

## *Piper crassinervium* Kunth vegetative propagation: influence of substrates and stem cuttings positions

### Abstract

*Piper crassinervium* Kunth. is considered as a potential species for economic exploitation due to the production of compounds with important biological activities, such as essential oils and antioxidants. Despite this, studies on its cultivation and propagation are scarce. Accordingly, the aim of the present study was to evaluate the viability of *P. crassinervium* vegetative propagation using cuttings collected from the apical, middle and basal positions of plagiotropic branches and different substrates for rooting. The cuttings were made with 10±1 cm, planted in plastic tubes containing vermiculite or Tropstrato® commercial substrate and kept in a greenhouse with intermittent mist. The stem cuttings remained in the greenhouse for 45 days until evaluation was carried out. Interaction between cuttings positions and substrates was not observed for none of the analyzed characteristics. Rooting percentage ranged from 18.8%, in basal cuttings, to 72.9% in middle ones. For rooting, survival and leaf retention, middle and apical cuttings obtained better performance, as well as the Tropstrato® substrate. Average roots number and shoots fresh weight did not vary according to treatments. It can be concluded that propagation through stem cutting is feasible for *P. crassinervium*. Apical and middle cuttings from plagiotropic branches as well as Tropstrato® substrate should be used for better rooting performance.

**Key Words:** jaborandi; rooting; Tropstrato®; vermiculite.

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### Resumo

## Propagação vegetativa de *Piper crassinervium* Kunth: influência do substrato e da posição de coleta das estacas

*Piper crassinervium* Kunth. é considerada uma planta com potencial para exploração comercial devido à produção de compostos com importantes atividades biológicas, como óleos essenciais e antioxidantes. Apesar disso, ainda são escassos estudos sobre seu cultivo e propagação. Nesse sentido, objetivou-se com o presente trabalho avaliar a viabilidade da propagação vegetativa de *P. crassinervium* utilizando estacas caulinares coletadas das porções apical, mediana e basal de ramos plagiotrópicos e diferentes substratos de enraizamento. Foram confeccionadas estacas com comprimento de 10 ±1 cm, que foram então plantadas em tubetes contendo vermiculita ou substrato comercial Tropstrato®, e mantidas em casa de vegetação com nebulização intermitente. A avaliação ocorreu 45 dias após a instalação do experimento. As porcentagens de enraizamento variaram de 18,8%, para estacas basais, até 72,9%, para estacas medianas. Para as variáveis de enraizamento, sobrevivência e retenção foliar, estacas medianas e apicais apresentaram melhores desempenhos, assim como o substrato Tropstrato®. O número médio de raízes e a massa fresca das brotações não variaram em função dos tipos de estacas ou substratos. Conclui-se que a propagação vegetativa de *P. crassinervium* via estaquia é viável. Estacas apicais e medianas de ramos plagiotrópicos e o substrato comercial Tropstrato® são recomendados para melhores índices de enraizamento.

**Palavras-chave:** enraizamento; jaborandi; Tropstrato®; vermiculita.

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## Resumen

### Propagación vegetativa de *Piper crassinervium* Kunth: influencia del sustrato y de la posición de recolección de las estacas

*Piper crassinervium* Kunth. se considera una planta con potencial para explotación comercial debido a la producción de compuestos con importantes actividades biológicas, como aceites esenciales y antioxidantes. A pesar de ello, todavía son escasos estudios sobre su cultivo y propagación. En este sentido, se objetivó con el presente trabajo evaluar la viabilidad de la propagación vegetativa de *P. crassinervium* utilizando estacas caulinares recogidas de las porciones apical, mediana y basal de ramas plagiotrópicas y diferentes sustratos de enraizamiento. Se han confeccionado estacas con una longitud de  $10 \pm 1$  cm, que luego se plantaron en tubos que contenían vermiculita o sustrato comercial Tropstrato®, y se mantuvieron en la casa de vegetación con nebulización intermitente. La evaluación ocurrió 45 días después de la instalación del experimento. Los porcentajes de enraizamiento variaron de 18,8%, para estacas basales, hasta 72,9%, para estacas medianas. Para las variables de enraizamiento, supervivencia y retención foliar, estacas medianas y apicales presentaron mejores desempeños, así como el sustrato Tropstrato®. El número medio de raíces y la masa fresca de las brotaciones no variaron en función de los tipos de estacas o sustratos. Se concluye que la propagación vegetativa de *P. crassinervium* vía estaquia es viable. Las estacas apicales y medianas de ramas plagiotrópicas y el sustrato comercial Tropstrato® se recomiendan para mejores índices de enraizamiento.

**Palabras clave:** enraizamiento; Jaborandi; Tropstrato®; vermiculita.

## Introduction

The genus *Piper* occupies a prominent position in Piperaceae family due to its ecological and agronomic importance. Several *Piper* species are used in folk medicine and many can produce economic valuable compounds, such as essential oils, in their structures. Some examples of species producing compounds with pharmaceutical interest are *Piper hispidinervium* C.D., *Piper aduncum* L., *Piper nigrum* L. and *Piper crassinervium* Kunth (GOGOSZ et al., 2012; KRINSKI and FOERSTER, 2016; SANINI et al., 2016).

*P. crassinervium*, commonly known as jaborandi in Brazil, is a 2-5 m high shrub that occurs throughout South America. The plant has light green color smooth cylindrical stems and alternate simple petiolate leaves with elliptical ovate limb (about 16 cm length and 7 cm width). Potential for commercial exploitation in this species lies on its use in folk medicine and essential oil production (ALBIERO et al., 2005).

Reports show differences in *P. crassinervium*. leaves essential oil composition and yield. Plants collected in northern Brazil yielded 0.2% (dry weight) after 4 hours extraction and the major compounds were identified as  $\beta$ -caryophyllene (17.7%),  $\gamma$ -elemene (14.4%) and  $\beta$ -elemene (10.9%)

(LUZ et al., 2003).  $\beta$ -pyrene,  $\alpha$ -pyrene, limonene and  $\alpha$ -terpinene were recorded by Sacchetti et al. (2005) as major compounds in plants collected in Ecuador. The authors also emphasized *P. crassinervium* antioxidant potential. Later, in a study with prenylated hydroquinones extracted from *P. crassinervium* fruits, the antioxidant activity was confirmed (YAMAGUCHI et al., 2006).

Prenylated hydroquinones from *P. crassinervium* leaves have also demonstrated fungicidal activity against phytopathogens *Cladosporium cladosporioides* and *C. sphaerospermum* (DANELUTTE et al., 2003). In effect, among the metabolites in different tissues of this plant, prenylated hydroquinones can be considered the most outstanding, both for their rare occurrence in higher plants and for the related biological activities. Prenylated hydroquinones biologic properties include, but are not restricted to, antifungals (LOSET et al., 2000; DANELUTTE et al., 2003), anti-inflammatory (FERRÁNDIZ et al., 1994) and anticancer activities (COTELLE et al., 1991).

Considering the exploitation potential related to this unique phytochemical profile, studies on suitable agronomic practices (such as propagation) for *P. crassinervium* are required in order to obtain a constant natural products output (quantitatively

and qualitatively) coupled with the sustainable use of plant genetic resources (i.e.: exploitation not restricted to extractivism). Among plant propagation methods, vegetative propagation is characterized by maintaining genetic stability in selected genotypes, especially regarding economically important secondary metabolites production and population uniformity (HARTMANN et al., 2002).

Tissue culture, grafting, cuttings and layering are the main vegetative propagation techniques. Plant propagation by cuttings is a widespread technique which consists in reconstituting a plant from a stock plant detached piece (JANICK, 1966). Propagating a large number of plants from a single stock plant in a short period of time, low cost and easy execution, in addition to plant juvenile period reduction are frequently listed as advantages from propagation through cuttings (FACHINELLO et al., 2005).

It can be affirmed that cuttings rooting is affected basically by factors related to the stock plant (intrinsic factors) and elements related to the environment (mesologic factors). The main environment-related factors are temperature, luminosity, humidity and rooting substrate. Intrinsic factors are those related to species genetic characteristics, phenologic phases, cuttings positions in stock plants and in the branches, flowering or fruiting, presence or absence of leaves, presence or absence of buds and hormones action (ZUFFELLATO-RIBAS and RODRIGUES, 2001).

Regarding environmental conditions, special attention should be given to the substrate, since this is the medium where the roots develop. It should have adequate porosity, drainage, homogeneity and be free of pathogens, besides being economically viable (MENDES et al., 2014).

The position in the branch from where stem cuttings are collected also has a strong influence on rooting, since it interferes with hormonal and juvenility issues. Stem cuttings from apical (distal) position in branches are closer to auxin synthesis sites and usually have lower tissue differentiation, in comparison to basal (proximal) ones. Because they normally have a more herbaceous consistence, however, they tend to be more susceptible to dehydration. Basal stem cuttings, on the other hand, despite the higher tissue maturity, have a greater capacity to provide reserves (mainly carbohydrates) required for roots and shoots emission and development (CUNHA et al., 2015; PIGATTO et al., 2018).

Given the above, this study aimed to evaluate *P. crassinervium* propagation feasibility by using apical, middle and basal cuttings from plagiotropic branches and two commercially available substrates.

## Material and methods

Branches with leaves of *P. crassinervium* were collected from a native plant population in the municipality of Adrianópolis, Parana state, southern Brazil (24°36'11"S 48°58'20" W - altitude 155 m ASL). Plant material collection was performed in December 15, late afternoon (between 5 - 6 pm). Plant material was moistened and packed in black polyethylene bags in order to be transported (about 3 hours trip) to greenhouse with intermittent misting (15 seconds every 30 minutes). The material was kept under intermittent misting during 12 hours until cuttings preparation took place.

*P. crassinervium* horizontal growth branches (called plagiotropics) were collected from stock plants apical segments. A voucher specimen was deposited Mato Grosso State University (UNEMAT) herbarium at Tangará da Serra Campus, under the identification TANG 1778.

To obtain the different cuttings types, plagiotropic branches were divided into three segments: apical (distal), middle and basal (proximal). Cuttings originated from each position were made with 10±1cm length with a bevelled (diagonal) cut on basis and straight cut above the last bud. Average diameter was 0.42, 0.54 and 0.69 cm for apical, middle and basal stem cuttings, respectively. One leaf reduced to a third of its original surface was kept at cuttings apex. Once prepared, stem cuttings were washed in running water for 5 minutes.

After washing, stem cuttings were planted at 5 cm depth in 120 cm<sup>3</sup> plastic tubes. The tubes were fulfilled with either fine granulometry vermiculite or Tropstrato HT® substrate. According to manufacturer informations, Tropstrato HT® is composed of pine bark, peat, expanded vermiculite and enriched with macro and micronutrients. The tubes with the cuttings were placed on plastic supports and kept at 1.20 m height from the ground in a greenhouse at the municipality of Curitiba, Parana State, Brazil. Cuttings were kept under intermittent misting (15 seconds every 30 minutes), with 21°C ± 2 °C temperature and air humidity higher than 80%.

The experiment was conducted under a completely randomized design in a 3x2 factorial

scheme (Three stem cuttings positions and two substrates). Each treatment was composed for four replicates, with 12 cuttings per plot, totaling 48 cuttings per treatment and 288 cuttings in the experiment.

After 45 days from planting, the stem cuttings were evaluated regarding rooting percentage (percentage of live cuttings with roots at least 1mm long), mortality (percentage of cuttings with necrotic tissues), sprouting (percentage of live cuttings that emitted new shoots), leaf retention (percentage of live cuttings that kept the leaf at the apex), average number of roots per cuttings, average length of three largest roots (cm), and average roots and shoots mass per cuttings. The roots and shoots were weighed immediately after being detached from the plant on an analytical precision scale (0.0001g).

Data were submitted to variance homogeneity analysis by the Bartlett's test and variance analysis (ANOVA). When significance was verified by the F

test, the means were compared by Tukey test at 5% probability. Statistical tests were performed using the Software Assisat (SILVA and AZAVEDO, 2016).

## Results and Discussion

Interaction between cuttings positions and substrates was not significant ( $P>0,05$ ) for none of the analyzed characteristics. This shows that these factors act independently on this species. The same behavior was observed for *Piper hispidum* Sw., *Piper aduncum* L. and *Piper amalago* L. stem cuttings (CUNHA et al., 2015; NUNES GOMES and KRINSKI, 2016a). In the comparison among treatment means, cutting position was statistically significant for rooting percentage, mortality, leaf retention, roots length and roots mass. The substrate as an isolate factor, significantly affected rooting percentage, mortality, leaf retention and sprouting (Table 1).

**Table 1.** Summary of variance analysis on rooting (ROT), mortality (MOR), leaf retention (LFR), sprouting (SPT), roots number (RNB), roots length (RLT), roots mass (RMS) and shoots mass (SMS), as a result of *Piper crassinervium* stem cuttings from three branch positions planted in two substrates.

Source of variation	F test values								
	D.F.	ROT	MOR	LFR	SPT	RNB	RLT	RMS	SMS
Substrates	1	6.50*	7.86*	5.01*	6.48*	0.16ns	0.21ns	0.04ns	1.07ns
Positions	2	11.20**	12.36**	26.77**	2.80ns	0.62ns	4.30*	10.73**	3.36ns
Interaction	2	1.25ns	1.13ns	0.05ns	2.14ns	0.17ns	0.10ns	0.14ns	0.38ns
Error	18	-	-	-	-	-	-	-	-
C.V. (%)	-	28.48	36.64	31.27	42.88	34.05	25.67	26.26	92.21

\*\*significant at 1% probability; \*significant at 5% probability; ns: non-significant. C.V.: coefficient of variation. D.F.: Degrees of freedom.

Higher rooting percentages, leaf retention and roots mass, as well as lower mortality was observed in apical and middle stem cuttings compared to basal ones. Average rooting in apical (66.7%) and middle (67.7%) cuttings did not differ from each other (Table 2). The rooting and survival rates observed in *P. crassinervium* apical and middle cuttings can be considered as satisfactory rates in view of the results observed in studies with other *Piper* species. Although some species do present high rooting percentages such as *Piper mikaniinum* (Kunth) Steudel (over 60%) (PESCADOR et al., 2007) and *P. hispidum* (over 85%) (CUNHA et al., 2015), most of them do not reach even 50%. In *P. amalago* stem cuttings, for example,

rooting percentages ranged from 2.08% in Plantmax® commercial substrate to 22.92% in sifted soil (NUNES GOMES and KRINSKI 2016a). *Piper umbellatum* L. stem cuttings, rooting percentages reached up to 37.5% in a study performed by Mattana et al. (2009). For the same species, percentages slightly higher (53.8%) were reported by using 15cm long leafless stem cuttings (NUNES GOMES and KRINSKI, 2016b). These differences in species ability for adventitious rooting are common and may be related to different genetic propensity for roots emission, to the presence or absence of endogenous auxins and co-factors, and also to the presence or absence of rooting anatomical barriers (ZUFFELATO-RIBAS and RODRIGUES, 2001).



**Table 2.** Means and standard deviation of rooting, mortality, leaf retention, sprouting, roots number, roots length, roots mass and shoots mass in *Piper crassinervium* stem cuttings from three branch positions and planted in two substrates.

Stem cutting positions	Variable	Apical	Middle	Basal	LSD
	Rooting (%)	66.7±5.9a	67.7±7.4a	34.4±22.1b	20.44
Mortality (%)	33.3±5.8b	30.2±10.3b	65.6±22.1a	20.13	
Leaf retention (%)	63.5 ±7.4a	52.1±8.8a	15.6±10.3b	17.46	
Sprouting (%)	28.1 ±1.5a	45.8±8.8a	33.3±23,5a	19.62	
Roots number (n)	13.1±0.3a	12.9±1.4a	11.0±0.3a	05.35	
Roots length (cm)	8.5±0.1a	7.7±0.6ab	5.8±0.2b	02.39	
Roots mass (g)	0.81±0.01a	0.77±0.04a	0.44±0.03b	0.226	
Shoots mass (g)	0.13±0.01a	0.21±0.08a	0.03±0.07a	0.304	
Substrates	Variable	Tropstrato®	Vermiculite	LSD	
	Rooting (%)	64.7±12.7a	47.9±25.2b	13.73	
Mortality (%)	34.0±14.2b	52.1±25.3a	13.53		
Leaf retention (%)	50.0±24.0a	37.5±26.0b	11.73		
Sprouting (%)	43.8±12.7a	27.8±11.5b	13.18		
Roots number (n)	12.0±0.9a	12.6±1.6a	03.60		
Roots length (cm)	7.1±1.5a	7.5±1.4a	01.61		
Roots mass (g)	0.68±0.23a	0.67±0.19a	0.152		
Shoots mass (g)	0.31±0.20a	0.21±0.10a	0.205		

\* Means followed by the same lower case letter in lines are not statistically different at 5% level of significance according to Tukey test. LSD: least significant difference.

The presence or absence of endogenous auxins may also interfere on rooting ability of different branch positions. In the present study, the poor basal cuttings performance in comparison to apical and middle ones might be related to the distance from the apices of branches and new leaves, which are known to be auxins producer sites. These hormones regulate cell expansion, division and tissue differentiation and are directly related to the adventitious root formation in plant species (TAIZ and ZEIGER, 2013).

*P. hispidum* cuttings from apical positions also had better rooting percentages compared to basal ones, in that species, however, basal cuttings performed better than middle ones (CUNHA et al., 2015). The authors justify this behavior due to apical cuttings proximity to the auxin production sites, and the cuttings greater availability of stem reserves, when compared to middle cuttings. In *P. crassinervium*, though, reserves availability did not seem to have influenced on basal cuttings performance, since they presented the larger diameter and still had the worse rooting and survival rates. A similar pattern was reported in *Stevia rebaudiana* BERT. stem cuttings rooting, which demonstrated to be better achieved by using apical cuttings rather than basal ones, despite their differences in diameter (PIGATTO et al., 2018).

In addition to lower auxin contents, the

presence of anatomical barriers may also play an important role on basal cuttings inability for rooting. According to Ono and Rodrigues (1996) the success for rooting in different species is inversely related to the sclerenchyma layers continuity in cuttings. Albiero et al. (2005) have related that discontinuous bands of annular colenchyma bellow the epidermis are observed in *P. crassinervium* stems. These bands, according to the authors, become continuous in older regions and undergo lignification. This process may constitute an anatomical barrier for rooting, especially in basal cuttings of this species.

The better results of apical and middle cuttings in comparison to basal ones were also observed for the ornamental species *Alternanthera dentata* Moench (BECKMANN-CAVALCANTE et al., 2014) and for the medicinal species *Stevia rebaudiana* Bert (ABDULLATEEF and OSMAN, 2011) stem cuttings. Salomão et al. (2002), however, reported worse performance of passion fruit plant apical stem cuttings when compared to the middle and basal ones. The authors attributed this response to the higher mortality rates observed in apical cuttings.

In fact, according to Hartmann et al. (2002), less lignified cuttings (i.e.: herbaceous cuttings) are more sensitive to dehydration and death. In this study, however, dehydration seemed not to affect *P. crassinervium* stem cuttings, since apical and

middle cuttings (less lignified) presented lower mortality rates than basal ones. *P. crassinervium* stem cuttings resistance to dehydration might be related to the plant anatomy. According to Albiero et al. (2005) a thick cuticle layer recovering the stem single-layered epidermis is observed in this species. Cuticle accumulation is one of the most well-known strategies to prevent water loss in plant tissues (TAIZ and ZEIGER, 2013). In addition, the greenhouse intermittent misting provided conditions of constant air humidity, preventing cuttings dehydration.

It is also possible to establish a relationship between rooting and leaf retention, since the highest values for this variable were verified in apical and middle cuttings (Table 2). Leaf maintenance in stem cuttings is important because such organs are capable to provide rooting hormones, cofactors and carbohydrates (BISCHOFF et al., 2017). More abundant carbohydrate reserves in stem cuttings correlate with higher rooting and survival percentages, because auxins require a carbon source for nucleic acid and protein biosynthesis (FACHINELLO et al., 1995).

Sprouting, roots number and shoots mass did not vary according to cuttings positions. Roots length and roots mass were higher in apical cuttings in comparison to basal ones (Table 2). The highest roots length and roots mass observed in the present study for apical cuttings in relation to basal ones were also observed by Bona et al. (2012) in *Lavandula dentata* L, and by Signor et al. (2007) in oregano (*Origanum vulgare* L.) stem cuttings, indicating the tendency to a better quality of plantlets produced from this type of cuttings.

Regarding substrates, Tropstrato® promoted higher rooting, sprouting and leaf retention percentages as well as lower mortality when compared to vermiculite. Roots number, length and mass and shoots mass did not differ as function of substrates (Table 2).

The most evident difference between Tropstrato® and vermiculite is nutrients availability, since vermiculite is an inert substrate. However, nutritional aspects did not seem to be a crucial factor for cuttings better performance on Tropstrato®, since substrates for use in stem cuttings rooting do not necessarily need to be a source of nutrients until the root system is established (JANICK, 1966).

Thus, better results obtained in Tropstrato® can be attributed to its physicochemical characteristics, such as the presence of organic matter, sufficient quantity of ionic charges, porosity and satisfactory moisture retention, as related for other commercial substrates in comparison to pure vermiculite (ZIETEMANN; ROBERTO, 2007; NUNES GOMES and KRINSKI, 2016b).

Nutrients availability, despite not fundamental for roots formation, seem to be important for shoots emission and development. For okra seedlings production, the supplementation of substrates with slow release fertilizer promoted higher biomass accumulation in shoots and roots (NUNES GOMES et al., 2017). In *Baccharis articulata* (Lam.) Pers. stem cuttings, higher sprouting percentages were found in Plantmax® commercial substrate in comparison to inert substrates (BONA et al., 2005). For *Mentha arvensis* L. stem cuttings, higher shoots number and shoots mass were obtained on a substrate made from soil, sand and bovine manure (AMARO et al., 2013). According to Nunes Gomes and Krinski (2016 a) nutrients availability is important for new shoots emission and development in *P. amalago* stem cuttings.

The present study is one of the first descriptions of agricultural practices related to *P. crassinervium*. Further studies evaluating a wider range of substrates, effects of seasonality on rooting and application of plant regulators are recommended.

## Conclusion

Vegetative propagation through stem cuttings is feasible for *P. crassinervium*. Apical and middle cuttings from plagiotropic branches as well as Tropstrato HT® substrate should be used for better rooting performance.

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