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Development of an *in vitro* culture of *Larrea divaricata* for biomass production

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INTRODUCTION

Larrea is an important genus widely distributed in the New World, including five well-known species, which are dominant bushes in extensive desertic areas. A native species to Argentina, *Larrea divaricata,* contains nordihydroguaiaretic acid (NDGA) together with other lignans and flavonoids in the resinous leaf exudates (Mabry and Bohnstedt, 1979). The main biological activities of these compounds are the following: antioxidant activity (Sinnott *et al.,* 1998), enzymatic inhibition (Whitman *et al.* 2002), antiviral properties (Larreastat, 2000; Konigheim *et al.,* 2005) as well as anticancer activity (Anesini *et al.,* 1999).

In vitro culture for obtaining active principles offers a possible alternative when thinking about the production of a secondary metabolite. The aim of the present work has been the optimization of the *in vitro* culture conditions for callus induction of *L. divaricata* (*Zigophyllaceae*) as a potential production source of the metabolites with pharmaceutical importance.

METHODOLOGY

Leaves from wild plants of *Larrea divaricata* of the Department of Punilla, province of Córdoba, Argentina, were used as explants for callus induction. Leaves were surface sterilized by immersion in tap water for 1 hour, 1% copper sulfate for 15 min, 70% ethanol for 10 min, and commercial 30% sodium hypochlorite for 15 min (1.5% active chlorine) with the addition of Tween 20, and rinsed several times with sterile distilled water. Thereafter, sterile explants were cultured in a culture basal medium of Murashige and Skoog (1962) (MS), supplemented with sucrose 30 g/L, agar 8 g/L, and adjusted to pH 5.6. Culture media were sterilized in autoclave at 121 °C and 1.05 atm for 15 minutes. Incubation was carried out under controlled conditions of temperature (25 ± 2°C) with a 16 h photoperiod (4.35 $W.m^2$). Treatments consisted on variations of the concentration and combination of growth regulators (Table l). Growth parameters analysed were fresh weight and dry (lyophillized) weight.

There were five replications for each treatment (total 36) in a randomized statistical design with an analysis of variance (ANOVA). Differences between mean values were analysed by the DGC test with a significance level of 5%.

RESULTS

Treatments 1, 2, 5, 6, 9, 11, 12, 13, 14, 18, 19, 20, 21, 22, 23, 24, 25, 26, and 27 were unable to induce callus formation.

Table 1. Combination of growth regulators used for callus induction of *Larrea divaricata*.

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Treatment	Growth regulators	
1	Pi 0.1 mg/L	BAP 0.1 mg/L
2		BAP 1 mg/L
3		BAP 2 mg/L
4	Pi 1 mg/L	BAP 0.1 mg/L
5		BAP 1 mg/L
6		BAP 2 mg/L
7	Pi 2 mg/L	BAP 0.1 mg/L
8		BAP 1 mg/L
9		BAP 2 mg/L
10	2,4 D 0.1mg/L	BAP 0.1 mg/L
11		BAP 1 mg/L
12		BAP 2 mg/L
13	2,4 D 1 mg/L	BAP 0.1 mg/L
14		BAP 1 mg/L
15		BAP 2 mg/L
16	2,4 D 2 mg/L	BAP 0.1 mg/L
17		BAP 1 mg/L
18		BAP 2 mg/L
19		Kin 0.1 mg/L
20		Kin 1 mg/L
21	Pi 0.1 mg/L	Kin 2 mg/L
22	Pi 1 mg/L	Kin 0.1 mg/L
23		Kin 1 mg/L
24		Kin 2 mg/L
25	Pi 2 mg/L	Kin 0.1 mg/L
26		Kin 1 mg/L
27		Kin 2 mg/L
28	2,4 D 0.1mg/L	Kin 0.1 mg/L
29		Kin 1 mg/L
30		Kin 2 mg/L
31	2,4 D 1mg/L	Kin 0.1 mg/L
32		Kin 1 mg/L
33		Kin 2 mg/L
34	2,4 D 0.1mg/L	Kin 0.1 mg/L
35		Kin 1 mg/L
36		Kin 2 mg/L

Pi: Picloran, 2,4-D: Phenoxyacetic acid; BAP: Benzylaminopurine, Kin: Kinetine.

Results of fresh and dry weight for the treatments which produced callus after three months growth are shown in Table 2.

Treatments showed significant differences to each other in relation with the growth parameter, fresh weight within the three months test, best

Table 2. Effect of different combinations and concentrations of growth regulators in the biomass induction and production of *L. divaricata* after three months growth

Treatment	Fresh weight	Dry weight
3	0.67 b	0.06 a
4	0.74 b	0.07 a
7	1.89 d	0.11 a
8	0.76 b	0.04 a
11	1.40 c	0.08 a
15	0.32 a	0.03 a
16	0.59 b	0.07 a
17	3.23 e	0.34 b
28	0.39 a	0.02 a
29	0.41 a	0.03 a
30	0.29 a	0.04 a
31	0.88 b	0.21 a
32	1.29 c	0.08 a
33	1.82 d	0.10 a
34	1.66 d	0.29 b
35	1.56 d	0.15 a
36	1.38 c	0.07 a

Different letters in the same column indicate significant differences ($p \leq 0,05$).

results being achieved at the third month for treatments 7 (Pi 2 mg/L: BAP 0.1 mg/L) and 17 (2,4-D 2 mg/L: BAP 1 mg/L). Concerning dry weight the best results were achieved for treatments 34 (2,4-D 0.1 mg/L: Kin 0.1 mg/L) and 17 (2,4D 2 mg/L: BAP 1 mg/L).

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CONCLUSIONS

According to the results, the most appropriate culture medium for development of *L. divaricata* callus taking into account fresh and dry weight was that supplemented with 2,4-D 2mg/L: BAP 1 mg/L, corresponding to treatment 17.

The selection of a suitable medium for biomass production was absolutely necessary for the continuation of the studies on *in vitro* culture of the plant.

Note: This study was presented at the 'I Reunión de Biotecnología aplicada a plantas medicinales y aromáticas' (First Biotechnology Meeting on Medicinal and Aromatic Plants), Córdoba, Argentina, 2006.

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