Rooting of cuttings and analysis of essential oils from wild and cultivated *Piper macedoi* Yunck

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ABSTRACT: *Piper macedoi* Yunck. is a shrub with selective habitat that grows in Southeastern Brazil in damp places, especially in swampy areas of forests. The aim of this study was to evaluate the influence of the diameter of stem cuttings on rooting and root growth as well as to analyze the chemical composition of the essential oil from leaves of *Piper macedoi*. For the rooting study was used a randomized design with four treatments and five replications, each plot consisted of five stem cuttings that were evaluated after 65 days. The essential oil was obtained from fresh leaves of *P. macedoi* from wild habitat (October 2011) and cultivated (September and October 2011) by hydrodistillation and analyzed by GC-FID and GC-MS. Stem cuttings with smaller diameter had lower root length, root dry weight, number of leaves and number of shoots. Only rooting percentage did not differ from stem cuttings diameter. The average rooting achieved was 75%. The chemical composition of essential oil from wild and cultivated plants was similar. Plants from cultivation in September showed a slight difference in chemical composition, once the arylpropanoid fraction was higher than wild habitat and cultivation in October. Comparison between wild and cultivation in October showed the main volatile fraction characterized as monoterpenes (wild 46.2%; cultivated 46.7%), being *trans*-β-ocimene (wild 28.5%; cultivated 24.9%) identified in higher content. The sesquiterpene fraction (wild 20.9%; cultivated 22.6%) showed to be diversified and arylpropanoids were also identified in great amounts (wild 25.3%; cultivated 23.5%). The results showed that *Piper macedoi* can be propagated by stem cuttings technique and the diameter of the stems does not influence in the rooting percentage. The analysis of the volatile components of this species showed large amount of *trans*-β-ocimene and sarisan, two compounds with large range of biological activities.

Key words: Piperaceae, rooting, stem cutting, terpene, arylpropanoid, *trans*-β-ocimene.

RESUMO: Enraizamento de estacas e análise do óleo essencial de *Piper macedoi* Yunck. silvestre e cultivada. *Piper macedoi* Yunck. é um arbusto com habitat seletivo e que cresce no Sudeste do Brasil em lugares úmidos, especialmente em áreas alagadas de matas. O objetivo desse estudo foi avaliar a influência do diâmetro das estacas em enraizamento e crescimento das raízes, assim como analisar a composição química do óleo essencial das folhas de *Piper macedoi*. Para o estudo de enraizamento foi utilizado um delineamento inteiramente casualizado, com quatro tratamentos e cinco repetições, sendo cada parcela constituída por cinco estacas que foram avaliadas após 65 dias. Os óleos essenciais foram obtidos a partir de folhas frescas de *P. macedoi* de habitat silvestre (outubro de 2011) e cultivado (setembro e outubro de 2011) por hidrodestilação e analisado por CG-DIC e CG-EM. Estacas com diâmetro menor apresentaram menor comprimento de raízes, peso seco de raiz, número de folhas e número de brotações. Somente a porcentagem de enraizamento não variou com o diâmetro da estaca. O enraizamento médio foi de 75%. A composição química do óleo essencial de plantas silvestres e cultivadas foi similar. As plantas de cultivo (coleta de setembro) apresentaram uma maior fração de arilpropanóide do que as do habitat silvestre e no cultivo (coleta de outubro). Em comparação

Recebido para publicação em 13/06/2016
Aceito para publicação em 20/03/2017

10.1590/1983-084X/0033

entre silvestre e cultivo (outubro) os monoterpenos aparecem como grupo majoritário (silvestre 46,2%; cultivado 46,7%), sendo o *trans*-β-ocimeno (silvestre 28,5%; cultivado 24,9%) o de maior concentração. A fração sesquiterpeno (silvestre 20,9%; cultivado 22,6%) apresentou-se mais diversificada e os arilpropanóides também foram identificados em grandes quantidades (silvestre 25.3%; cultivado 23,5%). Os resultados demonstram que *P. macedoi* pode ser propagada por estaquia e que o diâmetro do caule não interfere no percentual de enraizamento. A análise dos componentes voláteis dessa espécie demonstrou grande quantidade de *trans*-β-ocimeno e de sarisan, duas substâncias com grande espectro de atividades biológicas.

Palavras Chaves: Piperaceae, enraizamento, propagação, terpeno, arilpropanoide, *trans*-β-ocimeno.

INTRODUCTION

The Piperaceae family is mainly tropical, with great distribution on Central and South Americas, being found from North to South of Brazil, including in the Amazon and Atlantic Forest regions and less frequent in Brazilian savannah (Cerrado). The genus *Piper* is the most representative of the family, featuring more than 700 species of which about 170 grow natively in Brazil (Yunker, 1972; Quijano-Abril et al., 2006).

Regarding the volatile chemistry of the Piperarceae species, many scientific papers highlight rich fractions composed by monoterpenes, sesquiterpenes and arylpropanoids (Santos et al., 2001; Marques et al., 2010; Oliveira et al., 2013a,b). As rich as the volatile composition are the biological and pharmaceutical properties of many Piperaceae species, including *P. aduncum* L. (insecticide, larvicide, egg-hatching inhibition, antileishmanial and antimicrobial), *P. betle* L. (antidiabetic, antioxidant and antimicrobial), *P. methysticum* G. Fost (standardized extracts for the treatment of anxiety), *Peperomia blanda* Kunth. (antifungal), *Peperomia pellucida* (L.) HBK (anti-inflammatory and analgesic), *Peperomia sui* Lin & Lu (cytotoxic) and *Pothomorphe umbellate* L. (anti-inflammatory and analgesic) (Bernard et al., 1995; Torres-Santos et al., 1999; Santos et al., 2001; Morais et al., 2007; Marques et al., 2010; Sauter et al., 2012; Arrigoni-Blank, et al., 2004; Cordeiro et al., 2005; Perazzo et al., 2005; Cheng & Chen, 2008; Lara Júnior et al., 2012; Oliveira et al., 2013a; Oliveira et al., 2014).

Piper macedoi Yunck. is a shrub with selective habitat that grows in Southeastern Brazil in damp places, especially in swampy areas of forests. In the North of Minas Gerais State, *P. macedoi* grows naturally in areas of Footpaths, an important ecosystem in the Cerrado biome (savannah), considered wetland area of great environmental importance to the region (Alencar-Silva & Maillard, 2011). Although Footpaths are protected by laws dealing with the conservation of Permanent Preservation Areas (BRAZIL, 1989; CONAMA, 2002), they show a high degree of degradation due to the strong human pressure on its resources (Alencar-Silva & Maillard, 2011).

Many native species are lacking in technical production of seedlings, and in many cases, there are difficulties in sexual propagation. Thus, vegetative propagation is an alternative to multiplication, providing the production of good quality planting material in a short time with maintenance of genotypic characteristics. Studies on propagation techniques of native species provide subsidies for recovery of degraded areas, preservation of the environment and the selection and multiplication of plants that may exhibit characteristics of commercial interest, such as medicinal properties and/or ornamental (Oliveira et al., 2002; Tchoundjeu et al., 2004; Lü et al., 2012). Furthermore, asexual reproduction allows maintaining the constitution of a selected genotype from breeding program or from some individuals identified in the populations (Hartmann et al., 2002). Also, it is interesting to note that most studies of medicinal plants in Brazil are done with exotic species and much of the production comes from the extraction (Oliveira et al., 2013a). However, for obtaining raw material quality and sustainability of cultivation is very important to provide a standardized product (Jannuzzi et al., 2010). Studies on plant propagation are fundamental to the success of cultivation and domestication (Chagas et al, 2008; Carvalho Junior et al, 2009; Montanary Junior, 2010) and little is known about *in vitro* or *ex vitro* plant propagation of Piperaceae species. For example, some papers on this subject can be found regarding to black pepper (*Piper nigrum* L.), due its commercial importance, and regarding to *P. methysticum* G. Forst due its medicinal relevance (Thanuja et al., 2002; Zhang et al., 2008; Sharangi et al., 2010; Ahmad et al., 2013).

P. macedoi remains in the wild state in the Cerrado biome and there are no reports in the literature on agronomic (including propagation), biological and chemical points of view which motivated our study.

MATERIAL AND METHODS

Rooting of cuttings

Herbaceous stem cuttings of *Piper macedoi* Yunck. were collected in December 2010, in a foootpath area located near the city of Patis, North of Minas Gerais State, Brazil (GPS S 16 º05.622'/ W44 º12.088'; 782 meters high). Expert in Piperaceae Dr. Elsie Franklin Guimarães identified the plant material and voucher specimen was deposited at the Herbarium of the Botanical Garden of Rio de Janeiro (RB 501.330).

The study of propagation by stem cuttings started in December 2010, and were conducted in a greenhouse at Universidade Federal de Minas Gerais (UFMG), city of Montes Claros, Brazil (GPS: S16°44'02.8"/ W43°51'23.3"; 630 meters high). The stem cuttings were classified with the aid of digital calipers accurate to one hundredth of a millimeter, defining four treatments by intervals of diameters: 3.00 – 4.49 mm (D1); 4.50 – 6.49 mm (D2); 6.50 – 7.99 mm (D3) and 8.00 – 11.00 mm (D4). Leaves were removed in all treatments. It was used a completely randomized design with four treatments and five replications, five stem cuttings per plot. The stem cuttings were placed at a depth of 4 cm in plastic trays filled with commercial substrate Tropstrato HT (Vida Verde, Brazil).

The following parameters were measured after 65 days: (a) length of the bigger root and of the bigger shooting (cm); (b) number of shootings; (c) number of leaves; (d) rooting percentage; (e) shoot and root dry weight (g); (f) aerial part/ radicular system ratio (AP/RS). Graduated ruler was used to measure the roots and shoots. The seedlings were divided into roots and shoots, and the material weighed on a precision balance to obtain the fresh weight. Then, the material was stored in paper bags and transferred to a kiln to dry at a temperature of 65 °C until constant weight to determine the dry weight. The aerial part/ radicular system ratios (AP/ RS) were obtained by dividing the dry weight of shoots (DWS) by dry weight of roots (DWR). Data were subjected variance analysis and previously tested for homoscedasticity by Hartley test. Means were compared by Tukey test at a significance level of 5%. Software SISVAR 4.6 it was used for statistical analysis (Ferreira, 1998).

Essential oil chemical composition

The essential oils were extracted of fresh leaves of *P. macedoi* from wild habitat (October 2011) and from cultivated plants (September and October 2011) from the same stem cuttings used to rooting and root growth study. The plant material was subjected to hydrodistillation for 2h in a Clevenger-type apparatus. Extraction procedure was done at Aromatic and Medicinal Plants Laboratory of Universidade Federal de Minas Gerais, Montes Claros, Brazil.

The samples were subjected to analysis by gas chromatography coupled to flame ionization detector (HP-Agilent 6890 GC-FID) and by gas chromatography coupled to mass spectrometry (HP Agilent GC 6890 – MS 5973), at Analytical Platform of Farmanguinhos, Fiocruz, Rio de Janeiro, Brazil. Initially, the essential oil was diluted in dichloromethane (1.0 $mg.ml^{-1}$) and analyzed by GC-MS to obtain the mass spectra and to performer chemical characterization. Concomitantly, another sample of essential oil (0.5 $mg.ml^{-1}$) was analyzed by GC-FID for quantification of chemical constituents and to determine the retention indices (RI). Each essential oil component was quantified based only in the individual component's relative peak area in the chromatogram. The substances in the essential oil were identified by comparing their mass spectra with database registration (WILEY7n) and by comparison of Retention Indices (RI) calculated with those from literature records (Adams, 2007).

GC-FID parameters: HP-5ms column (30 m x 0.32 mm x 0.25 µm), temperature programming from 60 to 240 °C, with increase of 3 $°C.min^{-1}$, using the hydrogen and synthetic air as the carrier gases, with a flow rate of 1.0 $ml.min^{-1}$ and injection volume of 1µl. *GC-MS parameters:* HP-5ms column (30 m x 0.32 mm x 0.25 µm), temperature programming from 60 to 240 °C, with increase of 3 $°C.min^{-1}$, using helium as the carrier gas, with a flow rate of 1.0 $ml.min^{-1}$ and injection volume of 1µl.

RESULTS AND DISCUSSION

Rooting of cuttings

In accordance with our results, the stem cuttings diameter influence the rooting percentage of *Piper macedoi*. Our study showed that cuttings with smaller diameter (3.00 to 4.49 mm) had lower root length, root dry weight, number of leaves and number of shoots (Table 1). These results do not corroborate with those obtained by Dias et al. (1999), where the average cuttings diameters (9.4, 15.8 and 27.5 mm) of *Platanus acerifolia* (Aiton) Willdenow not influence the average number of roots, average root length and number of leaves.

The obtained rooting average was 75% which indicate the viability of propagation of this species by stem cuttings in greenhouse, without the use of growth regulators to stimulate root formation (Table 1).

Similar results were obtained by Biondi et al. (2008) that observed the same cuttings diameter did not influence the rooting of *Tecoma stans* (L.) Juss. ex Kunth, measured after 100 days after planting. Higher rooting percentages were obtained

TABLE 1. Number of leaves (NL), length of the bigger shooting (LBS), number of shootings (NS), length of the bigger root (LBR), dry weight of roots (DWR), dry weight of shoots (DWS) and rooting percentage (RP), in accordance with *Piper macedoi* stem cuttings diameters.

Diameter (mm)	NL*	LBS (cm)*	NS*	LBR (cm)*	DWR (g)*	DWS (g)*	RP%ns
3.00 – 4.49 (D1)	2.42b	2.72c	1.55b	11.65b	0.06b	0.13b	60.00
4.50 – 6.49 (D2)	4.34a	3.66bc	2.47ab	18.08a	0.11ab	0.16b	84.00
6.50 – 7.99 (D3)	4.31a	6.59ab	2.02ab	17.59a	0.14ab	0.35a	80.00
8.00 – 11.00 (D4)	4.19a	7.13a	2.01a	18.38a	0.15a	0.34a	76.00
Mean	3.81	5.02	2.01	16.42	0.11	0.24	75.00
RSD (%)	24.38	33.82	17.79	18.17	39.33	33.39	22.71

* Means followed by the same letter in the columns are not different by the Tukey test at a significance level of 5%. ns = no statistical differences.

for species of *Piper* genus in a study conducted by Magevski et al. (2011). They observed a survival rate of cuttings from *Piper arboreum* Aub. and *Piper amplum* Kunth., 45 days after transplanting, of 98% and 89%, respectively. Dousseau (2009) studied the rooting of *P. aduncum* L. from stem cuttings from apical, middle and basal branches of plagiotrophycal and orthotropic-like segment. The author found that the percentage of survival for the orthotropic branches ranged from 40 to 60%, while for plagiotrophycal branches ranged from 60 to 90%. These differences in rooting ability of cuttings of Piperaceae species may be related to the phases of development of the plant matrix, and/ or biochemical and physiological status of the stem cuttings (Leonel et al., 1991; Fachinello et al., 2005).

Garbuio et al. (2007) found that in the vegetative propagation, the sprouting percentage, average length of the three longest roots and number of roots per cuttings of patchouli (*Pogostemn cablin* Benth.) decreased from the apical to basal cuttings. However, Pacheco & Franco (2008), studying the rooting of thin (4.0 to 8.0 mm), medium (9.0 to 14.0 mm) and thick (15.0 to 26.0 mm) cuttings of *Luehea divaricata* Mart., found that larger diameter cuttings led to higher root length. They attribute this result to the higher amount of carbohydrates (the main source of energy available to root development) in these cuttings. According to Fachinello et al. (1995), larger diameter cuttings have higher levels of energy reserves, which promote rooting. However, larger diameter cuttings may also have high rates of shoots leading to less rooting rates, which was not observed in the cuttings of *P. macedoi*.

Stems cuttings with diameter D4 showed higher shoots length and dry weight of shoots that stems cuttings diameter D1 and D2 (Table 1). Similar results were found by Ferreira et al. (2010), where the larger diameter cuttings of *Manihot glaziovii* Muell. Arg. support the emission of shoots, number of roots and number of leaves of this species. In general, the thick cuttings have higher nutrient availability, which can support the development of the root system and stimulating the shoots. However, the fine cuttings may also have the highest concentration of endogenous auxins, since these compounds are stored close to their production zone (Tavares et al., 2012). In the present study we observed that the highest level of available reserves in the stem cuttings of *P. macedoi* supported the production of more vigorous seedlings. The highest number of leaves observed in cuttings of *P. macedoi* with diameter from 8.0 to 11.0 mm (D4) is probably related to the further development of the radicular system of these cuttings (Table 1).

According to Tchoundjeu et al. (2002), the rooting process is directly correlated with the increase in translocation of carbohydrates to the base of the stem or with the production of photosynthesis compounds, auxins and rooting cofactors by the younger leaves and its transport towards the base. Hartmann et al. (1990) emphasize that the leaves production is dependent of the emission of adventitious roots to guarantee supply of nutrition and water for the plant propagation. Thus, although in the present study there were no leaves at the start of propagation, it can be inferred that the development of vigorous leaves from cuttings of greater diameter (D4) resulted in the formation and development of vigorous roots.

The aerial part/ radicular system ratios (AP/ RS), that were obtained dividing the dry weight of shoots (DWS) by dry weight of roots (DWR), were not changed by the stems cut diameter (Figure 1). In agreement with Braun et al. (2007), stem cuttings with lower AP/ RS ratio have lower rate of water use and better tolerance of adverse conditions. However, cuttings of smaller diameter, such they are located near the top of the plants, are generally more susceptible to dehydration, which requires great care in the preparation and certainly would not survive without the condition of constant water supply by spraying (Biasi & Costa, 2003). Therefore, seedlings obtained from cuttings with smaller diameter require

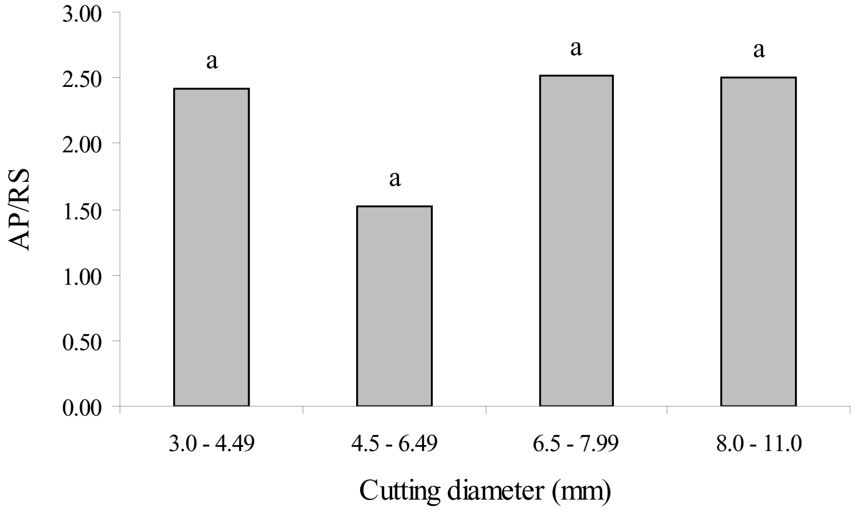

FIGURE 1. Aerial part/ radicular system ratio (AP/ RS) obtained from different stem cuttings diameters of *P. macedoi*, after 65 days of being placed in bed of rooting. Columns with the same brochure and letters indicate no statistical difference on Tukey test at a significance level of 5%.

greater care after transplanting. Also, it is interesting to note that cuttings with larger roots are more likely to loss or damage to seedlings transposition into another site (Biondi et al., 2008).

Essential oil chemical composition

This paper describes for the first time the chemical composition of the essential oil from leaves of *P. macedoi*, a species from Brazilian Cerrado biome (savannah). The yield of essential oil obtained by hydrodistillation of fresh leaves from cultivated and wild habitat plants was 0.40% (v/w).

The essential oil chemical composition of wild habitat and cultivated plants showed to be qualitatively and quantitatively similar, considering the main identified compounds (*trans*-β-ocimene, sarisan) and fractions of monoterpnes, sesquiterpenes and arylpropanoids. More than 90% of the volatile composition was characterized and a total of 49 different compounds were identified in the three samples (Table 2). The essential oil obtained from cultivated plants in September 2011 showed higher amounts of arylpropanoids, and sarisan (asaricin) was characterized as the main compound (24.0%). However, the *trans*-β-ocimene amount (21.0%) is quite the same of sarisan. Considering plants colleted in October from natural habitat and from cultivation, the main volatile fraction was identified as monoterpenes (wild 46.2%; cultivated 46.7%), being *trans*-β-ocimene (wild 28.5%; cultivated 24.9%) identified in higher amount. The monoterpene fraction of both samples showed high content of non-oxygenated compounds, but oxygenated one were also identified (linalool, α-terpineol, carvacrol and timol). The sesquiterpene fraction (wild 20.9%; cultivated 22.6%) showed to be very diversified and mainly composed by non-oxygenated compounds. Arylpropanoids were also identified in the samples from October 2011 in great amounts (wild 25.3%; cultivated 23.5%) (Table 2), being sarisan (wild 14.1%; cultivated 17.8%) characterized as the main compound of this fraction.

Comparison between arylpropanoid and monoterpenoid fraction, specifically considering the two main compounds identified, sarisan and *trans*-β-ocimene, suggest a dichotomy terpenoid / arylpropanoid. It is possible since these two substances have very different biosynthetic routes of formation. The monoterpene *trans*-β-ocimene is biosynthesized via acetate-mevalonate pathway, while the arylpropanoid sarisan is formed by the shikimate pathway (Dewick, 2009). This possible dichotomy was also suggested to *Piper dilatatum* L. C. Rich essential oils. Andrade et al. (2011) pointed out those essential oils of *P. dilatatum* from North of Brazil (Amazon region) as rich only in monoterpenes and sesquiterpenes, whereas other essential oils of *Piper* from the same region contain terpenoids or arylrpopanoids. However, the observed intra-specific dichotomy in *P. macedoi* essential oil must be proved in a seasonal study.

The two main compounds identified in the three samples, *trans*-β-ocimene and sarisan (asaricin), have a large range of biological activities. Essential oils rich fractions in *trans*-β-ocimene and sarisan have shown mainly antibacterial, antifungal, larvicidal, insecticidal, insect attractor, antioxidant, SNC stimulant and anti-inflammatory activities (Moreira et al., 2001; Morais et al., 2007; Pirbaloutia et al., 2013; Valente et al., 2013; Seyyedan et al.,

TABLE 2. Chemical Composition of the Essential Oil from Fresh Leaves of *P. macedoi.*

Constituents	RI_{ref}	RI_{exp}		Percentage (%)**		
		Wild*	Cultive#*	Wild*	Cultive#	Cultive*
Monoterpenes	-	-	-	46.2	36.1	46.7
α-pinene	939	926	928-929	1.0	0.9	2.1
β-pinene	980	972	973-973	2.0	2.3	5.0
β-myrcene	991	980	982-983	0.4	0.3	0.5
α-phellandrene	1002	1001	998-1003	0.7	0.6	0.9
limonene	1031	1027	1025-1024	1.2	0.6	0.7
β-phellandrene	1031	1028	1025-1029	9.0	6.5	7.9
trans-β-ocimene	1050	1041	1042-1042	28.5	21.0	24.9
linalool	1098	1092	1090-1095	2.7	3.2	2.7
perillene	1099	1103	1093-1098	0.2	0.1	0.2
not identified	-	x	1115-1117	x	0.4	0.5
α-terpineol	1189	1184	1184-1089	0.1	0.1	0.1
timol	1289	1285	1287-x	0.4	0.1	X
carvacrol	1298	x	x-1290	x	x	1.2
Sesquiterpenes	-	-	-	20.9	21.0	22.6
α-cubebene	1351	1342	1341-1341	0.4	0.9	0.6
α-copaene	1376	1367	1370-1370	0.1	0.8	1.2
β-cubebene	1387	1377	1383-1384	0.5	0.9	0.8
E-caryophyllene	1418	1415	1410-1414	2.2	1.2	3.1
β-cedrene	1419	1416	1414-1418	0.3	0.1	0.5
aromadendrene	1439	1432	1430-1433	0.3	0.2	0.6
α-humulene	1454	1450	1442-1450	1.2	0.7	1.1
γ-muurolene	1477	1470	1465-1470	0.6	0.3	0.4
germacrene D	1480	1477	1470-1480	3.7	2.3	3.2
β-selinene	1485	1480	1475-1483	0.2	0.1	0.6
cis-β-guaiene	1490	1486	1480-1490	0.1	0.1	0.1
valencene	1496	1489	1498-x	0.1	0.1	X
trans-β-guaine	1500	1490	1490-1493	0.2	0.1	0.4
γ-cadinene	1513	1500	1501-1502	0.1	0.1	0.1
cubebol	1514	1502	1502-1513	1.5	1.6	0.5
cis-γ-bisabolene	1515	1506	x-1505	0.1	x	0.2
germacrene B	1559	1545	1543-1552	0.2	0.3	0.2
nerolidol	1564	1557	1553-1558	1.4	1.5	1.2
germacrene D-4-ol	1574	1566	1565-1571	0.3	1.0	0.3
caryophyllene oxide	1581	1575	1570-1576	0.3	1.7	0.2
globulol	1583	1578	1575-1579	1.1	0.1	1.2
humulene epoxide II	1607	1601	1596-1605	0.4	0.8	0.4
1,10 di-*epi*-cubenol	1618	1609	1604-1608	0.3	0.6	0.4
epi-cubenol	1627	1620	1617-1620	0.5	0.6	0.5
cis-cadin-4-en-ol	1635	x	1622-1626	x	0.3	0.2
epi-α-cadinol	1638	1632	1628-1636	0.2	0.1	0.3
τ-muurolol	1642	1635	1631-1635	1.2	1.1	1.0
α-muurolol	1645	1637	1633-1638	0.3	0.3	0.3
α-cadinol	1653	1546	1642-1648	1.0	0.1	0.9

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TABLE 2. *Continuation*

Constituents	RI_{ref}	RI_{exp}		Percentage (%)**		
		Wild*	Cultive#*	Wild*	Cultive#	Cultive*
not identified	-	1662	1656-1662	0.1	1.0	0.1
α-bisabol	1686	1682	1678-1676	2.0	2.0	2.0
Arylpropanoids	-	-	-	25.3	41.6	23.5
methyleugenol	1403	1398	1397-1397	0.9	0.1	0.5
safrole	1285	1278	1278-1283	4.1	5.1	3.1
sarisan (asaricin)	-	1490	1485-1490	**14.1**	**24.0**	**17.8**
myristicin	1520	1512	1510-1512	5.0	9.7	0.1
elemicin	1554	1543	1545-1543	0.1	0.2	0.2
dillapiol	1620	1612	1611-1613	1.1	2.5	1.8
Other	-			0.2	0.0	0.2
anisol	1232	1218	x-1222	0.2	x	0.2
TOTAL %				92.6	98.7	93.0

RI_{ref} - Retention indices from literature[1]; RI_{exp} – Retention indices calculated based on GC-FID (see experimental); # September 2011; * October 2011; ** relative percentages based on the areas from GC-FID analysis. x = not present. [1]ADAMS (2001).

2013).

Studies on volatile components of Piperaceae species have revealed rich fractions of several components, especially monoterpenes and sesquiterpenes. It is worth to note the presence of arylpropanoids. Essential oils of Piperaceae have shown excellent biological activities. For example, the essential oil from leaves of *Piper claussenianum* (Miq.) C.DC. collected at Southeast Brazil, which is rich in nerolidol (ca. 80%), showed great activity against promastigotes of *Leishmania amazonensis* and against *Candida albicans* (Marques et al., 2010). The study of the volatile components of 10 Piperaceae species from the Atlantic Forest fragment near the city of Paraty, performed by Santos et al. (2001) revealed rich and diverse fractions in monoterpenes and sesquiterpenes. The majority of the identified sesquiterpenes were biosynthesized prior from the precursor *E*,*E*-farnesyl pyrophosphate. That study showed the absence of arylpropanoids, very common in essential oils from species of Piperaceae, in all analyzed samples. The low occurrence of arylpropanoids in Piperaceae species from the Atlantic Forest may be due to an adaptive function, since these substances are very common in Piperaceae species from the Amazon Rainforest (Andrade et al., 2009). Previous study on essential oil of *Piper aduncum* L. collected in the Cerrado area near of the city of Bocaiúva, North of Minas Gerais State (Southeast of Brazil), showed a main monoterpene fraction composed by monoterpenes (92.7%) in which 1,8-cineole was identified as the major compound (57.2%) (Oliveira et al., 2013a). Maia and colleagues (1998) showed that the essential oil from leaves of *P. aduncum* collected at different sites in Amazon region of Brazil (North region) is rich in dillaiole (30-90%). Chemical, agronomical and isoenzimatic analysis of *P. aduncum* from Cerrado region confirmed the existence of a new chemotype for this species (Oliveira et. al, 2013a). The essential oil composition of *P. macedoi* from Cerrado showed differences from that of *P. aduncum* because although encompassed as major substance a monoterpene (*trans*-β-ocimene) also had great amount of arylpropanoids (sarisan). These differences demonstrate the richness of the volatile components of Piperaceae species.

The results showed that *Piper macedoi* can be propagated by stem cuttings technique and the diameter of the stems does not influence in the rooting percentage. The analysis of the volatile components of this species showed large amount of *trans*-β-ocimene and sarisan, as well as a richness fraction of different sesquiterpenes. This found is very important once the growth of *P. macedoi* under controlled conditions in greenhouse did not affect the volatile composition, even considering the differences in soil, water supply and microclimate.

ACKNOWLEDGEMENTS

The authors thank to CAPES, FAPERJ, FAPEMIG and Farmanguinhos for the financial support and Dra. Elsie Franklin Guimarães for identification of the plant material.

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