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EFFECT OF PLANT GROWTH REGULATORS ON ORGANOGENESIS *OF SALVIA OFFICINALIS L.* PLANTS

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ABSTRACT

The traditional applications of sage herb in the domestic medicine are endless moreover, it is widely known as an ornamental plant. Nodal and leaf explants were cultured on MS medium supplemented with different combinations of TDZ, BA or Kin as a source of cytokinin and IAA or 2,4-D as an auxin. Supplemented growth medium with TDZ promoted callugensis of both types of explants however, callus did not develop when the medium contained only BA or Kin. Number of developing shoots was significantly varied among concentration of PGRs. Nodal explants cultured on medium with 1.5 mg/l TDZ + 0.1 or 0.5 mg/l IAA had the highest significant shoot number (7.2 shoots/explant) followed by IAA + TDZ at 1 mg/l which developed 5.5 and 5.8 shoots/explant, respectively. In many cases the adventitious shoots failed to develop into shoots even after subculture. The tallest shoots 9.6 cm were observed on medium with 1 mg/l of BA or Kin + 0.5 mg/l IAA. Adventitious shoots were perceived for all leaf explants that cultured on medium supplemented with (TDZ + IAA) and (1 mg/l TDZ + 0.1 mg/l 2,4-D). Unlike TDZ, Kin and BA had low efficiency to sustain shoot growth as the number of developing shoots was 0.4/explant. This method of regeneration will facilitate selection of *S. officinalis* plants more tolerant to environmental stress, their micropropagation, and the *in vitro* production of secondary products.

*Abbreviations:*2,4-D-dichlorophenoxyacetic acid; BA – 6-benzyladenine; IAA – indole 3-acetic acid; Kin -Kineten; MS – Murashige and SkoogTDZ – Thidiazuron;

INTRODUCTION

Salvia genus (sage) is one of the mint family Lamiaceae which includes more than 900 species (Pierozan *et al.*, 2009). Sage is nearly at the top of the list of household preparations for the relief of many diseases based on its wide variety of bioactivities (anti-bacterial, anti-mycotic, anti-inflammatory, antioxidant and others) (Abu-Darwish *et al.*, 2013).The major components of *S. officinalis* are 1, 8-cineole, camphor, borneol, bornyl acetate, camphene, α-and β-thujone, linalool, salvianolic acid, rosmarinic acid, carnosolic acid, ursolic acid and etc. (Avato *et al*., 2005 and Khan *et al*., 2011).

Actually, salvia species can be simply propagated by cuttings and in some cases by seeds (Grieve, 1994). However, plants do not last more than 3-4 years without degenerating so that, new plants should be planted at least every 4 years. Considerable progress has been achieved in *in vitro* production of various secondary metabolites from *Salvia* spp. (Duran *et al.*, 2013 and Petrova *et al.*, 2014). Nevertheless, there is growing interests in the development of effective protocols for the *in vitro* culture of some *Salvia* species, in order to find a relatively fast system for producing disease-free and true-to-type clonal uniform plants from outstanding genotypes (Kintzios, 2000).

In vitro culture can serve as a model system for studying the accumulation of antioxidative compounds under controlled conditions (Santos-Gomes et al., 2002). Total polyphenol content and antioxidant activity of S. officinalis in vitrocultures have been reported in several publications (Grzegorczyk et al., 2006 and Wielgus et al., 2011). Many researchers investigated the in vitro culture of S. officinalis for micropropagation or improvement their active substances content using different explants (Santos-Gomes et al., 2002; Gostin, 2008; Grzegorczyk and Wysokinska, 2008 and Wielgus et al., 2011). However, some of the serious limitations in protocols were inconsistent, e.g. low propagation frequency, and occurrence of hyperhydricity. Callus cultures, cell suspension, immobilized cell and hairy root cultures from S. officinalis were also established (Falk et al., 1990, Bolta et al., 2000, Tawfik Mohamed, 2007and Duran et al., 2013).

The efficiency of 6-benzyladenine (BAP) over other cytokinins in inducing multiplication of *S. officinalis* has been described (Avato *et al.*, 2005; Grzegorczyk and Wysokinska, 2008; Wielgus *et al.*, 2011 and Petrova *et al.*, 2014). The thidiazuron (TDZ) (0.5 or 1 mg/l) and IAA (0.1 mg/l) were also reported as appropriate for micropropagation, although some shoots showed

hyperhydricity (Avato *et al.*, 2005; Weielgus *et al.*, 2011).

Therefore, our study aimed to establish an efficient protocol for the regeneration of plantlets from single node or leaf explants of *S. officinalis* using different combinations of plant growth regulators.

MATERIALS AND METHODS:

Plant material and medium preparation

Salvia officinalis shoot tip segments (3-5 cm long) were collected at Juvenile field- grown plants in the Nursery of Ornamental and Medicinal Plants, Horticulture Department, Faculty of Agriculture, Minia University. Explants were prepared for culture initiation by surface sterilization using a routine method previously described by George (1993). The plant material was rinsed with a detergent solution, before being sterilized with 70% ethanol for 30s then Clorex solution contains 1.2% of active chlorine, for 20 min (few drops of liquid dish soap was added to the solution). Apical shoots 1-2 cm long were excised and individually cultured in jars containing Murashige and Skoog basal medium (MS) (Murashige and Skoog, 1962) supplemented with 30 g/l sucrose, and IAA 1.0 mg/l. The healthy grown plantlets were subcultured into fresh medium for another 4 weeks before using as a source for nodal and leaf explants.

For all culture media pH was adjusted to 5.8 and gelled with 8 g/l agar then autoclaved for 20 min at 121 °C and 1.06 kg cm^{-2}. The vessels (jam jar; 250 ml) containing the cultures were incubated in a culture room at 23±1 ^{o}C and 16 h photoperiod provided by Phillips TLD 50 W/ 84 HF fluorescent tubes giving a photon flux of approximately 40-50 µmol m^{-2} s^{-1}.

Regeneration of S. officinalis:

Nodal (about 1cm length) and leaf segments (complete leaf after removing both ends) from the *in vitro* grown plantlets (3-weeks old) were used as type of explants from aseptic culture. Explants were cultured on medium supplemented with different combination of auxins and cytokinins (0, 0.1 and 0.5 mg/l of IAA or 2,4-D) and (1.0 or 1.5 mg/l of TDZ, BA or Kin). Therefore, there were 30 treatments each one was repeated 6 times (6 jam jars; 250 ml) each contained 6 explants. After 4 weeks, cultures were transferred into a fresh medium. After another 4weeks, cultured explants with grown buds were subcultured into fresh medium supplemented with IAA at 0.5 to enhance shoots development.

Statistical analysis:

The significance of differences between the treatments in the frequency of explants which formed callus and organogenesis (shoots or roots) were calculated using the Tukey's t test (Zar, 1996) after transferring the percentage data to square root. Data of number and length of developing shoots were tested by multi-way factorial analyses of variance (Zar, 1996). This analysis was performed using M-stat C for Windows. The comparisons between any pair of data were calculated using Tukey's t.

RESULTS AND DISCUSSION

Callus formation and growth

Many of nodal and leaf explants showed a potential to develop callus after the 2nd week of culture. Callus was initiated from the base of the nodal explants however; it was developed from both ends of leaf explants when the medium included any PGRs combination rather than 2,4-D. However, callus developed from all cutting sides on medium contained 2,4-D. The percentage of explants which developed callus was significantly varied from 0 to 100% for both types of explants depending on the supplemented PGRs combinations (Tables 1 and 2). For all type of explants callus formation was significantly affected by type, concentration and the ratio of auxin/cytokinin. For example, leaf explants cultured on medium supplemented with (IAA at 0.0 or 0.1 mg/l) + (BA at 1.0 mg/l) or (Kin at 1.5 mg/l) failed to develop any callus however, all explants cultured on medium with TDZ and IAA or 2,4-D developed callus. Tables 1 and 2 show that supplemented growth medium with TDZ as a unique cytokinin source of PGRs promote callugensis of both types of explants however, callus did not develop once the medium contained BA or Kin without any auxin. Callus size was noticeably varied among treatments, generally medium enriched with 2,4-D developed the moderate or the biggest callus size regardless the cytokinin combination (Table 1 and 2).

Overall, there was no correlation between the high frequency of callus formation and its size which means some PGRs combinations had high ability to produce callus with a diameter less than 0.5 cm (Tables 1 and 2). Actually, regardless the type of explants, there was no clear pattern regarding the efficiency of PGRs combination on callus development and its growth. Callus colour was varied from green, to green with white or brown areas, or yellow with green areas without a clear effect following PGRs combinations.The effects of both PGRs and the explant type on *S. officinalis* callus formation and morphology are similar to those previously found by (Avato *et al.*, 2005; Wielgus *et al.*, 2011 and Petrova *et al.*, 2014). However, the present study showed very low percent of hyperhydricity unlike these reported by Avato *et al.* (2005) and Weielgus *et al.* (2011). Application of TDZ, BA and Kin induced a wide range of responses in sage explant tissues. This varies widely depending on the type and concentration of cytokines, cultured explant, and the type and concentration of the used auxin (George, 1993). The differential ability of cytokinins to induce shoots could be attributed to many factors e.g stability, mobility, and the rate of conjugation and oxidation of the

hormones (D'Onofrio and Morini, 2005). Thus, the hormonal conditions appear to be a determining factor for the successful enhancement of adventitious bud formation and shoots proliferation. Auxin/cytokinin ratio during *in vitro* tissue culture could play a critical role to induce the morphogenic response in higher plants. The expression of the morphogenic potential under *in vitro* culture could also depend on certain specific interactions between them (García *et al*., 2008).

Organogenesis

Rooting

Almost all nodal explants formed adventitious roots from the explant itself or the developed callus with all growth regulator combinations except those contained (TDZ with IAA or 2,4-D combination and or 1.5 g/l Kin + 0.5 mg/l 2,4-D (Table 1). However, only leaf explants cultured on medium with (Kin or BA + 2,4-D) had the ability to develop roots nevertheless, there were significant differences among these media (Table 2). Regarding the number of developing roots (Table 1) showed that nodal explants cultured on medium with 1.0 or 1.5 mg/l Kin+ 0.1 or 0.5 mg/l 2,4-D had the highest percentage of roots induction compared with the other treatments. Only leaf explants cultured on medium with BA or Kin and 2,4-D initiated adventitious roots. There were significant differences among these treatments as the percentage of rootability varied between 57 and 100% (Table 2). However, leaf explants which were cultured on medium with 1mg/l kin + 2,4-D had the highest number of roots compared with leaf explants which were cultured on medium with BA.

Shooting

All nodal explants except those cultured on medium with 0.5 mg/l 2,4-D + Kin or IAA +TDZ showed direct shoot regeneration which commenced after few days of culture. Yet, after the subculture adventitious shoots were regenerated from explants cultured on medium enriched with IAA as a source of auxin with TDZ or BA. Moreover, explants cultured on medium with 0.1 mg/l 2,4-D + TDZ or BA developed adventitious shoots. Direct regenerated shoot primordia which was observed on explants cultured on medium with TDZ + 0.5 mg/l 2,4-D lost the ability to grow and become necrotic, moreover, no adventitious shoots were further developed. Shoot induction percentage of nodal explant was significantly varied among the treatments (Table 3 and Figure 1). Where the lowest value 27% was found on medium supplemented with 1 mg/l Kin + 0.1 mg/l 2,4-D. Still, there were no significant differences among media containing any concentration of IAA regardless the supplemented cytokinins. As expected, the number of developing shoots was significantly varied among concentrations of PGRs (Table 3 and

Figure 1). Explants cultured on medium with 1.5 mg/l TDZ + 0.1 or 0.5 mg/l IAA had the highest significant shoot number (7.2 shoots/explant) followed by the same concentrations of IAA + TDZ at 1 mg/l which developed 5.5 and 5.8 shoots/explant, respectively. In many cases the adventitious shoots failed to develop into intact shoots even after subculture which means that the number of developing shoots was less than 3/explant. Overall, shoots developed on medium with TDZ was shorter than those regenerated on medium with BA or Kin. The tallest shoots 9.6 or 8.0 cm with no significant difference between them were observed on medium with 1.0 mg/l of BA or Kin + 0.5 mg/l IAA, respectively.

Adventitious shoots were perceived for all leaf explants that cultured was on medium supplemented with (TDZ + IAA). Direct shoot regeneration was observed from leaf explants prior to callus observation which was cultured on medium with (1.0 mg/l TDZ + 0.1 mg/l 2,4-D) and (1.5 mg/l BA + 0.5 mg/l IAA). There were significant variances on the shoot induction percentage among the treatments which ranged between 23 and 100% (Table 4). But, it should be emphasized that unlike TDZ, Kin and BA had low capacity to sustain shoot growth as the number of developing shoots was 0.4/explant. The lowest number of shoots was developed one medium with TDZ (3.8 shoots/explant) whereas, explants cultured on medium with 1.5 mg/l TDZ+0.1 mg/l IAA had the highest number of shoots (8/explant). The length of the developing shoots was significantly varied between 2.6 and 4.2 cm (Table 4). This observation confirms the results reported by Avato *et al.* (2005), Wielgus *et al.* (2011) and Petrova *et al.* (2014) on *S. officinalis*. However, the present study showed very low percent of hyperhydricity unlike those reported by Avato *et al.* (2005) and Wielgus *et al.* (2011). Thidiazuron is a substitute for phenylurea to stimulate axillary shoot proliferation in several plant species. It is less susceptible to the plant's degrading enzymes than other cytokinins, and is active at lower concentrations than the amino purine cytokinins (Huetteman and Preece, 1993). This was also consistent with the findings on potential applications of TDZ in adventitious shoot regeneration and synergistic effect of TDZ with auxins on inducing higher number of shoots (Babaei *et al.*, 2014).

Therefore, protocols were successfully established for the regeneration of plantlets from single node or leaf explants of *S. officinalis* using different combinations of plant growth regulators.

Table 1: Effect of plant growth regulators on callugensis and root induction of *Salvia officinalis* nodal segments after 8 weeks of culture.

PGR (mg/l)				Callus induction[1] (%)	Callus relative growth[2]	Callus color[3]	Root induction (%)	Root induction[4]
Cytokinin		Auxin						
TDZ	1	IAA	0	100 a	+	G	0 c	-
	1		0.1	100 a	+	G-W	0 c	-
	1		0.5	100 a	+	G-W	0 c	-
	1.5		0	100 a	+	G	0 c	-
	1.5		0.1	100 a	+	G-W	0 c	-
	1.5		0.5	100 a	+	G-W	0 c	-
	1	2,4-D	0.1	100 a	+	W-G	0 c	-
	1		0.5	100 a	++	W-G	0 c	-
	1.5		0.1	100 a	+	W-G	0 c	-
	1.5		0.5	70 d	++	W-G	0 c	-
BA	1	IAA	0	0 h	-	-	100 a	+
	1		0.1	93 abc	+	W-Y	100 a	+
	1		0.5	73 d	+	G-W	100 a	+
	1.5		0	0 h	-	-	100 a	+
	1.5		0.1	0 h	-	-	100 a	+
	1.5		0.5	46 e	+	W-G	100 a	+
	1	2,4-D	0.1	4 e	+	W-G	100 a	++
	1		0.5	100 a	+++	W-Y-B	100 a	++
	1.5		0.1	77 cd	++	W-G	100 a	++
	1.5		0.5	97 ab	+++	W	100 a	+
Kin	1	IAA	0	0 h	-	-	100 a	++
	1		0.1	73 d	+	W-Y	100 a	+
	1		0.5	0 h	-	-	100 a	++
	1.5		0	0 h	-	-	100 a	++
	1.5		0.1	27 f	+	W-G-B	100 a	++
	1.5		0.5	17 g	+	W-G	100 a	++
	1	2,4-D	0.1	90abc	++	W-Y	100 a	+
	1		0.5	80bcd	++	W-Y-B	70 b	+
	1.5		0.1	100 a	++	W -G	100 a	++
	1.5		0.5	100 a	++	W–Y-B	0 c	-

[1]Each presented value has been calculated based on 36 explants and percentage frequencies not followed by the same letter are significantly differ at p=0.05, Tukey test. [2]Callus diameter – no callus formation, (+) =< 0.5 cm, (++) = >0.5, [3]Callus color (G) green, (W) white, (Y) yellow and (B) brown,[4] Root induction – no root formation, (+) =less than 2 roots, (++) = 2-5 roots and (+++) => 5 roots.

Table 2: Effect of plant growth regulators on callugensis and root induction of *Salvia officinalis* leaf segments after 8 weeks.

PGR (mg/l)				Callus induction[1] (%)	Callus relative growth[2]	Callus color[3]	Root induction (%)	Root induction[4]
Cytokinin		Auxin						
TDZ	1	IAA	0	100 a	+	G-W	0 e	-
	1		0.1	100 a	+	G-W	0 e	-
	1		0.5	100 a	+	G-W	0 e	-
	1.5		0	86ab	+	W-G,b	0 e	-
	1.5		0.1	100 a	+	G-W	0 e	-
	1.5		0.5	100 a	+	W-G	0 e	-
	1	2,4-D	0.1	96ab	++	W-G	0 e	-
	1		0.5	93ab	++	G-W	0 e	-
	1.5		0.1	83b	++	G-W	0 e	-
	1.5		0.5	100 a	+++	G-W	0 e	-
BA	1	IAA	0	0 g	-	-	0 e	-
	1		0.1	0 g	-	-	0 e	-
	1		0.5	50 d	+	W-G	0 e	-
	1.5		0	0 g	-	-	0 e	-
	1.5		0.1	67 c	+	W-G	0 e	-
	1.5		0.5	40 e	+	W-G	0 e	-
	1	2,4-D	0.1	87ab	++	W	80 c	+
	1		0.5	100 a	+++	W-G	87 b	+
	1.5		0.1	100 a	+++	W-G	57 d	+
	1.5		0.5	87ab	++	W-G	60 d	+
Kin	1	IAA	0	23f	+	W-G	0 e	-
	1		0.1	63 c	+	W-G	0 e	-
	1		0.5	33 e	+	W-B	0 e	-
	1.5		0	0 g	-	-	0 e	-
	1.5		0.1	0 g	-	-	0 e	-
	1.5		0.5	83 b	++	W-G	0 e	+
	1	2,4-D	0.1	100 a	+++	W-G	100 a	+++
	1		0.5	100 a	++	W-G	100 a	++
	1.5		0.1	100 a	++	G	77 c	++
	1.5		0.5	100 a	+++	W-B	0 e	-

[1]Each presented value has been calculated based on 36 explants and percentage frequencies not followed by the same letter are significantly differ at p=0.05, Tukey test. [2]Callus diameter – no callus formation, (+) =< 0.5 cm, (++) = >0.5, [3]Callus color (G) green, (W) white, (Y) yellow and (B) brown,[4] Root induction – no root formation, (+) =less than 2 roots, (++) = 2-5 roots and (+++) => 5 roots.

Table 3: Effect of plant growth regulators on shoot induction of *Salvia officinalis nodal* segments. Shoot induction was recorded after 8 weeks of culture however, shoot numbers and length were observed after 4 weeks of subculture.

PGR (mg/l)				Shoot induction[1]		Shoot induction (%)	Shoot numbers/ explant	Shoot length (cm)
Cytokinin		Auxin		direct	advent			
TDZ	1	IAA	0	-	√√√	100 a	4.4 d	2.6 g
	1		0.1	-	√√√	97 a	5.5bc	3.2 fg
	1		0.5	-	√√	97 a	5.8 b	3.6fg
	1.5		0	-	√√	100 a	4.6 cd	2.6 g
	1.5		0.1	-	√√√	100 a	7.2 a	3 fg
	1.5		0.5	-	√√	100 a	7.2 a	3.2fg
	1	2,4-D	0.1	√	√√√	100 a	3.8 d	3.8fg
	1		0.5	√	-	0 e	0 i	0 h
	1.5		0.1	√	√√√	90 ab	4.2 d	4.2ef
	1.5		0.5	√	-	0 e	0 i	0 h
BA	1	IAA	0	√√	√	100 a	3.1 e	7.4bc
	1		0.1	√√	√	100 a	2 fg	6.6 bcd
	1		0.5	√√	√	100 a	2.1 fg	8 ab
	1.5		0	√	√√	100 a	2.2 fg	3.8 fg
	1.5		0.1	√	√√√	100 a	2.3 f	5.2 de
	1.5		0.5	√√	√	100 a	2.1 fg	7.4bc
	1	2,4-D	0.1	√√	√	100 a	2fg	5.8 cd
	1		0.5	√	-	47 c	0.5 h	2.8 g
	1.5		0.1	√√	√	100 a	1.8fg	6.4 bcd
	1.5		0.5	√	-	60 c	0.5 h	2.6 g
Kin	1	IAA	0	√	-	100 a	2.2 fg	6.2bcd
	1		0.1	√	-	100 a	2 fg	2.8 g
	1		0.5	√	-	100 a	2 fg	9.6 a
	1.5		0	√	-	100 a	2.3 f	5.4 de
	1.5		0.1	√	-	100 a	1.8fg	6.2bcd
	1.5		0.5	√	-	100 a	2 fg	7.7 bc
	1	2,4-D	0.1	√	-	27 d	0.3 hi	2.8 g
	1		0.5	-	-	0 e	0 i	0 h
	1.5		0.1	√	-	83b	1.4 g	6 cd
	1.5		0.5	-	-	0 e	0 i	0 h

[1] Shoot induction (–) no shoot formation, (√) poor, (√√) moderate, (√√√) massive

Table 4: Effect of plant growth regulators on shoot induction of *Salvia officinalis* leaf explants. Shoot induction was recorded after 8 weeks of culture however, shoot numbers and length were observed after one subculture and elongation.

PGR (mg/l)				Shoot induction (%)	Shoot induction[2]		Shoot numbers/ explant	Shoot length (cm)
Cytokinin		Auxin			direct	advent		
TDZ	1	IAA	0	87 b	-	√√√	5.8 b	2.8 cd
	1		0.1	94 ab	-	√√√	6.2 b	2.8 cd
	1		0.5	93 ab	-	√√√	6.2 b	4.2 a
	1.5		0	67 c	-	√√	3.8 c	2.8 cd
	1.5		0.1	100 a	-	√√√	8 a	3.2 bcd
	1.5		0.5	100 a	-	√√√	7.6 a	3.8ab
	1	2,4-D	0.1	40 d	√	√√	4.2 c	2.6 d
	1		0.5	0 f	-	-	0 e	0 e
	1.5		0.1	0 f	-	-	0 e	0 e
	1.5		0.5	0 f	-	-	0 e	0 e
BA	1	IAA	0	0 f	-	-	0 e	0 e
	1		0.1	0 f	-	-	0 e	0 e
	1		0.5	0 f	-	-	0 e	0 e
	1.5		0	0 f	-	-	0 e	0 e
	1.5		0.1	0 f	-	-	0 e	0 e
	1.5		0.5	23 e	√	-	0.4 d	3.6abc
	1	2,4-D	0.1	0 f	-	-	0 e	0 e
	1		0.5	0 f	-	-	0 e	0 e
	1.5		0.1	0 f	-	-	0 e	0 e
	1.5		0.5	0 f	-	-	0 e	0 e
Kin	1	IAA	0	0 f	-	-	0 e	0 e
	1		0.1	0 f	-	-	0 e	0 e
	1		0.5	0 f	-	-	0 e	0 e
	1.5		0	0 f	-	-	0 e	0 e
	1.5		0.1	0 f	-	-	0 e	0 e
	1.5		0.5	0 f	-	-	0 e	0 e
	1	2,4-D	0.1	0 f	-	-	0 e	0 e
	1		0.5	0 f	-	-	0 e	0 e
	1.5		0.1	0 f	-	-	0 e	0 e
	1.5		0.5	0 f	-	-	0 e	0 e

[1] *Number of explants*

[1] *Shoot induction (–) no shoot formation, (√) poor, (√√) moderate, (√√√) massive*

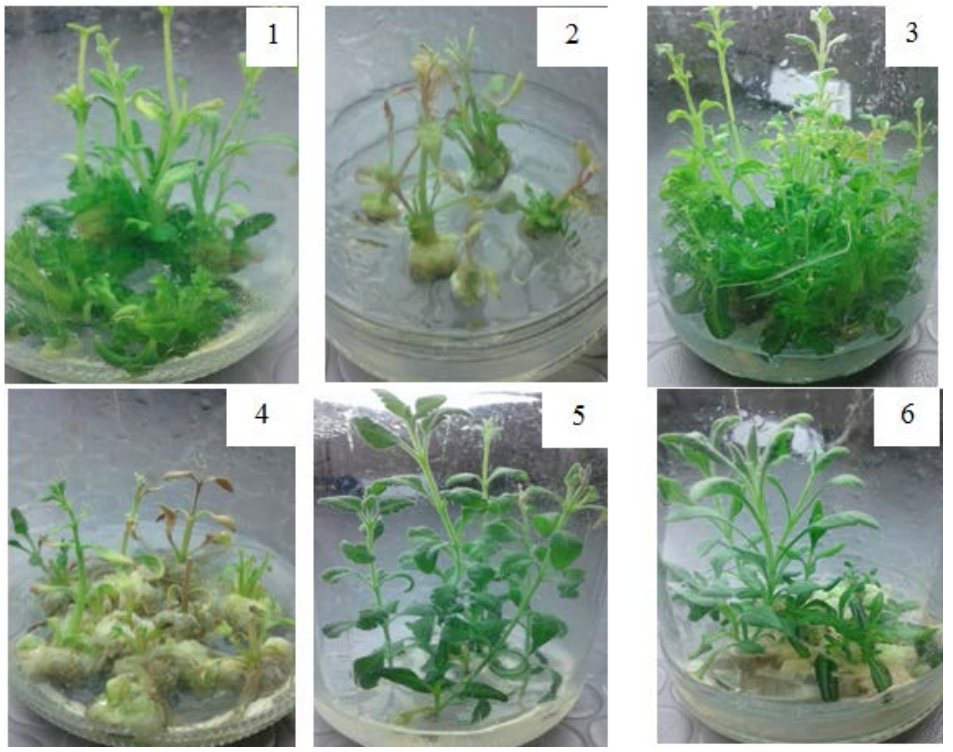

Fig. (1): Effect of plant growth regulators on shoot, callus and root induction of *Salvia officinalis* nodal segments. **1** (TDZ 1.5 mg/l + IAA 0.5 mg/l), **2** (1.5 mg/l + 2,4-D 0.5 mg/l), **3** (BA 1.5 mg/l + IAA 0.5 mg/l), **4** (BA 1.0 mg/l + 2,4-D 0.5 mg/l), **5** (KIN 1.0 mg/l) and **6** (Kin 1.5 mg/l + 2,4-D 0.5 mg/l)

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الملخص العربى

تأثير منظمات النمو على تكوين الاعضاء فى نباتات المريمية

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تعد التطبيقات العملية لعشب المريمية فى الطب الشعبى غير منتهية، علاوة على استخدام المريمية كنبات زينة. تم زراعة المستأصل النباتية من العقد الساقية والورقة فى الوسط المغذى MS المحتوى على خليط من منظمات النمو وهىTDZ أو BA أو Kin من السيتوكينينات بالاضافة إلى IAA أو 2,4-D من الاكسينات. واشارت النتائج إلى أن اضافة TDZ قد شجعت تكوين الكالس فى كلا المستاصلين النباتيين وعلى العكس من ذلك لم يتكون الكالوس فى الاوساط المحتوية على BA أو Kin. وقد تباين عدد الاشطاء المتكشف معنويا بين تركيزات منظمات النمو المستعملة. وكان أعلى عدد من الاشطاء المتكشفة (7.2/مستأصل نباتى) من العقد الساقية التى تم زراعتها فى وسط محتوى على 1.5 ملجم/لتر من TDZ + 0.5 ملجم/لتر من IAAمتبوعا بنفس التركيز من IAA مع تركيز 1 ملجم/لتر من TDZ. والذي نتج عنه 5.5 و5.8 فرع/مستأصل على التوالى. وفى العديد من الحالات فشلت البراعم المتكشفة فى مواصلة النمو وتكوين اشطاء حتى بعد النقل إلى مزرعة جديدة. وقد تبين أن اطول الأشطاء (9.6 سم) هى تلك المتكشفة فى بيئة مضاف إليها 1 ملجم/لتر من BA أو Kin المضاف معه 0.5 ملجم/لتر من IAA. وقد تم الحصول على اشطاء من كل المستأصلات الورقية التى تم زراعتها فى وسط محتوى على (TDZ + IAA) وكذلك 1 ملجم/لتر من TDZو 0.1 ملجم/لتر من 2,4-D. وعلى العكس من TDZ فإن كفأة Kin وBA فى تدعيم نمو الاشطاء كانت اقل حيث يلاحظ أن عدد الاشطاء كان 0.4 / مستأصل. ويمكن القول بأن البرنامج الذي تم الحصول عليه يمكن أن يساهم فى انتخاب نباتات مريمية مقاومة للإجهاد البيئى وكذلك للإكثار الدقيق كما يمكن أن يدعم عملية انتاج المركبات الثانوية معمليا.