

Impact of Various IBA concentrations on Rooting in Apical Shoot Cutting and stem cuttings of *Callistemon lanceolatus* (Sm.) Sweet in Poly tunnel Conditions

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 poly tunnel 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

Introduction

Callistemon lanceolatus, which belongs to the Myrtaceae family, native to Australia, is widely distributed in subtropical and tropical regions (Sowndhararajan *et al.*, 2021). This tree, characterized by its striking bright red flowers resembling bottlebrushes, is commonly cultivated in gardens throughout India as an ornamental plant (Kumar *et al.*, 2020). Referred to as the "lemon bottlebrush" due to its distinctive cylindrical brush-like red flowers (Singh *et al.*, 2020), this plant is known to possess various biological activities in its aerial parts, including antimicrobial (Nazreen *et al.*, 2020), antioxidant, antidiabetic (Ahmad *et al.*, 2018; Kumar *et al.*, 2011), anti-inflammatory (Kumar *et al.*, 2020), and anti-proliferative (Park *et al.*, 2018) properties. Notably, essential oils extracted from the leaves of *C. lanceolatus* exhibit antimicrobial and anti-inflammatory properties (Shukla *et al.*, 2012; Sudhakar *et al.*, 2004).

The propagation of ornamental plants has traditionally relied on seed germination and vegetative propagation techniques (Hartman *et al.*, 2002; Shokri, 2012). However, these conventional methods come with constraints related to time, labor, and genetic fidelity (Singh *et al.* 2013). Seed germination can be unpredictable and time-consuming, resulting in a wide range of progeny diversity due to genetic recombination (Müller-Starck *et al.*, 2005; Acquah, 2015). To address these challenges and meet the increasing demand for genetically identical, high-quality plant material, clonal propagation techniques have gained prominence (Muchugi *et al.*, 2023). 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 (Gradziel, 2012; Cardoso *et al.* 2023). Some species

necessitate vegetative propagation for short-term regeneration (Leakey, 2004; Mei *et al.*, 2023). 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 (Ciriello and Mori, 2015). However, the utilization of spontaneous or induced basal sprouts has yielded excellent rooting indexes, resulting in enhanced efficiency in vegetative proliferation (Rickli *et al.*, 2015). Clonal propagation is a simpler, quicker and more competent way to rapidly expand millions of cuttings from elite germplasms than seed propagation (Gonin *et al.*, 2019; Solgi *et al.*, 2022).

A special problem with *Callistemon 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, 2008; Shokri *et al.*, 2012; Parthiban *et al.*, 2020).

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 *et 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.*, 2002; Dominik and Gregor, 2007; Shokri, 2012). 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.*, 2012; Cirillo *et al.*, 2016; Chauhan *et al.*, 2017; Parthiban *et al.*, 2020).

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.

Materials and Methods

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%.

Experimental material: 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 Schwambach *et al.* (2008); Wendling *et al.* (2010). 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 (Packialakshmi and Sudhagar, 2019). 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 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 a 15 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.

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 (Table 1).

Table 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

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.

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, 2019; Smitha and Umesha, 2012 and Husen, 2011). The results of the study revealed that the maximum sprouting percent of *C. Lanceolatus* was observed in T₆ (80.87±0.86%), T₁₂ (79.15±3.43%), T₅ (75.73±4.53%), T₁₁ (73.13±2.89%) whereas the minimum sprouting percentage was noticed in T₁₃ (32.73±1.80%) (Table 1 & Fig 1). The study reported that the highest shoot length of *C. lanceolatus* was recorded in T₆ (12.72±0.73cm) followed by T₁₂ (11.68±0.05cm), T₅ (11.65±0.17cm) whereas the lowest was reported in T₁₃ as (8.23±0.36cm) (Table 2). The highest number of leaves per cutting was observed in T₆ as 9.45±0.26 followed by T₁₂ (8.56±0.03) and the lowest was reported in T₁₃ (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 T₁₂ (5.87±0.32cm), T₁₁ (5.74±0.24cm), T₆ (5.67±0.27cm) while the smallest leaf was noticed in T₁₃ (1.11±0.02cm).

Rooting efficiency

The results of the study depicted that the maximum rooting percentage was observed in T₆ (73.28±8.87%) and T₁₂ (72.27±1.97%), followed by T₁₁ (57.39±0.03%), while the minimum percentage was noticed in T₁₃ (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 T₁₂ (6.45±0.03), T₆ (6.45±0.36) whereas the minimum was recorded in T₁₃ (1.02±0.01). with regard to survival percentage the highest was reported in T₆ (69.84±0.65%) followed by T₁₂ (63.54±0.48%) and the lowest was registered in T₁₃ (20.45±0.20%) (Fig 1).

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 (Dirr & Heuser, 2006; Hartmann *et al.*, 2011). 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.*, 2013; Rahdari *et al.*, 2014; Costa *et al.*, 2015). 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 (Dirr & Heuser, 2006).

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 (Yusnita *et al.*, 2017; Husen & Pal, 2006).

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% (Kumar *et al.*, 2015); *Calliandra haematocephala* as 53.33% (Kaur and Grewal, 2017). 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.* (2017), *Syzygium malaccense* treated with 4000 ppm IBA grew by 3.30 cm; Sevik & Guney (2013) found that *Melissa officinalis* treated with 1000 ppm IBA grew by 6.71 cm; and Yeshiwas *et al.* (2015) 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, (2007); Guleria and Vashisht (2014), 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 (T₁₃) 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 (Sarkar *et al.*, 2023). 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 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 (Hartmann *et al.* 1997), metabolites, nutrients, season, position of cuttings and under this treatment (Husen and Pal, 2007). Joel *et al.* (2023) suggested that IBA at 400ppm gives maximum rooting success in *Chrysanthemum* (94.62%). Topacoglu *et al.* (2016); Sevik&Guney (2013); Suleiman *et al.* (2012) also observed better rooting percentages with IBA in *Ficus benjamina* (93.90%); *Melissa officinalis* (80.00%); *Capparis spinosa* (79.39%).

Table1.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 ^{def}	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±0.42 ^{cd}	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 \pm Standard deviation (SD).

Table2. Effect of IBA concentrations on rooting response of *Callistemon lanceolatus* cuttings under poly tunnel condition

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

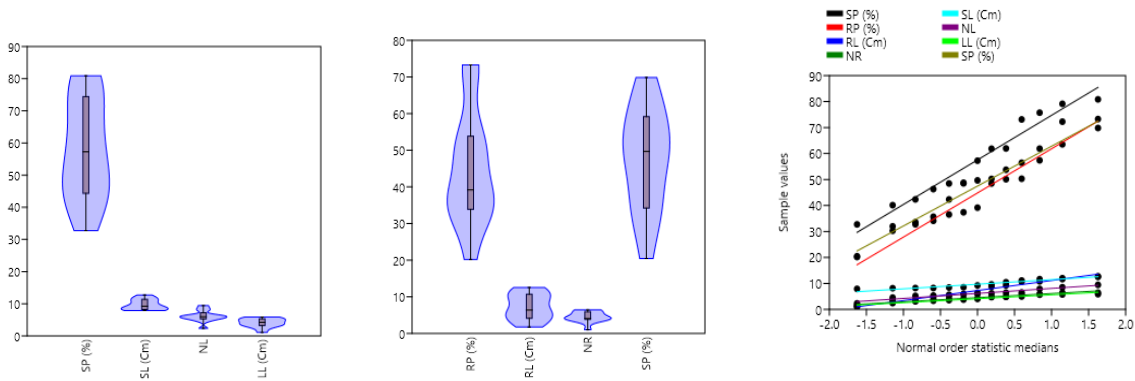


Figure 1. The data exhibit a normal distribution in relation to the growth parameters.

This violin plot illustrates the distribution of growth parameters among the different concentrations 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. For instance, the wider sections indicate that the maximum growth occurs at the IBA treatment.

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.

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*.

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- 1.

- 2.
- 3.

References

1. Abu-Zahra, T. R., Al-Shadaideh, A. N., Abubaker, S. M., & Qrunfleh, I. M. (2013). Influence of auxin concentrations on different ornamental plants rooting. *International Journal of Botany*, 9(2): 96-99.
2. Acquaah, G. (2015). Conventional plant breeding principles and techniques. *Advances in plant breeding strategies: Breeding, biotechnology and molecular tools*, 115-158.
3. Ahmad, K., Hafeez, Z. B., Bhat, A. R., Rizvi, M. A., Thakur, S. C., Azam, A., & Athar, F. (2018). Antioxidant and apoptotic effects of *Callistemon lanceolatus* leaves and their compounds against human cancer cells. *Biomedicine & Pharmacotherapy*, 106: 1195-1209.

4. Brophy, J. J., Craven, L. A., & Doran, J. C. (2013). *Melaleucas: their botany, essential oils and uses*. Australian Centre for International Agricultural Research (ACIAR): 415pp.
5. Cardoso, J. C., Vilcherrez-Atoche, J. A., Iiyama, C. M., Germanà, M. A., & Vendrame, W. A. (2023): *Breeding of Orchids Using Conventional and Biotechnological Methods: Advances and Future Prospects*. *Advances in Orchid Biology, Biotechnology and Omics*, 27-58.
6. Ciriello, E., and Mori, E. S. (2015). *Rooting of Guanandi (Calophyllum brasiliense CAMBESS) cuttings using Indole-butyric acid*. *Cerne*, 21: 641-648.
7. Costa, E. M., Loss, A., Pereira, H. P. N., & Almeida, J. F. (2015). *Rooting of Bougainvillea spectabilis Willd. stem cuttings using indolbutyric acid*. *Acta Agronomica*, 64(3): 205-210.
8. Dirr, M. A., & Heuser, Jr., C. W. (2006). *The reference manual of woody plant propagation: From seed to tissue culture (2nd ed.)*. Cary, North Carolina: Varsity Press, Inc.
9. Gonin, M., Bergougnot, V., Nguyen, T. D., Gantet, P., & Champion, A. (2019). *What makes adventitious roots*. *Plants*, 8(7): 240.
10. Gradziel, T. M. (2012). *Traditional genetics and breeding*. *Genetics, genomics and breeding of crop plants*, CRC Press, Boca Raton, 22-54.
11. Guleria, V., & Vashisht, A. (2014). *Rejuvenation and adventitious rooting in shoot cuttings of Tectona grandis under protected conditions in new locality of Western Himalayas*. *Universal Journal of Plant Science*, 2(6), 103-1006.
12. Hartmann HT, Kester DE, Davies JR FT, Geneve RL. 2011. *Plant propagation: principles and practices*. 8th Edition. Prentice-Hall, São Paulo, p. 915.

13. Hartman HT, Kester DE, Davies FT, Genev RL (2002). Plant Propagation, Principles and Practices. 7th Edn. Prentice Hall, Englewood Cliffs, New Jersey, USA.
14. Hartmann, H. T., Kester, D. E., Davies, F. T., & Geneve, R. L. (1997). Plant propagation: principles and practices.
15. Husen, A. (2011). Rejuvenation and adventitious rooting in coppice-shoot cuttings of *Tectona grandis* as affected by stock-plant etiolation. *American Journal of Plant Sciences*, 2(3), 370-374.
16. Husen, A., & Pal, M. (2006). Variation in shoot anatomy and rooting behaviour of stem cuttings in relation to age of donor plants in teak (*Tectona grandis* Linn. f.). *New Forests*, 31:57-73.
17. Husen, A., & Pal, M. (2007). Effect of branch position and auxin treatment on clonal propagation of *Tectona grandis* Linn. f. *New Forests*, 34, 223-233.
18. Joel, P. G., Bala, M., Kaur, A., & Dubey, R. K. (2023). Efficacy of rooting hormones on propagation and rooting potential of Japanese chrysanthemums (*Chrysanthemum morifolium* Ramat.). *Rhizosphere*, 27: 100740.
19. Kaur, R., & Grewal, H. S. (2017). Effect of growth regulator treatment on bud sprouting of hardwood cutting in different ornamental plants. *Journal of Applied and Natural Science*, 9(4): 2068-2072.
20. Kumar, R., Gupta, A., Singh, A. K., Bishayee, A., & Pandey, A. K. (2020). The antioxidant and antihyperglycemic activities of bottlebrush plant (*Callistemon lanceolatus*) stem extracts. *Medicines*, 7(3): 11.
21. Kumar, S., Kumar, V., & Prakash, O. (2011). Antidiabetic, hypolipidemic, and antioxidant activities of *Callistemon lanceolatus* leaves extract. *Journal of Herbs, Spices & Medicinal Plants*, 17(2): 144-153.

22. Kumar, V., Singh, M. K., Kumar, M., Prakash, S., Kumar, A., Rao, S., & Malik, S. (2015). Effect of different doses of IBA and rooting media on rooting of stem cutting of lemon (*Citrus limon* Burm) cv. Pant lemon-1. *Journal of Plant Development Sciences*, 7(7): 587-591.
23. Lamont, B. B., Pausas, J. G., He, T., Witkowski, E. T., & Hanley, M. E. (2020). Fire as a selective agent for both serotiny and nonserotiny over space and time. *Critical Reviews in Plant Sciences*, 39(2), 140-172.
24. Leakey, R. R. (2004). *Physiology of vegetative reproduction*. Academic Press.
25. Mei, G., Chen, A., Wang, Y., Li, S., Wu, M., Liu, X., & Hou, X. (2023). A simple and efficient in planta transformation method based on the active regeneration capacity of plants. *bioRxiv*, 2023-01.
26. Muchugi, A., Kalinganire, A., Chege, J., & Nyoka, I. (2023). **Commodity tree crop planting material infrastructures in Africa.**
27. Müller-Starck, G., Ziehe, M., & Schubert, R. (2005). Genetic diversity parameters associated with variability selection, reproductive efficiency, and growth in forest tree species. In *Forest diversity and function: Temperate and boreal systems* (pp. 87-108). Berlin, Heidelberg: Springer Berlin Heidelberg.
28. Nazreen, S., Mahboob Alam, M., Hamid, H., Ali, M., & Sarwar Alam, M. (2020). Chemical constituents with antimicrobial and antioxidant activity from the aerial parts of *Callistemon lanceolatus* (Sm.) Sweet. *Natural product research*, 34(22): 3275-3279.
29. Packialakshmi, M., & Sudhagar, R. J. (2019). Standardization of rooting hormone in mini clonal technology of *Tectona grandis* Linn. f. *International Journal of chemical studies*, 7(3), 4398-4401.

30. Park, S., Lim, W., Jeong, W., Bazer, F. W., Lee, D., & Song, G. (2018). Sideroxylin (*Callistemon lanceolatus*) suppressed cell proliferation and increased apoptosis in ovarian cancer cells accompanied by mitochondrial dysfunction, the generation of reactive oxygen species, and an increase of lipid peroxidation. *Journal of Cellular Physiology*, 233(11): 8597-8604.
31. Parthiban K. T. and Seenivasan. R (2017)a. A complete value chain approach. Scientific publisher. Jodhpur, p629.
32. Parthiban, K. T., Krishnakumar, N., & Devanand, P. S. (2020). *Tree breeding and improvement theory and technology*. Scientific Publishers.
33. Parthiban, K. T., Palanikumar, B., & Krishnakumar, N. (2017)b. Clonal Evaluation, Variability and Association Studies in *Casuarina junghuhniana*. Plantation and Agroforestry Pulpwood Value Chain Approach, 194.
34. Rahdari, P., Khosroabadi, M., Delfani, K., & Hoseini, S. M. (2014). Effect of different concentration of plant hormones (IBA and NAA) on rooting and growth factors in root and stem cuttings of *Cordyline terminalis*. *Journal of Medical and Bioengineering Vol*, 3(3).
- 35. Rickli, H.C., Bona, C., Wendling, I., Koehler, H.S., Zuffellato-Ribas, K.C. (2015). Origin of epicormic shoots and application of indolylbutyric acid in the rooting of *Vochysia bifalcata* Warm cuttings. *Forest science*, 25: 385-393.**
36. Sarkar, R., Mukherjee, S., Pradhan, B., Chatterjee, G., Goswami, R., Ali, M. N., & Ray, S. S. (2023). Molecular characterization of vermicompost-derived IAA-releasing bacterial isolates and assessment of their impact on the root improvement of banana during primary hardening. *World Journal of Microbiology and Biotechnology*, 39(12), 351.

37. Schwambach, J., Ruedell, C. M., de Almeida, M. R., Penchel, R. M., de Araújo, E. F., & Fett-Neto, A. G. (2008). Adventitious rooting of *Eucalyptus globulus* × *maidennii* mini-cuttings derived from mini-stumps grown in sand bed and intermittent flooding trays: a comparative study. *New Forests*, 36: 261-271.
38. Sevik, H., & Guney, K. (2013). Effects of IAA, IBA, NAA, and GA3 on rooting and morphological features of *Melissa officinalis* L. stem cuttings. *The Scientific World Journal*.
39. Shokri, S., Zarei, H., & Alizadeh, M. (2012). Evaluation of rooting response of stem cuttings and in vitro micro-cuttings of bottlebrush tree (*Callistemon viminalis*) for commercial mass propagation. *Journal of Agricultural Research*, 1(10): 424-428.
40. Shukla, R., Singh, P., Prakash, B., & Dubey, N. K. (2012). Antifungal, aflatoxin inhibition and antioxidant activity of *Callistemon lanceolatus* (Sm.) Sweet essential oil and its major component 1, 8-cineole against fungal isolates from chickpea seeds. *Food Control*, 25(1): 27-33.
41. Singh, P. P., Kumar, A., & Prakash, B. (2020). Elucidation of antifungal toxicity of *Callistemon lanceolatus* essential oil encapsulated in chitosan nanogel against *Aspergillus flavus* using biochemical and in-silico approaches. *Food Additives & Contaminants: Part A*, 37(9): 1520-1530.
42. Singh, S. R., Singh, R., Kalia, S., Dalal, S., Dhawan, A. K., & Kalia, R. K. (2013). Limitations, progress and prospects of application of biotechnological tools in improvement of bamboo—a plant with extraordinary qualities. *Physiology and Molecular Biology of Plants*, 19, 21-41.
43. Smitha, G. R., & Umesha, K. (2012). Vegetative propagation of Stevia [*Stevia rebaudiana* (Bertoni) Hemsl.] through stem cuttings. *Journal of Tropical Agriculture*, 50(1), 72-75.

44. Solgi, M., & Sahraei, F. (2022). Influence of cutting lengths and IBA on propagation of red willow ornamental-medicinal plant by stem cutting. *European Journal of Horticultural Science*, 87(2): 1-7.
45. Sowndhararajan, K., Deepa, P., & Kim, S. (2021). A review of the chemical composition and biological activities of *Callistemon lanceolatus* (Sm.) Sweet. *Journal of Applied Pharmaceutical Science*, 11(12): 065-073.
46. Sudhakar, M., Rao, C. V., Rao, A. L., Ramesh, A., Srinivas, N., Raju, D. B., & Murthy, B. K. (2004). Antinociceptive and anti-inflammatory effects of the standardized oil of Indian *Callistemon lanceolatus* leaves in experimental animals. *East and Central African Journal of Pharmaceutical Sciences*, 7(1), 10-15.
47. Suleiman Awwad, M. (2012). An application of the American Customer Satisfaction Index (ACSI) in the Jordanian mobile phone sector. *The TQM Journal*, 24(6): 529-541.
48. Topacoglu, O., Sevik, H., Guney, K., Unal, C., Akkuzu, E., & Sivacioglu, A. (2016). Effect of rooting hormones on the rooting capability of *Ficus benjamina* L. cuttings. *Šumarski list*, 140(1-2): 39-44.
49. Valsalakumari, P. K. (2008). *Flowering trees* (Vol. 12). New India Publishing.
50. Wen, D., Gong, B., Sun, S., Liu, S., Wang, X., Wei, M., ... & Shi, Q. (2016). Promoting roles of melatonin in adventitious root development of *Solanum lycopersicum* L. by regulating auxin and nitric oxide signaling. *Frontiers in plant science*, 7: 718.
51. Wendling, I., Brondani, G. E., Dutra, L. F., & Hansel, F. A. (2010). Mini-cuttings technique: a new ex vitro method for clonal propagation of sweetgum. *New Forests*, 39: 343-353.

52. Yeshiwas, T., Alemayehu, M., & Alemayehu, G. (2015). Effects of indole butyric acid (IBA) and stem cuttings on growth of stenting-Propagated rose in Bahir Dar, Ethiopia. *World Journal of Agricultural Sciences*, 11(4), 191-197.
53. Yusnita, Y., Jamaludin, J., Agustiansyah, A., & Hapsoro, D. (2017). A combination of IBA and NAA resulted in better rooting and shoot sprouting than single auxin on Malay apple [*Syzygium malaccense* (L.) Merr. & Perry] stem cuttings. *AGRIVITA, Journal of Agricultural Science*, 40(1): 80-90.
54. . Afzal, Muhammad, Hafsa Naeem, Muhammad Waqas, Isbah Akhtar, Razaullah, Imranullah, Sabeen Alam, Monaza Tehseen, and Sanaullah. 2023. "Effect of Indole Butyric Acid and Zinc Sulphate in Different Media on Rooting of Olive Cuttings". *Asian Journal of Soil Science and Plant Nutrition* 9 (4):104-11. <https://doi.org/10.9734/ajssp/2023/v9i4196>.
55. Guchhait, Pritam, Suraj Varma, Dhrubajyoti Banerjee, Sandeep Kumar, Rajdip Halder, and Ankit Dahiya. 2024. "Plant Growth Regulators and Rooting Media: A Viable Approach for Growth and Performance of Citrus". *Journal of Experimental Agriculture International* 46 (5):366-78. <https://doi.org/10.9734/jeai/2024/v46i52387>.
56. Wiesman Z, Markus A, Wybraniec S, Schwartz L, Wolf D. Promotion of rooting and development of cuttings by plant growth factors formulated into a controlled-release system. *Biology and fertility of soils*. 2002 Nov;36:330-4.
57. Wiesman Z, Lavee S. Enhancement of IBA stimulatory effect on rooting of olive cultivar stem cuttings. *Scientia Horticulturae*. 1995 May 1;62(3):189-98.