Standardization of vegetative propagation of fodder calliandra (*Calliandra calothyrsus)* using coppice cuttings in humid tropics of south India

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ABSTRACT: *Calliandra calothyrsus Meissn. is a leguminous fodder tree that has recently become the subject of intensive study and cultivation due to its protein-rich foliage and other uses including the provision of green manure, fuelwood, shade or support for perennial crops, land rehabilitation and erosion control, and the production of honey or shellac. Calliandra is usually propagated through seeds, however, the seed set is very poor under humid tropical conditions. Calliandra cannot be easily multiplied by planting stakes directly in the ground, and detailed studies on vegetative propagation are absent in this species. The present investigation was carried out at the College of Forestry, Vellanikkara to standardize a protocol for large-scale planting stock production of fodder calliandra from coppice cuttings, by assessing the influence of types of cuttings (softwood/semi-hardwood), type and concentration of growth regulators like IBA (Indole-3-butyric acid) and NAA (Naphthaleneacetic acid) (0, 50, 100, 200 and 250 mg L^{-1}); and the influence of season (rainy and summer) on the root and shoot development in vegetatively propagated calliandra. Two separate trials were carried out in both seasons with 19 treatments for each trial laid out in CRD (Completely Randomized Design), and one absolute control viz., calliandra planting stock raised from seeds, for relative comparison. Results indicate that calliandra can be vegetatively propagated from softwood coppice cuttings by overnight soaking with 50 ppm of NAA and using vermiculite as a rooting medium under controlled conditions of a mist chamber, preferably during the summer season. 50 ppm IBA was found to be the second-best treatment. The shoot and root parameters of vegetatively propagated calliandra planting stock were found to be on par with that of calliandra seedlings. Non-mist propagation method and soil-based rooting medium were found to be unsuccessful for the vegetative propagation of calliandra in humid tropical conditions of South India.*

1. INTRODUCTION

Livestock farming is a major means of subsidiary income for small farmers and agricultural labourers in the humid tropical conditions of South India. However, the farmers face acute shortages of feed and fodder, which hinder the development of livestock sectors due to enormous feed costs. Kerala's efforts to increase milk production were severely hampered by the inadequate production of fodder. According to studies conducted in Kerala, a 60:40 roughage-to-concentrate ratio is best for cattle and this percentage of concentrate is presently greater than 60% in Kerala. It is estimated that a humid tropical state like Kerala cultivates just over 2000 hectares of fodder, which produces only 46 per cent of the fodder requirements based on a 60:40 ratio and the remaining is met through purchased commercial feeds leading to less profit for farmers. An estimated 13000 hectares of fodder cultivation is at least required for Kerala to meet its roughage production target (Kerala Development Report, 2021).

Hence, in recent years, there has been a growing interest among farmers to cultivate protein-rich fodder trees as an alternative source of cheap and quality fodder against expensive commercial concentrates. Among fodder trees, calliandra (*Calliandra calothyrsus*), a leguminous fodder tree, is gaining popularity in Kerala owing to its protein-rich foliage, fast growth, higher biomass production and excellent adaptability to humid tropical climatic conditions and acidic soils of Kerala (Sagaran, 2017). The annual forage yield of calliandra is reported to be in the range of 7-10 t/ha dry matter (Ella *et al.*, 1989). Wiersum and Rika (1992) reported 22 per cent crude protein content, 30-70 per cent fibre content, 4-5 per cent ash and 2-3 per cent fat in dried leaves of calliandra. When calliandra was used as a supplement to cows (6 kg fresh leaves/day) to an existing basal diet of 2 kg dairy meal, it was observed that in addition to obtaining increased income, there was an improvement in cow's

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condition and improved butter content in the milk (Chakeredza *et al.,* 2007). Calliandra have relatively high protein (18.45 %), minerals, ether extract and energy content and thus have the potential to be a good alternate feed source for ruminant animals (Jayaprakash *et al.,* 2016).

It can withstand heavy pruning and has a good coppicing ability, which ensures a frequent and year-round supply of fodder, with proper management. Moreover, the tree exhibits good shade tolerance and can be integrated as fodder banks in the existing cropping systems of Kerala such as coconut gardens and homesteads as an agroforestry component (Sagaran, 2017; Patrick *et al.,* 2020).

The availability of quality planting material is an important factor that influences the large-scale adoption of a particular tree species. Calliandra is usually propagated through seeds, however, the seed set is very poor and normally restricted to some localized regions under higher altitudes during the winter season. In Kerala, calliandra seed production is confined to high ranges of the Idukki district over a short span from January to March, which is insufficient to meet the rising demand. In this context, vegetative propagation techniques offer the opportunity to produce a reliable and year-round supply of planting stock locally, for large-scale adoption in farmer fields. Calliandra cannot be easily multiplied by planting stakes directly in the ground, and a few low-cost vegetative propagation techniques have been attempted by other researchers.

Calliandra is a species with a relatively high rooting potential from coppice cuttings (Tchigio and Duguma, 1998) and cuttings from 5-month-old seedlings (Dick *et al*., 1996). Successful propagation by stem cuttings depends on factors such as the propagation environment, propagation medium, the type and concentration of auxins applied, the origin of the cuttings and the physiological state of the stock plant. Zeng *et al*. (2005) stated that there are positive responses of growth-regulating substances such as NAA and IBA on the rooting performance of cuttings. The root-promoting effect varied with auxin concentrations and types of auxins. However, detailed studies on vegetative propagation are absent in this species and especially under humid tropical conditions of Kerala. With this background, the present study is proposed to standardize a protocol for large-scale planting stock production of fodder calliandra from coppice cuttings, by assessing the influence of types of cuttings, type and concentration of growth regulators like IBA and NAA; and the influence of season on the root and shoot development in vegetatively propagated calliandra.

2. MATERIALS AND METHOD

The present study was conducted at the College of Forestry, Kerala Agricultural University, Vellanikkara, Thrissur district, Kerala located at latitude 10^0 32'N and longitude 76^0 26'E at an elevation of about 22 m above mean sea level. The study area experienced a warm humid tropical climate, having mean weekly minimum and maximum temperatures ranging from 22.6°C to 25.1°C and 29.3°C to 39.9°C respectively during the 2021-2022 planting season.

Rainy and summer season trials

The proposed nursery study was conducted as two separate trials during the rainy period (June 2021 to December 2021) and dry period (January 2022 to August 2022). An existing 4-year-old calliandra plantation was cut back at 30 cm height from the ground during June 2021 (rainy season trial) and during October 2021 (dry season trial) and allowed to coppice. Two-month-old coppice shoots were taken for vegetative propagation during both seasons.

Three- nodded softwood cuttings and semi hardwood having a length of 10-15 cm with 2 pairs of leaves intact were prepared from the apex and basal portions of the coppice shoot respectively. In order to minimize the transpiration rate, the leaf area was trimmed to half, retaining the apical bud intact (Hrideek *et al.*, 2019). To prevent fungal attack, cuttings were treated with 0.2% aqueous solution of bavistin for 30 minutes. The cuttings were treated with the solutions of two plant growth regulators NAA or IBA in different concentrations *i.e.*, 0, 50, 100, 200 and 250 mg L^{-1} overnight and kept in soil-based and vermiculite rooting mediums and under mist/non-mist propagation chambers for rooting. There were 19 treatments for each trial laid out in CRD design, which included all possible combinations of softwood/semi-hardwood cuttings treated with 4 concentrations of NAA/IBA along with untreated control, and one absolute control *viz.*, calliandra planting stock raised from seeds, for relative comparison (Table 1). Fifteen branch cuttings constituted one replication and there were three replications per treatment. The details of trials attempted for propagation of calliandra using low-tech and high-tech vegetative propagation techniques are presented in Table 2. All the treated cuttings failed to sprout and root under non-mist propagation conditions like that of shade house and polytunnels during both the seasons. Some additional trials with higher concentrations of IBA/NAA were also attempted for non-mist propagation, which also failed (Table 2).

Consequently, the treated cuttings were planted in vermiculite medium and kept in a mist chamber for rooting. Some of the treated cuttings produced enough sprouts and roots. Temperature inside the mist chamber was maintained at 28 ± 2 °C and relative humidity at 92%. Regular misting for 10 seconds was carried out at half an hour intervals. Then sprouted cuttings of different treatments were allowed to grow until successful rooting was observed (around 15 to 25 days according to treatments) and then transferred to polybags with rooting media of soil + coir pith compost + FYM (2:1:1) and kept again in mist chamber for 20 days for hardening. Successfully grown saplings were then shifted to a shade house with 50 per cent light availability and watered twice daily. The sprouted cuttings were allowed to grow for 3 months and various observations on shoot and root parameters were taken at the end of 3 months. The observations were also made on the sprouting percentage, shoot length, number of leaves, rooting percentage, number of roots, root length and fresh weight of root, stem, leaves and total fresh weight. Vegetatively propagated calliandra saplings were also compared with calliandra seedlings as absolute control to assess their relative performance during both seasons. Seeds of Calliandra were procured from Kerala Livestock Development Board, Dhoni Farm, Palakkad District. Calliandra seeds were soaked in water for 48 hours prior to planting to enhance

Table 1. Treatment Combinations

Treatment	Type of cuttings	Growth regulator	Concentration (mg l^{-1})
T1	Softwood cuttings	Control	0
T2	Softwood cuttings	IBA	50
T3	Softwood cuttings	IBA	100
T4	Softwood cuttings	IBA	200
T5	Softwood cuttings	IBA	250
T6	Softwood cuttings	NAA	50
T7	Softwood cuttings	NAA	100
T8	Softwood cuttings	NAA	200
T9	Softwood cuttings	NAA	250
T10	Semi-hardwood cuttings	Control	0
T11	Semi-hardwood cuttings	IBA	50
T12	Semi-hardwood cuttings	IBA	100
T13	Semi-hardwood cuttings	IBA	200
T14	Semi-hardwood cuttings	IBA	250
T15	Semi-hardwood cuttings	NAA	50
T16	Semi-hardwood cuttings	NAA	100
T17	Semi-hardwood cuttings	NAA	200
T18	Semi-hardwood cuttings	NAA	250
T19	Absolute control - Calliandra planting stock raised from seeds		

Table 2. Attempted trials

Experiments	
All 19-treatment combination planted in soil based rooting media (Soil + coir pith compost + FYM in the ratio 2:1:1) and kept in shade house	Failed
All 19-treatment combination planted in vermiculite rooting medium and kept in shade house	Failed
All 19-treatment combination planted in soil based rooting media (Soil + coir pith compost + FYM in the ratio 2:1:1) and kept in polytunnel	Failed
All 19-treatment combination planted in vermiculite rooting medium and kept in polytunnel	Failed
All 19-treatment combination planted in soil based rooting media (Soil + coir pith compost + FYM in the ratio 2:1:1) and kept in mist chamber	Failed
A few treatments combinations planted in vermiculite rooting medium and kept in mist chamber	Success
Additional Trials	
Cuttings treated with NAA or IBA in 500 ppm/1000 ppm/2000 ppm/ 8000 ppm by overnight soaking and quick dip method	Failed

germination and sown in nursery beds of standard size. Healthy and uniform seedlings were transplanted to polythene bags filled with potting mixture (soil: coir pith: FYM) in a 2:1:1 ratio, one month after sowing and transferred to the shade house. Seedlings were allowed to grow for 3 months and various observations on shoot and root parameters were taken at the end of 3 months.

The data were subjected to statistical analysis by analysis of variance (ANOVA) using the general linear model procedure in R software version 4.0.5 to ascertain the significance of various parameters. The Duncan's Multiple Range Test (DMRT) was used to test the differences among treatment means at a 5 per cent significance level.

RESULTS AND DISCUSSION

The study consisted of 19 treatments (Table 1) repeated both during the rainy and summer season. In the rainy season only 6 treatments resulted in successful shooting and rooting while in the summer season, 8 treatments were successful (Table 3). In both seasons T1 (softwood cuttings without any treatment (control)), T2 (softwood cuttings treated with 50 mg l^{-1} of IBA), T6 (softwood cuttings treated with 50 mg l^{-1} of NAA) and T19 (absolute control – Calliandra planting stock raised from seeds) were successful in rooting and shooting (Table 3). In both seasons softwood cuttings treated with lower concentration of growth hormones showed better success than higher concentration. Depending on the endogenous level of growth regulating substance, exogenous application of auxin may be promotive, ineffective or even inhibitory for the rooting and sprouting of cuttings (Hartmann *et al.,* 2002). In general softwood cuttings performed better than semi-hardwood cuttings. This may be because with increasing age endogenous auxin content decreases and lignification increases which inhibits rooting and other growth parameters (Leopold and Kriedemann,1975; White and Lovell, 1984). For vegetative propagation of calliandra, better success rate was obtained in the summer season compared to the rainy season. The environmental conditions during collection like light, temperature, humidity and rainfall play a significant role in the root induction of cuttings (Bunce, 1984; Karaguzel,1997) which may be related to endogenous plant growth regulator levels or carbohydrates (Day and Loveys,1998). The high carbohydrate concentration and C/N ratio of coppice cuttings during the summer season might have resulted in a better success rate during this season.

Shoot parameters of successful treatments

The results indicate that the shoot parameters of calliandra cuttings showed significant variation with respect to various treatments. Among different cuttings, softwood cuttings treated with the lower dose of 50 mg L^{-1} of NAA (T6) recorded the maximum

Table 3. Successful treatments of calliandra cuttings and seedlings 3 months after transplanting

Treatment	Type of cuttings	Growth regulator	Concentration (mg l^{-1})	Rainy season	Summer season
T1	Softwood cuttings	Control	0	Success	Success
T2	Softwood cuttings	IBA	50	Success	Success
T3	Softwood cuttings	IBA	100	Failed	Success
T4	Softwood cuttings	IBA	200	Failed	Failed
T5	Softwood cuttings	IBA	250	Failed	Failed
T6	Softwood cuttings	NAA	50	Success	Success
T7	Softwood cuttings	NAA	100	Failed	Success
T8	Softwood cuttings	NAA	200	Failed	Success
T9	Softwood cuttings	NAA	250	Failed	Failed
T10	Semi-hardwood cuttings	Control	0	Failed	Success
T11	Semi-hardwood cuttings	IBA	50	Failed	Failed
T12	Semi-hardwood cuttings	IBA	100	Success	Failed
T13	Semi-hardwood cuttings	IBA	200	Success	Failed
T14	Semi-hardwood cuttings	IBA	250	Failed	Failed
T15	Semi-hardwood cuttings	NAA	50	Failed	Failed
T16	Semi-hardwood cuttings	NAA	100	Failed	Failed
T17	Semi-hardwood cuttings	NAA	200	Failed	Failed
T18	Semi-hardwood cuttings	NAA	250	Failed	Failed
T19	Absolute control - Calliandra planting stock raised from seeds			Success	Success

sprouting percentage (84.44%) followed by softwood cuttings treated with 50 mg L^{-1} of IBA (64.44%, T2) during the rainy season (Table 4). In the summer season, a similar trend is followed, where T6 recorded 86.66% sprouting followed by T2 (71.11%) as presented in Table 4. While absolute control (T19, calliandra seedlings) recorded 100 per cent sprouting in both seasons. Similarly, the highest shoot length was also observed in softwood cuttings treated with 50 mg L^{-1} of NAA (66.5cm, T6) while it's on par with softwood cuttings treated with 50 mg L^{-1} of IBA (56 cm, T2) and also with absolute control – calliandra seedling (68.16cm, T19) during the rainy season (Table 4). In the summer season, T6 recorded 59cm shoot length and was significantly superior to other treatments as well as the calliandra seedlings(39cm). The highest number of leaves was recorded for softwood cuttings treated with 50 mg L^{-1} of IBA (12.33, T2) and was on par with many other treatments including T6 and calliandra seedlings during the rainy season (Table 4). Whereas in the summer T1 recorded the maximum number of leaves/plant (16.66) and it's on par with T6 (13.66) and with T19 (13).

In this study, softwood cuttings performed better than semi-hardwood cuttings. The variation in rooting potential of two types of cuttings seems to be due to the physiological nature of cuttings. This may be because with increasing age endogenous auxin content decreases and lignification increases which inhibits rooting and other growth parameters (Leopold and Kriedemann,1975; White and Lovell, 1984). Physiological ageing can also be a reason for it. The presence of large numbers of resin canals, sclerenchyma cells and branch traces in hardwood will reduce the number of parenchymatous tissues to such a low level that the potential primordial sites are limited (White and Lovell,1984). The rooting and sprouting potential of the cuttings is a juvenile characteristic that decreases after maturation, resulting in a reduced capacity to induce rooting and sprouting in mature shoot cuttings. Overall data shows that sprouting parameters were better for softwood cuttings treated with 50 ppm NAA followed by 50 ppm IBA states that lower concentration gives better results than higher concentration. This may be due to calliandra having some level of endogenous auxin and requiring only a minimum supplementation *i.e.* lower doses of IBA and NAA for sprouting and other growth parameters. High concentrations of auxin inhibit the growth of calliandra cuttings. Depending on the endogenous

Table 4. Shoot parameters of successful treatments of calliandra cuttings and seedlings 3 months after transplanting

Treatment	Type of cuttings	Growth regulator	Concentration (mg l^{-1})	Sprouting percentage (%)	Shoot length(cm)	Number of leaves/plant
Rainy season						
T1	Softwood cuttings	Control	0	42.22^{d}	55^{a}	10^{a}
T2	Softwood cuttings	IBA	50	64.44^{c}	56^{a}	12.33^{a}
T6	Softwood cuttings	NAA	50	84.44^{b}	66.5^{a}	9.33^{ab}
T12	Semi-hardwood cuttings	IBA	100	42.21^{d}	52^{a}	11.66^{a}
T13	Semi-hardwood cuttings	IBA	200	37.77^{d}	33.33^{b}	6.33^{b}
T19*	Absolute control - Calliandra planting stock raised from seeds			100^{a}	68.16^{a}	11.66^{ab}
	P value			<0.05	<0.05	<0.05
Summer season						
Treatment	**Type of cuttings**	**Growth regulator**	**Concentration (mg l^{-1})**	**Sprouting percentage (%)**	**Shoot length(cm)**	**Number of leaves/plant**
T1	Softwood cuttings	Control	0	44.44^{c}	49^{ab}	16.66^{a}
T2	Softwood cuttings	IBA	50	71.1^{b}	37^{bc}	7.66^{de}
T3	Softwood cuttings	IBA	100	51.10^{c}	26.33^{c}	5.33^{e}
T6	Softwood cuttings	NAA	50	86.66^{a}	59^{a}	13.66^{ab}
T7	Softwood cuttings	NAA	100	44.44^{c}	56.66^{a}	12^{bc}
T8	Softwood cuttings	NAA	200	42.21^{c}	50^{ab}	10^{bcd}
T10	Semi-hardwood cuttings	Control	0	35.55^{c}	35.66^{bc}	8.6^{cde}
T19*	Absolute control - Calliandra planting stock raised from seeds			100^{a}	39^{bc}	13^{ab}
	P Value			<0.05	<0.05	<0.05

Values with same superscript in a column do not differ significantly

p<0.05, significant at 5% level of significance

p>0.05, non-significant at 5% level of significance

*Germination percentage (%)

level of growth regulating substance, exogenous application of auxin may be promotive, ineffective or even inhibitory for the rooting and sprouting of cuttings (Hartmann *et al.,* 2002).

Root parameters of successful treatments

Looking into overall performance, the highest rooting percentage among different cuttings was recorded in softwood cuttings treated with 50 mg L^{-1} of NAA (68.88%, T6) followed by softwood cuttings treated with 50 mg L^{-1} of IBA (48.88%, T2) during the rainy season (Table 5). In the summer season also a similar trend is followed, among cuttings T6 recorded 80% rooting followed by T2 having a rooting percentage of 48.88% (Table 5). While T19 recorded a 100% rooting percentage in both seasons. However, with regard to the number of roots, the highest number of roots/plant among different cuttings was observed in semi-hardwood cuttings treated with 200 mg L^{-1} of IBA (66.33, T13) which was on par with softwood cuttings treated with 50 mg L^{-1} of NAA (55.66, T6) and softwood cuttings treated with 50 mg L^{-1} of IBA (46.66, T2) during the rainy season as presented in Table 5. While absolute control – Calliandra planting stock raised from seeds (T19) recorded 22.66 number of roots/plant. In the summer season among different cuttings, the maximum number of roots /plant (41.66) was observed in softwood cuttings in controlled condition (T1) which was on par with T6(36), T7(25), T3(26), T2(23.66) and T19(34.66). The highest root length among different cuttings was observed in softwood cuttings treated with 50 mg L^{-1} of IBA (33.83cm, T2) which was on par with softwood cuttings treated with 50 mg l^{-1} of NAA (25.33cm) and with absolute control – Calliandra seedlings (26.41cm, T19) in the rainy season as presented in Table 5. In the summer season among different cuttings, the maximum root spread (23.266 cm) was observed in softwood cuttings in controlled condition (T1) which was on par with T2 (19cm), T1 (23.26cm), T6 (19.66cm) and with T19 (25.66cm).

Tchigio and Duguma, (1998) reported a positive influence of auxins in rooting of calliandra coppice cuttings, with significant improvement in rooting percentage and number and size of roots. He recommends a high concentration of Seradix 3 (0.8% IBA) for mass propagation of calliandra through softwood cuttings under non-mist propagation. In contrast, De wolf and Jaenicke (1998) recommend minimal amounts of IBA (10 microgram/cuttings) as it increases the number of roots for successful hardening off and survival of calliandra cuttings. However, in our case non-mist propagation failed to initiate rooting in

Table 5. Root parameters of successful treatments of calliandra cuttings and seedlings 3 months after transplanting

Rainy season						
Treatment	**Type of cuttings**	**Growth regulator**	**Concentration (mg l^{-1})**	**Rooting percentage (%)**	**Number of roots/plant**	**Length of roots (cm)**
T1	Softwood cuttings	Control	0	28.88^{d}	12.33^{c}	27^{ab}
T2	Softwood cuttings	IBA	50	48.88^{c}	46.66^{abc}	33.83^{a}
T6	Softwood cuttings	NAA	50	68.88^{b}	55.66^{ab}	25.33^{ab}
T12	Semi-hardwood cuttings	IBA	100	31.10^{d}	40.33^{abc}	22.66^{b}
T13	Semi-hardwood cuttings	IBA	200	28.88^{d}	66.3^{a}	23.66^{ab}
T19	Absolute control - Calliandra planting stock raised from seeds			100^{a}	22.66^{bc}	26.41^{ab}
	P Value			<0.05	<0.05	<0.05
Summer season						
Treatment	**Type of cuttings**	**Growth regulator**	**Concentration (mg l^{-1})**	**Rooting percentage (%)**	**Number of roots/plant**	**Length of roots (cm)**
T1	Softwood cuttings	Control	0	31.10^{d}	41.66^{a}	23.266^{ab}
T2	Softwood cuttings	IBA	50	48.88^{c}	23.66^{ab}	19^{ab}
T3	Softwood cuttings	IBA	100	28.88^{de}	26^{ab}	15.33^{b}
T6	Softwood cuttings	NAA	50	80^{b}	36^{ab}	19.66^{ab}
T7	Softwood cuttings	NAA	100	24.44^{de}	25^{ab}	21^{ab}
T8	Softwood cuttings	NAA	200	31.10^{d}	20.33^{b}	17.5^{ab}
T10	Semi-hardwood cuttings	Control	0	22.14^{e}	20^{b}	21.66^{ab}
T19	Absolute control - Calliandra planting stock raised from seeds			100^{a}	34.66^{ab}	25.66^{a}
	P Value			<0.05	<0.05	<0.05

Values with same superscript in a column do not differ significantly

p<0.05, significant at 5% level of significance

p>0.05, non-significant at 5% level of significance calliandra and successful rooting was observed in the mist chamber at lower concentrations of NAA and IBA.

In both seasons softwood cutting treated in lower concentration give better result than in higher concentration. This supported Hentig and Gruber's (1987) hypothesis that the best rooting might be induced by hormone dosages when they were just below the hazardous limit. In the study done by Hossain and Urbi (2016) on shoot cuttings of *Andrographis paniculate*, it was discovered that 2.5 mM of NAA is highly effective in promoting root formation in *A. paniculate* shoot cuttings of young plants. Young apical shoot micro-cutting is more competent to induce roots compared to old apical shoot micro-cutting. The physiological makeup of cuttings appears to be the cause of the variance in rooting capacity between the two kinds of cuttings. This could be the case because softwood cuttings respond better to external NAA treatment, which promotes root growth. Additionally, the plant age, node locations, and clone differences in cuttings' rooting capacity (Yan *et al.*,2014). According to Kibbler *et al.* (2004), mature shoot cuttings have a diminished ability to induce rooting because the rooting capability of the cuttings is a juvenile trait that declines during maturation. Hossain and Urbi (2016) observed that adventitious root formation was greatly influenced by NAA in shoot cuttings obtained from young and old plants.

Calliandra is a species with a relatively high rooting potential from coppice cuttings (Tchigio and Duguma, 1998) and cuttings from 5-month-old seedlings (Dick *et al*., 1996). Zeng *et al.* (2005) stated that there are positive responses of growth-regulating substances such as NAA and IBA on the rooting performance of cuttings. The root-promoting effect varied with auxin concentrations and types of auxins. According to a review by Hartmann *et al.* (2010), the mechanisms by which IBA stimulates root formation in stem cuttings include its conversion to IAA, an increase in internal free-IBA, enhancement of tissue sensitivity to IAA, enhancement of endogenous IAA synthesis, or the action of IAA synergistically. In contrast, NAA's stimulatory effect on root formation was likely related to the inhibition of IAA-oxidase (IAAO) activity, which would prevent IAA degradation and increase its activity.

Fresh weight of sapling/seedling

In the present study during the rainy season, among different cuttings softwood cuttings treated with 50 mg L^{-1} of NAA (10.68 g $plant^{-1}$, T6) recorded the maximum fresh weight of stem which was on par with absolute control – Calliandra planting stock raised from seeds (11.67 g $plant^{-1}$, T19). In the summer season, no significant difference was observed in the fresh weight of the stem due to various treatments. In comparison, among different cuttings, the maximum fresh weight of stem (14.74 g $plant^{-1}$, T8) was recorded in softwood cutting treated 200 mg L^{-1} of NAA followed by T6 (12.42 g $plant^{-1}$). In absolute control – Calliandra planting stock raised from seeds (T19) recorded 13.84 g $plant^{-1}$fresh weight of stem (Fig 1). The shoot length in each cutting directly relates to the fresh weight of the stem. The highest fresh weight of root among different cuttings was observed in softwood cuttings treated with 50 mg L^{-1} of NAA (12.75 g $plant^{-1}$, T6) which is on par with softwood cuttings treated with 50 mg L^{-1} of IBA (9.9 g $plant^{-1}$, T2) and with absolute control – Calliandra seedlings (11.63 g $plant^{-1}$, T19) as presented in Fig 1. In the summer season, the maximum fresh weight of root (13.05 g $plant^{-1}$, T8) was recorded in softwood cutting treated with 200 mg L^{-1} of NAA which is on par with T6 (12.27 g $plant^{-1}$). Compared to absolute control (9.85 g $plant^{-1}$, T19), cuttings have performed well in both seasons. The number of roots, root spread and root length in each cutting directly relate to the fresh weight of the root. According to Milind (2008), an increase in the number of roots per cutting could have a direct impact on the fresh weight of the roots. Among which the highest fresh weight of leaves among different cuttings was recorded in softwood cuttings treated with 50 mg L^{-1} of NAA (10.62 g $plant^{-1}$, T6) (Fig 1). While absolute control – Calliandra planting stock raised from seeds (T19) recorded 21.58 g $plant^{-1}$fresh weight of leaves. In the summer season, among different cuttings, the maximum fresh weight of leaves (16.48 g $plant^{-1}$, T1) was recorded in softwood cuttings without any treatment (control) followed by T6 (14.93 g $plant^{-1}$). While absolute control – Calliandra planting stock raised from seeds (T19) recorded 8.14 g $plant^{-1}$ fresh weight of leaves. The number of leaves in each cutting directly relates to the fresh weight of the leaves. In the case of total fresh weight, among different cuttings softwood cuttings treated with 50 mg L^{-1} of NAA (34.06 g $plant^{-1}$, T6) recorded the maximum total fresh weight during the rainy season. While absolute control – Calliandra planting stock raised from seeds (T19) recorded 44.89 g $plant^{-1}$total fresh weight which was higher compared to that of cuttings. In cuttings, T6 (38.59 g $plant^{-1}$) and softwood cutting treated 200 mg L^{-1} of NAA (38.59 g $plant^{-1}$, T8) recorded the maximum total fresh weight (Fig 1). While T19 recorded 31.84 g $plant^{-1}$ total fresh weight which was lower than that of cuttings. In both seasons bigger plants with better weight were obtained from softwood cuttings compared to semi-hardwood cuttings. Better plant growth was observed in lower concentrations of growth hormones.

In general, plants raised from cuttings showed superior growth than that of seedlings during the summer season and the reverse trend was observed during the rainy season. The environmental conditions during collection like light, temperature, humidity and rainfall play a significant role in root induction of cuttings (Bunce, 1984 and Karaguzel, 1997), which may be related to endogenous plant growth regulator levels or carbohydrates (Day and Loveys, 1998). The season of collection of cuttings played a significant role in the successful rooting of cuttings and the cuttings collected during the summer recorded higher rooting as observed in our study. Similarly, better root induction was found in the summer season for tree species like *Saraca asoka*, *Oroxylum indicum, Embelia ribes* and bamboo (Surendran,1998; Saumya *et al*., 2013 and Raveendran *et al.,* 2010). Adventitious rooting in shoot cuttings of neem (*Azadirachta indica*) and karanj (*Pongamia pinnata*) also indicated that the maximum rhizogenesis coincided with the emergence of new sprouts in February and March. The high rooting percentage is attributed to high carbohydrate concentration and C/N ratio during the growing season.

In a nutshell, the best rooting attributes were observed in the softwood coppice cuttings collected during the summer season treated with 50 mg/L NAA. As the propagation through seed is not adequate to meet the large-scale planting stock requirement, vegetative propagation methods such as rooting of softwood cuttings, semi hardwood cuttings can be resorted for mass multiplication of this species. Perusal of the literature indicated that the rooting response of cuttings depends on species, type of cutting, season of collection, growth regulator and its concentration etc (Hartman *et al.,* 2002). Hence a species-specific protocol needs to be standardized after preliminary experiments considering all these parameters before going to large-scale planting stock production. The present investigation conducted with a view to standardizing a protocol for large-scale production of planting stock using cuttings revealed that rooting response can be as high as 80% in this species but it was obtained only on specific treatment combinations.

3. CONCLUSION

Based on the root and shoot parameters of treated cuttings it can be concluded that fodder calliandra can be vegetatively propagated from softwood coppice cuttings by overnight soaking with 50 ppm of NAA and using vermiculite as rooting medium under

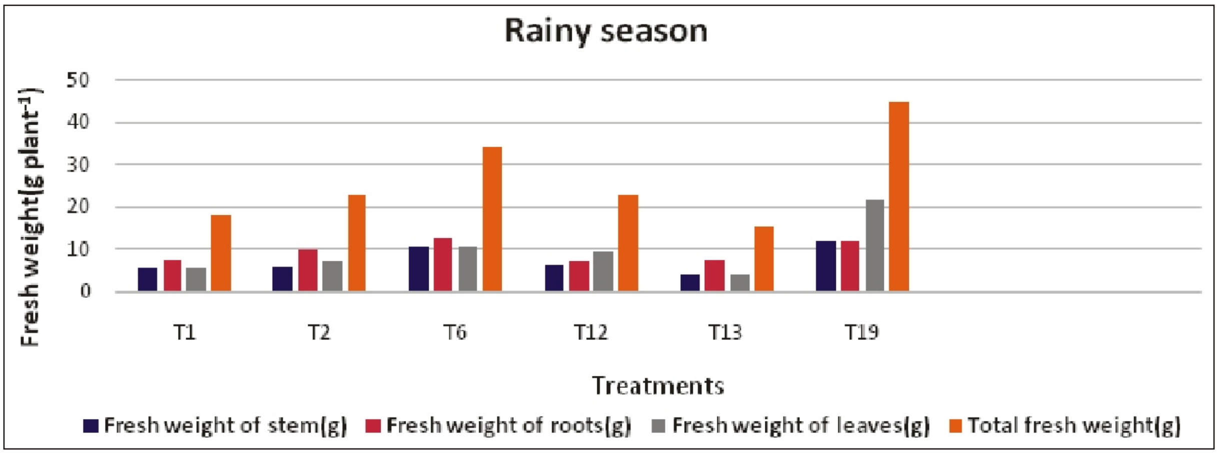

Fig 1. Fresh weight of shoots, roots and leaves and total fresh weight of calliandra cuttings and seedlings 3 months after transplanting during the rainy season

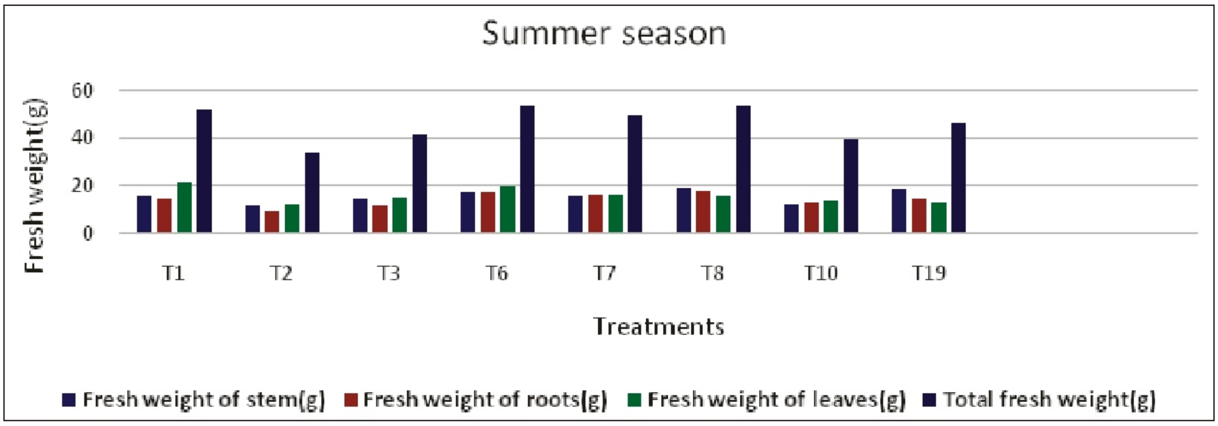

Fig 2. Fresh weight of shoots, roots and leaves and total fresh weight of calliandra cuttings and seedlings 3 months after transplanting during the summer season

controlled conditions of the mist chamber, preferably during the summer season. 50 ppm IBA was found to be the second-best treatment. Most of the shoot and root parameters of vegetatively propagated calliandra planting stock were found to be on par with that of calliandra seedlings. Non-mist propagation method and soil-based rooting medium were found to be unsuccessful for vegetative propagations of calliandra in humid tropical conditions of south India.

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