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EFFECT OF SOME FACTORS ON CAPER MICROPROPAGATION

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ABSTRACT

This study was carried out in Plant Tissue Culture Laboratory of Faculty of Environ. Agric. Sci .El-Arish, North Sinai, Suez Canal University during the period from April 2013 to August 2014.The objective of this study was to investigate the effect of growth regulators and some additives on *Capparis spinosa* L .micropropagation .The results indicated that the highest significant No. of axillary shoots/explant, axillary shoot length ،No. of leaves/shoot and main shoot length ٣.٤٠ ،٤.٢٢) cm ٢٣.٠٤ ،and ٤.٧٧cm ،respectively (were recorded when nodal explants were cultured on MS medium supplemented with0.5 mgl^{-1} BA combined with 0.1 mgl^{-1} NAA during establishment stage. While in multiplication stage BA at 0.5 mgl^{-1} combined with 0.1 mgl^{-1} NAA and 1.0 mgl^{-1} diphenyelurea gave the highest values of axillary shoot length ، No. of leaves/shoot and main shoot length ١٧.٧٤ ،٨.٨٨) cm and ١١.٦٦ cm ، respectively .(Moreover, addition of BA at 0.5 mgl^{-1} combined with 0.1 mgl^{-1} NAA and 2.0 mgl^{-1} phloroglucinol) PG (recorded the highest values of No. of axillary shoots/explant, axillary shoot length ،No. of leaves/shoot and main shoot length ١٢.٠٨ ،١١.٠٠)cm ٢٠.٠٤ ،and ١٤.٨٦ cm ،respectively .(Addition of sucrose to the medium at٥٠ gl^{-1} combined with Benzyl adenine at 0.5 mgl^{-1} and NAA at 0.1 mgl^{-1} recorded the highest growth parameters compared with other sucrose concentrations .Shoots were rooted successfully (70% rooting percentage) by addition of ٢.٠ mgl^{-1} IBA combined with 1.0 gl^{-1} activated charcoal to MS medium .Plantlets were successfully acclimatized %٩٢) survival) when cultured in peat moss and vermiculite (١:١ v/v)

Keywords:*Capparis spinosa* L., nodal explants, Phloroglucinol (PG), Diphenyelurea and IBA

INTRODUCTION

Caper (*Capparis spinosa* L.) is a summer perennial shrub specie belonging to the Capparidaceae family. It is a low perennial trailing shrub, with procumbent or pendulous branches. Leaves are greyish-green, sound, simple and thick. Flowers are white with red stamens. They appear from March to June. Each flower lasts for one day. Fruits with numerous light brown seeds (Sozzi, 2001) and (El-Tanbouly, 1990).

Caper has been used in traditional medicine against bacterial infection, which exploited its properties for several purposes. Also, roots are used as tonic, astringent and diuretic. Root bark is used as appetizer, purgative, anthelmintic, analgesic and applied externally as cataplasm for spleen troubles. Bark is used for treatment of gout, rheumatism, laxative, expectorant and for chest diseases. Infusion prepared from the stem and root bark for diarrhea and febrifuge. Flower buds and roots are utilized as renal

disinfectants, duiretic, tonic and for arteriosclerosis and chills, as well as compresses for the eyes. Leaves and fruits are carminative and aphrodisiac (El-Tanbouly, 1990).

The conventional method of propagation through seed is not preferred for multiplication of caper plant because it has low germination percentage this is probably because the seed coat contains inhibitors (Ölmez *et al*., 2004).On the other side, *C. spinosa* has not been propagated through vegetative cuttings because it has low rooting percentage (55%) with low success degree (30%) (Inocencio *et al*., 2002).

C. spinosa plants are disappeared gradually from Sinai natural environment so, there is a critical importance should be done to face this problem by using tissue culture technique.

Phloroglucinol (PG) a phenolic compound (1,3,5- trihydroxybenzene) is one of the degradation products of phloridzin known for its growth regulating property. There are some reports suggest that phloroglucinol enhances growth and rate of axillary shoot proliferation of some plants *in vitro* such as (Oliveria *et al*., 2003) on *Tabernaemontana fuchsiaefolia* L., Bhot *et al.* (2010) on three varieties of *Codiaeum variegatum* L.,(Steephen *et al*.,2010) on *Vitex negundo* L. plants and (Hassan, 2011) on *Balanites aegyptiaca* L. plants.

Diphenylurea addition success has been achieved by Genkov and Ivanova (1995) on carnation, Genkov *et al*. (1997) on *Dianthas caryophyllus* and El-Mekawy *et al*. (2012) on *Balanites aegyptiaca* L.

Carbohydrates are necessary in living cells as a source of energy and carbon skeleton for biosynthetic processes. The internal carbohydrate pool is suggested to have an important role in morphogenesis of several woody species (Kromer and Gamian 2000 and Li and Leung 2000).

The objective of present study was to investigate the effect of different concentrations of some growth regulators and the above mentioned additives on micropropagation of this plant. The main goal of this paper was to improve the efficiency and lowering the coast of caper micropropagation.

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) during the period from April 2013 to August 2014.

Explant source:

Wild plants of *Capparis spinosa* L. were collected from EL- Hasana region, North Sinai. Shoots about 3 cm were cut from mother plants and exposed to sterilization treatments.

Establishment stage:

Explant sterilization:

Shoots were washed under a running tap water for 1 hour. Then the explants were submerged in water with a few drops of liquid soap in a flask and shaked by hand for 15 min then rinsed in tap water to remove the soap. Explants were sterilized with 70% ethanol for 5 min followed by soaking for 15 min in 20% solution (v/v) commercial Clorox (5.25% sodium hypochlorite).

Explants were transferred to 0.1gl^{-1} mercuric chloride for 2 min. Explants were thoroughly rinsed three times with sterile distilled water after each previous step. Finally explants were cut into nodal and shoot tip explants (0.75 cm length).

Media preparation:

The sterilized nodal and shoot tips explants were cultured onMS medium (Murashige and Skoog, 1962) solidified with agar at 8 $gl^{-1}$and supplemented with glycin (2 mgl^{-1}), Myo–inositol (0.1 gl^{-1}) and sucrose (30 gl^{-1}). Medium was supplemented with different concentrations (0.0, 0.125, 0. 25, 0.5 or 1.0 mgl^{-1}) of benzyl adenine (BA) with NAA at 0.1mgl^{-1}. pH of the medium was adjusted at 5.7- 5.8. The medium has been cooked before distributed into the culture jars. Each jar contained 50 ml of the medium and the jars were immediately autoclaved at 121 C^{o} and 1.1 Kg cm^{2} for 20 min. Each treatment was consisted of 4 replicates each replicate contains 4 jars.

Multiplication stage:

In order to improve the efficiency of the best treatment (0.5mgl^{-1}BA + 0.1mgl^{-1} NAA) recorded in establishment stage, similar concentrations of these growth regulators were added to the medium combined with different concentrations of diphenyel urea (0.0, 0.5, 1.0 or 1.5 mgl^{-1}), phloroglucinol (0.0, 1.0, 2.0 or 4.0 mgl^{-1}) or sucrose (0.0, 30.0, 50.0 or 70 gl^{-1}).

After 6 weeks No. of axillary shoots/explant, axillary shoot length (cm), No. of leaves/shoot and main shoot length (cm) were recorded.

Rooting stage:

Similar shoots of *Capparis spinosa* L. (about 2-3 cm length) obtained from multiplication experiments were cultured on MS medium free without growth regulators for one month then shoots (about 2-3 cm length) were cultured on MS medium supplemented with different concentrations (0.0, 0.5, 1.0, 1.5 and 2.0 mgl^{-1}) of indole acetic acid (IAA) and indole butyric acid (IBA) with or without 1.0 gl^{-1} activated charcoal. After 4 weeks rooting percentage No. of roots/ shoot and root length (cm) were recorded.

All the above mentioned experiment cultures were incubated in growth room at 25 ± 2 C^{o} under 16 h/day photoperiod which provided by cool white fluorescent lamps with light intensity of 2000 Lux.

Acclimatization stage:

Rooted shoots (about 3-4 cm length) were acclimatized by transferring them to black polyethylene pots 8 cm diameter containing peat moss and vermiculite at rate (1:1, v/v). The cultured pots were covered with transparent polyethylene bags. After one week holes were made in covered bags. These holes were expanded gradually each week. After four weeks plantlets became suitable for transferring to the outside of green house.

Experimental design and statistical analysis:

Experiments were set up in simple completely 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:

This part of work was mainly designed to study the effect of benzyl adenine (BA) concentrations combined with NAA at 0.1 mgl^{-1} on shoot proliferation and growth of two explants types of *Capparis spinosa* L. during establishment stage.

Data presented in Table (1) showed that, when (BA) concentrations combined with NAA at 0.1mgl^{-1} were significantly increased axillary shoot length and number of leaves.

Also, number of axillary shoots / explant and main shoot length followed similar trend with the exception that the highest concentration (1.0 mgl^{-1}) BA did not significantly differ from control treatment.

Generally, the highest significant No. Of axillary shoots/explant, axillary shoot length, No. of leaves/shoot and main shoot length (4.22, 3.40 cm, 23.04 and 4.77 cm, respectively) were recorded when nodal explant was cultured on MS medium contained BA at 0.5 mgl^{-1} combined with 0.1 mgl^{-1} NAA.

It is worth to mention that there was no significant difference between using 0.25 or 0.5 mgl^{-1} of BA concerning No. of axillary shoots/explant and main shoot length.

Similar results were obtained by (Shehata, 2012) found that, one node cutting as starting materials for establishment stage of *Capparis spinosa* L. cultured on MS media supplemented with 0.50 mgl^{-1} (BA) in the combination with 0.05 mgl^{-1} (NAA) enhanced multiple shoots proliferation.

Cytokinin effects can become apparent. In some circumstances, cytokinins activate RNA synthesis, stimulate protein synthesis and the activities of some enzymes (Kulaeva, 1980).

Table(1): Effect of benzyladenine (BA) concentration combined with NAA at 0.1 mgl^{-1} on shoot proliferation and growth of two explants types of *Capparis spinosa* L. during establishment stage

Explants type	BA (mgl^{-1})	No. of axillary shoots/ explant	Axillary shoot length (cm)	No. of leaves/ shoot	Main shoot Length (cm)
Shoot tip	0.000	3.12 e	2.57 fg	18.33 f	3.67 e
	0.125	3.45 cd	2.79 de	19.45 e	3.90 cd
	0. 250	3.88 b	2.87 cd	20.67 cd	4.40 b
	0. 500	4.03 ab	3.23 b	21.8 b	4.63 ab
	1.000	3.33 de	2.35 h	20.1 d	3.89 de
Nodal explant	0.000	3.30 de	2.69 ef	20.42 d	3.77 de
	0.125	3.52 d	2.92 cd	21.18 c	4.12 c
	0. 250	4.06 ab	2.98 c	22.03 b	4.56 ab
	0. 500	4.22 a	3.40 a	23.04 a	4.77 a
	1.000	3. 50 cd	2.47 gh	21.11 c	3.95 cd

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

Multiplication stage:

Effect of different concentrations of diphenyl urea (DPU) combined with 0.5 mgl^{-1}BA and NAA at 0.1 mgl^{-1} on *Capparis spinosa* L. shoot proliferation and growth during multiplication stage:

Data illustrated in Fig.1& Plat. 1 showed that addition of DPU at any concentration to the medium containing 0.5 mgl^{-1} BA and NAA at 0.1 mgl^{-1} significantly increased the axillary shoot length, No. of leaves/shoot and main shoot length in most cases. On the other side, No. of axillary shoots/explant did not affect by supplementation the medium with DPU.

The highest values of axillary shoot length, No. of leaves/shoot and main shoot length (8.88 cm, 17.74 and 11.66 cm, respectively) were recorded with1.0 mgl^{-1}diphenyel urea combined with 0.5 mgl^{-1} BA and 0.1 mgl^{1} NAA.

These results were in agreement with those found by Genkov and Ivanova (1995) on carnation since they found that phenyl urea cytokinins caused higher rate of multiplication. Also, El-Shamy (2002) on Bougainvillea plant found that BA at 2 mgl^{-1}+ 0.25 mgl^{-1} IAA combined with 0.25, 0.50 or 1.0 mgl^{-1}diphenylurea in MS medium resulted in enhancing effect on axillary shoots number as the combination of 2.0 mgl^{-1} BA plus 0.25 mgl^{-1} IAA plus 1.0 mgl^{-1} diphenyl urea resulted in the highest axillary 1.0 mgl^{-1}diphenylurea as resulted in the highest axillary shoot number, shoot length and number of leaves number per shoot resulted from single node explant. Also 2.0 mgl^{-1} BA plus diphenyl urea was effective for shoot proliferation from shoot tip explant.

Positive effect of diphenyl urea and many others substituted ureas, because they have cytokinin activity and can promote the growth of dormant buds (Kefford *et al*., 1966), and induce cell division in cytokine dependent callus tissues (Bruce *et al*., 1965).

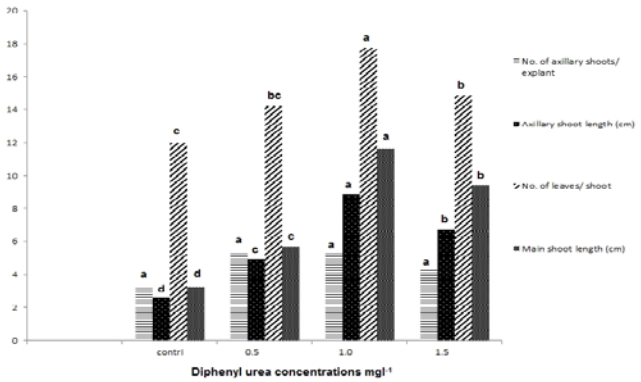
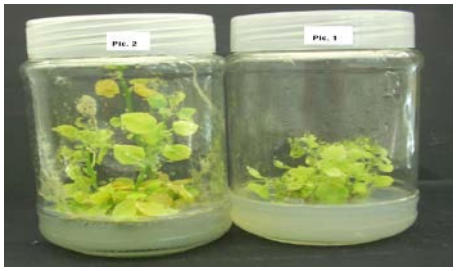


Fig.1.Effect of different concentrations of diphenyl urea (DPU) combined with 0.5 mgl^{-1}BA and NAA at 0.1 mgl^{-1} on *Capparis spinosa* L. shoot proliferation and growth during multiplication stage



Plat. 1. Effect of diphenyl urea (DPU) combined with 0.5 mgl^{-1}BA and NAA at 0.1 mgl^{-1} on *Capparis spinosa* L. shoot proliferation and growth

Pic. 1: Control (0.5 mgl^{-1} BA and 0.1 mgl^{-1} NAA).
Pic. 2:1.0 mgl^{-1}diphenyel urea combined with 0.5 mgl^{-1}BA and 0.1 mgl^{-1} NAA.

Effect of different concentrations of phloroglucinol (PG) combined with 0.5 mgl^{-1} BA and NAA at 0.1 mgl^{-1} on *Capparis spinosa* L. shoot proliferation and growth during multiplication stage:

The effect different concentrations of phloroglucinol (PG) combined with 0.5 mgl^{-1} BA and NAA at 0.1 mgl^{-1} on *Capparis spinosa* L. shoot proliferation and growth during multiplication stage are presented in Fig.2& Plat. 2.

All recorded parameters were increased as PG concentration increased up to 2.0 mgl^{-1}, while the highest concentration (4.0 mgl^{-1}) decreased most of these parameters. It is worth to mention that while axillary shoot length was enhanced significantly by addition of PG at 1.0 mgl^{-1} to the medium there was no significant differences were observed between PG concentrations in this regard.

Data clear that the highest values of no. of axillary shoots/explant, axillary shoot length, No. of leaves/shoot and main shoot length (11.00 cm,12.08, 20.04 and 14.86 cm, respectively) were obtained when 2.0 mgl^{-1} PG was combined with 0.5 mgl^{-1} BA and 0.1 mgl^{-1} NAA.

Positive effect of PG acted synergistically with BA in inducing early bud break and high frequency shoot proliferation of *Vitex negundo* L. an aromatic medicinal plant was reported by Steephen *et al*. (2010) Also, Debabrata and Prakash (2000) found that phloroglucinol fostered multiple shoot formation and promoted axillary shoot proliferation in potato.

PG has already used in micropropagation as a component that reduce vitrification and increase proliferation (Gaspar,1991 and Aklan *et al.*,1997).

Furthermore, phloroglucinol increased phosphorylation of ERK (Extracellular signal- regulated kinases), an important component of intracellular signaling cascades and activated ERK participates in a wide range of cellular programs including proliferation, differentiation and movement (Pages *et al*., 1991 & Robinson and cobb, 1997).

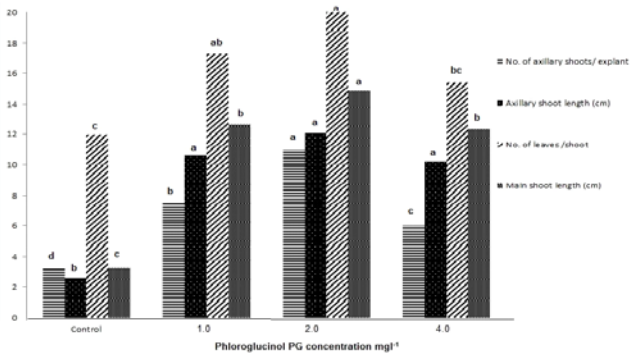
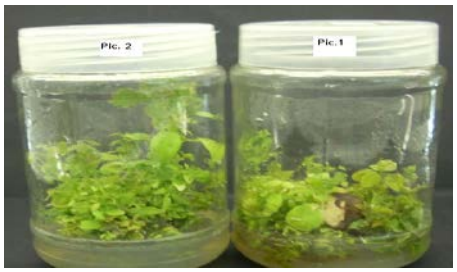


Fig.2.Effect of different concentrations of phloroglucinol (PG) combined with 0.5 mgl^{-1} BA and NAA at 0.1 mgl^{-1} on *Capparis spinosa* L. shoot proliferation and growth during multiplication stage



Plat. 2. Effect of phloroglucinol (PG) concentrations combined with 0.5 mgl^{-1}BA and 0.1 mgl^{-1} NAA on *Capparis spinosa* L. shoot proliferation

Pic. 1:Control (0.5 mgl^{-1} BA and 0.1 mgl^{-1} NAA).
Pic. 2:2.0 mgl^{-1} phloroglucinol combined with 0.5 mgl^{-1} BA and 0.1 mgl^{-1} NAA.

Effect of different sucrose concentrations combined with 0.5 mgl^{-1}BA and 0.1 mgl^{-1}NAA on *Capparis spinosa* L. shoot proliferation and growth during multiplication stage:

Data in Fig.3 show the effect of different sucrose concentrations combined with 0.5 mgl^{-1} BA and 0.1 mgl^{-1} NAA on *Capparis spinosa* L. shoot proliferation and growth during multiplication stage.

The data clear that supplementation the medium with sucrose significantly increased most of recorded parameters. There was no significant difference between using of 50 or 70 gl^{-1}sucrose. From the commercial point of view it is better to use 50 gl^{-1}sucrose which produced the highest values of No. of axillary shoots/explant, axillary shoot length, No. of leaves/shoot and main shoot length (5.33, 8.66 cm, 20.42 and 10.33 cm, respectively).

Several reports have proved that the carbon source affects the *in vitro* morphogenesis of different plant species (Fuentes *et al*., 2000). Carbohydrates control morphogenesis by acting as the energy source and by altering the osmotic potential of the culture medium, which in turn alters such

cell wall properties as extension, hardening and composition, followed by subsequent modification in morphogenesis (Pritchard *et al.*, 1991).

Gabryszewska (2011) has shown inhibitory effect of high concentration of sucrose on growth of axillary shoots of *Syringa vulgaris* this result is in harmony with our findings.

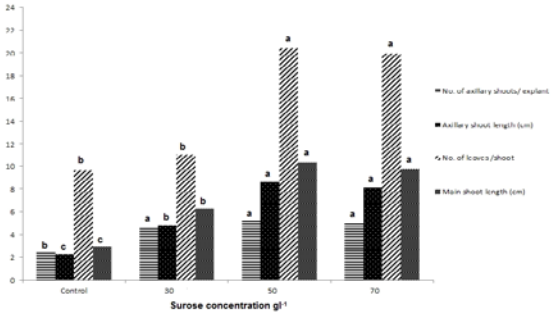
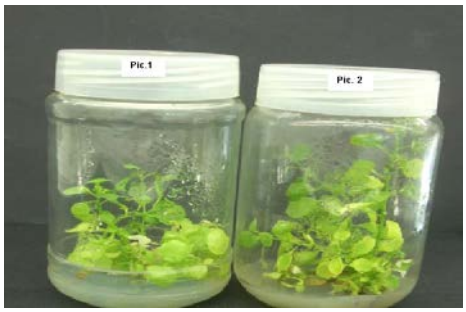


Fig.3.Effect of different sucrose concentrations combined with 0.5 mgl^{-1} BA and 0.1 mgl^{-1}NAA on *Capparis spinosa* L. shoot proliferation and growth during multiplication stage



Plat. 3. Effect of sucrose concentrations combined with 0.5 mgl^{-1} BA and 0.1 mgl^{-1} NAA on *Capparis spinosa* L. shoot proliferation and growth

Pic. 1:Control (0.5 mgl^{-1} BA and 0.1 mgl^{-1} NAA).
Pic. 2: sucrose at 50 gl^{-1} combined with 0.5 mgl^{-1}BA and 0.1 mgl^{-1}NAA.

Rooting stage:

Effect of auxin type, concentration and activated charcoal concentration on rooting of *Capparis spinosa* L. shoot during rooting stage:

Data inTable.2 clear that in most addition of IBA at 2.0 mgl^{-1} combined with 1.0 gl^{-1} activated charcoal recorded the highest values of all rooting parameters i.e. ; rooting percentage, No. of roots/shoot and root length (70.0, 3.55 and 7.01cm, respectively).

This result was in agreement with Sudipta *et al*. (2011) since they reported that maximum number of roots for *Leptadenia reticulate* was recorded when grown shoots were cultured on full strength MS media containing 2.00 mgl^{-1} IBA with 200 mgl^{-1} activated charcoal.

Table (2): Effect of auxin type, concentration and activated charcoal concentration on rooting of *Capparis spinosa* L. shoot during rooting stage

Treatments			Rooting percentage	No. of roots / Shoot	Root length(cm)
Activated charchol(gl^{-1})	Auxin type	Auxin con.			
Zero	IAA	0.0	0.0 l	0.00 k	0.00 k
		0.5	20 i	0.33 j	0.86 hi
		1.0	36 f	0.66 i	2.80 de
		1.5	55 d	1.77 c	2.18 g
		2.0	64 b	2.66 e	2.44 fg
	IBA	0.0	0.0 l	0.00 k	0.00 k
		0.5	6.0 k	0.40 j	0.28 jk
		1.0	30 g	1.33 g	2.36 g
		1.5	30 g	1.46 fg	3.60 c
		2.0	55 d	3.32 b	6.12 b
1.0	IAA	0.0	0.0 l	0.00 k	0.00 k
		0.5	25 h	0.55 ij	1.12 h
		1.0	40 e	1.00 h	3.15 de
		1.5	60 c	2.33 d	3.00 de
		2.0	63b	2.88 c	3.20 d
	IBA	0.0	0.0 l	0.00 k	0.00 k
		0.5	10 j	0.50 ij	0.55 ij
		1.0	35 f	1.67 f	2.76 e
		1.5	40 e	1.77 e	3.85 c
		2.0	70 a	3.55 a	7.01 a

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

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تأثير بعض العوامل على الإكثار الدقيق لنبات اللصف

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أجريت هذه الدراسة بمعمل زراعة الأنسجة النباتية بكلية العلوم الزراعية البيئية بالعريش، شمال سيناء، جامعة قناة السويس خلال الفترة من إبريل ٢٠١٣ إلى أغسطس ٢٠١٤. تهدف هذه الدراسة إلى دراسة تأثير بعض منظمات النمو والاضافات الاخرى على الإكثار الدقيق لنبات اللصف. وقد أوضحت النتائج أن أعلى زيادة معنوية فى عدد الأفرع، طول الأفرع الجانبية، عدد الأوراق/ فرع وطول الفرع الرئيسى (٤.٢٢، ٣.٤٠ سم، ٢٣.٠٤و٤.٧٧ سم) على التوالى تم الحصول عليها عند زراعة العقد الساقية على بيئة موراشيجى وسكوج تحتوى على البنزيل أدينين بتركيز ٠.٥ مجم/لتر مع ٠.١ مجم/لتر نفثالين حمض الخليك خلال مرحلة التأسيس. بينما فى مرحلة التضاعف أدت إضافة البنزيل ادينين بتركيز ٠.٥ مجم/لتر مع ٠.١ مجم/لتر نفثالين حمض الخليك بالإشتراك مع الداى فينيل يوريا بتركيز واحد مجم/لتر للحصول على أعلى طول للأفرع الجانبية، عدد الأوراق/ فرع وطول الفرع الرئيسى (٨.٨٨ سم، ١٧.٧٤و١١.٦٦ سم) على التوالى علاوة على ذلك أدى استخدام البنزيل أدينين بتركيز ٠.٥ مجم/لتر مع ٠.١ مجم/لتر نفثالين حمض الخليك مع ٢ مجم/لتر فلوروجلوسينول للحصول على أعلى عدد للأفرع، طول الأفرع الجانبية، عدد الأوراق/ فرع وطول الفرع الرئيسى (١١.٠، ١٢.٠٨ سم، ٢٠.٠٤و١٤.٨٦ سم) على التوالى فى حين أدى إضافة السكروز بتركيز ٥٠ جم/لتر مع البنزيل أدينين بتركيز ٠.٥ مجم/لتر و٠.١ مجم/لتر نفثالين حمض الخليك للحصول على أعلى قياسات النمو لنبات اللصف مقارنة بتركيزات السكروز الأخرى. كذلك تم تجذير الأفرع

الخضرية بنجاح (٧٠٪ نسبة تجذير) بإستخدام اندول حمض البيوترك بتركيز ٢ مجم/لتر مع إضافة ١ جم/لتر فحم نشط. وفى النهاية تم أقلمة النبيتات بنجاح بنسبة بقاء وصلت الى ٩٢٪ باستخدام خليط من البيتموس والفرموكوليت بمعدل إضافة (١:١حجما).