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Propagation

Rooting of herbaceous cuttings of *Malpighia emarginata* D.C. (CAMB-06 and APU-04 selections) associated with the use of indolebutyric acid and liquid extract of *Cyperus rotundus* L.

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Abstract - The objective of this work was to evaluate the rooting of herbaceous cuttings of CAMB-06 and APU-04 acerola cherry selections associated with the use of indolebutyric acid (IBA) and a concentration of aqueous extract of coco-grass. Cuttings were treated by immersion with different concentrations of IBA (0; 1,000; 2,000; 3,000; 4,000 mg L^{-1}) and one application of aqueous extract of coco-grass in proportion 100% and placed to root in plastic boxes containing vermiculite. The experimental design was a completely randomized in a 2×6 factorial arrangement (two acerola cherry selections and six solutions for rooting) with four replicates of ten cuttings. After 90 days of cutting, the following variables were evaluated: cutting survival (%); leaf retention (%); rooted cuttings (%); sprouting (%); number of roots per cutting (%); length of roots (cm) and dry mass of roots per cutting (g). It was concluded that the application of IBA at 4,000 mg L^{-1} presented a better root development, resulting in a higher percentage of rooted cuttings and higher number and dry mass of roots in relation to IBA at 0 mg L^{-1} for CAMB-06 selection, while the APU-04 selection can be efficiently multiplied by herbaceous cuttings without the use of IBA. Under the conditions that the trials was conducted, the application of 100% aqueous extract of coco-grass was not effective in promoting the rooting of cuttings of CAMB-06 and APU-04 acerola cherry selections.

Index terms: propagation, plant growth regulator, IBA, extract of coco-grass, acerola cherry.

Enraizamento de estacas de *Malpighia emarginata* D.C. (seleções CAMB-06 e APU-04) associadas ao uso do ácido indolbutírico e extrato aquoso de *Cyperus rotundus* L.

Resumo – O objetivo deste trabalho foi avaliar o enraizamento de estacas herbáceas das seleções de acerolas CAMB-06 e APU-04 associadas ao uso de ácido indolbutírico (AIB) e uma concentração de extrato aquoso de tiririca. As estacas foram tratadas por imersão com diferentes concentrações de AIB (0; 1.000; 2.000; 3.000 e 4.000 mg L^{-1}) e uma aplicação de extrato aquoso de tiririca, na proporção 100%, e dispostas para enraizar em caixas plásticas contendo vermiculita. O delineamento experimental foi inteiramente casualizado, em arranjo fatorial 2×6 (duas seleções e seis soluções para enraizamento) com quatro repetições de dez estacas. Após 90 dias da estaquia, foram avaliadas: sobrevivência das estacas (%); retenção foliar (%); estacas enraizadas (%); brotação (%); número de raízes por estaca (%); comprimento de raízes (cm) e massa seca de raízes por estaca (g). Concluiu-se que a aplicação de AIB na concentração 4.000 mg L^{-1} apresentou melhor desenvolvimento radicial, resultando em maior porcentagem de estacas enraizadas e maior número e massa seca de raízes em relação à concentração 0 mg L^{-1} de AIB para a seleção CAMB-06, enquanto a seleção APU-04 pode ser propagada por estaquia herbácea com eficiência, sem o uso de AIB. Nas condições em que o experimento foi conduzido, a aplicação de 100% de extrato aquoso de tiririca não foi efetiva na promoção do enraizamento de estacas das seleções de acerola CAMB-06 e APU-04.

Termos para indexação: propagação, regulador vegetal, AIB, extrato de tiririca, acerola.

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Introduction

The acerola (*Malpighia emarginata* DC) is a native fruit of the Caribbean Islands, Central America and North South America. Due to its high nutritional value, mainly as a source of vitamin C, it has dispersed to other regions of the world and found conditions in Brazil because of the favorable soils and climate for its cultivation, becoming one of the few countries that commercially cultivate acerola (RITZINGER; RITZINGER, 2011).

Although vegetative propagation presents some obstacles, such as low rooting percentage, it is still preferable to multiplicatef acerola, since obtaining cuttings by cutting is faster and simpler, ensuring the genetic characteristics of the plants, as well as early and uniform production (MENDES et al., 2012). Therefore, it is necessary that the orchards are formed from well-defined varieties, containing appropriate agronomic and technological characteristics.

The Acerola Breeding Program developed by the Agronomic Institute of Paraná (IAPAR) has obtained some selections adapted to subtropical conditions, which showed promising agronomic characteristics for consumption and industrialization, among them CAMB-06 and APU-04. However, few is known about the vegetative propagation by cutting of these selections.

In order to stimulate and accelerate root emission in cuttings, many nurserymen use chemical compounds such as auxins, which are plant growth regulators that potentiate nursery trees production, especially in hard-to-rooting species (ALCANTARA et al., 2010). Within this group, indolbutyric acid (IBA) is the most used in cuttings, being efficient for several species, little susceptible to the action of degradation enzymes and not toxic to most plants (PIRES; BIASI, 2003). However, depending on its concentration, it may present variable results, related to the species to be cultivated, physiological conditions of the mother plant, plant age and type of cutting, as well as the environmental conditions in which they are conducted, such as substrate, humidity, temperature, irrigation and luminosity (FACHINELLO et al., 2005).

In addition, to address the lack of use of alternative products to synthetic auxins, some researches have been conducted from the perspective of offering phytohormones obtained from coco-grass (*Cyperus rotundus* L.) (CÂMARA et al., 2016; KOEFENDER et al., 2017; CAVALCANTE et al., 2018). Coco-grass tuber extract, under appropriate conditions, produces several substances called secondary metabolites, which are mostly phenolic compounds, including polyphenols. These substances are essential to plants because their ability to stimulate root formation is due to a possible protective action on indolacetic acid (IAA) as a consequence of inhibition of IAA oxidase (QUAYYUM et al., 2000; HARTMANN et al., 2002).

Given the need for large-scale nursery production and the lack of information on the application of coco-grass extract as rooting promoters in the vegetative propagation of acerola, this work aimed to evaluate the rooting of herbaceous cuttings from the acerola selections CAMB-06 and APU-04 associated with the use of different concentrations of indolbutyric acid and an aqueous extract concentration of coco-grass.

Material and methods

The experiment was carried out from December 2018 to March 2019, at the Department of Agronomy, Fruit Sector, of the Agricultural Center Sciences of the State University of Londrina (UEL), Paraná, Brazil (23°23' S, 51°11' W, elevation of 566 m). Herbaceous cuttings were taken from the shoots of CAMB-06 and APU-04 acerola selections from the Paraná Agronomic Institute (IAPAR) of Londrina, PR.

The experimental design was completely randomized, with four replications of 10 cuttings per plot in a 2 × 6 factorial arrangement. The evaluated factors were: two acerola selections (CAMB-06 and APU-04) and six rooting solutions, in which five concentrations of indolebutyric acid – IBA, 99% pure SIGMA® (0, 1,000, 2,000, 3,000 and 4,000 mg L^{-1}) a 100% proportion of aqueous coco-grass extract.

The hydroalcoholic solution of IBA was prepared according to Colombo et al. (2018), in which 0.1 g of IBA was weighed and dissolved in 50 mL alcohol. After completely dissolving the IBA, the volume was made up to 100 mL with distilled water, giving the concentration of 1,000 mg L^{-1}. The aqueous extract of coco-grass was obtained according to the methodology described by Koefender et al. (2017). The coco-grass tubers used for the preparation of the extract were taken from coco-grass plants collected in the agricultural area and the extract was obtained in the proportion of 100 g of tubers plus 1,000 mL of distilled water to the concentration of 100% of the aqueous extract.

The acerola cuttings were obtained through a cut just below a bud and standardized with an average length of 10 cm, leaving two pairs of leaves at the apical end. During preparation, the cuttings were placed in a container with water to prevent dehydration (ROBERTO et al., 2004). Then the base of each cutting was immersed in aqueous IBA or coco-grass solutions for 10 seconds, depending on the treatments.

After immersion in the solutions, the cuttings (1/3 of the length) were placed in perforated plastic boxes (dimensions 44 × 30 × 07 cm) containing medium-grained vermiculite substrate. They were then placed in a timer-controlled intermittent mist chamber controlled by a solenoid valve. The valve was programmed to mist for 10 seconds every three minutes. The mist nozzle employed (Mist Model DanSprinklers, Israel) has a flow rate of 35 L hour^{-1}. The mist chamber was housed in a greenhouse with transparent polyethylene film cover and 30% shade.

After 90 days of conducting the experiment, the following variables were evaluated: cuttings survival (% of live cuttings); leaf retention (% of cuttings that did not lose leaves); rooted cuttings (% of cuttings that emitted at least one root); sprouting (% of cuttings that sprouted); number of roots per cutting (%); root length per cut (cm) and root dry mass per cut (g). The root dry mass was obtained by drying in an oven with forced air circulation at 78 ºC for 48 hours.

Data were subjected to analysis of variance and means compared by Tukey´s test at 5% probability with the aid of the *R* statistical software, ExpDes.pt package.

Results and discussion

There was no significant interaction between selections and concentrations of IBA and the aqueous extract of coco-grass regarding the percentage of survival and leaf retention of cuttings (Table 1), and no significant differences were observed between these factors, except for leaf retention between selections, in which APU-04 selection presented higher mean (84.6%) when compared to CAMB-06 (81.3%). In an experiment conducted with herbaceous cuttings of guava (*Psidium guajava* L.), no differences were observed regarding the percentage of survival and leaf retention at concentrations 0; 1,000; 2,000 and 3,000 mg L^{-1} of IBA (COLOMBO et al., 2008), as well as there was no difference in relation to these variables for ‘Paluma’ and ‘Seculo XXI’ guava cuttings, using concentrations 500; 1,000; 1,500 and 2,000 mg L^{-1} of IBA (ZIETEMANN; ROBERTO, 2007).

Table 1 - Survival and leaf retention of CAMB-06 and APU-04 acerola cherry cuttings (*Malpighia emarginata* D.C.) subjected to different concentrations of indolebutyric acid (IBA) and liquid extract of coco-grass (*Cyperus rotundus*) in mist chamber after 90 days of installation of the trial. Londrina-PR, 2019.

Acerola cherry selection (A)	Survival (%)	Leaf retention (%)
CAMB-06	94.6	81.3 b
APU-04	95.0	84.6 a
IBA concentration (mg L^{-1}) and liquid extract of coco-grass (B)		
0	97.5	82.5
1,000	91.3	83.8
2,000	96.3	83.8
3,000	93.8	85.0
4,000	95.0	82.5
CCG extract	95.0	80.0
F-value (A)	0.07^{ns}	4.57*
F-value (B)	1.36^{ns}	0.80^{ns}
F-value (A × B)	1.36^{ns}	2.00^{ns}
CV%	5.49	6.51

Means within columns followed by different letters differ significantly by Tukey’s test ($P < 0.05$). *Significant at $P < 0.05$. ^{ns}: non-significant. CCG extract: liquid extract of coco-grass. F-value: F test. CV: coefficient of variation.

Although there was no statistical difference between them, the evaluated cuttings presented leaf retention percentage ranging from 80.0 to 85.0% for the coco-grass extract and 3,000 mg L^{-1} IBA concentration, respectively (Table 1). The presence of leaves ensures the survival of cuttings and influences rooting, mainly due to their function in the production of carbohydrates in photosynthesis, and by providing auxins and other nutritive substances, which are transported to the bottom of the plant, accumulating at the base of the cuttings and stimulating the process of root formation (HARTMANN et al., 2002).

Regarding the rooting and sprouting percentage of cuttings (Table 2), it was found that the interaction between the selections and concentrations of IBA and the coco-grass extract was significant.

Table 2 - Rooted cuttings and sprouting (%) of CAMB-06 and APU-04 acerola cherry cuttings (*Malpighia emarginata* D.C.) subjected to different concentrations of indolebutyric acid (IBA) and liquid extract of coco-grass (*Cyperus rotundus*) in mist chamber after 90 days of installation of the trial. Londrina-PR, 2019.

	IBA concentration (mg L^{-1}) and liquid extract of coco-grass	Acerola cherry selection		F-value	CV%
		CAMB-06	APU-04		
Rooted cuttings (%)	0	82.5 Bab	92.5 Aa	6.12*	7.35
	1,000	85.0 Aa	82.5 Aab	0.38^{ns}	
	2,000	85.0 Aa	62.5 Bc	31.02*	
	3,000	92.5 Aa	72.5 Bbc	24.51*	
	4,000	92.5 Aa	65.0 Bc	46.34*	
	CCG extract	72.5 Ab	47.5 Bd	38.29*	
	F-value	6.74*	30.76*		
Sprouting (%)	0	95.0 Aa	90.0 Aa	1.02^{ns}	8.01
	1,000	87.5 Aa	90.0 Aa	0.25^{ns}	
	2,000	90.0 Aa	82.5 Aab	2.31^{ns}	
	3,000	87.5 Aa	80.0 Aab	2.31^{ns}	
	4,000	95.0 Aa	80.0 Bab	9.25*	
	CCG extract	95.0 Aa	72.5 Bb	20.82*	
	F-value	20.74^{ns}	1.81*		

Means followed by different capital letters within lines and lower case letters within columns differ significantly by Tukey's test ($P < 0.05$); *Significant at $P < 0.05$; ^{ns}: non-significant; CCG extract: liquid extract of coco-grass; F-value: F test; CV: coefficient of variation.

In the evaluation of rooted cuttings, differences were observed between the acerola selections (Table 2). The selection APU-04 showed cuttings with higher rooting potential compared to the selection of CAMB-06 for treatments without application of IBA and aqueous extract of coco-grass. The fact that APU-04 resulted in the highest rooting percentage without IBA application may indicate an appropriate endogenous auxin content to promote the root formation process, since the rooting of acerola cuttings varies as a function of the genetic characteristics of each cultivar (GOMES et al., 2000).

Comparing IBA concentrations and an aqueous extract of coco-grass extract for each of the selections (Table 2), it was observed that for CAMB-06 the highest rooting percentages (92.5%) were obtained when cuttings were treated with 3,000 and 4,000 mg L^{-1} of IBA, however they did not differ statistically from the concentrations of 1,000 and 2,000 mg L^{-1}, which showed 85.0% of rooting. In a study carried out with semi-hardwood acerola cuttings, the highest rooting percentages were obtained by applying 2,800 mg L^{-1} of IBA (GONTIJO et al., 2003). Similarly, acerola cuttings treated with IBA at concentrations 1,500 and 2,000 mg L^{-1}, after 60 days of application, presented higher rooting percentage when compared to the other concentrations (500 and 1,000 mg L^{-1}) (LOPES et al., 2003).

The existence of an adequate endogenous hormonal balance, especially between auxins, gibberellins and cytokines, is necessary for a balance between promoters and inhibitors of the process of root initiation. The most common way to promote this balance is by exogenous application of synthetic growth regulators (PASQUAL et al., 2001). Auxins are the most commonly used plant growth regulators in rooting studies, possibly because they stimulate ethylene synthesis and favor root emission (HARTMANN et al., 1990).

Regarding APU-04 selection (Table 2), the treatment without IBA resulted in the highest percentage of rooted cuttings (92.5%), but did not differ statistically from the concentration of 1,000 mg L^{-1} (82.5%).. On the other hand, applications with 2,000; 3,000 and 4,000 mg L^{-1} of IBA showed rooting averages of 62.5; 72.5 and 65.0%, respectively. Rooting stimulation occurs up to a certain regulator concentration, different for each variety, from which the effect is inhibited (ALVARENGA; CARVALHO, 1983). For Nascimento (1991), the IBA concentrations ranged between 2,000 and 8,000 mg L^{-1} have a detrimental effect on rooting of acerola cuttings.

In the two acerola selections evaluated, treatment with aqueous extract of coco-grass resulted in the lowest rooting averages (Table 2). Possibly, these results are related to the concentration of the coco-grass extract used and the immersion time of the cuttings.

For the APU-04 selection that presented only 47.5% of rooted cuttings, a hormonal imbalance may have occurred, because the higher the dose of coco-grass extract, the greater the amount of promoter substances and, consequently, there may be reduction or even inhibition of root development of cuttings, prone to toxicity (KOEFENDER et al., 2017). As well as the immersion time of 10 seconds, it may not have been sufficient to promote the greater root stimulation of the acerola cuttings.

IBA concentrations can have negative effects regarding cuttings rooting aspects, and this fact may be linked to the applied concentrations and the time of immersion of cuttings in the plant growth regulator solution (PAULA et al., 2009). Given the above, new experiments are necessary, with treatments composed by higher extract dilutions and longer immersion time in the solutions.

Regarding the percentage of sprouted cuttings between the two acerola selections (Table 2), only a difference was verified for the concentration of 4,000 mg L^{-1} of IBA and aqueous extract of coco-grass, being the highest averages observed for selection CAMB-06, which resulted 95.0% of sprouting for both treatments.

When evaluating sprouted cuttings among IBA concentrations and coco-grass extract for each of the acerola selections (Table 2), it was possible to observe significant differences among treatments only for APU-04. Concentrations at 0 and 1,000 mg L^{-1} of IBA presented the highest sprouting percentages (90.0%), while the aqueous extract of coco-grass resulted in the lowest average (72.5%). The reduction in the percentage of sprouting for this treatment may be related to the unbalance between auxins and cytokines, promoted by the high concentration of auxin present in the extract, because the excess auxins can inhibit the sprouting (TAIZ; ZEIGER, 2006).

Regarding the number of roots per cutting (Table 3), the mean of CAMB-06 acerola selection was higher when IBA at the concentrations 2,000 and 4,000 mg L^{-1} were employed, while the 3,000 mg L^{-1} concentration resulted in the highest mean for APU-04. There was no difference between the acerola selections among the remaining treatments.

Table 3 -Number of roots per cutting, length of roots and dry mass of roots per cutting of CAMB-06 and APU-04 acerola cherry cuttings (*Malpighia emarginata* D.C.) subjected to different concentrations of indolebutyric acid (IBA) and liquid extract of coco-grass (*Cyperus rotundus*) in mist chamber after 90 days of installation of the trial. Londrina-PR, 2019.

	IBA concentration (mg L^{-1}) and liquid extract of coco-grass	Acerola cherry selection		F-value	CV%
		CAMB-06	APU-04		
Number of roots per cutting	0	4.58 Ac	4.18 Ac	0.98^{ns}	9.91
	1,000	6.54 Aab	6.97 Aa	1.10^{ns}	
	2,000	7.33 Aa	6.21 Bab	7.56*	
	3,000	6.04 Bb	6.90 Aa	4.51*	
	4,000	7.28 Aa	5.44 Bb	20.35*	
	CCG extract	4.44 Ac	3.89 Ac	1.82^{ns}	
	F-value	19.63*	21.53*		
Length of roots (cm)	0	10.71 Ab	11.24 Aa	2.59^{ns}	4.80
	1,000	10.44 Abc	10.05 Ab	1.34^{ns}	
	2,000	9.56 Ac	8.74 Bc	6.07*	
	3,000	9.81 Abc	8.67 Bc	11.55*	
	4,000	10.03 Abc	8.15 Bc	32.07*	
	CCG extract	12.01 Aa	8.07 Bc	140.80*	
	F-value	27.61*	21.21*		
Dry mass of roots per cutting (g)	0	0.63 Ac	0.57 Ab	0.92^{ns}	15.18
	1,000	0.89 Ab	0.88 Aa	0.03^{ns}	
	2,000	0.70 Abc	0.50 Bbc	8.14*	
	3,000	0.79 Abc	0.45 Bbc	24.73*	
	4,000	1.13 Aa	0.33 Bcd	134.29*	
	CCG extract	0.61 Ac	0.24 Bd	28.08*	
	F-value	15.93*	20.60*		

Means followed by different capital letters within lines and lower case letters within columns differ significantly by Tukey's test ($P < 0.05$); *Significant at $P < 0.05$; ^{ns}: non-significant; CCG extract: liquid extract of coco-grass; F-value: F test; CV: coefficient of variation.

When comparing the treatments for each of the selections, significant differences in the number of roots were observed among IBA concentrations and the coco-grass extract (Table 3). For CAMB-06, IBA concentrations at 2,000 and 4,000 mg L^{-1} favored the highest number of roots per cutting, while the non-application of IBA and the aqueous extract of coco-grass resulted in the lowest averages. For APU-04, the highest percentage of roots was found in cuttings treated with 1,000 and 3,000 mg L^{-1}. However, as verified for the CAMB-06 acerola selection, the treatments with aqueous coco-grass extract and without IBA application presented the lowest number of roots per cutting. These results confirm that IBA interferes with the quality, quantity and weight of roots produced (PASQUAL et al., 2001; DUTRA et al., 2002), since the vigor of nursery trees is directly related to root size and quantity (CAMPOS et al., 2005).

Regarding root length and dry mass per cutting (Table 3), a significant interaction was observed between the acerola selections and concentrations of IBA and coco-grass. The CAMB-06 selection showed the highest averages for both variables, except for IBA at 0 and 1,000 mg L^{-1}, which did not differ between selections.

The application of aqueous extract of coco-grass resulted in the longest root length (12.01 cm), followed by treatment without application of IBA (10.71 cm) for CAMB-06. However, for root dry mass, these treatments presented the lowest averages (0.61 g and 0.63 g), respectively. The longer root length can be explained by the fact that the few roots present in the cuttings developed to obtain the nutrients necessary for their survival. On the other hand, probably, as observed for rooted cuttings and number of cuttings, these treatments did not present sufficient auxins to maximize rooting potential, or the concentration of auxins present in the extract may have been phytotoxic to acerola cuttings.

For the APU-04 selection, the treatment without IBA resulted in the longest root length, followed by the concentration of 1,000 mg L^{-1} IBA (Table 3). These treatments were also superior for the root dry mass variable, and the concentration of 1,000 mg L^{-1} presented the highest mean (0.88 g), followed by the concentration of 0 mg L^{-1} (0.57 g). Possibly, the longer root length may be related to the higher endogenous auxin concentration (ANTUNES et al., 2000), as the increase in root length may determine the increase in root dry matter mass (HARTMANN; KESTER, 1968).

The proper amount of exogenous auxins to stimulate rooting depends on the concentration already present in the cutting tissue (FACHINELLO et al., 2005). For APU-04, the applications of 2,000; 3,000; 4,000 mg L^{-1} of IBA and coco-grass extract had the lowest root lengths, demonstrating that the application of IBA above 1,000 mg L^{-1} and the 100% proportion of aqueous coco-grass extract may have caused phytotoxic effects to cuttings.

The presence of substances in the coco-grass tubers may stimulate the effect of IAA when applied at optimal concentrations, but at very high concentrations it may cause toxicity to plants (MEGURO, 1969). In addition, high concentrations of aqueous coco-grass extract may cause allelopathic action of substances that inhibit the rooting process (PORTILHO, 2006).

The wide variation among treatments for the analyzed variables may be related to the endogenous content of auxins and inhibitors in the cuttings, and consequently to the exogenous auxin concentrations applied and the immersion time used in the experiment. The morphological differences, determined by genetic characteristics of the acerola selections, may also result in differentiated responses regarding the effectiveness of IBA in herbaceous acerola cuttings.

Conclusions

The application of IBA at 4,000 mg L^{-1} presented a better root development, resulting in a higher percentage of rooted cuttings and higher number and dry mass of roots in relation to IBA at 0 mg L^{-1} for CAMB-06 selection, while the APU-04 selection can be efficiently multiplied by herbaceous cuttings without the use of IBA.

Under the conditions that the trials was conducted, the application of 100% aqueous extract of coco-grass was not effective in promoting the rooting of cuttings of CAMB-06 and APU-04 acerola cherry selections.

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