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Impact of Various IBA Concentrations on Rooting in Apical Shoot Cutting and Stem Cuttings of *Callistemon lanceolatus* (Sm.) Sweet in Polytunnel Conditions

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The study was undertaken to identify the optimal concentration of IBA and type of cuttings suitable for propagation of *Callistemon lanceolatus* and it was conducted at the Vanavarayar Institute of Agriculture, Pollachi, Coimbatore, India from December 2022 to September 2023. The experiment addressed the challenges of seed propagation, such as their seed capsules need to be activated by fire and the unpredictable nature of seed germination, which results in high progeny diversity due to genetic recombination. The investigation focused on apical and stem cuttings subjected to fifteen different treatments with three replications each; the Tukey test was used for statistical analysis; the cuttings were treated with various concentrations of Indole-3-butyric acid (IBA) and placed under polytunnel conditions; observations were made after 45 days, measuring sprouting percentage, rooting percentage, mean number of leaves, shoot length, mean number of roots, and root length per cutting. In light of these issues, the study explored clonal propagation as an alternative for mass multiplication of elite trees. The results of the study depicted that the maximum rooting percentage and root length were observed in T6 (73.28±8.87%) and (12.54±0.00cm) due to the exogenous application of auxin. On the whole, the result of this investigation suggest that apical shoot cuttings with IBA at 6000ppm can be made to induce maximum rooting and to produce high quality planting stock material for clonal propagation in *Callistemon lanceolatus*.

Keywords: Callistemon lanceolatus; rooting hormone; IBA; apical shoot cuttings; stem cuttings.

1. INTRODUCTION

Callistemon lanceolatus, which belongs to the Myrtaceae family, native to Australia, is widely distributed in subtropical and tropical regions [1]. This tree, characterized by its striking bright red flowers resembling bottlebrushes, is commonly cultivated in gardens throughout India as an ornamental plant [2]. Referred to as the "lemon bottlebrush" due to its distinctive cylindrical brush-like red flowers Singh et al., [3], this plant is known to possess various biological activities in its aerial parts, including antimicrobial [4], antioxidant, antidiabetic Ahmad et al., [5], Kumar et al., [6], anti-inflammatory Kumar et al., [2], and anti-proliferative [7] properties. Notably, essential oils extracted from the leaves of *C. lanceolatus* exhibit antimicrobial and anti-inflammatory properties [8,9].

The propagation of ornamental plants has traditionally relied on seed germination and vegetative propagation techniques [10,11]. However, these conventional methods come with constraints related to time, labor, and genetic fidelity [12]. Seed germination can be unpredictable and time-consuming, resulting in a wide range of progeny diversity due to genetic recombination [13,14]. To address these challenges and meet the increasing demand for genetically identical, high-quality plant material, clonal propagation techniques have gained prominence [15]. Clonal propagation enables the mass production of plants with identical genetic characteristics, ensuring uniformity in desirable attributes such as flower color, scent, and growth habits [16,17]. Some species necessitate vegetative propagation for short-term regeneration [18,19]. Over the past decade, vegetative propagation approaches have gained traction for the bulk propagation of enhanced genetic material, particularly for tree species with problematic seeds (Kantarli, 1993). Interestingly, some tropical trees have demonstrated the potential of vegetative propagation, which improves yield potential by reducing variation among the ramets (Parthiban et al., 2017a). This progress in vegetative propagation has evolved into clonal technology, emerging as an alternative to traditional seed-based propagation (Parthiban and Seenivasan, 2017b). When it comes to vegetative multiplication through cuttings, the use of crown branches of advanced ontogenetic age has been observed to reduce the percentage of propagule roots [20]. However, the utilization of spontaneous or induced basal sprouts has yielded excellent rooting indexes, resulting in enhanced efficiency in vegetative proliferation [21]. Clonal propagation is a simpler, quicker and more competent way to rapidly expand millions of cuttings from elite germplasms then seed propagation [22,23].

A special problem with *Calllistemon lanceolatus* is that their seed capsules need to be activated by fire, which can take an extended period of time, anywhere from a year to several years. Pre-treatment of the seeds is often necessary to disrupt this dormancy (Brophy et al., 2013; Lamont et al., 2020). Furthermore, these trees exhibit variable seed production in different regions, creating operational challenges in meeting annual planting material demands. The proposed clonal technology offers a solution to these challenges while ensuring uniformity in growth and production. It enables the large-scale multiplication of genetically identical ramets, promoting the propagation of elite genotypes and the bulk production of high-quality planting material for commercial ornamental trees. In this context, clonal technology is a game-changer in boosting the generation of improved genotypes in *Callistemon lanceolatus* trees, facilitating large-scale commercial production. (Valsalakumari, [24], Shokri et al., [11], Parthiban et al., [25].

Vegetative propagation of the bottlebrush plant poses challenges due to the inherent difficulty in rooting stem cuttings, which necessitates specific hormonal and environmental interventions to induce root formation (Zarinbalet al., 2005). Root induction in stem cuttings of challenging-to-root species has been addressed in numerous studies, employing methods such as auxin treatment, bottom heat, wounding and mist systems (Dawson and King, 1994; Hartman et al., [10], Dominik and Gregor, 2007; Shokri, [11]. The need for mass propagation of *C. lanceolatus* through cloning has become an important priority in horticulture. Clonal propagation techniques, which involve replicating individuals with identical genetic characteristics as the parent tree, hold the promise of preserving and proliferating the remarkable attributes of this species. This approach ensures that the enchanting qualities, both in terms of appearance and fragrance, remain consistent across the newly propagated species Shokri et al., [11], Cirillo et al., 2016; Chauhan et al., 2017; Parthiban et al., [25].

This paper aims to explore the methods of clonal propagation for *C. lanceolatus*, examining the different concentrations of IBA on the growth. By understanding and implementing these techniques, horticulturists and enthusiasts can ensure the continued cultivation and conservation of this iconic and culturally significant tree, which not only adds beauty to our surroundings but also holds a special place in our traditions and heritage.

2. MATERIALS AND METHODS

2.1 Study Site

The study was conducted at the Vanavarayar Institute of Agriculture, Pollachi, Coimbatore, India from December 2022 to September 2023. The location experiences a warm, humid climate with an average annual rainfall of about 2100 to 2500 mm. The average temperature ranges from 23.8°C to 34.6°C with a relative humidity of 51 to 82%.

2.2 Experimental Materials

The study was undertaken to standardize the optimal type of cuttings suitable for propagation. The cuttings collected from identified tree, were grouped into apical shoot cuttings and stem cuttings without an apical shoot). These cuttings were collected from the tree by conventional cutting methods [26,27]. The cuttings were harvested using sterile pruning scissors in the early morning. After harvesting, they were cut to the desired length (8- 10cm) by using a scale and diameter (2-4 mm) by using a calibrated vernier calliper, following which they were placed in an ice box to prevent damage during transportation to the laboratory. The cuttings were treated with an aqueous solution of 0.1% Bavistin for 15 minutes and subsequently washed with distilled water [28]. Since the discovery of natural plant rooting hormones for propagation, it has been intuitive to apply these substances to the basal end of cuttings to initiate new root formation. Six concentrations of aqueous rooting solutions *viz*. 1000, 2000, 3000, 4000, 5000 and 6000 mg L^{-1} IBA were prepared in 1N NaOH with distilled water. Untreated cutting was considered as control (cuttings were dipped in distilled water served as a control). Fifteen cuttings were prepared following the above procedure in each treatment with three replications.

After auxin treatments, the cuttings were planted in the polybags containing coir pith as a rooting medium. One third of the basal cut portion was inserted in the rooting medium. The medium was kept damp and the surface even. To insert

Chart 1. Treatment details of different concentrations of IBA

Treatments	
T_1	Apical shoot cuttings with IBA at 1000 ppm
T_2	Apical shoot cuttings with IBA at 2000 ppm
T_3	Apical shoot cuttings with IBA at 3000 ppm
T_4	Apical shoot cuttings with IBA at 4000 ppm
T_5	Apical shoot cuttings with IBA at 5000 ppm
T_6	Apical shoot cuttings with IBA at 6000 ppm
T_7	Stem cuttings with IBA at 1000 ppm
T_8	Stem cuttings with IBA at 2000 ppm
T_9	Stem cuttings with IBA at 3000 ppm
T_{10}	Stem cuttings with IBA at 4000 ppm
T_{11}	Stem cuttings with IBA at 5000 ppm
T_{12}	Stem cuttings with IBA at 6000 ppm
T_{13}	Control

cuttings without harming the cambium or losing the rooting hormone, holes should be punched into the medium. After the cuttings were stuck, the rooting medium was pressed slightly around them. The cuttings were kept in polytunnel for maintaining the relative humidity in the range of 60 to 80% and the optimal temperature (25-30°C / 15-20°C day/night). The cuttings were regularly watered and drenched with 0.1% Bavistin at a15 day interval to avoid desiccation damage and the attack of fungal pathogens. The rooting experiment was carried out for 60 days. Rooted cuttings were transferred to polythene bags (15 x 25 cm) containing soil + poultry manure (5:1) and kept in ashade house for 30 days.

2.3 Experimental Design and Data Collection

Statistical analysis: To compare the treatment means, the Tukey test was performed using the Statistical Package for Social Science data (SPSS) software, and the graphs were formed using Paleontological statistical software (PAST) version 3.The experiment included 12 treatments with three replications, each consisting of 15 cuttings per replication (Chart 1).

The biometric parameters of epicormic shoots such as sprouting percentage, rooting percentage, root length, number of roots per cutting, shoot length (cm), number of leaves, leaf length (cm), leaf width (cm), survival percentage and vigour index were measured and recorded.

3. RESULTS

The sprouting percentage can vary depending on the plant species, the quality of the plant parts, and the environmental conditions (such as temperature, humidity, and soil quality). It is an important metric in agriculture, horticulture, and plant propagation, as it indicates the success of sprouting process Packialakshmi and Sudhagar, [28], Smitha and Umesha, 2012 and Husen, [28]. The results of the study revealed that the maximum sprouting percent of *C. Lanceolatus* was observed in T_6 (80.87±0.86%), T12 (79.15±3.43%), T_5 (75.73±4.53%), T_{11} (73.13±2.89%) whereas the minimum sprouting percentage was noticed in T_{13} (32.73±1.80%) (Table 1 & Fig. 1). The study reported that the highest shoot length of *C. lanceolatus* was recorded in T_6 (12.72±0.73cm) followed by T_{12} (11.68±0.05cm), T_5 (11.65±0.17cm) whereas the lowest was reported in T_{13} as (8.23±0.36cm) (Table 2). The highest number of leaves per cutting was observed in T_6 as 9.45±0.26 followed by T_{12} (8.56±0.03) and the lowest was reported in T_{13} (2.35±0.06).The result of present investigation clearly exhibits that the leaf length of *C. Lanceolatus* varied significantly among the treatments, the longest leaf was observed in T_{12} (5.87±0.32cm), T_{11} (5.74±0.24cm), T_6 (5.67±0.27cm) while the smallest leaf was noticed in T13 (1.11±0.02cm).

3.1 Rooting Efficiency

The results of the study depicted that the maximum rooting percentage was observed in T_6 (73.28±8.87%) and T_{12} (72.27±1.97%), followed by T_{11} (57.39±0.03%), while the minimum percentage was noticed in T_{13} (20.19±1.27%). The highest root length was reported in T6 (12.54±0.00cm); T12 (12.01±0.29cm) and the lowest was recorded in T13 (1.76±0.02cm). The result of the study showed that the number of roots per cutting varied significantly between the treatments (Table 2). Among the treatments the maximum number of roots was noticed in T_{12} (6.45±0.03), T_6 (6.45±0.36) whereas the minimum was recorded in T_{13} (1.02±0.01).with regard to survival percentage the highest was reported in T_6 (69.84±0.65%) followed by T_{12} (63.54±0.48%) and the lowest was registered in T_{13} (20.45±0.20%) (Fig. 1).

4. DISCUSSION

Propagating plants from cuttings has several benefits, such as preserving the unique qualities of the parent plant, providing true-to-type plant materials, being straightforward to carry out, requiring less room than other propagation techniques like layering, grafting, or budding, and being less expensive. The success of plant propagation by cuttings is shown by root and shoot formation and growth which is well documented to be affected by several factors, including genotypes, physiological and ontogenetic age of cuttings, endogenous hormone contents, type of wood, carbohydrate contents, preconditioning treatment of cuttings and external factors such as micro-environment of cuttings and the use of root promoting substances [29-31]. One of the most important and widely reported factors is the use of auxins such as IBA as root promoting substances (Abu-Zahra et al., [32], Rahdari et al., [33], Costa et al., [34]. IBA at 6000ppm concentrations significantly stimulated adventitious root formation as shown by the marked increases in rooting percentage. It was also shown that regardless of the types of auxins applied, increasing concentrations from 1000 to 6000 ppm caused significant increases in the number of roots per cutting, all with a 73% rooting percentage (Fig. 1). These findings agree with the previous statement that for more than 70 years, IBA has been effectively used as a root promoting substance for cuttings from various species commercially, and has been the backbone of cutting propagation success [30].

Auxins are widely recognized for their significant role in promoting the development of adventitious roots in vegetative propagules. Both exogenously applied and endogenous auxins influence the rooting potential and the quantity of roots produced in cuttings (Wen et al., 2016; Gonin et al., 2019). Notably, the impact of externally administered growth hormones on the rooting process exhibits significant variation between the different concentrations of IBA. The sprouting percentage of stem cuttings refers to the proportion of stem cuttings from a plant species that successfully develop new sprouts or shoots, typically as a result of vegetative propagation. This percentage is a measure of the cloning or propagation success and can vary depending on factors such as the species, the specific method of propagation, and the conditions under which the cuttings are kept. Higher sprouting percentages indicate more successful propagation. It is an important metric in horticulture, agriculture, and forestry when reproducing plants asexually [35,36].

Days taken to start sprouting and days taken to attain fifty percent sprouting of cuttings were significantly affected by different growth regulators but the earliest sprouting of cuttings was noted in IBA at 6000ppm whereas, latest sprouting of cuttings was noted in control. The percentage of success of cuttings was also found significantly higher at IBA 6000ppm, whereas the minimum percentages of success of cutting were recorded in control. This may be due to the increased level of Auxins, which resulted in earlier completion of physiological processes in rooting and sprouting of cuttings. A plethora of workers have also reported similar values in *Citrus limonburm* as 81.90% [37]; *Calliandra haematocephala* as 53.33% [38]. IBA at 6000ppm produced the maximum shoot length, but the minimum shoot length was observed in control. The results of the study are in line with those of Yusnita et al. [35], *Syzygium malaccense* treated with 4000 ppm IBA grew by 3.30 cm; Sevik & Guney [39] found that *Melissa officinalis* treated with 1000 ppm IBA grew by 6.71 cm; and Yeshiwas et al. [40] found that rose stem cuttings treated with 2500 ppm IBA grew by 15.47 cm. The total number of leaves per cutting was also found to be significantly higher in IBA at 6000ppm, while the lowest number of leaves per cutting was noted in control (Table 1). It was found that the maximum was at IBA6000ppm, even though IBA had significant effects on the shoot length and maximum number of leaves per cutting. This may be due to the vigorous root system which increased nutrients uptake under this treatment. The present study falls in line with Husen and Pal, [41], Guleria and Vashisht [42], who reported that shoot length of *Tectona grandis* increased. This might be due to the thickness of the cutting, effect of branch position and auxin treatment.

Control (T_{13}) exhibited poor growth performance in terms of both the number of leaves and survival percentage. These results may be attributed to the fact that growth attributes in terms of root and shoot growth parameters are affected by the exogenous application of required growth regulators. This is depicted in lowest physiological activity for triggering root initiation and development and finally all other growth parameters of cuttings were seriously affected. This could be due to low activity of the growth substance and low physiological activity.

Rooting percentage is a critical indicator of the success of clonal propagation methods. It directly influences the number of new plants that can be produced from a mother plant, which can be crucial for agricultural, horticultural, and forestry practices [43]. As regards root characters like rooting percentage, root length, number of roots per cutting, the maximum value in IBA at 6000 ppm. Whereas, minimum rooting percentage, root length, number of roots per cutting were

Table 1. Effect of IBA concentrations on shoot growth response of *Callistemon lanceolatus* cuttings under poly tunnel condition

Treatments	SP (%)	SL (Cm)	NL(Nos)	LL (Cm)
T1	46.32±1.88^{c}	8.43±0.27^{de}	5.23±0.37^{fg}	2.43±0.00^{g}
T2	48.49±1.81^{c}	8.56±0.44^{de}	5.87±0.27^{cdef}	3.12±0.07^{f}
T3	48.52±1.32^{c}	9.24±0.45^{cde}	5.95±0.06^{cde}	3.78±0.06^{de}
T4	61.87±2.68^{b}	10.47±0.42^{cd}	6.52±0.22^{c}	4.89±0.09^{b}
T5	75.73±4.53^{a}	11.65±0.17^{ab}	7.89±0.09^{b}	4.97±0.16^{b}
T6	80.87±0.86^{a}	12.72±0.73^{a}	9.45±0.26^{a}	5.67±0.27^{a}
T7	40.14±0.42c^{d}	7.90±0.35^{e}	4.57±0.08^{g}	3.34±0.12^{ef}
T8	42.34±0.42^{c}	8.16±0.25^{e}	5.34±0.11^{ef}	3.58±0.22^{ef}
T9	57.25±0.20^{b}	8.24±0.51^{de}	5.65±0.10^{def}	4.23±0.04^{cd}
T10	61.98±0.69^{b}	9.64±0.11^{cd}	6.23±0.09^{cd}	4.76±0.12^{bc}
T11	73.13±2.89^{a}	11.13±0.52^{c}	6.43±0.32^{c}	5.74±0.24^{a}
T12	79.15±3.43^{a}	11.68±0.05^{ab}	8.56±0.03^{b}	5.87±0.32^{a}
T13	32.73±1.80^{d}	8.23±0.36^{de}	2.35±0.06^{h}	1.11±0.02^{h}
SEM	**2.19**	**0.39**	**0.19**	**0.17**
P (0.001)	**0.00**	**0.00**	**0.00**	**0.00**

[a] SEM: Standard Error of Mean. SP: Sprouting Percentage (%); SL: Shoot Length (cm); NL: Number of Leaves (Nos.); LL: Leaf Length (cm) According to the Tukey test, mean values with different superscript (abcdefgh) within a column are significantly different (p < 0.05). The data are expressed as mean±STANDARD Deviation (SD)

Table 2. Effect of IBA concentrations on rooting response of *Callistemon lanceolatus* cuttings under poly tunnel condition

Treatments	RP (%)	RL (Cm)	NR	SP (%)
T1	33.58±1.87^{de}	3.28±0.07^{f}	3.89±0.09^{c}	32.76±0.86^{g}
T2	34.12±0.81^{de}	5.87±0.15d^{e}	3.76±0.09^{c}	48.76±3.21^{de}
T3	37.40±0.03^{d}	6.39±0.23^{d}	4.09±0.27^{bc}	50.23±0.22^{cd}
T4	48.47±2.39^{c}	9.25±0.39^{c}	4.86±0.08^{b}	53.76±3.44^{cd}
T5	50.12±1.97^{c}	10.78±0.78^{b}	5.97±0.35^{a}	61.89±0.37^{b}
T6	73.28±3.87^{a}	12.54±0.00^{a}	6.45±0.36^{a}	69.84±0.65^{a}
T7	30.26±0.75^{e}	3.11±0.10^{f}	3.89±0.17^{c}	31.98±2.09^{g}
T8	36.53±0.04^{de}	4.98±0.24^{e}	3.76±0.01^{c}	35.67±1.40^{fg}
T9	39.18±2.53^{d}	5.23±0.21^{e}	4.09±0.03^{bc}	42.36±0.54^{ef}
T10	50.32±1.00^{c}	8.76±0.22^{c}	4.86±0.19^{b}	49.67±2.68^{cde}
T11	57.39±0.03^{b}	10.65±0.47^{b}	5.97±0.43^{a}	56.45±4.11^{bc}
T12	72.27±1.94^{a}	12.01±0.29^{a}	6.45±0.03^{a}	63.54±0.48^{ab}
T13	20.19±1.27^{f}	1.76±0.02^{g}	1.02±0.01^{d}	20.45±0.20^{h}
SEM	**1.79**	**0.31**	**0.21**	**2.04**
P (0.001)	**0.00**	**0.00**	**0.00**	**0.00**

[a] SEM: Standard Error of Mean. RP: Rooting Percentage (%); RL: Root Length (cm); NR: Number of Roots (Nos.); SP: Survival Percentage (%). According to the Tukey test, mean values with different superscript (abcdefgh) within a column are significantly different (p < 0.05). The data are expressed as mean±Standard Deviation (SD)

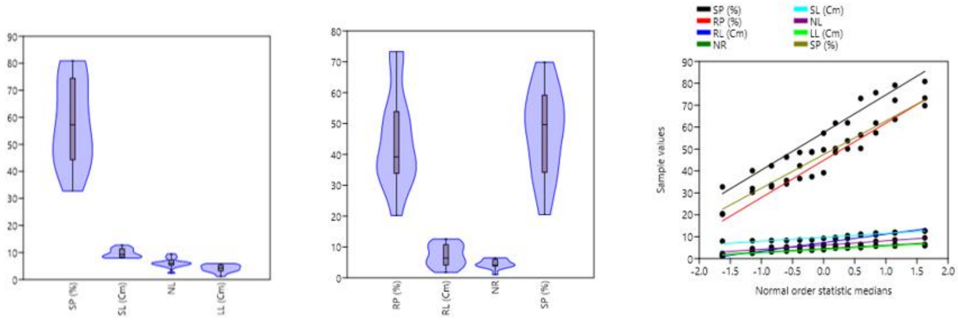

Fig. 1. The data exhibit a normal distribution in relation to the growth parameters

observed in control (Fig. 1). The longest root was noted in IBA 6000ppm, while the shortest root length was observed in control in this experiment. This could be due to the higher accumulation of photosynthates, accumulation of carbohydrates at the base of shoot [44], metabolites, nutrients, season, position of cuttings and under this treatment Husen and Pal, [41], Joel et al. [45] suggested that IBA at 400ppm gives maximum rooting success in *Chrysanthemum* (94.62%). Topacoglu et al. [46], Sevik&Guney [39], Suleiman et al. [47] also observed better rooting percentages with IBA in *Ficus benjamina* (93.90%); *Melissa officinalis* (80.00%); *Capparis spinosa* (79.39%) [48-50].

This violin plot illustartes the distribution of growth parameters among the different concentration of IBA viz., IBA 1000ppm, IBA 2000ppm, IBA 3000ppm, IBA 4000ppm, IBA 5000ppm, IBA 6000ppm. The width of the violin at different concentrations indicates the highest to lowest value of different growth parameters [51,52]. For instance, the wider sections indicate that the maximum growth occur at the IBA treatment [53-55].

The regression line showed that the effects of IBA concentrations on the shoot growth and root response of *Callistemon lanceolatus* cuttings grown in a polytunnel, this plot demonstrated that the highest to lowest values of the growth parameters depend on the percentage of sprouting, shoot length, number of leaves, leaf length, rooting percentage, root length, no. of roots/cutting, and survival percentage [56,57].

5. CONCLUSION

It is concluded that apical shoot cuttings with IBA at 6000ppm can be made to induce maximum rooting and to produce high quality planting stock material for clonal propagation in *Callistemon lanceolatus.*

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Authors have declared that no generative AI technologies such as large language models (ChatGPT, COPILOT, etc.) or text-to-image generators have been used during the writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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