

## Substrates on the cuttings rooting of black pepper genotypes

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### Abstract

The soil in the production of clonal seedlings of black pepper (*Piper nigrum* L.) contributes to fusarium and nematodes infestation in commercial crops, and thus the usage of alternative substrates might be a preventive control measure against these pathogens. The aim of this study was to investigate the rhizogenic behavior of the cultivars Bragantina, Iaçará and Guajarina of black pepper in different substrates. The experimental design was in randomized blocks in a 3x4 factorial design (cultivars: Bragantina, Iaçará and Guajarina x substrates: soil + organic compound (18%), vermiculite, carbonized rice hulls and commercial substrate), with four replications of 16 cuttings each. The evaluated characteristics were: survival (%); rooting (basal or nodal) (%); nodal rooting (%); basal rooting (%); number of nodal roots; number of basal roots; total number of roots; length of the largest nodal root (cm); basal root length (cm); nodal root volume (cm<sup>3</sup>); basal root volume (cm<sup>3</sup>); Total root volume (cm<sup>3</sup>); nodal root dry mass (mg); basal root dry mass (mg) and the total root dry mass (mg). There are differences within black pepper cultivars as to the characteristics related to the root system. The vermiculite substrate was the most suitable, and carbonized rice hull is not recommended for the adventitious rooting of cuttings of cultivars Bragantina, Iaçará and Guajarina.

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

### Introduction

Different biological activities are attributed to the black pepper (*Piper nigrum* L.), belonging to the Piperaceae family. In Brazil, this species presents several utilities, being a widely consumed spice; however, the tropical climate of the country favors the development of fungi of the type *Aspergillus* spp. which produce mycotoxins detected on the grain, such as the aflatoxin B<sub>1</sub>, causing a great problem for being a powerful hepatocarcinogen described in mammals, and is classified in the Group 1 (carcinogenic to humans) by the International Agency of Research on Cancer (Prado et al., 2008). Other utilities are attributed to the black pepper, such

as being a larvicide to the *Aedes aegypti*, of great importance for public health (Simas et al., 2007) and presents yet antibacterial property from its essence oil over food contaminants, in substitution to chemical additives (Trajano et al., 2009).

The two largest national producers of this spice, in decrescent order, are the state of Pará (North) and Espírito Santo (Southeast), highlighting the municipality of São Mateus-ES. Pará and Espírito Santo states presented, in 2015, a production of 32.414 (20.445 ha) and 13.863 (8.071 ha) tons and yield of 2.040 and 3.467 kg ha<sup>-1</sup>, respectively (IBGE, 2016). The black pepper producers in the Espírito Santo state received prices

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based in June 2015, by kilogram of the spice, of R\$ 27,00 (Conab, 2016). Among the existing black pepper cultivars, the most cultivated in the north of the Espírito Santo state and in Pará are the Bragantina, laçarará, Guajarina, Cingapure and Kottanadan. Currently, the commercial cultivar is the Bragantina, whose main characteristic lies on presenting long spikes, with good filling, large fruits and, consequently, culminating in a larger yield.

Even with the prices attractivity, some problems are faced by pepper producers, such as fusariosis (*Fusarium solanif. sp. piperis*) and the southern root-knot nematode (*Meloidogyne incognita*), which have, in part, being disseminated through cutting. This technique of vegetative propagation is the more utilized, currently, with the species *P. nigrum*, with good success in function of the maintaining of the characteristics of the selected genetic materials, and some works confirm the efficacy of this method (Serrano et al., 2012; Secundino et al., 2014), where the quality of the root system, such as number, volume and root dry mass is fundamental in the survival and acceptability of the seedling in the field.

The employment of alternative substrates in the production of clonal seedlings of black pepper might be a preventive measure in the control of fusariosis and nematosis. These substrates might also help to increase the quality of the seedling, by proportionating, for example, higher number and, consequently, higher volume of roots. The production of quality seedlings is also related with the choice of adequate substrates, such as the propagule source, propagation method, growth regulators, containers, irrigation systems and nursery ambience.

It was aimed with this work to analyze the rooting of black pepper cultivars cuttings in different substrates.

### Material and Methods

The work was conducted in the Centro Universitário Norte do Espírito Santo (CEUNES) of the Federal University of Espírito Santo (UFES), in the municipality of São Mateus-ES, BR 101 North, Km 60, 39° 51'22'' W and 18°40'16'' S.

The adopted experimental design was

in randomized blocks, in a factorial scheme of 3x4 [cultivars: Bragantina, laçarará and Guajarina x substrates: soil + organic compound (18%), vermiculite, semi-carbonized rice hull and commercial substrate], with four repetitions of 16 cuttings each.

The orthotropic herbaceous branches (branches of vertical growth, what is desirable for the vertical growth of the plant and higher production) of the black pepper plant (*P. nigrum*) of the cvs. Bragantina, laçarará and Guajarina of five years of age, of commercial crops, were utilized for the obtaining of the cuttings. The branches were placed in polystyrene boxes containing distilled water. Next, these branches were immediately transported to the nursery, where the individualization of the cuttings in 10 cm of length with two nodes and one leaf was performed, in which the base of each cutting was excised in simple bevel.

After the preparing of the cuttings, they had their stem immersed until the insertion of the leaf, with exception of the leaf, in talcum with the concentration of 4000 mg kg<sup>-1</sup> of indol-3-butyric acid (IBA) (Secundino et al., 2014). After the treatment with the auxin, the cuttings were planted with the leaf staying over the surface of the substrates soil (anthill ground) + organic compost – tanned bovine manure (18%), vermiculite with average particle size, carbonized rice hull and commercial substrate (Pinus bark and coconut fiber), disposed in polyethylene trays with cells of 200 cm<sup>3</sup> of volume each. Before the planting, in the referred substrates, orifices (openings) were made, approximately of the size of the cuttings and with higher diameter so that the planting would not remove the talcum with the auxin from the cutting surface. These substrates were irrigated before and after the planting of the cuttings.

The cuttings were kept in nursery covered with doubled layer of transparent polyethylene of 150 micra and irrigation of the intermittent fogging type, with automatic activation, every three minutes and thirty seconds, in which the climatic conditions presented an average of 30.2 °C and 84% of relative humidity.

The evaluated characteristics after 90 days of experiment placement were: survival

(%); rooting (independently if the root appeared in the basal or nodal region); nodal rooting (%); basal rooting (%); number of nodal, basal and total rootings; length of the largest nodal and basal root (cm) through a graduated scale; total volume of nodal, basal and total roots (cm<sup>3</sup>) with graduated cylinder of 250 mL through mass displacement; and dry mass of nodal, basal and total roots (mg) in greenhouse of forced circulation, regulated at 70 °C until constant weight.

The chemical and physical evaluation of the substrates was performed in the Laboratory of Soil, Leaf and Water Analysis (LAGRO) of the CEUNES/UFES (Table 1). The physical characteristics of the substrates were evaluated

in the Laboratory of Physical Analysis of the CEUNES/UFES, where the determination of the dry density and porosity was performed (MAPA, 2007). The particle size (Table 1) was determined through dry sieving, sifting 100g of each dry substrate in the air, coupled in a sieve compound with meshes > 4.75; 2.00 to 4.75; 1.00 to 2.00; 0.5 to 1.00; 0.25 to 0.5 and < 0.25 mm, and agitated for 5 minutes. The retained fractions in each sieve were weighed and the percentages over the total weight of the samples were calculated.

For the statistical analysis, the data were subjected to variance analysis and the averages were compared by Tukey's test, at 5% significance, through the Genes software (Cruz, 2013).

**Table 1.** Chemical and physical characteristics of the substrates soil + organic compound, vermiculite, carbonized rice hull and commercial substrate utilized in the rooting of *Piper nigrum* cuttings cvs. Bragantina, laçará and Guajarina

Characteristics	Substrates			
	S + CO	VERM	CRH	CS
<b>Chemicals</b>				
Total organic matter (dag kg <sup>-1</sup> )	3.1	0.8	11.2	8.5
pH in H <sub>2</sub> O	7.6	6.7	6.6	5.2
P Mellich (mg dm <sup>-3</sup> )	222.0	9.0	49.0	198.0
Na <sup>+</sup> (mg dm <sup>-3</sup> )	910.0	97.0	170.0	510.0
K <sup>+</sup> changeable (mg dm <sup>-3</sup> )	1920.0	160.0	570.0	990.0
Mg <sup>2+</sup> changeable (cmol)	1.7	0.5	0.3	3.2
Ca <sup>2+</sup> changeable (cmol)	5.0	0.5	0.6	7.8
Ca <sup>2+</sup> + Mg <sup>2+</sup> changeable (mmol <sub>c</sub> dm <sup>-3</sup> )	6.7	1.0	0.9	11.0
Al <sup>3+</sup> (cmol)	0.0	0.0	0.0	0.4
H <sup>+</sup> + Al <sup>3+</sup> (cmol)	1.0	0.9	0.8	4.7
Zn (mg dm <sup>-3</sup> )	14.1	1.0	3.0	15.2
Fe (mg dm <sup>-3</sup> )	83.0	299.0	23.0	206.0
Mn (mg dm <sup>-3</sup> )	85.0	18.0	29.0	45.0
Cu (mg dm <sup>-3</sup> )	0.9	1.7	0.5	2.4
B (mg dm <sup>-3</sup> )	1.15	0.30	0.55	1.22
CTC (t) (cmol)	11.6	1.4	2.4	13.9
Base saturation (%)	92.1	61.0	74.7	74.2
<b>Physicals</b>				
Dry density (kg m <sup>-3</sup> )	1202.45	186.15	123.27	303.59
Total porosity (%)	45.24	71.74	41.81	64.09
Particle sizes	Particle sizes distribution (%)			
> 4.75 mm	0.00	1.32	1.77	0.07
2.00 a 4.75 mm	6.41	17.14	62.73	34.56
1.00 a 2.00 mm	14.43	26.10	25.83	43.36
0.5 a 1.00 mm	19.67	22.19	7.33	18.78
0.25 a 0.5 mm	22.49	16.05	1.67	2.31
< 0.25 mm	37.01	17.21	0.67	0.92

<sup>1</sup>Substrates: S + CO (soil + organic compound); VERM (vermiculite); CRH (carbonized rice hull) and CS (commercial substrate).

## Results and Discussion

In the present work there was significant interaction between the factors cultivars and substrates for the variables nodal rooting, rooting, number of nodal roots and root volume (Table 1).

The higher survival percentage of cuttings was obtained in the vermiculite substrate with the cv. Guajarina (87.29%), which did not differ from the cv. laçará (78.12%) and, this, in its turn, did not statistically differ from the cv. Bragantina

(60.95%). The lowest survival percentage was found in the carbonized rice hull substrate, also in the cv. Guajarina (39.06%), which did not differ from cv. Bragantina (50%) and, this, in its turn, did not statistically differ from the cv. laçar (65.62%). In the commercial substrate, the highest survival rates were with the cultivars laçar (68.72%) and Guajarina (62.50%), and the lowest with the cv. Bragantina (40.62%) (Table 2). Magevski et al. (2011) obtained survival rates of 100% for

*Piper arboreum*, 98% for *Piper amplum* and 89% for *Piper sp.* at 45 days, in cuttings cultivated in the Bioplant® substrate. According to Cunha et al. (2015) the substrates washed sand and commercial are indicated for higher rooting percentages of *Piper hispidum* with 81.56% and 81.33%, respectively. However, for this species the substrates soil + bird manure and/or soil + guarana bark were the more indicated when the objective is achieving high quality seedlings.

**Table 2.** Rooting characteristics of the *Piper nigrum* cuttings cvs. Bragantina, laçar and Guajarina, in the substrates soil + organic compound, vermiculite, carbonized rice hull and commercial substrate

Evaluated characteristics	SUB	Cultivars		
		Bragantina	laçar	Guajarina
Survival (%)	VERM	60.95 Ab*	78.12 Aab	87.29 Aa
	CRH	50.00 Aab	65.62 Aa	39.06 Cb
	CS	40.62 Ab	68.72 Aa	62.50 Ba
Nodal rooting (%)	VERM	57.87 Ab	78.25 Aab	87.50 Aa
	CRH	50.00 Aab	62.50 Aa	39.25 Bb
	CS	40.75 Ab	68.75 Aa	54.87 Bab
Rooting (%)	VERM	60.93 Ab	78.25 Aab	87.50 Aa
	CRH	50.00 Aab	67.25 Aa	39.25 Bb
	CS	40.75 Ab	68.75 Aa	60.93 Bab
Number of nodal roots	VERM	4.47 Ab	12.59 Aa	6.68 Ab
	CRH	4.31 Aa	5.62 Ba	3.29 Aa
	CS	2.83 Ab	7.00 Ba	4.48 Aab
Volume of nodal root(cm <sup>3</sup> )	VERM	0.3750 Ab	0.9520 Aa	0.5350 Ab
	CRH	0.3125 Aa	0.3300 Ba	0.1725 Ba
	CS	0.3000 Ab	0.6250 Ba	0.4850 ABab

\*Averages followed by the same uppercase letter on the column and lowercase on the line do not differ within each other by Tukey's test, at 5% probability. !SUB (Substrates): VERM (vermiculite); CRH (carbonized rice hull) and CS (commercial substrate).

In the mixture soil + organic compound, comparing with the remaining studied substrates, it was verified that in spite of the best results of P, K, Mg, Ca, Ca + Mg, Zn, Mn, B and CTC, the highest density and lowest total porosity were observed with the highest distribution rate of particles in the smaller particle size <0,25 mm (37.01%) and in the particle size from 0.25 to 0.5 mm (22.49%) (Table 1).

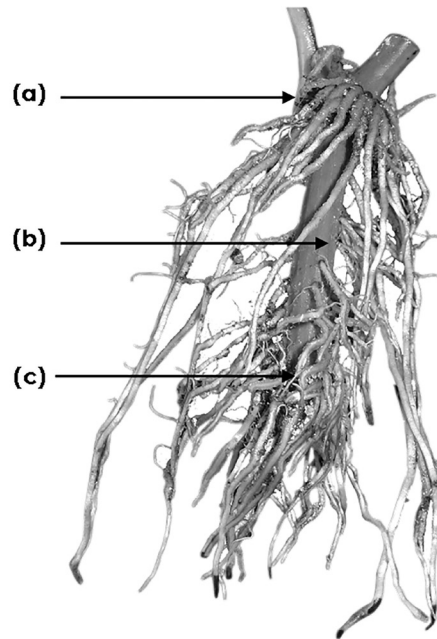
The mixture soil + organic compound resulted in the soaking and a sealant layer on the surface of the substrate, which likely resulted in a higher mechanical impedance. The substrate also proportioned the developed of algae in its surface and the darkening of tissues in the proximal region of the cuttings, resulting unto necrosis, what very likely might be related to infection by some phytopathogenicmicroorganism, where the sum of these negative factors resulted in the subsequent death of 100% of the cuttings.

Black peppers seedling producers from the North of the Esprito Santo state utilize the

soil in mixture with sand and organic matter, not purged, what turns possible the dissemination of important diseases in the orchards of that state, such as fusariosis and nematosis (Serrano et al., 2012). Substrate solarization would be a method of preventive control against the infection by phytopathogenic microorganisms and even sources of weed propagules, as observed by Thankaman et al. (2008), who verified the superiority of the solarized mixture soil + sand + corral manure (2:1:1 v/v), in the reduction of diseases and in the growth increase of *P. nigrum* roots, what indicates that, in mixtures involving soil and organic compounds, elimination techniques of phytopathogenic microorganisms are necessary. The usage of coconut fiber composted with vermicompost and Trichoderma in black pepper orchards minimizes the usage of chemical fungicides, in addition to the fact that the Trichoderma colonization of the root system of the seedlings helps in the prevention of field pathogen infections (Prasath et al., 2014).

The rooting of black pepper cuttings occurs in the nodal (leaf axil) and basal region (Figure 1), placed above the incised region in the distal portion. Nevertheless, in this experiment some roots appeared in the median region, between the nodal and basal regions, as observed in the cv. Guajarina (Figure 1). This is due to hormonal stimulus through total immersion

of the cuttings (Secundino et al., 2014) and the effect might have been potentialized by the cultivation of the cuttings in the vermiculite substrate. In this case, the usage of the auxin is necessary, since in its absence the rooting percentages for the Bragantina, laçar and Guajarina cultivars are low (Secundino et al., 2014).



**Figure 1.** Rooted cutting of *Piper nigrum* cv. Guajarina in the vermiculite substrate, with roots formed in the nodal (a), median (b) and basal (c) regions.

The rooting easiness is a function of several factors, and according to Mouri et al. (2011) it is dependent of the genetics of the mother plants. The black pepper, cv. Bragantina, presents difficulties in this process, what turns imperative the usage of the auxin (Secundino et al., 2014).

The nodal rooting and the rooting in the basal or nodal regions in the vermiculite substrate reached the same average value, being higher in the cvs. Guajarina (87.50%) and laçar (78.25%), where the cv. laçar did not statistically differ from the cv. Bragantina as to the nodal (57.87%) and basal (60.93%) rooting (Table 2).

Nevertheless, in the present study, the basal rooting was higher (62.25%) with the cv. laçar, followed by the cv. Guajarina (52.83%), independently of the substrate utilized (Table 3). The cv. Bragantina presented inferior results of nodal rooting and rooting in the basal or nodal

regions where the substrates did not statistically differ within each other, with averages of 49.54 and 50.56%, respectively. Although no statistical difference was found for the rooting in the basal or nodal regions in the cv. Bragantina within the studied substrates, the average values found for this characteristic in the vermiculite substrate (60.93%) compared to the semi-carbonized rice hull (50%) and to the commercial substrate (40.75%) was superior in 10.93 and 20.18%, respectively.

As to the quality of the root system in the vermiculite substrate, the characteristics number and volume of nodal root were higher in the cv. laçar. This cultivar, in the vermiculite substrate, produced an average of 12.59 roots, that is, approximately six roots more than the cv. Guajarina and eight roots more than the cv. Bragantina (Table 2). Cuttings of *Piper mikanianum* Kunth Stend var. *mikanianum* presented higher

number of roots in the vermiculite substrate, due to the good water retention and optimum space of air in the retention capacity, factors which turn possible the maintaining of an adequate supply of water for the rooting of the cuttings (Pescador et al., 2007).

The cv. laçará, independently of the substrate, also presented higher total root number, nodal and total root dry matter mass (Table 3). On the other hand, Serrano et al. (2012), by utilizing the mixture vermiculite + Pinus bark, verified that the cuttings of cv. Guajarina treated with 2000 mg kg<sup>-1</sup> of IBA, produced higher dry mass of the root system if compared with the cvs. laçará and Cingapura.

Independently of the cultivar, the vermiculite was superior to the remaining

substrates in increasing the characteristics basal rooting, basal and total root number and length of the bigger total and basal root (Table 4). Regarding the length of the largest nodal root, total and basal root volume, total, nodal and basal dry mass, the vermiculite did not statistically differ from the commercial substrate (Table 4). Oliveira et al. (2009) verified that the mixture perlite + vermiculite (1:1 v/v) incremented the average length of roots in olive cuttings (*Olea europaea* L.). According to Mattana et al. (2009), the cuttings of the Piperaceae *Pothomorphe umbellata* (L.) Miq. in the mixture soil + bovine manure + commercial substrate + vermiculite (3:1:1:1 v/v) presented higher values of dry and fresh root mass.

**Table 4.** Rooting characteristics of *Piper nigrum* cuttings in the substrates soil + organic compound, vermiculite, carbonized rice hull and commercial substrate

Evaluated characteristics	Substrates <sup>1</sup>		
	VERM	CRH	CS
Basal rooting (%)	66.35 a*	42.33 b	47.08 b
Basal root number	10.74 a	5.14 b	5.53 b
Total root number	18.66 a	9.55 b	10.30 b
Length of the largest basal root (cm)	3.70 a	1.67 b	2.55 b
Length of the largest nodal root (cm)	3.60 a	1.51 b	2.79 a
Length of largest total root (cm)	3.97 a	1.71 c	2.99 b
Basal root volume (cm <sup>3</sup> )	0.6583 a	0.3300 b	0.5550 a
Total root volume (cm <sup>3</sup> )	1.2775 a	0.6008 b	1.0233 a
Basal root dry mass (mg)	0.0316 ab	0.0200 b	0.0375 a
Nodal root dry mass (mg)	0.0358 a	0.0175 b	0.0383 a
Total root dry mass (mg)	0.0683 a	0.0358 b	0.0741 a

\*Averages followed by the same letter on the line do not differ within each other by Tukey's test, at 5% probability. <sup>1</sup>Substrates: VERM (vermiculite); CRH (carbonized rice hull) e CS (commercial substrate).

In the present work, the vermiculite and the commercial substrate were the ones which presented the highest total porosity (Table 1), what considerably contributed to the emission and growth of the root system. Dutra et al. (2012), also verified high porosity and low apparent density for the commercial substrate.

The vermiculite substrate presents low density (Table 1), which summed to its high porosity contributed to rate it as one of the best substrates, among those studied, concerning the root system characteristics. Higashikawa et al. (2010) also detected low density for the vermiculite substrate and, according to Abad et al. (2001), the perfect substrates density must be lower than 400 kg m<sup>-3</sup>, what was verified in the present work with the vermiculite (186. 15 kg m<sup>-3</sup>) (Table 1). The same was verified with the

commercial substrate (303.59 kg m<sup>-3</sup>) (Table 1), and another inferior to the recommended is the Plantmax<sup>®</sup> (278 kg m<sup>-3</sup>), as quoted by Fermino & Kämpf (2012). Dias et al. (2015) observed that the vermiculite proportioned to the mini-cuttings of *Anadenanthera macrocarpa* expressive root dry mass. These authors highlight that the weight of the root dry mass is directly correlated with the volume and quantity of roots, primordial factors for the better development of the seedlings after transplantation to the field.

The commercial substrate presents pH 5.2, situated in the recommended range for most crops according to Abreu et al. (2012) and also has an advantage of being one of the substrates with the best chemical composition regarding Mg, Ca, Ca + Mg, Zn, Cu, B, M.O. and CTC (Table 1).



The commercial substrate also presents as an important characteristic a good composition of M.O., which might likely be a source humic substances directly related with root formation. Sharangi & Kumar (2011) verified a better development of rooted cuttings of cv. Panniyur-1 when they receive, in field, a supplementation of organic matter (25%) with urea (75%).

The organic matter is very important in the production of seedlings through rhizogenic stimulation, considering that it presents, in its constitution, humic substances (HS) such as the humic (HA) and fulvic (FA) acids. Zandonadi et al. (2010) verified that the activation of the H<sup>+</sup>-ATPase of plasmatic membrane by HA essentially depends of mechanisms which utilize nitric oxide as messenger, specifically in the early development stages of the side roots.

In cuttings of *Codianeum variegatum* L. Rumph and hibiscus (*Hibiscus rosa-sinensis* L.) the concentrations of 579 and 970 mg L<sup>-1</sup> of IBA and 14 and 50 mmol L<sup>-1</sup> of C in the form of HA were those who promoted a higher accumulation of dry matter in the root system, respectively (Baldotto et al., 2012). Baldotto et al. (2013) also verified that the HA accelerates growth, anticipates and increases the flowering of gladiolus (*Gladiolus* L.).

The carbonized rice hull substrate was inferior compared to the remaining substrates in proportionating the characteristics length of largest total root, volume of basal and total root and total and nodal root dry mass. The carbonized rice hull also proportioned the worst results, similar to the commercial substrate, in the characteristics basal rooting, basal and total root number and length of the largest basal root. The carbonized rice hull also resulted in cuttings with inferior results of basal root dry mass, not differing of the vermiculite substrate (Table 4). The carbonized rice hull is a substrate which presented, as to its chemical characteristics the lowest CTC (2.4 cmol) (Table 1) and was also the substrate which presented the lowest total porosity (41.81%) (Table 1).

### Conclusions

The vermiculite is the most indicated substrate, and the mixture soil + organic compound is not recommended for the rooting of

cv. Bragantina, laçar and Guajarina cuttings.

As cvs. Guajarina e laçar apresentam alta capacidade rizognica no substrato vermiculite. E o substrato vermiculite  o mais adequado para a estaquia da cv. Bragantina.

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