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Micropropagation of Oregano (*Origanum syriacum* L.) Through Tissue Culture Technique

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ABSTRACT

This research was carried out at the Plant Tissue Culture Laboratory (PTCL) in Faculty of Environmental Agricultural Sciences, Arish University, Egypt during 2013 to 2016. The aim of this study was using tissue culture technique for the micropropagation of *Origanum syriacum* L. plant. One node cutting and shoot tip were cultured on Murashige and Skoog (MS), Nitsch and Nitsch (NN) and Gamborg (B5) media. The results showed that shoot tip cultured on the MS medium had the best combination for the establishment stage of mother plants. Shoots cultured on MS medium supplemented with0.50 mgl^{-1} Kin and 0.05 mgl^{-1}NAA produced the highest shoots developed during multiplication stage. MS medium supplemented with 0.40 mgl^{-1} thiamine achieved the best shoots development compared with the other additive treatments and control. Addition of 1 mgl^{-1}IBA to the full strength MS medium and 1.00 mgl^{-1} IAA to the half strength MS had the highest number of main roots and root lengths. Hardening the produced rooted plantlets were done in a greenhouse in pots containing mixture of peatmoss, vermiculite and washed sand at the rate (1:1:1) and after adjustment of mixture pH. Plantlets were successfully acclimated with 93 % survive.

Keywords: *Origanum.syriacum*. Micropropagation, explant, media and additives.

INTRODUCTION

Oregano (Origanum sp.) is an important aromatic perennial plant of the Lamiaceae family. The genus Origanum includes about 38 species native to the Mediterranean countries. Under Egypt conditions several species of Oregano are growing wild especially in Sinai regon including O. majorana, O. syriacum and O.vulgare (Soliman *et al.,* 2007). Origanum..syriacum subsp. sinaicum plant is grown in stony grounds in Sinai and commonly used in traditional medicine by Bedouins in this region. In folk medicine O. syriacum dry leaves used as spice, condiment and used as antitussives, expectorants, sedatives anti-parasitic, anti-rheumatics and gastrointestinal complaints (Dundar *et al.,* 2008). Also, this plant had antimicrobial, antifungal, insecticidal and antioxidant effects (Bakkali *et al.,* 2008 and Alma *et al.,* 2003).The chemical composition of the O.syriacum oils revealed the existence of thymol, thymol-carvacrol and carvacrol-thymolchemo types (El-Beyrouthy *et al.,* 2015).

Origanum syriacum plant disappeared gradually and consequently from Sinai natural environment. So, there is need for application of tools and techniques for multiplication and conservation of this species. Micropropagation considered a good tool for ex situ conservation programs for species with much reduced population or low seed production. This technique facilitates the rapid established of a large number of stock plants forming minimum impact on endanger wild plants. Few studies were conducted on in vitro propagation of O.syriacum (Arafeh *et al.,* 2003 and El-Beyrouthy*et al.,* 2015). Therefore this study aimed to establish an applicable protocol to save the endangered native Egyptian O. syriacum plant through in vitro micropropagation.

MATERIALS AND METHODS

This research was carried out at the Plant Tissue Culture Laboratory, Fac. of Environ. Agric. Sci., Arish University, Egypt to study the micropropagation of *O. syriacum* plant by using the different media (MS, NN, and B5) and different additives (thiamine, asparagines, and Glutamine)on the best medium (MS) with 0.50mgl^{-1} Kin + 0.05mgl^{-1} NAA.

Plant material: O. syriacum seeds from wild mature plants grown in North Sinai were collected and identified by the Research Station of El-Sheikh Zuwyed, Desert Research Center (DRC).

Seeds sterilization: Seeds of Organo were washed under running tap water for 60 minutes with a few drops of liquid soap. Seeds soaked in 70% ethanol for 30 sec and washed with sterilized distilled water for 5 minutes. The seeds were soaked for 20 minutes in 25% Clorox (containing 5.25 % sodium hypochlorite with two drops of Tween-20) then washed again with sterilized distilled water for 3-5 times to remove all traces of the disinfectant.10 seeds were placed on MS Medium in jars and incubated in a growth chamber at 18 ± 2°C in the dark. After 7 days these were transferred under 16/8 photoperiod.

Culture media: The MS, NN and B5 media containing macro and micro elements as well as vitamins, according to Murashige and Skoog (MS,1962), Nitsch and Nitsch (1969) and Gamborg et al.,(1968) media were used. The media were supplemented with 100 mgl^{-1} myo-inositol and 3 % sucrose. All media were adjusted to pH 5.70 – 5.80 using either 0.10 N NaOH or 0.10 N HCL before gelling with 7.00 gml^{-1} agar. The media were dispensed into glass tissue culture jars each jar contained 15 ml of culture medium. All media were autoclaved at 121^{o}c and 1.06kg/cm^2 for 20 min. The jars were transferred to the culture cabinet and left cool in a slanted position till they were used.

Establishment stage:

Medium type: The MS, NN and B5 media were used through this study to select the best medium type that induces the highest explants development.

Explants type: Shoot-tips and one -node cutting (0.50 – 1.00 cm) of 25 days old seedlings were excised and cultured on MS, NN and B5 media to select the best explants type which encourage the highest explants development.

Culture conditions: The sterilized explants were cultured on the media under complete aseptic conditions in the Laminar air flow cabinet, then the cultured explants were incubated under16hr of artificial light and 8hrs of dark at average temperature of 25±2°C provided by cool white fluorescent lamps (light intensity 2000 lux) for all experiments and the data were recorded after 4 weeks.

Multiplication stage: This stage aimed to increase the number of shoots, so that the growth obtained from the

establishment stage was used as explants during the multiplication experiments.

Effect of cytokinin type: Kinetin (Kin), 6-benzyladenine (BA) and 2-isopentenyl adenine (2iP) were studied at the rate of 1.00 mgl^{-1} to determine the best cytokinin that induces the highest multiplication.

Effect of different Kin concentrations: Different Kin concentrations (0.00, 0.05, 0.10, 1.50 and 2.00 mgl^{-1}) were evaluated to investigate the most suitable concentration that induces the highest multiplication.

Effect of Additives: Glutamine, asparagines and thiamine were added to the culture MS medium at the level of 0.20 mgl^{-1} to detect the most effective additive that maximize explants development and growth of O. syriacum. After the previous experiments the best additives were used at different concentrations. For O. syriacum thiamine was the best one at a concentration of 0.40 mgl^{-1}.

Rooting stage: The proliferated shoots of *O. syriacum* were used as explants and cultured on MS supplemented with 100 mgl^{-1} myo-inositol, 30.0 gl^{-1}sucrose and 7.00 gl^{-1}agar. Also, shoots were grown on plant growth regulators (PGRs) free MS for 4 weeks to eliminate any carry over effect of any PGRs that might inhibit/reduce rooting performance.

Effect of medium strength and auxin type: Shoots of 3-4 cm long were excised from the proliferated shoots and cultured on (full, 50 and 25 % strengths MS) basal medium supplemented with 1.00 mgl^{-1}IBA, 1.00 mgl^{-1}NAA and 1.00 mgl^{-1} IAA to determine which the best media strength and type of auxin that enhance the best root formation in O. syriacum.

Effect of IBA and IAA concentrations: Shoots were cultured on full strength and half strength of MS media with different concentrations (0.00, 0.50, 1.00, 1.50 and 2.00 mgl^{-1}) for both IBA and IAA for O. syriacum to investigate the suitable concentration which encourages the highest root formation.

Acclimatization stage: Well rooted plantlets of O. syriacum were subjected to the in vitro treatments. The selected plantlets were taken away from the jars. The roots of the chosen plantlets were washed thoroughly with running tap water to get rid of residues. The roots were washed with sterilized distilled water and planted in black polyethylene pots 8 cm in diameter filled with 1 : 1 : 1 (v/v/v) peat moss, vermiculite and sand for O. syriacum, then covered with white transparent bags having small holes which were made after one week and the size of these holes was increased gradually until the plantlet become suitable for transferring to the bigger pots (30 cm diameter) and when plantlets produced new leaves they were transferred from greenhouse eventually to field conditions.

Statistical analysis: The design of the experiments was completely randomized, sometimes in a layout of factorial according to the studied factors in every experiment. Data were tested using the analysis of variance (ANOVA) by the General Linear Models (GLMs) procedures using SAS (SAS, 2004).The significant differences were observed for the measured value, means were separated using Duncan's (DMRT) (Duncan, 1955) at level 5 % .

RESULTS AND DISCUSSION

Establishment stage:

Effect of medium type:

Data in Table, 1 show that the MS media achieved the best No. of shoots, shoot length, No. of leaves and number of leaves/shoot (1.83, 1.75 cm, 6.67 and 3.33, respectively) of O. syriacum plant as compared with the other two media tested. NN andB5 media provided the lowest number of shoots, shoots length, number of leaves and number of leaves/shoot (1.00, 1.00 cm, 1.00 and 1.00, respectively) for both media of O. syriacum plant. These results are in harmony with Arafeh *et al.,* (2003), Ozkum(2007), Oana et al.,(2008), Oluk and Cakir (2009) and El Beyrouthy *et al.,* (2015) they found that using MS medium provided the highest number of some parameters like No. of shoots number of leaves and number of leaves/shoot on different Origanum pecies. However, Arikat et al. (2004) found that B5 medium produced the shortest shoots compared with MS on Salvia fruticosa Mill.On the other hand, Yavuz (2015) found that Sideritis stricta plant cultured inB5 medium had highest shoots length than MS medium.

Table 1. Effect of medium type on number of shoots and shoot length, number of leaves/plant and number of leaves/shoot of *O.syriacum* plant.

Parameters Medium Type	No. Shoots	Shoot Length (cm)	No. Leaves/ plant	No. Leaves / Shoot
MS*	1.83 [a]	1.75 [a]	6.67 [a]	3.33 [a]
NN**	1.00 [b]	1.00 [b]	1.00 [b]	1.00 [b]
B5***	1.00 [b]	1.00 [b]	1.00 [b]	1.00 [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 tests

***Murashige and Skoog, **Nitsch and Nitsch, ***Gamborg B5**

Effect of explant type:

Data tabulated in Table, 2 indicated that the shoot tip achieved the highest number of shoots, shoot length, number of leaves and number of leaves/shoot (1.33, 1.33 cm, 3.78 and 2.22 , respectively) compared with one-node cutting of *O. syriacum* plant. These results are generally in a harmony with the results of Arafeh *et al.,* (2003), Ozkum (2007), Oluk and Cakir(2009), Yildirim (2013) and El Beyrouthy *et al.,* (2015) who found that the one-node cutting recorded the best number of shoots , shoots length, number of leaves and number of leaves/shoot on different *Origanum* species.

Table 2. Effect of explant type on number of shoots, shoot length, number of leaves/plant and number of leaves/shoot of *O.syriacum* plant.

Parameters Explant Types	No. Shoots	Shoot Length (cm)	No. Leaves/ plant	No. Leaves / Shoot
Shoot-tip	1.33 [a]	1.33 [a]	3.78 [a]	2.22 [a]
One-node cutting	1.22 [b]	1.17 [b]	2.00 [b]	1.33 [b]

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

Effect of media and explant type:

Data in Table 3 indicated that shoot-tip cultured on MS medium had the number of shoots, shoot length, number of leaves and number of leaves/shoot compared with other treatments of *O. syriacum* plant. However the

shoot -tips which were cultured on NN and B5media produced the lowest number of shoots, shoot length, number of leaves and number of leaves/shoot. These funding are harmony with Arafeh *et al.,* (2003), Yildirim (2013), El-Beyrouthy *et al.,* (2015) and Bakhtiar *et al.*,(2016) they found that the MS was the best medium with shoot-tip on Lamiaceae family plants.

Table 3. Effect of interaction between media and explant types on number of shoots and shoot length, number of leaves and number of leaves/shoot of *Origanum syriacum* plant.

Medium& Explant Type	Parameters	No. Shoots	Shoot Length (cm)	No. Leaves	No. Leaves / Shoot
MS*	Shoot tip	2.00[a]	2.00[a]	9.33[a]	4.67[a]
	One-node cutting	1.67[a]	1.50[b]	4.00[b]	2.00[b]
NN**	Shoot tip	1.00[b]	1.00[c]	1.00[c]	1.00[c]
	One-node cutting	1.00[b]	1.00[c]	1.00[c]	1.00[c]
B5***	Shoot tip	1.00[b]	1.00[c]	1.00[c]	1.00[c]
	One-node cutting	1.00[b]	1.00[c]	1.00[c]	1.00[c]

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.
***Murashige and Skoog, ****Nitsch and Nitsch, *****Gamborg B5**

Multiplication stage

Effect of cytokinin type:

Data in Table, 4and Fig 1 show significant effects for cytokinin types on all studied traits. The highest records were with Kin for all traits without significant differences between control and 2ip for shoot length. On the other hands, results are in harmony with the results of Arafeh *et al.,* (2003) who found that Kin at 0.4 mgl^{-1}gave the best number of shoots per explants and number of leaves for *O. syriacum*. On the other side, El-Beyrouthy *et al.,* (2015) observed that the BAP 1.5 mgl^{-1}leading to higher values of shoot length on *O. syriacum*.

Table 4. Effect of cytokinin type on number of shoots, average shoot length, number of leaves and number of leaves/shoot of*O. syriacum* plant.

Cytokinin type (1mgl^{-1})	Parameters No. Shoots	Shoot Length (cm)	No. Leaves	No. leaves /shoot
control	1.20[c]	2.17[a]	14.73[b]	8.25[b]
Kin	3.08[a]	2.85[a]	26.13[a]	12.87[a]
2ip	2.17[b]	2.20[a]	14.63[b]	6.73[b]
BA	2.20[b]	1.27[b]	13.07[b]	5,80[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.

Fig. 1. Effect of Cytokinin type on *Origanum syriacum* plant

Effect of kinetin (Kin) concentrations:

Data in Table 5 and Fig. 2 show significant effect for kinetin concentrations on all studied traits. Using 0.50$mgl^{-1}$of Kin resulted in the highest values for number of shoot, shoot length, number of leaves and number of leaves/shoot. The lowest values for all studied traits were recorded with control. These results are in a harmony with Arafeh *et al.,* (2003) who found that Kin at 0.4 mgl^{-1} gave the highest number of shoots per explants on *O. syriacum* plant. However, Ozudogru *et al.,* (2011) found that 1.00 mgl^{-1}Kin gave the highest number of shoots/ explants on *thymus vulgaris*.

Table 5. Effect of kinetin concentrations on number of shoots, average shoot length, number of leaves and number of leaves/shoot of *O. syriacum* plant.

kin Conc.mgl^{-1}	Parameters No. Shoots	Shoot Length (cm)	No. Leaves	No. leaves /shoot
control	1.63[d]	2.40b	28.53[c]	11.57[b]
0.50	9.63[a]	5.57a	64.59[a]	13.63[a]
1.00	6.40[b]	4.87a	51.40b	10.80[b]
1.50	4.67[c]	5.30a	54.67ab	11.73[b]
2.00	4.77[c]	5.17a	49.53b	11.13[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.

Fig. 2. Effect of kinetin concentrations on *Origanum syriacum* plant.

Effect of additive types:

Adding thiamine resulted in the highest values of number of shoots, shoot length, number of leaves and number of leaves/shoot (Table, 6). These were no significant differences among thiamine and control for number of leaves /shoot. The lowest recorded value was found with Glutamine. Similarly, Abdallah (2012) found that thiamine were effective in enhancing number of shoot, shoot length, number of leaves and number of leaves/shoot of *Capparis spinosa*. On the other hand, Ebrahem (2015) found that multiplying shoots from Jojoba (*Simmondsia chinensis*) plant on MS medium which supplemented with 1.00 mgl^{-1} BA in combination with 1.00 mgl^{-1} IAA plus 40 mgl^{-1} Adenine sulfate (Ads) was the best additives.

Table 6. Effect of different additive on number of shoots, average shoot length, number of leaves and number of leaves/shoot of *O. syriacum* plant.

Parameters / Additive types 1mgl^{-1}	No. Shoots	Shoot Length (cm)	No. Leaves	No. leaves /shoot
Control	2.18^{b}	2.55^{b}	18.93^{b}	11.02^{ab}
Thiamine	3.25^{a}	3.53^{a}	27.18^{a}	15.35^{a}
Asparagine	2.30^{b}	2.05^{bc}	17.25^{bc}	7.43^{bc}
Glutamine	1.90^{b}	1.27^{c}	10.17^{c}	6.27^{c}

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.

Effect of thiamine concentrations:

Using 0.40 mgl^{-1} of thiamine resulted in the highest values of number of shoots, shoot length, number of leaves and number of leaves/shoot (Table, 7).There were no significant differences among this concentration of thiamine 0.40 mgl^{-1} and 0.30 mgl^{-1}for number of shoots, shoot length, and number of leaves/shoot. The lowest values for all studied traits were recorded with control and 0.10mgl^{-1} of thiamine. Abdallah (2012) found that 0.30 mgl^{-1}of thiamine was highest number of shoots, shoot length, number of leaves and number of leaves/shoot of *Capparis spinosa*.

Table 7. Effect of different thiamine concentrations on number of shoots, average shoot length, number of leaves and number of leaves/shoot of *Origanumsyriacum* plant.

Parameters / Thiamine Con. (1mgl^{-1})	No. Shoots	Shoot Length (cm)	No. Leaves	No. leaves /shoot
Control	2.05c	2.33c	14.68c	9.78c
0.10	2.00c	2.23c	15.00c	7.47c
0.20	2.73bc	3.08bc	27.18bc	10.35bc
0.30	3.07ab	3.90ab	39.33b	14.67ab
0.40	4.00a	4.00a	71.33a	17.07a

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.

Rooting stage:

Effect of medium strength and auxin type:

Data in Table 8show significant effect for MS medium strength and type of auxin on number of shoots shoot length, number of main roots and root length of *O. syriacum*. The highest values were recorded with application of full strength medium with IBA or half strength medium with IAA. These results are in agreement with El-Beyrouthy *et al.,* (2015) who obtained high rooting response of shoots with IBA on full MS medium for *O. syriacum*. On the other hand Arafeh *et al.,* (2003), Oluk and Cakir (2009) observed that plants were rooted on 1/2 strength MS medium supplemented with 0.10 - 2.00 mgl^{-1} IBA for *Origanum* species. Also, Soni *et al.,* (2014) found that MS supplemented with IAA enhanced the roots parameters on *Lavandula aungustifolia*.

Effect of medium strength, auxin type and auxin concentrations:

Data in Table, 9 showed that the highest value of number of shoots, shoot length, number of main roots and root length were recorded with the application of full strength medium with 1.50mgl^{-1}IBA followed by half strength medium with IAA at 1.0 mgl^{-1} of *O. syriacum*. These results are in harmony with the findings of Oluk and Cakir (2009) on *Origanum sipyleum* found that full MS medium supplemented with 0.50 mgl^{-1} (IBA) had the highest root number and root length. However, Arafeh *et al.,* (2003) found that MS supplemented with 0.80 mgl^{-1} IAA achieved the highest roots number on *O. syriacum* plant.

Table 8. Effect of medium strength and type of Auxin on number of shoots, plant length, number of main roots, and root length of *Origanum syriacum* plant.

Treatment / Medium strength	Type of Auxin	No. shoots	Average of shoot Length (cm)	No. Main Roots	Root Length (cm)
Full	Control	1.00^{b}	5.00^{cd}	2.67^{de}	3.17^{bc}
	IAA	3.33^{b}	9.00^{b}	20.00^{abc}	2.33^{cde}
	IBA	7.00^{a}	11.75^{a}	33.00^{a}	5.25^{a}
	NAA	2.67^{b}	9.00^{b}	16.67^{bcd}	2.00^{de}
Half	Control	1.00^{b}	5.00^{cd}	2.33^{de}	3.33^{b}
	IAA	7.00^{a}	12.25^{a}	31.75^{ab}	5.00^{a}
	IBA	3.20^{b}	7.40^{bc}	15.60^{cde}	2.60^{bcd}
	NAA	1.25^{b}	5.75^{cd}	21,50^{abc}	1.50^{ef}
Quarter	Control	1.00^{b}	3.67^{d}	0.00^{e}	0.00^{g}
	IAA	1.67^{b}	5.00^{cd}	7.00^{cde}	1.67^{de}
	IBA	4.00^{b}	3.67^{d}	6.33^{cde}	1.33^{ef}
	NAA	1.00^{b}	6.50^{bcd}	5.67^{cde}	0.67^{fg}

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 9. Effect of medium strength and type of Auxin on number of shoots, plant length, number of main roots, and root length of *O. syriacum*.

Treatment / Medium type	Type of auxin	Auxin con.mgl^{-1}	No. shoots	Shoot length (cm)	No. main Roots	Root length (cm)
Full	IBA	Control	2.00^{c}	7.73^{ab}	9.67^{c}	3.00^{abc}
		0.50	3.33^{c}	7.13^{ab}	7.00^{c}	2.17^{c}
		1.00	3.33^{c}	6.83^{ab}	22.67^{bc}	3.10^{abc}
		1.50	15.67^{a}	9.50^{a}	46.67^{a}	4.20^{a}
		2.00	5.67^{bc}	6.23^{ab}	21.67^{bc}	3.73^{ab}
Half	IAA	Control	1.67^{c}	5.33^{b}	8.00^{c}	2.33^{bc}
		0.50	5.00^{bc}	5.17^{b}	11.33^{c}	3.33^{abc}
		1.00	11.33^{ab}	7.33^{ab}	36.00^{ab}	3.50^{abc}
		1.50	4.33^{bc}	8.07^{ab}	27.00^{abc}	3.23^{abc}
		2.00	3.33^{c}	8.33^{ab}	12.67^{c}	2.23^{c}

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.

Fig. 3. Effect of IBA concentrations (mgl^{-1}) on full MS medium on root formation of *Origanum syriacum* plant.

Fig. 4. Effect of IAA concentrations (mgl^{-1}) on half MS medium on root formation of *Origanum syriacum* plant.

Acclimatization stage:

O. syriacum was successfully acclimatized by using a combination of peatmoss, sand and vermiculite at rate (1:1:1) (v/v/v), respectively. Similarly, Arafeh *et al.,* (2003) and El-Beyrouthy *et al.,* (2015) found that the mixtures of peat and perlite at a ratio of 1:1 or 1:2 (v/v) were selected as the most suitable media for transplanting or adaptation stages of *O. syriacum* plants.

CONCLUSION

In this study a suitable protocol was developed for *in vitro* micropropagation of *O. syriacum*. Firstly, establishment stage of *in vitro* shoots from shoot tip explants on MS medium. Then multiply, the shoots on MS medium+ 0.5 mgl^{-1} Kin + 0.05 mgl^{-1} NAA**.** Moreover, Rooting the shoots on full strength MS medium with 1.50 mgl^{-1} IBA or half strength MS medium with 1.00 mgl^{-1} IAA**.** Finally, hardening stage of rooted shoots in plastic pots containing mixture of peatmoss, sand and vermiculite (1:1:1 v/v/v) in greenhouse.

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الإكثار المعملي الدقيق للزعتر الجبلي من خلال تقنية زراعة الانسجة

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تم إجراء هذه الدراسة في معمل زراعة الأنسجة النباتية – كلية العلوم الزراعية البيئية – جامعة العريش وذلك خلال الفترة من ٢٠١٣ – ٢٠١٦ . كان الهدف من هذه الدراسة هو استخدام تكنيك زراعة الأنسجة لإكثار نبات الزعتر الجبلي. تم زراعة القمة النامية والساق البرعمية علي بيئة موراشيج وسكوج ، ونيتش ونيتش وجامبورج. أظهرت النتائج أن زراعة القمة النامية علي بيئة موراشيج وسكوج أعطت أفضل نمو للنبات في مرحلة البداية . وكان أفضل تضاعف للقمة النامية باستخدام بيئة موراشيج وسكوج مضافاً اليها كينتين بمعدل ٠.٥ ملجم/لتر مع نفتالين حامض الخليك بمعدل ٠.٠٥ ملجم/لتر. اما أفضل إضافة فكانت الثيامين بتركيز ٠.٤ ملجم/لتر. حدث أفضل تجذير للنبتة مع استخدام البيئة الكاملة وإضافة ١.٥ ملجم/لترأندول حمض البيوتيريك(IBA) والنصف قوة للبيئة مع إضافة ١.٠ ملجم/لترأندول حمض الخليك(IAA) واجريت مرحلة الاقلمة في الصوبة في أوعية تحتوي علي ٣مخلوط من البيتموس والفيرمكيوليت والرمل بنسبه حجمية ١:١:١ .وكانت نسبة النجاح ٩٣%.