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In vitro Callus Induction and Plants from Stem and Petiole Explants of *Salvia canariensis* L.

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Abstract

Stem and petiole explant of *Salvia canariensis* L. were cultured on MS and B5 culture media supplemented with BAP, NAA, IBA. Callus formation on the surface of stems and petioles and also high and low concentrations of NAA alone and combined with BAP, stimulated the callus induction and direct shoot formation, respectively. However, concentrations of IBA used here, inhibited shoot induction. The shoots developed from both explants formed roots when transferred to half strength MS medium supplemented with lAA, NAA or IBA.

Introduction

Salvia canariensis L. is a shrub species endemic to the Canary Islands, Spain, and belongs to the Lamiaceae family. This valuable medicinal and ornamental plant is widely distributed in Canary Islands. *S. canariensis* is a perennial bush up to 2.0 m in height, it is abundantly branched in a disorderly fashion. Its older branches are woody, very rough and brittle, while the younger ones are soft and covered by a fine, soft fuzz. The leaves are simple and opposite, thick and petiolated lengthwise. It flowers in spring and winter. It is reproduced from seeds as well as from cuttings (Bramwell and Bramwell 2001). *S. canariensis* plants are generally propagated through cuttings and seeds. But high percentage of *S. canariensis* seeds usually lose their viability within 9 to 12 months from the date of their maturity (Personal communication). Therefore, several studies have centered on callus induction and organogenesis from different explants and embryos cultures of *S. canariensis* and *S. officinalis* (Mederos Molina, unplubished data). Several papers about *in vitro* tissue culture of this species have been published (Mederos 1991, Mederos-Molina et al. 1994, 1997). Micropropagation of *S. canariensis* is possible from juvenile

and mature shoots (Mederos-Molina 1991, Mederos-Molina et al. 1997) or very young seedlings are used for this purpose (Mederos-Molina, unpublished data). Due to the limited plant material of this species in its natural habitat, efforts have been directed to micropropagate this medicinal plant through *in vitro* culture with a view to producing secondary metabolites (Lucia et al. 1994). A reliable method for *S. canariensis* micropropagation will have considerable benefits (Mederos-Molina et al. 1997, Mederos-Molina 2002). Thus the potential of this species may be economically exploited as ornamental and medicinal shrubs. For this reason, Plant regeneration of *S. canariensis* from different explants are in progress. The aim of the present study was to determine for the first time, suitable culture media and growth regulators on callus induction and direct organogenesis.

Materials and Methods

The plant material of *Salvia canariensis* L. was collected in Gran Canaria, Canary Islands, Spain from the annual sprouts before the onset of flowering. The juvenile branches (two - five weeks old) were surface sterilized with 70 % (v/v) ethanol for 5 min followed by immersing them in a 0.05 % (w/v) $HgCl_2$ solution for 15 and 25 min, respectively. After this treatment, the short branches were rinsed five times with sterile distilled water. Petioles were separeted from the stems and were cut into 7 and 13 mm long pieces. They were placed in MS or B5 medium supplemented with 6% agar (Difco Bacto), 4% sucrose and various concentrations of plant growth regulators (Tables 1 and 2). Shoots formed directly from the petioles and stems. Shoots were transferred on half strength MS for *in vitro* rooting (Table 3). The pH of the culture medium was adjusted to 5.7 after adding agar. The cultures were maintained under a photoperiod of 16 h light and 8 h darkness and at 24 ± 2°C. The photosynthetic photon flux densities (PPFD) was 33 µmol m^{-2} s^{-1}. Light was provided by gro-Iux lamps (Sylvania, 20 W) or by 1 cool white fluorescent tube (GE, 80 W) and also in the dark.

The experiments were repeated only once. Results collected were subjected to analysis of variance. Least significant difference ($p < 0.05$) was used to compare the means.

Results and Discussion

Callus induction was observed on the cut surfaces of stems and petioles within two to three weeks. Horizontally placed explants produced callus or direct shoot more rapidly than the explant which were placed vertically (data not

shown). Frequency of callus formation is shown in Table 1. No callus was induced when explants were cultured on control MS and B5 media or supplemented with a low concentration of BAP. However, NAA alone or in combination with BAP was very effective in inducing callus in MS or B5. Eighty to 100% callus induction from stem or petiole was observed at high concentrations of BAP (8.88 µM) and NAA (21.48 µM). Significant differences were observed between the two basal media in callus induction ($p < 0.05$) (Table 1). Callus was induced more frequently from the petiole than the stem explants in MS medium. Furthermore, callus grown in MS and B5 media cotanining BAP and NAA was green and had a more compact texture. Similarly, callus grown on

Table 1. Frequency callus induction on stem (St) and petiole (Pet) explants of *Salvia canariensis*. Data were collected 45 days after culture.

BAP (µM)	NAA (µM)	IBA (µM)	% callus formation			
			MS		B5	
			St	Pet	St	Pet
0	0	0	0	0	0	0
4.44	0	0	0	0	0	0
8.88	0	0	16	28	8	16
0	10.74	0	72	80	60	62
0	21.48	0	76	80	62	68
0	0	9.8	72	76	58	62
0	0	19.6	70	76	58	64
4.44	10.74	0	84*	88*	64	68
4.44	21.48	0	88	92	76	76
8.88	10.74	0	92	96	68	76
8.88	21.48	0	96	100	80	80
4.44	0	9.8	64	68	62	62
4.44	0	19.6	72	76	64	64
8.88	0	9.8	68	76	60	68
8.88	0	19.6	68	76	64	64

IBA alone or in combination with BAP was invariably green but less friable than callus from the rest of the treatments. Moreover, induction of callus on media with IBA retained a rapid growth rate after 12 months (data not shown). On the other hand, direct shoot formation from stem and petioles was observed in MS supplemented with low concentration of BAP in combination with NAA (Tables 1 and 2). Good result was obtained from petiole explants in MS medium. The significant effect on the results obtained in MS and B5 media

may be due to the difference of salt concentrations. B5 medium nitrate was required in a low concentration. On the other hand, an addition of 2 mM ammonium sulphate led to an decrease in callus induction. Moreover, NH_4^+ added as the sole source of nitrogen did not support shoot formation. Similar results were reported when NO_3NH_4 was substituted for $(NH_4)_2SO_4$ (Mederos, unpublished data). MS is a very rich saline medium specially the macro elements in full concentrations, also stimulated the growth of the calli. Callus from the most successful culture media was subcultured up to five years at 33 - 35 days intervals and they reatined a rapid growth rate. The present study suggests that MS medium is suitable for callus initiation and its growth, although comparison between MS and B5 on *Salvia* explants has been reported before. Shoots were first observed five weeks after culture on the stems or on the

Table 2. Direct shoots formation frequency on stem and petiole explants of *S. canariensis*. Explants were cultured on MS supplemented with BAP and NAA. Data were collected 45 days after culture.

		Stem			Petiole		
BAP	NAA	%	Length (mm)	Observation	%	Length (mm)	Observation
4.44	2.69	28	17 (0.23)	Green	48	23 (0.19)	Green
4.44	10.71	16	15 (0.33)	Green	32	19 (0.21)	Green
8.88	2.69	8	10 (0.17)	Green-narrow	24	14 (0.17)	Green-yellow
8.88	10.71	4	7 (0.19)	Narrow	16	11(0.14)	Green-narrow

Table 3. Effect of half strength MS basal mediun supplemented with auxin combined with 4.44 µM BAP on *in vitro* rooting of adventitious shoots of *S. canariensis* L.

IAA*	NAA*	IBA*	% of shoots rooting	**Mean No. of root/shoot
0	0	0	8.33	1.0^{a} (0.00)
2.85	0	0	33.33	2.9^{b} (0.30)
5.71	0	0	41.67	$3.3^{c,b,d}$ (0,32)
0	2.69	0	50.00	$4.7^{g,f}$ (0,37)
0	5.37	0	58.33	$4.4^{t,g}$ (0,41)
0	0	2,46	75.00	$3.4^{d,c,e}$ (0.25)
0	0	4,92	66.67	$3.6^{e,d}$ (0.31)

*12 shoots were used per hormonal treatment. **Mean with the same letter in a column are not significantly different ($p < 0.05$). Numbers in parenthesis are ± standard error.

petioles. Table 2 shows the results that induced direct organogenesis of shoots from stem and petiole explants of *Salvia canariensis* L. There were considerable

differences between explant types and between culture media and hormonal treatments in the formation of callus (Table 1) and also, about direct organogenesis of shoots (Table 2). It was found that stem node explants of *S. canariensis* gave rise to a mass of friable callus, which interfered with the process of direct shoot formation if placed straightway onto a MS or B5 medium containing cytokinin and auxin (data not shown). The remaining calli cultured on MS medium also formed shoots and at present these experiments are in progress. On the other hand, when shoots were separately subcultured on MS containing 4.44 μM BAP combined with low concentation of NAA (2.7 μM), they formed multiple shoots. These results confirm the earlier reports (Hermann 1999, 2002, 2004; Mederos-Molina 2000, Srivastava et al. 2004). Root formation was obtained by transplanting shoots to half strength MS medium supplemented with IAA, NAA or IDA (Table 3). Preliminary experiments have shown that *in vitro* root growth and development were accelerated by the reduced MS salt concentration in the presence of auxin. These results are in agreement with those obtained in other woody plants. For instance, *in vitro* root formation was achieved by reduced MS macro- and micronutrient salts concentrations supplemented with auxin by Mederos-Molina (2003). Some differences, however, exist between these and earlier reports because, in this study shoots regenerated from explants of mature plants, whereas many other authors obtained direct organogenesis of shoots from seedling explants (Hermann 1999, 2002; Mederos-Molina 2003). Finally, the study on callus induction and/or direct shoot formation from different explants (shoot, stem and petiole) of *S. canariensis* offers the possibility to select different clones for further investigation on physiological, chemical and/or pharmaceutical aspects.

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