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Effect of Rooting Hormone on Stem-Cutting Propagation of Economically Important *Garcinia afzelii* Tree Species

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Abstract

Garcinia afzelii, a valuable non-timber forest product (NTFP), is utilised for its edible seeds, chewing sticks, and medicinal properties. However, its survival is threatened by overharvesting, slow growth, and limited natural regeneration, warranting its classification as vulnerable. To mitigate these risks, conservation efforts, including propagation, are essential. This study aimed to develop a vegetative propagation protocol for *G. afzelii* using stem cuttings treated with varying concentrations of Indole-3-Butyric Acid (IBA), hypothesising that IBA would improve rooting success. Seedlings were cultivated for 12 months in a nursery, after which semi-hardwood stem cuttings were treated with IBA concentrations of 0.0% (control), 0.1%, 0.3%, and 0.8%. These cuttings were propagated in non-mist propagators using river sand as the growth medium, arranged in a Completely Randomised Design (CRD) with five replications per treatment (10 cuttings each). Results indicated that IBA significantly ($P \leq 0.05$) enhanced root development, with the 0.8% IBA treatment yielding the longest roots (9.19 cm vs. 6.00 cm in control) and the highest number of lateral roots (59.31 cm vs. 39.46 cm in control). Although the untreated cuttings produced a higher number of roots, they accounted for only 27.90% of root formation, while the three IBA-treated cuttings contributed 72.10%. Conversely, the control cuttings recorded a higher survival rate (80.00%) than the IBA (70.00%). IBA-treated *G. afzelii* stem cuttings achieved a 62.10% shoot development rate, compared to 37.90% in the untreated cuttings. These findings suggest that IBA promotes shoot and root formation, making it an effective protocol for mass propagation. Thus, IBA application in stem-cutting propagation could optimise root establishment and resource mobilisation, supporting the conservation and cultivation of *G. afzelii.*

Keywords: Leafy Stem-Cutting, Indole-3-Butyric Acid, Shoot and Root Formation, Survival

Introduction

Garcinia afzelii is a member of the *Guttiferae* family, predominantly found in Africa (Cherry *et al*., 2016). This species is commonly utilised in the production of chewing sticks and flourishes particularly in ecotonal forest areas and fragmented woodland habitats (Hawthorne, 1998). As a species within the genus *Garcinia*, it serves multiple economic purposes, providing medicinal compounds, edible products, and durable wood suitable for construction purposes (Baruah *et al*., 2021). *G. afzelii* has been valued for its medicinal, pharmaceutical, and industrial uses. Notably, its twigs and stems are popular as natural chewing sticks for oral care (Waffo *et al*., 2006).

Its bark was used during World War II (1939–1945) in the tanning and dyeing industries, leading to substantial exports from Ghana (Irvine, 1961). Research in Liberia reveals that 14.8% of harvesters depend primarily on selling *G. afzelii* for income. However, overharvesting driven by high demand, particularly in Ghana's Ashanti and Kumasi areas, has pushed the species toward endangerment (Tally *et al*., 2022). Traders from other countries, mainly Ghana, have turned to Liberia to source *G. afzelii* (Tally *et al*., 2022) after depleting their genetic resources.

G. afzelii is an ecologically and economically valuable species, yet it exhibits weak natural regeneration, limited sprouting ability in forest understories, and slow seedling growth (Gyimah, 2000; Hawthorne, 1998). Overharvesting for chewing sticks and regenerative failure have pushed the species towards vulnerability, thus leading to its extinction (Hawthorne, 1998). The seeds are recalcitrant, prone to desiccation damage, and exhibit low viability, which complicates *ex-situ* conservation efforts (Baillie *et al*., 2004; Baldet *et al*., 2008). Cultivation is the most viable strategy to mitigate extinction risks (Peprah *et al*., 2009).

While studies have explored vegetative propagation in certain *Garcinia* species, particularly *G. kola* (Dao *et al*., 2020; Bhuyan *et al*., 2017; Kouakou *et al*., 2016; Takoutsing *et al*., 2014), limited research exists on *G. afzelii*, hindering its domestication and broader cultivation. Successful propagation is essential for both *in situ* and *ex-situ* conservation efforts, as it facilitates the rapid recovery and reintroduction of plant populations, particularly those that are endangered or rare. By optimizing propagation techniques, the rehabilitation of threatened species can be accelerated while maintaining genetic variability, which is crucial for ecosystem stability (Maunder *et al*., 2004).

Tree propagation plays a vital yet frequently underestimated role in conservation, enabling the swift regeneration of tree populations, particularly for at-risk species, while also safeguarding genetic diversity (Gupta *et al*., 2022; Nakhooda & Jain, 2016). Developing an effective propagation protocol for *Garcinia* species is key to integrating them into forestry and agroforestry systems, promoting sustainable cultivation practices (Kouakou *et al*., 2016). Additionally, vegetative propagation methods can support the conservation of species such as *Garcinia pedunculata* and *Morella* (Bhuyan *et al*., 2017).

Research indicates that applying rooting hormones enhances root and shoot formation in tree cuttings (Antwi-Wiredu *et al*., 2021; Bhuyan *et al*., 2017; Kouakou *et al*., 2016). Auxins or commercial rooting powders play a crucial role in triggering root initiation in woody species (Tchoundjeu *et al*., 2002; Teklehaimanot *et al*., 2004; Sanoussi *et al*., 2012). IBA promotes metabolic processes and supplies energy necessary for root and shoot development (Georges & De Klerk, 2007; Husen & Pal, 2007), as well as a synergy between auxins and carbohydrates for root morphogenesis (Sorin *et al*., 2005). IBA is a synthetic auxin known to induce cell elongation and division in root formation (Yoshida *et al*., 2014). The species' threatened status (vulnerability by IUCN) is due to various anthropogenic and biological factors, such as the overexploitation of bark for tannin extraction and textile dyeing, unsustainable harvesting for oral hygiene products, illegal logging, poor natural regeneration, seed desiccation, low viability, limited seed supply, a recalcitrant nature, and a lack of conservation measures. Consequently, asexual propagation using stem cuttings presents a more feasible alternative for raising materials for conservation and cultivation while maintaining desirable genetic traits. This study examined the effect of varying concentrations of the rooting hormone indole-3-butyric acid (IBA) on the propagation success of *G. afzelii* stem cuttings. The hypothesis posited that IBA treatment would substantially enhance cutting survival, root, and shoot development in this economically valuable species.

Materials and Methods

Study Site

The study was performed at the Council for Scientific and Industrial Research-Forestry Research Institute of Ghana (CSIR-FORIG), Fumesua, Ghana, situated within the Moist Semi-Deciduous Forest Zone (MSFZ) at coordinates 1°15′–1°45′ N and 6°15′–7°00′ W. The research utilised non-mist propagators housed in the CSIR-FORIG vegetative propagation facility, which maintains optimal microclimatic conditions for plant growth. The propagators minimise tissue transpiration and reduce desiccation risk, enhancing root development in cuttings (Longman, 1993). For the propagation of *G. afzelii* stem cuttings, the non-mist poly-propagators provided stable temperatures (27 ± 2°C) and relative humidity (80–90%), likely due to controlled misting. The local climate features an annual precipitation range of 900–1,500 mm, with mean temperatures varying from 25°C to 32°C. Rainfall follows a bimodal distribution, while relative humidity remains moderate but rises notably during wet periods (GSS, 2014; Hall & Swaine, 1981).

Plant Material

Healthy and vigorous growing one-year-old *Garcinia afzelii* seedlings, with an average stem diameter of 3.00–7.00 mm, were selected for the study. These seedlings were germinated from viable seeds in the CSIR-FORIG nursery and exhibited robust growth free from any pest or disease infestation. The seeds were initially sourced from Boamadumase village in Ghana's Juaben District (Ashanti Region) and pooled to ensure uniform germination (**Fig. 2**). Additionally, semi-hardwood shoots' cuttings, each with two leaves, were excised from the *G. afzelii* seedlings using sterilised secateurs. After collection, the cuttings were diligently sanitised, placed in zip-lock bags, lightly sprayed with water, and moved to a non-mist propagation chamber for experimental preparation (**Fig. 4**).

Experimental Design

Leafy Semi-Hardwood (Stem) Cutting Propagation of the *Garcinia afzelii* Tree Species

For the stem-cutting experiment, *Garcinia afzelii* seedlings served as the source material. Semi-hardwood stem cuttings (10.00 cm in length, 3.00–5.00 mm in diameter) (**Fig. 4**) were collected and treated with a 10% fungicide solution (9 water: 1 Shori 437.5SC, Dizengoff Ghana) to inhibit fungal growth. The basal ends of the cuttings were dipped in rooting hormone powder while shaking off any excesses. These cuttings were subjected to three Indole-3-Butyric Acid (IBA) concentrations: 0.1%, 0.3%, 0.8%, and a control treatment, with ten (10) cuttings per treatment. The experiment followed a Completely Randomised Design (CRD) with five (5) replicates per treatment to minimise bias, ensuring uniform distribution across experimental units. In total, two hundred (200) stem cuttings (50 per treatment × 4 treatments) were used to enhance statistical reliability.

The rooting hormone used was Hormodin® (OHP, Inc., Canada), which contains IBA as its active ingredient. After treatment, stem cuttings were placed vertically into river sand-filled non-mist propagators (P^H=6.5; Water holding capacity=17%), ensuring their apical ends were positioned upward. They were misted twice daily to maintain adequate moisture. Sprouting was indicated by visible shoot emergence, while rooting was confirmed when the longest root reached ≥1.00 cm. After 60 days, several growth metrics were recorded, including cutting survival, shoot number, leaf number, shoot length, root number, lateral root formation, and root length.

Data Analysis

The data were analysed using a one-way analysis of variance (ANOVA) with Origin® 9.1 Data Analysis and Graphing Software. Where applicable, Fisher's least significant difference (LSD) test was used to separate the means at a significance level of 5% ($\alpha = 0.05$). The means were calculated and displayed on bar graphs to compare the survival percentages of *G. afzelii* and the effects of IBA treatments on stem-cutting propagation. The average number of shoots, shoot lengths, and leaves produced by the stem cuttings of *G. afzelii*, influenced by the IBA application, were computed and presented in tables. The mean number and length of roots produced by stem cuttings were also calculated and shown in tables. Means bearing the same letter superscript indicate no significant difference at the 5% level. Bar graphs were employed to show the percentage of stem cuttings that sprouted after propagation.

Results

The stem cuttings that survived had two leaves intact and remained greenish irrespective of IBA level (**Fig. 1**). Although statistical analysis showed no significant difference ($P \leq 0.05$), the control exhibited a higher cutting survival rate (80.00%) compared to the IBA treatments (70.00%) (**Fig. 1**). The survival rate of the *G. afzelii* stem cuttings showed no significant difference ($P \leq 0.05$) among the four treatments, and the average survival rate of the stem cuttings was 72.5%. The control treatment produced the highest (80.00%) survival rate and cuttings with intact leaves. This was followed by 0.1% IBA (73.33%), and the lowest survival rate was recorded at 0.8% IBA (66.67%) (**Figs 1 & 5**).

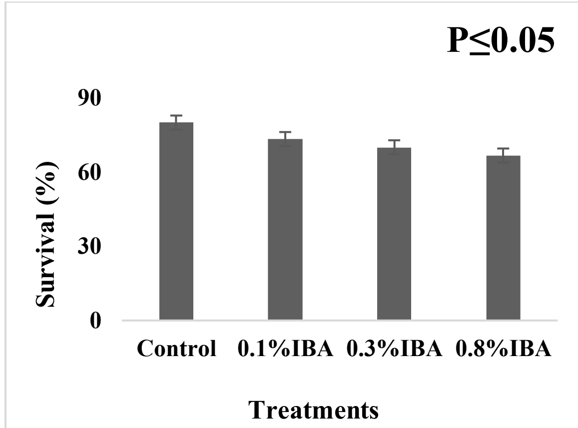

Figure 1. The percentage of *G. afzelii* stem cuttings with intact leaves and survival.

Figure 2. *G. afzelii* Tree with Ripe and Unripe Fruits in a Cocoa Farm at Boamadumase

Shoot formation of the *G. afzelii* stem cutting was significantly (P≤0.05) influenced by treatment application (**Fig. 3**). The control treatment produced the highest percentage (73.33%) of shoot development, and was significantly different from 0.3% IBA and 0.8%IBA levels, but was not significantly different from the 0.1%IBA level. Again, the development of shoots by the *G. afzelii* stem cuttings showed no significant difference (P≤0.05) between 0.3% IBA and 0.8%IBA (**Fig. 3**). The average IBA treatments (40.00%) on shoot formation was way lower as compared to the control-treated (73.33%) *G. afzelii* stem cuttings. Stem cuttings of *G. afzelii* treated with IBA exhibited a significantly higher shoot development rate (62.10%) than the untreated cuttings (37.90%).

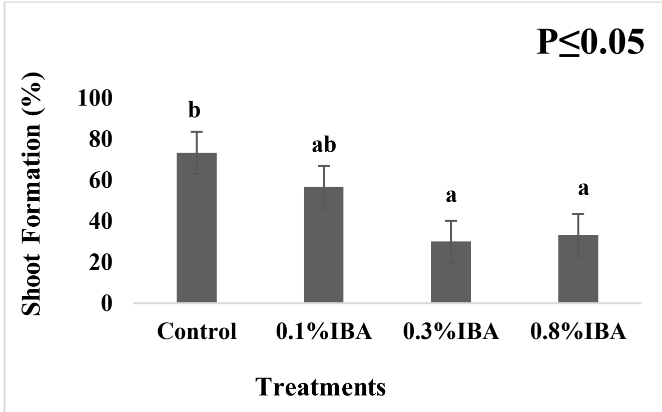

Figure 3. Shoot Formation of *G. afzelii* Stem Cuttings as Influenced by IBA Levels.

Figure 4. *G. afzelii* Leafy Stem-Cutting Setup in Non-Mist Propagator at the Nursery.

The number of shoots produced by *G. afzelii* stem cuttings showed slight significant differences (P≤0.05) among the treatments (**Tab. 1**). Each stem cutting of *G. afzelii* produced at least one (1) shoot irrespective of the treatments. On average, the control and 0.1% IBA treatments produced two (2) shoots per stem cuttings (**Fig. 5**), whereas 0.3% IBA and 0.8% IBA produced one (1) shoot. Shoot emergence occurred in 70.80% of IBA-treated stem cuttings, significantly higher than the 29.20% recorded in untreated cuttings. The control treatment was significantly different in shoot number from 0.3% IBA and 0.8%

IBA, but did not vary significantly from 0.1% IBA. All three IBA levels did not differ significantly in shoot number (**Tab. 1**).

The shoot length developed by the *G. afzelii* stem cuttings showed a significant difference ($P \leq 0.05$) between the control and the IBA treatments (**Tab. 1**). The non-IBA application (control) exhibited the highest shoot length of 3.34±0.697 cm of *G. afzelii* stem cuttings than the three IBA treatments (2.818±0.93cm). However, no significant difference ($P \leq 0.05$) existed among the three IBA concentrations in the *G. afzelii* stem-cutting shoot length.

Leaf development of *G. afzelii* stem cuttings was significantly affected by treatment application. The control treatment had the highest number of leaves (4.133±0.766), and the least number of leaves (3.500±1.240) was recorded at 0.3% IBA treatment (**Tab. 1**). The 0.1% IBA and 0.8% IBA treatments did not differ significantly ($P \leq 0.05$) from the control treatment. All the IBA concentrations showed no significant difference ($P \leq 0.05$) among themselves in the number of leaves. Besides the initial two leaves un-detached before propagating the *G. afzelii* stem cuttings, the cuttings developed new leaves. On average, each *G. afzelii* stem cutting produced four (4) leaves (**Tab. 1**). However, the control treatment (4.133±0.766) produced more leaves than all three IBA treatments put together (3.677±1.206).

Table 1. Mean Shoot Number, Shoot Length (cm), and Number of Leaves Produced by Stem Cuttings of *Garcinia afzelii* as Affected by IBA Application.

Treatments	Shoot Number	Shoot Length (cm)	Leaf Number
Control	1.733 ± 0.283^{b}	3.340 ± 0.697^{b}	4.133 ± 0.766^{b}
0.1%IBA	1.566 ± 0.360^{ab}	3.211 ± 0.765^{a}	4.000 ± 1.160^{ab}
0.3%IBA	1.300 ± 0.468^{a}	2.483 ± 0.981^{a}	3.500 ± 1.240^{a}
0.8%IBA	1.333 ± 0.480^{a}	2.760 ± 1.056^{a}	3.533 ± 1.220^{ab}

Means bearing the same letter superscript indicate no significant difference at the 5% level.

The number of roots produced by the *Garcinia afzelii* stem cuttings showed no significant difference (P≤0.05) among the four treatments. On average, each of the *Garcinia afzelii* stem cuttings produced at least three (3) roots irrespective of the levels of rooting hormones (**Tab. 2**). The untreated cuttings developed more roots than IBA-treated cuttings, yet contributed 27.90% as against 72.10% of the three IBA-treated cuttings for root formation. *G. afzelii* stem cuttings treated with 0.8%IBA were significantly different (P≤0.05) from the control treatments in the root length but did not significantly vary from 0.1% and 0.3%IBA levels (**Tab. 2 & Fig. 5**). No significant difference (P≤0.05) in the root length of the *G. afzelii* stem cuttings was among the control, 0.1%IBA and 0.3%IBA treatments. Although, the control treatment produced the highest number of roots, the IBA-treated stem cuttings produced the highest number of lateral roots and root length (**Tab. 2**). IBA treatments significantly enhanced root elongation by 40.3% (8.42 ± 0.88 cm vs. 6.00 ± 0.64 cm in controls), contributing 80.80% of the root length observed. The maximum root length (9.185±0.907cm) was recorded at 0.8% IBA and the minimum (6.000±0.637cm) was recorded at 0.0% IBA (control) (**Tab.2**).

The control treatment did not significantly vary from the 0.1% IBA and 0.3% IBA treatments, but did differ significantly from the 0.8% IBA treatment. The IBA treatments did not significantly differ among themselves (**Tab. 2**). The number of lateral roots produced by the *G. afzelii* was highest (59.310±7.787) at 0.8%IBA, whilst the control had the lowest (39.455±3.963) number of lateral roots (**Tab. 2 & Fig. 5**). IBA treatments significantly enhanced lateral root development in *G. afzelii* cuttings, yielding 55.28 ± 7.62 roots (84.85%). Conversely, controls produced only 39.46 ± 3.96 roots (15.15%), indicating IBA's pronounced stimulatory effect on root proliferation.

Table 2. Mean Root Number, Root Length (cm), and Lateral Root Number Formed by Stem Cuttings of *Garcinia afzelii* as Affected by IBA Application.

Treatments	Root Number	Root Length (cm)	Lateral Root Number
Control	3.367±0.433[a]	6.000±0.637[a]	39.455±3.963[a]
0.1%IBA	2.867±0.628[a]	8.000±0.770[ab]	51.000±7.634[ab]
0.3%IBA	2.833±0.652[a]	8.062±0.954[ab]	55.538±7.439[ab]
0.8%IBA	3.000±0.644[a]	9.185±0.907[b]	59.310±7.787[b]

Means bearing the same letter superscript indicate no significant difference at the 5% level.

Figure 5. Uprooted *G. afzelii* Stem Cuttings with Developed Roots, Leaves, and Shoots.

Discussions

The influence of IBA on the propagation of leafy semi-hardwood (stem) cuttings of *Garcinia afzelii* under a non-mist propagator system was highly successful. Over sixty percent (60%) of *G. afzelii* stem cuttings survived across the four treatments. The survival rate of the stem cuttings was unaffected by any treatments, yielding an average rate of 72.5%. However, the non-IBA-treated *G. afzelii* stem cuttings had a survival rate of 80%, outperforming the IBA-treated ones, indicating the strong potential of the endogenous auxins in *Garcinia* species. Root cuttings of *Paulownia* propagated without exogenous auxins also thrived, showcasing their natural auxin potency (Antwi-Wiredu *et al*., 2021).

Similar studies have revealed successful regeneration of *G. kola* from various plant parts aside from seeds (Kouakou *et al.*, 2016). The mean number of roots (2.900 ± 0.641) for *G. afzelii* stem cuttings treated with IBA is comparable to what was seen in *G. kola*, which had a mean number of roots of 2.600 ± 0.1 (Kouakou *et al.*, 2016). In contrast to *G. kola*, where IBA-treated cuttings produced significantly more roots than the control (Kouakou *et al.*, 2016), the control outperformed the *G. afzelii* IBA-treated cuttings. This highlights the species' dependency on auxin responsiveness during adventitious rooting. The ability to root is significantly influenced by species, clonally propagated plants (Antwi-Wiredu *et al.*, 2018), variations in endogenous auxins, phenolic compounds, and rooting cofactors (Leakey, 1985).

Exogenous auxins and fungicides markedly enhance the root initiation capability of stem cuttings by modulating physiological and biochemical pathways (Antwi-Wiredu *et al.*, 2018). Entire plants can be regenerated through stem cuttings even without exogenous auxins (Dao *et al.*, 2020). This suggests an inherent ability for adventitious root formation, likely facilitated by endogenous phytohormones and natural rooting stimulants present in the tissue. The environment, plant physiology (Leakey, 1983; Leakey & Longman, 1986), and the viability of plant materials are crucial for successful stem cutting propagation (Antwi-Wiredu *et al.*, 2018).

Throughout the propagation period, none of the surviving *G. afzelii* stem cuttings lost their original leaves (**Fig. 1**). The species retained green, photosynthetically active foliage year-round, aligning with its classification as a medium-sized evergreen tree (Tally *et al.*, 2022). *G. afzelii* stem cuttings showed robust root and shoot development in the non-mist propagators (**Tabs. 1 & 2**). Regardless of treatment, all surviving cuttings produced shoots, although the control outperformed each of the IBA treatments in shoot development.

G. afzelii stem cuttings displayed limited shoot development and length, likely due to the small diameter (3.00–7.00 mm) of the donor plant stems. Research suggests that both species and stem diameter are critical to shoot and root proliferation (Leakey, 1983; Dick *et al*., 2004; Kouakou *et al*., 2016). For instance, cuttings of *Populus deltoides* x *nigra* and *Salix matsudana* x *alba* with larger stem diameters exhibited increased root elongation and higher shoot numbers (Sulaiman *et al*., 2005). Thicker stems often enhance root production, improving the absorption of water and nutrients, which supports overall plant growth (Stenvall *et al*., 2009; Ky-Dembele *et al*., 2010).

While untreated *G. afzelii* cuttings produced the highest root number, the results were not significantly different from those treated with IBA. Nevertheless, IBA treatment notably impacted root elongation and lateral root formation (**Tab. 2**). Previous studies indicate that auxin treatments, including soaking cuttings in 2.5–5.0 g/L rooting hormone, improved root development in woody species (Tchoundjeu *et al*., 2002; Teklehaimanot *et al*., 2004; Sanoussi *et al*., 2012). For example, *Garcinia pedunculata* cuttings responded best to 2000 ppm IBA, while *Garcinia morella* required 4000 ppm for effective rooting (Bhuyan *et al*., 2017). Furthermore, the synergy between auxins and carbohydrates plays a crucial role in root initiation (Sorin *et al*., 2005; Georges & De Klerk, 2007), as IBA facilitates the breakdown of polysaccharides, driving metabolic pathways essential for root meristem activity (Georges & De Klerk, 2007; Husen & Pal, 2007).

For optimal rooting, cuttings must sustain photosynthetic activity to produce the assimilates needed for root primordia formation and subsequent elongation (Hartmann *et al*., 2002; Georges & De Klerk, 2007; Leakey, 2014). Rooting success is typically evaluated based on the proportion of cuttings that form roots, the average number of roots per cutting, and the rate of root emergence and growth (Leakey, 1985). The presence of both original and newly formed leaves on *G. afzelii* cuttings likely fostered enhanced root development

(**Tab. 2**). Improved rooting in softwood cuttings – even without hormone application – may be attributed to leaf retention, as photosynthesis and transpiration driven by foliage are critical for root initiation (Leakey & Storeton-West, 1992; Oboho & Iyadi, 2013; Takoutsing *et al*., 2014). Effective propagation relies on root formation, essential for acclimatisation and subsequent field establishment (Antwi-Wiredu *et al*., 2021). The findings of this study support the application of 0.8% IBA as an effective growth regulator for large-scale clonal propagation of *G. afzelii* using semi-hardwood stem cuttings, based on its considerable improvement of rooting parameters.

Conclusion and Recommendation

IBA treatment significantly improved shoot and root development in *Garcinia afzelii*, making it an effective method for mass propagation. The 0.8% IBA produced the longest roots and most lateral roots. However, untreated cuttings had a higher survival rate. The study also suggests that *G. afzelii* may naturally produce auxins, offering a cost-effective approach for conservation and cultivation. The findings support large-scale propagation efforts for restoring and conserving *G. afzelii* populations. Future research should investigate other growth regulators, auxin combinations (e.g., NAA-IBA), mature cuttings, growth media and field performance trials.

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