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Micropropagation for conservation of two rare *Capparis* species from Egypt

Ghada A. Hegazi[1], Saad R. Eid[1], Abd El Monem M. Sharaf[2]
[1]Tissue Culture Unit, Plant Genetic Resources Department, Desert Research Center, Cairo, Egypt
[2]Botany and Microbiology Department, Faculty of Science, Al-Azhar University, Cairo, Egypt

ABSTRACT

An efficient protocol for micropropagation was developed to conserve two rare Egyptian *Capparis* species; *Capparis orientalis* Duh. and *Capparis leucophylla* DC., using stem node sections and shoot tips. *In vitro* propagation of these two species has not been previously reported. Concerning *C. orientalis*; Murashige and Skoog (MS) medium containing 3 mg/L benzyl adenine (BA) with or without 0.2 mg/L 2-naphthalene acetic acid (NAA) were the most suitable media for the establishment of both stem node sections and shoot tips, and MS medium containing 3 mg/L BA was optimum for the multiplication of explants. Δ^2- isopentenyladenine (2iP) gave promising results in enhancing elongation of axillary shoots of *C. orientalis,* when added to MS medium at a concentration of 1 mg/L in addition to 3 mg/L BA. The highest rooting percentage of *C. orientalis* (60%) was obtained on MS medium supplemented with 1 mg/L of both indole-3-butyric acid (IBA) and NAA after 60 days of incubation. With respect to *C. leucophylla*; MS medium containing 1 or 3 mg/L BA and that containing 2.5 mg/L of both BA and 2iP were the best for the establishment of stem node sections and shoot tips. MS medium containing 1 or 3 mg/L BA were the most promising media for the proliferation of explants. Gibberellic acid (GA_3) at a concentration of 3 mg/L gave the best results in enhancing elongation of axillary shoots of *C. leucophylla* explants. The highest rooting percentage reached only 20% and was obtained on MS medium containing 2 mg/L IBA + 0.5 mg/L NAA after 60 days of incubation. An average of 92–98% of the acclimatized transplants of both *C. orientalis* and *C. leucophylla* survived after transferring into peatmoss:sand mixture (1:1 v/v) in the greenhouse conditions.

Key words: *Capparis orientalis*, *Capparis leucophylla*, *in vitro* propagation, stem node sections, shoot tips.

INTRODUCTION

The conservation of natural populations of plants is very important for maintaining biological diversity. It is necessary therefore to set priority for what should be conserved. The first priority is for rare and endangered species. *Capparis orientalis* Duh. and *C. leucophylla* DC. are two rare *Capparis* species from family Capparaceae. *Capparis* species are parts of the Egyptian plant diversity and are economically important with medicinal values. They are difficult in their normal propagation as they are poor in seed germination (Ben Salem *et al*., 2001; Yilidirim and Bayram, 2001; Soyler and Khawar, 2006 and 2007), and gave low rooting percentages in the propagation by cuttings (Ben Salem *et al*., 2001; Bhargava *et al*., 2006).

Caper (from the Arabic name Kaper) is the common name of the genus *Capparis*. Members of *Capparis* are shrubs containing glucocapparin (glucosinolates) which release isothiocyanate (mustard oils) when the plants are damaged (Chen and Andreasson, 2001). Isothiocyanates and glucosinolates compounds act as antitumor active chemoprotective agents against carcinogens by blocking the initiation of tumors in liver, colon, mammary gland, and pancreas (Krumbein *et al*., 2002; Keck and Finley, 2004). Glucosinolates and their breakdown products possess also chemoecological functions and serve not only as defense mechanisms against herbivores and pathogens, but also as attractant to specialized toxin tolerant insects (Mohn *et al*., 2007). They have long been known for their fungicidal, bacteriocidal, nematocidal and allelopathic properties (Fahey *et al*., 2001). Capers contain also considerable amounts of the anti-oxidant bioflavonoid rutin. In folk medicine caper plants are recorded as hepatic stimulants and protectors, improving liver function. They are used to treat arteriosclerosis and kidney disinfectants, and used as diuretics, vermifuges and tonic. Also, caper used to reduce flatulence and to be anti-rheumatic in effect. Infusions and decoctions from caper root bark have been traditionally used for dropsy, anemia, arthritis and gout (Ahmed *et al*., 1972). Fruits and young shoots with small leaves may also be pickled for use as a condiment (Alkire, 2001). They have a piquant flavour and add pungency, a peculiar aroma and saltiness to comestibles such as pasta sauces, pizza, fish, meats and salads. They are very decorative plants when flowering and would be suitable for gardens as ornamentals.

Efficient micropropagation has been reported earlier in many *Capparis* species such as *Capparis spinosa* L. (Ancora and Cuozzo, 1985; Rodriguez *et al*., 1990; Ben Salem *et al*., 2001; Yilidirim and Bayram, 2001; Chalak *et al*., 2003; Caglar *et al*., 2005; Chalak and Elbitar, 2006; Horshati and Jambor-Benczur, 2006; Xiaoxia *et al*, 2009). Also, the micropropagation of *Capparis cartilaginea* Decne (Hassanein *et al*., 2008) and *Capparis decidua* (Frosk.) Edgew. (Deora and Shekhawat, 1995; Tyagi and Kothari, 1997; Saxena *et al*., 2007; Tyagi *et al*., 2010) has been reported.

The objective of the present study was to conserve two rare Mediterranean species of *Capparis* (*C. orientalis* Duh. and *C. leucophylla* DC.) through *in vitro* propagation using stem node sections and shoot tips. The complete micropropagation of these two species is presented here for the first time, building on results obtained on the other *Capparis* species.

* *Corresponding Author: hegazighada@yahoo.com*

MATERIALS AND METHODS

The present study was conducted during 2009-2011, and the experiments were carried out in Plant Tissue Culture Unit, Plant Genetic Resources Department, Desert Research Center, Maryout Research Station, Alexandria, Egypt.

Explant collection and preparation

Explants of *C. orientalis* were obtained from Agyba's beach at Marsa Matrouh (Fig. 1a), while explants of *C. leucophylla* were collected from Marsa Matrouh-Siwa road (Fig. 2a). Actively growing shoots with terminal buds were excised, moistened and wrapped. Explants were washed under running tap water for 2-3 hours.

Explant sterilization

Surface sterilization was carried out under complete aseptic conditions in the Laminar Air Flow Hood. The stem node sections and shoot tips of *C. orientalis* and *C. leucophylla* were subjected to different sterilization treatments using commercial Clorox containing 5.25% sodium hypochlorite (NaOCl) for different durations. Following sterilization, the explants were rinsed 5-6 times in sterile distilled water. Stem node sections of both plants needed higher concentrations and durations of NaOCl application (1.5% for 12 min and 1% for 15 min, respectively) to give 100% of survived explants comparing to shoot tips which needed only 0.5% NaOCl application for short durations (7 and 10 min, respectively) to give 100% survival of explants.

The basic nutrient medium and culture conditions

Stem node sections (1.5-2 cm long) and shoot tips (0.5-1 cm long) of both plants, were dissected out of the cuttings and planted vertically on solid basal medium of Murashige and Skoog (MS) (Murashige and Skoog, 1962). The MS medium was supplemented with 30 g/L sucrose. Growth regulators such as benzyl adenine (BA), Δ^2- isopentenyladenine (2iP), gibberellic acid (GA_3), 2-naphthalene acetic acid (NAA), indole-3-butyric acid (IBA) and indole-3-acetic acid (IAA) were added to the medium independently or in combination, at different concentrations. Media were adjusted to pH 5.7- 5.8 before gelling with 2.7 g/L phytagel. Fifteen ml volumes of media were dispensed into 25×150 mm culture tubes or 30 ml volumes into large jars. Then closed with autoclavable polypropylene caps and autoclaved at 121°C under a pressured of 1.1 Kg/cm² for 20 min, then left to cool. The sterilized explants were cultured on the prepared media under complete aseptic conditions in the Laminar Air Flow Hood. Tissue culture tubes and jars were then placed in an air conditioned incubation room at a temperature of 26±2°C under a photoperiod of 16 hour with a light intensity of 2 Klux, provided by cool white fluorescent tubes.

Establishment stage

Explants were subjected to establishment medium supplemented with different concentrations of BA (1, 2, 3, 5 mg/L) individually or in combination with NAA (0.1, 0.2 mg/L) for *C. orientalis*, BA (0.5, 1, 1.5, 2.5, 3, 5 mg/L), 2iP (0.5 mg/L) and NAA (0.1 mg/L), with different combinations, for *C. leucophylla*, in addition to the control medium (MS nutrient medium without plant growth regulators). Percentage of survived explants (%), percentage of explants forming axillary shoots (%), mean number of axillary shoots/explant and mean length of axillary shoots (cm) were recorded after 6 weeks of culture.

Multiplication stage

Established shoots were subjected to be multiplied on MS medium containing BA (3 mg/L) individually or in combination with NAA (0.2 mg/L) for *C. orientalis*, and different concentrations of BA (0.5, 1, 2.5, 3 mg/L) and 2iP (2.5 mg/L) for *C. leucophylla*.

For further multiplication, the explants were subcultured five times on the same medium using large jars to obtain stock materials to be used in the following stages. Mean number and length (cm) of axillary shoots/explant were recorded after 6 weeks of each subculture.

Elongation stage

To enhance the elongation of the *in vitro* proliferated shoots of both studied plants, an experiment was carried out using MS medium supplemented with different concentrations of GA_3 (1, 2, 3 mg/L) (sterilized by filtration), and/or 2iP (0.5, 1 mg/L) in addition to BA (1, 3 mg/L). Mean length of axillary shoots (cm) were taken after 8 weeks of incubation.

Rooting stage

Elongated shoots of both plant species were tested for rooting on root induction media of full, half and quarter strengths of MS salts and vitamins containing 30 g/L sucrose, 2.7 g/L phytagel and different treatments of auxins; IBA (0.5, 1, 2 mg/L, NAA (0.5, 1, 2 mg/L) or both, in addition to the control medium (without plant growth regulators). Rooting percentage (%), mean number of roots/explant, mean length of roots (cm) and shoot height (cm) were taken after 8 weeks of culture.

Acclimatization stage

Rooted shoots (4-7 cm long) were washed from medium residues and treated with 0.2% topsin (w/v) solution as a fungicide, then hardened off inside the growth room in soilrite. After two weeks, they were transplanted into 0.2 L capacity pots filled with a soil mixture of sand and peat moss (1:1 v/v). Pots were covered with transparent polyethylene bags and placed in a greenhouse. One week later, the covers removed gradually within one month. The percentage of survived plantlets (%) was recorded.

Analysis of data

The experiments were subjected to the completely randomized design. Variance analysis of data was carried out using ANOVA program for statistical analysis. The differences among means for all treatments were tested for significance at 5% level by using Duncan´s multiple range test. Means followed by the same letter are not significantly different at $P \leq 0.05$.

RESULTS

Establishment and multiplication stages

C. orientalis stem node sections:

The *in vitro* establishment of *C. orientalis* stem node sections is presented in Table 1. Data revealed that the percentage of survived explants was high and insignificantly different in all tested media. With respect to the percentage of growth induction, it was maximum on three media; MS medium containing 3 mg/L BA, 0.2 mg/L NAA + 2 or 3 mg/L BA. Decreasing the concentration of BA in the presence of 0.2 mg/L NAA decreased the percentage of growth induction, mean number and length of axillary shoots. The highest values of mean number and length of axillary shoots reached 2.47 and 1.25 cm, respectively on MS medium supplemented with 3 mg/L BA. The concentration of 5 mg/L with or without NAA, and the media containing 1 mg/L BA with or without 0.1 mg/L NAA, in addition to the control medium without plant growth regulators, caused the complete inhibition of growth of the stem node sections of *C. orientalis*. It could be concluded that MS medium containing 3 mg/L BA in the presence or absence of 0.2 mg/L NAA were the most suitable media for the establishment of *C. orientalis* stem node sections (Fig. 1b).

Table (1): *In vitro* establishment of stem node sections of *C. orientalis* cultured on MS medium supplemented with NAA and BA. Results were taken after 6 weeks of culture.

Growth regulator conc. (mg/L)		% of survived explants	% of growth induction	Mean no. of axillary shoots/ explant	Mean length of axillary shoots (cm)
NAA	BA				
0.0	0	100^{a}	0.0	0.00	0.00
0.0	1	85.0^{a}	0.0	0.00	0.00
0.0	3	100^{a}	75^{a}	2.47^{a}	1.25^{a}
0.0	5	80.0^{a}	0.0	0.00	0.00
0.1	1	80.0^{a}	0.0	0.00	0.00
0.1	3	85.0^{a}	30^{b}	1.84^{b}	0.79^{c}
0.1	5	90.0^{a}	0.0	0.00	0.00
0.2	1	90.0^{a}	25^{b}	1.44^{d}	0.50^{d}
0.2	2	100^{a}	65^{a}	1.69^{c}	0.89^{b}
0.2	3	95.0^{a}	75^{a}	2.28^{a}	1.10^{a}

C. orientalis shoot tips

For the establishment of shoot tip explants of *C. orientalis* (Table 2), the maximum survival percentage reached 100% in the presence of 0.2 mg/L NAA in the medium, in addition to the plant growth regulators free medium.

The growth induction percentage reached the highest value (100%) by using 3 mg/L BA + 0.2 mg/L NAA, and decreased significantly with the decrease of BA concentration in the presence of 0.2 mg/L NAA. Reducing NAA concentration or its elimination, significantly reduced the percentage of growth induction

Table (2): *In vitro* establishment of shoot tips of *C. orientalis* cultured on MS medium supplemented with NAA and BA. Results were taken after 6 weeks of culture.

Growth regulator conc. (mg/L)		% of survived explants	% of growth induction	Mean no. of axillary shoots/ explant	Mean length of axillary shoots (cm)
NAA	BA				
0.0	0	100^{a}	00.0	0.00	0.00
0.0	1	20.0^{c}	20.0^{d}	0.15^{e}	0.12^{e}
0.0	3	90.0^{a}	75.0^{ab}	2.28^{b}	0.94^{b}
0.0	5	40.0^{bc}	00.0	0.00	0.00
0.1	1	70.0^{ab}	00.0	0.00	0.00
0.1	3	80.0^{a}	30.0^{cd}	1.33^{cd}	0.77^{cd}
0.1	5	80.0^{a}	00.0	0.00	0.00
0.2	1	100^{a}	60.0^{bc}	1.67^{c}	0.83^{c}
0.2	2	100^{a}	70.0^{ab}	1.11^{d}	0.80^{c}
0.2	3	100^{a}	100^{a}	2.78^{a}	1.10^{a}

with all tested BA concentrations, excluding the medium containing 3 mg/L BA. The mean number and length of axillary shoots reached the highest values of 2.78 and 1.1 cm, respectively, on MS medium supplemented with 3 mg/L BA + 0.2 mg/L NAA (Fig. 1c). Followed by the medium containing 3 mg/L BA. It is noticed that the mean number and length of axillary shoots increased with the increase of BA concentration for each NAA concentration, except the highest concentration of BA (5 mg/L) which gave a negative response either with or without NAA, in addition to the medium containing 1 mg/L BA + 0.1 mg/L NAA, and the plant growth regulators free medium.

It can be concluded from Table 1 and 2 that MS medium containing 3 mg/L BA with or without 0.2 mg/L NAA were the most suitable media for the establishment of both stem node sections and shoot tips of *C. orientalis*. Comparing MS medium containing 3 mg/L BA with that containing 3 mg/L BA + 0.2 mg/L NAA for further multiplication of *C. orientalis* shoots, through five successive subcultures; both media gave 100% survival percentage during all successive subcultures except the first and the fourth subcultures which gave 90% (Table 3).

It is noticed that the medium containing BA alone gave significantly higher mean number of short axillary shoots per explant, in contrast to the mean length of axillary shoots which was significantly higher in the medium containing NAA. It is also observed that on the medium containing 3 mg/L BA, the mean number of axillary shoots/explant increased with each subculture until

Table (3): Effect of MS medium supplemented with 3 mg/L BA with or without NAA on the multiplication of *C. orientalis* axillary shoots during 5 successive subcultures.

Growth regulator conc. (mg/L)		1st subculture			2nd subculture			3rd subculture			4th subculture			5th subculture		
BA	NAA	%EFS	MNS	MLS	%EFS	MNS	MLS	%EFS	MNS	MLS	%EFS	MNS	MLS	%EFS	MNS	MLS
3	0.0	90^{a}	7.7^{a}	0.41^{b}	100^{a}	8.9^{a}	0.45^{b}	100^{a}	9.5^{a}	0.44^{b}	90^{a}	7.7^{a}	0.41^{b}	100^{a}	8.9^{a}	0.45^{b}
3	0.2	90^{a}	4.0^{b}	1.14^{a}	100^{a}	4.1^{b}	1.25^{a}	100^{a}	4.3^{b}	1.15^{a}	90^{a}	4.0^{b}	1.14^{a}	100^{a}	4.1^{b}	1.25^{a}

*** %EFS**: Percentage of explants forming shoots. **MNS**: Mean number of axillary shoots/explant. **MLS**: Mean length of axillary shoots (cm).

reaching the third one (it reached the maximum value of 9.5) then decreased in further subcultures. While on the medium containing NAA, the number of axillary shoots ranged between 4 and 4.3. It could be concluded that MS medium with 3 mg/L BA was the more responsible for the multiplication of *C. orientalis* plant (Fig. 1d).

***C. leucophylla* stem node sections**

Table 4 shows the establishment of *C. leucophylla* stem node sections. The percentage of survived explants was high and insignificantly different in all tested media. With respect to the percentage of growth induction it was maximum on MS medium containing 3 mg/L BA, 2.5 mg/L BA + 0.5 mg/L 2iP and 2.5 mg/L BA + 2.5 mg/L 2iP. Decreasing the concentration of BA decreased the percentage of growth induction on the media containing BA individually or in combination with each 2iP concentration.

The optimum growth regulators combinations with respect to the mean number of axillary shoots per explant were 1 or 3 mg/L BA and 2.5 mg/L BA + 2.5 mg/L 2iP.

Decreasing BA concentration to 1.5 and 0.5 mg/L with 0.5 or 2.5 mg/L 2iP decreased the mean number of axillary shoots per explant. Concerning the mean length of axillary shoots, it was clear that MS medium containing 2.5 mg/L BA + 2.5 mg/L 2iP and 1 mg/L BA + 0.1 mg/L NAA were the optimum (1.64 and 1.60 cm, respectively), and both are insignificantly different. The concentration of 5 mg/L BA with or without NAA caused the complete inhibition of growth in the stem node sections of *C. leucophylla*, in addition to the MS medium free from plant growth regulators. It could be concluded that MS medium containing 1 and 3 mg/L BA and 2.5 mg/L BA + 2.5 mg/L 2iP were the most suitable media for the establishment of *C. leucophylla* stem node sections (Fig. 2b).

Table (4): *In vitro* establishment of stem node sections of *C. leucophylla* cultured on MS medium supplemented with NAA, BA and 2iP. Results were taken after 6 weeks of culture.

Growth regulator conc. (mg/L)			% of survived explants	% of growth induction	MNS	MLS
NAA	BA	2iP				
0.0	0.0	0.0	100^{a}	0.0	0.00	0.00
0.0	1.0	0.0	80.0^{a}	60^{ab}	2.67^{ab}	0.85^{bc}
0.0	3.0	0.0	100^{a}	70^{a}	3.00^{a}	0.96^{b}
0.0	5.0	0.0	80.0^{a}	0.0	0.00	0.00
0.1	1.0	0.0	100^{a}	20^{bcd}	2.10^{bc}	1.60^{a}
0.1	3.0	0.0	80.0^{a}	20^{bcd}	0.90^{d}	0.64^{cd}
0.1	5.0	0.0	80.0^{a}	0.0	0.00	0.00
0.0	0.5	0.5	100^{a}	50^{abc}	1.80^{c}	0.58^{d}
0.0	1.5	0.5	100^{a}	50^{abc}	2.20^{abc}	0.64^{cd}
0.0	2.5	0.5	80.0^{a}	70^{a}	1.94^{bc}	1.49^{ab}
0.0	0.5	2.5	100^{a}	10^{cd}	1.00^{d}	0.30^{d}
0.0	1.5	2.5	100^{a}	50^{abc}	2.20^{abc}	0.92^{b}
0.0	2.5	2.5	100^{a}	70^{a}	2.57^{ab}	1.64^{a}

***MNS**: Mean number of axillary shoots/explant. **MLS**: Mean length of axillary shoots (cm).

***C. leucophylla* shoot tips**

For the establishment of shoot tips of *C. leucophylla* (Table 5), the survival percentage of explants reached 100% on most of the tested media, it significantly reduced on the medium containing 5 mg/L BA with or without NAA and that containing 0.1 mg/L NAA + 1 mg/L BA. The growth induction percentage reached the highest value of 80% by using 2.5 mg/L BA + 2.5 mg/L 2iP, and then decreased with the decrease of BA concentration with both 2iP concentrations used.

The mean number of axillary shoots formed on the shoot tips reached the highest value of 3.13 on MS medium containing 2.5 mg/L BA + 2.5 mg/L 2iP, followed by the concentrations of 3 mg/L BA, 3 mg/L BA + 0.1 mg/L NAA and 0.5 or 2.5 mg/L BA + 0.5

Table (5): *In vitro* establishment of shoot tips of *C.leucophylla* cultured on MS medium supplemented with NAA, 2iP and BA. Results were taken after 6 weeks of culture.

Growth regulator conc. (mg/L)			% of survived explants	% of growth induction	MNS	MLS
NAA	BA	2iP				
0.0	0.0	0.0	100^{a}	0.0	0.00	0.00
0.0	1.0	0.0	100^{a}	70^{ab}	1.28^{c}	1.70^{a}
0.0	3.0	0.0	100^{a}	60^{abc}	2.89^{b}	0.73^{bc}
0.0	5.0	0.0	60.0^{b}	0.0	0.00	0.00
0.1	1.0	0.0	60.0^{b}	20^{cd}	1.80^{c}	0.63^{bc}
0.1	3.0	0.0	100^{a}	50^{abc}	2.70^{b}	0.55^{c}
0.1	5.0	0.0	60.0^{b}	0.0	0.00	0.00
0.0	0.5	0.5	90.0^{ab}	30^{bcd}	2.76^{b}	0.91^{b}
0.0	1.5	0.5	90.0^{ab}	40^{abcd}	1.35^{cd}	0.36^{d}
0.0	2.5	0.5	100^{a}	70^{ab}	2.71^{b}	0.31^{d}
0.0	0.5	2.5	100^{a}	40^{abcd}	1.25^{d}	0.27^{d}
0.0	1.5	2.5	100^{a}	70^{ab}	2.43^{bc}	0.94^{b}
0.0	2.5	2.5	100^{a}	80^{a}	3.13^{a}	0.84^{b}

***MNS**: Mean number of axillary shoots/explant. **MLS**: Mean length of axillary shoots (cm).

mg/L 2iP, which are all insignificantly different. Mean length of axillary shoots was superior on MS medium supplemented with 1 mg/L BA; it reached 1.7 cm. Followed by those cultured on 0.5 mg/L BA + 0.5 mg/L 2iP and 1.5 or 2.5 mg/L BA + 2.5 mg/L 2iP, which all are insignificantly different. As recorded for the stem node sections of *C. leucophylla*, it is also noticed that shoot tips did not give any response on the growth regulators free MS medium and the media containing a high concentration of BA (5 mg/L). It can be concluded that MS medium supplemented with 2.5 mg/L of both BA and 2iP was the best medium for the establishment of shoot tips of *C. leucophylla* (Fig. 2c).

In conclusion, MS medium containing 1 or 3 mg/L BA and that containing 2.5 mg/L of both BA and 2iP were the most suitable for the establishment of both *C. leucophylla* shoot tips and stem node sections.

For the multiplication of *C. leucophylla* axillary shoots, Table 6 shows that an increase in the mean number of axillary shoots/explant was observed on MS medium supplemented with 1 mg/L BA, during the successive subcultures, until reaching the fourth subculture (5.3) then began to decrease again in the fifth one. While, the mean length of axillary shoots on the same medium was stable. However, the MS medium containing 3 mg/L BA gave more or less the same mean number of axillary shoots/explant (4.3) during the successive subcultures, except the fourth subculture in which an increase in the mean number of axillary shoots was observed (5.1). The MS medium containing 3 mg/L BA was significantly better than that containing 1 mg/L BA regarding to the mean number and length of axillary shoots in the first subculture and they became insignificantly different in the rest of subcultures.

The combinations of 2iP and BA gave the highest mean number of axillary shoots in the first subculture and decreased during the successive subcultures then ceased completely in the fourth one. MS medium containing 0.5 mg/L BA and 2.5 mg/L 2iP gave the highest response regarding to the mean number and length of axillary shoots, but this response decreased after the second subculture.

It can be concluded from Table 6 that MS medium containing 1 mg/L BA or 3 mg/L BA were the most promising treatments for the continuous proliferation of *C. leucophylla* explants during successive subcultures (Fig. 2d).

Elongation stage

Capparis orientalis

MS medium containing 1 mg/L 2iP + 3 mg/L BA (Table 7, Fig. 1e) gave significantly the highest mean length of axillary shoots of *Capparis orientalis* (3.75 cm), comparing to the other tested media. MS medium containing 3 mg/L GA_3 gave 2.5 cm mean length of axillary shoots, which was superior if compared with the other tested GA_3 concentrations. The combination of GA_3 and 2iP gave the minimum response that makes this treatment not recommended for the elongation of *C. orientalis* axillary shoots.

Table (7): Elongation of *in vitro* proliferated shoots of *C. orientalis* cultured for 6 weeks on MS medium supplemented with different concentrations of GA_3 and/or 2iP in addition to BA.

Growth regulator conc. (mg/L)			Mean length of axillary shoots (cm)
GA_3	BA	2iP	
1	0	0.0	1.30^{d}
2	0	0.0	1.70^{cd}
3	0	0.0	2.50^{b}
1	0	0.5	1.80^{cd}
2	0	0.5	2.00^{bc}
3	0	0.5	2.25^{bc}
0	3	0.5	2.00^{bc}
0	3	1.0	3.75^{a}

Capparis leucophylla

Table 8 represents the influence of different concentrations of GA_3 and/or 2iP on the elongation of *in vitro* proliferated shoots of *C. leucophylla*. The highest mean length of axillary shoots was obtained on MS medium containing 3 mg/L GA_3 (Fig. 2e), it reached 3.1 cm. Followed by MS media containing 3 mg/L GA_3 + 0.5 mg/L 2iP and 1 mg/L BA + 0.5 mg/L 2iP, which are both insignificantly different. It can be noticed that increasing the concentration of GA_3 with or without 0.5 mg/L 2iP increased the axillary shoots elongation.

From the elongation experiments on both studies plants; *C. orientalis* and *C. leucophylla*, it is clear that they are different in their response to the growth regulators responsible for the elongation of axillary shoots, as 2iP was superior in case of *C. orientalis* and GA_3 gave the best elongation in case of *C. leucophylla*

Table (6): Effect of BA and 2iP on the multiplication of *C. leucophylla* axillary shoots during 5 successive subcultures. All tested media gave 100% of growth induction.

Cytokinin conc. (mg/L)		1st Subculture		2nd Subculture		3rd Subculture		4th Subculture		5th Subculture	
BA	2iP	MNS	MLS	MNS	MLS	MNS	MLS	MNS	MLS	MNS	MLS
1.0	0.0	3.1^{b}	0.53^{b}	3.7^{ab}	0.53^{a}	4.7^{a}	0.58^{a}	5.3^{a}	0.68^{a}	4.1^{a}	0.53^{a}
3.0	0.0	4.3^{a}	0.63^{ab}	4.3^{ab}	0.60^{a}	4.3^{a}	0.54^{a}	5.1^{a}	0.60^{a}	4.0^{a}	0.58^{a}
0.5	2.5	4.9^{a}	0.73^{a}	4.5^{a}	0.58^{a}	1.8^{b}	0.53^{a}				
2.5	2.5	4.5^{a}	0.65^{ab}	3.4^{b}	0.46^{ab}	1.6^{b}	0.51^{a}				

***MNS**: Mean number of axillary shoots/ explant. **MLS**: Mean length of axillary shoots (cm).

Rooting and acclimatization stages

Capparis orientalis

Data in Table 9 reveal that the highest rooting percentage of *C. orientalis* (60%) was obtained on MS medium supplemented with 1 mg/L IBA + 1 mg/L NAA after 60 days of incubation (Fig. 1f). This treatment also gave the significantly highest mean root number (6) and length (1.42 cm), in addition to the maximum shoot height of 5.67 cm. Forty percent of rooting was achieved when explants were cultured on MS medium supplemented with 1 mg/L IBA + 0.5 mg/L NAA or 0.5 mg/L IBA + 1 mg/L NAA. Then, 30% of rooting was obtained when explants treated with 2 mg/L of either IBA or NAA. Since, the high concentrations of IBA proportionally encouraged tissue lignifications, leading to considerable decrease in rooting ability.

Capparis leucophylla

Data in Table 10 show the poor rooting response of *C. leucophylla* axillary shoots. The highest rooting percentage reached only 20% and was obtained on MS medium containing 2 mg/L IBA + 0.5 mg/L NAA after 60 days of incubation (Fig. 2f). The mean number and length of roots and shoot height were also superior on this medium.

For both studied *Capparis* species, the combination of IBA and NAA gave the highest rooting percentages than IBA or NAA alone. Also, the use of half or quarter strengths of MS medium show negative response with all tested auxins concentrations and combinations, in both studied plants, and the control MS medium free from plant growth regulators did not show any rooting response.

Rooted plantlets of *C. orientalis* and *C. leucophylla* survived when were hardened off inside the growth room in soilrite for two weeks. An average of 92–98% of the acclimatized plantlets survived after two weeks of transferring into the peatmoss: sand mixture (1:1 v/v). All the *in vitro* derived transplants displayed normal development as that of the mother plants (Fig. 1g and 2g).

DISCUSSION

The results of the establishment of both stem node sections and shoot tips of *C. orientalis* are in agreement with those obtained in *Brassica napus* by Hong *et al***.** (2008), where the highest differentiation rates were observed in the medium containing 3 mg/L BA + 0.2 mg/L NAA. A lot of references described the joint effect of a cytokinin such as BA and an auxin such as NAA**.** Vyas *et al*. (2005) reported that the effect of NAA combined with BA evoked a significant response in terms of number of shoot buds of *Feronia limonia* (Family Rutaceae). Also, in *Capparis cartilaginea*, MS medium containing 3 mg/L BA + 0.1 mg/L NAA was the most suitable medium for establishment and multiplication of shoot tip explants (Hassanein *et al*., 2008). Keng *et al*. (2009) found that no induction of multiple shoot formation of *Gynura procumbens* (Lour.) Merr. was observed on control MS medium, as shown in the present study. Also, the inhibition of growth observed on the media containing 5 mg/L BA has been found in other plants; Karonda and peach showed vitrification at higher BA concentrations (Chiariotti and Antonelli**,** 1988)

Table (8): Elongation of *in vitro* proliferated shoots of *C. leucophylla* cultured for 6 weeks on MS medium supplemented with different concentrations of GA_3, BA and 2iP (0.5 mg/L).

Growth regulator conc. (mg/L)			Mean length of axillary shoots (cm)
GA_3	BA	2iP	
1	0	0.0	1.6^{ef}
2	0	0.0	1.9^{c}
3	0	0.0	3.1^{a}
1	0	0.5	1.5^{f}
2	0	0.5	1.7^{de}
3	0	0.5	2.1^{b}
0	1	0.5	2.2^{b}
0	3	0.5	1.8^{cd}

Table (9): Effect of solid MS medium containing 30 g/L sucrose and different treatments of IBA and/or NAA, on the rooting of *C. orientalis* axillary shoots.

Auxin conc. (mg/L)		Rooting %	Mean no. of roots/ explant	Mean length of roots (cm)	Mean shoot height (cm)
IBA	NAA				
0.0	0.0	0.0	0.00	0.00	0.00
0.5	0.0	20^{b}	2.50^{b}	1.13^{b}	4.00^{ab}
1.0	0.0	10^{b}	4.00^{b}	1.12^{b}	3.50^{b}
2.0	0.0	30^{ab}	4.67^{b}	1.21^{b}	4.33^{ab}
0.0	0.5	20^{b}	3.00^{b}	0.83^{b}	3.50^{b}
0.0	1.0	20^{b}	4.00^{b}	0.87^{b}	3.25^{b}
0.0	2.0	30^{ab}	4.33^{b}	0.96^{b}	3.83^{b}
0.5	0.5	20^{b}	3.50^{b}	0.86^{b}	5.00^{a}
1.0	0.5	40^{ab}	4.25^{b}	0.85^{b}	4.75^{ab}
2.0	0.5	20^{b}	4.00^{b}	0.69^{b}	4.50^{ab}
0.5	1.0	40^{ab}	4.75^{ab}	1.18^{ab}	4.50^{ab}
1.0	1.0	60^{a}	6.00^{a}	1.42^{a}	5.67^{a}
2.0	1.0	20^{b}	3.50^{b}	0.71^{b}	4.00^{ab}

Table (10): Effect of solid MS medium containing 30 g/L sucrose and different treatments of IBA and/or NAA, on the rooting of *C. leucophylla* axillary shoots.

Auxin conc. (mg/L)		Rooting %	Mean no. of roots/ explant	Mean length of roots (cm)	Mean shoot height(cm)
IBA	NAA				
0.0	0.0	0.0	0.00	0.00	0.00
0.5	0.0	0.0	0.00	0.00	0.00
1.0	0.0	0.0	0.00	0.00	0.00
2.0	0.0	10^{b}	4.00^{b}	0.75^{a}	3.33^{b}
0.0	0.5	0.0	0.00	0.00	0.00
0.0	1.0	10^{b}	4.00^{b}	0.87^{a}	3.25^{b}
0.0	2.0	0.0	0.00	0.00	0.00
0.5	0.5	0.0	0.00	0.00	0.00
1.0	0.5	10^{b}	4.25^{b}	0.85^{a}	3.75^{a}
2.0	0.5	20^{a}	7.00^{a}	0.98^{a}	4.00^{a}
0.5	1.0	0.0	0.00	0.00	0.00
1.0	1.0	0.0	0.00	0.00	0.00
2.0	1.0	10^{b}	3.60^{b}	0.82^{a}	4.00^{a}

Moreover, shoot proliferation and growth decreased considerably with the increase of BA level in Guava (Loh and Rao, 1989) and Ber (Rathore *et al*, 1992). Also, Rai and Misra (2005) demonstrated that increase in BA level reduced the proliferation rate of shoots of *Carissa carandas* cv. Pant Sudarshan. In this respect, Keng *et al*. (2009) indicated that BA played an important role in induction of multiple shoot formation of *Gynura procumbens* (Lour.) Merr. and was very effective in shoot proliferation. However, BA at higher concentrations not only reduced the number of shoots formed but also resulted in stunted growth of the shoots. Lately, Thomas and Yoichiro (2010) found that high BA concentration did not improve shoot proliferation of the rare medicinal plant *Justicia gendarussa* Burm. f.

Similar results of the multiplication of *C. orientalis* have been reported in *Capparis decidua* by Tyagi *et al*. (2010) who found that shoots should be transferred to MS medium containing 3 mg/L BA for growth and proliferation. Also, these results are in harmony with those obtained by Tyagi and Kothari (1997) who found that continuous presence of higher levels of BA in the medium resulted in shoot length suppression in *Capparis decidua.* In this respect, Aasim *et al*. (2009) reported that the maximum mean number of Turkish cowpea (*Vigna unguiculata* L.) shoots were obtained on MS medium containing 1 mg/L BA and longer shoots were recorded on MS medium containing various concentration of BA + 0.1 mg/L NAA compared to those containing BA singly. Also, in *Gynura procumbens* (Lour.) Merr., the MS medium supplemented with 2 mg/L BA induced the highest number of shoots from the nodal segments. These shoots appeared to proliferate from the node *via* axillary branching of buds from the explants. The addition of a little NAA into the MS medium with the presence of BA had reduced tremendously the formation of multiple shoots (Keng *et al*., 2009).

The positive effect of 2iP as a cytokinin in the enhancement of the elongation of *in vitro* formed shoots is in agreement with the observations of Hassanein *et al* (2008) who found that 2iP gave promising results in enhancing elongation of axillary shoots of *Capparis cartilaginea* by using 0.5 or 1 mg/L.

The results of the establishment of both *C. leucophylla* shoot tips and stem node sections are agreed with Huang *et al*. (2000) who claimed that the optimum BA concentration for shoot proliferation of *Garcinia mangostana* (Family Clusiaceae) ranged from 1 to 3 mg/L. Kale (2005) stated that BA at 2.5 mg/L recorded the maximum number of *Capsicum annuum* shoots produced *in vitro*. This result is agreed with that obtained by Ghareb *et al*. (2007) who worked on *Ochradenus baccatus* from family Resedaceae, where stem node sections were superior in enhancing formation of axillary shoots on MS medium supplemented with both BA and 2iP.

According to earlier reports, the aptitude for proliferation of *Capparis* species was limited due to basal disorganization and vitrification of axillary shoots in the presence of BA (Rodrigues *et al*., 1990; Chalak *et al*., 2003; Chalak and Elbitar, 2006). The results of the multiplication of *C. leucophylla* are agreed with those obtained by Huang *et al* (2000) who mentioned that the optimum BA concentration for shoot proliferation of *Garcinia mangostana* ranged from 1 to 3 mg/L. Also, such result is in harmony with Rai and Misra (2005) who demonstrated that shoot proliferation of *Carissa carandas* was the highest on MS basal medium supplemented with 3 mg/L BA. Also, in *Coccinia indica* (Family Cucurbitaceae), MS medium supplemented with 1 mg/L BA induced shoot buds (Venkateshwarlu, 2008). It is well known that the cytokinin promotes cell division by activating DNA synthesis, promoting the growth of lateral buds and inducing shoot formation.

The positive effect of GA_3 on the elongation of *C. leucophylla* is in agreement with the results obtained by Figueiredo *et al*. (2001) who mentioned that GA_3 was necessary for the elongation of shoots of *Rollinia mucosa* (Jacq.) Baill. Also, Tian *et al*. (2010) found that GA_3 was beneficial for shoot and stem elongation of *Paeonia lactiflora* Pall.

For both studied *Capparis* species, the superiority of using a combination of IBA and NAA than one of them singly, is supported by Jusaitis (1997) who developed rooting for *Swainsona formosa* (G. Don) J., where roots initiated *in vitro* following a treatment with 0.1% IBA and 0.1% NAA. Rai and Misra (2005) found that shoots of *Carissa carandas* cv. rooted on ½ MS nutrient medium supplemented with a combination of NAA and IBA at 0.8 mg/L IBA and 0.2 mg/L NAA. The negative effect of the control media without auxins on the rooting of shoots of both studied plants is supported the important role of auxins in enhancing root initiation. In this respect, it is known that auxin's effect on inducing root formation depends in addition to many factors, upon the type of auxin and the concentration used. Synthetic auxins such as NAA and IBA are frequently used for *in vitro* root initiation and increase roots number and length. References are full of reports, some of which favour the use of IBA, others claimed the superiority of NAA, while a third group ascertained that both auxins has the same impact or had to be applied together. The same problem of tissue lignifications, leading to decrease rooting ability, encouraged when using high concentrations of IBA, for rooting, was also encountered in *Capparis spinosa* (Ben Salem *et al.,* 2001).

Further studies on the rooting of *Capparis leucophylla* should be carried out to enhance the rooting response of this valuable and rare plant species. This recommendation is supporting that of Hasssanein *et al*. (2008) who found that *in vitro* produced explants of *Capparis cartilaginea* did not initiate rooting in a high percentage using the tested treatments, and it needs more studies.

In conclusion, a successful and efficient micropropagation protocol has been developed and described here for the first time, and it will be very useful for the conservation and clonal propagation of the rare *C. orientalis* and *C. leucophylla* plants.

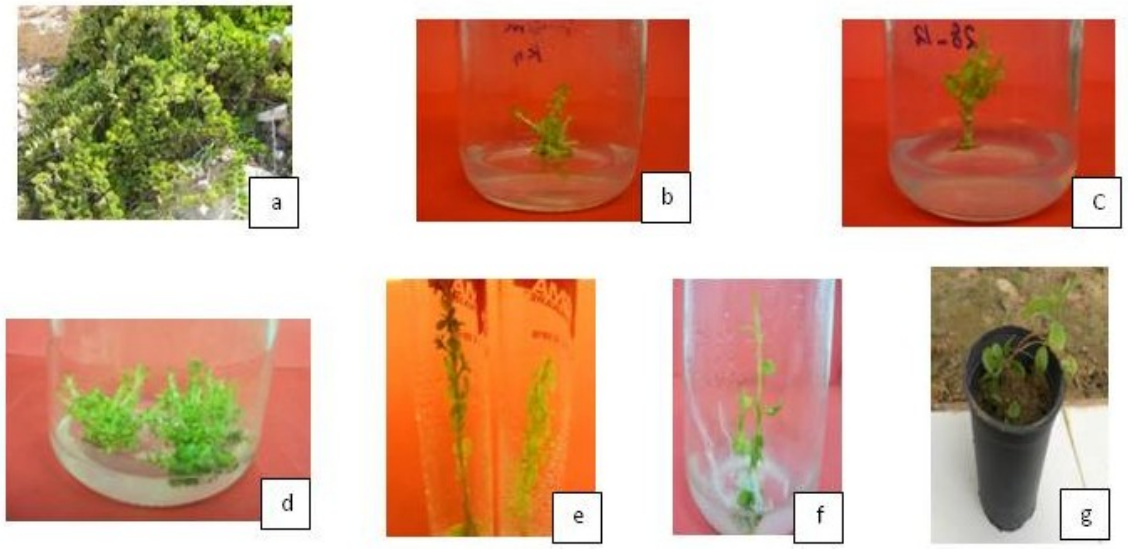

Fig. (1): Micropropagation of *Capparis orientalis*; **a** *C. orientalis* from Agyba's beach at Marsa Matrouh; **b** Establishment of stem node sections; **c** Establishment of shoot tips; **d** Proliferation of axillary shoots after 3 subcultures; **e** Elongation of axillary shoots; **f** Rooting of explants after 60 days of incubation; **g** Hardened in *vitro* derived plantlets.

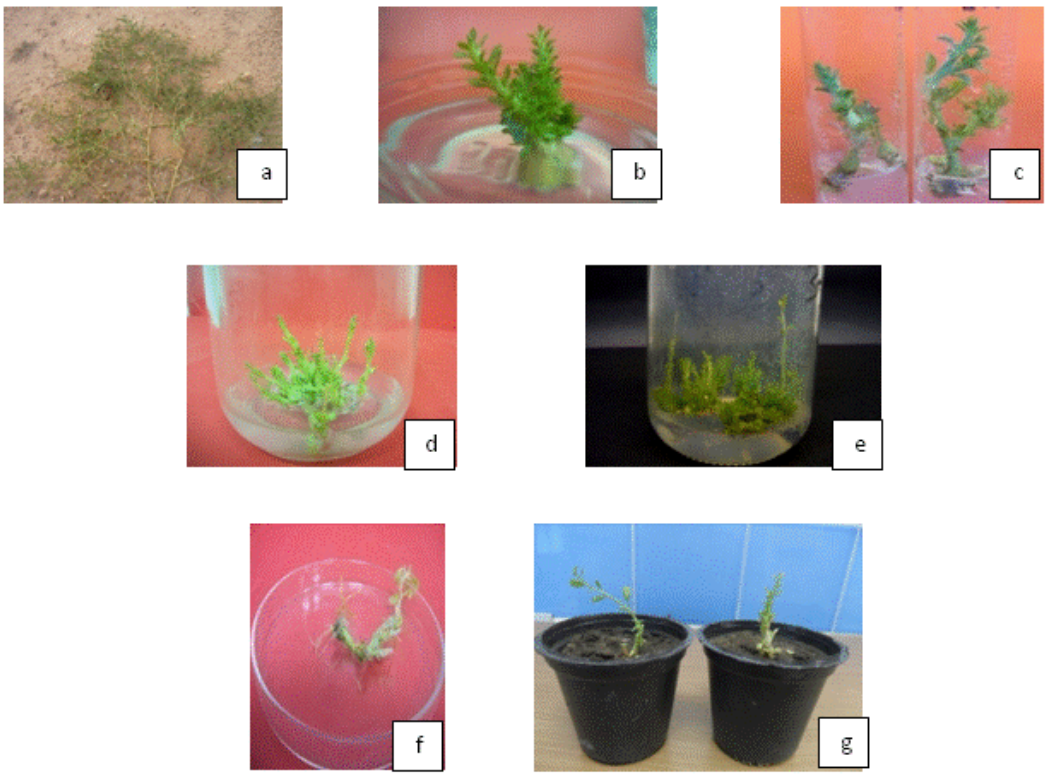

Fig. (2): Micropropagation of *Capparis leucophylla*; **a** *C. leucophylla* from Marsa Matrouh- Siwa road; **b** Establishment of stem node sections; **c** Establishment of shoot tips; **d** Proliferation of axillary shoots after 3 subcultures; **e** Elongation of axillary shoots ; **f** Rooting of explant after 60 days of incubation; **g** Hardened *in vitro* derived plantlets.

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الإكثار الدقيق للحفاظ على نوعين نادرين لنبات اللصف من مصر

غاده عبدالمنعم حجازي[1]، سعد رمزي عيد[1]، عبدالمنعم محمد شرف[2]
[1]وحدة زراعة الأنسجه، قسم الأصول الوراثيه النباتيه، مركز بحوث الصحراء، القاهره، مصر
[2]قسم النبات والميكروبيولوجي، كلية العلوم، جامعة الأزهر (فرع البنين)، القاهره، مصر

الملخص العربي

يهدف البحث إلى الحفاظ على نوعين من نبات اللصف (*Capparis*) الذي يتبع العائلة Capparaceae وهما *C. orientalis* و *C. leucophylla* من خلال الإكثار الدقيق، حيث أن هذين النوعين من النباتات الطبيعية النادرة والتي تنمو بالصحراء المصريه، وهما من النباتات ذات القيمة الإقتصادية العالية وبالأخص من الناحية الطبية والغذائية، ويصعب إكثارهما بالطرق التقليدية سواء بالعقلة أو بالبذرة. تم دراسة مدى استجابة النباتين وقدرتهما على النمو من خلال الزراعة المعملية بإستخدام القمم النامية والأجزاء الساقية البرعمية. بالنسبة لنبات *C. orientalis* في المرحلة البادئة والتضاعف أعطت بيئة موراشيجي وسكوج المضاف اليها ٣ مجم/لتر بنزيل أدينين (BA) و ٠,٢ مجم/لتر نفثالين حمض الخليك (NAA) وكذلك البيئة المضاف اليها ٣ مجم/لتر BA أفضل النتائج لكلاً من القمم النامية والأجزاء الساقية البرعمية. كما وجد أن إستخدام البيئة التي تحتوي على ٣ مجم/لتر BA في تضاعف الأفرع أدى الى أعلى زيادة في متوسط عدد النموات الجانبية للمنفصل النباتي (٩,٥) بعد النقلة الثالثة. بالنسبة إلي مرحلة الإستطالة وجد أن البيئة التى تحتوى على ١ مجم/لتر أيزوبنتينيل أدينين (2iP) بالإضافة إلى ٣ مجم/لتر BA كانت أفضل بيئة تحدث إستطالة للأفرع (٣,٧٥ سم). أما التجذير فقد وصل إلى أعلى نسبة وهي ٦٠٪ عند إضافة ١ مجم/لتر من IBA و NAA. أما بالنسبة لنبات *C. leucophylla*، في المرحلة البادئة و التضاعف أعطت بيئة موراشيجي وسكوج المضاف اليها ٣ مجم/لتر BA و ٢,٥ مجم /لتر من كلاً من BA و2iP أفضل النتائج للقمم النامية و الأجزاء الساقية البرعمية. وكذلك وجد أن البيئات التي تحتوي على ١ و ٣ مجم/لتر BA هي الأفضل فى مرحلة التضاعف خلال خمسة عمليات نقل متعاقبة. بينما أعطى حمض الجبريليك (GA_3) التأثير الأفضل لحدوث إستطالة فى أفرع نبات *C. leucophylla* حيث وصل متوسط طول الفرع إلى ٣,١ سم. كانت أفضل نسبة تجذيرلهذا النبات هي ٢٠٪ عند إستخدام بيئة موراشيجي وسكوج تحتوي على ٢ مجم/لتر IBA + ٠,٥ مجم/لتر NAA. تمت أقلمة النباتات الناتجة لكلا النباتين تدريجياً وتراوحت نسبة نجاح الأقلمة ما بين ٩٢-٩٨٪.