 3.73).

Keywords: Aluminium, Acid stress, Butterfly pea (*Clitoria ternatea* L.), Tissue culture

Introduction

Feed is an important factor in the maintenance and productivity of ruminants. According to Prayitno *et al*. (2018), one alternative that can be done is using legumes as a source of natural protein because legume plants can grow anywhere and have not been utilized optimally. One type of legume with good nutritional content especially high protein is butterfly pea.

Butterfly pea flower is a plant originating from Central South America and has spread to the tropics since the 19th century, especially to Southeast Asia, including Indonesia. It is one of the shrubs that commonly grows in Indonesia open spaces along roads and slopes (Cook *et al*., 2005). Butterfly pea has the characteristics of a perennial plant and has herbaceous habits, which has pinnate leaves, leaves with long petioles, pod-shaped fruits, and dark blue, purple, or white flower (Kosai *et al*., 2015). Butterfly pea is a source of leguminous forage, which has the potential as a source of forage for livestock because it has a high level of protein and digestibility (Sutedi, 2013).

Butterfly pea grows on various soil types, especially sandy soil and red clay with a pH range of 5.5-8.9 (Cook *et al*., 2005). The area of acid-dry land in Indonesia is around 108.8 million/ha, and around 62.6 million/ha has the potential as agricultural land. Various land conditions are caused by acid stress, so the land experiences changes in soil physiochemical status and nutrient deficits, which cause plant growth to be not optimal. (Mulyani and Sarwani 2013). Acidic soils generally has pH characteristics of 4.6 – 5.5, as well as fairly high clay content and relatively low potassium levels ranging from 0.1 – 0.2 me/100 g of soil (Putra and Hanum, 2018).

According to Kasno (2020), the main limiting factors for production on dry land are soil acidity and high Al content and low C-organic. Intensive management of acid-dry land without considering nutrient balance can lead to low C-organic content, primary and secondary macronutrients such as phosphorus (P), calcium (Ca), and magnesium (Mg), as well as increase soil acidity, and Al can be exchanged so that can cause toxins and disrupted plant growth so that plant productivity decreases significantly. Butterfly pea is a plant with low diversity, so it has not developed in other areas (acid soil and saline soil). For this reason, it is necessary to determine the tolerance limit of the butterfly pea plant to acid stress with tissue culture.

Tissue culture is a method for isolating plant parts such as protoplasm, cells, tissues, and organs and growing them under aseptic conditions so that these parts can multiply and regenerate into whole plants again (Ziraluo, 2021). Butterfly pea

has a relatively low diversity of production in Indonesia. Acid stress in the soil is caused by the high aluminium content, resulting in a decrease in the nutrients needed by plants, so their availability for plant growth and development is hampered. Butterfly pea has the potential to be developed as animal feed in dry and acidic areas, but the tolerance level to acid stress was unknown. This study aims to analyze the tolerance level to acid conditions in the butterfly pea through tissue culture techniques.

Materials and Methods

Research material

The tools used in this research are laminar air flow, autoclave, hot plate, magnetic stirrer, bunsen burner, calliper, 500 mL and 1 L beaker glass, 1 L plastic measuring cup, 100 mL capacity tissue culture bottles, pH meter, analytical scale, digital scale, pipette filler, mohr pipette, 200 mL glass bottle, scalpel, heat-resistant plastic, spatulas, blower, petri dishes, tweezers, Munsell color chart, and stationery.

The material used in this research was the seeds of butterfly pea obtained from BPTHMT (Balat Pembibitan Ternak dan Hijauan Makanan Ternak) Serading, Sumbawa, West Nusa Tenggara. Then the material was aluminum foil, sponge, Murashige Skoog (MS), 70% alcohol, white agar (agarose), BAP (Benzyl Amino Purine) stock solution, sugar, aquadest, and $AlCl_3$ stock solution.

Research procedures

Seed sterilization. The research was started by sterilizing the seeds using liquid detergent, Clorox solution, and sterile distilled water. The sterile seeds were placed on a petri dish lined with tissue sterilized in an autoclave, and then the sterile seeds were planted in bottles containing Murashige Skoog (MS) media.

Acid media creation. Preparation $AlCl_3$ stock solution First weighed 1 g of $AlCl_3$ crystals. Second, put $AlCl_3$ into the crystal beaker and distilled water to a volume of 1 L or 1000 mL, then homogenized 1 g of $AlCl_3$ and 1 L of distilled water using a magnetic stirrer. The stock solution was used according to the level of acid treatment ($AlCl_3$ solution 100 mL/L for 100 ppm, 200 mL/L for 200 ppm, 300 mL/L for 300 ppm, 400 mL/L for 400 ppm, and 500 mL/L for 500 ppm).

The manufacture of acidic media weighed 2.215 g of Murashige Skoog (MS) media, 15 g of sugar, and 3.5 g of agar using an analytical scale. Next, the Murashige Skoog (MS) media, sugar, the stock solution of 0 mL, 50 mL, 100 mL, 150 mL, 200 mL, and 250 mL $AlCl_3$ (according to treatment), and 0.5 mL of BAP stock solution were added for each treatment into a beaker glass. Then add distilled water so that the media reached a volume of 500 mL in each treatment. Homogenized the material that has been added. After that, the pH is checked using a pH meter. The finished media solution was removed and filled ±10 mL each into 35 bottles (each treatment), then closed the bottles using aluminium foil, and sterilized it using an autoclave at 121ºC, pressure 17.5 Psi for 15 min. The sterile media were arranged, stored in a tissue culture room at low temperature, and observed for one week. If there was contamination, they were not used as planting media.

Explant subculture on acid treatment media. Butterfly pea explants in the form of stems and shoots grown on germination media were transferred to the treatment medium through the subculture technique in laminar airflow. Each bottle contained one explant and there were 20 bottles in each treatment, so the total sample of explants was 120. The explants transferred to the treatment medium were then observed for 30 DAP (days after planting).

Research parameters

Acidity media changes. The measurement of changes in the acidity level of the media was carried out the difference between the acidity of the media last week (4^{th} week) and the acidity of the 0^{th} week of media using a pH meter.

Plant height gain. Measurement of plant height from the surface of the media to the highest point was measured using a caliper every 3 days from 0 to 30 DAP. Calculation of the increase in height was done by calculating the difference between the last observation of plant height and the previous observation. Data analysis was carried out for each result of the difference in days (increase in plant height on day 3 = plant height on day 3 – plant height on day 0).

Number of leaves. The number of leaves was calculated at the end of the observation at 30 DAP, on each explant by counting the number of leaves formed in each treatment.

Number of branches. The number of branches was calculated at the end of the observation on 30 DAP in each explant, by counting the number branches formed in each treatment.

Number of tillers. The number of tillers was calculated at the end of the observation on 30 DAP, by counting the number of tillers formed in each treatment.

Percentage of leaves withering. Leaves withering was obtained at 30 DAP observations by counting withering leaves in each explant. Leaves withering is calculated by the following formula (Number of withered leaves for individual in each treatment / Number of leaves for each treatment x 100%)**.**

Leaf color. Leaf was obtained by measuring the color change at the beginning and end of the observation using a leaf color application, namely the Munsell Color Chart for plants version 1.0.1.1.

Research methods and data analysis

This experiment was arranged by one factor (different levels of acid treatment). With 20 replications and the treatment consisting of different levels of $AlCl_3$ were 0 ppm (pH 6.05), 100

ppm (pH 5.05), 200 ppm (pH 4.32), 300 ppm (pH 3.73), 400 ppm (pH 3.50), and 500 ppm (pH 3.33).

The data were analyzed by analysis of variance (ANOVA) and if there was significantly different ($p<0.05$), then tested further by the Duncan's New Multiple Range Test using SPSS 22 software.

Results and Discussion

Acidity media changes

An increase in pH indicates the ability of plants to grow. Measurement of pH is done by measuring the initial pH and final pH. Changes in the pH of the media are presented in Table 1.

Table 1. Changes in the pH of the butterfly pea plant (*Clitoria ternatea* L.)

$AlCl_3$ (ppm)	Initial pH	Final pH	ΔpH media
0	6.05±0.00	6.49±0.12^{a}	0.44±0.12^{a}
100	5.05±0.00	5.39±0.33^{b}	0.35±0.33^{ab}
200	4.32±0.00	3.79±0.20^{c}	-0.53±0.20^{c}
300	3.73±0.00	3.49±0.11^{cd}	-0.24±0.11^{bc}
400	3.50±0.00	3.21±0.53^{cd}	-0.29±0.53^{c}
500	3.33±0.00	3.16±0.04^{d}	-0.17±0.047^{bc}

$AlCl_3$ = aluminium chloride; ^{a,b,c,d} Different superscript on the same column indicate statistical differences ($p<0.05$) among treatments ($p<0.05$).

Based on the results of the analysis of variance, it shows that different levels of $AlCl_3$ gave significantly ($p<0.05$) different results on changes in the pH of the butterfly pea planting media. Further tests using Duncan's test showed that the 0 ppm treatment was not significantly different from the 100 ppm treatment but significantly higher in increasing the pH of the media than the 200 ppm, 300 ppm, 400 ppm, and 500 ppm treatments. This indicates that the butterfly pea plant could neutralize the pH at 0 ppm (pH 6.49) and increase the pH at 100 ppm (pH 5.39) so that the plant could absorb nutrients well for plant growth and metabolism compared to under conditions of acid stress. Treatments of 200 ppm (pH 3.79), 300 ppm (pH 3.49), 400 ppm (pH 3.21), and 500 ppm (pH 3.16) at the end of the observation experienced a decrease in the pH of the media indicating an adaptation mechanism to acid stress by increasing the use of cations which function to maintain plant growth and the availability of anions in large quantities which causes the pH of the media to decrease.

According to Firmansyah and Sumarni (2013), decreasing and increasing pH were related to the ability of roots to absorb cations under acid stress conditions of each different variety. If many cations were absorbed by the roots (NH^{4+}), then many H^{+} ions would come out of the roots into the soil to become more acidic. If many anions were absorbed by the roots (NO^{3-}), then a lot of HCO^{3} released by the roots goes into the soil to become more alkaline. An increase in pH affected the low solubility of Al^{3+} so that P, which was previously bound to Al^{3+}, became available in P form. The activity of Al in binding P occurred when the pH lowed because Al was in the form of Al^{3+}. When the pH increased to ≥ 5.5, Al was in the form of oxide or hydroxide (precipitates), so it was not active in the P binding process (Fahrunsyah *et al*., 2023).

Plant height gain

Plant height, as the main parameter of plant morphology is visible when experiencing acid stress. The response of the butterfly pea plant height increase to acid stress for 30 days is presented in Table 2.

The analysis of variance showed that the application of different $AlCl_3$ levels shows significant ($p<0.05$) different decreased the increase in the height of the butterfly pea plant on the 6th and 9th days. Further tests using Duncan's test showed that the treatment of 100 to 500 ppm significantly differed in reducing plant height gain compared to the control. This is caused by aluminium inhibiting the absorption of phosphorus by a plant, so the plant tissue becomes smaller and unable to grow properly. According to Zulputra *et al*. (2014), the high solubility of Al was toxic to plants and caused growth and development to be disrupted. In acid soils, symptoms of Ca, Mg, P, K, and N deficiency were often found and Al poisoning occurs. High Al content in acid soils has been shown to inhibit plant growth, such as plant height and stem diameter (Damayanti *et al*., 2017). pH also affected other factors, such as nutrient availability. Al and Fe solubility were also affected by soil pH. At an acidic pH, Al and Fe element's solubility was high. As a result, at very low pH, plant

Table 2. Plant height gain of butterfly pea (*Clitoria ternatea* L.) acid treatment for 30 DAP

Age (day)	$AlCl_3$ (ppm)					
	0	100	200	300	400	500
	……..mm 3 days $^{-1}$……..					
3	7.85±5.83^{ab}	9.95±5.01^{a}	8.92±7.08^{a}	4.97±2.49^{bc}	3.63±1.42^{c}	4.33±2.83^{bc}
6	12.04±7.16^{a}	5.22±4.23^{b}	3.64±4.01^{b}	4.62±3.48^{b}	4.19±5.92^{b}	2.97±3.30^{b}
9	4.65±4.70^{a}	1.12±1.10^{b}	1.63±1.77^{b}	2.16±2.76^{b}	1.36±1.28^{b}	1.11±1.11^{b}
12	2.78±4.95	1.05±1.63	1.13±1.08	0.60±0.62	0.98±0.96	0.76±0.76
15	1.50±1.86	0.67±0.56	0.57±0.51	0.60±0.74	1.07±1.47	1.34±2.47
18	1.09±0.93	0.82±0.58	0.47±0.51	0.73±1.61	0.60±1.02	0.42±0.55
21	1.17±1.16	1.26±2.17	0.89±1.62	1.00±1.29	0.67±1.07	0.64±0.78
24	2.05±2.34	0.85±0.70	1.49±2.14	0.70±0.78	1.33±1.81	2.07±2.87
27	1.60±1.85	0.79±0.79	1.29±2.92	0.40±0.57	1.16±1.43	1.19±1.26
30	1.12±1.03	0.56±0.44	0.76±0.75	0.69±0.84	0.95±1.49	0.67±1.01

$AlCl_3$ = aluminium chloride; $AlCl_3$ 0 ppm (pH 6.05); $AlCl_3$ 100 ppm (pH 5.05); $AlCl_3$ 200 ppm (pH 4.32); $AlCl_3$ 300 ppm (pH 3.73); $AlCl_3$ 400 ppm (pH 3.50); $AlCl_3$ 500 ppm (pH 3.33).

^{a,b,c,d} Different superscript on the different column indicate statistical differences ($p<0.05$) among treatments ($p<0.05$).

growth would be stunted or abnormal (Karamina *et al*., 2017).

Plants undergo a homeostatic process or adaptation from the 12th to the 30th day to an acid-stress environment, making the added value of plant height insignificant. This is because plants begin to tolerate the presence of Al by chelating the symplast (internal) in plant tissues to undergo cell division. According to Kochian *et al.* (2015), mechanism plant tolerance internally, where the plant could tolerate the presence of Al in in tissues by secreting organic acids that could binds to Al to form non-toxic complexes, compartmentalization of Al by vacuoles, and increased antioxidant capacity, which could play an important role in tolerating the presence of Al in plants, organic acids, or organic ligands. According to Hue *et al.* (1986), organic acids that have a strong influence in reducing the toxic power of aluminium are citric and oxalic acids, organic acids that have moderate ability to reduce the toxicity of aluminium are malic acid and salicylic acid and organic acids which have a weak ability to reduce the toxicity. Blum (1996), suggested that plants can adapt to high Al due to efficiency in the absorption, reduction, translocation, and redistribution of nutrients.

Number of leaves

The number of leaves, number of branches, number of tillers, withering leaves, showed the physiological and morphological response of the butterfly pea plant to acid stress. The results of measurements of the growth of the butterfly pea plant against acid stress at 30 DAP are presented in Table 3.

Based on the analysis of variance, different levels of $AlCl_3$ gave results that were not significantly different on the growth of the number of leaves of the butterfly pea plant. This is thought to be caused by an internal tolerance mechanism to bind the accumulation of Al elements in the leaf organ tissues so that the leaves grow well and plants can reduce H^+ poisoning. This follows Santosa *et al.* (2016), the butterfly pea plant's mechanism in tolerating Al's presence is through an internal mechanism, namely tolerating the presence of Al in the tissue and then transporting and accumulating it in the leaf vacuole compartment.

Number of branches

The results of statistical analysis it was shown that giving $AlCl_3$ levels of 200 ppm, 400 ppm, and 500 ppm to the butterfly pea plant was significantly different ($p<0.05$) in reducing the number of branches compared to the 0 ppm level indicating extreme acid stress. However, the levels of 200 ppm, 400 ppm, and 300 ppm gave results that were not significantly different compared to the 100 ppm and 300 ppm level. This showed that $AlCl_3$ treatment at low levels could already cause inhibition of organ development in plants because acidic metals would bind nutrients essential for plant growth. The higher concentration of Al stress could affect cell division and enlargement, so growth in branches was inhibited. According to Silva *et al*. (2020), aluminum was a type of metal that has toxicity for plants which could adversely affect its biochemical and morphophysiological processes. High concentrations of aluminum in acidic soils caused disturbances in morphophysiological and biochemical processes so that plants experienced inhibition of the development of plant organs.

Number of tillers

Based on the analysis of variance, it was shown that the addition of $AlCl_3$ levels of 400 ppm and 500 ppm was significantly different ($p<0.05$) in reducing the number of tillers of the butterfly pea plant compared to the 0 ppm treatment, but not significantly different from the 100 ppm, 200 ppm, and 300 ppm treatments. This was because the pH of the 400 ppm and 500 ppm treatments in Table 3 has a very acidic pH <4.00 and was also due to the inhibition of absorption of phosphorus elements because high aluminum concentrations will bind phosphorus nutrients. The binding of phosphorus nutrients would also occur if the acid metal $AlCl_3$ is present, even at a low level. This was followed Zulputra *et al.* (2014) if the Al uptake was high, the P uptake of the plant was low so the number of tillers decreased. The increase in P uptake was due to the phosphorus plants need in cell division and as energy in every plant's metabolic process.

Withering leaves

The analysis of variance showed that giving different levels of $AlCl_3$ gave significantly ($p<0.05$) different results on leaves withering of the butterfly pea plant. Further tests used Duncan's test showed that leaves withering in the treatment of 300 ppm and 500 ppm was significantly higher than in the treatment of 0 ppm and 200 ppm. This showed that plants experiencing acid stress will be experience changes due to physiological responses so that the

Table 3. Growth response of butterfly pea (*Clitoria ternatea* L.) acid treatment for 30 DAP

$AlCl_3$ (ppm)	Variable			
	Number of leaves (unit)	Number of branches (unit)	Number of tillers (unit)	Withering leaves (%)
0	2.91±2.39	3.00±1.41^{a}	2.40±0.84^{a}	0.00±0.00^{a}
100	3.78±1.39	1.80±0.80^{abc}	2.29±0.73^{ab}	23.28±22.86^{ab}
200	4.60±4.09	1.67±0.71^{bc}	2.27±0.64^{ab}	14.05±18.06^{a}
300	4.88±2.95	2.80±0.84^{ab}	2.22±0.67^{ab}	47.51±24.40^{b}
400	5.00±3.58	1.33±0.58^{c}	1.60±0.55^{bc}	23.57±37.27^{ab}
500	4.00±3.32	1.60±0.90^{bc}	1.60±0.55^{bc}	50.86±45.77^{b}

$AlCl_3$ = aluminium chloride; $AlCl_3$ 0 ppm (pH 6.05); $AlCl_3$ 100 ppm (pH 5.05); $AlCl_3$ 200 ppm (pH 4.32); $AlCl_3$ 300 ppm (pH 3.73); $AlCl_3$ 400 ppm (pH 3.50); $AlCl_3$ 500 ppm (pH 3.33).

^{a,b,c} Different superscript on the same column indicate statistical differences ($p<0.05$) among treatments ($p<0.05$).

leaves will be turned yellow and die. This followed Zaeni *et al*. (2021), growth disturbances in plants were caused because plants have absorbed metals from the growing media and have translocated them to other parts of plant tissues. After Al enters plant tissue, Al could bind to nutrients that could cause disturbances in plant metabolism. This could cause the growth process in plants to be disrupted. This condition could be seen in the symptoms found in plants through the process of chlorosis on the leaves. The tips of the stems blacken, and the plants will wither and die.

Leaf color

Changed in the leaf color of the butterfly pea plant (*Clitoria ternatea* L.) to acid stress were presented in Table 4. Resulted from observations on the response of leaf color at the beginning and end of observations to acid stress showed that some plants were wilted so that the color changed from green to yellowish and even brownish. Based on the table, the different levels of $AlCl_3$, 100 ppm, and 200 ppm, experienced a decrease in the green color of the leaves compared to the control becoming yellowish by 82% and 70%, respectively. In comparison, the treatment of 300 ppm, 400 ppm, and 500 ppm of the dominant leaf color experienced decreased green color and changed to brownish and yellowish color compared to control as much as 100%.

This showed that acid stress could cause the butterfly pea plant to experience leaf damage visually as a survival defense against toxicity by decreased leaf transpiration, stomata opening, and pigment degradation. This followed Santosa *et al.* (2016), which stated that the conspicuous symptoms seen were yellowing of the leaves, which indicated low chlorophyll content (N deficiency), and necrosis at the leaves' tips, indicating Mg deficiency. High Al stress on plants could cause the edges of the leaves to turn yellow and brown and then dry (Damayanti *et al*., 2017). The higher the concentration of aluminum stress treatment, were more yellow leaves were seen (Septiani *et al*., 2017).

Table 4. Response to changes in leaf color of butterfly pea (*Clitoria ternatea* L.) to acid stress at the beginning and end of observation for 30 DAP

$AlCl_3$ (ppm)	Initial			Final		
	Color code	Color	%	Color code	Color	%
0 ppm	5gy 8/10		82	5gy 8/10		82
	7,5gy 7/12		9	7,5gy 7/12		9
	7,5gy 6/12		9	7,5gy 6/12		9
100 ppm	7,5 gy 6/10		31	7,5 gy 6/12		8
	7,5 gy 6/12		23	5 gy 7/8		23
				2,5 gy 5/8		8
				5 gy 5/8		15
				7,5 y 5/4		8
				2,5 gy 8/4		23
				2,5 y 9/4		15
200 ppm	7,5 gy 5/10		10	7,5 gy 5/10		20
	7,5 gy 6/10		20	7,5 gy 6/10		10
	7,5 gy 7/12		20	7,5 gy 5/8		10
	7,5 gy 8/12		20	5 gy 6/10		20
	5 gy 6/10		30	2,5 gy 6/8		10
				2,5 gy 9/4		20
				10y 9/6		10
300 ppm	7,5 gy 5/10		13	5gy 6/10		13
	7,5 gy 6/10		25	5gy 4/6		13
	7,5 gy 6/12		50	5gy 5/8		31
	7,5 gy 7/12		13	5gy 6/8		25
	5 gy 6/8			5 gy 7/10		13
				5 gy 5/6		13
400 ppm	7.5 gy 4/8		80	7,5 gy 5/8		10
	7,5 gy 7/12		10	5gy 5/8		60
	7,5 gy 7/10			5 gy 6/8		10
			10	7,5 gy 5/8		10
				5 gy 6/8		10
				5 gy 7/10		20
500 ppm	7,5 gy 5/10		10	5 gy 6/10		10
	7,5 gy 6/10		10	5 gy 5/8		10
	7,5 gy 6/12		60	2,5 gy 4/6		10
	7,5 gy 7/12		10	2,5 gy 5/8		30
				2,5 gy 6/8		20
				5y 6/6		10

$AlCl_3$ = aluminium chloride; $AlCl_3$ 0 ppm (pH 6.05); $AlCl_3$ 100 ppm (pH 5.05); $AlCl_3$ 200 ppm (pH 4.32); $AlCl_3$ 300 ppm (pH 3.73); $AlCl_3$ 400 ppm (pH 3.50); $AlCl_3$ 500 ppm (pH 3.33).

Conclusions

Butterfly pea plant has mechanism of adaptation to acid stress on the parameters of plant height gain and number of leaves at the end of the observation. However, the level of plant tolerance on the parameters of the number of branches and the number of tillers was ≤ 300 ppm (pH 3.73).

References

Blum, A. 1996. Crop responses to drought and the interpretation of adaptation. Plant Growth Regulation. 20: 135-148.

Cook, B. G., B. C. Pengelly, S. D. Brown, J. L. Donnelly, D. A. Eagles, M. A. Franco, J. Hanson, B. F. Mullen, I. J. Partridge, and M. Peters. 2005. Tropical Forages. CSIRO. Brisbane.

Damayanti, F., Suharsono, I. Marika, and A. Tjahjoleksono. 2017. *Agrobacterium tumefaciens*-mediated transformation of MmCuZn-SOD gene to sugarcane (*Saccharum officinarum* CV. PS864) for acidic soil stress tolerance. International Journal of Agriculture and Biology 19: 1489-1496.

Fahrunsyah. A.T. Batubara, R. Jannah, and A. A. Utama. 2023. Perubahan pH, aluminium dapat tukar dan fosfor tersedia ultisol karena pemberian pupuk organik batang pisang. Jurnal Agroekoteknologi Tropika 6: 1-6.

Firmansyah, I. and N. Sumami. 2013. Pengaruh dosis pupuk N dan varietas terhadap pH tanah, N-total tanah, serapan N, dan hasil umbi bawang merah (*Allium ascalonicum* L.) pada tanah entisols-Brebes Jawa Tengah. Jurnal Hortikultura 23: 358-364.

Hue, V., G. R. Cradock, and F. Adams. 1986. Effect of organic acids on alumunium toxicity in subsoil. SSSAJ. 50: 28-34.

Karamina, H., W. Fikrinda, and A. T. Murti. 2017. Kompleksitas pengaruh temperatur dan kelembaban tanah terhadap nilai pH tanah di perkebunan jambu biji varietas kristal (*Psidium guajava* L.). Jurnal Kultivasi 16: 430-434.

Kasno, A. 2020. Perbaikan tanah untuk meningkatkan efektivitas dan efisiensi pemupukan berimbang dan produktivitas lahan kering masam. Jurnal Sumberdaya Lahan 13: 27-40.

Kochian, L. V., M. A. Pineros, J. Liu, and J. V. Magalhaes. 2015. Plant adaptation to acid soils: the molecular basis for crop alumunium resistance. Annual Review of Plant Biology 66: 571-598.

Kosai, P., K. Sirisidthi, and W. Jiraungkoorsku. 2015. Review on ethnomedical uses of memory boosting herb, Butterfly Pea, *Clitoria ternatea*. J. Nat. Remedies. 15: 71-76.

Mulyani A. and M. Sarwani. 2013. Karakteristik dan potensi lahan sub optimal untuk pengembangan pertanian di Indonesia. Jurnal Pengelolaan Sumber Daya Alam dan Lingkungan 7: 46-57.

Prayitno, R. S., F. Wahyono, and E. Pangestu. 2018. Pengaruh suplementasi sumber protein hijauan leguminosa terhadap produksi amonia dan protein total ruminal secara. Jurnal Peternakan Indonesia 20: 116–123.

Putra, I. A. and H. Hanum. 2018. Kajian antagonism hara K, Ca, dan Mg pada tanah inceptisol yang diaplikasikan pupuk kandang, dolomit, dan pupul KCl terhadap pertumbuhan jagung manis (*Zea mays saccharata* L.). Journal of Islamic Science Technology 14: 23-44.

Santosa, H. R., C. Suherman, and S. Rosniawaty. 2016. Respons pertumbuhan tanaman kopi robusta (*Coffea robusta* L.) tercekam aluminium di lahan reklamasi bekas tambang batubara bervegetasi sengon (Periode El Nino). Jurnal Agrikultura 27: 124-131.

Septiani, N. M. N., Mukarlina, and E. R. P. Wardoyo. 2017. Pertumbuhan dan karakter anatomi mimosa air (*Neptunia oleracea* Lour.) pada air yang terpapar logam aluminium (Al). Jurnal Protobiont 6: 75 – 82.

Silva, C. O., D. S. Brito, and A. A. Silva. 2020. Differential accumulation of aluminum in root tips of soybean seedlings. Brazillian Journal of Botany 43: 99-107.

Sutedi, E. 2013. Potensi kembang telang (*Clitoria ternatea*) sebagai tanaman pakan ternak, Wartazoa 23: 51-62.

Zaeni, A., S. Ambardini, A. Sartinah, A. N. Ramadhani, Sartini, A. Amin, G. W. Patiung, and P. E. Susilowati. 2021. Bioakumulasi logam crom (Cr), seng (Zn) dan nikel (Ni) pada tanaman obat binahong (*Anredera cordifolia* (Ten). Steenis. Akta Kimindo 6: 12-28.

Ziraluo, Y. P. B. 2021. Metode perbanyakan tanaman ubi jalar ungu (*Ipomea batatas* poiret) dengan teknik kultur jaringan atau stek planlet. Jurnal Inovasi Penelitian 2: 1037-1046.

Zulputra, Wawan, and Nelvia. 2014. Respon padi gogo (*Oryza sativa* L.) terhadap pemberian silikat dan pupuk fosfat pada tanah ultisol. Jurnal Agroekoteknologi Tropika 4: 1-10.