Propagation of *Piper carniconnectivum* through leaf cuttings

Mauricio Reginaldo Alves Dos Santos, Eric Jonisson Rios Bisi, Leormando Fortunato Dornelas Júnior

Universidade Federal de Rondônia, Brazil

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 carniconnectivum, a plant whose compounds have great potential use in medicine and agriculture. Leaves were cut in halves (apical and petiolar) by transverse cutting in the middle of the leaf blade. The petiolar parts were immersed into a solution of indole 3-butyric acid (IBA) at 1000 ppm for 30 seconds, or not submitted to the hormone. Then the cuttings were planted in soil, using two positions of the petiolar halves: petiole down and petiole up. A factorial design was used – 2 times of immersion in IBA x 2 leaf half position x 3 blocs x 6 replications. After 145 days the number of shoots, shoot length, leaf area, dry matter of aerial part, root volume and root dry matter were evaluated. The highest number of shoots was observed in the petiole down position without IBA. In relation to the other aerial characteristics – shoot length, leaf area and dry matter of the aerial part, the highest values were observed both in the petiole down position without IBA and in the petiole up position with immersion in IBA. The characteristics related to the root – root volume and root dry matter were both highest in the petiole up position with immersion in IBA. Leaf cuttings can be a practical method to propagate P. carniconnectivum vegetatively. Both petiole up cutting with immersion in IBA and petiole down cutting without immersion in hormone can be used as propagules.

Keywords: Leaf cuttings. Leaf rooting. Adventitious shooting. Clonal propagation.

1. Introduction

The botanical family Piperaceae has tropical and subtropical distribution, with nearly 2,500 species and five genera, from which 500 species and four genera are found in Brazil (Magevski et al., 2011). The genus *Piper* is composed by more than 700 species, distributed in tropical regions around the world, with 170 species native to Brazil. These species are notable producers of secondary compounds with proven biological effects on insects, fungi, bacteria, trypanosomes (Navickiene et al., 2003; Dyer et al., 2004; Danelutte et al., 2006; Balbuena et al., 2009) and can also affect human health, such as analgesics, antidepressants, cytoprotectors, antiulceratives, anticonvulsants, anti-inflammatories and antioxidants (Ahmad et al., 2010). 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 carniconnectivum* C. DC., known in Brasil as pimenta-longa (long-pepper), is endemic to the Amazon region of Northern Brazil (Freitas et al., 2014), occurring in Amazonas, Amapá and Pará states;

in transition forest with occasional rock outcrops, low forest seasonally flooded, and occasionally on disturbed area, between altitudes of 150 and 200 m (Monteiro, 2018).

Traditional propagation of *Piper* species is not 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 fungal 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 & 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. carniconnectivum*, by promoting rooting in leaf cuttings and the subsequent shoot formation, aiming at the establishment of a simple method for propagation of this species.

2. Material and Methods

The experiments were carried out at Embrapa (Brazilian Agricultural Research Corporation) in Porto Velho, Rondônia state, Brazil. The leaves were collected from two years old stock plants of *P. carniconnectivum* grown in a greenhouse with 50% shading and sprinkler irrigation three times a day for 30 minutes. Leaves were cut in halves (apical and petiolar) by transverse cutting in the middle of the leaf blade. The petiolar parts of the halves were immersed into a solution of the hormone indole 3-butyric acid (IBA) at 1000 ppm for 30 seconds, or not submitted to the hormone. After that, the cuttings were planted in vertical position, individually, in plastic cups (400 mL) containing soil, according to the method described by Basak et al. (2014). Two positions of the petiolar leaf halves were used: petiole down (i.e. with the cross section up), and petiole up (i.e. with the cross section down). A total of 72 petiolar leaf halves were used, in a factorial design – 2 times of immersion in IBA x 2 leaf half positions x 3 blocs x 6 replications. After 145 days the number of shoots, shoot length, leaf area, dry matter of aerial part, root volume and root dry matter were evaluated. Variance analyses and Tukey test ($P<0.05$) were performed by using the Assistat 7.5 program.

3. Results and Discussion

The highest number of shoots was observed in the petiole down position without IBA (Table 1). In relation to the other aerial characteristics – shoot length, leaf area and dry matter of the aerial part, the highest values were observed both in the petiole down position without IBA and in the petiole up position with immersion in IBA. The characteristics related to the root – root volume and root dry matter were both highest in the petiole up position with immersion in IBA. It seems like the absence of buds in the cut surface was compensated by the presence of exogenous auxin. 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. Furthermore, the treatment of the surface of the cut with an auxin solution can be used in order to enhance this effect.

Table 1. Averages of shoot number (SN), shoot length (SL), leaf area (LA), dry matter of aerial part (DMAP), root volume (RV), and root dry matter (RDM) of *P. carniconnectivum* in relation to the positions of petiolar leaf halves – petiole down and petiole up, and immersion or not in IBA, at 145 days of cultivation.

Treatments	SN	SL (cm)	LA (cm^2)	DMAP (g)	RV (mL)	RDM (g)
Petiole down, without IBA	5.55 a	37.2 a	2,024 a	0.873 a	120.9 b	15.33 b
Petiole down, with IBA	4.50 b	29.5 b	1,734 b	0.754 b	108.0 b	14.19 b
Petiole up, without IBA	3.72 c	27.7 b	1,725 b	0.694 b	129.2 b	17.3 ab
Petiole up, with IBA	4.70 b	34.0 a	2,172 a	0.956 a	147.1 a	20.46 a

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

One pattern observed in the current research is that, in the petiole up position, the immersion in IBA was positive in relation to every aspect evaluated. The hormone probably induced rooting first, and then the abundant roots promoted the growth of the aerial part. The opposite was observed by Dornelas Júnior et al. (2018), studying the propagation of *P. hispidum* through leaf cuttings. The authors tested three positions of the leaf halves – basal (petiole down), inverted basal (petiole up), and apical, and the immersion in IBA (1000 ppm) for 5 and 20 minutes. They observed 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. After all, they recommend the use of basal and inverted basal leaf halves, without IBA.

On the other hand, the immersion in IBA was not positive in the petiole down position, both in relation to rooting and growing of the aerial parts. In relation to the aerial parts, the petiole down cuttings not subjected to the hormone had a better performance. In relation to the rooting, the subjection of these leaf portions to the hormone had no effect. In an entire plant, the apex and leaves of the plants produce auxins, which are transported to all growing tissues (Mercier, 2008), what can explain the presence of roots in cuttings not treated with hormone in the present study.

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.

In the present study it was clear that leaves of *P. carniconnectivum* 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 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.

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. 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.

4. Conclusion

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