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SOME FACTORS AFFECTING MICROPROPAGATION OF *Haplophyllum tuberculatum* L.

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ABSTRACT: This study was carried out at Plant Tissue Culture Laboratory, Faculty of Environmental Agricultural Sciences, El-Arish University, North Sinai Governorate, Egypt during 2014 to 2017. Effect of three medium types, Murashige and Skoog (MS), Linsmaier and Skoog (LS) and Lloyd and McCown (WPM), two explant types (shoot-tip and single node cutting), three cytokinin types (Benzyladenine, Kinetin and 2-isopenteny1 adenine), two additives; *viz.*, s (thiamine and pyridoxine hydrochloride) and amino acids (asparagine and arginine) and two gelling agents (guar seed powder and locust bean seed powder) combined with agar on micropropagation of *Haplophyllum tuberculatum* were investigated. For rooting, the effect of three auxins (Indole acetic acid, Indol butyric acid and Naphthalene acetic acid) were studied in combination with three medium strengths (full, half and quarter) combined with the best mixture (0.50 mgl^{-1} Kin + 6 gl^{-1} agar with 2 gl^{-1}guar seed powder) of gelling agents. Obtained results showed that Murashige and Skoog (MS) was the best medium with single node explant as starting material during establishment stage. Multiple shoots were obtained on MS medium supplemented with 1.0 mgl^{-1} Kinetin (Kin) combined with 0.1 mgl^{-1} α-naphthalene acetic acid (NAA). Thiamine and arginine were the best vitamins and amino acid at 0.1 mgl^{-1} and 0.50 mgl^{-1}, respectively. Agar at 6 gl^{-1} + guar seed powder at 2 gl^{-1} was the best gelling agent treatment. Half strength MS medium with 0.50 mgl^{-1} Kin + 0.5 mgl^{-1} NAA and 6 gml^{-1} agar with 2 gml^{-1} guar seed powder was the best combination for rooting were successfully (85% survival) acclimatized in mixture of peatmoss, vermiculite, washed sterilized sand and perlite (1:1:1:1, *V/V/V/V*).

Key words: *Haplophyllum tuberculatum*, micropropagation, plantlets, vitamins, kinetin, auixun, multiplication, rooting, acclimatization.

INTRODUCTION

Haplophyllum tuberculatum L. belongs to Rutaceae family and an indigenous perennial of most Mediterranean countries (**Elmaghrabi *et al*., 2017**). In North Sinai, the plant is known as 'dharret rieh, or um-jeneinah and naturally found in the course, sandy and gravelly soil (**El-Naggar *et al*., 2014**). Plant is used in the Egyptian folk medicine as a remedy for headaches and arthritis, the juice is applied as a wart removal, skin discoloration, infections and parasitic diseases (**Mechehoud *et al*., 2014**).

H. tuberculatum L. plant is about 60 cm in height and covered with tiny raised glands. The stem is yellowish- green to white, and the leaves are alternate and elliptic to obviate. The flowers are in loose corymbose terminal panicles, with five free ovate sepals (**Täckholm, 1974**).

H. tuberculatum L. naturally propagated by seeds in North Sinai. However, due to overgrazing of this plant, it failed to propagate naturally by seeds which led this species to endanger and there is a need to keep this genetic resource (**Elmaghrabi *et al*., 2017**).

Conservation of the endemic or threatened plant species is carried out using different strategies. Micropropagation a powerful tool for ex situ conservation programs of this rich flora especially for species with much reduced population. Also, Micropropagation facilitates the rapid establishment of the large number of

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stock plants forming minimum impact on endanger wild population (**Nizar, 2001**).

Few studies have been conducted on *in vitro* propagation of *H. tuberculatum* L **Elmaghrabi *et al.*, 2017**). So that this study aimed to establish an applicable protocol to save the endangered native *Haplophyllum tuberculatum* plant through *in vitro* micropropagation technique.

MATERIALS AND METHODS

This study was carried out at Plant Tissue Culture Laboratory, Faculty of Environmental Agricultural Sciences, El-Arish University, North Sinai Governorate, Egypt during the period from 2014 to 2017.

Establishment Stage

Plant materials

Shoot tip explants and single node cuttings from mature plants were collected from different locations of Rafah region (Balaa, Yamet, and Al matallah) on February and March 2014. Plant materials were wrapped with wet papers and transferred in ice box to the Tissue Culture Lab.

Explants sterilization

Explants (shoot tip and single node cuttins) were quickly rinsed with a few drops of liquid soap for 5 minutes and rinsed again under running tap water for 60 minutes to remove all the remaining detergent and washed with sterilized distilled water. The explants were soaked for 25 minutes in 25% Clorox (containing 5.25% sodium hypochlorite) then washed again with sterilized distilled water for 3-5 times to remove all traces of Clorox. Explants were quickly dipped in 70 % ethanol for 20 sec. and washed with sterilized distilled water for 3-5 times. The explants were then immersed in 0.1% mercuric chloride ($HgCl_2$) solution and again rinsed thrice in sterile distilled water. All steps of the sterilization had been done under aseptic condition inside the culture cabinet by using sterilized instruments. The purpose of this procedure was to disinfect the plant tissue from fungi, bacteria and other contamination without harming the regenerative capacity of the explants.

Culture media

MS, LS, WPM media containing macro and micro elements as well as vitamins, according to **Murashige and Skoog (1962), Linsmaier and Skoog (1965) and Lloyd and McCown (1980)**, were used through this study. The media were supplemented with 100 mgl^{-1} myo-inositol and 3% sucrose. All media pH were adjusted to be 5.7- 5.8 by using either 0.10 N NaOH or 0.10 N HCl prior to addition of 7.00 gl^{-1} agar. The media were dispensed into glass tissue culture jars and tube each one contained 15 ml of culture medium. All media were autoclaved at 121^{o}c and 1.06 kg/ cm^2 for 20 min. The jars and slant tubes were transferred to the culture cabinet and left cool till they were used.

Medium type

MS, LS and WPM media were tested in this study to select the best medium type that induces the highest explant development rate.

Explant type

Shoot tip or single node cuttings (0.5 - 1.00 cm) were excised and cultured on MS, LS and WPM media to select the best explant type for development.

Culture conditions

Explants were cultured on the media under complete aseptic conditions in the laminar air flow cabinet. All cultures were incubated for 4 weeks under controlled conditions in the growth chamber. The incubation temperature was 25± 2^{o}C. The photoperiod was 16 hr light/8 hr dark. Iliumination intensity was 3000 lux for all experiments. Shoot length (cm), number of shoots/explant and number of leaves/shoot were recorded after four weeks from culturing.

Multiplication Stage

This stage aimed to increase the number of proliferated shoots. The shoots obtained from the establishment stage were used as explants during multiplication experiments.

Cytokinin type

Kinetin (kin), 6-benzyladenine (BA) and 2-isopentenyl adenine (2iP) were used at the rate of 1.0 mgl^{-1} to detect the best cytokinin that induced the highest multiplication rate.

Kinetin concentration

Different Kin concentrations (0.00, 0.50, 1.00, 1.50, 2.00 mgl^{-1}) combined with 0.1 mgl^{-1}

NAA were evaluated to investigate the most suitable concentration that induces the highest multiplication rate.

Additives

Amino acids (Asparagine and Arginine) and vitamins [Thiamine hydrochloride (B_1) and Pyridoxine hydrochloride (B_6)] were added to the culture MS medium at the level of 0.10 mgl^{-1} to detect the most effective additive that maximize explants development and growth rate of *Haplophyllum tuberculatum.* Arginine and thiamine were the best amino acid and vitamin, respectively which were used for multiplication stage.

Kin plus NAA with some alternative gelling agents

Seeds of guar (GP) and locust bean (LBP) were separated from pods and ground in a blender. 2, 4, 6 and 8 g of powders from both seeds were mixed with agar to be used as a gelling agent. Powder mixed with full strength MS medium + 1.00 mgl^{-1} Kin + 0.10mgl^{-1} NAA as follows:

1) Agar 8 gl^{-1}; 2) 6 gl^{-1} Agar + 2 gl^{-1}GP; 3) 4 gl^{-1} Agar + 4 gl^{-1} GP; 4) 2 gl^{-1}Agar + 6 gl^{-1} GP; 5) 6 gl^{-1} Agar + 2 gl^{-1} LBP; 6) 4 gl^{-1} Agar + 4 gl^{-1} LBP and 7) 2 gl^{-1} Agar + 6 gl^{-1} LBP. After four weeks from inoculation date *i.e.,* shoot length (cm), number of shoots/explant and number of leaves/shoot) were recorded for all the above mentioned multiplication stage experiments.

Rooting Stage

The proliferated shoots 4 cm length of *Haplophyllum tuberculatum* were used as explants and cultured on MS supplemented with 100 mgl^{-1} myo-inositol, 30.0 gl^{-1} sucrose and 7.0 gl^{-1} agar.

Medium strength and auxin type

Shoots (about 1 cm length) obtained from multiplication stage were excised from the proliferated shoot and cultured on full, half or quarter strengths of MS basal medium supplemented with 0.50 of mgl^{-1} IAA, IBA, and NAA for each medium.

NAA concentration

Shoots (about 1 cm length) were cultured on ½ MS solid media with different concentration of NAA (0.00, 0.30, 0.50 and 0.70 mgl^{-1}) combined with 0.5 mgl^{-1} Kin to determine the suitable concentration which encouraged the highest root formation rate.

Acclimatization Stage

Rooted plantlets of *Haplophyllum tuberculatum* were taken out from the jars. The roots of the chosen plantlets were washed thoroughly with running tap water to get rid of residues. Then, the roots were washed with sterilized distilled water after removing of dead leaves and dry shoots of plantlets. Plantlets were surface sterilized by soaking in a fungicide solution of Rizolex (1 mgl^{-1}) for 3-5 min., then planted in black polyethylene bags diameter (8 cm) filled with peatmoss, vermiculite, washed sterilized sand, and perlite 1:1:1:1 (*V/V/V/V*). Plantlets were covered with white transparent bags having small holes which were made after one week and these holes were expanded each week gradually for four weeks until the plantlet became suitable for transferred to the bigger pots (30 cm). Survival percentage was calculated after 30 days from acclimatization.

Statistical Analysis

The statistical design of the above mentioned experiments was simple completely randomized design. Data were analyzed with analysis of variance (ANOVA) using SAS statistical software (**SAS, 2004**). Differences between means were compared by using Duncan's multiple range test (DMRT) (**Duncan, 1955**) at the 0.05 % level of probability.

RESULTS AND DISCUSSION

Establishment Stage

Effect of interaction between medium and explant types

Results in Table 1 show that use of MS with single node cutting had the highest values of all studied traits compared with the other treatments. This result is supported by the findings of **Sudipta *et al.* (2011), and Elmaghrabi *et al.* (2017)**. They found that one node cutting is the

Table 1. Effect of interaction between media and explant type on explant development of *Haplophyllum tuberculatum* plant during establishment stage

Media type	Explant type	Shoot length (cm)	Number of shoots/explant	Number of leaves/shoot
Murashige and Skoog (MS)	**Single node cuttings**	2.97a	2.67a	9.67a
	Shoot tip	2.17ab	1.33b	5.00b
Linsmaier and Skoog (LS)	**Single node cuttings**	1.30bc	1.00b	1.33c
	Shoot tip	0.93c	1.00b	1.67c
Woody Plant Medium (WPM)	**Single node cuttings**	1.70bc	1.00b	1.67c
	Shoot tip	1.00c	1.00b	1.00c

Means followed by the same letter within each column are not significantly different at 0.05 level of probability according to Duncan's multiple range test.

best source for the highest shoot induction obtained on MS medium on *Leptadenia reticulate*, *Atalantia monophylla* and *Haplophyllum tuberculatum,* respectively. On the other side, **Ozudogru *et al.* (2011) and Aicha *et al.* (2013)** found that MS was the best medium with shoot-tip from mother plants for *Thymus vulgaris* and *Thymus satureioides*.

Multiplication Stage

Effect of Cytokinin type

Results in Table 2 indicate that addition of kin to the medium increased number of shoots/ explant compared with control and other cytokinin types. Concerning shoot length and number of leaves/shoots, there were no significant differences between control and Kin treatments. Also, in most cases there were no significant differences between control and BA treatment in this regard.This result may be due to the effect of cytokinin which is required in optimal quantity for shoot proliferation in many genotypes as reported by **Sharma *et al.* (1993), Shasany *et al.* (1998) and Rout *et al.* (2000)**. This result is in agreement with those of **Elmaghrabi *et al.* (2017)** who found that amongst two cytokinins tested Kin proved to be more effective than BA for initiating shoots and a higher yield of shoots per explant on *Haplophyllum tuberculatum*.

Effect of different kinetin concentrations combined with 0.1 mgl^{-1}NAA

Results in Table 3 show that Kinetin at 1.00 mgl^{-1} combined with 0.1 mgl^{-1} NAA recorded the highest value for each of number of shoots/ explant and number of leaves. On the other side, shoot length did not significantly affected by increasing kin concentration in most cases. This result is in agreement with the findings of **Hussey and Falavigna (1980)** who found that kinetin together with NAA, IAA or 2,4-D stimulated *Allium cepa* shoot bud formation to a greater extent than kinetin alone. Also, **Parihar *et al.* (2010)** found that maximum shoot proliferation was obtained with 2 mgl^{-1} Kin on *Aegle marmelos* (L.). In addition, **Abdallah *et al.* (2017)** found that MS supplemented with 0.5 mgl^{-1} Kin and 0.05 mgl^{-1} NAA produced the highest shoot proliferation on *Origanum syriacum*. Moreover, **Elmaghrabi *et al.* (2017)** found that Kin at 1.00 mgl^{-1} + MS medium was the best concentration for multiplication of shoots of *Haplophyllum tuberculatum*.

Effect of additives

Effect of vitamins

Results in Table 4 and Photo 1 show that addition of 0.1 mgl^{-1} thiamine to the culture medium resulted in significant increase in all investigated parameters compared with control and pyriodoxine. This result is in agreement with **Abdallah (2012) and Abdallah *et al.* (2017)** since they found that thiamine was effective in enhancing number of shoots, shoot length, and number of leaves of *Capparis spinosa* and *Origanum syriacum,* respectively.

Table 2. Effect of cytokinin type on growth and development of *Haplophyllum tuberculatum* plant during multiplication stage

Cytokinin type at 1 mgl^{-1}	Shoot length (cm)	Number of shoots/explant	Number of leaves/shoot
Control (MS free)	2.87ab	1.67b	11.00ab
Kin	3.67a	2.67a	13.67a
BA	1.33c	1.33b	6.67b
2ip	1.93bc	1.67b	9.33ab

Means followed by the same letter within each column are not significantly different at 0.05 level of probability according to Duncan's multiple range test.

Table 3. Effect of different kinetin concentrations combined with 0.1 mgl^{-1} NAA on growth and development of *Haplophyllum tuberculatum* plant during multiplication stage

Kin (mgl^{-1})	Shoot length (cm)	Number of shoots/explant	Number of leaves/shoot
Control (MS free)	1.66 ab	2.63 c	5.33b
0.50	1.66 ab	3.66b	5.33b
1.00	2.66 a	5.13a	7.33a
1.50	1.33 b	2.10 cd	5.00bc
2.00	1.00 b	1.40d	1.40d

Means followed by the same letter within each column are not significantly different at 0.05 level of probability according to Duncan's multiple range test.

Table 4. Effect of vitamins on growth and development of *Haplophyllum tuberculatum* plant during multiplication stage

Vitamin (0.1 mgl^{-1})	Shoot length (cm)	Number of shoots/explant	Number of leaves/shoot
Control (MS + 1mgl^{-1} Kin + 0.1 mgl^{-1} NAA)	2.95b	1.83b	14.16b
Thiamin (B1)	4.53a	3.18a	17.00a
Pyridoxine (B_6)	2.14C	1.54c	14.50c

Means followed by the same letter within each column are not significantly different at 0.05 level of probability according to Duncan's multiple range test.

Photo 1. Effect of vitamins (0.1 mgl^{-1}) on shoot growth and development of *Haplophyllum tuberculatum* plant during multiplication stage

Effect of thiamine concentration

Results in Table 5 show that only the concentration of 0.10 mgl^{-1} of thiamine could significantly enhanced the values of all traits. This result is in harmony with **Abdallah (2012)** who found that thiamine was effective in enhancing number of shoots, shoot length, and number of leaves of *Capparis spinosa* and **Abdallah *et al.* (2017)** who reported that thiamine at 0.4 mgl^{-1} was the best additive for shoot development on *Origanum syriacum* L. On the other hand, **Ali (2017)** found that MS medium supplemented with 0.30 mgl^{-1} pyridoxine achieved the best shoot development compared with the other additive treatments on *Marrubium vulgare* L. This result may be due to that thiamine is basically required by all cells for growth as reported by **Ohira *et al.* (1976)**. Also, **Robbins (1951) and Linsmaier and Skoog (1965)** found that thiamine was essential for proper growth, metabolism, and division of plant cells in culture.

Effect of amino acids

Results in Table 6 and Photo 2 show that applying arginine resulted in the highest number for each of shoots/ explant, number of leaves and shoot length. This result may be due to the effect of amino acids which provide plant cells with an immediately available source of nitrogen, which generally can be taken up by the cells more rapidly than inorganic nitrogen as reported by **Thom *et al.* (1981)**.

Effect of arginine concentration

Results in Table 7 show that 0.5 mgl^{-1} arginine significantly improved shoot length compared with control and other arginine treatments. While, there were no significant differences between control treatment and different tested arginine concentrations concerning number of shoots/explan. Regarding number of leaves/shoot, only 0.50 mgl^{-1} concentration could enhance this character compared with control treatment, without significant differences among different investigated concentrations. This result may be due to the effect of amino acids which play a key role in plant growth and development because they are a good source of nitrogen as reported by **Kirby et *al.* (1987) and Shanjani (2003)**.

Effect of some alternative gelling agents combined with 1 mgl^{-1} Kin plus 0.1mgl^{-1} NAA

Results in Table 8 show that applying 2 gml^{-1} guar + 6 gml^{-1} agar combined with1mgl^{-1} Kin and 0.10 mgl^{-1} NAA recorded the highest values of all studied traits. This result is in agreement with the findings of **Jain- Raina and Babbar (2011)** who found that the highest shoot length was achieved when plant cultured on MS media supplemented with 2.7% guar gum and 0.3% agar compared with control (0.9% Agar) of *Albizzia lebbeck*. Also, **Clapa *et al.* (2011)** found that the highest number of multiple shoots was obtained with guar gum at 20 gml^{-1} as

Table 5. Effect of thiamine concentration on growth and development of *Haplophyllum tuberculatum* plant during multiplication stage

Thiamine conc. (mgl^{-1})	Shoot length (cm)	Number of shoots/ explant	Number of leaves/shoot
Control(MS + 1mgl^{-1} Kin +0.1 mgl^{-1} NAA)	1.13b	1.33b	2.66b
0.10	3.23a	3.00a	11.33a
0.30	1.13b	1.33b	2.33b
0.50	1.13b	1.33b	4.00b
1.00	1.90b	1.60ab	3.00b

Means followed by the same letter within each column are not significantly different at 0.05 level of probability according to Duncan's multiple range test.

Table 6. Effect of amino acids on growth and development of *Haplophyllum tuberculatum* plant during multiplication stage

Amino acid (0.1 mgl^{-1})	Shoot length (cm)	Number of shoots/explant	Number of leaves/shoot
Control (MS + 1 mgl^{-1} Kin + 0.1mgl^{-1}NAA)	2.95b	1.83b	14.17b
Asparagine	2.78c	1.36c	13.40c
Arginine	4.46a	4.94a	25.80a

Means followed by the same letter within each column are not significantly different at 0.05 level of probability according to Duncan's multiple range test.

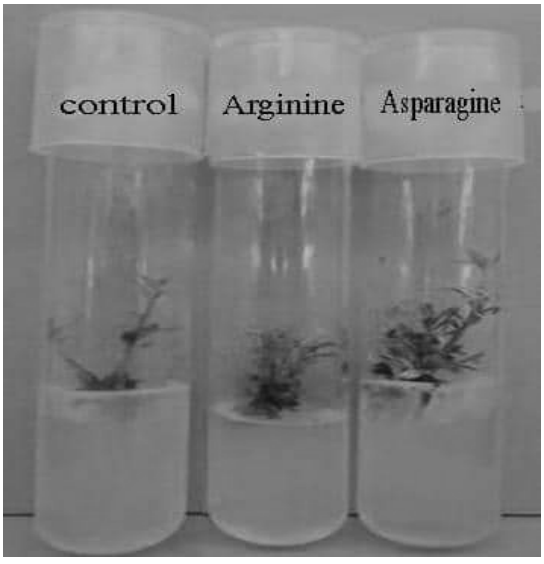

Photo 2. Effect of amino acid on growth and development of *Haplophyllum tuberculatum* plant during multiplication stage

Table 7. Effect of arginine concentration on growth and development of *Haplophyllum tuberculatum* plant during multiplication stage

Arginine conc. (mgl^{-1})	Shoot length (cm)	Number of shoots/explant	Number of leaves/shoot
Control (MS + 1mgl^{-1} Kin + 0.1 mgl^{-1}NAA)	1.13d	1.33ab	2.66b
0.10	3.10b	1.00b	4.33ab
0.30	1.96c	1.00b	6.00ab
0.50	4.86a	2.00a	8.00a
1.00	2.00c	1.33ab	7.33ab

Means followed by the same letter within each column are not significantly different at 0.05 level of probability according to Duncan's multiple range test.

Table 8. Effect of some alternative gelling agents combined with 1mgl^{-1}Kin plus 0.1 mgl^{-1} NAA on shoot proliferation of *Haplophyllum tuberculatum* plant during multiplication stage

Gelling agent	Shoot length (cm)	Number of shoots/explant	Number of leaves/shoot
Control (8 gl^{-1} Ag)	1.00 d	1.00 c	2.33 b
6 gl^{-1} Ag + 2 gl^{-1} GP	9.87 a	9.33 a	29.33 a
4 gl^{-1} Ag + 4 gl^{-1} GP	4.86 b	1.00 d	8.66 b
2 gl^{-1}Ag+ 6 gl^{-1} GP	4.67 b	4.33 cb	7.50 b
6 gl^{-1} Ag + 2 gl^{-1} LBP	9.66 b	2.33 cd	2.63 cb
4 gl^{-1} Ag + 4 gl^{-} LBP	4.67 b	6.00 b	7.00 b
2 gl^{-1} Ag + 6 gl^{-1} LBP	4.73 b	6.00 b	6.66 b

Means followed by the same letter within each column are not significantly different at 0.05 level of probability according to Duncan's multiple range test

Ag: Agar; GP: Guar Powder: LBP: locust bean Powder

gelling agents of *Pinguicula vulgaris*. Moreover, **Dobránszki and Tabori (2011)** found that the highest number of shoots per explant for Galaxy apple and black locust were obtained when agar/phytagel or agar/guar gum were used as gelling agents. However, **Hassan *et al.* (2016)** found that the combination between agar at 4gl^{-1} and locust bean (LBG) at 4gl^{-1}as gelling agent produced the maximum growth values on *Philodendron selloum*. On the other hand, **Babbar *et al.* (2005)** found that axillary shoot proliferation was better on guar gum-gelled media than on agar media.

Rooting Stage

Effect of medium strength and auxin type

Results in Table 9 and Photo 3 indicate that using half strength MS medium with NAA was the best combination which resulted in the highest value for each of studied traits (shoot length, number of shoots/explant and number of roots/ clump). This result is in agreement with findings of **Samarina *et al.* (2010)** who found that the best results for rooting of shoots was obtained on half strength MS medium supplemented with 0.5 mgl^{-1} NAA alone on lemon.

Tabal 9. Effect of medium strength and auxin type at 0.5 mgl^{-1} Kin on shoot and root growth of *Haplophyllum tuberculatum* plant during rooting stage

Medium strength	Auxin type	Shoot length (cm)	Number of shoots/explant	Number of roots/clump
Full	Control (MS +0.5 mgl^{-1} Kin)	1.75 c	1.50 bc	16.50 cbd
	NAA	4.25 a	2.50 bc	8.50 cfd
	IBA	2.25 bc	1.50 bc	11.50 edf
	IAA	2.50 bc	1.50 bc	11.00 edf
Half	Control (MS +0.5 mgl^{-1} Kin)	2.80 bc	1.50bc	13.50 cefd
	NAA	4.26 a	4.66 a	37.33 a
	IBA	2.20bc	2.50bc	24.00 b
	IAA	3.30 ab	2.00 bc	14.00 cedf
Quarter	Control (MS +0.5 mgl^{-1} Kin)	1.70 c	1.50 bc	16.00 cebd
	NAA	3.25 ab	3.00 b	22.00 cb
	IBA	2.25 bc	1.50 bc	7.00 ef
	IAA	1.85 c	1.00 c	5.50 f

Means followed by the same letter within each column are not significantly different at 0.05 level of probability according to Duncan's multiple range test.

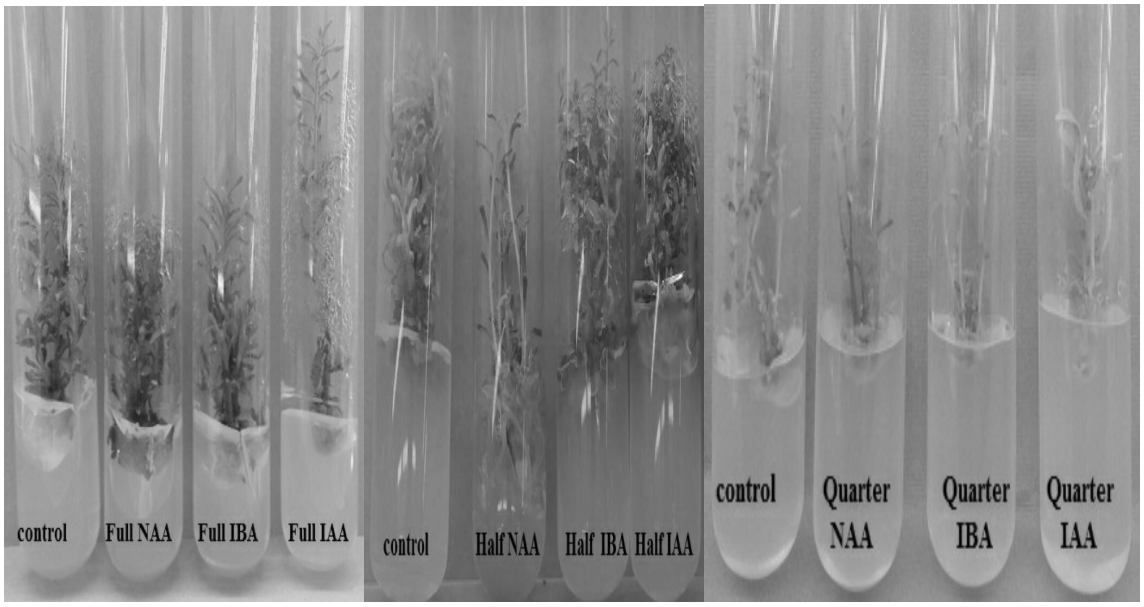

Photo 3. Effect of medium strength and auxin type at 0.5 mgl^{-1} Kin on shoot and root growth of *Haplophyllum tuberculatum* plant during rooting stage

Also, **Hiregoudar *et al.* (2005)** compared efficiency of NAA, IBA and IAA for root induction in *Feronia limonia* and reported that the highest rooting ability for NAA, while IBA caused callusing at the shoot base and IAA showed a low rooting rate. Also, **Praveena and Veeresham (2014)** found that the highest rooting percentage, average number of roots/ shoot and average root length were obtained on half strength MS medium containing IBA and NAA on *Toddalia asiatica*.

Effect of NAA concentration combined with 0.5 mgl^{-1} Kin + 2 gl^{-1}guar seed powder and 6 gl^{-1} agar (as gelling agent)

Results in Table 10 and Photo 4 show that using of half strength MS medium with 0.50 mgl^{-1} NAA + 1 mgl^{-1} Kin + 2 gl^{-1} guar seed powder + 6 gl^{-1} agar (as gelling agent) was the best combination which resulted in the highest value for each of the studied traits followed by descendingly by control, 0.10, 0.30 and 0.70 mgl^{-1} NAA. This result is supported with the findings of **Praveena and Veeresham (2014)** On *Toddalia asiatica* who found that half strength MS medium fortified with different concentrations of NAA (0.50, 1.0, 2.0, 3.0 mgl^{-1}) resulted in the best rooting. Also, **Esmaeilnia and Dehestani (2015)** on *Citrus sinensis* revealed that rooting percentage was influenced by the cytokinin concentration of the preceding bud induction medium, with the highest effects for Kin rather BA, independently of the auxin concentration in the rooting media. The highest rooting rates were recorded for media supplemented with 0.5 mgl^{-1} or 1.0 mgl^{-1}NAA, respectively. In the same direction, **Babbar *et al.* (2005)** found that the elongation of roots for *Crataeva nurvala* was much better on guar gum-gelled medium than on agar medium. Also, **Almazrooeia and Aldwai (2017)** on *Ochradenus baccatus* found that maximum root induction with high mean root length was produced from shoots subcultured on MS medium supplemented with low concentration of NAA (0.5 mgl^{-1}). At higher concentration of NAA (1.5-2.0 mg l^{-1}) a decrease in root production was observed. On the other hand, roots were not observed when shoots were subcultured on hormone-free medium. However, **Elmaghrabi *et al.* (2017)** found that *Haplophyllum tuberculatum* shoots were rooted well after six weeks on MS medium supplemented 1.0 mgl^{-1} Kin.

Acclimatization Stage

Plantlets were successfully acclimatized in the mixture of peatmoss, vermiculite, sand, and perlite since it resulted in 85% survivability.

Table 10. Effect of NAA concentration combined with 0.5 mgl^{-1} Kin + 2 gl^{-1} guar powder and 6 gl^{-1}agar on *Haplophyllum tuberculatum* plant during rooting stage

NAA conc. (mgl^{-1})	Shoot length (cm)	Number shoots/explant	Number of roots/clump
Control*	2.33 b	4.00ab	0.00 b
0.1	3.40 b	2.00b	0.00 b
0.3	2.80 b	2.00b	0.00 b
0.5	4.65 a	6.66a	2.08 a
0.7	3.10 b	2.33b	0.00 b

Means followed by the same letter within each column are not significantly different at 0.05 level of probability according to Duncan's multiple range test.

* Control=0.5 mgl-l Kin + 2 gml-1guar powder and 6gml-1agar

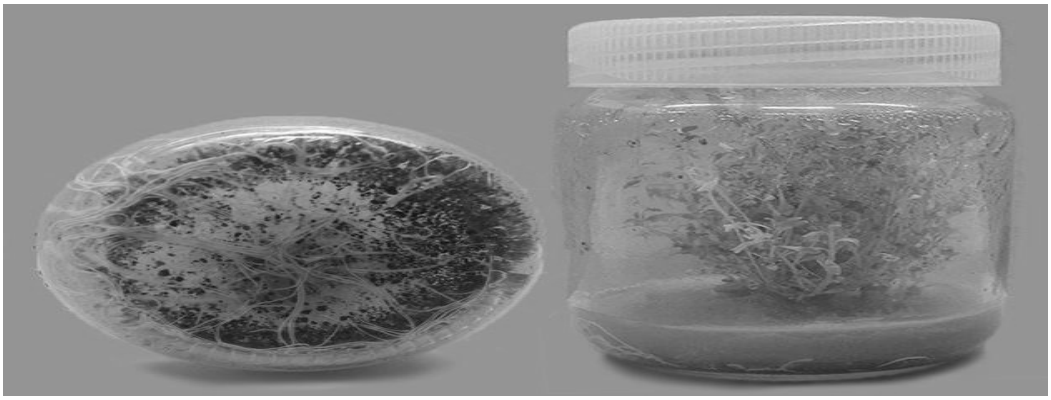

Photo 4. Effect of NAA concentration combined with 0.5 mgl^{-1} Kin + 2 gl^{-1} guar powder and 6 gl^{-1} agar on *Haplophyllum tuberculatum* plant during rooting stage

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بعض العوامـل المـؤثـرة فى الإكثـار الدقيـق لنبـات أم جنينـه

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أجريت هذه الدراسة في معمل زراعة الأنسجة النباتية – كلية العلوم الزراعية البيئية– جامعة العريش محافظه شمال سيناء- مصر وذلك خلال الفترة من ٢٠١٤–٢٠١٧م بهدف إكثار نبات أم جنينه باستخدام تقنيه زراعه الأنسجة، حيث تم إختبار تاثير ثلاث بيئات هى بيئة موراشيج وسكوج، وبيئة لنسمير وسكوج و بيئه ليلويد وماك كوين ونوعين من الأجزاء النباتية (القمم النامية – القطع ذات العقدة الواحدة)، وثلاث أنواع من السيتوكينين (الكينتين-البنزيل أدينين-أيزوبنتانايل أدينين)، ونوعين من الإضافات هما الفيتامينات (الثيامين - البيرودكسين)، والأحماض الأمينية (الأسبارجين-الأرجنين)، كما اختبر كذلك إستخدام نوعين من مواد التصلب (مسحوق بذور الجوار و مسحوق بذور الخروب) مع الأجار على الإكثار الدقيق لنبات أم جنينيه، كما تم خلال مرحلة التجذير دراسة تأثير ثلاث أكسينات (إندول حامض الخليك-إندول حامض البيوتيرك – نفثالين حامض الخليك) مع ثلاث قوى مختلفة من بيئة موراشيج وسكوج (كامله، ونصف و ربع قوه) علاوة على إضافة أفضل معاملة تصلب (٦جم/لتر أجار + ٢جم/لتر مسحوق بذور الجوار) بالإشتراك مع الكينتين بمعدل ٠,٥٠ ملليجرام/لتر، وقد اشارت النتائج المتحصل عليها أن أفضل بيئة هى بيئه موراشيج وسكوج مع القطع ذات العقدة الواحدة كما كان أفضل سيتوكينين هو الكينتين بمعدل ٠,٥٠ ملليجرام/لتر، وافضل معاملة تصلب هي ٦ جم/لتر أجار + ٢ جم/لتر مسحوق بذور الجوار. وكان الثيامين أفضل فيتامين بينما كان الأرجنين افضل حمض امينى بمعدل ٠,١ ملليجرام/لتر و١,٠٠ ملليجرام/لتر على التوالى. كذلك كانت أفضل توليفه للتجذير هى إستخدام بيئة موراشيج وسكوج ذات النصف قوه مع أفضل توليفه تصلب و هي ٦ جم/لتر أجار + ٢ جم/لتر مسحوق بذور الجوار، كما امكن اقلمة الشتلات الناتجة بنجاح (٨٥% نسبة بقاء) بزراعتها فى مخلوط بيئه يتكون من البيتموس والفيرميكيوليت والرمل والبيرليت بنسب حجمية متساوية.

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