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Multiplication, morphogenesis and foliar abscision of *Schinopsis brasiliensis* Engl. *in vitro*

[Multiplicación, morfogénesis y abscisión foliar de *Schinopsis brasiliensis* Engl. *in vitro*]

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Abstract: The objective was to evaluate plant growth regulators and ethylene inhibitors on the development and leaf abscission of *Schinopsis brasiliensis* Engl. Zeatin (ZEA) was evaluated in concentrations combined with concentrations of indolacetic acid (IAA), naphthalene acetic acid (NAA) and indolbutyric acid (IBA). ZEA and 6-benzylamino purine (BAP) were evaluated in concentrations plus a control. Ethylene inhibitors, silver nitrate and cobalt chloride were evaluated in four concentrations. The addition of 0.2 µL-1 of NAA to 0.4 µL-1 of ZEA promotes a greater number of baraúna sprouts. At concentrations of 5 and 10 µM, cobalt chloride is more efficient than silver nitrate for reducing leaf abscission in baraúna. IAA is the most suitable auxin to be associated with ZEA for higher shoot length and number of buds. Silver nitrate from a concentration of 20 µM completely avoids leaf abscission while cobalt chloride has a maximum reduction in abscission at a concentration of 40 µM.

Keywords: Auxins; Cytokinins; Cobalt chloride; Ethylene; Silver nitrate.

Resumen: El objetivo fue evaluar reguladores de crecimiento e inhibidores de etileno sobre el desarrollo y abscisión foliar en *Schinopsis brasiliensis* Engl. La zeatina (ZEA) se evaluó en concentraciones combinadas con concentraciones de ácido indolacético (IAA), ácido naftaleno acético (NAA) y ácido indolbutírico (IBA). Se evaluaron ZEA y 6-bencilamino purina (BAP) en concentraciones más un control. Se evaluaron inhibidores de etileno, nitrato de plata y cloruro de cobalto, en cuatro concentraciones. La adición de 0.2 µL-1 de NAA a 0.4 µL-1 de ZEA promueve un mayor número de brotes de baraúna. A concentraciones de 5 y 10 µM, el cloruro de cobalto es más eficaz que el nitrato de plata para reducir la abscisión de las hojas en baraúna. IAA es la auxina más adecuada para asociar con ZEA para una mayor longitud de brotes y número de brotes. El nitrato de plata a partir de una concentración de 20 µM evita completamente la abscisión de las hojas, mientras que el cloruro de cobalto tiene una reducción máxima en la abscisión a una concentración de 40 µM.

Palabras clave: Auxinas; Citoquininas; Cloruro de cobalto; Etileno; Nitrato de plata.

INTRODUCTION

Schinopsis brasiliensis Engl., popularly known as baraúna, is a species native to Brazil (Medeiros *et al*., 2018) belonging to the Anacardiaceae family (Carvalho, 2009). It is widely used in traditional medicine (leaves, bark, stem, resin and fruits) for the most diverse purposes. Among the uses, its anticoagulant action (Gomes & Bandeira, 2012), relief of gastric disorders (Ribeiro *et al*., 2014) and slower progression of some cancerous diseases such as colorectal cancer can be highlighted (Santos *et al*., 2017; Luz *et al*., 2018).

Currently, baraúna is considered a noble tree and, due to the almost depletion of its reserves, the collection, transportation, storage, handling, processing and commercialization of this species are prohibited (Brasil, 2014; Brito *et al*., 2018). In addition to the gradual loss of genetic variability, the establishment of mechanisms for the preservation and multiplication of native species is a strategy for maintaining species of remarkable economic and environmental potential (Santos *et al*., 2017) such as baraúna.

Among the different forms of multiplication and conservation of plant species, the use of artificial cultivation in an *in vitro* environment has been widely studied, especially in situations of difficulty in propagation by conventional routes or due to risk of species extinction. Plant tissue culture techniques can be used for germplasm conservation and large-scale multiplication of cultures with agronomic characteristics, both for productivity and quality, with emphasis on secondary metabolites of high biological value (Shahzad *et al*., 2017). Micropropagation enables rapid and asexual propagation in a controlled environment (Al-Khayri & Naik, 2017). This is possible due to the expressive plasticity of the plants and the high and continuous adaptation to the environment in which it grows, in response to exogenous and/or endogenous signals (Fehér, 2015).

In the multiplication phase, the assumption is that the success of *in vitro* cultivation depends on factors associated with the induction of morphogenesis and organogenesis (regeneration of shoots and roots) and it is necessary that the nutrient medium provides substances essential to the growth of plant *in vitro* (Morais *et al*., 2014).

The molecular mechanisms that govern the programming and reprogramming of plant cells and cells with totipotence (Ikeuchi *et al*., 2016) and direct the plant development are activated through plant hormone signaling. Endogenous hormone levels regulate explant differentiation processes, being the sensitivity to the hormone concentrations variable between species (Kumari *et al*., 2018).

The low proportion of auxin/cytokinin tends to induce the regeneration of the shoot, while a high proportion of these hormones promotes root regeneration. Furthermore, the high auxin concentration can promote somatic embryogenesis from explants (Horstman *et al*., 2017; Iwase *et al*., 2018).

The development of plants in a closed environment promotes the formation and accumulation of the hormone ethylene, with concentration dependent on the intensity of light, biosynthesis of pigments from explants, CO_2 concentration, humidity, air temperature, conditions of the culture medium, etc. Ethylene in high concentrations induces leaf abscission and affects the growth, differentiation and aging of *in vitro*-grown plants (Ha *et al*., 2020).

It is essential that the concentrations of plant growth regulators are in balance, in the proper proportion between auxin and cytokinin to optimize *in vitro* propagation (Emara *et al*., 2017; Prudente *et al*., 2019).

The *in vitro* cultivation of native species presents great challenges, mainly related to the adaptation of the culture media, physical factors and growth regulators (Prudente *et al*., 2016). In the literature, there is a scarcity of studies carried out with baraúna evaluating *in vitro* multiplication and the effect of exogenous hormones, i.e., plant regulators added to the culture medium. Considering the importance of the species, the relevant economic potential and the high risk of extinction, the objective was to induce the *in vitro* multiplication of baraúna and evaluate the effect of plant regulators on the growth and development of explants.

MATERIALS AND METHODS

Three experiments were conducted at the Biotechnology Laboratory of Embrapa Semiárido, Petrolina - PE, Brazil, with seeds from the Seed Laboratory of the same institution. To obtain nodal segments, the seeds were placed in polystyrene trays with 128 cells filled with commercial substrate, using one seed per cell. The trays were kept in a greenhouse where they received water daily through watering cans for 120 days. The plants received an *in vivo* pretreatment with three consecutive sprays with systemic fungicide (thiophanate-methyl) and bactericide (gentamicin). The day after the last

spraying, the young and non-lignified plants were sectioned to extract nodal segments approximately 2 cm long without leaves that were used as explants for the *in vitro* experiment.

The explants were taken to the laboratory, washed under running water and subjected to the asepsis process in a laminar flow chamber. The disinfestation occurred by immersing the explants in a 2% sodium hypochlorite solution in agitation for 10 minutes, followed by three washes with distilled and autoclaved water and in the last wash the water was added with 1 g L^{-1} of PVP (polivilpyrrolidone).

The containers used to place the explants were 200 ml-transparent glass vials containing 30 ml of WPM culture medium (Lloyd & McCown, 1980) added to the treatments. The medium was added with 30 g L^{-1} of sucrose, 8 g L^{-1} of agar and 2 g L^{-1} of activated carbon. The pH was measured to 5.7 before autoclaving. After inoculation of the explants in the referred treatments, the flasks were kept in a growth room with an average temperature of 25 ± 2°C and a photoperiod of 16 hours, with a luminous intensity of 25 µmol m^{-2} s^{-1} (measured with a lux meter) provided by cold white fluorescent lamps.

The first experiment was carried out to evaluate the effect of four concentrations of zeatin (0.2; 0.4; 0.8 and 1.0 µM) associated with auxins IAA, NAA and IBA, each of them in the concentrations of: 0.1; 0, 2; 0.4 and 0.5 µM and a control (without the addition of regulators). The experimental design used was completely randomized with 13 treatments and 5 replicates. Each experimental plot consisted of a flask with four explants each. The evaluations were carried out 30 days after the installation of the experiment and the variables analyzed were the number of shoots and the number of buds, by means of visual counting and the shoot length using a millimeter ruler.

In the second experiment, five concentrations (0.1, 0.2, 0.4, 0.8 and 1.0 µM) of zeatin and five concentrations (0.1, 0.2, 0.4, 0.8 and 1.0 µM) of BAP+ a control (without the addition of regulators) were evaluated. The experimental design used was the completely randomized in factorial 5 x 2 + 1 with five doses, two regulators and an additional control treatment totaling eleven treatments with five repetitions. Each experimental plot consisted of a flask with four explants each. After 30 days, the number of shoots and the number of buds were evaluated.

The third experiment was carried out using two abscission inhibitors (silver nitrate and cobalt chloride) in four concentrations (5, 10, 20 and 40 µM), forming a 4 x 2 factorial scheme, in a completely randomized design with three replicates. The evaluations were performed after 60 days, recording the length of the shoots measured with a caliper, the number of buds and the senescence of explants by means of visual counting.

The data were tested for the assumptions of normality of residues and homogeneity of variances, by the Shapiro-Wilk and Levene tests, respectively, at 1% probability, using the statistical software SPSS (IBM, 2013). After the assumption tests, the variables were subjected to analysis of variance using the SISVAR program (Ferreira, 2014). In the first experiment, the means were compared using the Scott-Knott test at 5% probability. In the second experiment, the qualitative means were compared using the Tukey test at 5% probability and quantitative using polynomial regression curves. For comparison between treatments and the control (additional treatment), Dunnett's test was performed using the statistical software Assistat (Silva & Azevedo, 2016). In the third experiment, qualitative means were compared by Tukey's test at 5% probability and quantitative by means of polynomial regression.

RESULTS

Auxines added to zeatin in different concentrations

The highest shoot lengths were observed when IAA was added, regardless of concentration, as well as when NAA was added in the lowest concentration and IBA in the highest concentration. All of these cases differed significantly from the control (Table No. 1). As for the number of buds, higher values were observed in the treatments that received IAA added to the ZEA. The association of NAA with ZEA at doses 0.2 and 0.4 μL^{-1} and 0.4 and 0.8 μL^{-1}, respectively and IBA 0.1 μL^{-1} with ZEA 0.2 μL^{-1} resulted in a number of buds statistically equal to the control treatment (Table No. 1).

Cytokinins at different concentrations in in vitro multiplication of baraúna

Number of shoots was not influenced by the interaction between cytokinins and the tested concentrations, by isolated factors or by the absence of plant regulator (Table No. 2).

Table No. 1
Shoot length, number of shoots and number of buds of baraúna regarding the different concentrations of auxins added to zeatin

Treatment	Auxin concentration (μL^{-1})	Zeatin concentration (μL^{-1})	Shoot length (cm)	Number of shoots	Number of buds
Control	0.0	0.0	0.39 b	1.0 b	2.95 b
IAA	0.1	0.2	0.65 a	1.0 b	4.05 a
	0.2	0.4	0.62 a	1.0 b	3.85 a
	0.4	0.8	0.58 a	1.0 b	3.30 a
	0.5	1.0	0.58 a	1.0 b	3.60 a
NAA	0.1	0.2	0.58 a	1.0 b	3.62 a
	0.2	0.4	0.36 b	2.0 a	2.90 b
	0.4	0.8	0.44 b	2.0 a	2.65 b
	0.5	1.0	0.41 b	2.0 a	3.50 a
IBA	0.1	0.2	0.36 b	1.0 b	2.90 b
	0.2	0.4	0.37 b	1.0 b	3.35 a
	0.4	0.8	0.45 b	1.0 b	3.45 a
	0.5	1.0	0.56 a	1.0 b	3.90 a
W: F_{Lev}			**0.979;1.158**	0.659;0.0	**0.980;1.772**
CV (%)			26.91	0.0	16.13

Means within each column followed by a different letter differ from one other according to Scott-Knott test ($p<0.05$). W and Flev.: statistics from Shapiro-Wilk and Levene's test (values in boldface indicate residuals with normal distribution and homogeneous variances at 1% probability, respectively). CV (%): coefficient of variation

Table Nº2
Shoot length, number of shoots, number of buds and leaf abscission of baraúna (*Schinopsis brasiliensis*) regarding the different cytokinins and their concentrations added to the medium

Concentration (μL^{-1})	Shoot length (cm)		Number of shoots		Number of buds		Leaf abscission (%)	
	ZEA	BAP	ZEA	BAP	ZEA	BAP	ZEA	BAP
0.0	0.395*		1.00		3.65		5.00*	
0.1	0.941* a	0.370 b	1.00	1.00	4.50	2.80	35.00*b	0.00 a
0.2	0.537 a	0.360 a	0.95	1.00	3.97	2.70	10.00 a	0.00 a
0.4	0.535 a	0.450 a	0.95	1.00	3.62	3.00	15.00 a	0.00 a
0.8	0.395 a	0.450 a	0.95	1.00	2.90	3.65	15.00 a	25.00 a
1.0	0.410 a	0.395 a	0.95	1.00	3.85	2.90	50.00*b	25.00 a
Mean	0.563	0.405	0.96	1.00	3.77 a	3.00 b	25.00	10.00
W: F_{Lev}	**0.25**	**1.18**	**3.28**	4.70	**3.70**	3.00	**0.88**	**2.70**
CV (%)	30.62		19.27		29.86		82.4	

Means within each row followed by a different letter differ at 5 % probability according to Tukey's test. *: different at 5% probability Dunnett's test. W and Flev.: statistics from Shapiro–Wilk and Levene's tests (values in boldface indicate residuals with normal distribution and homogeneous variances at 1 % probability, respectively). CV (%): coefficient of variation

Shoot length was favored by the use of ZEA at 0.1 μL^{-1} concentration compared to not using cytokinins and BAP at the same concentration. The evaluation of the number of buds did not demonstrate an effect of the interaction between plant regulators and concentrations, however when the effects were evaluated separately, ZEA provided a greater quantity of buds than BAP (Table No. 2).

The results regarding leaf abscission proved a significant effect of the interaction between type of cytokinins and concentrations (Table No. 2). The addition of ZEA promoted a higher rate of leaf abscission in concentrations 0.1 and 1.0 μL^{-1}, compared to BAP in the same concentrations and also the non-application of plant regulators.

In the presence of ZEA, the shoot length showed a quadratic behavior with reduced growth in response to the increase in the concentration of the plant regulator, reaching a minimum point in the concentration 0.74 μL^{-1} (Figure No. 1).

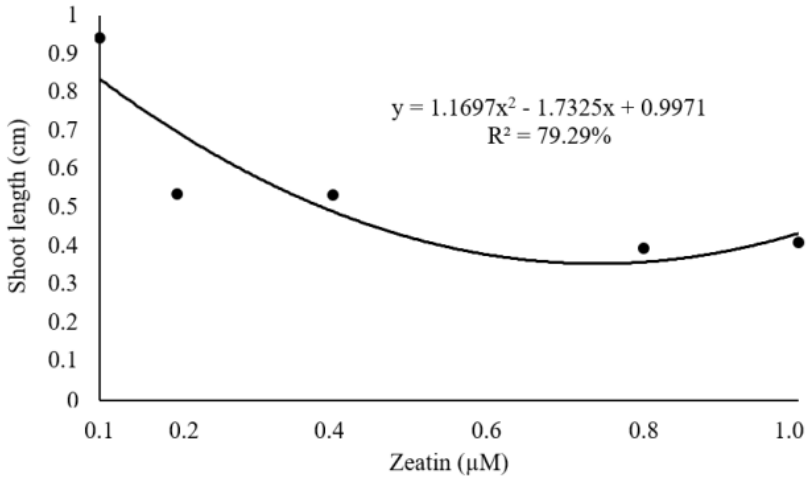

Figure Nº1
Shoot length (cm) of baraúna as a function of different concentrations of zeatin

The behavior of leaf abscission as a function of the concentration of ZEA obtained by regression showed a quadratic adjustment with a lower abscission in the concentration of 0.49 μL^{-1} for an expected abscission of 5%, a value equivalent to not using cytokinins. From of this concentration the abscission increased up to 50% (Figure No. 2).

The use of BAP showed a linear behavior in response to the increase in the dose, as shown in Figure No. 3.

Silver nitrate and cobalt chloride in the morphogenesis and leaf abscission of baraúna

The shoot length and number of buds were not influenced by the interaction inhibitor x concentration or by isolated factors (Table No. 3). Through the data referring to leaf abscission, we observed that there was a significant interaction between the concentration and ethylene inhibitor factors. At concentrations of 5 and 10 µM, cobalt chloride was more effective in reducing leaf abscission, while increasing the concentration of inhibitors to 20 µM favored silver nitrate. When using a concentration of 40 µM, both silver nitrate as chloride cobalt were efficient to prevent leaf abscission of baraúna plants *in vitro*.

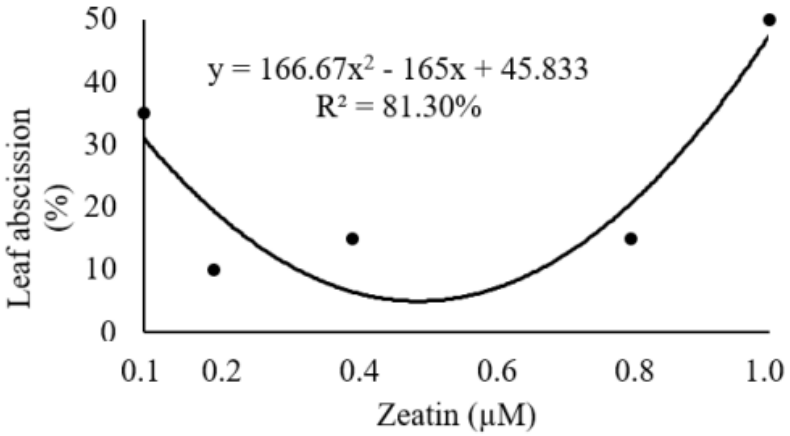

Figure No. 2
Leaf abscission (%) of baraúna as a function of different concentrations of zeatin

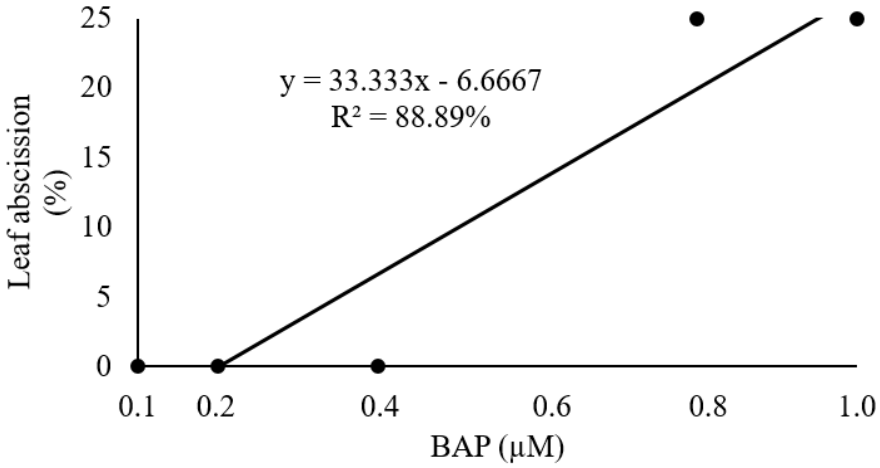

Figure Nº3
Leaf abscission (%) of baraúna as a function of different concentrations of BAP

Table No. 3
Shoot length (cm), number of buds and leaf abscission of baraúna seedlings treated with ethylene inhibitors – silver nitrate (SN) and cobalt chloride (CC)

Concentration (µM)	Shoot length		Number of buds		Leaf abscission (%)	
	SN	CC	SN	CC	SN	CC
5	0.500 a	0.608 a	3.417 a	4.194 a	25.00 b	8.33 a
10	0.392 a	0.383 a	2.583 a	3.167 a	10.71 b	8.33 a
20	0.485 a	0.617 a	3.617 a	3.583 a	0.00 a	8.33 b
40	0.517 a	0.383 a	4.000 a	3.167 a	0.00 a	0.00 a
Mean	0.473	0.498	3.404	3.528	8.929	6.250
W: F_{Lev}	**0.978;3.410**		**0.968;1.343**		0.454;16.00	
CV (%)	39.73		30.66		11.14	

Means within each row followed by a different letter differ from each other at 5% probability according to Tukey's test. W and Flev.: statistics from Shapiro–Wilk and Levene's tests (values in boldface indicate residuals with normal distribution and homogeneous variances at 1% probability, respectively). CV (%): coefficient of variation

Baraúna plants responded in a quadratic manner to silver nitrate concentrations with minimal leaf abscission calculated through the regression equation with a concentration of 29.31 µM (Figure No. 4).

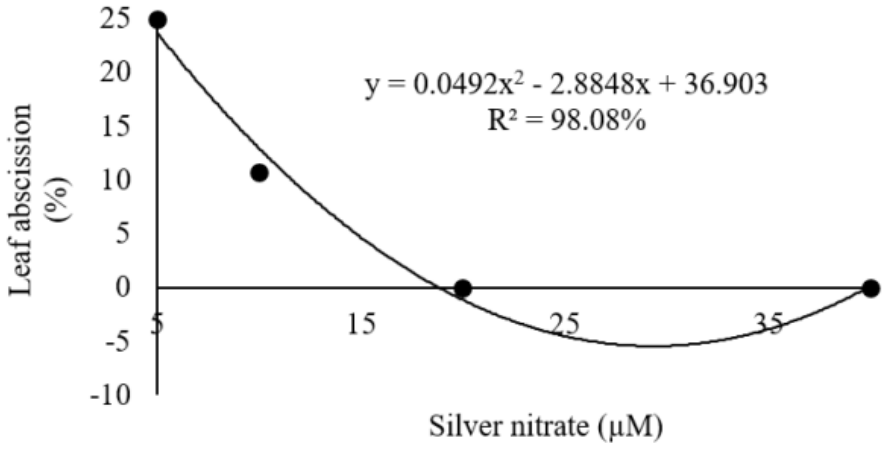

Figure Nº4
Leaf abscission (%) of baraúna as a function of different concentrations of silver nitrate

For cobalt chloride, the reduction in abscission occurred in a linear manner in response to an increase in concentrations, reaching an absence of abscission at the concentration of 40 µM (Figure No. 5).

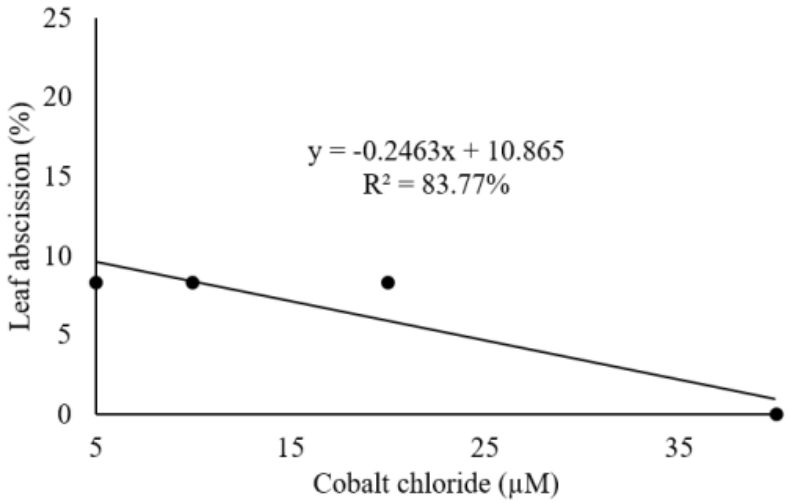

Figure No. 5
Leaf abscission (%) of baraúna as a function of different concentrations of cobalt chloride

DISCUSSION

In the first experiment, the use of NAA and ZEA from concentrations of 0.2 and 0.4 μL^{-1}, respectively, resulted in a greater number of shoots. Several factors can influence the process of organogenic induction, such as concentration, types of plant regulators, age of explant, genotype, physical conditions of cultivation, composition and consistency of the culture medium (Dobránszki & Silva, 2010). Kwon *et al.* (2017), observed that the number of shoots and the shoot length decreased notably with the increase in NAA concentrations in *Platycodon grandiflorum* explants. According to these authors, it is assumed that the difference in the number of shoots is an effect of auxins in the mix of regulators.

The literature presents variations in terms of concentration and combination of hormones according to species. Other species of the Anacardiaceae family such as *Pistacia lentiscus* and *Pistacia vera* performed better with IBA (Tilkat & Onay, 2009; Yıldırım, 2012). Metivier *et al.* (2007), also observed that IBA was the most effective auxin, followed by IAA and lastly NAA to *Cotinus coggygria* rooting.

In the other hand, auxins and cytokinins are synergistically necessary to induce cell division and growth in plant tissue cultures (Hegde *et al*., 2017), as can be seen in Table No. 1. De Klerk *et al.* (1997) pointed out that each type of auxin has a different absorption speed, which determines varying concentrations of these substances inside cells for the same concentration in the culture medium, causing different responses on explants, as can be seen in Table No. 1 between IAA, NAA and IBA. The IBA can use independent transport systems and move to a specific destination location as an inactive precursor, avoiding auxin responses during transport and maintaining IAA levels where necessary. The effectiveness of rooting in response to different auxins is also affected by the amount of endogenous auxins, the amount of free auxin exposed to cells and the affinity of the auxin receptors involved in the rooting response (Park *et al*., 2017).

De Souza *et al*. (2014), in a work testing regulators for *in vitro* establishment of *S. brasiliensis* observed that cytokinin was more effective than auxin in the development of shoots and buds, which collaborates with the present study. Costa *et al.* (2015), concluded that there was no effect of treatments on the number of shoots or an increase in the number of buds of *S. brasiliensis* treated with BAP and ZEA.

In short, Grokinsky and Petrasek (2019), state that auxins and cytokinins are considered essential regulators with numerous functions, being responsible for several regulatory complexes at different levels in plants. These two phytohormones have intimate interactions at the cellular level that are still highly investigated to improve understanding of their roles in plants (Schaller *et al*., 2015).

Few differences were observed in the second experiment, the use of ZEA (0.1 μL^{-1}) being one of them. The differences between cytokinins are related to translocation rates to the responsive regions, differential uptake, varied effects on metabolic processes and the ability to change the level of endogenous cytokinins (Rathore *et al*., 2016). The efficiency of ZEA may be related to the ability of plant tissues to metabolize natural growth regulators quickly and to be associated with other cellular components of the explant, which may vary depending on the cytokinin used.

Cappelletti *et al.* (2016), mentioned that ZEA is one of the best cytokinins to induce blueberry proliferation. The efficiency of ZEA can be associated to an association and synergism with other components and minerals present in the growth medium (Ortiz-Rojas *et al*., 2017). Singh *et al*. (2016), related to the cytokinin the reason behind the increase in the number of shoots and, due to the role of cytokinins, the growth of axillary shoots occurs. According to the authors, cytokinin acts in the regeneration of the aerial part, stimulating the accumulation of auxin in meristematic places where shoots are initiated.

The ability to delay leaf senescence varies widely among different types of cytokinins (Oliveira *et al*., 2007). BAP is directly linked to a high rate of shoot multiplication and has been found to be efficient in the multiplication of several species (Rodrigues *et al*., 2016; Emer *et al*., 2018; Enkhbileg *et al*., 2019). El-Bagoury *et al*. (2018), and Freitas *et al*. (2016), indicated the concentration of 1 ppm BAP as the best performance for multiplication of *Vangueria edulis* and *Annona emarginata*, respectively. At this dose there was the maximum abscission content, despite being half of the abscess found in ZEA, it is admitted that for baraúna, BAP doses below 0.8 are favorable to avoid abscission.

Cappelletti *et al.* (2016), in a study with strawberry and blueberry observed that the concentrations of BAP (2.0 mg L^{-1}) in combination with ANA (1.0 mg L^{-1}) significantly influenced the formation of shoots. Fauziah *et al.* (2019), have also

successfully identified associations between ANA and BAP (0.3 and 0.75 mg L^{-1}, respectively) in *Lilium longiflorum*.

Cytokinins act to induce the breakdown of apical dominance and proliferation of axillary buds. Although BAP is one of the most commonly used plant regulators and with the lowest acquisition cost (Dziedzic & McDonald, 2016), this cytokinin does not always have a positive effect for all species, as in the present study, where IAA stood out. The high concentration of cytokinin promoted by the addition of high doses of BAP can cause stress in explants and this can activate genes that alter the endogenous hormonal balance, especially those related to the synthesis of cytokinins, oxidases and dehydrogenases (Kopečný *et al.*, 2016).

In relation to the third experiment, we reported that only leaf abscission presented data with significant differences for the tested inhibitors. Unlike the results observed, Nepomuceno *et al.* (2009), working with angico (*Anadenanthera colubrina*) found that both silver nitrate and cobalt chloride contributed to increases in the number of buds and shoots. Silver nitrate supported the *in vitro* growth and the effective prevention of the abscission of *M. oleifera* leaves compared to the control (Ravi *et al*., 2019) and suppressed excessive stem elongation and increased leaf expansion in *A. andraeanum* cv. Alabama and Dakota (Cardoso, 2019).

The addition of silver nitrate and cobalt chloride, known ethylene inhibitors, may have led to a lower accumulation of ethylene in the atmosphere inside the culture test tubes, which stimulated the multiplication of *Andrographis paniculata* (Das & Bandyopadhyay, 2020). This effect was also raised in the production of *Passiflora gibertii* (Faria *et al*., 2017) and *Glycyrrhiza glabra* (Tahoori *et al*., 2018). Sarropoulou *et al.* (2016), observed direct effects of silver nitrate, silver sulfate and cobalt chloride on the proliferation of shoots *in vitro* and on the rooting of cherry. Kumar *et al.* (2016), attributed the efficiency in the multiplication of cotton explants to silver nitrate, relating the increase in plant growth to the increase in polyamine biosynthesis and inhibition of ethylene action.

The response to ethylene occurs through its connection to a specific receptor, responsible for sending the signal for its activation. The response of the plant tissue to ethylene is accompanied by the self-catalytic induction of the hormone itself, that is, the exposure of the tissue to ethylene stimulates its biosynthesis, due to the increase in the enzymes 1-carboxylic acid-1-aminocyclopropane synthase (ACCsintase) and 1-carboxylic acid-1-aminocyclopropane oxidase (ACCoxidase).

The cobalt ion acts by inhibiting synthesis of the plant regulator, blocking the conversion of ACC into ethylene, which is performed by ACC oxidase. This can happen from small concentrations, while the silver ion has a more specific action and competes for the ethylene receptor. Due to this, low concentrations may not be enough to inhibit the signaling that leads to the action of this plant regulator, which even in minimal amounts can have harmful effects (Nowak *et al*., 1991).

Sarropoulou & Maloupa (2017), indicated the 2.5 µM of cobalt chloride as the appropriate for the multiplication of *Sideritis raeseri*. Panchal & Patel (2017), found lower leaf abscission and higher number of leaves for *Annona squamosa* when used in the culture medium 5 mg L^{-1} (10 mg L^{-1} being phytotoxic and with a negative effect on leaf production). Although silver nitrate and cobalt chloride initially differed in reducing leaf abscission, these same compounds when used in the highest concentration (40 µM) proved to be equally effective in controlling this phenomenon in this experiment.

It is important to highlight that *in vitro* regeneration, growth and multiplication is highly dependent on genotype, type of explant, plant growth under concentrations of culture medium and levels of exogenous regulators. Therefore, it is essential to verify the optimal conditions for the growth of the desired species (Hegde *et al*., 2017), and this work directs some concentrations for the cultivation of baraúna.

CONCLUSIONS

The addition of 0.2 µL^{-1} of NAA to 0.4 µL^{-1} of ZEA promotes a greater number of baraúna shoots. IAA is the most suitable auxin to be associated with ZEA for higher shoot length and number of buds.

At concentrations of 5 and 10 µM, cobalt chloride is more efficient than silver nitrate for reducing leaf abscission in baraúna. Silver nitrate from a concentration of 20 µM completely avoids leaf abscission while cobalt chloride has a maximum reduction in abscission at a concentration of 40 µM.

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