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EVALUATION OF ROOTING IN LATERAL SHOOTS AND NODAL STEM CUTTINGS OF *LEUCAENA* (*L. LEUCOCEPHALA* AND *L. DIVERSIFOLIA*) CLONES FOR CLONAL PROPAGATION

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Clonal propagation method was developed for *Leucaena* clones (*L. leucocephala* and *L. diversifolia*) using lateral shoots which gave many advantages over the conventional method of using nodal stem cuttings. Lateral shoots of 25–30 cm length with apical buds emerging at 30–60° angle from stock plants maintained in sand beds gave higher rooting than nodal stem cuttings. Anatomical configuration of stem cuttings taken at positions 1–4 from shoot apex differed significantly between lateral and nodal cuttings and *E. camaldulensis* used as control. Rooting was significantly higher (87%) at 25–30 cm length (position 4) in lateral shoots (where vessel frequency was highest) compared with other positions (28–43%) and nodal cuttings (16–53%), whereas *Eucalyptus* stem cuttings had higher frequency of vessels of lower diameter and > 80% rooting at all positions. Rooting occurred in *Leucaena* lateral shoots within 20 days of misting with roots emerging all around the stem along a length of 2 cm from base of the cutting. Lateral cuttings produced significantly higher number of roots (22 roots per plant) than nodal cuttings (6 roots per plant) with significant reduction in nursery period (60 days), better rooting (> 80%) and root architecture.

Keywords: Clonal forestry, nursery management, juvenility, stem anatomy, vegetative propagation, rooting hormone, pulpwood

INTRODUCTION

Leucaena leucocephala and *Eucalyptus* are widely used raw materials for the pulp and paper industry in India. More than 100,000 acres of *Leucaena* plantations are raised in farmlands in Telangana and Andhra Pradesh. Majority of the plantations (97%) are of seed origin with low productivity and high variability in growth. Clonal forestry can improve wood yield by at least 50%, giving greater return to farmers and enhancing wood supply to paper mills. Though *Leucaena* is a popular tree crop in the black soils of Telangana and Andhra Pradesh, clonal forestry of this species has not picked up as in *Eucalyptus* due to poor rooting and lodging compared with seed-origin plantations. Clones generally give rise to very uniform plants compared with seedlings, but it also depends on adventitious root development which is influenced by several factors such as quality and juvenility of vegetative shoots and propagation process followed in nursery. While rooting of stem cuttings is well established in *Eucalyptus*, *Leucaena* stem cuttings often have problems such as poor rooting, one-sided rooting and mortality during hardening. Vegetative propagation of *Leucaena* is difficult although several methods have been attempted (Litzow & Shelton 1991). Rooting is reported to vary (9–71%) in *Leucaena* hybrids depending on the clone, position of cutting in stem, rooting hormone and season (Victorio et al. 1998).

Nodal stem cuttings of stock plants maintained in clonal multiplication area (CMA) in open field was conventionally used for rooting *Eucalyptus*, which was also employed in *Leucaena* (Dick et al. 1999, Shi & Brewbaker 2006). There is however substantial variation in rooting and root distribution depending on the type of cutting used. These limitations affect the genetic expression of clones resulting in intra-clonal growth variation. Unbalanced root distribution and absence of leading root cause windthrow due to poor anchoring in soil.

Studies of mass multiplication in hybrids and clones of *Leucaena leucocephala* have shown that leaf area and type of stem cutting had significant impact on rooting (Shi & Brewbaker 2006), which varied between clones (Victorio et al. 1998), and exogenous application of rooting hormone was essential for good rooting (Dick et al. 1999). Etiolation treatment in stock plants increased rooting rate and root length (Shi & Brewbaker 2006). Most propagation experiments of *Leucaena* focused on multiplying cultivars for fodder production. The current study was aimed at developing a vegetative propagation method for pulpwood production. *Leucaena* clones (variety K636 of *L. leucocephala* and *L. diversifolia*) propagated using nodal stem cuttings have rapid growth but are prone to windthrow. There is also very high mortality during hardening stage, making it difficult to meet the demand for planting stock. A detailed study of the problem revealed that most propagules had less than 3–4 roots with imbalanced distribution. Histological investigation revealed that shoots growing in vertical and lateral orientation to main stem had different anatomical structure, quite different from that of a typical eucalypt stem cutting. A study was taken up to understand the variation in anatomical structure and rooting in *Leucaena* cuttings depending on growth orientation and maturity. The hypothesis that rooting in *Leucaena* stem cuttings was influenced by anatomical configuration of the cutting was tested in different types of cuttings in three clones. Four major objectives were identified to develop a robust multiplication protocol: (1) evaluate impact of hormone application on rooting in different types of *Leucaena* cuttings, (2) compare rooting in three commercial clones, (3) evaluate rooting in lateral and nodal cuttings at different stem positions in *Leucaena*, and (4) compare anatomical structure of *Leucaena* with *Eucalyptus* stem cuttings at different positions and evaluate the impact of anatomical structure on rooting. This paper describes a technique developed for production of desirable stem cuttings to achieve successful rooting and uniform root distribution in vegetative propagules of *Leucaena*.

MATERIALS AND METHODS

The study was conducted in ITC clonal multiplication nursery at Paperboards and Specialty Papers Division, Bhadrachalam in Khammam district of Telangana state (17° 40' N, 81° 00' E, 100 m elevation) from February 2015 to May 2017. The nursery site has tropical humid climate with average annual temperature of 35 °C. Maximum temperature in summer reaches 49 °C in May and minimum temperature is 10 °C in winter with 70–80% relative humidity. Stock plants of three commercial *Leucaena* clones, i.e. two *L. leucocephala* clones (LL-1 and LL-2) and one *L. diversifolia* clone (LD) were used for the study. The study was conducted in two phases: Phase 1: Stock plants of *Leucaena* (3 years old) established in sand beds on raised platforms in covered shade house (Figure 1a), and those planted in open field CMA (Figure 1b) were used in March to December 2015. A commercial *E. camaldulensis* clone of the same age maintained in sand beds adjoining *Leucaena* in the shade house was used as control to compare rooting and anatomy of cuttings. Phase 2: New stock plants (4 months old) established in sand beds

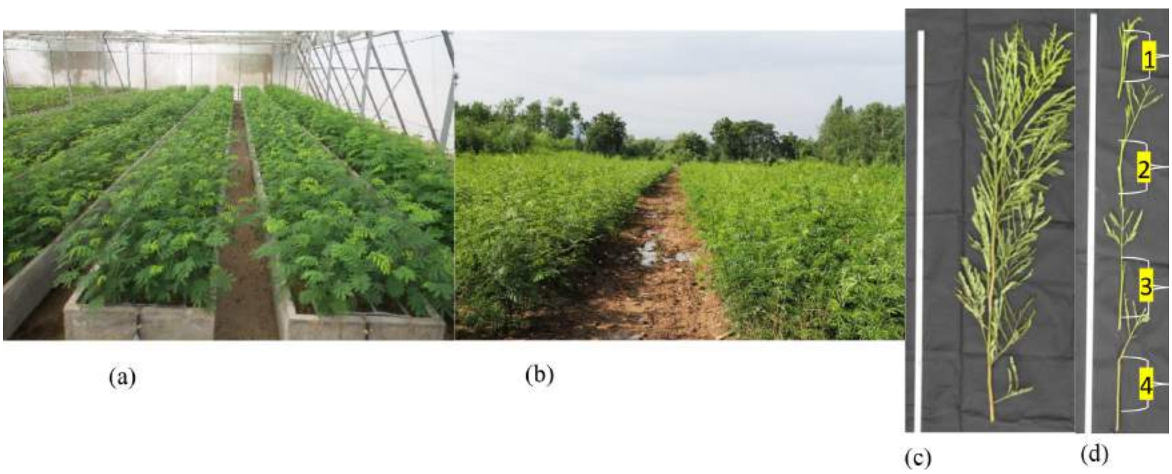
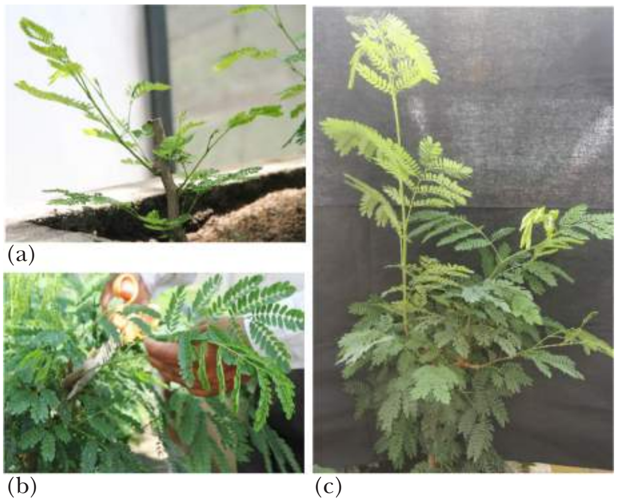


Figure 1 Stock plants are maintained in (a) sand beds in covered shade house and (b) field clonal multiplication area; nodal cuttings are harvested from (c) coppice shoots at positions (d) 1–4 from shoot apex

on raised platforms in covered shade house were evaluated in June 2016 to May 2017.

In phase 1, the stock plants in sand beds were divided into two portions whereby 50% was pruned at a height of 15 cm to produce lateral shoots and the other half maintained to produce nodal cuttings from coppice shoots. Lateral shoots were also produced in *Leucaena* field CMA by pruning stock plants at 15 cm height. An exploratory study was taken up in this phase to evaluate the impact of rooting hormone (IBA 5000 ppm) in *Leucaena* cuttings (combined for three clones) from sand beds and CMA using 120 cuttings each in three replications (3 trays of 40 cells in each replication) for each type of cutting. Subsequent studies in phases 1and 2 were conducted only in sand beds. Nodal and lateral cuttings (with apical bud) harvested from stock plants were planted in rooting medium of vermiculite and coir pith (2:1 ratio) in polypropylene root trainers (93 cc × 40 cells) and subjected to misting for 20 days in a mist chamber (holding capacity of 30,000 cuttings) where temperature (35–37 °C) and relative humidity (80–85%) were maintained by fogging and cooling pad. Misting (1 min) was applied from 9.30 a.m. to 5.30 p.m. at intervals of 30 min. In the peak of summer, misting was carried out two or three times at night when ambient temperature was more than 45 °C. Light transmission into the chamber through polythene cover was controlled by a shade net provided below the polythene cover. Rooted cuttings were shifted for hardening in shade for 15 days and later in open condition for 30 days. Every month 1000 cuttings each of lateral and nodal cuttings were harvested for large-scale evaluation from the two sets of stock plants in sand beds (three clones combined) (200 × 5 replications, 5 trays of 40 cells in each replication).

Phase 2 was carried out to fine tune the technique for large-scale commercial production. New stock plants were planted in sand beds at a spacing of 20 cm × 15 cm and all mother plants pruned after growth of 3 months to induce lateral shoot production (Figure 2a). Stock plants were provided nourishment (Table 1) every week through drip fertigation to get sustained production of cuttings. In phase 2, 1000 lateral cuttings (200 × 5 replications, 5 trays of 40 cells in each replication) were harvested each month from stock plants of three clones to evaluate rooting.



(a) (b) (c)

Figure 2 Lateral shoots emerging at an angle to main stem are harvested for vegetative multiplication (a–c)

Specifics, rooting and anatomy of cuttings

In phase 1, *Leucaena* stock plants in sand beds were assessed for the following parameters using 120 cuttings per clone: (a) length and diameter of each type of cutting (shoot positions 1–4 were combined in nodal cuttings, Figures 1c and d), (b) angle of emergence of lateral shoots and rooting (%), and (c) number of roots per plant and root distribution (scale 1–5, Table 2) recorded by carefully removing the growth medium 25 days after misting. Root emergence at the base of cutting was observed in 10 random samples at different periods during misting and hardening stages. Time required for production of healthy clonal plants was computed for 1000 cuttings collectively for all clones in lateral shoots and nodal cuttings.

Transverse sections of stem were taken at each shoot position (1–4) from 15 stems (three clones combined) each for lateral and coppice shoot (5 × 3 replications) to compare the anatomical structure of lateral and nodal cuttings with that of typical minicutting (position 1) and nodal cuttings (positions 2–4) of *E. camaldulensis* as control. *Eucalyptus camaldulensis* is a good control because, unlike *Leucaena*, its nodal cuttings are successful when subjected to same nursery conditions. Transverse sections (20–25 µm) were taken using a sledge microtome after fixing samples in formaldehyde–acetic acid–ethanol. Sections were stained with safranin (0.5%) and passed through ethanol series and xylene. Anatomical parameters were recorded using light

Table 1 Nutrients applied to stock plants for sustained production of cuttings

Chemical	Quantity (g) 1000 L^{-1}
N:P:K (19:19:19)	480
Muriate of potash	200
Magnesium sulfate	480
Ferrous sulphate	250
Calcium nitrate	550
Boric acid	20
EDTA	25
Manganese chloride	35
Copper sulphate	20
Zinc sulphate	25
Ammonium molybdate	15

Table 2 Procedure followed for evaluation of rooting pattern in *Leucaena* cuttings

Scale	Class	Root distribution	No. of roots
1	Poor rooting	Nil/one side	0–1
2	Slight rooting	One side	2–4
3	Moderate rooting	Two sides	5–10
4	Good rooting	2–3 sides	11–15
5	Heavy rooting	All around	> 15

microscope and image analysis software Leica QWin Plus (version 3.5.0).

A separate study was taken up to record rooting in cuttings from each stem position using 120 cuttings (1 tray of 40 cells × 3 replications) from each position in lateral shoots, nodal cuttings (combined for all clones) and *E. camaldulensis*. Shoot tip was retained in lateral shoots of *Leucaena* and minicuttings (position 1) of *Eucalyptus*. Rooting success in *Leucaena* was recorded after hardening in phases 1 and 2 by observing root emergence from the base of the root trainer in 1000 cuttings every month.

Experimental design and data analysis

Since the experiments were conducted over different periods, randomised complete block design was used and data from the following experiments were analysed using analysis of variance (ANOVA) as described by Shi & Brewbaker (2006) and Wendling et al. (2015):

(1) rooting with hormone treatment in cuttings from sand bed and field CMA, and between lateral shoots and nodal cuttings from different shoot positions (1–4) was analysed using one-way ANOVA,
(2) rooting and cutting parameters were analysed in three clones (clone × cutting type) using two-way ANOVA in phase 1 as there was no significant interaction between clone and cutting type,
(3) anatomical traits and rooting across different shoot positions were analysed using one-way ANOVA to compare three types of cuttings: lateral shoots, nodal cuttings and *Eucalyptus* cuttings, and
(4) rooting (%) in phases 1 and 2 across different months (combined for three *Leucaena* clones) was evaluated using one-way ANOVA.

Mean values were compared using Tukey's HSD test when significant differences ($p < 0.05$) were obtained in ANOVA. All percentage data were subjected to arcsine transformation

($\sqrt{x}/100$) and count data transformed using formula $\sqrt{x+1}$ (Snedecor & Cochran 1980). The statistical package R (2013) was used for data analysis and Tukey's HSD test was performed using the package Agricolae (2014).

RESULTS

Comparison of lateral and nodal cuttings

Average dimensions of cuttings harvested from stock plants for each clone in sand beds are given in Table 3. Length of lateral cuttings ranged from 17–34 cm with a mean of 24 and 27 cm for LD and LL clones respectively) and diameter ranged from 2.1–3.6 cm. Nodal cuttings were significantly thicker with diameter range of 2.5 to 6.5 cm, and only half the length. Lateral cuttings harvested at the same time across clones were significantly shorter in LD clone. As nodal cuttings were harvested almost once a month from coppice shoots of stock plants they grew vertically whereas angle of lateral shoot emergence (ranging from 30–60°) did not vary significantly between clones. Rooting rate across seasons was significantly higher in lateral shoots (range 70–95%) than in nodal cuttings (30–45%) and LL clones showed significantly higher rooting than LD. Lateral shoots (all clones) had an average of 22 roots (Figure 3, whereas in nodal cuttings, the numbers ranged from 0 to 11 roots (average 5) with no significant variation between clones (Table 3).

Rooting rate and distribution

There was no significant impact of rooting hormone in lateral shoot cuttings from sand beds as rooting was high (85%) in both hormone treated and untreated cuttings across the three clones (Figure 4). Hormone application did not have impact on rooting in nodal cuttings from sand beds which was similar to that in untreated lateral cuttings from field CMA (38%). Lateral cuttings from field CMA showed significantly higher rooting (55%) with IBA treatment (Figure 4). Lateral cuttings from sand beds showed early root development within 10 days of misting (Figure 5b) whereas in nodal cuttings

Table 3 Specifics and rooting in lateral and nodal cuttings of *Leucaena* clones

Clone	Cutting length (cm)	Cutting diameter (mm)	No. of roots	Rooting (%)	Shoot angle (Deg)
Lateral cutting					
LL-1	27a (25–32)	2.9^{a} (2.5–3.6)	22.5^{a} (19–28)	84^{a} (78–95)	53^{a} (40–60)
LL-2	27.5^{a} (22–34)	3.1^{a} (2.7–3.6)	21.5^{a} (18–26)	82^{a} (76–92)	46^{a} (30–60)
LD	24^{b} (17–30)	2.7^{a} (2.1–3.4)	20.5^{a} (17–25)	77^{b} (70–86)	47^{a} (35–60)
Nodal cutting (Combined position 1–4)					
LL-1	9^{c} (7.0–11.0)	4.6^{b} (2.6–6.3)	6^{b} (0–11)	42^{c} (40–45)	NA
LL-2	10.7^{c} (7.0–13.0)	4.5^{b} (2.5–6.5)	5.5^{b} (0–10)	40^{cd} (35–44)	NA
LD	9.8^{c} (6.0–13.0)	3.9^{b} (2.9–6.1)	4.3^{b} (0–9)	38^{d} (30–45)	NA
Cutting type					
Lateral cutting	26^{a}	2.88^{a}	21^{a}	81^{a}	48.7
Nodal cutting	9.9^{b}	4.4^{b}	5.2^{b}	40^{b}	NA
Significance					
Clone (C)	*	ns	ns	***	ns
Cutting type (CT)	***	*	***	***	
C × CT	***	ns	ns	ns	

ns = Non-significant, *significant at $p < 0.05$, ***significant at $p < 0.001$, NA = not applicable, mean values of each trait with same letter do not vary significantly ($p < 0.05$); range of values in parentheses root formation was not evident even after 14 days (Figure 5c). By the 25th day, lateral shoots had well developed roots compared with nodal cuttings (Figure 5d).

Number of roots and their distribution also differed between nodal and lateral cuttings. Thick nodal cuttings with prominent leaf pulvinus showed callus formation and poor root distribution (Figure 5e) whereas lateral shoots had root distribution > 4 in a scale of 1–5 (Figure 3). As seen from Figure 6a the majority of propagules from nodal cuttings had poor to slight rooting with average 5–7 roots. Nodal cutting from position 1 was not suitable for rooting whereas cuttings from positions 2–4 had moderate rooting with roots in only one or two directions (Figure 3). Plants developed from lateral shoots had more than 15 roots (average 22) distributed around the stem along a length of 2 cm from base of the cutting (Figure 5d). Nursery period for production of planting stock reduced by 40 days in lateral shoots over the conventional method using nodal cuttings. There was a reduction in misting time by 10 days and hardening time by over 30 days to produce well developed planting material (Figures 6b and 7).

Stem anatomy

The proportion of pith was significantly higher near the shoot apex in positions 1 and 2 than the lower positions 3 and 4 in both lateral and nodal cuttings of *Leucaena* (Table 4). Vessel frequency increased significantly from position 1 to almost twice the number in position 4 in lateral shoots whereas in nodal cuttings, the number of vessels per mm^2 at position 4 (54) was 1.5 times lower than the corresponding position in lateral cuttings (85). *Eucalyptus camaldulensis* cuttings had substantially higher vessel frequency (range 120–206) than *Leuceana* at all positions and significantly lower pith area (46–48%) in positions 1 and 2 (Figure 8) than *Leucaena* (59–70%) (Table 4). Lateral shoots of *Leuceana* had significantly lower vessel diameter than nodal cuttings at different positions, which was higher than that of *E. camaldulensis* cuttings at shoot positions 3 and 4. In *Leucaena* sclerenchyma tissues occurred in patches in lateral shoots just above the cambial region but roots emerged easily through the openings (Figure 5a).

Impact of stock plant age and season on rooting

Rooting of above 80% was consistently achieved in lateral cuttings across all seasons in both 3- and 1-year-old mother plants. Rooting rate was sustained across seasons in the 3-year-old stock plants (Figure 9) despite being located at a remote site and needing more processing time after harvest of cuttings. High rooting of 95% was obtained in October, significantly higher

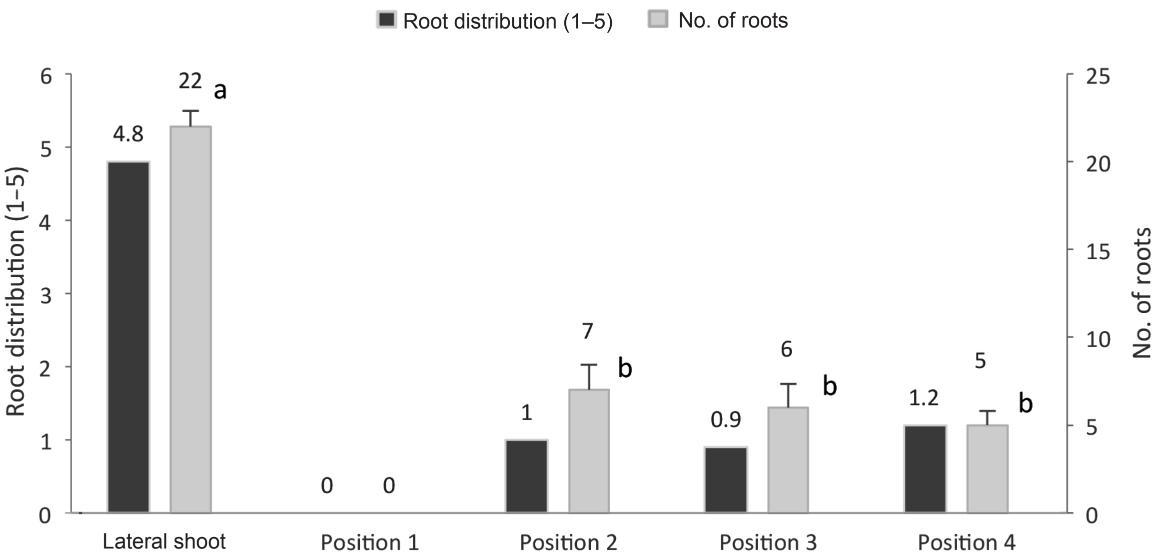


Figure 3 Root distribution and number of roots in lateral and nodal stem cuttings; mean values (average ± standard error) sharing the same letter do not differ significantly ($p < 0.05$) (Tukey adjusted comparisons)

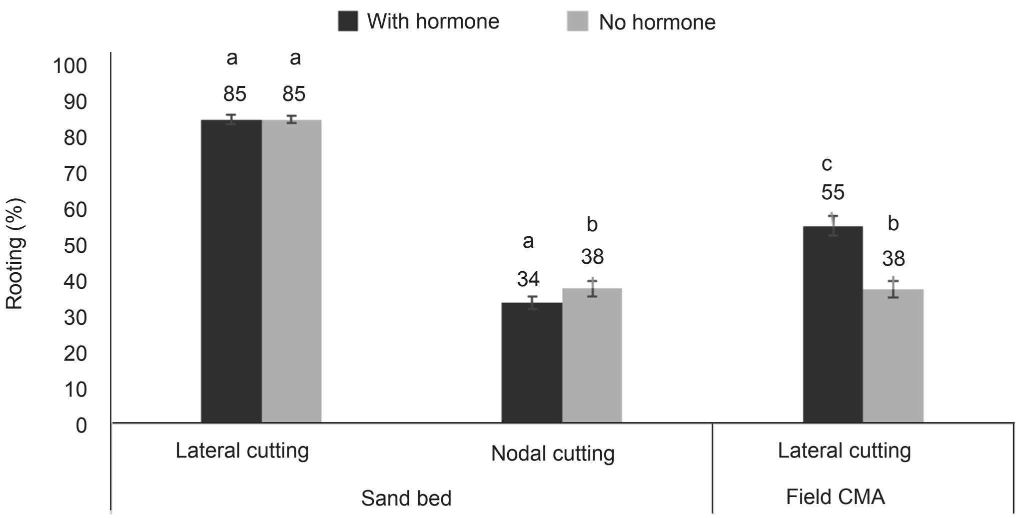


Figure 4 Lateral and nodal cuttings from sand bed and field clonal multiplication area CMA differ in rooting response to IBA treatment; mean values (average ± standard error) sharing the same letter do not differ significantly ($p < 0.05$) (Tukey adjusted comparisons)

Figure 5 (a) Root emergence through sclerenchyma (S) patches in a transverse section of lateral shoot (LS) and (b) root growth after 10 days in mist chamber, lateral shoots (LS) have better root system than nodal cuttings (N) after (c) 14 days and (d) 25 days; callus formation is seen in (e) thick nodal cuttings with prominent pulvinus (Pv)

(a) (b)

Figure 6 Lateral cuttings (L) have larger root system after (a) 15 days hardening in shade and better shoot growth after (b) 30 days hardening in open compared to nodal (N) plants

Table 4 Anatomical parameters in lateral and nodal cuttings of *Leucaena* and *E. camaldulensis*

Cutting type	Pith %	Vessel frequency mm^{-2}	Vessel diameter (μm)	Rooting %
Leucaena:lateral shoot (length 22–34 cm)				
1	70.5^{a}	38^{g}	25.0^{ab}	28^{ab}
2	61.3^{b}	40^{g}	26.4^{b}	35.5^{bc}
3	43.6^{cd}	54^{e}	36.5^{c}	43.5^{cd}
4	34.5^{e}	85^{d}	37.6^{c}	87.5^{e}
Leucaena:coppice shoot (length 6–13 cm)				
1	69.1^{a}	36^{g}	33.5^{bc}	15.8^{a}
2	58.8^{b}	46^{f}	56.0^{d}	17^{a}
3	41.1^{cde}	49^{ef}	59.4^{d}	21.3^{ab}
4	37.3^{de}	54^{e}	60.3^{d}	53^{d}
E. camaldulensis:coppice shoot (length 25 cm)				
1	48.0^{c}	120^{c}	17.5^{a}	82.6^{e}
2	46.8^{c}	180^{b}	26.4^{b}	85.5^{e}
3	45.7^{c}	186^{b}	26.0^{ab}	83.6^{e}
4	35.3^{e}	206^{a}	26.9^{b}	84.3^{e}
Significance of cutting type	***	***	***	***

***Significant at $p < 0.001$, mean values of each trait with same letter do not vary significantly ($p < 0.05$)

than that in other months due to favourable temperature and humidity at the nursery as seen in 1-year-old stock plants (Figure 10).

DISCUSSION

Vegetative propagation methods have evolved in forest trees depending on the requirement of each species. In eucalypts, minicuttings are widely used over conventional nodal cuttings after the pioneering work by Assis et al (2004). Even in species classified as difficult to root, inhibiting factors affecting rooting may be overcome by identifying the right position of the stem with optimum maturity of tissues for propagation (Wilson 1993). Exogenous application of auxin is often used to enhance rooting in woody stem cuttings whereas in minicuttings, apical buds,

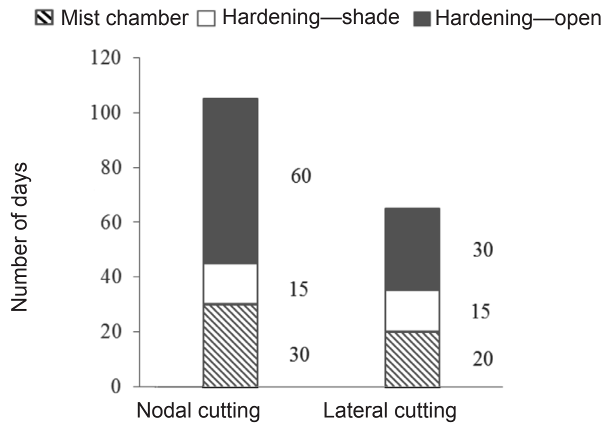


Figure 7 Nursery period for vegetative propagation with nodal and lateral cuttings

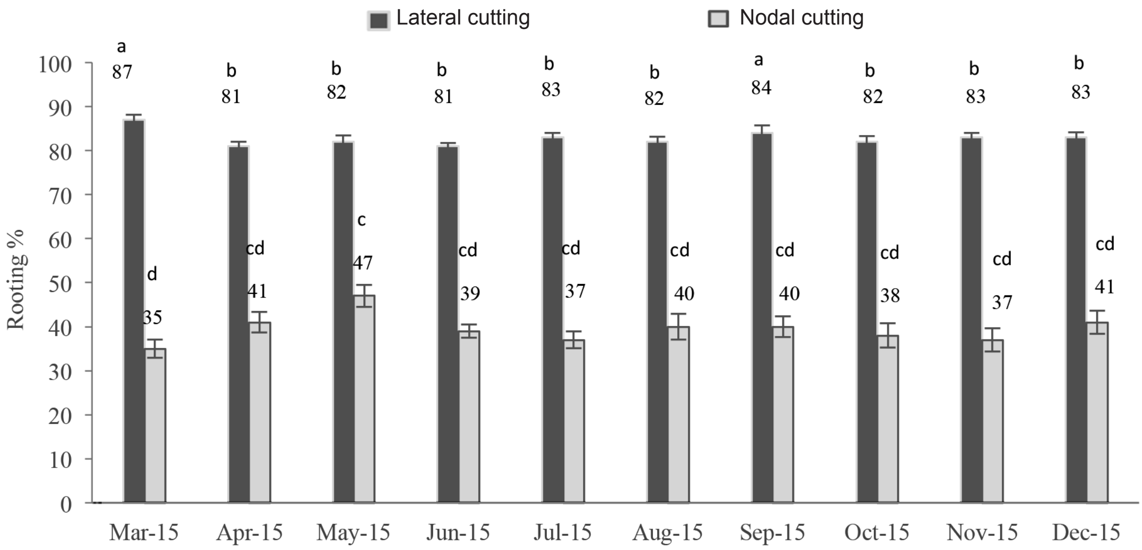


Figure 8 Rooting (%) in lateral cuttings and nodal cuttings in 3-year- old *Leucaena* stock plants (N = 1000/ month); mean values (average ± standard error) sharing the same letter do not differ significantly ($p < 0.05$) (Tukey adjusted comparisons)

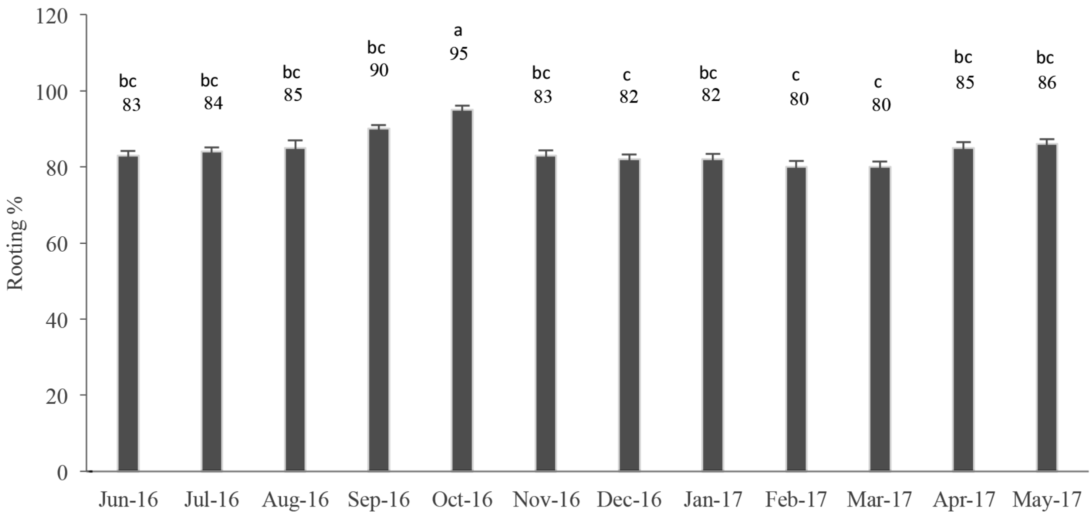


Figure 9 Good rooting (%) is achieved across seasons in lateral cuttings from 1-year-old *Leucaena* stock plants (N = 1000/month); mean values (average ± standard error) sharing the same letter do not differ significantly ($p<0.05$) (Tukey adjusted comparisons)

which are the source of IAA production (Aloni et al. 2003) play a major role in development of primary and lateral roots (Gehlot et al. 2014).

Lateral shoot production

Lateral shoots emerge from buds or shoot primodia that remain latent under the bark of stems. In the presence of an actively growing apical bud, these latent buds remain dormant but elongate with xylem growth and emerge only when the apical bud is cut back. The emergence of lateral shoots is determined by the extent to which apical dominance is inhibited in the plant. Severe pruning to prevent apical growth would be necessary to promote sustained lateral shoot production. When apical dominance is high, lateral shoots do not emerge but remain as latent bud. When the dominance of apical bud is reduced, the buds emerge as lateral shoots (Cline 1997). Shoots tend to grow fast if they have vertical orientation, normally with sufficient apical dominance whereas lateral shoots that grow at an angle to the vertical stem grow at a slower rate. The angle of growth decides the apical control exerted by the shoot (Wareing & Nasr 1961). Thus, when apical dominance is suppressed by cutting back the vertical growth, more and more lateral shoots emerge. If they are not harvested at the right time, the lateral shoots start growing vertically, competing to be the lead growth and take over the role of an apical shoot. Pruning and training help to produce the desired type of shoot by directing plant growth and exploit the natural response of plant to balance the competitive ability of apical and lateral growth. Growth vigour of the plant will decide the extent to which apical growth can be restricted. *Leucaena* stock plants established in open field (CMA) developed good root system prompting vigorous growth and even with severe pruning several lower shoots emerged with different levels of apical dominance. Stems growing vertically had larger diameter than those growing in lateral orientation as seen in Table 3. Nodal cuttings are generally taken from vertically growing coppice shoots (Victorio et al. 1998, Dick et al. 1999) and lateral shoots have not been reported in *Leucaena* propagation.

Anatomy of stem cuttings

Vigorous growth of plants may lead to maturation and related changes in anatomy of the stem which impacts rooting capacity (Wendling et al. 2015). In *E. globulus*, rooting and survival of rooted plants is dependent on the position of cutting in the stem. While rooting reduced with distance from the stem apex in nodal and apical cuttings due to increase in woodiness at the base, survival increased with woodiness at the base in apical cuttings (Wilson 1993).

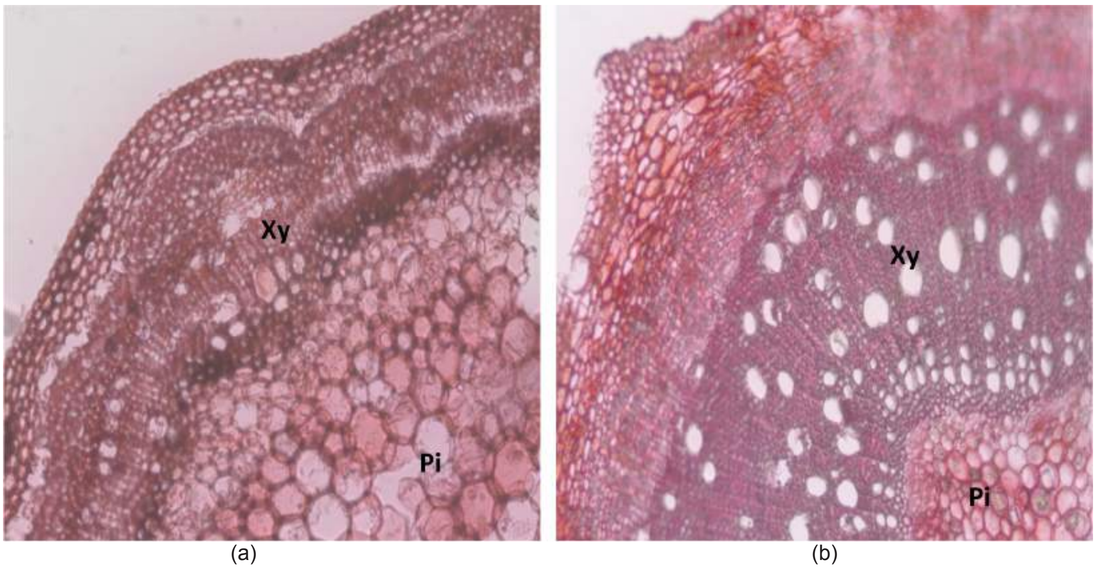


Figure 10 Transverse section of minicutting (10-cm length) in (a) *Leucaena* with narrow xylem (Xy) and larger proportion of pith (Pi) and (b) *Eucalyptus camaldulensis* depicting larger area of xylem and smaller pith region

Minicuttings of 6–10 cm length are normally used in eucalypts (Santana et al. 2013) but in *Leucaena* such cuttings do not perform well as they are quite soft and anatomically quite different from that of a typical *Eucalyptus* minicutting (10 cm length). At positions 1 and 2 from shoot apex both nodal and lateral stem cuttings of *Leuceana* had larger proportion of pith and lower rooting rate compared with eucalypt cuttings of the corresponding positions. Lateral shoot cuttings of 25–30 cm length (position 4) had lower pith area, similar to *Eucalyptus*, and a combination of low vessel diameter and high frequency compared with nodal cuttings, which enabled higher rooting than smaller sized cuttings. *Eucalyptus* cuttings from all positions (both mini and nodal) had large number of low diameter vessels and low or comparable pith proportion as *Leucaena*, enabling good rooting and survival. Based on the anatomical configuration and rooting potential the optimum length of lateral shoot cutting was standardised as 25–30 cm with two to three pairs of leaves. In *Azadirachta indica*, minicuttings did not root well when 30–35 cm long cuttings of less than 0.5 cm diameter were used (Gehlot et al. 2014). *Casuarina equisetifolia* had better rooting response in lateral shoot cuttings compared with terminal shoots (Rout et al. 1996). Lateral shoot cuttings in *Leucaena* had lower diameter than nodal cuttings, and different anatomical configuration than vertically growing shoots. Dick et al. (1999) reported good rooting in nodal cuttings taken from nodes 5–13 from the apex with stem diameter ranging from 2.9 to 5.5 mm, which was within the range observed in the current study. However, rooting was quite variable (9–71%) possibly due to variability in anatomy of the cuttings. A continuous ring of sclerenchyma formation around the site of adventitious root formation is often observed in woody plants as a barrier to root emergence (White & Lovell 1984) but with rejuvenation the sclerenchyma layer can be reduced into broken patches, enabling root emergence (Pijut et al. 2011) as observed in lateral cuttings of *Leucaena*.

Juvenility induction is associated with changes in anatomy such as increase in proportion of pith and width of vessel elements (Husen & Pal 2006, Wendling et al. 2014). In *Leuceana* juvenility was induced when stock plants were pruned, but rooting capacity was influenced by the difference in anatomical structure of vertically growing coppice shoots compared with lateral shoots. These results are in line with the conclusion drawn by Bryant and Trueman (2015) that anatomical structure of stem determines the number of roots formed in cuttings.

Rooting and root architecture

Retaining shoot tip for supply of endogenous auxin is crucial for successful rooting in *Leucaena* compared with exogenous application in nodal stem cuttings. In many eucalypt species, exogenous application of IBA (5000 ppm) sufficiently provides the hormone required for root induction when shoot apex is removed. Hence, *E. grandis*, *E. camaldulensis* and *E. saligna* have been successfully propagated using nodal cuttings (Fogaca & Fett-Neto 2005). Minicutting with shoot tip provides superior quality plants with tap root-like root system and uniformity in propagules (Assis et al. 2004). Time taken for root development may vary with species, but if shoot tip is removed in minicutting the rooting period is generally extended. If root induction is delayed due to desiccation, then rooting would be affected in nodal cuttings as the applied exogenous hormone may have side effects on rooting (Pop et al. 2011). Minicuttings of *E. benthamii* × *E. dunnii* hybrids without shoot tip only developed callus after 14 days, and first adventitious root emerged after 21 days extending up to 56 days (Brondani et al. 2012). In the current study, white soft roots emerged from the bark of *Leucaena* lateral cuttings in 8–10 days, along 2-cm length from the base. Root primodia emerged from the region between bark and cambium without callus formation. In nodal cuttings, rooting was delayed ranging from 20 to 25 days with callus formation seen in some cuttings. Wilson (1993) reported similar situation in *E. globulus* when rejuvenation increased rooting with direct emergence of roots whereas in older cuttings rooting was indirect with callus formation. Nodal cuttings suffer physiological shock resulting from loss of water in tissues and transpiration depending on the time taken for processing cuttings (Mesén et al. 1997). *Leucaena* cuttings need to be treated with care and processed in the shortest time possible as the leaves droop with loss of turgor due to the pulvinus action. Lateral shoots harvested with shoot tip were comparatively thin and impact of pulvinus on physiology of cuttings was less and leaves recovered quickly with misting in all three clones. Endogenous auxin level appeared to be adequate since exogenous IBA application did not cause any significant impact on rooting in lateral cuttings.

In the current study exogenous hormone application reduced rooting in nodal cuttings (though not statistically significant) because longer persistence of hormone in free form would inhibit rooting (Fogaca & Fett-Neto 2005). The presence of shoot tip however improved rooting in combination with exogenous application of rooting hormone in lateral cuttings of CMA. Exogenous application might help in attaining peak auxin concentration in thick cuttings with shoot tip, leading to improved rooting success although it was still low compared with lateral cuttings from sand beds.

Shi and Brewbaker (2006) suggested retaining the entire leaf in nodal cuttings to reduce the time needed for rooting in *Leucaena* hybrids, but the time they reported for root initiation was 24 days. The authors also reported high correlation of mortality of cuttings with leaf shedding, which affected not only root initiation but also root development. Leaf shedding is common in nodal cuttings if roots do not emerge within two weeks. The same trend of extended mortality of nodal cuttings during hardening stage was seen in the current study. It is quite common to see new shoots emerging from nodal cuttings during misting but they do not contribute to root development if the original leaves are shed (Shi & Brewbaker 2006). The authors reported that nodal cuttings produced an average 7–8 roots which was in line with that obtained in the current study. The presence of shoot tip in lateral cuttings along with 2–3 leaves not only increased the number of primary roots by almost three times but also improved the distribution. The propagation method with lateral shoots not only gave high rooting rate but also significantly higher number of roots along greater length of the cutting.

CONCLUSIONS

Mass vegetative propagation of *Leucaena* could be achieved as in *Eucalyptus* by lateral shoot production through controlled intensive management of stock plants in sand beds. This technique gave superior quality plants without use of rooting hormone and minimum seasonal effect. Mortality and lodging in plantations can be reduced by this propagation method as lateral cuttings produced three times more roots with balanced distribution compared with nodal cuttings. Lateral shoots of 25–30 cm length had different anatomical configuration of lower pith area and higher numbers of low diameter vessels which reduced post-harvest shock, enabling early root induction compared with conventional nodal cuttings that had fewer large diameter vessels and higher mortality during propagation.

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