DOI: 10.5965/223811711732018435

Revista de Ciências Agroveterinárias 17(3): 2018
Universidade do Estado de Santa Catarina

Rooting of apical, median and basal stem cuttings of *Piper aduncum* L. on different substrates

Enraizamento de estacas apicais, medianas e basais de Piper aduncum L. em diferentes substratos

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Submission: 28/07/2017 | Acceptance: 13/06/2018

ABSTRACT

Piper aduncum L. (Piperaceae) is an aromatic species native from Tropical Americas. The elevated dillapiole content in its essential oil attributes great economic potential to the species, due to its proven insecticidal action against important agricultural pests. The domestication and cultivation of this species, seeking the standardization of the essential oil quality, goes through the stage of plant propagation, which presents scarce technical and scientific information. Thus, the objective of this study was to evaluate the rooting of stem cuttings collected from the apical, median and basal positions of *P. aduncum* plagiotropic branches, in sieved soil, commercial substrate (composed of pine bark, peat, expanded vermiculite and enriched with macro and micronutrients), and vermiculite of medium granulometry. The cuttings were kept under intermittent misting for 45 days until the experimental evaluation. Higher rooting percentages were observed in apical cuttings (17.4%) when compared to basal ones (6.9%). There were also higher number, length and fresh mass of roots in apical cuttings. The substrate did not influence the rooting of *P. aduncum* stem cuttings.

KEYWORDS: dillapiole, Piperaceae, spiked pepper, vegetative propagation.

RESUMO

Piper aduncum L. (Piperaceae), é uma espécie aromática nativa da América Tropical. O alto teor do composto dilapiol no óleo essencial atribui à espécie grande potencial econômico em função de sua comprovada ação inseticida em importantes pragas agrícolas. A domesticação e cultivo da espécie, visando a padronização da qualidade do óleo essencial, passa pela etapa de produção de mudas, na qual são escassas as informações técnicas e científicas. Nesse sentido, objetivou-se com o presente trabalho avaliar o enraizamento de estacas caulinares coletadas das posições apical, mediana e basal de ramos plagiotrópicos de *P. aduncum* em solo peneirado, substrato comercial (composto de casca de pinus, turfa, vermiculita expandida e enriquecido com macro e micronutrientes) e vermiculita de granulometria média. As estacas foram mantidas sob nebulização intermitente por 45 dias até a avaliação do experimento. Não houve interação significativa entre substratos e tipos de estaca. Avaliando os fatores isoladamente, observaram-se maiores porcentagens de enraizamento em estacas apicais (17,4%) em relação às basais (6,9%). Verificou-se também maiores número, comprimento e massa fresca de raízes nas estacas da porção apical. O substrato não influenciou o enraizamento das estacas de *P. aduncum.*

PALAVRAS-CHAVE: dilapiol, Pimenta-de-macaco, Piperaceae, Propagação vegetativa.

Piper aduncum L. (Piperaceae), popularly known in Brazil as “pimenta-de-macaco” and “aperta-ruão”, is a shrub that can grow to 8 meters high, easily recognizable by its arched spikes and hanging leaves with scabrid adaxial surface, which are rough to the touch. The species is native from Tropical Americas, where it grows spontaneously in the subsurface of several forest formations, preferably in soils with high organic matter content and humidity. In Brazil, the plant is found in the states of Acre, Pará, Mato Grosso, Ceará, Bahia, Minas Gerais, Espírito Santo, Rio de Janeiro, São Paulo and Paraná (FAZOLIN et al. 2006, LORENZI & MATOS 2008, SARNAGLIA JUNIOR et al. 2014).

The main economic interest in this species derives from the high essential oil content (2.5 to 4.0%) produced in its leaves, which presents useful biological properties, mainly for the agrochemical industry of natural products (GAIA et al. 2010, SILVA et al. 2013). The use of the essential oil of *P. aduncum* in several biological tests presented useful activities in the agricultural segment, particularly as a fungicide, larvicide, insecticide and molluscicide. These effects can be attributed to phenylpropanoid dillapiole, a major compound in the essential oil of this species (SOUSA et al. 2008, PACHECO et al. 2016, KRINSKI & FOERSTER 2016, SANINI et al. 2017).

Several studies with agricultural pests of high economic interest have shown the potential of using *P. aduncum* as a natural insecticide (KRINSKI & FOERSTER 2016, TURCHEN et al. 2016, SANINI et al. 2017). Despite the potential for exploring natural products from the species, little is known about the agronomic practices needed to enable its cultivation. The ability to sustain the production of high-quality plant materials quality under controlled conditions is important, given the need to produce standardized compounds (PACHECO et al. 2016).

One of the primary information to introduce plant crops is defining the most viable propagation method. Considering the need to standardize the quality of *P. aduncum* essential oil, especially the selection of clones with high dillapiole contents, vegetative propagation is one of the most indicated methods for the maintenance of specific genetic characteristics. Among vegetative propagation methods, propagation by stem cuttings is one of the most viable techniques to enable the multiplication of genotypes of interest, crop uniformity, and present relatively low cost when compared to other methods (NUNES-GOMES & KRINSKI 2016a, BISCHOFF et al. 2017). However, there are few scientific studies reporting the propagation of *P. aduncum* by stem cuttings.

Many factors can affect the adventitious rooting of stem cuttings. The position from where the cuttings are collected in the branch and the substrates used for planting the cuttings are some of the most important (ZUFFELLATO-RIBAS & RODRIGUES 2001). Studies evaluating the best combinations of mixtures in substrates for the rooting of medicinal plant species through stem cuttings are still scarce (MATTANA et al. 2009), especially in aromatic species from the genus *Piper.* Regarding the position of stem cuttings, cuttings from the apical position of branches have greater endogenous auxin content; however, low survival rates may occur due to the greater predisposition of these stem cuttings to dehydration (LIMA et al. 2010, HARTMANN et al. 2011).

Given this context, the objective of this study was to evaluate the rooting of stem cuttings collected from the apical, median and basal positions of plagiotropic branches of *P. aduncum* on different substrates.

The collection of the plant material for preparing the stem cuttings was conducted on December 29th, 2015, in the afternoon, from the median part of 10 stock plants. Branches with leaves of *P. aduncum* were collected in the municipality of Adrianápolis (24°36'S 48°58'W – 150 m), Paraná, Brazil. The plants were moistened and conditioned in polyethylene black bags for transportation to a greenhouse in Curitiba, Paraná. A voucher specimen of the plant material used can be found in the Herbarium of the Universidade do Estado de Mato Grosso (UNEMAT) at the campus of Tangará da Serra, under the identification “TANG 1779”.

The experimental design was completely randomized in a 3x3 factorial scheme, with three substrates and three stem cutting positions in the branch. Each treatment was composed of four replicates, with 12 cuttings as sample unit, totalizing 432 cuttings in the experiment. The analyzed substrates were vermiculite of medium granulometry, commercial substrate for vegetables Tropstrato HT® and sieved soil. According to the manufacturer, the commercial substrate is composed of pine bark, peat, expanded vermiculite and enriched with macro and micronutrients. According to chemical analysis, the sieved soil presented pH 4.7; 1.9 cmol dm^{-3} of aluminum; 2.60 cmol dm^{-3} of calcium; 1.5 cmol dm^{-3} of magnesium; 3.2 mg dm^{-3} of phosphorus; and 16.7 g dm^{-3} of carbon.

To prepare the stem cuttings, horizontal growth branches, called plagiotropic, were selected and stratified into apical (distal), median and basal (proximal) segments. The cuttings were made with 15 cm in length, with a diagonal cut (bevel) on the base and a straight cut on the apical region. A leaf reduced to half of its original area was maintained in the apical region of the cuttings.

The cuttings were planted in 120 cm^3 plastic tubes filled with the substrates. The tubes were placed on plastic supports and kept in a greenhouse under intermittent misting (5 seconds every 30 minutes) at 1.20 m from the ground. The cuttings were planted at 5 cm depth.

The rooting percentage (considering the number of live cuttings with roots of at least 1 mm in length), mortality (relative number of cuttings with all tissues necrotic), sprouting (cuttings with new shoots), cuttings with maintenance of the original leaf (leaf retention), average number, length and fresh mass of roots and fresh mass of shoots were evaluated 45 days after the start of the experiment. Roots length was measured with a graduated ruler and the result showed as the arithmetic mean of the three largest roots lengths in each cutting within the replicate. Dry mass of roots and shoots was obtained by drying the fresh material in a forced air circulation oven at 65 °C until constant weight. The material was weighed on an analytical precision scale (0.0001 g).

Data were submitted to homogeneity analysis using Bartlett's test and analysis of variance (ANOVA). Significance was verified by the F test; the means were compared by Tukey's test at 5% significance. Statistical analyses were performed on data transformed into LogX + 10. Statistical tests were performed using the Assistat Software (SILVA & AZAVEDO 2016).

Interaction between the cuttings positions and the substrates was not observed for none of the analyzed characteristics. Analyzing the factors separately, it was found that the tested substrates did not differ for any of the characteristics related to the rooting or sprouting of *P. aduncum* stem cuttings. However, the cutting position in the branch was significant for the rooting percentage, leaf retention, number, length and fresh mass of roots (Table 1).

Table 1. Rooting (%), mortality (%), leaf retention (%), sprouting (%), average number (n), length (cm) and fresh mass of roots (g) and fresh mass of shoots (g) on cuttings from different positions on plagiotropic branches of *Piper aduncum* L. and in different substrates. Curitiba, PR (2016).

	Characteristics	Soil	Tropstrato	Vermiculite	CV (%)
Substrates	Rooting (%)^{ns}	15.3	8.3	10.4	17.23
	Mortality (%)^{ns}	84.0	91.7	85.4	2.82
	Leaf Retention (%)^{ns}	7.7	6.9	5.6	12.61
	Sprouting (%)^{ns}	9.0	3.5	7.6	17.57
	Roots number (n)^{ns}	8.6	6.5	7.2	12.38
	Roots length (cm)^{ns}	5.6	3.2	3.3	8.73
	Fress mass of roots (g)^{ns}	0.26	0.19	0.15	0.91
	Fress mass of shoots (g)^{ns}	0.05	0.02	0.04	0.23
	Variable	Apical	Median	Basal	
Stem cutting position	Rooting (%)	17.4 a	9.7 ab	6.9 b	17.23
	Mortality (%)^{ns}	82.6	88.9	89.6	2.82
	Leaf Retention (%)	13.9 a	4.2 b	2.1 b	12.61
	Sprouting (%)^{ns}	3.5	5.6	11.1	17.57
	Roots number (n)	13.2 a	6.3 b	2.7 b	12.38
	Roots length (cm)	7.9 a	3.2 b	1.0 b	8.73
	Fress mass of roots (g)	0.40 a	0.16 b	0.04 b	0.91
	Fress mass of shoots (g) ^{ns}	0.01	0.05	0.05	0.23

Means followed by different letters in the lines significantly differ according to Tukey' test at 5% of probability. ^{ns}: non-significant. CV: Coefficient of variation.

Although presenting contrasting characteristics (nutrients content, composition and structure), the substrates did not promote differences in the rooting of *P. aduncum* cuttings. These results differ from those observed by DOUSSEAU (2009) in the rooting of stem cuttings of this species. The author reports differences between the substrates sand, vermiculite and carbonized rice husk, with the worst performance observed in vermiculite (18.3% mean survival rate). The results of this study are also different from the values reported for *Piper umbellatum* L. – a species of the same genus, where the commercial substrate Plantmax® promoted greater root system vigor (NUNES-GOMES & KRINSKI 2016b). However, in a previous experiment for the same species, MATTANA et al. (2009) stated that there were no differences among substrates for rooting stem cuttings. These findings show the diversity in the response of species within the genus *Piper*, and even among different populations of the same species regarding the best substrates for rooting.

Considering the stem cuttings position on the branches, apical cuttings obtained higher rooting percentages (17.4%) when compared to basal ones (6.9%). The cuttings of the apical region were also superior in the characteristics related to the root system vigor: number, length and fresh root mass (Table 1). This behavior can be attributed to the proximity of auxin synthesis sites and the lower tissue differentiation in apical cuttings, something that was also observed for propagules of *Piper hispidum* Sw. (CUNHA et al. 2015). According to HARTMANN et al. (2011), stem cuttings harvested from the apical position of branches have less lignification, meristematic cells with more active metabolism and absence/fewer phenolic compounds, which facilitates rooting and sprouting.

Tissue lignification and the presence of anatomic barriers are aspects related to the lower percentage of rooting in basal cuttings. In *P. hispidum*, it was observed that basal cuttings presented a ring of sclerenchyma between the phloem and the cortex, which may constitute a barrier to roots emission (CUNHA et al. 2015). In *Piper amalago* L. stem cuttings, one of the factors used to justify the low percentages of rooting was the possibility of lignification of a continuous ring of angular collenchyma present in the cortex of the branches (NUNES-GOMES & KRINSKI 2016a). However, an anatomic study of apical, median and basal stem cuttings of *P. aduncum* no significant anatomical differences were found between the types of cuttings, which indicates that the difference observed in its rhizogenic potential is more related to physiological factors, mainly endogenous auxin contents (DOUSSEAU, 2009).

Although not affected by the treatments, the high mortality values in the stem cuttings of this experiment should be highlighted (Table 1). The values are similar to those observed for *P. amalago* stem cuttings, where the low survival rates were attributed to abscission in the node region, a phenomenon that separates the buried base of the cutting from the apex containing the leaf (NUNES-GOMES & KRINSKI 2016a). As reported by the cited authors, only cuttings that did not undergo abscission survived and were rooted in this study.

The separation of the cutting base from the region containing the leaf has direct impact on rooting and survival. Leaves are critical for the stimulation and induction of rhizogenesis, since the production of hormones and photoassimilates translocated to the base of the cuttings occurs in these organs (BISCHOFF et al. 2017). In fact, it is possible to observe that higher percentages of leaf retention occurred in apical stem cuttings when compared to basal ones (Table 1), evidencing its relation with the higher rooting percentages and the root system vigor (number, length and fresh mass of roots).

Considering the conditions in which this experiment was conducted, it can be affirmed that the use of sieved soil, commercial substrate Tropstrato HT® or vermiculite does not affect the rooting of *P. aduncum* stem cuttings. Regarding the cuttings positions in the branches, apical cuttings are recommended due to presenting higher rooting percentages and root system vigor.

Despite the low rooting percentages observed, this study is one of the first scientific reports on the vegetative propagation of *P. aduncum* and presents important results to support future studies. First, it is recommended that future research should involve different compositions of substrates, the application of plant regulators and effects of seasonality on the adventitious rooting of the species, seeking the development of more technically and economically feasible propagation protocols.

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