Lahuradewa Lake, Sant Kabir Nagar District, Uttar Pradesh, India. *Man Environment*, 2009, **XXXIV**(1), 88–100.
35. Saraswat, K. S., Sharma, N. and Saini, D. C., Plant economy at ancient Narhan (ca. 1300 BC–300/400 AD). In *Excavations at Narhan – 1984–1989* (ed. Singh, P.), Banaras Hindu University, Varanasi, 1994, pp. 255–346.
36. Saraswat, K. S. and Pokharia, A. K., Annual Report 2003–2004, Birbal Sahni Institute of Palaeobotany, Lucknow.
37. Harvey, L. E. and Fuller, D. Q., Investigating crop processing using phytolith analysis: the example of rice and millets. *J. Archaeol. Sci.*, 2005, **32**, 739–752.
38. Fuller, D. Q., Agricultural origins and frontiers in South Asia: A working synthesis. *J. World Prehist.*, 2006, **20**, 1–86.
39. *Indian Archaeology: A Review*. 1962–1963, pp. 6–8; 1968–1969, pp. 5–6; 1969–1970, pp. 3–4; 1970–1971, pp. 6–7; 1971–1972, pp. 6–7.
40. Varma, B. S., Black-and-red ware in Bihar. In *Potteries in Ancient India* (ed. Sinha, B. P.). The Department of Ancient Indian History, Culture and Archaeology, Patna University, Patna, 1969, pp. 102–111.
41. Vishnu-Mittre, Neolithic plant economy at Chirand. *Palaeobotanist*, 1972, **21**, 18–22.
42. Singh, B. P., Early farming communities of Kaimur foothills. *Puratattva*, 1990, **19**, 6–18.
43. Singh, P., *Excavations at Narhan (1984–1989)*, Banaras Hindu University, Varanasi, 1994.
44. Singh, P., Archaeological excavations at Imlidih Khurd – 1992. *Pragdhara*, 1993, **3**, 21–35.
45. Singh, B. P., Khairadih – a Chalcolithic settlement. *Puratattva*, 1989, **18**, 28–34.
46. Singh, P. and Singh, A. K., Trial excavations at Waina. *Pragdhara*, 1996, **6**, 41–61.
47. Tewari, R., Srivastava, R. K., Saraswat, K. S. and Singh, K. K., Excavations at Malhar 1999: a preliminary report, District Chandauli (UP). *Pragdhara*, 2000, **10**, 69–98.
48. Saraswat, K. S., Plant economy in ancient Malhar. *Pragdhara*, 2004, **4**, 137–171.
49. Misra, V. D., Pal, J. N. and Gupta, M. C., Further excavations at Jhusi. *Pragdhara*, 2000, **10**, 23–30.
50. Pokharia, A. K., Pal, J. N. and Srivastava, A., Plant macro-remains from Neolithic Jhusi in Ganga Plain: evidence for grain-based agriculture. *Curr. Sci*., 2009, **97**(4), 564–571.
51. Tewari, R., Srivastava, R. K. and Singh, K. K., Dadupur excavations 1999–2000–2001, District Lucknow, Uttar Pradesh: Preliminary report. *Pragdhara*, 2002, **12**, 99–116.
52. Anon., *The Wealth of India: Raw Materials* and *Industrial Products, Vol. III* (Revised): Ca–Ci, CSIR, New Delhi, 1992.
53. Anon., *The Wealth of India: Raw Materials, Vol. VI: L-M*, CSIR, New Delhi, 1962.
54. Anon., *The Wealth of India: Raw Materials, Vol. III.: D-E*, CSIR, New Delhi, 1952.
55. Anon., *The Wealth of India: Raw Materials, Vol. IX: Rh-So*, CSIR, New Delhi, 1972.
56. Anon., *The Wealth of India: Raw Materials, Vol. II: C*, CSIR, New Delhi, 1950.
57. Anon., *The Wealth of India: First Supplement Series* (*Raw Materials*), *Vol. 5: R-Z*, CSIR, New Delhi, 2004.

ACKNOWLEDGEMENTS. I thank the Director, BSIP, Lucknow for encouragement and providing the necessary facilities. I also thank the Director, UP State Archaeology Department, Lucknow for providing an opportunity to collect botanical remains during the course of excavations.

Received 31 March 2011; revised accepted 29 November 2011

Clonal propagation in *Eucalyptus camaldulensis* using minicutting technique

H. Bindumadhava[1,*], Jagdish Tamak[2], K. Mahavishnan[1], A. P. Upadhyay[1], Mohan Varghese[1] and N. Sharma[1]

[1]Corp R&D, ITC R&D Centre, ITC Ltd, Peenya Industrial Area, Peenya, Bangalore 560 058, India
[2]PSPD Unit, ITC Ltd, Sarapaka, Bhadrachalam 507 128, India

Efficient nursery management with rapid and cost-effective clonal propagation is a prerequisite for successful plantation. Mass propagation has become an important tool for increasing the competitiveness of the forest-based industry. However, in several hardwood species, most notably in eucalypts, the popular stem-cutting method poses limitations in rooting behaviour, such as rapid loss of rooting competence, intra-clonal variation and poor rooting quality which collectively negates genetic expression of some useful clones thereby hindering field deployment.

To overcome production barrier, a study was initiated using novel minicutting-based propagation with a primary objective of reducing the nursery duration from six to four months and in the process improving its productivity. To cater to this need, the hydroponic-aided minicuttings production technique for *Eucalyptus camaldulensis* has been standardized in India. The success lies in the plant nutrition management to get maximum harvestable sprouts. Further, as an imperative step to get vigorous saplings from minicutting sets, an efficient, ecosand-based growing medium was employed to boost survival rate, rapid rooting and early establishment.

Keywords: Clonal propagation, coppice, ecosand, hydroponics, minicuttings.

RED GUM (*Eucalyptus camaldulensis* L.) is renowned globally for its fast growth, high levels of drought tolerance and adaptability to diverse climatic conditions and soils, which makes it popular among eucalypt tree growers. Clonal propagation is an extensively used strategy to gain economic potential of eucalypt species/hybrids by multiplying desirable types. With moderate degree of sophistication in most forest nurseries, it is performed to strategically improve the productivity. Since yields from eucalypt forestry will continue to increase with improved clones and silvicultural methods, the availability of a highly reliable and cost-effective propagation technique is required[1]. The conventional (stem-cutting) technique, though the most common and widely used propagation method, suffers due to intrinsic genetic and physiological limitations. For instance, poor rooting and rapid loss of

*For correspondence. (e-mail: bindu.madhava@itc.in)

rooting competence due to ontogenetic aging, intra-clonal variation resulting from topophysis, and poor quality of the root system, negatively affect genetic expression of some clones, which consequently affects their deployment in the planting programme[2].

Further, poor rooting of certain clones derived from conventional cuttings is a major constraint to cloning, and has been attributed to the maturation degree of the plant material[3,4]. Since demand is burgeoning and nursery production units are unable to supply vigorous seedlings from the conventional coppice-producing stream (time constraint), surrogate propagation methods are being looked into.

Amongst such *in vitro* techniques[5–8], the minicutting technique[9,10] led to considerable gains, mainly through increasing the proportion of rooted plants and cutting short production time. Pioneer work by Assis *et al.*[11] led to the development and application of minicutting technique for *Eucalyptus* propagation.

Minicuttings have shown great potential in offering technical and economic advantages not available from conventional stem-cuttings. The minicutting system is based on the rooting of auxiliary shoots from rooted stem-cuttings. Field clonal hedges are replaced by indoor hydroponic mini-hedges, which provide plantlets or rooted cuttings with a high degree of juvenility. The success of the system is also dependent on optimal nutrient status in the resulting minicuttings. Compared to stem-cuttings, the minicutting system has improved rooting potential and speed coupled with quality at reduced costs.

Currently, this technique is being expanded rapidly and is most widely used by the forestry companies[9] under commercial operation in Brazil, Australia and South Africa[12,13] for species *E. grandis*, *E. globulus*, *E. nittens*, etc. Another important consideration for adopting minicuttings in clonal propagation is the space requirement for maintaining hedge plants. It is minimal compared to coppice produced in the field. For example, to meet the demand of 20 million cuttings per annum through the coppice route, the land required will be approximately 55 acres, whereas the minicutting route requires just two acres.

Additionally, this system offers propagules with high uniformity and low topophysis effects. The development of this super-intensive cloning system has set a stage for the new generation of mass vegetative propagation in eucalypts and other hardwood species[14]. Subsequent to minicuttings production, these young saplings need to be nurtured in proper growing/rooting medium for higher survival and development of vigorous early root system. An ideal growing medium used in a commercial nursery should not only be cost-effective, but also free from weeds and diseases. Further, the medium should be light in weight, well drained and yet retain sufficient moisture. Once a novel and proper growing medium is identified, high-yielding clones can be mass-multiplied and used for commercial forestry, where continuous and reliable supply of stock is the need of the hour.

In this backdrop with an objective to improve the nursery production and reduce its cycle (duration), a comprehensive study in selected clones and hybrids of Red Gum species was conducted at the Clonal Production Centre, PSPD Unit, ITC Ltd, Bhadrachalam, Andhra Pradesh, India.

First, 60–65-day-old eucalyptus plants from the open nursery (produced from conventional coppice shoot method) were selected and kept in a nutrient (mix) tank for 25 days (housed in a polythene enclosure with air temperature 33–35°C, relative humidity (RH) 60–65% and natural sunlight with 30% cut-off). These hedge stumps practically act as the source of minicuttings. The nutrient mix (NM) was fed continuously (cycled and recycled) using motorized pump (to create aeration; Figure 1). The DO (dissolved oxygen), pH and EC (electrical conductivity) of the NM were maintained continuously (using portable DO, pH/EC meters) to facilitate proper ion exchange, nutrient uptake and respiration by the roots. The NM was replaced once every fortnight. On the 25th day, minicuttings from these hedge plants were harvested/cut and continued till 30th day as and when sets were ready for harvest. A total of 25,000 hedge plants were maintained in 20 nutrient tanks to produce 1.5 lakh minicuttings in a span of five months. These sets

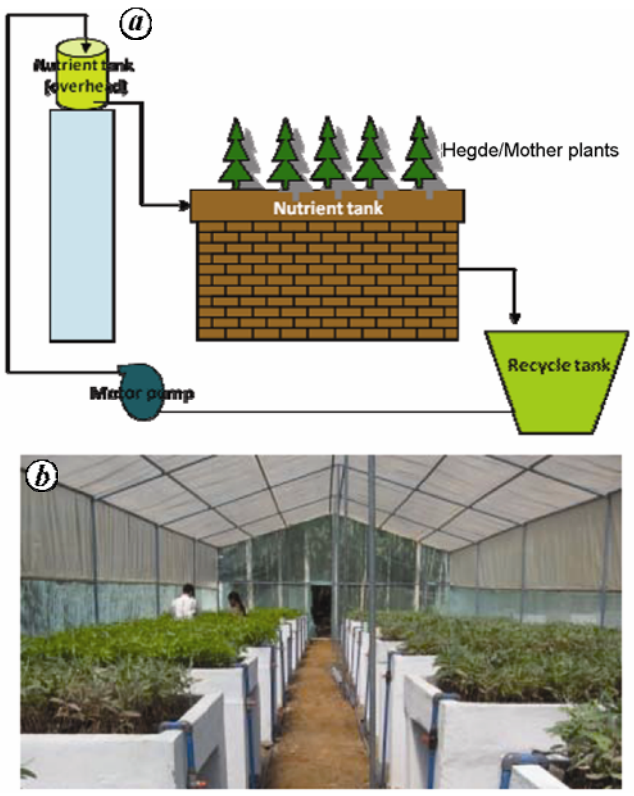

Figure 1. ***a***, Initiating the process of minicuttings production; ***b***, actual production area.

were placed immediately in rooting trays containing ecosand (E) : vermiculite (V) : compost (C) mix, and housed in mist chamber (regulated misting to maintain a temperature of 32–35°C and RH of 80–85%) for another 25 days. Subsequently these seedling trays were shifted to hardening area (under 50 : 50 shade with regulated water sprinklers) for 10 days. The hardened seedlings were moved to the open (nursery) area (completely under natural environment) and irrigated twice daily until they were lifted for field planting. After 60–70 days in the open nursery, 100 saplings derived from both minicutting and coppice source were planted in the field to examine their acclimatization and growth behaviour.

As plants require all 16 essential elements to complete their life cycle, the usage of different nutrients depends on the plant type. In tree species (perennials) due to its hardy wood, there is an alteration in tissue constituents, which leads to either higher dose of nutrient requirement or preferential uptake, i.e. need of one or more elements in higher dosage at a particular growth stage. Thus a few modifications of the standard Hoagland solution (SHS) were essential, similar to those required for *Eucalyptus* species such as *globulus*, *grandis* or *nitens*.

Generally, in commercial clonal multiplication system through hydroponics, SHS is used, which is more directed towards improving growth of annual crops (agriculture/horticulture) and its nutrient composition is mostly suited to tissue composition/constituents of crop plants (Table 1). However, in the present study, we have modified SHS and come out with a unique NM specifically for *E. camaldulensis* that provided improved hedge plant sprouts and lateral growth.

The rooting growth medium, ecosand, has the unique ability to absorb, hold, release and exchange different nutrients/ions. It is a mineral with infinite, three-dimentional, honey-comb-like structure that allows losing and gaining water reversibly. Being negatively charged (by nature) makes it attract certain cations. The added benefit is it does not break down over time, but remains in the soil to help in improve nutrient and water retention.

Table 1. Unique nutrient mix for hedge plants and minicuttings

Ions/molecules	Standard Hoagland solution (g/1000 l)	Unique nutrient mix (g/1000 l)
KNO_3	505	1010–1050
$CaNO_3$	1180	900–1000
KH_2PO_4	136	505–550
$MgSO_4$	490	600–650
$FeSO_4$	15	50–75
EDTA	20	25–40
$MnCl_2$	1.81	35–40
$CuSO_4$	0.08	15–25
$ZnSO_4$	0.22	25–50
H_3BO_3	2.86	15–25
Ammonium molybdate	0.01	10–20

Acting as a natural wetting agent, it is an excellent amendment for non-wetting sands and assists water distribution through the soils. The ecosand contains high concentration of silica, which provides physical strength to growing saplings at the early stages itself (Table 2). Properties such as higher cation exchange capacity (CEC) and bulk density facilitate enhanced water retention and slow nutrient release. In the present study, ecosand was used as a supplement to vermiculite in *Eucalyptus* nursery for realizing its potential.

In the present study a mixture of ecosand with vermiculite and leaf compost as a rooting medium was used for minicuttings made from hedge plants for rooting and early establishment. A set of control stem cuttings (wherein only vermiculite and leaf compost were used) from the coppice source was also kept for comparison.

To quantify variations in physiological traits, assimilation rate (A), stomatal conductance (g_s) and transpiration rate (T) were recorded using a portable photosynthesis system (LiCOR 6400, Nebraska, Lincoln, USA), in both coppice and minicutting-grown plants. On 30th day after tray planting, the third, fully expanded leaf from the apex from three different plants of the same species (three-replicates) was selected for measurement. After this, chlorophyll content was recorded on the same leaf, using Chlorophyll meter (SPAD-502, Minolta, USA), which is also considered as an indirect measure of leaf nitrogen and protein content[15–17].

Total soluble protein in the leaves of open nursery and field-grown plants was extracted and estimated using the simple protein-dye binding method of Bradford[18]. About 0.5 g of fresh leaf material was ground to a thin paste and soluble proteins were extracted with 1.5 ml Tris

Table 2. Chemical and physical properties of ecosand (natural zeolite)

		Composition (%)
Chemical properties		
Silica (SiO_2)		68.10
Alumina (Al_2O_3)		10.70
Potassium (K_2O)		4.30
Calcium (CaO)		2.20
Iron (Fe_2O_3)		1.70
Sodium (Na_2O)		0.60
Magnesium (MgO)		0.50
Loss on ignition (H_2O)		11.50
Clinoptilolite (%)		75–90
Cation exchange capacity (meq/100 g)		
Zeolite method		150–180
Soils method		80–110
Physical properties		
Bulk density		56 lb/cubic ft
Particule size	Size (mm)	Per cent
	1.0–1.4	30
	0.5–1.2	55
	0.25–0.5	10

buffer (0.1 M Tris, 0.02 M sodium sulphite, 5 mM β-mercaptoethanol, 200 mM benzidamine, 200 mM PMSF, PVPP-4%). The extract was centrifuged in cold at 10,000 rpm for 10 min. Then 5 µl of the cell-free extract of leaf proteins was added to 3 ml of Bradford reagent and mixed immediately. Next, 5 µl of buffer was added to Bradford reagent instead of cell-free extract for control (blank). The absorbance of the solution was recorded at 595 nm, after 3 min and within 30 min using a spectrophotometer (GENESYS 2, Milton Roy, USA). The data were statistically analysed using standard ANOVA technique[19].

The hedge plants were grown in liquid nutrient medium to harvest minicuttings. Formulation of the nutrient medium was done to get vigorous growth of hedge plants in order to increase harvest cycles. The nutrient solution mix is a modified SHS, which is normally (in hydroponics) a blend of nutrients (containing essential elements) largely recommended for crop plants (annuals). In the present study, the unique NM provided excellent results in inducing more number of new roots and proliferation, root and shoot growth with better leaf area and greenness under commercial nursery. Apart from this, plants fed with NM showed improved intrinsic physiological traits such as more leaf nitrogen content, carbon fixation rates, carboxylation efficiency and maintained higher leaf water status than SHS-fed plants.

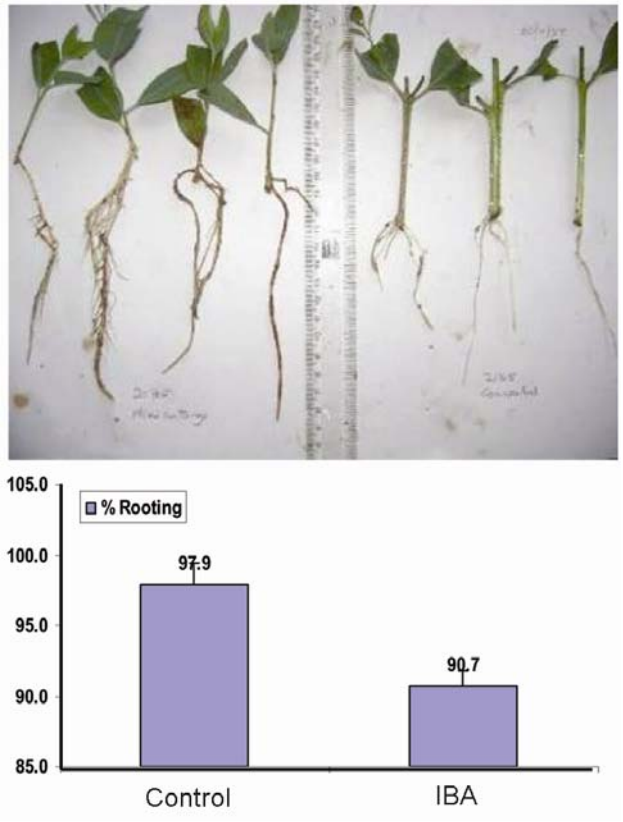

Figure 2. Efficient rooting behaviour of minicutting-derived plants and rooting percentage in minicuttings and coppice-based plants.

As plants require all the 16 essential elements to complete their life cycle, their usage will be plant-dependent. In tree species (perennials) due to its hardy wood nature, there is an alteration in tissue constituents which leads to either higher dose of nutrient requirement or preferential uptake, i.e. need of one or more elements in higher dosage at a particular growth stage[20]. This is the basis for modifying SHS to meet the demand of nutrients of *E. camaldulensis* in a particular combination. We found that this modification led to continued production (5–6 harvest cycles) of minicuttings from the hedge plants (source), whereas normal HS could support minicutting production for only 2–3 harvest cycles. The percentage of rooting was also significant (Figure 2), suggesting that native auxin level was sufficient to induce rooting. Since the root system developed in minicuttings during initial organogenesis is similar to tap roots, when planted in field they grow vigorously leading to early establishment.

Minicuttings made from the hedge plants showed efficient rooting behaviour without any hormone treatment (6000 ppm indole butyric acid (IBA) was used; Figure 3). The efficient, intrinsic, physiological and biochemical traits further corroborated an apparent improvement in growth rates.

In general, there is no physiological difference between plants derived from hydroponic and/or soil source. In the soil, both organic and inorganic components must be broken into inorganic elements, such as calcium, magnesium, potassium, phosphorus, iron, etc. before they are available to the plants. These elements adhere to the soil particles and are exchanged with the soil solution where they are absorbed by the plants (H. Bindumadhava, pers. commun.).

In hydroponics, plant roots are drenched in the nutrient solution containing these elements. The subsequent

Figure 3. Root and plant growth behaviour of minicuttings with and without hormone treatment (6000 ppm indole butyric acid).

Table 3. Percentage rooting and survival in regular (control) and ecosand (growth) media

Treatment	Rooting (%)		Survival (%)	
	11 DAP	20 DAP	11 DAP	20 DAP
Control (vermiculite : compost) 8 : 2	25.0 ± 1.1	50.0 ± 0.8	75.0 ± 1.1	67.6 ± 0.3
V : E : C (3 : 1 : 1)	40.0 ± 1.6	73.7 ± 1.0	96.7 ± 2.1	93.5 ± 0.9
V : E : C (1 : 1 : 1)	37.5 ± 0.9	76.8 ± 1.9	96.8 ± 2.5	93.4 ± 0.7
V : E : C (1 : 1 : 1)	35.0 ± 1.3	60.5 ± 0.4	94.2 ± 1.1	89.8 ± 0.6
F-test	*	**	*	**
CD at $P = 0.05$	2.11	6.31	5.55	3.03

V, Vermiculite; E, Ecosand; C, Compost. Each value is an average of five replicates. CD represents the critical difference between control and treatment means at a probability level of 0.05.

Table 4. Early growth vigour difference in control and ecosand-derived plants

Treatment	Number of leaves/plant		Leaf area (cm^2/plant)		Shoot length (cm/plant)	
	30 DAP	75 DAP	30 DAP	75 DAP	30 DAP	75 DAP
Control (vermiculite : compost) 8 : 2	$4.5^a \pm 0.09$	$7.6^a \pm 0.08$	$6.1^a \pm 0.03$	$42^a \pm 1.1$	$6.4^a \pm 0.09$	$8.7^a \pm 0.02$
V : E : C (3 : 1 : 1)	$8.0^b \pm 0.07$	$11.0^b \pm 0.09$	$27.3^b \pm 0.02$	$98^b \pm 1.9$	$8.9^b \pm 0.08$	$16.7^b \pm 0.07$
V : E : C (2 : 1 : 1)	$8.0^b \pm 0.03$	$11.0^b \pm 0.1$	$30.5^b \pm 0.09$	$94^b \pm 2.1$	$8.5^b \pm 0.05$	$16.3^b \pm 0.04$
V : E : C (1 : 1 : 1)	$8.3^b \pm 0.01$	$10.0^b \pm 0.03$	$30.3^b \pm 0.10$	$87^b \pm 2.0$	$8.0^b \pm 0.01$	$15.5^b \pm 0.05$
Percentage increase over control						
V : E : C (3 : 1 : 1)	77.8	43.8	347.5	133.3	39.1	92.0
V : E : C (2 : 1 : 1)	77.8	43.8	400.0	123.8	32.8	87.4
V : E : C (1 : 1 : 1)	84.4	30.7	396.7	107.1	25.0	78.2

Values were statistically significant at $P = 0.01$. Each value is an average of five replicates. All the treatments at each observation period (as represented by different alphabets) showed more or less similar significant effect over control in the present study.

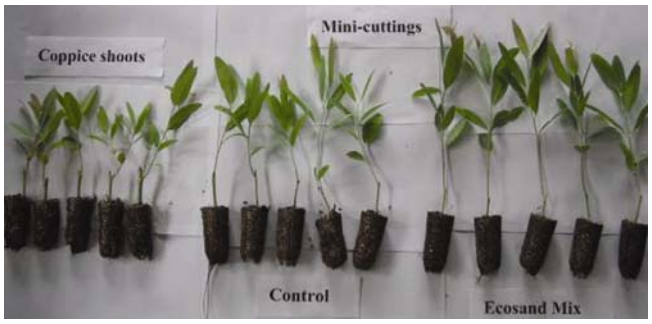

Figure 4. Effect of the ecosand mix on initial growth of minicutting (saplings from the same stage were used for field planting).

mineral uptake by the plants is the same. The growth difference between soil and hydroponics media is mainly due to the following reasons: (1) Hydroponically grown plants get balanced nutrient solutions during early growth stage, which are available to the roots directly at the right time. (2) For hydroponic plants, all nutrients are present in the liquid mixture, and so root system does not have to be as extensive as for soil-based plants. Hence plants can devote more nutrients and energy in improving above-ground parts. However in the soil, root systems need to spread all around to increase surface area. Hence the chances for plants to absorb minerals are relatively low[21].

As emphasized earlier, usage of ecosand as the growing medium in eucalypts is itself unique and novel. Apart from having unique features of higher water holding capacity (WHC) and ion exchange, ecosand also aids in the slow release of nutrients when blended with other rooting/growing media. Young saplings had early rooting and establishment compared to contemporary medium, thereby making them healthy to utilize available nursery resources efficiently. There was an improvement of 20% in seedling survival rate over contemporary medium (Figure 4).

The saplings were initially grown under NM solution (hydroponically). Once the hedge plants were ready, minicuttings were made and planted in ecosand mix medium for rooting for survival. Different combinations of ecosand with vermiculite and leaf compost have been tried to get the desired effect on rooting percentage and sapling survival. Though the experiments were conducted up to 30 DAP (days after planting), as initial survival is the crucial part in nursery management, we present data of 20 DAP. Between 20 and 30 DAP, a marginal difference in rooting and survival percentage was observed (Table 3).

The number of leaves, leaf area and shoot length also showed marked increase in the ecosand-based mix. Once survival is assured, the effect needs to be observed until

saplings move out of nursery. Hence data up to 75 DAP have been provided (Table 4).

Modified NM strengthens the growth and vigour of hedge plants by providing enough nourishment, which facilitates the harvest of more minicuttings in less time. Ecosand helps in improving the rooting ability and survival rates by holding water for long (wetting agent) and releasing required nutrients slowly[22]. Together, these two interventions make a viable blend in improving overall vigour of seedlings, thus reducing the nursery duration by two months, which has enormous potential from the point of view of nursery production scale and cost benefits in the *Eucalyptus* industry[23].

Further, in order to examine the reason behind the growth rate differences among plants derived from minicuttings (enriched with ecosand) and coppicing, various intrinsic physiological traits were determined. The hedge plants treated continuously with nutrient solution and the minicuttings made out of them (13–15 days after setting), showed considerable improvement in intrinsic carbon fixation (photosynthesis) and associated physiological traits along with leaf nitrogen status compared to coppice plants, thus indicating their elevated intrinsic physiological efficiencies (Figures 5 and 6). On comparison, the performance of minicuttings appreciably outplayed the coppice plants (similar age; Table 5) in overall growth behaviour. The minicuttings resembled a miniature plant with more vitality (ready to plant in the field; Figures 4 and 7 *a*).

To substantiate further, total soluble protein (TSP) content from each set plant was extracted and determined. The values suggested that leaves of microcuttings had higher TSP both in the open nursery as well as in the field conditions (Figure 8) compared to their coppice-shoot-grown plants.

Plants grown using minicutting technique possess largely all the intrinsic physiological and biochemical efficiencies, thus facilitating them to acclimatize early and perform better under any given natural field conditions. Hence they are superior compared to conventional coppice plants.

Field-established plants (from minicuttings) were compared with plants of the same age grown from coppice shoots at an interval of every month for six months. Once the saplings (whether from minicuttings or coppice shoot)

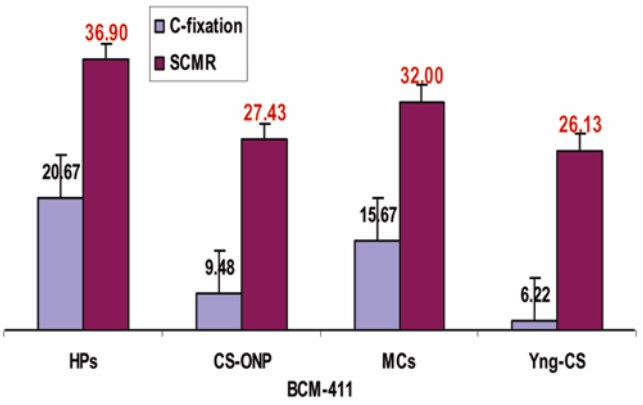

Figure 5. Percentage difference in net photosynthesis rate (*Pn*) and leaf chlorophyll index (measured through SPAD) in hedge, open nursery, coppice and minicutting plants.

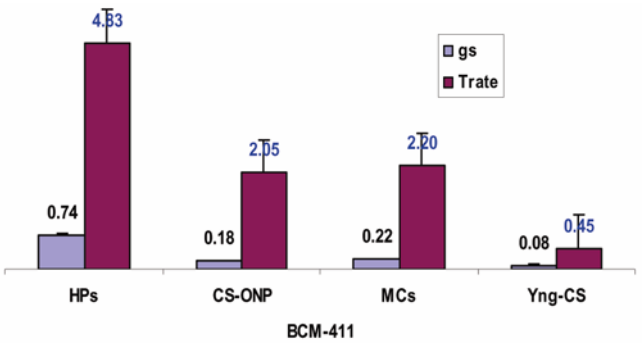

Figure 6. Percentage difference in stomatal conductance (g_s) and transpiration rate (*T*) in hedge, open nursery, coppice and minicuttings.

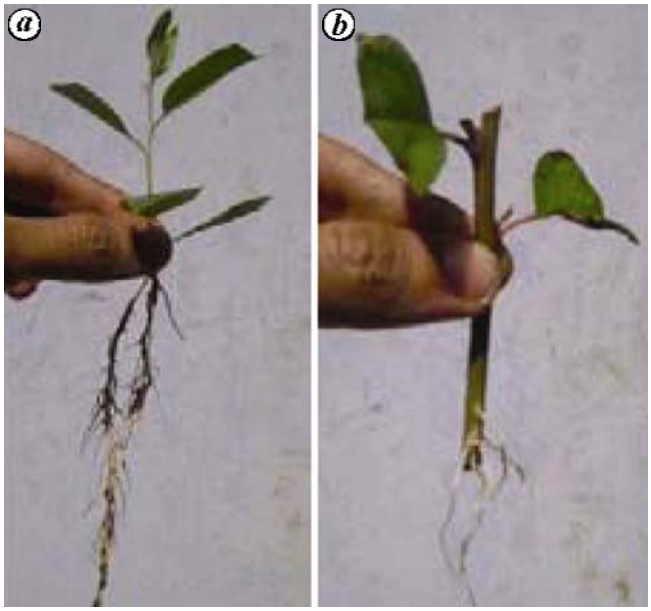

Figure 7. Miniature plant from minicutting (25-day-old) with tap roots (***a***) and coppice plant (25-day-old) with lateral roots (***b***).

Table 5. Percentage increase in physiological traits in hedge plants and minicuttings over coppice plants

	Percentage increase		
	C-fixation	SCMR	*T*-rate
Hedge plants	118	35	125
Minicuttings	151	22	193

C-fixation, Carbon fixation (μmol m^{-2} s^{-1}), SCMR, SPAD Chlorophyll Meter Reading, a unit-less, invasive index for leaf nitrogen content; *T*-rate; Transpiration rate (mmol m^{-2} s^{-1}).

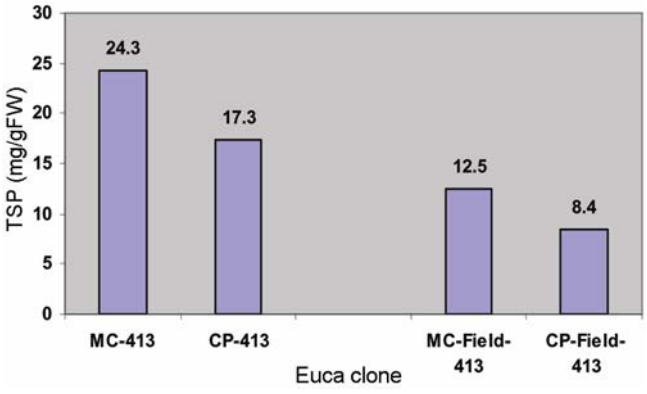

Figure 8. Total soluble protein content (mg/g fresh wt.) in minicutting plants (MC) and coppice-grown plants (CP).

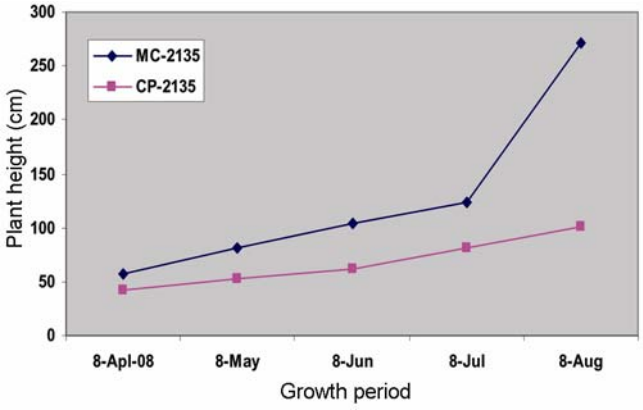

Figure 9. Increment in plant height (cm) of field-grown MC and CP.

Box 1. Process overview

Regular practice (conventional method):

Coppice shoots ⇨ Mist Chambe ⇨ Hardening ⇨ Open nursery ⇨ Plantable propagules

50–60 days + 30–35 days + 10 days + 75–90 days = 6 months

Game changer practice (minicutting method):

Minicuttings ⇨ Mist chamber ⇨ Hardening ⇨ Open nursery ⇨ Plantable propagules

25 days + 25 days + 10 days + 60 days = 4 months

were planted in the field, no external supply of nutrient solution was given to the minicuttings (native soil nutrients were the only source). Since minicuttings had luxurious consumption of nutrients during production stage, they probably continued to retain the same ability of higher intrinsic physiological traits during the initial period of field establishment also compared coppice-grown plants.

Increment in plant height and leaf area of saplings derived from minicuttings is considerably high in relation to coppice plants of similar age (composite of all plant sets planted at different time-periods) during similar growth period (3 months after planting; Figure 9). To examine the reasons for improved performance, intrinsic physiological traits were determined, which indicated marked differences in carbon fixation traits coupled with transpiration associated water relation traits. The extent of enhancement of C-fixation rates and leaf N traits was to a tune of 48–33% respectively. Similar increment in transpiration rates (represents water harnessing ability of roots) was around 28%.

Minicuttings have the following several operational, technical, economical, environmental and quality advantages over conventional stem cuttings.

- Operationally, many field activities like soil preparation, fertilization, irrigation, cultivation, weeding, pest and disease control, transportation of cuttings, etc. are replaced by intensive activities in smaller indoor areas.
- Cost incurred on labour, fertilizer, irrigation and chemicals in an extensive outdoor clonal hedge management system will be reduced.
- Managing indoor clonal hedges will be much easier than managing large area in field conditions.
- The rooting ability of minicuttings is much higher than stem-cuttings.
- Increased rooting due to higher levels of juvenility and optimal nutritional content in the tissues, which improves rooting predisposition and speed of root initiation.

- The rooting speed of minicuttings has important consequences in a commercial cloning programme because the time spent by the plants in the mist chamber is usually reduced significantly compared to rooting stem-cuttings, thus considerably improving the effective use of the infrastructure available.
- Minicuttings produce a robust root system with a tendency for a taproot-like system in contrast to the predominant lateral root system in the stem-cuttings.
- Reduced time – plants come out of the mist chamber quickly (fewer disease problems) and the time taken from setting to production of plantable seedlings is much shorter than stem-cuttings.
- Apparently the link between root and stem tissues in minicuttings is more suitable due to lower lignification of connecting tissues, and hence more responsive to fertilization.
- Seedling quality – higher ability to produce roots, better root and stem systems.

Box 1 provides an overview of the process. Minicuttings take 25 days for rooting in the mist chamber and 55–60 days in open nursery to attain the desired growth for planting in the field, thus offering an added advantage of two full months of nursery duration.

1. Zobel, B. J., Clonal forestry in the *Eucalyptus*. In *Clonal Forestry: Conservation and Application* (eds Ahuja, M. R. and Libby, W. J.), Springer-Verlag, Budapest, 1993, vol. 2, pp. 139–148.
2. Xavier, A., Comerio, J. and Iannelli, C. M., Eficiência da microestaquia e da micropropagação na clonagem de *Eucalyptus* spp. In Proceedings of the IUFRO Conference on Silviculture and Improvement of Eucalypts, Salvador, 1997, Embrapa Florestas, Colombo, 1997, vol. 4, pp. 40–45.
3. Gomes, Propagação clonal: princípios e particularidades. Vila Real: Universidade de Trás-os-Montes e Alto Douro (Série Didáctica, Ciências Aplicadas 1); Greenwood, M. S. and Hutchison, K. W., Maturation as a development process. In *Clonal Forestry: Genetics and Biotechnology* (eds Ahuja, M. R. and Libby, W. J.), Springer-Verlag, Budapest, 1987, pp. 14–33.
4. Hackett, W. P., Juvenility and maturity. In *Cell and Tissue Culture in Forestry* (eds Bonga, J. M. and Durzan, D. J.), Kluwer, Dordrecht, 1987, vol. 1, pp. 216–231.
5. Struve, D. K. and Lineberger, R. D., Restoration of high adventitious root regeneration potential in mature *Betula papyrifera* Marsh. Softwood stem cuttings. *Can. J. For. Res.*, 1988, **18**, 265–269.
6. Gupta, P. K. and Durzan, D. J., Micropropagation and phase specificity in mature, elite Douglas fir. *J. Am. Soc. Hortic. Sci., Alexandria*, 1987, **112**, 969–971.
7. George, E. F., *Plant Propagation by Tissue Culture: The Technology*, Exegetics, London; Gomes, A. L., Propagação clonal: princípios e particularidades. In *Clonal Forestry: Genetics and Biotechnology* (eds Ahuja, M. R. and Libby, W. J.), Springer-Verlag, Budapest, 1993, pp. 14–33.
8. Hartmann, H. T., Kester, D. E., Davies Jr, F. T. and Geneve, R. L., *Plant Propagation: Principles and Practices*, Prentice-Hall, New Jersey, 1997, 6th edn, p. 770.
9. Xavier, A. and Wendling, I., Miniestaquia na clonagem de *Eucalyptus*. *Viçosa*, 1998, p. 10.
10. Wendling, I., Xavier, A., Gomes, J. M., Pires, I. E. and Andrade, H. B., Propagação clonal de híbridos de *Eucalyptus* spp. por miniestaquia. *Rev. Árvore, Viçosa*, 2000, **24**, pp. 181–186.
11. Assis, T. F., Rosa, O. P. and Goncalves, S. I., Propagaçãopor microestaquia. In Congresso Florestal Estadual, 7, Nova Prata, Anais, Santa Maria, UFSM, 1992, pp. 824–836.
12. Titon, M., Propagação clonal de *Eucalyptus grandis* por miniestaquia e microestaquia. 2001. 65p. Dissertação (Mestrado em Ciência Florestal) – Universidade Federal de Viçosa, Viçosa, 2001, Recebido em 02/06/2004 Aceito para publicação em 25/05/2006.
13. Titon, M., Xavier, A. and Otoni, W. C., Clonal propagation of *Eucalyptus grandis* using the mini-cutting and micro-cutting technique. *Sci. For.*, 2006, **71**, 109–117.
14. Assis, T. F., Propagação vegetativa de *Eucalyptus* pormicroestaquia. In Proceedings of IUFRO Conference on Silviculture and Improvement of Eucalypts, Salvador, 1997. Embrapa Florestas, Colombo, 1997, vol. 1, pp. 300–304.
15. Nageswara Rao, R. C., Talwar, H. S. and Wright, G. C., Rapid assessment of specific leaf area and leaf nitrogen in peanut (*Arachis hypogaea* L.) using chlorophyll meter. *J. Agron. Crop Sci.*, 2001, **189**, 175–182.
16. Bindumadhava, H., Sheshshayee, M. S., Shankar, A. G., Prasad, T. G. and Udaya Kumar, M., Use of SPAD Chlorophyll meter to assess transpiration efficiency in peanut. In ACIAR Proceedings 112, Breeding of Drought-Resistant Peanuts, 2003, pp. 3–9.
17. Sheshshayee, M. S., Bindumadhava, H., Rachaputi, N. R., Prasad, T. G., Udaya Kumar, M., Wright, G. C. and Nigam, S. N., Leaf chlorophyll concentration relates to transpiration efficiency in Peanut. *Ann. Appl. Biol.*, 2005, **148**, 7–15.
18. Bradford, M., A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*, 1976, **72**, 248–254.
19. Sunderraj, N. and Nagaraj, S., *Design and Analysis of Field Experiments*, 1972, pp. 1–412.
20. Bonga, J. M., Vegetative propagation in relation to juvenility, maturity and rejuvenation. In *Tissue Culture in Forestry* (eds Bonga, J. M. and Durzan, D. J.), Martinus Nijhoff Publishers, Boston, 1982, pp. 387–412.
21. Taiz, L. and Zeigr, E., *Plant Physiology*, Sinauer Associates Inc., Sunderland, Massachusetts, USA, 2010, 5th edn.
22. Ecosand and its usage in agricultural fields, New Bulletin, FAO, 2002.
23. Transfer of Technology Document, Novel Nursery Technique for *Eucalyptus* clonal propagation, ITC R&D Centre, ITC Ltd, Hyderabad, 2008.

ACKNOWLEDGEMENTS. We thank Dr C. C. Lakshmanan, Chief Scientist, Innovation and Technology, Corp R&D, ITC Ltd, Bangalore for his continued support during the study. We acknowledge the technical and infrastructure facility extended by Dr H. D. Kulkarni, VP (Plantations) and Dr Sanjay Singh, CEO, PSPD Unit, Bhadrachalam. We thank the technical and office staff of the Plantation Division and CPA, PSPD for timely support. B.H. thanks Priya and Sravanthi for help during initial standardization of the nutrient mix. Editorial and other useful tips from the in-house manuscript committee are appreciated. Overall support from team Corp R&D is appreciated.

Received 25 May 2011; revised accepted 29 November 2011