

Response to Growth and Yield of Gandarusa (*Justicia Gendarussa*) on Different Stem Origin and Auxin Concentration

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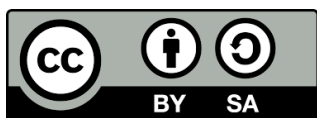
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Keywords:

Origin of Cuttings; Auxin;
Justicia Gendarussa; Growth;
Vegetative Propagation

ABSTRACT

This study aimed to evaluate the effects of stem cutting origin, auxin concentration, and their interaction on the growth and yield of gandarusa (*Justicia gendarussa*) plants. The experiment was conducted in Karang Tanjung Village, Alian District, Kebumen Regency, Central Java, Indonesia, using a factorial randomized complete block design (RCBD) with two factors: cutting origin (middle-stem and basal-stem cuttings) and 1-naphthaleneacetic acid (NAA) concentrations (0, 50, 75, and 100 ppm). The experiment consisted of eight treatment combinations, four replications, and five plants per experimental unit, resulting in 160 plants. The observed parameters included plant height, stem diameter, number of leaves, number of branches, root length, number of roots, fresh weight, dry weight, and cutting survival percentage. Data were analyzed using analysis of variance (ANOVA) at a 5% significance level, followed by Duncan's multiple range test (DMRT) when significant differences were detected. The results indicated that cutting origin significantly affected stem diameter and cutting survival percentage, while auxin concentration significantly influenced stem diameter. A significant interaction between cutting origin and auxin concentration was observed for stem diameter at 35 days after planting (DAP) and fresh weight at 56 DAP. The B2A1 treatment combination (basal-stem cuttings treated with 100 ppm NAA) produced the highest stem diameter (6.011 mm) at 35 DAP and the highest fresh weight (6.150 g) at 56 DAP. These findings suggested that the combination of basal-stem cuttings and 100 ppm NAA was the most favorable treatment for improving stem diameter and fresh biomass production in gandarusa under the experimental conditions.



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INTRODUCTION

Indonesia is a tropical country that receives high levels of solar radiation throughout the year (Arwiyadi, 2016). Excessive exposure to ultraviolet (UV) radiation can cause various skin disorders, including premature skin aging resulting from changes in skin structure and composition (Putra et al., 2012). These conditions have increased interest in

natural ingredients with potential health benefits, particularly medicinal plants that can be cultivated in tropical environments. The growing demand for medicinal plant materials, supported by developments in the phytopharmaceutical industry, has highlighted the importance of identifying and cultivating plant species with potential therapeutic applications, including gandarusa (*Justicia gendarussa*).

Gandarusa (*Justicia gendarussa*) belongs to the family Acanthaceae and is widely distributed in Indonesia, where it has potential medicinal applications (Ashari, 2006). Ethnopharmacological reports have documented its traditional use in managing diabetes in India (Yusmaini, 2009). Preclinical investigations involving alloxan-induced diabetic mice and the brine shrimp lethality test (BSLT) indicated that methanolic extracts of gandarusa leaves exhibited antihyperglycemic activity without significant cytotoxic effects under the experimental conditions (Putra et al., 2012). These findings suggested the potential value of gandarusa as a source of bioactive compounds. Consequently, the increasing demand for medicinal plant materials has emphasized the need for efficient propagation techniques capable of producing sufficient quantities of high-quality planting material.

Vegetative propagation through stem cuttings is a relatively simple and efficient technique for producing planting material within a shorter period than many conventional propagation methods (Hartmann & Kester, 2010). Stem cuttings are generally classified into hardwood, semihardwood, softwood, and herbaceous categories based on their physiological characteristics and degree of tissue maturity (Ashari, 2006). Gandarusa is an herbaceous to shrubby plant that can be propagated using cuttings obtained from different stem positions, including apical, middle, and basal sections (Ashari, 2006). Differences in stem maturity, carbohydrate reserves, endogenous hormone concentrations, and physiological activity may influence cutting survival, adventitious root formation, and subsequent seedling vigor.

Previous studies involving other plant species have demonstrated that cutting origin can influence propagation success. Apical cuttings of stevia exhibited higher survival and rooting capabilities than cuttings obtained from middle and basal stem sections (Yusmaini, 2009). Similarly, differences in cutting origin and planting techniques influenced the productivity of cat's whiskers (*Orthosiphon aristatus*) (Rahmania & Kurniawati, 2014). These findings indicated that the physiological characteristics associated with cutting position could affect root development and subsequent plant growth. However, information regarding the effects of cutting origin on the propagation performance of gandarusa remains limited, making it difficult to identify the most suitable stem section for producing vigorous planting material.

The successful propagation of gandarusa may also be influenced by the application of auxin-based plant growth regulators, which promote adventitious root initiation and development. Rootone-F is a commercial rooting formulation containing auxin-related compounds, including naphthaleneacetamide (NAD), methyl 1-naphthaleneacetate (MNAA), methyl 1-naphthaleneacetamide (MNAD), and indole-3-butyric acid (IBA), together with the fungicide thiram (Manurung, 1987). The effectiveness of auxin treatments varies according to plant species, cutting characteristics, and application concentration, making appropriate concentration selection essential for successful propagation (Hartmann & Kester, 2010). Auxin concentrations ranging from 20 to 200

ppm have been reported in propagation applications, although the optimal concentration depends on the plant species and treatment conditions (Hartmann & Kester, 2010). Nevertheless, specific information regarding the appropriate concentration of 1-naphthaleneacetic acid (NAA) for promoting root development and growth in gandarusa cuttings remains limited.

These research gaps highlighted the need to investigate the combined effects of cutting origin and auxin concentration on gandarusa propagation. Therefore, this study aimed to evaluate the effects of cutting origin (middle-stem and basal-stem cuttings) and NAA concentrations (0, 50, 75, and 100 ppm), as well as their interaction, on the growth and biomass production of gandarusa (*Justicia gendarussa*). The study also sought to identify the treatment combination that produced the most favorable growth responses under the experimental conditions. Its contribution lay in systematically examining the interaction between cutting origin and NAA concentration in gandarusa propagation, an area for which empirical evidence remains limited. The findings were expected to provide a scientific basis for improving vegetative propagation practices, supporting sustainable gandarusa cultivation, and increasing the availability of planting material for medicinal plant production and phytopharmaceutical development in Indonesia.

METHOD

Research Design

The study was conducted in Karang Tanjung Village, Alian District, Kebumen Regency, Central Java, Indonesia, using a factorial randomized complete block design (RCBD). This design was selected to account for potential environmental variability across the experimental field by grouping experimental units into relatively homogeneous blocks, thereby minimizing experimental error and improving the precision of treatment comparisons.

The experiment involved two treatment factors: 1-naphthaleneacetic acid (NAA) concentration and cutting origin. Factor A consisted of four NAA concentrations: A0 = 0 ppm, A1 = 100 ppm, A2 = 75 ppm, and A3 = 50 ppm. Factor B consisted of two cutting origins: B1 = middle-stem cuttings and B2 = basal-stem cuttings. The factorial arrangement produced eight treatment combinations, each replicated four times, resulting in 32 experimental units. Each experimental unit contained five plants, yielding a total of 160 plants. The treatment combinations are presented in Table 1.

Table 1. Combination of Cutting-Origin Treatment and Auxin Concentration

Auxin Concentration (NAA)	B1 (Middle Stem)	B2 (Rootstock)
A0 (0 ppm)	B1A0	B2A0
A1 (100 ppm)	B1A1	B2A1
A2 (75 ppm)	B1A2	B2A2
A3 (50 ppm)	B1A3	B2A3

Research Implementation

The planting medium is prepared by mixing soil, sand, and compost in a ratio of 1:1:1. The media is weighed proportionally, sifted to remove gravel and large impurities, and then mixed until homogeneous before being put into polybags (Prasetyo, 2019). A

mixture of media with a loose texture and rich in organic matter is known to promote root development and vegetative growth in various medicinal plants propagated by cuttings (Waman et al., 2019), while media with good aeration and drainage supports the formation of leaf and stem biomass in tropical leaf plants (Ebel et al., 2023).

Cuttings are taken from healthy, uniform gandarusa parent plants with a length of $\pm$ 15 cm and 3–4 segments, then grouped based on stem origin into middle stem cuttings (B1) and rootstock cuttings (B2) (Pandey & Husen, 2022). The leaf area is reduced by leaving 1–2 leaves to reduce transpiration. The position of origin of cuttings affects the content of food reserves and the degree of tissue differentiation which ultimately affects the rootability and growth of shoots (Arisoesilaningih et al., 2023). Research on other leaf plant propagation also shows that cuttings from the middle and lower stems tend to produce plants with higher vigor than upper cuttings (Ebel et al., 2023).

The base of the cuttings is soaked in an auxin solution according to the treatment concentration (0, 100, 75, and 50 ppm) for 3–5 minutes to stimulate the formation of adventitious roots, as the absorbed auxin helps accelerate the root initiation process (Alamsyah et al., 2021). Soaking cuttings in auxin solution has been shown to increase the root percentage, root count, and root length in various wood and medicinal plants (Khan et al., 2020), and at optimal concentrations it can increase the percentage of life, plant height, and biomass compared to treatments without auxin (Lee et al., 2024). The cuttings are then planted upright into a polybag 3–5 cm deep and compacted (Khan et al., 2020). Each polybag is labeled with a combination of treatments to prevent misidentification (Patil, 2022) and in the initial phase is placed under light shade to reduce light stress and retain moisture (Waman et al., 2019).

Maintenance includes watering 1–2 times a day or adjusted to weather conditions so that the medium remains moist but not flooded (Alam et al., 2016; R. (R. Hidayat & Prasetyo, 2019), manual weeding to avoid competition with water, nutrients, and light (Paturkar et al., 2022); Waman et al., 2019), and pest and disease control mechanically or with biological materials without synthetic chemical pesticides so that phytochemical quality is not contaminated with residues (Samanta et al., 2023). Observations were carried out until the plants were 56 HST old, then the plants were harvested to measure fresh and dry biomass. Uniform harvest timing is important so that differences between treatments are not covered by variations in the physiological age of plants (Putri, 2019; Samanta et al., 2023).

Data Observation and Analysis Parameters

The observation parameters included plant height, stem diameter, number of leaves, number of branches, root length, number of roots, wet weight, dry weight, and percentage of cuttings life. The observation data were tabulated and checked for completeness, then analyzed using variety analysis (ANOVA) according to the experimental design to test the influence of the origin of cuttings, auxin concentration, and interaction of the two. Treatments that showed real or very real effects were then tested using *Duncan's Multiple Range Test* (DMRT) at the level of 5%. All statistical analysis was carried out using SPSS software.

RESULTS AND DISCUSSION

Recapitulation of Variance Analysis Results

The observation data were analyzed to determine the growth response and yield of gandarusa plants (*Justicia gendarussa*) to the difference in the origin of cuttings and auxin concentration. The recapitulation of the results of the analysis of all variables is presented in Table 2.

Table 2. Recapitulation of Results of Variance Analysis (ANOVA) of All Growth Variables and Outcomes

Variable	Age (HST)	Cuttings	Auksin	Interaction (B×A)
Plant Height	14, 21, 28, 35, 42, 49, 56	tn	tn	tn
Rod Diameter	14	tn	tn	tn
	21	**	tn	tn
	28	**	tn	tn
	35	**	*	*
	42	**	*	tn
	49	tn	tn	tn
	56	**	*	tn
Number of Leaves	14, 28, 35, 42, 49, 56	tn	tn	tn
Number of Branches	56	tn	tn	tn
Root Length	56	tn	tn	tn
Number of Roots	56	tn	tn	tn
Wet Weight of Plants	56	tn	tn	*
Dry Weight of Plants	56	tn	tn	tn
Cuttings Life Percentage	56	*	tn	tn

Ket: (**) very real ($P < 0.01$); (*) real ($P < 0.05$); (tn) not real.

Based on Table 2, most of the observation parameters were not significantly influenced by the origin of the cuttings, auxin concentration, or the interaction of the two. The height of the plants at the entire age of observation, the number of leaves, the number of branches, the length of the roots, the number of roots, and the dry weight of the plants did not differ significantly between treatments. These results indicated that the early vegetative growth of gandarusa plants was more influenced by genetic factors and physiological conditions of the cuttings than by the treatment given. In the early stages of cuttings growth, endogenous carbohydrate and hormone reserves stored in plant tissues play a dominant role in the formation of shoots and roots before the plant is able to optimally absorb nutrients from the growing medium, and the success of growth is also determined by the ability of the tissue to maintain metabolic balance during the formation of new organs (Li, 2021).

The effect of treatment is mainly seen on the diameter of the stem. The origin of cuttings has a very noticeable effect on the diameter of the stem at the age of 21, 28, 35, 42, and 56 HST, the concentration of auxin has a real effect at the age of 35, 42, and 56 HST, while the interaction of the two has a real effect on the age of 35 HST. Auxin functions to regulate cell division, enlargement, and differentiation in meristematic and cambium tissues, so that the increase in auxin in stem tissue can stimulate vascular

cambium activity and accelerate the formation of secondary xylem and phloem. The real influence of cuttings origin is thought to be related to differences in nutrient reserves and the level of tissue maturity of each cutting material (Kartika, 2022). In addition, the interaction of cuttings origin and auxin has a real effect on the wet weight of plants, while the origin of cuttings has a real effect on the survival percentage of cuttings. Wet weight reflects the total fresh biomass which is greatly influenced by the plant's ability to absorb water and nutrients (Pratama, 2019), so the combination of cuttings material has the potential to root well with auxin at the appropriate concentration can increase biomass accumulation (Satriowibowo, 2014). The survival success of cuttings is more determined by the quality of the planting material, because cuttings with the right level of physiological maturity contain endogenous carbohydrates, proteins, and hormones that support the formation of new roots and shoots (A. Hidayat et al., 2020).

Plant Height

Table 3. Results of Plant Height Variety Analysis at the Age of 14–56 HST

Age	F Stitch (df=1)	Sig.	F Auksin (df=3)	Sig.	F Interaction (df=3)	Sig.
14 HST	0,154	0,698	1,113	0,363	0,434	0,730
21 HST	2,502	0,127	0,425	0,718	1,246	0,315
28 HST	1,767	0,196	0,734	0,542	1,191	0,334
35 HST	1,505	0,232	0,734	0,542	1,227	0,321
42 HST	2,806	0,107	0,675	0,576	2,093	0,128
49 HST	3,665	0,068	0,888	0,461	0,898	0,456
56 HST	2,450	0,131	0,948	0,433	0,895	0,458

Based on Table 3, the origin of cuttings showed a significance value greater than 0.05 at the entire age of observation, namely 0.698 (14 HST), 0.127 (21 HST), 0.196 (28 HST), 0.232 (35 HST), 0.107 (42 HST), 0.068 (49 HST), and 0.131 (56 HST), so that the origin of the cuttings did not have a significant effect on the height of the plant. This condition is suspected to be caused by the uniformity of the physiological ability of each part of the cuttings in accumulating energy reserves for initial growth. Internal factors that affect the growth of cuttings include genes, photosynthesis ability, food reserves, and respiration, while external factors include light, humidity, temperature, water, and nutrient availability; with uniform environmental conditions, differences in the origin of cuttings do not result in a noticeable high response of the plant (Pandey & Husen, 2022).

In the early stages of growth (14–35 HST), the plant is still adapting and forming a root system, so metabolic energy is directed more to root initiation than stem elongation. Carbohydrate reserves are the source of energy for root and bud formation before plants are able to photosynthesize optimally, while auxins stimulate adventitious root formation. At the age of 42–56 HST, plants enter more active vegetative growth so that height gains are determined by photosynthesis ability and assimilate allocation; relatively uniform environmental conditions cause the response between treatments to remain relatively the same (Husen, 2022).

Auxin concentration also has no real effect on plant height because auxin primarily plays a role in root initiation and elongation, not directly in stem elongation. Auxin

transport occurs polarly so that it forms a concentration gradient that regulates growth patterns, and the presence of auxins determines the success of root formation in cuttings (Debitama et al., 2022). The absence of a real interaction can also be caused by the dominance of genetic factors of gandarusa as a shrub plant that grows upright, has a woody stem, branches, and can reach a height of 0.8–2 meters, so the combination of treatments has not been able to modify the height growth pattern during 14–56 HST (Amalia et al., 2022).

Table 4. Average Plant Height at Age 14–56 HST

Treatment	14 HST	21 HST	28 HST	35 HST	42 HST	49 HST	56 HST
B1A0	11.78a	12.10a	12.34a	12.90a	13.40a	14.30a	15.96a
B1A1	13.18a	13.22a	13.54a	13.90a	14.30a	14.62a	15.42a
B1A2	11.50a	11.68a	11.94a	12.48a	12.98a	13.80a	15.64a
B1A3	9.575a	6.88a	7.00a	7.18a	7.52a	7.90a	8.80a
B2A0	11.28a	6.72a	6.82a	7.00a	7.22a	6.35a	7.94a
B2A1	10.92a	11.00a	11.20a	11.56a	11.94a	12.30a	13.22a
B2A2	10.38a	4.58a	4.60a	4.64a	4.76a	4.90a	5.20a
B2A3	11.20a	7.12a	7.26a	7.44a	7.54a	7.64a	8.44a

Ket: Numbers followed by the same letter in the same column show no significant difference based on the DMRT test of 5%.

Based on Table 4, the high response of plants differed in each combination of cuttings origin and auxin concentration, although statistically no significant difference. At the end of observation (56 HST), the highest value was obtained at B1A0 at 15.96, while the lowest value was obtained at B2A2 at 5.20. In general, treatment with B1 cuttings origin tends to result in better tall growth than B2. These differences can be related to the physiological condition of the cuttings material, endogenous hormone content, and the ability of tissues to form adventitious roots. Otiende et al. (2021) explained that the position of the origin of cuttings is related to the balance of auxins and cytokinins that affect the formation of adventitious roots, and Zheng et al. (2023) stated that auxin is one of the main factors regulating adventitious root formation in cuttings.

Rod Diameter

Table 5. Results of Analysis of Stem Diameter Variety at the Age of 14–56 HST

Age	F Stitch (df=1)	Sig.	F Auksin (df=3)	Sig.	F Interaction (df=3)	Sig.
14 HST	2,321	0,141	0,902	0,455	1,309	0,295
21 HST	33,335	0,000	2,214	0,113	2,597	0,076
28 HST	32,992	0,000	2,650	0,072	2,429	0,090
35 HST	29,670	0,000	4,155	0,017	3,007	0,050
42 HST	28,553	0,000	3,234	0,040	2,154	0,120
49 HST	2,174	0,153	0,731	0,544	1,744	0,185
56 HST	34,312	0,000	3,890	0,021	2,982	0,051

Based on Table 5, the origin of cuttings has no significant effect on stem diameter at age 14 HST (sig. 0.141). In the early phases, the plant is still adapting to the environment and newly planted cuttings focus energy on root formation, so that upper growth including

stem enlargement is still relatively slow (Hartmann & Kester, 2010). At the ages of 21, 28, 35, 42, and 56 HST, the origin of cuttings has a very pronounced effect with a significance value of 0.000 (< 0.01). The better the quality of the cuttings material, the greater the reserves of endogenous carbohydrates and hormones available to support vegetative growth. Stem diameter is closely related to cell division and enlargement activity and the availability of photosynthates, and larger stems indicate more optimal water and nutrient transport capabilities (Albornoz et al., 2020).

Auxin concentrations had a significant effect on stem diameter at ages 35 HST (sig. 0.017), 42 HST (sig. 0.040), and 56 HST (sig. 0.021), but had no significant effect at ages 14, 21, 28, and 49 HST. Auxins regulate growth through increased cell wall plasticity so that cells can develop faster, but their effectiveness is greatly influenced by the concentration, age of the plant, and the physiological conditions of the planting material so that the response that appears is not always consistent at every observation time (Taiz et al., 2017). The interaction of cutting-origin and auxin has a noticeable effect only at age 35 HST (sig. 0.050), while at other ages it is not real. This shows that at most observation times the two factors work independently and that the origin of cuttings is more dominant in influencing the diameter of the stem (Luta, 2023).

Table 6. Advanced Test of DMRT Rod Diameter (mm) on Cutting-Origin Treatment

Cuttings Treatment	21 HST	28 HST	35 HST	42 HST	56 HST
B1 (Middle Stem)	3,908b	3,913b	3,880b	3,912b	4,047b
B2 (Rootstock)	4,760a	4,761a	4,957a	5,051a	5,161a

Ket: Numbers followed by the same letter in the same column show no significant difference based on the DMRT test of 5%.

Table 6 summarizes the results of the stem diameter DMRT test at the cuttings origin treatment and at the same time presents graph data of the stem diameter of the cuttings treatment in the form of a table. B2 cuttings produced larger stem diameters and were significantly different from B1 at the entire age of observation, namely 4,760 mm vs 3,908 mm (21 HST), 4,761 mm vs 3,913 mm (28 HST), 4,957 mm vs 3,880 mm (35 HST), 5,051 mm vs 3,912 mm (42 HST), and 5,161 mm vs 4,047 mm (56 HST). The stem diameters of the two treatments tended to increase with age, with B2 consistently higher than B1. Different letters followed by treatments in the DMRT test showed a marked difference between treatments at the confidence level used (Muhammad et al., 2022).

The high stem diameter in B2 is thought to be due to cuttings that have more food reserves and more active meristem tissue. The success of cuttings growth is greatly influenced by the content of food reserves and the balance of hormones in the plant material; the greater the food reserve, the better the ability of the cuttings to form roots and shoots which further supports stem growth (Hartmann & Kester, 2010). The physiological conditions of the planting material such as tissue age, cuttings position, carbohydrate content, and endogenous hormone balance determine the root formation ability as well as the growth of the upper part of the plant (Druege et al., 2019). Once the roots develop well, the absorption of water and nutrients increases so that photosynthesis takes place more optimally and photosynthates can be allocated for stem growth.

The increase in stem diameter in dicot plants such as gandarusa is controlled by the activity of vascular cambium that divides laterally resulting in secondary xylem inward and

secondary phloem outward (Turley & Etchells, 2022). Stem diameter growth is affected by intensive cell division, enlargement, and differentiation processes in meristematic tissue (Sun et al., 2025). Cuttings from more woody parts (B2) also have sclerenchymal tissue with thicker secondary walls and contain lignin so that the stems formed tend to be sturdier and larger in diameter. Stem cuttings are divided into root, middle, and upper stem cuttings or shoots, with different tissue characteristics and food reserves from each other (Luta, 2022).

Table 7. Advanced Test of DMRT Rod Diameter (mm) on Auxin Concentration Treatment

Auxin Concentration	35 HST	42 HST	56 HST
A0 (0 ppm)	4,297ab	4,421ab	4,501ab
A1 (100 ppm)	5,014a	5,038a	4,347b
A2 (75 ppm)	4,125ab	4,179b	5,159a
A3 (50 ppm)	4,238ab	4,289ab	4,409ab

Ket: Numbers followed by the same letter in the same column show no significant difference based on the DMRT test of 5%.

Table 7 presents the results of the DMRT test of the rod diameter in the auxin concentration treatment as well as graph data of the shaft diameter of the auxin treatment in the form of a table. At the age of 35 HST, A1 produced the highest rod diameter of 5,014 mm, while A0, A2, and A3 were 4,297 mm, 4,125 mm, and 4,238 mm respectively with ab notation. At the age of 42 HST, A1 again produced the highest diameter of 5,038 mm and was significantly different from the lowest A2 (4,179 mm), while A0 (4,421 mm) and A3 (4,289 mm) were not significantly different from the two. At the age of 56 HST, the A2 produced the highest diameter of 5,159 mm and differed markedly from the A1 which decreased to 4,347 mm, while the A0 (4,501 mm) and A3 (4,409 mm) did not differ markedly from the two.

The high stem diameter at A1 to 42 HST is thought to be because the auxin concentration of 100 ppm is at a level capable of stimulating cambium activity, division, and cell enlargement so that xylem and phloem formation takes place better. The highest shift to A2 at age 56 HST suggests that the increase in auxin concentration does not necessarily increase the stem diameter linearly because growth hormone acts specifically at a specific concentration range, so giving too low or too high can decrease its effectiveness (Rademacher, 2021). The decrease in diameter at A1 at the end of the observation is thought to be related to decreased cambium activity and photosynthetic allocation to other organs, because the higher the rate of photosynthesis, the greater the carbohydrate that can be allocated for stem tissue formation (Sharma et al., 2021). The difference between A0 and A3 with other treatments shows that plants are still able to produce enough endogenous auxin to support stem growth, because the response to exogenous hormones is influenced by interactions with endogenous hormones, physiological, genetic conditions, and the growing environment (Davies, 2023).

Table 8. Advanced Test of DMRT Rod Diameter (mm) Age 35 HST on Interaction of Stem Origin and Auxin

Treatment	Rod Diameter (mm)
B1A0	4,098cd
B1A1	4,017cd
B1A2	3,746d
B1A3	4.818b
B2A0	4,496bc
B2A1	6,011a
B2A2	4,504bc
B2A3	4.818b

Ket: Numbers followed by the same letter in the same column show no significant difference based on the DMRT test of 5%.

Based on Table 8, there was an interaction between the origin of the cuttings and the concentration of auxin on the diameter of the stem age of 35 HST. The combination of B2A1 (rootstock cuttings with auxin 100 ppm) produced the highest stem diameter of 6,011 mm and was significantly different from all other combinations, while B1A2 (middle stem cuttings with auxin 75 ppm) produced the lowest diameter of 3,746 mm. The high stem diameter in B2A1 is thought to be due to the synergy between rootstock cuttings that have more carbohydrate, nutrient, and cambium tissue reserves and 100 ppm auxin which stimulates cambium activity so that vascular tissue formation takes place more intensively. In contrast, the low diameter in B1A2 is thought to be due to lower carbohydrate reserves and auxin concentrations that have not yet matched the physiological needs of cuttings, given that the effectiveness of growth regulators depends on the concentration, type of plant, physiological conditions of the planting material, and its interaction with endogenous hormones (Rademacher, 2021). The combination of B1A3 and B2A3 results in a diameter of 4,818 mm, while B1A0, B1A1, B2A0, and B2A2 show a moderate response, indicating that the diameter of the stem is jointly affected by the concentration of auxin and the origin of the cuttings material (Davies, 2023).

Number of Leaves

Table 9. Results of Analysis of Variety of Leaf Count at the Age of 14–56 HST

Age	F Stitch (df=1)	Sig.	F Auksin (df=3)	Sig.	F Interaction (df=3)	Sig.
14 HST	0,323	0,575	0,343	0,794	0,308	0,819
21 HST	1,037	0,319	0,136	0,938	1,165	0,344
28 HST	0,615	0,441	0,329	0,804	1,150	0,349
35 HST	1,378	0,252	0,212	0,887	1,333	0,287
42 HST	1,273	0,270	0,174	0,913	1,380	0,273
49 HST	1,234	0,278	0,396	0,757	1,007	0,407
56 HST	0,855	0,364	0,742	0,570	1,406	0,265

Ket: the whole value of Sig. > 0.05 (not real).

Based on Table 9, the origin of cuttings, auxin concentration, and the interaction of the two had no significant effect on the number of leaves at the entire age of observation. The origin of cuttings had an F value between 0.323 (14 HST) and 1.378 (35 HST) with a

Sig. value of 0.252–0.575, which is thought to be due to the relatively uniform regeneration ability of both types of cuttings because gandarusa is classified as a plant that easily regenerates vegetatively. The formation of leaves in cuttings is more influenced by the carbohydrate and nitrogen content in the cuttings material than the position of the cuttings, especially if the metabolite reserves are relatively evenly distributed throughout the stem (Hartmann et al., 2023).

Auxin concentrations also had no significant effect with F-values ranging from 0.136 (21 HST) to 0.742 (56 HST), in line with the more dominant physiological function of auxin spurring adventitious root initiation and elongation than the formation of new shoots and leaves. Leaf formation is more controlled by cytokinins and intrinsic genetic factors of plants (Weijers & Wagner, 2021), and even auxins at high concentrations can inhibit bud development due to apical dominance effects (Roni et al., 2022). An unreal interaction (F 0.308–1.406) suggests that the two factors work independently in influencing leaf count (Mahadi et al., 2021).

Table 10. Average Number of Leaves at the Age of 14–56 HST

Treatment	14 HST	21 HST	28 HST	35 HST	42 HST	49 HST	56 HST
B1A0	4.4a	4.4a	5.2a	6.0a	7.0a	7.6a	9.2a
B1A1	2.4a	2.8a	2.8a	4.0a	4.4a	5.2a	6.8a
B1A2	2.0a	2.0a	3.2a	3.2a	5.0a	5.2a	6.4a
B1A3	3.6a	4.2a	4.8a	6.2a	7.2a	8.6a	10.6a
B2A0	8.0a	4.0a	4.8a	5.2a	5.8a	7.0a	7.0a
B2A1	2.4a	2.4a	3.8a	3.8a	5.0a	5.4a	7.6a
B2A2	3.4a	3.8a	5.4a	5.8a	7.4a	7.8a	9.4a
B2A3	3.4a	3.4a	5.0a	5.6a	7.4a	7.8a	10.2a

Ket: Numbers followed by the same letter in the same column show no significant difference based on the DMRT test of 5%.

Based on Table 10, B2A0 showed the highest initial value at 14 HST of 8.0, but in 56 HSTs the highest value was obtained B1A3 of 10.6, followed by B2A3 of 10.2 and B2A2 of 9.4, while B1A2 produced the lowest value of 6.4. The number of leaves in most treatments increased with the age of the plant, indicating the continuation of vegetative development during the observation period. The difference in response is thought to be influenced by the physiological condition of the tissue and the hormonal balance of each cutting origin (Otiende et al., 2021; Zheng et al., 2023).

Number of Branches, Root Length, Number of Roots, and Dry Weight of the Plant

Table 11. Results of Analysis of Various Variables of Observation at the Age of 56 HST

Variable	F Stitch (df=1)	Sig.	F Auksin (df=3)	Sig.	F Interaction (df=3)	Sig.
Number of Branches	0,108	0,746	0,406	0,750	1,122	0,360
Root Length	0,027	0,872	0,212	0,887	0,105	0,956
Number of Roots	0,062	0,806	0,184	0,906	0,647	0,592
Wet Weight of Plants	0,091	0,766	1,372	0,275	3,292	0,038
Dry Weight of Plants	0,061	0,807	0,734	0,542	2,047	0,134
Cuttings Life Percentage	6,982	0,014	0,364	0,780	1,382	0,272

Based on Table 11, the origin of cuttings has no significant effect on the number of branches (sig. 0.746), as well as auxins (sig. 0.750) and their interactions (sig. 0.360). Branch formation is part of vegetative growth that is influenced by cell division activity, photosynthesis ability, nutrient availability, and genetic factors; if these conditions are relatively the same across treatments, the number of branches is less likely to differ significantly (Sharma et al., 2021). Physiologically, auxins regulate apical dominance that inhibits lateral shoot growth, so exogenous auxins do not necessarily increase the number of branches and their effects depend on interactions with cytokinins that stimulate lateral shoots (Li et al., 2022).

Root length and root count were also not significantly affected by the treatment. The significance values of root length for cuttings, auxins, and interactions were 0.872, 0.887, and 0.956, respectively, while for root counts were 0.806, 0.906, and 0.592 ($P > 0.05$). Root elongation and formation are more influenced by the physiological conditions of the planting material such as carbohydrate reserves, root meristem activity, and endogenous hormone balance, as well as environmental factors such as growing media, humidity, and temperature (Raza et al., 2023). Auxins do play a role in initiating root formation, but their effectiveness depends on the concentration and balance between exogenous and endogenous hormones; the administration of auxins is suspected to have not reached optimal concentrations or that the plant already has sufficient endogenous auxins (Li et al., 2022).

The dry weight of plants is also not significantly affected by the origin of cuttings (sig. 0.807), auxin (sig. 0.542), or their interactions (sig. 0.134). Dry weight reflects the accumulation of photosynthesis results stored in plant tissues (Sharma et al., 2014), so if the treatment has not been able to significantly increase physiological activity, dry weight is not significantly different. Increased vegetative growth due to auxins is not necessarily followed by an increase in dry weight if the concentration is not optimal, because the accumulation of dry matter is more influenced by the efficiency of photosynthesis and the distribution of photosynthetics (Li et al., 2022; Rahman, 2020).

Table 12. Average Number of Branches, Root Length, Number of Roots, and Dry Weight of Plants Age 56 HST

Treatment	Number of Branches	Root Length	Number of Roots	Dry Weight
B1A0	4.4a	13.02a	13.4a	3.0a
B1A1	3.0a	12.52a	12.4a	1.8a
B1A2	2.0a	7.74a	6.8a	2.0a
B1A3	2.2a	9.00a	8.4a	1.2a
B2A0	1.8a	5.26a	4.2a	0.6a
B2A1	2.8a	7.78a	8.0a	2.0a
B2A2	2.0a	7.56a	8.2a	2.0a
B2A3	4.0a	13.56a	11.2a	3.0a

Ket: Numbers followed by the same letter in the same column show no significant difference based on the DMRT test of 5%.

Based on Table 12, the highest number of branches was obtained by B1A0 at 4.4, followed by B2A3 at 4.0, while the lowest at B2A0 was 1.8. The highest root length was

obtained by B2A3 at 13.56, followed by B1A0 at 13.02, and the lowest at B2A0 at 5.26. The highest number of roots was obtained in B1A0 at 13.4 and the lowest in B2A0 at 4.2, while the highest dry weight was obtained at B1A0 and B2A3 at 3.0 and the lowest at B2A0 at 0.6. A consistent pattern was seen in all four variables, namely in the origin of B1 cuttings treatment without auxin (A0) tended to give the highest value, while in the origin of B2 cuttings treatment A3 gave the highest value and B2A0 always the lowest. This suggests that the plant's response to auxin depends on the origin of the cutting's material and the physiological conditions of the tissue, as the position of the cutting's origin affects the endogenous auxin content and adventitious root formation ability, while the administration of auxin from the outside can increase the rooting response in cuttings with lower rooting ability (Otiende et al., 2021; Li, 2021).

Wet Weight of Plants

Based on Table 11, the origin of cuttings (sig. 0.766) and the concentration of auxins (sig. 0.275) alone had no significant effect on the wet weight of the plant, while the interaction of the two had a significant effect (sig. 0.038; $P < 0.05$). Thus, the wet weight of the plant was not affected separately by each factor, but by the combination of the two factors (Prasetyo, 2006). The plant's response to auxin depends on the origin of the cuttings used; the right combination is able to create better physiological conditions thereby accelerating the growth of roots and shoots and increasing water accumulation and photosynthetic yields in plant tissues (Taiz et al., 2017).

Table 13. Advanced Test of DMRT Wet Weight of Plants (g) on Interaction of Stem Origin and Auxin

Treatment	Wet Plant Weight (g)
B1A0	5,350ab
B1A1	4,650bc
B1A2	4,350c
B1A3	4,475bc
B2A0	2,550d
B2A1	6,150a
B2A2	4,350c
B2A3	5,950A

Ket: Numbers followed by the same letter in the same column show no significant difference based on the DMRT test of 5%.

Based on Table 13, the combination of B2A1 (rootstock cuttings with auxin 100 ppm) resulted in the highest wet weight of 6,150 g and was not significantly different from B2A3 (5,950 g), but it differed markedly from the other combinations, whereas B2A0 (rootstock cuttings without auxin) resulted in the lowest wet weight of 2,550 g. The high wet weight of B2A1 is thought to be due to the rootstock cuttings having higher carbohydrate and nutrient reserves thus supporting the growth of roots, shoots, and leaves (Hartmann et al., 2022), while auxin 100 ppm stimulates root formation and development so that water and nutrient absorption increases (Zhao, 2021). The low wet weight of B2A0 suggests that without auxin the endogenous hormone is not sufficient to stimulate root formation and vegetative growth to the maximum extent even when using lower cuttings,

whereas exogenous growth regulators can promote growth when given at concentrations appropriate to the physiological needs of the plant (Rademacher, 2021).

Cuttings Life Percentage

Based on Table 11, the origin of cuttings has a significant effect on the percentage of cuttings life (sig. 0.014 < 0.05), while auxin (sig. 0.780) and its interaction (sig. 0.272) have no real effect. These results show that the condition of cuttings material, such as tissue age, food reserve content, and endogenous hormone balance, greatly determines the success of plant life. The absence of auxin is suspected because the concentration used is not optimal or the environmental conditions are sufficiently supportive without the addition of hormones. The success of propagation by cuttings is greatly influenced by the condition of the planting material and its physiological factors (Hartmann, Kester, & Davies, 1997).

Table 14. DMRT Advanced Test of Cuttings Life Percentage in Cutting-Origin Treatment

Cuttings Treatment	Cuttings Life Percentage (%)
B1 (Middle Stem)	77.50a
B2 (Rootstock)	57.50b

Ket: Numbers followed by the same letter in the same column show no significant difference based on the DMRT test of 5%.

Based on Table 14, mid-stem cuttings (B1) produce the highest survival percentage of 77.50% and are significantly different from rootstock cuttings (B2) of 57.50%. The high survival percentage in B1 is thought to be due to the younger tissues so that metabolic and cell division activities are more active and the content of endogenous hormones, especially auxin, is higher. Cuttings derived from juvenile tissues generally have higher regeneration capabilities than older tissues (Hartmann et al., 2022), and faster root formation increases the ability to absorb water and nutrients so that the chances of cuttings surviving are greater (Taiz et al., 2017). In contrast, rootstock cuttings have older tissue and have undergone lignification so that cell division activity is slower and root and bud formation takes longer (Davies, 2023). Environmental factors such as humidity, temperature, light intensity, and planting media also affect the success of cuttings, so proper environmental management is the key to increasing the success of plant propagation through cuttings.

CONCLUSION

The study concluded that cutting origin, auxin concentration, and their interaction produced different growth and biomass responses in gandarusa (*Justicia gendarussa*). Cutting origin significantly affected stem diameter at 21, 28, 35, 42, and 56 days after planting (DAP), as well as cutting survival percentage, whereas auxin concentration significantly influenced stem diameter at 35, 42, and 56 DAP. A significant interaction between cutting origin and auxin concentration was observed for stem diameter at 35 DAP and plant fresh weight at 56 DAP. However, plant height, number of leaves, number of branches, root length, number of roots, and dry weight were not significantly affected by the treatments. The B2A1 treatment combination (basal-stem cuttings treated with 100 ppm 1-naphthaleneacetic acid [NAA]) produced the highest stem diameter (6.011 mm) at 35 DAP and the highest fresh weight (6.150 g) at 56 DAP. In contrast, middle-stem cuttings (B1) exhibited the highest survival percentage (77.50%). These findings suggested

that basal-stem cuttings treated with 100 ppm NAA were preferable for promoting stem diameter and fresh biomass accumulation, whereas middle-stem cuttings were more suitable for improving cutting survival. Further research should investigate a broader range of NAA concentrations and longer observation periods to determine the optimal treatment for each cutting origin, particularly regarding root development and dry biomass accumulation, which did not exhibit significant treatment responses in this study.

FUNDING

The author declares that this research received no specific financial support from any governmental institution, private organization, or other entity for the conduct of the research and/or the publication of this article.

CONFLICT OF INTEREST

The author declares that this research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

AUTHOR CONTRIBUTIONS

The author was responsible for all stages of the research, including conceptualization, methodology development, literature search and selection, data analysis, interpretation of the results, manuscript preparation, and final revision of the article. The author has read and approved the final version of the manuscript for publication.

GENERATIVE ARTIFICIAL INTELLIGENCE USE STATEMENT

The author declares that generative artificial intelligence (Generative AI) was not used to generate research data, conduct scientific analyses, or replace the author's intellectual contributions in the preparation of this manuscript. If used during manuscript preparation, AI was employed only to a limited extent to assist with language refinement and improvement of the writing structure. The author remains fully responsible for the accuracy, integrity, and final content of the manuscript.

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