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Rhizogenic behavior of black pepper cultivars to indole-3-butyric acid

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ABSTRACT. Little information is available regarding vegetative propagation of the species *Piper nigrum* L. to generate technical recommendations for the production of seedlings on a commercial scale. The purpose of this study was to investigate the rhizogenic behavior of cultivars of this species regarding indol-3-butyric acid (IBA). The experiment was performed at a vegetation house equipped with an intermittent nebulization irrigation system. The experimental site was located in the University Center of Northern Espírito Santo (CEUNES) of the Federal University of Espírito Santo (UFES), Brazil. The experimental design consisted of randomized complete blocks design arranged in a 3 x 5 factorial scheme: three cultivars (Bragantina, Iaçará and Guajarina) x five IBA concentrations (0; 1,500; 3,000; 4,500 and 6,000 mg kg^{-1}), with four repetitions of 16 cuttings each. Total immersion of the cuttings in IBA is recommended for the Iaçará and Guajarina cultivars, and immersion of only the basal region is recommended for cv. Bragantina. The recommended IBA concentration for these cultivars is 4,000 mg kg^{-1}.

Keywords: *Piper nigrum* L., genotypes, propagation, cutting.

Comportamento rizogênico de cultivares de pimenta-do-reino ao ácido indol-3-butírico

RESUMO. Há pouca informação disponível sobre a propagação vegetativa da espécie *Piper nigrum* L. para gerar recomendações técnicas para a produção de mudas em escala comercial. O objetivo deste estudo foi o de investigar o comportamento rizogênico de cultivares desta espécie em relação ao indol-3-butírico (IBA). O experimento foi realizado em casa de vegetação equipada com sistema de irrigação do tipo nebulização intermitente. A área experimental foi localizada no Centro Universitário do Norte do Espírito Santo (CEUNES) da Universidade Federal do Espírito Santo (UFES), Brasil. O delineamento experimental foi o de blocos completos casualizados, dispostos em esquema fatorial 3 x 5: três cultivares (Bragantina, Guajarina e Iaçará) x cinco concentrações de AIB (0, 1500, 3000, 4500 e 6000 mg kg^{-1}), com quatro repetições de 16 estacas cada. A imersão total das estacas em AIB é recomendado para as cultivares Iaçará e Guajarina, e a imersão de apenas a região basal é recomendado para a cv. Bragantina. A concentração de AIB recomendada para essas cultivares é de 4000 mg kg^{-1}.

Palavras-chave: *Piper nigrum* L., genótipos, propagação, estaquia.

Introduction

In Brazil, black pepper, in addition to being a valuable spice, has shown other applications in research as a larvicide to fight *Aedes aegypti* (SIMAS et al., 2007; GRZYBOWSKI et al., 2012) and for the bactericide properties of the essential oil of its seeds (TRAJANO et al., 2009).

Black pepper is very important both socially and economically for the northern region of the State of Espírito Santo, especially for its principal producing city, São Mateus. In this State, black pepper is grown on 2,725 ha, producing up to 6,371 ton, with an average yield of 2,723 kg ha^{-1}; this State is second in the national production ranking, surpassed only by the State of Pará, where black pepper production occupies an area of 17,605 ha, producing up to 38,472 ton, with an average yield of 2,185 kg ha^{-1} (IBGE, 2011). The position of the State of Espírito Santo relative the State of Pará, even though the planted area is significantly smaller, still indicates that we can improve in a few aspects, including the quality of seedlings. Improving the seedling quality starts by selecting the vegetal material and implies the maintenance of the mother plant characteristics, such as high productivity, vigor, absence of diseases, etc.

Black pepper is propagated through cuttings, which, unlike sexual reproduction, maintain an identical genetic constitution in the descendants of the mother plant, with essentially no signs of segregation and genetic combination (ARAÚJO;

BRUCKNER, 2008). Vegetative propagation is an important strategy to improve plants because it reduces the time spent in this process. Among the various methods of vegetative propagation, cutting is the most commonly used because it is an easy technique; thus, it is applied to multiply selected genotypes to maintain the characteristics of the matrix plant, reduce the juvenile period, and increase the plant's production (HARTMANN et al., 2011). Vegetative propagation process through cutting occurs via the appearance of adventitious roots, defined as post embryonic, which appear from the stem and leaves. Various exogenous and endogenous factors regulate the formation of adventitious roots, including auxins, Ca^{2+}, sugars, polyamines, ethylene, nitric oxide, hydrogen peroxide, carbon monoxide, cyclic guanosine monophosphate (cGMP), mitogen-activated protein kinase (MAPKs), peroxidase, phenols, etc. Such mediators seem to function in the signalization route during adventitious root formation (QADDOURY; AMSSA, 2003; LI et al., 2009; LI; XUE, 2010; LIMA et al., 2011a).

Auxins have aided in clonal propagation in various situations, such as declines in the formation of adventitious roots with increasing plant age (HUNT et al., 2011; SINGH et al., 2011), recalcitrant rooting genotypes (LEMAY et al., 2009) and low rooting during the cold seasons of the year (LEITE; MARTINS, 2007).

Only a few scientific papers can be found regarding the vegetative propagation of *P. nigrum* species (FRANCIS et al., 1993; THANUJA et al., 2002; THANKAMAN et al., 2008; SHARANGI; KUMAR, 2011). Although cutting is a routine technique among producers, no scientific research was found regarding the vegetative propagation of cvs. Bragantina, Iaçará and Guajarina.

Scientific information is scarce regarding this cloning technique and cultivars of *P. nigrum* in Brazil. Thus, the purpose of this research was to identify the ideal IBA concentration for rhizogenesis in cuttings of three cultivars that are economically significant in Northern Espírito Santo.

Material and methods

The work was conducted at a vegetation house equipped with an intermittent nebulization irrigation system at a relative humidity of 85 ± 5ºC. The site was part of the Biologic and Agricultural Sciences Department of the Northern Espírito Santo University Center (CEUNES) of the Federal University of Espírito Santo (UFES), São Mateus, Espírito Santo State. The experiment was conducted from 9/29/2011 to 11/30/2011.

Three-year-old orthotropic herbal branches from adult plants in the field were used for the cuttings, which were performed using a pruning shear. Each removed branch was immersed in a styrofoam box containing distilled water to keep the tissue hydrated and prevent desiccation and thus avoid compromising the quality of the grafts in the rooting process. After collecting a sufficient number of branches, the branches were immediately transported to the vegetation house. Next, the branches were sanitized; in this process, cuttings with two knots and one leaf were individualized, and a simple bezel was made in the basin of the selected cuttings. After preparing a sufficient number of cuttings, the cuttings, with the exception of the inert talc sheets, were completely soaked with different concentrations of indole-3-butyric acid (IBA). For each treatment, the auxin was weighed on an analytical balance and immediately mixed with inert talc. After each stake was treated for three seconds with different concentrations of the auxin and inert talc formulation, the cuttings were planted in polyethylene trays containing vermiculita (Dimy®, Paraná State, Brazil) substrate [particle size distribution (%): > 4.75 mm (1.32%), 2.00 at 4.75 mm (17.14%), 1.00 at 2.00 mm (26.10%), 0.5 at 1.00 mm (22.19%), 0.25 at 0.5 mm (16.05%) and < 0.25 mm (17.21%)]. The substrate was irrigated before the cuttings were planted and was subsequently irrigated by the irrigation system. The cuttings were cultivated in a covered vegetation greenhouse for a period of 60 days.

The characteristics assessed in this experiment were survival (%); nodal, basal and total rooting (%); number of nodal, basal and total roots; length of nodal, basal and total roots (cm); volume of nodal, basal and total roots (cm^3); and dry matter mass from the nodal, basal and total roots (mg).

The design of the experiments used randomized blocks with a 3 x 5 factor system: three cultivars (Bragantina, Iaçará and Guajarina) x five IBA concentrations (0; 1,500; 3,000; 4,500 and 6,000 mg kg^{-1}), with 4 repetitions of sixteen cuttings each.

The averages of the variables were statistically analyzed using the F test, regression test and Tukey average test. The analyses were performed using the GENES (CRUZ, 2013) computer program.

Results and discussion

The Bragantina, Iaçará and Guajarina cultivars presented a high survival capacity of more than 99.3% with the use of an estimated IBA concentration of 3561 mg kg^{-1} (Figure 1). Sharangi et al. (2010) achieved a higher survival rate in *P. nigrum* for median cuttings with two knots treated with 100 mg L^{-1} of IBA. Working with the *P. amplum*, *P. arboreum* and *P.* sp., Magevski et al. (2011) also observed high rates of survival (98, 100 and 89%, respectively) regardless of the IBA concentration. Various factors are responsible for the maintenance of live cuttings during the adventitious rooting process, including genotype; high rooting percentage; the quality of the radicular system, including the number, volume and dry matter mass; and other factors, such as tissue consistency and reduced contamination of the vegetal material from the field, its proper disinfection or even the physical structure and the irrigation system of the greenhouse. According to Mateja et al. (2007), the physiologic stress of the vegetal material can be minimized by using an intermittent nebulization irrigation system to enable a higher survival rate, as observed in this paper.

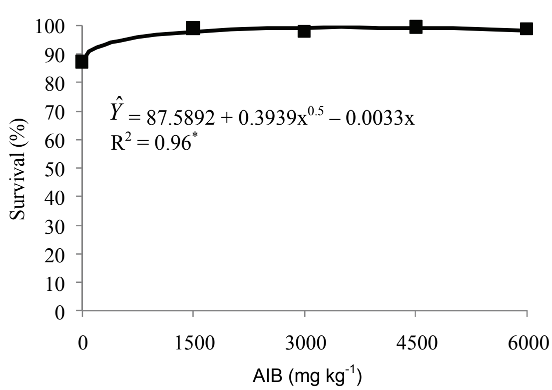


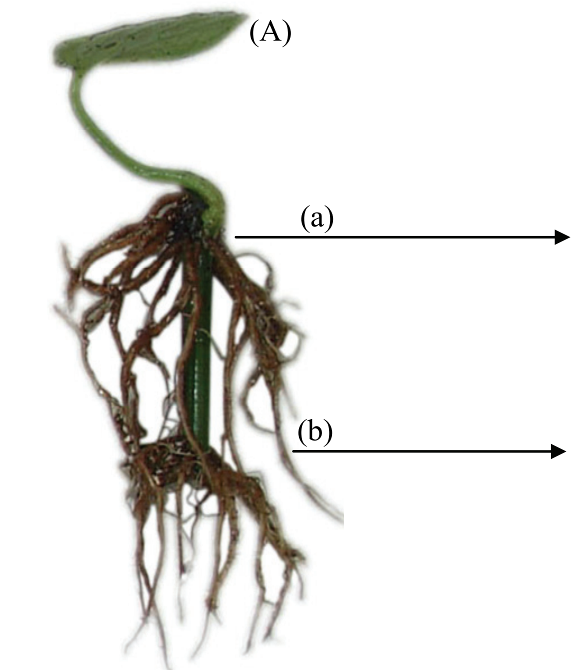
Figure 1. Survival (%) of *Piper nigrum* cvs. Bragantina, Iaçará and Guajarina cuttings treated with different concentrations of IBA. São Mateus, Espírito Santo State, 2012. *Significant at 5% probability by the F test.

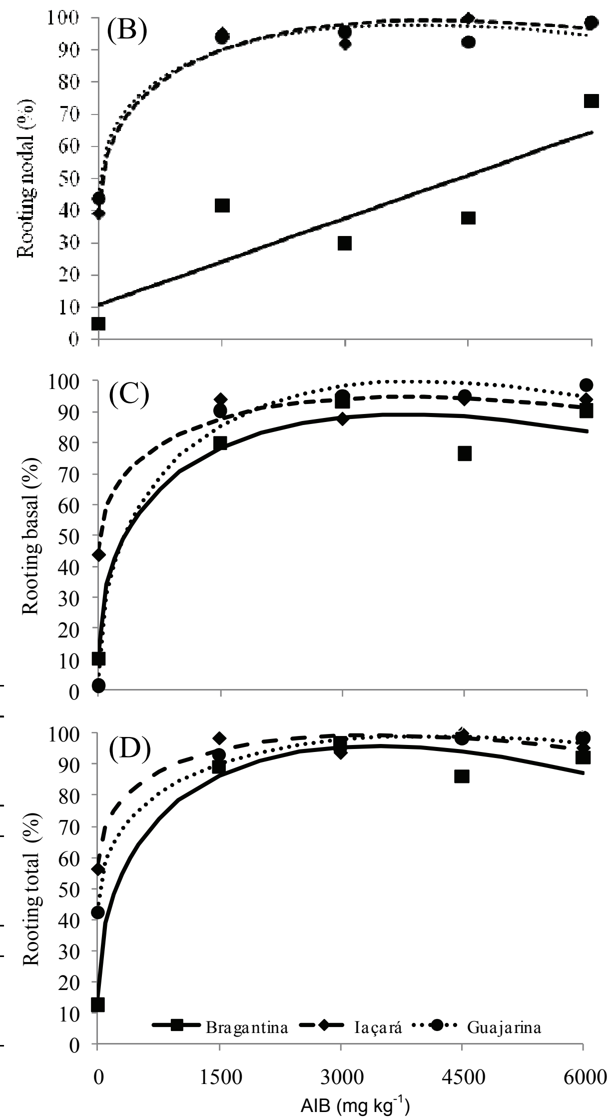
Another factor that may be beneficial for the survival of *P. nigrum* cultivar cuttings is the presence of potential endodermal cells in the stem, as observed in other Piperaceous species, such as *Piper aduncum* (VIANNA; AKISUE, 1997), *Piper crassinervium* (ALBIERO et al., 2004), *Piper diospyrifolium*, *Peperomia dahlstedtii* (SOUZA et al., 2004) and *Peperomia parnassiflora* (OLIVEIRA et al., 2008). According to Mauseth (1988), these endodermic cells are important for controlling the water output cortex through foliar transpiration or water input provoked by the root pressure. This occurs mainly in the case of an abrupt loss of the leaves or during the preparation of the cuttings through the reduction of the foliar limbs to induce adventitious rooting, as observed in the present paper.

In *P. nigrum*, roots appear in the nodal area (leaf axil) (Figure 2Aa) and basin area slightly above the cut region (Figure 2Ab). In the Bragantina, Iaçará and Guajarina cultivars, the nodal regions of the cuttings present preformed roots, as was also observed by Souza et al. (2009) for the species *Piper amalago* and other species, such as *Citrus medica* and *Ribes* sp., which present latent preformed primitive roots that will form roots when placed in favorable conditions (FACHINELLO et al., 2005). The development of axillary gems is regulated by a positive signal produced by the roots, along with the inhibitive influences of the preexisting apex gems (apex dominance) (THOMAS; HAY, 2010). According to the authors, in the absence of nodal roots, the basal radicular system is unable to maintain the continuous growth of the sprout. According to Thomas and Hay (2008), stoloniferous plants present a physiologic link between the growth of nodal roots and the development of stem tissues.

Nodal rooting resulted in a crescent linear behavior for cv. Bragantina with maximum rooting (64.5%) achieved with 6,000 mg kg^{-1} of IBA (Figure 2B). This means that for this cultivar, higher IBA concentrations can increase the rooting in this region of the cutting. However, it would most likely be necessary to use even higher concentrations of the auxin to achieve maximum rooting, which may present an inconvenience. The Iaçará (98.7%) and Guajarina (91.8%) cultivars presented high percentages of nodal rooting that exhibited square root behavior and maxima at 3,351 and 3,866 mg kg^{-1} IBA, respectively (Figure 2B). Singh and Singh (1989) have also identified the node effect in *P. nigrum* cultivar Panniyur-1 cuttings, in which the presence of two nodes enabled maximum rooting. In other species, such as *Bambusa vulgaris,* nodal cuttings exhibited better rooting results when treated with 8,000 mg L^{-1} IBA (ISLAM et al., 2011). The presence of nodes in rose cuttings was also positive for adventitious rooting (PARK et al., 2010).

The basal rooting of the cuttings presented a square root behavior and maxima at 3,154; 3,166 and 3,351 mg kg^{-1}, with 88.5, 94.3 and 93.3% rooting for the Bragantina, Iaçará and Guajarina cultivars, respectively (Figure 2C). The most pronounced effect of IBA in the basal region of the Bragantina cultivar cuttings may have occurred due to the increased cellular plasticity in this location, possibly because of the increased biosynthesis of cellulase (RAVEN; CURTIS, 1975). This also means that if the IBA concentration is increased in the Bragantina cultivar to improve the effect in the nodal region, it can negatively interfere in the basal region, most likely due to phytotoxicity.





(B) Rooting nodal	R^2
$\hat{Y}_{Bragantina} = 10.7479 + 0.008963x$	0.73^{*}
$\hat{Y}_{Iaçará} = 39.9648 + 1.8609x^{0.5} - 0.0146x$	0.97^{*}
$\hat{Y}_{Guajarina} = 44.4511 + 1.7161x^{0.5} - 0.0138x$	0.97^{*}
(C) Rooting basal	R^2
$\hat{Y}_{Bragantina} = 10.5799 + 2.5613x^{0.5} - 0.0209x$	0.95^{*}
$\hat{Y}_{Iaçará} = 44.6172 + 1.6325x^{0.5} - 0.0133x$	0.95^{*}
$\hat{Y}_{Guajarina} = 2.3444 + 3.1052x^{0.5} - 0.0247x$	0.98^{**}
(D) Rooting total = (B) + (C)	R^2
$\hat{Y}_{Bragantina} = 13.1445 + 2.8224x^{0.5} - 0.0241x$	0.97^{*}
$\hat{Y}_{Iaçará} = 56.7599 + 1.4654x^{0.5} - 0.0126x$	0.96^{*}
$\hat{Y}_{Guajarina} = 42.6264 + 1.7527x^{0.5} - 0.0136x$	0.99^{**}

Figure 2. Rooted cutting of cv. Iaçará (A) with roots formed in the nodal (a) and basal (b) regions. Nodal (B), basal (C) and total (D=B+C) (%) rooting of cuttings of *Piper nigrum* cvs. Bragantina, Iaçará and Guajarina treated with different concentrations of IBA. São Mateus, Espírito Santo State, 2012. $^{**},^{*}$ Significant at 1 and 5% probability levels by the F test, respectively.

Total rooting in the absence of auxin was low for cvs. Bragantina (12%), Iaçará (56%) and Guajarina (42%) (Figure 2D and Table 1). Thanuja et al. (2002) also observed a low rooting value (25.75%) for the species *P. nigrum* cv. Panniyur-1 when growth regulators were not used. Cardoso and Santos (1981) reported that *P. nigrum* cuttings presented easy rooting but did not state whether growth regulators were needed.

In some Piperaceous plants, such as *Peperomia parnassifolia* species, vegetative propagation has been reported to occur naturally by stolon, defined as fresh plagiotropic stem aerial branches formed in the base of the plant. These stolons can be the origin of new individuals and may or may not detach from the mother plant (OLIVEIRA et al., 2008). In other species, vegetative propagation occurs through preexisting aerial adventitious roots in the nodal region of the vegetative branches, as observed by Souza et al. (2009) in the species *Piper amalago*. In such processes, the xylem and phloem, which are rich in amillaceous cellular content, are important for the emission and growth of the roots. The authors observed the same behavior for other Piperaceous plants, such as *Piper xylosteoides*, *Piper arboreum*, *Peperomia parnassiflora* and *Ottonia martiana*. A wide variety of amide grains have also been observed in the cells of the medullar parenchyma of *Piper crassenervium* (ALBIERO et al., 2004) and *Piper hispidum* (ALBIERO et al., 2006) stems.

It is observed that the use of IBA is important for the adventitious rooting of cuttings from all the studied cultivars of *P. nigrum*, with square root behavior and maxima at 3,428 (Bragantina), 3,381 (Iaçará) and 4,152 mg kg^{-1} IBA (Guajarina). The average rooting was 98.0% (Figure 2D). The smallest concentration of IBA employed (1,500 mg kg^{-1}) resulted in a high value of total rooting for Bragantina (89%), Iaçará (98.4%) and Guajarina (98.4%) cultivars (Table 1). There are relatively unsuccessful reports dated 50 years ago regarding the use of IBA-based products, such as Serradix® and Vigortone® (CARDOSO, 1961). Thanuja et al. (2002) achieved 60.58% rooting using 50 mg L^{-1} IBA. The IBA concentration depends on the species and also on the studied cultivar; for *Piper* sp., the adventitious rooting presented a linear behavior at the optimal concentration of 5,000 mg L^{-1} (87.5%) (SILVA et al., 2004). For *Piper mikanianum* var. *mikaniamum,* the ideal IBA concentration was 1,500 mg L^{-1} (65%) (PESCADOR et al., 2007), whereas for *P. nigrum* cv. Panniyur 1 (FRANCIS et al., 1993) and *P. longum* (SUNDHARAIYA et al., 2000), the ideal concentration was 1,000 mg L^{-1}, which resulted in a higher percentage of rooting and better quality of the radicular system.

In cocoa tree clones, different responses to IBA with regard to adventitious rooting have been reported (SANTOS JÚNIOR et al., 2008). Cuttings of *Arbutus unedo* and *Taxus baccata* treated with 8,000 and 10,000 mg L^{-1} (IBA, AIA and ANA) presented superior adventitious rooting in crescent order with IBA > AIA = ANA and IBA > AIA > ANA, respectively (METAXAS et al., 2004). *Pongamia pinnata* presented an efficiency of AIB > ANA > AIA (KESARI et al., 2009). The positive effect of IBA is most likely due to its higher chemical stability, and according to Fogaça and Fett-Neto (2005), this feature is related to rooting favoring, mainly in recalcitrant species. IBA was also superior to AIA and ANA in *Camellia sinensis* (ROUT, 2006), to ANA in *Tectona grandis* (HUSEN; PAL, 2007) and to AIA in *Cloezia buxifolia* and *Cloezia aquarum* (LEMAY et al., 2009). According to Husen and Pal (2007), IBA and ANA increased the total soluble sugar, amide, and protein levels and the peroxidase activity in the rooting zone. Sugar has also been observed to accumulate in the stem base of Ulmus vilosa (BHARDWAJ; MISHRA, 2005) and *Dianthus caryophyllus* (AGULLÓ-ANTÓN et al., 2011). According to Correa et al. (2011), the total levels of sugar and phenolic compounds and the peroxidase activity are important biochemical markers in the adventitious rooting process.

Table 1. Characteristics of the adventitious rooting of cuttings of *Piper nigrum* cvs. Bragantina, Iaçará and Guajarina treated with different concentrations of IBA. São Mateus, Espírito Santo State, 2012.

Caracteristics evaluated	Cultivars	AIB (mg kg^{-1})				
		0	1500	3000	4500	6000
Rootting nodal (%)	Bragantina	5.00 b	41.56 b	29.75 b	37.75 b	64.52 b
	Iaçará	39.25 a	95.25 a	92.00 a	100.00 a	98.50 a
	Guajarina	43.75 a	94.00 a	95.50 a	92.50 a	98.50 a
Rooting basal (%)	Bragantina	10.00 b	80.00 b	93.50 a	76.50 b	90.31 a
	Iaçará	43.75 a	93.75 ab	87.50 a	93.87 a	93.75 a
	Guajarina	1.50 b	98.50 a	98.50 a	100.00 a	100.00 a
Rooting total (%)	Bragantina	12.50 b	89.06 a	96.87 a	85.93 a	92.18 a
	Iaçará	56.25 a	98.43 a	93.75 a	100.00 a	95.31 a
	Guajarina	42.18 a	98.43 a	98.43 a	100.00 a	100.00 a
Number of roots nodal	Bragantina	0.05 b	2.07 b	0.70 c	1.03 c	1.01 c
	Iaçará	2.14 ab	7.27 a	13.20 a	16.12 a	14.69 a
	Guajarina	2.87 a	5.34 a	7.70 b	10.68 b	9.51 b
Number of roots basal	Bragantina	0.30 a	5.57 b	8.03 a	5.92 c	8.09 b
	Iaçará	1.31 a	10.30 a	10.95 a	11.23 b	10.57 ab
	Guajarina	0.01 a	8.93 a	9.30 a	15.23 a	12.52 a
Number of roots total	Bragantina	0.35 a	7.65 b	11.00 c	7.23 b	12.01 b
	Iaçará	3.45 a	17.57 a	24.15 a	27.35 a	25.26 a
	Guajarina	2.89 a	14.28 a	17.00 b	25.92 a	22.03 a
Length of the longest nodal root (cm)	Bragantina	0.12 b	3.21 b	8.85 b	3.01 b	5.93 c
	Iaçará	1.55 ab	7.33 a	9.04 a	9.47 a	7.86 b
	Guajarina	2.67 a	8.00 a	9.91 a	9.35 a	10.18 a
Total volume of roots (m^3)	Bragantina	0.0019 a	0.3384 b	0.5885 b	0.3865 b	0.9135 b
	Iaçará	0.0865 a	0.5577 b	0.7404 b	0.6539 b	0.5442 b
	Guajarina	0.0154 a	0.9327 a	1.2693 a	2.0096 a	1.5673 a
Dry mass of root nodal (mg)	Bragantina	0.0000 a	0.0150 b	0.0087 c	0.0177 c	0.0331 c
	Iaçará	0.0039 a	0.0566 a	0.1387 a	0.1435 a	0.1331 a
	Guajarina	0.0092 a	0.0419 ab	0.0665 ab	0.0735 b	0.0716 b
Dry mass of root basal (mg)	Bragantina	0.0021 a	0.0289 a	0.0614 a	0.0450 b	0.0594 a
	Iaçará	0.0039 a	0.0427 a	0.0519 a	0.0502 b	0.0704 a
	Guajarina	0.0000 a	0.0510 a	0.0510 a	0.0835 a	0.0612 a
Dry mass of root total (mg)	Bragantina	0.0021 a	0.0439 b	0.0700 c	0.0627 b	0.0925 b
	Iaçará	0.0077 a	0.0993 a	0.1906 a	0.1937 a	0.2035 a
	Guajarina	0.0092 a	0.0929 a	0.1175 b	0.1569 a	0.1327 b

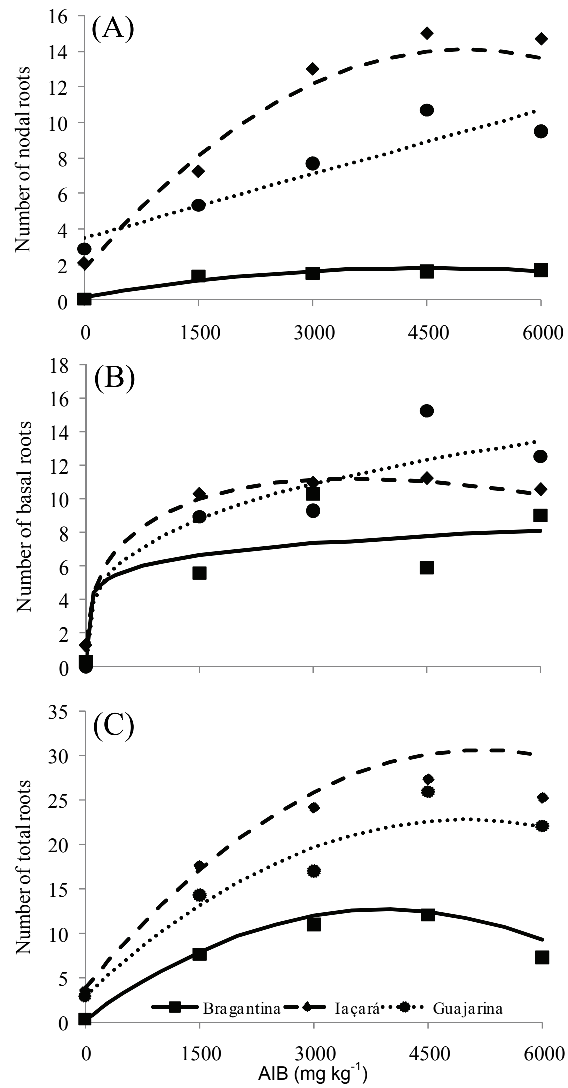
*Averages followed by the same letter in the column between cultivars within each IBA concentration are not different by the Tukey Test at a 5% significance level.

However, species such as *Sapium glandulatum* exhibit difficulties in the adventitious rooting process even when large IBA concentrations (8,000 mg kg^{-1}) are used (FERREIRA et al., 2009).

The quality of the radicular system is very important to the effectiveness of the plantlet's growth; an indicator of the quality of the radicular system is the number of roots. It was, therefore, observed that very few roots formed in the nodal region of the cuttings of cv. Bragantina (1.82), with the maximum at 4,531 mg kg^{-1} IBA (Figure 3A), while only 8 basal roots were formed when the highest IBA concentration (6,000 mg kg^{-1}) was used (Table 1 and Figure 3B). In *Psidium guajava*, a larger number of roots were also produced when higher concentrations of IBA (8,000 mg L^{-1}) were used (ABDULLAH et al., 2006). The higher number of roots of cv. Bragantina formed in the basal region of the cuttings indicates less waste of the auxin, as the immersion of the cuttings in powder can be performed for this region only. The same cannot be said for cvs. Iaçará and Guajarina, for which full immersion of the cuttings in powder is needed because 10 to 12 roots are generally formed in the nodal region (Table 1 and Figure 3A).

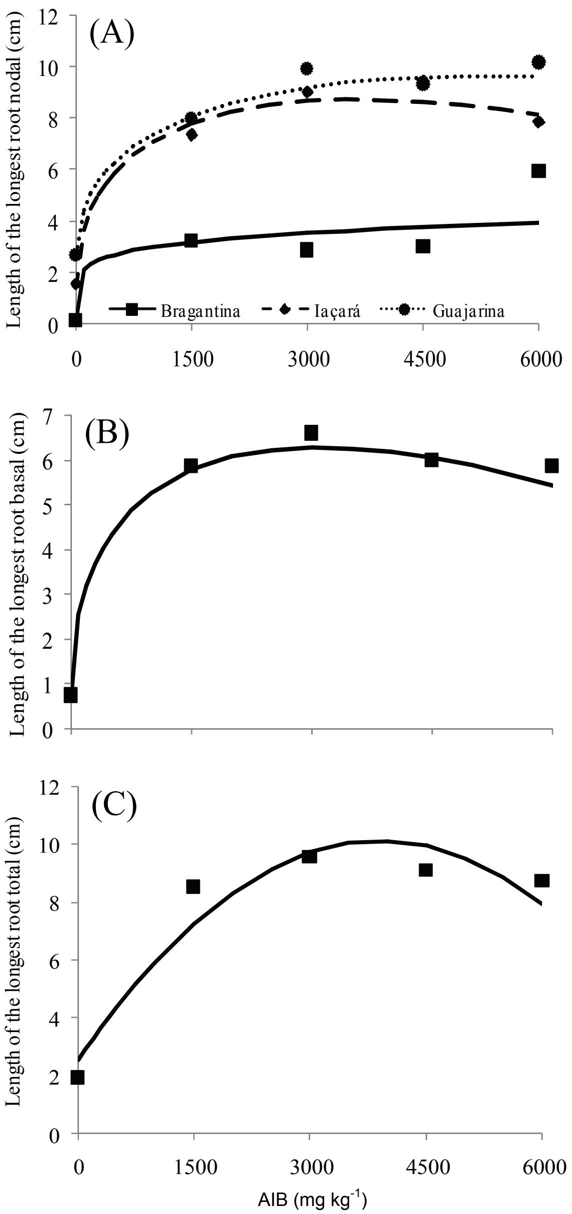
The largest total numbers of roots were 12, 30 and 22 at estimated IBA concentrations of 3,937; 5,187 and 4,980 mg kg^{-1} for cvs. Bragantina, Iaçará and Guajarina, respectively (Figure 3C). It is observed that cv. Iaçará presented the largest number and the highest total dry matter mass of roots for all the studied IBA concentrations (Table 1), which indicated that this cultivar is very responsive with regard to the formation of roots; furthermore, this responsiveness can be influenced by various aspects, such as the presence of new leaves, degree of lignification of the cuttings, amide, hormonal balance, etc.

The nodal roots of cv. Bragantina grew at lower rates than those of the other studied cultivars, but the longest length (4 cm) was achieved at an IBA concentration of 6,000 mg kg^{-1}. IBA also influenced the growth of *Camellia sinensis* L. (LIMA et al., 2011b) roots. According to Corrêa et al. (2005), auxins regulate the rooting process and carbohydrates increase the root growth. The Iaçará (8.73 cm) and Guajarina (9.64 cm) cultivars presented a higher growth rate for their roots, with maxima at 3,639 and 5,374 mg kg^{-1} IBA, respectively (Figure 4A). The length of the roots in the basal region of the cuttings presented square root behavior in all cultivars, with a maximum at 3,117 mg kg^{-1} IBA (6.29 cm) (Figure 4B). The total length (average length in the nodal and basal regions) of the largest root was 10.1 cm when an estimated IBA concentration of 3,900 mg kg^{-1} was used (Figure 4C). A paper published by Ansari and Singh (2008) reported an interaction among half-siblings in the family of *Albizia procera* Benth with IBA, with a significant increase in the root length of four out of seven families. In *Dalbergia melanoxylon*, cuttings treated with IBA presented a higher rooting percentage and a superior number and length of roots compared to untreated cuttings.



(A) Number of nodal roots	R^2
$\hat{Y}_{Bragantina} = 0.178286 + 0.000725x - 0.00000008x^2$	0.92^*
$\hat{Y}_{Iaçará} = 1.7668 + 0.004972x - 0.0000005x^2$	0.98^{**}
$\hat{Y}_{Guajarina} = 3.506 + 0.0012x$	0.86^*
(B) Number of basal roots	**R^2**
$\mathrm{Log}\hat{Y}_{Bragantina} = 0.3555 + 0.1467\log x$	0.97^{**}
$\hat{Y}_{Iaçará} = 1.3460 + 0.3320x^{0.5} - 0.0028x$	0.99^{**}
$\mathrm{Log}\hat{Y}_{Guajarina} = -0.0130 + 0.3019\log x$	0.99^{**}
(C) Number total roots = (A) + (B)	**R^2**
$\hat{Y}_{Bragantina} = 0.2406 + 0.0063x - 0.0000008x^2$	0.99^*
$\hat{Y}_{Iaçará} = 3.772571 + 0.010375x - 0.000001x^2$	0.99^{**}
$\hat{Y}_{Guajarina} = 2.9600 + 0.007968x - 0.0000008x^2$	0.94^*

Figure 3. Nodal (B), basal (C) and total (C=A+B) root numbers of cuttings of *Piper nigrum* cvs. Bragantina, Iaçará and Guajarina treated with different concentrations of IBA. São Mateus, Espírito Santo, 2012. **,* Significant at 1 and 5% probability level by the F test, respectively.

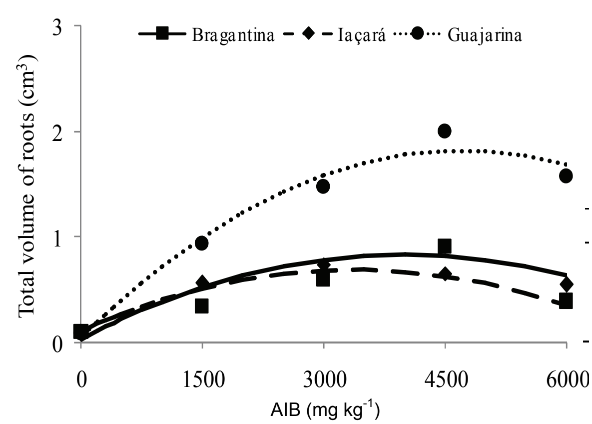


(A) Length of the longest root nodal	R^2
Log $\hat{Y}_{Bragantina} = 0.0142 + 0.1535\log x$	0.97^{**}
$\hat{Y}_{Iaçará} = 1.4609 + 0.2413x^{0.5} - 0.002x$	0.97^{*}
$\hat{Y}_{Guajarina} = 2.6631 + 0.1906x^{0.5} - 0.0013x$	0.98^{*}
(B) Length of the longest root basal	R^2
$\hat{Y} = 0.7343 + 0.2001x^{0.5} - 0.0018x$	0.99^{**}
(C) Length of the longest root total	R^2
$\hat{Y} = 2.532 + 0.0039x - 0.0000005x^2$	0.91^{**}

Figure 4. Length of the largest nodal (B), basal (C) and total (C=A+B) (cm) roots of cuttings of *Piper nigrum* cvs. Bragantina, Iaçará and Guajarina treated with different concentrations of IBA. São Mateus, Espírito Santo State, 2012. **,* Significant at 1 and 5% probability level by the F test, respectively.

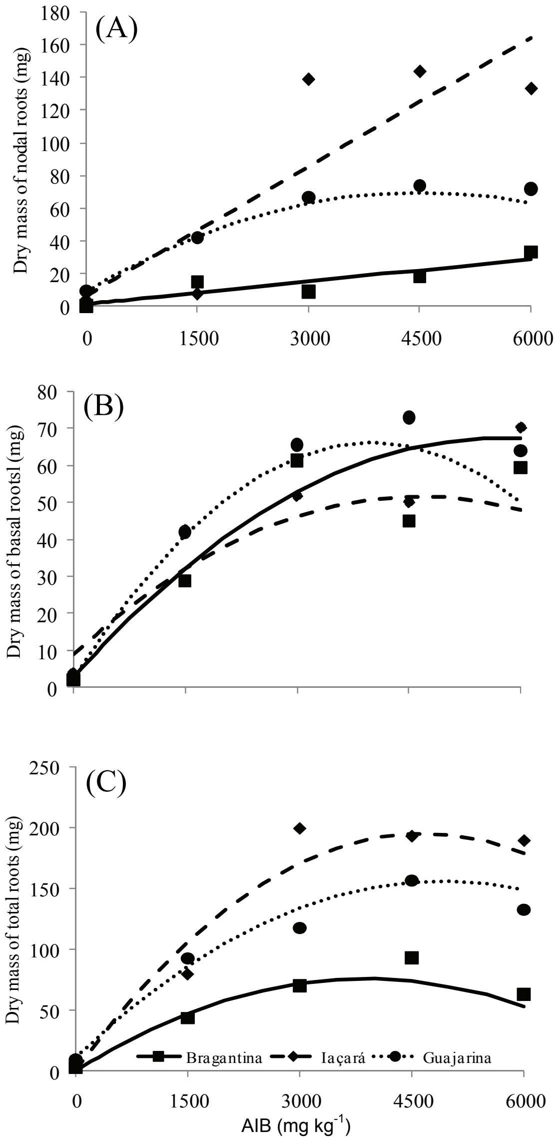
The total volume of roots presented a quadratic behavior in all cultivars, with maxima at 4,000; 3,397 and 4,714 mg kg^{-1} IBA, achieving values of 0.828, 0.687 and 1.793 cm^3 for the Bragantina, Iaçará and Guajarina cultivars, respectively (Figure 5). The Guajarina cultivar presented a higher total volume of roots for all the IBA concentrations studied (Table 1), and it can be inferred that this cultivar can be used as rootstock, considering the higher exploitation of its radicular system and its consequently greater effectiveness of water absorption and use of mineral substrates; this was observed in a paper by Veloso et al. (2000), in which only the Guajarina cultivar responded to phosphorus application.

Root dry matter mass is associated with the rooting rate and the number of roots (HOLANDA et al., 2012). With regard to this characteristic, the Bragantina cultivar was 2.34 times superior in its basal region (67.38 mg) when 5,687 mg kg^{-1} IBA was used compared to the nodal region (28.78 g) when 6,000 mg kg^{-1} IBA was used (Figure 6A and B, respectively). For the other cultivars, the nodal dry matter mass was superior to the basal dry matter mass, and the Iaçará and Guajarina cultivars presented 164.3 and 69.61 mg of roots, respectively, with maxima at 6,000 and 4,500 mg kg^{-1} IBA (Figure 6A). The total dry matter masses of the roots were 75.71 mg for Bragantina, 195.5 mg for Iaçará and 153.82 mg for Guajarina, with maxima at 3,890; 4,650 and 4,316 mg kg^{-1} IBA, respectively (Figure 6C). Once again, it is observed that the rhizogenic capacity of the Bragantina cultivar is lower than those observed in the Iaçará and Guajarina cuttings.



Total volume of roots	R^2
$\hat{Y}_{Bragantina} = 0.0289 + 0.0004x - 0.00000005x^2$	0.76^{*}
$\hat{Y}_{Iaçará} = 0.110314 + 0.0003397x - 0.00000005x^2$	0.96^{*}
$\hat{Y}_{Guajarina} = 0.041714 + 0.00075438x - 0.00000008x^2$	0.96^{*}

Figure 5. Total volume of roots (cm^3) of cuttings of *Piper nigrum* cvs. Bragantina, Iaçará and Guajarina treated with different concentrations of IBA. São Mateus, Espírito Santo, 2012. *Significant at 5% probability level by the F test.



(A) Dry mass of nodal roots	R^2
$\hat{Y}_{Bragantina} = 1.18 + 0.0046x$	0.79*
$\hat{Y}_{Iaçará} = 6.5 + 0.0263x$	0.73*
$\hat{Y}_{Guajarina} = 8.86 + 0.027x - 0.000003x^2$	0.99*
(B) Dry mass of basal roots	R^2
$\hat{Y}_{Bragantina} = 2.691429 + 0.02275x - 0.000002x^2$	0.86*
$\hat{Y}_{Iaçará} = 8.8486 + 0.0185x - 0.000002x^2$	0.88*
$\hat{Y}_{Guajarina} = 2.148571 + 0.032009x - 0.000004x^2$	0.99*
(C) Dry mass of total roots = (A) + (B)	R^2
$\hat{Y}_{Bragantina} = 0.0549 + 0.0389x - 0.000005x^2$	0.95*
$\hat{Y}_{Iaçará} = 0.9071 + 0.0837x - 0.000009x^2$	0.94*
$\hat{Y}_{Guajarina} = 10.951 + 0.059x - 0.000006x^2$	0.97*

Figure 6. Nodal (B), basal (C) and total (C=A+B) dry root mass (mg) of cuttings of *Piper nigrum* cvs. Bragantina, Iaçará and Guajarina treated with different concentrations of IBA. São Mateus, Espírito Santo State, 2012. *Significant at 5% probability level by the F test.

Conclusion

The total immersion of cuttings in IBA is recommended for the cvs. Iaçará and Guajarina, whereas immersion of only the basal region is recommended for cv. Bragantina because the nodal region produces virtually no roots. The recommended concentration for these cultivars is 4,000 mg kg^{-1}.

References

ABDULLAH, A. T. M.; HOSSAIN, M. A.; BHUIYAN, M. K. Clonal propagation of guava (*Psidium guajava* Linn.) by stem cutting from mature stockplants. **Journal of Forest Research**, v. 17, n. 4, p. 301-304, 2006.

AGULLÓ-ANTÓN, M. A.; SÁNCHEZ-BRAVO, J.; ACOSTA, M.; DRUEGE, U. Auxin of sugars: wat makes the difference in the adventitious rooting of stored carnation cuttings? **Journal of Plant Growth Regulation**, v. 30, n. 1, p. 100-113, 2011.

ALBIERO, A. L. M.; PAOLI, A. A. S.; SOUZA, L. A.; MOURÃO, K. S. M. Morfoanatomia dos órgãos vegetativos de *Piper crassinervium* H.B. & K. (Piperaceae). **Acta Botânica Brasílica**, v. 19, n. 2, p. 305-312, 2004.

ALBIERO, A. L. M.; PAOLI, A. A. S.; SOUZA, L. A.; MOURÃO, K. S. M. Morfoanatomia dos órgãos vegetativos de *Piper hispidum* Sw. (Piperaceae). **Revista Brasileira de Farmacognosia**, v. 16, n. 3, p. 379-391, 2006.

ANSARI, S. A.; SINGH, S. Genetic difference in adventitious rhizogenesis in *Albizia procera* Benth. with IBA treatment. **Journal of Forest Research**, v. 13, n. 2, p. 79-82, 2008.

ARAÚJO, R. C.; BRUCKNER, C. H. Biologia reprodutiva de fruteiras. In: BRUCKNER, C. H. (Ed.). **Fundamentos do melhoramento de fruteiras**. Viçosa: Editora UFV, 2008. p. 1-38.

BHARDWAJ, D. R.; MISHRA, V. K. Vegetative propagation of *Ulmus villosa*: effect of plant growth regulators, collection time, type of donor and position of shoot on adventitious root formation in stem cuttings. **New Forest**, v. 29, n. 2, p. 105-116, 2005.

CARDOSO, M. Sobre o enraizamento de estacas de pimenta-do-reino. **Bragantia**, v. 20, n. 16, p. 529-531, 1961.

CARDOSO, M.; SANTOS, R. R. Pimenteira-do-reino: produtividade segundo o tipo de muda. **Bragantia**, v. 40, n. 9, p. 221-224, 1981.

CORRÊA, L. A.; PAIM, D. C.; SCHWAMBACH, J.; FETT-NETO, A. G. Carbohydrates as regulatory factors on the rooting of *Eucalyptus saligna* Smith and *Eucalyptus globulus* Labill. **Plant Growth Regulation**, v. 45, n. 1, p. 63-73, 2005.

CORREA, L. R.; STEIN, R. J.; FETT-NETO, A. G. Adventitious rooting of detached *Arabidopsis thaliana* leaves. **Biologia Plantarum**, v. 56, n. 1, p. 25-30, 2011.

CRUZ, C. D. Genes: a software package for analysis in experimental statistics and quantitative genetics. **Acta Scientiarum. Agronomy**, v. 35, n. 3, p. 271-276, 2013.

FACHINELLO, J. C.; HOFFMANN, A.; NACHTIGAL, J. C.; KERSTEN, E. Propagação vegetativa por estaquia. In: FACHINELLO, J. C.; HOFFMANN, A.; NACHTIGAL, J. C. (Ed.). **Propagação de plantas frutíferas**. Brasília: Embrapa Informação Tecnológica, 2005.

FERREIRA, B. G. A.; ZUFFELLATO-RIBAS, K. C.; CARPANEZZI, A. A.; TAVARES, F. R.; KOEHLER, H. S. Metodologias de aplicação de AIB no enraizamento de estacas semilenhosas de *Sapium glandulatum* (Vell.) Pax. **Revista Brasileira de Plantas Medicinais**, v. 11, n. 2, p. 196-201, 2009.

FOGAÇA, C. M.; FETT-NETO, A. G. Role of auxin and its modulators in the adventitious rooting of *Eucalyptus* species differing in recalcitrant. **Plant Growth Regulation**, v. 45, n. 1, p. 1-10, 2005.

FRANCIS, G.; ARAVINDAKSHAN, M.; GOPIKUMAR, K.; VALSALAKUMARI, P. K. Effect of growth substances on rooting of planting materials in black pepper (*Piper nigrum* L.). **Journal of Tropical Agriculture**, v. 31, n. 1, p. 71-76, 1993.

GRZYBOWSKI, A.; TIBONI, M.; SILVA, M. A. N.; CHTOLINA, R. F.; PASSOS, M.; FONTANA, J. D. The combined action of phytolarvicides for the control of dengue fever vector, *Aedes aegypti*. **Revista Brasileira de Farmacognosia**, v. 1, n. 1, p. 15-23, 2012.

HARTMANN, H. T.; KESTER, D. E.; DAVIES JR., F. T.; GENEVE, R. L. **Plant propagation**: principles and practices. 8. ed. New Jersey: Prentice-Hall, 2011.

HOLANDA, F. S. R.; VIEIRA, T. R. S.; ARAÚJO FILHO, R. N.; SANTOS, T. O.; ANDRADE, K. V. S.; CONCEIÇÃO, F. G. Propagation through cutting technique of species occurring in the lower São Francisco in Sergipe Stage with different concentrations of indolbutiric acid. **Revista Árvore**, v. 36, n. 1, p. 75-82, 2012.

HUNT, M. A.; TRUEMAN, S. J.; RASMUSSEN, A. Indole-3-butyric acid accelerates adventitious root formation and impedes shoot growth of *Pinus elliottii* var. *elliottii* x *P. caribaea* var. *hondurensis* cuttings. **New Forests**, v. 41, n. 3, p. 349-360, 2011.

HUSEN, A.; PAL, M. Metabolic changes during adventitious root primordium development in *Tectona grandis* Linn. F. (teak) cuttings as affected by age of donor plants and auxin (IBA and NAA) treatment. **New Forest**, v. 33, n. 3, p. 309-323, 2007.

IBGE-Instituto Brasileiro de Geografia e Estatística. **Levantamento sistemático de produção agrícola**: pesquisa mensal de previsão e acompanhamento das safras agrícolas no ano civil. Rio de Janeiro: IBGE, 2011. v. 24, n. 11, p. 82.

ISLAM, M. S.; BHUIYAN, M. K.; HOSSAIN, M. M.; HOSSAIN, M. A. Clonal propagation of *Bambusa vulgaris* by leafy branch cuttings. **Journal of Forest Research**, v. 22, n. 3, p. 387-392, 2011.

KESARI, V.; KRISHNAMACHARI, A.; RANGAN, L. Effect of auxins on adventitious rooting from stem cuttings of candidate plus tree *Pongamia pinnata* (L.), a potential biodiesel plant. **Trees - Structural Function**, v. 23, n. 3, p. 597-604, 2009.

LEITE, J. B. V.; MARTINS, A. B. G. Efeito do ácido indolbutírico e época de coleta no enraizamento de estacas semi-lenhosas do cacaueiro. **Revista Brasileira de Fruticultura**, v. 29, n. 2, p. 204-208, 2007.

LEMAY, V.; GÂTEBLÉ, G.; McCOPY, S. Vegetative propagation of two endemic species of *Cloezia* Brongn. & Gris for conservation and mining revegetation activities in New Caledonia. **New Forest**, v. 37, n. 1, p. 1-8, 2009.

LI, S. W.; XUE, L. The interaction between H_2O_2 and NO, Ca^{2+}, cGMP, and MAPKs during adventitious rooting in mung bean seedlings. ***In Vitro* Cellular and Developmental Biology – Plant**, v. 46, n. 2, p. 142-148, 2010.

LI, S. W.; XUE, L.; XU, S.; FENG, H.; NA, L. Mediators, genes and signaling in adventitious rooting. **The Botanical Review**, v. 75, n. 2, p. 230-247, 2009.

LIMA, D. M.; BIASI, L. A.; ZANETTE, F.; ZUFFELLATO-RIBAS, K. C.; BONA, C.; MAYER, J. L. S. Capacidade de enraizamento de estacas de *Maytenus muelleri* Schwacke com a aplicação de ácido indol butírico relacionada aos aspectos anatômicos. **Revista Brasileira de Plantas Medicinais**, v. 13, n. 4, p. 422-438, 2011a.

LIMA, J. D.; LIMAS, A. P. S.; BOLFARINI, A. C. B.; SILVA, S. H. M. G. Enraizamento de estacas de *Camellia sinensis* L. em função da época de coleta de ramos, genótipos e ácido indolbutírico. **Ciencia Rural**, v. 41, n. 2, p. 230-235, 2011b.

MAGEVSKI, G. C.; CZEPAK, M. P.; SCHMILDT, E. R.; ALEXANDRE, R. S.; FERNANDES, A. A. Propagação vegetativa de espécies silvestres do gênero *Piper*, com potencial para uso como porta enxertos em pimenta-do-reino (*Piper nigrum*). **Revista Brasileira de Plantas Medicinais**, v. 1, n. especial, p. 559-563, 2011.

MATEJA, Š.; DOMINIK, V.; FRANCI, Š.; GREGOR, O. The effects of a fogging system on the physiological status and rooting capacity of leafy cuttings of woody species. **Trees - Structure and Function**, v. 21, n. 4, p. 491-496, 2007.

MAUSETH, J. D. **Plant anatomy**. Menlo Park: The Benjamin/Cummings Publishing Company, 1988.

METAXAS, D. J.; SYROS, T. D.; YUPSANIS, T.; ECONOMOU, A. S. Peroxidases during adventitious rooting in cuttings of *Arbutus unedo* and *Taxus baccata* as affected by plants genotype and growth regulator treatment. **Plant Growth Regulation**, v. 44, n. 3, p. 257-266, 2004.

OLIVEIRA, J. H. G.; SOUZA, L. A.; IWAZAKI, M. C. Estruturas de reprodução de *Peperomia parnassifolia* Miq. (Piperaceae). **Acta Scientiarum. Biological Sciences**, v. 30, n. 1, p. 1-7, 2008.

PARK, S. M.; WON, E. J.; PARK, Y. G.; JEONG, B. R. Effect of node position, number of leaflets left, and light intensity during cutting propagation on rooting and subsequent growth of domestic roses. **Horticulture, Environement, and Biotechnology**, v. 52, n. 4, p. 339-343, 2010.

PESCADOR, R.; VOLTONI, A. C.; GIRARDI, C. G.; ROSA, F. A. F. Estaquia de pariparoba-do-Rio Grande do Sul sob efeito do ácido indol-butírico em dois substratos. **Scientia Agraria**, v. 8, n. 4, p. 391-398, 2007.

QADDOURY, A.; AMSSA, M. Endogenous phenolic contents, peroxidase and polyphenoxidase activities in date palm (*Phoenix dactylifera* L.) off shoots related to rooting ability. **Acta Physiologiae Plantarum**, v. 25, n. 4, p. 417-421, 2003.

RAVEN, P. H.; CURTIS, H. **Integración del crescimento**: fitohormonas. Biología vegetal. Barcelona: Omega, 1975. p. 162–168.

ROUT, G. R. Effect of auxins on adventitious root development from single node cuttings of *Camellia sinensis* (L.) Kuntze and associated biochemical changes. **Plant Growth Regulation**, v. 48, n. 2, p. 111-117, 2006.

SANTOS JÚNIOR, A. J.; ALMEIDA, A. A. F.; SILVA, D. C.; FARIA, J. C.; MIELKE, M. S.; GOMES, F. P. Enraizamento de estacas, crescimento e respostas anatômicas de mudas clonais de cacaueiro ao ácido indol-3-butírico. **Revista Brasileira de Fruticultura**, v. 30, n. 4, p. 1071-1082, 2008.

SHARANGI, A. B.; KUMAR, R. Performace of rooted cuttings of black pepper (*Piper nigrum* L.) with organic substitution of nitrogen. **International Journal of Agricultural Research**, v. 6, n. 9, p. 673-681, 2011.

SHARANGI, A. B.; KUMAR, R.; SAHU, P. K. Survivability of black pepper (*Piper nigrum*) growing media. **Journal of Crop and Weed**, v. 6, n. 1, p. 52-54, 2010.

SILVA, J. M. M.; RAPOSO, A.; SOUZA, J. A.; MIRANDA, E. M. Indução de enraizamento em estacas de joão-brandinho (*Piper* sp.) com ácido indolbutírico. **Revista Ciência Agronômica**, v. 35, n. especial, p. 248-252, 2004.

SIMAS, N. K.; LIMA, E. C.; KUSTER, R. M.; LAGE, C. L. S.; OLIVEIRA FILHO, A. M. Potential use of *Piper nigrum* ethanol extract against pyrethroid-resistant *Aedes aegypti* larvae. **Revista da Sociedade Brasileira de Medicina Tropical**, v. 40, n. 4, p. 405-407, 2007.

SINGH, S. S.; SINGH, S. Effect of nodal cuttings and rooting media on the propagation of black pepper under South Andaman conditions. **Indian Cocoa Anecanut and Spices Journal**, v. 12, n. 4, p. 122-123, 1989.

SINGH, B.; YADAV, R.; BHATT, B. P. Effect of mother tree ages, different rooting mediums, light conditions and auxin treatment on rooting behaviour of *Dalbergia sissoo* branch cuttings. **Journal Forestry Research**, v. 22, n. 1, p. 53-57, 2011.

SOUZA, L. A.; MOSCHETA, I. S.; MOURÃO, K. S. M.; ALBIERO, A. L. M.; IWAZAKI, M. C.; OLIVEIRA, J. H. G.; ROSA, S. M. Vegetative propagation in Piperaceae species. **Brazilian Archieves of Biology Technology**, v. 52, n. 6, p. 1357-1361, 2009.

SOUZA, L. A.; MOSCHETA, I. S.; OLIVEIRA, J. H. G. Comparative morphology and anatomy of the leaf and stem of *Peperomia dahlstedtii*, *Ottonia martiana* and *Piper diospyrifolium* (Piperaceae). **Gayana Botánica**, v. 61, n. 1, p. 6-17, 2004.

SUNDHARAIYA, K.; PONNUSWAMI, V.; JASMINE, A. J. Effect of growth regulators in the propagation of sarkaraikolli (*Gymnema sylvestre*), medicinal coleus (*Coleus forskohlii*) and thippili (*Piper longum*). **Journal South Indian Horticulture**, v. 48, n. 1-6, p. 172-174, 2000.

THANKAMAN, C. K.; DINESH, R.; EAPEN, S. J.; KUMAR, A.; KANDIANNAN, K.; MATHEW, P. A. Effect of solarized potting mixture on growth of black pepper (*Piper nigrum* L.) rooted cuttings in the nursey. **Journal of Spices and Aromatic Crops**, v. 17, n. 2, p. 103-108, 2008.

THANUJA, T. V.; HEDGE, R. V.; SREENIVASA, M. N. Induction of rooting and root growth in black pepper cuttings (*Piper nigrum* L.) with the inoculation of arbuscular mycorrhizae. **Scientia Horticulturae**, v. 92, n. 3-4, p. 339-346, 2002.

THOMAS, R. G.; HAY, M. J. M. Adaptive variation in physiological traits underpinning stem elongation responses among nodally-rooting stoloniferous herbs. **Evolutionary Ecology**, v. 22, n. 3, p. 369-381, 2008.

THOMAS, R. G.; HAY, M. J. M. The role of nodal roots in prostate clonal herbs: 'phalanx' versus 'guerrilla'. **Evolutionary Ecology**, v. 24, n. 6, p. 1489-1504, 2010.

TRAJANO, V. N.; LIMA, E. O.; SOUZA, E. L.; TRAVASSOS, A. E. R. Propriedade antibacteriana de óleos essenciais de especiarias sobre bactérias contaminantes de alimentos. **Ciência e Tecnologia de Alimentos**, v. 29, n. 3, p. 542-545, 2009.

VELOSO, C. A. C.; CARVALHO, E. J. M.; MALAVOLTA, E.; MURAOK, T. Resposta de cultivares de pimenta-do-reino aos nutrientes NPK em um latossolo amarelo da Amazônia Oriental. **Scientia Agricola**, v. 57, n. 2, p. 343-347, 2000.

VIANNA, W. O.; AKISUE, G. Caracterização morfológica de *Piper aduncum* L. **Lecta**, v. 15, n. 1-2, p. 11-62, 1997.

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