EFFECT OF EXPLANT TYPE AND BENZYLADENINE CONCENTRATION ON *in vitro* MULTIPLICATION OF *Salvia tomentosa*

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Abstract

Salvia tomentosa Mill. (Lamiaceae) is a Mediterranean perennial sage species, part of the macchia vegetation. Aiming to impove and promote the species for ornamental and medicinal use, in the present work the effect of explant type and benzyladenine (BA) concentration on *in vitro* blastogenesis of the species was studied. Shoot tip or single-node explants from microshoots originated from explants excised from *in vitro* grown seedlings were used. The explants were cultured on MS medium either without plant growth regulators (control) or enriched with 0.4 or 0.8 or 1.6 or 3.2 mg L^{-1} BA in combination with 0.01 mg L^{-1} naphthalynacetic acid (NAA). Shoot tip explants responded at higher percentage (59-66%) to form shoots compared to nodal ones (27-48%), at BA concentration 0.4-1.6 mg L^{-1}. In the control, both explant types produced shoots at the same higher percentage (67-69%), whereas at 3.2 mg L^{-1} BA the lowest percentage of shoot production was observed (16-21%). The percentage of explants that produced hyperhydrated shoots was 13-33%, depending on the treatment. More shoots (1.6-1.7) were produced at 1.6 mg L^{-1} BA from shoot tip explants and at 0.0-0.8 mg L^{-1} BA from nodal explants, whereas the number of hyperhydrated shoots was highest (2.1-2.9) at 1.6 (only for nodal explants) or 3.2 mg L^{-1} BA. The highest multiplication rate and the longest shoots (5.1-5.8 cm) with the highest node number (4.8-5.0) were observed in the control for both explant types, followed by the response at 0.4 mg L^{-1} BA. In conclusion, the increase of BA concentration resulted in an increase in the number of produced shoots, but hyperhydricity was increased simultaneously.

Keywords: *benzyladenine (BA), hyperhydricity, Mediterranean sage, micropropagation, native plant*

Introduction

Salvia tomentosa Miller (Lamiaceae), Balsamic sage, is a strongly aromatic, medicinal, perennial semi-woody herbaceous plant (Hedge, 1982), up to 80 cm, which grows in areas of maquis vegetation and on limestone slopes. Its geographical distribution extends from South Eastern Europe to Transcaucasia (Guner *et al.*, 2000). In Greece, it spreads in North-Eastern Greece and in the North-Eastern and Eastern Aegean Islands (Dimopoulos *et al.*, 2013). In traditional medicine, *S. tomentosa* is used to heal wounds (Aşkun *et al.*, 2010) and relieve stomach and abdominal pain (Ulubelen and Miski 1979), while it is consumed as an herbal tea in some Mediterranean countries (Dincer *et al.*, 2013). The aerial parts of the plant have antimicrobial and antioxidant properties, due to the significant quantities in secondary metabolites such as phenolics and terpenoids (Haznedaroglu *et al.*, 2001; Tepe *et al.*, 2005; Aşkun *et al.*, 2010).

Modern biotechnological methods like *in vitro* micropropagation technique have the benefits of the large scale multiplication of disease-free plants, faster cloning and the conservation of desired genotypes, in a very short span of time, along with the potential for the production of high-quality plant-based medicine (Máthé *et al.*, 2015). Micropropagation is also used in the

propagation of medicinal and aromatic plants (MAPs) as a tool to conserve rare, threatened, and valuable MAPs, and to massively produce high-value plant material for cultivation without seasonal constraints (Grigoriadou *et al.*, 2019). Micropropagation protocols are worked out for many plant species cultured *in vitro* to obtain high regeneration rates, aiming to facilitate commercially feasible micropropagation and enable their possible sustainable use (Máthé *et al.*, 2015; Grigoriadou *et al.*, 2019).

To the best of our knowledge, no studies have been performed on the *in vitro* propagation of *S. tomentosa*. Therefore, a first approach was made to its micropropagation with the aim of improving and promoting the species for ornamental and medical use. In the present work the effect of explant type and benzyladenine (BA) concentration on *in vitro* blastogenesis of the species was studied.

Materials and Methods

Shoot tip or single-node explants excised from microshoots of *in vitro* cultures of *S. tomentosa* initiated from *in vitro* grown seedlings were used. Explants were cultured on MS medium (Murashige and Skoog, 1962) with 30 g L^{-1} sucrose either without plant growth regulators (control) or supplemented with benzyladenine (BA), at four concentrations, i.e., 0.4, 0.8, 1.6, or 3.2 mg·L^{-1} in combination with 0.01 mg L^{-1} naphthalynacetic acid (NAA).

All media were solidified with 8 g L^{-1} agar and their pH was adjusted to 5.7 – 5.8 before agar addition and autoclaving (121 ^{o}C for 20 min). The cultures were maintained at 25 ^{o}C with a 16 h photoperiod at 37.5 μmol m^{-2} s^{-1} fluorescent light, provided by cool-white fluorescent lamps. Data were collected after 30 d of culture.

The "multiplication index" of each culture was calculated by multiplying the percentage of explants that produced shoots by the mean number of shoots per responding explant, and by the mean node number per shoot.

The completely randomized design was used. The significance of the results was tested by either one- or two-way analysis of variance (ANOVA) and the means of the treatments were compared by Student's *t* test at $P < 0.05$ (JMP 13.0 software, SAS Institute Inc., Cary, NC, 2013, USA).

Results and Discussion

The 2-way ANOVA in most of the parameters measured revealed significant interaction of the two nain factors of the experiment, i.e., BA concentration in the medium and explant type. Only the length and the node number of the shoots were affected by the BA concentration, as the inrease in the BA concentration resulted in a decrease in both of these parameters (Table 1).

Shoot tip explants responded at higher percentage (59-66%) to form shoots without hyperhydricity compared to nodal ones (27-48%), at BA concentration 0.4-1.6 mg L^{-1}, result that has also been reported for *S. officinalis* (Vlachou *et al.*, 2021). In the control, both explant types produced shoots at the same higher percentage (67-69%), whereas at 3.2 mg L^{-1} BA the lowest percentage of shoot production was observed (16-21%) (Table 1). The percentage of explants that produced hyperhydrated shoots was 13-33%, depending on the treatment (Table 1).

The presence of BA at high concentrations in the medium increased the number of shoots (normal and hyperhydrated) produced per explant. More shoots (1.6-1.7) were produced at 1.6 mg L^{-1} BA from shoot tip explants and at 0.0-0.8 mg L^{-1} BA from nodal explants, whereas the number of hyperhydrated shoots was highest (2.1-2.9) at 1.6 (only for nodal explants) or 3.2 mg L^{-1} BA (Table 1, Figure 1).

The highest multiplication rate and the longest shoots (5.1-5.8 cm) with the highest node number (4.8-5.0) were observed in the control for both explant types, followed by the response at 0.4 mg L^{-1} BA (Table 1, Figure 1). In previous studies on micropropagation of various *Salvia* species, such as *S. fruticosa* (Arikat *et al.*, 2004), *S. officinalis* (Petrova *et al.*, 2015), *S. sclarea* (Grigoriadou et al., 2020) or *S. wagneriana* (Ruffoni *et al.*, 2016), respectively low BA concentration (0.2-0.5 mg L^{-1}) favored shoot proliferation, in most cases combined with low auxin concentration as in the present study. Regarding the effect of the explant type on proliferation, in previous works on other *Salvia* spp, i.e., *S. fruticosa* (Arikat *et al.*, 2004), *S. sclarea* (Ghanbar *et al.*, 2016), *S. valentina* and *S. blancoana* subsp. *mariolensis* (Cuenca and Amo-Marco, 2000), maximum shoot proliferation has been reported from nodal explants compared to the shoot tips, whereas in the present study the explant type did not have such a significant effect on shoot proliferation of *S. tomentosa*. This is probably due to the fact that single node explants were used in this work, while in previous works nodal explants bear more nodes (Arikat *et al.*, 2004).

Table 1. Effect of BA concentration on shoot multiplication of shoot tip or nodal explants excised from *S. tomentosa* seedlings grown in vitro, in the presence of 0.01 mg L-1 NAA.

BA concn (mg L^{-1})	Shoot production [1] (%)	Shoot production [2] (%)	Mean NSh[Ŧ] number	Mean NSh length[Ŧ] (cm)	Mean node number[Ŧ]	Mean HSh[ŦŦ] number	Multiplication index
Shoot tip explant							
0.0 (Hf[ŦŦŦ])	69 a	28 cd	1.0 c	5.1 ab	4.8 a	0.3 f	3.3 b
0.4 BA	64 ab	28 cd	1.0 c	4.6 abc	3.8 b	0.4 f	2.4 cd
0.8 BA	66 a	27 d	1.3 bc	3.4 cde	3.4 bc	0.3 f	2.9 bc
1.6 BA	59 b	29 b	1.7 a	2.9 de	2.6 c	0.5 ef	2.6 bc
3.2 BA	21 ef	25 ef	1.3 bc	3.1 cde	3.1 bc	2.6 a	0.8 e
Nodal explant							
0.0 (Hf[ŦŦŦ])	67 a	25 ef	1.6 ab	5.8 a	5.0 a	0.6 de	5.4 a
0.4 BA	48 c	26 de	1.6 ab	4.2 bcd	3.4 bc	0.7 d	2.6 bc
0.8 BA	27 de	33 a	1.7 a	2.3 e	2.3 c	1.1 c	1.1 e
1.6 BA	29 d	17 f	1.3 bc	3.1 cde	3.4 bc	2.1 b	1.3 de
3.2 BA	16 f	13 g	1.3 bc	2.3 e	2.8 bc	2.9 a	0.6 e
$F_{\text{concn BA}}$	-	-	-	***	***	-	-
F_{explant}	-	-	-	NS	NS	-	-
$F_{\text{concn BA x expl}}$	***	***	**	NS	NS	***	***
$F_{\text{one-way}}$	***	***	***	***	***	***	***

[z]Mean separation in columns by Student's *t*, $P \leq 0.05$.
NS: not significant or *, **, ***: significant at $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$, respectively, n=30.
Multiplication Index = Shooting (%) x mean shoot number[Ŧ] x Mean node number[Ŧ]
[1]The explants produced normal and hyperhydrated shoots
[2]The explants produced hyperhydrated shoots only
[Ŧ]NSh = normal shoot
[ŦŦ]HSh = hyperhydrated shoot

$^{\overline{T}\overline{T}\overline{T}}$Hf = hormone free

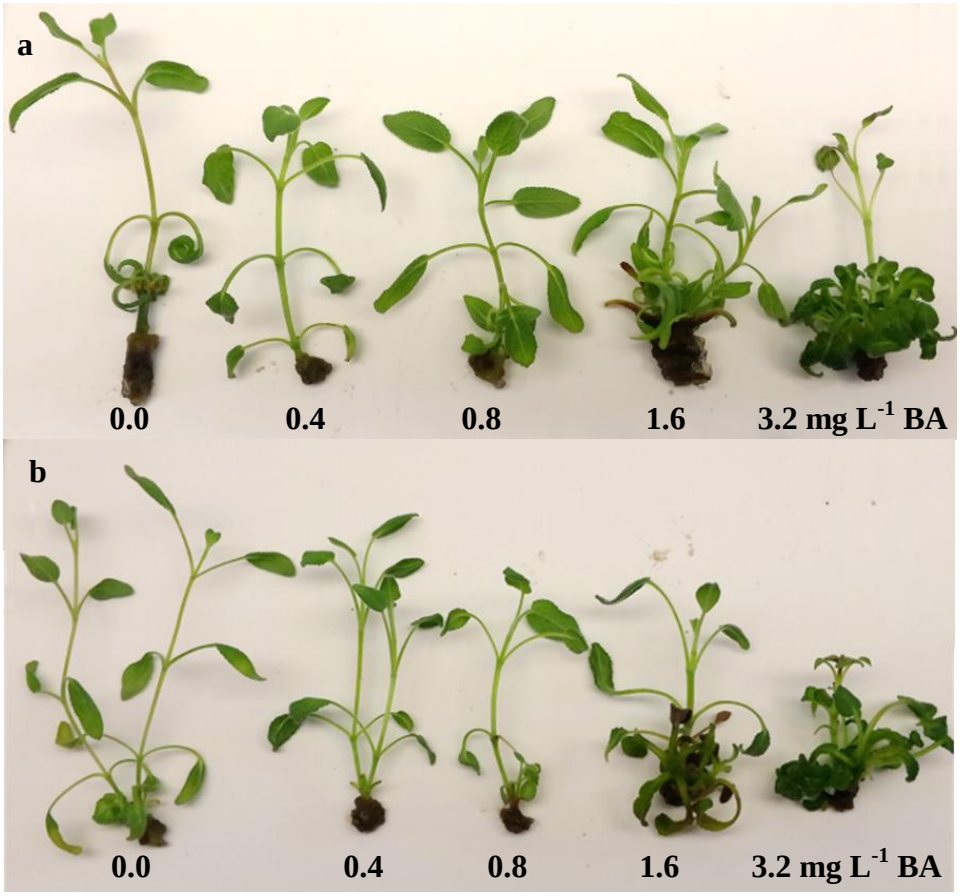

Figure 1. Typical response of shoot tip (a) and nodal (b) explant of *S. tomentosa* cultured *in vitro* on MS medium with marked BA concentration (mg L^{-1}) in the presence of 0.01 mg L^{-1} NAA.

Conclusions

The increase of BA concentration in the medium resulted in an increase in the number of produced shoots, but hyperhydricity was increased simultaneously.
Higher multiplication rates along with low hyperhydricity were achieved when either shoot tip or nodal explants were cultured on MS medium without plant growth regulators or enriched with the lowest BA concentration tested, 0.4 mg L^{-1}, in combination with 0.01 mg L^{-1} NAA.

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INTRODUCTION

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MATERIALS AND METHODS

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RESULTS

Shoot tip explants responded at higher percentage (59-66%) to form shoots without hyperhydricity compared to nodal ones (27-48%), at BA concentration 0.4-1.6 mg L^{-1}, result that has also been reported for *S. officinalis* (Vlachou *et al.*, 2021). In the control, both explant types produced shoots at the same higher percentage (67-69%), whereas at 3.2 mg L^{-1} BA the lowest percentage of shoot production was observed (16-21%) (Table 1). The percentage of explants that produced hyperhydrated shoots was 13-33%, depending on the treatment (Table 1).

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CONCLUSIONS

The increase of BA concentration in the medium resulted in an increase in the number of produced shoots, but hyperhydricity was increased simultaneously.

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1.6 BA	59 b	29 b	1.7 a	2.9 de	2.6 c	0.5 ef	2.6 bc
3.2 BA	21 ef	25 ef	1.3 bc	3.1 cde	3.1 bc	2.6 a	0.8 e
Nodal explant							
0.0 (Hf[ŦŦŦ])	67 a	25 ef	1.6 ab	5.8 a	5.0 a	0.6 de	5.4 a
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0.8 BA	27 de	33 a	1.7 a	2.3 e	2.3 c	1.1 c	1.1 e
1.6 BA	29 d	17 f	1.3 bc	3.1 cde	3.4 bc	2.1 b	1.3 de
3.2 BA	16 f	13 g	1.3 bc	2.3 e	2.8 bc	2.9 a	0.6 e
$F_{\text{one-way ANOVA}}$	***	***	***	***	***	***	***

[z]Mean separation in columns by Student's *t*, $P \leq 0.05$.
NS: not significant or *, **, ***: significant at $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$, respectively, n=30.
Multiplication Index = Shooting (%) x mean shoot number[Ŧ] x Mean node number[Ŧ]
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[2]The explants produced hyperhydrated shoots only
[Ŧ]NSh = normal shoot
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[ŦŦŦ]Hf = hormone free

Figure 1. Typical response of shoot tip (a) and nodal (b) explant of *S. tomentosa* cultured *in vitro* on MS medium with marked BA concentration (mg L^{-1}) in the presence of 0.01 mg L^{-1} NAA.

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