BOSQUE 43(2): 169-177, 2022 DOI: 10.4067/S0717-92002022000200169

Emission of epicormic shoots and *in vitro* establishment of *Cordia trichotoma* selected adult trees

Emisión de brotes epicórmicos y establecimiento *in vitro* de *Cordia trichotoma* árboles adultos seleccionados

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SUMMARY

Cordia trichotoma, popularly known in Brazil as 'louro-pardo', has numerous environmental and timber-related applications. This species is naturally propagated by seeds; however, this form of propagation results in restricted seedling production due to high genetic variability associated with a low degree of forest improvement. Thus, the cloning of selected adult trees is an alternative for producing high-quality plants. The aims of this work were to evaluate the emission of epicormic shoots from pruned branches and to define a protocol for the *in vitro* establishment of *C. trichotoma* selected adult trees. Propagules used in these experiments were derived from the selection of four *C. trichotoma* adult trees. The branches were placed in a greenhouse to induce epicormic shoots. Number of shoots according to the climatic season (autumn and spring) and shoot collection time were evaluated. Three sodium hypochlorite concentrations were evaluated for five minutes (C_1 = 0.58 % active chlorine, C_2 = 1.12 % active chlorine, and C_3 = 2.12 % active chlorine) for *in vitro* establishment. Results indicated that the epicormic shoot technique could be considered viable for shoot emission of *C. trichotoma,* regardless of the climatic season (autumn or spring). Sodium hypochlorite solution applied for 5 minutes (1.12 % of active chlorine) was effective for the *in vitro* establishment of nodal segments from epicormic shoots, resulting in 61.3 % of established explants. These results are promising for the cloning of adult trees during the mature stage of development in the field without the need to cut the selected tree.

Keywords: louro-pardo, *in vitro* cultivation, plant propagation, plant cloning.

RESUMEN

Cordia trichotoma, conocida popularmente en Brasil como "louro-pardo", tiene numerosas aplicaciones ambientales y madereras. Esta especie se propaga naturalmente por semillas; sin embargo, esta forma de propagación resulta en una producción restringida de plántulas por la alta variabilidad genética asociada con un bajo grado de mejoramiento forestal. La clonación de árboles adultos seleccionados es una alternativa para producir plantas de alta calidad. El objetivo de este trabajo fue evaluar la emisión de brotes epicórmicos de ramas podadas y definir un protocolo para el establecimiento *in vitro* de árboles adultos seleccionados. Los propágulos utilizados se derivaron de la selección de cuatro árboles adultos y las ramas se colocaron en un invernadero para inducir brotes epicórmicos. Se evaluó el número de brotes según la época climática (otoño y primavera), el tiempo de recolección de los brotes, y tres concentraciones de hipoclorito de sodio durante cinco minutos (C1 = 0,58 % de cloro activo, C2 = 1,12 % de cloro activo y C3 = 2,12 % de cloro activo) para el establecimiento *in vitro*. Los resultados indicaron que la técnica de brotes epicórmicos podría considerarse viable para la emisión de brotes independientemente de la estación climática. La solución de hipoclorito de sodio (1,12 % de cloro activo) fue eficaz para el establecimiento *in vitro* de segmentos nodales de brotes epicórmicos, resultando en un 61,3 % de explantes establecidos. Los resultados son prometedores para la clonación de laureles durante la etapa madura de desarrollo en el campo sin necesidad de cortar el árbol seleccionado.

Palabras clave: louro-pardo, cultivo *in vitro*, propagación de plantas, clonación de plantas.

INTRODUCTION

Having little knowledge on the potential use of native tree species and associated technologies, whether for productive or environmental purposes, has been one of the factors preventing the development of propagation techniques, especially regarding the cloning of superior individuals (*i.e.* selected plants in the field). This includes not only the

propagation process but also the appropriate selection of genetic material with sufficient quality and genetic variability levels to fulfil silvicultural needs (Stuepp *et al*. 2018).

Among the native species with great potential for afforestation is *Cordia trichotoma* (Vell.) Arrab. ex Steud., in the family Boraginaceae, which is popularly known in Brazil as 'louro-pardo' or 'freijó'. This species occurs naturally from Ceará to Rio Grande do Sul in the semi-deciduous Atlantic rainforest and the Cerrado, and it is considered important for environmental restoration and noble timber production (Berghetti *et al*. 2015).

Cordia trichotoma reproduces naturally by seed; however, this form of propagation results in specific restrictions in seedling production due to the high unevenness at the seed germination stage and the fact that sexual propagation confers high genetic variability to new generations, hampering commercial plantations, in which homogeneity is highly important (Tambarussi *et al*. 2017, Bisognin *et al.* 2020).

The rescue of selected adult trees in the field may be an alternative for vegetative propagation, in which the selection of trees is based on phenotypic traits of interest towards increasing forest productivity and improving the quality of timber as raw material (Tambarussi *et al*. 2017, Bisognin *et al*. 2020, Abiri *et al*. 2020). Among the various existing techniques, the vegetative rescue method based on epicormic shoots is an alternative for the cloning of adult trees and is considered an excellent tool for vegetative propagation without the need to cut the selected tree (Baccarin *et al*. 2015, Trueman *et al*. 2018, Avelar *et al*. 2020).

Studies that seek to improve large-scale vegetative propagation techniques of native species for use in homogeneous plantations, degraded area recovery, genetic improvement and germplasm conservation have become fundamental from a strategic point of view, considering the increased exploitation of natural resources (Avelar *et al*. 2020).

Micropropagation technique is considered an *in vitro* vegetative propagation (*e.g.* microcutting technique), and compared to other techniques, such as cutting and mini-cutting, it has advantages in terms of tissue rejuvenation and propagule rooting (Hartmann *et al*. 2011). Micropropagation consists of different stages, and *in vitro* establishment is the most critical for most woody plants (Souza *et al*. 2020, Molinari *et al*. 2020) considering the high losses of materials due to microorganism contamination (*e.g.* fungal and/or bacterial contamination) or tissue oxidation. These losses justify the need for studies seeking to minimize these obstacles during native species propagation.

Considering the above context, the aims of the work were to evaluate the epicormic shoot emission, and to define a protocol for the *in vitro* establishment of nodal segments from *C. trichotoma* selected adult trees.

METHODS

Study site and experimental material. The experiments were conducted at the Forest Nursery and the Laboratory of *in vitro* Culture of Forest Species, both located in the Department of Forestry Sciences of the Federal University of Lavras (Universidade Federal de Lavras – UFLA) in the municipality of Lavras, Minas Gerais, Brazil (21° 14' 43" S and 44° 59' 59" W).

The experimental material used to conduct the tests was derived from the selection of four *C. trichotoma* adult trees, which were selected according to their trunk shape (straight), diameter at breast height (21.4 to 23.9 cm) and health (absence of diseases and pest attacks), that originated from a commercial stand belonging to the company "Symbiosis Investimentos e Participações S.A." in Trancoso, Distrito de Porto Seguro, Bahia, Brazil. The trees were identified as tree A, B, C and D. Branch collection was realized considering two seasons, autumn (06/08/2017) and spring (10/10/2017).

Pruned branches were transported to the city of Lavras, Minas Gerais, and standardized to a length of 45 cm without the presence of shoots (*i.e*. axillary or apical shoot) and leaves. The branches were placed in a greenhouse with controlled temperature (T < 35 °C) and relative humidity (RH > 80 %), maintained by an intermittent mist system with high-pressure, low flow rate nozzles and automatically controlled by a humidistat and thermostat. The branches were placed vertically in polyethylene pots (5 L) containing washed medium sand according to Baccarin *et al*. (2015) (figure 1 A – D).

The branches remained in the greenhouse for 50 days during the experimental period. The mean temperature was 16.4 °C in the autumn, and 21.9 °C in the spring. Mean cumulative rainfall levels were 29 mm and 227.4 mm in the autumn and spring, respectively. Climatological data were obtained from the website of the National Institute of Meteorology (Instituto Nacional de Meteorologia – INMET) according to the meteorological station OMM: 83687 located in Lavras, Minas Gerais, Brazil.

Epicormic shoot emission: genotype vs climatic season. The experiment was set up in a completely randomized factorial design (2 × 4), with two climatic seasons (autumn and spring) and four selected trees (trees A, B, C and D). The experiment consisted of eight treatments with 12 replicates, in which each pot was considered an experimental unit. At 20 days after the start of the experiment, the percentage of emitted epicormic shoots and the number of shoots were evaluated.

Epicormic shoot emission: climatic season vs shoot collection time. The experiment was organized in a completely randomized factorial design (2 × 4), which addressed the climatic season (*i.e.* autumn and spring) and the shoot collection time (20, 30, 40 and 50 days). The experiment consisted of eight treatments with 36 replicates, in which each pot was considered one experimental unit. The percentage of epicormic shoot emission and the number of shoots were evaluated at 20, 30, 40 and 50 days after the start of the experiment.

Figure 1. Detail for *Cordia trichotoma* branches under greenhouse conditions. A – transport of branches in a plastic bag, B – standardization of pruned branches to 45 cm, C – filling of pots with sand, and D – placement of pruned branches vertically in pots with medium sand, 48 hours after the branch collection. Bar = 10 cm.

Detalle de las ramas de *Cordia trichotoma* en condiciones de invernadero. A – transporte de ramas en bolsa plástica, B – estandarización de ramas podadas a 45 cm, C – llenado de macetas con arena, y D – colocación de ramas podadas verticalmente en macetas con arena media, 48 horas después de la recolección de ramas. Bar = 10 cm.

In vitro establishment. The epicormic shoots of pruned branches from four *C. trichotoma* individuals were used to obtain explants. Prior to collection (72, 48 and 24 hours), a dimethyl 4.4' - (o-phenylene) bis (3-thioallophanate) fungicide was applied at a concentration of 0.5 g L^{-1}. Shoots measuring 3 to 5 cm in length were collected after 30 days in the greenhouse, stored in plastic bags moistened with autoclaved water and transported to the laboratory.

Prior to *in vitro* inoculation, the explants (*i.e.* nodal segments) were standardized to contain two axillary buds and no leaves and washed in running water for 10 minutes. Next, the aseptic procedure was started, during which the explants were washed in a 70 % (v / v) hydroalcoholic solution in a horizontal laminar flow chamber. Subsequently, disinfection was initiated by immersion in a sodium hypochlorite (NaClO) solution for five minutes at three different concentrations; namely C_1 = 0.58 % active chlorine, C_2 = 1.12 % active chlorine and C_3 = 2.12 % active chlorine, followed by triple washing with deionized and autoclaved water.

The explants were inoculated vertically, under aseptic conditions, in 2 × 10 cm test tubes containing 10 mL of MS culture medium (Murashige and Skoog 1962). The tubes were sealed with polyvinyl chloride (PVC) - based plastic film and placed in a growth room at 24 °C (± 1 °C) for a 16 - hour photoperiod and 40 μmol m^{-2} s^{-1} irradiance. During the entire process, the equipment was disinfected with 70 % hydroalcoholic solution. The culture medium was supplemented with 30 g L^{-1} of sucrose and 6 g L^{-1} of agar and prepared in deionized water, and the pH value of solution was adjusted to 5.8 (± 0.05) with NaOH (0.1 M) and HCl (0.1 M). The culture medium was autoclaved at 127 °C and 1.5 kgf cm^{-2} pressure for 20 minutes.

Twenty-eight days after the *in vitro* inoculation of the explants, the percentage of fungal and bacterial contamination, tissue oxidation and establishment were evaluated. The experiment was conducted in a completely randomized design, with three treatments (*i.e.* active chlorine concentration: C_1, C_2 and C_3), 50 replicates and one explant per replicate.

Data analyses. Data collected during the experiments were analyzed for the homoscedasticity and normality of the residual distribution using the Hartley ($P > 0.05$) and Shapiro-Wilk ($P > 0.05$) tests, respectively. When non-parametric, the data were Box-Cox transformed and subjected to an analysis of variance (ANOVA, $P < 0.05$). The quantitative data were subjected to a polynomial regression analysis ($P < 0.05$), and the qualitative data were compared using Tukey's test ($P < 0.05$). The analyses were performed in R (R Core Team, 2018) using the ExpDes package, version 1.1.2 (Ferreira *et al*. 2013).

RESULTS

Epicormic shoot emission: genotype vs climatic season. There was effect of the selected plant and climatic seasons

on the shoot emission and the number of shoots on the pruned branches. The highest mean shoot emission values in the autumn were observed in tree A (100.0 %) and D (91.7 %). In the spring, the highest means were observed for tree C (66.7 %) and D (83.3 %); in contrast, tree B did not produce shoots during the evaluated seasons (figure 2A). This result demonstrates that the shoot emission is also genotype-dependent and related to the season.

Considering tree A, the highest mean shoot emission occurred in the autumn (100.0 %), and for B, there was no shoot emission (0.0 %). Tree C had the highest mean during the spring (66.7 %), and D showed no effect from the interaction at the different evaluated seasons. The observed means were 91.7 % in autumn, and 83.3 % in spring. The highest mean number of shoots emitted in the autumn were found in tree A (11.3 shoots per branch) and D (8.3 shoots per branch). In the spring, the highest mean was observed in tree D (12.8 shoots per branch) (figure 2B). For the interaction between seasons and selected adult plants, in tree A, the highest mean number of emitted epicormic shoots was observed in the autumn (11.3 shoots per branch). For trees C and D, the highest means were observed in the spring, with mean values of 6.5 and 12.8 shoots per branch, respectively (figure 2B). The epicormic shoots emitted in autumn and spring in trees A, C and D were considered viable at 20 days after the start of the experiment (figures 3A - F).

Epicormic shoot emission: climatic season vs shoot collection time. The percentage of shoot emission (53.3 %) and the number of shoots (9.6 shoots) throughout the evaluation time were highest at 20 days of evaluation (figures 4A – B).

The best time interval for collecting the shoots was 20 days after setting up the experiment in a greenhouse, considering that the highest shoot emission and number of shoots were recorded during this period. There was a reduction in the percent shoot emission and number of shoots over time and no viable shoots, and at the 50-day evaluation, there was no emission of viable shoots for the *in vitro* introduction.

In vitro establishment. Active chlorine treatments significantly influenced the *in vitro* establishment of *Cordia trichotoma* nodal segments at 28 days after inoculation

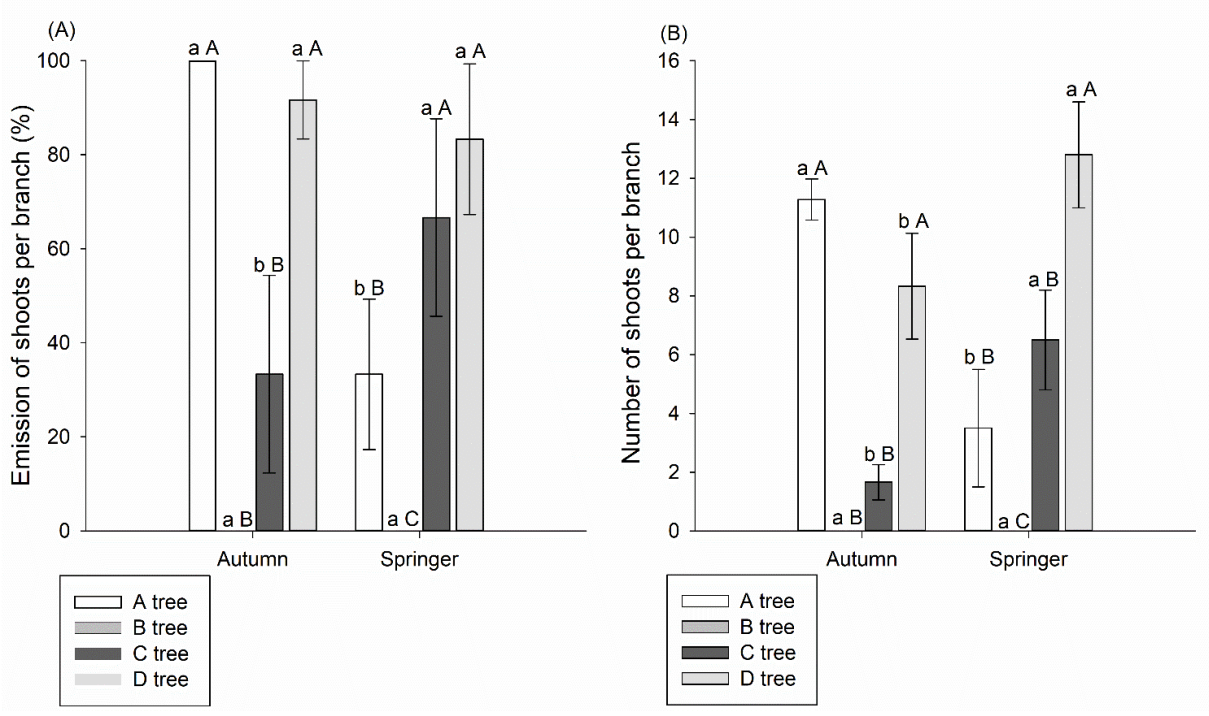

Figure 2. Percentage of epicormic shoot emission and number of shoots from pruned branches of *Cordia trichotoma* at 20 days. A – Percentage of shoot emission observed as a function of the selected plant and climatic season, and B – Number of shoots observed as a function of the climatic season and selected plant. Means followed by the same uppercase letter show no effect of the interaction of seasons and means followed by the same lowercase letters show no effect of the interaction of selected plant. The data are presented as the means ± standard error.

Porcentaje de emisión de brotes epicórmicos y número de brotes de ramas podadas de *Cordia trichotoma* a los 20 días. A - Porcentaje de emisión de brotes observados en función de la planta seleccionada y estación climática, y B - Número de brotes observados en función de la estación climática y planta seleccionada. Las medias seguidas de la misma letra mayúscula no muestran ningún efecto de la interacción de las estaciones y las medias seguidas de las mismas letras minúsculas no muestran ningún efecto de la interacción de la planta seleccionada. Los datos se presentan como la media ± error estándar.

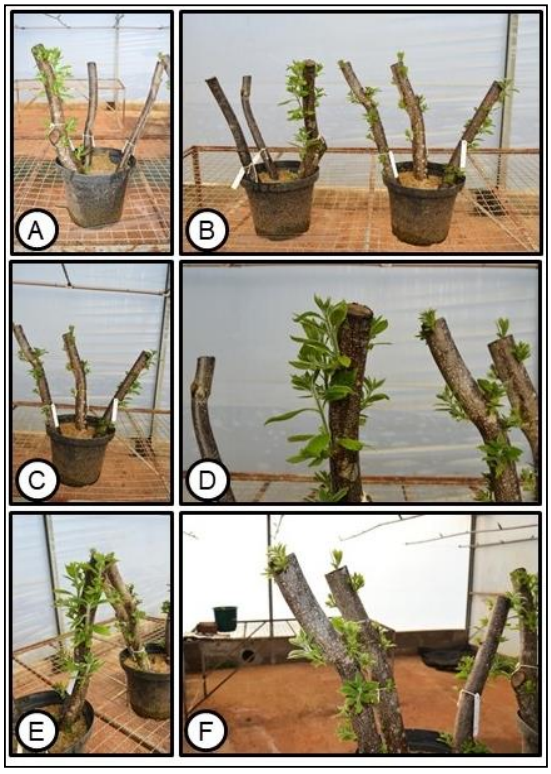

Figure 3. Detail on the shoot emission in pruned *Cordia trichotoma* branches at 20 days after placement in a greenhouse. A - C – branch of tree A, C and D in the fall, and D - F – branch of trees A, C and D in the spring. Bar = 10 cm.

Detalle de la emisión de brotes en ramas de *Cordia trichotoma* podadas a los 20 días de su colocación en invernadero. A - C - rama de un árbol, C y D en el otoño, y D – F – rama de los árboles A, C y D en la primavera. Bar = 10 cm.

(C_1 = 0.58 % active chlorine, C_2 = 1.12 % and C_3 = 2.12 %) (figure 5A - D).

The highest fungal contamination was 55.1 %, as observed in treatment C_1 with 0.58 % active chlorine. In contrast, the lowest mean values were observed in treatments C_2 and C_3 at active chlorine concentrations of 1.12 % and 2.12 %, respectively, which did not differ significantly (figure 5A). Regarding bacterial contamination, as shown in figure 5B, the lowest means were found in treatments of 1.12 % to 2.12 % active chlorine (C_2 and C_3); the lowest mean for this variable was observed in treatment C_1 (0.58 % active chlorine). The pattern of tissue oxidation for the explants (figure 5C), a direct relationship between active chlorine concentration and percent oxidation is observed. The highest value for this variable was 96.8 %, with a concentration of 2.12 % active chlorine. The highest percentage of *in vitro* establishment (61.3 %) occurred in the treatment in which 1.12 % active chlorine was used (figure 5D), resulting in the emission of viable shoots to be used for the other micropropagation stages (figures 6A - 6D).

DISCUSSION

Epicormic shoot emission. The results of the present study suggest that epicormic shoots from the pruned branch propagation technique was efficient regarding the shoot emission and number of shoots for *C. trichotoma.* By definition, epicormic shoots are branches that sprout from dormant buds, which elongated over a previous growth period (Andrew *et al*. 2012). These shoots can be used for the vegetative propagation of forest species, to rescue

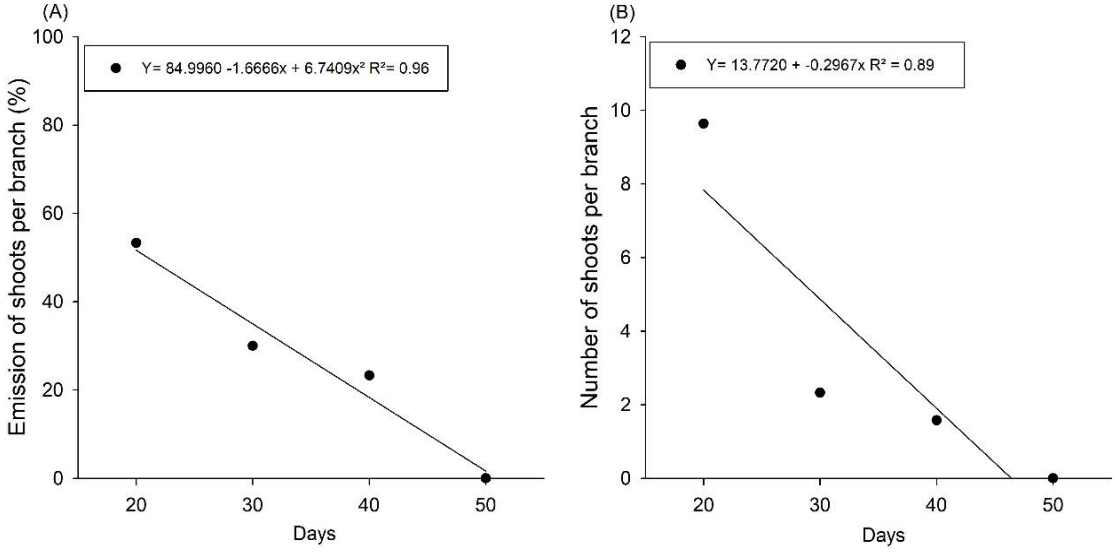

Figure 4. Percentage of shoot emission and number of shoots in *Cordia trichotoma* branches over time. A – Percentage of shoot emission over time, and B – Number of shoots over time.

Porcentaje de emisión de brotes y número de brotes en ramas de *Cordia trichotoma* a lo largo del tiempo. A – Porcentaje de emisión de brotes a lo largo del tiempo y B – Número de brotes a lo largo del tiempo.

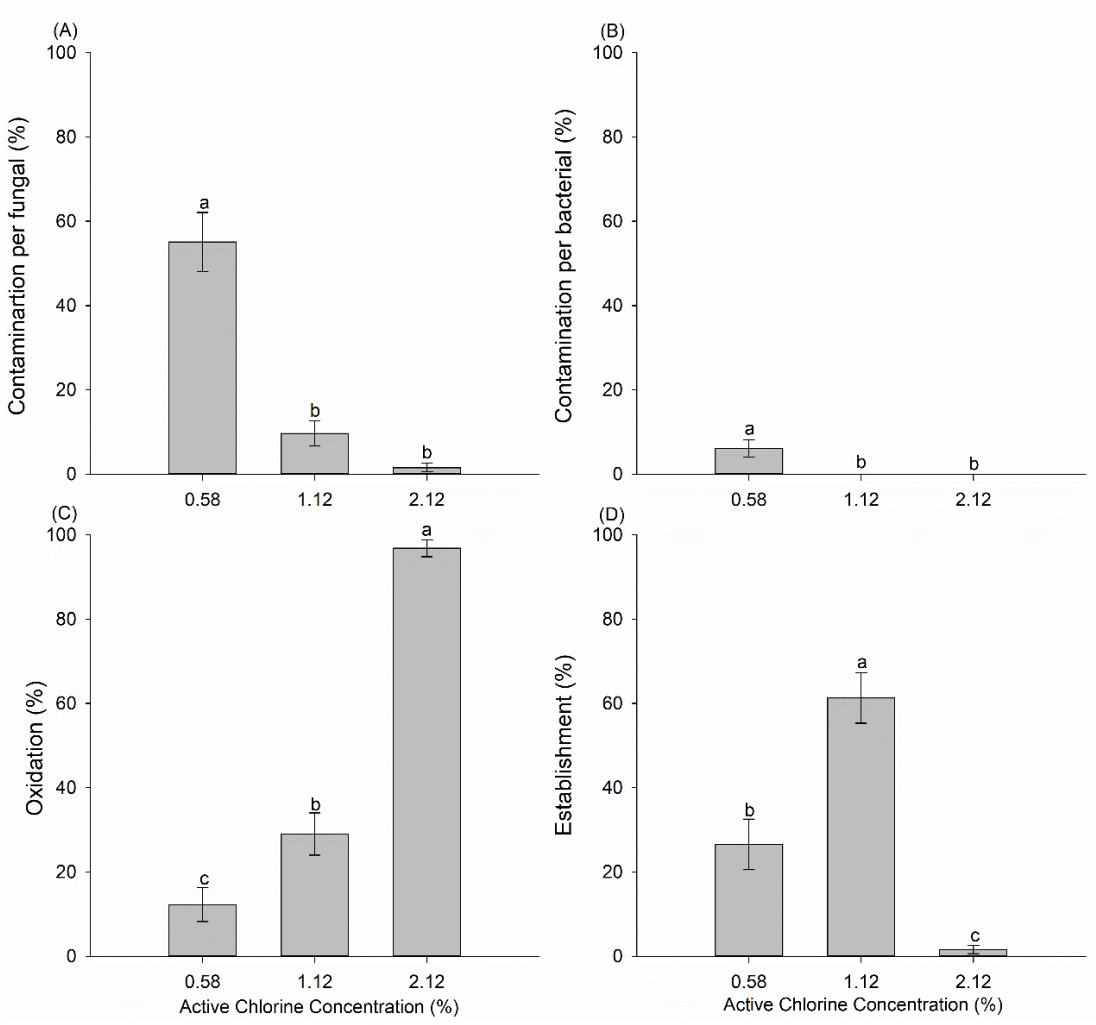

Figure 5. Variables evaluated during the *in vitro* establishment of *Cordia trichotoma* at 28 days**.** A - Percentage of fungal contamination, B - Percentage of bacterial contamination, C - Percentage of tissue oxidation and D - Percentage of *in vitro* establishment. Means followed by the same letter do not differ significantly according to Tukey's test. Data are presented as the means ± standard error.

Variables evaluadas durante el establecimiento *in vitro* de *Cordia tricotoma* a los 28 días. A - Porcentaje de contaminación por hongos; B - Porcentaje de contaminación bacteriana; C - Porcentaje de oxidación tisular; y D - Porcentaje de establecimiento *in vitro*. Las medias seguidas de la misma letra no difieren significativamente según la prueba de Tukey. Los datos se presentan como la media ± error estándar.

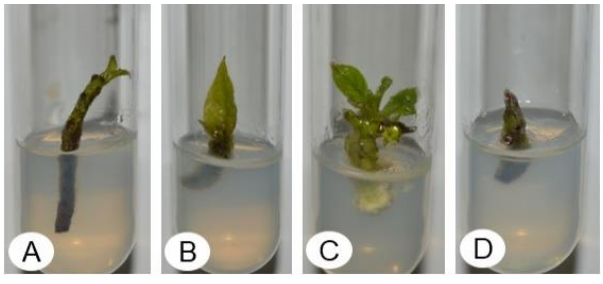

Figure 6. Details of matrix A of *Cordia trichotoma* explants subjected to 1.12 % active chlorine, considered *in vitro* established. A - D – nodal segments established at 28 days. Bar = 1.0 cm.

Detalles de la matriz A de explantes de *Cordia tricotoma* sometidos a cloro activo al 1,12 %, considerados establecidos *in vitro*. A - D: segmentos ganglionares establecidos a los 28 días. Bar = 1,0 cm.

the adult individuals selected in the field. This mechanism was important for the study species *C. trichotoma*, which is considered a species with high commercial potential, given that during the seedling production stage, the use of seeds results in certain restrictions, primarily because sexual propagation confers high genetic variability to new generations (Kielse *et al.* 2015).

Several similar studies have indicated the efficiency of this vegetative rescue method using epicormic shoots, such as in *Ilex paraguariensis* (Wendling *et al*. 2013), *Eucalyptus benthamii* (Baccarin *et al*. 2015), *E. cloeziana* (Oliveira *et al*. 2015) and *E. pilularis* (Avelar *et al*. 2020). According to Kuppusamy *et al*. (2019) and Esposito-Polesi *et al*. (2020), the use of this technique is especially important for germplasm conservation and

the selection of superior individuals, in which a vegetative rescue is typically performed at the adult stage and propagation may be limited due to the ontogenetic age. Kratz *et al*. (2016) reported that in situations in which vegetative rescues are performed in plants with high genetic value, the application of this method is indicated, considering the low interference of branch collection from the selected plant and ensuring the highest possible survival of the selected individuals.

However, the physiological principle for bud induction in epicormic shoots is related to changes in the balance of the plant growth regulation (auxin / cytokinin), favoring shoot emergence (Trueman *et al*. 2018, Abiri *et al*. 2020). The vegetative propagation of adult trees may be facilitated by epicormic shoot induction to obtain shoots with a higher degree of juvenility, usually with good propagation potential (Oliveira *et al*. 2015, Souza *et al*. 2020). These changes may vary with the genotype, resulting in considerable shoot emergence by favoring rejuvenation opposite to the base of the shoot from the shoot collected from the tree branches. In the present study on *C. trichotoma*, the selected tree directly influenced the emission of epicormic shoots, and it is important to account for the genotype factor of the selected plants, which may influence the success of this technique.

The use of branches to obtain epicormic shoots has also been employed as a source of explants for the micropropagation of *E. benthamii* (Baccarin *et al*. 2015), *Ilex paraguariensis* (Wendling *et al*. 2013), *E. cloeziana* (Olveira *et al*. 2015) and *E. pilularis* (Avelar *et al*. 2020). According to Abiri *et al*. (2020), there are many gaps in our knowledge of epicormic shoots; therefore, developing a larger understanding of the physiological, ontogenetic and environmental factors that support the development of these shoots is still necessary. However, for *C. trichotoma,* regardless of the climatic season (autumn or spring) in which the pruned branches were collected, it is possible to obtain epicormic shoots, which can be used in propagation techniques such as grafting, cutting and micropropagation. In addition, it is possible to use this method to rescue other native species without the need to fell the selected tree, although the genotype factor must be considered because it can also influence the technique success.

In vitro establishment. The use of branches to obtain epicormic shoots has been employed as a source of explants for the micropropagation of several forest species, such as, *E. benthamii* (Baccarin *et al*. 2015), *E. cloeziana* (Olveira *et al*. 2015) and *E. pilularis* (Avelar *et al*. 2020). For *C. trichotoma*, this technique has also been shown to be efficient for the preparation and establishment of explants and is recommended as an alternative for the *in vitro* production of *C. trichotoma* propagules.

As previously reported, *C. trichotoma* showed appropriate behavior in terms of *in vitro* establishment; however, it is necessary to develop adequate measures for the use of chemicals during disinfection, defining the factors, time and ideal concentration for better success in obtaining established explants (Salles *et al*. 2017, Zorz *et al.* 2020), especially when the sources of explants originate from adult selected plants, selected in the field due to the high percentage of contamination under these conditions (Postemsky and Curvetto 2016).

The use of active chlorine in micropropagation is an inexpensive and effective method for the disinfection of explants and its use is widespread in several species, such as *E. cloeziana* (Oliveira *et al*. 2015), *Cochlospermum regium* (Gavilan *et al*. 2018) and *E. grandis* × *E. urophylla* (Miranda *et al*. 2020). In the present study, the lowest percentages of fungal and bacterial contamination occurred with the use of active chlorine at concentrations of 1.12 % and 2.12 %, showing the effectiveness of using this disinfection agent for the successful initial *in vitro* establishment of *C. trichotoma* from epicormic shoots.

For micropropagation to be successful, it is necessary for only a few explants to emit shoots free of contamination, because the start of *in vitro* propagation is the primary limiting stage (Trueman *et al*. 2018). In addition, the metabolic pathway of microorganisms may be subject to regulation by the genotype through differential genetic expression, in carotenoid biosynthesis and hyphal aggregation (Abiri *et al*. 2020).

Phenolic oxidation has been a problem that is associated with the micropropagation of woody species. In the present study on *C. trichotoma*, the oxidation percentage increased directly in relation to the use of sodium hypochlorite; the higher the concentration of the disinfecting agent, the higher the percentage of oxidation, thus indicating that the tissues of this species exhibited high sensitivity to chemical treatment. Similar results have been reported in studies on *E. cloeziana* (Oliveira *et al*. 2015), *E. benthamii* (Baccarin *et al*. 2015), *Corymbia citriodora* × *C. torelliana* and *C. torelliana* × *C. citriodora* (Souza *et al*. 2018), and *E. grandis* × *E. urophylla* (Molinari *et al*. 2020).

The highest *in vitro* establishment percentage (61.3 %) at 28 days was observed when 1.12 % active chlorine was used, which was considered an adequate value denoting the importance of high rates of shoot induction. Different results are found in literature, in which the response varied according to plant material (genotype) and culture conditions. In *E. cloeziana*, Oliveira *et al*. (2015) obtained, on average, 51.2 % of established explants *in vitro* with the use of sodium hypochlorite. Souza (2020) studied the *in vitro* establishment of *Eucalyptus grandis* × *E. urophylla* and achieved 95.0 % establishment by this method. The results obtained here provide a source of information for future research on *C. trichotoma* for continuing on to the other micropropagation stages, including bud multiplication, shoot elongation and adventitious rooting.

CONCLUSIONS

The vegetative propagation technique of using epicormic shoots in pruned branches was considered viable for *C. trichotoma*, regarding the percent emission and number of shoots, regardless of the collection season (autumn or spring); however, the genotype factor should be considered because it may influence the success of this technique. The use of active chlorine at a concentration of 1.12 % for five minutes was effective for *in vitro* establishment of nodal segments derived from epicormic shoots of *C. trichotoma*, resulting in 61.3 % establishment at 28 days.

ACKNOWLEDGEMENTS

The present study was conducted with the support of the Brazilian Federal Agency for the Support and Evaluation of Graduate Education (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES) - Funding Code 001; National Council for Scientific and Technological Development (Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq) and the Minas Gerais Research Support Foundation (Fundação de Amparo à Pesquisa do Estado de Minas Gerais – FAPEMIG). We are thankful to our laboratory technician, Dr. Ana Luiza Oliveira Timbó, for assistance in conducting the experiments and to Dr. Renato Moraes de Jesus, Dr. Felipe Garbelini Marques and Dr. Bruno Mariani for the financial support and acquisition of the genotypes from Symbiosis Investimentos e Participações S.A.

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Recibido: 08.05.21
Aceptado: 10.03.22