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PROPAGATION OF *PIPER HISPIDUM* THROUGH LEAF CUTTINGS

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ABSTRACT

The regeneration of roots and shoots through leaf cuttings is a feasible technique for plant clonal propagation, using a quite available organ, which can be collected without great damage to the plant. A protocol of propagation through leaf cuttings was defined to Piper hispidum, a plant whose compounds have great potential use in medicine and agriculture. Leaves of three sizes were used, according to their length: small (4-6 cm), medium (7-9 cm) and large (10-12 cm). The leaves were cut in halves (apical and petiolar) by transverse cutting in the middle of the leaf blade. The cut parts were immersed into a solution of indole 3-butyric acid (IBA) at 1000 ppm for 5 or 20 minutes, or not submitted to the hormone. Then the cuttings were planted in a mixture of sand and soil (1:1), using three positions of the leaf halves: apical, basal and inverted basal. A factorial design was used – 3 sizes x 3 times of immersion in IBA x 3 leaf half position x 3 blocs x 3 replications. After 82 days the number of roots and shoots, length of roots and shoots were evaluated. There was no significant interaction among the three factors. Small leaves produced longer roots and more numerous and longer shoots than the medium or large ones. IBA induced rooting, but the number and length of shoots were higher where there was no immersion in the hormone. The apical portion generated far more numerous and longer roots, but the basal and inverted basal cuttings produced more shoots, which gave rise to their own roots afterwards. Leaf cuttings can be a practical method to propagate P. hispidumvegetatively. Small leaves can be used as source of basal and inverted basal cuttings, without treatment with auxin.

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INTRODUCTION

The genus *Piper* (Piperaceae) is a Pantropical group with nearly 2000 species, constituting an important element of montane and lowland forests. It is one of the ten greater genera of basal Angiosperms found in the tropics, with approximately 1300 species in the Neotropics and an estimated 700 species in the Old World tropics (Quijano-Abril *et al*., 2006). Throughout the tropics, various *Piper* species are used for many purposes such as foods, spices, perfumes, oils, fish poisons, insecticides, hallucinogens and medicines (Michel *et al*., 2010). *Piper hispidum* Swingle is a shrub native to the lowlands of Mexico, of pan-tropical occurrence. It is distributed throughout the Antilles, and Central and South America, including all Brazilian geographic regions, where is known as “jaborandi” and “falso-jaborandi”.

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Its seeds germinate shortly after natural or man-made disturbance but it is also common in the understory of transitional forests and may persist into the primary forest (Walters & Field, 1987). In tropical South America its occurrence includes both disturbed and forest sites (Michel *et al*., 2007; Orlandelli *et al*., 2012). In eastern Mexico it is found principally in light gaps and in the understory of the evergreen rainforest (Mooney *et al*., 1983). The species is used in South American traditional Pharmacopeias for many purposes. In Peru, its leaves are traditionally used by the Chayahuitas, an Amazonian ethnic group, as poultices for healing wounds and to treat the symptoms of cutaneous leishmaniasis (Estevez *et al*., 2007). In Guatemala, it is known as “puchuq” and used by the Q’eqchi people as a tea to treat dysmenorrhea, amenorrhea, and body aches. In Nicaragua, it is known as “cordoncillo” and also used to ease aches and pains, in Peru to regulate menstruation, and in the Amazon to treat urinary infections (Michel *et al*., 2007). In Colombia, a leaf decoction is used to treat malaria (Morton, 1981). Its use as an astringent, diuretic, stimulant, for unblocking the liver and stopping hemorrhages has also been described (Orlandelli *et al*., 2012). In Brazilian Amazon, *P. hispidum* is known by the vernacular names of "matico" and "aperta-ruão", and its leaf infusion is used in the folk medicine as a diuretic and anti-hemorrhagic (Silva *et al*., 2014). In terms of chemical constituents, *P. hispidum* is reported to contain amides, benzenes, benzoic acids, flavonoids and volatile oils, which have significant antifungal, antimicrobial, antiplasmodial, leishmanicidal and insecticidal activities (Michel *et al*., 2010; Silva *et al*., 2014). The potential use of *P. hispidum*compounds in medicine and agriculture has encouraged chemical and pharmacological researches. However, collection of *Piper* species from the wild for folk medicine purposes, in addiction to deforestation of tropical forests has diminished their populations and even threatened their very existence, leading to a depletion of the irreplaceable genetic diversity (Ahmad *et al*., 2011; Rani and Dantu, 2012; Basak *et al*., 2014). Traditional propagation of *Piper* species has not proved to be efficient, due to poor seed viability, seed recalcitrance, low rates of germination, and scanty or delayed rooting of cuttings, evidencing the need of alternative methods of propagation (Abbasi *et al*., 2010; Ahmad *et al*., 2014; Padham, 2015). *In vitro* techniques have been also used to propagate *Piper* species. However, serious fungi and bacterial contamination of the explants is peculiar to this genus, and to overcome this problem surface sterilization has been made by using mercury chloride (Bhat *et al*., 1992; Bhat *et al*., 1995; Kelkar *et al*., 1996; Zhang *et al*., 2008; Ahmad *et al*., 2011; Rani and Dantu, 2012; Ahmad *et al*., 2010; Maju&Soniya, 2012; Ahmad *et al*., 2014; Padham, 2015; Umadevi *et al*., 2015), a compound whose toxic effects on environment, human and animal systems are well known (Micaroni *et al*., 2000; Rao & Sharma, 2001; Issa *et al*., 2003; Pandey *et al*., 2005). The objective of the present research was the regeneration of plants of *P. hispidum*, by promoting rooting in leaf cuttings and the subsequent shootformation, aiming at the establishment of a simple and rapid method for propagation of this species.

MATERIAL AND METHODS

The experiments were carried out during September-December 2017 at Embrapa (Brazilian Agriculture Research Corporation) in Porto Velho, Rondônia state, Brazil. The leaves were collected from two years old stock plants of *P. hispidum* grown in a greenhouse with 50% shading and sprinkler irrigation three times a day for 30 minutes. Leaves of three sizes were used, according to their length: "small"(4-6 cm), "medium"(7-9 cm) and "large"(10-12 cm). The leaves were cut in halves (apical and petiolar) by transverse cutting in the middle of the leaf blade. The cut parts of the halves were immersed into a solution of the hormone indole 3-butyric acid (IBA) at1000 ppm for 5 or 20 minutes, or not submitted to the hormone. After that, the cuttings were put at 45 degree angle individually in plastic cups (400 mL) containing a mixture of sand and soil (1:1), according to the method described by Basak *et al*. (2014). Three positions of the leaf halves were used: "apical" - apical half with the cross section down, "basal" - petiolar half with the cross section up (i.e. petiole inside the substrate), and "inverted basal" - petiolar half with the cross section down (i.e. petiole up) (Fig. 1). A total of 243 leaf halves were used, in a factorial design – 3 sizes x 3 times of immersion in IBA x 3 leaf half position x 3 blocs x 3 replications. After 82 days the number of roots and shoots, length of roots and shoots were evaluated. Variance analyses and Tukey test ($P<0.05$) were performed by using the Assistat 7.5 statistical program.

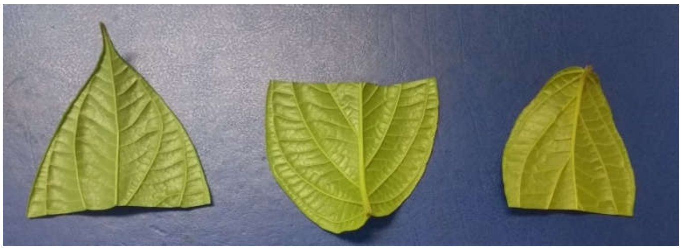

Figure 1. Leaf cuttings: apical, basal and inverted basal

RESULTS

There was no significant interaction among the three factors, so they were evaluated separately. In Table 2 are presented the averages of number and length of shoots and roots of *P. hispidum* in relation to the different sizes of leaves, leaf portions and time of immersion in IBA.

Table 2. Averages of number and length of shoots and roots of *P. hispidum* in relation to leaf sizes, leaf portions and time of immersion in IBA, at 82 days of cultivation

Factors	Treatments	Shoots		Roots	
		Number	Length	Number	Length
Leafsize	P	1.03 a	6.31 a	6.29 a	49.65 a
	M	0.41 b	2.61 b	6.25 a	39.77 a
	G	0.07 b	0.20 b	5.74 a	39.33 a
Leafportion	A	0.04 b	0.05 b	13.40 a	64.66 a
	B	0.67 ab	4.00 a	2.85 b	34.92 b
	BI	0.81 a	5.07 a	2.03 b	29.16 b
Immersion in IBA	0	0.70 a	5.35 a	5.07 b	42.58 a
	5'	0.48 a	2.59 ab	6.52 ab	40.94 a
	20'	0.33 a	2.18 a	6.70 a	45.24 a

*Letters indicate significance among treatments, within each factor (Tukey test 5%).

Figure 2. Plant (130 days old) produced from a basal leaf cutting (arrow) of *P. hispidum*

Immersion in IBA did not affect the number of shoots on the leaf cuttings, nor did the number and length of the roots. However, the length of the shoots was inversely proportional to the time of immersion in IBA, being higher where there was no immersion in the hormone. The apical portion generated more numerous and longer roots in comparison to the basal and inverted basal portions. Nevertheless, the apical portion did not produce expressive number of shoots and consequently resulted in reduced shoot length. Basal and inverted basal cuttings produced more numerous and longer shoots in comparison to the apical cutting. Small leaves did not differ from the medium and large ones in relation to the number and length of roots, but produced more numerous and longer shoots. In Figure 2 is possible to observe an entire plant originated from a basal leaf cutting.

DISCUSSION

In the present study it was clear that the leaf can function as a cutting, alike a stem cutting. This is possible because the leaf has all the structures present in the stem, including those meristematic ones, like procambium and cambium (leaves of some species), which give origin to the primary and secondary xylem and phloem in the stem, respectively. According to Raven *et al*. (2007), the pattern formed by the vascular bundles reflects the close structural and developmental relationship between the stem and the leaves. As the leaf primordium grows in length, the procambial bundles also differentiate toward it. From the beginning, the procambial system of the leaf is continuous with that of the stem. At each node, one or more vascular bundles diverge from the cylinder of stem strands, cross the cortex and enter the sheet. Thus, the mesophyll of the leaf is completely covered by a system of veins or vascular bundles, which is continuous with the vascular system of the stem. The median rib and sometimes the larger caliber veins show secondary growth in some leaves of dicotyledons. One pattern observed in the current research is the inverse production of roots or shoots in relation to the exposure to auxin. The number of roots was higher when the cuttings were immersed into a solution of IBA, but this hormonal treatment had a negative effect in relation to the length of the shoots. As stated by Mercier (2008), the rooting of leaves or stem cuttings occurs due to the accumulation of auxin in the portion immediately above the cut, since the polar transport of auxin is interrupted in this region and, in order to enhance this effect, the surface of the cut can be treated with an auxin solution. At induction, auxin acts as the signal for the initiation of cell division and formation of the new meristem. During growth, the apex and leaves of the plants produce the hormone, which is transported to all growing tissues, what can explain the presence of roots in cuttings not treated with hormone in the present study.

In relation to the leaf portions used as cuttings, the number and lengths of the roots were also contrary to the number and lengths of shoots. Apical cuttings gave rise to a great number of roots, but practically failed in the production of shoots. On the other hand, basal and inverted basal cuttings produced relatively low number of roots, but originate more numerous and longer shoots. It is important to observe that the all the shoots gave rise to their own roots afterwards. In order to propagate the species using this technique, it is recommended to use either basal or inverted basal cuttings. Regarding the size of the leaves used to produce the cuttings, there was no significant difference in relation to the number and length of the roots. However, small leaves resulted in the highest number and length of shoots. Verstraeten *et al*. (2013) remark that a plant has to maintain a certain level of plasticity to be able to engage in the formation of new body shapes and organs. Through this plasticity, a plant has the capacity to tolerate changing environmental conditions via morphological adaptations. The authors mention as an example of plasticity the initiation of roots on stem cuttings. It is possible that young leaves, and consequently the small ones, have more plasticity than the older and bigger leaves, what could explain their higher capacity to regenerate plants as observed in this study.

It is interesting to observe that is still unclear the tissue which gives origin to the adventitious roots in cuttings, even in stem cuttings, widely used in horticulture. As stated by Haissig (1986), most information concerning metabolism during rooting describes the rooting zone but not events in the precise location of primordium initiation. At present, histochemical tests offer the only hope of describing biochemical differentiation within root primordium initials and their progenitor cells. According to Verstraeten *et al*. (2013), adventitious roots are defined as roots that develop on non-root tissue, such as leaves, hypocotyls, stems, and shoots. This process is distinct from other organogenesis processes as it involves the *de novo* initiation of a meristem and is rather rare in the absence of human intervention, but it is a commonly used industrial handling to reproduce elite cultivars. These authors carried out an experiment using adventitious root induction in *Arabidopsis thaliana* as a model for root organogenesis and observed that the adventitious roots emerged from cells that are located at the center of the stem structure, and histological sections pointed to cambial/phloem cells that start dividing upon auxin application.

The first researches on rooting of isolated leaves were performed by Gregory & Samarantai (1950) and Samarantai & Kabi (1953), who studied the *in vitro* rooting of leaves of several species (*Hedera helix, Phaseolus vulgaris, Ipomoea batatas, Helianthus annuus, chenopodium album, Amaranthusgangeticus, Cephalandraindica, Boerhaaviadiffusa* and *Pogostemonplectranthoides*), testing the immersion of entire leaves in diverse concentrations of rooting hormones. These studies showed that meristematic cells originate at the back of the vascular bundles in the petioles of *Phaseolus vulgaris* and *Hedera helix*. These cells form the root primordia and later on, when the root grows out, the xylem and the phloem of the root establish connections with those of the petioles. Leaf rooting, or detached leaves technique, has been used on *in vitro* phytopathological studies of *Helianthus annuus, Brassica campestris, Brassica napus, Phaseolus vulgaris, Viciafaba* and *Vignaunguiculata* (Rios *et al*., 1994) and *Arachis* (Subrahmanyam & Moss, 1983); on molecular studies of root development in *Phaseolus vulgaris* (Brown & Mangat, 1970); or to obtain root tips for chromosome observation in *Glicine max* and *Arachis* species (Blomgren *et al*., 1988; Fávero *et al*., 2005). Rooting of detached leaves was induced by Fisher (1992) to address physiological aspects of *Guareaglabra*and *G. guidonea*leaf autonomy. Also, some plants can normally be propagated by leaf cuttings, like African violets (*Saintpaulia*sp.) (Mithila *et al*., 2003) or plants of the family Cactaceae (Zaiden &Valio, 1977). Basak *et al*. (2014) also used leaf cuttings (apical and basal portions) in order to propagate *P. longum*, without or with immersion for 30 seconds in IBA (1000 ppm), NAA (1000 ppm) or both hormones together IBA (1000 ppm) + NAA (1000 ppm). The authors observed that basal cuttings, treated with IBA (1000 ppm) + NAA (1000 ppm) resulted in the highest number of roots and shoots, percentages of rooting and shooting, root length and survival of the cuttings. These authors mention that this method can be adopted with minimum capital to produce quality planting material. Besides, leaves can be obtained with very little damage to the plant.

Conclusion

Leaf cuttings can be a practical method to propagate *P. hispidum* vegetatively. Small leaves can be used as a source of basal and inverted basal cuttings, without previous immersion in auxin.

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