



## Minicutting - A powerful tool for the clonal propagation of the selected species of the *Eucalyptus* hybrid clones based on their pulpwood studies

Sowmya Kuppusamy<sup>a,b</sup>, Seenivasan Ramanathan<sup>b</sup>, Subramanian Sengodagounder<sup>b</sup>, Chinnaraj Senniappan<sup>b</sup>, Kathirvel Brindhadevi<sup>c</sup>, Thamaraiselvi Kaliannan<sup>a,\*</sup>

<sup>a</sup> Laboratory of Molecular Bioremediation and Nanobiotechnology, Department of Environmental Biotechnology, School of Environmental Sciences, Bharathidasan University, Tiruchirappalli - 620 024, Tamil Nadu, India

<sup>b</sup> Research and Development Division, Tamil Nadu Newsprint and Papers Limited, Kagithapuram, Karur - 639 136, Tamil Nadu, India

<sup>c</sup> Innovative Green Product Synthesis and Renewable Environment Development Research Group, Faculty of Environment and Labour Safety, Ton Duc Thang University, Ho Chi Minh City, Viet Nam

### ARTICLE INFO

#### Keywords:

Raw material  
Minicutting technology  
Microcutting  
Indoor sand bed hydroponics  
Clonal hedges  
*Eucalyptus* plantations

### ABSTRACT

In this study, five improved clones of the *Eucalyptus* hybrid species namely, *E.pellita* X *E.camaldulensis* (Clone No.9), *E.camaldulensis* X *E.pellita* (Clone No. 21), *E.camaldulensis* X *E.tereticornis* (Clone No.4), *E.camaldulensis* X *E.pellita* (Clone No. 16) and *E.tereticornis* X *E.urophylla* (Clone No. 41) were selected. All of these clones had an efficient micropropagation protocol which was already developed and pulping properties were studied earlier. In order to develop these clones in large scale, miniclinal gardens were developed using minicutting technology. The microcuttings derived from these micropropagated plantlets of the five different clones of the *Eucalyptus* hybrid species were grown on the sand bed. Its growth was found to be efficient through the application of the nutrient solution. Minicuttings were produced from the microcuttings taken from the micropropagated plantlets of these clones. All these clones were found to adapt the minicutting technology which was evident from the statistical analysis of its growth parameters.

### 1. Introduction

Raw material is a very important factor in an industry. Its availability should be easy and cost effective. In paper industry the supply of the raw material could not meet the demand. The raw materials constitute the wood fibers and straw fibers. *Eucalyptus* has a lot more fibers - millions - per gram, fibers which are interlinked and with a better relation of physical proportions than other fibers, which overall improves the formation of the sheet of paper. Due to urbanization and high population, the availability of land is less and so the maximum production of *Eucalyptus* is required in a limited available area. Land requirement will be less if the biomass of the *Eucalyptus* is increased. Conventional breeding can improve the production in terms of biomass and fiber quality. Improved clones can be mass propagated. Mass propagation of improved genetic material (selected genotype) or clone, produced through breeding and cloning programs are highly desirable.

Repeated production of stem cutting of *Eucalyptus* species by conventional macro propagation slowly reduces the rooting per cent in the successive generations, resulting low productivity in large scale

commercial nursery (Saya et al., 2008). This problem has been successfully solved by adopting unique method viz. indoor mini clonal gardens, where the clonal mother plants were grown in a controlled environment and nutrient management. The method is considered to be an effective and economically viable for faster and easier multiplication of the *Eucalyptus* clones (Goulart and Xavier, 2008). For the miniclinal garden, mother plants are derived from micropropagation. Micropropagated plants are the mother plants for miniclinal garden and are considered as superior for sand beds because it improves the rooting per cent of the minicutting which are grown for making pulp.

The cuttings taken from the micropropagated plantlet was referred to as the microcutting (Stuepp et al., 2017). For the propagation of *Eucalyptus* on large scale, micropropagation process is integrated with minicutting method. The micropropagated plants on the sand bed serves as the mother plants for the indoor hydroponic mini hedges (Chinnaraj and Malimuthu, 2011). These hedges provide young vegetative material for the production of minicuttings. In other words, the cuttings derived from the microstumps on the sand bed was referred to as the minicuttings. Minicuttings that are rooted are transferred from the hedges to the

\* Corresponding author.

E-mail addresses: [kathirvelbrindhadevi@tdtu.edu.vn](mailto:kathirvelbrindhadevi@tdtu.edu.vn) (K. Brindhadevi), [kthamaraiselvi@bdu.ac.in](mailto:kthamaraiselvi@bdu.ac.in) (T. Kaliannan).

<https://doi.org/10.1016/j.bcab.2019.101357>

Received 10 August 2019; Received in revised form 10 September 2019; Accepted 23 September 2019

Available online 24 September 2019

1878-8181/© 2019 Elsevier Ltd. All rights reserved.

**Table 1**List of *Eucalyptus* hybrid clones selected for minicutting studies.

Reference No.	Clone name (Male)	Species name	Clone name (Female)	Species name	Types of hybrid
9 ( <i>E.pellita</i> X <i>E.camaldulensis</i> )	E29	<i>E.pellita</i>	IFTGB EC1	<i>E. camaldulensis</i>	Inter
21 ( <i>E.camaldulensis</i> X <i>E.pellita</i> )	TNPL 103	<i>E.camaldulensis</i>	EP29	<i>E. pellita</i>	Inter
4 ( <i>E.camaldulensis</i> X <i>E.tereticornis</i> )	53	<i>E.camaldulensis</i>	186	<i>E. tereticornis</i>	Inter
16 ( <i>E.camaldulensis</i> X <i>E.pellita</i> )	44	<i>E.camaldulensis</i>	EP29	<i>E. pellita</i>	Inter
41 ( <i>E.tereticornis</i> X <i>E.urophylla</i> )	186	<i>E.tereticornis</i>	EU8	<i>E. urophylla</i>	Inter

**Table 2**

List of nutrients applied to the sand bed for the plant growth.

S.No.	Nutrients	Per plant dose	Unit
1.	Calcium ammonium nitrate	0.08	g
2.	UREA	0.04	g
3.	NPK (19:19:19)	0.004	g
4.	Phosphoric acid	0.008	ml
5.	Boric acid	0.02	ml
6.	Zinc sulphate	0.002	g
7.	Ferrous sulphate	0.002	g
8.	Sulphate of potash	0.08	g
9.	Muriate of potash	0.03	g
10.	ALL19	0.004	g

plantations (Jain and Ishii, 2003).

The cuttings taken for the minicutting method should be from the juvenile plant material for the successful rooting (Naghmouchi et al., 2008). The seedling production in earlier days was possible only with vegetative stem cutting and other traditional methods. These difficulties today were overcome by minicutting and microcutting (Xavier, 2002).

Minicutting technique is a cost effective technique which when integrated with micropropagation. It is a fast method to develop the clones

with higher pulp yield and good field performance (Xavier and Wendling, 1998). It is found to improve the rooting ability of the plants. The plants produced are of higher quality. Minicuttings are developed in indoor sand bed clonal minihedges. It also improves the rooting potential when compared to stem cuttings and the quality of rooting is also found to be superior (Wendling et al., 2000).

Minicutting plants develop tap root system which helps the plants to withstand heavy winds and they require minimal nutrients and irrigation (Hartmann and Kester, 1975). About 90% rooting occurs in minicutting whereas only 40% rooting is seen in stem cutting. Minicutting method is suitable for mass propagation of *Eucalyptus* species (Chinnaraj and Malimuthu, 2011; Titon et al., 2006). The technique was developed in early 1990's in Brazil. The rooting ability of the stem cuttings were found to be lower when compared to the rooting percentage of the minicutting (Higashi et al., 2000). Maximum value of the rooting potential was achieved in the minicuttings from cotyledons because of the high level of juvenility and the results were found to be similar in all of the species tested (de Assis et al., 2004; Greenwood and Hutchison, 1993).

The present study was initiated to check the adaptability of the five improved *Eucalyptus* hybrid clones (Table 1), to the miniclinal garden. The shoot and root quality of the minicuttings and the percentage of the



**Fig. 1.** Propagation of clones on the sand bed. (A) Clone no. 9; (B) Clone no. 21; (C) Clone no. 41; (D) Clone no. 4; (E) Clone no. 16.

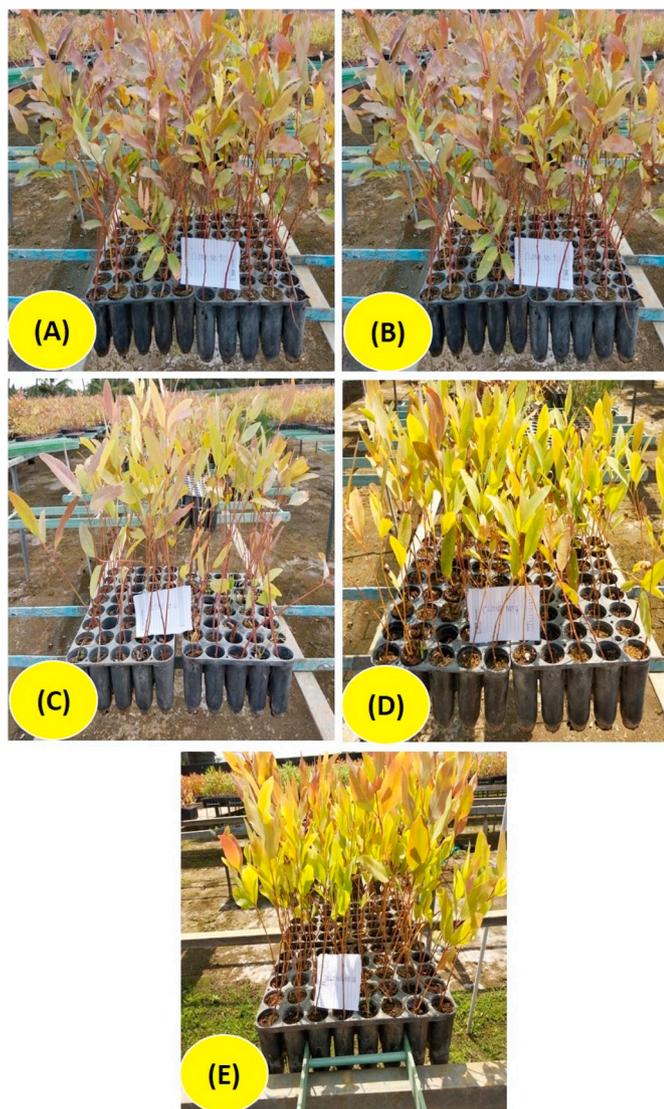


Fig. 2. Rooted mini cuttings. (A) Clone no. 9; (B) Clone no. 21; (C) Clone no. 41; (D) Clone no. 4; (E) Clone no. 16.

minicutting rooting of all the five clones were studied.

## 2. Materials and methods

### 2.1. Development of the microcuttings on the sand beds

The cuttings derived from the tissue cultured plant material is referred to as microcutting. The clonal hedges were set up using the microcutting plant materials (Ferreira et al., 2004). The micro stumps of 3–5 cm were grown in sand beds formed using bricks. The spacing of 10 × 10 cm<sup>2</sup> was followed. The irrigation regime and mineral nutrition were supplied manually. The nutrient solution was prepared and its pH was adjusted to 5.5 with 1 M NaOH or HCl. The nutrient solution (Table 2) was given to the rooting zone with the help of a rocker sprayer once a day. In hydroponic system it is not possible for the clones to obtain these nutrients from the soil, as the plantlets were planted in the sand beds filled with sand. So a nutrient solution prepared for an indoor hydroponic system should ensure the presence of all elements in it (Leite and Oliveira, 2002). The sand bed nutrients were applied to obtain the growth of the microcuttings. The microcuttings were maintained on the sandbed for a period of about two months. The nutrient solution applied to the plantlet should be in accurate concentration. Micro stumps were irrigated with tap water sprinkle system. The characteristics of the tap water which was analysed chemically consisted of 1.4 mg L<sup>-1</sup> N-NO<sub>3</sub><sup>-</sup>; 0.4 mg L<sup>-1</sup> N-NH<sub>4</sub><sup>+</sup>; 0.93 mg L<sup>-1</sup> P; 1.44 mg L<sup>-1</sup> K; 26.08 mg L<sup>-1</sup> Ca; 5.07 mg L<sup>-1</sup> Mg; 0.4 mg L<sup>-1</sup> Cu; 0.09 mg L<sup>-1</sup> Fe; 0.04 mg L<sup>-1</sup> Mn and 0.04 mg L<sup>-1</sup> Zn.

### 2.2. Preparation of minicuttings

The shoots of the microcuttings about 12 cm long were excised initially from the sand bed and further cut into 2.5 cm containing 2 nodes which were termed as minicuttings. Young side shoots were cut from stock plants with clean scissors and taken in a bucket filled with water to avoid dehydration. In the processing area minicuttings were cut

Table 3  
Mini cutting studies of selected pulpwood clones.

Clone No	No. of cuttings	No. of cuttings rooted	Rooting and Survival%	No. of shoots/cutting	Quality of the shoots
9	120	85	71	5	Good
21	120	77	64	6	Good
41	120	105	87.5	7	Excellent
4	120	98	82	6	Excellent
16	120	103	86	8	Excellent

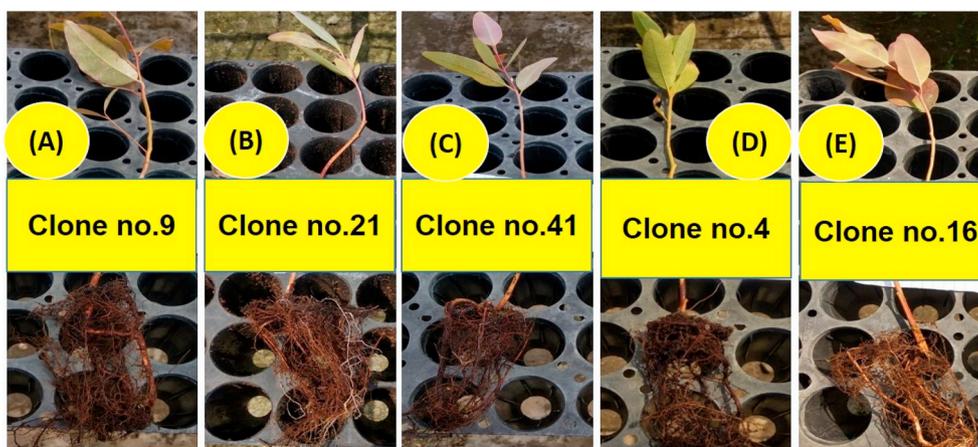


Fig. 3. Roots of the *Eucalyptus* hybrid. (A) Clone no. 9; (B) Clone no. 21; (C) Clone no. 41; (D) Clone no. 4; (E) Clone no. 16.

smaller consisting of two nodes and one internode to maintain the uniformity of minicuttings. These minicuttings were placed in the rooting substrate in the green house within 15 min of their preparation in the root trainers. The rooting substrate consisted of soil: coir pith: boiler ash: sand in the ratio 2:2:1:1. Root trainer technology has introduced root trainers for rooting of *Eucalyptus* sp. cuttings and other forest species. Root trainers should have appropriate dimensions for the root development and moisture retention (Alfenas et al., 1997; Rawat and Dhiman, 2002). An ideal rooting media for the root trainers should be in such a way that it does not hold excessive moisture, should be inert and lightweight. In South India many nurseries started using coconut coir waste as it is cheap and easily available (George, 1993; Landis et al., 2010).

The minicuttings were kept in the hydro pit (Average relative humidity 85% and temperature 38 °C) for about two weeks, after rooting the plants were transferred to shade house for a period of 10 days with 50% light intensity and finally placed under full sunlight.

### 2.3. Rooting of minicuttings

Rooting assessments were done six weeks after the minicuttings were placed. To evaluate the success of rooting of minicuttings, its growth parameters like the percentage of the rooting of the clones and the number of shoots per cutting formed were monitored and calculated statistically. The quality of roots of the minicuttings of each clones were rated on a 4 point scale.

## 3. Results and discussion

### 3.1. Clonal propagation of microcuttings

The clonal hedges were well established using the well grown microcutting plant material of the five *Eucalyptus* hybrid clones on the raised sand beds. Application of the nutrients on the sand beds for all the five clones resulted in healthy micropropagated stock plants, which was evident from the healthy shoots produced (Fig. 1). The maintenance of these stock plants served as a source material for micropropagation and for the production of minicuttings. If the source material for micropropagation is taken from these healthy mother plants it was found to reduce the contamination during the in vitro regeneration process rather than collecting the explants from the field grown mother plants. The cuttings produced from these plants will also be easy to root (Aumond et al., 2017). Propagation of the clones on the sand beds resulted in the highest survival rates of the clones, with no loss of the plantlets. For these purposes maintenance of the micropropagated plants in the sand beds are essential (Schwambach et al., 2008).

### 3.2. Rooting assessment of the minicuttings

Minicutting method is an efficient method for the propagation of the clones (Zobel, 1993). The adaption of all the clones towards the miniclinal garden were significantly good (Fig. 2). This was because of the good environmental conditions which resulted in the efficient rooting of the clonal material obtained. Juvenile plant material gives a good rooting for the cuttings (Bonga, 1982; Bonga and Durzan, 2012). The use of juvenile material resulted in higher rooting percentage of the clones, ranging from 60 to 90. The spacing 10 × 10 cm<sup>2</sup> between the plants did not interfere with the rooting ability of the minicuttings of the different clones. The quality of rooting of all the clones of the five *Eucalyptus* hybrid species was recorded to a score of 4 on 4 which was evident from Fig. 3. Clone No.41, 4 and 16 was found to have higher rate of rooting percentage which was 87.5, 82 and 86, respectively while the rooting percentage of 21 and 9 were found to be 64 and 71, respectively (Table 3). The quality of the shoots of clone No.4, 41 and 16 were found to be excellent while the shoot quality of 9 and 21 was also good.

Mass propagation of all the clones will be possible because of its

higher survival rate during the propagation process. The survival of the minicuttings depends on how the cuttings were maintained in the green house. Climatic oscillations also play an important role in the survival of the cuttings (Pires et al., 2017). Type of species, nature of the clones, origin of cuttings, conditions in which they are grown are the determining factors for rooting of cuttings (Dhiman and Gandhi, 2005). Many forestry based woody plants which are difficult to root have been clonally propagated successfully using indoor hydroponic system (Lewis et al., 2017). Clonal seedlings developed through these methods are commercially deployed to develop quality pulpwood plantations.

## 4. Conclusion

The successfully grown clones in the miniclinal gardens were further developed in the *Eucalyptus* plantations. For the production of homogeneous raw materials, uniform forests has been developed which provides a unique genetic characteristic of a selected plant. When compared to the forest plantations produced by the seeds, minicutting provides uniformity of forests, maximizes the production of the forest based companies in terms of quality and quantity Profits can be calculated and accurate yields can be predicted with the help of the clonal forests. So development of clonal hedges is necessary. Consequently, every effort is made to ensure that optimal rooting is achieved, from the preparation of harvested cuttings, to the environment in which they are subsequently housed. The rooting speed of minicuttings will be faster compared to the rooting speed of stem cuttings. These minicuttings will be grown in the clonal hedges and subsequently in plantations. These managed plantations supply raw material for the industries. They not only supply raw material but also make contribution in the improvement of the environment.

## Acknowledgements

The authors also acknowledge UGC for providing SAP (UGC-SAP: No. F.5-4/2016/DRS-1 (SAP-11)) and DST for providing FIST (FST: SR/FST/LSI-687/2016), New Delhi, India, to the Department of Environmental Biotechnology, Bharathidasan University, Tiruchirappalli, Tamil Nadu, India.

## References

- Alfenas, A., Stowasser, E., da Silveira, S., 1997. Current status and control strategies of diseases associated to clonal propagation of eucalytus in Brazil. In: IUFRO Conference on Silviculture and Improvement of Eucalypts, Salvador (Brazil). EMBRAPA, pp. 24–29. Aug 1997.
- Aumond Jr., M.L., de Araujo Jr., A.T., de Oliveira Junkes, C.F., de Almeida, M.R., Matsuura, H.N., de Costa, F., Fett-Neto, A.G.J.F., 2017. Events associated with early age-related decline in adventitious rooting competence of *Eucalyptus globulus* Labill, 8, 1734.
- Bonga, J., 1982. Vegetative Propagation in Relation to Juvenility, Maturity, and Rejuvenation, Tissue Culture in Forestry. Springer, pp. 387–412.
- Bonga, J.M., Durzan, D.J., 2012. Cell and Tissue Culture in Forestry: Volume 2 Specific Principles and Methods: Growth and Developments. Springer Science & Business Media.
- Chinnaraj, S., Malimuthu, C., 2011. Development of micro-propagation and mini cutting protocol for fast growing Melia, Dalbergia and Eucalyptus clones for pulpwood and bio-energy plantations. BMC Proc. BioMed Central P131.
- de Assis, T.F., Fett-Neto, A.G., Alfenas, A.C., 2004. Current Techniques and Prospects for the Clonal Propagation of Hardwoods with Emphasis on Eucalyptus. Plantation Forest Biotechnology for the 21st Century. Research Signpost, Trivandrum, India, pp. 303–333.
- Dhiman, R., Gandhi, J., 2005. Preliminary results of making plywood from Eucalyptus species. J. Timber Dev. Assoc. India 51, 35.
- Ferreira, E.M., Alfenas, A.C., Mafia, R.G., Leite, H.G., Sartorio, R.C., Penchel Filho, R.M., 2004. Determination of the optimum time for rooting of mini-cuttings of Eucalyptus spp. clones. Rev. Árvore 28, 183–187.
- George, E.F., 1993. Plant Propagation by Tissue Culture. Part 1: the Technology. Exegetics limited.
- Goulart, P.B., Xavier, A., 2008. Efeito do tempo de armazenamento de minestacas no enraizamento de clones de *Eucalyptus grandis* x *E. urophylla*. Rev. Árvore 32, 671–677.
- Greenwood, M., Hutchison, K., 1993. Maturation as a Developmental Process, Clonal Forestry I. Springer, pp. 14–33.

- Hartmann, H.T., Kester, D.E., 1975. *Plant Propagation: Principles and Practices*. Prentice-Hall.
- Higashi, E., Silveira, R., Gonçalves, A., 2000. Monitoramento nutricional e fertilização em macro, mini e microjardim clonal de Eucalyptus. GONÇALVES, JLM; BENEDETTI, V. *Nutrição e fertilização florestal*. IPEF, Piracicaba, pp. 191–217.
- Jain, S.M., Ishii, K., 2003. *Micropropagation of Woody Plants and Fruits*.
- Landis, T.D., Dumroese, R.K., Haase, D.L., 2010. *The Container Tree Nursery Manual: Volume 7, Seedling Processing, Storage, and Outplanting*. Agric. Handbook No. 674, vol. 199. US Department of Agriculture, Forest Service, Washington, DC, p. 674.
- Leite, H., Oliveira, F., 2002. Statistical method to test the identity of analytical methods. *Commun. Soil Sci. Plant Anal.* 6, 22.
- Lewis, V., Kester, S., Geneve, R., 2017. Developing a modified hydroponic stock plant system for redbud®. *Proceedings of the 2017 Annual Meeting of the International Plant Propagators' Society* 1212, 177–180.
- Naghmouchi, S., Khouja, M.L., Rejeb, M.N., Boussaid, M., 2008. Effect of growth regulators and explant origin on in vitro propagation of *Ceratonia siliqua* L. via cuttings. *Biotechnol. Agron. Soc. Environ.* 12, 251–258.
- Pires, P.P., Wendling, I., de Souza, A.M., Coelho, A.S.G., 2017. Climatic Oscillations in the Production of *Eucalyptus Benthamii* X *E. Dunnii* Shoots in Mini-Clonal Hedge.
- Rawat, G., Dhiman, R., 2002. Silviculture research in India. *Indian For.* 128, 715–725.
- Saya, A.R., Mankessi, F., Toto, M., Marien, J.-N., Monteuis, O., 2008. Advances in Mass Clonal Propagation of *Eucalyptus Urophylla* X *E. grandis* in Congo, pp. 15–26.
- Schwambach, J., Ruedell, C.M., de Almeida, M.R., Penchel, R.M., de Araújo, E.F., Fettes Neto, A.G., 2008. Adventitious rooting of *Eucalyptus globulus* × *maidennii* mini-cuttings derived from mini-stumps grown in sand bed and intermittent flooding trays: a comparative study. *N. For.* 36, 261.
- Stuepp, C., Wendling, I., Trueman, S., Koehler, H., Zuffellato-Ribas, K.J.F., 2017. The use of auxin quantification for understanding clonal tree propagation, 8, 27.
- Titon, M., Xavier, A., Otoni, W.J.S.F., 2006. *Clonal Propagation of Eucalyptus Grandis Using the Mini-Cutting and Micro-cutting Techniques*, vol. 71, pp. 109–117.
- Wendling, I., Xavier, A., Gomes, J., Pires, I., Andrade, H., 2000. Efeito do regulador de crescimento AIB na propagação de clones de *Eucalyptus* spp. por miniestaquia. *Rev. Árvore* 24, 187–192.
- Xavier, A., 2002. *Silvicultura clonal I: princípios e técnicas de propagação vegetativa*. UFV, Viçosa.
- Xavier, A., Wendling, I., 1998. *Miniestaquia na clonagem de Eucalyptus*. SIF, Viçosa, MG.
- Zobel, B., 1993. *Clonal Forestry in the Eucalypts, Clonal Forestry II*. Springer, pp. 139–148.