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# Effect of indolebutyric acid on *in vitro* root production of *Psychotria ipecacuanha* (Brot.) Stokes (Rubiaceae)

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## Abstract

*Psychotria ipecacuanha* (Brot.) Stokes (Rubiaceae) is a medicinal plant commonly known as ipecac, which contains two alkaloids of high pharmacological value (emetine and cephaeline) with anti-diarrheal, amebicidal, expectorant and anti-inflammatory properties. The risk of extinction and the difficulty of the conventional cultivation of the species combined with great market demand has generated a need for studies of cultivation methods that allow the economic exploitation of this species. This work presents data on the development of an *in vitro* root culture protocol for *P. ipecacuanha*. Leaf, nodal, internodal and root segments were used, which were introduced in culture media containing different concentrations (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg.mL$^{-1}$) of indolbutyric acid (IBA). The best treatment for root formation included the use of nodal segments in culture media containing 2.5 and 3.0 mg.mL$^{-1}$ of IBA, which gave rise to 100% root formation at 60 days of culture. Regarding the number of roots per explant, the best result (13:1) were obtained with the use of internodal segments in medium supplemented with 2.5 mg.mL$^{-1}$ IBA and, the best root length was obtained in root segments developed in medium enriched with 1.5 mg.mL$^{-1}$ IBA, with an average increment of 0.87 cm per root.

**Keywords:** Ipecac. Root culture. *In vitro* culture. Plant biotechnology.

## Introduction

*Psychotria ipecacuanha,* a perennial shrub that is popularly known as *ipeca*, ipecacuanha, poaia, ipeca-verdadeira, poaia cinzenta, poaia legítima, ipeca-preta and ipeca-do-Mato-Grosso. This species belongs to the family Rubiaceae, whose center of origin is Brazil; it has the highest occurrence in the State of Mato Grosso, mainly in the city of Cáceres(1). The species is distributed along the Atlantic, Amazonian, Central American and Colombian ranges(2).

It is a medicinal species of great economic potential due to the pharmacological properties of the roots, which contain high concentrations of emetine and cephaeline[3-4]. These compounds show activity against bronchial diseases through anti-inflammatory properties, can combat fever and malaria, and show amebicidal activity[5-7]. These medicinal properties are well-known and have been used by native populations since before the discovery of Brazil. This knowledge was passed on to the European settlers, contributing to the integration of the species in the list of tropical products exported by Brazil in the colonial period, and it was considered as the species with the highest medicinal value at that time[8-9].

Brazil is among the main exporters of ipeca followed by Panama and Costa Rica[10]. The Brazilian ipeca is preferred in the world market because it contains higher concentration of emetic alkaloids, which has led Brazil to become one of the main exporters of this product[11].

The roots of ipeca are obtained mostly by extractivism, and the species has become increasingly rare in natural habitat such as sub-forests of tropical forests[1-2]. *P. ipecacuanha* is a species threatened with extinction due to having undergone intense extraction in the past two centuries, which opened new agricultural frontiers while reducing areas of natural occurrence for this species[12]. The slow growth, the low percentage of production and germination of seeds, in addition to the loss of viability of the seeds after storage create challenges for cultivation[2]. Another relevant factor for production is deforestation, which has reduced the availability of adequate habitat and contributed to uncontrolled collection by native populations; this has further accelerated the demographic decline of wild populations of ipeca[2,6,13]. It is important to point out that the long period (3-4 years) for cultivating crops with a higher concentration of root emetic alkaloids also contributes to variation in the demand of the pharmaceutical industry[14].

The use of natural products as a model for drug synthesis or even common use by the population is a growing trend, and many studies evaluating these products have been carried out[15]. Literature review reveals high medicinal and commercial value of the roots of *P. ipecacuanha*, but there is strong variability in the production of the chemical compounds of interest (emetine and cephaeline) and little information on the standardization of raw plant material for increasing the concentration of these constituents[1].

Due to high economic and pharmacological value of this species and associated market demand combined with the risk of extinction and the difficulty of conventional cultivation methods, the objective of this work was to develop an *in vitro* root culture protocol for *P. ipecacuanha*. These results will contribute to the future chemical characterization of this species in order to obtain standardized extracts that may help to meet the demands of the pharmaceutical industries for this product.

## Materials and Methods

The experiments took place at the Coordenação de Biotecnologia Vegetal do Centro de Biotecnologia da Amazônia (CBA) in Manaus, AM.

For the establishment of the micropropagation protocol of *P. ipecacuanha*, *in vitro* cultures from the Embrapa Amazônia Oriental germplasm bank (already identified by the IAN Herbarium - Embrapa Amazônia Oriental) were used as donors of explants for tests.

In order to select the best organogenetic expression for rooting *in vitro*, we used different sources of propagules (leaf fragments, nodal, internodal and root segments) that were inoculated in semi-solid culture media with a basic composition, without growth regulators (MS0) and supplemented with 0.5; 1.0; 1.5; 2.0; 2.5 and 3.0 mg.mL$^{-1}$ of indolebutyric acid (IBA) plus 3% sucrose[16]. The pH was adjusted to 5.8 and the media was sterilized by autoclaving at 120 °C and 1.1 kg/cm$^2$ for 15 minutes. Glass vials containing 40 ml of culture medium and 30 copies of each explant type were used for each treatment. Cultures maintained at 25°C, illuminated with fluorescent lamps (Sylvania, Phillips/daylight) with intensity of 30.0 moles -2 e -1, and kept under a 16-hour photoperiod. Cultures nutrient medium was changed every four weeks, and the analysis of development was performed in the same period by counting the percentage of root formation per explant, the number of roots emitted by the explants, and by measuring the length of the roots (cm).

The effect of different concentrations of IBA on root development per explant were evaluated by analysis of variance (ANOVA) and the means were compared by the Tukey-Kramer test at a significance level of 5%. These evaluations were performed using Graph Pad in Stat version 3.01. In the analysis of the rooting percentages according to the medium used, the percentage difference test (p1 and p2) at the 5% level of significance was run using Statistical for Windows TM Software. The experimental design was completely randomized and used 30 explants per treatment performed in triplicate.

## Results and Discussion

The different types of explants of ipeca were submitted to the different concentrations of IBA (0; 0.5; 1.0; 1.5; 2.0; 2.5 and 3.0 mg.mL$^{-1}$). ANOVA indicated a significant interaction between IBA dose and the origin of explant groups (leaf, nodal, internodal and radicular segments) (**Tables 1-4**), showing significant differences between the explants for the percentage of root formation, the number of roots and average length. In addition, significant differences were observed for both parameters evaluated, being influenced by the increase of IBA doses.

After 8 weeks of cultivation, using the leaves as explants, the best root formation (46%) resulted from the treatment with 3.0 mg.mL$^{-1}$ of IBA, which produced 3 roots per explant with a mean length of 0.25 cm (**Table 1**).

**TABLE 1:** Effect of different concentrations of indolebutyric acid (IBA) on root formation using leaves explants of *Psychotria ipecacuanha*.

| Culture Medium | Root Formation (%) | Number of Roots | Root Length (cm) |
|---|---|---|---|
| MS0 | 0 | 0 | 0 |
| 0.5 mg.mL$^{-1}$ IBA | 20$^{d}$ | 2.0$^{c}$ | 0.12$^{d}$ |
| 1.0 mg.mL$^{-1}$ IBA | 20$^{d}$ | 2.0$^{c}$ | 0.25$^{a}$ |
| 1.5 mg.mL$^{-1}$ IBA | 24$^{c}$ | 1.0$^{d}$ | 0.17$^{b}$ |
| 2.0 mg.mL$^{-1}$ IBA | 28$^{b}$ | 2.0$^{c}$ | 0.16$^{c}$ |
| 2.5 mg.mL$^{-1}$ IBA | 24$^{c}$ | 4.0$^{a}$ | 0.12$^{d}$ |
| 3.0 mg.mL$^{-1}$ IBA | 46$^{a}$ | 3.0$^{b}$ | 0.25$^{a}$ |

a-d: Equal letters in the same column indicate that there was no significant difference between the values.

For nodal segments, the best culture media were those supplemented with 2.5, 3.0 and 2.0 mg.mL$^{-1}$ of IBA, yielding 100, 100 and 96% root emission with 11, 7 and 7 roots each measuring 0.23, 0.41, and 0.2 cm, respectively (**Table 2**).

**TABLE 2:** Effect of different concentrations of indolebutyric acid (IBA) on root development in nodal segments of *Psychotria ipecacuanha*.

| Culture Medium | Root Formation (%) | Number of Roots | Root Length (cm) |
|---|---|---|---|
| MS0 | 0 | 0 | 0 |
| 0.5 mg.mL$^{-1}$ IBA | 52$^{d}$ | 2.0$^{e}$ | 0.25$^{b}$ |
| 1.0 mg.mL$^{-1}$ IBA | 88$^{b}$ | 6.0$^{c}$ | 0.25$^{b}$ |
| 1.5 mg.mL$^{-1}$ IBA | 80$^{c}$ | 5.0$^{d}$ | 0.41$^{a}$ |
| 2.0 mg.mL$^{-1}$ IBA | 96$^{a}$ | 7.0$^{b}$ | 0.2$^{d}$ |
| 2.5 mg.mL$^{-1}$ IBA | 100$^{a}$ | 11.0$^{a}$ | 0.23$^{c}$ |
| 3.0 mg.mL$^{-1}$ IBA | 100$^{a}$ | 7.0$^{b}$ | 0.41$^{a}$ |

a-e: Equal letters in the same column indicate that there was no significant difference between the values.

For internodal segments, the best culture medium was supplemented with 2.5 mg.mL$^{-1}$ IBA, with 96% root emission and 13 roots per explant (mean length 0.32 cm) (**Table 3**).

**TABLE 3:** Effect of different concentrations of indolebutyric acid (IBA) on root development in internodal segments of *Psychotria ipecacuanha*.

| Culture Medium | Root Formation (%) | Number of Roots | Root Length (cm) |
|---|---|---|---|
| MS0 | 0 | 0 | 0 |
| 0.5 mg.mL$^{-1}$IBA | 76 | 7.0$^{d}$ | 0.38$^{a}$ |
| 1.0 mg.mL$^{-1}$IBA | 80$^{c}$ | 5.0$^{e}$ | 0.29$^{d}$ |
| 1.5 mg.mL$^{-1}$IBA | 44$^{d}$ | 5.0$^{e}$ | 0.25$^{e}$ |
| 2.0 mg.mL$^{-1}$IBA | 88$^{b}$ | 11.0$^{b}$ | 0.36$^{b}$ |
| 2.5 mg.mL$^{-1}$IBA | 96$^{a}$ | 13.0$^{a}$ | 0.32$^{c}$ |
| 3.0 mg.mL$^{-1}$IBA | 92$^{a}$ | 8.0$^{c}$ | 0.24$^{e}$ |

a-e: Equal letters in the same column indicate that there was no significant difference between the values.

The best results with the use of root segments were observed in MS medium plus IBA 1.5 mg.mL$^{-1}$, with an increase in root growth of 0.87 cm, 96% secondary root formation, and mean production of five secondary roots for each main root (5:1).

Regarding the best treatment to stimulate the production of secondary roots, the most efficient treatment was 2.5 mg.mL$^{-1}$ IBA, with 100% formation and average production of 11 secondary roots for each primary root (11:1) (**Table 4**).

**TABLE 4:** Effect of different concentrations of indolebutyric acid (IBA) on the growth of root segments and the development of secondary roots in *Psychotria ipecacuanha*.

| Culture Medium | Increase in root length (cm) | Formation of secondary roots (%) | Number of secondary roots |
|---|---|---|---|
| MS0 | 0.38$^{f}$ | 44$^{e}$ | 1.0$^{h}$ |
| 0.5 mg.mL$^{-1}$ IBA | 0.44$^{e}$ | 88$^{b}$ | 7.0$^{c}$ |
| 1.0 mg.mL$^{-1}$ IBA | 0.73$^{c}$ | 76$^{c}$ | 4.0$^{e}$ |
| 1.5 mg.mL$^{-1}$ IBA | 0.87$^{a}$ | 96$^{a}$ | 5.0$^{d}$ |
| 2.0 mg.mL$^{-1}$ IBA | 0.81$^{b}$ | 96$^{a}$ | 10.0$^{b}$ |
| 2.5 mg.mL$^{-1}$ IBA | 0.6$^{d}$ | 100$^{a}$ | 11.0$^{a}$ |
| 3.0 mg.mL$^{-1}$ IBA | 0.7$^{c}$ | 64$^{d}$ | 3.0$^{f}$ |

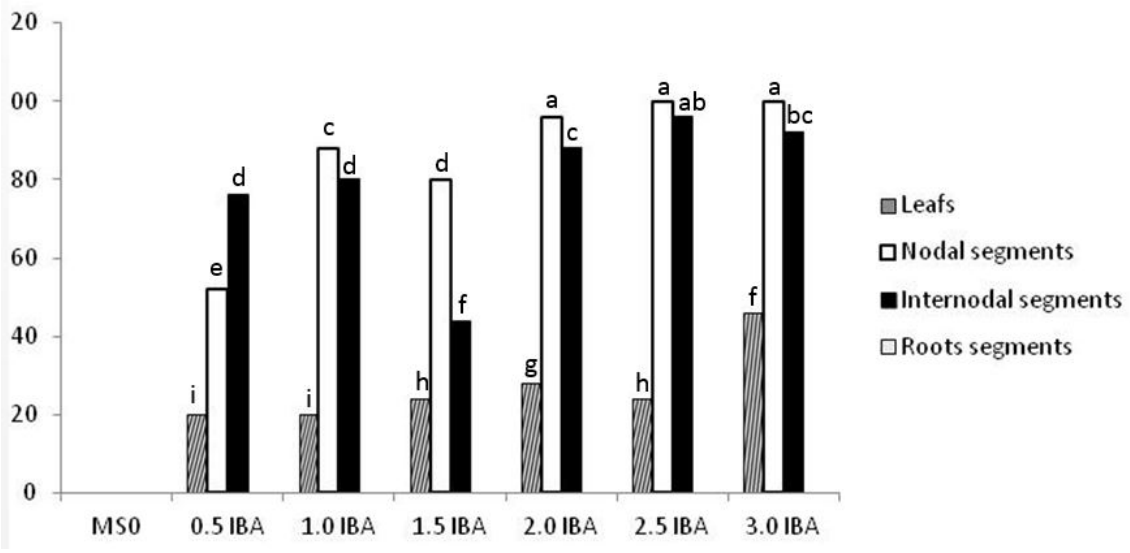
a-h: Equal letters in the same column indicate that there was no significant difference between the values.

These results show that the best results for root formation (100%) observed using the nodal segments as explant with the addition of 2.5 and 3.0 mg.mL$^{-1}$ of IBA to the MS medium. However, these results were statistically similar to those obtained with MS medium plus IBA 2.0 mg.mL$^{-1}$ and for internodal segments in medium supplemented with IBA 2.5 mg.mL$^{-1}$ (96% in both treatments) (**Figure 1**).

The use of plant growth regulators induces the root formation process, increasing the percentage of rooted cuttings, the number and quality of roots formed, and the uniformity of rooting[17]. Auxins are the plant regulators indicated for induction of rooting, and indolebutyric acid (IBA) is one of the most commonly used and most efficient due to photostability and resistance to biological activity[18].

In a study with *Ixora coccinea* L., was observed that the IBA treatment was five times more efficient than the control, with rooting percentage increasing from 8.89% to 44.44%; this was also confirmed in a study in which the *Ixora* rooting rate increased by more than 35.55% using IBA[19-20].

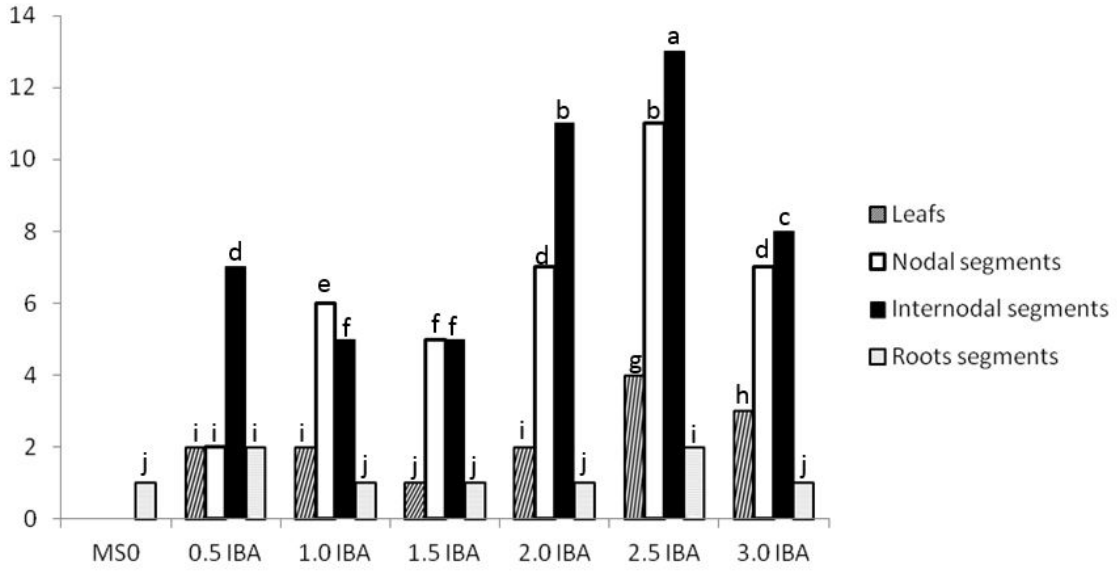
**FIGURE 1:** Effect of different *Psychotria ipecacuanha* explants and indolebutyric acid (IBA) concentrations on root development.




** Means indicated by the same letter do not differ significantly from each other by the Tukey test at 5% probability.

Regarding the number of roots per explant, the best result was obtained with the use of internodal segments in a semi-solid medium plus 2.5 mg.mL$^{-1}$ IBA, with the formation of 13 roots per explant, followed by semi-solid culture medium plus 2.0 mg.mL$^{-1}$ IBA, which gave rise to 11 roots per explant (**Figure 2**). However, with the dose of 3.0 mg.mL$^{-1}$ of IBA in the culture medium there was a decrease in root production in both explants used. This behavior may be related to the fact that high concentrations of auxin may be toxic to the plant, affecting root formation[21].

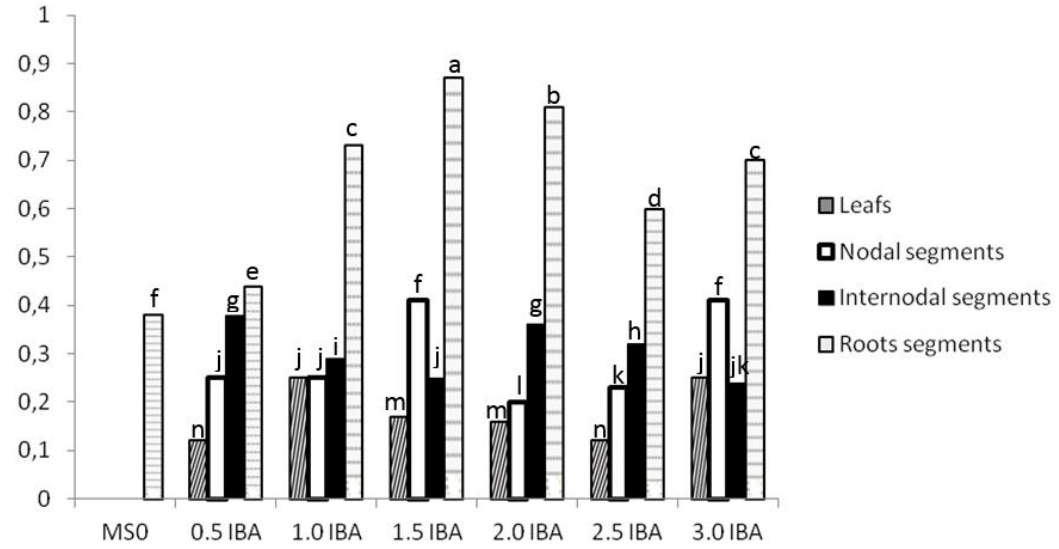
**FIGURE 2:** Effect of different *Psychotria ipecacuanha* explants and indolebutyric acid (IBA) concentrations on the number of roots.




** Means indicated by the same letter do not differ significantly from each other by the Tukey test at 5% probability.

With respect to root length, root segments in MS + 1.5 mg.mL$^{-1}$ IBA medium were found to be more efficient, with an additional growth of 0.87 cm compared to the initial length (fragments of 1.0 cm) at 60 days of culture (**Figure 3**).

**FIGURE 3:** Effect of different *Psychotria ipecacuanha* explants and indolebutyric acid (IBA) concentrations on root length.




** Means indicated by the same letter do not differ significantly from each other by the Tukey test at 5% probability.

Auxins are essential in the rooting process, possibly by stimulating the synthesis of ethylene, which favors the emission of roots[22]. The *in vitro* culture of plants allows the evaluation of the performance of the crops under certain conditions, such that it is possible to evaluate the role of several agents on various aspects of plant developmental physiology. Thus, it is possible to work with significant quantities of plants in limited space and over a relatively short time period compared to field cultivation conditions. Because the plants are genetically standardized, the interference of genetic variability in the results can eliminated. Consequently, the results obtained are effects of the variables introduced into the process as part of the experimental design.

The use of adequate growth regulator dosages is very important because the optimum concentrations vary by species and with various types of synthetic inductors[23]. After treatment of the stem base with the root-inducing growth regulator, the carbohydrates translocated to the treated area, increasing the rate of respiration and resulting in transformation of the carbohydrates and organic nitrogen compounds. The growth regulator can accelerate normal metabolism and increase the number of primordial radicles.

The application of auxin promotes faster formation, and higher quality and uniformity of the root system[24]. Root emission in greater number and length is fundamental when the objective is the production of seedlings on a commercial scale, since these factors are fundamental in the success of implantation in field areas[25]. In addition, a well-formed root system increases the harvested soil area, favoring the absorption of nutrients and water, which promotes better seedling development in the field[26].

## Conclusion

Under the current study conditions, our results indicate that the type of explant and the concentration of indolebutyric acid in the culture media influence the percentage of root formation and root development. The use of indolebutyric acid increased rooting efficiency, providing benefits in terms of increased production and root length in ipeca.

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