Different propagules and auxin concentration on rooting of passionflower sandbank

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ABSTRACT

Passiflora mucronata Lam. is resistant to *Fusarim oxysporum* f. *passifloraceae* and therefore can be used as rootstock for the species *Passiflora edulis* Sims. f. *flavicarpa*. The rootstocks in this case can be vegetatively propagated through cuttings. The objective of this study was to evaluate the effect of different types of cuttings and different concentrations of indole-3-butyric acid (IBA) on the adventitious rooting of *P. mucronata*. The experiments were arranged in a completely randomized design with four replications of 16 cuttings each. In Experiment 1, the treatments consisted of the different types of cuttings from mother plants grown in protected environment: shoot tips; two leaves and two nodes; one leaf and one node; leafless with two nodes; leafless with one node. In Experiment 2, the cuttings were taken from field plants and treated with the following IBA concentrations: 0; 1000 mg kg^{-1} (0.0036456 mol L^{-1}); 1500 mg kg^{-1} (0.0054684 mol L^{-1}) and 2000 mg kg^{-1} (0.0072912 mol L^{-1}). The characteristics evaluated in the experiments 1 and 2 were: survival (1 and 2); budding (1 and 2); shoot number (2), shoot length (2), number of shoot leaves (2);shoot dry mass (2); callusing (1); rooting (1 and 2); root number (1 and 2), largest root length (1 and 2), root volume (1 and 2), and root dry mass (1 and 2). We found that, instead the shoot tips, the cuttings of the type leafless with two nodes are the best for *P. mucronata* rooting. Neither the cuttings from greenhouse plants nor the cuttings from field plants require growth regulators for rooting of *P. mucronata* cuttings of the type leafless with two nodes.

Keywords: *Passiflora mucronata* Lam.; vegetative propagation; cutting; auxin.

RESUMO

Diferentes propágulos e concentrações de auxina no enraizamento de maracujazeiro-de-restinga

A espécie *Passiflora mucronata* Lam. é identificada como resistente ao *Fusarim oxysporum* f. *passifloraceae e*, portanto, pode ser utilizada como porta-enxerto para a espécie *Passiflora edulis* Sims. f. *flavicarpa* Deg., e a obtenção desses porta-enxertos pode dar-se por propagação vegetativa por estaquia. Objetivou-se, com este trabalho, avaliar diferentes tipos de estacas e diferentes concentrações do ácido indol-3-butírico (AIB), no enraizamento adventício de *P. mucronata*. Os experimentos foram realizados em delineamento inteiramente casualizado, com quatro repetições, de 16 estacas cada. No primeiro experimento, os tratamentos foram os diferentes tipos de estacas de plantas-matrizes de ambiente protegido: apical; de duas folhas e dois nós; de uma folha e um nó; sem folhas e dois nós; sem folhas e um nó. No segundo experimento, as estacas foram retiradas de campo e os tratamentos foram as concentrações de AIB: 0; 1000 (0,0036456 mol L^{-1}); 1500 (0,0054684 mol L^{-1}) e 2000 mg kg^{-1} (0,0072912 mol L^{-1}). As características avaliadas no primeiro (1º) e no segundo (2º) experimentos foram: sobrevivência (1º e 2º); brotação (1º e 2º); número (2º), comprimento (2º),

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número de folhas (2º) e massa seca do broto (2º); calejamento (1º); enraizamento (1º e 2º); número (1º e 2º), comprimento (1º e 2º), volume (1º e 2º) e massa seca de raiz (1º e 2º). Recomenda-se usar estacas sem folhas e com dois nós, e não as apicais, para o processo de enraizamento de *P. mucronata*. O enraizamento das estacas sem folhas e com dois nós de *P. mucronata* dispensa o uso de reguladores de crescimento, tanto para as estacas provenientes de casa de vegetação quanto para as de campo.

Palavras-chave: *Passiflora mucronata* Lam.; propagação vegetativa; estaquia; auxina.

INTRODUCTION

Passiflora mucronata Lam. has been detected in the predominantly sandbank vegetation in the states of Espírito Santo and Rio de Janeiro (Magnago *et al*., 2011). This species is best adapted to more fertile soils (Lawrence Junior *et al*., 2013). Morphologically, it is considered an herbaceous (non-woody) vine with slender stem, twining, with touch-sensitive tendrils that coil around a support (Barros et al., 2009). The species *P. mucronata* has great ornamental potential such as phosphorescent white flowers, with nocturnal anthesis for bat pollination, intense and almost continuous all year flowering (Meletti *et al*., 2011).

The species has medicinal properties and is used as sedative to treat insomnia, calming agent, anthelmintic and anti-hemorrhoidal (Boscolo & Valle, 2008). Agriculturally, it stands out for its resistance to leaf bacterial blight and to be highly resistant to anthracnose of fruits and branches (Junqueira *et al*., 2005).

Passifloraceae species are kept in in field germplasm banks , and can be propagated by seeds or vegetatively, depending on the species.

The success of the propagation by cuttings depends on the capacity of forming roots, the quality of the root system and the subsequent plant development. Among the exogenous factors that affect rooting, the physiological conditions such as hormonal balance are considered the most important (Fachinello *et al*., 1995). However, morphological factors such as the presence or absence of nodes and leaves in the cuttings are also important. According to Hartmann *et al*. (2002), leaves and buds are sites of carbohydrates and auxin synthesis, respectively, which are key factors in stimulating the initiation of roots.

There is little literature information on the vegetative propagation of *P. mucronata*: we found only one published study with grafting reporting that the combination scion-rootstock (*P.edulis* f. *flavicarpa*/*P. mucronata*) resulted in over 90% graft success, greater shoot height and greater internode growth than with the rootstocks *P. alata*, *P. cincinnata* Mast., *P. gibertii* N.E.Br., with autograft (*P. edulis* f. *flavicarpa* / *P. edulis* f. *flavicarpa*) and the ungrafted *P. edulis* f. *flavicarpa* (Morgado *et al*., 2015). In a study using mini-grafting, *P. mucronata* was grafted on *P. edulis* f. *flavicarpa* with 80% graft success (Alexandre *et al*., 2013a), showing the new method efficiency.

The use of *P. mucronata* as rootstock for species *P. edulis* f. *flavicarpa* is very important, considering its resistance to *Fusarim oxysporum* f. *passifloraceae* (Oliveira *et al*., 2013). Rootstocks can be obtained by seeds or vegetative propagation, the latter being very important when resistant individuals are detected and the intention is to maintain their characteristics. Propagating *P. mucronata* vegetatively is important for several factors: the species does not bear fruit all year round; it has a small number of seeds per fruit; and its recalcitrant seeds (Santos *et al*., 2012a). According to Santos *et al*. (2012b), germination decreases over time, from zero to four months of storage.

Regarding the propagation by cuttings, only Alexandre *et al*. (2014) investigated the rooting ability of native *P. mucronata* plants, thus the need of more studies on the vegetative propagation of this species as an alternative of clonal rootstocks for *P. edulis* f. *flavicarpa*. Therefore, the objective of this study was to evaluate the effect of different types of cuttings and different concentrations of indole-3-butyric acid (IBA) on the adventitious rooting of *P. mucronata*.

MATERIAL AND METHODS

The experiments were conducted in a greenhouse at the Experimental Farm of the Centro Universitário Norte do Espírito Santo (CEUNES), belonging to the Universidade Federal do Espírito Santo (UFES). Two experiments were carried out: the first, developed with plant cuttings grown in a greenhouse and the second, with cuttings of field plants.

Experiment 1: Adventitious rooting of different types of adult cuttings from *P. mucronata* cultivated in greenhouse

The plant material was obtained from *Passiflora mucronata* mother plants derived from vegetative propagation and grown in a greenhouse of the CEUNES/ UFES. This experiment complements the study developed by Alexander *et al*. (2014), with cuttings grown in the greenhouse.

The treatments consisted of different types of cuttings: shoot tips; two leaves and two nodes; one leaf and one node; leafless with two nodes; leafless with one node. In this experiment, the cuttings were not treated with phytohormones. The leaves of the cuttings had their limbs halved and their botton ends were cut into a simple bevel.

Experiment 2: Adventitious rooting of mature cuttings from field plants of *P. mucronata* and treated with IBA

In this experiment, the type of cutting was chosen based on the results from experiment 1, leafless cuttings with two nodes.

The treatments consisted of different concentrations of IBA [0; 1000 mg kg^{-1} (0.0036456 mol L^{-1}); 1500 mg kg^{-1} (0.0054684 mol L^{-1}) and 2000 mg kg^{-1} (0.0072912 mol L^{-1})], using inert talc as vehicle, applied to the simple beveled base of leafless cuttings with two nodes.

In both experiments, the cuttings were planted in Bioplant® substrate filled into black polyethylene trays with the following cell dimensions: 54.4 cm long; 28.8 cm width; 12.5 cm and six-liter volume, divided into 32 equal sized cells.

The experiments were arranged in a completely randomized design with four replications of 16 cuttings each, totaling 64 cuttings per treatment. The characteristics evaluated in experiments 1 and 2 were: survival (1 and 2); budding (1 and 2); shoot number (2), shoot length (2), number of shoot leaves (2); shoot dry mass (2); callusing (1); rooting (1 and 2); root number (1 and 2), largest root length (1 and 2), root volume (1 and 2), and root dry mass (1 and 2).

Data were examined by analysis of variance and the means of the different types of cuttings (shoot tips; two leaves and two nodes; one leaf and one node; leafless with two nodes; leafless with one node (experiment 1)) were compared by the Tukey test and phenotypic correlation. The different concentrations of IBA [0; 1000 mg kg^{-1} (0.0036456 mol L^{-1}); 1500 mg kg^{-1} (0.0054684 mol L^{-1}) and 2000 mg kg^{-1} (0.0072912 mol L^{-1})] (experiment 2) were subjected to regression analysis, and the models were selected by the biological behavior and significance of betas, at 5% probability using the Genes software (Cruz, 2013).

RESULTS AND DISCUSSION

Experiment 1: Adventitious rooting of different types of adult cuttings from *P. mucronata* cultivated in greenhouse

The statistical analysis showed significant differences ($p > 0.01$) between the means of the different cuttings for all but one characteristic (survival).

The leafless cuttings with two nodes of *P. mucronata* showed the highest survival (90.6%), rooting (85.9%), root number (6.6), largest root length (8.0 cm) and more budding (85.9%) (Table 1), which show its potential for the production of plants, considering its greater efficiency in the quality of the root system (root number, root length, root volume and root dry mass per cutting) and the production of buds for shoot formation. The ability of this species to root without leaves on the cuttings is a very important characteristic, for preventing the cutting water loss by dehydration, which compromises their survival, with the advantage of not needing an irrigation system, by intermittent misting, which increases costs of the process. With the species *Ligustrum sinense* Lour. the presence of leaves in the cuttings is not necessary for the adventitious rooting process (Bona et al., 2002).

According to Alexandre *et al*. (2014), cuttings with one leaf and one node of *P. mucronata* grown in a greenhouse had 65.47% rooting and 2.36 roots with 5.98 cm long, without using growth regulators. In this work, rooting of *P. mucronata* cuttings with one leaf and one node showed 59.3% rooting and 2.1 roots with 4.8 cm long without application of auxin (Table 1). However, Alexandre *et al*. (2014) reported that the use of IBA increased linearly up to the concentration of 1000 mg kg^{-1} (or 0.0036456 mol L^{-1}), the rooting (86.07%), number (12.26) and the length (8.48 cm) of roots.

The leafless cuttings with two nodes showed less callusing on its bottom end (12.5%), indicating that the rooting process had more direct than indirect effect (Table 1). However, there is the assumption that, in shoot tip cuttings, the large amount of calli (70.3%) formed on the bottom end (Table 1) is of the non-friable type, which does not allow the formation of organogenic structures, either from vegetative origin or, the more expected, from root origin. This cell disruption can interfere with the rooting process by constricting the xylem and phloem and thus restraining root emission, even for longer time than the cutting survival.

Rooting of leafless cuttings with two nodes of *P. mucronata* is possible without growth regulators (Table 1). Stem cuttings of ‘Boysenberry’ (hybrid between ‘Marionberry’ and raspberry) also need not be treated with IBA to stimulate adventitious rooting (Tiberti *et al*., 2012). The rooting of blackberry root cuttings also occurs without the use of IBA (Campagnolo & Pio, 2012). According to Silva *et al*. (2012), stem cuttings of raspberry give better results than root cuttings and do not require treatment with IBA. In other species such as *Pinus taeda* (Alcantara *et al*., 2008) and *Rubus fruticosus* L. (Tadeu *et al*., 2012), the rooting percentage decreases with the supply of IBA.

Corroborating our findings, cuttings without and with leaves of other species, for example, *Dalbergia sissoo*, presented 72.0% and 35.0% of rooting, respectively (Singh *et al*., 2012). In other Passifloraceae species such as *P. actinia* Hook., *P. serrato-digitata* L., and *P. setacea* DC, the presence of two or three nodes did not result in statistically significant differences in the number of rooted and budded cuttings (Braga *et al*., 2006). On the other hand, cuttings with three leaves and three nodes of *P. nitida* Kunth, *P. giberti*, *P. edulis*, and *P. setacea* presented 21.32; 37.21; 26.48 and 6.76% of rooting, respectively, without the use of auxin (Roncatto *et al*., 2008).

The cuttings with two leaves and two nodes, despite the low rooting percentage (58.3%), had one of the best results for root number (6.0), root volume (0.77 cm^3), and root dry mass (0.73g) and were not significantly different from the leafless cuttings with two nodes (Table 1). The high response of volume and dry mass of roots found in cuttings with two leaves and two nodes is probably due to the presence of leaves on the cutting, which maintains the photosynthetic activity and produces assimilates for the formation and growth of the root system and also promotes the growth of secondary roots, increasing its volume.

In african mahogany (*Khaya anthotheca* (Welw.) C.DC. and *K. ivorensis* A. Chev., cuttings with one node and leaves of larger leaf area (30-50 cm^2) showed greater rooting (Opuni-Frimpong *et al*., 2008). Santos *et al*. (2012a) reported that *P. cincinnata* cuttings with two or three knots and half of the leaf per cutting planted in Bioplant® substrate showed the greatest root length. The same substrate was used in this study with *P. mucronata* species and was also efficient for the growth of roots (Table 1).

The phenotypic correlation coefficients of the characteristisc in Table 2 were rated on the basis of the scales given by Carvalho *et al*. (2004) as follows: budding (%) vs. rooting (%) with f = 0.992, as very strong; budding (%) vs. root number with f = 0.927, as very strong; budding (%) vs. mean root length (cm) with rf = 0.995, as very strong; and budding (%) vs. root volume (cm^3) with rf = 0.804, as strong. These results allow us to infer that for the experimental conditions applied, the increase of budded cuttings also increases rooting and the number, length and volume of roots. Moreover, through visual observation (bud emissions), it is possible to predict the rooting behavior of *P. mucronata* cuttings nondestructively, which is very important to ensure the success of the propagation process.

Thus, it is likely that the production of buds has not competed for the carbohydrate sources of cuttings and

Table 1: Survival (SUR,%), callusing (CALL, %), rooting (ROOT,%), number of roots per cutting (NRC), length of largest root (LLR, cm), shoots (SHOOT, %), root volume (RV, cm^3), and root dry mass (MDM, g) for different types of *P. mucronata* cuttings

	Characteristics evaluated							
Cuttings	**SUR (%)**	**CALL (%)**	**ROOT (%)**	**NRC**	**LLR (cm)**	**SHOOT (%)**	**RV (cm^3)**	**MDM (g)**
Apex	87.5 a*	70.3 a	17.1 c	0.5 b	2.1 c	1.5 b	0.063 d	0.041 c
2 leaves and 2 node	93.7 a	45.8 a	58.3 b	6.0 a	5.1 b	64.5 a	0.77 a	0.73 a
1 leafand 1 node	87.5 a	56.2 a	59.3 ab	2.1 b	4.8 b	25.0 b	0.41 bc	0.35 bc
Without leavesand 2 node	90.6 a	12.5 b	85.9 a	6.6 a	8.0 a	85.9 a	0.59 ab	0.47 ab
Without leavesand 1 node	75.0 a	8.3 b	70.8 ab	2.3 b	5.6 ab	68.7 a	0.23 cd	0.092 c
SDM	20.8	31.5	27.2	3.1	2.6	32.3	0.27	0.316
CV (%)	10.9	37.3	21.4	41.1	23.3	30.1	30.9	42.84

* Means followed by the same letter in the column are not significantly different by the Tukey test at 5% probability.

Table 2: Phenotypic correlation coefficients between the characteristics survival (SUV,%), callusing (CALL, %), rooting (ROOT,%), number of roots per cutting (NRC), length of largest root (LLR, cm), shoots (SHOOT, %), root volume (RV, cm^3), and root dry mass (RDM, g) with different types of *P. mucronata* cuttings

Characteristics evaluated	**SUV (%)**	**CALL (%)**	**ROOT(%)**	**NRC**	**LLR (cm)**	**SHOOT (%)**	**RV (cm^3)**	**RDM (g)**
SUV (%)	-	0.678^{ns}	-0.571^{ns}	-0.149^{ns}	-0.507^{ns}	-0.508^{ns}	0.088^{ns}	0.475^{ns}
CALL (%)		-	-0.987^{**}	-0.826^{*}	-0.975^{**}	-0.976^{**}	-0.659^{ns}	-0.306^{ns}
ROOT (%)			-	0.891^{**}	0.996^{**}	0.992^{**}	0.757^{*}	0.459^{ns}
NRC				-	0.922^{**}	0.927^{**}	0.963^{**}	0.816^{*}
LLR (cm)					-	0.995^{**}	0.797^{*}	0.508^{ns}
SHOOT (%)						-	0.804^{*}	0.528^{ns}
RV (%)							-	0.952^{**}
RDM (g)								-

^{ns}Non significant, *, **significant at 5 and 1% probability by the t test, respectively.

has not caused a negative effect on adventitious rooting. Bud emission is actually an important signal to measure the efficiency of *P. mucronata* cuttings in producing and promoting root growth. In addition, the meristematic region of shoots and buds is the site of auxin synthesis, which can be redirected to the bottom end of the cuttings and promote adventitious rooting (Taiz & Zeiger, 2009).

Experiment 2: Adventitious rooting of mature cuttings of *P. mucronata* field plantstreated with IBA

Cuttings of *P. mucronata* field plants treated with IBA had lower survival rates, lower rooting, and smaller length and root dry mass (Figure 1A, B, D and E, respectively). Salomão *et al*. (2002) found for basal and medium cuttings

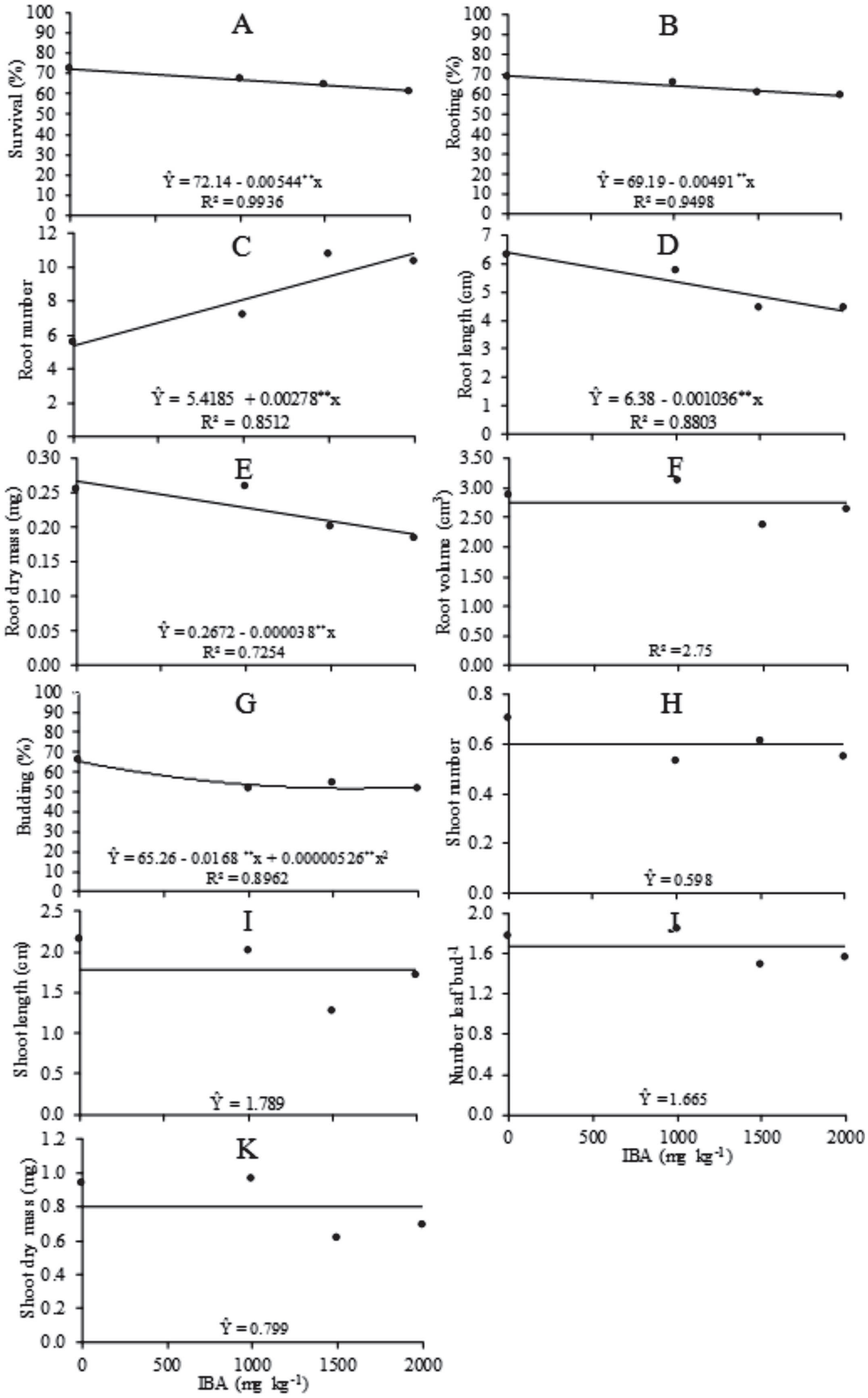

Figure 1: Survival (%, A), rooting (%, B), root number (C), root length (cm, D), root dry weight (mg, E), root volume (cm^3, F), budding (%, G), shoot number (H), shoot length (cm, I), number leaf bud^{-1} (J) and shoot dry mass (mg, K) of *P. mucronata* cuttings treated with IBA. **Significant at 1% probability.

that were not treated with auxins rooting percentages of 93 and 94%, for *P. alata*, and 96% for *P. edulis* f. *flavicarpa*, respectively. Alexandre *et al*. (2013b) and Sousa *et al*. (2014) reported that IBA is indispensable for rooting cuttings of *P. alata*. In *Ficus carica* L. there is also no need of IBA for the rooting of cuttings (Sousa *et al*., 2013). Kollaróva *et al*. (2005) corroborate the finding of this study when they reported that galactoglucomannans oligosaccharides inhibited the length of adventitious roots in mung bean treated with indole acetic acid (IAA). According to these authors, this fact is dependent on the type of the auxin. Mutui *et al*. (2010) found that IBA reduced the total growth of roots and the root fresh weight in *Pelargonium zonale* (L.) L'Hér. ex Aiton, which may be related to reduction in ethylene biosynthesis.

The IBA concentrations did not affect the root volume (Figure 1F). Baul *et al*. (2011) observed that the root number and length of the largest root of *Litsea monopetala* (Rouxb) cuttings was not significantly different for the different concentrations of IBA. However, the root number was higher with increasing concentrations of IBA, and, in the absence of IBA or at the concentration of 2000 mg kg^{-1} (0.0072912 mol L^{-1}) the production was 5 and 10.97 roots, respectively (Figure 1C). We found that the use of IBA is harmful and not necessary for *P. mucronata* cuttings mainly based on the characteristics mass and volume of roots (Figure 1E and F, respectively).

The percentage of budding in *P. mucronata* cuttings was lower when using IBA (Figure 1G). The number and length of shoots, number of leaves per shoot and shoot dry mass were not affected by the treatment of *P. mucronata* cuttings with IBA (Figure 1 H, I, J and K, respectively). The shoot characteristics were not benefited by the use of auxin, which may have contributed to reduce the endogenous cytokinin/auxin ratio.

CONCLUSIONS

Leafless cuttings with two nodes are recommended for the propagation of *P. mucronata*.

The rooting of leafless cuttings with two nodes of *P. mucronata* is possible without the use of growth regulators.

The use of shoot tip cuttings is not recommended for adventitious rooting of *P. mucronata*.

IBA is dispensable for the production of *P. mucronata* plants from cuttings.

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