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In vitro propagation of *Psychotria ipecacuanha* (Brot.) Stokes under different concentrations of Indoleacetic Acid

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Abstract

Psychotria ipecacuanha is a medicinal plant that is native to the Americas with the largest area of occurrence in the State of Mato Grosso in Brazil. It is critically endangered due to overexploitation of natural populations. Due to difficulties in conventional propagation, the aim of this study was to evaluate the effect of different concentrations of the growth regulator indoleacetic acid (IAA) on *in vitro* propagation of this species. Stem node explants were cultured in Murashige and Skoog medium (MS) without growth regulators (control) and supplemented with four concentrations (0.05, 0.5, 1.5 and 2.0 $mg.L^{-1}$) of indoleacetic acid (IAA) in semisolid media. After 60 days of cultivation, nodal segments (n=30) grown in Murashige & Skoog (MS) medium supplemented with 0.05 $mg.L^{-1}$ of IAA produced on average 4.56 nodal segments per explant. The seedlings were successfully acclimatized without detection of any morphological anomalies or variation.

Keywords: Plant biotechnology. Tissue culture. Micropropagation. Rubiaceae. Ipecac.

Introduction

Psychotria ipecacuanha Brot. Stokes (Rubiaceae), commonly known as ipecac, is recognized worldwide as a medicinal plant and is officially listed in the pharmacopoeia of several countries, such as Brazil, India, Japan, England, the United States, and Portugal(1). It is a species that is distributed in bands along the extension of the Atlantic, the Amazon, Central America and Colombia(2). Its economic importance is due to the pharmacological properties present in its roots, where emetine and cephaeline are concentrated(3-5). These constituents give the plant emetic activity against bronchial diseases through anti-inflammatory properties, and makes them useful for combating fevers and malaria. They also show amoebicidal activity via acting as inhibitors of protein and DNA synthesis(6-7).

P. ipecacuanha is threatened by genetic erosion and is in the process of extinction due to intense extraction over the past two centuries, opening new agricultural frontiers while reducing areas of natural occurrence[7]. The cultivation of the species is hampered by slow growth, low percentage of production and seed germination, and loss of seed viability after storage[8]. Another relevant aspect is attributed to the long period (3-4 years) required to produce higher concentrations of the emetic alkaloids in the roots[9-10]. These factors contribute to oscillations in meeting the demands of the pharmaceutical industry.

In order to commercialize the product of Brazilian origin, licensing by IBAMA is required, attesting that the material comes from cultivation and not extractivism. *P. ipecacuanha* is currently one of the most promising agricultural crops, with India being one of the largest producers. Thus, better methodology for asexual propagation of the species is one of the primary needs for successful commercial cultivation[11].

Given the commercial value of this plant, some initiatives for cloning and micropropagation in the laboratory have already been carried out, but there is still a need to optimize these protocols to test different dosages of growth regulators to increase seedling productivity, ensuring the economic viability of this cultivation technique[12].

Due to the high economic and pharmacological value of the species and associated market demand combined with the extinction risk and the difficulty of the conventional cultivation, the objective of this study was to evaluate the effect of different dosages of the regulator (IAA) on the *in vitro* development of the plant. The purpose of this study is to establish an efficient protocol for large-scale production of seedlings to allow economic exploration and consequently, the creation of new productive chains.

Materials and Methods

Plant material

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 with different concentrations of the indoleacetic acid growth regulator (IAA) in semisolid culture medium.

The explants (nodal segments) were introduced into glass flasks (250 ml) containing 40 ml of MS semi-solid medium without growth regulators (MS0) and supplemented with different concentrations (0.05, 0.5, 1.5 and 2.0 $mg.L^{-1}$) of IAA plus 3% sucrose and solidified with 2% phytagel[13]. The pH was adjusted to 5.8 and the media was sterilized by autoclaving at 120 °C and 1.1 $kgf.cm^2$ for 15 minutes. Each explant or phytomer was composed of an internodal region without leaves with a size of approximately 1 cm.

Cultures were maintained at 25 °C and were illuminated with fluorescent lamps (Sylvania, Phillips/daylight) with intensity of 30.0 moles.m-2.s^{-1} and a 16-hour photoperiod. The parameters evaluated for the effects of the different IAA concentrations were the multiplication rate, seedling height, and rooting after two months of cultivation.

Statistical analysis

The effects of the different concentrations of indoleacetic acid on *in vitro* seedling development were analyzed by analysis of variance (ANOVA), and the means were compared using the Tukey-Kramer test at a significance level of 5%. These analyses were performed using Graph Pad in Stat version 3.01. For the analysis of rooting percentages according to the means, a percentage difference test (p1 and p2) at the 5% significance level was carried out using Statistica for Windows TM version 5.0[14].

The experimental design was completely randomized and consisted of five treatments with 30 explants per treatment, which were performed in triplicate.

Acclimatization

After being withdrawn from the culture flasks, the seedlings obtained *in vitro* were washed in running water for complete removal of the culture medium and packed in polypropylene bags (1 kg) containing substrate. The plants were kept in a greenhouse for 30 days with a nebulization irrigation system. The plants were then transferred to another greenhouse with a microaspersion irrigation system, where they remained for additional 23 months.

Results and Discussion

Data on the *in vitro* development of *P. ipecacuanha* with different concentrations of indoleacetic acid (IAA) were compared with those obtained in MS0. After 60 days of *in vitro* culture, the multiplication rate was not significantly affected by the presence of the IAA. Although the highest multiplication rate (4.56:1) was obtained in MS medium plus 0.05 mg.L^{-1} of IAA, this result did not differ statistically from the other treatments tested, including the control (**TABLE 1**). However, the action of IAA in the *in vitro* development of the plant was in accordance with known data for the effects of such hormones[15-16].

TABLE 1: *In vitro* development of *Psychotria ipecacuanha* under different concentrations of indoleacetic acid (IAA).

Culture medium	Multiplication Rate	Seedling Height (cm)	Rooting Rate (%)	Number of Roots	Root Height (cm)
MS0	4.52^{a}	1.70^{b}	15^{d}	2.25^{ab}	0.60^{a}
0.05 mg.L^{-1} IAA	4.56^{a}	1.79^{ab}	52^{c}	2.00^{b}	0.27^{b}
0.5 mg.L^{-1} IAA	3.41^{a}	1.54^{c}	86^{a}	3.15^{ab}	0.25^{b}
1.5 mg.L^{-1} IAA	3.50^{a}	1.80^{a}	70^{b}	2.93^{ab}	0.24^{b}
2.0 mg.L^{-1} IAA	3.33^{a}	1.63^{b}	71^{b}	4.27^{a}	0.27^{b}

Values in the same column followed by the same letter do not differ statistically from each other (significance level 5%).

The formation of 5.95 and 5.41 new shoots using internode segments of *P. ipecacuanha* sized 1.0 and 1.5 cm, respectively, was observed in MS medium plus 2.0 mg.L^{-1} of 6-benzylaminopurine (BAP) alternated with the same culture medium with the addition of 0.5 mg.L^{-1} of gibberellic acid (GA_3) [17]. When cultivating nodal segments of *P. ipecacuanha*, some authors report the importance of using juvenile explants, mainly in *in vitro* culture establishment, as an ideal material for micropropagation and genetic manipulation[10,18-19]. In addition to serving as an alternative tool for a fast multiplication and conservation of species for which propagation by conventional methods is challenging, this technique is especially advantageous for the preservation of genotypes producing compounds of interest[20].

Regarding the height of the seedlings, the best result was obtained with the use of 1.5 mg.L^{-1} of IAA in the culture medium, providing an average growth of 1.8 cm for seedlings; this result was statistically similar to that obtained with 0.05 mg.L^{-1} IAA = 1.79 cm (**TABLE 1**).

The *in vitro* rooting of *P. ipecacuanha* was significantly affected by the addition of IAA to the culture medium (**TABLE 1**). Statistical analysis showed a greater increase in rooting percentage (86%) with a mean of 3.15 roots per plantlet with a height of 0.25 cm when 0.5 mg.L^{-1} of IAA was used.

Among the main factors related to rooting of *in vitro* cultivated plants are the endogenous auxin levels and the inherent conditions of the parent plant such as juvenility and genotype, among others[21]. Endogenous IAA acts as a gene activator, boosting the early formation of the primordial radicals, and the application of synthetic auxins favors conjugation of endogenous IAA and amino acids that promote the synthesis of specific proteins necessary for the formation of the initial roots[21].

The acclimatization process used for *in vitro* cultivation of *P. ipecacuanha* was successful, resulting in a survival rate of 100% (n = 450). This demonstrates that the protocol developed in this study is appropriate for the regeneration of ipecac.

The adaptation of *in vitro* seedlings to natural conditions is crucial for the success of any protocol. This is due to the fact that there are large differences between the growing conditions in the growing room, greenhouse conditions, and the natural conditions in terms of quantity and quality of light, nutrients and substrate and relative humidity[22].

Conclusion

The results of the present work demonstrate that the micropropagation of *P. ipecacuanha* using IAA is feasible for mass propagation and conservation. This species demonstrated strong *in vitro* rooting ability, and the seedlings were successfully acclimatized.

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