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ANATOMICAL AND PHYSIOLOGICAL CHARACTERIZATION OF THE VEGETATIVE PROPAGATION OF *Piper aduncum L.*

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ABSTRACT

Piper aduncum L., has great impotence in folk medicine and agriculture (insecticide and fungicide) due to the presence of essential oil in its structures, being mainly in the stem and leaves. Despite the commercial importance of this species, little is known about its propagation mechanisms. In this context, the objective of this study was to evaluate the influence of different substrates on the rooting of P. aduncum cuttings, taken from different portions of the plant, opposing these results with the anatomical characteristics. Stakes of plagiotropic (ortho) type plagiars were collected in three positions each (apical, median and basal), with only the plag cuttings being maintained with leaf. Three types of substrates (medium sand, charcoal rice bark and medium vermiculite) were evaluated and rooting was evaluated after 2 months. The cuttings used in rooting were evaluated for the presence of possible barriers to rhizogenesis, based on anatomical analysis and histochemical tests. Based on the results obtained, it was possible to verify the existence of biochemical and anatomical differences in the different cuttings used, being possible to constitute barriers to rhizogenesis.

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INTRODUCTION

Piper aduncum L., also known as the pimenta de macaco, is a shrub native to tropical America and widely distributed across the Brazilian territory, mostly in the Southeast, occurring spontaneously in pastures and forests borders (Lorenzi and Matos, 2002). Of its shoots an essential oil, with high levels of the phenylpropanoid dilapiol, may be extracted. The aqueous extracts of leaves and roots of *Piper aduncum* show insecticidal activity (Fazolin *et al*., 2007; Pereira *et al*., 2008) and fungicide (Navickiene *et al*., 2006; Guerrini *et al.,* 2009). The low environmental impact during the collection and practicality of obtaining the raw material favors the recommendation of the aqueous extract of leaves in alternative control programs (Silva *et al*., 2007). Depending on the dosage of *P. aduncum* extract, there is a high margin of safety with minimal toxic effects on hematological and biochemical parameters (Sousa *et al.,* 2008). Phytochemical tests have shown that the ethanolic extract of *P. aduncum* stems have alkaloids, volatile coumarins, saponins, tannins, triterpenes, flavonoids and free anthracenes (Aires and Lima, 2014), essential compounds in folk medicine and agriculture. The type of environment and container are factors that directly influence the concentration of the oil content (Negreiros *et al.,* 2017). This species is considered an opportunistic plant that invades deforested areas after logging, because of its high rusticity and high resistance to climate change (Aires and Lima, 2014). However, information on cultivation techniques in the Piper genus is scarce. Studies have shown that basal and median cuttings promote a greater length and mass of shoots (Cunha *et al*. 2015), favoring the production of quality seedlings. The formation of adventitious roots is a key step in vegetative propagation of plant species and problems associated with the rooting often results in significant economic losses (Ferreira and Ferrari, 2010; Oliveira *et al*., 2010). The formation of adventitious roots is a complex process regulated by a combination of different pathways, including hormonal biosynthesis and primary metabolism (Ahkami *et al.,* 2009). Vegetative propagation plants have the same genetic characteristics as the parent plant. In addition to the external factors (environment, substrate, temperature, humidity, luminosity and water availability), internal factors influence the vegetative development of cuttings (Vernier and Cardoso, 2013). Plants of good origins and quality are vital to the achievement of a lasting and quality production (Júnior *et al.,* 2013). Mayer *et al.* (2006) studying *Vitis* L. and Peixe et al. (2007) studying *Olea europaea* L., found that anatomical features may also influence the expansion or emergence phase of adventitious roots in stem cuttings, since these cuttings may contain fibers, or even a continuous ring of sclerenchyma in the cortical region, mechanically blocking the growth of the formation of root primordia. In this work, the influence of different substrates on the rooting of cuttings collected from maturing plants at different vegetation stages was studied, due to the scarcity of studies on the physiological and anatomical characteristics of the asexual propagation of *P. aduncum* plant, from January to March, contrasting these results with the anatomical characteristics.

MATERIALS AND METHODS

The cuttings were collected from vegetative plants at the Universidade Federal de Lavras (UFLA), located in the micro region of Lavras (MG), from January to March 2008. Exsicatas were made and these were found deposited in the Herbarium of the Federal University of Lavras, under registration number HUM 9137. Cuttings were collected from plagiotropic shoots (plag), which develop horizontally and may originate leaves, flowers and infructescenses, and orthotropic shoots (ortho), which develop vertically, and produces only the plagiotropic shoots. Three types of cuttings of both shoots were collected, and each obtained the following specifications. The cuttings of the plag shoots were removed from the median region of the plant and standardized at about 10 to 12 cm in length, containing two buds and a leaf split in half, in the direction perpendicular to the longitudinal axis of the leaf (Fig. 1a). The node immediately below the apical bud was called node 0 and the following 1, 2, 3, 4, 5 and 6. Three types of cuttings of these shoots were collected, as represented in Fig. 1a. These cuttings were called apical plag cuttings (containing the nodes 1 and 2, and about 2.5 mm in diameter), median plag cutting (containing the nodes 3 and 4, and about 3.75 mm in diameter) and basal plag cuttings (containing the nodes 5 and 6, and about 5.0 mm in diameter). In the orthotropic shoots the cuttings were standardized in size from 20 to 25 cm in length and contained two buds (Fig. 1b). The top node divides into two plag and was called node 0 and the following 1, 2, 3, 4, 5 and 6. The cuttings were called apical ortho cuttings (containing the nodes 1 and 2, and about 8.95 mm in diameter), median ortho cuttings (containing the nodes 3 and 4, and about 10.74 mm in diameter) and basal ortho cuttings (containing the nodes 5 and 6, and about 12.53 mm in diameter). The potential of rooting of these cuttings was evaluated against three types of substrates (medium sand, charcoal rice bark and medium vermiculite), in expanded polystyrene trays with 72 cells, kept in a growth room at a temperature of 25 ± 2 ° C and 12-hour photoperiod under fluorescent white light, with 78.5 $\mu mol.m^{-2}.s^{-1}$ of photosynthetically active radiation (RFA) incident on the leaf surface was measured by means of a quantum sensor LI 1600M coupled to the porometer. After 2 months of rooting, it was evaluated the leaf retention (LR), and for the leaves that remained after cutting, it was evaluated the number (SN) and length (SL) of shoots, number of leaves of each shoot (NLS), number of roots (NR) and length of the longest root (LLR). It was also calculated the percentage of rooting and cuttings survival (Surv). The experimental design was completely randomized, with 4 replicates of 5 cuttings each in 3×3 factorial arrangement, consisting of the three types of cuttings and three substrates for each of the ortho and plag shoots. The data were subjected to analysis of variance (ANOVA) and the means compared by a Tukey test ($p < 0.05$), using the statistical software SISVAR (Ferreira, 1999).

The same cuttings used in the rooting experiment were used to evaluate the presence of possible barriers to rhizogenesis. The fragments were collected in the region just below the basal bud of the cuttings. The specimens were fixed in FAA70, afterwards, they were cross-sectioned with the free hand, being stained with safrablau (safranin and astra blue 7.5: 2.5) (Kraus and Arduin, 1997), then submitted to histochemical tests to identify the presence and location of different chemical compounds (Ascensão, 2004). For identification of starch, lugol (Johansen, 1940) was used; comassie blue R250 (Fisher, 1968) for total proteins; floroglucinol (Johansen, 1940) in acidic medium for lignins; Sudan III (Sass, 1951) for total lipids; osmium tetroxide (Ganter and Jollés, 1969) for unsaturated lipids; sulfuric acid (Geissman and Griffin, 1971) for sesquiterpene lactones; dinitrophenylhydrazine (Ganter and Jollés, 1969) for carbonyl group terpenoids; ferric chloride (Johansen, 1940) and potassium dichromate (Gabe, 1968) for general phenolic compounds (ortho dihydroxyphenols); and hydrochloric vanillin (Mace and Howell, 1974) for tannins. The sections were mounted on semi-permanent slides with glycerol water (1:1) or the proper medium, depending on the specification of the method, analyzed and photographed with a microscope Olympus model BX 60 with a Canon A630 digital camera attached. The scales for the illustrations were obtained using a micrometric slide in the same optical conditions used in each case.

RESULTS AND DISCUSSION

The stakes of plag (Table 1) and ortho (Table 2) responded differently to different types of substrates and collection positions in the branch. For stakes of plag branches, a significant interaction between substrates and collected positions was observed for all variables analyzed (Table 1). It was verified that the rooting of the different types of cuttings was influenced by the substrates used. Similar results were found by Lima *et al.* (2007), who also observed interaction between these factors, indicating that they are not independent and that rooting is dependent on the effect of the types of substrates applied. By observing the effect of the substrate in each type of stake, it was possible to verify that in the sand occurred the highest survival rate in stakes taken from the median portion and the lowest in stakes, however, both did not differ from the apical stakes, the stakes of the region median survival rates on average 50% higher than the basal cuttings. The median and apical stakes had a higher number of roots, whereas basal and minor stakes had the highest number of roots (Table 1). For leaf retention, root length, number of shoots, number of leaves and shoot length, the results did not differ in this substrate. According to Cunha *et al.* (2015), the fact that the sand substrate is more favorable for the rooting of *Piper hispidum* SW., may be related to the higher aeration requirement than to the substrate wetting, since sand because it has a larger amount of macropores facilitates aeration at the base of the stakes, reducing cutting mortality and facilitating rooting In the charred rice bark, the highest survival rates and leaf number of shoots were observed in cuttings removed from the medial and basal portions of the branch, followed by apical. The leaf retention, the length of the largest root and the number of shoots were higher in the median and inferior stakes in the apical stakes, however, both did not differ from the basal stakes. Regarding the number of roots in the median stakes practically doubled in relation to the apical and basal stakes, which did not differ between them. The length of the largest sprout did not differ from each other. In vermiculite, the performance of cuttings removed from the apical and medial portions were superior and did not differ among them, regarding the survival rate, number of roots, length of the largest root, number of shoots and number of leaves of shoots. The leaf retention did not differ. However, the length of the shoot in the medium stakes was practically double in relation to the apical and basal stakes, which did not differ. The sprouting issue is an important characteristic for the formation of quality seedlings, since the presence of new leaves is essential for the plant nutrition after the depletion of the seed reserves (Gomes and Krinski, 2016). The substrates of rice husk and vermiculite did not influence the rooting of the blackberry cuttings (Yamamoto *et al*., 2013). Thus, in a wide analysis it was verified that medium cuttings presented better performance in relation to the others and that the sand substrate favored the rooting. cuttings was markedly reduced. It is also verified that in vermiculite all basal cuttings died (Table 1). Contrary to what was observed in this study for *P. aduncum*, the rooting of *Piper mikanianum* var. (Fig 1). The presence of the microorganisms in the vermicompost was associated with a higher number of leaves, roots and shoots in relation to sand (Pescador *et al.,* 2007). However, *P. hispidum* showed higher rooting in the sand-like substratum than commercial (Cunha *et al.*, 2015). Even brandegean *Justicia brandegeana* Wassh. & L. B. SM. presenting difficult rooting, it was observed that in vermiculite there was 50% of rooting. This indicates that the substrate may influence the rate of rhizogenesis (Rodrigues *et al.,* 2017). In the present work the vermiculite substrate did not influence the rooting of *P. aduncum* when compared to the other two substrates. Both sand and vermiculite are nutrient-free substrates, however, they present very distinct characteristics mainly as regards water retention capacity and weight. The sand presents higher density and smaller values of porosity, air spaces and water retention capacity when compared to vermiculite (Stumpf *et al.,* 1999; Lima *et al.,* 2007; Kratz *et al.,* 2013). The bark of charred rice has a lower density than sand, however, greater than vermiculite. It has higher porosity, aeration space and readily available water than sand and vermiculite (Stumpf *et al*., 1999; Lima *et al.,* 2007). The higher density of the sand may have contributed to the favoring of the rhizogenesis of *P. aduncum* cuttings in this substrate, which was probably due to the increase of the contact between the substrate and the stake, as well as the reduction of the oxygen content the middle one. High levels of oxygen can favor oxidation of the base of the stake, which can reduce rooting. Another important factor among the substrates that can influence the rooting of the cuttings is the pH. According to Hartmann *et al.* (2002), the appropriate substrate for rooting depends on the species, the type of cutting, the season, the propagation system, the cost and the availability of its components.

Table 1. Decomposition of the interaction between substrate (Sub.) (Sand, CRH = carbonized rice husk and Verm. = vermiculite), and position of the collection of cuttings in plagiotropic shoots (Pos) being (AP = apical, ME = median and BA = basal), evaluating the survival percentage (Surv), leaf retention (LR), number of roots (NR), length of the longest root (LLR) in mm, number of shoots (NS), number of leaves of shoots (NLS) and the highest shoot length (HSL) in cm

Sub.	Pos.	Surv	LR	NR	LLR	NS	NLS	HSL
Sand	AP	80 abA	1,00 aA	14 aA	17,37 aA	1 aA	1 aB	0,59 aA
	ME	90 aA	1,00 aA	14 aA	19,46 aA	1 aA	0 aB	0,33 aB
	BA	60 bA	0,75 aA	07 bA	10,00 aA	1 aA	1 aA	0,64 aA
CRH	AP	10 bB	0,00 aB	02 bB	1,78 bB	0 bB	0 bB	0,25 aA
	ME	45 aB	1,00 bA	16 aA	16,43 aA	1 aA	1 aAB	1,10 aB
	BA	50 aA	0,50 abAB	07 bA	10,52 abA	0 abAB	0 bA	0,17 aA
Verm.	AP	25 abB	0,25 aB	10 aAB	10,56 aAB	1 aA	2 aA	1,04 bA
	ME	30 aB	0,25 aB	10 aA	13,29 aA	1 aA	2 aA	2,50 aA
	BA	00 bB	0,00 aB	00 bA	0,00 bB	0 bB	0 bA	0,00 bA

Means followed by same letter in column do not differ by Tukey test ($p < 0.05$). Lowercase letters refer to the decomposition of the interaction compared to the positions of cuttings collection on each substrate, whereas capital letters, compare the substrates in cuttings taken from the different portions of the shoot.

Table 2. Orthotropic shoot cutting in function of the type of substrate (Sub.) and position of cutting collection (position), evaluating the survival percentage (Surv), number of roots (NR), length of the longest root (LLR), number of shoots (NS), number of leaves of shoots (NLS) and the highest shoot length (HSL).

Treatments		Surv	NR	LLR	NS	NLS	HSL
Type of substrate	Sand	48 a	7,23 a	8 a	1 a	2 a	1,76 a
	Husk	28 a	3,82 a	6 a	1 a	2 a	1,67 a
	Verm	00 b	0,00 b	0 b	0 b	0 b	0,00 b
Position	Apical	27 a	4,62 a	8 a	1 a	2 a	1,76 a
	Median	28 a	4,79 a	5 ab	1 a	2 ab	1,00 ab
	Basal	22 a	1,63 a	2 b	0 b	1 b	1,00 b

Means followed by same letter do not differ, considering the three types of cuttings and the three substrates

According to Stumpf *et al.* (1999), the pH of the rice hull is more alkaline (9.1) than that of vermiculite (7.9) or sand (6.6). For *Syzygium cumini* (L.) Skeels (Myrtaceae), Lima *et al.* (2007) observed similar results to that of this study. They found that the best rooting occurred on the middle-branch cuttings planted in the sand when compared to vermiculite. For mint, cuttings removed from the apical part have better percentages of glue and vigor of seedlings, and the presence of leaves on the cuttings, regardless of the cutting position, is fundamental to increase the percentage of glue and to improve the development of *Mentha* sp. (Costa *et al.,* 2016). Stake length and substrate type have a great influence on the mass of roots and shoots, and can be explained by the joint effect between the higher amount of seed stocks and greater availability of nutrients. Stakes withdrawn from the different portions of the plant generally have distinct physiological and anatomical characteristics that can induce distinct responses as to the factors that affect rhizogenesis. The apical cuttings usually have tissues with less lignification, therefore, they are more tender, which can favor the excessive loss of water by transpiration and lead to death of the cuttings (Lima *et al.,* 2007).

Also, the amount of organic and inorganic nutrient reserves in apical cuttings may be lower, thus contributing to the failure of cutting (Nicoloso *et al.,* 1999). In the present study, it seems that these factors did not affect the rhizogenesis in the plagiotropic cuttings, considering that in the sand and the vermiculite, the rooting did not differ between the apical and median stakes. This situation can be explained by the plagiotropic orientation of the branches collected for these stakes, because, with horizontal arrangement, the nutrients may have less difficulty to reach the apical region, since, in this case, gravity does not act negatively, hampering the flow of the sap. Basal stakes may present more lignified tissues, constituting an anatomical barrier to rhizogenesis. Another fact that may have influenced, may be the low concentration of endogenous auxins, which implies in reducing the number of primordia initialized in view of the distance of the production site in the plant. Possibly some of these factors are involved in the rhizogenesis of *P. aduncum*, since the number of roots emitted per pole was practically double in the median stakes, in relation to the basal ones. Similar results were found by Garbuio *et al.* (2007), on cuttings of *Pogostemon cablin* (Lamiaceae), whose rooting was decreasing from the apical cuttings to the basal cuttings.

Among the characteristics analyzed in this experiment, NR, CR and Sobr are the most important for the development of cuttings, because these characteristics explain how the cuttings are established, and direct their development to the survival in the new environment. High survival rates allow a great use of the transplanted cuttings, and thus greater yield. The NR was doubled in the medium stakes and superior in the sand substratum, allowing the development of a large area for nutrient absorption and, together with the CR, allowed to reach deeper regions of the substrate, demonstrating the superiority of the median roots and the substrate for the production of cuttings in this species. Regarding the cuttings removed from ortho branches, no interaction was observed between the studied factors (substrates and types of cuttings), for the characteristics analyzed. As for the substratum used in rooting, it was verified that all cuttings planted in the vermiculite died (Table 2), while in the sand and carbonized rice husk, the results did not differ for any analyzed variables.

The orthogonal branches responded in a general way, similar to what was observed for the plag branches, that is, better performance in the sand and worse in vermiculite, proving to be a requirement for the species under the conditions used in these experiments. For the type of stake, no significant difference was observed in the survival rate, length of the highest root and number of shoots. The number of roots, number of leaves and length of shoots were higher in the apical and inferior stakes in the basal, however, did not differ from the median stakes. It is worth mentioning that in basal stakes, the NR and CR were at least 2.5 times smaller than the other stakes, being the inhibition to rooting notorious. Possibly, as with the plag branches, there are anatomical barriers to rhizogenesis as it approaches the base of the plant. In general, the ortho branches had a worse performance in relation to the plag branches. Possibly, besides the anatomical barriers to rhizogenesis, the absence of leaves in the ortho piles is one of the factors that may have contributed to this reduction.

In this type of stake, in addition to these factors, the influence of the age of the branches may still occur, since ortho branches (which give rise to the plag branches) are older and, therefore, may have fewer auxins or even less hormonal balance suitable for root development. Thus, all these factors may have contributed to the fact that the ortho branches had a lower performance than the plag branches. The presence of leaves is one of the factors, which exerts great stimulus to the initiation of roots. This effect is related to the translocation of carbohydrates to the base of the cutting (Ahkami *et al.,* 2009), besides being source of endogenous auxins (Garrido *et al.,* 2002) and other co-factors important for rooting (Hartmann *et al.,* 2002). About 5 days after planting, a few abscission processes in the node region begin (Fig. 1a). Later these stakes develop normally (Fig. 1c). The adventitious root system formed in the pile is similar to the pivotal system, in which one root develops more sharply than the others, and becomes, to some extent, the main root of the pile (Fig. 1d). However, some roots formed have plagiotropic growth (Fig. 1d), even emerging from the substrate and penetrating into another cell of the tray (Fig. 1b). This type of growth was observed in adventitious roots of adult plants of other species of the genus Piper, developing in natural environment, such as *Piper amalago* Var. medium L. (Rosa and Souza, 2004) and *P. hispidum* (Albiero *et al.,* 2006).

The external morphology of the rooted cuttings was similar in all the substrates used, only varying according to the type of branch (plag or ortho) and the collected portion (apical, median and basal). However, they differed from the basal stakes, which had much thinner and scarcer roots (Fig. 1f), demonstrating an impediment to rhizogenesis, in these plagic and medial cuttings of plag branches, showing the same appearance after rooting (Fig. 1d and 1e) cuttings. All the cuttings of orthopedic branches have scarce and thin roots (Fig. 1g-i), compared to the plag branches, indicating some type of difficulty to rhizogenesis. Analyzing the cross sections of the base of the different cuttings, used for rooting, in this study, one can observe distinct anatomical characteristics, especially between cuttings of plag and ortho branches. All the cuttings already presented secondary growth, with increasing stage of development, from the apical portion of the plag to basal branches of the orthopedic branches (Fig. 2). The plag apical cuttings have unstrapped and densely pilose epidermis, having mainly multicellular tectonic trichomes, with sparse unicellular tectonic trichomes and, more rarely, saculiform glandular trichomes (Fig. 2a). The pilosity decreases with the evolution of the secondary structure, being practically nonexistent in the orthogonal branches of the medial and basal portion (Fig, 2e and 2f). In the plag stakes (Fig. 2a to c), the cortical parenchyma is reduced, while the chlorophyll parenchyma contains discontinuous bands of angular colenquimm associated with the vascular bundles, with thickening composed of lignin, facing the phloem. Crystalline idioblasts of the acicular type are present, although rare,

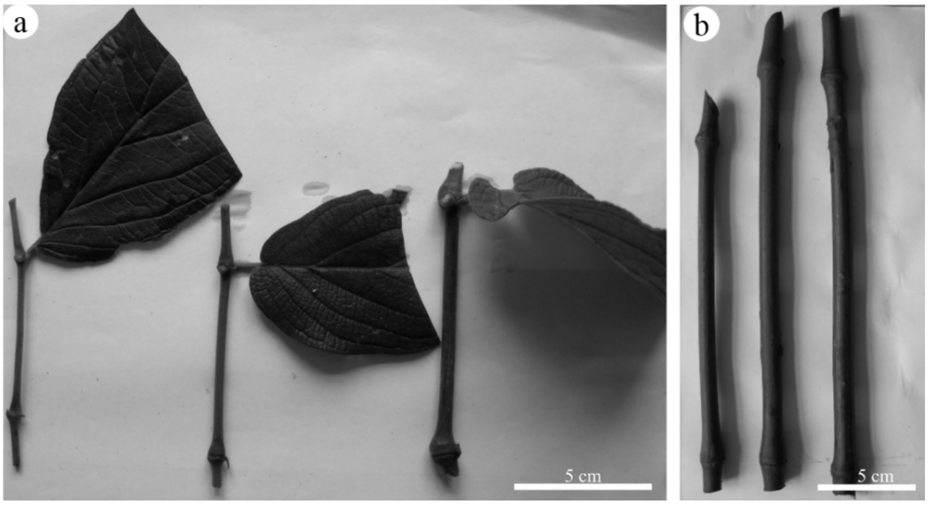

Fig. 1. Types of P. aduncum cuttings. A Plagiotropic shoot cutting, from the left to right are shown the apical plag, medium plag and basal plag cuttings, B Orthotropic shoot cuttings, from the left to right are shown the apical ortho, medium ortho and basal ortho cuttings

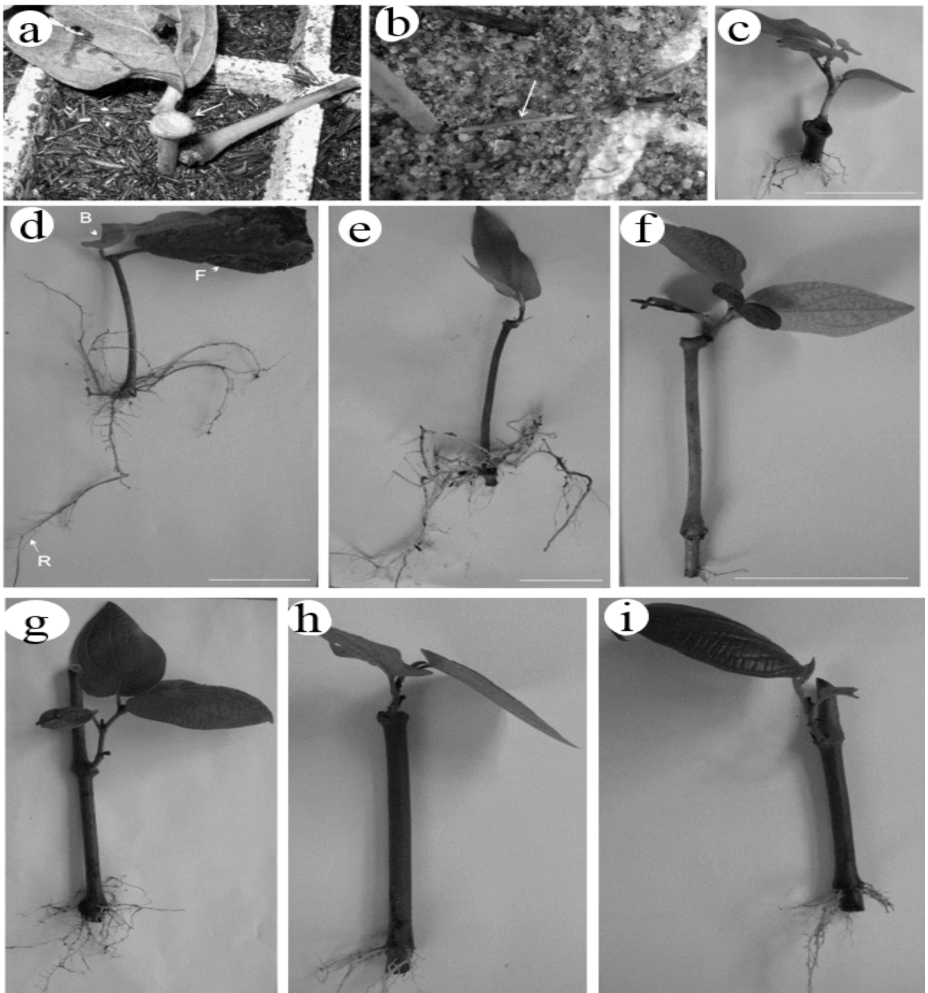

Fig. 2. Different types of cuttings, featuring the adventitious roots of Piper aduncum, depending on the position of collection of cuttings in plag (A to F) and ortho shoots (G to I). A Detail of cutting in the tray, 5 days after planting, showing the abscission in the node region (arrow), B Details of the cutting in the tray, 60 days after planting, showing adventitious roots emerging on the surface of the substrate (arrow), C Details of a cutting that suffered abscission, D Apical plag shoot cutting, E Median plag shoot cutting, F Basal plag shoot cutting, G Apical ortho shoot cutting, H Median ortho shoot cutting, I Basal ortho shoot cutting. Bar = 5 cm

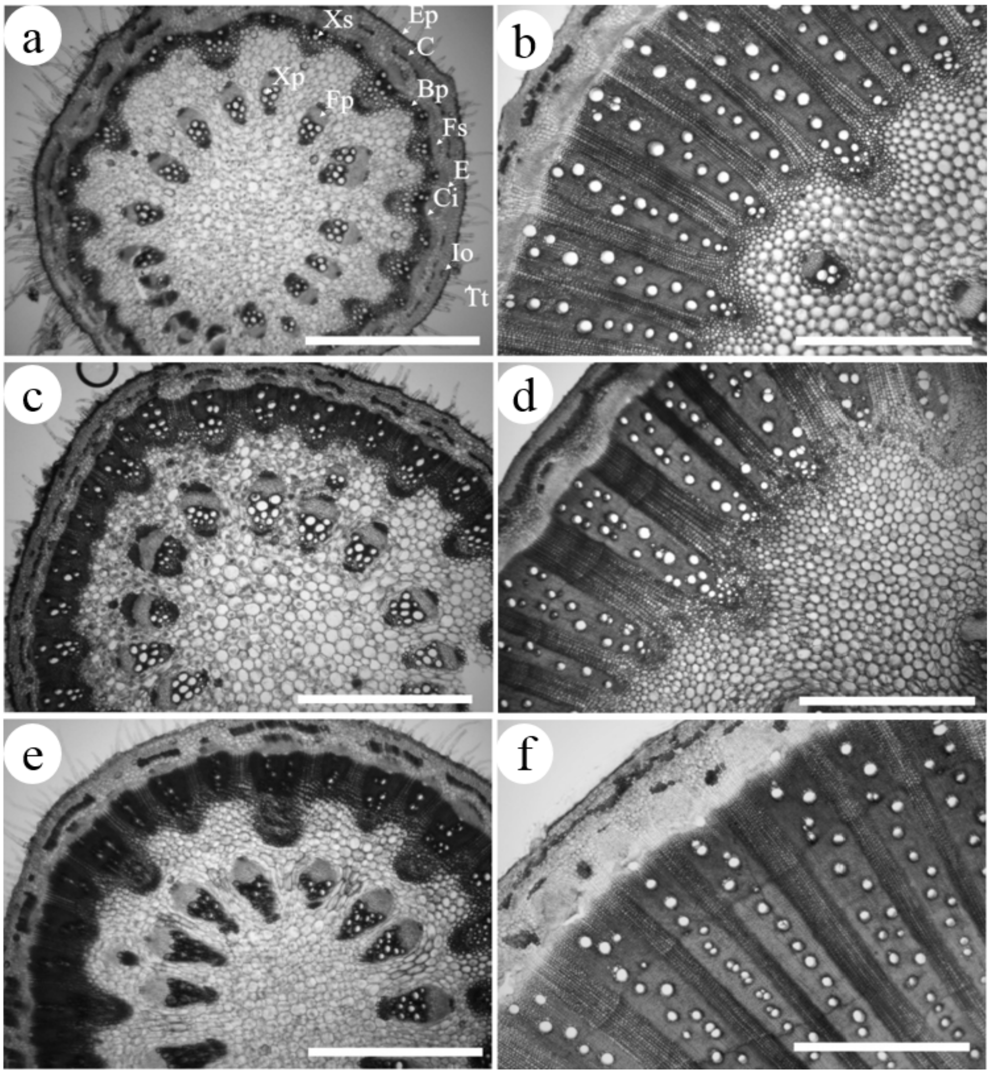

Fig. 3. Transversal sections of cuttings of Piper aduncum collected from plagiotropic (A, C and E) and orthotropic shoots (B, D and F), where A and B apical cuttings, C and D median cuttings, E and F basal cuttings. Scale: bar equals 1 mm. Legend: Ep epidermis, C- Collenchyma, Xs secondary xylem, Fs secondary phloem, Bp perivascular sheath, E endodermis, Ci interfascicular exchange, Io oil idioblasts, Tt trichomes, Xp primary xylem and Fp primary phloem

whereas the oil idioblasts are distributed throughout the cortical region. These idioblasts present a positive reaction for sesquiterpene lactones and ortho dihydroxyphenols. It also contains numerous starch grains, terpene compounds with a carbonyl group and the presence of proteins. The endoderm is located just below the colenchyma and forms a continuous band, consisting of a layer of cells, with amiliferous composition. The vascular tissue is of the open collateral type and is organized in two concentric circles. The outer circle is delimited externally by the endoderm and internally by the perimedular sheath sclerified in all plag stakes. The internal vascular bundles are immersed in the medullar parenchyma forming a definite circle. In the medullary parenchyma a large amount of acicular and rarely prismatic crystals can be observed, as well as starch grains, oil idioblasts, terpene compounds with carbonyl groups and proteins. These characteristics of the cells of the medullary parenchyma confirm the intense metabolic nature of these cells. The intense metabolism may be associated with the ability of these stakes to preserve living cells, with the presence of total proteins, which encompass enzymes, among others, which make up all the machinery for the survival of cells, and the maintenance of the cell cycle. Terpenic compounds, observed in the cuttings, may play an important role in protecting the cuttings, being important in the preservation against oxidations and pathogens, such as fungi. The starch grains observed in the stakes are important indicators of the health and the potential of the cuttings, since they can be used as substrate for energy production or as carbon skeletons for the development of adventitious roots (Nanda and Jain, 1972). Basal cutting in some species have a higher lignification content and are often of woody consistency having large dimensions, and according to Hartmann *et al.* (2002), these characteristics make possible a greater amount of reserves, which are translocated for an adventitious root formation (Hartmann *et al.,* 2002). Costa *et al.* (2007) and Sousa *et al.* (2013) reiterate that basal stakes is the increased availability of carbohydrates and starch. Plag stakes differ little between them, except for the advance of secondary growth. However, the number of adventitious roots in basal cuttings was about half the number of apical and median cuttings (Table 1). No anatomical impediments were observed at the exit of adventitious roots, since there is no formation of the continuous ring of lignified tissues. If there are no anatomical impediments, probably the difference in rhizogenesis is due to the low concentration of endogenous auxins in view of the distance of the production site in the plant. On the other hand, the plag and orto cuttings are quite different. The orthotic stakes have a larger number of xylem vessels, especially the secondary ones, the cambium and the cortex are very small, there is more lignification in the colenchyma region, there are practically no oil idioblasts in the cortical parenchyma, which possibly influenced the rhizogenesis. However, there is a greater starch deposition in ortho stakes, especially in the basal ones, a fact that probably helped to compensate for the lack of leaves in the initial stages of rooting. As far as orthotic stakes are concerned, they differ little (Fig. 2d to f). The epidermis has rare trichomes and thin periderm associated only with sparse lenticels. The cortex contains less quantity of oil idioblasts, the vascular change is smaller than in the plag cuttings and the inner cells of the colenchyma suffer lignification. In ortho stakes, the ring of lignified tissues is also not continuous, yet it is present in a larger number of cells, probably causing some effect on rhizogenesis. In a study of the anatomy of *Averrhoa carambola* L. cuttings. Bastos (2006) observed that herbaceous and semilenous cuttings present primary phloem fibers as cells with thicker walls, located just below the cortical parenchyma. This continuous sheath of fibers can hinder the passage of root beginnings, making difficult the formation of adventitious roots. Evaluating the rooting capacity of cuttings of four cultivars of Vitis, only V. rotundifolia Michx. cv. Topsail showed difficulty in rooting, differing from the others by maintaining the fiber caps on the primary phloem, by the presence of reduced secondary phloem with radial bands of fibers and also less developed xylem, with narrower rays and smaller diameter vessel elements. Thus, the difficulty of rooting this cultivar may be related to anatomical barriers (Mayer *et al.,* 2006). According to Husen and Pal (2006), the increase in the number of potting elements has an inverse relation with the rhizogenesis in cuttings, a fact that may have contributed to the reduction of rooting in orthogonal stakes in relation to the plag and in the basal stakes. In these cases, where the increase in the number of vessels is associated with an increase in the thickness of the secondary xylem, they impede the exit of the root primordia from the interior of the tissues, and their communication with the other tissues. On the other hand, the reduction in the amount of oil idioblasts may have led to a decrease in the amount of ortho dihydroxyphenol, such as caffeic acid, catechol and chlorogenic acid, which according to Hartmann *et al.* (2002), are compounds that favor the rhizogenesis in cuttings, since they interact with the auxins inducing the initiation of the adventitious roots.

Conclusion

Cuttings taken from the middle or apical portions of plagiotropic shoots, planted in the sand have better rooting capacity and should be used for the success of rhizogenesis. Biochemical and anatomical differences exists between the different types cuttings used, which may constitute barriers to the rhizogenesis.

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