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Propagation of Umbuzeiro (*Spondias tuberosa* Arr. Cam.), a native plant to Brazilian semi-arid regions, using ethephon and indolebutyric acid (IBA)

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Abstract

Umbuzeiro (*Spondias tuberosa* Arr. Cam.) is a plant native to Brazilian semi-arid regions. Currently, there is no protocol for propagation for this species. In this sense, the aim of this study was to evaluate the propagation of Umbuzeiro by cuttings, using ethephon and indolebutyric acid (IBA). In this study, a completely randomized statistical design was used with the doses of ethephon (0, 14.5, 50, 85.5, 100 mg L^{-1}) and IBA (0, 872.35, 3000, 5127.65, 6000 mg L^{-1}) combined by the matrix "Central Compound Box" in 4 replicates. After application of ethephon and IBA on plants, cuttings with 20 cm length were prepared and planted in tubes with sand and organic compound (1:1 v/v) and placed in a greenhouse at 50% light under misting. The percentage of sprouted cuttings, length and diameter of the shoots, the percentage of rooted cuttings, live cuttings with and without calyx, dead cuttings, number of roots and length, dry matter of roots, leaf and total dry matter were evaluated. A combination of the doses of 100 mg L^{-1} of ethephon and 6000 mg L^{-1} of IBA increased the percentage of sprouted cuttings, number of shoots per cutting, number of roots per cuttings and promoted the rooting of 47.20% of the Umbuzeiro cuttings. The shoot formation and dry matter of Umbuzeiro cuttings were not influenced by the doses of ethephon. The IBA doses increased shoot formation and dry matter production of the umbuzeiro cuttings under greenhouse conditions.

Keywords: *Spondias tuberosa* Arruda Câmara, vegetative propagation, growth regulators.

Introduction

The Umbuzeiro (*Spondias tuberosa* Arr. Cam.) is a plant species belongs to the Anacardiaceae family. It is characterized as a xerophytic and deciduous plant. It is originally from the Brazilian semi-arid region and northern of the Minas Gerais state (Neves et al., 2007). In addition, this species is a source of income for the farmers in the semi-arid region of Brazil, though, the Umbuzeiro production is seasonal and the fruits are very perishable (Oliveira et al., 2012). In 2014 and 2015, the quantity of Umbuzeiro produced in Brazil was 7,466 and 8,904 t, respectively (Ibge, 2016).

Although it presents a great economic and environmental potential in agriculture and in the forest sector, there is no protocol for the production of Umbuzeiro to recommended (Cruz et al., 2016). Another problem in the commercial planting of this species is the delay in the time for production, when the plants are sexually propagated. Therefore, the vegetative propagation is an alternative to the natural production aiming to economic exploitation of the Umbuzeiro (Dutra et al., 2012). One of the advantages of the vegetative propagation is the multiplication of plants that are identical to the parent plant, reduction of juvenility, increase in uniformity and vigor of production. In addition, vegetative propagation may be a strategy for difficult rooting species (Tosta et al. 2012) like the Umbuzeiro. Currently, no acceptable rooting percentage of Umbuzeiro cuttings has been reported. This way, the formed seedlings do not present high quality for the formation of commercial plantations (Souza and Costa, 2010).

As an alternative to stimulate rooting of the cuttings, growth regulators have been used, such as indolebutyric acid (IBA), which efficiency has been proven in numerous studies (Rios et al., 2012; Tosta et al., 2012; Paula et al., 2007; Lima et al., 2002). Paula et al. (2007) found a low percentage of rooting on woody and herbaceous Umbuzeiro cuttings and observed a percentage of 33.3% of rooted herbaceous cuttings with the application of 500 mg L^{-1} of IBA immersed for 10 min. Rios et al. (2012) verified a low percentage of rooting (33.33%) in Umbuzeiro cuttings of 20 cm length, treated with IBA (6000 mg L^{-1}) planted in March.

Another alternative to increase the rooting of low percentage species is the use of ethylene. This plant hormone has a great importance in the rooting process, since it stimulates root formation and development. The ethylene synthesis occurs when auxin is applied and may increase the percentage of rooting of the cuttings (Fachinello et al., 2005). In addition, the interaction between auxin and ethylene may be synergistic or antagonistic in the rooting process (Muday et al., 2012).

Souza (2007) observed that the treatment of Cajazeira and Umbuzeiro with ethephon, 30 days before the preparation of the cuttings did not increase the percentage of rooting in these two species. Marco et al. (1998), found that the 47.22 mg L^{-1} dose of ethephon promoted the best result for percentage of rooted cuttings in guava, obtaining the mean value of 40.66% of rooted cuttings. In plum cuttings (*Prunus salicina* Lindl), Dutra et al. (1997) observed 90% of rooting in 'Frontier' cultivar applying 100 mg L^{-1} dose of ethephon.

The cultivation of the Umbuzeiro still faces several problems, mainly the formation of high quality seedlings. Thus, studies on vegetative propagation are needed as well as the obtaining of a protocol for rooting of the cuttings forthis species, aiming to creation of commercial orchards with desirable productive characteristics. Based on that, the aim of this study was to evaluate the propagation of Umbuzeiro (*Spondias tuberosa* Arr. Cam.) using cutting and application of ethephon and indolebutyric acid (IBA).

Results and Discussion

Shoots formation

The percentage of sprouted cuttings adjusted to a response surface (Figure 1A). The doses of ethephon presented a linear effect, whereas the IBA doses presented a quadratic effect. The highest percentage of sprouted cuttings was 60.35% with combined doses of 100 mg L^{-1} ethephon and 6000 mg L^{-1} of IBA. It was also observed that the minimum point of 7.83% of sprouted cuttings was obtained at the doses of 2554.30 mg L^{-1} of IBA and 72.42 mg L^{-1} of ethephon.

These results were satisfactory compared to those observed by Lima et al. (2002). They studied the vegetative propagation using cuttings of umbu-cajazeira (*Spondias* sp.), under conditions of intermittent misting, in a riparian chamber with 50% shading, in the municipality of Mossoró-RN, Brazil, and obtained a percentage of 30% of sprouted cuttings. The above mentioned behavior was also observed by Yamamoto et al. (2013) in "Xavante" mulberry, who observed that the sprouted cuttings percentage did not show any statistical difference with application of indolebutyric acid treatments.

The doses of ethephon presented a quadratic effect, whereas the doses of IBA presented a linear effect on the number of shoots per cutting. According to Figure 1B, the highest estimated number of shoots per cutting occurred in the combination of the doses of 100 mg L^{-1} of ethephon and 6000 mg L^{-1} of IBA with approximately 3.10 shoots per cutting. It was also observed that the doses of 3170.97 mg L^{-1} of IBA and of 43.28 mg L^{-1} ethephon provided the minimum point of sprouts per cutting of 2.43.

Different result was verified by Rios et al. (2012) in Umbuzeiro cuttings, where they observed no significant effect for the number of shoots per cutting as a function of IBA concentration. These authors emphasized that the best response was obtained when the cuttings were collected in the month of September and this possibly can be attributed to more favorable balance of phytohormones such as the cytokinins to bud formation. This period may be identified when the plant leaves are between the vegetative dormancy and beginning of the bud formation. In this period higher responses verified by the longer cuttings length (20 cm), due to the amount of reserves that they contained.

Tosta et al. (2012) observed an increase in the number of shoots of umbu-cajazeira (*Spondias* sp.) as the IBA concentration increased (on average, until up to 3500 mg L^{-1}). Above this concentration, there was a decrease with a maximum value of 2.9 shoots per cutting. Similarly, Gomes et al. (2005) studied the influence of the IBA on the number of shoots of umbu-cajazeira cuttings and found that the use of IBA promoted a significant increase in the number of shoots, evidencing that IBA positively influences the number of shoots of umbuzeiro. The same effects were also observed in this work.

The increase in the number of shoots can result in a decrease in the rooting, when the cuttings reserves are used for this purpose, in detriment of rooting. However, when there is enough time to produce and send auxins to the base of cuttings, they can favor the rooting process in a basipetal movement (from the apex downward to the base).

No significant effects of the ethephon x IBA interaction or for the application of ethephon were observed, presenting the mean values for shoot length (Figure 2A) and shoot diameter (Figure 2C), of 4.69 cm and 1.81 mm, respectively. Although it did not present a significant difference in length and diameter, the dose of 100 mg L^{-1} of ethephon was different from the other doses, presenting values of 1.5 cm and 0.75 mm, respectively.

The IBA doses influenced the length (Figure 2B) and shoot diameter (Figure 2D) of the Umbuzeiro cuttings. As the IBA dose was increased, an increase in the length and diameter of shoots occurred. For example, the maximum value of 13 cm and 2.62 mm was observed in the cuttings treated with 6000 mg L^{-1} of IBA. Also, minimum value of 3.08 cm in the cuttings treated with 3000 mg L^{-1} and 1.35 mm with the dose of 5127.65 mg L^{-1} were observed.

In umbu-cajazeira, Tosta et al. (2012) observed that the IBA doses increased the length of the shoots, whereas the increase of IBA concentration enhanced the mean of shoot length, with a maximum value of 5.6 cm at the estimated concentration of 3045 mg L^{-1} of IBA. Gomes et al. (2005) verified that there was a significant influence of IBA on the diameter of the umbu-cajazeira shoots.

A higher number of shoots may be an indicative of rooting of the cuttings, since the aerial part acts as a source of photoassimilates and, among them, soluble sugars and possibly other substances synergistic to rooting. Thus, the sprouting of shoots may be a source of photo-assimilates and/or substances that induce the rooting process and can be used for the formation of adventitious roots. However, the occurrence of sprouts before rooted cuttings can negatively affect the rooting since the reserves that would be used for it can be deviated to the sprouting process (Dias et al., 2011).

Survival of the cuttings without callus and dead cuttings

The ethephon x IBA interaction and the ethephon doses did not present a significant effect on the survival percentage of the cuttings with callus and dead cuttings, presenting the mean percentage of 53.47 (Figure 3B) and 46.52% (Figure 3D), which may have been influenced by the time not enough for the cuttings to start rooting. Although it did not present a significant difference, we was observed that the dose of 100 mg L^{-1} of ethephon differed from the other doses with the percentages of 20 and 77.08%, respectively, when the Dunnet test compared the survival percentage of cuttings with callus and dead cuttings.

For the IBA doses, there was a significant increase in the survival percentage of cuttings without callus with the application of IBA (Figure 3A), reaching the minimum point with the dose of 2930 mg L^{-1}, corresponding to the percentage of 36.90%. The cuttings of umbuzeiro presented a higher survival rate from cuttings callus (77.08%) using the lowest dose of IBA (0 mg L^{-1}).

The increase in the IBA doses promoted significant effects on the percentage of dead cuttings (Figure 3D), reaching a maximum of 63.09% at the dose of 2930 mg L^{-1}. Paula et al. (2007) found that herbaceous cuttings of umbuzeiro without the IBA treatment had the highest results for survival percentage (69.44%). The callus formation on the cuttings as well as their survival is a parameter that indicates the environmental conditions, in which the cuttings were maintained, resulting in an increase in the percentage of rooting, if the permanence of the cuttings in greenhouse occurred for a longer period (Oliveira and Ribeiro, 2013).

Rooting

For the percentage of rooted cuttings, we observed a significant effect for the ethephon x IBA interaction, with a linear effect for the ethephon doses and quadratic effect for the IBA doses (Figure 4A). The highest percentage of estimated rooted cuttings of 47.20% at the doses of 100 mg L^{-1} of ethephon and 6000 mg L^{-1} was reached. It was also observed that the minimum point of 7.88% of rooted cuttings was obtained at the doses of 2423.44 mg L^{-1} of IBA and 5.30 mg L^{-1} of ethephon.

Rios et al. (2012), observed that the highest percentage of rooting (30%) in Umbuzeiro occurred at the concentration of 6000 mg L^{-1} of IBA in cuttings collected in March and September, which may have been influenced by the semi-arid climate of Juazeiro-BA city. Paula et al. (2007) worked with woody and herbaceous cuttings of umbuzeiro and verified a percentage of 33.3% of rooted herbaceous cuttings with the application of IBA at the dose of 500 mg L^{-1}, immersed for 10 min. Tosta et al. (2005) showed that the increase of the IBA doses resulted in an increase in the percentage of rooted cuttings of umbu-cajazeira, where the maximum estimated concentration of 5400 mg L^{-1} promoted the highest rooting.

Dutra et al. (1998) in plum tree (*Prunus salicina* Lindl), observed that the application of 50 mg L^{-1} of ethephon provided the highest percentage of rooting of plum cv. Ace cuttings. They obtained 56.51% of rooted cuttings using this dose. Mori et al. (2011) found that the rooting of *Pinus thunbergii* cuttings was not significantly influenced by the treatment with ethephon (7.0%), compared to the control (2.0%). Marco et al. (1998) observed that the dose of 47.22 mg L^{-1} of ethephon promoted the best result for the percentage of rooted cuttings, obtaining a value of 40.66% of rooted cuttings.

One of the causes that may have influenced the percentage of rooted cuttings is the interaction of phytohormones, since, auxins play a central role in the regulation, growth and development of the plants. In addition, the auxin and ethylene hormones present a classic and complex interaction and may induce rooting (Rahman, 2013). However, there may be a positive or negative effect between these two hormones, which may act in an antagonistic or synergistic way in the regulation of various developmental processes, such as in the formation and apical and root stretching, besides the development of lateral roots and leaf abscission (Muday et al., 2012; Shkolnikinbar and Bar-zvi, 2010; Belin et al., 2009).

The doses of ethephon and IBA presented a quadratic behavior for the number of roots per cuttings. We verified that the highest number of roots was obtained without the treatment with ethephon, but with the application of 6000 mg L^{-1} of IBA, with an estimated maximum value of roots per cutting of 2.67. The minimum point of 0.84 roots per cuttings was obtained at the doses of 2596.51 mg L^{-1} of IBA and 54.74 mg L^{-1} of ethephon (Figure 4B).

Souza et al. (2007) in Umbuzeiro and Cajazeira cuttings, verified that the treatment of the parent plant with ethephon did not increase the rooting of Umbuzeiro and cajazeira cuttings. Dutra et al. (1998) observed that the application of 100 mg L^{-1} of ethephon in a plum tree (*Prunus salicina* Lindl) provided the highest number of roots per cuttings of the plum cv. Frontier, where they obtained 10.49 roots per cuttings in this dose. Marco et al. (1998) found that the dose of 46.8 mg L^{-1} of ethephon provided the best result for the number of roots per cuttings, obtaining a value of 4.06 roots per cuttings.

Rios et al. (2012), studied the cutting propagation of Umbuzeiro and observed that there was an increase in the number of roots due to the increase of IBA concentration, obtaining 1.25 roots per cutting at the maximum dose (6000 mg L^{-1}). This is inferior to what was found in this study. Tosta et al. (2012), verified that the number of roots per cutting had an increase in Umbu-cajazeira as the IBA doses were increased, obtaining the highest number of roots (3.8 units) in the maximum concentration (8545 mg L^{-1} of IBA) and that superior doses promoted a reduction in the number of roots.

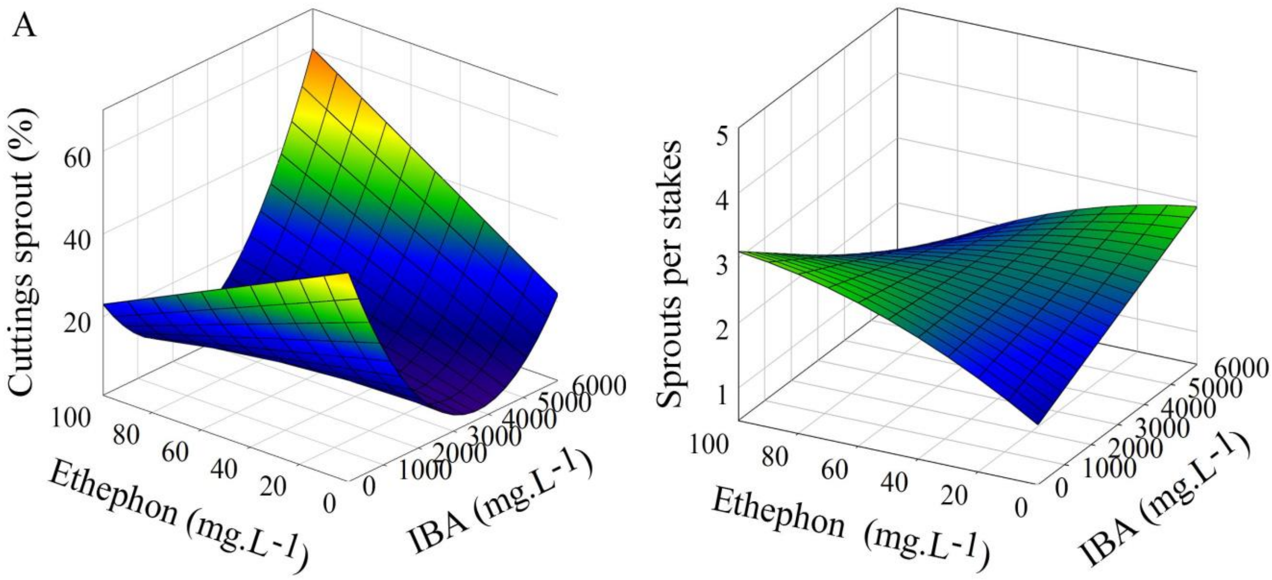
Dry matter

No significant effect of the ethephon x IBA interaction on root length and dry matter was observed. The root length (Figure 5A and B) presented no statistical difference when related to the ethephon and IBA doses, presenting mean values of 2.31 cm for root length. The dry matter of the roots also did not present statistical difference when subjected to the doses of ethephon and IBA (Figure 5C and D), showing a mean value of 0.12 g. Although, there was no statistical difference, the dose of 6000 mg L^{-1} of IBA differed from the other doses when the Dunnet test was performed for root length and root dry matter, presenting the values of 7.67 cm and 0.69 g, respectively. Although no significant effect was found, the values observed for root length are higher than those obtained by Tosta et al. (2012), when verifying that the root length of the cajaraneira cuttings increased until up to the dose of 1,295.2 mg L^{-1} of IBA, by which a value of 5.0 cm was observed, and the higher doses promoted a decrease for this variable.

Table 1. Levels and doses of Ethephon (ET) and indolebutyric acid (IBA) used in the Umbuzeiro experiment (*Spondias tuberosa* Arr. Cam.), according to Central Compound Box (Mateus et al., 2001), Areia – PB, Brazil.

Treatments/combinations									
	1	2	3	4	5	6	7	8	9
Ethephon Levels	-1	+1	-1	+1	+α	-α	0	0	0
IBA Levels	-1	-1	+1	+1	0	0	+α	-α	0
ET Doses ($mg.L^{-1}$)	14.5	85.5	14.5	85.5	100	0	50	50	50
IBA Doses ($mg.L^{-1}$)	872.35	872.35	5127.65	5127.65	3000	3000	6000	0	3000

Number of treatments = $2^k + 2K + 1$, Where K = number of factors, NT = $2^2 + 2.2 + 1 = 9$; $\alpha = \sqrt{2}$.



A=51.528-0.,026^{ns}IBA-0.285^{ns}ET + 0.0000035**IBA² + 0.00011*IBAxET R² = 0.76

A=1.421+0.000250^{ns}IBA+0.028^{ns}ET-0.000115** ET² - 0.00000579**IBAxET R² = 0.88

Fig 1. Percentage of sprouted cuttings (A) and number of shoot per cuttings (B) of Umbuzeiro under the effect of ethephon doses as function of the application of indolebutyric acid in the cuttings.

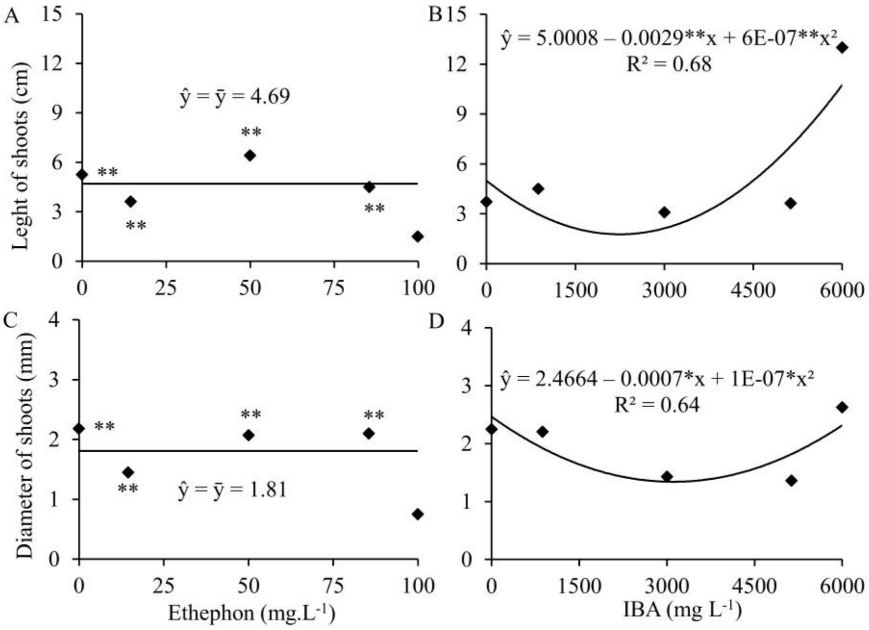


Fig 2. Length (A and B) and diameter of shoots (C and D) of Umbuzeiro cuttings under the effect of ethephon doses as function of the application of indolebutyric acid in the cuttings. ** Significant difference in the dose of 6000 mg L^{-1} of IBA according to the the Dunnett test at 5% of probability.

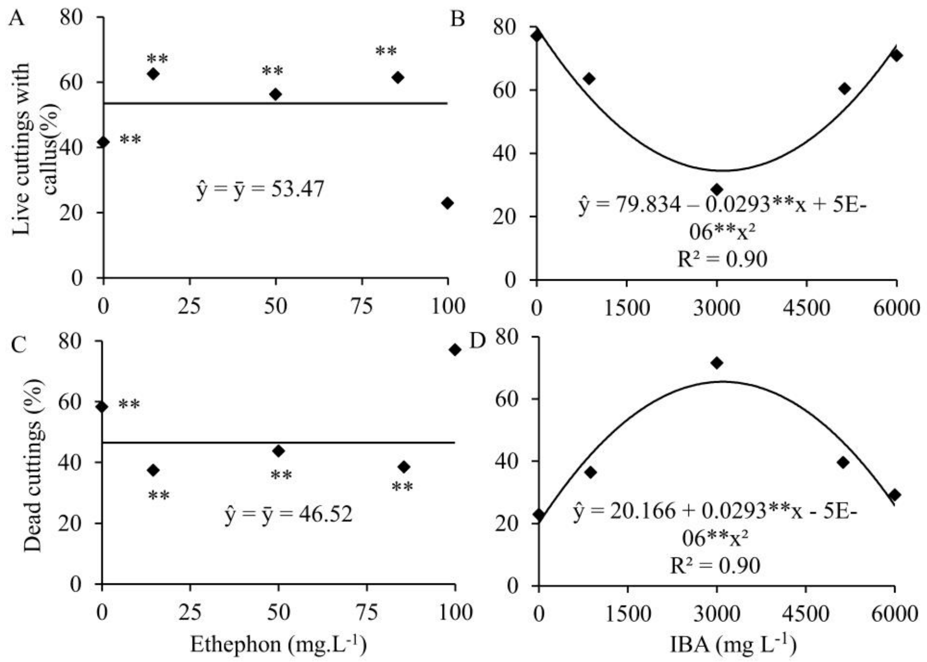


Fig 3. Survival percentage of the cuttings without callus (A and B) and dead cuttings (C and D) of Umbuzeiro cuttings under the effect of ethephon doses as function of the indolebutyric acid application in the cuttings. ** Significant difference in the dose of 6000 mg L^{-1} of IBA according to the Dunnett test at 5% of probability.

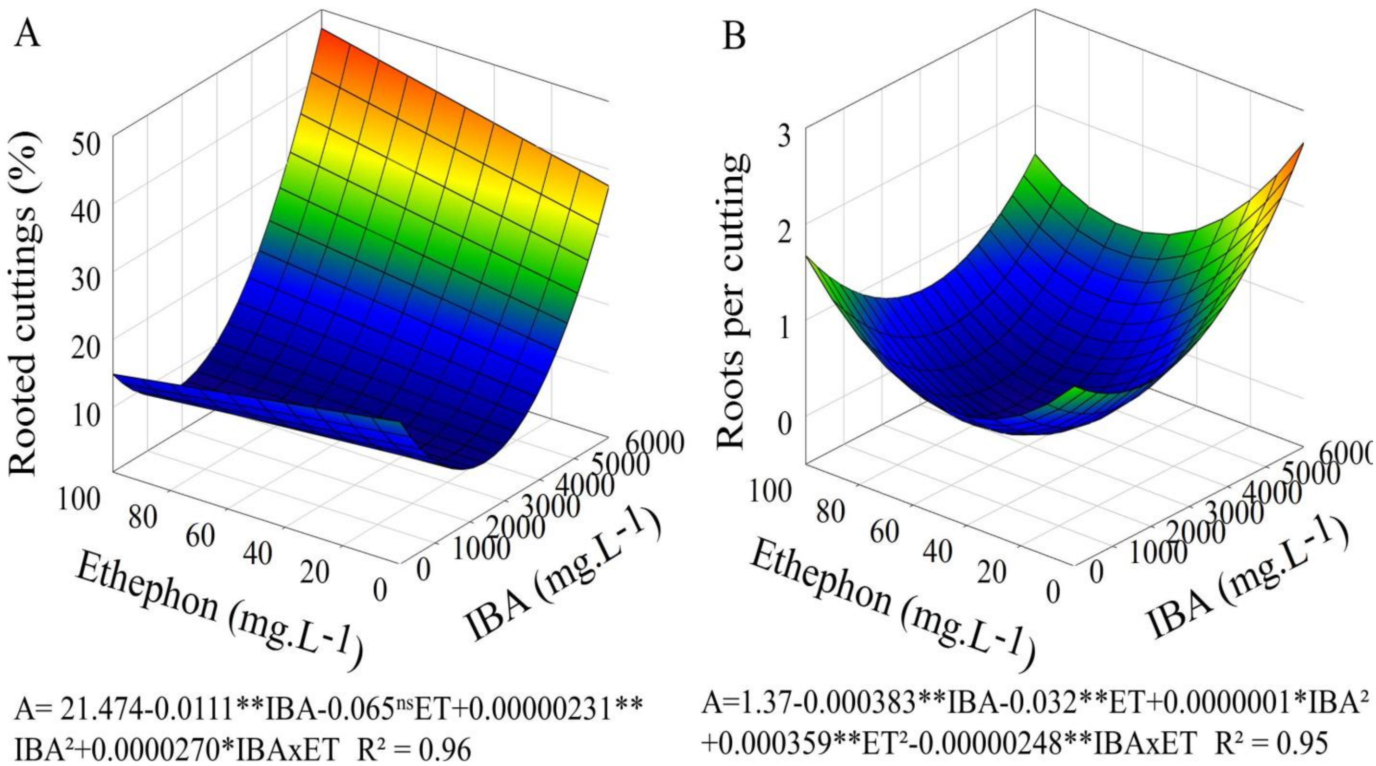


Fig 4. Percentage of rooted cuttings (A) and number of roots per cutting (B) of Umbuzeiro under the effect of ethephon doses as function of the application of indolebutyric acid in the cuttings.

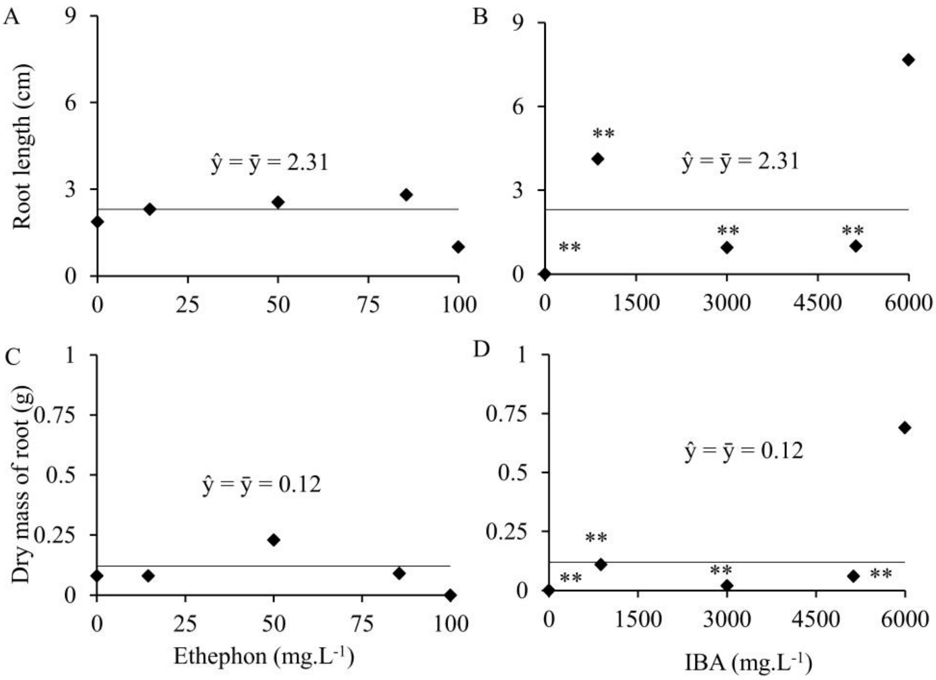


Fig 5. Root length (A and B) and root dry matter (C and D) of Umbuzeiro cuttings under the effect of ethephon doses as function of the application of indolebutyric acid in the cuttings. **Significant difference in the 6000 mg L^{-1} dose of IBA according to the Dunnett test at 5% of probability.

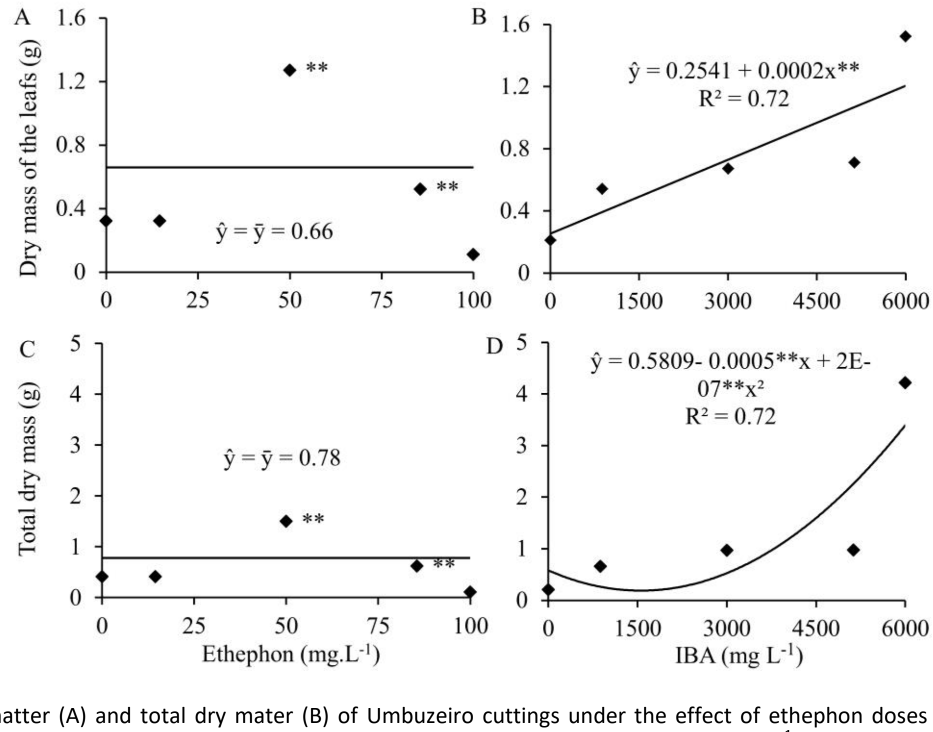


Fig 6. Leaf dry matter (A) and total dry mater (B) of Umbuzeiro cuttings under the effect of ethephon doses as function of the indolebutyric acid application in the cuttings. ** Significant difference in the dose of 6000 mg L^{-1} of IBA according to the Dunnett test at 5% of probability.

Paula et al. (2007) also did not observe significant statistical difference in dry matter of roots of Umbuzeiro cuttings when submitted to the doses of IBA. The same result was observed by Yamamoto et al. (2013) in plum tree (*Prunus salicina* Lindl), where they found that root length and root dry matter of Xavante mulberry cuttings did not present differences in the treatments with indolebutyric acid.

The length and dry matter of the root, also did not present a significant effect for the ethephon x IBA interaction nor for the ethephon doses for leaf dry matter and total dry matter, presenting values of 0.66g and 0.78g, respectively. Although it did not present a significant difference, we observed that the dose of 100 mg L^{-1} of ethephon differed from the doses of 50 and 85.5 mg L^{-1} of ethephon when applying the Dunnet test for leaf dry matter and total dry matter, presenting the values of 0.31 and 0.11 g, respectively (Figure 6A and 6B).

The IBA doses had a significant effect on the leaf dry matter (Figure 6B) of Umbuzeiro cuttings. As the IBA doses increased, there was an increase in leaf dry matter reaching the estimated maximum value of 1.5 g in the cuttings treated with the dose of 6000 mg L^{-1} of IBA. The application of IBA in the Umbuzeiro cuttings influenced the total dry matter, where an increase with a quadratic adjustment was observed, by which under dose of 1,250 mg L^{-1} the minimum point of 0.26 g promoted (Figure 6D).

Materials and Methods

Localization

The experiment was carried out from September of 2015 to January of 2016 in the fruticulture nursery of the Universidade Federal da Paraíba, in the city of Areia-PB, Brazil, located in the geographical coordinates 6º51'47 " and 7º02'04" South latitude and 35º34 ' 13 "and 35 ° 48'28" west longitude of the Greenwich meridian.

Vegetal material

The woody branches of Umbuzeiro trees (*Spondias tuberosa* Arr. Cam.) were selected from the municipality of Pocinhos-PB, Brazil. The branches for the cuttings preparation were selected from Umbuzeiro plants with 40 to 60 years old. The experimental plot consisted of 2 cuttings per treatment.

Experimental design

The experimental design was a completely randomized with the ethephon doses of (0, 14.5, 50, 85.5 and 100 mg L^{-1}) and the IBA doses of (0, 872.35, 3000, 5127.65 and 6000 mg L^{-1}) combined by the Central Compound Box (Mateus et al., 2001), with 4 replicates. The treatments consisted of the doses and the combinations adopted by Souza et al. (2007), which are specified in the Table 1.

Ethephon application

The treatment with ethephon was prepared with the commercial product Ethrel®, and sprayed on the Umbuzeiro plants before the removal of the branches. The doses of ethephon treatments were diluted according to the commercial indication of the product with 240 g of ethephon L^{-1}, from where the specific dose for each treatment was prepared. The ethephon was sprayed with an approximate volume of 4 L/plant.

Cuttings preparation

Seven days after the application of the ethephon, branches with 3 to 4 mm in diameter and approximately 30 cm length were collected and placed in moist paper, stored in plastic bags and transported to the fruticulture nursery of the Universidade Federal da Paraíba, municipality of Areia-PB, Brazil. They were then taken to the misting chamber, where the cuttings were prepared, with the top cut straight and the basal cut bevelled, with 20 cm length.

Application of the indolebutyric acid (IBA) and planting of the cuttings

The IBA doses were prepared from the dissolution of IBA in a hydroalcoholic solution (distilled water and alcohol 96°). After the cuttings preparation, they were grouped by the replicates and had 1.0 cm of the base inserted in the hormonal solution for 5 seconds, for each treatment.

Subsequently, they were planted in tubes with internal diameter of 26 mm and external diameter of 33 mm and volume of 55 cm³, filled with substrate of sand and organic compound, in the proportion 1:1 (v/v); being placed under shading of 50% light and in a greenhouse under 10 seconds misting and with intervals of 15 minutes.

Evaluated variables

Ninety days after the installation of the experiment the following variables were evaluated: the percentage of sprouted cuttings, the length of shoots, the diameter of the shoots, the percentage of rooted cuttings, the survival percentage of cuttings with and without callus, the percentage of dead cuttings, the number of root per cutting, root dry matter, leaf dry matter and total dry matter.

The percentage of sprouted cuttings was obtained by counting and transformed to percentage (%); the length of shoots in cm was obtained by the measurements made with a graduated ruler; the diameter of the shoots, in mm was obtained with the use of the pachymeter; the percentage of rooted cuttings was obtained from the manual count and then transformed into percentage (%), for this the tube was carefully removed, and the cutting was rinsed in order to obtain the intact root system, considering that rooted cuttings were those that had at least one root ; the survival percentage of cuttings with callus and without callus was obtained by counting and transformed to percentage (%); the percentage of dead cuttings was obtained by counting and transformed to percentage (%); the number of roots per cutting was obtained by counting; root length was obtained by measuring the length of the roots with a graduated ruler in centimeters; the dry matter of the leaf, root and total dry matter were obtained by washing the material that were then placed into the paper bags and taken to an stove at 65 ºC until the constant weight and then weighed on a precision scale to obtain the dry matter in grams.

Statistical analysis

The results were subjected to the analysis of variance by the F test ($p < 0.05$). For the variables with effect of the significant interaction they were adjusted to the response surface, and if the interaction was not significant a polynomial regression analysis was performed. The Dunnett test was applied at 5% of significance to compare the dose of 100 mg L^{-1} of ethephon with the other treatments and to compare the dose of 6000 mg L^{-1} of IBA with the other treatments. The analysis were performed using the statistical software SAS® (Cody, 2015).

Conclusion

The combination between the doses of 100 mg L^{-1} of ethephon and 6000 mg L^{-1} of IBA increased the percentage of sprouted cuttings, number of shoots, number of roots per cuttings and promoted the rooting of 47.20% of the umbuzeiro cuttings. The production of shoots and dry matter of the umbuzeiro cuttings was not influenced by ethephon treatment. The IBA doses increased shoot formation and dry matter production of the Umbuzeiro cuttings.

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