IN VITRO MASS PROPAGATION OF *SALVIA CANARIENSIS* BY AXILLARY SHOOTS

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ABSTRACT

During the establishment of shoots of *Salvia canariensis* L., five environmental factor treatments were applied. For each axillary node two shoots grew well when explants were incubated at continued light for 15 days followed by 16 hrs photoperiod by 30 days. Shoots multiplication was improved on a modified Murashige and Skoog (MS) (1962) medium – MS + 825 mg/l NH_4NO_3 – supplemented with 10^{-7} M BA and 10^{-7} M NAA. The shoots produced well developed root systems within three weeks after transfer to the same culture medium supplemented with 5×10^{-7} M NAA.

Key words: axillary shoots, micropropagation, medicinal plant, *Salvia canariensis* L.

INTRODUCTION

Published research on *in vitro* culture of *Salvia canariensis* L. is limited (Mederos 1991, Mederos et al. 1994). This species is pharmacologically important plants and for this reason we intended to propagate. The purpose of this study was to develop an rapid *in vitro* propagation system of *S. canariensis* L. This paper describes an *in vitro* method which results in large numbers of axillary shoots providing material suitable for research secondary metabolites of this species (Luis J.G. et al. 1992, Lucía San Andrés et al. 1994).

MATERIALS AND METHODS

Plant material and sterilization

Juvenile branches (three month-old) were collected from mature 13 years-old plants gathered at Gáldar, Gran Canaria (voucher specimen on file in the Herbarium of the University of La Laguna No. 25252) and used for this study. Explants were excised and immersed for 4 min in 70% (v/v) ethanol and then were sterilized by immersing for 10 min in 1 g l⁻¹ benomyl – benlate – and for 30 min in a solution of 40 g l⁻¹ $\text{Ca}(\text{ClO})_2$ followed by four successive rinses in sterile desionized water. Cultures were then initiated from axillary stems segments 15 mm long.

Establishment of shoots

Explants were cultured on modified Quoirin and Lepoivre macroelement solution – QL. 4 medium + 900 mg l⁻¹ KNO_3 –

(Mederos et al. 1991, 1994) supplemented with Fe-EDTA and the microelement formula described by Murashige and Skoog (1962) plus 1 mg l⁻¹ thiamine-HCl and pyridoxine-HCl respectively, 100 mg l⁻¹ myo-inositol, 30 g l⁻¹ sucrose and 10^{-8} M BA plus 10^{-8} M NAA. Each tube (150x25 mm) contained one explant with two axillary buds by node and all cultures were incubated at 24 °C. The pH in the culture medium was adjusted to 5.8 ± 0.3 prior to autoclaving and solidified with 6 g l⁻¹ agar (Sigma Chemical Co.). With the objective to stimulating two axillary buds by explant this culture medium was added without or with 10^{-7} or 2×10^{-7} M GA_3 , and different experiments were performed:

- Explants incubation at continued light for 45 days.
- Explants incubation at continued light for 15 days followed by 12 hrs photoperiod by 30 days.
- Explants incubation at continued light for 15 days followed by 16 hrs photoperiod by 30 days.
- Explants incubation at 12 hrs photoperiod by 45 days.
- Explants incubation at 16 hrs photoperiod by 45 days.

For these four experiments illuminations was supplied by cool white fluorescent tubes with a light intensity of $25 \mu\text{E m}^{-2} \text{s}^{-1}$. Forty eight explants were used for each treatment.

Mass propagation of axillary shoots

Basal part of forty eight shoots with two axillary node and 20 mm in length, were cultured on a modified Murashige and Skoog medium (MS) (1962) – MS + 825 mg/l NH_4NO_3 – (Mederos 1991) supplemented with 40 g l⁻¹ sucrose, 10^{-7} M GA_3 and combinations of 10^{-7} M NAA with BA or kinetin at

TABLE 1. Effect of GA₃ and environmental factors on shoot development of *S. canariensis* in a modified Quoirin and Lepoivre medium (QL 4) after forty five days of culture. Forty eight explants were used for each treatment. 1 = Explants incubation at continued light for 15 days followed by 16 hrs photoperiod. 2 = Explants incubation at 16 hrs photoperiod by 45 day.

GA ₃ [M]	% of explants developing two axillary shoots per node		Mean lenght of shoots [mm]	
	1	2	1	2
0	29.2	14.6	15 ^a ± 0.4	10 ^a ± 0.3
10 ⁻⁷	83.3	31.2	54 ^c ± 0.3	34 ^c ± 0.5
2x10 ⁻⁷	79.2	25.0	50 ^b ± 0.5	28 ^b ± 0.5

Each value represents the mean of data from parameter studied with standar error. Means in columns followed by the same letter are not significantly different at 0.01 level of confidence (Duncans Test).

10⁻⁷ and 10⁻⁶ M concentrations. The pH in the culture medium was adjusted to 5.8 ± 0.3 prior to autoclaving and solidified with 6 g l⁻¹ agar (Sigma Chemical Co.). Shoots were incubated at continued lighth for 15 days followed by 16 hrs photoperiod with a light intensity of 25 µE m⁻² s⁻¹. The shoots were subcultured every three weeks.

Rooting of axillary shoots

The rooting medium differed of the medium used in the stage before only as regards the growth regulator NAA was added (between 10⁻⁶ and 10⁻⁸ M) and BA, kinetin and GA₃ was eliminated. Each treatment consisted of forty eight shoots which were incubated for the first seven days at 24°C in total darkness and later incubated a 16 hrs photoperiod under low intensity of lighth (13 µE m⁻² s⁻¹) for 53 days.

All culture media were adjusted to pH 5.7 with 1 N NaOH prior the addition of 0.6% Difco-Bacto agar and then autoclaved for 20 min at 121°C. The experiments were repeated three times.

RESULTS AND DISCUSSION

Plant material and sterilization

Surface sterilization of explants was effective and 100% of all explants cultured remained uncontaminated during the course of the experiment.

Establishment of shoots

The explant cultured on GA₃ at continued light for 15 days followed by 16 hrs photoperiod by 30 days or at 16 hrs photoperiod by 45 days, stimulated the development of two axillary shoots by node and significantly difference (P = 0.01) was observed between environmental factors treatments (Table 1). These results proved significant difference at 0.01 level with the rest of the environmental factor treatments because treatments a, b and d, caused inhibition on shoot development (between 65% and 80%). The effect of giberellic acid on shoots growth and development was also observed by other workers (Kara, 1995; Islam et al. 1994). Photoperiods appears to be an important factor modulating shoot growth and elongation of *Salvia canariensis* L. cultured *in vitro*. This result agrees with that Morini et al. (1991) and Wang (1992) who observed that control of growth in species rootstocks are partially under photoperiod regulation. Recently we observed that the 8 hrs photoperiod can not be used to induced the growth and development of shoots of *S. canariensis* because gave a much lower rate (1.0) of these by explant and this behaviour has also been observed in the plum rootstock (Morini et al. 1991).

Mass propagation of axillary shoots

Preliminary study with casein hydrolysate (between 75 and 300 mg l⁻¹) did not improve the axillary shoots propagation of *S. canariensis*. Also, Roy et al. (1994) observed that the

TABLE 2. Effect of cytokinins and auxin on shoot development and shoot proliferation from nodal bud explants of *S. canariensis* after 45 days of culture. Forty eight explants were used for each treatment.

Hormones [M]			% of explants developing shoots	No. of shoots per explant	Lenght of shoots [mm]
BA	Kin	NAA			
0	0	0	28.0	2.8 ^a ± 0.5	10 ^a ± 0.5
10 ⁻⁷	0	0	64.0	9.0 ^g ± 0.5	21 ^d ± 0.4
10 ⁻⁶	0	0	56.0	8.0 ^{f, g} ± 0.3	21 ^d ± 0.4
0	10 ⁻⁷	0	33.3	3.6 ^b ± 0.5	15 ^{c, b} ± 0.6
0	10 ⁻⁶	0	44.5	4.7 ^{c, b} ± 0.6	13 ^b ± 0.5
10 ⁻⁷	0	10 ⁻⁷	95.0	25.0 ⁱ ± 0.3	55 ^g ± 0.3
10 ⁻⁶	0	10 ⁻⁷	83.3	19.0 ^h ± 0.5	58 ^{h, g} ± 0.4
0	10 ⁻⁷	10 ⁻⁷	38.9	5.0 ^{d, c} ± 0.7	25 ^{c, d} ± 0.6
0	10 ⁻⁶	10 ⁻⁷	50.0	6.1 ^{e, d} ± 0.5	28 ^f ± 0.7

Each value represents the mean of data from parameter studied with standar error. Means in columns followed by the same letter are not significantly different at 0.01 level of confidence (Duncans Test).

Figure 1. Shoot of *Salvia canariensis* L. rooted on a modified Murashige and Skoog medium containing $825 \text{ mg l}^{-1} \text{ NH}_4\text{NO}_3$ and $5 \times 10^{-7} \text{ M}$ NAA after three weeks in culture.

effect of this organic nitrogen compound was not found to be satisfactory for shoots multiplication. On the other hand, from preliminary experiments with plant growth regulators, when concentrations between 10^{-7} M and 10^{-5} M of 2-iP and zeatin were added to the modified Murashige and Skoog medium – $\text{MS} + 825 \text{ mg l}^{-1} \text{ NH}_4\text{NO}_3$ – (Mederos 1991) only a few percentage of explants responded (7 and 13% respectively). The behaviour of multiple axillary shoots depended on the plant growth regulators combination added to this culture medium (table 2). From this table it is clear that the best percentage of explants developing shoots, number of shoots per explant and length of shoots were achieved with combination of 10^{-7} M BA and 10^{-7} M NAA. The highest concentration of BA (between 2×10^{-6} and 10^{-5} M) inhibited one of the two bud per node whereas the highest NAA concentration (from 2×10^{-7} to 10^{-6} M) inhibited both buds development but stimulated callus formation (data not included in Table 2). In another plant study, Roy et al. (1994) showed that BA or Kinetin alone inhibited the induction of shoots. Moreover, Islam et al. (1994) achieved that cytokinin (BAP or Kinetin) alone also stimulated on shoot development from mature plant species but the percentage of response and number of shoots per explant were significantly low.

Rooting of axillary shoots

Roots development was induced by separating the multiple shoots and growing them on a modified Murashige and

Skoog medium – $\text{MS} + 825 \text{ mg l}^{-1} \text{ NH}_4\text{NO}_3$ – (Mederos 1991) supplemented with 2×10^{-7} or $5 \times 10^{-7} \text{ M}$ of NAA. An average of 22 and 33 roots respectively (63 and 94 mm mean roots length) per shoot was produced after three weeks of culture (Figure 1). Multiple roots formation required the presence of NAA in the culture medium, which is in agreement with previous findings in other medicinal species (Herman 1993).

The present study provides a method that ensures a multiple shoots induction from axillary bud explants of mature plant of *Salvia canariensis* L. In our acknowledgement, no reference to *in vitro* mass propagation of *Salvia* species is to be found in the literature.

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MASOWE ROZMNAŻANIE *IN VITRO* *SALVIA CANARIENSIS* Z PĘDÓW PACHWINOWYCH

STRESZCZENIE

W okresie tworzenia się pędów u *Salvia canariensis* L. zastosowano pięć zabiegów czynnikami środowiskowymi. W każdym węźle pachwinowym dwa pędy rozwijały się dobrze jeśli przeszczepy inkubowane były przy ciągłym świetle przez okres 15 dni, po których następował 16 godzinny czas naświetlania przez trzydzieści dni. Rozmnażanie pędów było lepsze na zmodyfikowanej pożywce Murashige i Skooga – MS + 825 mg/l NH_4NO_3 – uzupełnionej o 10^{-7} M NAA. Pędy wytwarzały dobrze rozwinięte systemy korzeniowe w ciągu trzech tygodni po przeniesieniu ich do tej samej pożywki uzupełnionej o 5×10^{-7} M NAA.

SŁOWA KLUCZOWE: pędy pachwinowe, mikropropagacja, roślina lecznicza, *Salvia canariensis* L.