Some Factors Affecting Micropropagation of Some Wild Medicinal Plants 1- *Ecballium elaterium* L. Plant

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Abstract: *Excised embryos of Ecballium elaterium L.(squirting cucumber, wild cucumber) was cultured on MS medium supplemented with 2, 4-D at 0, 1 or 2 mg/l and kinetin at 0, 1 or 2 mg/l. Kinetin alone did not induce callus formation. Using 2, 4-D at 1 or 2 mg/l alone show the less response of callus fresh weight while the effective combinations of 2, 4-D and kinetin were 1 mg/l 2, 4-D combined with 1 mg/l kinetin or 2 mg/l 2, 4-D combined with 2 mg/l kinetin which resulted in the highest fresh weight of callus after 30 and 60 days from embryos culture without significant differences between the two treatments. Similar excised pieces of callus cultured on MS medium supplemented with combinations of benzyl adenine (BA) at 0.5 or 1.0 or 1.5 mg/l and naphthalene acetic acid (NAA) at 0.1, 0.2 or 0.3 mg/l showed the less differentiations of shoot number with NAA alone, but the best combination which resulted in the highest shoots number was 0.5 mg/l BA combined with 0.1 mg/l NAA or 1.5 mg/l BA combined with 0.2 mg/l NAA. Shoot length resulted in the highest value with the later combination. Where, the highest number of leaves/ shoot was obtained from 0.5 mg/l BA combined with 0.1, 0.2 or 0.3 mg/l NAA. The excised shoots showed the highest number of roots and root length on $^1/_4$ MS medium strength supplemented with 0.3 mg/l indole-3-butyric acid (IBA).The obtained plantlets were successfully acclimatized on peat moss: sand (1:1) medium with 90% survival plants.*
Key words: *squirting cucumber, embryos culture, callus formation, plantlets and acclimatization.*

INTRODUCTION

Ecballium elaterium L. A. Rich (Fam. Cucurbitaceae), a powerful hydragogue cathartic known as squirting cucumber. Perennial plant some what fleshy monoecious. Stems 30-100 cm. long, rough hairy, prostrate, branched from base, tendrils absent. Leaves 6-10 cm. long petiold, triangular to heart-shaped, rough on the upper surface, density white hairy on the lower surface, 3-lobed or with irregularly wansy-dentate margin. Flowers greenish-yellow; female flowers in the leaf axils, usually solitary; male flowers in racemes. Fruit a large juicy berry, 3 – 5 cm anote-oblong, nodding, detaching it self explosively at maturity scattering seeds and juice. Flowering, March and September or all year (Tackholm 1974). In folk medicine the roots were used as analgesic and in treatment of hemorrhoids, fruits in sinusitis Jaundice, nocturia, lumbago and otalgia (Toker *et al.*, 2003).

The *Ecballium elaterium* L. plant is one of the in danger plants due to that the growth of plant depend upon the available rain fail in Sinai conditions in addition to intensive grazing may cause the lost of this plant. So this work was conducted aiming to investigate the propagation of this plant through micro propagation techniques, since it can obtain high number of plants through this technique regardless environmental conditions in order to conserve this important medicinal plant.

MATERIALS AND METHODS

This work was carried out in the Plant Tissue Culture Laboratory in Faculty of Environmental Agricultural Sciences, El-Arish, Suez Canal University during the period from 2002 to 2005.

Seeds of *Ecballium elaterium* L were collected from Rafah region, North Sinai. The embryo of *Ecballium elaterium,* L seeds was isolated and used as starting explants in the following experiments:

1- The first experiment:

This experiment was conducted aiming to obtain callus culture from excised embryos. The plant seeds were soaked for 10 minutes in sulphuric acid (98 %), the seed cover was removed. The internal parts of the seed was soaked for 3 minutes in chlorox (10 %) then washed three times with sterilized distilled water in laminar air flow hood and the embryos were excised under septic conditions. The excised embryos were cultured on Murashige and Skoog basal medium (1962) supplemented with the combinations of 2, 4-dichlorophenoxy acetic acid (2, 4-D) at 0, 1 or 2 mg/l and kinetin at 0, 1 or 2 mg/l. The culture was conducted in a jars (150 cm^3) contains 30 ml of the medium and subjected to 3000 lux photoperiod for 16 hrs fluorescent light and 8 hrs. dark. The temperature was 25±2°C. After 30 days, from represented samples the callus fresh weight was determined and again reculture on the same medium for another 30 days then the fresh weight was again determined.

2-The second experiment:

The experiment was conducted aiming to enhance the regeneration of callus. The MS medium was used which supplemented with a combinations of benzyl adenine (BA) at 0.0, 0.5, 1.0, 1.5 ml/l and naphthalene acetic acid (NAA) at 0.1, 0.2 or 0.3 mg/l. Similar pieces of callus about (1 gm) weight were cultured in a jars as mentioned before with the same conditions. After six weeks the following data were recorded: The number of

proliferated shoots, shoot length (cm) and leaves number/shoot.

3-The third experiment:

The experiment was conduced aiming to enhance rooting for the obtained shoots. The excised shoots were cultured in jars contains 30 ml of $^1/_4$ or $^1/_2$ MS strength medium supplemented with indole-3- butyric acid (IBA) at 0.0, 0.1, 0.2 or 0.3 mg/l. The jars were incubated under the above mentioned conditions in room culture. After eight weeks the number of roots/ shoot and root lengths were determined.

The obtained plantlets after eight weeks were transferred to acclimatization in 1 peat moss: 1 sand medium covered with polyethylene bags in a shade place at 25±2°C, after two weeks the polyethylene cover was removed as the acclimatization process showed a success survival percentage of 90%.

All the above mentioned experiments were conducted in complete randomized block design with three replicates for each treatments and each replicate represented by 10 jars. Rooting experiment was factorial experiment between media strength and IBA concentration. The obtained data was subjected to statistical analysis according to Snedecor and Cochran (1990). Mean separations were done by using MSTATC computer program V.4 (1986) and least significant differences (LSD) was computed for means separation of all parameters obtained in these studies.

RESULTS AND DISCUSSION

1-Effect of 2, 4-D and kinetin concentrations on callus fresh weight of *Ecballium elaterium*, L plant

Data in Table (1) indicate that the medium lacking of 2, 4-D even amend with kinetin up to 2 mg/l did not show any callus induction. On the other side, using 2, 4-D at 1 or 2 mg/l alone shows the less response of callus weight (0.60 and 0.61 mg/explants).

The more effective combinations of 2, 4-D and kinetin were 1mg/l 2, 4-D combined with 1 mg/l kinetin or 2 mg/l 2, 4-D combined with 2 mg/l kinetin. Both treatments resulted in the highest fresh weight of callus after 30 and 60 days with subculture. There was no significant difference between them (photo 1).

Table (1): Effect of 2, 4-D and kinetin concentrations on callus fresh weight (gm) of *Ecballium elaterium*, L plant

2,4-D concentrations (mg/l)	Kinetin concentrations (mg/l)	Culture age	
		30 days	60 days
0	0	0.00	0.00
	1.0	0.00	0.00
	2.0	0.00	0.00
1	0	0.60	3.90
	1.0	1.23	8.65
	2.0	0.70	4.88
2	0	0.61	4.31
	1.0	1.03	7.24
	2.0	1.38	9.69
L.S.D. at 0.5%		0.30	2.38
L.S.D. at 0.1%		0.41	3.21

The above mentioned trend are in harmony with Wakhlu and Barna (1989), they found that the best initiation and growth of callus on *Plantago ovata* Forssk. cv. explants was achieved on MS medium containing 2,4-D at 1mg/l and kinetin at 1 mg/l. Likewise, Azza and Noga (2002) induced callus formation from hypocotyls and primary leaf explants of *Cuminum cyminum* L plant on MS medium containing 0.8 mg/l 2,4-D alone or plus 0.4 or 0.8 mg/l kinetin. The embryogenesis callus was developed within 2 weeks after transferring the callus to medium lacking plant growth regulators. The presence of kinetin with 2, 4-D enhanced callus formation on the explants.

2-Effect of BA and NAA concentrations on number of shoots of *Ecballium elaterium*, L plant:

Data in Table (2) show that NAA alone at 0.1 and up to 0.3 mg/l resulted in the less differentiation of shoots number from callus. On the other side, the highest shoots number was obtained from the combination of 0.5 mg/l BA and 0.1 mg/l NAA or 1.5 mg/l BA and 0.2 mg/l NAA. The other combinations of BA and NAA came in between the above mentioned treatments (photo 2).

In this regard Skoog and Miller (1957) demonstrated that the key variables in the chemical regulation of in vitro organogenesis was the ratio of auxin - cytokinins presence in the medium. Since there protocols of regeneration have been elucidated for many plant genera. The herein obtained results clear that the high BA and less NAA ratio as (0.5: 1 or 1.5: 0.2) was inducing for shoot regeneration from callus of *Ecballium elaterium*, L moreover NAA alone was not largely effective comparing to all combinations of BA and NAA. Similar findings was previously mentioned by Azza and Noga (2001) on cumin hypocotyl and stem internodes explants since, they found that the best

Table (2): Effect of BA and NAA concentrations on shoot number, shoot length and leaves number/shoot of *Ecballium elaterium*, L plant

BA Conc. (mg/l)	NAA Conc. (mg/l)	Number of shoots	Shoot length (cm)	Number of leaves/shoot
	0.1	1.40	1.80	0.20
0.0	0.2	1.60	2.00	0.40
	0.3	1.40	1.20	0.40
	0.1	4.20	3.60	3.20
0.5	0.2	3.80	3.80	3.80
	0.3	3.80	3.40	3.60
	0.1	2.60	2.80	2.20
1.0	0.2	1.40	1.80	0.20
	0.3	3.00	3.00	1.80
	0.1	3.60	3.60	1.40
1.5	0.2	4.80	5.00	1.60
	0.3	3.40	3.00	3.40
L.S.D. at 0.5%		0.94	1.38	0.76
L.S.D. at 0.1%		1.26	1.84	1.02

response towards shoot initiation on MS medium was obtained with 0.56 mg/l benzyl adenine (BA), while no shoot initiation could de obtained on medium lacking BA. Also, Ebrahimie *et al.* (2003) obtained multiple shoot regeneration of cumin impeded embryo cultures in a short period (30-50 days) on B5 medium (Gamborg *et al.* 1968) containing 1.0 mg/l BA, 0.2 mg/l NAA and 0.4 mg/l IAA with an average of 140 shoots per explanted. Similarly, Lee *et al.* (1994) found that BA concentrations as 0.1 and 0.3 mg/l induced shoots formation on excised leaf sections of *Lance coreopsis* regardless of the presence of NAA.

Moreover, El- Sawy *et al.* (2000) showed that the type of cytokinins is important factor for bud shoot development; BA was the most effective cytokinins for promoting shoot production of *Dracaena marginata*. In harmony with the obtained results Sakr *et al.* (1999) mentioned that the highest concentration of BA (5.0 mg/l) and NAA (0.5 mg/l) was the beneficial treatment for increasing shoots number of *Yucca elephantipes* and *Yucca elephantipes* cv. varigata. On the other hand, lowering the concentration of NAA (0.125 mg/l) in the medium containing low concentration of BA (1.25 mg/l) was more effective in inducing more shoot formation than did the high concentration. This means that the effect of NAA on shoot proliferation depends greatly on the added concentration of BA to the medium.

3- Effect of BA and NAA concentrations on shoot length of *Ecballium elaterium*, L plant:

Data in Table (2) show that the least shoot length was obtained from using NAA alone up to 0.3 mg/l. On the other side the highest shoot length was obtained from the combination of 1.5 mg/l BA and 0.2 mg/l NAA. In harmony with the obtained results, Saker *et al.* (1999) mentioned that culturing yucca explants on MS medium containing 5 mg/l BA plus 0.5 mg/l NAA was the most effective treatment in promoting the growth of shoots. The absence of BA+ NAA produced the shortest shoots.

4- Effect of BA and NAA concentrations on leaves number/shoot of *Ecballium elaterium*, L plant:

Data in Table (2) show that the least number of leaves/shoot was obtained using NAA up to 0.3 mg/l. On the other side the highest number of leaves/shoot was obtained from the concentrations of 0.5 mg/l BA combined with NAA up to 0.3 mg/l and or 1.5 mg/l BA combined with 0.3 mg/l NAA without significant differences between these combinations. Similar findings were mentioned by Sakr *et al.* (1999) since; they found that BA (5 mg/l) plus NAA (0.125 mg/l) was the more effective treatment in enhancing leaf formation on yucca plantlet. Doubling the concentration of NAA greatly reduced the process of leaf formation, regardless of BA concentration.

5-Effect of media strength and IBA concentrations on rooting of *Ecballium elaterium*, L plant:

Data in Table (3) show that using $^1/_4$ strength of MS medium resulted in high roots number/shoot and root length.

The main effect of IBA clear that using IBA at 0.2 or 0.3 mg/l decreased roots number and root lengths, where 0.1 mg/l was not significantly differ than the medium without IBA addition.

The interaction treatments between medium strength and IBA concentration in Table (3) indicate that the promising treatment was the combination of $^1/_4$ MS strength and 0.3 mg/l IBA. This treatment resulted in the highest roots number/shoot and root length. In this regard Tiwari *et al.* (1999) on *Rubia cordifolia* found that adventitious rooting of *in vitro* raised shoots was induced in half strength MS medium containing 0.51 mg/l IBA. Also, Rady and Saker (2000) on *Lavandula officinalis* L obtained increase in roots number and root length by increasing IBA concentration in MS medium. Also, Hunalut (1984) observed that the number of roots of fennel (*Foeniculum vulgare* Miller) increased with increasing IBA concentration. However, Mohamed (2003) found that root formation was achieved at low concentration of IBA for *Stevia rebaudiana*.

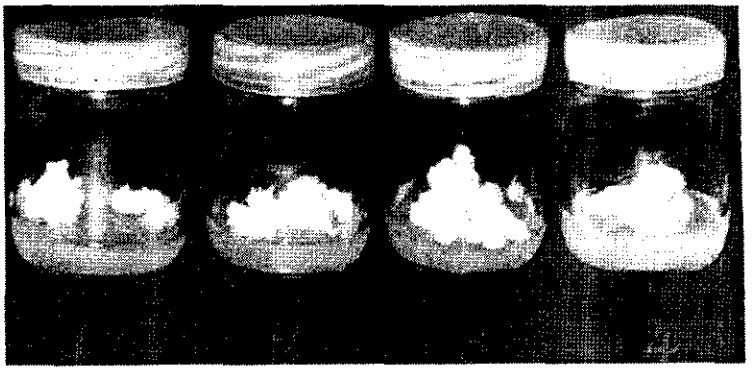

Photo. (1): Effect of 2, 4-D and kinetin on callus fresh weight of *Ecballium elaterium* L.

Photo. (2): Effect of BA and NAA on shooting growth of *Ecballium elaterium* L.
1-1.0 mg/l 2, 4-D +0.1 mg/l kinetin
2-1.0 mg/l 2, 4-D +0.2 mg/l kinetin
3-2.0 mg/l 2, 4-D +0.1 mg/l kinetin
4-2.0 mg/l 2, 4-D +0.2 mg/l kinetin

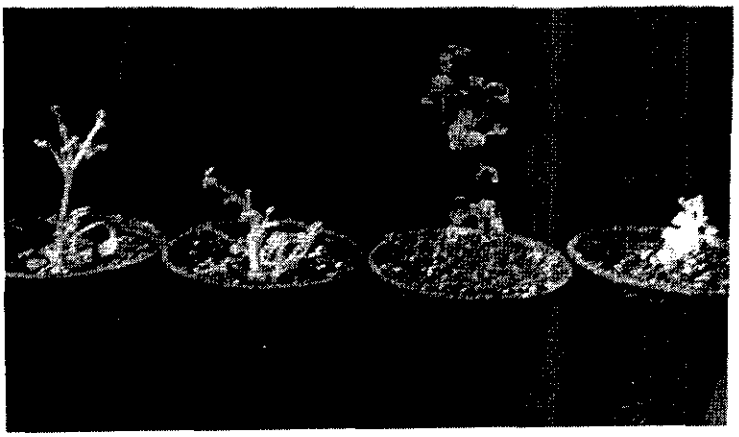

Photo. (3): Seedling of *Ecballium elaterium* L. after 60 days from acclimatization

Table (3): Effect of medium strength and IBA concentrations on roots number/plantlet and root length of *Ecballium elaterium*, L plant.

Medium strength	Roots number/shoot				Medium average	Root length (cm)				Medium average
	IBA Conc.(mg/l)					IBA Conc.(mg/l)				
	0.0	0.1	0.2	0.3		0.0	0.1	0.2	0.3	
1/2 MS	1.50	1.95	1.80	0.30	1.38	1.35	1.75	1.50	0.34	1.23
1/4 MS.	2.85	2.50	1.65	2.90	2.47	3.00	2.90	2.10	3.50	2.87
IBA average	2.17	2.22	1.72	1.60		2.17	2.32	1.80	1.92	
L.S.D. Medium at 0.5%				0.17					0.11	
L.S.D. Medium at 0.1%				0.24					0.17	
L.S.D. IBA at 0.5%				0.25					0.18	
L.S.D.IBA at 0.1%				0.36					0.27	
L.S.D. Interaction at 0.5%				0.35					0.25	
L.S.D. Interaction at 0.1%				0.51					0.36	

6- The acclimatization:

The plantlets were successfully acclimatized on peat moss: sand (1:1) medium with 90% survival percentage of plants (photo 3).

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بعض العوامل المؤثرة على الإكثار الدقيق لبعض النباتات الطبية البرية
١- نبات قثاء جحا

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قسم الإنتاج النباتي و وقايته – كلية العلوم الزراعية البيئية بالعريش- جامعة قناة السويس

فصلت أجنة بذور نبات قثاء جحا(تفاحة جحا، بلحة جحا، قثاء الحمير) و زرعت في بيئة موراشيج وسكوج تحتوى على ٢، ٤ ثناني كلورو فينوكسى حمض ألخليك بتركيزات صفر، ١، ٢ مليجرام/لتر والكينيتين بتركيزات صفر، ١، ٢ مليجرام/لتر. أوضحت النتائج أن الكينيتين بمفردة لم يؤثر على تكوين الكالس، و استعمال ٢، ٤ ثناني كلورو فينوكسى حمض ألخليك بتركيزات ١ أو ٢ مليجرام/لتر اظهر استجابة طفيفة في زيادة الوزن الطازج للكالس بينما التأثيرات المشتركه من٢، ٤ ثناني كلورو فينوكسى حمض ألخليك والكينيتين بتركيزات ١، ٢ مليجرام/لتر كانت أكثر تأثيرا حيث أعطت أعلى وزن طازج للكالس بعد ٣٠ و٦٠ يوم من زراعة الأجنة بدون فروق معنوية بين المعاملتين.

أظهرت زراعة الكالس على بيئة موراشيج وسكوج مضافا إليها بنزيل أدينين بتركيزات صفر، ٠,٥، ١، ١,٥ مليجرام/لتر ونفثا لين حمض ألخليك بتركيزات ٠,١، ٠,٢، ٠,٣ مليجرام/لتر الذي اظهر بمفردة اقل تكشف لعدد الفروع حيث أعطت معاملات التفاعل عند تركيز ١,٥ مليجرام/لتر بنزيل أدينين متحدا مع ١ أو ٢ مليجرام/لتر نفثالين حمض ألخليك اكبر عدد من الفروع. اكبر عدد من الأوراق/فرع تم الحصول علية من تركيز ٠,٥ مليجرام بنزيل أدينين متحدا مع ١ أو ٢ أو ٣ مليجرام/لتر نفثالين حمض ألخليك.

كانت الجذور أطول وأكثر عددا عند الزراعة في بيئة قوتها ½ موراشيج وسكوج أضيف إليها ٠,٣ مليجرام أندول حمض البيوترك.

تمت أقلمة البادرات بعناية على بيئة مكونة من بيت موس ورمل (١:١) حيث كانت نسبة النباتات الحية ٩٠%.