Effects of the phenological stage, type of cutting and plant growth regulators on the propagation by stem cutting of *Poiretia latifolia* Vogel, a brazilian native medicinal plant

Efectos de la etapa fenológica, tipo de corte y reguladores del crecimiento de las plantas en la propagación por corte del tallo de *Poiretia latifolia* Vogel, una planta medicinal nativa de Brasil

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Poiretia latifolia cutting.
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ABSTRACT

Poiretia latifolia Vogel has bioactive potential that includes antibiotic properties for human uses. The aim of this research was to develop an efficient method for vegetative propagation of P. latifolia by using cuttings as an aid to the selection and multiplication of interesting genotypes. In the first experiment, the cuttings were collected in vegetative and reproductive stages and treated with six plant growth regulators and distilled water. In the second experiment, the treatments consisted of cuttings from the apical, median, and basal branch segments. The treatment with the immersion of cuttings in 4,000 mg L⁻¹ of IBA provided greater number and length of the roots, while high dilution of Calcarea phosphorica at 20CH produced longer branches. The cuttings of the middle and basal part of the branch were found to be more viable. It was achieved 79.17% of cuttings rooting using Calcarea phosphorica 20CH. The vegetative propagation employed in this work is a feasible option for the domestication, preservation, and cultivation of Poiretia latifolia.
Additional key words: auxin; high dilution; medicinal plants; erva-de-touro; domestication.

RESUMEN

Poiretia latifolia Vogel tiene un potencial bioactivo que incluye propiedades antibióticas para usos humanos. El objetivo de esta investigación fue desarrollar un método eficiente para la propagación vegetativa de P. latifolia utilizando esquejes como ayuda para la selección y multiplicación de genotipos interesantes. En el primer experimento, los esquejes se recolectaron en etapas vegetativas y reproductivas y se trataron con seis reguladores del crecimiento y agua destilada. En el segundo experimento, los tratamientos consistieron en estacas de rama apical, mediana y basal. El tratamiento con la inmersión de esquejes en 4,000 mg L⁻¹ de IBA proporcionó un mayor número y longitud de las raíces, mientras que la alta dilución de Calcarea phosphorica a 20CH produjo ramas más largas. Se encontró que los esquejes de la parte media y basal de la rama eran más viables. Se logró el 79,17% de los esquejes de enraizamiento utilizando Calcarea phosphorica 20CH. La propagación vegetativa empleada en este trabajo es una opción factible para la domesticación, preservación y cultivo de Poiretia latifolia.

Palabras clave adicionales: auxina; alta dilución; plantas medicinales; erva-de-touro; domesticación

INTRODUCCIÓN

Poiretia latifolia Vogel (Fabaceae), popularmente conocida como "erva-de-touro", es tradicionalmente utilizada en el tratamiento de problemas estomacales y urinarios, como un sabor para el mate tradicional y como afrodisíaco (Müller, 1984; Amorim and Boff, 2009). Nanoemulsión, basada en su aceite esencial, tiene el potencial de ser utilizado como agente antidermatofítico y anti-inflamatorio (Porto et al., 2010; Pérez-Zamora et al., 2016; Paim et al., 2018). Adicionalmente, Poiretia latifolia podría ser una alternativa de ingreso para los agricultores ya que su aceite esencial es rico en componentes importantes para la industria de los sabores y fragancias, como carvona, dihidrocarvona y limoneno (Porto et al., 2010; Fernandes and Boff, 2017).

Poiretia latifolia naturalmente se encuentra en el Plateau del Sur de Santa Catarina, Brasil Sur. La diversidad de plantas de esta región tiene el potencial para numerosos usos, como ganadería, alimentación, medicinales y cosméticos. Sin embargo, este potencial ha sido amenazado por actividades antropogénicas como ganadería, huertos de frutas, agricultura intensiva...
cultivation of soybean and corn, and reforestation with *Pinus* sp. and *Eucalyptus* sp. (Zank and Hanazaki, 2012; Pereira *et al*., 2006). In order to strengthen this plant diversity and enhance its potential, the knowledge of the most appropriate propagation technique for plants such as *Poiretia latifolia* is essential (Nogueira and Nogueira-Filho, 2011; Sarasan *et al*., 2011; Vodouhè *et al*., 2011).

*Poiretia latifolia’s* seeds present germination rate above 80%, however, its production is severely damaged by insects which destroy 54% of its flowers and 88% of its seeds (Maass *et al*., 2018). Vegetative propagation could overcome this problem and even facilitate the selection of the best plant characteristics (Rasmussen *et al*., 2015; Kaviani and Nagahdar, 2017; Wetzstein *et al*., 2018). However, vegetative propagation knowledge of *P. latifolia* are limited and unsuccessful (Teixeira *et al*., 2011; Amorim *et al*., 2012). The success of this technique relies on the adventitious rooting which is influenced by several factors such as plant phenological stage, type of cutting and levels of inhibitors and/or promoters of plant growth in the propagule (Guo *et al*., 2009; Zerche and Druege, 2009; Ludwig-Müller, 2011; Pacurar *et al*., 2014; Pigatto *et al*., 2018).

The exogenous plant growth regulators are broadly used in vegetative propagation and they can be synthetic or natural. However, when working with medicinal plants, natural alternatives are preferable. Yamashita *et al*. (2017) found that both, the extract of *Cyperus rotundus* (natural) and indol-3-butyric acid - IBA (synthetic) improved the cuttings rooting of *Myrciaria cauliflora* and *Psidium guajava*. High dilutions are another alternative which aids the production, minimizing the residues generation and reducing the costs (Moreno, 2017). The homeopathic preparations *Phosphorus* 3CH and *Kali muriaticum* 12CH, selected based on the nutrients commonly used in plant fertilization, promoted increased root growth of eucalyptus seedlings. (Moraes *et al*., 2018). Cassol *et al*. (2017) also reported that the use of both, IBA and *Arnica montana* 12 CH, stimulated the rooting of *Cuphea gracilis*.

In order to enhance the knowledge of *Poiretia latifolia* vegetative propagation the objective of this study was to evaluate the influences of the phenological stages, segments of the branch and plant growth regulators on the vegetative propagation of *P. latifolia* using stem cutting.
MATERIALS AND METHODS

Localities of collecting and conduction

The work was conducted at the Lages Experimental Station of the EPAGRI (Agricultural Research and Rural Extension Agency of Santa Catarina State, Brazil). The vegetative material used in the experiments was collected from a natural population of Poiretia latifolia in the region of Vacas Gordas, district of Capão Alto, Santa Catarina (27°58'33.1”S and 50°30'26.5”W). Immediately after sampling, the herbaceous shoots were kept immersed in water and transported in polystyrene boxes to avoid the dehydration of the vegetal material.

Experimental design

In the first experiment, seven treatments with plant growth regulators were evaluated: immersion of the base of the cuttings for 20 s in four concentrations of indole-3-butyric acid (IBA) – 1,000, 2,000, 3,000, and 4,000 mg L\(^{-1}\); aqueous extract of Cyperus rotundus, concentration 10%; high dilution of Calcarea phosphorica at 20CH (CH = hahnemannian centesimal dilution order); pure water (control). Each treatment had three replicates of 20 cuttings, in a completely randomized block design. The experiment was carried out in both phenological stage of P. latifolia, vegetative (14 December, 2016) and reproductive (18 January, 2017). In total, 840 cuttings were used in the experiment.

In the second experiment, the treatments consisted of the cutting type, in relation to the segment of the P. latifolia branch. They were divided into basal, median, and apical thirds. Each treatment had four replicates of 20 cuttings in a randomized block design totalling 240 cuttings in the experiment. The cuttings were collected at the reproductive stage (22 February, 2017).

Obtaining and applying plant growth regulators

The IBA was previously dissolved in 1N NaOH and then adjusted to the use concentrations with distilled water. The aqueous extract of Cyperus rotundus was obtained by blending 100 g fresh plant bulbs in 1,000 mL of pure water in a blender for 10 min. The high dilution of Calcarea phosphorica was obtained from a pharmacy and the last two levels of dynamization were made in pure water, following the methodology described in the Brazilian Homeopathic Pharmacopoeia (2011). Plant growth regulators were applied by immersion for 20 seconds of 1/3 of the base of the
Cuttings preparation and planting

The herbaceous shoots of *P. latifolia* were segmented into cuttings with two lateral gems. In the first experiment, all the segments of the branch were used and placed randomly inside the blocks, producing cuttings with varied length and diameter. In the second experiment, the diameter and length of the cuttings varied according to the characteristics of the segment evaluated, basal, median, and apical.

The preparation of the cuttings for all the experiments and treatments followed the protocol described by Bettoni *et al.* (2014). At the base of the cuttings, a transverse cut was made, with 20% of the total length of the cutting remaining below the lateral gem. At the top end of the cutting, an angle cut was made, with the remaining 30% of the total length of the cutting above the lateral gem. Afterwards, at the base of the cuttings, two lesions were made in the longitudinal direction, with approximately 30% of the total length of the cuttings. A leaflet was kept attached at each cutting.

After preparation, the cuttings were transplanted into styrofoam honeycomb tray of 60 cells with sand and vermiculite substrate (1:1, v/v). The trays were arranged in plastic boxes (60 x 40 x 20 cm) and covered with a transparent plastic film to maintain moisture saturation. They were watered by spray twice per day, and then they were kept in a greenhouse at 25°C. No control for pests and diseases were carried out.

Statistical analysis and assessment

Survival rate, rooting rate, shoot sprouting rate, the number of roots and shoots, length of the four largest roots, and of the two largest shoots were assessed 35 days after planting the cuttings. In order to analyse survival, rooting, and sprouting, generalized linear models were used, using the binomial model or the binomial model with a dispersion parameter (quasibinomial), both considering logit link function. The means were compared through confidence intervals (95%) for
Tukey contrasts of the linear predictors of the fitted model. All analyses were performed using the R environment v 3.2.1 (The R Foundation, 2015).

RESULTS AND DISCUSSION

The joint analysis of the experiment with plant growth regulators in the vegetative and reproductive phases of *P. latifolia* plants showed the simple effect of the treatments (Tab. 1). This means that the plant growth regulators performed similarly in the two phenological, vegetative, and reproductive stages.

**Table 1. Descriptive levels (p-values) of the joint analysis of the propagation by cutting of Poiretia latifolia with plant growth regulators in the vegetative and reproductive stages, Lages, SC, 2018.**

| Factors    | SURV. | R   | R/C | LR   | S    | S/C  | LS   |
|------------|-------|-----|-----|------|------|------|------|
| PE         | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | 0.0430 | 0.3806 |
| PE/Block   | 0.0371 | 0.0230 | 0.1738 | 0.0085 | 0.0352 | 0.7835 | 0.1011 |
| GR         | 0.0417 | 0.0293 | <0.0001 | <0.0001 | 0.0369 | 0.0438 | 0.0069 |
| GR*PE      | 0.0724 | 0.0699 | 0.6407 | 0.5178 | 0.1897 | 0.9826 | 0.2279 |

PE = phenological stage, GR = growth regulators, SURV. = survival rate of cuttings, R = rooting rate, R/C = roots per cutting, LR = mean length of the four largest roots, S = sprouting rate, S/C = shoots per cuttings, LS = mean length of the two largest shoots.

The phenological stage of *P. latifolia* plants influenced the cuttings viability, with the rate of surviving, rooted, and sprouted cuttings lower in the vegetative stage (Tab. 2). The plant growth regulators did not increase the cuttings viability. Cuttings from medial and basal branch sections overcame the apical section. The sprouting rate was still better for the basal section than for the median section (Tab. 2).

The vegetative stage, in contrast with what was found in viability (Tab. 2), increased the cuttings number of roots, average length of the four largest roots, number of shoots, and average length of the two bigger shoots (Tab. 3).

IBA concentrations of 3,000 and 4,000 mg L\(^{-1}\) showed higher root numbers than the treatments without synthetic auxin application. IBA at 4,000 mg L\(^{-1}\) was superior to the 2,000 mg L\(^{-1}\) and did not differ from the 3,000 mg L\(^{-1}\) in relation to the length of the four largest roots (Tab. 3). The raise in IBA concentration increased the number of roots per cutting and the average length of the four largest roots. This response occurred inversely with the cuttings shoot number and the length of the two largest shoots (Tab. 3).
Table 2. Viability of *Poiretia latifolia* cuttings collected in different phenological stages, treated with plant growth regulators and originated from different sections of the branch. Lages, SC, 2018.

| Treatments                        | Survival (%) | Rooting (%) | Sprouting (%) |
|-----------------------------------|--------------|-------------|---------------|
| Phenological stages of plant collection ¹ |              |             |               |
| Vegetative                        | 53.81 ± 3.16 b | 55.71 ± 3.01 b | 49.52 ± 3.25 b |
| Reproductive                      | 81.90 ± 2.22 a | 82.62 ± 2.09 a | 70.00 ± 2.50 a |
| Plant growth regulators ¹         |              |             |               |
| Control                           | 68.33 ± 5.43 ab | 68.33 ± 5.11 ab | 60.83 ± 3.52 ab |
| 1,000 mg L⁻¹ IBA                  | 57.50 ± 9.20 b | 58.33 ± 8.63 b | 47.67 ± 8.82 b |
| 2,000 mg L⁻¹ IBA                  | 66.67 ± 8.72 ab | 70.00 ± 8.66 ab | 63.33 ± 8.23 ab |
| 3,000 mg L⁻¹ IBA                  | 64.17 ± 7.46 ab | 67.50 ± 6.42 ab | 60.83 ± 8.41 ab |
| 4,000 mg L⁻¹ IBA                  | 72.50 ± 6.68 ab | 74.17 ± 7.00 ab | 60.00 ± 4.83 ab |
| *C. rotundus*                      | 66.67 ± 10.46 ab | 66.67 ± 9.80 b | 64.17 ± 10.36 ab |
| *Calc. phos.* 20CH                | 79.17 ± 5.54 a | 79.17 ± 5.39 a | 72.50 ± 4.23 a |
| Sections of the branch ²          |              |             |               |
| Apical                            | 2.50 ± 1.44 b  | 2.50 ± 1.44 b  | 5.00 ± 0.00 c  |
| Median                            | 16.25 ± 1.25 a | 32.50 ± 2.50 a | 17.50 ± 1.44 a |
| Basal                             | 12.50 ± 2.50 a | 46.25 ± 2.39 a | 12.50 ± 2.50 b |

Control = distilled water, *C. rotundus* = aqueous extract of *Cyperus rotundus*, *Calc. phos.* 20CH = High dilution of *Calcarea phosphorica* at 20CH (CH = Hahnemannian centesimal dilution order). Different letters in the columns present a statistical difference between treatments, Tukey's contrast (P≤0.05); Means ± standard error; 1 Trials analyzed together; 2 Independent trial.

The growth regulators did not influence the number of shoots per cutting. However, *Calcarea phosphorica* 20CH increased the length of the two largest shoots of the cuttings but did not differ from IBA concentrations of 1,000 and 2,000 mg L⁻¹ (Tab. 3). The different sections of the branch did not show difference in the quality of the viable *P. latifolia* cuttings (Tab. 3).

During the plant collection, it was observed branches more lignified in the reproductive stage than in the vegetative stage. Cuttings from older plants, that are more lignified, besides having more nutrients reserve, owing to its tissue consistency, are more resilient to environmental stresses such as temperature and humidity (Villa et al., 2017). This may explain the better cuttings viability for the reproductive stage and medial and basal branch segment of *P. latifolia* plants (Tab. 2).

However, the vegetative stage showed better cuttings quality than the reproductive stage, the opposite of what was found in the cuttings viability. According to Maia et al. (2008), more lignified cuttings may present some kind of rooting impediment. They observed a better rooting in less lignified cuttings of the apical segment of the *Hyptis suaveolens* branch. Even having lower nutritional reserves and less resistance to stresses that prejudice the cutting viability, cuttings from
vegetative stage may have better hormonal balance improving the rooting quality in the surviving cuttings (Ludwig-Müller, 2011; Pacurar et al., 2014).

Table 3. Quality of viable Poiretia latifolia cuttings collected in different phenological stages, treated with plant growth regulators and originated from different sections of the branch. Lages, SC, 2018.

| Treatments                     | R/C (n°) | LR (cm) | S/C (n°) | LS (cm) |
|--------------------------------|----------|---------|----------|---------|
| Phenological stages of plant collection |          |         |          |         |
| Vegetative                     | 21.46 ± 0.82 a | 18.39 ± 0.65 a | 1.35 ± 0.06 a | 8.18 ± 0.46 a |
| Reproductive                   | 16.77 ± 0.63 b | 15.67 ± 0.49 b | 1.19 ± 0.04 b | 7.78 ± 0.32 b |
| Plant growth regulators        |          |         |          |         |
| Control                        | 16.97 ± 1.35 bc | 15.35 ± 1.22 b | 1.25 ± 0.10 ab | 7.59 ± 0.43 b |
| 1,000 mg L\(^{-1}\) IBA        | 19.58 ± 1.24 abc | 17.46 ± 1.14 ab | 1.27 ± 0.11 ab | 8.04 ± 0.71 ab |
| 2,000 mg L\(^{-1}\) IBA        | 20.90 ± 1.26 ab | 17.57 ± 0.41 b | 1.31 ± 0.08 ab | 8.27 ± 0.79 ab |
| 3,000 mg L\(^{-1}\) IBA        | 22.47 ± 1.54 a | 17.66 ± 0.94 ab | 1.07 ± 0.09 b | 6.81 ± 0.67 b |
| 4,000 mg L\(^{-1}\) IBA        | 21.63 ± 1.42 a | 20.46 ± 1.07 a | 1.10 ± 0.06 ab | 6.79 ± 0.52 b |
| C. rotundus                    | 15.83 ± 1.81 c | 14.16 ± 1.37 b | 1.40 ± 0.07 ab | 8.13 ± 0.68 b |
| Calc. phos. 20CH               | 16.42 ± 1.14 c | 16.58 ± 0.61 b | 1.52 ± 0.11 a | 10.26 ± 0.67 a |

| Sections of the branch         |          |         |          |         |
| Apical                         | 4.00 ± 2.00 a | 11.90 ± 9.10 a | 1.00 ± 0.00 a | 3.00 ± 2.00 a |
| Median                         | 7.58 ± 0.64 a | 13.12 ± 1.76 a | 1.17 ± 0.10 a | 5.04 ± 0.83 a |
| Basal                          | 7.37 ± 1.40 a | 9.63 ± 1.07 a | 1.12 ± 0.12 a | 3.12 ± 1.16 a |

Control = distilled water, C. rotundus = aqueous extract of Cyperus rotundus, Calc. phos. 20CH = high dilution of Calcarea phosphorica at 20CH (CH = Hahnemannian centesimal dilution order), R/C = roots per cutting, LR = mean length of the four largest roots, S/C = shoots per cuttings, LS = mean length of the two largest shoots. Different letters in the columns present a statistical difference between treatments, Tukey’s contrast (\(P\leq0.05\)); Means ± standard error; 1 Trials analyzed together; 2 Independent trial.

The IBA effects on root quality obtained in this work are aligned with the findings of Bettoni et al. (2014) and Amorim et al. (2012). They observed that although IBA did not change the cuttings viability of grapevine rootstocks and Poiretia latifolia cuttings, it improved the quality of the surviving cuttings. Amorim et al. (2012) suggest studies with higher IBA concentrations and with other rooting inducers. However, the application of IBA or increases its doses, does not necessarily promote the quality of the cuttings. Benzylaminopurine (BAP) concentrations from 0.2 to 3.0 mg L\(^{-1}\) did not enhance Poiretia latifolia vegetative propagation and, despite it did not differ statistically, the treatment with 2,000 mg L\(^{-1}\) of AIB promoted better rooting rate than the treatment with 4,000 mg L\(^{-1}\) (Teixeira et al., 2011).

The high dilution of Calcarea phosphorica at 20CH promoted shoots growth at the same time that it did not interfere with root quality (Tab. 3). Calcarea phosphorica, when applied since a
young age, strengthens the plants improving their nutrients absorption as well as its resilience to cope with hydric stress (Moreno, 2017). However, the choice of a high dilution that provides the desired effect is somewhat complex and depends on several factors, such as method of preparation and potency, application, concentration and nature of the preparation (Hanif and Dawar, 2015; Giesel et al., 2017). Giesel et al. (2017) report that both the potency and the dynamization method affect the effect of the high dilution on the foraging of leaf-cutting ants (Acromyrmex laticeps Emery). According to Broetto et al. (2011), the quality of the seedlings is associated with the development of both the aerial and the root. Thus, more studies are needed to achieve results similar to the study by Pulido et al. (2014) that was able to stimulate both aerial growth and cabbage roots. The homeopathic preparations Phosphorus 3CH and Kali muriaticum 12CH, selected based on the nutrients commonly used in plant fertilization, promoted increased root growth of eucalyptus seedlings (Moraes et al., 2018). Cassol et al. (2017) also reported that the use of both, IBA and Arnica montana 12 CH, usually used for help the organism to cope with traumatic events such the cutting process, stimulated the rooting of Cuphea gracilis.

The results obtained from P. latifolia cuttings treated with C. rotundus extract were similar to those of Yamashita et al. (2017), which tested five concentrations of the extract (0, 25, 50, 75, and 100%) and did not observe difference in the number of roots of Myrciaria cauliflora and Psidium guajava. The authors suggest that the auxin concentration in the aqueous extract of C. rotundus is not sufficient to increase the number of roots. Dias et al. (2012) also warn that depending on the cuttings’ immersion time, the extract may provide toxicity effects.

Even though there was no increase in the rooting rate with plant growth regulator treatments, it is evident that the application of 4,000 mg L⁻¹ of IBA provides a better development of the root system of P. latifolia cuttings. In the same way, the high dilution of Calcarea phosphorica at 20CH provides better development of the aerial part of P. latifolia cuttings. It is important to note that the methodology applied for the cuttings collection and preparation as well as the conduction period of rooting provided a higher rooting rate (79.17%) when comparing with previous works (around 10 and 40%) (Amorim et al., 2012; Teixeira et al., 2011).

**CONCLUSION**

With Calcarea phosphorica, it was achieved a rooting rate of 79.17%. The high dilution improved the aerial part of P. latifolia cuttings while the AIB enhanced the quality of the rooting.
More studies with these two plant growth regulators, both individually and combined, should be carried out to improve even more the vegetative propagation of this specie. More lignified *Poiretia latifolia* cuttings are more viable, both due to the phenological stage and to the position of the branch segment. The vegetative propagation employed in this work is a feasible option for the domestication, preservation, and cultivation of *Poiretia latifolia*.

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