THE EFFICIENCY OF SHOOT AND PLANTLET FORMATION OF *Cephaelis ipecacuanha* AFTER THREE SUBCULTURES *IN VITRO*

EFICIÊNCIA NA REGENERAÇÃO DE BROTOS E FORMAÇÃO DE PLÂNTULAS DE *Cephaelis ipecacuanha* EM TRÊS SUBCULTIVOS *IN VITRO*

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SUMMARY

Multiple adventitious shoot formed from internodal segments of *Cephaelis ipecacuanha* cultured 25 days on Gamborg basal medium (GAMBORG et al., 1968) supplemented with 6.66μM 6-benzylaminopurine there was a maximum of nine shoots per segment and an average of five shoots per segment formed. The presence of gibberellic acid in the subculture media promoted shoot elongation in all treatments. The shoots attained 3cm in height and rooting of 100% after 35 days of culturing upon Murashige and Skoog's basal medium (MS), added with 4.92μM indole-3-butyric acid, 0.87μ gibberellic acid and 0.1% activated charcoal. Further growth was accelerated after the transfer to 1/2 MS without growth regulators. Rooted plantlets transferred to potting soil could be successfully established.

Key words: medicinal plant; micropropagation, Ipecac.

RESUMO

Brotos adventícios foram regenerados de segmento internodal de *Cephaelis ipecacuanha* em três subcultivos. Após 25 dias de cultivo em meio básico de Gamborg (GAMBORG et al., 1968), o meio contendo 6,66μM, 6-benzilaminopurina (BAP) induziu até 9 brotos por segmentos na cultura inicial e uma média de 5 brotos no subcultivo. O ácido giberélico no meio de cultura promoveu um alongamento em todos os tratamentos. Os brotos alcançaram uma altura de 30mm e 100% de enraizamento após 35 dias de cultivo no meio basal de Murashige & Skoog (MURASHIGE & SKOOG, 1962) - MS, suplementado com 4,92μM ácido indolbutírico, 0,87μM ácido giberélico e 0,1% de carvão ativado. Houve um crescimento acelerado após cultivar os brotos em meio 1/2 MS sem regulador de crescimento. As plântulas foram transferidas para vaso e aclimatadas com sucesso.

Palavras-chave: planta medicinal, micropropagação, Cephaelis.

INTRODUCTION

Cephaelis ipecacuanha (Ipecac) is a perennial herb with medicinal use, grows in humid forests of Brazil. The roots of this plant contain emetine and cephaline and are widely used in folk medicine,

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mainly as an expectorant, a diaphoretic, and most importantly, an excellent amoebacid (GUPTA, 1971).

The propagation of ipecac by seeds in rarely used because of the large amount of time required for germination, at least six months. Precious attempts to propagate *C. ipecacuanha* through root cuttings managed to increase the percentage but failed to improve root growth (GATONNI, 1959; KALYANASUNDARAM, 1968).

Two studies have been reported on the propagation of *C. ipecacuanha* through tissue culture (IKEDA et al., 1988; YOSHIMATSU & SHIMOMURA, 1991). This paper describes the results of an investigation on shoot and plantlet formation of *C. ipecacuanha* (Ipecac) after three subcultures *in vitro*.

MATERIALS AND METHODS

The explant sources were internodal segments of ipecac plantlets obtained from preliminary experiments *in vitro* incubated in Gamborg medium B5 (GAMBORG et al., 1968). The segments, approximately 5mm long were excised and incubated in 25 x 100mm test tubes containing 10ml of Gamborg medium, salts and vitamins (B5) supplemented with 0.22, 2.22, 6.66 or 13.22μM 6-benzylaminopurine (BAP) and 2% (w/v) sucrose. The liquid media were (filter paper bridge) adjusted to pH 5.8 before autoclaving at 121°C for 15 minutes. All cultures were maintained at 28 ± 1°C under a 16h light photoperiod at 20-25Wm^{-2} for 4 weeks. The shoots obtained were subcultured three times at 3-week intervals under the same conditions and the media were supplemented with 0.87μM gibberellic acid (GA_3).

To measure root induction and growth, the shoots that had attained 1cm in height were transferred individually into 100ml Erlenmeyer flasks containing 30ml of Murashige and Skoog's (MURASHIGE & SKOOG, 1962) basal medium (MS), supplemented with combinations of 4.92, 14.76μM indole-3-butyric acid (IBA), 0.87μM GA_3, 0.1% or 0.3% activated charcoal. Finally, after 15 days the shoots were transferred to 1/2 MS solid medium, consisted of a half macro salt formulation and 3% (w/v) sucrose and supplemented with 0.1% activated charcoal and without activated charcoal and without growth regulators. The regenerated plantlets were transferred to polyethylene bags containing a mixturer of soil, manure and sawdust (1:1:1) and cultivated under shady conditions. Each experiment was consisted of 20 explants per treatment. Duncan's multiple range test was used to compare treatment means.

RESULTS

The effect of BAP on shoot multiplication are shown in Table 1. After 4 weeks of incubation, shoots were observed on all media in use. The most effective basal medium for shoot multiplication was B5 medium containing 6.66μM BAP which induced a mean of 5.4 shoots per segment. The shoots induced on medium containing higher concentration of BAP were smaller in height than the shoots induced in lower concentration (data not shown). The presence of GA_3 in the subculture media promoted shoot elongation in all treatments (Table 2). The subcultures were less efficient for shoots multiplication than initial culture for all media, decreased gradually until the second subculture, except for medium containing 0.22μM BAP.

The effects of combination off IBA, GA_3 and activated charcoal on plantlet formation are shown in Table 2. Root induction was observed 10 to 15 days after incubation. The media containing 4.92μM IBA were the most effective for root formation. The combination of 14.76μM IBA, 0.87μM GA_3 and 0.3% activated charcoal was the least efficient for root formation. After 5 weeks of culturing the plantlets formed on medium containing 4.92μM IBA, 0.87 GA_3 and 0.1% activated charcoal attained 30mm in height, were more easily rooted and produced more roots per shoot. The root growth was accelerated after the transfer to new medium with half macro salt formation without growth regulators. All rooted plants showed over 95% of establishment efficiency when transplanted to soil.

Table 1 - Effect of 6-benzylaminopurine (BAP) on shoot multiplication from internodal segments of *C. ipecacuanha* after three subcultures.

BAP (μM)	Initial culture		Subculture		
	Mean ± SD number of shoots/internode segment	Range number of shoots	Mean ± SD number of shoots		
			First	Second	Third
0.22	2.3 ± 0.8a*	2 - 5	2.4±0.8a	2.5±0 9a	2.3±0.8a
2.22	2.7 ± 1.0a	2 - 5	2.6±1.0a	2.4±0.8a	2.5±0.9a
6.66	5.4 ± 1.4c	4 - 9	5.2±1.2c	5.0±1.0c	5.3±1.3c
13.22	4.6 ± 1.2b	3 - 8	4.2±1.0b	3.6±1.0b	4.0±1.1b

* Treatments with same letter in each column do not differ among them by the Duncan's multiple range test $P < 0.05$.
SD = Standard Deviation

Table 2 - Effects of combination of indole-butyric acid (IBA) and activated charcoal (AC) on ipecac plantlet formation at end of 5 weeks of culture, supplemented with 0.87μM GA_3.

IBA (μm)	AC (%)	Rooting (%)	Number of root Mean ± SD	Range root lenght (mm)	Height of plantlet (mm)
4.92	0.1	100	15.5 ± 2.4	3 - 13	30
4.92	0.3	80	8.2 ± 1.5	3 - 10	20
14.76	0.1	50	1.6 ± 0.6	2 - 7	30
14.76	0.3	30	1.5 ± 0.4	1 - 3	25

SD = Standard Deviation

DISCUSSION

The higher concentrations of BAP (6.66 and 13.22μM) resulted in greater efficiencies of shoot multiplication than the lower concentrations. It was not observed the inhibition of shoot formation by cytokinin concentration over 2.32μM as reported by YOSHIMATSU & SHIMOMURA (1991). IKEDA et al. (1988) also did not observe this inhibition when 13.94 and 23.23μM BAP or kinetin were used in combination with 0.05μM napthaleneacetic acid (NAA) in nodal segments of ipecac.

It was observed that shoots formed on medium supplemented with lower concentration of BAP in the initial incubation were taller and had larger leaves than the shoots regenerated on higher BAP concentration. These observations were noted under similar conditions by YOSHIMATSU & SHIMOMURA (1991). In this study the level of GA_3 used in the subcultures promoted shoot elongation in all media, which made the establishment of subsequent subculture easier. The level used in this experiment (Tabel 2) is among the concentrations tested for shoot elongation by other researchers (PALEG, 1965; MERTZ, 1966). Occasionally, a low level of GA_3 may be useful since it permits some extension growth (KRIOKORIAN, 1982). The gradual decrease in shoot multiplication observed during subcultures demonstrated that the multiplication capacity of certain species decreased rapidly after several subcultures and the morphogenetic potential may eventually be fully lost (HU & WANG, 1983).

The better efficiency for root regeneration and growth is obtained on low concentration of auxin and the elongation is stimulated when the level of auxin and gibberellin are balanced (MERTZ, 1966). The results obtained with the levels of auxin and gibberellin used in this investigation confirm this efficiencies. The higher concentration of IBA inhibited root formation, although the height of plantlet had not been affected. In the experiment of IKEDA et al. (1988) this inhibition was not observed in concentrations over 5.71μM of indole-3-acetic acid (IAA). However, EVANS (1976) reported that the root formation could be inhibited when shoots were cultivated for long periods under auxin or higher concentrations of growth regulators. The results obtained with the higher concentrations of IBA in this investigation confirm this report.

For plantlet formation the presence *in vitro* of activated charcoal can have inhibitory or stimulatory effects on growth (FRIDBORG et al., 1978). In this study the combination of activated charcoal, IBA and GA_3 in adequate levels stimulated this effect and the root number obtained was more than the obtained by IKEDA et al. (1988).

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