

Soybean (*Glycine max* L.) Response to Aluminum Stress in The Vegetative Phase

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Abstract. One of the problems in increasing soybean crop production in Indonesia is the need for more suitable land for soybean cultivation. One solution is to utilize coastal land to expand the planting area. The issue with coastal land is its low pH and high Al content. Consequently, it is essential to determine soybean cultivars tolerant to Al stress. This study aimed to explain the response of soybean growth to Al stress in the vegetative phase and to assess soybean varieties that are tolerant to aluminum (Al) stress. The research was conducted from March to May 2022 in the Greenhouse and Agronomy Laboratory, Department of Agricultural Cultivation, Faculty of Agriculture, Bengkulu University, Indonesia. The study used three repetitions of a two-factorial, Completely Randomized Design (CRD). The first factor was soybean varieties consisting of the Deja and Gepak Kuning varieties. The second factor was the concentration of Aluminum Stress which consisted of three levels, namely 0 μ M (control), 74 μ M, and 148 μ M. The results showed that Al stress did not affect the growth of soybean plants in the vegetative phase. Soybean variety Deja was more tolerant than Gepak Kuning in both Al stress concentrations of 74 μ M and 148 μ M.

Keywords: Soybean Cultivation, Aluminum Stress, Soybean Varieties, Tolerance, Coastal Land Expansion

1 Introduction

Indonesia's main issue with developing soybean production (*Glycine max*) is the need for designated land for soybean development [1]. According to Yolinda et al. (2015), Indonesia has not been able to become self-sufficient in soybeans due to a variety of factors such as limited cultivable area, low productivity, high production costs, a once-a-year planting frequency, and farming profits and efficiency. Marginal land may be utilized to increase soybean production. [2] Indonesia has 148 million ha of dry land, with an estimated 102.8 million ha of acid soil. Low productivity is a limitation of soybean growing on acidic soils.

One of the major issues with growing plants in acidic soils is the toxicity of aluminum (Al) [3]. Al toxicity in acid soils is caused by Al's high solubility, which makes it toxic to plants. On the other hand, soybeans have different Al tolerances between varieties [4]. Plants on Ultisol land are exposed to the possibility of Al poisoning and nutrient

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deficiencies of N, P, K, Ca, and Mg, resulting in stunted growth and low productivity (Indrayani et al. 2017). High Al saturation in the growing medium will limit root penetration to absorb nutrients. Furthermore, because roots cannot absorb nutrients effectively, they do not develop and produce nutrient deficiencies in plants [5].

To overcome Al stress, acid-tolerant soybean varieties should be developed [6]. Studying soybean lines at various Al concentrations can be used to develop soybean varieties that are tolerant to Al toxicity. Determination of tolerance to Al stress in soybean varieties can be carried out by testing various Al concentrations. Reported that, under 0.5 mM Al stress, the Tanggamus and Cikuray varieties were more tolerant of Al stress than the Ceneng variety, which was indicated by longer roots. This study aimed to explain the response of soybean growth to Al stress in the vegetative phase and to determine soybean varieties that were tolerant to Al stress.

2 Methodology

2.1 Research site and time

This study was carried out at the Greenhouse of the Agronomy Laboratory, Faculty of Agriculture, University of Bengkulu, Indonesia, from March to May 2022. The study site is 200 meters above sea level at -3,7566625°S and 102,2725781°E.

2.2 Research design

A Completely Randomized Design (CRD) was used in the study, which included two factors with 3 replication, Soybean Varieties, and Aluminum (Al) Stress. The first factor was soybean varieties, consisting of the V₁: Deja variety and V₂: Gepak Kuning variety. The second factor is Aluminum Stress, measured using an AlCl₃ solution with three levels, namely A₀: Control, A₁: 74 µM, and A₂: 148 µM. Each treatment combination was repeated three times with five plants per replicate.

2.3 Seeding

Soybean seeds were sown on trays with a planting medium of a mixture of compost + roasted husks in a ratio of 1:1 (v/v). The germinated seeds were transplanted into nutrient culture media seven days after sowing (DAS).

2.4 Nutrient Culture Preparation

The nutrient culture medium used was an AB mix solution consisting of stock A and B. Nutrient solutions were prepared as needed. Nutrient culture solutions were prepared by dissolving them in water.

2.5 Transplanting

The transplanting was conducted once the soybean seeds had reached 7 DAS. Seedlings were cultivated for seven days in a 32 cm × 38 cm experimental tank with nutrient culture. Styrofoam was used to cover the top of the experimental tank, creating planting holes for soybean seeds.

2.6 Treatment application

After seven days of nutrient culture, the solution was replaced and treated with Al stress by adding AlCl_3 at 74 μM and 148 μM concentrations. The nutrient solution and stress treatment were replaced with as much as 7 liters per experimental unit every seven days. The pH of the growing medium was controlled and kept at a neutral level of 7, while the pH of the treatment was 4.

2.7 Variable observation

Growth variables and root anatomy were examined seven days after nutrient culture transplant and 5, 10, and 15 days after treatment (DAT). In addition to observations on the growth of soybean seedlings, observations on the growing environment were performed. Growth variables observed were shoot length (cm), leaves number, shoot fresh weight (g), shoot dry weight (g), primary root length (cm), lateral root length (cm), fresh root weight (g), and root dry weight (g); The physiological variables observed include stomata density (mm^{-2}) and leaves greenness while the environmental data are temperature and humidity.

2.8 Stress tolerance index (SI)

Soybean tolerance to Al stress was assessed using the stress sensitivity index (Fischer and Maurer, 1978) with the abbreviation:

$$SI = \frac{(1-Y / YP)}{(1-X / XP)} \quad (1)$$

Noted :

- SI : Stress tolerance index
- Yp : The average of a varieties that was subjected to stress
- Y : The average of a varieties that was not subjected to stress
- Xp : The average of all varieties that was subjected to stress
- X : The average of all varieties that was not subjected to stress

The fresh and dried weights of shoots and roots were used to calculate the stress sensitivity index. If the value of $SI < 0.5$ is included in the tolerant varieties, $0.5 < SI < 1.0$ medium susceptible varieties, and $SI > 1.0$ sensitive varieties [7]

2.9 Data analysis

The data were examined using the F test and analysis of variance (ANOVA). Duncan's Multiple Test (DMRT) was performed at a significance level of 5% for parameters with a significant effect.

3 Results and discussion

3.1 Research Overview

The average temperature during the study from March to May 2022 was 28.34OC, and the humidity was 71%. The temperature required for soybean growth is 22-27°C [8] with an optimum humidity of 75% - 90% (Adisarwanto, 2008) and a soil pH of 6.8. [9] Soybeans

etiolate as a result of high temperatures and low humidity. The pH of the solution in the experimental control bath was always adjusted in neutral conditions (6.9-7.1) by adding NaOH, while the pH of the Al stress treatment was adjusted at pH 3.9-4.1 by adding HCl. The AB mix solution used for this study was 35 ml of A stock and 35 ml of B stock, with a solution volume of 7 L in each experimental bath. The solution on the experimental bath was replenished every week.

During the study, the plants were attacked by the fungus *Sclerotium rolfsii*, which killed them. The dry weight of the shoot was affected by dead plants. *S. rolfsii*, can infect plants from germination through harvest. Disease infection at the sprouting phase causes the sprouts to wilt and die, but disease infection in mature plants causes brown to black lesions at the base of the stem [10]

3.1.1 The effect of soybean cultivars on plant growth at various stages

Varieties and Al stress treatment significantly affected several parameters of soybean growth. There was no interaction between types and Al stress on all observed parameters. Stress treatment at Al concentrations of 74 μ M and 148 μ M had a significant effect on the fresh weight of shoots and roots at 0 to 15 DAT, shoot dry weight at 0 DAT, 5 DAT, and 15 DAT, while the number of leaves only had a significant effect on 15 DAT. Differences in soybean cultivars affect plant vegetative growth, shown in Tables 1, 2, 3, and 4.

Table 1. Effect of Varieties on soybean growth at 0 DAS

Cultivars	SL (cm)	LN	PRL (g)	LRL (g)	LG
Deja	17.43 b	1.22	6.4	16.28	26.08 b
Gepak Kuning	19.76 a	1.07	4.56	13.31	27.18 a
Cultivars	SFW (g)	SDW (g)	RFW (g)	RDW (g)	SD
Deja	2.32 a	0.26 a	0.674 a	0.037	95.68
Gepak Kuning	1.80 b	0.22 b	0.52 b	0.036	88.89

Table 2. Effect of Varieties on soybean growth at 5 DAS

Cultivars	SL (cm)	LN	PRL (g)	LRL (g)	LG
Deja	30.4	4.19	6.69	17.45 b	34.47
Gepak Kuning	29.91	4.03	5.61	23.06 a	33.74
Cultivars	SFW (g)	SDW (g)	RFW (g)	RDW (g)	SD
Deja	8.15 a	1.13 a	2.49 a	0.16 a	129.65
Gepak Kuning	6.33 b	0.84 b	1.80 b	0.11 b	113.8

Table 3. Effect of Varieties on soybean growth at 10 DAS

Cultivars	SL (cm)	LN	PRL (g)	LRL (g)	LG
Deja	67.9	7.63	6.99	23.81	33.28
Gepak Kuning	66.02	7.29	6.49	20.95	32.01
Cultivars	SFW (g)	SDW (g)	RFW (g)	RDW (g)	SD
Deja	23.86 a	3.06	4.83 a	0.27 a	133.05
Gepak Kuning	16.12 b	2.74	2.67 b	0.15 b	120.03

Table 4. Effect of Varieties on soybean growth at 15 DAS

Cultivars	SL (cm)	LN	PRL (g)	LRL (g)	LG
Deja	111.007	12.85 a	7.10	18.14	34.9
Gepak Kuning	101.27	10.92 b	5.68	14.92	34.56
Cultivars	SFW (g)	SDW (g)	RFW (g)	RDW (g)	SD
Deja	35.87 a	5.25 a	7.19 a	0.51 a	164.77
Gepak Kuning	27.77 b	3.92 b	4.68 b	0.34 b	150.04

Note: shoot length (SL), leaves number (LN), primary root length (PRL), lateral root length (LRL), leaves greenness (LG), shoot fresh weight (SFW), shoot dry weight (SDW), fresh root weight (FRW), and root dry weight (RDW), stomata density (SD). Numbers followed by different letters in the same column are significantly different on the DMRT test.

At 0 DAT (control treatment), the shoot length and leaves greenness of the Gepak Kuning variety were higher, but the shoot fresh and dry weight was lower than that of the Deja variety. The Deja variety's soybean canopy is larger than the Gepak Kuning variety's, whereas the Gepak Kuning variety has a small, elongated plant canopy. Therefore, although Gepak Kuning has a higher plant, its weight is lower than that of the Deja variety[11].

This study's findings align with [12] results, which showed that each variety had different genetic characteristics, resulting in additional production. [13],[14] Stated that genetic factors determine differences in growth between types in adapting to the environment. Plants will experience physiological and morphological changes in a direction suitable to their new environment.

Al stress of 5, 10, and 15 DAT did not affect the number of leaves and root length of the two varieties studied. However, the fresh and dry weight of the shoot and roots of the Deja variety was higher than that of the Gepak Kuning variety (Tables 2, 3, and 4). Thus the Deja variety is more susceptible to Al stress. This study's findings align with the results of [10] who reported that a the variety's ability determines the variety's ability to generate roots and shoot to adapt to high Al stress. Each plant has its mechanism for regulating the harmful effects of Al, ensuring that nutrient and water uptake is not affected.

3.1.2 The effect of Al stress at various concentrations on plant growth at various stages

Tables 5, 6, 7, and 8 show that Al stress treatment at 74 μ M and 148 μ M had no significant effect on all variables observed except plant height at 0 DAS. The late application of Al stress enables it not to affect plant growth. Al stress was applied to the plants after being in nutrient culture for seven days in this study.

Table 5. Effect of Al stress on soybean growth at 0 DAS

Concentration	SL (cm)	LN	PRL (g)	LRL (g)	LG
0	18.45 ab	1.11	5.40	14.46	26.40
74 μ M	19.87 b	1.11	5.72	14.71	26.85
148 μ M	17.51 a	1.22	5.31	15.22	26.63
Concentration	SFW (g)	SDW (g)	RFW (g)	RDW (g)	SD
0	2.05	0.24	0.62	0.27	91.72
74 μ M	2.09	0.24	0.57	0.06	95.97
148 μ M	2.95	0.24	0.61	0.27	89.17

Table 6. Effect of Al stress on soybean growth at 5 DAS

Concentration	SL (cm)	LN	PRL (g)	LRL (g)	LG
0	30.58	4.22	6.15	19.18	35.08
74 μ M	29.25	3.95	6.41	19.31	33.60
148 μ M	30.64	4.17	5.90	22.29	33.63
Concentration	SFW (g)	SDW (g)	RFW (g)	RDW (g)	SD
0	7.18	0.89	2.19	0.13	111.25
74 μ M	7.24	0.99	1.91	0.15	135.03
148 μ M	7.30	1.07	2.33	0.14	118.90

Table 7. Effect of Al stress on soybean growth at 10 DAS

Concentration	SL (cm)	LN	PRL (g)	LRL (g)	LG
0	69.18	7.78	7.90	28.88	31.53
74 μ M	62.48	7.39	7.12	20.67	33.25
148 μ M	69.23	7.22	5.21	21.60	33.17
Concentration	SFW (g)	SDW (g)	RFW (g)	RDW (g)	SD
0	19.32	2.46	4.22	0.22	117.20
74 μ M	19.98	3.75	3.45	0.22	119.75
148 μ M	20.67	2.50	3.58	0.20	142.68

Table 8. Effect of Al stress on soybean growth at 15 DAS

Concentration	SL (cm)	LN	PRL (g)	LRL (g)	LG
0	101.93	12.33	6.02	19.92	33.57
74 μ M	113.45	12.42	7.00	15.17	37.28
148 μ M	103.05	10.91	6.16	14.49	33.33
Concentration	SFW (g)	SDW (g)	RFW (g)	RDW (g)	SD
0	31.28	3.91	6.65	0.40	153.72
74 μ M	31.60	5.16	5.62	0.43	170.70
148 μ M	32.56	4.68	5.53	0.45	147.77

Note: shoot length (SL), leaves number (LN), primary root length (PRL), lateral root length (LRL), leaves greenness (LG), shoot fresh weight (SFW), shoot dry weight (SDW), fresh root weight (FRW), and root dry weight (RDW), stomata density (SD). Numbers followed by different letters in the same column are significantly different on the DMRT test.

The delay in applying Al stress causes the plants to no longer respond to Al stress. According to [15] the application time of Al stress determines its effect on root growth. Al stress applied to soybean plants immediately after being transferred to nutrient culture (7 days old seedlings) resulted in shorter soybean roots than the control treatment (not Al stress).



A

B

Fig. 1. Roots of 15 HSP (A) Deja variety, (B) Gepak Kuning variety (30 cm scale)

The major limiting factor for plant growth in acidic soils is Al stress [16] According to [17] root growth suppression is a common sign of Al stress in plants. [18] Root growth is inhibited because the root tip (root cap, meristem, and elongation zone) can accumulate more Al than other parts of the root. This study, however, showed that Al stress did not affect soybean root growth. The root growth of Deja and Gepak Kuning soybean cultivars did not differ significantly between Al stress concentration treatments.

Although not significantly different, the primary root of the Deja variety (10.50 cm) was longer than that of the Gepak Kuning variety (8.68 cm). In comparison, the lateral roots of the Gepak Kuning variety (27.90 cm) were longer than that of the Deja variety (25.20 cm) (Figure 4). [19] [20] The ability to develop longer roots defines the Tanggamus variety's tolerance level to Al stress, even though aluminum stressed the root growth of the soybean variety Tanggamus.

Soybean plant height continues to rise as the plant ages. However, the height of the soybean plants was not significantly different from the control (without stress) at 5, 10, and 15 HSP with Al stress concentrations of 0.74 M and 148 M. (Tables 7, 8, 9). This study's results align with [21] finding that Al stress had no significant effect on maize plant height after in vitro selection for resistance to $AlCl_3$ stress.

3.1.3 Aluminum Stress Tolerance Index (SI)

The aluminum stress tolerance index, also known as the Sensitivity Index (SI), demonstrates that the values vary between 74 M and 148 M. In general, the value of SI increases with increasing stress concentration. Determination of the SI value for each stress treatment was carried out to obtain a threshold concentration of soybean tolerance to Al

stress [22] Tables 10 and 11 show the stress tolerance index 15 days after stress treatment for the variables shoots fresh and dry weight and root fresh and dry weight.

Table 9. Stress tolerance index (SI) of two soybean varieties at a concentration of 74 μ M

Varieties	SFW	SDW	RFW	RDW	Average	Classification
Deja	-0.60	0.54	-2.80	0.07	- 0.70	T
Gepak Kuning	-1.15	18.83	-0.31	-1.14	4.06	S

Table 10. Font styles for a reference.

Varieties	SFW	SDW	RFW	RDW	Average	Classification
Deja	-0.30	0.52	-1.30	0.55	- 0.13	T
Gepak Kuning	-1.37	26.32	0.13	-0.29	6.20	P

Notes: Shoot Fresh Weight (SFW), Shoot Dry Weight (SDW), Root Fresh Weight (RFW), Root Dry Weight (RDW), Tolerant (T), Sensitive (S). SI value <0.5 is classified as tolerant, and SI value > 1.0 is classified as sensitive.

The grouping of soybean varieties tolerant to Al stress (SI) differs among researchers. [23] Grouped the tolerance of soybean varieties to Al stress into two groups, tolerant and sensitive. Meanwhile, [24] [25] the criteria for determining the level of stress tolerance are divided into three groups, namely, if the value of SI SI<0.5 is defined as tolerant, 0.5<SI<1.0 susceptible medium, and SI > 1.0 as sensitive. In this study, plant tolerance to Al stress was classified into three classes [26]

Stress tolerance is the plant's ability to grow and survive to complete its life cycle. Tables 10 and 11 show that at an Al concentration of 74 M, the SI for the Deja variety is -0.70 and for the Gepak Kuning variety is 4.06, indicating that the Deja variety is more susceptible than the Gepak Kuning variety because the SI Deja value is <0.5. Similarly to the concentration of 74 M, the Deja variety was more tolerant than the Gepak Kuning variety at 148 M since the SI value for the Deja variety was -0.13 or SI<0.5, and the SI value for the Gepak Kuning variety was 6.20 or SI>1.0. Plants that are tolerant to Al toxicity can reduce the adverse effects of Al poisoning. [27] Classified plants that are tolerant to aluminum stress into three categories: roots that can continue to grow and root tips that are not damaged, roots can change the pH in the root area, and plants with a specific mechanism in which Al cannot inhibit the absorption of Ca, Mg, and K so that plants can still meet their nutrient needs.

4 Conclusion

Al stress did not affect soybean plant growth in the early vegetative phase due to the late application of Al stress, which caused soybean plants not to respond to Al stress. The soybean variety Deja was more tolerant to Al stress than the Gepak Kuning variety.

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