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Growth of vanilla cuttings under modified acid soil media and application of several plant growth regulators

Abstract. This research aimed to optimize the growth of vanilla (*Vanilla planifolia*) cuttings in acid soils by modifying the growing media and applying several types of plant growth regulators (PGRs). Several types of PGRs, including synthetic compounds such as indole-3-acetic acid (IAA) and 6-benzylaminopurine (BAP), as well as natural extracts from shallot (*Allium ascalonicum* L.) and moringa leaves (*Moringa oleifera*), were tested to enhance plant performance in acid-growing conditions. The primary objectives were to determine the optimal soil amendment formula and the most effective PGR type for promoting vanilla growth, and to develop a measurable, easy-to-use PGR formulation accessible to farmers. The study was conducted experimentally using a randomized complete block design (RCBD) with the first factor being modified acid soil media and the second factor being the type of PGRs. The results showed that both 100 ppm BAP (Z2) and 10% shallot extract (Z3) significantly outperformed other PGR treatments across all parameters and observation times. For instance, Z2 produced the highest recorded values for internodes (15.11 internodes) at 90 DAP, number of leaves (10.26 leaves) at 60 DAP, total leaf area (413.74 cm^2) at 90 DAP, and aerial roots (12.22 roots) at 90 DAP. Conversely, Z3 demonstrated superior performance in the number of leaves (15.56 leaves) at 90 DAP and total leaf area (421.95 cm^2) at 90 DAP. This treatment can be recommended as an effective strategy for enhancing the establishment of vanilla cuttings in acidic soil conditions.

Keywords: 6-benzylaminopurine · Indole-3-acetic acid · PGRs · Shallot · *Vanilla planifolia*.

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Introduction

Vanilla (*Vanilla planifolia*) is a high-value plantation commodity in both local and export markets. Its high economic value has led to vanilla being called "green gold." Indonesia is the second-largest producer of vanilla in the global market. According to data from World Population Review (2024), the three largest vanilla-producing countries in 2023 were Madagascar (3.11 thousand tons), Indonesia (1.83 thousand tons), and Mexico (508 tons). Indonesia's production decreased from 1.97 thousand tons in 2022. Indonesian grade A vanilla is highly valued, with a price range of USD140-200/kg, exceeding the global market average price of EUR175.56/kg for whole vanilla and EUR270.40/kg for vanilla extract in 2022 (Munarso et al., 2024). Indonesian vanilla production from 2015 to 2024 (in terms of dried pods) has fluctuated, but showed a downward trend. Production in 2015 reached 1.74 thousand tons and was projected to decline to 1.56 thousand tons in 2024, with an average annual growth rate of -1.25% (Pertanian Sekretariat Jenderal Kementrian, 2024)

There is a need to increase the supply of vanilla planting materials and to ensure the availability of healthy seedlings. The availability of healthy planting materials is one of the requirements for successful large-scale vanilla cultivation (Murthy et al., 2010). Propagating vanilla by seed is less efficient because germination is slow, resulting in inconsistent plants. Therefore, stem cutting is more commonly used. This method accelerates plant production. Its advantages include producing plants with characteristics identical to those of the parent plant and lower costs (Ayyubi et al., 2019).Although the demand for vanilla is high, production remains limited due to farmers' challenges in managing agronomic conditions and the scarcity of fertile land.

It is necessary to explore marginal acidic land, one of the types of marginal land commonly found in Indonesia (99.56 million ha) (Hidayat & Mulyani, 2002). Acid soils are characterized by infertility, with a low pH of <5.5. High acidity levels result from the weathering of sulphide minerals, the leaching of base cations (Ca^{2+}, Mg^{2+}, K^{+}), and the decomposition of organic matter, which releases H^{+} (Brady & Weil, 2017; Food and Agriculture Organization of the United Nations, 2020). Vanilla grows optimally in a medium with a neutral pH of 6.5-7 (Divakaran et al., 2024; Havkin-Frenkel & Belanger, 2018). Acidic soils cause nutrient deficiencies in plants due to the binding and leaching of nutrients. High Al^{3+} ratios (>60%) interfere with Ca^{2+} and Mg^{2+} absorption (Food and Agriculture Organization of the United Nations, 2020). Al^{3+} inhibits root elongation, reducing nutrient and water absorption (Kochian et al., 2015). As a result, plants experience physiological stress, affecting their growth response.

At low pH (<5), plants have reduced activity of auxin-synthesizing enzymes, which are essential for cell differentiation (Taiz et al., 2015). The main method of vanilla propagation is through cuttings, but the roots of cuttings are susceptible to stress in acidic soils. Vanilla cuttings rely on adventitious root growth, which is sensitive to Al^{3+}. Exposure to Al^{3+} not only reduces root respiration and disrupts the uptake and transport of Ca^{2+}, Mg^{2+}, and H_2O, but also, upon root absorption, induces the production of reactive oxygen species (ROS), including the superoxide anion (O_2^-), which subsequently damages biomolecules such as lipids and nucleic acids (Berndt, 2024). Cuttings experience stress in acidic soils, resulting in plants failing to reach physiological maturity in the vegetative phase, with low biomass and limited carbohydrate reserves.

This study was based on the urgent need to optimize acidic land as a strategy for utilizing marginal land in vanilla cultivation. Acidic soil media were modified by incorporating organic matter and dolomite. Organic matter helps reduce Al toxicity and Fe2+, increase soil pH, and improve phosphorus availability in acidic soils (Suyanto, 2023). The application of dolomite increases the pH of acidic soil, with fine-textured dolomite showing higher effectiveness in neutralizing soil acidity (Wu et al., 2021). The application of exogenous PGRs and phytohormones is used to enhance the growth of vanilla cuttings in acidic soils. Shallot extract at 300 g/L (P_1) consistently produced the highest vegetative growth in vanilla cuttings under both elicitor treatments, namely *Coleus scutellarioides* extract (E1) and *Amaranthus spinosus* extract (E_2), with the respective values for P_1E_1 and P_1E_2 as follows: shoot length (44.97 cm and 46.60 cm), leaf number (7.67 and 7.33), leaf length (8.25 cm and 7.43 cm), leaf width (2.71 cm and 2.70 cm), and stem diameter (0.79 cm and 0.87

cm), compared to banana weevil extract (P_2), bamboo shoot extract (P_3), coconut water (P_4), and sprouts extract (P_5) (Rampe et al., 2024).

Moringa leaf extract as a biostimulant consistently improved the growth of black orchid (*Coelogyne pandurata*) plantlets across all growing media, increasing sapling height (up to 3.66 cm compared to 2.82 cm in the control on wood sawdust), leaf number (up to 4.80 compared to 3.40 on cocopeat), leaf length (up to 2.48 cm compared to 2.13 cm on coconut coir), leaf width (up to 0.64 cm compared to 0.53 cm on cocopeat), root number (up to 7.60 compared to 4.80 on cocopeat), and root length (up to 1.80 cm compared to 0.91 cm on sugarcane waste) (Zakiah & Turnip, 2023). In the orchid tissue culture study by Rathnayaka et al. (2023), moringa leaf extract at ¼ KnC + 15 ml/l produced the highest number of leaves (5.2 per plantlet) and roots (6 per plantlet), while at full KnC + 5 ml/l it yielded the highest number of shoots (3.5 per plantlet) (Unit & Garden, 2023).

Aluminium stress increases auxin accumulation in the root apical meristem and transition zone but decreases it in the elongation zone, whereas exogenous IAA partially restores auxin distribution, reduces Al accumulation in the cell wall, and alleviates toxicity (Li et al., 2018; Tao et al., 2023). Exogenous IAA application in the elongation zone can partially restore this inhibition by minimizing the disruption of auxin transport under Al toxicity. Exogenous IAA alleviates aluminium toxicity by reducing Al accumulation in the cell wall of alfalfa, whereas in tea plants, moderate Al supply promotes root growth through IAA accumulation, indicating that optimal IAA levels support root development under Al exposure (Gao et al., 2022; Wang et al., 2016). Modification of the growing medium and application of PGRs were done to stimulate the physiological performance and growth of vanilla plants in acidic soils.

Materials and Methods

Plant material. The research was conducted in Karangbanjar Village, Bojongsari District, Purbalingga (7°21'00"S 109°20'18" E) at an altitude of 148 meters above sea level. The research was conducted from November 2025 to February 2026. The vanilla cultivar used in this study was Vania 1. The cuttings had three nodes and two to three leaves. They were planted in a planter bag as a growing medium at a depth of 10 cm. One month after planting, the cuttings were tied to 130-cm-tall climbing poles made of coco fiber to support climbing. The average environmental conditions were 26.5°C, 83.63% humidity, 4.16 mm rainfall, and 15,957 lux light intensity.

The extraction of shallot and moringa was carried out as follows. Fresh shallot bulbs and fresh moringa leaves were separately washed, cut into small pieces, and then homogenized using an electric blender. For each extract, 100 g of plant material was blended with 1 L of distilled water (10%, w/v) at room temperature (24–26 ± 2°C) for 5 minutes until a homogeneous slurry was obtained. The resulting mixture was filtered twice through cheesecloth (pore size ± 0.5 mm) to remove coarse debris, and the clear filtrate was collected. The filtrate was stored in sterile amber glass bottles and used within 24 hours of preparation to prevent degradation of bioactive compounds.

Experimental design and treatments. The experiment was conducted under 75% shading net. The planter bags had a capacity of 35 L, and they were spaced 35 × 35 cm apart on acidic soil with a pH of 4.947. This study employed a factorial randomized complete block design (RCBD) with two factors: modified acidic soil media (three levels) and type of plant growth regulators (five levels), resulting in 15 treatment combinations. The experiment was arranged in three blocks as replicates, yielding 45 experimental units. Each experimental unit consisted of three planter bags (subsamples), with one plant per bag, giving a total of 135 plants. Data from the three subsamples within each experimental unit were averaged prior to statistical analysis. The first factor was modified acid soil media: acid soil (S_1), acid soil + 36 g of dolomite (S_2), and acid soil + 250 g of goat manure fertilizer + 36 g of dolomite (S_3). The second factor was the type of PGRs: control without PGRs (Z_0), 100 ppm IAA (Z_1), 100 ppm BAP (Z_2), 10% shallot extract (Z_3), and 10% moringa leaf extract (Z_4). The observation variables included tendrils length, number of internodes, number of leaves, total leaf area, and number of aerial roots. Observation data were statistically analyzed using analysis of variance (ANOVA) at the 5% significance level to determine treatment effects. When a significant effect was detected, Duncan's Multiple Range Test (DMRT) at the 5% level and regression analysis were conducted for further evaluation.

Tendrils length (cm). Tendrils length was measured using a tape measure from the base of the stem at ground level to the tip of the longest tendril (growing point). Measurements were taken at 60 days after planting (DAP) and at the end of the study, at 90 DAP.

Number of internodes. The number of internodes was measured by counting all fully formed nodes along the main stem of the plant. Measurements were taken at 30, 60, and 90 DAP.

Number of leaves. The number of leaves was recorded by counting all fully expanded, healthy leaves per plant. Leaf number was measured at 30, 60, and 90 DAP.

Total leaf area (cm²). Total leaf area was measured first by assessing the sample leaf area using a non-destructive computer vision method through the Petiole Pro application (version 24.10.01) on a smartphone (Figure 1). A calibration sheet containing eight markers was printed from the application at 100% scale and used as a reference. Small leaves were placed on the calibration sheet, whereas large leaves were positioned next to it on a contrasting surface, ensuring that the entire leaf and all eight markers were captured within a single frame. Images were taken under uniform lighting to avoid shadows. The application then automatically processed the image and displayed the leaf area per leaf (cm²).

Total leaf area was calculated according to Campbell and Norman (1998), as follows.

$$\text{Total leaf area} = \left(\frac{\textit{total leaf area of sampled leaves}}{\textit{Number of leaf samples}}\right) \text{x total leaves per plant}$$

Number of aerial roots. The number of aerial roots was counted on all visible roots emerging from each stem segment above the surface of the growing medium. Counts were recorded at 30, 60, and 90 DAP.

Results and Discussion

Before discussing the growth responses of vanilla cuttings, it is essential to first describe the changes in soil properties resulting from the applied treatments, as soil quality is a key determinant of rooting success in acidic soils. In this study, the initial acidic soil was characterized by low pH (<5.0), high exchangeable Al^{3+}, and very limited phosphorus availability. The application of organic matter and dolomite significantly improved soil chemical properties, including increasing soil pH to near-neutral levels, reducing soluble Al^{3+} and Fe^{2+} concentrations, and enhancing available phosphorus, although the magnitude of improvement varied among treatment combinations. Whether these improvements in soil quality significantly affected vanilla growth or not should be discussed, as they may determine the physiological response of cuttings to the modified root-zone environment.

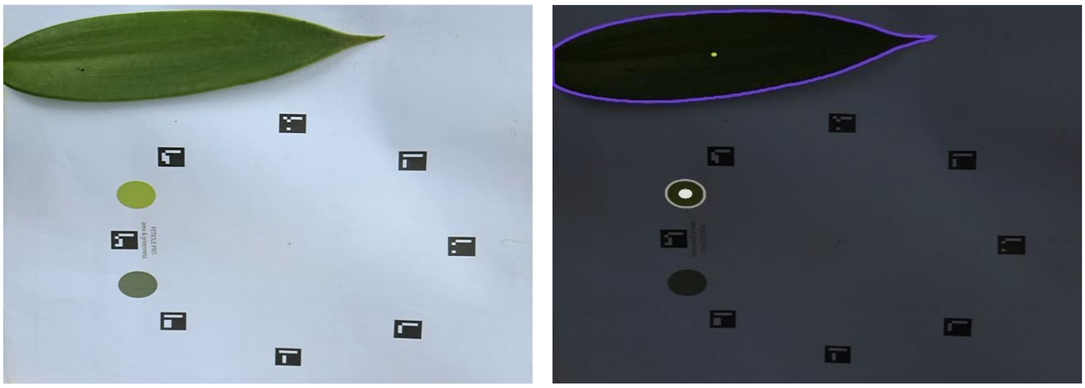

Figure 1. Assessing the sample leaf area using a non-destructive computer vision method through the Petiole Pro application (version 24.10.01)

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Table 1. The effect of modified acid soil media and type of plant growth regulator on tendrils length

Treatments	Tendrils Length at 60 DAP (cm)	Tendrils Length at 90 DAP (cm)
Modified acid soil media (S)		
S_1 (acid soil)	65.17±13.02a	77.80±18.13a
S_2 (acid soil + 36 g dolomite)	66.40±16.14a	78.93±21.23a
S_3 (acid soil + 250 g goat manure fertilizer + 36 g dolomite)	62.23±12.63a	82.80±9.25a
Type of PGRs (Z)		
Z_0 (Control)	49.56±3.27b	61.11±13.06b
Z_1 (100 ppm IAA)	64.39±16.80ab	84.11±15.54ab
Z_2 (100 ppm BAP)	74.50±6.83a	87.67±8.57a
Z_3 (10% shallot extract)	70.06±13.56ab	86.67±17.58a
Z_4 (10% moringa leaf extract)	64.50±12.13ab	79.67±15.95ab

Data are expressed as the mean of determination ± SD in 3 replicates. Within each column, means followed by the same letter are not significantly different among treatments at $p<0.05$.

Table 2. The effect of modified acid soil media and type of plant growth regulator on number of internodes

Treatments	Number of Internodes at 30 DAP	Number of Internodes at 60 DAP	Number of Internodes at 90 DAP
Modified acid soil media (S)			
S_1 (acid soil)	7.57±1.12a	8.98±1.29a	14.00±2.01a
S_2 (acid soil + 36 g dolomite)	8.53±1.38a	10.28±1.38a	13.93±2.13a
S_3 (acid soil + 250 g goat manure fertilizer + 36 g dolomite)	8.17±1.13a	9.52±1.20a	13.67±1.65a
Type of PGRs (Z)			
Z_0 (Control)	6.46±0.45b	8.22±0.40b	12.11±1.02b
Z_1 (100 ppm IAA)	8.30±1.57ab	9.39±2.02ab	13.22±2.22ab
Z_2 (100 ppm BAP)	8.85±0.62a	10.70±0.94a	15.11±1.07a
Z_3 (10% shallot extract)	8.56±0.51a	10.00±0.73ab	14.78±2.22a
Z_4 (10% moringa leaf extract)	8.28±1.22ab	9.65±1.20ab	14.11±1.35ab

Data are expressed as the mean of determination ± SD in 3 replicates. Within each column, means followed by the same letter are not significantly different among treatments at $p<0.05$.

Table 3. The effect of combined treatments of modified acid soil media and type of plant growth regulator on number of internodes

Treatments	Number of Internodes at 30 DAP	Number of Internodes at 60 DAP	Number of Internodes at 90 DAP
Modified acid soil media (S) x Type of PGRs (Z)			
S_1Z_0	6.00±1.73a	7.78±2.02a	12.33±1.53bc
S_1Z_1	7.44±2.00a	8.11±2.17a	12.67±2.52bc
S_1Z_2	8.17±0.76a	9.78±0.48a	14.33±2.52abc
S_1Z_3	9.00±0.50a	10.83±1.04a	17.33±1.53a
S_1Z_4	7.22±0.69a	8.39±1.34a	13.33±3.51abc
S_2Z_0	6.50±1.80a	8.56±0.36a	11.00±1.00c
S_2Z_1	10.11±1.64a	11.72±0.75a	15.67±0.58ab
S_2Z_2	9.39±1.51a	11.67±1.53a	16.33±2.08ab
S_2Z_3	8.67±2.33a	9.67±2.60a	13.33±2.08abc
S_2Z_4	8.00±2.08a	9.78±2.83a	13.33±2.08abc
S_3Z_0	6.89±1.26a	8.33±0.58a	13.00±1.00bc
S_3Z_1	7.33±1.20a	8.33±2.08a	11.33±2.89c
S_3Z_2	9.00±0.67a	10.67±0.88a	14.67±1.53abc
S_3Z_3	8.00±0.00a	9.50±0.87a	13.67±0.58abc
S_3Z_4	9.61±0.10a	10.78±1.07a	15.67±0.58ab

Data are expressed as the mean of determination ± SD in 3 replicates. Within each column, means followed by the same letter are not significantly different among treatments at $p<0.05$

Based on Table 1, the type of PGRs influenced the tendrils length growth parameter. At 60 DAP, the 100 ppm BAP treatment (Z_2) produced the highest mean tendrils length (74.50 cm), which was 50.32% greater than the control (Z_0; 49.56 cm). The highest mean tendrils lengths at 90 DAP were 87.67 cm and 86.67 cm in the 100 ppm BAP (Z_2) and 10% shallot extract (Z_3) treatments, respectively. Modified acidic soil medium had no statistically significant effect on tendrils length of vanilla cuttings, where the treatment mean square (68.72) was substantially smaller than the residual mean square (255.90). This indicates that variation among soil treatments was negligible relative to random experimental error, leading to failure to reject the null hypothesis. The interaction between soil medium modification and PGR type was also not significant for tendrils length.

The type of PGR treatment affected the number of internodes (Figure 2A and 3). Based on Table 2, at 30 DAP, the highest mean number of internodes was observed in the 100 ppm BAP (Z_2) and 10% shallot extract (Z_3) treatments, with values of 8.85 and 8.56 internodes, respectively. These values were 37.00% and 32.51% higher than the control (Z_0), which had 6.46 internodes. The highest number of internodes at 60 DAP was recorded in the 100 ppm BAP treatment (Z_2), with 10.70 internodes, which was 30.17% higher than the control (Z_0), which had 8.22 internodes. At 90 DAP, the 100 ppm BAP (Z_2) and 10% shallot extract (Z_3) treatments produced the highest numbers of internodes, with 15.11 and 14.78 internodes, respectively, representing increases of 24.77% and 22.05% over the control (Z_0).

The modified acidic soil medium had no effect on the number of internodes in this study. Modified acidic soil medium did not have a statistically significant effect on the number of internodes at both 60 DAP ($p = 0.100$) and 90 DAP ($p = 0.887$). At 60 DAP, the p-value approached but did not reach the 0.05 significance threshold, while at 90 DAP, the effect was clearly negligible. The very low F-value at 90 DAP (0.120) indicates that variation among soil treatments was far smaller than residual error (MS treatment = 0.467, MS residual = 3.890). Consequently, we fail to reject the null hypothesis, meaning that under the conditions tested, modifying the acidic soil did not lead to measurable changes in internode formation. However, the interaction between the modified acidic soil medium and the type of PGR significantly affected the number of internodes at 90 DAP. The highest number of internodes at 90 DAP was observed in the acidic soil plus 10% shallot extract treatment (S_1Z_3), with 17.33 internodes, representing a 40.55% increase over the control (S_1Z_0), which had 12.33 internodes (Table 3).

Table 4 indicate that the type of PGR significantly influenced the number of leaves. At 30 DAP, the highest mean leaf counts were observed in the 100 ppm BAP (Z_2) and 10% shallot extract (Z_3) treatments, with 8.52 and 8.80 leaves, respectively. These values represented increases of 32.92% and 37.29%, respectively, compared with the control (Z_0). At 60 DAP, the 100 ppm BAP treatment (Z_2) produced the highest leaf count, with 10.26 leaves, representing a 25.27% increase over the control (Z_0). At 90 DAP, the 10% shallot extract treatment (Z_3) produced the highest leaf count, with 15.56 leaves, representing a 27.33% increase over the control (Z_0). The number of leaves was not affected by the modified acidic soil medium. In addition, the interaction between the modified acidic soil medium and PGR type had no significant effect on leaf number. Modified acidic soil medium did not significantly affect leaf number at any observation time (30 DAP: $p = 0.536$; 60 DAP: $p = 0.070$; 90 DAP: $p = 0.357$). Similarly, the interaction between modified acidic soil medium and PGR type was not significant across all time points (30 DAP: $p = 0.856$; 60 DAP: $p = 0.167$; 90 DAP: $p = 0.114$). These results indicate that variation in leaf number was primarily explained by PGR type, which showed a significant effect at all observation times ($p < 0.05$), whereas soil modification and its interaction with PGRs contributed negligibly under the tested conditions.

Table 4. The effect of modified acid soil media and type of plant growth regulator on number of leaves

Treatments	Number of Leaves at 30 DAP	Number of Leaves at 60 DAP	Number of Leaves at 90 DAP
Modified acid soil media (S)			
S_1 (acid soil)	7.56±1.09a	8.91±1.04a	13.60±2.01a
S_2 (acid soil + 36 g dolomite)	8.16±1.16a	10.17±1.38a	14.73±2.13a
S_3 (acid soil + 250 g goat manure fertilizer + 36 g dolomite)	8.07±0.92a	9.63±1.09a	14.40±1.55a
Type of PGRs (Z)			
Z_0 (Control)	6.41±0.43b	8.19±0.54b	12.22±0.84b
Z_1 (100 ppm IAA)	7.87±0.84ab	9.65±1.70ab	13.78±2.52ab
Z_2 (100 ppm BAP)	8.52±0.45a	10.26±0.72a	15.11±1.35ab
Z_3 (10% shallot extract)	8.80±0.31a	10.06±0.63ab	15.56±1.26a
Z_4 (10% moringa leaf extract)	8.04ab±0.97ab	9.70±1.50ab	14.56±1.68ab

Data are expressed as the mean of determination ± SD in 3 replicates. Within each column, means followed by the same letter are not significantly different among treatments at p<0.05.

Table 5. The effect of modified acid soil media and type of plant growth regulator on total leaf area

Treatments	Total Leaf Area at 60 DAP (cm^2)	Total Leaf Area at 90 DAP (cm^2)
Modified acid soil media (S)		
S_1 (acid soil)	260.47±71.26a	270.73±48.55b
S_2 (acid soil + 36 g dolomite)	319.17±82.51a	377.11±85.52a
S_3 (acid soil + 250 g goat manure fertilizer + 36 g dolomite)	338.06±129.84a	403.20±80.10a
Type of PGRs (Z)		
Z_0 (Control)	194.29±23.03b	274.71±71.00b
Z_1 (100 ppm IAA)	301.90±103.92ab	289.23±41.17ab
Z_2 (100 ppm BAP)	341.21±28.27a	413.74±100.32a
Z_3 (10% shallot extract)	328.78±19.41a	421.95±78.90a
Z_4 (10% moringa leaf extract)	363.33±163.88a	352.09±76.57ab

Data are expressed as the mean of determination ± SD in 3 replicates. Within each column, means followed by the same letter are not significantly different among treatments at p<0.05.

Table 6. The effect of combined treatments of modified acid soil media and type of plant growth regulator on total leaf area and leaf greenness

Treatments	Total Leaf Area at 60 DAP (cm^2)	Total Leaf Area at 90 DAP (cm^2)
Modified acid soil media (S) x Type of PGRs (Z)		
S_1Z_0	173.02±85.19d	207.38±65.91a
S_1Z_1	237.91±60.68bcd	244.46±29.47a
S_1Z_2	319.98±53.26bcd	302.10±56.26a
S_1Z_3	346.30±27.79bcd	331.83±33.46a
S_1Z_4	225.16±136.03cd	267.87±46.55a
S_2Z_0	191.09±82.35cd	267.85±112.79a
S_2Z_1	421.81±88.17ab	325.44±68.54a
S_2Z_2	330.35±83.22bcd	442.79±186.23a
S_2Z_3	332.14±85.74bcd	478.58±187.89a
S_2Z_4	320.44±146.71bcd	370.86±121.51a
S_3Z_0	218.74±66.27cd	348.89±33.01a
S_3Z_1	245.99±61.27bcd	297.80±70.45a
S_3Z_2	373.30±123.82abc	496.32±144.52a
S_3Z_3	307.91±50.90bcd	455.44±158.40a
S_3Z_4	544.39±136.43a	417.52±10.94a

Data are expressed as the mean of determination ± SD in 3 replicates. Within each column, means followed by the same letter are not significantly different among treatments at p<0.05.

Based on Table 5, the modified acidic soil medium had no effect on total leaf area at 60 DAP. However, it significantly affected total leaf area at 90 DAP. The highest total leaf area at 90 DAP was observed in the acid soil + 36 g dolomite (S_2) and acid soil + 250 g goat manure fertilizer + 36 g dolomite (S_3) treatments, with values of 377.11 and 403.20 cm², respectively, representing increases of 39.29% and 49.93% over the control (Z_0). The type of PGR affected total leaf area. At 60 DAP, the highest mean total leaf area was observed in the 100 ppm BAP (Z_2), 10% shallot extract (Z_3), and 10% moringa leaf extract (Z_4) treatments, with values of 341.21, 328.78, and 363.33 cm², respectively. These values were 32.92% and 37.29% greater than those of the control (Z_0; 194.29 cm²). At 90 DAP, the highest total leaf area was recorded in the 10% shallot extract treatment (Z_3), at 421.95 cm², which was 53.60% greater than that of the control (Z_0).

The interaction between the modified acidic soil media and PGR type significantly affected total leaf area at 60 DAP, but not at 90 DAP. Based on Table 6 the highest total leaf area at 60 DAP was recorded in the treatment combining acidic soil, 250 g goat manure fertilizer, 36 g dolomite, and 10% moringa leaf extract (S_3Z_4), with a value of 544.39 cm². This was 214.65% higher than that of the acidic soil treatment without PGRs (S_1Z_0). The significant F-value for the interaction (p = 0.024) confirms that the effect of modified acidic soil medium on total leaf area depends on the type of PGR applied.

Table 7. The effect of modified acid soil media and type of plant growth regulator on number of aerial roots

Treatments	Number of Aerial Roots at 30 DAP	Number of Aerial Roots at 60 DAP	Number of Aerial Roots at 90 DAP
Modified acid soil media (S)			
S_1 (acid soil)	5.04±1.10a	6.49±1.21a	10.53±1.73a
S_2 (acid soil + 36 g dolomite)	5.42±1.64a	7.04±1.43a	11.67±2.82a
S_3 (acid soil + 250 g goat manure fertilizer + 36 g dolomite)	5.18±1.28a	6.36±1.55a	10.87±1.50a
Type of PGRs (Z)			
Z_0 (Control)	3.76±0.52b	4.87±0.45b	8.44±1.17b
Z_1 (100 ppm IAA)	5.37±1.48ab	6.87±1.63ab	11.22±2.34a
Z_2 (100 ppm BAP)	6.06±0.87a	7.22±0.39a	12.11±1.64a
Z_3 (10% shallot extract)	5.70±0.79a	7.37±1.11a	12.56±1.07a
Z_4 (10% moringa leaf extract)	5.19±1.66ab	6.81±1.36ab	10.78±1.39ab

Data are expressed as the mean of determination ± SD in 3 replicates. Within each column, means followed by the same letter are not significantly different among treatments at $p<0.05$.

Table 8. The effect of combined treatments of modified acid soil media and type of plant growth regulator on number of aerial roots

Treatments	Number of Aerial Roots at 30 DAP	Number of Aerial Roots at 60 DAP	Number of Aerial Roots at 90 DAP
Modified acid soil media (S) x Type of PGRs (Z)			
S_1Z_0	4.11±1.64abcd	5.33±1.20a	9.67±1.15bcde
S_1Z_1	5.17±2.25abcd	6.44±1.50a	9.00±2.65cde
S_1Z_2	5.44±1.50abcd	6.83±1.26a	11.00±3.00abcde
S_1Z_3	6.61±0.35abc	8.33±1.89a	13.33±0.58ab
S_1Z_4	3.89±1.54acd	5.50±1.32a	9.67±3.21bcde
S_2Z_0	3.17±1.04ad	4.83±2.52a	7.33±0.58e
S_2Z_1	6.94±1.73ab	8.67±1.26a	13.67±0.58a
S_2Z_2	7.06±1.55a	7.22±1.44a	14.00±4.04a
S_2Z_3	5.33±1.76abcd	7.78±2.41a	13.00±3.00ab
S_2Z_4	4.61±1.78abcd	6.72±2.66a	10.33±3.21abcde
S_3Z_0	4.00±2.00bcd	4.44±1.39a	8.33±2.52de
S_3Z_1	4.00±1.73bcd	5.50±2.50a	11.00±4.04abcde
S_3Z_2	5.67±0.67abcd	7.61±1.27a	11.33±2.31abcd
S_3Z_3	5.17±0.29abcd	6.00±0.87a	11.33±2.08abcd
S_3Z_4	7.06±0.42a	8.22±0.84a	12.33±1.15abc

Data are expressed as the mean of determination ± SD in 3 replicates. Within each column, means followed by the same letter are not significantly different among treatments at $p<0.05$.

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The type of PGR treatment significantly affected the number of aerial roots (Table 7 and Figure 2B). At 30 and 60 DAP, the highest mean numbers of aerial roots were observed in the 100 ppm BAP (Z_2) and 10% shallot extract (Z_3) treatments, with 6.06 and 5.70 roots, respectively, compared with 4.76 roots in the control (Z_0), representing increases of 27.31% and 19.75%. At 90 DAP, the highest mean numbers of aerial roots were recorded in the 100 ppm IAA (Z_1), 100 ppm BAP (Z_2), and 10% shallot extract (Z_3) treatments, with 11.22, 12.11, and 12.56 roots, respectively, which were 32.94%, 43.48%, and 48.82% higher than the control (Z_0; 8.44 roots). The modified acidic soil medium had no effect on the number of aerial roots in this study.

The interaction between modified acidic soil media and PGR type significantly affected the number of aerial roots at 30 DAP and 90 DAP. At 30 DAP, the highest number of aerial roots (7.06 roots) was observed in both the acid soil + 36 g dolomite + 100 ppm BAP (S_2Z_2) treatment and the acid soil + 250 g goat manure fertilizer + 36 g dolomite + 10% moringa leaf extract (S_3Z_4) treatment. Each represented a 71.78% increase over the acidic soil treatment without PGRs (S_1Z_0), which had 4.11 roots. At 90 DAP, the highest numbers of aerial roots were recorded in the acid soil + 36 g dolomite + 100 ppm IAA (S_2Z_1) and acid soil + 36 g dolomite + 100 ppm BAP (S_2Z_2) treatments, with 13.67 and 14.00 roots, respectively. These values were 41.36% and 44.78% higher than those of the control (S_1Z_0), which had 9.67 roots (Table 8).

Vanilla plant growth is inhibited in soils with low pH. Soils with a pH of 4.0–5.0 are classified as very strongly acidic. The primary factor limiting plant growth in acidic soils is aluminum toxicity. According to Blum et al. (1989), at pH values below 5.0, aluminum dissolves as Al^{3+}, which is toxic to plant roots, rapidly inhibiting root elongation and damaging the root system. Because vanilla roots are shallow and highly susceptible to injury, such conditions can disrupt water and nutrient uptake. Research by Wan et al. (2018) found that Al increases proton (H^+) release and plasma membrane H^+-ATPase activity in roots of *Camellia sinensis*, which is associated with higher Al accumulation and excess cation uptake relative to anion uptake as Al concentration increases. Excessive H^+ release acidifies the rhizosphere and lowers soil pH. In addition, an imbalance between cation and anion uptake disrupts ion homeostasis in root cells. Because elevated H^+-ATPase activity requires substantial energy, and Al accumulation inhibits root cell division and elongation, the combined effects of acidification, nutrient imbalance, and root damage can cause systemic disruption and ultimately reduce plant growth.

The soil's capacity to resist changes in pH (soil buffering capacity) is important for maintaining nutrient availability. The addition of dolomite and organic fertilizers, such as goat manure, as soil amendments improves both the physical and chemical properties of the soil (Głąb et al., 2023; Juniardi et al., 2022). Organic fertilizers such as goat manure increase soil nutrient content, particularly nitrogen, which plays a role in vegetative growth, and improve soil aggregation and microbial activity (Salim et al., 2023). Soarizafy et al. (2024) reported that the application of organic compost derived from agricultural waste (straw and manure) to vanilla at a dose of half a 5-L bucket promoted rapid vine growth, reaching up to 100 cm, which was significantly greater than the control without compost, which reached only 60 cm. In the present study, modified acidic soil media affected vanilla growth, as indicated by total leaf area at 90 DAP. The acid soil + 36 g dolomite (S2) and acid soil + 250 g goat manure fertilizer + 36 g dolomite (S3) treatments resulted in total leaf areas of 377.11 and 403.20 cm^2, respectively, compared with 270.73 cm^2 in the control (Z0) (Table 4).

The acidic soil used in this study (pH 4.947) imposed abiotic stress on vanilla plants. PGRs were applied to enhance plant survival under unfavourable environmental conditions, including acidic soil, through various physiological and biochemical mechanisms. Regarding tendrils length, PGR type had a significant effect at 60 DAP, with the 100 ppm BAP treatment (Z_2) producing the highest value (74.50 cm). At 90 DAP, the highest tendrils lengths were recorded in the 100 ppm BAP (Z_2) and 10% shallot extract (Z_3) treatments, at 87.67 and 86.67 cm, respectively. BAP is also known to stimulate lateral shoot growth and branching, thereby directly increasing the number of nodes (Mawaddah et al., 2021).

To properly interpret the growth responses of vanilla cuttings, it is essential first to establish the fundamental soil constraints, as an acidic environment with a pH below 5.5 typically induces high levels of phytotoxic Al^{3+} and Fe^{2+} alongside limited phosphorus availability, which collectively impair root development (Sun et al., 2025). The applied dolomite functions through acid-base neutralization to elevate soil pH and reduce heavy metal ion activity, while the addition of organic matter helps detoxify Al^{3+} primarily through complexation mechanisms where functional groups such as carboxyl and hydroxyl bind with the toxic ions (Lijuan et al., 2025). The integrated application of dolomite and organic amendments has been demonstrated to outperform single applications in mitigating subsoil acidification, a conclusion supported by the synergistic effects observed when dolomite is combined with organic materials based on their titratable alkalinity (Iticha et al., 2024). Even when these chemical improvements did not translate into a statistically significant increase in vanilla stem length during this study, they represent a net improvement in soil quality for sustainable root development and long-term cultivation.

The type of PGR treatment significantly affected the number of internodes, number of leaves, and number of aerial roots at 30, 60, and 90 DAP, whereas total leaf area was affected at 60 and 90 DAP. The most effective PGR treatments were 100 ppm BAP (Z_2) and 10% shallot extract (Z_3). As a cytokinin, BAP promotes cell division and bud formation. As shown in Table 2, the highest number of internodes at 30 DAP was recorded in the 100 ppm BAP treatment (S_2), with 8.85 internodes, whereas the highest value at 90 DAP was observed in the 10% shallot extract treatment (S_3), with 8.56 internodes. At 60 DAP, the highest number of internodes was recorded in the 100 ppm BAP treatment (S_2), with 10.70 internodes. Mahardhini et al. (2023) found that vanilla shoot length did not differ significantly among the 0.6, 1.2, and 1.8 mg L^{-1} BAP treatments, although all three differed significantly from the 2.4 mg L^{-1} treatment. The BAP and control (0 mg L^{-1}) treatments were used for comparison.

The highest number of leaves at 30 DAP was observed in the 100 ppm BAP (S_2) and 10% shallot extract (S_3) treatments, with 8.52 and 8.80 leaves, respectively. At 60 DAP, the highest leaf number was recorded in the 100 ppm BAP treatment (S_2), with 10.26 leaves, whereas at 90 DAP, the highest value was observed in the 10% shallot extract treatment (S_3), with 15.56 leaves (Table 4). Total leaf area was highest at 60 DAP in the 100 ppm BAP (Z_2), 10% shallot extract (Z_3), and 10% moringa leaf extract (Z_4) treatments, with values of 341.21, 328.78, and 363.33 cm^2, respectively. At 90 DAP, the highest total leaf area was 421.95 cm^2 in the 10% shallot extract treatment (Z_3). Greater leaf number and leaf area indicate improved plant health and photosynthetic capacity. (Kusbianto et al., 2024). Cytokinin is involved in cell differentiation and organ formation, including in leaves (Kusbianto et al., 2022). The application of synthetic or natural cytokinins mobilizes nutrients to the treatment site. Cytokinin treatment retards chlorophyll degradation and downregulates the expression of genes associated with chlorophyll degradation, thereby delaying leaf senescence (Li Liu et al., 2016).

In addition, the highest numbers of aerial roots at 30 and 60 DAP were recorded in the 100 ppm BAP (S_2) and 10% shallot extract (S_3) treatments, with 6.00 and 5.70 roots, respectively. At 90 DAP, the highest numbers of aerial roots were also observed in the 100 ppm BAP (S_2) and 10% shallot extract (S_3) treatments, with 12.11 and 12.56 roots, respectively. However, the 100 ppm IAA (S_1) treatment also produced a high number of aerial roots, with 11.22 roots (Table 5). The number of aerial roots was also significantly affected by the interaction between the modified acidic soil medium and PGR type at 30 and 90 DAP. Auxin plays a key role in root growth initiation (Aditania et al., 2023). The interaction at 30 and 90 DAP indicates that the stimulatory effect of PGRs on root formation was more pronounced in media with improved physical and chemical properties, allowing the PGRs to act more effectively and efficiently (Juniardi et al., 2022; Salim et al., 2023). Auxins are essential for regulating plant growth and development by governing the formation of primary, secondary, and adventitious roots. The application of rooting hormones enhances adventitious root initiation, improves root quantity and quality, and increases overall rooting percentage (Saini & Anmol, 2024).

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(A) (B)

Figure 2. Growth of shoots that will become new internodes (A) and aerial roots (B) of vanilla plants in the first week

(A) (B)

Figure 3. Vanilla plant shoot growth has formed new internodes in the second week (A) and third week (B) of the plant

Acidic soil conditions, characterized by low pH, aluminum toxicity, and nutrient deficiency, represent major constraints to plant growth. PGRs may mitigate these adverse effects through several mechanisms. IAA and BAP enhance stress tolerance by activating antioxidant enzymes that neutralize reactive oxygen species (ROS). Increased activity of these enzymes is important, as superoxide dismutase (SOD) converts superoxide radicals (O_2^-) into hydrogen peroxide (H_2O_2), whereas catalase (CAT) further decomposes H_2O_2 into water and oxygen. Aluminum stress in acidic soils increases ROS production, which can damage cell membranes, as reflected by elevated malondialdehyde (MDA) levels. Conversely, a decrease in MDA indicates reduced lipid peroxidation and membrane damage.

Auxins such as IAA plays an important role in plant growth, including root formation and biomass accumulation (Kecis et al., 2023; Ullah et al., 2023). Exogenous application of IAA can increase tolerance to heavy metal stress, such as reducing arsenate toxicity in eggplant roots through endogenous H_2O_2 signaling (Alamri et al., 2021). Auxin carriers such as LAX3 are involved in stress tolerance by affecting stomatal opening and salt tolerance in tobacco(Li, Hong, et al., 2022). Biofertilizer cyanobacteria is also produced IAA and can improve atrazine stress in rice field cyanobacteria (Ahmad et al., 2022). In corn, plant smoke containing IAA and IBA improves root architecture and reduces the effects of the auxin inhibitor 2,3,5-Triiodobenzoic acid (TIBA) (Ullah et al., 2023). Other auxins, such as NAA, help grape growth under drought conditions through physiological and hormonal modifications (Khandani et al., 2024). The protective effects of auxins are generally associated with the modulation of antioxidant enzymes and the suppression of ROS, thereby reducing oxidative damage (Alamri et al., 2021; Khandani et al., 2024).

The application of IAA under Al stress conditions requires caution due to the potential for counterproductive effects. In this study, the IAA treatment did not produce the highest values for all growth variables, namely tendrils length, number of internodes, total leaf area, number of leaves, and number of aerial roots at 30 and 60 DAP; however, it did produce the highest value for the number of aerial roots at 90 DAP. Research on *Citrus limonia* proves that high concentrations of endogenous IAA due to Al stress are correlated with plant susceptibility, where IAA transport-related genes are not differentiated so that IAA accumulates but is not well distributed to root cells (Silva et al., 2019). A study on Arabidopsis thaliana revealed the molecular mechanism, whereby Al inhibits the endocytosis of the PIN2 protein (auxin transporter) by limiting plasma membrane fluidity, which disrupts the basipetal transport of IAA from the transition zone to the root elongation zone, thereby inhibiting cell elongation (X. Li et al., 2018). Based on this evidence, before applying IAA to vanilla plants in acid soil, it is necessary to determine their tolerance level to Al; if vanilla is classified as sensitive, exogenous IAA application carries a high risk of exacerbating Al accumulation in the roots and oxidative damage.

Synthetic cytokinin such as BAP, plays an important role in cell division, differentiation, and shoot regeneration, including in *Vanilla planifolia* (Duri, 2022; L. Li, Li, et al., 2022; Salfiani et al., 2021). Cytokinins such as BAP and cis-zeatin are key regulators that control plant growth and defense systems by regulating antioxidant mechanisms. BAP has been shown to increase salt stress tolerance in grapes by modulating antioxidant genes such as Catalase (CAT) and Ascorbate Peroxidase (APX) (Ahmad et al., 2022; Mahardhini et al., 2023; Ullah et al., 2023). The application of IAA and BAP on Mentha rotundifolia also increases biomass and phytochemical parameters correlated with stress resistance (Alamri et al., 2021; Rivas et al., 2021).

The interaction between IAA and BAP is crucial in stress tolerance, involving the activation of antioxidant defense. Plant hormones form a frontline network that integrates abiotic and biotic stress responses through convergent crosstalk pathways (Ku et al., 2018), which ultimately relies on the management of oxidative stress by the antioxidant system (Ahmad et al., 2022; Khandani et al., 2024; Mahardhini et al., 2023; Ullah et al., 2023). Although direct molecular studies on *Vanilla planifolia* are still limited, this plant is susceptible to stress that induces oxidative stress and naturally possesses an antioxidant system (Alamri et al., 2021; Khandani et al., 2024). Therefore, the exogenous application of IAA and BAP has the potential to enhance the inherent antioxidant system of vanilla to strengthen its resistance to environmental stresses, similar to the mechanisms that occur in other plants.

Natural PGRs derived from natural extracts such as shallot and moringa leaf have the potential to provide synergy because they contain a combination of auxins, cytokinins, and gibberellins simultaneously. These phytohormones also control plant responses to various stimuli such as water availability, light, nutrients, abiotic, and biotic stress. Phytohormones, as plant secondary metabolites, act as chemical messengers that regulate growth, development, reproduction, survival, and senescence; they coordinate signalling pathways for abiotic stress adaptation, and their endogenous fluctuations modulable by exogenous application before or during stress affect all aspects of plant growth (Amoanimaa-Dede et al., 2022). Research by Tini et al. (2022) found that shallot bulbs contain 251.76 ppm IAA; 75.54 ppm kinetin; 23.77 ppm zeatin; and 594.12 ppm GA_3, with the highest GA_3 content compared to banana stems, moringa leaves, sweet corn seeds, and mung bean sprouts. The application of shallot extract to vanilla cuttings resulted in the highest average shoot length (46.60 cm), number of leaves (7.67), and leaf length (8.25 cm) (Rampe et al., 2024). Moringa leaves contain 662.17 ppm IAA; 161.37 ppm kinetin; 55.5 ppm zeatin; and 417.88 ppm GA_3, with the highest levels of IAA and kinetin compared to other sources (Tini et al., 2022). The application of plant growth regulators (PGRs) supports plant growth under specific environmental conditions. PGRs are natural organic compounds in higher plants that are active in small amounts, inducing growth,

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physiology, and yield in plants (Desta & Amare, 2021).

The discussion comparing synthetic plant growth regulators (BAP and IAA) with natural plant growth regulators, such as shallot extract and moringa leaf extract, is quite interesting, as recent findings provide a clearer picture of the underlying mechanisms. While synthetic IAA is a standard auxin, natural extracts often work through complementary pathways. For instance, shallots are rich in quercetin, a flavonoid that can modulate the distribution of natural auxins in plant tissues (Singh et al., 2021). In comparison, plant-based alternatives such as aloe vera gel can substitute for synthetic auxins in promoting in vitro rooting, indicating that the actions of these complex mixtures often extend beyond a single hormone (Madhushani & Rohanadheera, 2025). Similarly, compared to synthetic cytokinin, Moringa leaf extract owes its efficacy to its zeatin content, a naturally occurring cytokinin promoting cell division and growth. Thus, these extracts mimic or alter natural hormone actions, and their bioactive compounds can also enhance stress resilience, leading to a stronger growth response (Singh et al., 2024; Zia et al., 2021). The comparable or superior performance of these natural extracts on certain parameters likely stems from their multifaceted composition, which provides more balanced hormonal and protective effects.

Based on this mechanism, the application of PGRs to vanilla plants cultivated in acid soil (pH 4.0–5.0) can be specifically targeted. Root system expansion is promoted by IAA for optimal nutrient absorption, while BAP maintains leaf greenness and the photosynthesis rate of tendrils, which influence growth parameters such as tendrils length and internodes of vanilla plants. In addition, natural PGs are an economical alternative with safer, gradual effects. However, specific research on vanilla is still needed to determine the appropriate type of PGR, concentration, and application frequency, considering that plant response to hormones is greatly influenced by species, stress levels, and endogenous hormonal balance (Ahammed & Yu, 2016).

Conclusion

Based on the evaluation of various combinations of modified acidic soil media and types of plant growth regulators (PGRs), the treatment consisting of acid soil + 250 g goat manure fertilizer + 36 g dolomite (S_3) combined with 100 ppm BAP treatment (Z_2) and 10% shallot extract (Z_3) treatments, with consistently produced the highest total leaf area, tendrils length, number of internodes, number of leaves, and number of aerial roots in vanilla cuttings. This specific treatment outperformed all other combinations across the measured growth parameters. Therefore, this treatment can be recommended as an effective strategy for enhancing the establishment of vanilla cuttings in acidic soil conditions. The findings serve as a practical reference for researchers and growers seeking to optimize vanilla cultivation on marginal acidic lands.

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