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## VEGETATIVE PROPAGATION OF *Cordia trichotoma* (VELL.) ARRAB. EX STEUD. BY CUTTINGS FROM SHOOTS AND ROOTS

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### HIGHLIGHTS

Adult *Cordia trichotoma* plants do not provide cuttings responsive to propagation.

Both shoot and root cuttings do not root when they come from adult plants.

Young plants of *Cordia trichotoma* provide cuttings that root without using auxin.

*Cordia trichotoma* plants can be produced by root cuttings.

### ABSTRACT

*Cordia* (*Cordia trichotoma* (Vell.) Arrab. ex Steud.) is a native species that has high quality wood and potential for the establishment of commercial plantations. However, its propagation by seeds is difficult, and vegetative propagation is a good alternative. The aim of this study was to evaluate the vegetative propagation of cordia by cuttings from shoots and roots. In one experiment, cuttings from shoots of adult plants were treated with 0 and 40 mM of indolbutyric acid (IBA). In other experiment, cuttings from roots of adult plants were classified by diameter as thick (2.0-3.0 cm) and thin (<2.0 cm) and treated with 0, 20, 40 and 60 mM of IBA. In other experiment, cuttings from roots of three-year-old plants were treated with 0, 20 and 40 mM of IBA. In all experiments, the percentages of survival, rooting and shooting, the number and length (cm) of shoots and roots were evaluated. Cuttings from roots and shoots of adult plants were not responsive to propagation, because they produced new shoots without rooting. Cuttings from roots of young plants differentiate roots, even without AIB application, enabling the production of cordia new plantlets.

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## INTRODUCTION

Belonging to the Boraginaceae family, *Cordia trichotoma* (Vell.) Arrab. ex Steud., commonly referred as cordia, is a tree species, semi-heliophile, polygamous that can grow up to 35 meters high and 100 cm in diameter (Carvalho, 2003). From initial secondary to late secondary succession group, cordia can be found in Submontane Semidiciduous Seasonal Forest, Deciduous Seasonal Forest, Dense Ombrophylous Forest and Subtropical Ombrophylous Forest (Reitz et al., 1988). Cordia has potential for commercial forest plantation, because the species presents good trunk form and excellent wood quality (Carvalho, 2002) presenting average levels of ash and plant extractives (Wille et al., 2017).

In addition to the potential to compose a forest stands, the choice of a native species for commercial plantations depends strongly, among other factors, on the knowledge of silvicultural aspects related to the production of seedlings. In this context, Kielse et al., (2013) states that the propagation of cordia via seeds has been hampered by the rapid loss of viability, caused by the recalcitrant behavior to storage. Mafra (2019) also mentions that there are differences between the parent plants in relation to the potential for seed emergence and advises that cordia seeds should be planted soon after collection.

As for vegetative propagation, studies show that cloning this species is a challenge, and it was not possible to define an efficient plantlet production protocol so far (Fick, 2007; Heberle, 2010; Kielse, 2015; Kielse et al., 2013). The most expressive result was obtained by Kielse et al. (2013) who observed 45% rooting of root cuttings treated with 30 mM of indolebutyric acid (IBA).

The rooting of root cuttings of some species, such as cordia, occurs due to the presence of buds in the root system that have cells capable of de-differentiating at points of meristematic growth and develop new shoots and roots (Hartmann et al., 2011). These buds in the roots can be of two types: additional buds, formed in the undisturbed root system and of endogenous origin, or repairing buds, formed in response to senescence, anthropogenic lesions or other types of rupture (Appezato-da-Gloria and Carmello-Guerrero, 2006). Both buds are capable of differentiating new shoots. Additional shoots, during the growth of the secondary root, can become perennial if they grow simultaneously with the exchange rate, so that vascular traces are produced in the secondary xylem. On the other hand, repairing shoots are exogenous in origin and vascular traces may be absent or, if present, may not reach the root center (Appezato-da-Gloria and Carmello-Guerrero, 2006).

The production of plantlets from root cuttings is not a common practice, due to the difficulty in collecting roots from mature trees and the unfeasibility of the operational process. This technique has been studied for some species due to the high potential of morphogenetic response of this propagule (Snedden et al., 2010; Ky- Dembele et al., 2010) and it seems to be a viable strategy for the production of clonal cordial plantlets. Thus, considering this aspect, as well as the low competence for adventitious rooting, the potential for commercial cordia forest plantations and the possibility of producing new plants by cuttings with proven genetic origin were the rationality for motivating this research.

The objective of this study was to evaluate the vegetative propagation of cordia by shoot cuttings of adult plants and root cuttings of young and adult plants.

## MATERIAL AND METHODS

The experiments were carried out at the Center of Plant Breeding and Vegetative Propagation, Plant Science Department of the Federal University of Santa Maria (UFSM), Santa Maria, RS, Brazil (29° 43' S, 53° 43' W and altitude of 95 m). The experiments were carried out with young (three-year old) and adult plants (with unknown age) of cordia that belongs to UFSM.

Three experiments were carried out which used the same methodology to prepare IBA solutions and the same culture conditions described below. The indolebutyric acid (IBA) solution was prepared by dissolving the auxin in 50 mL of ethyl alcohol (98 °GL) and the final volume adjusted to 100 mL with distilled water. The control treatment consisted of only distilled water and ethyl alcohol in the ratio of 1:1 (v/v). Cuttings were cultivated vertically in polyethylene plastic trays (55 x 34 x 15 cm) containing sand, pine bark-based commercial substrate and vermiculite (1:1:1 v/v) (Pimentel et al., 2017). Cuttings were cultivated in a humid chamber, located inside the greenhouse, with relative humidity of about 85%, maintained by nebulizers automatically activated six times a day for 15 min.

### Shoot cuttings of adult plants

This first experiment, shoots of three adult plants were collected and had their total length divided into two portions. The first, closest to the central axis of the plant, was called basal and the most distal, called apical. These portions of the shoot were then sectioned on cutting (basal and apical) of 12 cm in length were

immersed in IBA solution of 0 and 40 mM for 10 s. The experiment was a factorial 2 x 2 (cutting position and IBA concentration) in the complete random design, with three replications of 30 cuttings. Cuttings were evaluated at 40 days of cultivation in the humid chamber for the percentages of survival, rooting and shooting, and the number and length (cm) of roots and shoots.

### Root cuttings of adult plants

In the second experiment, five adult plants had the soil around them excavated in a radius of 2 m with the aid of a shovel to collect the superficial roots. The roots were identified as to the distal and proximal position of the trunk and packed in plastic bags for transport to the laboratory. The roots were then cut into 5 cm cuttings and classified according to their diameters, as thick (2.0-3.0 cm) and thin (<2.0 cm). The cuttings were immersed in IBA solution of 0, 20, 40 and 60 mM for 10 s. The experiment was a factorial 2 x 4 (diameter classes and IBA concentration) in the complete random design, with five replications of four cuttings. Cuttings were evaluated at 180 days of cultivation in the humid chamber for the same variables described above.

### Root cuttings of young plants

The third experiment, cuttings from roots of three-year-old (young) plants were prepared with 5 cm in length and 1.6 to 2.5 cm in diameter and immersed in IBA solution of 0, 20 and 40 mM for 10 s. The experiment was carried out in the complete random design, with six replications of five cuttings. Cuttings were evaluated at 90 and 120 days of cultivation in the humid chamber for the same variables described above.

### Statistical analysis

To assure the normality assumption, the percentage data were transformed to arcsine  $\sqrt{x/100}$  and that of counting to  $\sqrt{x} + 0.5$ . Data were subjected to analysis of variance and the treatment means were compared by Tukey test at 5% error probability. All analysis were done with the aid of the RBio (Bhering, 2017).

## RESULTS AND DISCUSSION

### Shoot cuttings of adult plants

There was no significant effect of the interaction, not even of the independent factors for all variables analyzed. Semi-woody basal and apical cuttings from branches of adult cordia plants did not form roots,

even when treated with 40 mM of IBA. At 40 days of cultivation, 100% of cuttings survived suggesting that the environmental condition in the humid chamber was adequate to maintain the vegetative material throughout this period. In this evaluation it was also observed the formation of shoots in 49.5% of the cuttings, regardless of the treatment evaluated at 40 days of cultivation (Table 1).

At 80 days of cultivation, 100% mortality was verified, which might be related to cutting desiccation, once root absence impedes water absorption, while leaves and sprouts lose water through transpiration (Lima et al. 2011). Similar results were obtained in a rooting study with semi-woody cuttings of amoreira preta *Morus nigra* L. treated with different concentrations of IBA and kept in greenhouse with an automatic misting system, in which 100% mortality was observed at 20 days of cultivation (Canesin et al., 2016). In semi-woody cuttings of *Copaifera langsdorffii* (Desf.) and *Tibouchina stenocarpa* (DC. Cogn.), 100% mortality also occurred at 60 day-assessment. In *Calophyllum brasiliense* (Camb.), however, a low-rooting capability was observed in semi-woody cuttings despite showing high survival rate (Rios and Ribeiro, 2014).

**TABLE 1** Endemic plants in the phytogeographical regions of Turkey (MFWA, 2014).

Trials	Shooting (%)	Shoot number	Shoot length (cm)
Harvesting position			
Basal	53.0 a*	1.85 a	0.62 a
Apical	46.0 a	1.30 a	0.61 a
CV (%)	13.30	15.02	22.96
IBA (mM)			
0	46.0 a	1.52 a	0.65 a
40	53.0 a	1.63 a	0.58 a
CV (%)	5.83	1.87	30.38

\* Means followed by the same letters do not differ by the Tukey test at 5% probability.

The switch from juvenile to adult phase has significant importance in vegetative propagation of woody species, since, generally, it is difficult to induce adventitious roots in mature or ligneous tissue (Pijut et al., 2011). This is due to the fact that some species might present a continuous sclerenchyma ring, which comes to be a physical barrier to root emergency (Peixoto, 2017), or because of cellular inability in organizing itself to form root meristems in response to auxin, caused by the lack of specific-rooting receptors (Overvoorde et al., 2010).

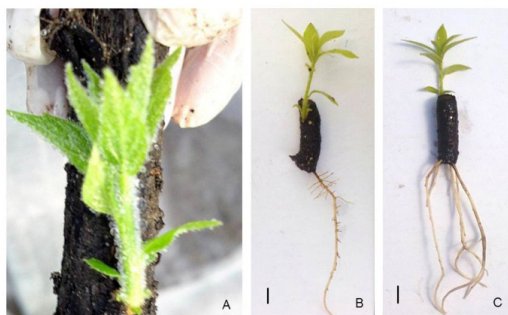
Normally, difficult-to-root species present low level of endogenous auxin (Hartmann et al., 2011), being necessary the use of exogenous plant regulators for root formation in cuttings. The mechanisms that trigger or regulate the initiation and development of adventitious roots in woody species is a complex physiological

process, which can also be influenced by genetic and environmental factors that, to a large extent, are still unknown (Pijut et al., 2011).

In a study of rooting of woody cuttings treated with IBA (0.5, 1.0, 1.5 mM) it was observed that *Cestrum laevigatum* (Schlecht.) and *Salix humboldtiana* (Willd.) rooted even without IBA treatment, but the same result was not found for the species *Tapirira guianensis* (Aubl.), *Dedropanax cuneatus* (DC. Decne. & Planch.), *Sebastiania commersoniana* (Baill. LB Sm. & Downs.), *Erythrina falcate* (Benth. Voucher), *Inga marginata* (Willd.), *Umbellata myrsine* (Mart.), and *Casearia sylvestris* (Swartz), since they did not differentiate any roots in the assessed treatments (Santos et al. 2011). The cutting of woody and semi-woody branches is a commonly observed practice for rescuing woody species. However, in the case of cordia, cuttings from semi-woody shoots did not root even when treated with IBA, suggesting that the selected genotypes or propagules used did not have natural capability for rooting.

### Root cuttings of adult plants

No roots were observed in root cuttings of adult cordia plants at 180 days of cultivation. Shoots formation occurred in cuttings of larger diameter (2.0-3.0 cm) not treated with IBA (Figure 1A), but the low occurrence (1.25% shooting) and the low survival (1.25%) made it impossible to run statistical analysis of this experiment (Table 2).



**FIGURE 1** Shoots in thick (2.0-3.0 cm) cutting from roots of *Cordia trichotoma* adult plants at 180 days of cultivation (A); shoot and root in cutting from root of three-year-old plant without IBA at 90 days of cultivation (B) and with 20 mM IBA application at 120 days of cultivation in the humid chamber (C). Reference measurement: 1 cm.

In adult root cuttings of cordia, there was a low capacity for shoot formation and no roots were observed, suggesting that the content of carbohydrates and auxins was insufficient to promote this response. This is because root cuttings were collected when

the cordia was at the end of the fruiting period (fall), a period in which the reserves were mobilized to form fruit, resulting in a lower carbohydrate content in the plant's rooting system. Han et al. (2009) stated that carbohydrate reserves may be correlated to the rooting capacity of root cuttings, corroborating this hypothesis. However, this statement is still controversial.

While for Zem et al. (2015) synthetic plant regulators induce adventitious rooting, for Rios et al. (2012) reserves are used for the growth of adventitious organs. In addition, the formation of sprouts works as a strong drain on the reserves of carbohydrates and nitrogen compounds, leading to the depletion of propagule reserves that affect rooting and, therefore, cutting survival (Vignolo et al., 2014).

**TABLE 2** Shooting percentage, number and length of shoots in cuttings from roots of *Cordia trichotoma* adult plants at 180 days of cultivation in a humid chamber.

Trials	Survival (%)	Shooting (%)	Shoot number	Shoot length (cm)
Diameter				
Thick (2.0 – 3.0 cm)	1.25	1.25	0.1	2.36
Thin (< 2.0 cm)	0.00	0.00	0.0	0.00
IBA (mM)				
0	1.25	1.25	0.1	2.36
20	0.00	0.00	0.0	0.00
40	0.00	0.00	0.0	0.00
60	0.00	0.00	0.0	0.00

The phenotypic condition of the plant donor of vegetative propagules was pointed as a factor that can influence the regeneration of root cuttings. This is caused by the influence of seasonal variation in the physiological processes, such as photosynthesis and substance transport, affecting the availability of auxins and carbohydrates in the plant (Ky-Dembele et al., 2010; Snedden et al., 2010). In other tree species, the carbohydrate content of the propagules differs and might affect rooting responses of cuttings. In *Detarium microcarpum* (Guill. And Perr.), it was observed that the levels of carbohydrates present in the roots collected in the dormant period were different from those obtained during the vegetative growth phase (Ky-Dembele et al., 2010). In *Populus tremuloides* (Michx), donor plants of root propagules were grown in an open environment and in a greenhouse, with a higher content of non-structural carbohydrates in the roots maintained in external environmental conditions (Snedden et al., 2010). In this case, the carbohydrate content could affect cutting survival and rooting capacity (Fachinello et al., 2013).

### Root cuttings of young plants

This experiment, root cutting of three-year-old plant of cordia formed roots and shoots (Figures 1B and

1 C), but no significant differences were verified among IBA concentrations for all evaluated characteristics (Tables 3). Root cuttings of three-year-old plant of cordia developed roots even without IBA application, indicating that this type of material possesses competence to rooting. These results are possibly due to the greater youthfulness of these propagules, since maturity is a limiting factor for adventitious rooting of cuttings (Wendling et al, 2014). Corroborating this, Kielse et al., (2015) observed that when using mini-cuttings, which are theoretically more youthful than the mini-stump, the rooting was not influenced by the application of naphthalenoacetic acid (NAA). However, when using propagules without any form of rejuvenation/invigoration, the use of different concentrations of IBA (0, 10, 20 and 30 mM) favors the rooting of root cuttings of cordia (Kielse et al., 2013) and of other species, such as *Spondias* spp. and *Caesalpinia ferrea* (Novelli et al., 2019; Santos et al., 2019).

**TABLE 3** Percentage of survival and rooting, number and length of roots formed in cutting from roots of three-year-old plants of *Cordia trichotoma* at 90 and 120 days of cultivation in the humid chamber.

IBA (mM)	Survival (%)	Rooting (%)	Root number	Root length (cm)
At 90 days of cultivation				
0	56.6 a*	40.0 a	0.76 a	3.52 a
20	46.6 a	36.6 a	0.53 a	6.80 a
40	80.0 a	70.0 a	1.60 a	11.71 a
Mean	61.1	48.8	0.96	8.01
CV (%)	32.14	39.97	28.14	39.13
At 120 days of cultivation				
0	53.3 a*	36.6 a	1.67 a	10.16 a
20	36.6 a	33.3 a	0.33 a	11.29 a
40	76.6 a	70.0 a	1.56 a	14.07 a
Mean	55.5	46.6	1.18	11.84
CV (%)	31.16	38.95	27.19	37.25

\* Means followed by the same letters do not differ by the Tukey test at 5% probability.

In this study, it was also observed that IBA did not influence shooting of juvenile root cuttings, being found a mean of 46.6 % of rooted cuttings at 120 days of cultivation in the humidity chamber (Table 3). Even keeping the root cuttings until 120 days of cultivation at the humidity chamber, it did not improve rooting. Therefore, new experiments may be evaluated only at 90 days, to reduce risks of disease that negatively affect survival.

Some studies have shown that cuttings with shoot is strongly influenced by its size and diameter, which are characteristics that define the content of reserves present in the cuttings (Fachinello et al., 2013; Snedden et al., 2010; Souza et al., 2017). In a study of vegetative propagation of *Spondias mombin* L., it was observed that adult root cuttings with 10 cm long are indicated for its propagation (Souza et al., 2017). For *Detarium microcarpum* (Guill. & Perr), both the length and the

diameter were important on the regeneration of adult root cuttings, with the best response for rooting with cuttings from 10 to 15 cm long and from 1.5 to 6.0 cm in diameter (Ky-Dembele et al., 2010). According to the same authors, root cuttings of *D. microcarpum* with 5 cm long were inadequate in propagating the species, due to the inefficiency at forming shoots.

In woody species, the rooting competence of cuttings derived from branch is strongly associated with the maturation level, being observed that plants in juvenile phase provide propagules with higher rooting capacity compared with propagules collected in mature individuals (Hartmann et al., 2011). For root cuttings, however, Bonga (1982) mentions that this type of vegetative material can conserve its juvenility, enabling high regeneration capacity of cuttings even when harvested from trees of advanced chronological age. In this study, root cuttings of adult cordia did not develop roots; however, juvenile root cuttings were capable of rooting, suggesting that juvenility of radicular propagules can determine morphogenetic responses of this species.

The present study consists of the first attempt to propagate cordia from root cuttings collected from adult plants. Information on cloning of adult plants is extremely relevant for the rescue of selected plants, especially woody ones, which may be recalcitrant to adventitious rooting in response to ontogenetic aging. This seems to be the reason why it was not possible to obtain plantlets by this type of propagule, nor of the branches collected in adult plants. Thus, the information presented here indicates that cordia is a species strongly affected by the ontogenetic age and that propagules collected from juvenile plants are more responsive to factors inducing adventitious rooting. The juvenility of the propagules can be by serial mini-cuttings, corroborating with Kielse et al. (2015), which state that plantlets derived from root cuttings collected in juvenile plants can be established in clonal mini-gardens. In addition, it opens new possibilities of studies focusing on the effects of juvenility/maturation of the stock plants, genotypes, as well as on the conditions favoring vegetative propagation by cuttings of cordia.

## CONCLUSIONS

Cuttings from roots and shoots of adult plants were not responsive to propagation, because they produced new shoots without rooting. Cuttings from roots of young plants differentiate roots, even without AIB application, enabling the production of cordia new plantlets.

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