

## *In vitro* ESTABLISHMENT OF *Eucalyptus* AND *Corymbia* SPECIES FROM EPICORMIC SHOOTS

Maria Lopes Martins Avelar<sup>2\*</sup>, Denys Matheus Santana Costa Souza<sup>2</sup>, Eduardo Henrique Macedo<sup>3</sup>,  
Leticia Vaz Molinari<sup>4</sup> and Gilvano Ebling Brondani<sup>5</sup>

<sup>1</sup> Received on 29.03.2019 accepted for publication on 25.06.2020.

<sup>2</sup> Universidade Federal de Lavras, Programa de Pós-Graduação em Engenharia Florestal, Lavras, MG-Brasil. E-mail: <maria.lma@hotmail.com> and <dmscsouza@gmail.com>.

<sup>3</sup> Klabin, Planejamento Florestal, Telêmaco Borba, PR-Brasil. E-mail: <eduardo.hmacedo@gmail.com>.

<sup>4</sup> Universidade Federal de Lavras, Programa de Pós-Graduação em Agronomia, Lavras, MG-Brasil. E-mail: <leticia\_vaz03@hotmail.com>.

<sup>5</sup> Universidade Federal de Lavras, Departamento de Ciências Florestais, Lavras, MG-Brasil. E-mail: <gilvano.brondani@ufla.br>.

\*Corresponding author.

**ABSTRACT** – The importance of *Eucalyptus* and *Corymbia* has been evident in forestry programs, mainly due to their adaptation to various environmental conditions and the multiple products that can be obtained. As the selection and cloning of superior individuals are usually performed in adulthood, the development and adaptation of the vegetative propagation techniques that enable the rescue and *in vitro* establishment of species are necessary. Thus, the present study aimed to induce epicormic shoots from pruned branches of adult trees and to promote the *in vitro* establishment of nine species: six of *Eucalyptus* and three of *Corymbia*. The material used to obtain the explants came from the selection of two 44-year-old mother plants of each species, from species, and provenance tests. The number of buds and shoots of each branch was evaluated at 15, 25, 35, and 45 days after the beginning of the experiment in a greenhouse. According to the results, the induction of epicormic shoots from pruned branches was considered a viable and efficient technique to obtain propagule sources from adult mother plants of all species, presenting a gradual increase in the evaluated characteristics over time. Also, *in vitro* culture is an effective alternative to improve the propagation of the evaluated species, with an establishment of up to 62.5%. However, for *Corymbia citriodora* (Hook.) K.D. Hill & L.A.S Johnson and *Eucalyptus tereticornis* Smith it is necessary to develop further studies to enable the *in vitro* establishment.

**Keywords:** Shoot induction; *In vitro* culture; Vegetative propagation.

## **ESTABELECIMENTO *in vitro* DE ESPÉCIES DE *Eucalyptus* E *Corymbia* POR MEIO DE BROTAÇÕES EPICÓRMICAS**

**RESUMO** – A importância de espécies dos gêneros *Eucalyptus* e *Corymbia* vem sendo evidenciada nos programas de silvicultura, principalmente pela adaptação em diversas condições ambientais e pelos múltiplos produtos que podem ser obtidos. Como a seleção e a clonagem de indivíduos superiores geralmente são realizadas na fase adulta, o desenvolvimento e a adequação de técnicas de propagação vegetativa que possibilitem o resgate e o estabelecimento *in vitro* de espécies desses gêneros são necessários. Dessa forma, o presente estudo teve como objetivo induzir brotos epicórmicos a partir de ramos podados de árvores adultas e promover o estabelecimento *in vitro* de nove espécies, sendo seis do gênero *Eucalyptus* e três de *Corymbia*. O material utilizado para a obtenção dos explantes foi proveniente da seleção de duas matrizes de cada espécie com 44 anos de idade, oriundas da implantação de um teste de espécies e procedências. Foram avaliados o número de gemas e brotos de cada ramo aos 15, 25, 35 e 45 dias após o início do experimento em casa de vegetação. De acordo com os resultados, a indução de brotações epicórmicas a partir de ramos podados foi considerada uma técnica viável e eficiente para obter fontes de propágulos de matrizes adultas de todas as espécies, apresentando um aumento gradual das características avaliadas ao longo do tempo. Além disso, o cultivo *in vitro* é uma alternativa eficaz para estabelecer propágulos a partir de brotações epicórmicas de árvores adultas da maioria das espécies avaliadas, com estabelecimento de até 62,5%. Contudo, para *Corymbia citriodora* (Hook.) K.D.



Hill & L.A.S Johnson e *Eucalyptus tereticornis* Smith é necessário o desenvolvimento de mais estudos para viabilizar o estabelecimento *in vitro*.

Palavras-Chave: Indução de brotos; Cultivo *in vitro*; Propagação vegetativa.

## 1. INTRODUCTION

In the mid-1970s, eucalypts species and provenance tests were implemented in Brazil for testing potential species for industrial applications, aimed at possibly forming future forest plantations. The implementation of these tests was carried out through the 'Projeto de Desenvolvimento e Pesquisa Florestal (PRODEPEF)', together with the 'Instituto Brasileiro de Desenvolvimento Florestal (IBDF)' and 'Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA)' (IPEF, 1984).

One of the last remaining and well-preserved test sites is in Lavras, Minas Gerais, in the Forest Nursery, Department of Forestry Sciences, Federal University of Lavras (UFLA), where seedlings produced from seeds from different regions of Australia were planted, comprising twenty-seven species in total, of the genera *Eucalyptus* and *Corymbia*.

The cloning of superior individuals, which aims to increase the homogeneity and productivity of stands or species conservation, involves steps that begin with the selection of genotypes, followed by the selection of vegetative propagation techniques. In most cases, this selection is only performed in the adult stage, where vegetative propagation has shown certain limitations, such as reduced rooting ability of cuttings due to the advanced maturity of plant tissues, which is linked to ontogenetic age (Wendling et al., 2014).

Several propagation techniques have been adopted to promote tissue rejuvenation and/or juvenile shoot induction in adult plants, such as girdling (Hartmann et al., 2011), cutting (Trueman et al., 2013), mini-cutting (Brondani et al., 2012a), coppicing (Xavier et al., 2013), epicormic shoot induction (Wendling et al., 2013) and micropropagation (Brondani et al., 2012b; Brondani et al., 2018; Souza et al., 2019a). These techniques can be used to rescue and clone selected trees (Baccarin et al., 2015).

Some of these techniques become infeasible due to the impossibility of slaughter or girdling of the mother plants in species and provenance tests. Thus, the

induction of epicormic shoots from pruned branches of selected mother plants provides a feasible alternative for obtaining juvenile shoots (Wendling et al., 2013; Baccarin et al., 2015; Oliveira et al., 2015). These buds originate from latent axillary buds, called epicormic buds, are used to reconstitute the plant crown (Wendling et al., 2013) and can contribute to the propagation and multiplication of selected trees.

Thus, the objective of this study was to induce epicormic shoots from pruned branches of adult trees of species and provenance test and promote the *in vitro* establishment of nine species: six from the genus *Eucalyptus* and three from the genus *Corymbia*.

## 2. MATERIALS AND METHODS

### 2.1. Study site and experimental material

The experiments were conducted at the Forest Nursery and Laboratory of *In Vitro* Culture of Forest Species, belonging to the Department of Forestry Sciences of the Federal University of Lavras (UFLA), located in the municipality of Lavras, Minas Gerais (MG) state, Brazil (21°14'S latitude; 44°59'W longitude and 919 m altitude).

The material used to obtain the explants (nodal segments) originated from two 44-year-old mother plants of each species (Table 1), with six *Eucalyptus* species (*Eucalyptus microcorys* F. Muell., *Eucalyptus grandis* W. Hill ex Maiden, *Eucalyptus camaldulensis* Dehnh., *Eucalyptus cloeziana* F. Muell., *Eucalyptus pilularis* Smith and *Eucalyptus tereticornis* Smith), and three *Corymbia* species (*Corymbia citriodora* (Hook.) K.D. Hill & L.A.S Johnson, *Corymbia maculata* (Hook.) K.D. Hill & L.A.S Johnson and *Corymbia torelliana* (F. Muell.) K.D. Hill & L.A.S. Johnson), derived from *Eucalyptus* and *Corymbia* species tested and with provenance in the Forest Nursery of UFLA in 1974 (IPEF, 1984).

### 2.2. Collection and preparation of branches for the induction of epicormic shoots

The mother plants were selected based on visual criteria, ensuring that the trunk was as straight as

**Table 1** – Provenances and location of the selected mother plants in the species and provenance test of the nine species of *Eucalyptus* and *Corymbia*.**Table 1** – Procedências e localização das matrizes selecionadas no teste de espécies e procedências das nove espécies de *Eucalyptus* e *Corymbia* utilizadas.

Species	Mother plants	Field	Identification	Provenances	Lat (S)	Long (E)	Alt (m)
<i>E. microcorys</i>	M1	B-8	8717	Gympie	26°11'	152°40'	100
<i>E. microcorys</i>	M2	B-8	8717	Gympie	26°11'	152°40'	100
<i>E. grandis</i>	M1	B-1A	9753	Coff's Harbour	30°18'	153°08'	91
<i>E. grandis</i>	M2	B-1A	9753	Coff's Harbour	30°18'	153°08'	91
<i>E. camaldulensis</i>	M1	B-1A	10266	Petford	17°17'	145°59'	460
<i>E. camaldulensis</i>	M2	B-1A	10266	Petford	17°17'	145°59'	460
<i>E. cloeziana</i>	M1	B-1C	9785	SW Kennedy	18°17'	145°55'	122
<i>E. cloeziana</i>	M2	B-1C	9785	SW Kennedy	18°17'	145°55'	122
<i>E. pilularis</i>	M1	B-1E	9492	Gallangowan	26°30'	152°20'	580
<i>E. pilularis</i>	M2	B-9(Y)	6183	Mte. Glorius – W Brisbane	27°15'	152°40'	600
<i>E. tereticornis</i>	M1	B-1C	10054	Atherton Dist.	18°17'	145°58'	15
<i>E. tereticornis</i>	M2	B-1C	10054	Atherton Dist.	18°17'	145°58'	15
<i>C. citriodora</i>	M1	B-1C	10268	W Herbeton	17°24'	145°20'	853
<i>C. citriodora</i>	M2	B-1C	10268	W Herbeton	17°24'	145°20'	853
<i>C. maculata</i>	M1	B-9(Y)	6168	W Brisbane	27°15'	152°40'	550
<i>C. maculata</i>	M2	B-9(Y)	6168	W Brisbane	27°15'	152°40'	550
<i>C. torelliana</i>	M1	B-1D	10466	Atherton Area	17°12'	145°35'	694
<i>C. torelliana</i>	M2	B-1D	10466	Atherton Area	17°12'	145°35'	694

Lat: latitude; Long: longitude; Alt: altitude. Source: Adapted from IPEF (1984).  
 Lat: latitude; Long: longitude; Alt: altitude. Fonte: Adaptado de IPEF (1984).

possible, free of pathogen attacks and had branches in the lower portion of the crown to minimize the effects of ontogenetic age (Almeida et al., 2007) and to facilitate the cutting and collection of the branches (Figure 1A).

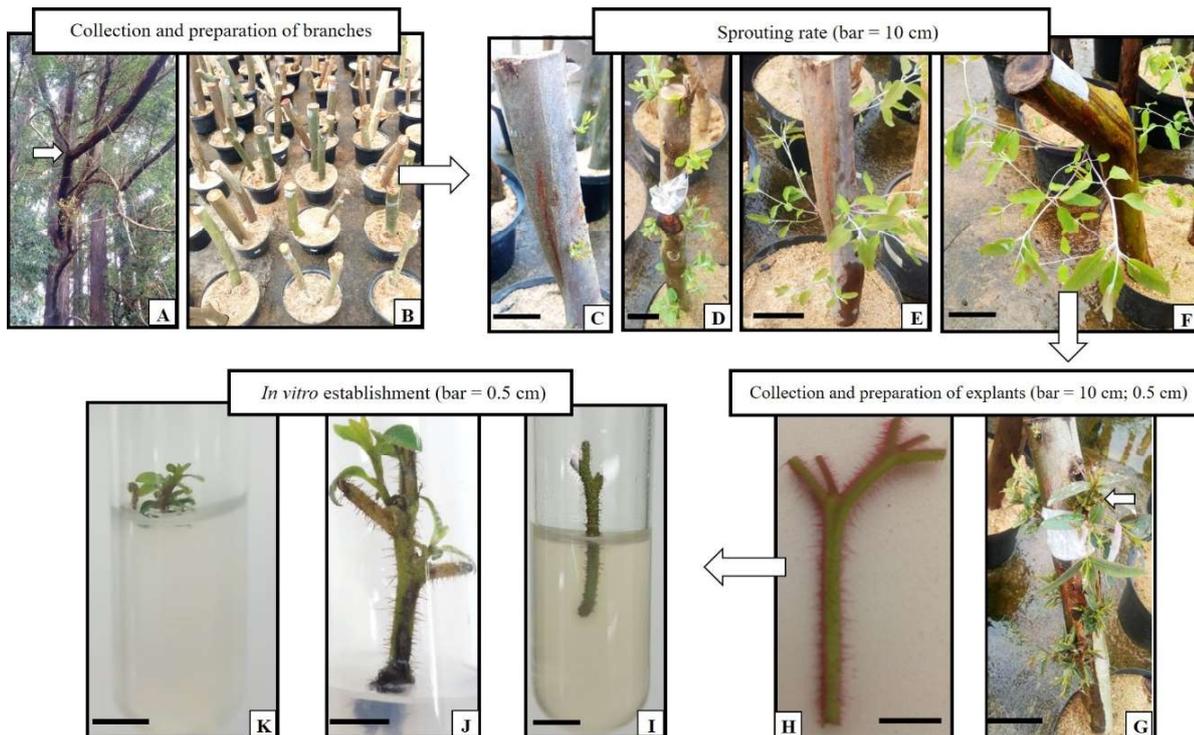
The branches were sectioned into lengths of approximately 50 cm and placed in a greenhouse with controlled relative humidity and temperature (RH > 80%; temperature between 20 and 35°C) and irrigation by an intermittent misting system with high-pressure and low-flow nozzles, controlled automatically by a humidistat. These branches were placed vertically in polyethylene pots (5 L), which were filled with washed sand, without fertilization, for induction of epicormic shoots, for 45 days since collection, performed on the same day for all species (Figure 1B).

The experiment was arranged in a randomized block design, with six species of *Eucalyptus* and three species of *Corymbia* (i.e., nine species in total), with six replicates composed of plots with two branches per pot, totalling 108 branches. Total number of epicormic buds and the total number of shoots per branch at 15, 25, 35 and 45 days after installation of the experiment were evaluated.

### 2.3. In vitro establishment

Shoots (Figure 1G) were collected after the branches stayed for 45 days in the greenhouse, being applied dimethyl 4,4'- (o-phenylene) bis (3-thioallophanate) fungicide 48 h before the shoot collection (i.e., concentration of 0.5 g L<sup>-1</sup>). Epicormic shoots with 4 to 5 cm of length were collected, immersed in autoclaved deionized water and transported to the laboratory.

The nodal segments (standardized with two axillary buds, without leaves, and 2 to 3 cm of length) were used as explants (Figure 1H); these explants were washed in running water for 5 min. Subsequently, they were immersed in 70% alcohol solution (v/v) for 30 s with constant agitation inside a horizontal laminar flow hood. They were then immersed in Clarix® NaOCl solution (1.00-1.50% of active chlorine) for 10 min. The nodal segments were washed in autoclaved deionized water three times after immersion in alcohol and NaOCl and inoculated vertically under aseptic conditions in test tubes (15.0 cm × 2.5 cm) containing 10 mL of MS culture medium (Murashige and Skoog, 1962). Throughout the entire process, the equipment used was disinfected with 70% alcohol solution (v/v).



**Figure 1** – Steps to obtain propagules from the selection and pruning of selected adult tree branches to the *in vitro* establishment of nodal segments prepared from epicormic shoots. (A) Selected mother plant, highlighting the portion used to obtain the branches; (B) Branches after standardization and arranged in a greenhouse; (C) Detail of *Eucalyptus grandis* shoots at 15 days; (D) Detail of *Eucalyptus grandis* shoots at 25 days; (E) Detail of *Eucalyptus grandis* shoots at 35 days; (F) Detail of *Eucalyptus grandis* shoots at 45 days; (G) Epicormic shoots recently collected from the branches, highlighting the obtaining of the explants; (H) Nodal segment after preparation; (I) Explant introduced in MS culture medium; (J) Beginning of *in vitro* establishment with the appearance of the first shoots; (K) *In vitro* established explant.

**Figura 1** – Etapas para obtenção de propágulos desde a seleção e poda de ramos de árvores adultas selecionadas até o estabelecimento *in vitro* de segmentos nodais preparados a partir de brotações epicórmicas: (A) Matriz selecionada, destacando a porção utilizada para obtenção dos ramos; (B) Ramos após a padronização e dispostos em casa de vegetação; (C) Detalhe da brotação de *E. grandis* aos 15 dias; (D) Detalhe da brotação de *E. grandis* aos 25 dias; (E) Detalhe da brotação de *E. grandis* aos 35 dias; (F) Detalhe da brotação de *E. grandis* aos 45 dias; (G) Brotação epicórmica recém coletada dos ramos, destacando a obtenção dos explantes; (H) Segmento nodal após preparo; (I) Explante introduzido em meio de cultura MS; (J) Início do estabelecimento *in vitro* com o aparecimento das primeiras brotações; (K) Explante estabelecido *in vitro*.

The time from explant collection in the field until inoculation in culture medium was less than 2 h. During the collection, transport and intervals between disinfection and inoculation, the explants were kept immersed in autoclaved deionized water to prevent dehydration.

The culture medium was supplemented with 30 g L<sup>-1</sup> of sucrose (Synth Ltda) and 6 g L<sup>-1</sup> of agar (Merck S.A.), without adding of plant growth regulators. The culture medium was prepared using deionized water, and the pH was adjusted to 5.80 ± 0.05 with NaOH (0.1 M) and HCl (0.1 M) before autoclaving and the addition of the agar. The culture medium was autoclaved at 127°C and 1.5 kgf cm<sup>-2</sup> of pressure for 20 min.

After inoculation, the explants were kept in a growth room at 24 ± 1°C, a photoperiod of 16 h and irradiance of 40 μmol m<sup>-2</sup> s<sup>-1</sup> (quantified by radiometer, LI-COR®, LI-250A Light Metre) for 35 days.

Regarding the *in vitro* establishment (Figure 1K), the experiment was performed in a completely randomized design, with nine species constituting the treatments with forty replicates composed of plots with one explant per test tube. At 35 days after inoculation for *in vitro* establishment, the following characteristics were evaluated: mean percentage of fungal and/or bacterial manifestation (i.e., *in vitro* contamination), oxidation of tissues, non-responsive explants (i.e., explants that showed green coloration and absence of oxidation,

however absence of bud and/or shoot emission), *in vitro* establishment (i.e., explants with absence of contamination, oxidation and which bud and/or shoot emission), length of shoots (> 0.5 cm) and mean number of shoots per explant (>0.5 cm).

## 2.5. Data analysis

The analyses were performed using R Core Team software (2018), using the ExpDes package, version 1.1.2 (Ferreira et al., 2013). The data collected from the treatments were used to perform the polynomial regression analysis of the variables evaluated according to the evaluation times for each species in a greenhouse, and to perform statistical analysis (ANOVA) and Tukey's test at 5% of probability for *in vitro* establishment of explants.

## 3. RESULTS

### 3.1. Induction of epicormic buds and shoots

The data observed between species of *Eucalyptus* and *Corymbia* for the number of buds and shoots in relation to the time of evaluation were better adjusted in polynomial curves of the second degree, in which they presented higher coefficient of determination ( $R^2$ ) (Figure 2).

The number of buds (Figure 2), i.e. protruding meristems observed in the branches, from which the shoots were emitted, *E. camaldulensis* had the highest values (39 and 70 buds at 15 and 25 days in a greenhouse, respectively). However, in the first evaluation, at 15 days, *E. cloeziana*, *C. citriodora*, *C. maculata*, *C. torelliana* and *E. tereticornis* did not show epicormic buds. Subsequent evaluations resulted in a higher number of buds for *E. grandis* (80 and 87 buds at 35 and 45 days, respectively) and lower values for *E. tereticornis* (7 and 10 buds at 35 and 45 days, respectively) (Figure 2).

Among the species evaluated, regarding the sprouting rate (Figure 3), it was observed that, on the first evaluation (15 days), *E. grandis* had the highest number of shoots (54 shoots), while *E. cloeziana*, *C. citriodora*, *C. maculata*, *C. torelliana* and *E. tereticornis* did not show epicormic shoots. The largest number of shoots (159, 188 and 219 shoots at 25, 35 and 45 days, respectively) was observed for *E. pilularis*, with the lowest values observed for *E. tereticornis* in all evaluations, resulting in 14 shoots at 45 days. Throughout the experiment, a gradual increase was observed for the

number of epicormic buds and shoots in relation to the different evaluation times (15, 25, 35 and 45 days after the beginning of the experiment).

### 3.2. In vitro establishment

At 35 days after inoculation in culture medium, the species exhibited significant differences for percentages of contamination, with the lowest mean (20.0%) being observed for *E. grandis* (Figure 4A), which differed statistically from the species. On the other hand, *E. tereticornis* and *C. citriodora* reached, on average, 75.0% of contamination (Figure 4A), being one of the factors that limited the *in vitro* establishment, however they did not differentiate from *E. cloeziana*, *E. microcorys* and *C. maculata*.

Regarding phenolic oxidation in the tissues, the lowest means, 5 and 8%, were observed, respectively, for *E. pilularis* and *E. microcorys* (Figure 4B), which differed significantly from the other species.

The amount of non-responsive explants *in vitro* (explants that showed green coloration and no oxidation, however absence of bud and shoot emission – Figure 1C) was statistically similar for *E. camaldulensis*, *C. citriodora* and *E. tereticornis*, in which all the explants were responsive, and for *E. pilularis* and *E. grandis*, in which both presented 5.0% of non-responsive explants.

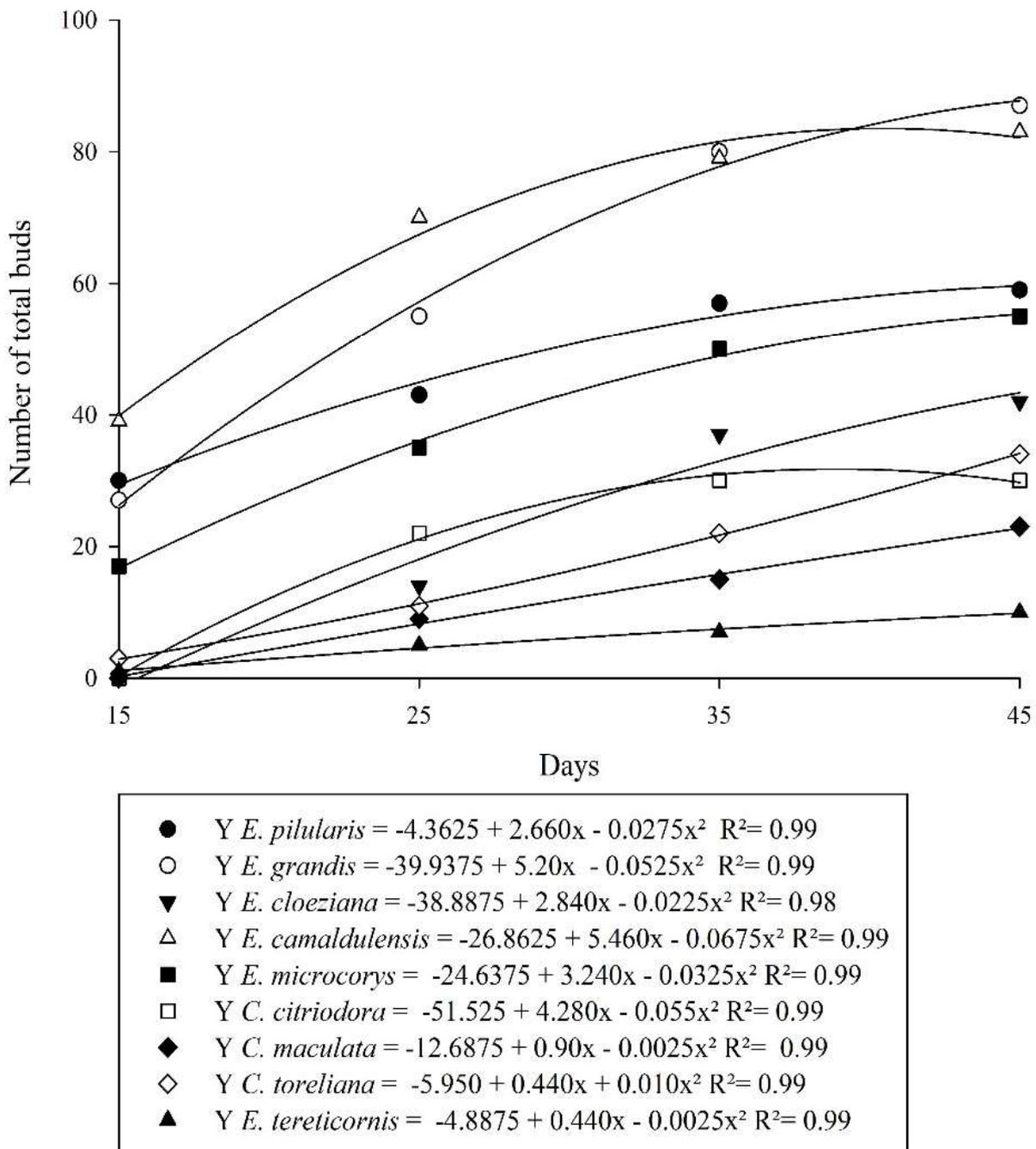
Regarding the *in vitro* establishment (Figure 1D), *E. pilularis* (60.0%) and *E. grandis* (62.5%) showed the highest values. In contrast, no explants were established for *C. citriodora* and *E. tereticornis*.

The numbers of shoots induced from the explants (Figure 4E) in *E. grandis* (mean of 2.2 shoots), *E. cloeziana* (mean of 2.4 shoots), *E. microcorys* (mean of 1.9 shoots) and *C. torelliana* (mean of 2.5 shoots) were the highest observed, showing a significant difference from the other species. For the length of the shoots (Figure 4F), *C. torelliana* had the highest values (mean of 0.95 cm), differing statistically only for species that could not be *in vitro* established.

## 4. DISCUSSION

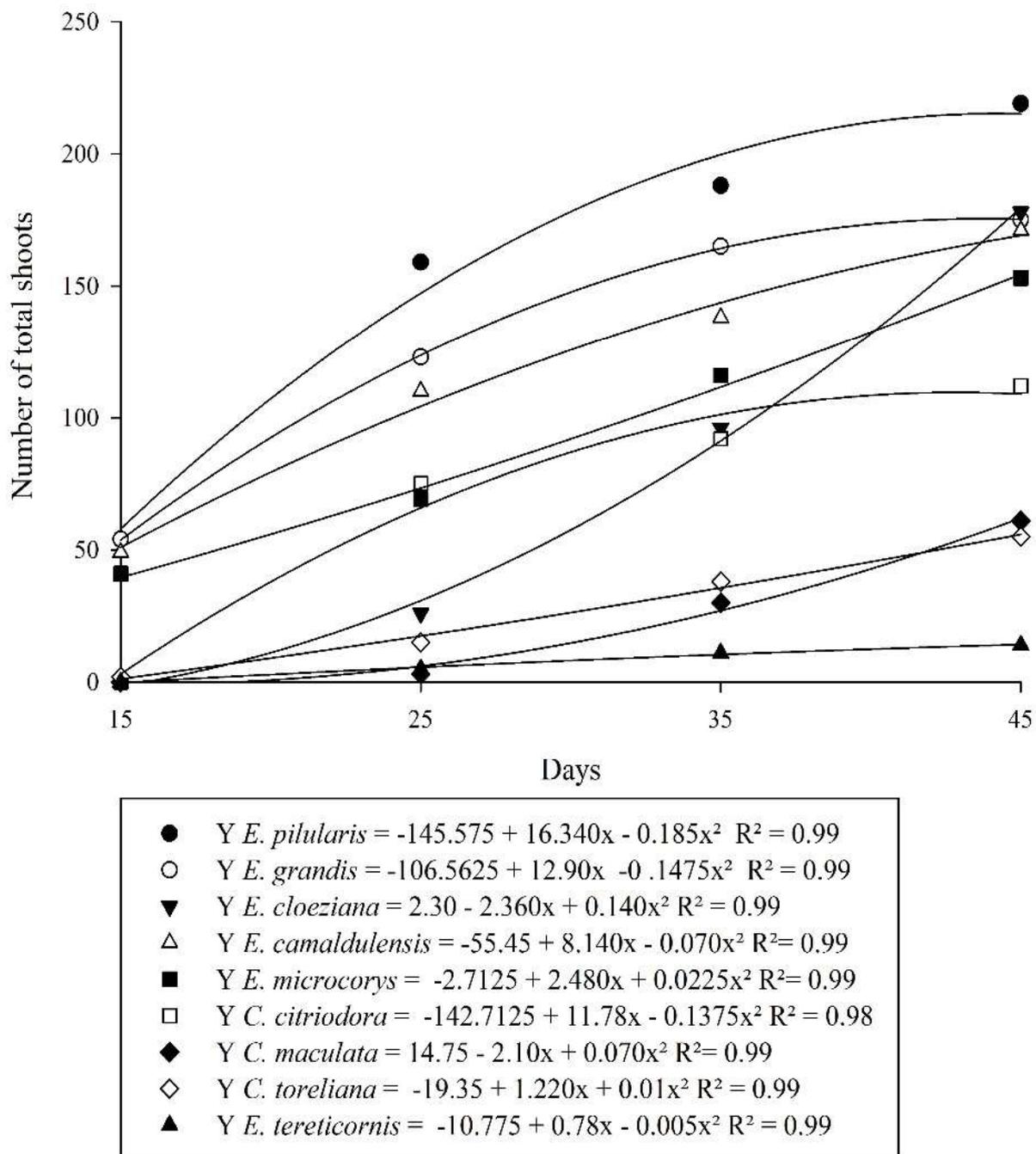
### 4.1. Induction of epicormic buds and shoots

The induction of epicormic buds and shoots from pruned branches was considered a viable technique for all species studied, showing a gradual increase in the characteristics evaluated over time. Several



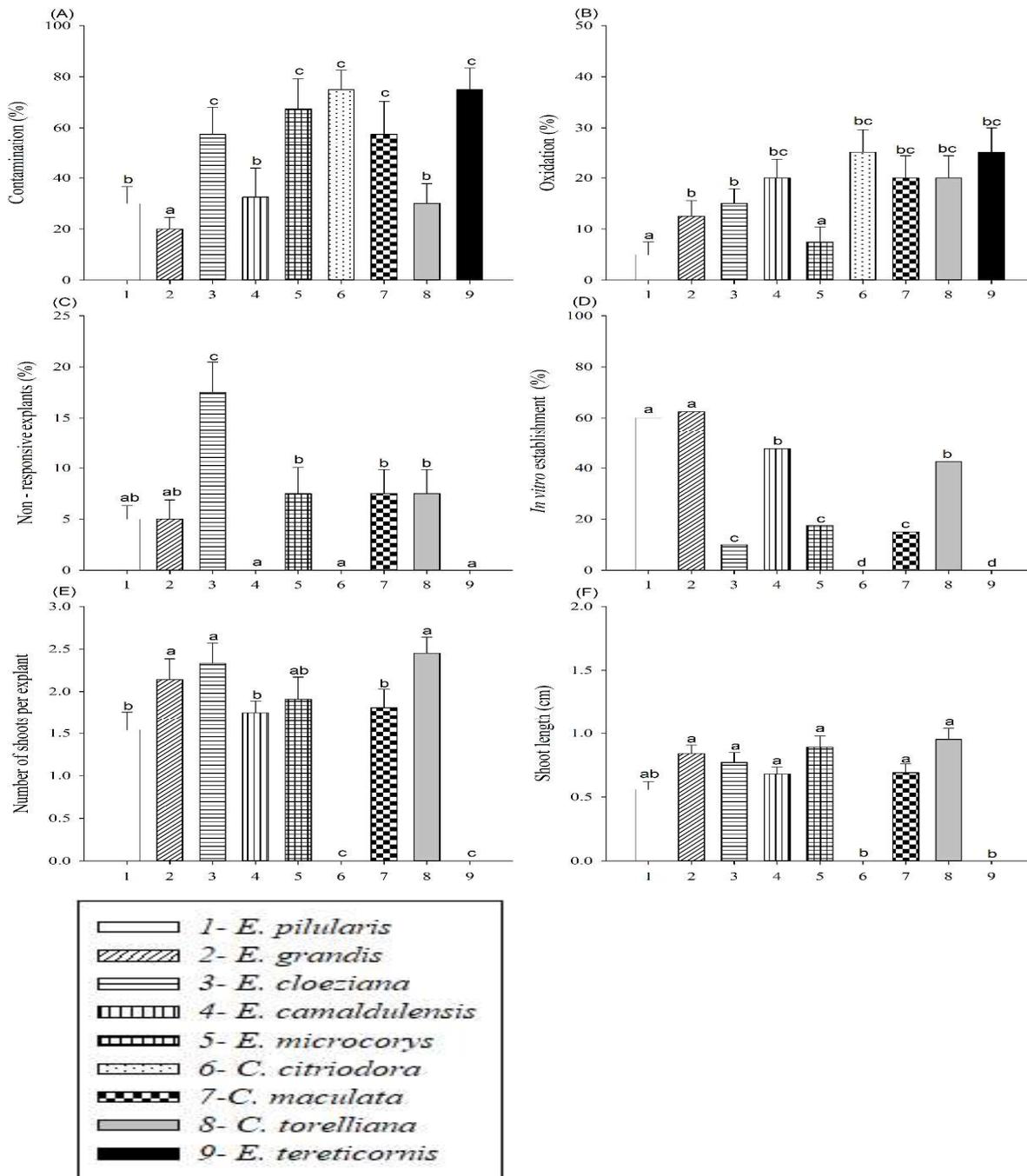
**Figure 2** – Number of buds observed in the pruned branches according to the evaluations (15, 25, 35 and 45 days after the beginning of the experiment in a greenhouse) of the nine species of eucalypts (*Eucalyptus* and *Corymbia*).

**Figura 2** – Número de gemas observadas nos ramos podados em função das avaliações (15, 25, 35 e 45 dias após o início do experimento em casa de vegetação) das nove espécies dos gêneros *Eucalyptus* e *Corymbia*.



**Figure 3** – Number of shoots observed in the pruned branches according to the evaluations (15, 25, 35 and 45 days after the beginning of the experiment in a greenhouse) of the nine species of eucalypts (*Eucalyptus* and *Corymbia*).

**Figura 3** – Número de brotos observados a partir de gemas epicórmicas em função das avaliações (15, 25, 35 e 45 dias após o início do experimento em casa de vegetação) das nove espécies dos gêneros *Eucalyptus* e *Corymbia*.



**Figure 4** – Variables evaluated in the in vitro establishment of the nine species of eucalypts (*Eucalyptus* and *Corymbia*). (A) Percentage of contamination; (B) Percentage of oxidation; (C) Percentage of non-responsive explants; (D) Percentage of *in vitro* establishment; (E) Number of shoots per explant; (F) Shoot length. Mean with the same letter do not differ statistically from each other by the Tukey's test at 5% of probability.

**Figura 4** – Variáveis avaliadas no estabelecimento *in vitro* das nove espécies dos gêneros *Eucalyptus* e *Corymbia*: (A) Porcentagem de contaminação; (B) Porcentagem de oxidação; (C) Porcentagem de explantes não responsivos; (D) Porcentagem de estabelecimento *in vitro*; (E) Número de brotos por explante; (F) Comprimento de brotos. Médias com mesmas letras acima das colunas não diferem estatisticamente entre si pelo teste de Tukey à 5% de probabilidade de erro.

similar studies have indicated the efficiency of this rescue method, including plants of *Ilex paraguariensis* with 19-year-old (Wendling et al., 2013), *Eucalyptus benthamii* with 13-year-old (Baccarin et al., 2015) and *Eucalyptus cloeziana* with 26-year-old (Oliveira et al., 2015).

Wendling et al. (2013) used branches from adult individuals of *Ilex paraguariensis* to induce epicormic shoots and observed the emission of six shoots per pruned branch at 50 days. Nascimento et al. (2019) and Duarte et al. (2019), also working with *Ilex paraguariensis*, observed that the emission of epicormic shoots was efficient in the propagation of the species. In addition, Maggioni et al. (2020) reported the use of epicormic shoots as a way to promote higher rooting rates in *Araucaria angustifolia*.

According to Wendling et al. (2013) and Pires et al. (2013), the use of this technique is especially important for germplasm conservation and selection of superior individuals, for which rescue is normally performed in the adult stage when propagation may be limited due to ontogenetic age. The physiological principle for the induction of epicormic buds and shoots in pruned branches is related to the alteration of the balance in plant growth regulation (e.g., auxin and cytokinin), favoring the bud and shoot induction (Wendling et al., 2013).

The vegetative propagation of adult trees can be facilitated by the epicormic shoots induction, where tissues with a higher degree of juvenility can be obtained (Hartmann et al., 2011; Oliveira et al., 2015). However, in adulthood, vegetative propagation has shown limitations, such as reduced of adventitious rooting due to the advanced maturity of plant tissues (Wendling et al., 2014).

#### 4.2. In vitro establishment

Epicormic shoots, obtained from pruned branches, have also been used as a source of explants for the micropropagation of *Eucalyptus grandis* (Ikemori, 1987), *Quercus robur* (Vieitez et al., 1994), *Eucalyptus maidenii* (Sotelo and Monza, 2007), *Arbutus unedo* (Gomes and Canhoto, 2009), *Eucalyptus benthamii* (Baccarin et al., 2015), *Ilex paraguariensis* (Wendling et al., 2013) and *Eucalyptus cloeziana* (Oliveira et al., 2015).

At 35 days after *in vitro* inoculation of *Eucalyptus* and *Corymbia* tissues, variations were observed through

epicormic shoots. According to Trueman et al. (2018) the success of micropropagation is dependent on only a few explants forming shoots free of contamination, because the onset of *in vitro* propagation is a limiting phase.

However, when a large amount of micropropagated plants is required, higher rates of explants with shoots can be necessary to rapidly increase the number of clones produced. The different percentages of *in vitro* contamination demonstrate that the metabolic pathway of microorganisms may be regulated by the genotype, through differential genetic expression in carotenoid biosynthesis and hyphal aggregation (Postemsky and Curvetto, 2016).

As for phenolic oxidation, the percentages were considered low, despite the fact that these are mother plants with advanced ontogenetic age, from which higher oxidation values were expected. Phenolic oxidation has been a problem associated with the micropropagation of woody species, as reported in several studies with *E. cloeziana* (Oliveira et al., 2015), *E. benthamii* (Brondani et al., 2012b; Baccarin et al., 2015), *Eucalyptus dunnii* (Navroski et al., 2014), *Corymbia citriodora* × *Corymbia torelliana* and *Corymbia torelliana* × *Corymbia citriodora* (Souza et al., 2019b), which reported similar response.

This allows advancement to the multiplication phase, showing the importance of high induction rates. In *E. cloeziana*, Oliveira et al. (2015) obtained, on average, 51.2% explants with shoots. For *Eucalyptus urophylla* × *E. globulus*, Borges et al. (2012) observed 95.0% of explants with shoots. Thus, different results are observed according to the genotype and the culture conditions.

The use of epicormic shoots as a source of explants for micropropagation allows the rejuvenation/reinvigoration of tissues from mother plants in the field, which are usually found in the adult stage, when the tree tissues already show a certain level of maturity (Trueman et al., 2018) In this context, epicormic shoots is an effective alternative for vegetative propagation of adult trees through *in vitro* culture.

## 5. CONCLUSION

The epicormic shoots from branches pruned is an efficient technique for the rescue of eucalypts mature trees with 44-year-old.

*In vitro* culture is an alternative technique to improve the vegetative propagation of eucalypts adult trees, with establishment of 62.5%, except for *C. citriodora* and *E. tereticornis*, which need further studies to enable *in vitro* establishment.

## 6. ACKNOWLEDGMENTS

We thank the National Council for Scientific and Technological Development, Brazil (Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq), Coordination for Improvement of Higher Education Personnel, Brazil (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES – Código de Financiamento 001), and Foundation for Research of the State of Minas Gerais, Brazil (Fundação de Amparo a Pesquisa do Estado de Minas Gerais - FAPEMIG).

## 7. REFERENCES

- Almeida FD, Xavier A, Dias JM. Propagação vegetativa de árvores selecionadas de *Eucalyptus cloeziana* F. Muell por estaquia. *Revista Árvore*. 2007;31(3):445-53. doi: doi.org/10.1590/S0100-67622007000300010
- Baccarin FJB, Brondani GE, Almeida LV, Vieira IG, Oliveira LS, Almeida M. Vegetative rescue and cloning of *Eucalyptus benthamii* selected adult trees. *New Forests*. 2015;46(4):465-83. doi: doi.org/10.1007/s11056-015-9472-x
- Borges SR, Xavier A, Oliveira LS, Lopes AP, Otoni WC, Takahashi EK, et al. Estabelecimento in vitro de clones híbridos de *Eucalyptus globulus*. *Ciência Florestal*. 2012;22(3):605-16. doi: doi.org/10.5902/198050986626
- Brondani GE, Baccarin FJB, Wit Ondas HW, Stape JL, Gonçalves AN, Almeida M. Low temperature, IBA concentrations and optimal time for adventitious rooting of *Eucalyptus benthamii* minicuttings. *Journal of Forestry Research*. 2012a;23(4):583-92. doi: doi.org/10.1007/s11676-012-0298-5
- Brondani GE, Oliveira LS, Konzen ER, Silva ALLD, Costa JL. Mini-incubators improve the adventitious rooting performance of *Corymbia* and *Eucalyptus* microcuttings according to the environment in which they are conditioned. *Anais da Academia Brasileira de Ciências*. 2018;90(2 supl.1):2409-23. doi: doi.org/10.1590/0001-3765201720170284
- Brondani GE, Wit Ondas HW, Baccarin FJB, Gonçalves AN, Almeida M. Micropropagation of *Eucalyptus benthamii* to form a clonal micro-garden. *In Vitro Cellular & Developmental Biology – Plant*. 2012b;48(5):478-87. doi: doi.org/10.1007/s11627-012-9449-9
- Duarte MM, Mireski MC, Oliszeski A, Wendling I, Stuepp CA. Rooting of yerba mate cuttings with different lengths. *Revista Eletrônica Científica da UERGS*. 2019;5(1):5-11. doi: doi.org/10.21674/2448-0479.51.6-12
- Ferreira EB, Cavalcanti PP, Nogueira DA. ExpDes: experimental designs package. R package version 1.1.2. 2013.
- Gomes F, Canhoto JM. Micropropagation of strawberry tree (*Arbutus unedo* L.) from adult plants. *In Vitro Cellular & Developmental Biology – Plant*. 2009;45(1):72-82. doi: doi.org/10.1007/s11627-008-9164-8
- Hartmann HT, Kester DE, Davies Junior FT, Geneve RL. *Plant propagation: principles and practices*. 8th ed. São Paulo: Prentice-Hall; 2011. ISBN 9780135014493.
- Ikemori YK. Epicormic shoots from the branches of *Eucalyptus grandis* as an explant source for *in vitro* culture. *Commonwealth Forestry Review*. 1987;66(4):351-56.
- Instituto de Pesquisas e Estudos Florestais - IPEF. Procedências de *Eucalyptus* spp introduzidas no Brasil por diferentes entidades. *Boletim Informativo*. 1984;10(29):259. Disponível em: <http://www.ipef.br/publicacoes/boletim\_informativo/bolinf29.pdf>. Acesso em: 03 de dezembro de 2018.
- Maggioni RA, Tomasi JC, Zuffellato-Ribas KC, Wendling I. *Araucaria angustifolia*: ácido indolbutírico e diferentes clones no enraizamento de estacas. *Advances in Forestry Science*. 2020;7(1):861-66. doi: doi.org/10.34062/afs.v7i1.7429
- Murashige T, Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum*. 1962;15(3):473-97. doi: doi.org/10.1111/j.1399-3054.1962.tb08052.x
- Nascimento B, Sá ACS, Souza G, Pereira

- MO, Navroski MC. Nitrogenated fertilization favors vegetative rescue and propagation of *Ilex paraguariensis*. *Cerne*. 2019;25(1):76-83. doi: doi.org/10.1111/j.1399-3054.1962.tb08052.x
- Navroski MC, Reiniger LRS, Araújo MM, Curti AR, Pereira MO. In vitro establishment and multiplication of genotypes of *Eucalyptus dunnii* Maiden. *Cerne*. 2014;20(1):139-46. doi: doi.org/10.1590/S0104-77602014000100017
- Oliveira LS, Brondani GE, Batagin-Piotto KD, Calsavara R, Gonçalves AN, Almeida M. Micropropagation of *Eucalyptus cloeziana* mature trees. *Australian Forestry*. 2015;78(4):219-31. doi: doi.org/10.1080/00049158.2015.1073211
- Pires PP, Wendling I, Brondani G. Indol-butyric acid and orthotropism in minicutting of *Araucaria angustifolia*. *Revista Árvore*. 2013;37(3):393-99. doi: doi.org/10.1590/S0100-67622013000300002
- Postemsky PD, Curvetto NR. In vitro studies of secondary metabolite-related responses in some species of genus *Grifola* (Agaricomycetes) from Argentina. *International Journal of Medicinal Mushrooms*. 2016;18(4):355-63. doi: doi.org/10.1615/IntJMedMushrooms.v18.i4.90
- R Development Core Team. R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2018. Available at: <URL <https://www.R-project.org/>>
- Sotelo M, Monza J. Micropropagation of *Eucalyptus maidenii* elite trees. *Agrociencia*. 2007;11(1):81-89.
- Souza DMSC, Fernandes SB, Avelar MLM, Frade SRP, Molinari LV, Gonçalves DS, et al. Mixotrophism effect on *in vitro* elongation and adventitious rooting of *Eucalyptus dunnii*. *Cerne*. 2019a;25(4):394-01.
- Souza DMSC, Xavier A, Otoni WC, Miranda NA, Maggioni JH. Light quality in the *in vitro* introduction of *Corymbia* hybrid clones. *Revista Árvore*. 2019b;42(6):1-9. doi: doi.org/10.1590/1806-90882018000600004
- Trueman SJ, Hung CD, Wendling I. Tissue culture of *Corymbia* and *Eucalyptus*. *Forests*. 2018;9(2):1-42. doi: doi.org/10.3390/f9020084
- Trueman SJ, McMahon TV, Bristow M. Production of cuttings in response to stock plant temperature in the subtropical eucalypts, *Corymbia citriodora* and *Eucalyptus dunnii*. *New Forests*. 2013;44(2):265-79. doi: doi.org/10.1007/s11056-012-9315-y
- Vieitez AM, Sánchez MC, Amo-Marco JB, Ballester A. Foreed flushing of branch segments as a method for obtaining reactive explants of mature *Quercus robur* trees for micropropagation. *Plant Cell, Tissue and Organ Culture*. 1994;37(3):287-95. doi: doi.org/10.1007/BF00042342
- Xavier A, Wendling I, Silva RL. *Silvicultura clonal: princípios e técnicas*. Viçosa: Editora UFV; 2013. ISBN 9788572694698.
- Wendling I, Brondani GE, Biassio A, Dutra LF. Vegetative propagation of adult *Ilex paraguariensis* trees through epicormic shoots. *Acta Scientiarum. Agronomy*. 2013;35(1):117-25. doi: doi.org/10.4025/actasciagron.v35i1.15958
- Wendling I, Trueman SJ, Xavier A. Maturation and related aspects in clonal forestry – part II: reinvigoration, rejuvenation and juvenility maintenance. *New Forests*. 2014;45(4):473-86. doi: doi.org/10.1007/s11056-014-9415-y

**ERRATA**

**NO ARTIGO “IN VITRO ESTABLISHMENT OF EUCALYPTUS AND CORYMBIA SPECIES FROM EPICORMIC SHOOTS.”, PUBLICADO NO VOLUME 44, DA REVISTA ÁRVORE, ONDE SE LÊ:**

***In vitro* ESTABLISHMENT OF *eucalyptus* AND *corymbia* SPECIES FROM EPICORMIC SHOOTS**

***ESTABELECIMENTO in vitro DE ESPÉCIES DE eucalyptus E Corymbia POR MEIO DE BROTAÇÕES EPICÓRMICAS***

**L eia- s e:**

***In vitro* ESTABLISHMENT OF *Eucalyptus* AND *Corymbia* SPECIES FROM EPICORMIC SHOOTS**

***ESTABELECIMENTO in vitro DE ESPÉCIES DE Eucalyptus E Corymbia POR MEIO DE BROTAÇÕES EPICÓRMICAS***