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In vitro culture of *Peumus boldus* Molina via direct organogenesis

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ABSTRACT

Boldo (*Peumus boldus* Molina) is an endemic plant from Chile. Its leaves have been traditionally used to regulate liver function, due to the presence of the aporphine alkaloid boldine. In order to propagate boldo *in vitro*, 9 hormonal treatments were used, consisted in the combination of three concentrations of benzylaminopurine (BAP) and three of indole butyric acid (IBA). The results show that the effect of the indole butyric acid hormone is significant and that both the interaction and the presence of the hormone benzylaminopurine are not significant in relation to the organogenic responses of number of leaves and buds. The best conditions for the response in terms of number of buds were in the treatments where 0.1 mg/L of indole butyric acid was used. The effect of the hormone benzylaminopurine was not significant. In conclusion, the best conditions for the response in terms of number of leaves were in a treatment where 0.1 mg/L of indole butyric acid and 0.5 mg/L of benzylaminopurine were used.

Keywords: boldo, micropopagation, benzylaminopurine, indol butyric acid.

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Introduction

Boldo (*Peumus boldus* Molina) is an endemic plant from Chile. Its leaves have been traditionally used to regulate liver function, as well as for their hepato-protector, cholagogue, antispasmodic, sedative and antioxidant properties, due to the presence of the aporphine alkaloid boldine (Muñoz *et al.* 2001). Its propagation from seeds is made difficult due to an imbalance of the gibberellic and abscisic acid promoter-inhibitor system and reaches a 13% germination rate a year after sowing (Vogel *et al*., 2008).

The demand for exported dry leaves reached US$1,700,000 in Chile towards the year 2007, the main destinations being Argentina, Brazil, Paraguay and France (INFOR-CORFO, 2008). Given the above mentioned, the study of alternatives to help satisfy the demand shows that *in vitro* culture is an attractive option given that the propagated tissues do not present pathogens, do not require the participation of the plant's reproductive organs and that the growth factors are controlled (Simon *et al*., 2001).

Experimental

Nodal segments of *Peumus boldus* Mol., were obtained from mother plants cultivated in Las Tres Pascualas campus of Universidad San Sebastián, Concepción, Chile.

A protocol of sterilization kept under continuous shaking (150 rpm) was then applied as follows: wash with distilled water and 3 drops of commercial liquid soap for 10 min., rinse with distilled water for 10 min., polyvinyl pyrrolidone 2g/L- sucrose 20g/L for 60 min., captan 1.9g/L-benomyl 3 g/L for 120 min. Then under a laminar flow hood, in sterile conditions and under continuous shaking (150 rpm), 5% commercial chlorine was applied with 3 drops of commercial liquid soap for 15 min., citric acid 0.3g/L-ascorbic acid 0,3 g/L for 2 min. and finally cefotaxime 1g/L for 60 min. The tissues were cultivated in a Murashige & Skoog medium (1962). The macronutrients were reduced in a quarter and supplemented with sucrose 30g/L, agar 8g/L, with a pH adjusted to 5.8. The culture media were sterilized in an autoclave at 121 °C and 1 atm for 15 min. The incubation was carried out at 23± 2ºC with a 16 hour photoperiod (3,000 lux). The 9 treatments consisted in the combination of three concentrations of benzylaminopurine (BAP) and three of indole butyric acid (IBA), generating a 3x3 factorial design as shown in Table 1. The growth parameters analyzed were the number of leaves and the number of buds.

Table 1. Combination of growth regulators for the induction of direct organogenesis in *Peumus boldus* Molina

Treatments	Growth regulators	
	IBA (mg/L)	BAP (mg/L)
1	0	0
2		0.5
3		1.0
4	0.05	0
5		0.5
6		1.0
7	0.1	0
8		0.5
9		1.0

Results

The propagation response, in terms of the mean number of leaves and the mean number of buds after 28 days of incubation is shown in Table 2 for each of the treatments evaluated.

The results show that the effect of the indole butyric acid hormone is significant as shown in Table 3, and that both the interaction and the presence of the hormone benzylaminopurine are not significant in relation to the organogenic responses of number of leaves and buds.

In Table 3: *p*: probability value, S: significant, I: interaction between BAP and IBA.

Table 2. Effect of different combinations of growth regulators on the organogenic response in *Peumus boldus* Molina after 28 days of incubation

Treatment	Organogenic response	
	Nº buds	Nº leaves
1	1.89 a	0.89 a
2	1.93 a	1.14 a
3	1.53 a	0.53 a
4	2.10 a	3.10 b
5	2.50 a	3.20 b
6	2.11 a	1.67 a
7	2.15 a	1.31 a
8	4.00 b	3.91 d
9	4.00 b	2.06 c

Different letters in the same column indicate significant differences ($p \leq 0.05$)

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Table 3. ANOVA (95% confidence interval) in the organogenic responses in *Peumus boldus* Molina after 28 days of incubation

	Nº buds		Nº leaves	
	p	S	*p*	S
I	0.0821	No	0.5030	No
BAP	0.0927	No	0.1385	No
IBA	$p<0.0001$	Yes	0.0198	Yes

The number of repetitions for each treatment was: 9, 14, 15, 10, 10, 9, 13, 11 and 17 for treatments 1 to 9, respectively. The results were evaluated by means of a two-way analysis of variance (ANOVA) with a Bonferroni post test for 95% confidence intervals.

Conclusions

The best conditions for the response in terms of number of buds were in the treatments T8 and T9 where 0.1 mg/L of indole butyric acid was used. The effect of the hormone benzylaminopurine was not significant.

The best conditions for the response in terms of number of leaves were in treatment T8 where 0.1 mg/L of indole butyric acid and 0.5 mg/L of benzylaminopurine were used. The effect of the latter was not significant.

Note: Part of this study was presented at the 'II Reunión de Biotecnología aplicada a plantas medicinales y aromáticas' (Second Biotechnology Meeting on Medicinal and Aromatic Plants), Córdoba, Argentina, 2009.

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