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Micropropagation of thyme plant (*Thymus vulgaris*)

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

ABSTRACT

The current research was conducted at Vegetable and Floriculture Department in the experimental station and tissue culture laboratory, Agriculture Faculty, Mansoura University. The objective of this research was to develop a direct plant regeneration of a valuable aromatic and medicinal plant (*Thymus vulgaris*) by *in vitro* culture of shoot tips or nodal segments from mature plants on MS medium supplemented with different cytokinin type at different concentrations. Nodal segments were found to be more efficient than shoot tips for *Thymus vulgaris* shoots regeneration on MS medium (Murashig and Skoog 1962) supplemented with 6-benzyladenine (BA) at 2 mg/L. The best elongation was obtained on MS medium supplemented with BA at 2 mg/L in combination with GA_3 at 0.5 mg/L. The best rooting of shoots was obtained on MS medium augmented with α-naphthaleneacetic acid (NAA) at 1.5 mg/L. Regenerated plants were successfully established in pots filled with mixture of soil: peat moss (1: 1 v/v). *In vitro Thymus vulgaris* plants had a survival rate of 100 %, and showed healthy and uniform growth.

INTRODUCTION

Thymus vulgaris called common thyme, German thyme or garden thyme belongs to the most valuable genera in the family *Lamiaceae*. The genus consists of about 400 species of perennial shrubs or subshrubs of aromatic and medicinal plants. It is found in several Mediterranean regions which can describe as the native of the genus (Sáez 2001; Morales 2002). *Thymus spp.* have been reported as the natural source of oleoresins, phenolic oils, fresh and dried herbs (Lawrence and Tucker 2002) which are widely used all over the world, both for medicinal and non-medicinal purposes. Because of their carminative, antiseptic, antimicrobial and antioxidative properties (Letchamo *et al.* 1995; Baranauskiene et al. 2003). For many centuries *Thymus spp.* has been used in folk medicine (Stahl-Biskup 2002). On the other hand, non-medicinal use of the plant provides a great economic importance in the world market. For example, essential oils extracted from herbs are used widely in cosmetic and trophic industries, serving as a preservative and aromatic additive for foods and pharmaceuticals. Also, fresh or dry Leaves of thyme are used in food to add flavor (Senatore 1996; Simon *et al.* 1999).

Populations of naturalist thyme are away from being capable to support such a large, and even growing, demand for its products. Furthermore, concern of the cosmetic and food industries in mainly a few chemotypes, lead to the forfeit of other species, such as *Thymus cariensis*, *T. cilicicus, T. sipyleus, and T. cherlerioides* in nature (Rey and Sáez 2002). Recently advanced micropropagation mechanism can be utilized to rapidly multiply cultivars with desirable traits and create healthy, disease-free plants without seasonal constraints for plants of horticultural, economic and medicinal importance (Pati *et al.* 2006). However, regardless of the potential applications of the *in vitro* culture methods, to date only a few researchers have used them with *Thymus* genus. Furmanova and Olzsowska (1992) worked on *Thymus vulgaris in vitro* propagation, using axillary buds and apical collected from plants grown in the field to initiating *in vitro* cultures and multiplying nodal segments on semi-solid Nitsch and Nitsch medium (Nitsch and Nitsch 1969) containing kinetin and IBA or NAA. Also, Ozudogru *et al.* (2011) reported that cultured shoots tips of *Thyums vulgaris* on Murashige and Skoog medium augmented with 1 mg/L kinetin and 0.3 mg/l GA_3 gave the best percentage of shoot proliferation (97%), with 8.6 shoots per explant. Additional reports provide protocols of micropropagation or organogenesis in *Thymus mastichina* L. (Fraternale *et al.* 2003), *T. sipyleus* Boiss. (Baba Erdag and Yurekli 2000) and *T. piperella* (Sáez *et al.* 1994). Therefore, a great efficient *in vitro* propagation protocol that can be widely applied to the genus is still lacking.

Hence, the aim of current work was to improve an effective *in vitro* propagation methodology of *Thyums vulgaris* which was selected for it is valuable content of bio-active compounds. This paper describes the procedure used to induce direct regeneration of shoots and subsequently plantlets production from young shoots excised from mature plants.

MATERIALS AND METHODS

Plant material:

Young healthy shoots of *Thyums vulgaris* were collected from an adult plant growing in the farm of Medicinal and Aromatic plant, Faculty of Agriculture, Mansoura University. The shoots were cut into shoot tips and nodal segments (about 1 – 1.5 cm long). Explants were thoroughly washed with tap water containing a few amount of household detergent for one hour. The surface sterilization was done with sodium hypochlorite at 3 % for 12 and 15 min for shoot tips and nodal segments, respectively then explants washed with sterile distilled water four times for 3 min each.

Media and culture conditions:

Murashig and Skoog nutrient medium (1962) 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.

Stage I: Growth and *in vitro* multiplication.

Experiment 1: Effect of explants type and cytokinin type on multiplication rate.

To investigate the effect of the two types of explant (shoot tips and nodal segments) on the production of multiple shoots, the surface sterilized shoot tips and nodal segments were cultured on Murashig and Skoog medium (MS) supplemented with 0.5, 1.0, 2.0 and 3.0 mg/L 6-benzyladenine (BA), kinetin (KIN), thidiazuron (TDZ) or 2-isopentenyladenine (2iP). After 30 day of culture, data were recorded on shoot proliferation. A factorial experiment in a randomized complete block design was used with 4 replicates included 12 jars for each treatment.

Experiment 2: Shoot elongation.

In vivo shoots were collected from an adult plant and cut into pieces containing a single node (about 1-1.5 cm) and were cultured on MS medium supplemented with BA (6-benzyladenine) at 2 mg/L in combination with varied concentration of GA_3 at 0.25, 0.50 and 1.00 mg/L. After 30 day of culture, data were recorded on shoot proliferation. A completely randomized design was used with 4 replicates included 12 jars for each treatment.

Stage II: Induction of rooting and acclimatization.

For root induction, individual *in vitro* raised micro-shoots (3 – 4 cm long) were excised from 4 weeks old shoot clusters and transferred to MS basal medium full strength supplemented with different auxin types, i.e., indole-3-butyric acid (IBA), a-naphthalene acetic acid (NAA) or indole-3-acetic acid (IAA) at various concentrations 0.0, 0.25, 0.5, 1 and 1.5 mg/L. A completely randomized design was used with 4 replicates included 12 jars for each treatment. Micropropagated plantlets were getting out from the medium, washed under tap water to remove all traces of media and then single plants were relocated to plastic pots filled with soil: peat moss (1: 1 v/v).

Statistical analysis:

Data of all experiments were 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- Multiplication stage:

1- Effect of explants type and cytokinin type at different concentrations on shoot formation of *Thymus vulgaris*.

This experiment was conducted to test the effect of explant type, different cytokinin (BA, Kin, TDZ and 2ip) at various concentrations 0.5, 1.0, 2.0 and 3.0 mg/L) as well as their interactions on shooting percentage, shoots number per explant and shoot length of thyme shoots. The results were recorded after 4 weeks of culture on MS medium and are shown in Tables (1, 2 and 3).

Effect of explant type (A) on shooting behavior:

Concerning the effect of explant type on shooting percentage, shoots number and length, data in Tables (1, 2 and 3) clearly indicated that nodal segments gave the highest shooting percentage (70.8 %), shoots number per explant (5.06 shoots) along with the highest shoot length of 1.60 cm.

Effect of cytokinin type (B) on shooting behavior:

Regarding the effect of cytokinin type on shooting percentage as shown in Table (1), of the four cytokinins tested, BA was most effective in inducing bud break. The highest recorded percentage of 71.9 % was obtained with BA followed with 69.8 % for Kin without significant difference. The lowest value of shooting percentage (45.8 %) was obtained with TDZ.

Table 1. Effect of explant type, cytokinin type, concentrations and their interactions on response (%) of Thymus vulgaris.

Explant type (A)	Cytokinin type (B)	Cytokinin conc. (mg/L) (C) 0.5	1.0	2.0	3.0	Mean of (A)	Mean of (B)	Mean of (A×B)
Shoot tip	BA	41.7	66.7	75.0	58.3	56.2	71.9	60.4
	Kin	66.7	83.3	58.3	41.7			62.5
	TDZ	41.7	41.7	50.0	33.3		69.8	41.7
	2ip	58.3	58.3	66.7	58.3			60.4
Mean of (A×C)		52.1	62.5	62.5	47.9			
Nodal segment	BA	58.3	91.7	100.0	83.3	70.8	45.8	83.3
	Kin	75.0	91.7	83.3	58.3			77.1
	TDZ	50.0	50.0	58.3	41.7		66.7	50.0
	2ip	66.7	75.0	83.3	66.7			72.9
Mean of (A×C)		62.5	77.1	81.2	62.5		LSD at 5%	
Mean of (B×C)	BA	50.0	79.2	87.5	70.8		A 11.1	
	Kin	70.8	87.5	70.8	50.0		B 24.4	
	TDZ	45.8	45.8	54.2	37.5		C 14.4	
	2ip	62.5	66.7	75.0	62.5		AB 25.8	
							AC 16.8	
Mean of (C)		57.3	69.8	71.9	55.2		BC 33.3	
							ABC 36.7	

Concerning the effect of cytokinin type on shoots number per explant and shoot length, the highest value of shoots number (5.59 shoot/ explant) was obtained when MS medium was supplemented with BA, while the highest value of the shoot length (1.92 cm) was recorded with Kin (Tables 2 and 3).

Effect of cytokinin concentration (C) on shooting behavior:

Regarding the effect of cytokinin concentration on shooting percentage as shown in Table (1), the obtained results showed a positive relationship between cytokinin concentrations and shooting percentage, it was noticed that every increase in cytokinin concentrations from 0.5 mg/L up to 2.0 mg/L gradually increased shooting percentage. The highest recorded percentage (71.9 %) was obtained with cytokinin at 2.0 mg/L and increasing cytokinin concentration to 3 mg/L significantly reduced shooting percentage to 55.2 %

Concerning the effect of cytokinin concentration on shoots number per explant and shoot length, the highest value of shoots number (4.81 shoots/ explant) and shoot length (1.62 cm) was obtained when MS medium was supplemented with cytokinin at 2.0 mg/L (Tables 2 and 3).

Table 2. Effect of explant type, cytokinin type, concentrations and their interactions on shoot number formation of Thymus vulgaris.

Explant type (A)	Cytokinin type (B)	Cytokinin conc. (mg/L) (C) 0.5	1.0	2.0	3.0	Mean of (A)	Mean of (B)	Mean of (A×B)
Shoot tip	BA	3.50	4.21	5.29	3.25	3.19	5.59	4.06
	Kin	3.50	4.41	2.75	3.12			3.45
	TDZ	2.50	2.00	3.37	1.08		4.64	2.24
	2ip	2.58	3.08	3.79	2.66			3.03
Mean of (A×C)		3.02	3.42	3.80	2.53			
Nodal segment	BA	5.37	6.25	9.66	7.16	5.06	2.70	7.11
	Kin	6.21	7.62	5.41	4.12			5.84
	TDZ	3.16	4.62	2.87	2.00		3.57	3.17
	2ip	3.54	3.91	5.29	3.71			4.11
Mean of (A×C)		4.57	5.60	5.81	4.25		LSD at 5%	
Mean of (B×C)	BA	4.44	5.23	7.48	5.21		A 0.34	
	Kin	4.85	6.02	4.08	3.62		B 0.27	
	TDZ	2.83	3.31	3.12	1.54		C 0.24	
	2ip	3.06	3.50	4.54	3.19		AB 0.41	
							AC 0.38	
Mean of (C)		3.80	4.51	4.81	3.39		BC 0.48	
							ABC 0.71	

Table 3. Effect of explant type, cytokinin type, concentrations and their interactions on shoot length (cm) formation of Thymus vulgaris.

Explant type (A)	Cytokinin type (B)	Cytokinin conc. (mg/L) (C) 0.5	1.0	2.0	3.0	Mean of (A)	Mean of (B)	Mean of (A×B)
Shoot tip	BA	0.84	1.40	1.24	1.36	1.24	1.34	1.21
	Kin	1.35	1.44	1.90	1.51			1.55
	TDZ	0.64	0.67	1.00	0.52		1.92	0.71
	2ip	1.44	1.81	1.52	1.18			1.49
Mean of (A×C)		1.07	1.33	1.42	1.08			
Nodal segment	BA	1.26	1.51	1.40	1.69	1.60	0.77	1.47
	Kin	2.13	2.32	2.80	1.90			2.29
	TDZ	0.54	0.90	1.25	0.65		1.66	0.83
	2ip	1.79	2.08	1.86	1.58			1.83
Mean of (A×C)		1.43	1.70	1.83	1.46		LSD at 5%	
Mean of (B×C)	BA	1.05	1.46	1.32	1.53		A 0.11	
	Kin	1.74	1.88	2.35	1.71		B 0.07	
	TDZ	0.59	0.79	1.12	0.59		C 0.07	
	2ip	1.61	1.95	1.69	1.38		AB 0.12	
							AC 0.12	
Mean of (C)		1.25	1.52	1.62	1.30		BC 0.13	
							ABC 0.20	

Effect of the interaction on shooting behavior:

Concerning the interaction between explant type and cytokinin type (A×B), as shown in Tables (1, 2 and 3), the interaction between nodal segments and cytokinin type showed a highly significant differences in all cases. The best significant interaction effect on shooting percentage and shoots number per explant was

achieved when nodal segments was cultured on MS medium supplemented with BA, while the highest value of shoot length was recorded with nodal segments and Kin.

It appeared from the interaction between explant type and cytokinin concentrations (A×C) as shown data in the Table (1) that the highest percentage of shooting (81.2 %) was recorded with the interaction of nodal segments and cytokinin concentration at 2 mg/L. Also, culturing the shoot tips on the MS medium containing 3 mg/L of cytokinin produced precisely the lowest values of shooting percentage (47.9 %) when compared with the other treatments.

Concerning the interaction between explant type and cytokinin concentrations, the obtained results in Tables (2 and 3) showed that the highest significant values of shoots number per explant (5.81 shoots/ explant) and shoot length (1.83 cm) were achieved when the nodal segments were cultured on MS medium supplemented with 2 mg/L of cytokinin. On the other hand, the weakest interaction effect was recorded when shoot tips were cultured on MS containing 3 mg/L of cytokinin, since they were 2.53 shoots and 1.08 cm, respectively.

Regarding the interaction between the cytokinin type and cytokinin concentrations (B×C), data in Table (1) showed that no significant effect was detected on shooting percentage. The highest record of shooting percentage (87.5 %) was achieved when MS medium supplemented with BA at 2 mg/L or Kin at 1 mg/L.

Concerning the effect of this interaction on shoots number per explant and shoot length (Tables 2 and 3), the highest significant shoots number per explant was recorded with MS medium supplemented with BA at 2 mg/L (7.48 shoots) while the tallest shoot length (2.35 cm) was obtained with Kin at 2 mg/L.

As for the interaction among explant type, cytokinin type and cytokinin concentration (A×B×C) on shooting percentage, data presented in Table (1) showed that shooting percentage of 100 % was obtained when nodal segments were cultured on MS medium supplemented with BA at 2 mg/L

Concerning the effect of this interaction on shoots number per explant (Table 2), it was found that nodal segments cultured on MS medium supplemented with BA at 2 mg/L significantly produced the highest significant value of 9.66 shoots per explant as shown in Fig. 1A. As for shoot length, data in Table (3) showed that the highest shoot length (2.80 cm) was recorded with nodal segments cultured on MS media supplemented with Kin at 2 mg/L.

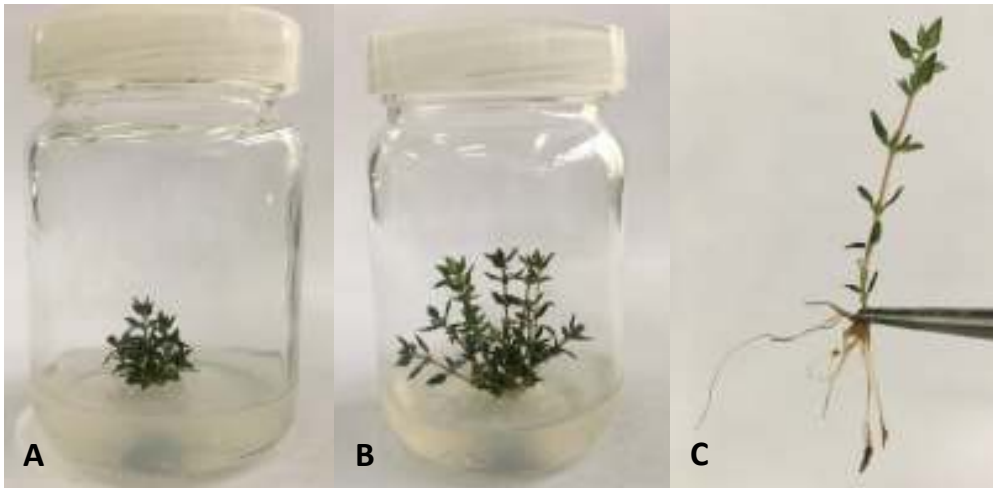

Figure 1. *In vitro* propagation of *Thymus vulgaris*. A) Multiple shoots, obtained by culturing nodal segments on MS medium supplemented with 2 mg/L of BA. B) Elongated shoots on MS medium with BA at 2 mg/L and GA_3 at 0.5 mg/L. C) Rooted shoots on MS medium supplemented with NAA at 1.5 mg/L.

Following these results, the strength of *T. vulgaris* explants to compose new shoots varied with the explant type and hormones. In this work, the most effective explant type in shoot formation was found to be nodal segments. Similar results on different plants were obtained by Arikat *et al* (2004), Zuzarte *et al.* (2010) and Aicha *et al.* (2013) whom showed that nodal segments explants gave better results than the apical explants in micropropagated plants of *Th*ymus *hyemalis*. The different responses of both types of explant were probably due to the endogenous hormone balance in the plant tissue Grattapaglia and Machado (1998).

Results obtained clearly indicated that Shoots number was affected by application of cytokinin type and concentration to the nutrient medium, since BA at 2 mg/L gave the highest significant shoots number per explant compared to Kin, TDZ and 2ip. These results are in general agreement with Coelho *et al.* (2012), Fraternale *et al.* (2003) and Sáez *et al.* (1994). This may due to the observe of Kieber (2002) who mentioned that 6-benzyl aminopurine (BAP) is one of the plant growth substance (cytokinins) that promote cell division and shoot morphogenesis. On the contrary, convenient effect of Kin was found in *T. vulgaris* (Furmanowa and Olszowska 1992; Ozudogru *et al.*, 2011).

Shoot length was also affected by application of cytokinin type and concentration to the nutrient medium, Kin was found to be most effective in inducing

shoot length. This result is in agreement with those obtained by Ozudogru *et al.* (2011) and Nordine *et al.* (2013).

2- Effect of gibberelic acid (GA_3) at different concentrations in combination with 6-benzyladenine (BA) on development of *Thymus vulgaris* nodal segments.

Since the shoots were relatively short (1.4 cm) on proliferation medium (BA at 2 mg/L), promoting the length of shoots was the aim of this phase of the experiment before rooting and acclimatization stage. The effect of different concentrations of GA_3 (0.25, 0.5 and 1.0 mg/L) in combination with BA at 2 mg/L was evaluated. Because of the superior *in vitro* response of the nodal segments explants compared to the apical shoot tips, only the former were used in this experiment.

Concerning the shoot length, data in Table (4) showed that application of GA_3 to the MS medium at all concentrations had a positive effect on this character. Also, there was significant differences among all treatments were detected on the characters of shoot formation. The highest significant shoots number per explant (12.16 shoots), shoot length (3.7 cm) and leaves number per shoot (8.66 leaves/ shoot) was obtained with BAP at 2 mg/L + GA_3 at 0.5 mg/L (Fig. 1B). While, there was non-significant difference on shooting response percentage between all treatments.

The promoted effect of GA_3 on shoot formation was recorded by Ozudogru *et al.* (2011) on *Thymus vulgaris* and Nordine *et al.* (2013) on *Thymus saturioides*. The effective of GA_3 on shoot formation may be due to that lot of physiological activities in plants can be enhance by Gibberellins (GAs), including seed germination, dormancy breakage, stem elongation flowering and fruit development through the increase of cell division, cell wall formation and expansion (Huttly and Phillips 1995).

Table 4. Effect of gibberellic acid (GA_3) at different concentration in combination with BA on Thymus vulgaris nodal segments after 4 weeks of culture.

Treatments		Response %	Shoots Number/ Explant	Shoot Length (cm)	Leaves number/ shoot
Cytokinin type mg/ L	GA_3 conc. (mg/L)				
	0.25	100	9.58	3.39	7.25
BA at 2.0	0.5	100	12.16	3.70	8.66
	1.0	91.7	7.79	3.53	7.41
L.S.D. at 5%		15.4	0.914	0.15	0.21

Stage II: Induction of rooting and acclimatization.

1- Effect of auxin type at different concentrations on *in vitro* rooting of *Thymus vulgaris* shoots.

Elongated shoots were cultured on MS medium supplemented with different auxins (IBA, NAA and IAA) at various concentrations 0.00, 0.25, 0.50, 1.0 and 1.5 mg/L. According to the results obtained in Table (4) all tested auxins can induce rooting in *Thymus vulgaris*. For both NAA and IBA, the rooting percentage increased as the concentration of the growth regulators increased, reaching a maximum (100 %) at the highest concentration tested (1.5 mg/L). Use of this maximum concentration of NAA also provided a significant increase in roots number per explant (16.75 roots/ shoot). On the other hand, the weakest interaction effect was recorded when shoots were cultured on MS containing IAA at 0.25 mg/L, since it were 58.3 and 4.66 roots per shoot.

Concerning the effect of this interaction on root length, it was found that highest values root length (4.35 and 3.82 cm) were recorded with MS media supplemented with NAA at 1.0 and 1.5 mg/L, respectively.

Table 4. Effect of auxin type at different concentrations on *in vitro* rooting of *Thymus vulgaris* shoots.

Auxin type	Auxin concentration mg/ L	Rooting percentage (%)	Roots number/ shoot	Root length (cm)
	0.0	75.0	5.37	1.74
	0.25	75.0	5.37	1.33
IBA	0.5	83.3	6.00	1.65
	1.0	91.7	10.91	2.66
	1.5	100	14.33	3.64
	0.0	75.0	5.37	1.74
	0.25	83.3	10.00	2.71
NAA	0.5	91.7	9.29	3.22
	1.0	100	15.50	4.35
	1.5	100	16.75	3.82
	0.0	75.0	5.37	1.74
	0.25	58.3	4.66	1.21
IAA	0.5	66.7	7.62	0.97
	1.0	83.3	8.33	1.77
	1.5	75.0	6.04	1.46
L.S.D. at 5%		29.1	0.64	0.25

In the present study, it was a matter of importance to notice that MS free medium of auxin also gave 75 % rooting percentage, 5.37 roots number per shoot and 5.37 cm root length. However, in comparison to the treatment with 1.5 mg/L NAA, medium free auxin produced a lower mean root number and the roots were very thin without secondary roots, both considered to be important parameters for success of the following acclimatization step. In the same line Lê (1989) reported that the highest rooting of *T. vulgaris* was obtained when shoots were cultured on MS medium free hormone.

Results obtained clearly indicated that of the three tested auxins (IBA, NAA and IAA), NAA at 1.5 mg/L was found to be most effective in inducing roots. About 100 % of the excised shoots developed (16.75 roots/ shoot) with root length averaging 3.82 cm. This result was similar with those obtained by Aicha *et al.* (2013) in *Thymus hyemalis*. While, in the same species (*T. vulgaris*), Ozudogru *et al.* (2011) studied it's *in vitro* rooting by using different auxins [IAA, IBA,

NAA and 2,4 dichlorophenoxyacetic (2,4-D)] and they observed that 2,4-D gave the best result of rooting. Also, Furmanowa and Olszowska (1992) found that *T. vulgaris* rooted easily in medium with IBA. In *T. lotocephalus*, the best rooting was achieved with IAA (Coelho *et al.* 2012). The variance in response of rooting among thyme species cited above could be related to multiple agents, such as the endogenous cytokinin/auxin ratio, the genotype, the influence of shoot multiplication medium and the sensitivity of tissues to absorb or use the exogenous auxin, among others (De Klerk *et al.* 1999; De Klerk 2002).

After 30 day of *in vitro* rooting, healthy regenerated plantlets with perfectly developed leaves and well- advanced roots were cleaned with water several times to take of all trace of culture medium. The rooted plants were successfully established in plastic pots containing soil and peat moss (1:1). Four weeks after transfer about 100 % of plantlets were surviving.

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الاكثار الدقيق لنبات الزعتر (*Thymus vulgaris*)

هبة يوسف البنا

قسم الخضر و الزينة ـ كلية الزراعة ـ جامعة المنصورة

اجري هذا البحث بمعمل زراعة الانسجة بقسم الخضر و الزينة- كلية الزراعة جامعة المنصورة و يهدف البحث للوصول الى بروتوكول لاكثار سريع و مباشر علي نطاق واسع لنبات طبي و عطري هام و هو الزعتر بواسطة تكنيك زراعة الانسجة بزراعة القمة النامية او قطع ساقية برعمية صغيرة علي بيئة موراشيج و سكوج محتوية على انواع مختلفة من السيتوكينين بتركيزات مختلفة و كانت اهم النتائج ما يلي: استخدام القطع العقدية الساقية كان اكثر كفاءة عن القمة النامية علي بيئة موراشيج و سكوج التي تحتوي علي ٢ مللجم / لتر من البنزيل ادينين. وقد ادي استخدام البنزيل ادينين بتركيز ٢ مللجم/ لتر الي الحصول علي اعلي النتائج بالمقارنة السيتوكينينات المختلفة. و تم الحصول على افضل استطالة للافرع بالزراعة علي بيئة موراشيج و سكوج مزودة بي ٢ مللجم / لتر من البنزيل ادينين و ٠.٥ مللجم / لتر جبريلك اسيد.أما بالنسبة للتجذير كانت اعلي ما يمكن باستخدام بيئة موراشيج و سكوج مزودة بنفثالين 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 ١٠٠% قبل نقل النباتات لزراعتها خارج المعمل.