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DIRECT MICROPROPAGATION OF ENGLISH LAVENDER (*Lavandula angustifolia* Munstead) PLANT.

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ABSTRACT

The present investigation was carried out to study the *in vitro* shoot proliferation, root formation and *ex vitro* acclimatization of English lavender (Munstead). Nodal explant showed a good response for producing the highest survival percentage, shoots and leaves number comparing with the shoot tip one. Among the tested cytokinins, TDZ at 0.20 mg/L recorded the highest shoots number (30.55 shoots), followed by BAP at 0.80 mg/L (16.50 shoots). The weakest effects on shoots number were recorded for media supplemented with all Kin concentrations, however it tabulated the tallest shoots length. Also, MS at full strength supplemented with sucrose as a carbon source at concentration of 30.00 or 40.00 g/L, significantly recorded the highest number of shoots and leaves. But, the lowest vitrification percentage was obtained when media strength started to decrease, as $^1/_4$ strength medium which supplemented with sucrose or glucose at 40 g/L recorded 0.00% vitrification. Rooting which was achieved on half strength medium fortified with NAA at 1.00 mg/L significantly recorded the highest roots number of 21.25 roots, comparing with 11.86 roots only when IBA was added into the medium at 2.00 mg/L, but it was noticed that NAA concentrations decreased the roots length comparing with IBA one. Transplanting the rooted shoots into a mixture of peat moss + vermiculite + loamy soil (1:1:1 v) resulted in the highest survival percentage of 90.00% and a increase in shoots length of 12.71cm.

Abbreviations: 2,4-D; 2,4-dichlorophenoxyacetic acid, BAP; 6-benzylamino purine, IBA; 3-indolebutyric acid, MS; Murashige and Skoog (1962) basal medium, NAA; Naphthaleneacetic acid, TDZ; Thidiazuron, Kin; N6-furfuryladenine (Kinetin) and AC; Activated charcoal.

INTRODUCTION

Lavandula angustifolia "Munstead" (English lavender) family *Lamiaceae* (*Labiatae*) is a hardy perennial shrubs rich in aromatic essential oils and is valuable for its pharmaceutical, aromatic and culinary properties. A total of 32 species of *Lavandula* have been described in the literature, plus a number of infraspecific taxon and hybrids (Upson, 2002). There is confusion with the naming of lavenders round the world, owing to differences in their appearance under different climatic and/or husbandry conditions (Lis-Balchin, 2002 a). This genus is relatively rich in phenolic constituents, with 19 flavones and 8 anthocyanins (Harbourne and Williams, 2002).

On the other hand, (Bertram 1995) illustrated that *Lavandula angustifolia* is stated to be a carminative, spasmolytic, tonic, antidepressant, nervous headache, neuralgia, rheumatism, depression, insomnia, windy colic, fainting, toothache, sprains, sinusitis, stress and migraine. Aromatherapy involves massage using a very diluted essential oil or mixture of essential oil to the bath or a basin of hot water, or using burners (Lis-Balchin, 2002 b). Also, it is a very beautiful ornamental pot plant and has ability for cut and formation.

Lavandula angustifolia plants are commercially propagating by stem cuttings, but have a poor rooting ability (Andrade *et al.* 1999). Also, the seeds have a poor germination percentage (Takano *et al.* 1990), however these methods are not efficient enough to produce mass production needed. Direct micropropagation offers a potential to deliver large quantities of disease-free, true-to type healthy stock within a short span of time. Several species of lavender have been used, like *L. dentata* (Echeverrigaray *et al.* (2005), *L. vera* (Andrade *et al.* 1999), *L. latifolia* (Panizza and Tognoni, 1991), but the maximum number of the regenerated shoots in most of cases was not increased than 20 shoots per explant. So, the aim of this study is a trial to develop a commercial protocol for direct micropropagation of this aromatic and medicinal plant.

MATERIALS AND METHODS

The current research was conducted at the experimental station and tissue culture laboratory of the Vegetable. and Floriculture Department, Faculty of Agriculture, Mansoura University during the seasons of 2008-2010.

Preparation of explant: The plant material was obtained from a Farm in the North Coast of Egypt on February, 20 2008. The plants were cultured in the Farm of Medicinal and Aromatic plants, Faculty of Agriculture, Mansoura University. After some preliminary experiments, apical shoots with length of 10cm were obtained from six month old *Lavandula angustifolia* plants. Then, these explants were washed under running tap water for 60 minutes and were surface sterilized under aseptic conditions inside the culture cabinet laminar air flow by using ethyl alcohol 50% for 1 minute and 2% NaOCl (44.4% Commercial Clorox) for 20 minutes with two drops of Tween 20. All traces of the used disinfectant were removed by rinsing the explants four times in sterilized distilled water.

Shoots proliferation: The two explant types (shoot tip and nodal) were taken from the previous sterilized shoots at length of 1-1.5cm approximately. Then, each explant type was cultured on (MS) Murashige and Skoog (1962) full strength basal medium in three experiments. Media were fortified with different concentrations of BAP, Kin or TDZ at (0.0, 0.05, 0.1, 0.2, 0.4 or 0.8 mg/L) for each. Activated charcoal was either not added (0.0) or added at (2.0 g/L). The pH of the used media was adjusted to 5.8 by using HCl or NaOH, 0.1M and solidified by difco bacto agar (7 g\L). The medium was cooked and distributed into 250 ml screw cap jars containing 25 ml of the nutrient medium. Jars were covered and autoclaved at 121°C at 1.5 kg/cm^2 for 20 min. Data were recorded after 4 weeks for:

1- Survival%= (Number of the grown explants / All number of the cultured explants) x 100.
2- Shoots number per explant.
3- 3- Shoots length (mm).
4- Leaves number per explant.

Each of these experiments included 24 treatments each of which consisted of 4 replicates of 3 jars per replicate.

For studying the influence of media strength, sugar type and sugar concentration in proliferative shoots, nodals with a pair of axillary buds were taken from the *ex vitro* mother plant with length of 5cm and the same sterilization procedure as mentioned before was performed. Then, nodals were cut again at length of 1–1.5cm approximately inside the culture cabinet. MS medium has been used with (full, ¾, ½ or $^1/_4$ strength) and supplemented with two types of carbon source (sucrose and glucose) at different concentrations (20, 30 or 40 g/L). The other conditions of media were the same as in the previous experiments. TDZ, was added to the media at concentration of 0.20 mg/L. Data were recorded after 4 weeks for:

1- Vitrification%=(Number of abnormal grown shoots/All number of the cultured shoots)× 100.
2- Shoots number per explant.
3- Shoots length (mm).
4- Leaves number per explant.

This experiment included 24 treatments each of which consisted of 4 replicates of 3 jars for each replicate.

***In vitro* rooting formation:** Shootlets which were cultured on MS full strength medium containing TDZ at 0.20 mg/L and sucrose at 30 g/L (the best proliferation treatment) were grown on a growth regulators-free medium for 2 weeks to eliminate any carry over effects of TDZ that might inhibit or reduce rooting and used as explant material. The nutrient medium was tested at two strength (full and half strength) and supplemented with NAA or IBA at concentrations of (0.0, 0.5, 1.0, and 2.0 mg/L) for each alone and distributed into clean jars where each jar contained 25 ml of the medium. Data were recorded after four weeks for:

1- Rooting %= (Number of the rooted shoots / All number of the cultured shoots) x 100.
2- Roots number per explant. 3- Roots length (mm).

This experiment included 16 treatments each of which consisted of 4 replicates of 3 jars per replicate.

***Ex vitro* establishment:** This experiment was conducted to study the effect of transplanting media on the growth and survival percentage of plantlets which were propagated *in vitro*. Well developed rooted plantlets were taken out of the culture jars and washed with warm water at 30°C to eliminate any medium surplus, then transferred into plastic pots containing one of the following autoclaved media; [(Peat moss + Loamy soil (1:1), Vermiculite + Loamy soil (1:1) and Peat moss + vermiculite + Loamy soil (1:1:1) by volume)]. Data were recorded after 3 weeks for; survival% and average increase in shoots length (cm).

The experiment included 3 treatments (media) each of which consisted of 3 replicates of 3 pots for each. Every pot contained one plantlet and covered with transparent plastic bag to maintain high relative humidity around plants. The plastic bags were bored gradually starting from the second day of transplanting in order to get rid of excess humidity as well as expose the plantlets to the normal atmosphere condition. On the fifth day from

transplanting, these bags were removed completely. The plantlets were irrigated with tap water whenever needed.

Incubation conditions:

All the previous experiments were incubated at temperature of 25 ºC ± 2 °C, photoperiod also was adjusted to 16 hours light and 8 hours dark, controlled automatically. Illumination intensity was 1500 lux at top culture vessels level from fluorescent lamps (60cm long).

Statistical analysis:

A complete randomize design for each 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. Data given in percentages were subjected to arcsine ($\sqrt{X}$) transformation according to (Snedecor and Cochran, 1967) before statistical analysis.

RESULTS AND DISCUSSION

Shoots proliferation:

Effect of the interaction between explant type, BAP and AC concentrations on survival %, shoots [number and length (mm)] and leaves number of lavender plantlets.

The concerned results in Table (1) showed that culturing shoot tip explant or nodal ones on medium supplemented with BAP at 0.00 mg/L and AC at any concentration significantly resulted the highest survival % if they were compared with most of the other treatments. Such super survival % in case of nodal explant was valued 90.00% with BAP at 0.00 with either the two concentrations of AC, followed by the same value 90.00% with the same explant type (nodal), which cultured on MS medium supplemented with BAP at 0.05 mg/L and the presence of AC at 2.00 g/L. The corresponding values with the shoot tip explants were 83.44 and 90.00% when they were cultured on MS medium supplemented with BAP at 0.00 mg/L and AC at 0.00 or 2.00 g/L, respectively. The recorded survival % for the rest interactions of BAP and AC concentrations on both explant types were significantly fewer, as compared with the above said values .

The same data also revealed that the combination of BAP and AC at a highest concentration for both explant types obviously exhibited a poor effect on this shoot character. In most cases they recorded a less survival % values on both types of explant fewer than those recorded in their control medium (0.0 mg/L BAP and 0.0 g/L AC). In the same line, it could be concluded that among the tested combinations, BAP at 0.80 mg/L and AC at 0.00 or 2.00 g/L combinations had the lowest effect with the shoot tip explant, since they counted the least survival percentage of 70.68 and 71.81%, respectively. The same was true with the nodal explant with the same combination, since these combinations tabulated the percentage of 71.70 and 76.42%, respectively. These results confirm the results of (Chebel *et al.*, 1998) who revealed that greater amount of hyperhydricity were found in

cultures containing higher concentrations of BAP and this case directly reduce the shoot survival %. Also, our result was in agreement with (Lee and Chan, 2004) who stated that the survival rate of nodal segments of *Orthosiphon stamineus* decreased with increasing concentration of BAP on MS medium.

Table (1): Effect of the interaction between explant type, BAP and AC concentrations on survival %, shoots [number and length (mm)] and leaves number of lavender plantlets.

Explant type (A)	BAP mg/L(B)	Survival %		Shoots number		Shoots length (mm)		Leaves number	
		AC g/L (C)							
		0.0	2.0	0.0	2.0	0.0	2.0	0.0	2.0
Shoot tip	0.00	83.44	90.00	1.00	1.00	80.00	78.72	6.81	4.23
	0.05	79.12	80.12	3.53	2.31	71.30	73.81	10.42	9.76
	0.10	77.96	80.12	3.62	3.27	73.23	69.43	12.30	13.52
	0.20	78.71	78.52	5.13	4.20	68.14	68.00	15.67	13.41
	0.40	75.42	77.08	6.41	4.54	45.51	51.86	18.75	15.62
	0.80	70.68	71.81	8.72	6.31	36.62	38.73	27.90	24.53
Nodal	0.00	90.00	90.00	2.33	2.14	75.22	73.40	8.32	6.54
	0.05	82.05	90.00	4.60	3.24	68.41	70.33	12.71	10.51
	0.10	81.86	90.00	4.63	5.47	65.67	63.07	14.35	18.23
	0.20	80.68	78.30	9.55	9.42	59.83	57.33	24.22	26.82
	0.40	76.66	77.26	13.13	12.33	43.27	48.70	32.61	34.74
	0.80	71.70	76.42	16.70	14.45	34.42	37.93	35.42	38.60
LSD at 5% (A×B×C)		7.40		3.52		4.94		4.48	

In addition, data in Table (1) and Photo (1) indicated that culturing both types of explant on MS nutrient medium supplemented with the highest concentration of BAP (0.80 mg/L) and AC at the two concentrations significantly resulted in the greatest shoots number per explant. But, it may be noted that culturing the nodal treated with these concentrations were significantly higher (more than the double) than the shoot tip ones. Since, they were (16.70 and 14.45 shoots) for the nodal explants. These results disagreed with (Andrade *et al.*, 1999) who stated that the optimal BAP concentration for shoots productions was 0.50 mg/L with *Lavandula vera* explant. But, this may be due to the difference in plant genotype, as our species was *Lavandula angustifolia* L. In this respect, (Dronne *et al.*, 1999) revealed that all steps of lavandin micropropagation were cultivar dependent.

The interaction between shoot tip or nodal explants with BAP at 0.80 mg/L and AC at 0.00 or 2.00 g/L significantly recorded the shortest shoots length of 36.62, 38.73, 34.42 and 37.93mm, respectively and there were no significant differences between them. On the other hand, BAP-free medium supplemented with AC at 0.00 or 2.00 g/L recorded the tallest shoots length values with the shoot tip and the nodal explant, since they were 80.00, 78.72, 75.22 and 73.40mm, respectively. Concerning leaves number, the greatest values of 35.42 and 38.60 leaves were recorded for nodal explants which were cultured on MS media fortified with BAP at 0..80 mg/L and AC at 0.00 or

2.00 g/L., respectively. This finding coincided with (Begum *et al.*, 2002) on *Ocimum basilicum*. as they found that nodal explant was significantly higher than shoot tip one in this shoot characteristic.

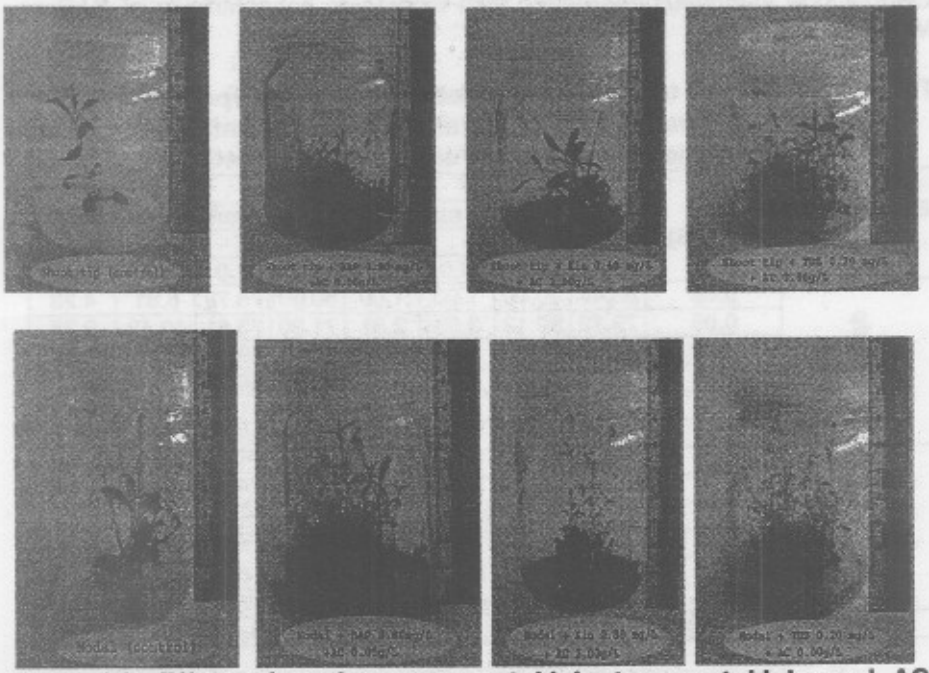

Photo (1): Effect of explant type, cytokinin type, cytokinin and AC concentrations on average shoots number of lavender plantlets.

Effect of the interaction between explant type, Kin and AC concentrations on survival %, shoots [number and length (mm)] and leaves number of lavender plantlets.

Considering the effect of this interaction on survival %, data in Table (2) indicated that in general evaluation culturing the two explant types on nutrient media supplemented with most of Kin and AC concentrations were higher than BAP + AC, concentrations (table 1), since in case of using shoot tip explant, the survival % ranged from 81.86 to 90.00%. The corresponding values for the nodal ones also ranged from 80.46 to 90.00%. But, it was obvious that culturing the nodal explant on Kin-free medium supplemented with or without AC concentration recorded the highest survival % value of 90.00% for each. Also, the same value were obtained when the same explant type was cultured on media had Kin at 0.05, 0.10 and 0.40 mg/L interacted with AC at 2.00 g/L. On the other hand, the same value of 90.00% resulted when the shoot tip explant was cultured on some Kin concentrations with or without AC concentrations. However, culturing the shoot tip explants on media received Kin at 0.80 mg/L and AC at 2.00 g/L resulted the lowest value

of 81.86% and a significant difference was found when compared with the previous value (90.00%)

Regarding shoots number, nodal explants which were cultured on MS media supplemented with Kin at (0.40 or 0.80 mg/L) and AC at (0.00 or 2.00 g/L), recorded the highest shoots number values of (7.53, 8.70, 9.82 and 10.76 shoots), respectively (photo1). The corresponding values for the shoot tip ones were (3.60, 4.73, 3.91 and 2.40 shoots), respectively. But, it was noticed that nodal explant had the upper hand in that respect when compared with the shoot tip one. Also, it was quite clear that shoot tip and nodal explant which were cultured on the control medium counted the lowest shoots number of (1.00 and 2.33 shoots), respectively. This result confirm the results of (Andrade *et al.*, 1999) who stated that BA or TDZ were more effective in promoting shoot development than those without growth regulators or supplemented with Kin.

Table (2): Effect of the interaction between explant type, Kin and AC concentrations on survival %, shoots [number and length (mm)] and leaves number of lavender plantlets.

Explant type (A)	Kin mg/L(B)	Survival %		Shoots number		Shoots length (mm)		Leaves number	
		AC Con. g/L (C)							
		0.0	2.0	0.0	2.0	0.0	2.0	0.0	2.0
Shoot tip	0.00	83.44	90.00	1.00	1.00	80.00	78.72	6.81	4.23
	0.05	90.00	83.44	2.23	2.50	77.54	74.30	6.44	6.50
	0.10	90.00	90.00	2.74	2.34	75.41	74.22	10.31	8.24
	0.20	81.96	90.00	2.81	3.42	70.83	72.71	12.70	12.93
	0.40	85.37	83.44	3.60	4.73	67.13	69.53	16.92	12.04
	0.80	82.05	81.86	3.91	2.40	65.32	68.14	16.94	14.24
Nodal	0.00	90.00	90.00	2.33	2.14	75.22	73.40	8.32	6.54
	0.05	83.46	90.00	2.50	3.81	64.37	68.86	10.46	10.71
	0.10	82.05	90.00	2.14	3.42	61.42	67.24	8.32	12.87
	0.20	80.46	83.44	4.21	5.53	58.74	62.91	12.70	10.54
	0.40	82.05	90.00	7.53	8.70	53.60	55.23	18.54	20.60
	0.80	83.44	85.37	9.82	10.76	49.34	52.62	22.33	24.76
LSD at 5% (A×B×C)		4.90		2.80		4.73		4.53	

Results in Table (2) revealed that culturing the shoot tip and the nodal explants on media supplemented with Kin at 0.00 mg/L and AC at 0.00 g/L measured the tallest shoots length of (80.00 and 75.22mm), respectively. But, it was obviously observed that the shoot tip explants measured a tallest lengths comparing with the nodal one and this could be due to the apical meristem, suppression effect found in the shoot tip explant and responsible for the apical dominance. The weakest effect of interactions was cleared with nodal explant cultured on medium supplemented with Kin at 0.80 mg/L and AC at 0.00 g/L, since it was 49.34mm only.

As regarding leaves number, it was noticed that nodals which were cultured on media supplemented with Kin at 0.80 mg/L and AC at 0.00 or 2.00 g/L counted the highest values, since they were 22.33 and 24.76 leaves, respectively. The corresponding values for the shoot tip explants were 16.94

and 14.24 leaves. On the other hand, it may be noted in general that leaves number was in ascending order with Kin concentrations increase.

Effect of the interaction between explant type, TDZ and AC concentrations on survival %, shoots [number, and length (mm)] and leaves number of lavender plantlets.

The recorded results in Table (3) indicated that culturing the shoot tip or the nodal explants on nutrient media supplemented with TDZ at 0.05 mg/L and AC at 0.00 or 2.00 g/L significantly recorded the highest survival % of (90.00%) for all of them. Also, the same value was recorded for shoot tip or nodal explants cultured on TDZ-free medium supplemented with AC at 2.00 g/L. while, it was noticed that culturing the two types of explant on MS medium supplemented with TDZ at 0.80 mg/L and AC at 2.00 g/L resulted the lowest survival % of 66.53% for both of them.

It appears from the data presented in the same Table (3) and illustrated in Photo (1) that culturing the shoot tip explant or the nodal on media supplemented with TDZ at 0.20 mg/L and AC at 0.00 g/L significantly counted the highest shoots number of (26.52 and 30.55 shoots), respectively when compared with the effect of the control medium. The interaction between TDZ at 0.00 mg/L and AC at 0.00 or 2.00 g/L., obviously appeared a poorest effect in this character when compared with the other treatments . Since, shoot tip or nodal which were cultured on that interaction recorded 1.00 and 2.14 shoots, respectively. These results were partially in the same line with the results of Poovaiah *et al.* (2006) on scotch spearmint.

Table (3): Effect of the interaction between explant type, TDZ and AC concentrations on survival %, shoots [number and length (mm)] and leaves number of lavender plantlets.

Explant type (A)	TDZ mg/L(B)	Survival %		Shoots number		Shoots length (mm)		Leaves number	
		AC Con. g/L (C)							
		0.0	2.0	0.0	2.0	0.0	2.0	0.0	2.0
Shoot tip	0.00	83.44	90.00	1.00	1.00	80.00	78.72	6.81	4.23
	0.05	90.00	90.00	7.21	8.76	60.73	65.38	18.31	14.04
	0.10	90.00	82.05	22.43	18.32	42.60	47.16	32.52	30.67
	0.20	85.37	81.95	26.52	26.41	35.21	33.43	38.23	37.27
	0.40	74.49	69.05	23.10	21.45	23.17	20.61	26.40	23.57
	0.80	70.54	66.53	15.54	13.63	15.29	17.25	20.13	16.38
Nodal	0.00	90.00	90.00	2.33	2.14	75.22	73.40	8.32	6.54
	0.05	90.00	90.00	10.52	7.33	56.18	62.74	22.44	16.04
	0.10	85.37	90.00	24.24	22.70	48.45	55.37	34.03	29.62
	0.20	83.44	85.37	30.55	27.71	32.62	30.00	54.17	38.24
	0.40	69.37	71.60	21.43	17.20	21.40	23.21	23.53	25.73
	0.80	64.95	66.53	17.68	14.45	11.72	13.46	18.29	22.14
LSD at 5% (A×B×C)		7.93		3.52		6.33		4.85	

As for shoots length, the results in the same Table (3) revealed a negative relationship between TDZ concentrations and this character with the two explant types. Also, AC did not play a vital role in that respect. Since, culturing the shoot tip on TDZ-free medium supplemented with or without AC concentration significantly measured the tallest shoots of 78.72 and

80.00mm, respectively when compared with all of the other treatments except for the corresponding values for the nodal explants, as they were 73.40 and 75.22mm, respectively.

On the other hand, shoot tip cultured on media contained the highest TDZ concentration of 0.80 mg/L and AC at 0.00 or 2.00 g/L measured drastically the shortest shoots values of 15.29 and 17.25mm only, respectively. As for the nodal ones, a similar trend was followed, since they were 11.72 and 13.46mm, respectively. Concerning the leaves number, data in the same table revealed that the highest leaves number of 54.17 leaves was obtained when the nodal explant was cultured on medium augmented with TDZ at 0.20 mg/L without AC. Data in the same line was obtained from the shoot tip explant when it was cultured in the same previous medium, since it was 38.23 leaves. While, a great extent less leaves number of 4.23 and 6.54 leaves were obtained from the shoot tip and the nodal explants which were cultured on TDZ-free medium interacted with AC at 2.00 g/L, respectively.

Effect of the interaction between media strength, sugar (type and concentrations) on vitrification %, shoots [number and length (mm)] and leaves number of lavender plantlets.

Dealing with the effect of this interaction, data in Table (4) cleared that there was a decrease in vitrification % when the media strength started gradually to decrease. Since, using $^1/_4$ strength medium fortified with sucrose or glucose at 40 g/L were significantly recorded the lowest value of 0.00% for each when compared with most of the other interactions. Also, no vitrification was recorded with $^1/_2$ strength medium supplemented with sucrose at 40 g/L. On the other hand, media at full strength supplemented with sucrose or glucose at 20 g/L significantly tabulated the highest vitrification percentages of 42.13 and 45.00%, respectively. This may be due to the high osmotic stress of sucrose when comparing with the glucose one, which decrease the free water into the nutrient medium, so the explant could not absorb it, by that the vitrification % start to decrease. On the other hand, the high concentrations of the micro and macro-elements in the full strength media play as a compressor with the plant cell which make its walls feeble and thick and this coincide with the results of (Kozai *et al.*, 1995) who cleared that the uptake rate per plantlet from NO_3, NH_4, P, K, Ca and Mg generally increased with increasing volume and initial strength of the nutrient medium. So, the osmotic stress increase inside the plant cell which make it absorb a high percentage of the medium water and in the end the hyperhydricity appears.

In addition, as shown in Table (4) and Photo (2) it may be noticed that, the shoots number was affected by the interaction between the three factors of media strength, sugar type and sugar concentration. Since, full strength medium supplemented with sucrose at 40 g/L significantly counted the highest shoots number of 32.11 shoots when compared with most of the other cases. This value was followed by the same medium strength which supplemented with 30 g/L sucrose, as it was 30.55 shoots and it was clear that no significant difference was detected between them. One fourth strength media augmented with sucrose or glucose at any concentration resulted in

the lowest values of shoots number which ranged from 2.37 to 7.43 shoots only. Similar finding was recorded by (Villamor, 2010) on *Zingiber officinalis*, who illustrated that the highest number of shoots was recorded on full strength medium and decreasing the media strength significantly decreased this shoot parameter. Also, (Kawa-Miszczak *et al.*, 2009) on *Hemerocallis*, tested different types of sugar and found that the suitable one was sucrose in producing the highest shoots number comparing with the glucose and glactose ones.

Table (4): Effect of the interaction between media strength, sugar (type and concentrations) on vitrification %, shoots [number and length (mm)] and leaves number of lavender plantlets.

Media strength (A)	Sugar type (B)	Vitrification %			Shoots number			Shoots length (mm)			Leaves number		
		Sugar conc. (g/L) (C)											
		20	30	40	20	30	40	20	30	40	20	30	40
Full	Sucrose	42.13	20.22	10.87	18.30	30.55	32.11	45.00	35.62	31.14	28.13	54.17	58.72
	Glucose	45.00	23.57	15.24	12.15	22.07	27.27	38.34	26.12	27.53	22.24	44.02	54.63
3/4	Sucrose	38.10	15.32	9.54	15.03	26.45	27.32	41.17	33.32	30.16	36.22	52.54	48.37
	Glucose	40.39	16.99	9.97	8.48	17.72	19.24	35.40	23.11	22.13	20.73	34.15	36.25
1/2	Sucrose	27.94	11.28	0.00	8.31	14.04	12.23	33.24	27.68	29.25	22.18	28.42	20.61
	Glucose	29.33	12.04	9.88	5.14	8.22	7.53	29.47	19.04	16.37	14.43	18.23	14.44
1/4	Sucrose	18.05	9.54	0.00	4.51	5.31	7.43	22.19	20.23	24.28	12.64	10.03	12.75
	Glucose	19.31	9.97	0.00	2.37	3.02	5.12	15.40	10.73	7.92	6.81	6.86	10.92
LSD at 5% (A×B×C)		3.06			3.35			4.35			3.65		

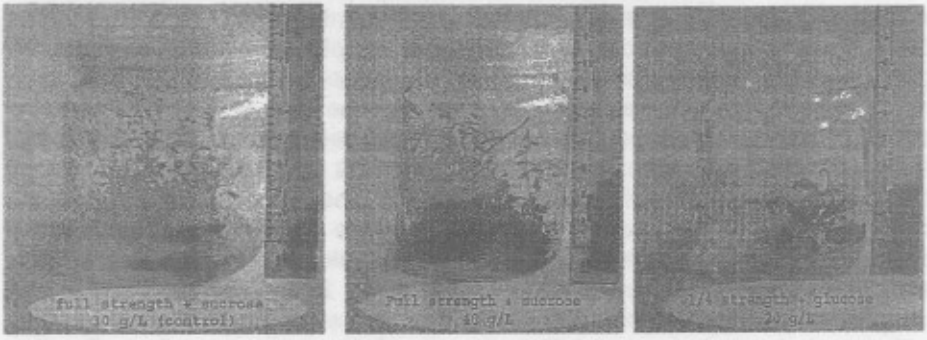

Photo (2): Effect of the interaction between media strength, sugar (type and concentration) on shoots number.

Full strength medium supplemented with sucrose at 20 g/L induced significantly a highest value of 45.00mm when compared with all of the other interactions except for explants cultured on medium at $^{3}/_{4}$ strength fortified with sucrose at the same concentration, as it was 41.17mm. One fourth strength media supplemented with glucose at 20, 30 and 40 g/L significantly measured the shortest values of 15.40, 10.73 and 7.92mm, respectively when compared with most of the other treatments. In general, it was noticed that the sugar concentration was shoots length dependent, as increasing the sugar concentration, decreased the shoots length at most of the tested media

strength and the two types of sugar. These results were in agreement with (Kumar *et al.*, 2010) who noticed an increase in shoots growth of *Chlorophytum borivilianum* when they were cultured on nutrient media supplemented with sucrose at concentrations of 20 g/L and increasing the concentration than this range produced a dwarf shoots.

Using MS full strength medium supplemented with sucrose at 40 g/L promoted remarkably leaves number (58.72 leaves), as it was significantly the highest value. Also, full strength media supplemented with sucrose at 30 g/L or glucose at 40 g/L recorded values of 54.17 and 54.63 leaves, respectively. But, it was extremely noticed that using $^1/_4$ strength medium significantly reduced the leaves number to 6.81, 6.86 and 10.92 leaves only for media supplemented with glucose at 20, 30 and 40 g/L, respectively.

***In vitro* rooting formation:**

Effect of the interaction between media strength, auxin (type and concentration) on rooting %, roots [number and length (mm)] of lavender plantlets.

As for the interaction between media strength, auxin type and auxin concentration, data presented in Table (5) showed obviously that culturing the micro-shoots on MS full strength medium or half strength medium which were supplemented with NAA at 2.00 mg/L significantly achieved the highest rooting percentage of 90.00% for each, when compared with all of the other interactions. The same result was obtained by (Nobre, 1996) on *Lavandula stroechas*, who found that the rooting percentage increased with the increasing of the auxin concentration. Also, our findings were in agreement with (Dias *et al.*, 2002) on *Lavandula viridis*, as they found that the best rooting frequency (100%) was obtained with NAA.

The percentage of 80.03% came in order for that respect when MS half strength medium supplemented with IBA at 2.00 mg/L was used. As for the lowest rooting percentage of 0.00, it was observed when MS full strength-free auxin media used. While, 14.48% for MS half strength-free auxin media tested. But , it was a matter of importance to notice that the interacted effect of auxin type and auxin concentration with full strength media were significantly weaker than these interactions between auxin type and auxin concentration with the half strength media in most of cases. This result agreed with (Chabukswar and Deodhar, 2005) on *Garcinia indica*, as they found that half strength medium significantly produced the highest rooting percentage comparing with full strength medium.

The lowest roots number of 0.00 root in the same table and photo 3 were obtained when MS full strength media-free auxin (control) were used. Also, lowering the media strength to the half without adding auxins increased the roots number to some extent (2.17 roots only). On the other hand, it was a matter of interest to observe that roots number dramatically raised up to 21.25 roots when MS half strength medium supplemented with 1.00 mg/L NAA was tested compared with all of the other treatments. In general, the interaction effect between half strength media, the auxin type and auxin concentration was observed higher than using full strength media combined with the same other two factors, as they ranged from 2.17 to 21.25 roots and

from 0.00 to 15.54 roots, respectively. So, it was clear that the media strength had the upper hand in that respect, followed by auxin type. The indicator which our suggestion was built based on, is the control treatments, as when we started with the lower media strength, we obtained a value of 2.17 roots without adding auxins. Though, NAA showed good response, the roots formed at all concentrations of it were stumpy, thick and brown in color. These roots get easily detached from the microshoots during transplantation and the same result was recorded by (Lakshmi *et al.*, 2010) on *Hoya wightii* Hook. So, we recommend to use IBA than NAA one.

As for roots length, data in Table (5) and Photo (3) indicated that the tallest length of roots was obtained when half strength medium which supplemented with IBA at 2.00 mg/L was evaluated, since it measured 60.63mm.

Table (5): Effect of the interaction between media strength, auxin (type and concentration) on rooting %, roots [number and length (mm)] of lavender plantlets.

Media strength (A)	Auxin type (B)	Rooting %				Roots number				Roots length (mm)			
		Auxin conc. (mg/L) (C)											
		0.00	0.50	1.00	2.00	0.00	0.50	1.00	2.00	0.00	0.50	1.00	2.00
Full	NAA	0.00	60.00	68.87	90.00	0.00	9.43	15.54	7.22	0.00	5.1	9.04	3.13
	IBA	0.00	46.00	61.89	73.57	0.00	4.47	5.61	6.53	0.00	17.42	32.11	51.14
1/2	NAA	14.48	69.73	75.46	90.00	2.17	12.05	21.25	15.26	6.43	8.23	15.76	8.54
	IBA	14.48	55.12	71.19	80.03	2.17	6.24	8.03	11.86	6.43	25.34	45.74	60.63
LSD at 5% (A×B×C)		2.38				3.45				4.29			

Photo (3): Effect of the interaction between media strength, auxin (type and concentration) on roots number.

Also, it was observed that full strength medium combined with IBA at the same concentration (2.00 mg/L) came somewhat less in that respect, as it was 51.14mm, but a significant difference was detected between them. Full strength-free auxin medium produced the shortest roots length of 0.00mm, followed by 3.13mm for full strength medium received NAA at 2.00 mg/L. In general, it was noticed that IBA at any concentration had the upper hand in that respect, since adding it with any media strength significantly induced taller roots in most of cases, when compared with the corresponding NAA

ones. The same results were obtained by (Badawy *et al.*, 2003) on *Lavandula officinalis* and (Fadel *et al.*, 2010) on *Mentha spicata*.

***Ex vitro* establishment:**

Effect of transplanting medium on survival % and increase in shoots length (cm) of lavender transplants.

The benefit of any micropropagation system can, however, is fully realized only by the successful transfer of plantlets from tissue culture vessels to the ambient conditions found *ex vitro*. Most species grown *in vitro* require an acclimatization process in order to ensure that sufficient number of plants survive and grow vigorously when transferred to soil. So, the aim of this experiment was conducted to study the effect of transplanting media on survival % and transplantlets length. It was clear from data in Table (6) that culturing the plantlets of English Lavender on loamy soil augmented with peat moss and vermiculite (1:1:1 by volume) significantly increased the survival percentage, as it reached the highest significant value of 90.00%. On the other hand, using loamy soil mixed with peat moss or vermiculite recorded the lowest values of 60.70 and 63.40%, respectively and no significant differences were detected between them. These results were in agreement with these obtained by (Makunga and Van Staden, 2008) on sage transplantlets.

Data in the same Table (6) and Photo (4) showed that using the mixture of peat moss, vermiculite and loamy soil stimulated the plantlets length, as it reached 12.71cm, followed by 7.00cm when the mixture of loamy soil and vermiculite was used.

Table (6): Effect of transplanting medium on survival % and increase in shoots length (cm) of lavender transplants.

Transplanting media	Survival %	shoots length (cm)
Peat moss + Loamy soil (1:1)	60.70	5.40
Vermiculite + Loamy soil (1:1)	63.40	7.00
Peat moss + Vermiculite + Loamy soil (1:1:1)	90.00	12.71
LSD at 5% (A×B×C)	7.11	7.74

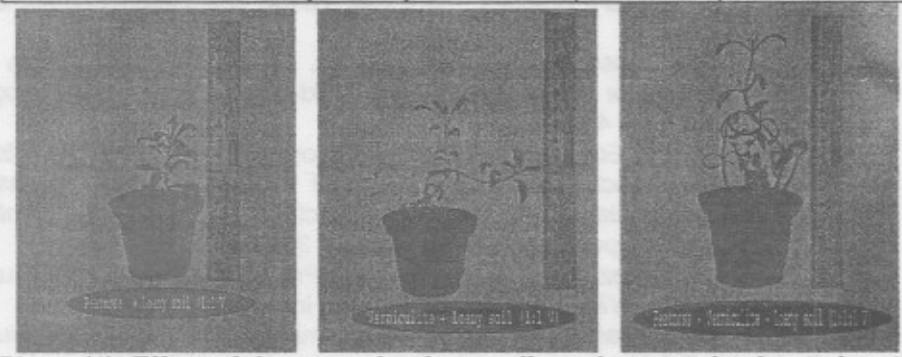

Photo (4): Effect of the transplanting media on increase in shoots length (cm).

But, no significant difference was found between them. Using the mixture of loamy soil and peat moss tabulated the shortest plantlets of 5.40cm. These results may be attributed to the interacted effects of the three transplanting media between them, as presence of loamy soil and vermiculite saved the nutrient elements (containing potassium and magnesium for the last one) in an available form. In addition, presence of peat moss increased the aeration around the transplantlets roots.

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الإكثار الدقيق المباشر لنبات اللافندر الإنجليزى

على منصور حمزة ، أميمة محمد عبد الكافى و محمود مكرم قاسم
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أجرى هذا البحث بمعمل زراعة الأنسجة بقسم الخضر والزينة – كلية الزراعة – جامعة المنصورة خلال الفترة من ٢٠٠٨ – ٢٠١٠. وقد هدف البحث اجراء التضاعف العددى المباشر والتجذير لنبات اللافندر الإنجليزى معمليا وكذلك أقلمة النباتات الناتجة للظروف الخارجية. ولهذا فقد تم دراسة تأثير كلا من نوع الجزء النباتى (القمة النامية و العقدة المحتوية على برعمين جانبيين) المستخدم فى مرحلة التضاعف (انتاج الأفرع الخضرية) وكذلك دراسة تأثير ثلاثة من السيتوكينينات (البنزيل امينو بيورين ، الكينيتين و الثيديازيورون) كلا منهم على حدة بتركيزات (صفر، ٠.٠٥، ٠.١٠، ٠.٢٠، ٠.٤٠ و ٠.٨٠ ملليجرام/اللتر) مقترنا مع تركيزين للفحم النشط (صفر و ٢ جرام/اللتر) وذلك لتقيم تأثير تلك العوامل على النسبة المئوية للبقاء، عدد الأفرع/الجزء النباتى، عدد الأوراق وطول النموات الناتجة. أيضا تم زراعة أفضل جزء نباتى متحصل عليه من التجارب السابقة على بيئات موراشيج وسكوج مختلفة القوى (كاملة، ٣/٤، ١/٢ و ١/٤ قوة) مع نوعين من مصادر الكربون (السكروز و الجلوكوز) بثلاثة تركيزات (٢٠، ٣٠ و ٤٠ جرام/اللتر) وذلك لدراسة تأثير هذه العوامل على النسبة المئوية للتزجج، عدد الأفرع/الجزء النباتى، عدد الأوراق وكذلك طول النموات الخضرية بعد أربع اسابيع من تاريخ الزراعة. للتجذير المعملى صممت تجربة لدراسة تأثير قوة البيئة (كاملة و نصف قوى) ونوع الأكسين المستخدم (نفثالين حمض الخليك و اندول حمض البيوتريك) وتركيز كلا منهما (صفر، ٠.٥٠، ١.٠ و ٢.٠ ملليجرام/اللتر) على النسبة المئوية للتجذير ، عدد و طول الجذور. كما انه لإجراء الأقلمة تم دراسة تأثير ثلاث مخاليط من بيئات الزراعة (بيتموس + تربة طمية ، فيرميكيوليت + تربة طمية و بيتموس + فيرميكيوليت + تربة طمية).

وكانت أهم النتائج كالتالى:

١- أفضل عدد للأفرع الخضرية (٣٠.٥٥ نمو خضرى) وكذلك عدد الأوراق (٥٤.١٧ ورقة) نتج من عقدة محتوية على برعمين جانبيين والنامية على بيئة موراشيج وسكوج المضاف اليها ثيديازورون بتركيز (٠.٢٠ ملليجرام/اللتر). أعطى اضافة البنزيل امينو بيورين بتركيز (٠.٨٠ ملليجرام/اللتر) وبدون فحم نشط للبيئة المغذية عدد ١٦.٧٠ نمو خضرى وعدد أوراق ٣٥.٤٢ ورقة. كان أكبر طول للنموات الخضرية المتكونة عند اضافة الكينيتين الى بيئة النمو مع معظم تركيزاته بالمقارنة مع السيتوكينينات الأخرى. أيضا استخدام الفحم النشط لم يبين أى تأثير مع معظم الصفات المدروسة.

٢- سجلت بيئة موراشيج وسكوج كاملة القوى المضاف اليها السكروز كمصدر للكربون بتركيز ٣٠ أو ٤٠ جرام/اللتر اكبر عدد من النموات الخضرية والأوراق. ولكن بيئة ربع قوى والتى زودت بالسكروز أو الجلوكوز بمعدل ٤٠ جرام/اللتر سجلت أقل نسبة مئوية للتزجج (٠.٠ %).

٣- أكبر عدد للجذور (٢١ جذر) سجل لبيئة موراشيج وسكوج نصف قوى تحتوى على ١.٠ ملليجرام/اللتر نفثالين حامض الخليك ولكن لم يتعدى طول الجذور عن ١٥.٧٦ ملليمتر وكانت الجذور سميكة وضعيفة وذات لون بنى سهلة القصف عن الجزء النباتى بالمقارنة مع الجذور الناتجة من استعمال اندول حمض البيوتريك بتركيز ٢.٠ ملليجرام/اللتر والمضاف لبيئة نصف قوى، حيث كان متوسط عددها ١١.٨٦ جذر ومتوسط طولها ٦٠.٦٣ ملليمتر.

٤- أقلمة النباتات الناتجة فى المخلوط الثلاثى (البيتموس + الفرميكيوليت + التربة الطمية) بنسبة (١:١:١ بالحجم) كان الأفضل فى اعطاء اعلى نسبة بقاء وكذلك اكبر زيادة فى طول الأفرع ولكن لم يكن هناك فروق معنوية بين الثلاثة مخاليط فى الصفة الأخيرة.

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