Effect of different concentrations of IBA on rooting and growth factors in apical shoot cuttings of *Callistemon lanceolatus* under polytunnel condition

Abstract. The study was undertaken to standardize the optimal concentration of rooting hormone 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. Thirteen different treatments were tried *viz.*, T_1- Apical shoot cuttings with IAB at 1000 ppm; T_2- Apical shoot cuttings with IAB at 2000 ppm; T_3- Apical shoot cuttings with IAB at 3000 ppm; T_4- Apical shoot cuttings with IAB at 4000 ppm; T_5- Apical shoot cuttings with IAB at 5000 ppm; T_6- Apical shoot cuttings with IAB at 6000 ppm; T_7- stem cuttings with IAB at 1000 ppm; T_8- stem cuttings with IAB at 2000 ppm; T_9- stem cuttings with IAB at 3000 ppm; T_{10}- stem cuttings with IAB at 4000 ppm; T_{11}- stem cuttings with IAB at 5000 ppm; T_{12}- stem cuttings with IAB at 6000 ppm; T_{13}- control (treated with distilled water). The studies were conducted in a completely randomized block design (CRD). The results of the study depicted that the maximum rooting percentage and root length were observed in T_6 (73.28±8.87%) and (12.54±0.00cm) due to the exogenous application of auxin.

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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*., 2011), 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; Acquaah, 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). Notably, some tropical trees have demonstrated the potential of

vegetative propagation, which improves yield potential by reducing variation among the ramets (Parthiban *et al*., 1999). This progress in vegetative propagation has evolved into clonal technology, emerging as an alternative to traditional seed-based propagation (Parthiban and Seenivasan, 2017). 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). Compared to seed propagation, clonal propagation is a simpler, faster, and more efficient way to rapidly expand millions of cuttings from elite germplasms (Gonin *et al*., 2019; Solgi *et al*., 2022).

Callistemon lanceolatus trees pose a unique challenge as they require a substantial amount of time for seed release, ranging from one year to several years, with some even requiring fire to activate the capsules. 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.

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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 (Zarinbal *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 imperative for mass propagation of *C. lanceolatus* through cloning has emerged as a significant priority in the realms of horticulture and conservation. 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.

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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 size 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 screened for desired length (8- 10cm) by using scale) and diameter (2-4 mm) by using a calibrated vernier calliper following which they were placed in

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ice box to prevent damage during transportation to laboratory. The cuttings were treated with an aqueous solution of 0.1% Bavistin for 15 minutes and subsequently washed with distilled water (Sabari, 2017). 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 which served as a control). 15 cuttings were prepared following the above procedure in each treatment with 3 replications.

After auxin treatment, the cuttings were planted in the polybags containing coir pith as a rooting medium. One third of the basal cut portion was inserted in different rooting mediums. The medium has to be kept damp and the surface even. Holes should be punched into the medium to allow the insertion of the cuttings without damaging the cambium or removing the rooting hormone. 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 a shade house for 30 days.

Experimental Design and Data Collection

Thirteen different treatments were tried *viz.*, T_1- Apical shoot cuttings with IAB at 1000 ppm; T_2- Apical shoot cuttings with IAB at 2000 ppm; T_3- Apical shoot cuttings with IAB at 3000 ppm; T_4- Apical shoot cuttings with IAB at 4000 ppm; T_5- Apical shoot cuttings with IAB at 5000 ppm; T_6- Apical shoot cuttings with IAB at 6000 ppm; T_7- stem cuttings with IAB at 1000 ppm; T_8- stem cuttings with IAB at 2000 ppm; T_9- stem cuttings with IAB at 3000 ppm; T_{10}- stem cuttings with IAB at 4000 ppm; T_{11}- stem cuttings with IAB at 5000 ppm; T_{12}- stem cuttings with IAB at 6000 ppm; T_{13}- control (treated with distilled water). The studies were conducted in a completely randomized block design (CRD) with three replications and 15 cuttings per treatment per replication.

The biometric parameters of epicormic shoots such as sprouting per cent, rooting per cent, root length, number of roots per cutting, shoot length (cm), number of leaves, leaf length (cm), leaf width (cm), survival per cent 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. The results of the study revealed that the maximum sprouting percent of *C. lanceolatus* was observed in T_6 (80.87±0.86%), T_{12} (79.15±3.43%), T_5 (75.73±4.53%), T_{11} (73.13±2.89%) whereas the minimum sprouting per cent was noticed in T_{13} (32.73±1.80%) (Table 1). Shoot length refers to the measurement of the length of the above-ground part of a plant, specifically the stem or shoots. It is a common parameter used in plant biology and agriculture to assess the growth and development of plants. Measuring shoot length can provide valuable information about a plant's response to various environmental factors, such as light, water, and nutrients, and can be an important indicator of overall plant health and productivity. 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 number of leaves per cutting can vary widely depending on the plant species, the size of the cutting, and the specific growth conditions. Some cuttings may have only a few leaves, while others can have several leaves. It's essential to consider the type of plant and its

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specific requirements when propagating through cuttings, as the number of leaves can influence the success of propagation. 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) whereas 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).

Rooting efficiency

Rooting percentage often referred to as "rooting success" or "rooting efficiency," is a crucial metric in horticulture and plant propagation. It measures the success rate of stem cuttings developing roots, a key step in clonal propagation. Rooting percentage is the proportion of stem cuttings that successfully form roots compared to the total number of cuttings used. 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. 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 per cent 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 4-&5).

Discussion

Plant propagation by cuttings has several advantages including maintenance of the specific characteristics of the plant from which the cutting was collected, giving true-to-type plant materials, being simple and easy to conduct, it does not need a large space and being a cheaper propagation method compared to layering, grafting or budding. 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&2). 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

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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 current study was consistent with the findings of Sevik & Guney (2013) in *Melissa officinalis* as 6.71cm when treated with IBA at 1000ppm. The study is consistent with the findings of Yusnita *et al*. (2017) in *Syzygium malaccense* as 3.30cm treated with IBA at 4000ppm; Yeshiwas *et al*. (2015) in rose stem cuttings treated with IBA at 2500ppm (15.47cm). 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. Although, growth regulators significantly influenced the shoot length and maximum number of leaves per cutting it was observed that the maximum was at IBA6000ppm. This may be due to the vigorous root system which increased nutrients uptake under this treatment. It is in accordance with the findings of Yusnita *et al*. (2017) in *Syzygium malaccense* as and it was lower than the earlier reports of Yeshiwas *et al*. (2015) in rose stem cuttings as 58.50; Damar *et al*. (2014) in *Punica granatum* treated with IBA at 2000ppm (37.77). Poor performance of cuttings as regards the percentage of success and number of leaves per shoot and per cuttings were found to be under control (T_{13}). 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 may 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 per cent, root length, number of roots per cutting, the maximum value in IBA at 6000 ppm. Whereas, minimum rooting per cent, root length, number of roots per cutting were observed in control (Fig. 3). The longest root was noted in IBA 6000ppm, while the shortest root was observed in control in this experiment. This may be due to the higher accumulation of photosynthates, metabolites and nutrients under this treatment. 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%).

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Table1. Effect of IBA concentrations on shoot growth response of *Callistemon lanceolatus* cuttings under poly tunnel condition

Treatments	SP (%)	SL (Cm)	NL	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}
MSE	**2.19**	**0.39**	**0.19**	**0.17**
P (0.001)	0.00	0.00	0.00	0.00

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Write in the text whether or not there were significant differences between treatments and comment on what this means.

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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±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]
MSE	**1.79**	**0.31**	**0.21**	**2.04**
P (0.001)	0.00	0.00	0.00	0.00

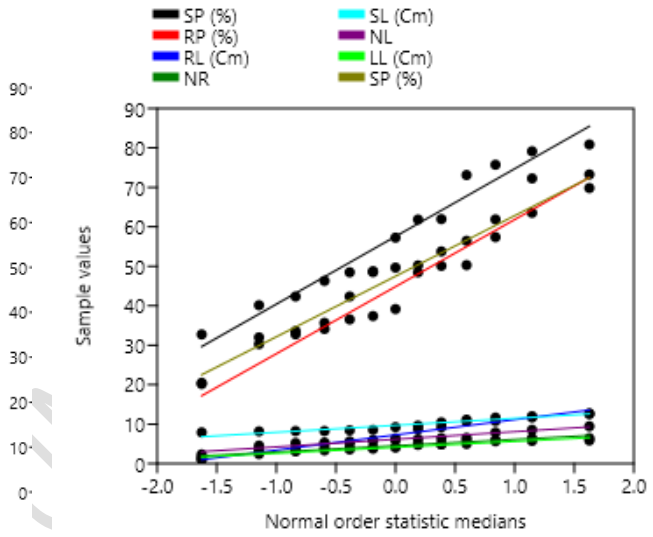

Figure 1. *The data exhibit a normal distribution in relation to the growth parameters.*

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Figure 2. Shoot growth response of *Callistemon lanceolatus* cuttings under poly tunnel condition

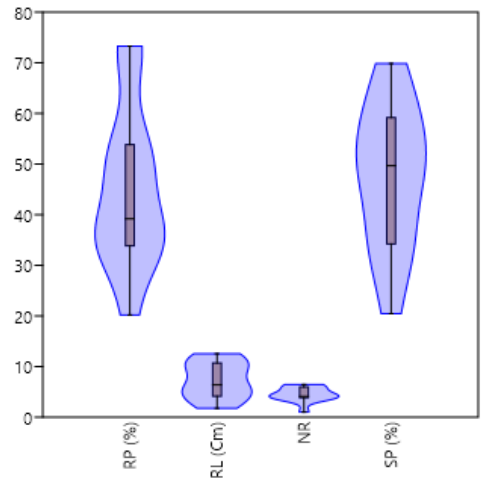

Figure 3. Rooting response of *Callistemon lanceolatus* cuttings under poly tunnel condition

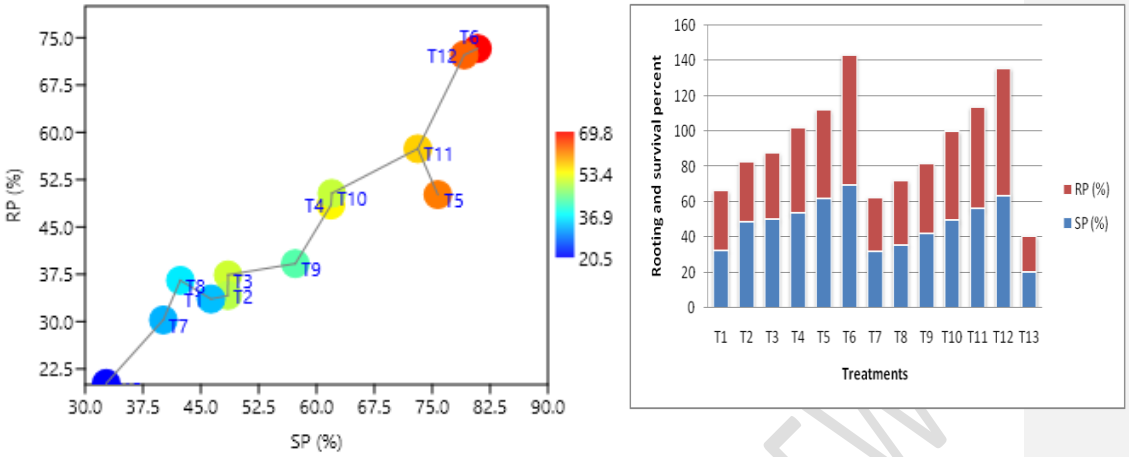

Figure 4. The rooting percentage of both apical shoot cuttings and stem cuttings is directly correlated with the survival percentage of the cuttings.

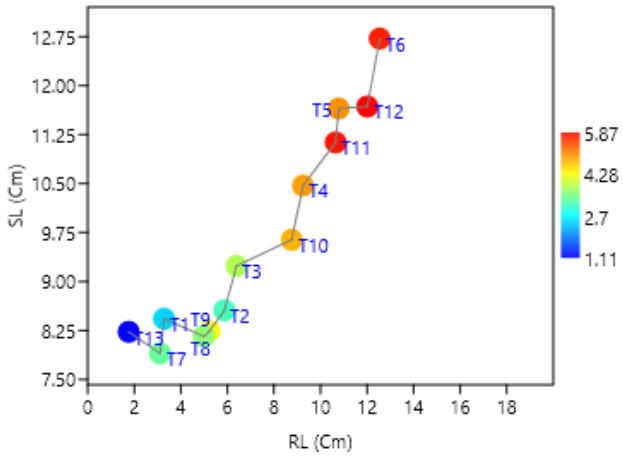

Figure 5. The rooting performance of the cuttings positively correlates with the shoot growth performance of the cuttings.

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