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Developing an Efficient Protocol for Micropropagation of an Endangered Medicinal Plant, *Cleome droserifolia* (Forssk.) Del.

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ABSTRACT

An efficient protocol for micropropagation of an endangered medicinal plant, *Cleome droserifolia* (Forssk.) Del. which belongs to Family Cleomaceae. A wild perennial herb, was conducted to test the effect of different concentrations of sodium hypochlorite (clorox) 10, 15, 20,and 25 (v/v) for different durations (15, 20, 25and 30 min.) on seeds sterlization and adenine sulphate (Ads) addition at (0, 10 , 20 and 30 mgl^{-1}) with benzyel adenine (BA) at 0.0, 0.5, 1 and 1.5 mgl^{-1} on micropropagation of the two type *Cleome droserifolia* explants shoot tip and nodel explants. The obtained results showed the highest survival percentage was 80% which was achieved by using 20% (v/v) for 30 min. While the highest number of axial shoots was resulted from shoot tip explants with (BA) at 1.0 mgl^{-1}.While, using adenine sulphate at 30 mgl^{-1}(Ads) with benzyel adenine (BA) at 1.0 mgl^{-1} resulted the highest axial shoot length. Shoots were rooted using full strength MS medium fortified with 1.5 mgl^{-1} IBA. Soil mixture of sand and peat moss (1:1) were used for seedling acclimatization.

Key words: *Cleome droserifolia,* Benzyel adenine (BA), Adenine sulphate (Ads). Micropropagation , indole acetic acid (IAA).

Introduction

Cleome droserifolia (Forssk.) Del. belongs to Family Cleomaceae. A wild perennial herb about 60 cm high, much branched. The plant carries cauline leaves. The leaves have a characteristic slightly disagreable odour and a bitter taste. Flowers are yellow. Täckholm (1974), Batanouny (1999).

Cleome species are generally used in folk medicine as stomachics, rubefacients and in the treatment of scabies, rheumatic fever and inflammation Boulos (2000). The dried herb of *C. droserifolia*, locally known as Samwah, Afein, Reeh-El-Bard, is used by herbalists in Egypt as a hypoglycemic agent, and its decoction is widely used by the Bedouins of the southern Sinai for the treatment of diabetes Abdel-Hady(1998).

The growth and multiplication of shoots in *in vitro* condition are affected by many factors. The effect of adenine sulphate is known in the tissue cultures at many plant species. Hassan (2011), Siwach *et al*., 2012, Singh and Patel (2014).

In the last decade, the plant has been subject to severe overexploitation to be used in folk medicine for diabetes. It has been eradicated from vast areas, especially in the Sinai and the Eastern Desert. However, in the far south of the Eastern Desert, the plant is still flourishing and is growing in many wadis in hot desert areas. So the conservation of this species is urgent. Therefore, the present study was undertaken to assess protocol for *in vitro* micropropagation of *Cleome droserifolia* (Forssk.) Del.

Materials and Methods

This study was carried out in plant Tissue Culture Laboratory, Faculty of Environmental Agricultural (FEAS) El-Arish, North Sinai, Suez Canal University (SCU), Egypt during the period from 2012 to 2014.

Establishment stage

Seed sterilization and germination

Samwah (*Cleome droserifolia* (Forssk.) Del.) seeds were collocated from North Sinai Research Station (Gene Bank) (El-Sheikh Zuwyed), Desert Research Center (DRC), Mataria, Cairo and washed under a running tap water for 1 hour, then washed with sterile distilled water after that the explants were transferred to the laminar air flow hood and exposed to different concentrations of sodium hypochlorite (clorox) for different

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durations as shown in Table (1). At the end of each treatment the seeds were rinsed 3-5 times with sterile distilled water .

Media Preparation

Murashige and Skoog basel medium (Murashige and Skoog, 1962) supplemented with 100 mgl^{-1} myo-inositol and 3 % sucrose. All media were adjusted to pH 5.7 – 5.8 using either 0.10 N NaOH or 0.10 N HCL depending upon high or low before gelling with 7.00 gl^{-1} agar in all stages and sterilized in autoclave at 121° C and 1.1Kg cm^2 for 20 min. Seeds cultured on this media and incubated in darkness till germinated .

Table 1: Different concentrations and durations of sodium hypochlorite (clorox) for *Cleome droserifolia* (Forssk.) seeds.

Clorox con.(v/v)	10	15	20	25
Duration (min)	15	15	15	15
	20	20	20	20
	25	25	25	25
	30	30	30	30

Incubation of culture

Culture condition in all stages was incubated in culture room at 25±2 ºC under 16 hr light and 8 hr dark provided by cool florescent light intensity of 2500 lux.

Multiplication stage

Explant type and benzyle adenine(BA) concentrations

This stage aims to study two types of explants (shoot tip and nodal segments explant). Four benzyl adenine (BA) concentration at 0.0,0.5,1.0 and 1.5 mgl^{-1} and *In vitro* germinated shoots were used as explants which were one and half month age and were cut into shoot tips and nodal segments (1-1.5 cm).

Adenine sulphate concentrations

This experiment aimed to study the effect of (BA) at 1 mgl^{-1} with different concentrations of adenine sulphate on multiplication of samwah on soild MS medium at (0.0, 10, 20 and 30 mgl^{-1}).

Rooting stage

Shoots were excised from each culture passage and transferred to full-strength and half-strength (1/2 MS) MS medium containing 3% (w/v) sucrose and 0.8% (w/v) agar. The medium was further supplemented with (0,0.5,1,1.5 and 2) mgl^{-1} Indole Acetic Acid (IAA) or (0,0.5,1,1.5 and 2) mgl^{-1} Indole Butyric Acid (IBA) or (0,0.5,1,1.5 and 2) mgl^{-1} Naphthalene Acetic Acid (NAA) individually.

Acclimatization and field establishment

The objective of this experiment was to adapt the plantlets obtained *in vitro* to the free living conditions. Plantlets were washed with a current tap- water, then soaked in a fungicide solution (1gl^{-1} of rizolex) for 3-5 min. Then the plantlets were transferred into plastic pots (10 cm diam.) containing a soil mixture of sand and peat moss (1:1). The pots were covered with polyethylene bags to maintain a high relative humidity around the plantlets. The pots were placed in the greenhouse and after 10 days, small holes were poked in the plastic covers for air circulation and remained in the pots for another 10 days. Gradually, relative humidity was reduced and the covers were completely removed.

Measurements

The following determinations were carried out:
For sterilization experiment
- Percentage of survived explants.

For multiplication stage
- Number of axial shoot / plantlet, Axial shoot length (cm),Plantlet length (cm) and Number of leaves / plantlet

For rooting stage
- Rooting percentage, Number of roots/shoot and Root length (cm)

Statistical analysis

Data were statistically analyzed by using a randomized complete block design (RCBD) in factorial arrangement in four replicates according to Snedecore and Cochran, (1990) by using SPSS computer program V.10 (1999). For mean separations, least significant differences (L.S.D, Duncan, 1955) was used.

Results and Discussion

Establishment stage:

Seed sterilization and germination:

Data in Table (2) showed the effect of sodium hypochlorite solution on the survival percentage of *Cleome droserfolia* seeds .The highest survival percentage was 80% which was achieved by using 20%(v/v) for 30 min. However increasing concentration of sodium hypochlorite up to 25 % (v/v) and duration of soaking up to 15 min decreased the survival percentage by increasing death of the explants. Also decreasing sodium hypochlorite concentration or duration increased the contamination of seeds. These results were in agreement with Guanih *et al.,* (2004) on *Dryobalanops lanceolata* Burck. The auther showed that, seeds of Dryobalanops lanceolata Burck pretreated with 70% alcohol and sterilized with 30% commercial Clorox for 5 minutes and cultured in half-strength of Murashige Skoog (MS) without seed coat showed less than 20% contamination.

Table 2: Effect of surface sterilization with different concentration and durations of sodium hypochlorite (Clorox) solution on the survival percentage of *Cleome droserfolia* seeds.

Clorox con.(v/v) Duration(min)	10	15	20	25
15	0.00	20	30	20
20	20	30	40	0.00
25	30	60	70	0.00
30	30	60	80	0.00

Multiplication stage:

Effect of explant type and benzyle adenine(BA) concentrations:

Data in Table (3) show the effects of explant type and benzyle adenine (BA) on shoot multiplication of *Cleome droserfolia* after 6 weeks:

Number of axial Shoot number / plantlet

The data clear that the highest number of axial shoots / explant was belong to shoot explants with BA at 1.0 mgl-1, while lowest number of axial shoots was belongs to nodal explants without using BA. Similar results were obtained by Ali, *et al.*, (2004) they cultured that shoot tip of *Mentha arvensis* as explant in Murashige and Skoog's (MS) medium containing various concentrations of auxins and cytokinins. they found that Shoots were induced in the MS medium containing 1.0 mg BAP [benzyl adenine] /liter.

Axial shoot length (cm)

Data in Table (3) clear that the highest axial shoot length after 6 weeks was resulted from shoot tip explants with BA at 1.0 mgl^{-1}.On the other side the least axial shoot length was belong to nodal explants with BA at 1.5 mgl^{-1}.These results were in a harmony with Aggarwal and Barna(2004) they developed a protocol for *Aloe vera,* through enhanced axillary branching was standardized. Murashige and Skoog medium containing 1 mg l-1 BA and 0.2 mg l-1 IBA gave highest multiplication.

Plantlet length (cm)

The highest plant length (4.02cm) was belong to tip explants with BA at 1.0 mg^{-1}.In the second order came nodal explants with BA at 1.0 mgl^{-1} .On the other side the least plantlet length was resulted from nodal explants with BA at 1.5 mgl-1 (2.18 cm). Similar results were obtained by Abd Alhady (2011) indicated that the shoot-tips for *Solenostemma arghel* on MS medium. He found that high proliferation of shoots and shoot length was obtained on (MS) basal medium supplemented with a combination of 0.50 mgl^{-1}BA plus 0.50 mgl^{-1}NAA.

Number of leaves / plantlet

Data in Table (3) indicate that number of leaves / plantlet significantly increased by using shoot tip explants and the highest leaves number / plantlet after 6weeks was belong to BA at 1.0 mgl^{-1} ., On the other side the lowest leaves number (8.33) was belong to nodal explants with BA at 1.5 mgl^{-1}

Table 3: Effect of explant type and benzyle adenine(BA)concentrations on multiplication *Cleome droserfolia* .

Treatments		No. of axial shoot / plantlet	Axial shoot length(cm)	Plantlet length (cm)	No. of leaves / plantlet
Explant type	BA con. mgl^{-1}				
Shoot tip Explant	0.0(Control)	3.00a	2.33b	3.33bc	11.33bc
	0.5 mgl^{-1}	3.11a	2.29b	3.44bc	10.67bc
	1.0 mgl^{-1}	3.89a	3.24a	4.02a	14.22a
	1.5 mgl^{-1}	3.22a	2.12b	2.24d	10.15cd
Nodal explant	0.0(Control)	2.77a	2.10b	3.01c	10.22cd
	0.5 mgl^{-1}	2.89a	2.18b	3.11c	9.89cd
	1.0 mgl^{-1}	3.66a	3.09a	3.77ab	12.27b
	1.5 mgl^{-1}	3.17a	2.02b	2.18d	8.33d

Treatment means followed by different letters in their superscript are significantly different from each other ($p<0.05$); comparison by DMRT.

Effect of adenine sulphate concentrations

Data in Table (4) show the effects of adenine sulphate on shoot multiplication of *Cleome droserfolia* after 6 weeks. Data in Table (4) indicate that the highest number of axial shoots (3.22 shoot) was obtained from addition of adenine sulphateat 30 mgl^{-1} + BA 1 mgl^{-1}.

As for axial shoot length in Table (4) the highest axial shoot length was belong to adenine sulphateat 30 mgl^{-1} +BA1 mgl^{-1} (3.12cm). While the other concentrations of adenine sulphate with BA at 1 mgl^{-1} resulted in less significant axial shoot length.

As for both plantlet length and number of leaves / plantlet there is no significant increase in addition of adenine sulphate combined with BA at 1 mgl^{-1}.

The effect of adenine sulphat alone or combined with BA on shoot multiplication from shoot tip or nodal mgl^{-1} explant was investigated by El-Shamy (2002) on Bougainvillea plant, the author found that adenine sulphat alone did not induce shoot initiation for either two explants types, while adenine sulphat at 40 mgl^{-1} plus2.0 mgl^{-1} BA was effective in this regard, increasing adenine sulphate to 80 or 120 mgl^{-1} was less effective. Also Hassan (2011) on *Balanites aegyptiaca*,L.reported that, using adenine sulphate alone at 40 and 80 mgl^{-1} did not affect significantly shoot number in either two explant types comparing to control. However, the highest number of shoots was obtained from 1 mgl^{-1}kinetin, NAA 0.2 mgl^{-1}+ 40 mgl^{-1}adenine sulphate from shoot tip explant after six weeks and a similar constant trend was obtained after 12 weeks.

Table 4: Effect of adenine sulphate (Ads) concentrations on multiplication *Cleome droserfolia.*

Adenine sulphate (mgl^{-1}) (Ads)	No. of axial shoot / plantlet	Axial shoot length(cm)	Plantlet length (cm)	No. of leaves / plantlet
Zero (Ads)+ BA 1.0 mgl^{-1}	2.22a	2.18b	2.97a	8.22a
10 mgl-1(Ads) + BA 1.0 mgl^{-1}	2.45a	2.46ab	3.07a	8.45a
20 mgl-1(Ads) + BA 1.0 mgl^{-1}	2.88a	2.54ab	3.22a	8.71a
30 mgl-1(Ads) + BA 1.0 mgl^{-1}	3.22a	3.12a	3.48a	9.66a

Treatment means followed by different letters in their superscript are significantly different from each other ($p<0.05$); comparison by DMRT.

Rooting stage

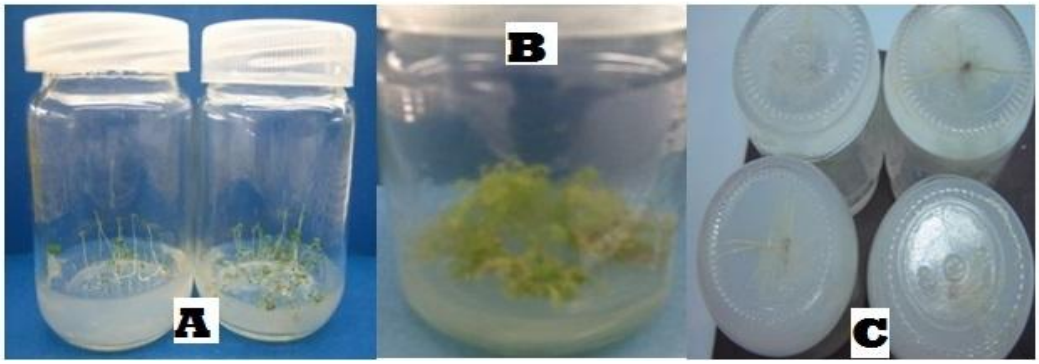
Effect of auxins on rooting of shoots

Data in Table (5) show the effect of half strength and full strength MS medium supplemented with all concentrations of auxins. Data showed that all concentrations of auxins induced roots from shoots within 6 weeks days of culture. Among the three auxins tested, the number of roots and root length varied in both medium. Full strength MS medium fortified with 1.5 mgl^{-1} IBA shows better rooting percentage when compared to half strength MS medium with 1.5 mgl^{-1}. Full strength MS medium significantly promoted lengthy roots and strengthened root induction. In half strength MS medium, IBA was found to be more effective for root induction than IAA and NAA. Full strength MS medium supplemented with IBA (1.5 mgl^{-1} IBA) was more effective for root induction than IAA and NAA. However, .IAA and NAA formed slender roots in both medium. IBA was more effective for root induction in both types of medium than IAA or NAA. Similar responses were observed

in different plant species (Sahoo and Chand 1998, Komalavalli and Rao 2000 and Sivakumar, and Krishnamurthy 2000).

Acclimitization stage

A diluted fungicide solution 1 gl-1 (rizolex) prior to transfer to the soil to avoid contamination. Cleome droserfolia was successfully acclimatized by using combination of peat moss peat moss and sand 1:1 (v/v). Similar responses were observed in *Thymus capitatus* was successfully acclimatized by using combination of peat moss peat moss and sand 1:1 (v/v). Nishawy (2008).



A: *Cleome droserfolia* germination, **B:** Shoot tip explants with BA at 1.0 mg^{-1} , **C:** Full MS strength medium + IBA (1.5 mgl-1 IBA)

Table 5: Effect of MS strength and different auxins concentrations on rooting of *Cleome droserfolia*.

Growth regulators (mgl-1)	Rooting percentage	Number of roots/shoot	Root length (cm)
Full strength MS + IAA			
0	55.6d	4.0de	2.0c
0.5	59.0bc	6.3d	2.6bc
1	61.5b	10.3bc	3.1a
1.5	66.4a	14.9a	2.8ab
2	53.3de	11.3b	2.6b
Full strength MS + IBA			
0	77.0de	13.6d	4.2e
0.5	89.4d	25.3bc	5.1c
1	86.0bc	27.4b	6.0a
1.5	93.3a	33.5a	5.7ab
2	88.5b	29.7ab	4.9cd
Full strength MS + NAA			
0	64.7de	9.4d	3.0de
0.5	75.0c	16.0bc	3.7b
1	81.6ab	20.0ab	4.2a
1.5	87.0a	23.3a	3.5bc
2	68.2d	19.3b	3.1d
Half strength MS + IAA			
0	47.6d	2.2d	1.7d
0.5	56.0b	2.9cd	2.1bc
1	57.0ab	4.6c	2.4b
1.5	59.0a	9.0a	2.7a
2	51.3c	7.8ab	2.0c
Half strength MS + IBA			
0	63.0cd	11.0e	3.6c
0.5	68.0c	15.8d	4.0b
1	72.2b	21.4ab	4.2ab
1.5	83.0a	22.0a	4.3a
2	69.0bc	19.9c	3.8bc
Half strength MS + NAA			
0	56.0d	7.7d	2.2de
0.5	59.0c	10.2bc	2.3d
1	64.4b	12.9ab	3.1bc
1.5	73.0a	13.2a	3.8a
2	57.7cd	11.0b	3.2b

Treatment means followed by different letters in their superscript are significantly different from each other ($p<0.05$); comparison by DMRT.

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