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Micropropagation of Caper (*Capparis spinosa* L.) from Wild Plants

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ABSTRACT

Caper bush is grown commercially to obtain unopened flower buds (capers) used as a condiment in salads and sauces and in the manufacture of cosmetics and medicines. Capers play an important role in the food industry and have become a costly and a commercially valuable product. The effect of various treatments on the behavior of *in vitro* consecutive micropropagation stages of *Capparis spinosa* was studied and a micropropagation protocol was developed. Many media were tested; Murashige and Skoog (MS) at different basal salt strengths, modified MS (½MSD) and woody plant medium (WPM) for the establishment stage. WPM was found to be the best medium for establishment of mother plants. Multiple shoots were obtained on WPM medium supplemented with 0.8 mgL^{-1} kinetin in combination with 0.05 mgL^{-1} indole-3-butyric acid and 0.1 mgL^{-1} gibberellic acid. High frequency (80%) of rooting was obtained on ½MS medium supplemented with 5 mgL^{-1} indole-3-acetic acid. Regenerated plantlets were successfully acclimatized with 63% survival.

Keywords: capers, *in vitro* propagation, tissue culture, woody plant medium (WPM)
Abbreviations: BAP, 6-Benzylaminopurine; **GA_3**, gibberellic acid; **IAA**, indole-3-acetic acid; **IBA**, indole-3-butyric acid; **ICARDA**, International Center for Agricultural Research in the Dry Areas; **IPGRI**, International Plant Genetic Resources Institute; **Kin**, kinetin; **MS**, Murashige and Skoog; **½MSD**, half-strength nitrates stock but doubled calcium chloride and magnesium sulphate; **NAA**, α-naphthaleneacetic acid; **WPM**, woody plant medium; **Zea**, zeatin

INTRODUCTION

Capparis spinosa is a wild and cultivated bush which grows mainly in the Mediterranean countries. Caper shrubs are generally well adapted to dry areas receiving less than 200 mm rainfall annually, as most economically important commercial crops cannot be grown under such conditions without irrigation, therefore caper shrubs are good candidates for cultivation (Vidaeus 2002). Many countries and institutions have given recently greater attention to caper plant, by listing at least one of the *Capparis* spp. on their research program. IPGRI mentioned caper as a wild species with high economic potential both as aromatic plant and as a vegetable (IPGRI 2001). India also considered *C. spinosa* as an important arid zone spice, and a micropropagation protocol for its cultivation have been scaled up (Ministry of Science and Technology 2002). In Egypt both *C. spinosa* and *C. sinaica* are considered as vulnerable over-used species that have cultivation potential (El-Ashry 2001). Based on the global and local importance of the species and due to high endemic species rate, Turkey has given *C. spinosa* higher priority for conservation (Kaya *et al.* 1997). In Italy, there is a high interest for caper cultivation for several reasons; among them are the possible use of marginal lands, low initial costs, higher profit margins as compared to other local crops, synergisms of the crop with tourism activities, and alternative use of capers in cosmetics and pharmacology (Infantino *et al*. 2008). The International Center for Agricultural Research in the Dry Areas (ICARDA) also considered caper as a medicinal plant that can fill in a gap in the domestic needs of folk medicine and the pharmaceutical industry in their new proposal (Abou Hadid *et al.* 2004).

The conventional method of propagation through seed is not preferred for multiplication of caper plant mainly for two reasons: the low germination percentage (Ölmez *et al.* 2004) due to seed dormancy and the cross pollination behavior of the plant that result in high degree of heterozygosity in its seed (Rivera *et al.* 2003). Therefore, with the need to increase production, and in keeping with the demand of farmers, vegetative methods are now preferred for the propagation of selected varieties of caper. Conventional methods of propagation by vegetative cutting have low rooting ability (55%) and low degrees of success (30%) (Sozzi 2006). Therefore, using micropropagation would be beneficial in accelerating large-scale multiplication, improvement and conservation of caper plant.

Despite its economic and ecological importance, only few studies have been carried out on *C. spinosa* propagation; almost all research has only focused on its medicinal properties. Archeobotanical information and ancient literary sources traced the use of caper (seeds, leaves and roots) for medicinal purposes and cosmetics back to ancient Greece (Infantino *et al*. 2008). As a medicinal plant, *C. spinosa* contains several chemically active constituents, but one of the most important classes of compounds present is the flavonoids. These flavonoids display a remarkable role in various pharmacological activities including antiallergic, antiinflammatory and antioxidant effects (Ageel *et al*. 1986; Germano *et al*. 2002; Domenico *et al*. 2005; Panico *et al*. 2005). Furthermore, flavonoids have been suggested to affect the function of the immune system (Middleton and Kandaswami 1992; Middleton 1998). The major components of flavonoids in capers were rutin with average wet weight values 0.23% for raw and 0.13% for pickled capers, respectively and kaempferol-3-rutinoside with average wet weight values of 0.19% for raw and 0.053% for pickled capers, respectively (Giuffrida *et al*. 2002). The presence of the flavonoid rutin makes caper a valuable medicinal herb, as it improves capillary function and as a general antioxi-

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dant (Spiteri 1998; Inocencio *et al*. 2000).

Development of a protocol for micropropagation of *C. spinosa* is an important contribution as this could be applied for cloning plants selected for higher yield of both buds and flavonoids content. Therefore, the objective of this study was to develop a protocol for *in vitro* propagation of *C. spinosa*.

MATERIALS AND METHODS

Chemicals

Indole-3-butyric acid (IBA), gibberellic acid (GA_3) (90%), indole-3-acetic acid (IAA) (98%), α-naphthaleneacetic acid (NAA, 97%), 6-benzylaminopurine (BAP), kinetin (Kin) (commercial grade, plant cell culture tested), zeatin (Zea) (95%), vitamins, macro and micro-nutrients (AR grade), *myo*-inositol, and activated charcoal (100-400 mesh) were purchased from Sigma–Aldrich (Steinheim, Germany). Agar was obtained from Bacto-agar, Bio basic Inc., Ontario, Canada. Sucrose and Tween-80 purchased from Tedia, Fairfield, OH, USA.

Collection of plant material and sterilization procedure

Primary explants used in this study were collected from wild caper (*C. spinosa*) in its native habitat in Irbid, in May 2006. Nodal shoot segments (approx. 2 cm) were cut off from mature selected shrubs of caper. Explants were washed with 0.5% (v/v) solution of tween-80 for 20 min and then with sterile water. Explants were surface sterilized with 70% (v/v) ethanol for 1 min followed by 15% bleach containing 5.25% sodium hypochlorite (NaOCl) for 5 min. The explants were then thoroughly washed with sterile distilled water 6 times, each one for 10 min under a laminar air flow cabinet.

Culture medium

Stock solutions of media used in this study were prepared by dissolving the required amount of nutrients or vitamins in 1 L of distilled water and kept in dark-colored bottles in a refrigerator. The recommended amount of each stock solution was added to distilled water up to 75% of the final volume required for the medium preparation. The pH of the medium was adjusted to 5.8 using 1 N NaOH or 1 N HCl. The Murashige and Skoog medium (MS) (Murashige and Skoog 1962) and woody plant medium (WPM) (Lloyd and McCown 1981) were solidified, unless otherwise mentioned, with 8 and 6 gL^{-1} agar, respectively. Autoclaving was carried out at 126°C and 105 kg/cm^{-2} pressure for 20 min. The laminar air flow cabinet was sterilized by exposing to ultraviolet radiation for at least 1-2 h before explant inoculation in media. The laminar flow hood and the autoclaved utensils were further sterilized with 70% ethanol before use to avoid any contamination.

In vitro multiplication of mother stock

To examine the best medium for establishment of caper, explants were cultured on 5 different media: different strength of basal salts MS (full MS, ¾MS and ½MS), half-strength nitrates and doubled calcium chloride and magnesium sulphate (½MSD) and WPM gelled with agar. Antioxidants (ascorbic acid, citric acid and polyvinylpyrrolidone: 50 and 100 mgL^{-1} (w/v)) were examined to decrease oxidation process during the establishment stage.

The explants were placed vertically in 25 × 150 mm culture tubes. Cultures were transferred to a growth room and maintained at 24 ± 2°C and 16-h photoperiod. Light was supplemented using white fluorescent tubes at a photosynthetic photon flux density (PPFD) of 40-45 µmol m^{-2} sec^{-1}. Explants were subcultured every 3 weeks. After 6 weeks of subculture, data were collected on number of proliferated shoots, shoot length, number of nodes $shoot^{-1}$, leaves number, and callus formation (presence or absence). The treatments were arranged in a completely randomized block design (CRBD). For each medium 30 shoots were employed (10 explants × 3 replications).

Shoot multiplication

Uniform shoot segments (approx. 2 cm) were subcultured in Erlenmeyer flasks (250 ml) containing 75 ml of solid WPM media containing 0.05 mgL^{-1} IBA and 0.1 mg l^{-1} GA_3. The following experiments were performed to study the best cytokinin type and concentration for maximizing shoot multiplication. Different concentrations (0.4, 0.8, 1.2, 1.6 or 2.0 mgL^{-1}) of BAP, Kin or Zea were studied in separate experiments. Two control treatments C_1 and C_2 were included; the medium of C_1 did not contain IBA while C_2 contained 0.05 mgL^{-1} IBA (C_2 was include in the experiment as control because IBA was used as basal hormone for all treatments). The shoots were cultured under the same conditions as defined above.

Data were collected after 6 weeks on number of proliferated shoots, shoot length, callus formation and the plant performance (plant color and shape). Experiments were arranged in a CRBD with 10 replications (5 flasks with 2 explants $flask^{-1}$).

In vitro rooting

Shoots were grown on a plant growth regulator (PGR)-free WPM for 4 weeks to eliminate any carry-over effect of any PGR that might inhibit or reduce rooting. Individual shoots, approximately 10 mm long were excised and transferred to 25 × 150 mm culture tubes containing 10 mL agar-gelled ½MS rooting medium supplemented with 500 mgl^{-1} activated charcoal (AC) (based on preliminary trials) and different concentrations (0.25, 2.5, 5.0, 7.5) of IAA, IBA or NAA. A control treatment was included in which the medium contained no PGRs. Six weeks later, cultures were evaluated for root formation. Data were recorded on percent root formation, number and length of roots and shoots $explant^{-1}$. The treatments were arranged in a CRBD with 10 replications (culture tube) and a single $shoot\ replicate^{-1}$.

In a separate set of experiments, the shoots were pulse treated with IBA (0, 10, 25, 50, 100 and 200 mg l^{-1}) in ½MS basal salts liquid medium for 4 h in the dark. The pulse-treated shoots were transferred onto agar-gelled ½MS medium supplemented only with 500 mg l^{-1} AC in the dark for 6 days, and then under continuous light (PPFD of 40-45 µmol m^{-2} sec^{-1}). After 5 weeks in the light, cultures were evaluated for root formation. The treatments were arranged in a CRBD with 10 replicates (culture tube) per treatment and a single shoot per replicate.

Statistical analyses

Data for each experiment were subjected to analysis of variance (ANOVA) by the general linear models (GLMs) procedure using Statistica software v 7.0 (StatSoft, Inc. 2004). Mean comparison was performed using the least significant difference (LSD) method. A significance level of 5% was used for all statistical analyses. Percent data were arcsine transformed before performing ANOVA.

RESULTS AND DISCUSSION

Establishment of mother stock

Successful *in vitro* establishment of *C. spinosa* plant was achieved in this study. The procedure used for sterilization was found to be effective, while the percentage of contamination was less than 5%. Phenolic browning was observed during establishment stage, but adding 100 mgL^{-1} (w/v) citric acid to the medium effectively decreased phenolic oxidation (data not shown). The pre-experiment showed that nodal cutting can be considered a good starting material for *in vitro* establishment of *C. spinosa*. Usually plant material taken directly from their natural habitat, require higher concentration of disinfectant material. In order to reach better sterilization in the culture, different concentrations of commercial sodium hypochlorite (5.25% active ingredient) were tested (data not shown), but the best one was found to be 15% (v/v). This high concentration was found to be more suitable for the nodal cutting, but not for the shoot tips where higher concentration was destructive.

Generally establishment of mother plants *in vitro* from

shoot tips is more preferable than nodal cutting (Razdan 1992) as it has comparably less contamination. But, shoot tips of woody plants are more liable than those of herbaceous species to release undesirable phenolic substances when first placed onto a growth medium (George *et al.* 2008). Moreover, in this experiment, shoot tips can withstand only lower concentration of disinfectant material which was not enough to prevent infection. Komalavalli and Rao (2000), studied micropropagation of a perennial medicinal woody plant (*Gymnema sylvestre*) mentioned that the performance of nodal segments is much better than that of shoot tips.

Along with choosing the appropriate explant type (shoot tip or nodal cutting), using a suitable nutrient medium is also necessary for ensuring good establishment of mother stocks. Callus was not occurred in the different tested media. Among the five different media tested, WPM was found to be the best basal medium for establishment of mother plants. WPM and ½MSD media gave satisfactory shoots length, number of nodes and number of leaves per shoot (**Table 1**).

Moreover, WPM was found to be the best basal medium for shoot elongation (1.89 cm), number of nodes (4.07) and leaves (8.45), followed by ½MSD medium. The shoot buds sprouted on MS medium showed only limited development even if they were maintained for longer period in culture. Between different strengths of MS medium, ½MSD medium gave more shoot length (1.73 cm) and greater average number of node per shoots (3.42 cm), than full-strength, ¾MS and half strength MS media.

By establishment of plant from seed, Chalak *et al.* (2003) reported an *in vitro* culture of *C. spinosa* L. when solid modified 1/2 MS media was used. Rodriguez *et al.* (1990) experimented both MS and modified MS media to test for the best basic media for shoot growth, and achieved successful *in vitro* shoot growth on modified MS over MS media. Thus, the degree of growth varied considerably with medium. The need of WPM and ½MS salts for shoot growth showed negative effect of high salt concentration on shoot development of *C. spinosa*. Therefore, even the MS medium is very popular, and most plants react to it favorably, it is not necessarily to be always optimal for all plants growth and development since the salt content is so high (Pierik 1987). Based on the above results, WPM media was chosen for the proliferation stage in this study.

Shoot multiplication

Only one or two axillary shoots were developed from nodal explants on medium devoid of cytokinins. No significant difference appeared between control treatments C_1 and C_2 during the incubation period, and also no proliferation occurred in C_1 or C_2. The potential of nodal explants was enhanced with the use of cytokinins (**Tables 2-4**).

The best multiplication parameters and growth performance of *C. spinosa* were obtained at (1.2 mgL^{-1}) BAP, (0.8 mgL^{-1}) Kin and (1.6 mgL^{-1}) Zea. At these concentrations number of shoots was (4.6-4.8) and shoot length ranged from 1.78 to1.82 cm. Maximum number of shoots produced per explant in presence of Kin and Zea were about five shoots. More shoots (about seven) could be produced on media containing (2.0 mgL^{-1}) BAP, but these shoots were produced in a compact cluster, very short (0.5-1 cm long) and did not elongate. They did not survive after separation from the parent node. Less shoots (4.70) were produced at 1.2 mgL^{-1} of BAP, but the decrease in shoot number was compensated by increased shoots length (1.82 cm) and these shoots were found to be suitable for root induction. Therefore among the different concentration of BAP tested, 1.2 mgL^{-1} was decided to be the best concentration for axillary shoot proliferation. For Kin the best concentration was 0.8 mgL^{-1}. Zea gave the maximum shoot length (2.44 cm) at lower concentration (0.4 mgL^{-1}) but the number of shoots was low (2.1) at such concentration. When Zea concentration was increased the explants produced shoots with unusual appearance and short in length, then it became brown and died.

It was observed that when the number of shoots per explant was increased this led to decrease in individual shoot length and decrease in percent shoot survival. The same observation was made in *C. decidua* (Deora and Shekhawat 1995) and *Melissa officinalis* (Tavares *et al.* 1996), where, increase in shoot number resulted in decrease in shoot length. The type and concentration of the cytokinin was the determining factor for multiple shoot induction. Statistically, significant differences were found with respect to shoot

Table 1 Effect of different basal media on shoot proliferation of *Capparis spinosa*.

Basal medium	Number of shoots	Shoot length (cm)	Number of nodes	Number of leaves
MS*	1.61 a**	0.93 cd	2.04 c	4.42 c
¾ MS	1.59 a	1.04 c	2.16 c	4.64 c
½ MS	1.68 a	0.91 d	2.10 c	4.78 c
½ MSD	1.73 a	1.73 b	3.42 b	7.13 b
WPM	1.80 a	1.89 a	4.07 a	8.45 a
Mean	1.68	1.30	2.76	5.88

*Means within each column followed by the same letter(s) are not significantly different at $P \leq 0.05$ according to LSD.
Abbreviations: MS: Murashige and Skoog medium, WPM: woody plant medium, MSD: half-strength nitrates stock but doubled calcium chloride and magnesium sulphate.

Table 2 Effect of various concentrations of BAP supplemented to WPM on shoot multiplication of *Capparis spinosa*.

Concentration (mgL^{-1})	Number of shoots	Shoot length (cm)	Callus (%)
C_1	1.9 d*	0.97 cd	0 b
C_2	1.7 d	0.90 de	10 b
0.4	2.1 d	2.07 a	10 b
0.8	3.9 c	1.90 b	10 b
1.2	4.7 c	1.82 b	20 b
1.6	6.0 b	1.07 c	90 a
2.0	6.9 a	0.80 e	100 a

*Means within each column followed by the same letter(s) are not significantly different at $P \leq 0.05$ according to LSD.
Abbreviations: BAP: 6-benzylaminopurine, WPM: woody plant medium, C_1: control without plant growth regulators, C2: control with 0.05 mgL^{-1} indole-3-butyric acid.

Table 3 Effect of various concentrations of kinetin supplemented to WPM on shoot multiplication of *Capparis spinosa*.

Concentration (mgL-1)	Number of shoots	Shoot length (cm)	Callus (%)
C1	1.8 b*	0.97 d	0 b
C2	1.8 b	0.92 d	0 b
0.4	2.2 b	1.87 a	0 b
0.8	4.6 a	1.82 a	0 b
1.2	5.3 a	1.70 ab	0 b
1.6	5.3 a	1.46 bc	10 b
2.0	5.1 a	1.32 c	90 a

*Means within each column followed by the same letter(s) are not significantly different at $P \leq 0.05$ according to LSD.
Abbreviations: WPM: woody plant medium, C_1: control without plant growth regulators, C_2: control with 0.05 mgL^{-1} indole-3-butyric acid.

Table 4 Effect of various concentrations of zeatin supplemented to WPM on shoot multiplication of *Capparis spinosa*.

Concentration (mgL^{-1})	Number of shoots	Shoot length (cm)	Callus (%)
C_1	2.0 c*	0.97 d	0 b
C_2	1.8 c	1.13 d	0 b
0.4	2.1 c	2.44 a	0 b
0.8	2.4 c	2.13 b	0 b
1.2	3.4 b	1.71 c	10 ab
1.6	4.8 a	1.78 c	30 a
2.0	-	-	-

*Means within each column followed by the same letter(s) are not significantly different at $P \leq 0.05$ according to LSD.
Abbreviations: WPM: woody plant medium, C_1: control without plant growth regulators, C_2: control with 0.05 mgL^{-1} indole-3-butyric acid.

Table 5 Effect of various concentrations of IAA supplemented to half-strength MS medium containing 500 mgl^{-1} activated charcoal on *in vitro* rooting of *Capparis spinosa*.

Concentration (mgL^{-1})	% Rooting	Root length (mm)	Root number	Shoot length (cm)	Callusing (%)
0.00	0	0.0 c*	0.0 b	2.2 b	0 b
0.25	30	6.1 b	1.0 b	3.4 a	0 b
2.50	70	11.9 a	2.8 a	3.7 a	30 ab
5.00	80	15.5 a	4.2 a	3.9 a	40 a
7.50	20	2.3 bc	0.9 b	2.3 b	60 a

*Means within each column followed by the same letter(s) are not significantly different at $P \leq 0.05$ according to LSD.
Abbreviations: IAA: indole-3-acetic acid, MS: Murashige and Skoog medium.

Table 6 Effect of various concentrations of IBA supplemented to half-strength MS medium containing 500 mgl^{-1} activated charcoal on *in vitro* rooting of *Capparis spinosa*.

Concentration (mgL^{-1})	% Rooting	Root length (mm)	Root number	Shoot length (cm)	Callusing (%)
0.00	0	0.0 b*	0.0 b	2.23 c	10 b
0.25	20	2.1 ab	0.5 b	3.21 b	10 b
2.50	50	4.3 a	1.8 a	3.16 b	40 ab
5.00	20	2.0 ab	0.9 ab	4.34 a	30 ab
7.50	0	0.0 b	0.0 b	1.85 c	70 a

*Means within each column followed by the same letter(s) are not significantly different at $P \leq 0.05$ according to LSD.
Abbreviations: IBA: indole-3-butyric acid, MS: Murashige and Skoog medium.

Table7 Effect of various concentrations of NAA supplemented to half-strength MS medium containing 500 mgl^{-1} activated charcoal on *in vitro* rooting of *Capparis spinosa*.

Concentration (mgL^{-1})	% Rooting	Root length (mm)	Root number	Shoot length (cm)	Callusing (%)
0.00	0	0.0 a*	0.0 a	2.37 b	0 c
0.25	20	1.6 a	0.5 a	3.26 a	30 bc
2.50	20	1.0 a	1.0 a	3.79 a	50 ab
5.00	10	0.6 a	0.7 a	2.36 b	70 a
7.50	0	0.0 a	0.0 a	1.95 b	70 a

*Means within each column followed by the same letter(s) are not significantly different at $P \leq 0.05$ according to LSD.
Abbreviations: NAA: α-naphthaleneacetic acid, MS: Murashige and Skoog medium.

number and shoot length demonstrated that amongst the three types of cytokinins incorporated into the medium, only Kin and BAP were effective enough to give a good induction of shoots number and length. Even though more number of shoots were formed at higher concentration level, most of these shoots were not satisfactory for elongation and root induction.

Lower callusing formation is generally occurred when explants were incubated in medium supplemented with Kin and Zea. Callusing was increased when BAP was used especially at higher levels (1.6-2.0 mgL^{-1}). Therefore, among the different cytokinins types and levels used, Kin at (0.8 mgL^{-1}) gave the highest and the best one, followed by BAP (1.2 mgL^{-1}). Rodriguez *et al.* (1990) who did not test Kin in their study in micropropagation of *C. spinosa* showed successful culture of explants on modified MS media supplemented with BAP (0.9 mgL^{-1}), IAA (0.05 mgL^{-1}) and GA_3 (0.1 mgL^{-1}).

In vitro rooting

There were significant variations among and within the various growth regulators and concentrations used in rooting experiment of *C. spinosa* (**Tables 5-7**). Media that contained IAA enhanced the root formation of *C. spinosa* and the highest root percentage (80%), root length (15.5 mm) and roots number (4.2) were obtained at the concentration 5.0 mgL^{-1} (**Table 5**). At 2.5 mgL^{-1} IBA the highest root percentage (50%), root length (4.3 mm) and roots number (1.8) with good shoot length (3.16 cm) and little callus were obtained (**Table 6**). When the media were supplemented with NAA no remarkable enhancement in root formation was recorded. At the best concentrations (0.25-2.5 mgL^{-1}) NAA, the highest root percentage did not exceeded 20%, root length 1.6 mm and only one root was formed (**Table 7**).

Callusing occurred when levels of auxins increased above 2.5 mgL^{-1} in the rooting media. But NAA was found to be the most inducing to callus. In agreements with other groups (Rodriguez *et al.* 1990; Deora and Shekhawat 1995) the results show that when the microshoot formed callus, it failed to produce or develop long functional roots. This is because roots differentiated in callus frequently do not have vascular connection with the stem (Deora and Shekhawat 1995).

In the current study, the best auxin and concentration for rooting of *C. spinosa* may be weighted to be IAA at the level 5.0 mgL^{-1}. Although this concentration did not give the longest shoots but it gave the best root number and length as well as little callus formation. Rodriguez *et al.* (1990) found that the best *in vitro* root induction and elongation of *C. spinosa* microshoot was obtained when IAA (5.25 mgL^{-1}) was used. However, Carra *et al.* (2007) who regenerated plant of *C. spinosa* from unfertilized ovules obtained the best result in rooting step when the explants were incubated in presence of 2.04 mgL^{-1} IBA for six days in the dark and then transferred in hormone free medium under light.

In the pulse experiment, the shoots fail to root using a 4 h pulse treatment at the low concentration of IBA (0-50 mgL^{-1}) (data not shown). At the higher concentration of IBA (100-200 mgL^{-1}), root length and root number was not statistically significant from the other concentrations of IBA. However, shoot length and callus formation found to vary significantly. The high concentration of IBA (100-200 mgL^{-1}) increase both shoot length and callus formation. Based on the results of this study, we don't recommend using pulse treatment for 4 h period with IBA hormone in the rooting stage of *C. spinosa* micropropagation. However, pulse treatment with IBA succeeded with other species of caper like *C. deciduas* (Deora and Shekhawat 1995). Using IAA pulse treatment for 4 h period in darkness, Chalak *et al.* (2003) have successfully rooted shoots of *C. spinosa* but the survival rate of acclimatized plantlets was only 40%.

Acclimatization

In vitro rooted plantlets were acclimatized by transferring to plastic pots containing soil mix with perlite and plant compost (1:1:1) under controlled growth conditions. The mixture was initially moistened with sterile quarter-strength MS medium without sucrose. To maintain high relative humidity, pots were placed in plastic bags. Relative humidity

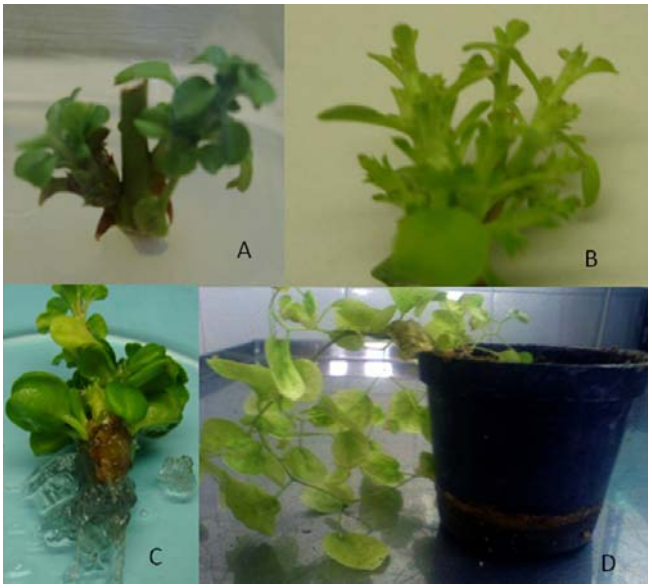

Fig. 1 Micropropagation of *Capparis spinosa* from mature plant. (**A**) *In vitro* shoot establishment from nodal explants on WPM medium + 100 mgL^{-1} (w/v) citric acid; (**B**) Multiplication of shoots on WPM medium + 0.8 mgL^{-1} kinetin + 0.05 mgL^{-1} IBA + 0.1 mgL^{-1} GA_3; (**C**) Development of roots on ½ MS + 5 mgL^{-1} IAA+500 mgL^{-1} activated charcoal; (**D**) Plant raised after transplantation to potting mixture.

was reduced gradually and complete removal of plastic bag took place after two weeks of placement. The pots with the plantlets were kept in greenhouse for three weeks for acclimatization. Normal growth of the potted plants was visible 10–15 days after transfer to field conditions.

CONCLUSIONS

The protocol defined in this study as outlined below and is demonstrated in **Fig. 1**, was found to be efficient and can be utilized for cloning of selected wild plant of this species. Firstly, establishment of *in vitro* shoots from nodal explants on WPM medium + 100 mgL^{-1} (w/v) citric acid. Then, multiply the shoots on WPM medium + 0.8 mgL^{-1} Kin + 0.05 mgL^{-1} IBA + 0.1 mgL^{-1} GA_3. After that, rooting the shoots on ½ MS + 5 mgL^{-1} IAA + 500 mgL^{-1} AC. Finally, hardening the rooted shoots in the greenhouse in pots containing a mixture of soil, perlite and plant compost (1:1:1).

The findings have several implications for managing the diversity of this species, as well as restoration of its degradation. The developed protocol can be used to produce uniform and desirable plants for cultivation in order to reduce pressure on the wild populations. It also offers a potential system that should be used for improvement, conservation and mass propagation of *C. spinosa*. Conservation of this drought-tolerant plant can be a challenge as well as a powerful tool to reduce desertification, improve farmers livelihoods and enhance biodiversity.

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