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Optimizing Vegetative Propagation of *Litsea garciae* Vidal: Effects of Cutting Source and Rootone-F Soaking Duration on Root and Shoot Development

Hanna Artuti Ekamawanti[*], Sania Hazelyna, Reine Suci Wulandari

Faculty of Forestry, Tanjungpura University, Pontianak, Indonesia
[*]Corresponding author. E-mail address: hannaartuti@fahutan.untan.ac.id

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ABSTRACT

Litsea garciae Vidal, commonly known as *engkala*, is an endemic medicinal plant of Borneo with promising pharmacological potential. Vegetative propagation through shoot cuttings is a practical alternative to overcome the challenges of seed-based propagation. This study investigated the effects of cutting material source (seedlings and saplings) and soaking duration in 50% Rootone-F (0, 15, 30, 45, and 60 minutes) on the rooting and shoot growth of *L. garciae* shoot cuttings. A factorial experiment with a completely randomized design was applied, involving ten treatment combinations with five replications each. Results showed that cuttings derived from seedlings achieved 100% survival and rooting rates regardless of Rootone-F treatment. In contrast, sapling cuttings achieved optimal rooting only after 15 minutes of soaking. The interaction of cutting source and soaking duration significantly influenced root development, while shoot growth varied only with individual treatments. Notably, seedling cuttings produced roots that were 1.3 times longer than those from sapling cuttings; however, the dry weight of sapling roots was 1.5 times greater. The dry weight of roots soaked in Rootone-F for 15 minutes significantly increased by 1.3 to 2.8 times compared to the dry weight of roots soaked for other durations. The seedling cuttings produced 1.2 times more shoots than saplings. Interestingly, cuttings without soaking treatment in Rootone-F produced the same number of new leaves as those soaked for 60 minutes but significantly more than those soaked for other durations. These results are vital for optimizing the vegetative propagation technique of *L. garciae* by selecting the appropriate cutting source and timing for Rootone-F immersion.

1. Introduction

Litsea garciae Vidal, also known as *engkala*, which belongs to the Lauraceae family, is one of the endemic fruit-producing plant species found on the island of Borneo (Hassan et al. 2013). *L. garciae* has a habitus as a broad-leaved and evergreen tree with a low canopy and produces fruit once a year (Amit and Zinyin 2021). Like other species in the genus Litsea, *L. garciae* is often found in open mixed dipterocarp forests, especially in riparian areas and hillsides, and can adapt to various types of soil, from sandy soil to clay-rich soil, and thrives in humid tropical environments (Setiawan et al. 2021; Srinivas and Krishnamurthy 2019). *L. garciae* also has economic value related to traditional pharmacological benefits (Goh et al. 2024). Wulandari et al. (2018) proved that *L. garciae* plants contain secondary metabolites, including alkaloids, flavonoids, tannins, and coumarins (essential oils). Goh et al. (2024) found that *L. garciae* plants contain specific phytochemical compounds that act as antioxidants, fighting free radicals and chronic diseases such as cancer and heart failure. Therefore, the success of *L. garciae* propagation is critical to increase the significance of its pharmacological and economic benefits.

Propagation of *L. garciae* can be done using generative techniques. However, the challenge of propagation using generative techniques is that seeds are somewhat difficult to obtain because the *L. garciae* tree only bears fruit once a year and has a relatively high water content (recalcitrant). Therefore, vegetative propagation is a more feasible option. One method of vegetative propagation is the use of shoot cuttings, which can develop roots and shoots (Saini and Anmol, 2024). The ability to shoot cuttings to root is a challenge to the success of vegetative propagation of *L. garciae*. Internal factors, such as the type of cutting material, endogenous hormone levels, food reserves in the cutting material, and the age of the parent plant, determine the ability of cuttings to root (Kaushik and Shukla 2020). This study focused on the source of cutting material from the shoots of mother trees at the seedling and sapling stages.

One of the external factors that can affect root growth is the application of exogenous hormones, particularly growth regulators that contain auxins (Gehlot et al. 2014), which stimulate the rooting of cuttings. The application of exogenous growth regulators or auxins has a positive effect on the rooting and growth of the mulberry tree (*Morus alba*) stem cuttings (Sourati et al. 2022) and the propagation of *Bambusa blumeana* (Saddoy et al. 2022). One of the widely used growth regulators is Rootone-F, which contains synthetic auxins, including 1-naphthalene acetamide (NAA) at 0.067%, 2-methyl-1-naphthalene acetamide (NAD) at 0.013%, 2-methyl-1-naphthalene acetate at 0.33%, indolebutyric acid (IBA) at 0.057%, and oyster shell at 4.00%. One factor determining the effectiveness of Rootone-F is the duration of soaking, as it affects the amount of exogenous auxin absorbed by the plant tissue. Several previous studies to assess the growth and root formation of shoot cuttings of forest plant species have been conducted, including Eucalyptus pellita (Ekamawanti et al. 2021), *Eriobotria japonica* (Surya et al. 2022), and *Cotylelobium melanoxylon* (Susilowati et al. 2020), have shown variations in response to the application of growth regulators containing synthetic auxins. The growth regulator, Rootone-F, with a concentration range of 25-100% w/v, has also been used to stimulate root formation of *L. garciae* seedling shoot cuttings with the application of arbuscular mycorrhizal fungi (Ekamawanti et al. 2023). However, knowledge about how long the cuttings must be soaked in Rootone-F is still limited. Therefore, research is needed to explore the interaction effect of the source of cutting material and the duration of soaking in Rootone-F on root formation and shoot growth of *L. garciae* shoot cuttings.

2. Materials and Methods

2.1. Experimental Design and Treatments

A factorial experiment based on a completely randomized design was used in this study, with two treatment factors, namely the source of shoot cuttings (B) and the duration of immersion in Rootone-F (W). The cutting material factor consisted of two levels, namely seedling stage seedlings (b1) with a height criterion of <150 cm and sapling stage seedlings (b2) with a height criterion of >150 cm with a stem diameter of <10 cm. The duration of the immersion factor consisted of five levels: without immersion (w0), 15 minutes of immersion (w1), 30 minutes of immersion (w2), 45 minutes of immersion (w3), and 60 minutes of immersion (w4). Each treatment combination was repeated five times, resulting in 50 experimental units being tested.

2.2. Preparation of Planting Media

This study used a mixture of cocopeat and rice husk charcoal in a 1:1 v/v ratio. These planting media materials were obtained from flower traders in Pontianak city. Initially, the cocopeat was soaked in clean water for 24 hours to dissolve dirt and excessive tannins that could inhibit root growth. After soaking, the cocopeat was washed several times until no residue remained and dried. The advantages of using cocopeat as a planting medium for cuttings are that it is light and provides good air circulation and moisture retention. The advantages of rice husk charcoal include its permeable and draining properties. The cocopeat and rice husk charcoal mixture is filled into polytubes in tray pots and stored in a propagation box.

2.3. Preparation of Shoot-Cutting Material

The cuttings were obtained from natural shoots of *L. garciae* found in the Green Open Space of the Faculty of Forestry, Tanjungpura University. Selected shoots were taken from seedling and sapling stage shoots, each about 10 to 15 cm long. Only upright shoots (orthotropic shoots) are chosen for this process. After selection, the cutting material was cut by cutting the top part 5–7 cm. It is cut at a 45° angle at the base, leaving two previously cut leaves with 1/3 of each remaining. Each leaf on the cuttings was trimmed to minimize evaporation during the planting process. The base of the cuttings was then cut at a 45° angle using pruning shears and submerged in water before being placed into the planting medium.

2.4. Preparation of Growth Regulators

To prepare a 50% concentration of Rootone-F, 50 g of powder was dissolved in 100 mL of distilled water. The IBA content in 50% Rootone-F is equivalent to 0.03% or 285 ppm IBA. The powder was initially mixed with water to form a paste, which facilitated the dissolution process. The blend was stirred gently until the Rootone-F was thoroughly combined with the water, resulting in a uniform solution. This consistent mixture is crucial for even application in growth experiments. The concentration of 50% Rootone-F was selected based on the results of preliminary research, where the percentage of survival of *L. garciae* shoot cuttings soaked in 50% Rootone-F for 15 minutes and planted in a cocopeat: rice husk charcoal (1:1 v/v) medium reached 100%.

2.5. Planting and Maintenance

The cuttings were soaked in the Rootone-F solution for varying periods: 15 minutes, 30 minutes, 45 minutes, and 60 minutes, respectively. The control cuttings, which did not receive Rootone-F, were soaked in distilled water for 60 minutes. After soaking, the cuttings were planted in potting media prepared in pot trays, according to the designated treatment codes. The planted cuttings were placed in a propagation box and kept in a screen house. Watering is done with a hand sprayer twice daily, at 9:00 a.m. and 5:00 p.m. Weeding out wild plants (grass) growing in the planting medium was also conducted.

2.6. Survival Percentage and Growth Variables Measurement

The survival percentage of shoot cuttings was calculated by counting the number of surviving cuttings for each treatment combination at the end of a 12-week observation period. The survival percentage was calculated using Equation 1.

$$\text{Survival percentage (\%)} = \frac{\text{number of surviving shoot cuttings}}{\text{number of cuttings planted}} \times 100\% \quad (1)$$

Several variables were evaluated to assess the root growth of the cuttings, including the percentage of rooted cuttings, the number of primary and secondary roots, the length of primary and secondary roots, and the fresh and dry weights of the roots. The percentage of rooted cuttings was calculated using Equation 2.

$$\text{The percentage of rooted cuttings (\%)} = \frac{\text{number of rooted cuttings}}{\text{number of cuttings planted}} \times 100\% \quad (2)$$

Root length was measured from the base of the cutting to the tip using a calibrated ruler, and all results were carefully recorded. Both the number of roots and their lengths were meticulously documented. Fresh weight was measured immediately after the plants were harvested and carefully cleaned of any adhering dirt. The samples were placed in an oven at 103 ± 2°C to determine the dry weight of the shoots and roots. They were kept in the oven for three days until they reached a constant weight, indicating that the sample weight no longer decreased. The constant weight achieved on the third day is the dry weight of the sample.

The observed and measured variables of shoot growth include the number of shoots, the number of new leaves, and the fresh and dry weight of shoots. Measuring the fresh and dry weight of shoots is equivalent to measuring the weight of root samples.

2.7. Data Analysis

The experimental data were analyzed using Minitab 19 software. Before conducting a variance analysis, we performed a test for the homogeneity of variances using the Bartlett test. If the p-value was greater than $\alpha = 0.05$, we accepted the null hypothesis (H0), indicating that the variances were homogeneous. However, we needed to transform the data if the variances were non-homogeneous. Once we achieved homogeneity of variances, we carried out the analysis of variance. Finally, we compared the average values between the two treatments using the least significant difference (LSD) test.

3. Results and Discussion

The findings of this study indicate that both the source of the cutting material and the duration of soaking in Rootone-F significantly affect the success of vegetative propagation in *L. garciae* plants. The analysis of variance regarding the growth of *L. garciae* shoot cuttings shows that the interaction between the source of cutting material and the soaking duration in Rootone-F significantly impacts root fresh weight (**Table 1**). Treating the cutting material influences primary root size, root fresh weight, root dry weight, and the number of shoots produced. Additionally, the soaking duration in Rootone-F has a positive effect on root fresh weight, root dry weight, and the number of new leaves, although it does not significantly influence other variables. The *L. garciae* shoot-cutting research results demonstrate strong growth and successful root and new shoot development.

Table 1. Recapitulation of the analysis of variance of the effect of the source of cuttings material and the duration of Rootone-F soaking on the shoot-cuttings of *L. garciae* growth

Variables	Treatments		
	Source of material (B)	**Soaking duration (W)**	**Interaction (B × W)**
Root fresh weight	20.58**	5.41**	5.24**
Root dry weight	5.08*	3.53*	3.62^{ns}
Number of primary roots	2.14^{ns}	2.49^{ns}	2.34^{ns}
Number of secondary roots	0.55^{ns}	1.23^{ns}	2.05^{ns}
Primary root length	6.37*	1.90^{ns}	2.22^{ns}
Secondary root length	0.27^{ns}	0.64^{ns}	1.17^{ns}
Number of buds	4.87*	2.30^{ns}	1.48^{ns}
Number of new leaves	0.45^{ns}	2.67*	1.01^{ns}
Shoot fresh weight	2.11^{ns}	1.53^{ns}	1.63^{ns}
Shoot dry weight	0.00^{ns}	1.76^{ns}	1.66^{ns}

Notes: **=very significant, *=significant, ns=not significant.

3.1. Survival Percentage of L. garciae Shoot Cuttings

The overall survival rate of cuttings reached 84%, with cuttings taken from seedlings achieving an impressive 100% survival and rooting percentage across all Rootone-F soaking treatments (**Table 2**). However, saplings only achieved 100% survival when soaked in Rootone-F for 15 minutes; those soaked for 30 minutes had the lowest survival rate. Cuttings taken from seedlings exhibit greater juvenile properties than those from saplings. This finding is consistent with research by Abidin and Metali (2015), which showed that cuttings of *Dillenia suffruticosa* from younger sources are more responsive to hormone treatment than those from older sources. The factor of juvenility is crucial for successful propagation across various plant species.

Table 2. The survival and rooted-cutting percentage of *L. garciae* shoot cuttings with the treatments of the source of cuttings material and Rootone-F soaking duration for 12 weeks after planting

Source of material	Soaking duration (minutes)				
	Without	**15 min**	**30 min**	**45 min**	**60 min**
	---------- Survival percentage of shoot cuttings (%) ----------				
Seedling	100	100	100	100	100
Sapling	80	100	40	80	80
	---------- Rooted-cuttings percentage (%) ----------				
Seedling	100	100	100	100	100
Sapling	80	100	40	80	80

In studies on the pitaya plant, Cavalcante and Martins (2008) found that juvenile cuttings produced 35% more root formation than adult cuttings. They emphasized the importance of juvenility as a rooting factor for red pitaya cuttings, supporting the existence of a "cone of juvenility" in plants, where lower regions retain juvenile characteristics that facilitate root formation. Similar results were also obtained by Bijalwan and Thakur (2010), where juvenile cuttings of Jatropha curcas achieved a rooting percentage of 90.5% higher than cuttings from more mature materials of 60.8% under optimal conditions. Several research results have proven a strong relationship between juvenile cuttings and the level of viability and ability to form adventitious roots, involving supporting mechanisms such as water-holding capacity and nutrient translocation efficiency. Juvenile tissue contains 15–30% more water, which reduces the risk of desiccation during the early stages of cutting development and can mobilize carbohydrates 2–3 times faster than mature tissue (Rencoret et al. 2011). The diversity of responses in juvenile cuttings is possible due to their high metabolic activity, cell plasticity, and hormonal profile, which promote tissue regeneration (Abu-Abied et al., 2014; Haapala et al., 2004; Liu et al., 2022). Therefore, selecting cutting material is an important factor to consider for successful vegetative propagation.

The survival percentage and rooting ability of cuttings showed more variability than those from sapling material when treated with the exogenous hormone Rootone-F (**Table 2**). A 100% rooting success rate was achieved for both seedling and sapling cuttings when soaked in Rootone-F for 15 minutes. It indicates that the 0.03% IBA treatment on cuttings from seedlings and saplings effectively stimulates the initiation of adventitious roots in *L. garciae*. Several studies have shown that the rooting ability response varies by cuttings derived from seedling material, depending on the plant species and the concentration of IBA given. Stem cuttings of *Pouteria adolfi-friederici* seedlings were given 0.2% IBA and could form adventitious roots and shoots by 70% (Bahru and Derero (2023). Stem cuttings of *Aidia racemosa* exhibited a similar response after being treated with a commercial growth regulator containing 0.3% IBA, resulting in the highest percentage of survival and rooting compared to other treatments (Kamis et al. 2016). Meanwhile, applying exogenous hormones to Norway spruce shoot cuttings has significantly increased adventitious root growth (OuYang et al. 2015). The results of these studies suggest that the application of exogenous hormones should be carried out appropriately.

3.2. Root Growth of L. garciae Shoot Cuttings

The root growth variables measured included the percentage of rooted cuttings, the number of primary roots, the number of secondary roots, the length of primary roots, the length of secondary roots, the fresh weight of roots, and the dry weight of roots. The source of shoot cuttings and the duration of immersion in Rootone-F significantly affected the fresh weight of the root, the dry weight of the root, and the primary root length, both in interaction and in single effects (**Table 1**). Adventitious roots formed by cutting are essential for the successful vegetative propagation of important forest species (Greenwood 1986). Utilizing shoot cuttings from forest tree species is a straightforward and effective method for promoting adventitious root formation. The successful development of these roots depends on various external factors, such as growth regulators and internal factors, including the source of the cutting material.

The root fresh weight variable revealed a significant interaction between the source of cutting material and the duration of immersion in Rootone-F. Seedlings did not exhibit significant differences across various soaking times (**Table 3**), indicating that endogenous hormones are sufficient for root development. Among the sapling cuttings, those treated with a 15-minute Rootone-F soak exhibited the highest root fresh weight, significantly outperforming seedlings. These results suggest that using sapling material and soaking for 15 minutes can have a positive influence on root fresh weight. This study's results align with the research of Ali et al. (2017), which demonstrated that the optimal immersion duration in a specific concentration of IBA, combined with the appropriate cutting material condition, can enhance root cell division and elongation. Furthermore, increasing the cell division and elongation process will increase root weight. Wang et al. (2021) also proved that the growth of *Pyracantha angustifolia* cuttings can be increased by soaking the cuttings in 9 g/L IBA dissolved in phenolic solution for 15 minutes. It was confirmed by Bijalwan and Thakur (2010) and Ebeid (2016) that applying plant growth regulators at specific concentrations and the right immersion duration can support the successful formation of cutting roots from more mature materials. The findings of these studies confirm the importance of the right duration of immersion in plant growth regulators at specific concentrations to stimulate adventitious root formation, especially in more mature cuttings material.

Table 3. The root fresh weight of *L. garciae* shoot cuttings is influenced by the interaction of the source of cuttings material and Rootone-F soaking duration for 12 weeks after planting

Soaking duration (minutes)	Source of material	
	Seedling	Sapling
Without	0.18^{a} (A)	0.71^{b} (B)
15	0.23^{a} (A)	1.19^{a} (B)
30	0.16^{a} (A)	0.22^{c} (A)
45	0.27^{a} (A)	0.24^{c} (A)
60	0.24^{a} (A)	0.49^{bc} (A)

Notes: Significant differences (LSD 0.05 = 0.36) between the means of source of cuttings material in each treatment of soaking duration are indicated by different uppercase letters. Significant differences between the means of soaking time in each treatment of the source of cuttings material are indicated by different lowercase letters.

Furthermore, the findings of this study indicate that the dry weight of the roots, the length of the primary roots, and the number of shoots are significantly affected by the source of the shoot-cutting material (**Table 1**). The dry weight of the roots of cuttings from saplings can increase by 1.5 times higher than that of the cutting roots from seedlings (**Fig. 1**). It shows that cuttings from more mature material (saplings) have greater energy to produce root biomass than cuttings from young material (seedlings). Plants at the sapling level are thought to have more carbohydrate reserves from photosynthesis to stimulate better adventitious root formation. Wang et al. (2024) explained that in mature tissue, there is a higher accumulation of lignin, tannin, and other structural compounds, such as cellulose, compared to young tissue. Consequently, the accumulation of these compounds causes the thickening of cell walls and an increase in the density of the parenchyma in mature plants, serving as a source of energy for the formation of cutting roots (Aghdaei et al. 2019).

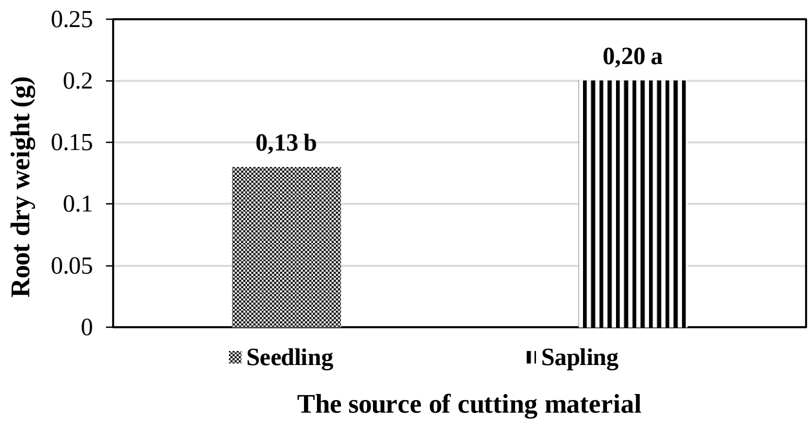

Fig. 1. The root dry weight of *L. garciae* cuttings at each source of cutting material for 12 weeks after planting (LSD 5% = 0.05).

In addition, the root dry weight was also significantly affected by the duration of immersion in Rootone-F (**Table 1**). Shoot cuttings soaked in 50% Rootone-F for 15 minutes produced higher root dry weight than those soaked for other durations, both in cuttings from seedlings and saplings (**Fig. 2**). The duration of immersion may affect the amount of auxin absorbed by plant tissue. In *Hemarthria compressa* grass cuttings, optimal auxin absorption occurred in a combination of 200 mg/L NAA concentration and 20-minute immersion duration, which caused an increase in root dry weight of 0.4 g per cutting (Yan et al. 2014). Several previous studies have shown that the optimal duration of immersion with an optimal auxin concentration is a crucial factor in increasing adventitious root biomass. This can be explained through the mechanism of increasing the activity of peroxidase and polyphenol oxidase enzymes (Yan et al., 2014), the regulation of gene expression involved in auxin transport (An et al., 2020), and the decreased levels of ABA, which is antagonistic to auxin (Chen et al., 2023).

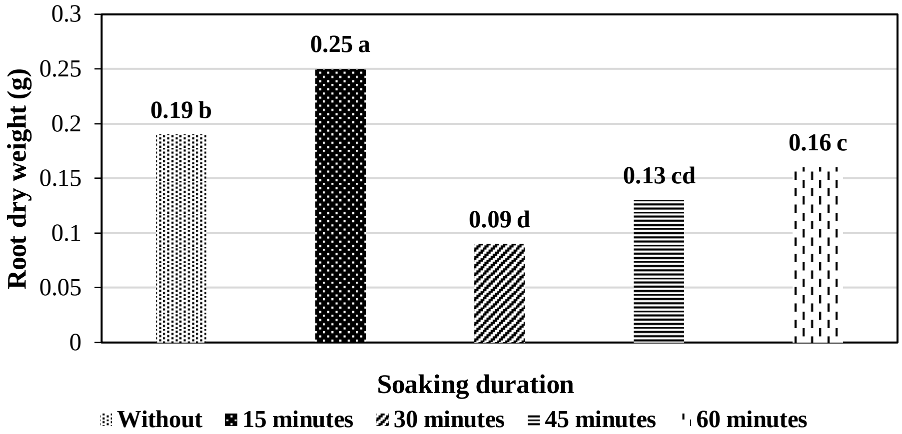

Fig. 2. The root dry weight of *L. garciae* cuttings at each soaking duration in Rootone-F for 12 weeks after planting (LSD 5% = 0.09).

The results of the study also showed that the source of cuttings had a significant effect on the length of primary roots, where cuttings from seedlings produced an average primary root length 1.3 times longer than cuttings from saplings (**Fig. 3**). This difference is thought to be due to endogenous hormones in shoot cuttings from seedlings being sufficient to stimulate better primary root elongation. Gehlot et al. (2014) stated that giving exogenous hormones can increase the effectiveness of endogenous hormones in plants. Aghdaei et al. (2019) noted that young tissues, such as seedlings, have thinner cell walls, higher water content, and higher concentrations of auxin hormones, which support longer primary root growth. Younger (juvenile) tissues tend to allocate energy more towards cell elongation rather than cell thickening, resulting in longer roots (Wang et al. 2024).

3.3. Shoot Growth of L. garciae Shoot Cuttings

We observed several factors related to shoot cuttings of *L. garciae*, including the sprouting percentage, number of shoots, number of new leaves, shoot fresh weight, and shoot dry weight. The analysis revealed that the source of cutting material and immersion duration in Rootone-F significantly affected the number of shoots and new leaves (**Table 1)**. However, neither a single factor nor its interactions had a significant influence on the wet and dry shoot weights. Moreover, the source of seedling material showed a significant difference compared to saplings according to the 5% LSD test (**Fig. 4**). The results indicated that seedlings produced more shoots than saplings, demonstrating a better potential for shoot growth in the seedling material. This finding aligns with the research by Bahru and Derero (2023), which found that cuttings from seedling material of *Pouteria adolfi-friederici* treated with IBA at a concentration of 0.2% had a higher success rate of the shoot and root growth compared to cuttings from branches. Seedling material exhibited a more favorable growth response, with greater root length and increased shoot numbers.

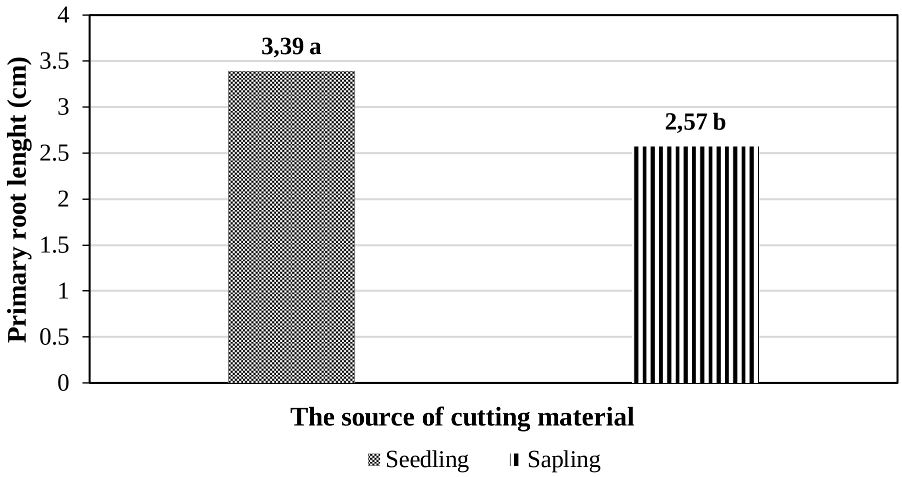

Fig. 3. The primary root length of *L. garciae* shoot cuttings at each cutting material source for 12 weeks after planting (LSD 5% = 0,69)

This study supports the notion that juvenile cuttings outperform mature cuttings in root and shoot regeneration, while older cuttings tend to produce more stable biomass despite slower growth (Aghdaei et al. 2019; Wang et al. 2024). The differences in results may be attributed to complex interactions between the juvenile stage and resource allocation strategies. The juvenile stage influences hormone profiles and regeneration capacity, while the resource allocation strategy in seedlings optimizes primary growth, as saplings tend to prioritize structural resilience.

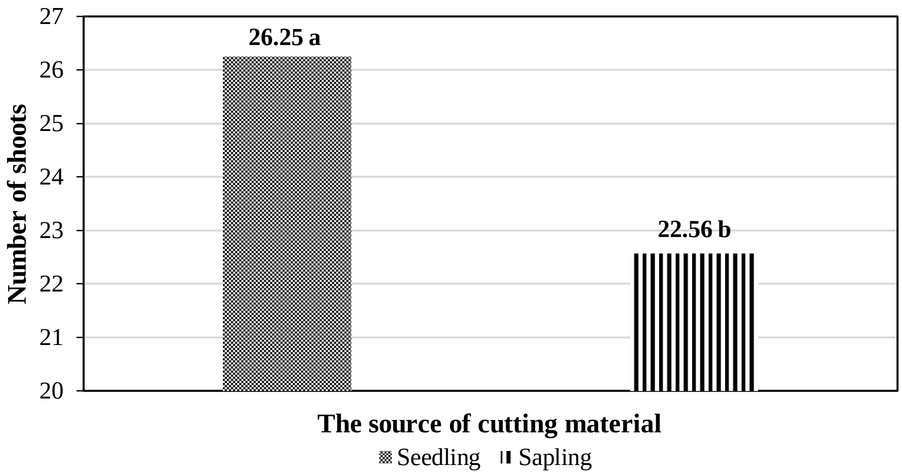

Fig. 4. The number of shoots of *L. garciae* shoots cuttings at each source of cutting material for 12 weeks after planting (LSD 5% = 3.62).

The single-factor treatment related to the source of cutting material, the duration of Rootone-F immersion, and the interaction between these factors did not significantly affect the shoot's fresh or dry weight. As shown in **Fig. 5**, the number of leaves on cuttings not soaked in Rootone-F and those soaked for 60 minutes was significantly higher than those for other durations. It indicates that cuttings not treated with Rootone-F can still produce many new leaves, suggesting that their natural physiological processes are functioning well. However, when Rootone-F is applied to *L. garciae* shoot cuttings, a soaking time of 60 minutes is optimal for stimulating the formation of new leaves, assuming that environmental conditions are also favorable.

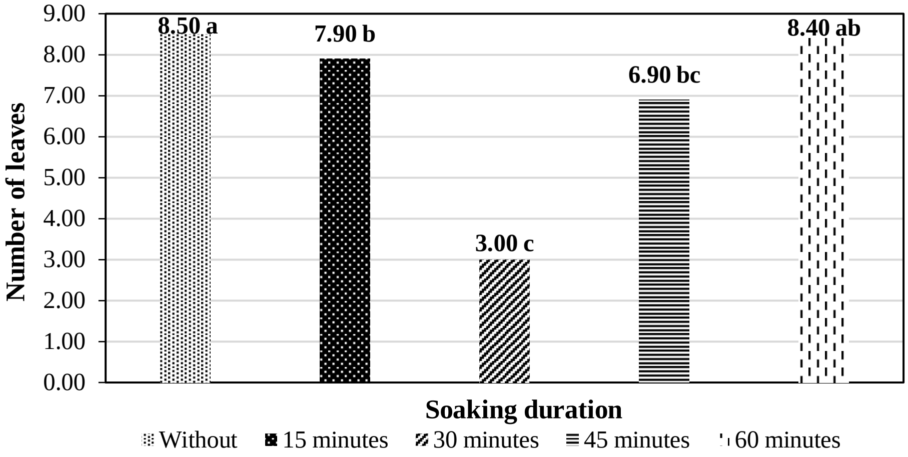

Fig. 5. The number of new leaves of *L. garciae* shoots cuttings at each soaking duration in Rootone-F for 12 weeks after planting (LSD 5% = 4.00).

Shoot growth, particularly the number of shoots and new leaves on *L. garciae* shoot cuttings, was significantly influenced by the cutting material used and the duration of immersion in Rootone-F. Similar to the impact of these two treatment factors on root growth, the age of the cuttings (juvenile factor) and their hormonal profile also played a crucial role in shoot and leaf development (**Table 4**). These findings align with research by Manohar et al. (2022), which demonstrated that the duration of soaking in Rootone-F significantly affected the number of new leaves on *Stevia rebaudiana*. Aghdaei et al. (2019) and Wang et al. (2024) reported that high metabolic activity and auxin concentration in seedling cuttings, along with an optimal cytokinin-auxin ratio, can stimulate shoot formation.

Based on the research findings, using seedlings as a source of shoot-cutting material and immersion in 50% Rootone-F for 15 minutes can be recommended as an effective protocol for the vegetative propagation of *L. garciae*. Theoretically, these results support the "cone of juvenility" theory proposed by Hartmann et al. (2014), which states that plants go through juvenile, transitional, and adult phases at different levels, varying from the bottom to the top of the plant.

Table 4. Correlation coefficient between root and shoot growth variables

Growth variables	Number of buds	Number of new leaves	Shoot fresh weight	Shoot dry weight
	-------------------- Correlation coefficient (r)* --------------------			
Number of primary roots	0.46	0.70	0.74	0.78
Number of secondary roots	0.33	0.72	0.72	0.74
Primary root length	0.46	0.66	0.66	0.75
Secondary root length	0.27	0.73	0.73	0.76
Root fresh weight	0.15	0.41	0.73	0.70
Root dry weight	0.23	0.58	0.79	**0.80**

Notes: *Correlation values 0.00–0.19 (very weak), 0.20–0.39 (weak), 0.40–0.59 (moderate), 0.60–0.79 (strong), 0.80–1.00 (very strong).

Table 4 demonstrates a strong relationship between root and shoot dry weight, while the correlation between root fresh weight and the number of buds is very weak. The robust correlation between the two variables suggests a closely related relationship. This relationship explains the complex physiological interaction between the root and shoot systems, which is important in successful vegetative propagation. The study by Lailaty et al. (2024) emphasized the positive correlation between root and shoot growth of Staurogyne elongata cuttings influenced by the balance of auxin and cytokinin hormones. Maintaining this hormonal balance is crucial for effective root formation (rhizogenesis) and shoot growth in the context of shoot cuttings. Well-developed roots enhance the efficiency of nutrient and water absorption, promoting healthier shoot growth (Hartmann et al. 2014). The growth response of *L. garciae* shoot cuttings tested in this study still requires further investigation, particularly through biochemical and molecular-based studies using cuttings of varying levels of juvenility. It is based on the findings of Osterc and Štampar (2011) and Rencoret et al. (2011), which showed that physiological and genetic factors are the primary drivers of aging and impact rhizogenesis.

4. Conclusions

The objective of the study, to examine the effect of the interaction between the source of cutting material and the duration of immersion in Rootone-F on the growth of *L. Garciae* cutting seedlings, has been achieved. Root fresh weight was significantly affected by the interaction between the source of cutting material and the duration of immersion in the growth regulator (Rootone-F). Cuttings from seedling material can survive and root more than sapling material. In addition, the growth of primary root length and number of shoots of cuttings from seedling material was also significantly better than that from sapling material. The growth of cutting roots, especially the dry weight of roots from sapling material, can be increased by immersion in a 50% Rootone-F solution for 15 minutes. It indicates that applying appropriate exogenous hormones can reduce the limitations of cuttings from sapling material. The selection of appropriate cutting material and the application of optimal growth regulators are practical approaches that can be used in the vegetative propagation of *L. garciae*. It has significant implications for the conservation and sustainable use of this species. Further research focusing on the interaction between the cutting material and growth regulators at the physiological and genetic levels may provide deeper insights into the mechanisms driving the growth responses of cuttings.

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Author Contributions

H.A.E.: Conceptualization, Methodology, Data Curation, Writing – Review and Editing, Supervision; S.H.: Investigation, Data Analysis, Writing – Original Draft Preparation, Software, Visualization; R.S.W.: Supervision, Writing – Review and Editing.

Conflict of Interest

The authors declare no conflict of interest.

Declaration of Generative AI and AI-Assisted Technologies in the Manuscript Preparation

During the preparation of this work, the authors utilized Perflexity Pro and Grammarly Edu to enhance writing quality, including correcting grammar, refining writing style, and selecting the most effective words to convey their ideas more clearly and effectively. After using this tool/service, the authors reviewed and edited the content as needed and took full responsibility for the publication's content.

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