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# Propagation of three native Brazilian *Piper* species by cuttings and indole butyric acid

## Propagación de tres especies nativas brasileñas de *Piper* usando estacas y ácido indolbutírico



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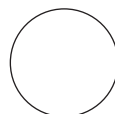
**Propagated Brazilian species of *Piper*.**

Photo: A.P. Ferriani

### ABSTRACT

Native *Piper* species present significant repellent, antimicrobial, insecticidal, anti-tumor and anti-protozoal biological activities. Studies on new species can discover unpublished potentialities and vegetative propagation for the development of cultivation protocols and reduce the natural extraction. The objective of this study was to evaluate the rooting of *P. arboreum*, *P. cernuum* and *P. diospyrifolium* stem cuttings with different treatments containing doses of indole butyric acid (0, 500; 1,000; 1,500; 2,000 and 3,000 mg L<sup>-1</sup>). Branches were collected in the “Bom Jesus Biological Reserve”, Parana state, Brazil. The cuttings, with a length of 12 cm and average diameter of 6 mm, were placed in 53 cm<sup>3</sup> plastic tubes with the commercial substrate Tropstrato HP® and intermittent misting. After 90 days, the survival, mortality, cuttings with new shoots, number and length of the three main roots were evaluated. The percentages of rooting reached an average of 67.5% for *P. arboreum*, 51.6% for *P. cernuum* and 50.4% for *P. diospyrifolium*. A positive effect in the treatments containing the plant regulator was observed for rooting development, percentage of cuttings with shoots and number of roots per propagule, but there were variations in the responses of each species. Leaf retention was an important factor for the adventitious rooting formation in all evaluated species.

**Additional key words:** species domestication, biological activity, essential oil, plant growth regulators, plant propagation.



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## RESUMEN

Las especies nativas de *Piper* presentan importantes actividades biológicas repelentes, antimicrobianas, insecticidas, antitumoral y anti-protozoario. Los estudios sobre nuevas especies representan potenciales descubrimientos sin publicar y la propagación vegetativa es necesario para el desarrollo del protocolo de cultivo; además, reduce la extracción natural. El objetivo de esta investigación fue evaluar el enraizamiento de esquejes de *P. arboreum*, *P. cernuum* y *P. diospyrifolium* con diferentes tratamientos que contienen dosis del ácido indolbutírico (0, 500, 1.000, 1.500, 2.000 y 3.000 mg L<sup>-1</sup>). Ramas fueran colectadas en la Reserva Biológica Bom Jesus, estado de Paraná, Brasil. Los cortes con una longitud de 12 cm y el diámetro promedio de 6 mm se instalaron en tubos de plástico de 53 cm<sup>3</sup> usando el sustrato comercial Tropstrato HP® y riego por nebulización intermitente. Después de 90 días se evaluaron las tasas de supervivencia, mortalidad y esquejes con nuevos brotes, número y longitud de las tres raíces principales. Los porcentajes de enraizamiento alcanzaron un promedio de 67,5% para *P. arboreum*, 51,6% para *P. cernuum* y 50,4% para *P. diospyrifolium*. Se encontró respuesta positiva de los tratamientos que contienen el regulador vegetal para las variables de enraizamiento, porcentaje de esquejes con brotes y número de raíces por estaca, pero hubo variaciones en las respuestas de cada especie. La permanencia de hojas fue un factor importante para el proceso de enraizamiento adventicio en las especies evaluadas.

**Palabras clave adicionales:** domesticación de especies, actividad biológica, aceite esencial, reguladores de crecimiento vegetal, propagación de plantas.

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## INTRODUCTION

The *Piper* genus has 291 species, of which 184 are endemic to Brazil (Flora do Brasil, 2018). These species have been studied because of the production of essential oil with different biological activities (Pereira *et al.*, 2008; Lopes *et al.*, 2012).

*Piper arboreum* Aubl., known as “long pepper”, “rosemary-angola”, “fruit-of-bat”, “jaborandi” and “jaborandi-from-river”, is a perennial shrub with wide distribution, especially in the dense ombrophylous segment of Atlantic Forests (Guimaraes *et al.*, 2006).

Several biological activities in *P. arboreum* result from the essential oil composition, such as a leishmanicidal action attributed to sesquiterpenes (Bernuci *et al.*, 2016). The main constituent, sesquiterpene bicyclogermacrene, was reported in plants from the Federal District area by Potzernheim *et al.* (2006), whereas the oxygenated sesquiterpenes 1-epi-Cubenol, Spathulenol,  $\alpha$ -Cadinol and epi- $\alpha$ -Muurolol were observed in plants collected from Antonina-PR (Bernuci *et al.*, 2016).

*Piper cernuum* Vell., known as “pariparoba”, is a native non-endemic species found in primary and secondary

forests in Atlantic Tropical Areas (Flora do Brazil, 2018), which has been used in traditional medicine for stomach, liver, kidney and circulatory treatments (Mariot *et al.*, 2007).

The essential oil produced by this species has the monoterpenes  $\alpha$ -pinene and  $\beta$ -pinene and the oxygenated sesquiterpenes spathulenol, epi- $\alpha$ -muurolol,  $\alpha$ -muurolol, caryophyllene oxide and  $\alpha$ -cadinol, which have shown high leishmanicidal activity and moderate anti *M. tuberculosis* activity (Bernuci *et al.*, 2016). The sesquiterpenes dihydro- $\beta$ -agarofuran and 10-epi- $\gamma$ -eudesmol, besides the monoterpenes  $\alpha$ -pinene and camphene, were the main constituents and have shown antibacterial activity (Perigo *et al.*, 2016).

Furthermore, Girola *et al.* (2015) verified induced apoptosis in melanoma cells by a camphene compound isolated from *P. cernuum* essential oil.

*Piper diospyrifolium* Kunth., a semi-heliophilous perennial shrub regionally known as “joao-borandi”, is found in humid areas of dense low mountain forests and in lowland forests in Rio de Janeiro, São Paulo and Parana (Brazil) (Guimaraes *et al.*, 2006). The

essential oil composition presented the sesquiterpenes selin-11-en-4- $\alpha$ -ol, germacrene B and  $\alpha$ -selinene, with inhibitory growth potential of *Leishmania* forms (Perigo *et al.*, 2016). Sesquiterpenes were also related to antifungal potential against *Candida* species (Vieira *et al.*, 2011).

These species have a common characteristic, the high environmental regulation of essential oil production (Bergo, 2010; Oliveira *et al.*, 2013; Silva *et al.*, 2013; Perigo *et al.*, 2016), which results in differences in yield and composition according to the plant collection location. Even in the same location, high variability can be found in the essential oil production of *Piper* species because of natural propagation through seeds. The development of vegetative protocols could be very useful for these species in order to maintain the essential oil yield and quality of selected plant materials for cultivation.

Some vegetative propagation protocols have been developed for *Piper* using different types of propagules (Cunha *et al.*, 2015; Gomes and Krinski, 2016a), substrates, luminosity and seasonality (Mesquita *et al.*, 2005; Dousseau, 2009; Gomes and Krinski, 2016b; Pacheco *et al.*, 2016; Gasparetto *et al.*, 2017). However, there is a lack of information on propagation for the great majority of species, especially for the three species included in this study.

The objective of this study was to evaluate the root production of *Piper arboreum*, *P. cernuum* and

*P. diospyrifolium* after treatment with indole butyric acid to develop cultivation protocols and reduce extractivism.

## MATERIALS AND METHODS

The botanical specimens used in this study were collected in April, 2016 in the “Biological Reserve Bom Jesus”, Parana state (Brazil), located at 25°29'69.3" S and 49°00'84.4" W, at sea level. Samples of the botanical species were deposited in the herbarium of the “Botanical Museum”, located in the “Botanic Garden of Curitiba” under the numbers MBM 396412 (*Piper arboreum* Aubl.), MBM 396416 (*Piper cernuum* Vell.) and MBM 396413 (*Piper diospyrifolium* Kunth.).

The plant material was collected and moistened in the field until the stem cutting preparation. Cuttings with a length between 10-12 $\pm$ 2 cm and a diameter of 6 $\pm$ 2 mm were prepared maintaining one pair of leaves with half of the leaf area. Before the rooting treatments, the stem cuttings were kept in running water for 5 min.

This experiment was conducted with a completely randomized design for each species, comparing five treatments [immersion in hydro alcoholic solutions containing 0, 500; 1,000; 1,500; 2,000 and 3,000 mg L<sup>-1</sup> of indole butyric acid (IBA) for 10 s], with four replications with 10 cuttings each. After the treatment, the stem cuttings were transferred to polypropylene

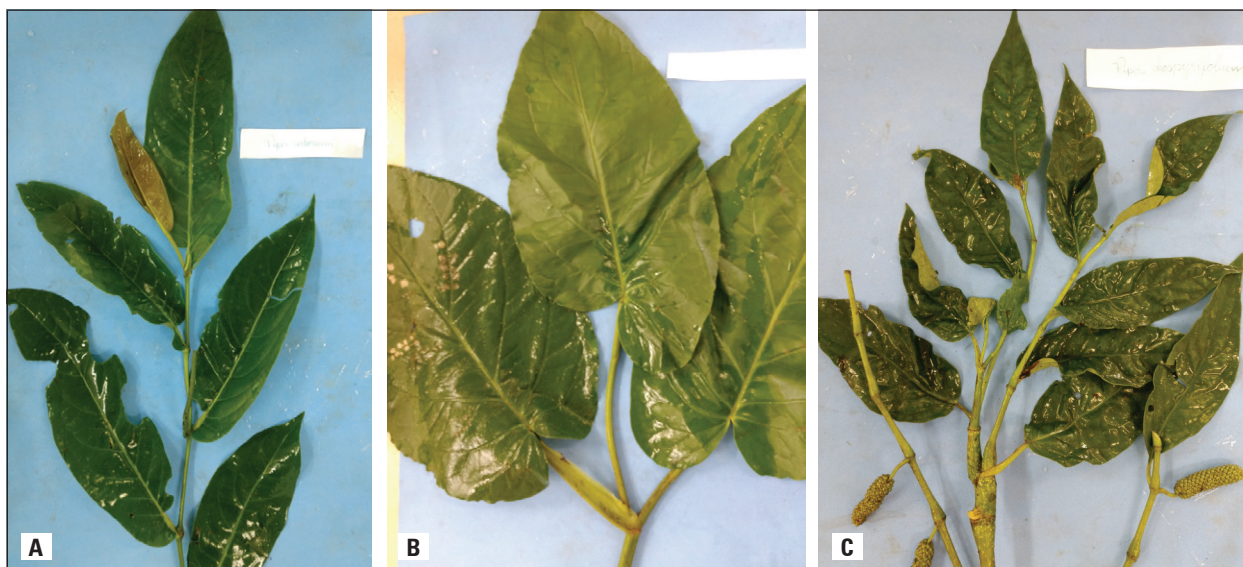


Figure 1. Semihardwood stems from A. *P. arboreum*; B. *P. cernuum*; and C. *P. diospyrifolium* (Ferriani, 2016).



tubes (53 cm<sup>3</sup>) containing the commercial substrate Tropstrato HP® maintained in a greenhouse with intermittent misting, relative humidity around 80% and temperatures ranging for 20-30°C for 90 d.

The rooting percentages, number and average length of the three main roots, foliar retention, sprout and shoot mortality were evaluated. The data was submitted to homogeneity analysis with Bartlett's test and the analysis of variance and regression using the statistical software Assistat (Silva *et al.*, 2016).

## RESULTS AND DISCUSSION

In all analyzed species, the rooting response were positively affected by the plant growth regulator indole butyric acid. *P. arboreum* reached 70% rooting percentage of cuttings when the highest IBA concentration was used (Fig. 2).

Variables related to rooting response, such as leaf retention percentages, number and length of roots were negatively affected by the IBA concentrations over 1,500 mg L<sup>-1</sup>, which may indicate a phytotoxicity effect. These results may be related to the intrinsic capacity of natural vegetative propagation with stolons, as pointed by Souza *et al.* (2009).

In this species, leaf loss at high concentrations of an exogenous regulator affect adventitious rooting because mature leaves act as a source of carbohydrates and cofactors as verified in *P. hispidum*, which presented higher numbers of sprouts and roots in cuttings that maintained a higher number of leaves (Cunha *et al.*, 2015). The rooting of the *P. cernuum* cuttings showed an upward behavior with a maximum of 45% in 3,000 mg L<sup>-1</sup> of IBA, and it was possible to verify that the highest concentrations also promoted the highest percentages of this variable, limited by the plant regulator treatments (Fig. 3).

The percentage of sprout shoots showed a linear increase as a function of the higher IBA concentration. It was not possible to associate the behavior of the leaf retention variable to any treatment whose overall mean was 25.4%. However, for the number and root length variables, it was possible to verify the tendency to increase the values up to the limit of 2,000 mg L<sup>-1</sup>. These results suggest that doses containing higher concentrations may trigger phytotoxic effects, leading to a reduction of the quality of the adventitious root system.

In comparison with other species from the same family, *Piper cernuum* presents a greater leaf area (almost five times greater), which represents higher metabolic intakes and losses. Thus, in this case, the leaf loss was associated with an increase in the percentage of shoots that promoted the translocation of photoassimilates to a critical level, from which there was a reduction in the number and length of the roots.

The *P. diospyrifolium* cuttings reached a percentage of rooting of 70%, being influenced by the application of IBA treatments up to the concentration of 1,500 mg L<sup>-1</sup>, since there was a reduction in the means of this variable with the higher IBA concentrations (Fig. 4).

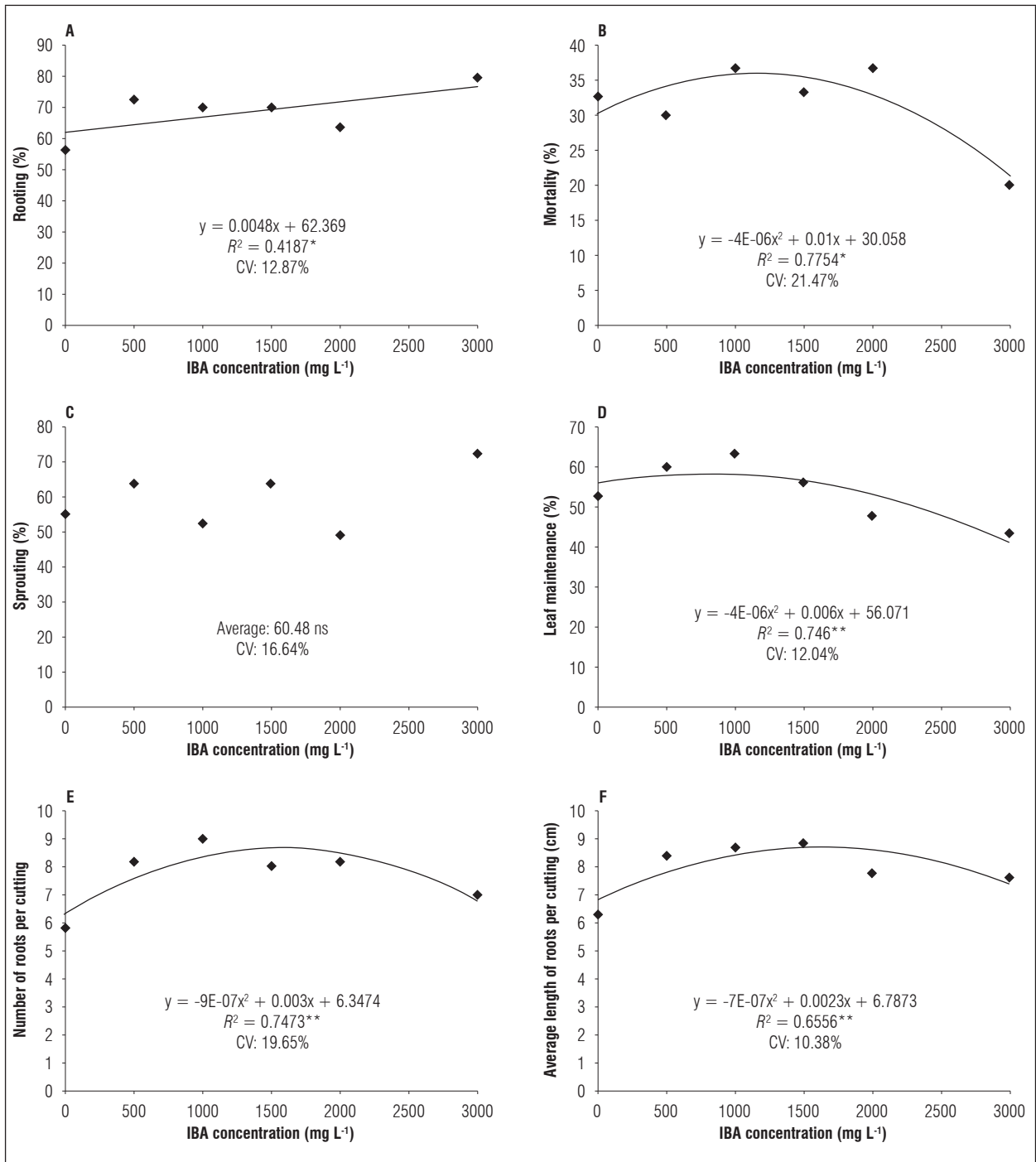
The percentages of sprouts and leaf retention indicate that high doses of the plant regulator may have caused foliar abscission, interfering in propagule sprouting and, consequently, in root induction and the root length variable. Vignolo *et al.* (2014) verified a similar behavior for the cuttings of three blackberry (*Rubus spp.*) cultivars, where the presence of leaves positively affected the sprouting and rooting in all of the evaluated cultivars.

The number of roots varied linearly as a function of the plant regulator treatments. In *Piper mikanianum*, Pescador *et al.* (2007) related an increase in the rooting rate with the foliar maintenance and sprout formation in the cuttings. According to Hartmann *et al.* (2002), mature leaves and sprouting development increase the synthesis of cofactors related to the rooting process.

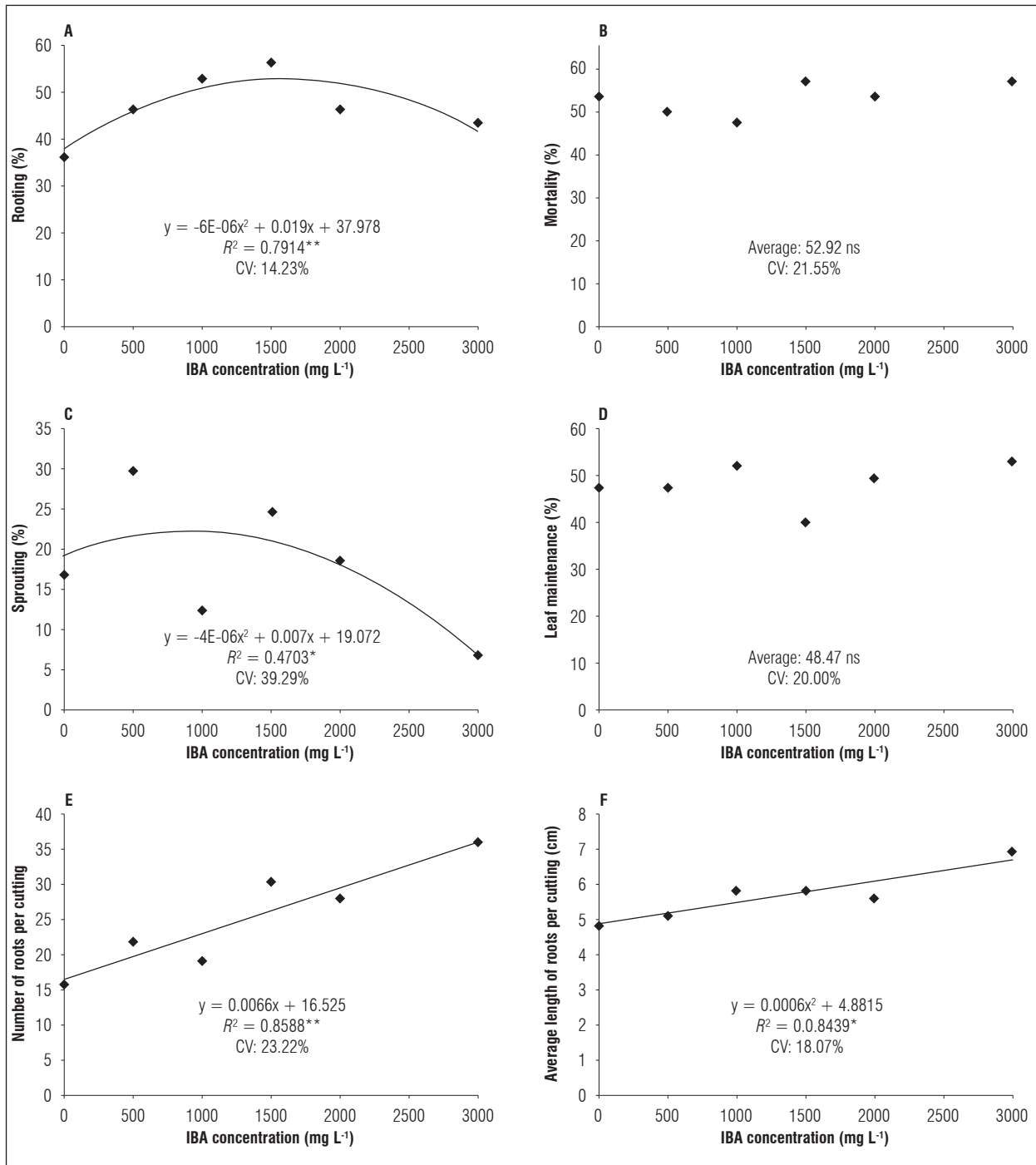
These results show the importance of the specific evaluation of rooting production for each *Piper* species since they present different responses in root induction and development.

The formation of an adventitious rooting system is dependent on the endogenous level of hormones and other rooting promoters (Hartmann *et al.*, 2002). In *P. hispidum* for example, the rooting of stem cuttings reached percentages greater than 80% without the application of exogenous auxins (Cunha *et al.*, 2015), and stem cuttings from *P. amalago* reached only 22% under the same auxin concentration (Gomes and Krinski, 2016a).

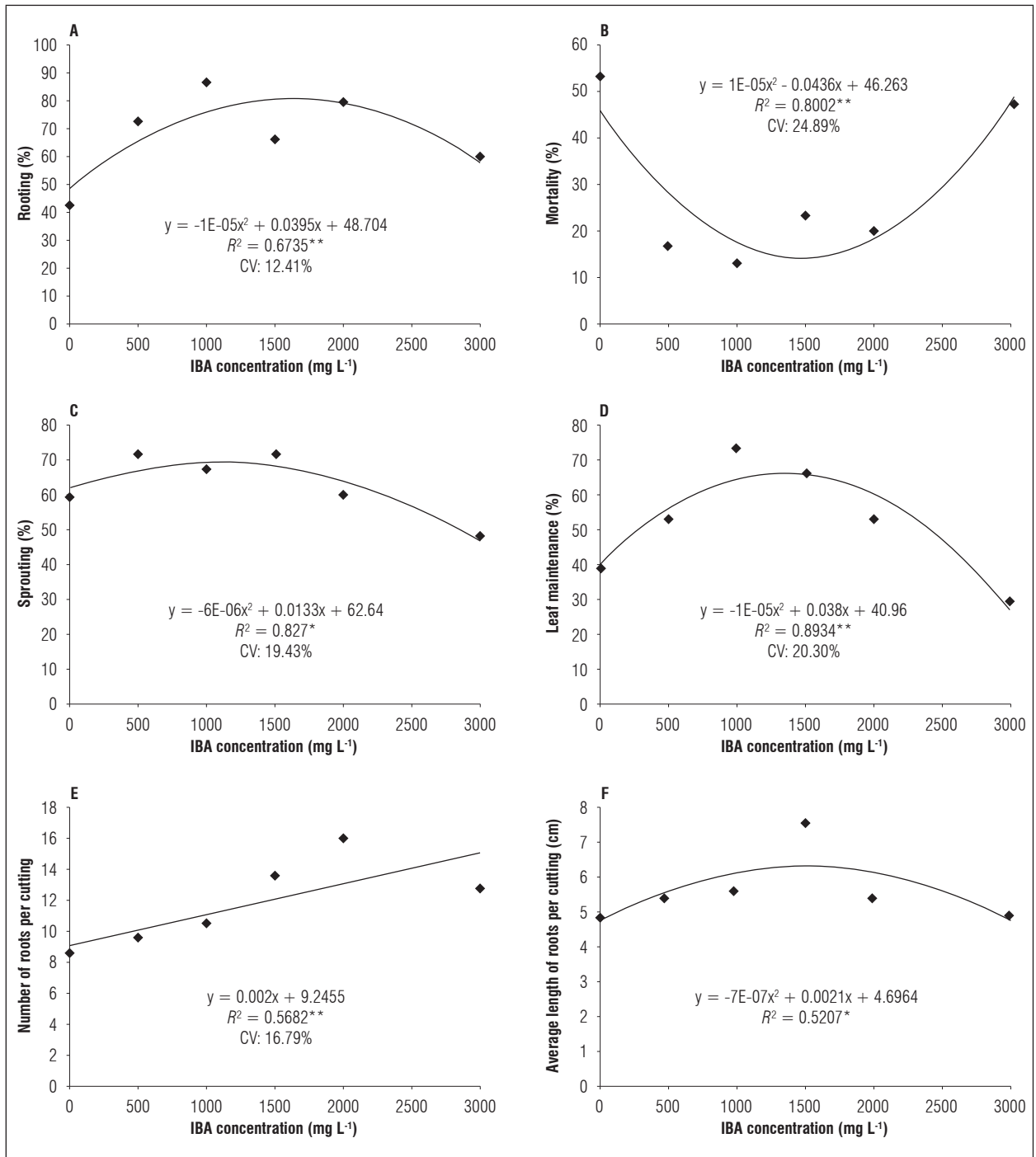
Other studies that consider the seasons for plant collection are recommended for the root production of these species.



**Figure 2.** Rooting percentage, mortality, sprouting, leaf retention, number and average length of roots in the *P. arboreum* cuttings. **\*\***Significant at 1% probability according to polynomial regression analysis; **\***significant at 5% probability according to polynomial regression analysis. ns: not significant. CV: coefficient of variation.



**Figure 3.** Rooting percentage, mortality, sprouting, leaf retention, number and average length of roots in the *P. cernuum* cuttings. \*\*Significant at 1% probability according to polynomial regression analysis; \*Significant at 5% probability according to polynomial regression analysis. ns: not significant. CV: coefficient of variation.



**Figure 4.** Rooting percentage, mortality, sprouting, leaf retention, number and average length of roots in the *P. diospyrifolium* cuttings. \*\*Significant at 1% probability according to polynomial regression analysis; \*Significant at 5% probability according to polynomial regression analysis. ns: not significant. CV: coefficient of variation.

The present study provides unprecedented information on the propagation of these *Piper* species, which will help establish protocols that guarantee sustainable cultivation practices since the current use of raw material is exclusively obtained with extractivism.

## CONCLUSIONS

All of the evaluated species presented an increase in root production after treatment with indole butyric acid. The rooting percentages reached averages of 67.5% for *P. arboreum*, 51.6% for *P. cernuum* and 50.4% for *P. diospyrifolium*.

These positive results show the importance of specific studies on the domestication of *Piper* native species for crops development.

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**Conflict of interests:** the manuscript was prepared and reviewed with the participation of the authors, who declare that there exists no conflict of interest that puts the validity of the presented results at risk.

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