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Growth of vanilla (*Vanilla planifolia*) roots in different internodes of stem cuttings with NAA (Naphthaleneacetic Acid) treatments

WIDYA MUDYANTINI, YARIFA NURUL HUDA, ARI PITOYO♥
Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sebelas Maret. Jl. Ir. Sutami 36A Surakarta 57 126, Central Java, Indonesia. Tel./fax.: +62-271-663375, ♥email: aripitoyo@staff.uns.ac.id

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Abstract. *Mudyantini W, Huda YN, Pitoyo A. 2024. Growth of vanilla (*Vanilla planifolia*) roots in different internodes of stem cuttings with NAA (Naphthaleneacetic Acid) treatments. Cell Biol Dev 8: 13-21.* Vanilla (*Vanilla planifolia* Andrews) is a plantation commodity with high economic value. Unstable production of vanilla pods causes a decrease in vanilla pod exports. One of the obstacles is the limited availability of seeds. This study aims to determine the effect of combining two treatments on root growth in vanilla stem cuttings. The first treatment was a variation of NAA concentrations of 0, 50, 100, and 150 ppm. The second treatment was the cuttings' age on the third, fifth, and seventh nodes of vanilla stem cuttings. The planting material was soaked in a solution of NAA hormone and was carried out 3 repetitions for 60 minutes. Cuttings were planted in the growing medium for 90 days. The research parameters measured were root emergence time, first root length, number of root branches, first root diameter, root branch length, number of leaves, and shoot height. The results showed that soaking vanilla cuttings with an NAA concentration of 150 ppm accelerated root emergence and increased primary root length. The seventh node of the vanilla cuttings soaked in 150 ppm of NAA hormone solution was the best combination for the time of root emergence and root length, while the fifth node without being soaked in NAA hormone solution was the best combination for shoot height and the number of leaves. Soaking vanilla cuttings with NAA hormone solution did not affect increasing root diameter, number of roots, and length of root branches.

Keywords: Auxin, Naphthaleneacetic acid, soaking method, stem cuttings, *Vanilla planifolia*

INTRODUCTION

Indonesia has a tropical climate that is favorable for plant growth, one of which is vanilla (*Vanilla planifolia* Andrews). Vanilla is used as a mixture of food, beverages, and cosmetics industry, which makes its high economic value. Indonesia is the largest vanilla producer in the world, along with Madagascar, Papua New Guinea, and India (Frenkel and Belanger 2019). North Sumatra, Lampung, Central Java, West Java, East Java, Bali, West Nusa Tenggara, East Nusa Tenggara, North Sulawesi, and South Sulawesi are areas that cultivate vanilla plants and even become vanilla production centers (Ruhnayat 2003). According to the report of the Vanilla Plantation Statistic Directorate General of Plantations (2010), in 2004-2005, the price of dried vanilla pods was from Rp 2,000,000 to Rp 3,000,000/kg, but in 2007-2008, the price dropped from Rp 200,000 to Rp 300,000. This can happen because the quality of the vanilla fruit is low and does not meet the criteria for the international market (Baharudin et al. 2023).

Vanilla farmers in Indonesia tend to cultivate vanilla vegetatively through cuttings rather than seeds because vanilla seeds are difficult to germinate due to the immature endosperm of the plant (Umesha et al. 2011). Vegetative propagation is an alternative for farmers in the vanilla nursery process. Propagation by cuttings is easy, inexpensive, and produces the same tillers as the parent plant. According to Kartikawati and Rosman (2018), short vanilla cuttings consisting of 2 nodes with 1 node can be an alternative to limited seeds. Sources of seeds from shortcuttings take 4-6 months to be transferred to planting land (Hadipoentyanti et al. 1998). This problem hinders vanilla production, which can impact decreasing vanilla exports. In addition, the production of vanilla seeds that have been certified by the Minister of Agriculture in Indonesia is still limited due to the small number of vanilla mother farms in Indonesia (Balfas 2022). According to the Directorate General of Plantations (2021), the vanilla main gardens established in Indonesia are spread across five provinces with a total of 16 main gardens.

Vanilla planting material using short cuttings needs to pay attention to the mother tree's age, which indicates the stem's maturity on the cuttings and the vanilla stem's hardness (Sukarman 2011). According to Hartman and Kester (1975), good cutting material can be determined from the hardness of the stem. Carbohydrate distribution depends on the plant's age; young cuttings contain relatively low carbohydrates, while old cuttings contain relatively high carbohydrates (Aldrete-Herrera et al. 2019). Somantri and Evizal (1987) stated that vanilla cuttings were able to germinate due to the support of well-developed and growing roots. The hormone contained, i.e., auxin, functions in root formation by stimulating cell elongation and division in the cambium tissue.

Naphthalene-acetic Acid (NAA) is an auxin hormone consisting of a white amorphous synthetic organic compound. NAA stimulates cell division, enlargement, cell differentiation, and protoplasmic flow in the vegetative growth of plants, including root organs (Widiastoety 2014). NAA is applied for plant vegetative propagation, topically

as a paste or soaking. The concentration and duration of immersion affect the level of absorbed auxin. The longer the immersion and the higher the concentration, the greater the auxin absorbed by plant cells (Supardi and Seda 2010). Research by Yan et al. (2014) on *Hemarthria compressa* (L.f.) R.Br. plants treated with an NAA concentration of 200 ppm by immersion method for 20 minutes resulted in a root growth percentage of 97%, the number of adventitious roots per cutting, and dry weight of roots per cutting. Jamaludin's research (2019) showed that single-node vanilla cuttings treated with NAA and IBA at a concentration of 500 ppm produced a shoot height of 14 cm and a lower root length of 25 cm at 16 weeks after planting. This study used 0 ppm, 50 ppm, 100 ppm, and 150 ppm in the concentration of NAA as an exogenous auxin hormone and different stem cuttings to determine the growth of vanilla cuttings.

MATERIALS AND METHODS

Study area

The research was carried out from December 2022 to May 2023 at the Biology Laboratory Department of Biology, Universitas Sebelas Maret, Indonesia, and Universitas Sebelas Maret Integrated Laboratory Green House, Indonesia.

Tools and materials

The tools used were pruning shears for cutting cuttings, a plastic bucket for soaking cuttings, a 25 × 25 cm polybag for cuttings to grow in, a spatula for taking NAA powder, a 100 mL measuring cup for measuring water volume, a 1,000 mL beaker for preparation of hormone solutions, stir bars to stir hormone solutions, analytical balances to weigh ingredients, measuring tape to measure root length and shoot height, caliper to measure root diameter, Munsell Plant Tissue Color Chart (Munsell 2023), and plastic caps.

The materials used are Naphthaleneacetic Acid (NAA) powder, vanilla cuttings taken from the vanilla tree of the vania 2 variety with an age of ≥ 12 months that have not yet flowered from the Karavan Boyolali cooperative vanilla garden, aquadest, KOH, Dithane M45, manure, poor sand, and garden soil.

Procedures

Obtain vanilla cuttings

Obtaining vanilla cuttings refers to the Decree of the Minister of Agriculture Number 08/KPTS/KB.020/1/2018 concerning guidelines for the production, certification, distribution, and supervision of vanilla plant seeds. The vanilla plant of the Vania 2 variety selected for cuttings is a healthy mother tree, not nutrient deficient, aged ≥ 12 months, ± 1 meter long (8-10 nodes), and vines that have not yet produced flowers. Planting material was taken from the Karavan Boyolali cooperative vanilla garden, where the vines were cut using sharp pruning shears to prevent tissue damage into one node and two node cuttings according to the treatment.

Preparation of planting media

The planting medium used is a mixture of soil, sand, and manure with a ratio of 1:1:1 (Mariska et al. 1987; Supardi and Seda 2010). The garden soil is dried for about 3 days to reduce the water content and then sieved to obtain uniform soil size. Soil, sand, and manure are mixed with a ratio of 1:1:1 until blended. The planting media mixture is filled into a polybag measuring 25 × 25 cm as much as 1,500 grams. The center of the media is given a hole with a depth of 10 cm.

Preparation of NAA hormone solution

NAA hormone powder was dissolved according to the predetermined treatment, namely 0, 50, 100, and 150 ppm. To make a NAA 50 hormone solution, 100 and 150 ppm. Next, 50, 100, and 150 mg of NAA powder were dissolved in 1000 mL of distilled water each. Before being dissolved in distilled water, the NAA powder was dissolved with a few drops of concentrated KOH. Based on several studies that have been carried out previously, the use of NAA to stimulate roots using the soaking method uses a concentration range of 20-250 ppm, so in this study, a concentration variation was taken that was still in that range (Blythe et al. 2007).

Soaking cuttings in NAA hormone solution

Vanilla cuttings were soaked in Dithane M45 fungicide solution for 15 minutes and then dried overnight to avoid pathogens that can infect vanilla cuttings due to cuttings (Holis 2017). In the next stage, cuttings were immersed in a solution of the NAA hormone according to the treatment for 60 minutes. Each treatment had 3 (three) repetitions from each segment of the stem cuttings. Soaking is done in a shady place that is not exposed to direct light.

Planting in the growing media

The soaked vanilla cuttings are dried at room temperature for overnight. Furthermore, the cuttings are planted in polybags filled with media with the node's position closed with the planting medium.

Plant maintenance

Polybags are placed in the Universitas Sebelas Maret Integrated UPT Laboratory Green House with an altitude of 106 meters above sea level, 89% humidity, and temperatures ranging from 26-29°C. The plants are covered with a bamboo and plastic frame measuring 2.5 × 0.8 × 1 meter to keep the environment moist and avoid direct sunlight. Vanilla cuttings are watered with 150-300 mL water in the morning when the planting medium is dry. After three weeks of planting, vanilla cuttings were sprayed with Dithane M-45 fungicide to prevent the cuttings from rotting. In addition, weeding plant weeds is done if there are weeds in the soil media.

Observation variable measurement

Vanilla cuttings are grown for 90 days. Vanilla roots appeared on the 10^{th}, 20^{th}, 30^{th}, 60^{th}, and 90^{th} days after planting. Measurements of the number of leaves and the height of the growing shoots were carried out once a week

for 90 days after planting. Shoot height was measured from the base of the shoot to the tip using a measuring tape. Measurement of the first root length, number of root branches, root branch length, and first root diameter was carried out 30 days after planting by dismantling the media. Next, the length of the first root and the growing root branch were measured using a measuring tape, counting the entire number of growing root branches and measuring the root diameter using a caliper.

Data analysis

Qualitative data in this study were in the form of morphological and anatomical descriptions of vanilla roots. Quantitative data for measuring parameters on the 90th day obtained in this study were analyzed using the ANOVA test with a significance value of the difference P <0.05. If there is a significant effect, continue with Duncan's Multiple Range Test (DMRT) at a 95% confidence level presented in the table. If the DMRT test shows significant results, it is followed by a different letter symbol.

RESULTS AND DISCUSSION

Morphological structure and anatomy of vanilla root

The *V. planifolia* has two types of roots: terrestrial roots in the soil and aerial roots that appear at the stem nodes. Morphologically, vanilla is classified as a monocot plant with fibrous terrestrial roots (Ruhnayat 2003). Figure 1. A shows the morphology of vanilla terrestrial roots without treatments. The texture of the vanilla root is not woody (herbaceous) and has mucilage when cut. Vanilla root has fine root hairs; elongated cylindrical round root shape with a pointed tip. Terrestrial roots consist of the first root that appears on the cutting and the root branch that grows on the first root. Based on the Munsell Book Color Chart, the terrestrial root has a yellowish-white color (2.5Y 8/6) (Figure 2. A).

The morphology of aerial roots without treatments is shown in Figure 1. B. The air root of vanilla has a slippery texture, is not woody (herbaceous), and has mucilage when cut. Aerial roots do not have root hairs. Fine root hairs appear when aerial roots grow downward and touch the ground; elongated cylindrical round root shape with a blunt end. Aerial roots do not have root branches. Vanilla aerial roots are green (2.5GY 5/8) (Figure 2. B).

Figure 1. A. Terrestrial roots, B. Aerial roots. Scale bars=100mm (Photo credit: Nurul Huda)

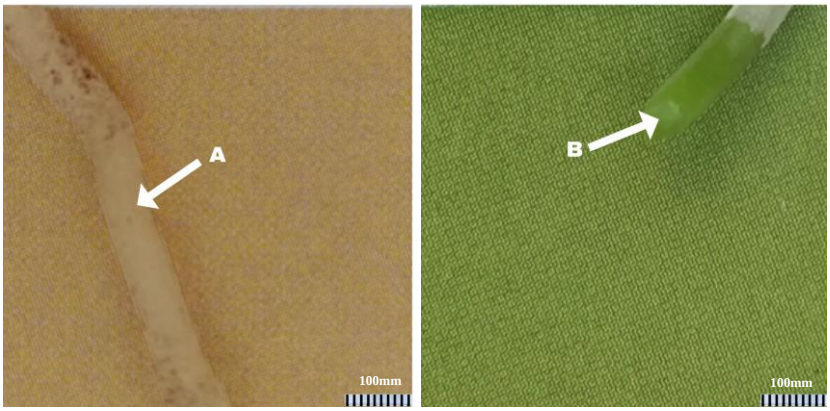

Figure 2. A. Terrestrial root color and B. Aerial root color of *Vanilla planifolia* Andrews. Scale bars= 100 mm (Photo credit: Nurul Huda)

Table 1. The average value of the first root appearance of vanilla (days) on different sections after damping with NAA

Cutting Segment	Concentration of NAA				Average
	0 ppm	50 ppm	100 ppm	150 ppm	
R3*	21.67±1.52^{a}	21.67±5.13^{a}	22.33±6.80^{a}	15.67±4.50^{abc}	20.33±5.03
R5*	15.33±3.05^{abc}	21.00±1.73^{ab}	19.67±1.52^{ab}	17.00±4.35^{abc}	18.25±3.38
R7*	14.33±4.93^{bcd}	19.33±0.57^{ab}	12.33±1.52^{cd}	8.33±2.51^{d}	13.58±4.81
Average	17.11 ± 4.56	20.67 ± 2.91	18.11 ± 5.73	13.67±5.26	17.39±5.20

Note: Means with the same letter along columns are not significantly different based on the DMRT test at a 95% confidence level. *R3: Third segment, R5: Fifth segment, R7: Seventh segment

Table 2. The average value of the first vanilla root length (cm) on different segments of age 90 days after planting after soaking with NAA

Cutting Segment	Concentration of NAA				Average
	0 ppm	50 ppm	100 ppm	150 ppm	
R3*	12.76±2.01^{def}	9.53±2.30^{ef}	14.66±2.65^{bcde}	18.06±3.34^{abc}	13.75±3.93
R5*	19.70±1,90^{ab}	12.03±1.35^{def}	12.06±2.05^{def}	21.70±3.38^{a}	16.37±4.97
R7*	12.36±1.26^{def}	9.10±5.31^{f}	13.53±2.37^{cdef}	17.06±3.55^{abcd}	13.01±4.19
Average	14.94±3.88	10.22±3.27	13.42±2.34	18.94±3.64	14.38±4.50

Note: Means with the same letter along columns are not significantly different based on the DMRT test at a 95% confidence level. *R3: Third segment, R5: Fifth segment, R7: Seventh segment

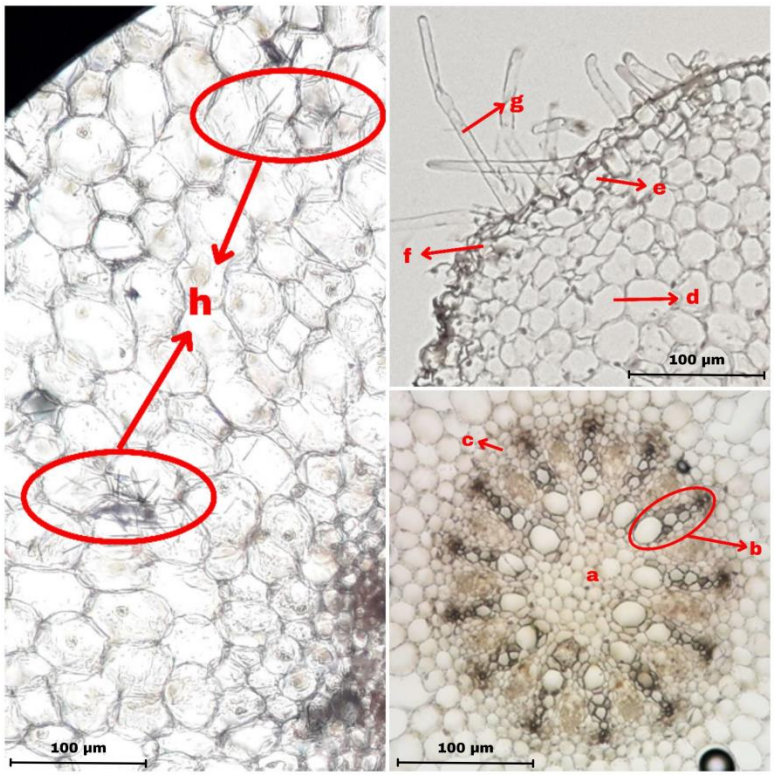

Figure 3. Anatomical structure cross-section of vanilla terrestrial root magnification 100×. a. Pith, b. Vascular bundle c. Endodermis, d. Cortex, e. Exoderm, f. Epidermis, g. Root hair, h. Rapidhes needle. Scale bars= 100 µm (Photo credit: Nurul Huda)

Anatomically, the cross-section of the vanilla terrestrial root consists of root hair, epidermis, exodermis, cortex, endodermis, vascular bundle, and pith (Figure 3). In this study, the structure of the valamen was not observed in the cross-section of the vanilla terrestrial root). The velamen structure is suspected to be only observed in cross-sections of vanilla aerial roots (Deseo et al. 2020). The structure of the epidermis terrestrial roots is thinner than the exodermis, causing the epidermis to collapse easily. The cortex structure consists of peripheral cells between the exodermis and endodermis. The cortex structure is also observed as an ergastic object that is rapid and needle-shaped. The endodermal structure consists of a single row of cells surrounding a vascular bundle consisting of the xylem and phloem. The pith was the deepest structure observed,

consisting of large, round, thick parenchyma cells (Stern and Judd 1999).

Time of first root emergence

Vanilla roots appeared on the 10th, 20th, 30th, 60th, and 90th days after planting. If roots appear before the observation day, the root emergence day is determined based on the root growth rate. ANOVA results show that variations in the concentration of the NAA hormone and the age of the cuttings, independently or in combination, significantly affect the time of appearance of the first vanilla roots.

Table 1 shows that soaking cuttings with NAA hormone was significantly different in accelerating the appearance of the first vanilla roots. The fastest roots were shown in 150 ppm NAA immersion, 13.67 days. Panjaitan et al. (2014) state that auxin stimulates root growth. This aligns with research by Agustiansyah et al. (2017) that using NAA can accelerate the emergence of roots in guava grafts by up to 73.3%. Auxin moves basipetal (from tip to base) to encourage root formation (Rashotte et al. 2000). Auxin makes it easier for cell walls to stretch so that wall pressure down the cell. Thus, cell flexing occurs so that cell elongation and enlargement occur and then encourage the formation of roots (Wijana and Lasmini 2021).

This study's treatment combinations significantly differed in the vanilla roots' emergence time. The fastest roots were shown in the combined treatment of the seventh segment of 150 ppm NAA immersion, 8.33 days. Table 1 shows that the roots of the seventh internode of vanilla cuttings appeared faster than the third and fifth internodes. It was shown that the roots appeared in 13.58 days. The seventh cutting segment is thought to have older cuttings, so they have sufficient food reserves. Root formation on cuttings requires energy in the form of carbohydrates and protein stored in the origin of the planting material (Suryanti et al. 2022).

First root length

The parameters measured were vanilla terrestrial roots. The ANOVA results showed that variations in the concentration of the NAA hormone and the age of the cuttings, independently or in combination, significantly affected the length of the first vanilla root.

Table 2 shows that soaking cuttings with different NAA hormones significantly increased the length of the first vanilla root. The NAA concentration of 150 ppm shows the longest root, 18.94 cm. Root elongation results from the enlargement of new cells in the apical meristem area due to continuous cell division (Khadr et al. 2020). Auxin induces cell division by regulating the cell cycle with the help of glucose. Auxin and glucose signals are needed to regulate cell proliferation, especially preparation for cell replication from the G1 phase to the S phase. Auxin and glucose induce the expression of the Cyclin D3;1 (CYCD3;1) gene in plants to increase the cell cycle (Sablowski and Dornales 2014). Activated cyclin D can still be blocked by the CDK inhibitor KRP. Auxin can reduce the expression of several KRP genes so that Cyclin D can still trigger transcriptional suppressor RBR phosphorylation and release complex transcription regulators E2FA/B and DPA. Auxin stabilizes the E2FA/B and DPA complexes, which trigger gene expression transcription for the initial S phase. Furthermore, after the S phase is complete, glucose plays a role again in starting the transition from the G2 phase to the M phase (Wang and Ruan 2013).

Table 2 shows that the combination treatment of the fifth node of vanilla cuttings and an NAA concentration of 150 ppm produced the longest root, 21.70 cm. The fifth segment of vanilla cuttings is suspected to be physiologically young (juvenile), so the meristem cells are still active (Suryanti et al. 2022). The root meristem is located at the distal part of the root, which continues to grow to produce new cells supported by the expression of the NAC1 gene (Xie and Ding 2022). Continuous cell division causes cell elongation, thereby promoting vanilla root elongation.

First root diameter

Root diameter is an important parameter to observe. Roots have carrier bundles for transporting nutrients and photosynthetic products. The first root diameter measurement using a caliper was measured on the 90th day after planting. ANOVA results show that variations in the concentration of NAA hormone and the age of the cuttings independently or in combination have no significant effect on the diameter of the first vanilla root.

Table 3 shows that soaking cuttings with NAA hormone did not show any significant difference in increasing the diameter of the first root. The treatment with the NAA hormone concentration of 50 ppm, which was 2.86 mm, showed the largest diameter of the vanilla root. Auxin plays a role in cell expansion. Auxin will stimulate H+-ATPase in the plasma membrane through upregulation of phosphorylation to pump H+ ions into the cell wall. H+ ions cause acidification of the apoplast in the plant cell wall. Expansion proteins work optimally at low pH to break the hydrogen bonds between the polymer walls. Plant cell walls contain cellulose and hemicellulose fibrils embedded in a pectin and protein matrix (Wolf et al. 2012). This allows slippage between cellulose microfibrils, resulting in cell wall elongation (Sablowski and Dornales 2014). Plant cell walls elongate, resulting in water entering by osmosis and active cell walls loosening proteins. This process causes enlargement of the cell wall. Cells continue to grow by re-synthesizing cell walls and cytoplasmic materials (Putra and Shofi 2015; Barbez et al. 2017; Majda and Robert 2018).

Number of root branches

Root branches that appeared on the first vanilla root were counted on the 90th day after planting. In general, from all treatments, root branches grew on the first root of the vanilla plant. The results of ANOVA showed that variations in the concentration of the NAA hormone and the age of the vanilla cuttings, either independently or in combination, had no significant effect on the number of root branches.

Table 3. The average value of the first root diameter of vanilla (mm) on different segments 90 days after planting after soaking with NAA

Cutting Segment	Concentration of NAA				Average
	0 ppm	50 ppm	100 ppm	150 ppm	
R3*	2.86±0.16	2.96±0.30	2.83±0.15	2.66±0.15	2.83±0.20
R5*	2.60±0.50	2.83±0.57	2.53±0.36	2.63±0.25	2.66±0.40
R7*	2.50±0.50	2.73±0.12	2.56±0.25	3.26±0.10	2.76±0.40
Average	2.65±0.39	2.86±0.34	2.64±0.27	2.85±0.34	2.75±0.34

Note: R3: Third segment, R5: Fifth segment, R7: Seventh segment

Table 4. The average value of the number of vanilla root branches (strands) at different ages 90 days after planting after soaking with NAA

Cutting Segment	Concentration of NAA				Average
	0 ppm	50 ppm	100 ppm	150 ppm	
R3*	3.67±1.52	3.33±2.51	6.67±2.08	5.00±4.35	4.67±2.77
R5*	6.67±3.05	7.00±4.58	8.33±2.08	3.67±3.21	6.42±3.37
R7*	5.33±1.52	3.67±3.21	4.33±3.05	9.67±4.50	5.75±3.69
Average	5.22±2.27	4.67±3.53	6.44±2.74	6.11±4.45	5.61±3.28

Note: R3: Third segment, R5: Fifth segment, R7: Seventh segment

Table 5. The average value of the length of the vanilla root branch (cm) at different ages of 90 days after planting after soaking with NAA

Cutting Segment	Concentration of NAA				Average
	0 ppm	50 ppm	100 ppm	150 ppm	
R3*	1.87±0.22	2.28±1.87	2.31±0.83	2.51±2.17	2.24±1.30
R5*	3.96±1.74	2.23±0.56	3.64±0.76	2.96±1.12	3.20±1.19
R7*	3.22±1.28	1.86±1.64	2.90±1.00	3.88±1.36	2.97±1.37
Average	3.01±1.42	2.12±1.29	2.95±0.95	3.12±1.52	2.80±1.32

Note: R3: Third segment, R5: Fifth segment, R7: Seventh segment

Table 4 shows that soaking cuttings with NAA hormone did not significantly differ from the number of vanilla root branches. Soaking NAA at a concentration of 100 ppm resulted in the highest average number of root branches, namely 6.44 strands. Root branches originate from pericycle cells that have differentiated during the root branch initiation process (Alarcon et al. 2016). Sufficient auxin levels can increase the number of root branches. In some plants, auxin does not induce root branching. The independent meristem initiation and branching mechanisms influence this; the structural differences in the roots cause no auxin-induced branching mechanisms (Fang et al. 2019). In this study, the combination of the seventh segment treatment and 150 ppm NAA immersion concentration had the highest average number of root branches, namely 9.67 strands. Exogenous auxin resulted in the number of root branches growing on the first root. The growth of root branches is affected by the length of the first root. The first roots grow well, and then the root branches have room to develop (Alarcon et al. 2019).

Research on the growth of corn root branches conducted by Alarcon et al. (2019) showed that applying NAA doses of 0.01µM and 0.05µM significantly increased the average number of root branches. The effect of stimulating root branch growth is lost when high doses are applied. This is not the case with vanilla root. NAA immersion at concentrations of 100 ppm and 150 ppm still showed the growth of root branches. It is suspected that there is a raphide needle structure in the cortex of the vanilla root. The presence of raphide needles causes cells to produce clear, homogeneous mucus. The mucus has a role in protecting the protoplasm from raphide spiky needles. It also functions to regulate osmotic pressure. The roots do not lose water when there is a high solution concentration (Smith 1923; Seker et al. 2016).

Long branch root

Root branch length was measured on the 90th day after planting the cuttings. The measured root branches are the root branches that appear on the first vanilla root. Root branches that grow are measured using a measuring tape. The results of ANOVA showed that variations in the concentration of NAA hormone and the age of vanilla cuttings, either independently or in combination, had no significant effect on the length of the root branches.

Root branches generally originate from the pericycle of the vascular cylinder. If the first root has developed well, the emergence of root branches will develop well, too. Based on Table 5, soaking cuttings with NAA hormone did not show a significant difference in the length of the vanilla root branches. NAA concentration of 150 ppm produced the best average root branch length of 3.12 cm. This shows that auxin can encourage root elongation through cell division.

Table 6. The average value of vanilla shoot height (cm) on different segments 90 days after planting after soaking with NAA

Cutting Segment	Concentration of NAA				Average
	0 ppm	50 ppm	100 ppm	150 ppm	
R3*	8.90±6.03^{abc}	5.56±6.49^{abc}	4.03±6.05^{bc}	15.53±14.09^{ab}	8.50±8.85
R5*	18.00±5.89^{a}	11.23±1.76^{abc}	1.10±1.15^{c}	10.16±9.22^{abc}	10.12±7.87
R7*	10.96±5.08^{abc}	0.00±0.00^{c}	0.43±0.75^{c}	6.50±8.04^{abc}	4.47±6.25
Average	12.62±6.42^{x}	5.60±5.91^{yz}	1.85±3.52^{z}	10.73±10.12^{xy}	7.70±7.88

Note: Means with the same letter along columns are not significantly different based on the DMRT test at a 95% confidence level. *R3: Third segment, R5: Fifth segment, R7: Seventh segment

Table 7. The average value of the number of vanilla leaves on different segments of age 90 days after planting after soaking with NAA

Cutting Segment	Concentration of NAA				Average
	0 ppm	50 ppm	100 ppm	150 ppm	
R3*	5.67±0.57^{ab}	1.33±2.30^{bc}	2.00±3.46^{abc}	4.67±4.16^{ab}	3.42±3.14
R5*	6.33±1.15^{a}	4.67±0.57^{ab}	0.00±0.00^{c}	3.33±2.87^{abc}	3.58±2.77
R7*	4.67±1.15^{ab}	0.00±0.00^{c}	0.00±0.00^{c}	2.33±4.04^{abc}	1.75±2.70
Average	5.56±1.13	2.00±2.39	0.67±2.00	3.44±3.39	2.92±2.92

Note: Means with the same letter along columns are not significantly different based on the DMRT test at a 95% confidence level. *R3: Third segment, R5: Fifth segment, R7: Seventh segment

The addition of exogenous auxin can stimulate existing endogenous auxin for cell division. In this study, combining the fifth node of vanilla cuttings and 0 ppm NAA concentration resulted in the best average root branch length of 3.96 cm. This is presumably because the fifth segment of vanilla cuttings has high meristem activity, so the endogenous auxin is still sufficient for cell division. If endogenous auxin is sufficient, plant cells do not need exogenous auxin (Tamba et al. 2019).

High shoots

New vanilla shoots start growing from the cutting nodes. The height of the growing vanilla shoots was measured on the 90th day. Shoots are measured from the base of the emergence of shoots to the tip using a measuring tape. ANOVA results show that the age of vanilla cuttings has no significant effect on shoot height, but variations in the concentration of NAA hormone independently and the combination of the two have a significant effect on shoot height.

The growth of shoots is an organogenesis carried out by meristem cells. The response is dependent on the phase of the G1 phase of the cell cycle. The formation of shoots requires the hormones auxin and cytokinins (Tyas et al. 2016). Table 6 shows that soaking cuttings with different NAA hormones significantly increased the height of vanilla shoots. Although auxin increased the height of vanilla shoots, optimal results were obtained by treating NAA at a concentration of 0 ppm, namely 12.62 cm. Dinarti et al. (2010) stated that if the concentration of cytokinins is higher than auxin, shoots are formed faster.

Number of leaves

Vanilla leaves are located alternately on each stem node. The number of leaves was counted on the 90th day after planting. The leaves that are counted are leaves that have opened or rolled. The results of ANOVA showed that the age of the vanilla cuttings did not significantly affect the number of leaves, but variations in the concentration of the NAA hormone independently and the combination of the two had a significant effect on the number of leaves.

Good root growth causes more nutrients to be absorbed, and it can increase plant growth. Good plant growth causes leaf growth so that photosynthesis is more optimal. The results of photosynthesis are used by plants for growth and development (Arat et al. 2021). Based on Table 7, soaking cuttings with different NAA hormones significantly increased the number of vanilla leaves. The highest number of leaves was shown in soaking NAA cuttings with a concentration of 0 ppm, namely 5.56 leaves. Wibowo et al. (2023) on vanilla plants stated that the highest number of leaves was shown in the treatment of natural auxin (Indole Acetic Acid) compared to synthetic auxin (Indole Butyric Acid) with an average value of 12 leaves. The hormone cytokinin influences the growth of shoots and leaves, while auxin inhibits shoots at high concentrations (Wulandari and Darwati 2015). In this study, it was suspected that plant tissue already contained sufficient cytokinins to help leaf growth.

In conclusion, treatment of varying concentrations of the NAA hormone increased the first root length, shoot height, number of leaves, and accelerated root emergence on each tested cutting segment. The higher the NAA concentration, the average root length, shoot height, and the number of leaves, also increased the appearance of the first roots of vanilla cuttings on each tested cutting, faster. Treatment of variations in cuttings increased the length of the first root and accelerated the appearance of roots on each segment of the cuttings tested. Medium age of stem cuttings increased the average value of first root length, while older stem cuttings accelerated the emergence of first roots of vanilla stem cuttings. The combination of treatments with variations in NAA concentrations and the age of vanilla cuttings increased first root length, shoot

height, number of leaves, and accelerated root emergence on each tested cutting segment. The most optimal length of the first root was obtained by combining the treatment of the fifth internode with an NAA concentration of 150 ppm. The optimal shoot height and number of leaves were obtained by combining the fifth node treatment with an NAA concentration of 0 ppm. The most optimal time for roots to appear was obtained by combining the treatment of the seventh segment with an NAA concentration of 150 ppm.

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