

**Indolebutyric acid and incision in *Maytenus ilicifolia* Mart. Ex. Reissek cuttings rooting****Ácido indolbutírico e incisão no enraizamento de estacas de *Maytenus ilicifolia* Mart. Ex. Reissek**

DOI:10.34117/bjdv6n7-701

Recebimento dos originais:03/06/2020

Aceitação para publicação:27/07/2020

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**ABSTRACT**

The “espinheira-santa” (*Maytenus ilicifolia* Mart. Ex. Reissek – Celastraceae) with great medicinal importance. The aim of the study was to test the effects of applying different concentrations of

indolbutyric acid (IBA) and incisions at the base of the cuttings in rooting of espinheira-santa. The study was conducted (July to November) in the community of São Cristóvão in 2018. Semi-hardwood cuttings 10 cm long were made, with and without incision at the base. The mini-cuttings bases were immersed in alcoholic solutions (50% v/v) of different concentrations of IBA (0, 1000, 2000, 3000, mg L<sup>-1</sup>), for ten seconds and planted in tubes of 53 cm<sup>3</sup> filled with vermiculite. The statistical design used was completely randomized, with four replications of 16 cuttings per plot, with a 4 x 2 factorial system (four concentrations of IBA and two types of cuttings, with and without incision), totaling 8 treatments. At 120 days after planting, variables were evaluated: percentage of rooted cuttings, livings, with sproutings, with calluses, dead ones, number and average length of roots formed per cutting (cm) and average number of sprouts. The species did not respond to the applied IBA concentrations, however the presence of incision contributes to the rooting of the cuttings.

**Keywords:** Espinheira-santa, Plant growth regulator, Vegetative propagation, Cutting.

## RESUMO

A espinheira-santa (*Maytenus ilicifolia* Mart. Ex. Reissek - Celastraceae) de grande importância medicinal. O objetivo do estudo foi testar os efeitos da aplicação de diferentes concentrações de ácido indolbutírico (AIB) e incisões na base das estacas no enraizamento da espinheira-santa. O estudo foi realizado (julho a novembro) na comunidade de São Cristóvão em 2018. Foram feitas estacas semilenhosas de 10 cm de comprimento, com e sem incisão na base. As bases de miniestacas foram imersas em soluções alcoólicas (50% v / v) de diferentes concentrações de AIB (0,1000, 2000, 3000, mg L<sup>-1</sup>), por dez segundos e plantadas em tubos de 53 cm<sup>3</sup> preenchidos com vermiculita. O delineamento estatístico utilizado foi o inteiramente casualizado, com quatro repetições de 16 estacas por parcela, com sistema fatorial 4 x 2 (quatro concentrações de AIB e dois tipos de estacas, com e sem incisão), totalizando 8 tratamentos. Aos 120 dias após o plantio, foram avaliadas variáveis: porcentagem de estacas enraizadas, viveiros, com brotações, com calos, mortos, número e comprimento médio das raízes formadas por estaca (cm) e número médio de brotos. A espécie não respondeu às concentrações aplicadas de AIB, porém a presença de incisão contribuiu para o enraizamento das estacas.

**Palavras-chave:** Espinheira-santa, Regulador de crescimento vegetal, Propagação vegetativa, Corte.

## 1 INTRODUCTION

The espinheira-santa is a native species of South America, occurring in Southern Brasil, in addition to Paraguay, Uruguay and Eastern Argentina. The species presents great economic importance and has been utilized in other countries. In the national flora, with applications in popular medicine, *Maytenus ilicifolia* or *Maytenus muelleri*, popularly known as espinheira-santa, receiving such name (holy-thornbush) due to the presence of leaves with thorny edges. For use of the medicinal properties of this species, the population use the leaves in the form of an infusion (CARVALHO-OKANO and LEITÃO FILHO, 2004; CARVALHO, 2006).

Studies reveal that this plant, as well as others from the *Maytenus* genre, contain antibiotic compounds that show potent anti-tumoral and anti-leukemic activity in very low doses (LORENZI and MATOS, 2008).

The propagation of espinheira-santa may occur sexually, by dissemination, or asexually, with sprigs born from roots and layering (SILVA JÚNIOR and OSAIDA, 2006), herbaceous stem cuttings (Silva 1999), minicuttings (LIMA, 2009) and also, through micropropagation (PEREIRA, 1998).

Cutting allows for direct definition of several genetic parameters, nutritional and phenological studies, in addition to contributing for understanding of the competition, given that by the homogeneity of genotypes it is possible to make a more precise handling of plantation. The root formation of cuttings involves direct regeneration of radicular meristems with vascular tissue association, possibly occurring by the formation of callous tissue located in the base of the cutting (XAVIER et al., 2013). Radicular regeneration depends on the species, *maturation* level of the donor plant and its genotype (XAVIER et al., 2013; HARTMANN et al., 2018).

Plant growth regulators such as auxins, acetic naphthalene acid (ANA) and indolebutyric acid (IBA) accelerate the normal plant metabolism and, thus, may increase the number of formed radicular primordia. These plant growth regulators interfere directly in the quality and uniformity of the radicular system (ALCÂNTARA et al., 2010; PRETI et al., 2016). The plant growth regulators support the cuttings' root formation, although other factors may interfere in this process. Size, age and carbohydrate reserves may make these cuttings insensitive to the use of these regulators (SANTOS et al., 2010).

The objective of the present study was studying the effects of application of different concentrations of indolebutyric acid (IBA) and incisions in the base of cuttings in the root formation of espinheira-santa in order to allow for production of cuttings through this technique.

## **2 MATERIAL AND METHODS**

The study was conducted in the Southeast of the State of Paraná, in the township of Dois Vizinhos, in the São Cristovão community in the period between July and November of 2018. The study matrixes are located in the Education and Research in Horticulture Unit (Unidade de Ensino e Pesquisa em Horticultura – UNEPE) in the experimental area of the Federal Technological University – Dois Vizinhos Campus (UTFPR/DV), latitude 25° 44' 01", longitude 53° 03' 26", 510m of altitude. According to the Köppen classification, the region's climate is subtropical humid, Cfa type, with temperatures in the coldest month between -3°C and 18°C, always humid, with rain every

month of the year and temperature in the warmest month superior to 22°C, with at least four months with temperatures greater than 10 °C (ALVARES et al., 2013).

The matrix plants of espinheira-santa gathered for material collection were randomly selected in the planting area, which consists of 57 espinheira-santa individuals, distributed in six lines, containing a variable number of plants per line. These plants come from the city of Santa Maria-RS and were implanted as seeds in October 2011, by the responsible person for the Horticulture UNEPE, Prof. Dr. Dalva Paulus.

Afterwards, semi-ligneous twigs were collected and cuttings of 10cm length were made, with presence and absence in the base, a pair of leaves reduced in half in the apical portion being kept. The cuttings underwent mechanical incisions with the help of a scalpel. The incisions were made with two sideway wounds at the base of the cutting, with about 1cm length, in which a barked portion was eliminated in order to expose a greater area in the cambial region.

After confection, the bases of the stakes were immersed in alcoholic solutions (50% v/v) of IBA in concentrations of 0, 1000, 2000 and 3000 mg L<sup>-1</sup>, for 10 seconds. The experimental outlining used was entirely randomized (DIC) with a factorial arrangement of 4 x 2 (four concentrations of IBA and two types of cuttings, with and without incision in the base), four repetitions and 16 cuttings per parcel, totaling 520 cuttings.

The planting was performed in narrow polypropylene tubes of 53 cm<sup>3</sup> containing fine granulometry vermiculite as its substrate, packed in trays and kept under shading in the UNEPE Viveiro Florestal of UTFPR-DV) in room temperature and black shade cloth of 50% solar transparency, with manual irrigation four times a day for 20 minutes. The physical analysis of the substrate was also made.

### **Substrate physical analysis**

The vermiculite substrate was stored in a kraft paper package and taken to the Silviculture Campus Lab of UTFPR-DV and placed in a drying greenhouse for 48 hours at a temperature of 60°C. After drying the physical analysis of this substrate was performed in the Microscopy I (UTFPR-DV) in accordance to the methodology by Fretz et al. (1979).

At 120 days after planting, an evaluation of the following variables was made: percentage of rooted cuttings (PEE), living cuttings (PEV), sprouting cuttings (PEB), with calluses (PEC) and dead cuttings (PEM), number (NRE) and average length of roots formed per cutting (cm) (CMRE) and average number of sprouts per cuttings (NBE).

The data of variables were previously submitted to a Shapiro-Wilk normality test ( $p\text{-valor} > 0,05$ ) and Bartlett test in order to check the homogeneity of variance, verifying that the variables ‘average number of roots’ and ‘average number of buddings’ per cutting were transformed by the function  $\ln(1+x)$ . The average scores with or without transformation were subject to variance analyses and to the Tukey test at 5% of probability. The analyses were performed by the Genes software version 1990.2018.57.

### 3 RESULTS AND DISCUSSION

It was verified in the present study there was no significant interaction for no variable among factors (IBA concentrations x two types of cuttings), this way the factors were analyzed separately, given that only a factor “types of cuttings” was significant (Table 1).

Table 1: Summary of the analysis of variance containing the values of the mean squares for the variables: percentage of rooted cuttings (PEE); live cuttings (PEV); average number of roots per cutting (NRE); average roots length per cutting (CMRE); percentage of sproutings cutting (PEB); average number of sprouts per cutting (NBE); percentage of dead cuttings (PEM), under different concentrations of IBA and types of cuttings (with and without incision) of *M. ilicifolia*. UTFPR, Dois Vizinhos - PR, 2018.

SV	DF	Medium square						
		PEE (%)	PEV (%)	NRE <sup>(T)</sup>	CMRE (cm)	PEB (%)	NBE <sup>(T)</sup>	PEM (%)
IBA	3	14.2 <sup>ns</sup>	99.3 <sup>ns</sup>	0.02 <sup>ns</sup>	5,2 <sup>ns</sup>	174.2 <sup>ns</sup>	0.09 <sup>ns</sup>	99.3 <sup>ns</sup>
TC	1	10557.9*	12700.2*	5.5*	30.7*	7050.8*	0.52*	12700.2*
IBA X TC	3	76.1 <sup>ns</sup>	118.8 <sup>ns</sup>	0.08 <sup>ns</sup>	0.46 <sup>ns</sup>	110.7 <sup>ns</sup>	0.03 <sup>ns</sup>	118.8 <sup>ns</sup>
Error	24	125.7	211.6	0.05	3.6	205.9	0.04	211.6
Total	31							
CV (%)		41.91	45.97	39.03	55.67	54.83	32.32	21.28

SV – Source of variation; DF – Degrees of freedom. \*Significant at 5 % of probability by the F test; <sup>ns</sup> not significant at 5 % of probability. IBA - concentrations of IBA (0; 1000; 2000 and 3000). TC - types of cuttings (with and without incision). <sup>(T)</sup> Data transformed by  $\ln(1 + X)$ . CV - Coefficient of Variation.

For the types of cuttings (with incision and without incision) the analysis of variance showed significance in most variables (Table 2), with exception of the “cuttings with calluses” variable, that even with the transformation of data did not present normality due to the excessive of zeroes. The low percentage of calli may be explained by the superiority of obtained of living cuttings. In *M. ilicifolia* cuttings, Lima (2008), obtained a good percentage of cuttings with calli (26.67%). In some species with difficulty to form roots, root formation happens over the callus, even if the callus formation is no safe indicator of future root formation (FACHINELLO et al., 2005; HARTMANN et al., 2018).

Table 2: Average data for variables: percentage of rooted cuttings (PEE); live cuttings (PEV); average number of roots per cutting (NRE); average roots length per cutting (CMRE); percentage of sproutings cutting (PEB); average number of sprouts per cutting (NBE); percentage of dead cuttings (PEM) for the factor (TE) types of cuttings (with and without incision) of *M. ilicifolia*. UTFPR, Dois Vizinhos - PR, 2018.

TE*	Variables <sup>1</sup>						
	PEE (%)	PEV (%)	NRE <sup>(T)</sup>	CMRE (cm)	PEB (%)	NBE <sup>(T)</sup>	PEM (%)
With Incision	39.00 a	16.55 a	1.03 a	4.42 a	41.01 a	0.82 a	44.44 b
Without Incision	8.89 b	14.70 b	0.19 b	2.45 b	11.33 b	0.56 b	76.41 a
CV%	41.91	45.97	39.03	55.67	54.83	32.32	21.28

<sup>1</sup>\* Significant at 5% probability for the isolated factor types of piles. CV - Coefficient of Variation. Means followed by the same letter in the column do not differ significantly by Tukey's test at 5% probability. <sup>(T)</sup> Data transformed by  $\ln (I + X)$ .

Concerning techniques used, it can be remarked on Table 2 that the method with incision at the base of cuttings presented better results for the analyzed variables, in relation to the non-incisive method, which presented the highest mortality of cuttings (76.41%). According to Biasi et al. (2000), in the area where the wound is made cellular activity is stimulated by the increase in respiratory rates, elevation in auxin rates, carbohydrates and ethylene, root formation occurring at the margins of the wound. Results obtained in this study show that the wound at the base (incision) of the cutting contributed in the root formation of the *M. ilicifolia* cuttings.

The incision method at the base of cuttings presented the highest rate of rooted stakes (39.00%) when compared to the non-incisive method (Table 2). To Tofanelli et al. (2005) the incision technique at the base of cuttings also benefited the root formation of peach trees, being 82%, superior to the cuttings which received no incision at the base. De Paiva et al. (2015) also obtained better results with the incision technique at the base of "Wonderful" pomegranate-tree cuttings, with which the authors obtained an increase in the survival rate with the use of incision (wound) in the base of the cuttings.

In auxin application, as well as for the used techniques (Table 2 and Table 3) the species did not respond well to root formation, being considered difficult to root. Results obtained by cutting using herbaceous propagules of *Psidium guinnensis* the best results for the analyzed variables were obtained when 4,000 mg L<sup>-1</sup> of IBA was applied to cuttings with leaves (CARVALHO et al., 2020). Difficulty in rooting may be associated to genetic variability present in the collected material, or factors related to the matrix plant, given that the plant could be found with blossoming flower buds, directing its metabolic roots to flower formation (FERRARI et al., 2004).

These flower, seed and growing fruit parts are considered consumption parts (draining) (FLOSS, 2011). Several experiments show that with the maintenance of a draining tissue, there is

occasionally an increase of translocation to alternate and competitive drains by photo-assimilates generating energetic deviations of roots and leaves to the flower, making rooting difficult (TAIZ et al., 2017).

For cuttings with buddings, stakes with incisions were significantly superior in development of buddings when compared to the absence of incision (Table 3). In studies with pomegranate-tree saplings (*Punica granatum* L.) propagated by cuttings, authors also had a higher number of buds in cuttings with incisions at the base (DE PAIVA et al., 2015).

Table 3: Non-significant average data for the variables: percentage of rooted cuttings; live cuttings; average number of roots per cutting; average root length per cutting; percentage of sproutings cuttings; average number of sprouts per cutting for the factor IBA concentrations in the cuttings of *M. ilicifolia*. UTFPR, Dois Vizinhos - PR, 2018.

	Rooted Cuttings (%)				Live cuttings (%)			
	Concentrations IBA (mg L <sup>-1</sup> )				Concentrations IBA (mg L <sup>-1</sup> )			
	0 <sup>ns</sup>	1000 <sup>ns</sup>	2000 <sup>ns</sup>	3000 <sup>ns</sup>	0 <sup>ns</sup>	1000 <sup>ns</sup>	2000 <sup>ns</sup>	3000 <sup>ns</sup>
Averages	25.00	27.00	26.50	28.00	35.90	32.00	31.20	27.30
CV (%)	41.91				45.97			
	Average number of roots				Average length of roots (cm)			
	Concentrations IBA (mg L <sup>-1</sup> )				Concentrations IBA (mg L <sup>-1</sup> )			
	0 <sup>ns</sup>	1000 <sup>ns</sup>	2000 <sup>ns</sup>	3000 <sup>ns</sup>	0 <sup>ns</sup>	1000 <sup>ns</sup>	2000 <sup>ns</sup>	3000 <sup>ns</sup>
Averages	0.53	0.57	0.67	0.64	2.55	4.52	3.32	3.35
CV (%)	39.03				55.67			
	Sprouting cuttings (%)				Average number of sprouts			
	Concentrations IBA (mg L <sup>-1</sup> )				Concentrations IBA (mg L <sup>-1</sup> )			
	0 <sup>ns</sup>	1000 <sup>ns</sup>	2000 <sup>ns</sup>	3000 <sup>ns</sup>	0 <sup>ns</sup>	1000 <sup>ns</sup>	2000 <sup>ns</sup>	3000 <sup>ns</sup>
Averages	31.2	28.1	25.0	20.3	0.81	0.72	0.67	0.55
CV (%)	54.83				32.32			

<sup>ns</sup> not significant at 5% probability. CV - Coefficient of Variation.

The mortality of cuttings has influenced results, may be caused by other external factors such as temperature, light, humidity, may have contributed to the failure of variables analyzed in the used techniques (with and without incision) as they relate to climate conditions occurred during the



experiment period that may have contributed to the cuttings' low root formation. The climate presents influence on root formation (DUTRA et al., 2002).

The time of material collection (June) was a period of lower temperatures, which caused the metabolic activity of the plants and the radicular growth to be lower. According to Taiz et al. (2017), temperature has a direct effect on plant metabolism, given that, the higher it is, the more accelerated the chemical reactions will be, which may not have favored the radicular development.

According to Hartmann et al. (2018), under appropriate conditions (high humidity and heat), root formation in cuttings is easy and it reaches high rates. Fachinello et al. (2005) informed that the increase in temperature favor cellular division in cuttings. With this, it is supposed that low temperature, as obtained in the collecting time in July, did not favor the rooting of the species. The season of the year in which the collection of the cuttings is performed influences their mortality, due to changes to their physiological balance in shorter or longer days (RAIBS, 1993; ZEM et al., 2015).

As aforementioned, the root formation of these cuttings may be influenced positive or negatively by internal factors. In this case these factors, such as espinheira-santa cuttings collections during flourishing may interfere on root formation process, where it was observed that cuttings coming from twigs with flower buds have a tendency of less rooting. In a study with *M. ilicifolia*, Lima (2008) has verified, through anatomical analyses that the internal factors make the species' rooting more difficult in different times of the year, although in the present study these anatomical analyses may have not been performed, as it may be considered as limiting factors to rooting as the species is the same.

Lima (2008), however, related the slow or difficult rooting of the espinheira-santa by the anatomical characteristics of the stem, which may justify the difficulty in the espinheira-santa cuttings rooting in the present study. Nevertheless, the best rooting of cuttings with incisions probably occurred by breach of the sclerenchymetic sheath that allowed the passage of radicular primordia.

Therefore, as the species has difficult rooting even when internal and external factors are adequate, greater care is needed with the time of material collection and physiological conditions of the mother plant, as well as the technique used for cutting confection, as even in the aforementioned restricted conditions, the incisive method at their base presented acceptable results concerning the species rooting, although the percentage was still low in order to make possible the production of saplings.



Concerning the tested IBA concentrations (0, 1000, 2000, 3000 mg L<sup>-1</sup>), they were not significant (Table 3 and Table 4), that is, the average scores of this factor were statistically the same for all analyzed variables, and therefore, the regression analysis was not performed for any variable.

Table 4: Percentage of dead cuttings (PEM) for the factor IBA concentrations in *M. ilicifolia* cuttings. UTFPR, Dois Vizinhos - PR, 2018.

	Dead cuttings (%)			
	Concentrations IBA (mg L <sup>-1</sup> )			
	0 <sup>ns</sup>	1000 <sup>ns</sup>	2000 <sup>ns</sup>	3000 <sup>ns</sup>
Averages	64.0	67.9	68.7	72.6
CV (%)	21.28			

<sup>ns</sup> not significant at 5% probability. CV - Coefficient of Variation.

It was verified, thus, that the exogen application of auxin did not influence the formation of roots in espinheira-santa cuttings in the used concentrations, as there was no response of the analyzed variables in none of the tested concentrations, although the auxins are growth regulators that induce the formation of roots in cuttings and, they may present little or no effect in species of difficult rooting (WILSON, 1994; PETRI et al., 2016, TAIZ et al., 2017, HARTMANN et al., 2018).

The studied species is considered difficult to root for presenting limiting factors such as the need to remain in controlled humidity and temperature conditions for a longer period, that is, permanence in greenhouses between 90 and 120 days of installation, time for the species to begin the cellular differentiation process and radicular emission.

Working with *Maytenus ilicifolia*, Lima (2009) verified that the presence or absence of auxin application, the process of induction and rooting in stem cuttings of the species needed a long period in the greenhouse, between 180 and 365 days, and even after 365 days in these conditions, the author verified that the species did not present sufficient rooting percentages for production in a commercial scale.

Considering the aforementioned factors, the espinheira-santa material collected for cutting confection may have suffered influence from internal factors. The material was flourishing, which may have caused lower performance of the initiation and development of the radicular system. The flourishing of espinheira-santa occurs between the months of August and November, and the fruitage of October to March (SILVA JÚNIOR, 2003).

Origin cuttings of twigs with floriferous buds tend to root less often than those obtained from vegetative twigs undergoing an active growth phase, even if one removes the floristic structures from the twigs, to which the cuttings were made. These may even have undergone a process of

antagonism between blossoming and rooting, since the plant had its physiological system geared towards flower formation, mobilizing the cutting's reserves and interfering in the process of root initiation (FACHINELLO et al., 2005).

As verified in the present study, Lima (2008) observed that the rooting of cuttings of *Maytenus ilicifolia*, in different seasons of the year, with concentrations of indolebutyric acid (AIB) and different forms of application (talcum and solution), a lower response to rooting was obtained when the cuttings were collected in the spring, time when the blossoming starts. Ziantonio et al. (2003), using different concentrations of IBA in rooting of espinheira-santa cuttings, a superior rooting (92.5%) was obtained in the absence of the regulator.

For rooting of rosemary cuttings (*Rosmarinus officinalis*), the application of six concentrations of IBA promoted a positive effect in rooting (PAULUS et al., 2016). In passionfruit cuttings (*P. mucronnata* Lam.), using six concentrations of IBA and two application forms (liquid and solid), the authors obtained a good rooting rate (86%) in IBA concentrations (1000 mg L<sup>-1</sup> or mg Kg<sup>-1</sup>) (ALEXANDRE et al., 2014).

However, it may be observed that despite no statistical difference for any variable, for percentage of living cuttings, percentage of cuttings with budding and bud number, higher survival, presence and number of buddings in cuttings with no auxin. For number and length of roots, the tendency of greater results with an increase of auxin.

Concerning the mortality of cuttings, no significant differences were observed in the used IBA concentrations (Table 4), with percentage of dead cuttings in the 3000 mg L<sup>-1</sup> (72.6%), 2000 and 1000 mg L<sup>-1</sup> (68.7%), respectively. It is important to highlight that even when auxin was not applied (additional check) the species obtained a good response to the analyzed variables. It is supposed therefore that the cuttings of espinheira-santa produce sufficiently quantity of endogenous auxins, that allow for a good response to the analyzed variables even when compared with the application of auxin.

In a study performed with *Maytenus aquifolium*, with indolebutyric acid (IBA), even in low concentrations, the author observed mortality of all cuttings after five weeks from the beginning of the experiment, with exception of the additional check, with 30% of survival (ALVES, 2015). Therefore, due to the species' difficulty in rooting, it would be necessary to remain for a period longer than 120 days in the rooting beds, in order to analyze the mortality.

The vermiculite substrate presented in the study a total porose space of (74.5%), a macroporosity of (8.44%) and microporosity of (66.07%) (Table 5).

Table 5: Physical analysis of the vermiculite substrate used in cutting *M. ilicifolia*. UTFPR, Dois Vizinhos - PR, 2018.

Substrate	DS (g/cm <sup>3</sup> )	DU (g/L)	EPT .....(%) .....	ARCC	EACC
VER	0.2374	237.4	74.5	66.07	8.44

DS – dry density; DU - wet density; EPT - total porous space; ARCC - water retention in field capacity (microporosity); EACC - air space in field capacity (macroporosity); (VER) vermiculite.

#### 4 CONCLUSION

It is possible to conclude that the concentrations of IBA used did not influence any of the analyzed variables.

The cuttings of espinheira-santa need to remain for a period longer than 120 days in the rooting beds, and the species' rooting may be considered difficult or slow.

In relation to the presence and absence of incision at the base of the cuttings, the species responded better to the method with incision, thus promoting rooting.

It was verified that the vegetative propagation of espinheira-santa through cutting technique must be improved for obtaining rooting of the cuttings and sapling production.

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