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Callusing, Cell Suspension Culture and Secondary Metabolites Production in Persian Oregano (*Origanum vulgare* L.) and Arabian Oregano (*O. syriacum* L.)

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ABSTRACT

In vitro cultures of Persian oregano (*Origanum vulgare* L.) and Arabian oregano (*O. syriacum* L.) were initiated from seeds on Murashige and Skoog (MS) medium containing 2.0 mg/l Gibberellic Acid (GA_3) and 15 g/l sucrose. Callus induction was experimented by culturing leaf discs at different levels (0.0, 0.1, 0.5, 1.0, 1.5 or 2.0 mg/l) of 2,4-dichlorophenoxyacetic acid (2,4-D). Best callus induction and fresh weight were obtained at lower levels (0.1 or 0.5 mg/l) of 2,4-D. Callus maintenance was tested on different levels (0.5, 1.0, 1.5, 2.0, or 2.5 mg/l) of N^6-Benzyladenine (BA) or Thidiazuron (TDZ) (with or without 0.5 mg/l 2,4-D). The largest callus diameter (12.5 mm) of *O. vulgare* L. was obtained when TDZ was at 1.0 mg/l without using 2,4-D. Adding 2,4-D at 0.5 mg/l was inhibitory on callus growth and diameter during 30 days of incubation when used in combination with TDZ or BA. BA gave less callus growth and smaller diameter than TDZ for the two species. *O. syriacum* L. callus was best grown when TDZ was 1.5 mg/l (with or without 0.5 mg/l 2,4-D). Callus from the third generation was friable and able to release cells in cell suspension culture. Cells were successfully subcultured every 17 days on liquid MS media supplemented with 1.0 mg/l TDZ for both *Origanum* spp. *O. syriacum* L. grown under greenhouse conditions, produced higher oil yield (1.76 %) than *in vitro* grown plants (1.17 %), whereas *O. vulgare* L. was a poor oil producing plant in this study. Callus and cells produced very low oil percentage compared to intact plants. Thymol was identified by gas chromatography analysis in *O. syriacum* L. intact plants. *Ex vitro* plants gave 13.1 % thymol while *in vitro* cultures gave 5.9 %. No thymol was identified in *O. vulgare* L. intact plants or in oil produced from callus and cells in both species.

KEYWORDS: *Origanum vulgare, Origanum syriacum*, Secondary Metabolites, Thymol.

1. INTRODUCTION

Origanum spp. are widely used all over the world as a popular spice (Simon, 1990). These species are of great economic importance which is not only related to their use as spices, but furthermore, for their traditional use in many ways due to their biological activities against a huge number of microorganisms (Kokkini, 1996).

In *Labiatae*, callus and cell culture were induced and subcultured to find new mutants (Kumari and Saradhi, 1992), or for expected secondary metabolites production (Svoboda *et al*., 1995). Kumari and Sradhi (1992) induced callus tissue of *O. vulgare* L. by using different explants from 15 day old seedling, and cultured on B5 semi-solid media. Callus tissue of *Thymus vulgaris* was induced from leaf explants on MS (Murashige and

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Skoog, 1962) media supplemented with 1.0 mg/l Naphthaleneacetic Acid (NAA) and 1.0 mg/l kinetin, or 1.0 mg/l 2,4 dichlorophenoxyacetic acid (2,4-D) and 1.0 mg/l kinetin (Tamura *et al.*, 1993).

Glandular trichomes, that produce essential oil, are widespread on leaves and flowers of the *Labiatae* in all the species that have been studied (Simon, 1990). Essential oil is a mixture of volatile compounds (terpenes and other derivatives) produced in small quantities as secondary metabolites from aromatic and medicinal plants (Manou *et al*., 1998; Oran, 1994; Simon, 1990). The essential oil and its constituents have multiple uses as food flavors (Simon, 1990), and in a recent study conducted by Crowell (1999), a number of dietary monoterpenes had antitumor activity and showed chemopreventive action against rodent mammary, skin, liver, lung, and forestomach cancers. Conner and Beuchat (1984) reported a strong inhibitory effect of *O. vulgare* L. and *T. vulgaris* L. oils on 13 food spoilage yeast strains. Thyme oil is obtained from leaves and flowering tops of *T. vulgaris* L. and other species of *Origanum*. *Origanum* oil was given a synonym for thyme oil. But true oil of *Origanum* is derived from *T. capitatus* L. and *Origanum* species, it may contain up to 75 % phenols (Reynolds, 1982). Kokkini (1996) mentioned that essential oils of *Origanum* members vary with respect to the total amount produced per plant as well as in their qualitative composition.

Thymol is a colorless crystal or white crystalline powder with a characteristic pungent aromatic odor and taste (Reynolds, 1982). Thymol is a more powerful disinfectant than phenol, used chiefly as a deodorant in mouth-washes and gargles and in dentistry to prepare cavities before filling (Reynolds, 1982). Gilman *et al.* (1985) reported that thymol is used at too low concentrations to be effective within any practical contact time in lotions. Dudai *et al.* (1985) reported a change in yield of essential oil and its constituents, which resulted from distillation and identification from different plant parts. They have suggested the presence of two major chemotypes of *O. syriacum* L., the first is carvacrol type (countains higher amount of carvacrol than thymol), and the second is thymol type (contains more thymol than carvacrol).

This investigation aimed at studying the essential oil content in intact plants (*in vitro* and *ex vitro*), callus, and cell cultures in *O. vulgare* and *O. syriaca*. Suitable protocols for callus induction, maintenance and establishment of cell culture system for the two species were investigated.

2. MATERIAL AND METHODS

2.1. *In vitro* Establishment of Plant Material

Seeds of *O. vulgare* L. var. Perenne, were purchased from La Semiorto-Sementi Salerno, (Italy). *O syriacum* L. seeds were collected from wild grown plants in the North of Jordan (Natfeh-Irbid). Seeds were washed thoroughly under running tap water for 15 min with few drops of mild detergent were added. Seeds were immersed in an antiseptic solution (5.0 % sodium hypochlorite plus two drops of Tween-20®) for 15 min under laminar flow cabinet. Seeds were rinsed with autoclaved distilled water for three times (5 min each) and then transferred into 70% (v/v) ethanol solution for 30 sec and rinsed with sterile distilled water 3 times (5 min each). Sterilized seeds of the two *Origanum* spp. were inoculated on the surface of half strength solid MS media (Murashige and Skoog, 1962). Germination media was supplemented with 2.0 mg/l Gibberellic Acid (GA_3) and 15 g/l sucrose. Seeds were kept in the dark at 21°C for 10 days until germination. Germinated seeds were transferred to growth chamber (16 h light/ 8 h dark photoperiod, at 24°C) for further growth. Internodal segments (10-12 mm) of both species were subcultured in Erlenmeyer flasks (250 ml) containing 50 ml solid MS media supplemented with 0.5 mg/l kinetin and 0.1 mg/l NAA, to establish sufficient mother stock cultures.

2.2. Callus Induction and Maintenance

Callus was induced in dark leaf discs. Explants were subcultured on the surface of gelled MS callus induction media in 9.0 cm sterile plates (25 ml each). Callus induction medium was composed by MS macro and micro-nutrients and supplemented with different levels

(0.0, 0.1, 0.5, 1.0, 1.5 or 2.0 mg/l) of 2,4-D. Data were recorded every week for one month after the inoculation of callus weight, color, and texture. Each treatment contained 40 replications. Induced calli were subcultured to fresh MS media supplemented with (0.5 mg/l) 2,4-D in combination with different levels (0.0, 0.5, 1.0, 1.5, 2.0 or 2.5 mg/l) of BA or TDZ. Each treatment contained 20 replications. Data were recorded after 30 days for callus diameter.

2.3. Cell Suspension Culture

Suspension cultures were initiated by inoculating 2.0 grams of fresh friable callus into 60 ml of MS liquid media. Media for suspension culture was supplemented with 1.5 mg/l TDZ. Cultures were incubated on a rotary shaker (100 rpm) under dark conditions at 24°C. After 10-15 days callus clumps larger than 2.0 mm were discarded and the remaining cultures were separate before subculture to fresh media supplemented with the same level of TDZ. Subsequent subcultures were performed at 14 days intervals by transferring 2.0 ml Settled Cell Volume (SCV) to liquid MS media supplemented with 1.0 mg/l TDZ. Flasks were closed with cotton plugs. Data were recorded every three days for cells fresh weight (mg) according to the procedure described by Shibli *et al*. (1999).

2.4. Oil Separation

O. vulgare L. and *O. syriacum* L. shoots were collected from greenhouse grown plants mid April (*ex vitro*), and from flasks after 8 weeks (*in vitro* grown on gelled MS media supplemented with 0.5 BA, 0.1 NAA and 30 g/l sucrose). Shoots were dehydrated in shade at room temperature for 10 days. Thereafter, they were weighed and ground by blender then macerated for 24-h in 500 ml distilled water at room temperature. Steam distillation was performed for 3 h by a modified steam distillation apparatus (Arafeh, 1999).

The essential oil was extracted from distillate by chloroform ($CHCl_3$). Chloroform layer was separated and then dried with Na_2SO_4. Chloroform was evaporated under reduced pressure (*in vacuo)* at 40°C by using a rotary evaporator. Essential oil content was determined gravimetrically on shoot dry matter basis. Oils extracted from callus and cells were determined on fresh weight basis.

Callus tissue, 14.6 g of *O. vulgare* L. and 12.0 g of *O. syriacum* L. respectively were immersed in 150 ml CH_2Cl_2 at 4°C for 24 h (Arafeh, 1999). The solvent was evaporated under reduced pressure and the oily residues were weighed and stored in dark at 4°C. Cells of the two *Origanum* species were collected after 15 days of incubation in liquid MS media supplemented with 1.5 mg/l TDZ and fresh weight was measured. Cells were mixed with chloroform and dried. Chloroform was evaporated under reduced pressure at 40°C, and the oily residues were weighed and stored in dark at 4°C.

2.5. Quantitative Analysis of Oils

The quantitative determination of different oil samples was done by using Gas Chromotography (GC) (Varian 3300 Gas Chromatograph) equipped by Flame Ionization Detector (FID) and coupled to an integrator (Unicam). For separation of thymol, a column of stainless steel (2m X 1/8") packed with 15% Carbo Wax 20 M, with W 80-100 mesh was used. The carrier gas was N_2 with a flow rate of 16 $ml.sec^{-1}$. Oven temperature was held at 180°C.

The injector and detector temperatures were held at 240°C and 250°C, respectively. Essential oil samples were diluted with n-hexane (extra pure), 4 µl from samples of callus and cell oil, and 2 µl from samples of greenhouse and *in vitro* grown plants were injected. Four oil samples of each treatment were analyzed separately. The area BC for thymol was averaged and thymol percentage was determined according to the calibration curve.

2.6. Experimental Design and Statistical Analysis

Each experiment was set up as a completely randomized design. Data were subjected to one-way ANOVA, data were statistically analyzed by MSTATC software (Michigan State University, 1988) and means were separated according to the Least Significant Difference (LSD) test at 0.01 level of probability.

3. RESULTS AND DISCUSSION

3.1 Callus induction and callus culture

Better callus texture, color and highest fresh weight were obtained when MS media was supplemented with low levels (0.1 to 0.5 mg/l) of 2,4-D (Table 1). Increasing 2,4-D levels (1.0 to 2.0 mg/l) caused callus to be gelatinous and brown in color, and overall fresh weight reduction was noticed (Table 1). No callus induction was obtained without the addition of 2,4-D. Kumari and Saradhi (1992) were able to initiate callus of *O. vulgare* L. on B5 media supplemented with 0.2 mg/l 2,4-D. Also 2,4-D was reported by Stafford and Warren (1991) to be essential to induce callus in dicotyledonous plants.

Callus induction in *O. vulgare* L . seems similar to that of *O. syriacum* L. The healthy and friable callus with high fresh weight was obtained at low levels of 2,4-D (0.1 or 0.5 mg/l). Induced calli transferred to MS media supplemented with 0.1 or 0.5 mg/l 2,4-D showed rhizogenesis (data not shown).

Media supplemented with 1.0 mg/l TDZ showed the highest callus diameter in *Origanum vulgare*. Addition of 0.5 mg/l 2,4-D to media containing TDZ had inhibitory effects on callus diameter (Table 2). Weak callus growth of *Origanum vulgare* L. was obtained on solid MS media supplemented with different levels of BA (Table 2). Calli grown on BA supplemented media (with or without 0.5 mg/l 2,4-D) were pale brown in color and compact in texture. Phenolic browning was observed on the media after two weeks of incubation.

When TDZ was used alone on *O. syriacum*, 1.5 mg/l gave the highest callus diameter (Table 2). Callus grown on 1.5 mg/l TDZ was friable, and successfully subcultured for 3 successive generations on the same media. The combination of 2,4-D (0.5 mg/l) with TDZ (1.5 mg/l) resulted in high callus diameter (Table 2). Higher concentrations of TDZ (2.0 or 2.5 mg/l) showed inhibitory effect on callus growth and no phenolic browning was observed on media with TDZ treatments (with or without 2,4-D). BA at 1.5 in combination with 0.5 mg\l 2,4-D gave the highest callus growth.

No root formation was observed in *O. vulgare* L. or *O. syriacum* L. in all treatments (BA or TDZ with or without 2,4-D). Tamura *et al.* (1993) found three callus colors, green, yellow and white callus in cell lines from *T. vulgari*s L. after 31 subcultures. In our current study, after few subcultures we obtained yellow callus from *Origanum vulgare* L. and white callus from *Origanum syriacum* L.

3.2. Cell Suspension Culture

Calli clumps from the third generation which had been grown on 1.5 mg/l TDZ were friable and able to provide acceptable cells to start cell suspension culture. Cell growth was demonstrated on liquid MS media supplemented with (1.0 mg/l) TDZ (Figure 1) by recording cell fresh weight every three days. Results showed the need for subculturing to new fresh media between day 16 and 17 of incubation. This is because the early stationary phase onsets at that time. Stafford and Warrren (1991) mentioned that it is better to subculture cells at the end of exponential growth phase. After linear growth stage, media became exhausted and toxic substances were produced by cells (Bhojwani and Razdan, 1983).

3.3. Essential Oils and Quantitative Analysis

Moisture and oil content of different tissues vary among the two species and within different tissues of the same species (Table 3). Essential oil (percentage) obtained from *in vitro* grown *O. vulgare* L. is 6 fold more than shoots of greenhouse grown plants. Callus and cells gave very little amount of oil compared to intact plants (*ex vitro* and *in vitro*) in *O. syriacum* L. *In vitro* grown *O. vulgare* L. plants produced the highest amount of oil compared to greenhouse plants, callus or cells.

Gas chromatographic analysis and thymol identification confirm the presence of thymol in *O. syriacum* L. (*in vitro* plants = 5.94% and *ex vitro* = 13.09%). No thymol was identified in the oil of callus or cells of *O. syriacum* L. Also , no thymol was identified in *Origanum vulgare* L. in all tissues analyzed.

Studies in different geographical areas reported high changes in oil percentage and its constituents produced from *O. syriacum* L. and *O. vulgare* L. for example:

distilled oil from *O. syriacum* L. in Lebanon averaged 3% of dry weight (Daouk *et al*., 1995). Thymol percentage in the same study was reported to be 4% during August and 14% during October. Dudai *et al*. (1988) reported a change in thymol percentage between two different chemotypes of *O. syriacum* L.; chemotype (A) yield 0.7% thymol and the other chemotype (B) yield 52.8%. Putievsky *et al.* (1996) reported thymol percentage in the essential oil to reach the highest level (57%) at June harvest and lowest (37%) at October harvest.

The presence of thymol in *O. syriacum* L. grown under *in vitro* conditions indicates that these plants are capable to produce thymol under controlled environment (nutrient media, light and temperature). Thus, changing thymol production can be achieved if any manipulation in the *in vitro* conditions happened (pH, nutrients, light) to attain the preferable environmental conditions of certain spp. The study of Economakis and Demetzos (1999) reported thymol to comprise 6.55% of the essential oil from wild *O. dictamnus* L. leaves. In the same study, leaves of hydroponically grown plants produced 3.5 % oil while wild plants produced 0.8 % from leaves. These results are similar to our study in *O. syriacum*, where thymol percentage in greenhouse grown plants exceeded the percentage of *in vitro* grown plants, also oil content was higher in *ex vitro* grown plants than *in vitro* grown ones.

Callus and cells in this study failed to produce thymol due to the lack of the special secretory structures (oil glands) needed to store monoterpenes such as thymol. Very few amounts (0.02%) of thymol were detected in oil of *T. vulgaris* L. callus tissue Tamura *et al*. 1993. Chang *et al.* (1998) improved menthol production up to 40 folds after elicitation with chitosan in cell suspension culture of *Mentha* spp. (*Labiatae*). This indicates the probability to increase the production of specific metabolite via elicitation. Furmanowa and Olszowska (1992) reported changes in callus oil quantity and quality after changing plant growth regulator and adding precursors (mevanolic acid 1 g/10 ml).

In conclusion**,** maintenance and subsequent growth of induced calli depend on cytokinins rather than 2,4-D, where using TDZ showed better callus growth and diameter than BA. Cell suspension can be maintained by subculturing every 17 days. Furthermore, *O. syriacum* L. can be considered a promising oil producing variety, different from *O. vulgare* L. where thymol was not detected.

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Table (1): Effect of different levels of 2,4-D on *O. vulgare* L. and *O. syriacum* L. callus fresh weight (mg).

	***O. vulgare* L.**					***O. syriacum* L.**				
2,4-D (mg/l)	**Week**					**Week**				
	0	1	2	3	4	0	1	2	3	4
	Callus fresh weight (mg)									
0.1	5.0 [1]	16.3	26.8	38.0	41.0	5.0	12.5	18.0	35.0	44.0
0.5	5.0	16.0	30.9	40.2	44.3	5.0	11.8	21.5	30.0	37.0
1.0	5.0	14.3	30.3	29.5	34.1	5.0	11.0	16.0	18.8	24.7
1.5	5.0	14.5	19.1	16.0	17.0	5.0	10.0	13.0	17.0	19.0
2.0	5.0	11.0	17.3	23.1	22.1	5.0	10.0	17.3	22.0	23.0
LSD			**7.0**					**6.6**		

[1] A control (0.0 mg/l 2,4D) was not shown as no growth occurred and callus turned brown after three weeks.

Table (2): Effect of different levels of TDZ or BA (with or without 0.5 mg/l 2,4-D) on callus diameter (mm) of *O. vulgare* L. and *O. syriacum* L. after 30 days of incubation on MS gelled media.

2,4-D (0.5 mg/l)	TDZ (mg/l)					BA (mg/l)				
	0.5	1.0	1.5	2.0	2.5	0.5	1.0	1.5	2.0	2.5
	***O. vulgare* L.**									
Without	10.7	12.5	11.7	10.0	10.0	8.0	8.5	8.9	8.0	7.8
With	8.6	8.8	9.2	8.6	8.2	9.1	9.8	9.0	8.6	8.1
	LSD 0.70					**0.84**				
	***O. syriacum* L.**									
Without	8.1	10.1	13.0	11.5	9.6	7.9	8.0	8.2	8.8	7.8
With	8.0	10.0	12.0	10.4	10.0	7.4	7.9	9.0	8.1	7.0
	LSD 0.80					**0.66**				

Table (3): Weight, moisture content, oil content and percentage and thymol percentage from different tissues of *O. vulgare* L. and *O. syriacum* L

Species	Greenhouse Grown plants	*In vitro* Grown Plants	Callus	Cells
***O. vulgare* L.**				
Fresh Weight (g)	226	197	14.6	5.522
Moisture Content (%)	90.0%	90.7%	92.3%	93.3%
Room Dehydrated Weight (g)	47.0	30.0	-	-
Moisture Content (%)	55.8%	39.0%	-	-
Dry Weight (g)	20.74	18.16	1.12	0.368
Oil Weight (mg)	20	80	14.2	3.4
Oil %	0.042	0.266	0.095	0.06
Thymol %	-	-	-	-
***O. syriacum* L.**				
Fresh Weight (g)	176	55	12.0	7.94
Moisture Content (%)	90.1%	91.4%	92.0%	94.8%
Room Dehydrated Weight (g)	36.36	7.8	-	-
Moisture Content (%)	57.0%	40.0%	-	-
Dry Weight (g)	15.62	4.68	0.96	0.410
Oil Weight (mg)	640	92	14.4	5.3
Oil %	1.76	1.17	0.11	0.06
Thymol %	13.09	5.94	-	-

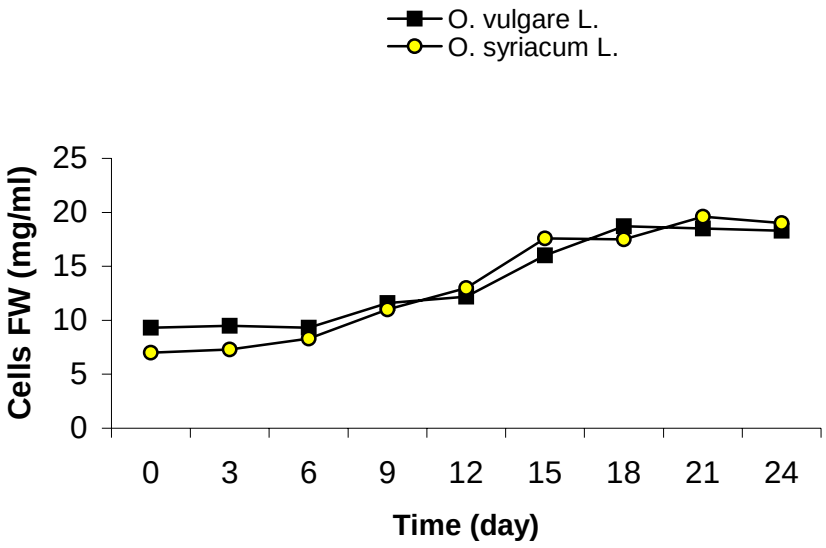

Figure 1. Growth of *O. vulgare* L. and *O. syriacum* L. cells during 24 days of incubation in MS media containing 1.0 mg/l TDZ.

REFERENCES

Arafeh, R. 1999. ***Factors Affecting In Vitro Propagation, Callusing, Cell Suspension Culture and Secondary Metabolites Production in Sweet Marjoram Origanum vulgare L. and Syrian Marjoram Majorana syriaca L. Rafin Origanum syriacum L***. M.S. Thesis, Jordan University of Science and Technology. Irbid, Jordan.

Bhojwani, S.S. and Razdan, M.K. 1983. ***Plant Tissue Culture: Theory and Practice***. Elsevier Science Publishing Company, Inc. 43-53.

Chang, J.H., Chin, J.H., Chung, I.S. and Lee, H.J. 1998. Improved Menthol Production from Chitosan-elicited Suspension Culture of *Mentha piperita*. ***Biotechnol. Lett***., 20, 1097-1099.

Conner, D.E. and Beuchat, L.R. 1984. Effects of Essential Oils from Plants on Growth of Food Spoilage Yeasts**. *J. Food Sci***., 49, 429-434.

Crowell, P.L 1999. Prevention and Therapy of Cancer by Dietary Monoterpenes. ***J. Nut***., 129, S775-S778.

Daouk, R.K., Dagher, S.M. and Sattout, E.J. 1995. Antifungal Activity of the Essential Oil of *Origanum syriacum* L. ***J. Food Prot***., 58, 1147-1149.

Dudai, N., Werker, E., Putievsky, E., Ravid, U., Palevitch, D. and Halevy, A.H. 1988. Glandular Hairs and Essential Oils in Leaves and Flowers of *Majorana syriaca*. ***Isr. J. Bot***., 37, 11-18.

Economakis, C., Demetzos, C., Anastassaki, T., Papazoglou, V., Gazouli, M., Loukis, A., Thanos, C.A. and Harvala, C. 1999. Volatile Constituents of Bracts and Leaves of Wild and Cultivated *Origanum dictamnus* L. ***Planta Medica***., 65, 189-191.

Furmanowa, M. and Olszowska, O. 1992. Micropropagation of Thyme *Thymus vulgaris* L. In: BAJAJ, Y.P.S(ed.), Biotechnology in Agriculture and Forestry. High Tech and Micropropagation III. Springer-Verlag Heildelberg, Berlin, 19: 230-234.

Gilman, A.G., Goodman, L.S., Rall, T.W. and Murad, F. 1985. ***The Pharmacological Basis of Therapeutics.*** 7th ed. Macmillan Publishing Company, P. 970.

Kokkini, S. 1996. Taxonomy, Diversity and Distribution of Origanum Species. In: Padulosi, S. (ed.), Ciheam, Valenzano, Oregano proceedings of the International Plant Genetic Resources Institute (IPGRI); International Workshop on Oregano, 8 – 12 May, 1996. Bari, Italy.

Kumari, N. and Saradhi, P.P. 1992. Regeneration of Plants from Callus Culture of *Origanum vulgare* L. ***Plant Cell Rep***., 11, 476-479.

Manou, I., Bouillard, L., Devleeschouwer, M. J. and Barel, A. O. 1998. Evaluation of the Preservative Properties of *Thymus vulgaris* L. Essential Oil in Tropically Applied Formulations under a Challenge Test***. J. App. Microbiol***., 84, 368-376.

Murashige, T. and Skoog, F. 1962. A Revised Medium for Rapid Growth and Bioassays with Tobacco Tissue Cultures***. Physiol. Plant***., 15, 473- 497.

Oran, S. 1994. Genetic Resources of Medicinal Plants in Jordan. Plant Genetic Resources of Jordan. International Plant Genetic Resources Institute (IPGRI)., Proceeding of National Seminar. Amman, Jordan, 117-126.

Putievsky, E., Dudai, N. and Ravid, U. 1996. Cultivation, Selection and Conservation of Oregano Species in Israel. In: Padulosi, S. (ed.). Ciheam, Valenzano, Oregano, proceedings of the International Plant Genetic Resources Institute (IPGRI); International Workshop on Oregano, 8 – 12 May, 1996. Bari, Italy.

Reynolds, J.E.F. 1982. ***Martindale the Extra Phrmacopoeia***. (29th ed.). The Pharmaceutical Press, London, pp. 1067-1068.

Shibli, R.A., Smith, M.A. and Kushad, M. 1999. Influence of Ethylene on the Production of Anthocyanins and Carotenoids from Callus Culture of *Vaccinium pahalae*. ***Dirasat***. 26(2), 240-247.

Simon, J.E. 1990. Essential Oils and Culinary Herbs. In: Janick, J. and J.E. Simon (eds.), Advances in New Crops. Timber Press, Portland, OR., pp.472-483.

Stafford, A. and Warren, G. 1991. ***Plant Cell and Tissue Culture***. Open University Press, Milton, Keynes.,7-23.

Svoboda, K.P., Finch, R.P., Cariou, E. and Deans, S.G. 1995. Production of Volatile Oils in Tissue Culture of *Origanum vulgare* L. and *Tanacetum vulgare*. ***Acta. Hort.***, 390, 147-152.

Tamura, H., Takebayashi, T. and Sugisawa, H. 1993. Thymus Vulgaris L. (Thyme): *in vitro* Culture and the Production of Secondary Metabolites. In: Bajaj, Y.P.S (ed), Biotechnology in Agriculture and Forestry. ***Medicinal and Aromatic Plants IV***, Springer-Verlag Heildelberg, Berlin, 21: 413-426.

تكوين الكلّس والخلايا المعلقة وإنتاج المواد الثانوية من الزعتر الفارسي (*Origanum vulgare* L.) والزعتر البلدي(*O. Syriacum* L.)

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ملخـص

لقد تم تأسيس نبات الزعتر الفارسي (*Origanum vulgare* L.) والزعتر العربي (*O. syriacum* L.) داخل الأنابيب من البذور في وسط موراشيك وسكوك (MS) يحتوي على 2 ملغم/ لتر من الجبرلين مضافاً إليه 15غم/لتر سكروز. تم تأسيس الكلس من الأوراق في نفس الوسط مضافاً إليه مستويات مختلفة من داي كلوروفينوكسي أستيك اسيد (2,4-D) (2, 1.5, 1.0, 0.5, 0.1, 0.0) وقد نتج اكبر حجم من الكلس عن إضافة مستويات متدنية من 2,4-D (0.5/0.1) ملغم/ لتر. كما درست متابعة نمو الكلس على مستويات مختلفة من البنزيل أدينين (BA) والثيادياز ورون (TDZ) مع أو بدون 0.5 ملغم/ لتر 2,4-D. وكان اكبر حجم قطري من الكلس (12.5 mm) من الزعتر الفارسي نتج بإضافة 1 ملغم/ لتر TDZ. إن إضافة 0.5 ملغم/ لتر2,4-D كان مثبطا لنمو الكلس بعد 30 يوماً من الحضن عند استعماله مع TDZ أو BA. وقد أعطى BA حجما اقل من الكلس مقارنة ب TDZ للصنفين. إن أفضل نمو للكلس الناتج من الزعتر الفارسي كان بإضافة 1.5 ملغم/ لتر TDZ مع أو بدون 2,4-D، وكان الكلس الناتج من الجيل الثالث سهل التفتيت وسهلا لنمو الخلايا المعلقة. وإن افضل إنتاج للخلايا المعلقة تم عند نقلها إلى أوساط جديدة كل 17 يوماً في وسط سائل يحتوي على 1 ملغم/ لتر TDZ للصنفين. لقد اعطت نباتات الزعتر العربي النامية في البيت الزجاجي زيتاً (1.76%) أعلى من النباتات النامية في الأنابيب (1.17%)، بينما كان انتاج الزعتر الفارسي قليلا للزيت. تم التعرف على وجود الثايمول بواسطة جهاز gas chromatography في نبات الزعترالعربي. كانت نسبة الثايمول في النباتات من خارج الأنابيب 13%، بينما أعطت النباتات النامية في الأنابيب 5.9%. ولم يتم التعرف على الثايمول في نبات الزعتر الفارسي.

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