J. Plant Production, Mansoura Univ., Vol. 8(11) :1241 - 1246, 2017

Indirect Micropropagation of *Thymus vulgaris* Plant.

El-Banna, H. Y.
Vegetable & Floriculture Department, Faculty of Agriculture, Mansoura University.

ABSTRACT

A protocol for indirect micro propagation of *Thymus vulgaris* was developed using shoots tips explants derived from *ex vitro* mother plants. Murashige and Skoog (MS) Medium with different concentrations of auxins (NAA, IAA and 2,4-D) alone or in combination with different cytokinins (BA and Kin) were used to induce callus formation. MS medium supplemented with 2,4-D at 2 mg/L in combination with Kin at 1 mg/L produced the highest callus formation percentage of 100 % and significantly counted spectacularly callus fresh weight of 9.56 g. The highest Callus regeneration percentage (100 %) commitment to the highest significant shoots number (23.42 shoots/ explant) were obtained on MS medium provided with BA at 2.5 mg/L. The preferable rooting of shoots was obtained on MS medium half strength augmented with 30 g/L sucrose and NAA at 1.5 mg/L. On this medium, 100 % of the shoots produced roots with a mean number of 19.83 roots per shoot. Shoots with well-developed roots were successfully established in pots filled with mixture of soil: peat moss (1: 1 v/v). The survived plantlets had a survival rate of 98 %.

INTRODUCTION

Aromatic and medicinal plants have played a vital function in the evolution of people's life worldwide. The use of herbal medicine showed significant increase in the last decade. A lot of modern medicines and many of new drugs are created from medicinal plants indirectly (Calixto 2000; Prasanth *et al.* 2014). Thyme (*Thymus vulgaris*) is very important an aromatic and medicinal plant, which belongs to the Lamiaceae family. It is a flowering plant growing up to 15-30 cm tall by 40 cm wide (Christopher 2008). The essential oil from *T. vulgaris* showed a low contents of monoterpene hydrocarbons (28.69 %) and high content of oxygenated monoterpenes (56.53 %), sesquiterpene hydrocarbons (5.04 %) and oxygenated sesquiterpenes (1.84 %) (Maher 2011). The natural terpenoid thymol and its phenol chemical compound carvacrol are the main composition of this oil (Thompson *et al.* 2003; Borugă *et al.* 2014). In addition to the flavoring properties determined by the constitutive active ingredients, the thyme essential oil exhibits significant antimicrobial activity (Dorman and Deans 2000; Rota *et al.* 2008) as well as strong antioxidant properties (Grigore *et al.* 2010).

In nature, the species propagates through seeds and stem cuttings. Based on preliminary investigations on propagation of thyme with seed is hindered due to poor germination. Ozudogru *et al.* (2011) found that after 45 days of sowing seeds of *Thymus vulgaris* in soil the germination was very poor about 0.80 % germination percentage. Also, Nordine *et al.* (2013) reported that a germination seed *ex vitro* was poor and produced 5.5 % germination rate only. While, propagation with vegetative cuttings need a lot of caring and the survival rate is very limited. Traditional propagation through seeds and vegetative cuttings is not enough to meet the demand of this important medicinal plant. Thus, for the swift multiplication and preservation of this plant alternative propagation method especially *in vitro* techniques are necessary. Tissue culture technique for plant has been known as an alternative method of plant propagation and production of great number of uniform, elite clones in a confined period of time (Kumar and Thomas 2012). Tissue culture is a collection of lab methods that rely on TotiPotency property of an organ, tissue, cell, or even part of a cell, produce a complete and consistent plant with the intended purpose (Piri and Firoozabadi 2006). It has two ways (direct and indirect methods), in the indirect methods the cultured explant is form callus tissues and this callus subculture into a new medium stimulating shoots proliferation. Indirect *in vitro* propagation through callus culture is considered to be the most efficient method for crop improvement by the production of somaclonal and gametoclonal variants (Ziba *et al.* 2016). Using this technology gave a great possibility to produce high quality plants and allows the isolation of plants with desirable traits like better disease resistance and stress tolerance (Brown and Thorpe 1995).

Hence, this research aim to describes successful an efficient protocol for indirect *in vitro* propagation of *Thymus vulgaris* to achieve high-frequency shoot induction and plant regeneration, starting from a callus culture of *T. vulgris*.

MATERIALS AND METHODS

Plant material:

Shoot tips from *ex vitro* mother plants of *Thyums vulgaris* were used as explants. Shoot tips about 1 cm in length were collected from an adult plant growing in the farm of Medicinal and Aromatic plant, Faculty of Agriculture, Mansoura University. Explants were thoroughly washed with tap water containing a few amount of household detergent for an hour. The surface sterilization was done with sodium hypochlorite at 3 % for 12 minutes and then washing three times with sterile distilled water for 4 minutes each. After that the shoot tips were cultured onto the culture medium.

Media and culture conditions:

Murashig and Skoog (1962) nutrient medium was used in all experiments supplemented with 3% (w/v) sucrose. The medium was solidified with 7g agar /l (w/v) and the pH of the medium was adjusted to 5.8 before autoclaving at 121° C for 20 min. All the cultured jars (250 ml) contained 30 ml of medium were incubated in plant growth room at 25 ± 2°C under constant fluorescent light of 2500 Lux for 16/8 h (light/ dark) photoperiod.

Callus induction:

First experiment was conducted in order to study the effects of various concentrations of different auxins on callus induction from the shoot tips, the MS basal medium was supplemented with different auxins a-naphthalene acetic acid (NAA), indole-3-acetic acid (IAA) and 2,4-Dichlorophenoxyacetic acid (2,4-D) at different

concentrations of 0.0, 0.5, 1, 1.5, 2 and 3 mg/L. A factorial experiment in a randomized complete block design was used with 4 replicates included 12 jars for each treatment. After 30 day of culture, data were recorded.

Second experiment was carried out in order to study the effect of combination between auxin and cytokinin on callus induction. The best single auxin results obtained from the first experiment (2,4-D at 2 mg/L and NAA at 1.5 mg/L) were further combined with three cytokinins; 6-benzyladenine (BA), kinetin (Kin) and thidiazuron (TDZ) at a concentration of 1 mg/L. A completely randomized design was used with 4 replicates included 12 jars for each treatment. After 30 day of culture, data were recorded.

Callus regeneration:

For plant regeneration, organogenic, friable callus derived from MS medium supplemented with 2,4-D at 2 mg/L in combination with Kin at 1 mg/L (the best result in the second experiment) were cut into piece of 1 × 1 cm^2 and inoculated on MS medium supplemented with two types of cytokinins (BAP and Kin) each alone at different concentrations of (1.0, 1.5, 2.0, 2.5 and 3.0 mg/L). Observations on the percentage of callus forming shoots, shoots number, shoot length and leaves number per responding cultures were recorded. After 4 weeks of culture, data were recorded. A completely randomized design was used with 4 replicates included 12 jars for each treatment.

Induction of rooting and acclimatization:

For root induction, the effect of MS basal medium strength (3/4 or 1/2 strength), two carbon source (sucrose or glucose) at different concentrations (20, 30 and 40 g/L) was studied. NAA was added to the media at concentration of 1.5 mg/L (the best concentration obtained for rooting in different researches). A factorial experiment in a randomized complete block design was used with 4 replicates included 12 jars for each treatment. The *in vitro* plantlets were bring out from the gars, rinsed under tap water to clean all traces of media and then single plantlets were cultured onto pots filled with soil: peat moss (1: 1 v/v).

Statistical analysis:

Data of all experiment was subjected to analysis of variance (ANOVA) by the general linear models (GLMs) procedure using (SAS) Statistical Analysis System (2000). Mean comparisons were performed using the least significant difference (LSD) method according to (Gomez and Gomez, 1984). A significance level of 5 % was used for all statistical analyses.

RESULTS AND DISCUSSION

I- Callus induction stage:

1- Effect of auxin type, auxin concentration and their interactions on callus formation of *Thymus vulgaris* shoot tips.

This experiment was conducted to test the effect of different auxins (NAA, IAA and 2,4-D) at various concentrations 0.0, 0.5, 1.0, 1.5, 2.0 and 3.0 mg/L as well as their interactions on callus formation percentage, callus weight. The results were recorded after 4 weeks of culture on MS medium and are shown in Table (1).

Effect of auxin type (A) on callus formation:

Concerning the effect of auxin type on callus formation percentage and callus weight, data in Table (1) showed that significant differences were noticed among the different used auxins, since medium supplemented with 2,4-D recorded the highest significant callus formation percentage (58.3 %) followed with media supplemented with NAA (48.5 %).

Table 1. Effect of auxin type, auxin concentration and their interactions on callus formation of *Thymus vulgaris*.

Parameters	Auxin type (A)	Auxin concentrations (mg/L) (B) 0.0	0.5	1.0	1.5	2.0	3.0	Mean of (A)
Callus induction (%)	NAA	0.0	41.7	58.3	75.0	66.7	50.0	48.6
	IAA	0.0	25.0	33.3	33.3	41.7	58.3	31.9
	2,4-D	0.0	58.3	66.7	66.7	83.3	75.0	58.3
Mean of (B)		0.0	41.7	52.8	58.3	63.9	61.1	
LSD at 5%			A 7.4		B 10.5		A×B 18.2	
Callus fresh weight (g)	NAA	0.0	3.75	4.65	6.69	6.31	5.66	4.50
	IAA	0.0	0.40	0.87	1.47	2.33	2.78	1.30
	2,4-D	0.0	4.43	5.63	5.38	6.80	5.70	4.70
Mean of (B)		0.0	2.90	3.70	4.50	5.20	4.70	
LSD at 5%			A 0.13		B 0.19		A×B 0.33	

It was clear from the same table that adding 2,4-D on the nutrient medium significantly produced the heaviest callus fresh weight of 4.7 g, when compared with adding NAA or IAA, since it was 4.5 and 1.3 g, respectively as shown in Fig. 1.

Effect of auxin concentration (B) on callus formation:

Regarding the effect of auxin concentration on callus formation percentage as shown in Table (1), the obtained results showed a positive relationship between auxin concentrations and callus formation percentage, it

was noticed that every increase in auxin concentrations from 0.5 mg/L up to 2.0 mg/L gradually increased callus formation percentage. The highest recorded percentage (63.9 %) was obtained with auxin at 2.0 mg/L and increasing auxin concentration to 3 mg/L reduced callus formation percentage to 61.1 %. In contrast, the control medium (free auxin medium) failed to perform callus. The results of Mahmoud *et al.* (2011) on lavender plant confirm our results. From a long time it was confirmed that the action of auxins was in relationship with the concentration applied.

Concerning the effect of auxin concentration on callus fresh weight, the highest significant value of callus fresh weight (5.2 g) was obtained when MS medium was supplemented with auxin at 2.0 mg/L.

Effect of the interaction between auxin type and auxin concentration (A×B) on callus formation:

Data presented in the same table revealed that adding 2,4-D on the nutrient media at the highest concentrations of 2 mg/L recorded the highest callus formation value of 83.3%. In contrast, the control medium (free auxin medium) did not record any callus formation.

Concerning the effect of the interaction between auxin type and auxin concentrations on callus fresh weight, data in Table (1) cleared that using medium supplemented with 2,4-D at 2 mg/L or NAA at 1.5 mg/L significantly recorded the heaviest callus fresh weight of 6.80 and 6.69 g, respectively. On the other hand, using IAA on medium at all different concentrations significantly recorded the lowest callus fresh weight.

Generally for callus induction, the presence of a strong auxin like 2,4-D or NAA is necessary. Simon and Petrasek (2011) referred that to the important roles of both auxin in cell enlargement, stimulating cell division and elongation by increasing the plasticity of the cell wall. When extensibility of the wall is increased, the wall pressure around the cell decreases and the turgor pressure caused by osmotic forces in the vacuolar sap causes water to enter the cell, resulting in cell enlargement. Also, Gautheret (1955) reported that for the culture of a number of callus tissues, auxin is the essential supplement which is needed to be added to the basal medium for supplying inorganic ions and sugars. In the present investigation incorporation of 2,4-D was found to have beneficial effect on callus formation and this was also reported in other medicinal plants such as *Ocimum sanctum* (Lim *et al.* 2009) and *Achyranthes aspera* (Sen *et al.* 2014).

2- Effect of the interaction between auxin type and cytokinin type on callus formation of *Thymus vulgaris* shoots tips.

The goal of this phase of the experiment was to study the effect of various auxins (NAA at 1.5 mg/L and 2,4-D at 2 mg/L) and various cytokinins (BA, Kin and TDZ at 1 mg/L) in combinations. Data in Table (2) showed that culturing the shoot tip on MS medium supplemented with NAA in combination with Kin recorded the lowest callus formation value of 75 %, when compared with all of the other cases. On the other hand, culturing shoots tips on media supplemented with 2,4-D in combination with BA or Kin produced the highest callus formation percentage of 100 %.

It could be noticed that, the callus fresh weight was affected by the combination between auxin type and cytokinin type. In this concern, 2,4-D at 2 mg/L in combination with Kin at 1 mg/L significantly counted spectacularly callus fresh weight of 9.56 g when compared with all of the other treatments (Table 2 and Fig. 2A). The next positive effect in that respect was 8.46 g, when using 2,4-D at 2mg/L in combination with BA at 1 mg/L.

Table 2.Effect of the interaction between auxin type and cytokinin type on callus formation of *Thymus vulgaris*.

Treatments		Callus induction (%)	Callus fresh weight (g)	Callus regeneration (%)	Shoots Number
Auxin type	Cytokinin type at 1 mg/ L				
NAA at 1.5 mg/L	BA	91.7	7.38	58.3	3.38
	Kin	75.0	6.84	41.7	1.62
	TDZ	91.7	7.21	16.7	0.75
2,4-D at 2 mg/L	BA	91.7	8.46	41.7	2.25
	Kin	100	9.56	33.3	1.50
	TDZ	100	8.19	0.0	0.00
L.S.D. at 0.05		20.2	0.17	21.0	0.85

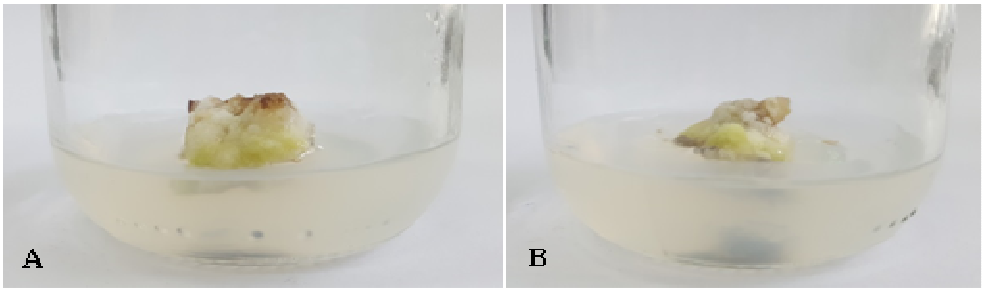

Figure 1. A) Callus formation of *Thymus vulgaris*. obtained by culturing shoot tip on MS medium supplemented with 2 mg/L of 2,4-D. B) Callus formation, obtained by culturing shoot tip on MS medium supplemented with 1.5 mg/L of NAA..

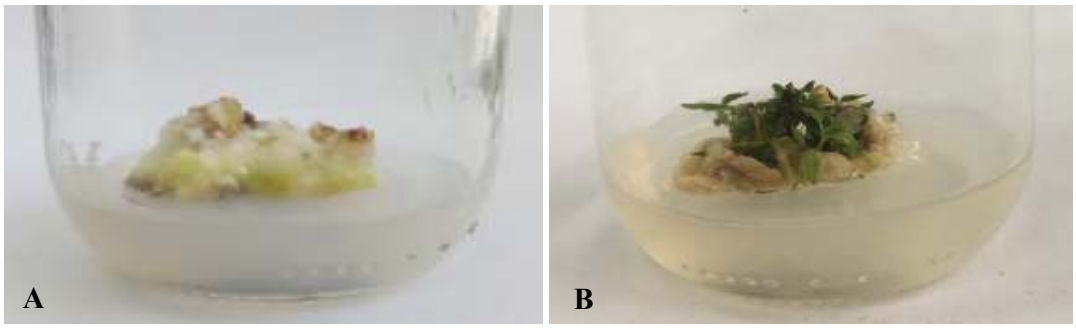

Figure 2. A) Callus formation of *Thymus vulgaris*. obtained by culturing shoot tip on MS medium supplemented with 2 mg/L of 2,4-D in combination with Kin. B) Callus formation, obtained by culturing shoot tip on MS medium supplemented with 1.5 mg/L of NAA in combination with BA.

In addition, it was a matter of importance to note a percentage of callus regeneration commitment with a positive relationship of shoots number. As, the highest callus regeneration percentage of 58.3 % and the highest significant shoots number of 3.38 shoot obtained when MS medium supplemented with NAA and BA at 1.5 and 1 mg/L, respectively as shown in Fig. 2B. While, it was cleared that no callus regeneration on MS medium supplemented with 2,4-D and TDZ.

Results obtained cleary indicated that callus formation was affected by the combination between auxin and cytokinin type. A ratio of auxins and cytokinins greater than one in the nutrient medium was effective for the induction of callus in different plants (Nikam and Savant 2009). In this study, 2, 4-D in combination with different cytokinins was found to be essential for callus induction. Our studies are in line with (Sen *et al.* 2014) on *Achyranthes aspera* (Akram *et al.* 2010) on *Thymus vulgaris* whom reported that that the maximum frequency of callus induction was obtained on MS medium containing 1:0.5 ratio of 2, 4 D:Kin (mg/L). The efficiency of 2,4-D in combination with cytokinins in induction of callus might due to their function in mitosis and DNA synthesis (Skoog and Miller 1957).

II- Callus regeneration stage:

1- Effect of cytokinin type at different concentrations on callus regeneration of *Thymus vulgaris*.

The callus formed from the previous experiment was cultured on MS medium supplemented with various concentrations (1.0, 1.5, 2.0, 2.5 and 3.0 mg/L) of BA or Kin for shoot regeneration (Table 3). The callus increased in bulk and after 4 weeks of culture on regeneration medium numerous shoots were appeared on the surface of the callus. Among the two cytokinins used, BA at different concentrations was comparatively superior in terms of Callus regeneration percentage and shoots number. The highest Callus regeneration percentage (100 %) commitment to the highest significant shoots number (23.42 shoots/ explant) were obtained on MS medium provided with BA at 2.5 mg/L (Fig. 3A). Also, MS medium supplemented with Kin at 3 mg/L gave 100 % Callus regeneration but with less shoots number (18.75 shoots/ explant).

As for shoot length and leaves number per shoot, data in Table (3) showed that for both cytokinin type (BA and Kin) there was a positive relationship between increments in concentrations and both characters. But, the highest significant shoot length (3.82 cm) and leaves number per shoot (9.25 leaves/ shoot) were recorded with callus cultured on MS media supplemented with Kin at 3 mg/L (Fig. 3B).

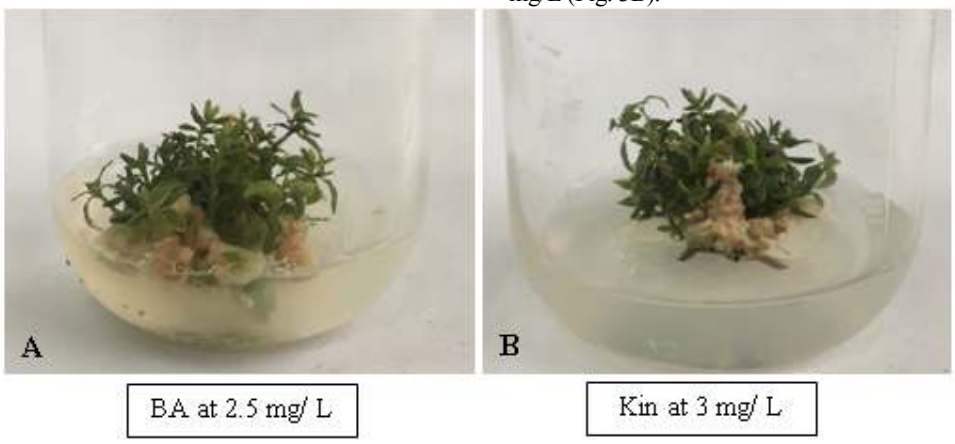

Figure 3. Effect of cytokinin type at different concentrations on callus regeneration of *T. vulgaris*.

Results are in agreement with data by Ziba *et al.* (2016) on *Thymus persicus* who reported that using BA or Kin in regeneration medium gave the same callus regeneration percentage but BA was more effective than Kin in inducing shoots number while Kin was more effective in shoot length. BA is a strong cytokinin and is

routinely used either alone or in combination with low amount of auxin for shoot induction from callus (Irvani *et al.* 2010). According to Sharma and Wakhlu (2003), the superior role of BA over other cytokinins may be due to the capability of plant cells and tissues to metabolize natural plant growth regulators more easily than synthetic hormones.

Table 3. Effect of cytokinin type at different concenterations on callus regeneration of *Thymus vulgaris*.

Treatments cytokinin type	cytokinin conc. mg/L	Callus regeneration (%)	Shoots Number/ explant	Shoot Length (cm)	Leaves Number/ shoot
BA	1.0	66.7	9.25	2.24	7.22
	1.5	83.3	14.21	2.54	7.43
	2.0	100	19.16	2.72	7.94
	2.5	100	23.42	2.91	8.13
	3.0	91.7	13.83	3.20	8.42
Kin	1.0	41.7	7.37	2.66	7.56
	1.5	50.0	9.375	2.82	7.81
	2.0	75.0	12.54	3.13	8.26
	2.5	83.3	15.12	3.28	8.60
	3.0	100	18.75	3.82	9.25
L.S.D. at 0.05		20.14	0.62	0.09	0.10

Induction of rooting and acclimatization:

1- Effect of media strength, sugar type at different concentrations on rooting behavior of *Thymus vulgaris* shoots.

This experiment was conducted to test the effect of MS media strength, suger type and sugar concentration on rooting parameters of *Thymus vulgaris* shoots, i.e., rooting percentage, roots number per shoot and root length. The results were recorded four weeks after culture and were presented in Table (4).

Data in Table (4) clearly showed that culturing the shoots on half strength MS medium supplemented with 20 or 30 g/L sucrose achieved the highest rooting percentage of 100 % for each, when compared with all of the other treatments. Also, it was a matter of importance to observe that the previous treatments gave the highest roots number per shoot (12.41 and 19.83 roots, respectively) with a significant differences between the two treatments. Also, the most efficient treatment in enhancing rooting with the highest roots number gave suitable root length (8.42 cm) (Fig. 4A). Generally increasing sugar concentrations from 20 g/L up to 40 g/L significantly in all treatments increased root length and the highest length of root 10.71 cm was achieved with MS medium half strength furthered with glucose at 40 g/L (Fig. 4B). On the other hand, the lowest treatment for rooting percentage (66.7 %) and roots number per shoot (5 roots) was ¾ strength MS medium supplemented with glucose sugar at 40 g/L. While, the shortest root length was obtained with ¾ strength MS medium supplemented with sucrose sugar at 20 g/ L.

The root initiation and growth were high energy requiring processes that could only occur at the expense of available metabolic substrates, which were mainly carbohydrates (Thorpe 1982). In this study, rooting of thyme shoots was affected by the carbon source. In general, sucrose induced the highest frequency of roots number per shoot. This is might be due to that sucrose is an important source of carbohydrate in the culture medium as well as acting as an osmoticum (Hartmann *et al.* 1997). Also, the effect of sugar concentration on root length might be due to carbohydrate osmotic contribution, since it controls morphogenesis by acting as energy source and also by altering the osmotic potential of the culture medium, which alters such cell wall properties as extension, hardening, and composition, followed by subsequent modification in morphogenesis (Pritchard *et al.* 1991). These results were in agreement with the findings of Mahmoud *et al.* (2011) who reported the preferable rooting of *Lavandula angustifolia* shoots were gained on MS medium half strength augmented with 30 % sucrose and NAA.

For the further establishment, *in vitro* grown well rooted plantlets were washed with tap water to remove agar traces and transferred to pots filled with the mixture of soil and peat moss (1:1 by volume) for hardening. The survival rate of 98 % was achieved after 4 weeks (Fig. 4C).

Table 4.Effect of media strength, sugar type at different concentrations on rooting behavior of *Thymus vulgaris* shoots.

Media strength	Sugar type	Rooting (%)			Roots number/ shoot			Root length (cm)		
		Sugar conc. (mg/L)								
		20	30	40	20	30	40	20	30	40
3/4 strength	sucrose	91.7	91.7	83.3	9.08	9.83	7.24	5.80	7.44	8.73
	glucose	75.0	83.3	66.7	6.33	7.41	5.00	7.35	8.75	10.71
1/2 strength	sucrose	100	100	91.7	12.41	19.83	10.58	7.55	8.42	9.28
	glucose	83.3	91.7	66.7	8.16	9.16	5.66	7.71	8.79	9.89
LSD at 5 %			20.8			0.54			0.36	

REFRENCES

Akram, Z., F. Rezanejad and A. Safarnejad (2010). *In vitro* selection for NaCl tolerance in *Thymus vulgaris* L. Journal of Cell and Molecular Research,2(2): 86-92.

Borugă, O., C. Jianu, C. Mişcă, I. Goleţ, A. T. Gruia and F.G. Horhat (2014). *Thymus vulgaris* essential oil: chemical composition and antimicrobial activity. Journal of Medicine and Life, Volume 7, Special Issue 3: 56-60.

Brown, D. C. W. and T. A. Thorpe (1995). Crop improvement through tissue culture. World Journal of Microbial & Biotechnology, 11: 409-415.

Calixto, J. B. (2000). Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines (phytotherapeutic agents). Braz. J. Med. Biol. Res. 33: 179-189.

Christopher, B. (2008). RHS A-Z encyclopedia of garden plants. Dorling Kindersley, United Kingdom.

Dorman, H. J. D. and S. G. Deans (2000). Antimicrobial agents from plants: antibacterial activity of plant volatile oils. Journal of Applied Microbiology, 88(2): 308-316.

Gautheret, R. J. (1955). The nutrition of plant tissue culture. Ann Rev Plant Physiol, 6: 433.

Grigore, A., I. Paraschiv, S. Colceru-Mihul, C. Bubueanu, E. Draghici and M. Ichim (2010). Chemical composition and antioxidant activity of Thymus *vulgaris* L. volatile oil obtained by two different methods. Romanian Biotechnological Letters, 15(4): 5436-5443.

Hartmann, H. D., D. E. Kester, F. T. Davies and R. L. Geneve (1997). Plant propagation: Principles and Practices, 6th ed. Prentice-Hall International (UK) Limited, London.

Irvani, N., M. Solouki, M. Omidi, A.R. Zare and S. Shahnazi (2010). Callus induction and plant regeneration in *Dorem ammoniacum* D., an endangered medicinal plant. Plant Cell Tissue Organ Cult, 100: 293–299.

Kasem, M. M. E. (2011). Biotechnological studies on lavender plant. Ph. D. Thesis, Faculty of Agriculture, Mansoura University.

Kumar, G. K. and T. D. Thomas (2012). High frequency somatic embryogenesis and synthetic seed production in *Clitoria ternatea* Linn. Plant Cell Tissue Organ Cult, 110:141–151.

Lim, Z. X., A. P. K. Ling and S. Hussein (2009). Callus induction of *Ocimum sanctum* and estimation of it's total flavonoids content. Asian Journal of Agricultural Sciences, 1: 55-61.

Maher, A. A., A. Maqtari, S. M. Alghalibi and E. H. Alhamzy (2011). Chemical composition and antimicrobial activity of essential oil of *Thymus vulgaris* from Yemen. Turk J Biochem, 36:342-349.

Murashige, T. and F. Skoog (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant, 15: 473-475.

Nikam, T. D. and R. S. Savant (2009). Multiple shoot regeneration and alkaloid cerpegin accumulation in callus culture of *Ceropegia juncea Roxb*. Physiol. Mol. Biol. Plants, 15(1): 71-77.

Nordine, A.; C. R. Tlemcani and A. EL Meskaoui (2013). Micropropagation of *Thymus satureioides* Coss. an endangered medicinal plant of Morocco. Journal of Agricultural Technology, 9(2): 487-501.

Ozudogru, E.A.; E. Kaya; E. Kirdok and S. Issever- Ozturk (2011). *In vitro* propagation from young and mature explants of thyme (*Thymus vulgaris* and *T. longicaulis*) resulting in genetically stable shoots. *In Vitro* Cellular and Developmental Biology – Plant. 47(2):309–320.

Piri, K. and F. N. Firoozabadi (2006). Plants tissue culture guide. Bu-Ali Sina University Press, Hamedan, Iran, p. 214.

Prasanth, R. V., R. V. Kandisa, P. V. Varsha and S. Satyam (2014). Review on *Thymus vulgaris* traditional uses and pharmacological properties. Medicinal and Aromatic Plants, 3:3.

Pritchard, J., R. G. Wyn-Jones and A. D. Tomos (1991). Turgor, growth and rheological gradients in wheat roots following osmotic stress. Journal of Experimental Botany, 42: 1043–1049.

Rota, M. C., A. Herrera, R. M. Martínez, J. A. Sotomayor and M. J. Jordán (2008). Antimicrobial activity and chemical composition of *Thymus vulgaris*, *Thymus zygis* and *Thymus hyemalis* essential oils. Food Control, 19(7): 681-687.

Sen, M. K., S. Nasrin, S. Rahman and A. H. M. Jamal (2014). *In vitro* callus induction and plantlet regeneration of *Achyranthes aspera* L., a high value medicinal plant. Asian Pac J Trop Biomed, 4(1): 40-46.

Sharma, R. K. and A. K. Wakhlu (2003). Regeneration of *Heracleum candicans* wall plants from callus cultures through organogenesis. Plant Biochem Biotechnol, 12:71–72.

Simon, S. and P. Petrasek (2011). "Why plants need more than one type of auxin". Plant Science. 180 (3): 454-460.

Skoog, F. and C. O. Miller (1957). Chemical regulation of growth and organ formation in plant tissues cultured *in vitro*. Symp Soc Exp Biol, 11: 118-130.

Thompson, J. D., J. C. Chalchat, A. Michet, Y. B. Linhart and B. Ehlers (2003). Qualitative and quantitative variation in monoterpene co-occurrence and composition in the essential oil of *Thymus vulgaris* chemotypes. Journal of Chemical Ecology, 29(4): 859-880.

Thorpe, T. (1982). Carbohydrate utilization and metabolism. In: Bonga, J.M., Durzan, D.J. (Eds.), Tissue Culture in Forestry. Martinus Nijhoff Publishers, London: 325-368.

Ziba, B., M. H. Mirjalili and A. Sonboli (2016). *In vitro* callus induction and micropropagation of *Thymus persicus* (Lamiaceae), an endangered medicinal plant. Crop Breeding and Applied Biotechnology 16: 48-54.

الاكثار الدقيق الغيرالمباشر لنبات الزعتر (*Thymus vulgaris*)

هبة يوسف البنا

قسم الخضر و الزينة ـ كلية الزراعة ـ جامعة المنصورة

اجري هذا البحث بهدف الوصول الى بروتوكول للاكثار الدقيق و الغير المباشر لنبات الزعتر باستخدام القمم النامية المفصولة من نباتات الامهات. استخدمت بيئة موراشيج و سكوج محتوية على تركيزات مختلفة من الاوكسينات وحدها او في تداخل مع سيتوكينينات مختلفة. بيئة موراشيج و سكوج التي تحتوي علي ٢،٤ داى كلوروفينوكسى حامض الخليك بتركيز ٢ مللجم / لتر في تداخل مع الكينيتين بتركيز ١ مللجم / لتر اعطت اعلى نسبة تكون للكالس ١٠٠ % و الوزن الطازج للكالس (٩.٥٦ جرام). أعلى نسبة مئوية لإعادة التكشف (١٠٠%) مرتبطة باعلى عدد افرع (٢٣.٤٢ فرع) تم الحصول عليها علي بيئة موراشيج و سكوج متحتوية علي ٢.٥ مللجم / لتر من البنزيل ادينين. أفضل تجذير تم الحصول عليها باستخدام بيئة موراشيج و سكوج نصف قوة مزودة بسكروز ٣٠ جرام/ لتر و نفثالين اسيتيك اسيد بتركيز ١,٥ مللجم / لتر.وقد تم اقلمة النبيتات بزراعتها علي بيئة مكونة من التربة و البيتموس بنسبة ١: ١ و قد ادت الي نجاح الاقلمة بنسبة ٩٨% .