COMUNICAÇÃO

TISSUE CULTURE PROPAGATION OF *Cephaelis ipecacuanha* A.Richard: EFFECT OF GROWTH REGULATORS ON PLANTLET ROOT FORMATION

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ABSTRACT - *Cephaelis ipecacuanha* A.Richard (Rubiaceae) was propagated *"in vitro"* at 28 ± 1°C under 16h light and 8h darkness. Shoots were grown from internodal segments on basic Gamborg medium to a height of 15mm within three weeks. By switching the segments to Murashige-Skoog medium containing indole-3-butyric acid, gibberellic acid, and activated charcoal, root formation was initiated. Conditions for 100% efficiency of root formation were found. Plantlets grew to about 30mm height within five weeks. The plantlets were then transferred to soil, where they established.

INDEX TERMS: *Cephaelis ipecacuanha*, medicinal plant; *"in vitro"*, growth regulator

EFEITO DE REGULADORES DE CRESCIMENTO NO ENRAIZAMENTO DE PLÂNTULAS DE *Cephaelis ipecacuanha* A. Richard EM CULTURA DE TECIDO

RESUMO - *Cephaelis ipecacuanha* A. Richard (Rubiaceae) foi propagada *"in vitro"* a 28 ± 1°C num fotoperiodo de 16 h de luz e 8 h de escuridão. Os brotos foram crescidos de segmento internodal no meio básico de Gamborg a uma altura de 15 mm dentro de 3 semanas. Na transferência para o meio básico de Murashige-Skoog contendo ácido indol butirico, ácido giberelico, e carvão ativado, o enraizamento foram iniciadas. Condições de 100% de enraizamento foi encontrado. Plântulas cresceram por volta de 30 mm de altura dentro de 5 semanas. As plântulas foram então transferidas para o solo, onde se aclimataram.

TERMOS PARA INDEXAÇÃO: *Cephaelis ipecacuanha,* planta medicinal, *"in vitro"*, regulador de crescimento

Cephaelis ipecacuanha A. Richard, ipecac, is an important medicinal plant native to Brazil. The roots of this plant contain emetine and cephaline as active principles and have been used for years as expectorants, emetics, and amoebacides (Gupta, 1971).

Ipecac seeds require at least six monhts for germination. To increase rooting and root growth, propagation methods using root cuttings have been established, but these methods are not very efficient (Gattoni, 1959; Kalyanasundaram, 1969). Also, propagation methods using tissue culture have been developed (Ikeda et al.1988; Yoshimatsu; Shimomura 1991; Lameira; Costa; Pinto, 1994). Our laboratory has started investigations to establish methods with higher efficiencies for plantlet formation of ipecac *"in vitro"*. This paper describes the effect of growth regulators on plantlet formation of ipecac in tissue culture

"In vitro" stock cultures of *Cephaelis ipecacuanha* from micropropagation of internode segments were used as a source for explants. The cells proliferated on a liquid medium containing the macro and micro nutrients and vitamins of Gamborg; Miller; Ojima (1968),

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supplemented with 0.67 μM benzylaminopurine and 2% sucrose. The cultures were maintained in a growth chamber at 28 ± 1° C in 16/8 h light/dark cycles with a light intensity of 25 $\mu mol.m^{-2}.s^{-1}$ irradiance.

The culture medium used for shoot elongation of the rosette-type was B5 mineral salts and vitamins (Gamborg; Miller; Ojima, 1968) supplemented with 0.27, 0.54 or 0.82 μM gibberellic acid. The medium was adjusted to pH 5.8 prior to autoclaving at 108 kpa at 121°C for 15 min. Culture tubes contained 10 ml of the medium.

After five weeks, the elongated shoots over 10 mm in lenght were separated individually and were transferred to 100 ml Erlenmeyer flasks containing 30 ml of Murashige and Skoog (1962) mineral salts and vitamin medium supplemented with 1.48, 4.92, or 14.76 μM indole-3-butyric acid, 0.82 μM gibberellic acid, and 0.1 or 0.3 % activated charcoal. All media contained 3 % (w/v) sucrose were solidified with 0.7 % (w/v) agar. After two weeks on this medium, for rooting the elongated shoots were transferred to half-strength Murashige-Skoog medium without growth regulators and maintained as described above. Data were submitted to analysis of variance and means were compared by Duncan's multiple range test at 0.1 % level. Each experiment was repeated three times with 10 replications per treatment and five shoots per repetition.

For shoot elongation all treatments were efficient. However, the medium containing 0.82 μM gibberellic acid was the most effective. Within three weeks of inoculation, the shoots grew to 15 mm height and showed two leaflets. Crozier (1981) showed that shoot elongation was best with adequate levels of gibberelin (1,0 mg/l). In our study, the levels of gibberelin tested were within the concentrations used for shoot elongation by others (Paleg, 1965; Mertz, 1966). Occasionally, a low level of gibberelin may be useful since it will permit some growth extension (Kriokorian, 1982).

The synergistic effect of indole-3-butyric acid, gibberelin, and activated charcoal in inducing plantlet formation is shown in Table 1. Mertz (1966) reported that root elongation is stimulated when the levels of auxin and gibberelin are balanced. After five weeks of culturing, our results revealed that the best conditions for plantlet formation were 4.92 μM indole-3-butyric acid, 0.82 μM gibberellin, and 0.1 % activated charcoal. Plantlets showed 100% root formation and grew to a height of about 30 mm. All media containing 4.92 μM indole-3-butyric acid were more effective in root formation than media containing 1.48 or 14.76 μM indole-3-butyric acid, although the plantlet height was not affectedby this hormone. It is possible that the root cells are more sensitive to auxin than the stem cells. The inhibition of root formation by high concentrations of auxin was observed in intact or in cut roots (Evans, 1976).

The medium containing only activated charcoal was the least effective for plantlet formation. The presence of activated charcoal can have inhibitory or stimulatory effects on growth *"in vitro"* (Fridborg et al. 1978). For plantlet formation, the inhibitory effect was only observed in media containing this adsorbent alone.

TABLE 1 - Synergistic effect of IBA(indole -3-butyric acid), GA(gibberelin) and activated charcoal (AC) on plantlet formation of ipecac after five weeks in culture.

IBA (μM)	GA (μM)	AC (%)	Rooting (%)	Average number of roots/segments	Average lenght root (mm)	Average height plantlet (mm)
1.48	0.82	0.3	20	1.2 c	5.6 a	25 b
4.92	0.82	0.3	80	3.2 b	5.8 a	20 c
4.92	0.82	-	60	2.1 c	4.8 ab	25 b
4.92	0.82	0.1	100	15.5 a	7.4 a	30 a
14.76	0.82	0.1	50	1.6 c	4.2 ab	30 a
14.76	0.82	0.3	30	1.5 c	2.0 b	25 b
-	-	0.3	10	0.9 c	2.0 b	7 d

Values within each row followed by the same letter are not significantly different (P = 0.01), by Duncan's Multiple Range Test.

However, the combination of activated charcoal with growth regulators is stimulatory and synergistic.

Plantlets removed from culture tubes and maintained seven to nine days in shade conditions, then transferred to natural conditions, survived and grew well (100%). Thus, the method describe here is useful for the fast and efficient propagation of ipecac.

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