BIOLOGIA PLANTARUM 40 (3): 465-468, 1997/98

BRIEF COMMUNICATION

Micropropagation of a medicinal plant, *Plantago major* L.

S. MEDEROS*, C. MARTIN**, E. NAVARRO*** and M.J. AYUSO**

*Facultad de Farmacia, Universidad de La Laguna, 38207, La Laguna, Tenerife, Canary Islands, Spain**
*Facultad de Farmacia, Universidad de Sevilla, 41013, Sevilla, Spain***
*Facultad de Medicina, Universidad de La Laguna, 38203, La Laguna, Tenerife, Canary Islands, Spain****

Abstract

An efficient micropropagation protocol was developed for an important medicinal plant, *Plantago major* L. For this purpose, it is recommended to culture shoot-tips on modified MS medium [412.5 mg dm^{-3} NH_4NO_3 and 340 mg dm^{-3} KH_2PO_4] supplemented with 50 g dm^{-3} glucose and 0.5 μM 6-benzylaminopurine. Maximum rooting frequency was obtained at 1 μM naphthaleneacetic acid.

Additional key words: glucose, growth regulators, *in vitro* plantlets.

Plantago major L. belongs to the family *Plantaginaceae*, which is reputed to be very effective in the treatment of tumors (Martín-Cordero *et al.* 1996). Only limited literature of *Plantago* tissue culture is available (Mederos *et al.* 1991, Mederos 1994, Martín-Cordero *et al.* 1996). So, the aim of the present study was to optimize tissue culture conditions for shoots formation from meristematic tissue.

Plantago major L. plants were grown on natural habitat and voucher specimen are in the Herbarium of the University of La Laguna. Segments of stems were collected and shoot-tips (10 mm) excised from five month-old plants were disinfected with 40 % ethanol (2 min) and 0.5 g dm^{-3} $HgCl_2$ (10 min), and washed three times with sterile distilled water. After surface sterilization, shoot-tips were transplanted to 20 cm^3 of modified Murashige and Skoog (1962) medium [412.5 mg dm^{-3} NH_4NO_3 and 340 mg dm^{-3} KH_2PO_4] (Mederos 1994) containing 100 mg dm^{-3} myo-inositol, 0.4 mg dm^{-3} pyridoxine-HCl, 30 g dm^{-3} sucrose, 8 g dm^{-3} agar (*Sigma Chemical Company*), 0.01 μM GA_3 and 0.01 μM BAP.

Received 22 May 1997, *accepted* 10 August 1997.
Abbreviations: BAP - 6-benzylaminopurine; 2iP - N-isopentenylaminopurine; IBA - indole-3-butyric acid; NAA - naphthaleneacetic acid.
Fax: (+34) 922 630095, e-mail: sdomingu@ull.es

For *in vitro* proliferation of shoots, stem explants of 12 mm in length with apical buds were aseptically cut and cultured in test tubes on 15 cm^{-3} containing 7 g dm^{-3} agar with the same nutrient salt composition as above, but supplemented with glucose and different concentrations of BAP (0.0 to 2.0 μM). Shoot proliferation was scored after 25 d of culture.

Elongated shoots were tranferred to half-strength modified MS medium (Murashige and Skoog 1962) supplemented with 5 g dm^{-3} agar with different concentrations of NAA or IBA. The rooting response was scored after 35 d of culture.

The pH of all nutrient media was adjusted to 5.8 before autoclaving. Explants were incubated at 24 °C with a 16-h photoperiod, the irradiance being 15 μmol m^{-2} s^{-1} provided by cool white fluorescent light tubes.

All data were analysed using Duncan´s new multiple range test.

Table 1. Effect of BAP on the proliferation of shoots from *Plantago major* L. The modified MS medium was supplemented with glucose (5 %) and BAP at the indicated concentration. Results were obtained after 25 d of culture (means of 2 repetitions with 30 explants each; values followed by different letters are significantly different at a 0.01 level of confidence).

BAP [μM]	Explants with shoots [%]	Shoots per explant	Shoot length [mm]
0.0	57	1^{a}	11^{a}
0.1	100	$2^{b,a}$	17^{b}
0.2	100	$4^{c,c}$	28^{c}
0.5	100	7^{d}	55^{d}
1.0	83	$3^{e,c}$	42^{e}
2.0	83	$2^{b,a}$	$30^{f,e}$

In an early attempt to grow axenic *Plantago major* L. plantlets, standard MS culture medium was used, which resulted in poor formation of shoots (less than 30 %); elongation and proliferation of shoots was also inhibited. Reduction of the NH_4NO_3 and KH_2PO_4 content in the culture medium to 412.5 mg dm^{-3} NH_4NO_3 and 340 mg dm^{-3} KH_2PO_4, respectively, induced from 57 % to 100 % explants with

Table 2. Effect of glucose on the proliferation of shoots from *Plantago major* L. explants. The modified MS medium was supplemented with BAP (0.5 mM). Results were obtained after 25 d of culture (mean value are given of two repetitions with 30 explants each; values followed by different letters are significantly different at a 0.01 level of confidence).

Glucose [g dm^{-3}]	Explants with shoots [%]	Shoots per explant	Shoot length [mm]
0	33	1^{a}	10^{a}
30	65	4^{b}	33^{b}
40	100	$3^{c,b}$	49^{c}
50	100	7^{d}	57^{d}
60	87	1^{a}	25^{e}

shoots and also the vigour and elongation of shoots was improved. Addition of BAP promoted shoot formation in a concentration dependent manner (Table 1). At concentration 0.1, 0.2, 1.0 and 2.0 μM rarely more than three shoots per explant was observed and addition to NAA inhibited the number of shoots (data not shown). However, this combination has frequently been found favourable for proliferation of shoots of the same and other species (Mederos *et al.* 1994, Mederos and Schöbert 1995, Mederos *et al.* 1996). Thus, 0.5 μM BAP in the culture medium was best: about 7 shoots were formed, which elongated to about 55 mm (Table 1). An increase in the glucose content in the medium to 50 g dm^{-3} stimulated shoot formation, shoot elongation and chlorosis was not observed (Table 2). It is known that glucose added into the culture medium not only stimulated shoot formation, but also shoot elongation in other species (George and Sherrington 1984, Mukherjee *et al.* 1991).

Table 3. Effect of NAA and IBA on the *in vitro* rooting of elongated shoots from *Plantago major* L. The modified MS medium was supplemented with glucose (50 g dm^{-3}). Results were obtained after 35 d of culture (mean value are given of two repetitions with 24 explants each; values followed by different letters are significantly different at a 0.01 level of confidence).

NAA [μM]	IBA [μM]	Shoots with roots [%]	Roots per shoot	Root length [mm]
0.0	0.0	0	0^{a}	0^{a}
0.5	0.0	100	22^{b}	22^{b}
1.0	0.0	100	28^{c}	39^{c}
10.0	0.0	74	10^{d}	34^{d}
100.0	0.0	75	8^{e}	30^{e}
0.0	0.5	100	$12^{f,i}$	$33^{f,d}$
0.0	1.0	100	$14^{g,f}$	28^{g}
0.0	10.0	63	$16^{h,d}$	$31^{h,e}$
0.0	100.0	75	$11^{i,d}$	$23^{i,b}$

Elongated shoots readily rooted in the presence of NAA or IBA; a maximum rooting frequencies were obtained at NAA concentrations of 0.5 and 1 μM (Table 3). Further investigations studying the cytotoxic activity are in progress.

References

George, E.F., Sherrington, P.D.: Plant Propagation by Tissue Culture. - Exegetics Ltd., Eversley - Basingstoke 1984.

Martín-Cordero, C., Mederos, S., Navarro, E., Ayuso, M. J.: Evaluation of *Plantago in situ* and *in vitro* extracts for cytotoxic activity. - Exp. clinic. Pharmacol. **18** (B): 144, 1996.

Mederos Molina, S.: [*In vitro* cultivation of stem apexes of *Plantago major* L.] - In: Proceedings das II Jornadas Ibéricas de Plantas Medicinais, Aromáticas e Oleos Essenciais. Vol. I. Pp. 107-110. Portugal 1994. [In Span.]

Mederos Molina, S., Schöbert, C.: Micropropagation of *Ricinus communis*. - J. Plant Physiol. **147**: 270-272. 1995.

Mederos, S., López-Bazzocchi, I., Ravelo, A., González, A.: Hypericin from clonal propagation of *Hypericum canariensis* L. - Plant Tissue Cult. **6**: 7-13, 1996.

Mederos, S., Luis, J.G., San Andrés, L.: [*In vitro* cultivation of *Salvia canariensis* L.] - Proceedings das II Jornadas Ibéricas de Plantas Medicinais, Aromáticas e Oleos Essenciais. Vol. I. Pp. 282-286. Portugal 1994. [In Span.]

Mederos, S., Méndez, M.: Tissue culture studies on *Plantago major* L.: Use of shoot-tip explants. *In vitro* plant. - *In vitro* cell. develop. Biol. **27** (3, part 2): 386, 1991.

Mukherjee, S., Rathinasabapathi, B., Narendra, G.: Low sugar and osmotic requirements for shoot regeneration from leaf pieces of *Solanum melongena* L. - Plant Cell Tissue Organ Cult. **25**: 13-16, 1991.

Murashige, T., Skoog, F.: A revised medium for rapid growth and bioassays with tobacco tissue cultures. - Physiol. Plant. **15**: 473-497, 1972.