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Optimisation of Propagation Structures and Indole-3-Butyric Acid Concentrations for Large-Scale Clonal Multiplication of *Hibiscus rosa-sinensis* Culture AccHR1

M. Visalakshi [a], S. V. Swathika [b], M. S. Swetha [b], M. Ganga [a], S. P. Thamaraiselvi [a], S. Ganesh [c] and Keerthivasan Ragupathi [a*]

[a] *Department of Floriculture and Landscape Architecture, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore - 641003, Tamil Nadu, India.*
[b] *Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore- 641003, Tamil Nadu, India.*
[c] *Department of Floriculture and Landscape Architecture, SRM College of Agricultural Sciences, SRM Institute of Science and Technology, Baburayanpettai, Chengalpattu-603201, Tamil Nadu, India.*

Authors' contributions

This work was carried out in collaboration among all authors. Author MV conceptualized and conducted the experiment, collected and analyzed the data, and prepared the manuscript. Authors SVS and MSS assisted in data collection and experimental observations. Author MG supervised the research work and contributed to the interpretation of results. Authors SPT and SG provided technical guidance and reviewed the manuscript. Authors KR designed the study, supervised the research, interpreted the results and finalized the manuscript. All authors read and approved the final manuscript.

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**Corresponding author: E-mail: keerthivasan0088@gmail.com;*

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Abstract

Effective vegetative propagation is essential for rapid multiplication and maintenance of genetic fidelity in ornamental crops such as *Hibiscus rosa-sinensis*. The success of clonal propagation through stem cuttings is strongly influenced by the propagation environment and the application of rooting hormones. The present investigation was conducted to standardise suitable propagation structures and identify an effective concentration of indole-3-butyric acid (IBA) for improving rooting and establishment of *Hibiscus rosa-sinensis* culture AccHR1 under protected conditions. The study was carried out during 2022-2023 at the Department of Floriculture and Landscape Architecture, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India. Semi-hardwood cuttings of *H. rosa-sinensis* culture AccHR1 were treated with IBA at 1000, 2000, 3000 and 4000 ppm and maintained under three propagation structures: mist chamber, shade net and poly tunnel. The experiment was laid out in a factorial randomised block design comprising eighteen treatments with three replications. Significant treatment effects were observed for all measured traits. The earliest sprouting was recorded in cuttings treated with 3000 ppm IBA under mist chamber conditions. However, superior vegetative growth and rooting characteristics were recorded in cuttings treated with 4000 ppm IBA under mist chamber conditions. This treatment combination produced greater plant height, higher leaf production, larger leaf area, improved root traits and better establishment of cuttings. Based on these findings, treatment of semi-hardwood cuttings with 4000 ppm IBA under mist chamber conditions may be recommended as an effective propagation strategy for rapid rooting and large-scale clonal multiplication of *H. rosa-sinensis* culture AccHR1.

Keywords: Hibiscus rosa-sinensis; indole-3-butyric acid; mist chamber; semi-hardwood cuttings; clonal multiplication; vegetative propagation; rooting performance.

Definitions, Acronyms, Abbreviations

Term		Definition
IBA	*:*	*Indole-3-Butyric Acid*
RBD	*:*	*Randomized Block Design*
DAP	*:*	*Days After Planting*
SED	*:*	*Standard Error Difference*
CD	*:*	*Critical Difference*
CV	*:*	*Coefficient of Variation*

1. Introduction

Hibiscus (*Hibiscus rosa-sinensis* L.), a member of the family Malvaceae, is an important ornamental flowering shrub widely cultivated across tropical and subtropical regions. The species is highly valued in landscape gardening because of its attractive blooms, prolonged flowering duration and adaptability to diverse environmental conditions. Beyond its ornamental value, hibiscus has substantial medicinal, nutraceutical and industrial significance owing to the presence of bioactive constituents such as flavonoids, anthocyanins, phenolics and other phytochemicals (Sivaraman & Saju, 2021; Sakhanokho et al., 2022; Mejía et al., 2023). Extracts obtained from different plant parts of *Hibiscus rosa-sinensis* have demonstrated various pharmacological activities, including antioxidant, antimicrobial, anti-inflammatory, antidiabetic and wound-healing properties. In addition, the flowers are extensively used in herbal drinks, cosmetics and traditional medicines due to their rich phytochemical profile and pharmacological effects (Mak et al., 2013; Abate & Belay, 2022).

Commercial multiplication of hibiscus through seeds is generally avoided due to the occurrence of high genetic variability and heterozygosity among progenies, which often results in non-uniform planting material. Thus, vegetative propagation through stem cuttings is considered the most reliable approach for ensuring genetic fidelity and true-to-type planting material in commercial hibiscus cultivation (Ali et al., 2025; Sereda et al., 2024). Successful rooting of stem cuttings is influenced by several factors, including the type of propagule used, environmental conditions, propagation techniques and the application of plant growth regulators. Among auxins, indole-3-butyric acid (IBA) is considered one of the most active rooting hormones for stimulating adventitious root formation in ornamental plants. IBA facilitates root development by enhancing cell division, stimulating cell proliferation and cambial activity, and improving carbohydrate translocation towards rooting zones (Shadparvar et al., 2011; Sereda et al., 2024). Previous studies have reported that IBA treatment significantly improves rooting percentage, root growth and subsequent survival of hibiscus cuttings under controlled propagation conditions. Higher levels of IBA have been reported to be effective in increasing root percentage, root length and survival rate in semi-hardwood cuttings of hibiscus (Ali et al., 2025). Positive effects of IBA application on rooting percentage, root length, shoot development and leaf production have also been recorded for jasmine genotypes, where auxin application considerably improved adventitious rooting and successful propagation (Keerthivasan et al., 2024). Propagation environments also play a crucial role in determining rooting success. Structures such as mist chambers, shade net houses and poly tunnels are commonly employed to create suitable environments for vegetative propagation. Among these, mist chambers are considered highly effective because they maintain elevated humidity levels, reduce transpiration-related water loss and provide a conducive micro-environment for root initiation and growth (Ali et al., 2025). However, the combined influence of propagation structures and graded IBA concentrations on the multiplication of the pre-release hibiscus culture AccHR1 under Coimbatore conditions remains insufficiently documented. Thus, the present investigation was undertaken to identify suitable propagation structures and optimal IBA concentrations for efficient large-scale multiplication of *Hibiscus rosa-sinensis* culture AccHR1.

2. Materials and Methods

The experiment was carried out during 2022-2023 at the Department of Floriculture and Landscape Architecture, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India. The experiment was undertaken to identify suitable propagation structures and optimal concentrations of indole-3-butyric acid (IBA) for enhancing the rooting, sprouting and vegetative growth of semi-hardwood cuttings of *Hibiscus rosa-sinensis* culture AccHR1 under protected environmental conditions. Semi-hardwood stem cuttings of *Hibiscus rosa-sinensis* culture AccHR1 were obtained from healthy, disease-free mother plants grown in the botanical garden nursery. Semi-hardwood cuttings are generally used in hibiscus propagation due to their superior rooting potential and greater reserve carbohydrate content, which contribute to successful establishment. Semi-hardwood stem cuttings measuring 12-15 cm in length and containing 3-4 nodes each were prepared using sterilised secateurs. A slanting cut was made at the basal end to facilitate the uptake of the growth hormone solution. Propagation was carried out using polybags (6 x 8 inches; 250-gauge thickness) filled with a rooting medium consisting of soil, sand and vermicompost in a 2:1:1 ratio. This medium was selected to provide an optimum balance of aeration, drainage and nutrient availability required for successful root initiation and establishment of semi-hardwood cuttings. Sand improved drainage and prevented waterlogging, while vermicompost enhanced nutrient supply and microbial activity, thereby promoting root growth and overall plant establishment. The components of the medium were thoroughly mixed to facilitate proper drainage, aeration and nutrient availability. Indole-3-butyric acid (IBA) solutions were prepared at concentrations of 1000, 2000, 3000 and 4000 ppm by initially dissolving the required quantity of IBA in a small volume of ethyl alcohol, followed by dilution with distilled water. Auxins such as IBA have been extensively used for root initiation from stem cuttings (Hartmann et al., 2011). The basal portions of the cuttings were treated using the quick-dip method for five seconds, following the procedure described by Shadparvar et al. (2011).

The treated cuttings were then planted in polybags and maintained under three different propagation structures: mist chamber, shade net and poly tunnel. The propagation structures employed for rooting and establishment of *Hibiscus rosa-sinensis* cuttings under protected conditions are presented in Fig. 1. Growth and establishment of *Hibiscus rosa-sinensis* semi-hardwood cuttings maintained under mist chamber, shade net and poly tunnel conditions are presented in Fig. 2. Standard cultural operations, including irrigation, weed management and plant protection measures, were carried out uniformly whenever required throughout the experimental period. The study was laid out in a Randomized Block Design (RBD) consisting of eighteen treatment combinations with three replications. Each replication consisted of five cuttings. The treatment combinations included four IBA concentrations evaluated under mist chamber, shade net and poly tunnel environments, along with separate control and water-treated cuttings maintained within each propagation structure.

Observations were recorded periodically for rooting and growth parameters, including days taken for sprouting, sprouting percentage, number of leaves, leaf area, plant height, root length, root diameter and survival percentage of rooted cuttings. Sprouting percentage was calculated based on the proportion of sprouted cuttings relative to the total number of cuttings planted. Leaf area was estimated from the largest fully expanded leaf using the following formula:

$$\text{Leaf Area} = \text{Leaf Length} \times \text{Leaf Width}$$

Mist Chamber **Shade Net** **Poly Tunnel**

Fig. 1. Propagation structures used for rooting and establishment of *Hibiscus rosa-sinensis* semi-hardwood cuttings under protected conditions

Mist Chamber **Shade Net** **Poly Tunnel**

Fig. 2. Growth and establishment of *Hibiscus rosa-sinensis* semi-hardwood cuttings under mist chamber, shade net and poly tunnel conditions

Root length was measured from the base of the root to the tip of the longest root using a measuring scale, while root diameter was measured using a vernier caliper. Experimental data obtained for growth and rooting characteristics were subjected to statistical analysis following the procedures outlined by Gomez and Gomez (1984). Treatment effects were tested at the 5% level of significance. The values of standard error difference (SEd), critical difference (CD) and co-efficient of variation (CV) were computed for the interpretation of experimental results.

3. Results and Discussion

3.1 Effect of Propagation Structures and IBA Concentrations on Sprouting Behaviour of *Hibiscus rosa-sinensis*

From the findings, it was evident that propagation structures and varying concentrations of indole-3-butyric acid (IBA) significantly influenced sprouting behaviour in semi-hardwood cuttings of *Hibiscus rosa-sinensis*. Significant differences among treatments were recorded for the minimum number of days required for sprouting, sprouting percentage, number of leaves, leaf area, plant height, root length and root diameter. The earliest sprouting was recorded in T_3 (3000 ppm IBA + mist chamber), where cuttings initiated sprouting within 4 days after planting. In contrast, delayed sprouting was recorded under poly tunnel conditions. T_{12} (4000 ppm IBA + poly tunnel) and T_{15} (control + poly tunnel) required 14 days for sprouting initiation, whereas T_9, T_{10}, T_{11} and T_{18} required 13 days. Shade net treatments receiving elevated IBA concentrations also exhibited delayed bud emergence, particularly T_7 and T_8, which recorded sprouting after 10 days. These observations indicate that the propagation environment exerted a substantial influence on bud break and the establishment of cuttings. The influence of propagation environments and IBA concentrations on sprouting characteristics of *Hibiscus rosa-sinensis* semi-hardwood cuttings is presented in Fig. 3. Significant differences among treatments were observed with respect to days taken for sprouting and sprouting percentage. Earlier sprouting was recorded in cuttings treated with 3000 ppm IBA under mist chamber conditions, while delayed sprouting was observed under poly tunnel conditions (Table 1).

Sprouting percentage also varied significantly among treatments. Maximum sprouting (100%) was recorded in T_{13} (control + mist chamber) and T_{17} (water treatment + shade net). Control cuttings maintained under shade net conditions (T_{14}) registered 80% sprouting. Among the remaining treatments, T_1, T_2, T_6 and T_{16} recorded 66% sprouting, while T_4 and T_8 registered 60%. The lowest sprouting percentage (14%) was observed in T_7 (3000 ppm IBA + shade net). Poly tunnel treatments generally exhibited lower sprouting percentages, ranging between 33 and 46%, suggesting comparatively poor establishment under this propagation structure. Early sprouting under mist chamber conditions could be attributed to the maintenance of high relative humidity, which minimizes transpirational water loss and maintains tissue hydration essential for metabolic activities associated with bud break. Favourable environmental conditions could have promoted physiological activation and carbohydrate utilisation required for early shoot emergence. Similar findings regarding the beneficial role of humid propagation environments in vegetative multiplication have been reported by Izadi & Zarei (2014). Furthermore, controlled humidity conditions reduce desiccation stress and improve the establishment of cuttings, thereby enhancing sprouting efficiency (Aziz et al., 2020; Uddin et al., 2024). Auxin application may also have contributed to improved cellular activity and physiological responses involved in early establishment.

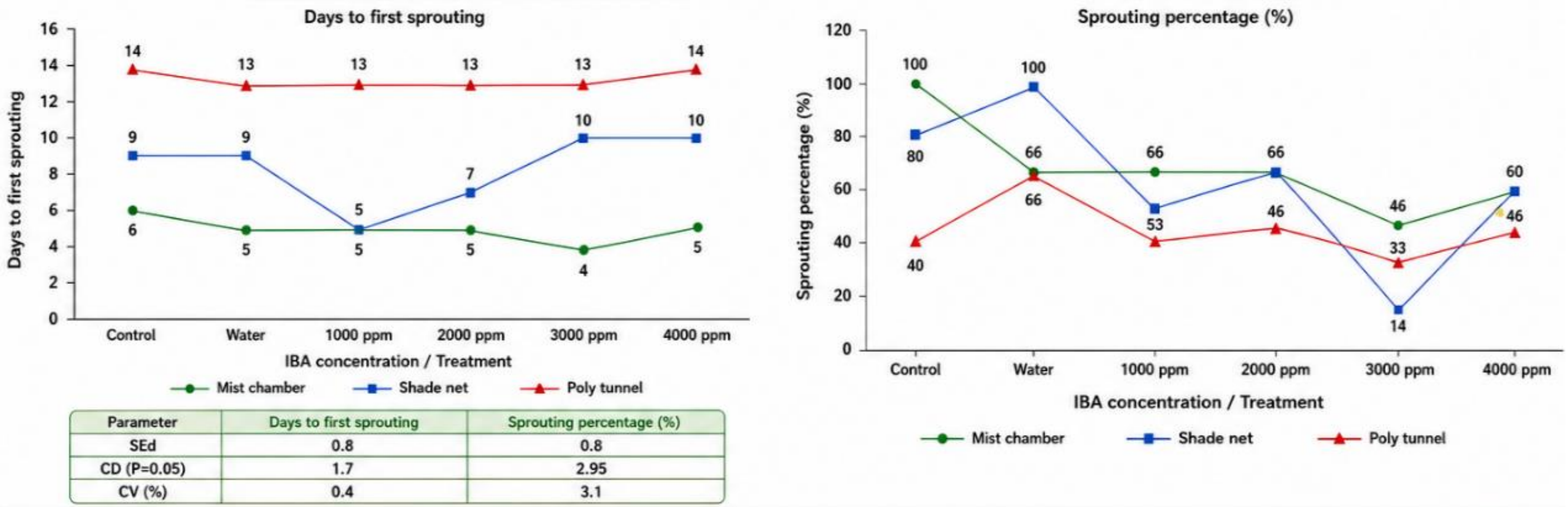
Table 1. Effect of propagation structures and IBA concentrations on sprouting characteristics of *Hibiscus rosa-sinensis* semi-hardwood cuttings

Treatment Code	Treatment Combination	Days to First Sprouting	Sprouting Percentage (%)
T_1	1000 ppm IBA + Mist chamber	5	66
T_2	2000 ppm IBA + Mist chamber	5	66
T_3	3000 ppm IBA + Mist chamber	**4**	46
T_4	4000 ppm IBA + Mist chamber	5	60
T_5	1000 ppm IBA + Shade net	5	53
T_6	2000 ppm IBA + Shade net	7	66
T_7	3000 ppm IBA + Shade net	10	**14**
T_8	4000 ppm IBA + Shade net	10	60
T_9	1000 ppm IBA + Poly tunnel	13	40
T_{10}	2000 ppm IBA + Poly tunnel	13	46
T_{11}	3000 ppm IBA + Poly tunnel	13	33
T_{12}	4000 ppm IBA + Poly tunnel	14	46
T_{13}	Control + Mist chamber	6	**100**
T_{14}	Control + Shade net	9	80
T_{15}	Control + Poly tunnel	14	40
T_{16}	Water treatment + Mist chamber	5	66
T_{17}	Water treatment + Shade net	9	**100**
T_{18}	Water treatment + Poly tunnel	13	66
SEd		**0.8**	**0.8**
CD (P=0.05)		**1.7**	**2.95**
CV (%)		**0.4**	**3.1**

(Note: DAP = days after planting; IBA = indole-3-butyric acid; SEd = standard error difference; CD = critical difference; CV = coefficient of variation)

3.2 Effect of propagation structures and IBA concentrations on vegetative growth parameters of *Hibiscus rosa-sinensis* cuttings

Vegetative growth parameters, including leaf production, leaf area, plant height, root length and root diameter, were significantly influenced by propagation structures and IBA concentrations. The influence of different propagation environments and IBA concentrations on the vegetative growth attributes of *Hibiscus rosa-sinensis* semi-hardwood cuttings is presented in Fig. 4. Cuttings maintained under mist chamber conditions exhibited superior vegetative growth, characterised by increased leaf production, larger leaf area and greater plant height, particularly at higher IBA concentrations. The interaction between favourable microclimatic conditions and auxin application significantly enhanced vegetative performance compared with cuttings maintained under shade net and poly tunnel conditions (Tables 2-4; Fig. 4).



Parameter	Days to first sprouting	Sprouting percentage (%)
SEd	0.8	0.8
CD (P=0.05)	1.7	2.95
CV (%)	0.4	3.1

Fig. 3. Influence of propagation environments and IBA concentrations on sprouting characteristics of *Hibiscus rosa-sinensis* semi-hardwood cuttings

Table 2. Influence of propagation environments and IBA concentrations on leaf production of *Hibiscus rosa-sinensis* semi-hardwood cuttings at different growth stages

Treatment	15 DAP	30 DAP	45 DAP	60 DAP	75 DAP
T_1 – 1000 ppm IBA + Mist chamber	4	7	5	5	8
T_2 – 2000 ppm IBA + Mist chamber	4	8	13	13	**21**
T_3 – 3000 ppm IBA + Mist chamber	4	7	10	12	17
T_4 – 4000 ppm IBA + Mist chamber	3	6	11	15	18
T_5 – 1000 ppm IBA + Shade net	3	4	6	6	6
T_6 – 2000 ppm IBA + Shade net	3	5	0	3	3
T_7 – 3000 ppm IBA + Shade net	2	3	2	0	0
T_8 – 4000 ppm IBA + Shade net	3	5	0	0	0
T_9 – 1000 ppm IBA + Poly tunnel	3	6	4	2	2
T_{10} – 2000 ppm IBA + Poly tunnel	2	5	4	0	0
T_{11} – 3000 ppm IBA + Poly tunnel	2	3	0	0	0
T_{12} – 4000 ppm IBA + Poly tunnel	2	4	0	0	0
T_{13} – Control + Mist chamber	5	7	5	5	4
T_{14} – Control + Shade net	1	4	0	0	0
T_{15} – Control + Poly tunnel	5	2	0	0	0
T_{16} – Water treatment + Mist chamber	4	7	6	6	5
T_{17} – Water treatment + Shade net	8	6	0	5	5
T_{18} – Water treatment + Poly tunnel	6	4	4	3	3
SEd	**0.68**	**1.9**	**2.1**	**2.3**	**2.5**
CD (P=0.05)	**4.5**	**4.8**	**5.1**	**5.1**	**5.3**
CV (%)	**2.4**	**3.0**	**2.7**	**2.8**	**3.9**

(Note: DAP = days after planting; IBA = indole-3-butyric acid; SEd = standard error difference; CD = critical difference; CV = coefficient of variation)

Table 3. Effect of propagation structures and IBA concentrations on leaf area (cm²) of *Hibiscus rosa-sinensis* semi-hardwood cuttings during establishment

Treatment	15 DAP	30 DAP	45 DAP	60 DAP	75 DAP
T_1 – 1000 ppm IBA + Mist chamber	2.6	4.0	4.0	4.3	4.9
T_2 – 2000 ppm IBA + Mist chamber	1.9	6.8	10.5	11.4	23.1
T_3 – 3000 ppm IBA + Mist chamber	2.0	6.4	8.9	15.3	27.6
T_4 – 4000 ppm IBA + Mist chamber	**3.5**	**10.7**	**14.5**	**20.1**	**34.3**
T_5 – 1000 ppm IBA + Shade net	1.7	2.0	4.5	4.9	4.9
T_6 – 2000 ppm IBA + Shade net	1.2	2.9	0	1.5	1.5
T_7 – 3000 ppm IBA + Shade net	1.5	1.7	1.7	0	0
T_8 – 4000 ppm IBA + Shade net	2.0	5.2	0	0	0
T_9 – 1000 ppm IBA + Poly tunnel	1.1	1.8	1.8	1.8	1.8
T_{10} – 2000 ppm IBA + Poly tunnel	1.0	1.5	1.5	0	0
T_{11} – 3000 ppm IBA + Poly tunnel	1.0	1.3	0	0	0
T_{12} – 4000 ppm IBA + Poly tunnel	0.3	0.5	0	0	0
T_{13} – Control + Mist chamber	2.8	3.0	3.5	3.6	3.6
T_{14} – Control + Shade net	0.8	1.9	0	0	0
T_{15} – Control + Poly tunnel	0.1	0.3	0	0	0
T_{16} – Water treatment + Mist chamber	0.3	2.6	6.0	6.0	6.0
T_{17} – Water treatment + Shade net	2.6	3.6	5.1	5.2	5.3
T_{18} – Water treatment + Poly tunnel	0.6	0.9	1.6	1.6	1.6
SEd	**2.8**	**3.0**	**2.9**	**3.1**	**3.2**
CD (P=0.05)	**5.1**	**4.8**	**6.2**	**6.6**	**6.8**
CV (%)	**2.8**	**2.9**	**3.1**	**3.1**	**3.2**

(Note: DAP = days after planting; IBA = indole-3-butyric acid; SEd = standard error difference; CD = critical difference; CV = coefficient of variation)

The number of leaves increased progressively in cuttings maintained under mist chamber conditions. The highest number of leaves at 75 days after planting was recorded in T_2 (2000 ppm IBA + mist chamber), which produced 21 leaves, while T_4 (4000 ppm IBA + mist chamber) produced 18 leaves. Shade net and poly tunnel treatments exhibited comparatively poor leaf retention and development. Complete loss of leaves at later growth stages was particularly evident in T_7, T_8, T_{10}, T_{11} and T_{12}, indicating reduced sustainability of vegetative growth under these conditions.

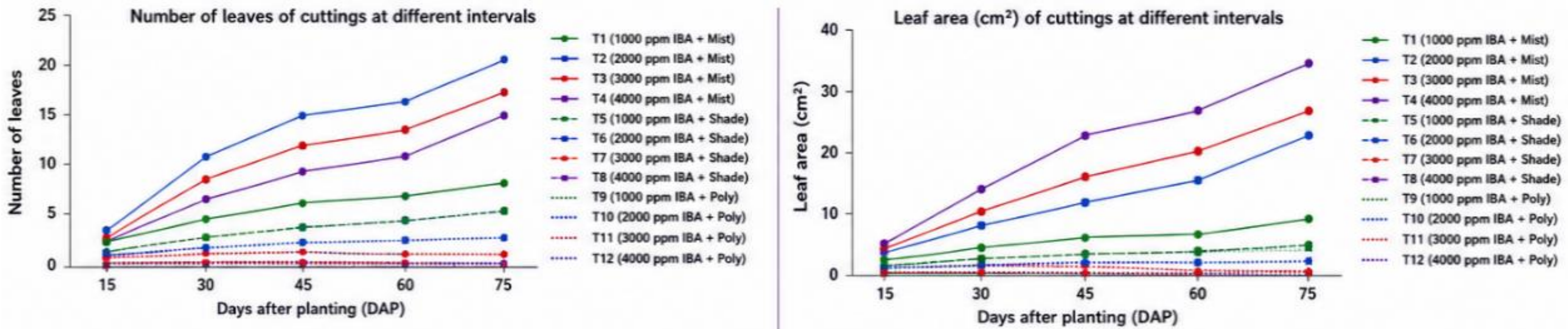
Table 4. Influence of propagation environments and IBA concentrations on plant height and rooting characteristics of *Hibiscus rosa-sinensis* semi-hardwood cuttings at 75 DAP

Treatment	Plant Height (cm)	Root Length (cm)	Root Diameter (mm)
T_1 – 1000 ppm IBA + Mist chamber	33.2	0	0
T_2 – 2000 ppm IBA + Mist chamber	40.0	**15.5**	**6**
T_3 – 3000 ppm IBA + Mist chamber	36.0	9.7	4
T_4 – 4000 ppm IBA + Mist chamber	**40.3**	10.9	**6**
T_5 – 1000 ppm IBA + Shade net	32.0	4.5	4
T_6 – 2000 ppm IBA + Shade net	30.0	5.3	4
T_7 – 3000 ppm IBA + Shade net	12.0	0	0
T_8 – 4000 ppm IBA + Shade net	12.0	0	0
T_9 – 1000 ppm IBA + Poly tunnel	25.1	0	0
T_{10} – 2000 ppm IBA + Poly tunnel	12.0	0	0
T_{11} – 3000 ppm IBA + Poly tunnel	12.0	0	0
T_{12} – 4000 ppm IBA + Poly tunnel	12.0	0	0
T_{13} – Control + Mist chamber	30.0	0	0
T_{14} – Control + Shade net	0	0	0
T_{15} – Control + Poly tunnel	12.0	0	0
T_{16} – Water treatment + Mist chamber	15.7	0	0
T_{17} – Water treatment + Shade net	14.0	0	0
T_{18} – Water treatment + Poly tunnel	15.0	0	0
SEd	**2.4**	**1.8**	**0.06**
CD (P=0.05)	**5.0**	**3.8**	**0.13**
CV (%)	**0.7**	**5.0**	**3.4**

(Note: DAP = days after planting; IBA = indole-3-butyric acid; SEd = standard error difference; CD = critical difference; CV = coefficient of variation)

Leaf area was also markedly affected by treatment combinations. At 75 DAP, T_4 (4000 ppm IBA + mist chamber) recorded the maximum leaf area (34.3 cm²), followed by T_3. Lower leaf areas were recorded under shade net and poly tunnel structures. Treatments T_7, T_8, T_{10}, T_{11}, T_{12}, T_{14} and T_{15} showed a complete decline in leaf area during the later stages of observation. Water-treated cuttings maintained under shade net conditions (T_{17}) retained moderate leaf area development and recorded 5.3 cm² at 75 DAP. Enhanced rooting and vegetative growth observed in cuttings treated with 4000 ppm IBA under mist chamber conditions at 75 DAP are presented in Fig. 5. This treatment combination resulted in superior root development, greater plant height, increased leaf production and improved establishment percentage. The improved rooting response may be attributed to the stimulatory effect of IBA on adventitious root formation, combined with the favourable humidity conditions maintained in the mist chamber, which reduced moisture stress and promoted rapid establishment of cuttings (Fig. 5).

Plant height and rooting characteristics also varied significantly across treatments. Maximum plant height was observed in T_4 (40.3 cm), followed by T_2 (40.0 cm) and T_3 (36.0 cm). Enhanced leaf production under mist chamber treatment may be associated with favourable microclimatic conditions that supported efficient root establishment, water uptake and nutrient translocation. Root development directly influences shoot growth by improving resource acquisition, thereby facilitating leaf production and canopy expansion. Similar observations were reported by Shadparvar et al. (2011), who emphasized the importance of root development in sustaining vegetative growth. Auxin-mediated stimulation of cambial activity and mobilisation of stored carbohydrates towards rooting zones may further explain the improved rooting response observed under optimal IBA concentrations (Izadi & Zarei, 2014; Shadparvar et al., 2011). The results obtained are consistent with previous findings in jasmine, where external treatment with IBA increased the number of roots, root length and rooting percentage by inducing adventitious root formation (Prasad et al., 2022; Keerthivasan et al., 2024). The larger leaf area observed at higher IBA concentrations under mist chamber conditions may be attributed to enhanced cell division and cellular expansion processes promoted by auxin activity, resulting in improved photosynthetic



DAP	15	30	45	60	75
SEd	0.68	1.9	2.1	2.3	2.5
CD (P=0.05)	4.5	4.8	5.1	5.1	5.3
CV (%)	2.4	3.0	2.7	2.8	3.9

DAP	15	30	45	60	75
SEd	2.8	3.0	2.9	3.1	3.2
CD (P=0.05)	5.1	4.8	6.2	6.6	6.8
CV (%)	2.8	2.9	3.1	3.1	3.2

Note: IBA – Indole-3-butyric acid; DAP – Days after planting; SEd – Standard error of difference; CD – Critical difference; CV – Coefficient of variation.

Fig. 4. Effect of propagation structures and IBA concentrations on vegetative growth attributes of *Hibiscus rosa-sinensis* semi-hardwood cuttings

4000 ppm cuttings under mist chamber on 75th day

Rooting of best grown cuttings under mist chamber

Multiplication of 4000 ppm cuttings under mist chamber

Fig. 5. Enhanced rooting and vegetative growth of *Hibiscus rosa-sinensis* semi-hardwood cuttings treated with 4000 ppm IBA under mist chamber conditions at 75 DAP

surface area and biomass accumulation (Shadparvar et al., 2011; Bisht et al., 2025). The superior performance of T_2 and T_4 demonstrates that the interaction between an appropriate propagation environment and an optimum auxin concentration is critical for maximising rooting and vegetative growth of *Hibiscus rosa-sinensis* semi-hardwood cuttings. Overall, the findings indicate that mist chamber conditions combined with suitable IBA concentrations considerably improve propagation efficiency and establishment success in hibiscus cuttings.

The enhanced rooting response observed at higher IBA concentrations may be associated with the role of auxins in stimulating cell division, cell elongation and differentiation of root primordia. Indole-3-butyric acid promotes the mobilisation of carbohydrates and endogenous metabolites towards the basal portion of cuttings, thereby facilitating adventitious root formation. The superior rooting characteristics recorded under 4000 ppm IBA combined with mist chamber conditions suggest a synergistic interaction between auxin treatment and favourable environmental conditions, resulting in improved establishment and vegetative growth of *Hibiscus rosa-sinensis* cuttings.

4. Limitations of the Study

The study was conducted at a single location, Coimbatore, Tamil Nadu, during 2022-2023, and the responses were evaluated only in the pre-release *Hibiscus rosa-sinensis* culture AccHR1. Therefore, the findings should be interpreted within the environmental and genetic scope of the experiment. Although three propagation structures and four IBA concentrations were compared, the study did not include additional rooting media, seasonal replication across different climatic periods or other hibiscus genotypes. The observations were recorded up to 75 days after planting, which provided useful information on early establishment but did not assess long-term field performance after transplanting. The treatment combination of 4000 ppm IBA under mist chamber conditions showed superior rooting and vegetative responses in this experiment; however, its consistency under varied nursery management practices requires further validation. Future studies involving multiple seasons, locations, genotypes and post-transplant evaluation would help determine the broader applicability of the protocol for commercial propagation systems overall.

5. Conclusion

The present investigation confirmed that propagation structure and indole-3-butyric acid (IBA) concentration significantly affected sprouting, rooting and vegetative growth of *Hibiscus rosa-sinensis* culture AccHR1. Among the treatment combinations, cuttings treated with 3000 ppm IBA under mist chamber conditions initiated sprouting earliest, requiring only 4 days for bud emergence. However, the most favourable overall performance was recorded in cuttings treated with 4000 ppm IBA under mist chamber conditions. This combination resulted in greater plant height, increased leaf production, larger leaf area and improved rooting traits, including root length and root diameter, at 75 days after planting. The response indicates that a humid protected environment, together with an appropriate auxin concentration, supports the establishment of semi-hardwood cuttings by reducing moisture stress and promoting adventitious root formation. Shade net and poly tunnel conditions were comparatively less effective for sustaining vegetative growth and rooting performance in this study. Based on the recorded responses, treating semi-hardwood cuttings with 4000 ppm IBA and maintaining them under mist chamber conditions may be considered an effective protocol for the rapid clonal multiplication of *Hibiscus rosa-sinensis* culture AccHR1. Further validation across seasons, locations and genotypes would strengthen the wider application of this propagation approach under broader production conditions elsewhere.

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Declaration of AI Use

This manuscript was prepared through the combined contributions of all author(s), including contributions to the study design, data, content development, results, interpretation, and related scholarly work. The author(s) acknowledge the use of Grammarly and ChatGPT to assist with grammar checking, language refinement, reference formatting. These AI-assisted tools were not used as authors and did not replace the intellectual contributions or scholarly judgment of the author(s). All AI-assisted outputs, including content, references, and interpretations, were carefully reviewed, revised, verified, and approved by the author(s). The author(s) accept full responsibility for the accuracy, integrity, and final content of the manuscript.

Competing Interests

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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