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EFFECT OF SOME GROWTH REGULATORS AND GELLING AGENT ON MICROPROPAGATION OF *Codiaeum varigatum*

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ABSTRACT

This study was carried out in Plant Tissue Culture Laboratory of the Faculty of Environ. Agric. Sci. El-Arish, North Sinai, Suez Canal University during the period from 2012 to 2015. The present studies were undertaken to look for additional cheap and easily available substitutes of agar to be used in tissue culture media. In order to enhance the micropropagation efficiency of *Codiaeum variagatum* different concentrations (0, 1, 2, 4, 8 and 16 mg l^{-1}) of benzyle adenine (BA) or Kinetin (Kin) were investigated during establishment stage. Results indicated that kin at 4 mgl^{-1} was the best treatment during this stage. During multiplication stage different concentrations (0.0, 0.1, 0.2 and 0.4 mgl^{-1}) of IAA or NAA combined with 4 mgl^{-1} Kin were tested. Recorded data referred that 0.4 mgl^{-1} NAA combined with 4 mgl^{-1} Kin gave the best values for shoots proliferation and growth. Toward searching for low cost gelling agent could be used as alternative for high price agar, different concentrations (10, 20, and 30 gl^{-1}) of guar seed powder or different combinations between locust bean (LB) and agar (6 agar + 2 LB, 4 agar + 4 LB, 2 agar+ 6 LB, 2 agar or 8 agar, gl^{-1}) were used as gelling agents. Recorded data indicated that 4 gl^{-1} agar +4 gl^{-1} LB gave the maximum values of shoot proliferation and growth. Since the produced shoots from multiplication stage were quite short, different concentrations (0.0, 1.0, 2.0 and 4 mgl^{-1}) of GA_3 combined with 4 mgl^{-1} Kin + 0.4 mgl^{-1} NAA were examined. Addition of GA_3 at 4 mgl^{-1} combined with the above mentioned growth regulators proved to be the treatment in this regard. In order to enhance root indication on obtained shoots bases different concentrations (0.0, 0.5, 1.0, 1.5 and 2.0 mg l^{-1}) of IBA, activated charcoal (0.0 and 1.0 mgl^{-1}) and MS strength (full and half) were evaluated .Recorded data stated that there were no significant differences among some of applied treatments, but the highest number of roots/explant and root length were obtained when shoots were cultured on full MS supplemented with 1.5 mgl^{-1} IBA without activated charcoal. Produced plantlets were successfully acclimatized (100% survivability) in vermiculite and peat (1:1, *V/V*) medium.

Key words: *Codiaeum varigatum*, kin, gelling agent, LB, Guar gum, IBA.

INTRODUCTION

Codiaeum variegatum, commonly known as Croton belongs to family Euphorbiceae, is one of the most popular ornamental plants because of vivid foliage colors and varied leaf shapes. Croton with their colorful, glossy foliage and variation of leaf types are one of the most popular plants.

More than 200 varieties of croton exist on the globe, available in different leaf sizes, shapes and color patterns. The agriculture strategy is now much onto the ornamental plants production for local and exportation. Croton can be propagated by various methods such as cutting, grafting, seed, air layering and shoot tip cuttings.

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One mother/stock plant can yield only 20 plants per year. Due to its slow rate of conventional multiplication, the plant is very high in demand. Micropropagation is a relatively new technology and application of innovative method have served to overcome barriers to progress in the multiplication of elite species and further improvements are anticipated **(Sana *et al.*, 2012)**.

Agar has remained the most frequently used gelling agent for culture media employed for both plants and microbes. The properties of agar, which make it a gelling agent of choice, are its stability, high clarity, its non-toxic nature, and resistance to metabolism during culture **(McLachlan, 1985; Henderson; Kinnersley, 1988)**.

Guar gum is derived from the endosperms of *Cyamopsis tetragonoloba*, Guar gum has a water-soluble fraction (85%) called guaran, which is a non-toxic polysaccharide made up of straight chain mannan, with relatively regular branching of every second mannose by a single galactose unit **(Anonymous, 2001)**. Guar gum, a creamy white-colored powder, hydrates easily to produce solutions possessing very high viscosity with pH ranging between 5.5 and 6.

Guar gum have been used as gelling agent for tissue culture media for many plants like, for *in vitro* seed germination of *Linum usitatissimum* and *Brassica juncea*, *in vitro* axillary shoot proliferation in nodal explants of *Crataeva nurvala* and rooting of regenerated shoots of these plants (**Babber *et al*., 2005).**

Locust bean gum also known as Carob bean gum is derived from the seeds of the leguminous plant *Ceratonia siliqua* Linn. (Leguminosae) **(Romano and Goncalves, 2005)**.

Cytokinin is a group of growth regulators that induce bud formation and cell multiplication .Most cytokinin are adinine (aminopurine) derivatives like BA (6-Benzladenine) and kin (N6-furfuryadinine). They have an essential role in shoot induction and plant regeneration in most plant species and also may stimulate cell division **(Huetteman and Preece, 1993)**.

The present studies were undertaken to validate the available data and to look for additional cheap and easily available substitutes of agar to be used in tissue culture media.

MATERIALS AND METHODS

This study was carried out in Plant Tissue Culture Laboratory, Faculty of Environmental Agricultural Sciences (FEAS), El-Arish, North Sinai, Suez Canal University (SCU) from 2012 to 2015. This study aimed to improve micropropagation of *Codiaeum varigatum* by addition of different growth regulators and some alternative gelling agents.

Establishment Stage

Plant Material and Explant Sterilization

Nodal explants were excised from growing *Codiaeum variegatum* plants grown in greenhouse from Floriculture and Medicinal Plants Farm, Faculty of Environmental Agricultural Sciences (FEAS), El-Arish, North Sinai. Nodal explants were washed under running tap water for 1 hr., with few drops of tween 20, followed by surface sterilization for 2 min in 70% ethanol, then rinsed (3-5 times) with sterile distilled water. On shaker, explants were sterilized by fungicide solution of Rizolex ($2gl^{-1}$) for 15 min., then washed (4-5 times) with sterile distilled water. Finally, colorox at 40% *V/V*) for 20 min were used for explant sterilization and explants were washed (3-5 times) with the sterile distilled water.

The sterilized explants were cultured on MS medium **Murashige and Skoog (1962)**

with 30 gl^{-1} sucrose and supplemented with different concentrations (0.0, 1.0, 2.0, 4.0, 8.0 or 16.0 mgl^{-1}) of benzyl adenine (BA) or kinetin (kin). Medium pH was adjusted to be 5.7- 5.8 and solidified with 8 gl^{-1} agar. Medium was cooked and distributed into glass jars (60×120 mm) every jar contained about 50 ml media. Finally jars were sterilized in autoclave at 121^0C and 1.1 Kg cm^2 for 20 min. Each treatment was consisted of 16 jars. Number of shoots/ explant, main shoot length (cm), shoot length (cm) and number of leaves/ shoot were recorded after 6 and 12 weeks from inoculation date. The best establishment treatment (4.0 mgl^{-1}kin) was repeated to obtain enough shoots for multiplication stage experiments.

Multiplication Stage

First experiment

Shoots (about 1.5 - 2.0 cm length) obtained from the best establishment stage treatment were transferred to MS medium contained 4.0 mgl^{-1} kin combined with different concentrations (0.0. 0.1, 0.2, or 0.4 mgl^{-1}) of naphthalene acetic acid (NAA) or indole acetic acid (IAA) Number of shoots/ explant, shoot length (cm), main shoot length (cm) and number of leaves/shoot were recorded after 6 and 12 weeks from inoculation date. The best multiplication treatment (4.0 mgl^{-1} kin + 0.4 mgl^{-1} NAA) was repeated to obtain enough shoots for the second multiplication experiment.

Second experiment

This experiment was conducted to determine the effect of two alternative gelling agents on shoot multiplication of croton by culturing shoots into MS medium contained (4.0 mgl^{-1} kin + 0.4 mgl^{-1} NAA) and solidified with two alternative gelling agents *i.e;* guar gum or locust bean (LB) as follows: (8gl^{-1} agar as control, 10, 20 and 30 gl^{-1} guar gum or 6 gl^{-1} agar + 2 gl^{-1} LB, 4 gl^{-1} agar + 4 gl^{-1} LB, 2 gl^{-1} agar + 6 gl^{-1} LB and 8 gl^{-1} LB. Number of shoots/explant, shoot length (cm), main shoot length (cm) and number of leaves/shoot were recorded after 6 and 12 weeks from inoculation date.

Third experiment

This experiment was conducted to study the effect of gibberellic acid GA3 concentration on shoot elongation during multiplication stage of croton by culturing shoots into MS medium contained (4.0 mgl^{-1} kin + 0.4 mgl^{-1} NAA) and combined with different concentrations of GA_3 at (0.0, 1.0, 2.0 and 4.0 mgl^{-1}). Number of shoots/ explant, main shoot length (cm), shoot length (cm) and number of leaves/shoot were recorded after 6 and 12 weeks from culture date.

Rooting Stage

Shoots about (3 cm length) obtained from the second multiplication experiment were cultured on full strength or half MS medium strength combined with or without activated charcoal at 1 mgl^{-1} and supplemented with (IBA) indol buteric acid at 0.0, 0.5, 1.0, 1.5 or 2.0 mgl^{-1}. Data *i.e*, rooting percentage (%), Number of roots/ explant and root length (cm), callus formation, callus size were recorded after 8 weeks from inoculation.

Culture Conditions

The above mentioned experiment cultures were incubated in growth room at 25 ± 2^oC under 16 hr./day photoperiod which provided by cool white fluorescent lamps with light intensity of 2000 Lux.

Acclimatization Stage

Plantlets (about 3-4 cm length) were acclimatized by transferring them into black pots (8 cm diameter) filled with five mixtures of planting media *i.e.,* vermiculite + sand (1:1, *V/V*), peatmoss + sand (1:1 *V/V*), vermiculite + peat (1:1 *V/V*), vermiculite + peat + sand (1:1:1 *V/V*) and sand alone. The cultured pots were covered with transparent polyethylene. After one

week holes were made in polyethylene covers, these holes were expanded gradually each week. Plantlets irrigated regularly with sterilized distilled water, quarter MS medium strength or half MS medium strength.

Experimental Design and Statistical Analysis

Experiments were set up in a complete randomized design (CRD). All collected data were analyzed with analysis of variance (ANOVA) procedure using MSTAT-C Statistical Software Package **(Michigan State University, 1983**). Differences between means were compared by using Duncan's multiple range test **(Duncan, 1955)**.

RESULTS AND DISCUSSION

Establishment Stage

Effect of different concentrations of BA and Kin on shoot proliferation and growth during establishment stage of (*Codiaeum variegatum*)

Data in Table 1 show the effect of different concentrations of BA and kin on shoot proliferation and growth during establishment stage of (*Codiaeum variegatum*).

Results cleared that supplementation medium with 4 mgl^{-1} kin proved to be the best treatment for shoot induction and growth either after 6 and 12 weeks. The maximum No. of shoots/explant, main shoot length, shoot length and No. of leaves/shoot (3.0, 4.67 cm, 2.83 cm and 11.67 cm) after 6 weeks as well as 3.67, 5.1 cm, 3.33 cm and 13.33 cm after 12 weeks, respectively were recorded with the above mentioned treatment. It is worth to mention that however there was no significant differences between 4 and 8 mgl^{-1} kin in most cases, but from the economical point of view it is better to use 4 mgl^{-1} kin than higher concentration (16 mgl^{-1}).

This result is in harmony with those obtained by **Sreekumar *et al.* (2001)** who emphasized the role of cytokinins in micropropagation stage, **George *et al.* (2008)** who reported that the effect of cytokinins is most noticeable in tissue cultures where they are used to encourage the growth of axillary buds, and reduce apical dominance in shoot cultures of broad leafed plants. Most demonstrations of a requirement for a particular cytokinin have been made with shoot cultures; they are dispersed over many species and **Vernosefadrani *et al.* (2009)** who obtained most proliferation of *Gerbera jamesonii* during establishment stage due to MS medium supplemented with 2.00 mgl^{-1} Kin.

Effect of 4 mgl^{-1} kin combined with different concentrations of NAA or IAA on shoot proliferation and growth during multiplication stage of (*Codiaeum variegatum*)

This experiment was conducted to study the effect of kin at 4 mgl^{-1} with different levels of NAA and their combinations on multipropagation of croton. Results presented in Table 2 show that cultured shoots of croton on MS medium supplemented with 0.4 NAA mgl^{-1} produced the highest growth values *i.e.,* number of shoots/explant, shoot length (cm), main shoot length (cm) and number of leaves/shoot, respectively (3.00, 8.00, 6.83, and 20.00 cm, respectively) after six weeks from culture date comparing with control.

However, 12 weeks period was not largely effective in enhancing growth parameters. Results cleared that the same trend was observed when nodales were cultured on MS medium supplemented with 4 mgl^{-1} with 0.4 NAA mgl^{-1} resulted in a significant increase in growth parameters *i.e.*, number of shoots/explant, shoot length (cm), main shoot length (cm) and number of leaves/shoot respectively (3.33, 10.33, 8.67 and 22.33 cm, respectively).

Table (1): Effect of different concentrations of BA and kin on shoot proliferation and growth of *Codiaeum variegatum* during establishment stage

Cytokinin conc. (mgl^{-1})	No. of shoots/ explant	Main shoot length (cm)	Shoot length(cm)	No. of leaves/shoot
After 6 weeks				
Control without growth regulators	1.00c	2.83e	1.83a	4.00f
BA 1.0	1.00c	3.67cd	1.83a	4.00f
BA 2.0	1.67bc	3.67cd	2.17a	4.00f
BA 4.0	2.00bc	3.33de	2.33a	7.00de
BA 8.0	2.00bc	3.83b-d	2.33a	7.67cd
BA 16.0	2.00bc	4.17a-c	2.50a	9.33bc
Kin 1.0	1.00c	3.17de	2.00a	4.00f
Kin 2.0	1.33bc	3.33de	2.17a	5.33ef
Kin 4.0	3.00a	4.67a	2.83a	11.67a
Kin 8.0	2.33ab	4.67a	2.67a	11.33ab
Kin 16.0	2.00bc	4.50ab	2.67a	10.00ab
After 12 weeks				
Control without growth regulators	1.00d	3.20e	2.03b	4.33f
BA 1.0	1.33cd	4.10cd	2.03b	4.33f
BA 2.0	2.00bc	3.97c-e	2.37b	4.33f
BA 4.0	2.67b	3.57de	2.50ab	7.33d
BA 8.0	2.33b	4.00cd	2.67ab	8.00d
BA 16.0	2.67b	4.40bc	2.77ab	10.00c
Kin 1.0	1.00d	3.50de	2.17b	4.33f
Kin 2.0	2.00bc	3.57de	2.43b	5.67e
Kin 4.0	3.67a	5.10ab	3.33a	13.33a
Kin 8.0	2.67b	5.30a	2.77ab	12.00b
Kin 16.0	2.33b	4.60a-c	2.87ab	10.67c

Means having the same letter(s) within the same column are not significantly different according to Duncan`s multiple range test at 5% level of probability.

Table (2): Effect of different types and concentrations of auxins with 4.0 mgl^{-1} kin on shoot proliferation and growth of *Codiaeum variegatum*

After 6 weeks				
Control (4 Kin)	1.00b	4.00c	3.33c	6.00d
0.1 IAA	1.00b	4.00c	3.50c	7.67cd
0.2 IAA	1.33b	5.00bc	3.50c	9.00c
0.4 IAA	1.67b	5.33b	4.13b	11.67b
0.1 NAA	1.00b	4.67bc	3.50c	11.00b
0.2 NAA	1.67b	5.33b	4.00b	12.67b
0.4 NAA	3.00a	8.00a	6.83a	20.00a
After 12 weeks				
Control (4 Kin)	1.00c	4.83d	4.00d	8.00d
0.1 IAA	1.00c	5.00cd	4.20cd	8.67d
0.2 IAA	1.67b	5.83b-d	4.53bc	10.67c
0.4 IAA	1.67b	6.33b	4.70b	14.00b
0.1 NAA	1.00c	5.33b-d	4.00d	14.00b
0.2 NAA	2.00b	6.00bc	4.00d	15.00b
0.4 NAA	3.33a	10.33a	8.67a	22.33a

Means having the same letter(s) within the same column are not significantly different according to Duncan`s multiple range test at 5% level of probability.

This result is agree with **Vinothkumar *et al.* (2011)** finding, who observed that highest shoot multiplication rate for wattakaka volubilis after 28 days of culture on MS basal medium supplemented with 0.6 mgl-1 BAP and 0.2 mgl^{-1} NAA. Also, agree with **Sana *et al.* (2012)** who regenerated three varieties of croton (*Codiaeum variegatum* L.) testing 8 types and concentrations of supplemented hormones to MS medium and they observed that NAA was recorded best shooting, during the multiplication stage of the variety pictum spot. As well as **El-Sheikh *et al.* (2013)** who interested the effectiveness of NAA combined with BAP on different concentrations on Dieffenbachia.

Effect of different gelling agents concentrations on shoot proliferation and growth of *Codiaeum varigatum* during multiplication stage

This study compared the potentials of agar, guar and locoust bean gelled media on *in vitro* shoot regeneration and propagation of croton. Data illustrated in Table 3 and Photo. 1 indicate that the combination between agar at 4 gl^{-1} and locoust bean (LB) at 4 gl^{-1} as gelling agent produced the maximum growth values *i.e.*, number of shoots/explant, shoot length (cm), main shoot length (cm) and number of leaves/ shoot (9.33, 9.33, 8.17 and 25.00, respectively) after six weeks from culture date. The same trend was observed after 12 weeks since MS medium solidified with agar at 4 gl^{-1} and locoust bean (LB) at 4 gl^{-1} gained the highest growth values.

These results are in harmony with those found by **Romano and Goncalves (2005)** since they used LB as a gelling agent in combination with agar for shoot multiplication and rooting of carob tree and Iberian rose shoots and they found that LBG can be used in combination with agar in culture medium as a gelling agent without negative effect on plant material and with the advantage of reducing medium cost.

Table (3): Effect of different gelling agents concentrations on shoot proliferation and growth of *Codiaeum varigatum* during multiplication stage

Gelling agent (gl^{-1})	No. of shoots/ explant	Main shoot length (cm)	Shoot length (cm)	No. of leaves/shoots
	After 6 weeks			
Control (8 agar)	1.00e	3.67e	2.00d	14.67c
10 guar	1.00e	3.33e	2.00d	9.67d
20 guar	1.67de	4.00e	2.33d	11.33cd
30 guar	2.67d	6.33cd	4.50c	14.00cd
6 agar + 2 LB	4.67c	7.33bc	6.17b	15.00c
4 agar + 4 LB	9.33a	9.33a	8.17a	25.00a
2 agar + 6 LB	6.00b	8.17ab	7.33a	25.67a
8 LB	5.33bc	5.50d	4.67c	19.67b
	After 12 weeks			
Control (8 agar)	1.00d	4.17e	2.50 e	16.67c
10 guar	1.00d	2.83f	2.17e	8.67f
20 guar	1.00d	3.50ef	2.50e	10.67ef
30 guar	2.67c	6.83cd	5.67c	13.33de
6 agar + 2 LB	4.00c	7.00c	6.33c	15.67cd
4 agar + 4 LB	10.33a	10.33a	9.17a	30.67a
2 agar + 6 LB	6.33b	8.50b	7.83b	27.00b
8 LB	6.00b	6.00d	4.83d	18.67c

Means having the same letter(s) within the same column are not significantly different according to Duncan`s multiple range test at 5% level of probability.

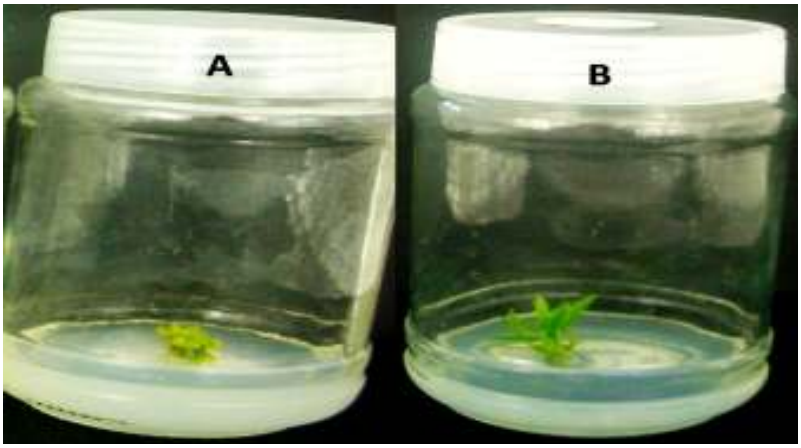

Photo (1): *Codiaeum varigatum* shoot proliferation and growth as affected by control [8.0 gl^{-1} agar (A)] and the best LB concentration [4 gl^{-1} agar + 4 gl^{-1} LB (B)] combianed with 4.0 mgl^{-1} kin +0.4 mgl^{-1} NAA during multiplication stage after 12 weeks.

Effect different concentrations of gibberellic acid (GA_3) combined with 4.0 mgl^{-1} kin + 0.4 mgl^{-1} NAA on shoot proliferation and growth of *Codiaeum varigatum*

Although the above mentioned experiment results showed that treatments gave a good multiplication rate, but obtained shoots still quite short. So that the following experiment was devoted to investigate some GA3 concentrations in order to stimulate shoot elongation, since it is well known that GA3 plays a key role in shoot elongation.

Data presented in Table 4 and Photo 2 show that cultured shoots of croton on MS medium supplemented with 4 mgl^{-1} + 0.4 NAA mgl^{-1} + 4 mgl^{-1} GA3 produced the highest growth values *i.e*., number of shoots/explant, main shoot length (cm), shoot length (cm) and number of leaves/ shoot (7.67, 8.17, 6.50 and 24.33, respectively) after 6 weeks from culture date comparing with control.

Results clear that the same trend was observed when nodales of croton were cultured on MS medium supplemented with 4 mgl^{-1} with 0.4 NAA mgl^{-1} with 4 mgl^{-1} GA3 since it produced the highest values of number of shoots/explant, main shoot length (cm), shoot length (cm) and number of leaves/shoot (9.33, 9.50, 8.00 and 30.00, respectively. These results are go in line with the findings of **Orlikowka *et al.* (2000)** on *Codiaeum varigatum* Blume var. Pictum Muell. Arg." Excellent", since they found that Murashige-Skoog medium containing 1.0 mgl-1 6-benzylamino-purine (BAP) and 2.0 mgl-1 gibberellic acid (GA3) doubled the number of axillary shoots in comparison to non-defoliated controls. Also results were published by **Bhot *et al.* (2010)** who used explants from field grown plants of three varieties of *Codiaeum variegatum* L. Blume viz Norwood Beauty, Undulatum during *In vitro* culture study for induction of multiple shoots node explants were inoculated on various induction media and proliferation media for the development of multiple shoots and the elongation enhanced by using GA_3.

Rooting Stage

Effect of MS medium strength, activated charcoal and IBA concentration on shoot rooting and growth during rooting stage *Codiaeum varigatum*

The presented data in Table 5 and Photo 3 clear that there was no effect for MS medium strength, activated charcoal and IBA concentration on rooting percentage since all applied treatments produced the same rooting percentage (100%). While the the maximum number of roots (3.34) was recorded when shoots of Philodendron were cultured on half MS medium supplemented with 0.5 mgl^{-1} IBA on the absence of activated charcoal. On the other side, full MS medium 0.5 and 1mgl^{-1} IBA without activated charcoal produced high significant increase in root length and recorded the highest root length (cm) (3.67) on both treatment.

These results are in a agreement with those obtained by **Pragya *et al.* (2012**) on gladiolus since they using half MS medium strength combianed with IBA during rooting stage. Also, **Roy and Hassan (2005)** on *Gloriosa superba* they mentioned that *In vitro* raised shoots rooted on half strength MS with 1.0 mgl^{-1} IBA+0.5 mgl^{-1} IAA added as a supplement. In addition, **Waseem *et al.* (2011)** on chrysanthemum, they found that the highest root initiation percentage (100%), roots per plantlet and root length was obtained in half strength MS media supplemented with 0.2 mg/l Indole butyric acid (IBA).

Table (4): Effect of different concentrations of gibberellic acid (GA_3) combined with 4.0 mgl^{-1} kin + 0.4 mgl^{-1} NAA on shoot proliferation and growth of *Codiaeum varigatum*

Gibberellic acid concentration (mgl^{-1})	No. of shoots/ explant	Main shoot length (cm)	Shoot length (cm)	No. of leaves/shoot
	After 6 weeks			
Control	3.33c	3.67c	2.50c	12.67c
0.1	4.00c	4.67c	3.00c	18.33b
0.2	5.67b	6.67b	4.17b	19.33b
0.4	7.67a	8.17a	6.50a	24.33a
	After 12 weeks			
Control	3.67c	4.50c	3.67c	15.67c
0.1	4.00c	5.33c	3.83c	20.00bc
0.2	7.00b	7.83b	6.00b	23.33b
0.4	9.33a	9.50a	8.00a	30.33a

Means having the same letter(s) within the same column are not significantly different according to Duncan`s multiple range test at 5% level of probability.

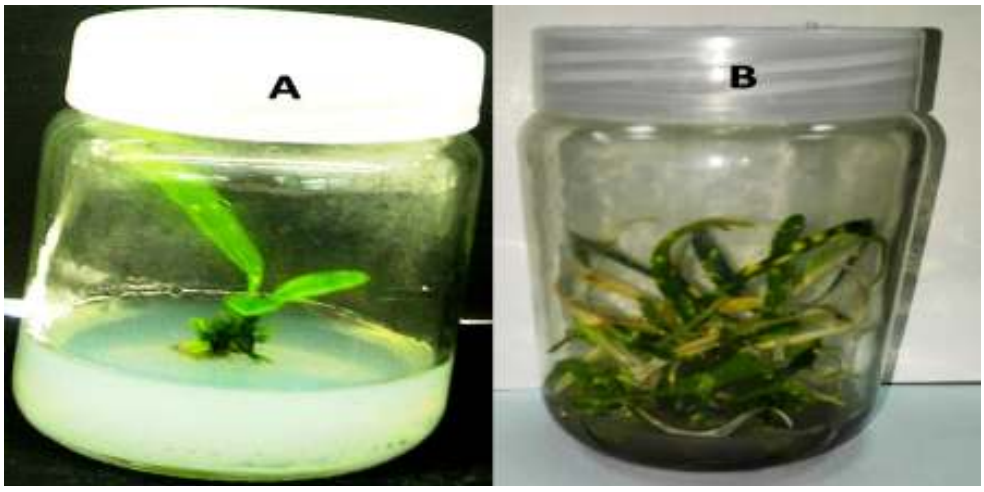

Photo (2): *Codiaeum varigatum* shoot proliferation and growth as affected by control treatment (without GA_3, A) and the best GA_3 concentration (4.0 mgl^{-1} GA_3, B) combined with 4.0 mgl^{-1} kin+0.4 mgl^{-1} NAA during elongation stage 12 weeks.

Table (5): Effect of MS medium strength activated charcoal, IBA concentrations on *Codiaeum variegatum* shoot rooting and growth during stage

MS Medium strength	Activated charcoal (Ac)	IBA (mgl^{-1})	Callus formation	Callus size	Rooting (%)	No. of root/ Explant	Root length (cm)
Full MS	Without activated charcoal	0.0	-	-	100	1.67d	1.67ef
		0.5	+	+++	100	2.33c	1.67a
		1.0	-	-	100	3.33a	2.33cd
		1.5	-	-	100	3.00ab	3.67a
		2.0	-	-	100	1.67d	1.67ef
	Activated charcoal (1 mgl^{-1})	0.0	-	-	100	1.67d	1.67ef
		0.5	+	+++	100	3.33a	3.67a
		`1.0	+	++	100	3.00ab	3.00b
		1.5	-	-	100	2.33c	3.67a
		2.0	-	-	100	3.33a	2.67ef
Half MS	Without activated charcoal	0.0	-	-	100	1.00e	1.00g
		0.5	+	+	100	2.33c	1.67ef
		1.0	+	++	100	1.67d	1.33fg
		1.5	-	-	100	2.33c	2.33cd
		2.0	+	+	100	2.67bc	2.00de
	Activated charcoal (1 mgl^{-1})	0.0	+	+	100	1.67d	1.00g
		0.5	+	+	100	3.34a	2.33cd
		1.0	+	+	100	2.33c	2.67bc
		1.5	-	-	100	3.00ab	2.67bc
		2.0	-	-	100	1.67d	2.67bc

Means having the same letter(s) within the same column are not significantly different according to Duncan`s multiple range test at 5% level of probability.

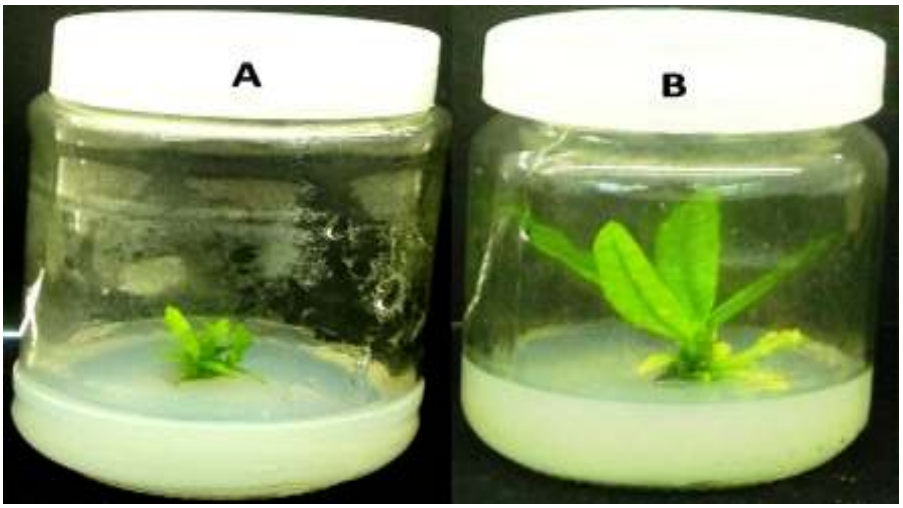

Photo (3): *Codiaeum variegatum* shoot rooting and growth as affected by control treatment (Full MS medium without auxin or activated charcoal, A) and the best treatment for root induction (Full MS with 0.5 mgl^{-1} IBA without activated charcoal, B).

Acclimatization Stage

Effect of some planting media mixtures on survival of *Codiaeum variegatum* plantlets during acclimatization stage

As shown in Table 6 results indicate that plantlets of croton were acclimatized successively and the highest survival percentage (100%) was achieved by using the media mixture of vermiculite: peat (1:1) and irrigating plants with quarter MS strength after six weeks.

On the other side, the lowest value of survivability percentage (35%) was belonged to vermiculite + peatmoss + Tap water. Feeding the plantlets with nutrient salt solution has been reported to beneficial for promotion of orchid survival and growth **(Mukherjee, 1983; Kumaria and Tandon, 1994)**. Also, **Shrotri and mukundan (2004)** on *Rubia cordifolia* plants they found that the potted plants were reported to have irrigated with 1/2 strength MS medium without vitamins and sucrose a week for 15 days for 30 days. Also, **Dep and Imchen (2010)** on raised plants they reported that irrigating newly potted plants with 1/10 MS salt solution at 1 week interval, proves to be beneficial for better growth.

Table (6): Effect of some planting media mixtures on survivability (%) of *Philodendron selloum* plantlets during acclimatization stage.

Planting medium	Irrigation	Survival percentage (%)
Vermiculite +Sand (1:1)	Irrigation tap water	70.00
Peatmoss+ Sand (1:1)		63.00
Vermiculite+ Peat (1:1)		35.60
Vermiculite+ Peat+ Sand (1:1:1)		58.00
Sand		50.00
Vermiculite +Sand (1:1)	irrigation 1/4 MS	80.00
Peatmoss+ Sand (1:1)		90.00
Vermiculite+ Peat (1:1)		100
Vermiculite+ Peat+ Sand (1:1:1)		52.00
Sand		72.00
Vermiculite +Sand (1:1)	irrigation 1/2 MS	75.00
Peatmoss+ Sand (1:1)		65.00
Vermiculite+ Peat (1:1)		40.00
Vermiculite+ Peat+ Sand (1:1:1)		5.00
Sand		75.00

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الملخص العربى

تأثير منظمات النمو وعامل تصلب البيئة على الإكثار الدقيق لنبات الكروتون

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أجريت هذه الدراسة بمعمل زراعة الأنسجة النباتية بكلية العلوم الزراعية البيئية، شمال سيناء، جامعة العريش خلال الفترة من ٢٠١٢ م إلى ٢٠١٥م، تم إجراء هذه الدراسة للبحث عن بديل رخيص ومتاح للآجار يمكن استخدامه في بيئات زراعة الأنسجة النباتية ولتحسين الإكثار الدقيق لنبات الكروتون تحت تركيزات مختلفة من البنزايل ادينين والكينتين وهى (صفر ، ١، ٢، ٤، ٨ و١٦) أثناء مرحلة التأسيس والنتائج تشير أن الكينتين بتركيز ٤ ملجم/لتر أعطت أفضل النتائج أثناء هذه المرحلة، أثناء مرحلة التضاعف بتركيزات من نفثالين حمض الخليك وأندول حمض الخليك بتركيزات (صفر، ٠,١، ٠,٢ و٠,٤ ملجم/لتر) مع ٤ ملجم/لتر من الكينيتن والنتائج أشارت بأن ٠,٤ ملجم/لتر من النفثالين حمض الخليك مع ٤ ملجم/لتر من الكينيتن أعطت أعلى القيم في نمو الفروع، استخدام بدائل الآجار رخيصة التكلفة مقارنة بالآجار غالى الثمن وبالتالي تم باستخدام تركيزات مختلفة من بذور نبات الجوار والتركيزات كالتالي (١٠، ٢٠ و٣٠ جم/لتر) وباستخدام تركيزات مختلفة من بذور نبات الخروب مع الآجار وهى كالتالي (٨ جم آجار، ٢ جم خروب + ٦ جم آجار، ٤ جم خروب + ٤ جم آجار، ٦ جم خروب + ٢ جم آجار) والتى استخدمت كبدائل الآجار، والنتائج المسجلة تشير بأن ٤ جم آجار + ٤ جم خروب أعطت أفضل النتائج في نمو الفروع، حيث أن نمو الفروع في مرحلة التضاعف كانت قصيرة تم استخدام تركيزات مختلفة من حمض الجبرلين مع ٤ ملجم/لتر الكينتين و ٠,٤ ملجم/لتر نفثالين حمض الخليك والتركيزات كالتالي (صفر، ١، ٢ و٤ ملجم/لتر) ووجد أن تركيز ٤ ملجم/لتر من حمض الجبرلين أعطت نتائج البحث أن لنمو الجذور بعد نمو الفروع يتم استخدام تركيزات مختلفة من اندول حمض البيوتيرك اسيد بتركيزات (٥,٥، صفر ، ١,٥ و ٢ ملجم/لتر) واستخدام الفحم النشط بتركيزات (٠,١ جم/لتر) مع قوة بيئة (قوة كاملة، نصف قوة) والنتائج المسجلة غير المعنوية بين المعاملات ولكن على عدد للجذور للمنفصل النباتي وطول الجذر المتحصل عليه مع قوة بيئة كاملة وبتركيز ١,٥ ملجم/لتر من اندول حمض البيوتيرك اسيد بدون فحم نشط وأقلمة النبيتات المنتجة كانت نسبة البقاء ١٠٠% في بيئة مكونة من البيتموس + الفيرموكوليت بنسبة ١:١.

الكلمات الإسترشادية: نبات الكروتون، هرمون الكينتين، بدائل الآجار، بذور نبات الجوار، بذور نبات الخروب، هرمون اندول حمض البيوتيرك اسيد.