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# Clonal propagation of *Eucalyptus grandis* using the mini-cutting and micro-cutting techniques

## Propagação clonal de *Eucalyptus grandis* por miniestaquia e microestaquia

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*Resumo*

O presente estudo objetivou avaliar a eficiência da propagação clonal de quatro clones de *Eucalyptus grandis* W. Hill ex Maiden pelo uso das técnicas de microestaquia e miniestaquia, analisando-se a sobrevivência, o enraizamento e o vigor das microestacas e miniestacas. O microjardim clonal foi constituído de microcepas oriundas de mudas rejuvenescidas por micropropagação, mediante subcultivos *in vitro*, e, no minijardim clonal, foram utilizadas minicepas obtidas pelo enraizamento de miniestacas oriundas de brotações de plantas propagadas pelo método da estaquia convencional. Os resultados observados para a sobrevivência após 21 ou 28 dias na casa de vegetação, enraizamento à saída da casa de sombra, sobrevivência, altura e diâmetro do colo das mudas aos 50 dias e peso de matéria seca de raízes das microestacas e miniestacas aos 28 dias, de modo geral, foram superiores na microestaquia em relação à miniestaquia. Essas diferenças foram maiores em clones com maior dificuldade de enraizamento, possivelmente, devido ao efeito de rejuvenescimento dos clones com o uso da microestaquia.

**Palavras-Chave**: Silvicultura clonal, Propagação vegetativa, Rejuvenescimento, Enraizamento

*Abstract*

Mini-cutting and micro-cutting techniques were used to assess the clonal propagation efficiencies of four *Eucalyptus grandis* W. Hill ex Maiden clones. The micro-clonal hedge consisted of micro-stumps from micropropagation-derived plants produced by *in vitro* subcultures, whereas the mini-clonal hedge consisted of micro-stumps obtained by rooting mini-cuttings from conventional cutting method-derived sprouts. Sequential steps during cutting production from axillary shoots, after removing shoot apices, were adopted for both techniques. Micro-cuttings and mini-cuttings survival, rooting and vigor were evaluated. Survival after 21 or 28 days in the greenhouse, rooting on leaving the shade house, survival, height and root collar diameter of the cuttings at 50 days and dry weight of root matter of the micro-cuttings and mini-cuttings at 28 days, were generally higher in the micro-cuttings than in the mini-cuttings. These differences were more noticeable in clones with low rooting ability, possibly due to the effect of "rejuvenation" of the clones with the use of micro-cuttings.

**Keywords**: Clonal forestry, Vegetative propagation, Rejuvenation, Rooting

## INTRODUCTION

Vegetative propagation of the genus *Eucalyptus* is carried out to a greater or lesser degree of sophistication in most of the forestry companies that have adopted clonal forestry, where it is considered strategic for improving forest yield and quality. As yields from Eucalyptus forestry will continue to increase as more improved clones are developed, matched with improvements silvicultural methods for growing on these plants for which they are well adapted (ZOBEL, 1993), the availability of a highly reliable and cost effective propagation techniques to support these developments are required. The cutting technique is the most widely used way for the propagation of *Eucalyptus* because of its ease of handling as compared to the micropropagation methods. However, this method presents some difficulties in the production process, such as poor rooting and plants formation in certain clones, which consequently affects their deployment in the planting programme (XAVIER *et al*., 1997).

The poor rooting of certain clones by using conventional cuttings is a major constraint to cloning, and has been attributed to the maturation degree of the plant material (GOMES, 1987; HACKETT, 1987), leading to the adoption of various techniques for "rejuvenating" the mature plants material into the "juvenile" stage

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(BONGA, 1982), amongst them is the *in vitro* techniques (STRUVE and LINEBERGER, 1988; GUPTA and DURZAN; 1987; GEORGE, 1993; HARTMANN *et al*., 1997). The development of the micro-cutting technique (ASSIS *et al*., 1992; XAVIER and COMÉRIO, 1996) and the mini-cutting technique (XAVIER and WENDLING, 1998; WENDLING *et al*., 2000) led to considerable gains, mainly derived from increasing the proportion of plants that rooted and shortening the time required to produce.

Pioneer studies by Assis *et al*. (1992) led to the development and application of micro-cutting technique for *Eucalyptus* propagation. *In vitro* rejuvenated plants are used as a source of propagules; stem apices are then excised from these plants and used as micro-cuttings. Micro-cuttings about three cm in size with two to three pairs of leaves are rooted in the greenhouse under controlled conditions, moisture and temperature (ASSIS, 1997).

The mini-cutting technique is similar to the micro-cutting one, but does not rely on the micropropagation stage and is therefore less costly to use. Shoots from plants propagated by conventional cutting are used as source of propagules. The use of the former technique has expanded rapidly and is currently the most widely used technique by the forestry companies (XAVIER and WENDLING, 1998).

Clonal propagation of *Eucalyptus grandis* is very important in intensive forestry. Micro-cutting and mini-cutting techniques have been developed and implemented in practice, but the advantages and/or disadvantages of these approaches have not been extensively evaluated. Once identified, high-yielding and/or high quality clones can be mass propagated and deployed for practical forestry purposes, where a continuous and reliable supply of stock plantation is highly demanded.

The objective of this study was to provide new insights on the usefulness and to compare the efficiencies of micro-cutting and mini-cutting techniques for the clonal propagation of four *Eucalyptus grandis* clones.

## MATERIALS AND METHODS

The four *Eucalyptus grandis* clones (CC1, CC8, CC11 and CC12) used in the study were supplied by Empresa Celulose Nipo-Brasileira S. A. (CENIBRA) located in Belo Oriente, state of Minas Gerais, Brazil. The clones were derived from seven-years-old trees that reached flowering stage that had been selected from a commercial plantation.

The plants used to set up the micro-clonal hedge were raised from micropropagated plants from axillary buds, as described by Titon (2001). The number of *in vitro* subcultures for each of the four clones are as follows: 7-9 subcultures for clone CC1, 9-10 for clone CC8, 12-13 for clone CC11, and 11-12 subcultures for clone CC12.

In vitro-derived elongated shoots were transplanted into pots and then placed in a greenhouse (average relative humidity 85% and CA 30ºC), in 55 cm³ containers (27 mm internal diameter, 125 mm height). The pots contained a 1:1 ratio potting mix of medium granulometry vermiculite and burnt rice husk. After rooting, the cuttings were transferred to a shade house (50% full light intensity), for acclimation for eight days, and then allowed to harden outdoors. No growth regulator was added. After approximately 60 days, when the rooted cuttings were 10 to 12 cm tall, the shoots were pruned to a height of 8 cm to obtain the micro-stumps. The latter supplied the micro-cuttings which formed the micro-clonal hedge (XAVIER and COMÉRIO, 1996).

Based on the mini-cutting technique (XAVIER and WENDLING, 1998; WENDLING *et al*. 2000) the mini-clonal hedge consisted of mini-stumps obtained by rooting mini-cuttings derived from the shoots the propagated plant by the conventional cutting method. The rooting under greenhouse conditions, acclimation in shade house and hardened outdoors was adopted as previously described. Likewise, after approximately 40 days when the rooted mini-cuttings were 10 to 12 cm tall, their shoot apices were pruned to 8 cm thus forming the mini-stumps, which supplied the sprouts (mini-cuttings) to carry out the experiment.

The efficiency of the mini-cutting and micro-cutting techniques under experimental conditions was assessed at the Forestry Nursery of the Empresa Celulose Nipo-Brasileira (CENIBRA), from September to December 2000.

The clonal hedges were set up using the micro-cutting and mini-cutting plant materials. The micro-cuttings and mini-cuttings were placed in containers of the same size and filled with the same mixture as described before. The clonal hedge management system was the same as that used operationally by the Company. The micro and mini-stumps were grown in rigid polypropylene plastic containers (595 mm length X 388 mm width X 25mm height) with 176 cells. In order to allow 100 cm² average spacing per stump, a total of 32 mini-stumps were inserted per tray, which were then placed into stainless steel bench trays (Figure

1A). The irrigation regime and mineral nutrition were supplied by an automated flooding irrigation system (two to three times daily, for 25 minutes each time); only the rooting system remained in contact with the nutrient solution. The fertilizer consisted of a mixture of calcium nitrate (555 g $m^{-3}$), ammonium sulphate (200 g $m^{-3}$), phosphoric acid (70 g $m^{-3}$), potassium chloride (210 g $m^{-3}$), magnesium sulphate (150 g $m^{-3}$), boric acid (3.33 g $m^{-3}$), zinc sulphate (0.15 g $m^{-3}$), copper sulphate (0.40 g $m^{-3}$), manganese sulphate (1.67 g $m^{-3}$), iron sulphate (5.22 g $m^{-3}$), EDTA (6.96 g $m^{-3}$) and sodium molybdenum (0.05 g $m^{-3}$). To minimize salt accumulation in the substrate, the micro-stumps and mini-stumps were irrigated with tap water sprinkler system once a week.

The micro-cuttings and mini-cuttings, 4-6 cm long, were harvested 15 days after pruning and placed immediately into recipients containing distilled water to avoid dehydration. The micro and mini-cuttings were placed in the rooting substrate in the greenhouse within 15 minutes of their preparation (Figure 1B).

The plants were fertilized with a 5 L solution per 100 L substrate of single super phosphate (8 Kg $m^{-3}$), ammonium sulphate (20 Kg $m^{-3}$), potassium chloride (3.33 Kg $m^{-3}$), zinc sulphate (0.22 Kg $m^{-3}$), copper sulphate (0.22 Kg $m^{-3}$) and boric acid (0.39 Kg $m^{-3}$). One batch of the micro and mini-cuttings were kept in the greenhouse for 21 days, while another batch was retained there for 28 days before being transferred to the shadehouse. The micro-cuttings and mini-cuttings were kept in the shadehouse (for eight days acclimation), and finally placed under full sun light until they were 50 days old (Figure 1C).

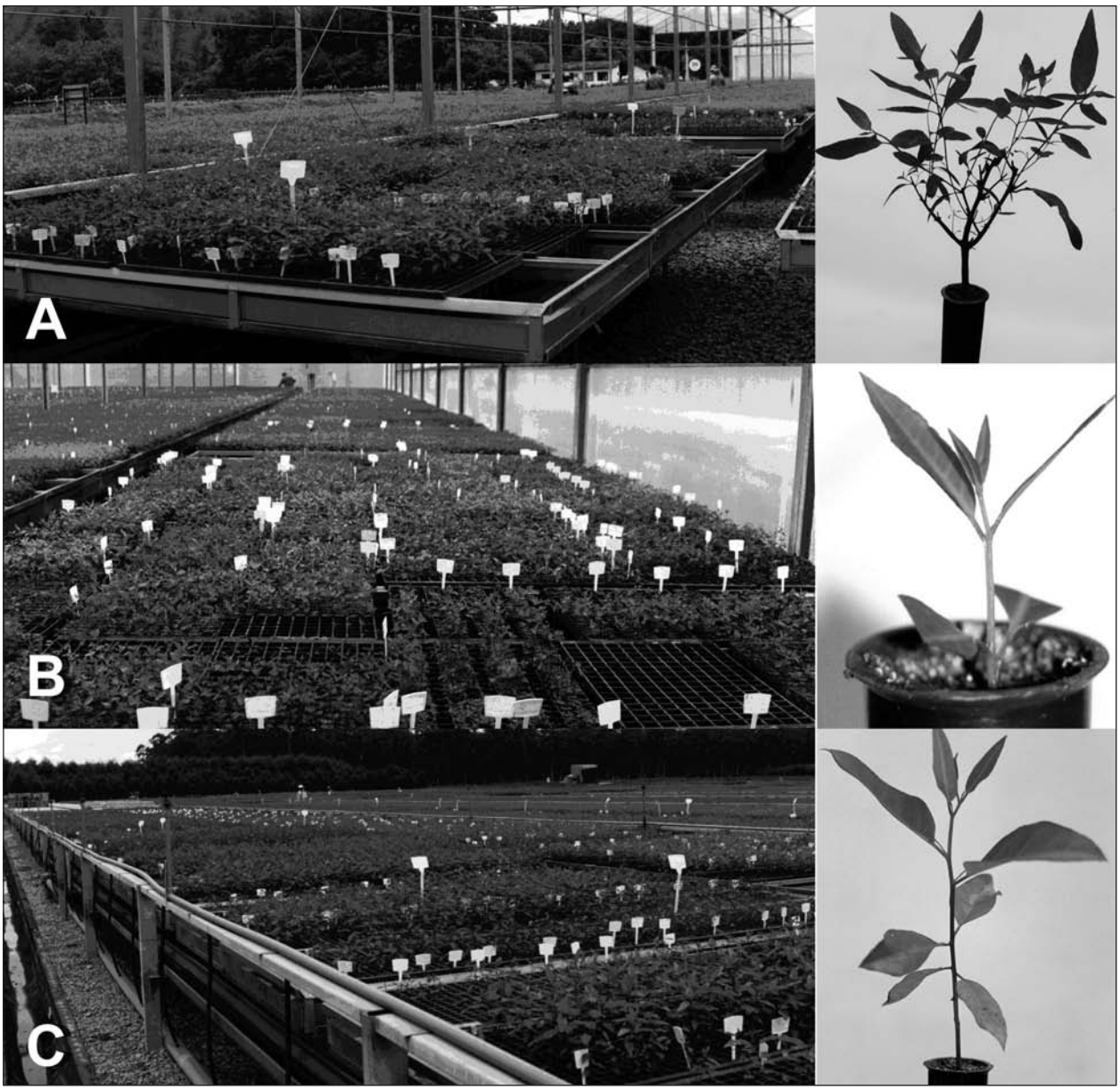


**Figure 1.** Detail of the micro-clonal and mini-clonal hedges (A), micro-cutting and mini-cutting rooting on the greenhouse (B) and development outdoors (C). (Detalhe do microjardim e minijardim clonal (A), enraizamento de microestacas e miniestacas em casa de vegetação (B) e desenvolvimento das mudas a pleno sol (C))

A completely randomized block design was used as a factorial scheme, consisting of two techniques (mini-cutting and micro-cutting), four clones (CC1, CC8, CC11 and CC12), and two periods of time in the greenhouse (21 and 28 days) with six replications, each plot consisting of 16 plants.

The following parameters were assessed: a) micro-cutting and mini-cutting survival after 21 and 28 days in the greenhouse; b) rooting percentage of the micro-cutting and mini-cutting 8 days acclimation in the shadehouse; c) survival, height and root collar diameter of the 50-day old rooted cuttings; and d) root dry weight of the micro-cuttings and mini-cuttings after 28 days. The data were analyzed following a full factorial ANOVA design and the means compared by the Tukey test, at 5% probability level.

## RESULTS AND DISCUSSION

A significant effect by the F test ($P < 0.05$) was detected for "Clone X Technique" and "Clone X Treatment" interactions on some assessed characteristics, indicating differentiated clonal responses in relation to the techniques and the remaining period in the greenhouse (Table 1).

There was no significant difference between the two techniques for the effect of rooting period (21 or 28 days) in the greenhouse (Figure 2). According to Xavier and Comério (1996), the maintenance period of the micro-cuttings in the greenhouse can vary from 10 to 30 days, and rooting usually occurs 7-20 days after cutting (ASSIS *et al*., 1992). Except for the clone CC11, 28 days after treatment, 82.3% of the plants survived for those produced using the mini-cutting technique, and all clones treated with the techniques had survival rates greater than 90% regardless of treatment. These results were generally more uniform and superior than those previously reported by Wendling *et al*. (2000).

Significant differences among the techniques used for rooting were detected only for the clone CC11 (Figure 3), similar to the results obtained for survival. There was no statistical difference between the treatments (21 or 28 days) which indicates that 21 days was sufficient for the rooting of the micro-cuttings and mini-cuttings. More than 87.5% of the plants initiated roots, the clone CC11 being an exception for the mini-cutting treatment. However, there was an increase of 19.2% and 34.7% in the rooting percentages for plants propagated by the micro-cutting technique compared with values obtained with the mini-cutting technique, after 21 or 28 days in the greenhouse, respectively. The average rooting percentages were 94% for micro-cutting and 88% for mini-cutting. Plants of all clones propagated better using the micro-cutting technique. While some clones (e.g. CC11) that were difficult to root performed very poorly when propagated using the mini-cutting technique, differences for other clones were small.

The better rooting response to micro-cutting compared to mini-cutting, could be possibly attributed to rejuvenation brought about by micropropagation (GEORGE, 1993; HARTMANN *et al*., 1997).

**Table 1.** Analysis of variance of the characteristics micro-cuttings and mini-cuttings survival on leaving the greenhouse (SGH), rooting on leaving the shade house (RSH), survival (SUR50), height (H50) and root collar diameter (RCD50) of the micro-cuttings and mini-cuttings at 50 days, and root dry matter weight (RDM) of the micro-cuttings and mini-cuttings at 28 days, assessed in two periods in the greenhouse (Treat), of the four *Eucalyptus grandis* clones (Clo) propagated by the micro-cutting and mini-cutting techniques (Tec). (Análise de variância das características de sobrevivência das microestacas e miniestacas na saída da casa de vegetação (SGH); enraizamento na saída da casa de sombra (RSH); sobrevivência (SUR50), altura (H50) e diâmetro do colo (RCD50) das mudas aos 50 dias de idade; e peso de matéria seca de raízes (RDM) das microestacas e miniestacas aos 28 dias, avaliadas em dois tempos de permanência em casa de vegetação (Treat), para os quatro clones (Clo) propagados por microestaquia e miniestaquia (Tec))

| SV | DF | Mean Square | | | | | |
|---|---|---|---|---|---|---|---|
| | | SGH[1] (%) | RSH[1] (%) | SUR50[1] (%) | H50 (cm) | RCD50 (mm) | RDM (mg) |
| Clone (Clo) | 3 | 0.3513** | 0.6255** | 0.7249** | 184.05** | 0.4038** | 3041.0** |
| Technique (Tec) | 1 | 0.1174* | 0.2387** | 0.3817** | 17.70* | 0.7905** | 551.04* |
| Treatment (Treat) | 1 | 0.0009 ns | 0.0038 ns | 0.0416 ns | 76.87** | 0.6035** | 1204.2** |
| Clo * Tec | 3 | 0.0169 ns | 0.1043** | 0.1389** | 11.78* | 0.1120** | 205.9 ns |
| Clo * Treat | 3 | 0.0295 ns | 0.0124 ns | 0.0216 ns | 29.77** | 0.1020* | 478.47* |
| Tec * Treat | 1 | 0.0004 ns | 0.0008 ns | 0.0085 ns | 0.1237 ns | 0.0079 ns | 126.04 ns |
| Clo * Tec * Treat | 3 | 0.0163 ns | 0.0139 ns | 0.0104 ns | 2.12 ns | 0.0015 ns | 151.74 ns |
| Error | 80 | 0.0184 | 0.0225 | 0.0215 | 4.3976 | 0.0335 | 123.75 |
| Overall mean | - | 95.05 | 90.95 | 89.58 | 15.14 | 1.97 | 41.86 |
| CV (%) | - | 9.54 | 11.22 | 11.13 | 13.85 | 9.31 | 26.57 |

*, ** = significant at 5 and 1% probability by the F test, respectively.
ns = not significant at 5% probability by the F test.
(1) data transformed in arc sin $\sqrt{x/100}$ .

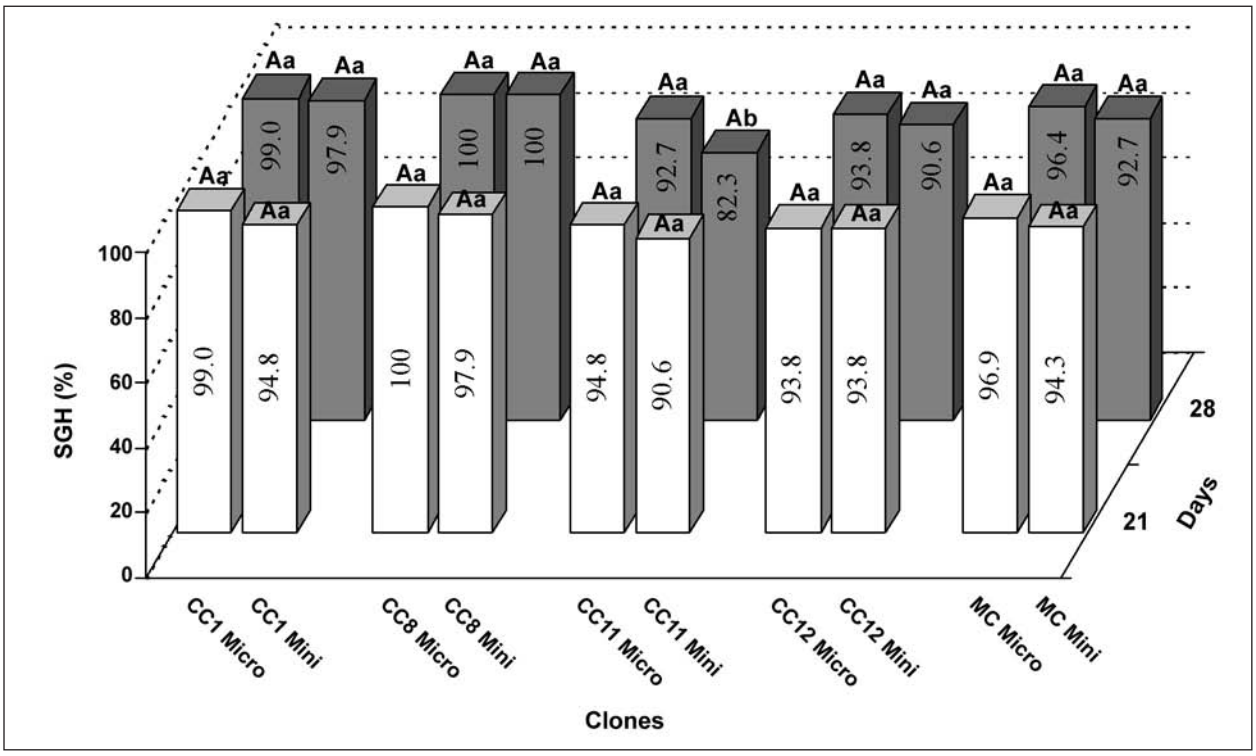


**Figure 2.** Micro-cuttings and mini-cuttings survival on leaving the greenhouse (SGH), at 21 or 28 days, of the four *Eucalyptus grandis* clones and overall mean of the clones (MC). The means followed by the same upper case letter within the same technique and among treatments and those followed by a lower case letter within the same treatment and among techniques, respectively, do not differ by the Tukey test (P < 0.05). (Sobrevivência das microestacas e miniestacas na saída da casa de vegetação (SGH), aos 21 ou 28 dias, dos quatro clones estudados e média dos clones (MC). As médias seguidas de uma mesma letra maiúscula dentro de uma mesma técnica e entre tratamentos e as seguidas de uma letra minúscula dentro do mesmo tratamento e entre técnicas, respectivamente, não diferem entre si, pelo teste de Tukey a 5% de probabilidade)

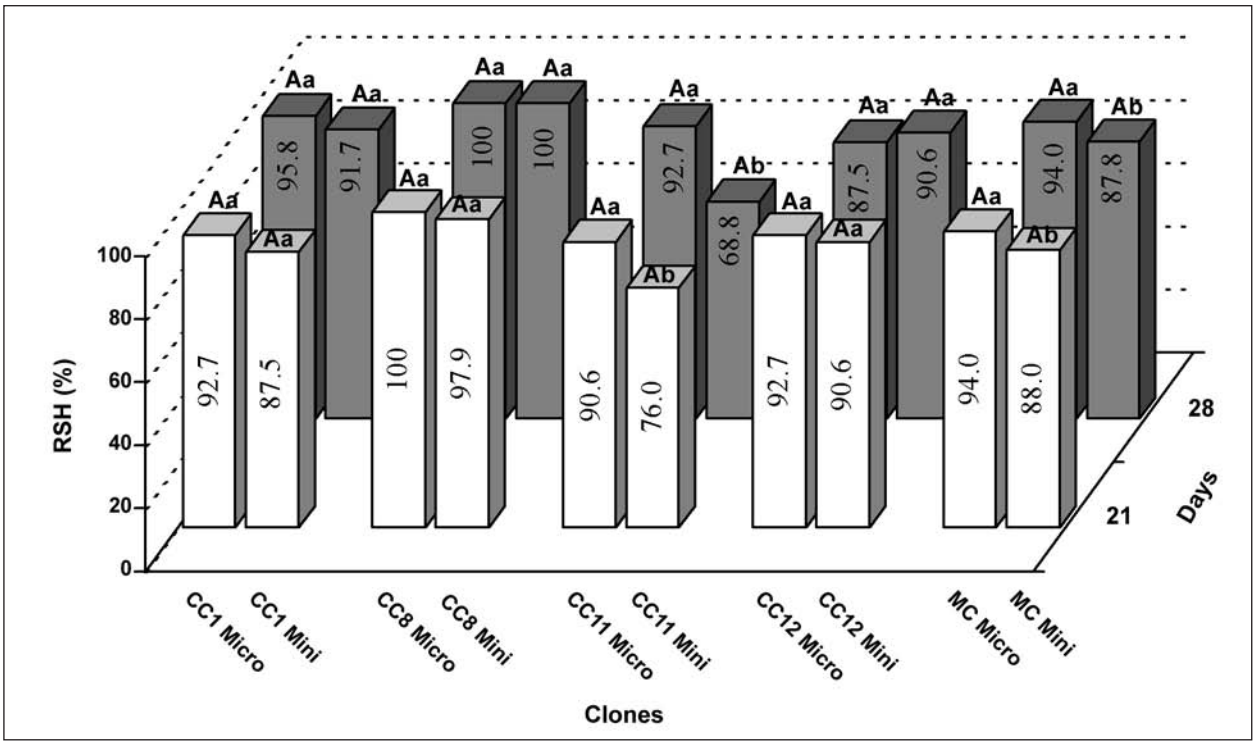


**Figure 3.** Micro-cuttings and mini-cuttings rooting on leaving the shade house (RSH) for the two periods in the greenhouse (21 or 28 days), of the four *Eucalyptus grandis* clones and overall mean of the clones (MC). The means followed by the same upper case letter within the same technique and among treatments and those followed by a lower case letter within the same treatment and among techniques, respectively, do not differ by the Tukey test (P < 0.05). (Enraizamento das microestacas e miniestacas na saída da casa de sombra (RSH), para os dois tempos de permanência em casa de vegetação (21 ou 28 dias), dos quatro clones estudados e média dos clones (MC). As médias seguidas de uma mesma letra maiúscula dentro de uma mesma técnica e entre tratamentos e as seguidas de uma letra minúscula dentro do mesmo tratamento e entre técnicas, respectivamente, não diferem entre si, pelo teste de Tukey a 5% de probabilidade)

For cutting survival at 50 days, clonal behavior was similar to that one of percentage rooting after leaving the shade house (compare Figure 4 with Figure 3). The mean cutting survival percentages of the four clones at 50 days, for the two techniques, were 93.2% (micro-cutting) and 84.4% (mini-cutting). This confirms that the micro-cutting technique is more efficient than the mini-cutting one, especially for clones that are difficult to root.

The heights of the micro-cuttings and mini-cuttings at 50 days varied with treatments and the techniques used (Figure 5). Height, after 50 days, was greater for cuttings (both micro and mini-cuttings) that were kept for 21 days (period treatment) in the greenhouse than for those staying longer 28 days. The longer period of exposure to full sunlight, for the plants that were in the greenhouse for 21 days, compared to those kept for 28 days probably contributed to this outcome. It is likely that a shorter period of time in the greenhouse might have improved the height growth of the plants, particularly for clones that rooted easily (e.g. CC8).

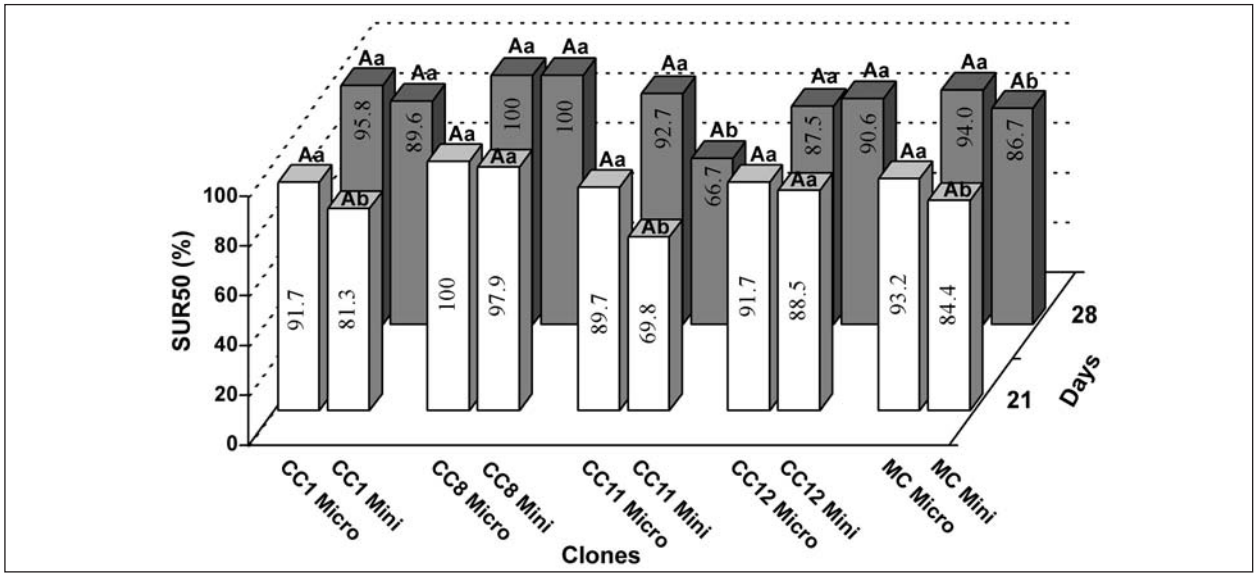


**Figure 4.** Survival of the micro-cuttings and mini-cuttings at 50 days (SUR50) for the two periods in the greenhouse (21 or 28 days), of the four *Eucalyptus grandis* clones and overall mean of the clones (MC). The means followed by the same upper case letter within the same technique and among treatments and those followed by a lower case letter within the same treatment and among techniques, respectively, do not differ by the Tukey test (P < 0.05). (Sobrevivência das mudas aos 50 dias de idade (SUR50), para os dois tempos de permanência em casa de vegetação (21 ou 28 dias), dos quatro clones estudados e média dos clones (MC). As médias seguidas de uma mesma letra maiúscula dentro de uma mesma técnica e entre tratamentos e as seguidas de uma letra minúscula dentro do mesmo tratamento e entre técnicas, respectivamente, não diferem entre si, pelo teste de Tukey a 5% de probabilidade)

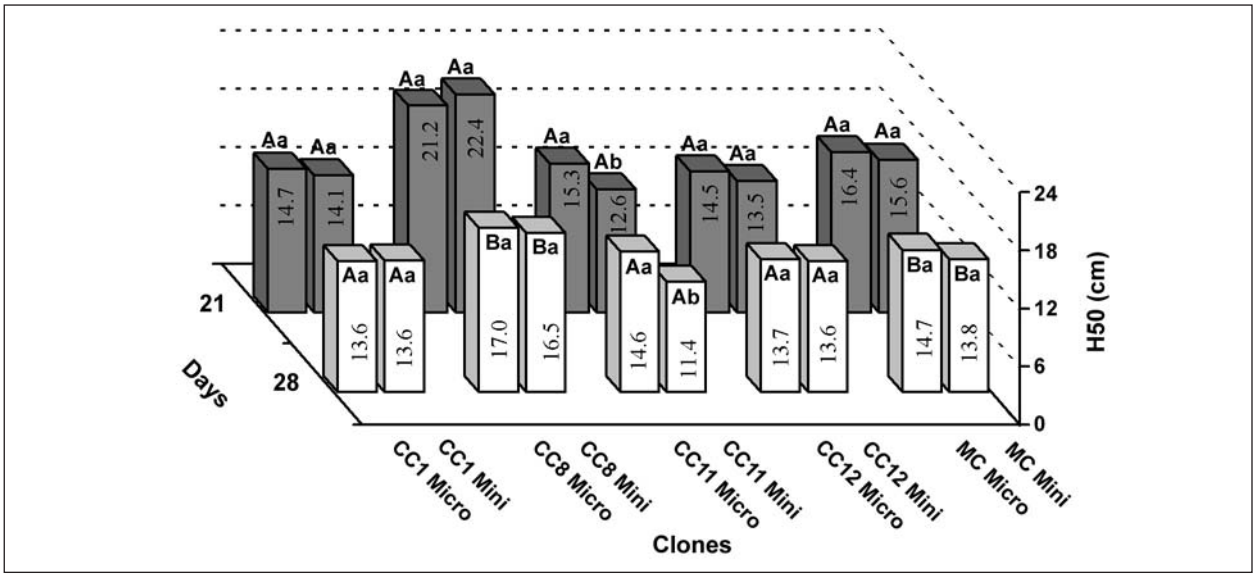


**Figure 5.** Micro-cuttings and mini-cuttings height at 50 days (H50) for the two periods in the greenhouse (21 or 28 days), of the four *Eucalyptus grandis* clones and overall mean of the clones (MC). The means followed by the same upper case letter within the same technique and among treatments and those followed by a lower case letter within the same treatment and among techniques, respectively, do not differ by the Tukey test (P < 0.05). (Altura das mudas aos 50 dias de idade (H50), para os dois tempos de permanência em casa de vegetação (21 ou 28 dias), dos quatro clones estudados e média dos clones (MC). As médias seguidas de uma mesma letra maiúscula dentro de uma mesma técnica e entre tratamentos e as seguidas de uma letra minúscula dentro do mesmo tratamento e entre técnicas, respectivamente, não diferem entre si, pelo teste de Tukey a 5% de probabilidade)

The cutting heights produced through micro-cutting and mini-cutting techniques were quite similar, with the exception of clone CC11. The micro-cutting technique increased height growth by 21.4% and 28.1% for the 21- and 28-day treatments, respectively, compared to that achieved using the mini-cutting technique. It is likely that tissues were rejuvenated during the micropropagation process; cuttings derived from the juvenile parts of the plant are usually more vigorous (GEORGE, 1993; GREENWOOD and HUTCHISON, 1993).

The results for root collar diameter (Figure 6) indicated similarity to those obtained for height, in that the micro-cutting technique presented equal or higher values to those for the mini-cutting technique, especially for the clones with rooting difficulty. The greatest diameters were generally obtained for cuttings that were given 21 days treatment in the greenhouse.

The micro-cuttings and mini-cuttings height and root collar diameter, were generally a little below the standard considered as ideal for planting in the field (CARNEIRO, 1995), except for the CC8 clone. However, these characteristics were assessed for plants that were only 50 days old.

Cuttings wich had been given the 28-days treatment in the greenhouse had the highest root dry matter weights (Figure 7), which may be due to the fact that the root system developed for a longer period under greenhouse environmental conditions and also possibly because the cuttings were subsequently air pruned in the shade house.

Plants propagated via the micro-cutting technique had larger root dry weights, reinforcing the suggestion that the plants were rejuvenated using this method (GEORGE, 1993; GREENWOOD and HUTCHISON, 1993). Juvenility improves vigor, which might increase root growth, and consequently also increase root dry matter.

There is little published information available on the use of mini-cutting and micro-cutting techniques. The results of this study showed that both techniques are reliable methods for the mass propagation of *Eucalyptus grandis* clones. The mini-cutting method is more widely used because it is cheaper and because it does not depend on tissue culture to supply stock plants. However, the micro-cutting technique can be used as an efficient and cost effective mode for clones that are difficult to root.

Interest in the application of genetic transformation technique to improve Eucalyptus yields is increasing, but the costs of these methods are likely to be very high. However, propagation techniques using the methods described in this study might provide the opportunity for exploiting any potential gains. The amount of improved material could be "multiplied up", thus helping to reduce costs.

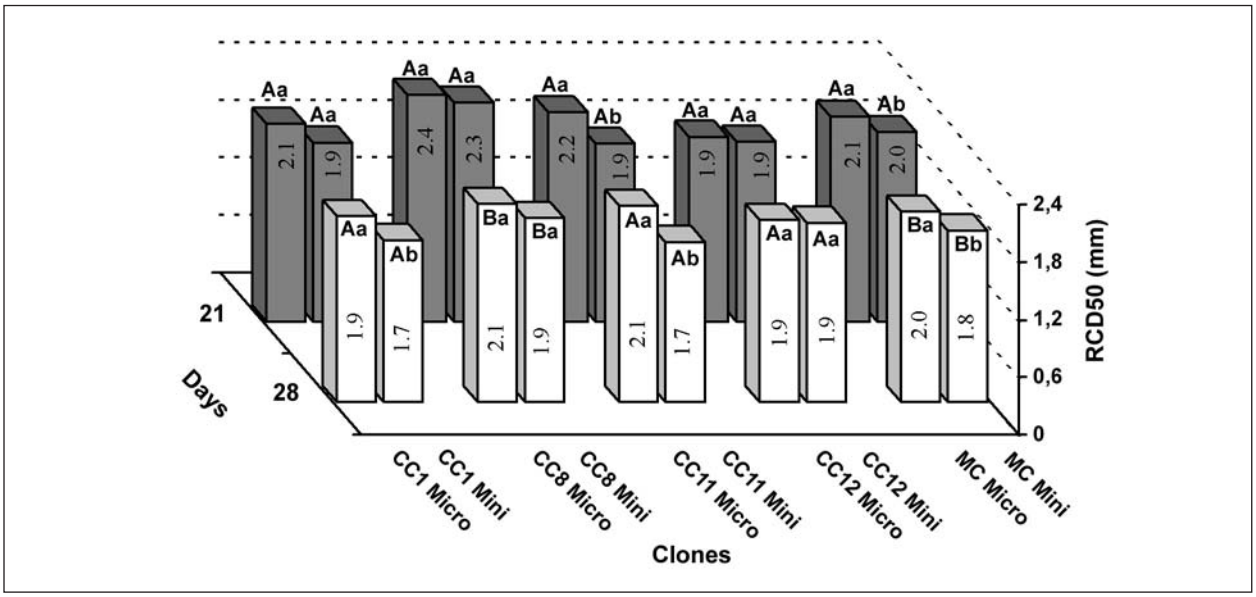


**Figure 6.** Root collar diameter at 50 days (RCD50) for the two periods in the greenhouse (21 or 28 days), of the four *Eucalyptus grandis* clones and overall mean of the clones (MC). The means followed by the same upper case letter within the same technique and among treatments and those followed by a lower case letter within the same treatment and among techniques, respectively, do not differ by the Tukey test ($P < 0.05$). (Diâmetro do colo das mudas aos 50 dias de idade (RCD50), para os dois tempos de permanência em casa de vegetação (21 e 28 dias), dos quatro clones estudados e média dos clones (MC). As médias seguidas de uma mesma letra maiúscula dentro de uma mesma técnica e entre tratamentos e as seguidas de uma letra minúscula dentro do mesmo tratamento e entre técnicas, respectivamente, não diferem entre si, pelo teste de Tukey a 5% de probabilidade)

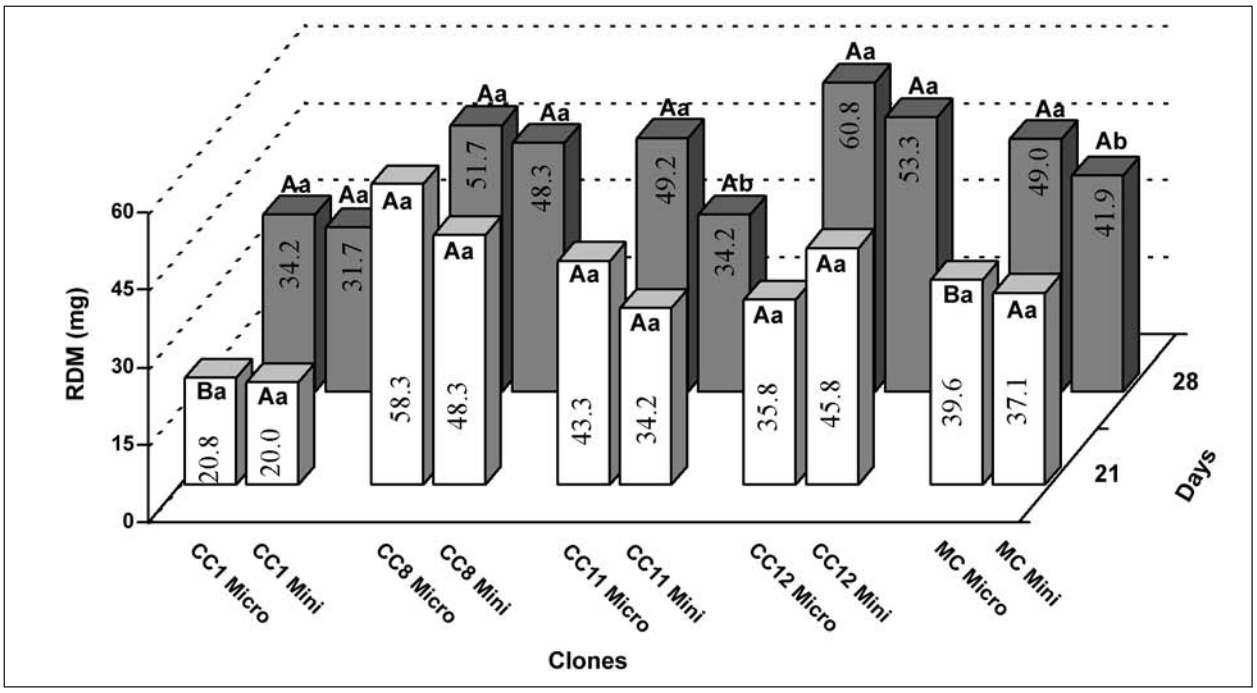


**Figure 7.** Micro-cutting and mini-cutting root dry matter weight at 28 days (RDM) for the two periods in the greenhouse (21 or 28 days), of the four *Eucalyptus grandis* clones and overall mean of the clones (MC). The means followed by the same upper case letter within the same technique and among treatments and those followed by a lower case letter within the same treatment and among techniques, respectively, do not differ by the Tukey test ($P < 0.05$). (Peso de matéria seca de raízes das microestacas e miniestacas aos 28 dias (RDM), para os dois tempos de permanência em casa de vegetação (21 ou 28 dias), dos quatro clones estudados e média dos clones (MC). As médias seguidas de uma mesma letra maiúscula dentro de uma mesma técnica e entre tratamentos e as seguidas de uma letra minúscula dentro do mesmo tratamento e entre técnicas, respectivamente, não diferem entre si, pelo teste de Tukey a 5% de probabilidade)

## CONCLUSION

The results suggest that micro-cutting technique possesses higher performance regarding to clonal propagation of *Eucalyptus grandis* as compared to mini-cutting, mainly for those difficult-to-root clones, as indicated by higher values of rooting and survival rates, height, root collar diameter and root dry matter weight.

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## REFERENCES

ASSIS, T.F. Propagação vegetativa de *Eucalyptus* por microestaquia. In: IUFRO CONFERENCE ON SILVICULTURE AND IMPROVEMENT OF EUCALYPTS, Salvador, 1997. **Proceedings**... Colombo: Embrapa Florestas, 1997. v.1, p.300-304.

ASSIS, T.F.; ROSA, O.P.; GONÇALVES, S.I. Propagação por microestaquia. In: CONGRESSO FLORESTAL ESTADUAL, 7, Nova Prata, 1992. **Anais**... Santa Maria: UFSM, 1992. p.824-836.

BONGA, J.M. Vegetative propagation in relation to juvenility, maturity and rejuvenation. In: BONGA, J.M.; DURZAN, D.J. (Eds.). **Tissue culture in forestry**. Boston: Martinus Nijhoff Publishers, 1982. p.387-412.

CARNEIRO, J.G.A. **Produção e controle de qualidade de mudas florestais**. Curitiba: UFPR/FUPEF, 1995. 451p.

GEORGE, E.F. **Plant propagation by tissue culture: the technology**. 6.ed. London: Exegetics, 1993. v.1, 574p.

GOMES, A.L. **Propagação clonal: princípios e particularidades**. Vila Real: Universidade de Trás-os-Montes e Alto Douro, 1987. 69p. (Série Didáctica, Ciências Aplicadas, 1).

GREENWOOD, M.S.; HUTCHISON, K.W. Maturation as a development process. In: AHUJA, M.R.; LIBBY, W.J. (Eds.). **Clonal forestry: genetics and biotechnology**. Budapest: Springer-Verlag, 1993. p.14-33.

GUPTA, P.K.; DURZAN, D.J. Micropropagation and phase specificity in mature, elite Douglas fir. **Journal of the American Society of Horticulture Science**, Alexandria, v.112, p.969-971, 1987.

HACKETT, W.P. Juvenility and maturity. In: BONGA, J.M.; DURZAN, D.J. (Eds.). **Cell and tissue culture in forestry**. Dordrecht: Kluwer Academic Publishers, 1987. v.1, p.216-231.

HARTMANN, H.T.; KESTER, D.E.; DAVIES JUNIOR, F.T.; GENEVE, R.L. **Plant propagation: principles and practices**. 6.ed. New Jersey: Prentice-Hall, 1997. 770p.

STRUVE, D.K.; LINEBERGER, R.D. Restoration of high adventitious root regeneration potential in mature *Betula papyrifera* Marsh. softwood stem cuttings. **Canadian Journal of Forestry Research**, Ottawa, v.18, p.265-269, 1988.

TITON, M. **Propagação clonal de *Eucalyptus grandis* por miniestaquia e microestaquia**. 2001. 65p. Dissertação (Mestrado em Ciência Florestal) – Universidade Federal de Viçosa, Viçosa, 2001.

WENDLING, I.; XAVIER, A.; GOMES, J.M.; PIRES, I.E.; ANDRADE, H.B. Propagação clonal de híbridos de *Eucalyptus* spp. por miniestaquia. **Revista Árvore**, Viçosa, v.24, p.181-186, 2000.

XAVIER, A.; COMÉRIO, J. Microestaquia: uma maximização da micropropagação de *Eucalyptus*. **Revista Árvore**, Viçosa, v.20, p.9-16, 1996.

XAVIER, A.; COMÉRIO, J.; IANNELLI, C.M. Eficiência da microestaquia e da micropropagação na clonagem de *Eucalyptus* spp. In: IUFRO CONFERENCE ON SILVICULTURE AND IMPROVEMENT OF EUCALYPTS, Salvador, 1997. **Proceedings**... Colombo: Embrapa Florestas, 1997. v.4, p.40-45.

XAVIER, A.; WENDLING, I. **Miniestaquia na clonagem de *Eucalyptus***. Viçosa: SIF, 1998. 10p. (Informativo Técnico SIF, 11).

ZOBEL, B.J. Clonal forestry in the *Eucalyptus*. In: AHUJA, M.R.; LIBBY, W.J. (Eds.). **Clonal forestry: conservation and application**. Budapest: Springer-Verlag, 1993. v.2, p.139-148.

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