Revista Brasileira de Ciências Agrárias
ISSN (on line) 1981-0997
v.14, n.2, e5650, 2019
Recife, PE, UFRPE. www.agraria.pro.br
DOI:10.5039/agraria.v14i2a5650
Protocolo 5650 - 04/07/2017 • Aprovado em 11/02/2019

Vegetative propagation of red mombin (*Spondias purpurea*) with immersion in indole-3-acetic acid

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ABSTRACT: The objective of this work was to evaluate the effect of different concentrations of indole-3-acetic acid (IAA) applications, in three immersion times on the vegetative propagation of red mombin (*Spondias purpurea*), using the cutting technique. The experimental design was the completely randomized, with three replicates, in a factorial scheme (3 x 6), three different immersion periods (8, 16 and 24 seconds), in six concentrations (0, 2, 4, 6, 8 and 10 g L^{-1}) of IAA, with ten cuttings per plot. The experimental period was 180 days and the characteristics percentage of cuttings survival, percentage of rooted cuttings, number of young shoots, number of roots per cuttings, general root length and total fresh mass. Data were submitted to analysis of variance by the F test and, later, fitted in regression equations. The vegetative propagation by red mombin cuttings was satisfactory to rooting and sprouting in the cuttings, with greater increases between the IAA concentrations of 6 to 10 g L^{-1}. The 10 g L^{-1} concentration, in 24 seconds of immersion time, was the one that induced greater increases in the red mombin variables.

Key words: Anarcadiaceae; fruit trees; growth regulator

Propagação vegetativa de seriguela (*Spondias purpurea*) com imersão em ácido indol-3-acético

RESUMO: Objetivou-se com este trabalho avaliar o efeito da aplicação de diferentes concentrações do ácido indol acético (AIA), em três tempos de imersão na propagação vegetativa de seriguela (*Spondias purpurea*), utilizando a técnica de estaquia. O delineamento experimental utilizado foi inteiramente causalizado, com três repetições, em esquema fatorial (3 x 6), três tempos de imersão (8, 16 e 24 segundos), em seis concentrações (0, 2, 4, 6, 8 e 10 g L^{-1}) do AIA, com dez estacas por parcela. O período de condução do experimento foi de 180 dias e foram avaliadas as características de porcentagem de sobrevivência das estacas, porcentagem de estacas enraizadas, número de brotações jovens foliares, número de raízes por estacas, comprimento geral das raízes e massa fresta total. Os dados foram submetidos à análise de variância pelo teste de F e posteriormente, ajustados em equações de regressão. A propagação vegetativa por estaquia de seriguela foi satisfatória ao enraizamento e a brotação nas estacas, com maiores incrementos entre as concentrações 6 a 10 g L^{-1} de AIA. A concentração de 10 g L^{-1} em tempo de imersão de 24 segundos foi a que induziu maiores incrementos nas variáveis de seriguela.

Palavras-chave: Anarcadiaceae; frutíferas; regulador de crescimento

Introduction

The *Spondias purpurea* L. species, belonging to the family of Anacardiaceas and the genus *Spondias*, has its origin center in Central America, but also with distribution in some areas of Mexico, the Caribbean and in several countries of the north region of South America, that probably were dispersed by the man (Mitchell & Daly, 2015). This species, whose common name is red mombin, is more cultivated in domestic orchards and has a reddish coloration, with pleasant taste and aroma, consumed *in natura* or as pulps for juice, jellies, popsicles and a range of sweets (Ferreira et al. 2015).

Red mombin trees requires selected clones with desirable agronomic characteristics, adapted and phenotypically stable for their commercial cultivation, as well as techniques that promote the best development of cuttings (Lira-Júnior et al., 2014). In this sense, for the red mombin production in commercial scale, the quality of the seedlings is essential, in order to guarantee a high productivity, fast fruiting and homogeneity in the planting, it is imperative to use vegetative propagation more efficiently. As seeds rarely propagate the *S. pupurea* species, its multiplication occurs mainly asexually, since the pollen produced by the species is mostly infertile (Santos & Oliveira, 2008).

Cutting is the most recommended method to propagate tropical species such as red mombin, however, the rooting ability of each cutting depends on the interaction of several factors, such as the phenological state of the stock plant, the environmental conditions and the treatment to be used (Souza & Lima, 2008). In order to favor hormonal balance, rooting and physiological performance of cuttings, the application of growth regulators is of utmost importance for improving the quality and quantity of seedlings by the cutting process (Gratieri-Sossella et al., 2008).

These plant regulators can be used alone or combined in the process of root induction as plant growth promoters, with the IAA (indole-3-acetic acid) auxin being the most used for this process. This regulator is mainly produced in the apical meristem of the plant stem and later transported through the cells in the parenchyma to the root system, having as main effect the promotion of the growth of roots and stems (Taiz & Zeiger, 2013).

However, there are few studies related to red mombin propagation using IAA, and there is a need for new studies aimed at identifying techniques that display superior performance in the early stages of development and that possibly perpetuate it to the reproductive stage (Lira-Júnior et al. al., 2014). Thus, this study aimed to evaluate the effect of the application of different IAA concentrations, in different immersion times, on the vegetative propagation of *Spondias purpurea* species by the cutting technique.

Materials and Methods

The experiment was conducted in a greenhouse composed by a 3.5 m tall, 30 m length and 7 m width metal structure, with a covering of 150 microns thick light diffusing polyethylene film and a shade net with 50% light passage at the sides, at Goiás State University, Ipameri – GO. The vegetative propagules were collected from adult stock trees at Estância Akenaton, located 18 km away from the Ipameri municipality core town in May 2016.

For woody cuttings, we adopted a length of 20 cm, with at least two lateral buds, with a straight cut at the top part and a bevel at the basal end, with pruning shears and manual defoliation.

The experimental design employed was the completely randomized, in a 6x3 factorial scheme (six concentrations of IAA in three immersion times), using three replicates, with ten cuttings per plot.

The concentrations were 0, 2, 4, 6, 8 and 10 g L^{-1} of indole-3-acetic acid (IAA – $C_{10}H_9NO_2$ – M.W. 175.18 – 99% purity) diluted in ethyl alcohol and distilled water in the proportion of 1:1, according to Souza & Costa (2010). Subsequently, the cuttings remained immersed for 8, 16 and 24 seconds in the different IAA concentrations. Soon after, the cuttings were placed in polypropylene pots with 12 L capacity filled with Dystrophic Red Latosol, classified according to Embrapa (2013), with corrected pH by dolomitic limestone, with relative power of total neutralization of 70%, previously, 28 days before installation, with constant irrigation, maintaining field capacity at 80%. Afterwards, they were fertilized with 6 g from the 8-28-16 NPK formulated, according to the soil chemical characteristics: 320 g kg^{-1} sand; 90 g kg^{-1} lime; 590 g kg^{-1} sand; 4.9 pH; 24.1 g dm^{-3} organic matter; 5 mg dm^{-3} P; 30.3 $mmol_c$ dm^{-3} H+Al; 4.1 $mmol_c$ dm^{-3} K; 18.2 $mmol_c$ dm^{-3} Ca; 7.5 $mmol_c$ dm^{-3} Mg; 27.8 $mmol_c$ dm^{-3} SB; 57.6 $mmol_c$ dm^{-3} CEC; 47.7 V (%).

In order to avoid the dryness of the soil contained in the pots and to keep the cuttings propagated in a higher humidity, we assembled humidification structures for each pot, made of bamboo and strings, and covered by transparent polyethylene light diffuser bags (40 microns) with a height of 1 m and cylindrical shape.

The experiment conduction period was of 180 days and divided into two stages, with the cuttings being conditioned in the protective structure for 90 days, and afterwards, in the second stage, the cuttings remained in the pots without protection for another 90 days. Irrigations were performed every four days to maintain the soil moisture, with 500 mL of water each week, in the first period, while in the second period it would be every two days, with 300 mL.

The evaluations were percentage of cuttings survival (PSUR), in which the surviving cuttings were considered those that presented alive wood, young shoots and/or rooting; percentage of rooted cuttings (PROC), where the cutting with induction of root beginnings greater than 1 mm was classified as rooted; number of roots (NRO), analyzed by the number of roots already formed and larger than 1 mm in each cutting, in units; General length of roots (GLR), that is, all roots from each cutting were measured with the aid of a graduated ruler and summed, in millimeters; Number of young leafy shoots

(NYS), obtained by the number of total shoots per cutting; Total fresh mass (TFM), when masses of the fresh roots, leaves and shoots were agglomerated, placed in plastic trays and weighed in precision scale, expressed in mg.

The data were submitted to analysis of variance and then fitted in regression equations, according to the different concentrations of IAA, with three immersion times in *Spondias purpurea* employing the SISVAR program (Ferreira, 2011).

Results and Discussion

The result of the analysis of variance indicates a significant difference ($p < 0.05$) for all evaluated characteristics (Table 1). These results denote the potential of the vegetative propagation technique for the red mombin when using IAA. These results were divergent from those observed by Muniz et al. (2015), which verified that there was a significant effect on the interaction time of evaluation and the concentrations of IAA, only for the number of roots per cutting of Canada goldenrod (*Solidago canadensis* L.).

We can observe in Figure 1A that there was a marked linear growth in the cuttings for the PSUR as the concentrations increased, with similar performance among the three tested immersion times. The average increment displayed by the cuttings was of 56, 74 and 49% between the 0 and 10 g L^{-1} concentrations, in the times of 8, 16 and 24 seconds, respectively. Silva et al. (2009) observed that there was a progressive and linear increase in the rooting of camu-camu [*Myrciaria dubia* (H.B.K.) Mc.Vaugh], when submitted to values of 1000 to 5000 mg L^{-1} of IAA, thus confirming the greater survival of vegetative propagules. This may also indicate that the species may have higher survival rate at higher concentrations, as verified in this study.

It is also observed that the highest auxin concentrations were more efficient for the PSUR variable, which presented an average increment of 1.2% for each 1 g L^{-1} of IAA, considering the three immersion times (Figure 1A). Alcantara et al. (2010) evaluated the naphthalene acetic and indole-butyric acids in the rooting of black plum [(*Syzygium cumini* L.) Skeels] and found that concentrations of approximately 1 g L^{-1}, in this case, had a higher number of live cuttings for these regulators.

Several authors affirm that the cuttings survival is linked to the rooting capacity of the vegetative materials, since the presence and the volume of the roots allow the cuttings to absorb more water and nutrients, with the roots being essential for the initial development of the seedling (Fachinello et al., 2005; Amaral et al., 2012). It was possible to detect that survival linked through the PSUR (Figure 1A) and the NRO (Figure 1C), as reported by these authors.

According to Figure 1B, the cuttings displayed a linear behavior at the immersion time of 24 seconds for the PERS variable, with a 42% increase between the control and the 10 g L^{-1} concentration. The cuttings immersed in 8 seconds showed an increase of 81%, in the 6.6 g L^{-1} concentration (maximum point) and three times higher at the 16 seconds time. According to Timm et al. (2015), this could be due to the excessive increase in the phytoregulators concentration, which can cause hormonal imbalance and reduce the percentage of rooting in the species. In *S. purpurea*, the immersion time was the determinant factor in the increase of the percentage of rooted cuttings, since high concentrations of IAA auxin presented the best results, at times of 16 and 24 seconds, indicating that the cutting should remain longer under immersion to promote greater rooting.

According to Hartmann et al. (2011), the adequate concentration of the hormone is dependent on the endogenous level present in the cuttings, which this balance was responsible for stimulating the growth and the cellular differentiation of the plant tissues, thus increasing the rooting percentage and, in the future, the development of the adult plant in the field.

In 16 seconds of immersion, we observed that the absence of indole-3-acetic acid provided values relevant to PERS (Figure 1B), with a value of 7%, however, when subjecting the cuttings to the phytoregulator, in the minimum concentration of 3.4 g L^{-1}, there was a reduction in the rooting percentage of the seedlings, and afterwards, a 65% increase from this point until the 10 g L^{-1} treatment, evidencing the importance of IAA concentrations for the satisfactory rooting of the species.

Meneguzzi et al. (2015) when evaluating IAA concentrations of 0, 1, 2, 3 and 4 g L^{-1}, found that the maximum efficiency concentration for Japanese pittosporum (*Pittosporum tobira*) was 2.2 g L^{-1}, with 57 % of rooted cuttings, however, with the concentration increase, there was a decrease in the percentage, around 30%. In contrast to that observed by these authors, the increase of IAA auxin concentrations, applied to the red mombin cuttings, stimulated the adventitious rooting in the vegetative material of the *S. purpurea* species, mainly in

Table 1. Mean squares of the variables: percentage of cuttings survival (PSUR), percentage of rooted cuttings (PROC), number of young shoots (NYS), number of roots (NRO), general length of roots (GLR) and total fresh mass (TFM), submitted to different immersion times and doses of indole-3-acetic acid, in red mombin.

VF	DF	PSUR	PROC	NRO	GLR	NYS	TFM
		(%)		(unit)	(mm)	(unit)	(mg)
Time	2	49.04**	46.21**	42.76**	158.72**	1.05*	5745.52**
Concentration	5	221.87**	74.71**	90.03**	105.19**	1.61**	266.87**
Time*Concentration	10	52.03**	272.31**	99.96**	73.11**	0.36**	2205.96**
Error	81	3.37	2.10	1.76	4.73	0.02	52.01
CV (%)		12.26	19.73	23.64	12.95	5.72	27.18

** - highly significant; * - significant; 1 and 5% of probability, by the F test

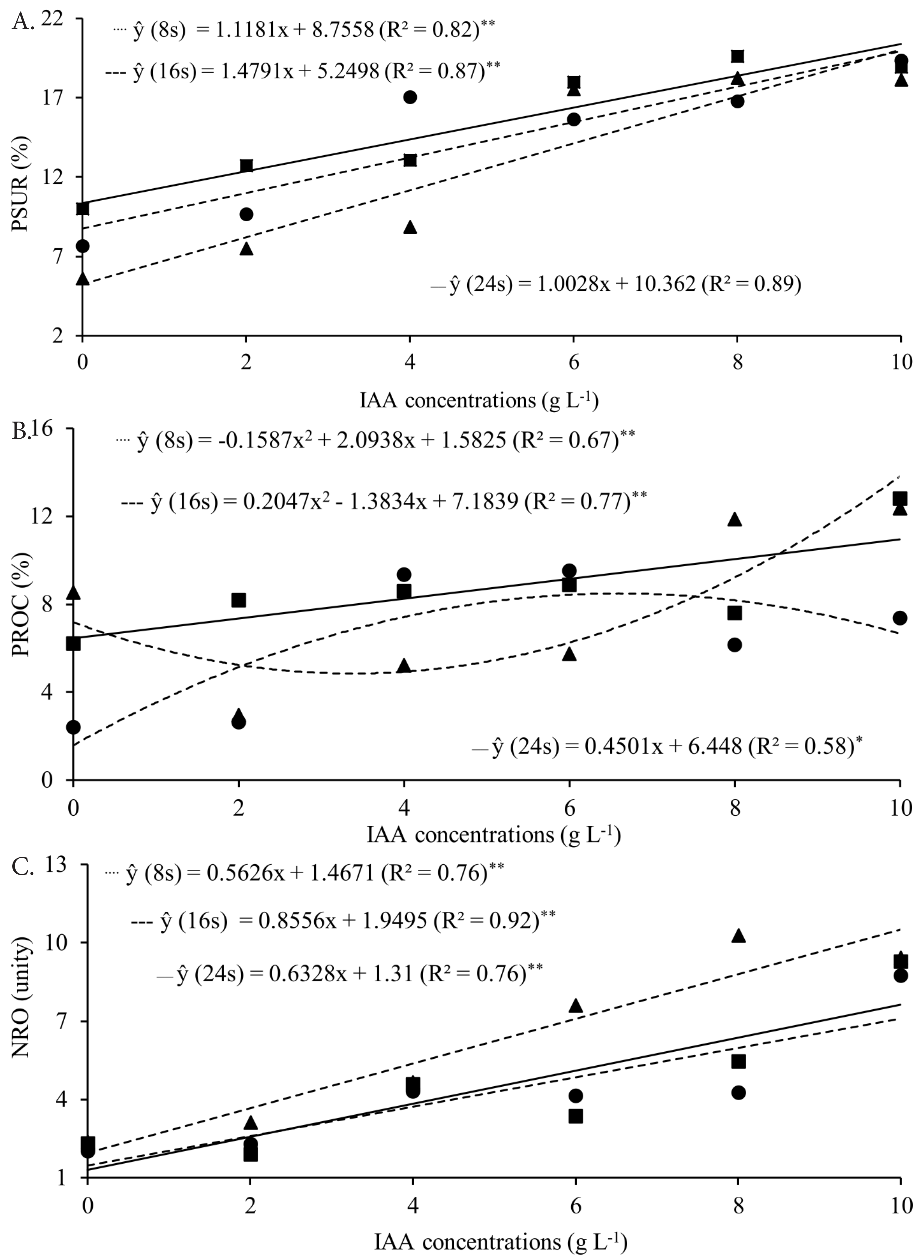

Figure 1. Percentage of cuttings survival (PSUR - A), percentage of rooted cuttings (PROC - B) and number of roots per cuttings (NRO - C) as a function of different concentrations of indole-3-acetic acid (IAA) on *Spondias purpurea*

the 16 and 24 second immersion times, confirming the results obtained in PSUR (Figure 1A) and in NRO (Figure 1C).

Suguino et al. (2009) observed that the immersion times of 0, 4, 8 and 16 seconds, in a IBA concentration of 1 g L^{-1} did not promote the formation of calluses and roots, in *Myrcia selloi* (Cambuí) cuttings, denoting the necessity of using other concentrations or other regulators to verify the influence on the propagation of this species. For *Spondias purpurea* species, the tested immersion times, together with the IAA concentrations, provided satisfactory values for the cuttings rooting, with the time of 16 seconds being considered the best immersion period, at the maximum tested concentration, with a more rapid growth from 4 g L^{-1}.

In Figure 1C, for the NRO variable, the cuttings roots presented linear and increasing behavior in the concentrations and in the immersion time of 16 seconds, the formation of new roots was more efficient, with approximately ten units of roots, and, the times of 8 and 24 seconds, with seven units values, in the IAA concentration of 10 g L^{-1}. The emplyment of growth regulators has the purpose of accelerating the emission of roots, increasing the number and the qualities of the formed roots, which as a consequence from this stimulus in the root system, further stimulates the rhizogenesis of the cuttings, as observed in this study. This occurs due to the intense synthesis of auxins, which, soon after, are transported to the vegetable base, as is the case with the IAA.

According to Mbagwu et al. (2017) in *Gongronema latifolium* Benth, the IAA concentration of 10 mg L^{-1} is able to increase the dry mass of the roots, the number of leaves, buds and roots, besides promoting changes in some secondary metabolites, such as alkaloids, flavonoids, tannins and saponins. According to Leandro & Yuyama (2008), in the vegetative propagation of cuttings, the ability to emit new roots is related to environmental and endogenous factors, with the adequate effect of the hormone on the species depending on the auxin concentration present in the tissue of the studied species, being of great importance for the development of the plant aerial part. According to the results observed in this study, the internal concentration present in the cuttings was low, since it gradually increased with the concentrations increase (Figure 1C).

The immersion for 24 seconds was the one that induced the linear and most expressive behavior for the GLR variable (Figure 2A), with an upper value of 5 and 2.5 mm approximately, at the immersion times of 8 and 16 seconds, respectively. Although the increment appears to be less expressive, in order to the seedling can reach certain nutrients, any increase may contribute to accelerated aerial part development, as confirmed by the similarity of the performance with the TFM (Figure 2C). The formation and development of adventitious roots are also influenced by the concentrations of several plant regulators, especially auxins, since they are responsible for activating the cambial cells, regulating plant growth and development (Taiz & Zeiger, 2013).

Muniz et al. (2015) verified that there was linear growth for the GLR of *Solidago canadensis* L. cuttings with the increase of IAA concentrations, the increment was of 37% in the general length of the roots in comparison with the control group, in the concentration of 3,000 mg kg^{-1}, with a difference of one cm between treatments. According to these same authors, it

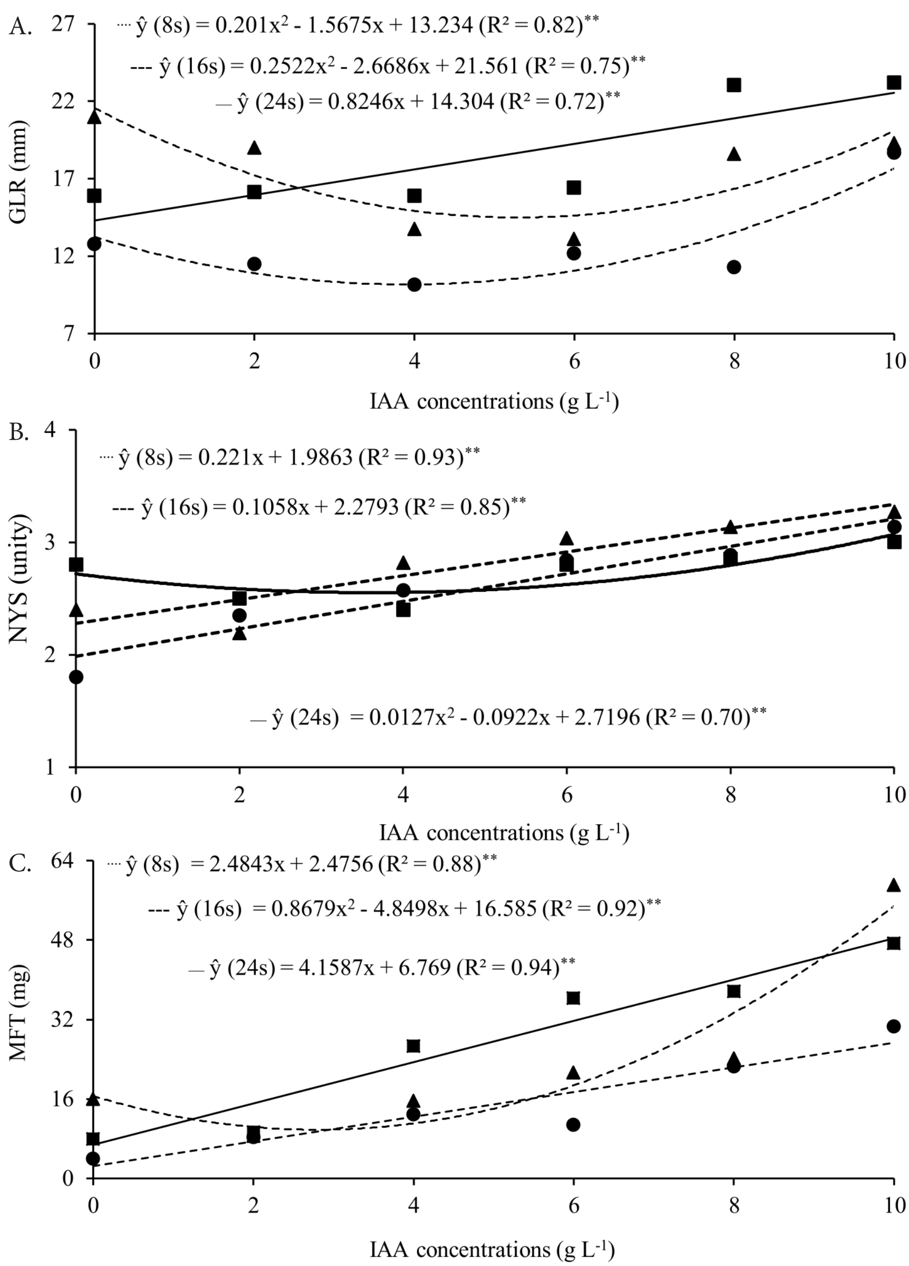

Figure 2. General length of roots (GLR - A), number of young shoots (NYS - B) total fresh mass (TFM - C) as a function of different concentrations of indole-3-acetic acid (IAA) on *Spondias purpurea*

is beneficial to the cuttings that the root system is as large as possible, since it improves the aggregation of the vegetal material to the substrate, increasing the stability and its sustentation and, thus, favoring its initial performance.

In Figure 2B, the IAA auxin employment linearly increased the average emission and the number of young shoots (NYS), in the red mombin cuttings, in 0.24 and 0.21 unit in every 2 g of increase in the concentrations, at the immersion times of 8 and 16 seconds, however, it is worth mentioning that the evaluation was carried out in a period of 90 days after the removal of the humidification protection, reducing the potential for the formation of new shoots, which were already developed.

The simple action of increasing the immersion time without the action of the regulator would already be feasible for NYS (Figure 2B), due to the values presented between the 0 and 10 g L^{-1} concentrations being close, which shows that the cuttings only immersed in water, but with a longer immersion period, may present methodological advantages and, also, in the production cost reduction of red mombin seedlings. Siddique et al. (2015) studied the synergistic effects of combinations of cytokinin and auxin on explants of mature plants and seedlings under different concentrations in the *Cassia angustifolia* Vahl species and detected an increase in the shoot number and length of shoot in the combination of 5.0 and 5.0 µM of BAP (6-benzyladenine) and IAA.

High concentrations of the IAA growth regulator under the different immersion times almost did not influence the NYS, which according to Pacheco & Franco (2008) this shoot production is extremely important for the maintenance and performance of the cuttings and, consequently, to increase the vegetal material survival since they are natural sources of carbohydrates, necessary for the plant development. Despite the small effect of generating new shoots, IAA immersion did not interfere negatively; however, this variable should not be analyzed in isolation because of its low contribution to seedling formation.

Hartmann et al. (2011) state that the presence of shoots and leaves on the cuttings are important in stimulating rooting, since IAA auxin is naturally produced in the aerial part and later transported to the base of the plants, but it is not the only co-factor of the rhizogenesis, since other substances produced in the leaves and physiologically active buds aid in the process of root formation, thus reaffirming the importance of shoots and leaves in the cuttings rooting.

Figure 2C shows a linear increasing effect on the TFM between the initial and final concentrations, in the immersion times of 8 and 24 seconds, which showed an increase of 90 and 86% in the propagules fresh mass, being that the IAA application in high concentrations can benefit the formation of shoots and roots. These results are confirmed by Taiz & Zeiger (2013), which explain that the vegetative regions of the cuttings produce and concentrate most auxin and this can induce the formation of leaves and roots in the plant material, thus raising the fresh mass values of cuttings, especially the foliar.

At the immersion time of 16 seconds, we observed that after the minimum point, which was approximately at the 3 g L^{-1} concentration, the red mombin cuttings showed an increase of TFM in 82% up to the maximum evaluated concentration, demonstrating that the different vegetative parts of the species were influenced by auxin (Figure 2C). In view of this, it was possible to associate the cuttings development with the fresh mass produced, since the capacity of the plant material to remain alive, through vegetative propagation, is mainly associated with the cutting type, composition of the tissues and also to the presence of “source” organs such as leaves and shoots (Sousa et al., 2014).

Scariot et al. (2017) observed that the cuttings of *Prunus persica* cv. ‘Chimarrita’ containing concentrations of 0.5; 1 and 1.5 g L^{-1} of the IAA growth regulator obtained an increase in dry mass of roots at 1.23; 1.42 and 1.52 g, differing statistically from the cuttings without the application of the regulator, confirming the importance of the application to promote the development of the fresh and dry mass of the plants, stimulating the faster development of the plants.

The results demonstrated that vegetative propagation by cuttings, linked with the application of IAA auxin, in red mombin, was a viable and satisfactory technique for most variables, except for GLR and NYS, with the increase of plant regulator concentrations. According to Hartmann et al. (2011), the concentrations, for rapid immersion of the cuttings base, vary between 0.5 to 10 g L^{-1}, being the highest concentrations, used in woody species and those of difficult rooting, as was the case of the vegetal material from the present study, which obtained better results of rooting, shoots and with leaves generation under the 10 g L^{-1} concentration. The increment obtained in these variables is of utmost importance for the survival and development of the plant, since these characteristics can confer to the species greater adaptability and satisfactory performance in the formation of a productive and quality adult plant.

The obtained results provide relevant contributions for the development of future research related to the *Spondias* genus; moreover, the employment of protection for the maintenance of humidity is shown as a viable and low cost technique for the vegetative propagation in commercial plantations.

Conclusion

The red mombin (*Spondias purpurea*) vegetative propagation by cutting, employing IAA at the 10 g L^{-1} concentration and the immersion time of 24 seconds, is satisfactory and effective for the rooting and induction of new shoots and leaves in the cuttings.

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