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Adventitious rooting on the Brazilian red-cloak and sanchezia after application of indole-butyric and humic acids

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ABSTRACT

Plant regulators, as well as bioactive fractions of the organic matter, can accelerate the development and increase the efficiency of adventitious rooting in ornamental plants. We evaluated rooting in the Brazilian red-cloak (*Megaskepasma erythrochlamys*) and sanchezia (*Sanchezia nobilis*) cuttings in response to application of indole-butyric acid (IBA) and humic acids (HA). Stem cuttings of both species treated with solutions of 0, 250, 500, 1000, 2000 mg/L of IBA and 0, 10, 20, 30, 40 mmol/L of C of HA were placed in 2.0 L plastic pots containing carbonized rice husk, kept in greenhouse. Survival of cuttings, number of leaves and shoot and root fresh and dry matter were evaluated 45 days after. Data were used to run an analysis of variance. We observed the average contrasts, calculated the regression equations and estimated the concentrations that provided the best efficiency in terms of root dry matter. For IBA, 185.4 and 66.8 mg/L were the concentrations that promoted the highest root dry matter accumulation in the Brazilian red-cloak and sanchezia, respectively, with increments of 140 and 22.5% in relation to the control. HA, at 33.6 mmol L^{-1} of C, increased shoot fresh and dry matter in the Brazilian red-cloak, but reduced root formation compared to the control. In sanchezia, HA at 19.5 mmol/L of C promoted increases of 35.9% in root dry matter in relation to the control. We concluded that IBA, in the abovementioned concentrations, increased adventitious rooting in cuttings of both the Brazilian red-cloak and sanchezia, while optimal doses of HA solutions are very likely to improve adventitious rooting in sanchezia, but not in the Brazilian red-cloak.

Keywords: *Megaskepasma erythrochlamys*, *Sanchezia nobilis*, vegetative propagation, humic substances, auxins.

RESUMO

Enraizamento adventício de justícia vermelha e sanquésia usando ácidos indolbutírico e húmico

O enraizamento adventício de plantas ornamentais pode ser acelerado por meio da aplicação de fitorreguladores e também de frações ativas da matéria orgânica. O objetivo deste trabalho foi avaliar o enraizamento adventício de justícia vermelha (*Megaskepasma erythrochlamys*) e sanquésia (*Sanchezia nobilis*) em resposta à aplicação de concentrações de ácido indolbutírico (AIB) e ácido húmico (AH). Estacas caulinares das duas espécies tratadas com soluções de 0, 250, 500, 1000, 2000 mg/L de AIB e 0, 10, 20, 30, 40 mmol/L de C de AH foram colocadas em vasos de plástico de 2,0 L, contendo casca-de-arroz carbonizada como substrato, mantidos em casa-de-vegetação. A sobrevivência das estacas, número de folhas e matéria fresca e seca da parte aérea e de raízes foram avaliadas 45 dias após. Os resultados foram submetidos à análise de variância. Foram observados os contrastes médios, calculadas as equações de regressão e estimadas as concentrações dos fitorreguladores de melhor eficiência em termos de matéria seca de raiz. As concentrações 185,4 e 66,8 mg/L de AIB promoveram os maiores acúmulos de matéria seca da raiz em estacas de justícia e sanquésia, respectivamente, com incrementos de 140 e 22,5% em comparação ao controle. A aplicação de AH na concentração de 33,6 mmol/L de C promoveu aumento no acúmulo de matéria fresca e seca da parte aérea em justícia, mas diminuiu a formação de raízes em relação ao controle. Em sanquésia, a aplicação de AH na concentração de 19,5 mmol/L de C promoveu incrementos de 35,9% no acúmulo de matéria seca da raiz em comparação ao controle. Concluiu-se que a aplicação de AIB nas concentrações citadas acima aumentou o enraizamento adventício de justícia e sanquésia e doses ótimas de soluções de AH provavelmente proporcionam aumentos no enraizamento adventício de sanquésia e diminuições em justícia.

Palavras-chave: *Megaskepasma erythrochlamys*, *Sanchezia nobilis*, propagação vegetativa, substâncias húmicas, auxinas.

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Brazil has a large and expanding potential to become a major producer and exporter of flowers and ornamental plants (Junqueira & Peetz, 2008), especially when it comes to tropical plants, which have a broad genetic diversity in the country and find favorable growing conditions (Anefalos *et al.*, 2010; Vera, 2008). The Brazilian red-cloak (*Megaskepasma erythrochlamys*) and sanchezia (*Sanchezia nobilis*), from the Acanthaceae family, are erect shrubs, 2-3 meter tall (Lorenzi & Souza, 2008), are ornamental plants that can be used in gardens and parks, either as isolated plants or in patches, in tropical and subtropical conditions. Both species are propagated through vegetative means, mainly by stem cuttings, best results being observed with cuttings harvested after flowering and rooted in protected environments (Lorenzi & Souza, 2008).

The propagation by cuttings favors the quick and massive production of plantlets with complete fidelity to the mother plant. However, for the propagation process to be successful, it is important to achieve a high rate of rooting. The main factors that influence adventitious rooting in cuttings are related to the propagation material (e.g. genotype, type of cutting) and the rooting environment (e.g. substrate, humidity) (Hartmann *et al*., 2011).

Growth regulators of the auxin type, such as the indole-butyric acid (IBA) accelerate the process of adventitious rooting and make room for a vigorous development of plantlets in many species, including several ornamental plants (Loss *et al*., 2009; Pizzatto *et al*., 2011). Nevertheless, IBA is an expensive chemical reagent produced by specialized companies. In addition, it presents toxicity to humans, causing skin irritation and eye and respiratory infection, if handled improperly (Sigma-Aldrich, 2014). It is also toxic to the environment, particularly aquatic environments (Sigma-Aldrich, 2014). Humic acids (HA), the bioactive fraction of the organic matter after humification, also promotes adventitious rooting in cuttings of ornamental plants (Baldotto *et al*., 2012), and with economic and environmental advantages in relation to IBA: HA are a renewable natural resource which can be extracted from different urban wastes such as garbage and sewage sludge (Canellas *et al*., 2000), as well as from agriculture residues, such as animal manures (Melo *et al*., 2008). However, plant response to HA application is not uniform and varies with the raw material which these compounds where extracted from and their concentration, and plant genotype (Baldotto *et al*., 2011, 2012).

HA, the most stable reactive fraction of humic substances, have dark color and varied chemical structure, with high content of aromatic rings and carboxylic, phenolic and quinone functional groups (Baldotto *et al*., 2007; Guerra *et al*., 2008). HA alter growth and development of various plants of agronomic interest, accelerating root growth and boosting plant biomass (Zandonadi *et al*., 2007; Baldotto *et al*., 2009; Lima *et al*., 2011; Arancón *et al*., 2012).

We aimed to study the adventitious rooting of apical stem cuttings of Brazilian red-cloak and sanchezia plants in response to the application of different concentrations of IBA and HA.

MATERIAL AND METHODS

Apical cuttings were taken from branches of the Brazilian red-cloak (*Megaskepasma erythrochlamys*) and sanchezia (*Sanchezia nobilis*) mother plants grown at the Universidade Federal de Viçosa, *Campus* Florestal. Cuttings were collected in September 2011 and standardized to 15-cm length and two apical leaves cut crosswise in half.

The experimental matrix [2x (5+5)] consisted of two ornamental species (the Brazilian red-cloak and sanchezia), five concentrations (0, 250, 500, 1000, 2000 mg/L) of indole-butyric acid (IBA) and five concentrations (0, 10, 20, 30, 40 mmol/L C) of humic acids (HA) previously isolated from vermicompost by Baldotto *et al*. (2012). Cuttings were placed in 50 mL plastic cups containing HA solutions for 24 hours (Baldotto *et al*., 2009) and in those with IBA solutions for 10 seconds (Lima *et al*., 2008). Following, cuttings were transferred to 2.0 L black plastic pots filled with substrate (carbonized rice husk), and kept in greenhouse. The experimental units consisted of a five-cutting pot. The experiment was carried out in a randomized block design, with five replications, totaling 100 experimental units.

Forty-five days after, the following characteristics were assessed: number

Table 1. Means, average contrasts, relative increases, error mean square and coefficient of variation for survival of cuttings (SC), number of leaves (NL), leaf fresh (LFM) and dry matter (LDM), root fresh (RFM) and dry matter (RDM) of the Brazilian red-cloak plants in response to application of indole-butyric acid (IBA) and humic acids (HA), data from 5 replicates of 5 cuttings per pot [médias, contrastes, incrementos relativos, quadrado médio do resíduo e coeficiente de variação para sobrevivência das estacas (SC), número de folhas (NL), matérias fresca (LFM) e seca das folhas (LDM), matérias fresca (RFM) e seca das raízes (RDM) de plantas de justícia-vermelha em resposta à aplicação de ácido indol butírico (IBA) e ácidos húmicos (HA), dados de 5 repetições de 5 estacas por vaso]. Florestal, UFV, 2011.

Treatments	SC	NL	LFM	LDM	RFM	RDM
	(unit)		(g)			
IBA0	5	27	8.92	1.26	1.83	0.15
IBA250	5	29	10.59	1.77	4.02	0.36
IBA500	5	31	13.89	1.92	3.83	0.35
IBA1000	5	26	11.17	1.71	2.68	0.25
IBA2000	5	26	12.84	1.79	2.98	0.30
HA0	5	26	18.81	2.45	4.56	0.40
HA10	5	27	19.58	2.56	3.16	0.33
HA20	5	30	11.28	1.71	1.26	0.12
HA30	5	27	9.67	1.58	1.02	0.10
HA40	5	34	10.63	1.62	1.52	0.13
Contrast (IBA x HA)	-0.80^{0}	5.60	12.57*	1.06^{0}	-3.80	-0.32
RI (%)[1]	3.23	3.89	17.96	14.68	24.79	22.89
MS	0.10	22.77	13.39	0.25	3.12	0.23
CV (%)	6.59	16.89	28.73	27.28	65.73	62.07

* and ^{0}= significant at p<0.05 and 0.01 respectively (significativo, p<0,05 e 0,01, respectivamente); Relative increases = 100 (x-y)/y, where x and y correspond to the means for the treatment with the highest and lowest values respectively (incrementos relativos = 100 (x-y)/y, sendo x e y as médias dos tratamentos de maior e menor valor respectivamente).

of surviving cuttings per pot (SC), total number of leaves per experimental unit (NL), leaf fresh (LFM) and dry matter (LDM) and root fresh (RFM) and dry matter (RDM). Dry matter was obtained by drying leaves and roots in an oven under forced ventilation at 60°C, for seven days.

Results were used to run an analysis of variance. The effects of treatments were deployed unfolded on average contrasts, as indicated by Alvarez & Alvarez (2006). We also adjusted regression equations using the means of the characteristics studied and IBA and HA concentrations. The F test was applied to the unfolded factors at 10, 5 and 1% of probability. The slopes of the regression equations were tested when presented coefficient of determination greater than 0.60. The regression equations were used to estimate the concentration of maximum physical efficiency for root dry matter as function of IBA and HA concentrations.

Table 2. Means, average contrast, relative increases, error mean square and coefficient of variation for survival of cuttings (SC), number of leaves (NL), leaf fresh (LFM) and dry matter (LDM), root fresh (RFM) and dry matter (RDM) of sanchezia plants in response to application of indole-butyric acid (IBA) and humic acids (HA), data from 5 replicates of 5 cuttings per pot [médias, contrastes, incrementos relativos, quadrado médio do resíduo e coeficiente de variação para sobrevivência das estacas (SC), número de folhas (NL), matérias fresca (LFM) e seca das folhas (LDM), matérias fresca (RFM) e seca das raízes (RDM) de plantas de justícia-vermelha em resposta à aplicação de ácido indol butírico (IBA) e ácidos húmicos (HA), dados de 5 repetições de 5 estacas por vaso]. Florestal, UFV, 2011.

Treatments	SC	NL	LFM	LDM	RFM	RDM
	(unit)		(g)			
IBA0	5	29	12.54	1.95	10.60	1.03
IBA250	5	27	14.11	2.00	12.99	1.22
IBA500	5	25	12.99	1.89	11.91	1.16
IBA1000	5	28	13.30	1.98	13.84	1.28
IBA2000	5	28	12.47	1.78	15.15	1.25
HA0	4	23	9.57	1.43	9.43	0.62
HA10	4	24	9.07	1.29	8.05	0.75
HA20	5	29	11.80	1.78	8.72	0.93
HA30	4	24	9.67	1.43	7.22	0.70
HA40	4	24	8.41	1.22	5.72	0.63
Contrast (IBA x HA)	-3.00**	-13.80^0	-16.89**	-2.43**	-25.34**	-2.31^0
RI (%)	12.00	10.03	25.83	25.33	39.29	38.94
MS	0.40	27.01	12.91	0.28	11.57	0.92
CV (%)	13.45	19.88	31.54	31.58	32.82	31.72

* and ^{0}= significant at p<0.05 and 0.01 respectively (significativo, p<0,05 e 0,01, respectivamente); Relative increases = 100 (x-y)/y, where x and y correspond to the means for the treatment with the highest and lowest values respectively [incrementos relativos = 100 (x-y)/y, sendo x e y as médias dos tratamentos de maior e menor valor respectivamente].

RESULTS AND DISCUSSION

In this study we established the concentrations of indole-butyric acid (IBA) and humic acids (HA) that promoted growth and adventitious rooting of cuttings of the Brazilian red-cloak and sanchezia, aiming at speeding up and improving the efficiency of vegetative propagation in these species.

The application of mounting concentrations of IBA and HA significantly alter rooting performance and shoot growth in cuttings of the Brazilian red-cloak and sanchezia (Tables 1 and 2). The same tables contain the average contrasts for qualitative data. Leaf fresh (LFM) and dry matter (LDM) in plants of the Brazilian red-cloak treated with HA increased significantly when compared to those treated with IBA, with LFM and LDM values 17.9 and 14.6% higher in the former than in the later (Table 1). Biomass accumulation in shoots in response to HA application in nursery material had already been reported for other species of agronomic interest such as pineapple (Baldotto *et al*., 2009), croton, and hibiscus (Baldotto *et al*., 2012). However, in eucalyptus there was no significant response (Pinheiro *et al*., 2010), findings that encourages research towards elucidating HA mechanisms of action if these compounds are intended to be developed into a sustainable agricultural input.

HA mode of action was not investigated in this work. However, other studies have suggested the existence of different mechanisms (Zandonadi *et al*., 2013). It has been proposed that HA stimulate root growth in an auxin like manner, i.e., by modulating the H^+-ATPases in the cell membrane (Canellas *et al*., 2002; Façanha *et al*., 2002). ATPases are enzymes that acidify the apoplast, and thus activate enzymes that degrade the cell wall and make cells more susceptible to the action of the vacuolar turgor pressure, resulting in cell expansion (Rayle & Cleland, 1992). HA also act in the regulation of nutrient uptake (Nardi *et al*., 2002; Sondergaard *et al*., 2004; Lima *et al*., 2011) and further increases in the accumulation of photosynthesizing pigments (Baldotto *et al*., 2009). Such mechanisms may explain the growth in shoot fresh and dry matter we observed in cuttings of the Brazilian red-cloak.

IBA promoted increments in all traits in sanchezia (Table 2). Survival of cuttings (SC), number of leaves (NL), leaf fresh (LFM) and dry matter (LDM), and root fresh (RFM) and dry matter (RDM) had increases of 12, 10, 26, 25, 39 and 39% respectively, compared to the treatment with HA. IBA is a plant regulator with typical auxin hormonal action. It is commercially available in liquid and solid forms and it is used to stimulate adventitious root development, since it favors the formation of meristematic tissue and cell elongation (Hartmann *et al*., 2011). IBA efficiency in promoting adventitious rooting in cuttings of ornamental plants

Table 3. Regression equations for survival of cuttings (SC), number of leaves (NL), leaf fresh (LFM) and dry matter (LDM), root fresh (RFM) and dry matter (RDM) of the Brazilian red-cloak and sanchezia plants in response to application of indole-butyric acid (IBA) and humic acids (HA) {equações de regressão para sobrevivência das estacas (SC), número de folhas (NL), matérias fresca (LFM) e seca das folhas (LDM), matérias fresca (RFM) e seca das raízes (RDM) de plantas de justícia-vermelha em resposta à aplicação de ácido indol butírico (IBA) e ácidos húmicos (HA)}. Florestal, UFV, 2011.

Characteristics	Unfolding	Regression equation	R^2
		The Brazilian red-cloak	
SC (unit)	IBA	$\hat{y} = 4.990 + 0.000088\ x - 0.00000009^{*}\ x^2$	0.9863
	HA	$\hat{y} = 4.596 + 0.273^{*}\ x^{0.5} - 0.04288\ x$	0.9832
NL (unit)	IBA	$\hat{y} = 26.889 + 0.0165\ x - 0.000026\ x^2 + 0.000000009^{*}\ x^3$	0.8871
	HA	$\hat{y} = 26.071 + 0.5607\ x - 0.0391\ x^2 - 0.00075^{o}\ x^3$	0.8241
LFM (g)	IBA	$\hat{y} = 8.535 + 0.018\ x - 0.000022\ x^2 + 0.000000007^{o}\ x^3$	0.8263
	HA	$\hat{y} = 20.263 - 0.4651\ x + 0.00505^{(P<0.20)}\ x^2$	0.7909
LDM (g)	IBA	$\hat{y} = 1.291 + 0.0379^{(P<0.15)}\ x^{0.5} - 0.000625\ x$	0.8506
	HA	$\hat{y} = 2.591 - 0.0424\ x + 0.000401^{(P<0.21)}\ x^2$	0.7851
RFM (g)	IBA	$\hat{y} = 1.818 + 0.3825\ x^{0.5} - 0.0188\ x + 0.00024^{(P<0.14)}\ x^{1.5}$	0.9869
	HA	$\hat{y} = 4.731 - 0.2379\ x + 0.3889^{*}\ x^2$	0.9689
RDM (g)	IBA	$\hat{y} = 0.149 + 0.0349\ x^{0.5} - 0.00169\ x + 0.00002^{(P<0.15)}\ x^{1.5}$	0.9867
	HA	$\hat{y} = 0.424 - 0.0187\ x + 0.000278^{o}\ x^2$	0.8980
		Sanchezia	
SC (unit)	IBA	$\hat{y} = \bar{y} = 5$	
	HA	$\hat{y} = \bar{y} = 4$	
NL (unit)	IBA	$\hat{y} = 29.054 - 1.671^{(P<0.29)}\ x^{0.5} + 0.248\ x$	0.7063
	HA	$\hat{y} = \bar{y} = 23$	
LFM (g)	IBA	$\hat{y} = 12.581 + 0.7719^{(P<0.26)}\ x^{0.5} - 0.125\ x$	0.7392
	HA	$\hat{y} = 0.9551 - 3.5322\ x^{0.5} + 1.6749\ x - 0.1821^{(P<0.25)}\ x^{1.5}$	0.7523
LDM (g)	IBA	$\hat{y} = \bar{y} = 1.917$	
	HA	$\hat{y} = 1.432 - 0.01356\ x - 0.0017^{o}\ x^2$	0.7381
RFM (g)	IBA	$\hat{y} = 10.73 + 0.1878^{(P<0.18)}\ x^{0.5} + 0.7142\ x$	0.8159
	HA	$\hat{y} = 9.136 - 0.0136\ x - 0.0017^{o}\ x^2$	0.8769
RDM (g)	IBA	$\hat{y} = 1.039 + 0.0556^{(P<0.17)}\ x^{0.5} - 0.0034\ x$	0.8316
	HA	$\hat{y} = 0.614 + 0.0231\ x - 0.00059^{(P<0.12)}\ x^2$	0.7433

*, º and ^{P}= significant at p<0.10, 0.05 and 0.01 respectively (significativo, p<0,10, 0,05 e 0,01, respectivamente).

have been reported to allamanda (Loss *et al*., 2008; Pereira *et al*., 2012), croton (Baldotto *et al*., 2012), oleander (Pivetta *et al*., 2012), hibiscus (Pizzatto *et al*., 2011; Baldotto *et al*., 2012) and wax mallow (Loss *et al*., 2009). Besides stimulating root formation, exogenous application of auxins also increases rooting speed and uniformity (Hartmann *et al*., 2011), contributing to production of vigorous plantlets in less time. However, IBA is potentially harmful to health. It presents acute toxicity when ingested and causes a irritation of skin, eyes and the respiratory tract if inhaled (Sigma-Aldrich, 2014).

Once the regression equations for plant growth characteristics as function of IBA and HA concentrations were obtained (Table 3), we calculated the concentrations of maximum physical efficiency for RDM for the Brazilian red-cloak and sanchezia (Table 4).

In plants of the Brazilian red-cloak, maximum physical efficiency for RDM was achieved at 185.42 mg/L of IBA and 33.6 mmol/L of C of HA (Table 4). Such IBA concentration resulted in an increment 140% in RDM in relation to the control. As no effect of HA has been significant, HA should not be used in the Brazilian red-cloak to replace auxins. IBA, on its turn, resulted in significant increases in root growth, corroborating Ferriani *et al*. (2006), who affirmed that IBA is one of the most effective rooting inducing substances.

In sanchezia, the maximum physical efficiency for MSR was achieved with 66.8 mg/L of IBA and 19.5 mmol/L of C for HA (Table 4), which resulted in root dry matter 35.9 and 22.3% higher than the control, respectively (Table 4). These results allow the conclusion that HA can be an alternative to the use of synthetic auxin in sanchezia. Baldotto *et al*. (2012) also observed HA satisfactory results in the adventitious

Table 4. Root dry matter (RDM) of the Brazilian red-cloak and sanchezia plants on condition of maximum physical efficiency (MFE) in response to the application of indole-butyric acid (IBA) and humic acids (HA) {matéria seca da raiz (RDM) de plantas de justícia-vermelha e de sanquésia na condição de máxima eficiência física (MEF) em resposta à aplicação de ácido indol butírico (IBA) e ácidos húmicos (HA)}. Florestal, UFV, 2011.

Species	Treatment	MFE Concentration for RDM	Average for the control (g)	Average for MFE (g)	Difference (%)
The Brazilian red-cloak	IBA	185.42 mg/L	0.15	0.36	140
	HA	33.63mmol/L	0.40	0.11	-72
Sanchezia	IBA	66.85 mg/L	1.03	1.27	22
	HA	19.58 mmol/L	0.62	0.84	36

Difference = 100 (x-y)/y, where x and y correspond to the means for the treatment with the highest and lowest values respectively [diferença = 100 (x-y)/y, sendo x e y as médias dos tratamentos de maior e menor valor respectivamente].

rooting of hibiscus. There are several studies with horticultural crops such as the perennial ryegrass (Silva *et al*., 2000), caisin (Wangen *et al*., 2013), gladiolus (Baldotto & Baldotto, 2013), melon (Pinto *et al*., 2008) and tomato (Bernardes *et al*., 2011; Lima *et al*., 2011) reporting positive effects on the development and production of plants grown in open field and protected environments in response to application of humic substances, either as isolate compounds or as constituents of organic fertilizers or biofertilizers. These studies point to a potential new technological option for farmers, especially for those seeking a more sustainable agriculture, in which the use of synthetic inputs is reduced, while the recycling of organic waste is levered.

Our results indicate that the application of IBA and HA in apical cuttings of the Brazilian red-cloak and sanchezia, in the concentrations evaluated, accelerates plantlet production. This result is relevant, since the efficiency of the vegetative propagation interferes on the production and commercialization of ornamental plants.

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