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Effect of amino acid on growth of durian seedling cv. Monthong under drought condition

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Abstract Results showed that moderate concentrations of amino acids (500–1000 ppm) significantly improved soil pH, promoted leaf development, increased dry leaf biomass, and enhanced nutrient uptake efficiency compared to controls. Treatments at higher concentrations was not benefited and sometimes decreased performance. Amino acid supplementation was significantly affected the trunk diameter or height, but did increase plant resilience, especially in new leaf formation and chlorophyll content. The findings are supported that foliar amino acid application is a promising strategy for enhancing drought tolerance, seedling establishment, and productivity in durian cultivation under water-limited conditions. These insights are provided practical guidance for durian growers using biostimulants to optimize plant health and survival during dry periods.

Keywords: Durian, Amino acid, Growth, Drought

Introduction

Durian (*Durio zibethinus* L.) is one of the most economically valuable tropical fruits in Southeast Asia, especially the Monthong cultivar, which is prized domestically and internationally for its thick arils, distinctive aroma, and sweet-creamy flavor (Husin *et al*., 2018; Ho and Bhat, 2015; Charoenkiatkul *et al*., 2016; Haruenkit *et al*., 2010). Cultivation of Monthong durian requires precise water management, but during the dry season rainfall often falls below optimal levels, leading to reduced seedling survival, underdeveloped root systems, and significantly impaired photosynthetic efficiency (Ismail *et al*., 1994; Farooq *et al*., 2009).

Plant responses to drought stress encompass multiple layers of adaptation, including osmotic adjustment, activation of antioxidative enzymes, and reprogramming of primary metabolism, notably the accumulation of specific

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amino acids that function as osmolytes and signaling molecules (Batista-Silva *et al.,* 2019; Yang *et al*., 2020). Exogenous application of amino acids such as proline and branched-chain amino acids (BCAAs) has been shown to enhance drought tolerance in various crops such as proline maintains osmotic balance, stabilizes proteins, and scavenges reactive oxygen species (Hayat *et al*., 2012), while BCAAs serve as alternative energy reserves and help balance cellular metabolites under stress (Hildebrandt *et al*., 2015).

Other amino acids also exhibit protective effects such as glycine betaine stabilizes membranes and mitigates oxidative damage during water deficit (Chen *et al*., 2025), and methionine regulates cellular redox status to improve stress resilience (García-García *et al*., 2020). Foliar applications of amino acid biostimulants at optimal concentrations significantly increase plant height, chlorophyll content, and productivity of broccoli (El-Beltagi *et al*., 2023). Previous studies on tree seedlings demonstrated that amino acid treatments can improve growth and leaf water relations under drought conditions (Fetouh, 2017), highlighting the need for well-controlled experiments to validate their efficacy in durian.

Studies on other tree seedlings, such as *Cupressus macrocarpa*, demonstrate that amino acid treatments improve root and shoot growth and leaf water relations under drought conditions (Fetouh, 2017). However, empirical data on the effects of amino acid applications in Monthong durian seedlings remain scarce, despite the heightened sensitivity of young durian plants to water stress. Therefore, this study aims to investigate the effects of amino acid applications on the growth and physiological responses of durian seedlings cv. Monthong under drought stress conditions. The research was evaluated various growth parameters, physiological indicators, and biochemical responses to determine the potential of amino acids as effective stress-mitigating agents in durian cultivation.

Materials and methods

The experiment was arranged in a Completely Randomized Design (CRD) with five replications, each consisting of one durian seedling. The treatments consisted of a control (T1:water only) and four amino acid concentrations 500 (T2), 1000 (T3), 1500 (T4), and 2000 (T5) ppm. The experiment was conducted at the Faculty of Agricultural Technology, Rambhai Barni Rajabhat University, Chanthaburi Province. Twenty-five 1–2-year-old Monthong durian seedlings, each averaging 85.88 ± 7.12 cm in height, were selected. Seedlings were planted in cylindrical containers 60 cm in diameter and 45 cm deep, filled with a growing medium consisting of field soil mixed with raw rice hulls at a 3:2 ratio. Seven

days after transplanting, the prescribed amino acid treatments were applied once weekly for seven weeks, totaling 49 days. Throughout the experiment, seedlings were irrigated 5 days interval to simulate the drought condition, and the soil moisture content was weekly determined. All seedlings also received pest-control sprays following recommendations of the Chanthaburi Provincial Agricultural Extension Office (2013). At the end of the experiment, trunk diameter, plant height, leaf number, and chlorophyll content were recorded. Seedlings were separated into roots, stems and leaves then they were placed in the dry oven at 70 °C for 72 hours. Soil pH and nutrient concentrations (nitrogen, phosphorus, and potassium) were analyzed at the start and end of the experiment. All data were subjected to analysis of variance (ANOVA), and mean comparisons were performed using Duncan's multiple range test (DMRT) at the 95% confidence level.

Results

Soil moisture percentage

Soil moisture analysis showed that the 1000 ppm amino acid treatment (T3) achieved the highest mean soil moisture percentage (42.00%), followed by the control without amino acid treatment (T1: 41.67%) and 2000 ppm treatment (T5: 39.33%), whereas 500 ppm and 1500 ppm treatments (T2 and T4) maintained lower soil moisture levels (34.00% and 35.00%, respectively), with no significant differences detected ($p>0.05$) (Table 1).

Table 1. Average of soil moisture throughout the experiment and soil pH, nitrogen concentration, phosphorus concentration and potassium concentration in soil at the end of experiment

Amino acid concentration (ppm)	Soil moisture (%)[1]	Soil pH[2]	Nitrogen concentration in soil (%)[2]	Phosphorus concentration in soil (mg/kg)[1]	Potassium concentration in soil (mg/kg)[1]
0	41.67±1.48	4.85±0.09^{c}	0.14±0.01^{a}	45.43±4.43	336.93±41.55
500	34.00±2.92	5.14±0.11^{b}	0.09±0.02^{b}	43.37±2.54	314.37±24.82
1000	42.00±1.73	5.34±0.02^{a}	0.10±0.01^{b}	55.13±6.67	396.80±72.90
1500	35.00±1.63	5.08±0.08^{b}	0.11±0.03^{b}	52.10±3.98	315.33±20.35
2000	39.33±3.13	5.20±0.02^{b}	0.09±0.01^{b}	37.60±4.67	368.47±20.86

[1]/ Means without letters in each column are not significantly different according to DMRT.
[2]/ Means with different letters in each column are significantly different according to DMRT.

Soil pH

The experiment demonstrated a clear response of soil pH to amino acid treatments. Soil pH increased from 4.85 (T1, control) to maxima at T3 (5.34; 1000 ppm), with intermediate values for T2 (5.14), T4 (5.08), and T5 (5.20). The between-group difference was statistically significant ($p<0.05$) (Table 1).

Nitrogen concentration in soil

Soil nitrogen analysis revealed statistically significant treatment effects ($p<0.05$) with the control without amino acid treatment (T1) showing the highest mean nitrogen content (0.1433%), while amino acid treatments showed lower values: T4 (1500 ppm: 0.1100%), T3 (1000 ppm: 0.1000%), T2 (500 ppm: 0.0967%), and T5 (2000 ppm: 0.0933%) (Table 1).

Phosphorus concentration in soil

The soil phosphorus content analysis showed that the 1000 ppm amino acid treatment (T3) achieved the highest mean phosphorus level (55.13 mg/kg), followed by 1500 ppm treatment (T4: 52.10 mg/kg), control without amino acid treatment (T1: 45.43 mg/kg), 500 ppm treatment (T2: 43.37 mg/kg), and 2000 ppm treatment (T5: 37.60 mg/kg), though differences were not statistically significant ($p>0.05$). The trend suggests optimal phosphorus availability at moderate amino acid concentrations (1000-1500 ppm) (Table 1).

Potassium concentration in soil

The analysis of soil potassium concentration demonstrated that the 1000 ppm amino acid treatment (T3) achieved the highest mean potassium level (396.80 mg/kg), followed by 2000 ppm treatment (T5: 368.47 mg/kg), control without amino acid treatment (T1: 336.93 mg/kg), 1500 ppm treatment (T4: 315.33 mg/kg), and 500 ppm treatment (T2: 314.37 mg/kg), though no statistically significant differences were detected ($p>0.05$) (Table 1).

Height increment

The analysis of height increments in Monthong durian seedlings revealed that the 500-ppm amino acid treatment (T2) exhibited the highest mean height (9.42 cm) compared to the control group receiving no amino acid treatment (T1: 5.30 cm), although the difference was not statistically significant ($p>0.05$). The other concentrations showed intermediate responses: T3 (1000 ppm) at 7.78 cm, T4 (1500 ppm) at 6.74 cm, and T5 (2000 ppm) at 7.04 cm (Table 2).

Table 2. Height increment, trunk diameter increment, new leaf number and new shoot number of durian seedlings under drought condition

Amino acid concentration (ppm)	Height increment (cm)/1	Trunk diameter Increment (cm)/1	New leaf number (leaf)/2	New shoot number (shoot)/1
0	5.30±2.90	1.72±0.54	12.67±0.58[b]	17.33±1.15
500	9.42±1.52	1.35±0.59	30.33±1.37[a]	18.50±2.31
1000	7.78±2.41	1.71±0.44	40.33±1.02[a]	18.33±2.04
1500	6.74±1.83	1.44±0.70	29.00±1.73[a]	22.00±3.55
2000	7.04±1.50	1.89±0.42	14.33±1.57[b]	18.33±2.23

1/ Means without letters in each column are not significantly different according to DMRT.
2/ Means with different letters in each column are significantly different according to DMRT.

Trunk diameter increment

Trunk diameter measurements showed that the highest amino acid concentration treatment (T5: 2000 ppm) had the greatest mean increase (1.898 mm), followed by the control (T1: 1.726 mm) and 1000 ppm treatment (T3: 1.712 mm), while 500 ppm treatment (T2) exhibited the lowest increase (1.350 mm). No significant differences were detected ($p>0.05$) (Table 2).

New leaf number

The experiment found that amino acid foliar application significantly affected new leaf formation in durian seedlings. The highest mean new leaf number occurred at 1000 ppm (T3: 40.33 leaves), followed by 500 ppm (T2: 30.33) and 1500 ppm (T4: 29.00), while control (T1: 12.67) and 2000 ppm (T5: 14.33) showed lowest values. ANOVA confirmed a statistically significant treatment effect ($p<0.05$) (Table 2).

New shoot number

Shoot counts revealed that the 1500 ppm amino acid treatment (T4) achieved the highest mean number of new shoots (22.00), with 500 ppm treatment (T2) at 18.50, and both 1000 ppm and 2000 ppm treatments (T3 and T5) at 18.33, versus the control (T1) at 17.33, though differences were not significant ($p>0.05$) (Table 2).

Dry weight of old leaves

The dry weight analysis of old leaves revealed that the 1500 ppm amino acid treatment (T4) produced the highest mean dry weight (13.84 g), while other treatments showed similar values: T2 (500 ppm) at 12.89 g, T1 (control) at 12.87 g, T3 (1000 ppm) at 12.81 g, and T5 (2000 ppm) at 12.35 g, with no significant differences detected ($p>0.05$) (Table 3).

Dry weight of new leaves

Foliar application of amino acids greatly influenced the dry weight of new leaves in durian seedlings. The highest mean dry weight occurred with 1000 ppm treatment (T3: 11.11 g), closely followed by 500 ppm (T2: 9.92 g) and 1500 ppm (T4: 9.80 g), whereas control (T1: 5.16 g) and 2000 ppm (T5: 3.69 g) were remarkably lower. ANOVA showed a significant difference among treatments ($p<0.05$), indicating effective enhancement at moderate concentrations (Table 3).

Chlorophyll a content in old leaves

Chlorophyll a content followed a similar trend based on amino acid foliar spray levels. The 500 ppm treatment (T2) led to the highest mean (21.95 $\mu g/cm^2$), with the control (T1: 20.81 $\mu g/cm^2$) higher than the higher concentrations (T3: 19.24 $\mu g/cm^2$; T4: 18.96 $\mu g/cm^2$; T5: 19.27 $\mu g/cm^2$) (Table 3).

Chlorophyll b content in old leaves

The pattern in this result confirmed the findings: 500 ppm amino acid application (T2) resulted in the highest chlorophyll b (8.75 $\mu g/cm^2$), compared to the control (T1: 8.31 $\mu g/cm^2$) and higher concentrations (T3: 7.86 $\mu g/cm^2$; T4: 7.54 $\mu g/cm^2$; T5: 7.98 $\mu g/cm^2$) (Table 3).

Table 3. Dry weight of old and new leaves of leaves and chlorophyll a, b and a+b content in old leaves of durian seedling under drought condition at the end of experiment

Amino acid concentration (ppm)	Dry weight of old leaves (g)/1	Dry weight of new leaves (g) /2	Chlorophyll a content in old leaves (µg/cm²)/1	Chlorophyll b content in old leaves (µg/cm²)/1	Chlorophyll a+b content in old leaves (µg/cm²)/1
0	12.86±1.57	5.16±0.61[b]	20.81±0.47	8.30±1.15	29.11±1.56
500	12.88±3.21	9.92±2.90[a]	21.95±1.55	8.74±0.85	30.35±2.54
1000	12.80±2.61	11.11±2.32[a]	19.24±1.89	7.86±0.48	27.09±2.31
1500	13.84±2.10	9.79±2.91[a]	18.96±2.24	7.54±0.48	26.49±1.70
2000	12.35±2.87	3.69±0.63[b]	19.27±1.65	7.98±0.45	27.24±2.08

[1]/ Means without letters in each column are not significantly different according to DMRT.
[2]/ Means with different letters in each column are significantly different according to DMRT.

Chlorophyll a+b content in old leaves

The highest chlorophyll ab content in old durian leaves was recorded for the 500-ppm group (T2: 30.35 µg/cm^2), with the control (0 ppm, T1: 29.11 µg/cm^2) and higher concentrations (T3: 27.09 µg/cm^2; T4: 26.50 µg/cm^2; T5: 27.24 µg/cm^2) showing lower values (Table 3).

Chlorophyll a content in new leaves

The analysis of chlorophyll a content in new durian leaves demonstrated that foliar application of amino acids at 500 ppm (T2) produced the highest mean chlorophyll a level (19.24 µg/cm^2), compared to the control without amino acid treatment (T1: 12.61 µg/cm^2), 1000 ppm treatment (T3: 16.96 µg/cm^2), 1500 ppm treatment (T4: 15.03 µg/cm^2), and 2000 ppm treatment (T5: 15.21 µg/cm^2), though no statistically significant differences were detected ($p>0.05$) (Table 4).

Chlorophyll b content in new leaves

Chlorophyll b content in new leaves showed the highest mean value with 500 ppm amino acid treatment (T2: 6.98 µg/cm^2), compared to 2000 ppm treatment (T5: 6.33 µg/cm^2), 1000 ppm treatment (T3: 6.19 µg/cm^2), 1500 ppm treatment (T4: 5.63 µg/cm^2), and the control (T1: 5.62 µg/cm^2), though differences were not statistically significant ($p>0.05$) (Table 4).

Chlorophyll a+b content in old leaves

The total chlorophyll (a+b) content analysis revealed that 500 ppm amino acid treatment (T2) achieved the highest mean value (26.21 µg/cm^2), followed by 1000 ppm treatment (T3: 23.15 µg/cm^2), 2000 ppm treatment (T5: 21.54 µg/cm^2), 1500 ppm treatment (T4: 19.49 µg/cm^2), and the control (T1: 18.31 µg/cm^2), with no significant statistical differences ($p>0.05$) (Table 4).

Table 4. Chlorophyll a, b and a+b content in new leaves of durian seedling under drought condition at the end of experiment

Amino acid concentration (ppm)	Chlorophyll a content in new leaves (µg/cm^2)$^{/1}$	Chlorophyll b content in new leaves (µg/cm^2)$^{/1}$	Chlorophyll a+b content in new leaves (µg/cm^2)$^{/1}$
0	12.60±2.28	5.61±3.37	18.31±1.58
500	19.23±3.44	6.98±1.85	26.20±2.26
1000	16.96±3.87	6.18±2.16	23.15±2.98
1500	15.02±2.60	5.62±0.27	19.49±3.61
2000	15.21±2.82	6.33±2.18	21.54±2.99

[1]/ Means without letters in each column are not significantly different according to DMRT.

Discussion

Foliar application of amino acids to Monthong durian seedlings under drought stress generated clear treatment-dependent responses at both soil and plant levels, with moderate concentrations (500–1000 ppm) consistently outperforming the control and the highest dose. This pattern is consistent with the concept of amino acids acting as biostimulants that enhance nutrient uptake and utilization efficiency and support plant growth and development, rather than simply serving as additional nutrient inputs (Al-Karaki and Othman, 2023; García-García *et al*., 2020).

At the soil level, the most prominent effect of amino acid application was the increase in soil pH from strongly acidic conditions in the control to slightly higher values in all amino acid treatments, with the maximum pH observed at 1000 ppm. Even a modest rise in pH can be agronomically important in acidic tropical soils, where aluminum and iron toxicities and low nutrient availability often limit growth. Previous studies have shown that amelioration of soil acidity improves root growth and nutrient uptake (Neina, 2019), and that soil pH strongly shapes rhizosphere microbial community structure in subtropical systems (Xia *et al*., 2024), while optimal soil conditions have been demonstrated to directly influence growth and physiological performance of durian trees

(Ismail *et al*., 1994). The current findings indicate that repeated foliar spraying, likely through canopy leachate and indirect rhizosphere changes, can slightly moderate soil acidity within a relatively short experimental period.

In contrast to pH, residual soil nitrogen was highest in the untreated control and lower in all amino acid treatments, despite identical soil and irrigation conditions. This pattern suggests enhanced nitrogen uptake and assimilation by durian seedlings receiving amino acids, consistent with findings in rice where amino acid-enriched fertilizer simultaneously reduced nitrogen loss/residue patterns in soil and increased nitrogen accumulation and utilization efficiency in plant tissue (Cheng *et al*., 2025).

Soil phosphorus and potassium did not show statistically significant differences among treatments but exhibited clear numerical trends. Phosphorus concentrations tended to be highest at 1000 and 1500 ppm, while potassium peaked at 1000 ppm and remained relatively high at 2000 ppm. These patterns are consistent with previous reports that amino acid-based biostimulants and protein hydrolysates can increase phosphorus and potassium uptake and overall nutrient-use efficiency by altering rhizosphere processes and enhancing plant physiological responses, particularly under stress conditions (Sun *et al*., 2024; Henderson *et al*., 2025; Pranckietienė *et al*., 2015). The decline in soil P at 2000 ppm suggests that excessive amino acid inputs may disturb P dynamics or increase plant P removal without a proportional growth benefit, in agreement with observations that amino acid-based foliar treatments do not always produce positive or dose-dependent effects in cereals (Ramazanova *et al*., 2025). Overall, the soil data indicate that moderate amino acid rates favor improved N utilization and tend to support higher P and K availability without exhausting soil reserves.

Growth measurements revealed that height and trunk diameter responded only weakly to amino acid treatments, with no significant differences among concentrations. This is not unexpected in a 49-day experiment on perennial tree seedlings under stress, where structural traits change slowly and are strongly genotype-dependent. More sensitive indicators were new leaf number, new shoot number, and dry weight of new leaves. Amino acid application at 500, 1000, and 1500 ppm significantly increased new leaf number compared with the control and 2000 ppm, with 1000 ppm producing the highest value. Similar concentration-dependent stimulation of vegetative growth by amino acids has been reported in faba bean under seawater salinity stress (Sadak *et al*., 2015) and faba bean under NaCl salinity stress (Abdeen and Hefni, 2023). Increased leaf production is particularly important for young durian seedlings, as it expands photosynthetic surface area and enhances carbon capture during recovery periods after drought.

New shoot number showed a numerical maximum at 1500 ppm but was not significantly different among treatments. This suggests that branching may be less responsive than leaf initiation to foliar amino acids under the conditions tested, although amino acid-driven stimulation of shoot growth has been documented in other species (Fetouh, 2017). Taken together, the shoot data indicate that amino acids primarily promoted leaf initiation and expansion rather than major changes in height or stem thickness during the experimental timeframe.

The biomass data support this interpretation. Old leaf dry weight did not differ significantly among treatments, whereas new leaf dry weight increased markedly at 500–1500 ppm and declined at 2000 ppm compared with the control. This pattern indicates that amino acids preferentially supported the growth of newly formed leaves, while older leaves remained relatively stable in mass. Differential responses between mature and developing leaves to stress and nutrient supply are well documented; new leaves often show stronger metabolic and morphological adjustments, while older leaves maintain structural integrity (Cechin *et al*., 2010; González-Villagra *et al*., 2022). In the present study, durian seedlings at 500–1000 ppm produced more and heavier new leaves, consistent with findings in lettuce where foliar amino acid fertilizers increased leaf dry weight and yield (Al-Karaki and Othman, 2023; Keskin *et al*., 2025).

Chlorophyll measurements in old and new leaves further clarify how amino acids improved canopy function. In old leaves, chlorophyll a, b, and total chlorophyll were highest at 500 ppm, slightly lower at higher concentrations, and intermediate in the control, although differences were not statistically significant. These results suggest that a low to moderate amino acid supply is sufficient to maintain or slightly enhance pigment stability in mature foliage under drought. Similar improvements in chlorophyll content at optimal amino acid doses have been reported under low-temperature and other stress conditions (Abd EL-All and Saeed, 2024; Kalisz *et al*., 2024; Ibrahim *et al*., 2023).

In new leaves, the responses were more pronounced 500 ppm produced the highest chlorophyll a, b, and total chlorophyll, followed by 1000 ppm, with lower values in the control and 2000 ppm. This pattern closely parallels the differences in new leaf dry weight and indicates that amino acids not only stimulated the formation of new leaves but also enhanced their photosynthetic capacity. Amino acid supplementation can increase leaf nitrogen allocation, chlorophyll biosynthesis, and photosynthetic nitrogen use efficiency, as demonstrated in Arabidopsis and fruit trees (Perchlik and Tegeder, 2018; Sheng *et al.*, 2020). Enzymes and regulatory networks involved in chlorophyll formation, such as chlorophyll a oxygenase and stay-green proteins, are sensitive to nitrogen and amino acid status (Tanaka *et al*., 1998; Jiao *et al*., 2020), and coordinated changes

in amino acid pools are known to influence pigment levels (Foyer and Noctor, 2005; Batista-Silva *et al*., 2019). The present results are consistent with these mechanisms and indicate that moderate amino acid rates optimized pigment formation in new durian leaves under drought.

Across all variables, the 2000 ppm treatment tended to perform worse than 500–1000 ppm and, in some cases, only slightly better than the control. Excessive amino acid supply may cause osmotic or metabolic imbalances, disrupt endogenous nitrogen signaling, or alter rhizosphere processes in ways that do not translate into improved growth (Calvo *et al.,*2014). For a sensitive species such as durian, this emphasizes the need for careful dose selection when using amino acid-based products.

In summary, foliar application of amino acids at 500–1000 ppm under drought conditions improved soil pH, enhanced nitrogen utilization, increased new leaf production and biomass, and promoted higher chlorophyll contents, particularly in newly developed leaves. These responses collectively indicate improved canopy structure and function and suggest that moderate amino acid supplementation can enhance drought resilience and establishment of Monthong durian seedlings in nursery systems. Higher concentrations (2000 ppm) did not confer additional benefits and may negatively affect some traits, highlighting the importance of using optimized, rather than maximal, doses of amino acid biostimulants in durian cultivation.

Amino acid foliar applications at 500–1000 ppm markedly improved growth, leaf development, and nutrient uptake efficiency in Monthong durian seedlings under drought stress. These treatments raised soil pH and optimized phosphorus and potassium availability, while higher doses (1500–2000 ppm) offered no added benefit and sometimes reduced effectiveness. Overall, moderate amino acid supplementation is a practical strategy to enhance durian seedling establishment and resilience in water-limited conditions.

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Conflicts of interest

The authors declare no conflict of interest.

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