Egypt. J. Plant Breed. 13: 1 – 9 (2009)

IN VITRO PROPAGATION OF CAPER, *Capparis spinosa*

M. M. Abd-Alla, Mahdia F. Gabr and M.A. Ahmed

Desert Research Center, Genetic Resources Dept. Tissue Culture Unit.
Mataria, Cairo,Egypt

ABSTRACT

***Capparis spinosa* is a wild economic plant which had a problem in natural propagation in South Sinai. Shoot tip and stem segment explants of Caper were used for in vitro propagation. The proliferation of shoots was high in autumn. MS medium supplemented with 0.5 mg/l BA or 0.5 mg /l 2IP produced 7.3 and 7.0 shoot/explant, respectively. But combination of 0.5 mg BA with 0.5mg /L 2iP in MS medium was more effective in proliferating shoots (8 shoots/explant) and shoot length (6.41 cm). Hairy roots were obtained with BA and NAA. Rooted plants were transferred for acclimatization in the green house.**

Keywords: ***Capparis spinosa, Micropropagation, Growth regulators.***

INTRODUCTION

Capparis spinosa L. is one of the family *Capparidaceae*, which has a common name of caper. This shrub is an important multipurpose plant because of its medicinal effects; the fruits are consumed fresh and also as a dry vegetable and pickled. Seeds of *Caper* have short viability in germination so that it became an endangered species.

For micropropagation shoot tip and stem segment explants were used by Sudha *et al* (1998). Concerning the genus *Capparis*, multiple shoots were developed from nodal shoot segments taken from field grown *Capparis spinosa* plants (Rodriguez *et al* 1990). Moreover, multiple shoot formation was obtained on basal nodes cultured on medium supplemented with BA and NAA after 8 weeks (Sudha *et al* 1998, Das *et al* 1999, Kulkarni and Rao 1999 and Sumana *et al* 1999. Moreover Singh *et al* 1999, Ali *et al* 2004 and Neeti and Kothari 2005 multiplied shoots from shoot tips on MS medium supplemented with 0.5-1.0 mg/l BAP within 6-7days of culture. Skala and Wysokinska (2004) found that the best shoot proliferation was obtained from shoot tips on MS medium supplemented with 8.9 μ M 6-BAP and 2.9μM IAA. Husain and Mohamed (2006) found that the highest shoot regeneration frequency as well as the maximum number of shoots were recorded on MS medium supplemented with BA (5 μ M) + NAA (0.5 μ M), while Elangomathavan *et al* (2003) showed that multiple shoots were obtained on MS medium containing 2.22 μ M BA, from nodal explants of a medicinally valuable plant. Lee and Chan (2004) found that the highest number of regenerated shoots were obtained on MS medium supplemented with 6.7μ M BA.

For rooting stage, Rodriguez *et al* (1990) mentioned that higher rooting response of *Capparis spinosa* was obtained after 20 days of incubation in darkness on solid half-strength MS medium plus IAA (30μM), followed by a subsequent 20-day culture period on half- strength MS medium. Ndoye *et al* (2003) achieved rooting of *in vitro* formed shoots on medium containing 20 mg /l of IBA. Andrade *et al* (1999) reported that roots were induced on shoots of *Lavandula vera* transferred to MS medium supplemented with IBA or NAA (٤-٠mg/l). Micropropagated plants were successfully transferred to soil. They also reported that shoots of *Majorana hortensis* were rooted on MS medium supplemented with IBA at ٠,٢mg/l. Singh and Sehgal (1999) found that about ٩٢% of the *in vitro* regenerated shoots of *Ocimum sanctum* rooted on MS hormone-free medium within 2-3 weeks of culture and 85% of the micro propagated plantlets were successfully established in soil, where they grew normally. Hembrom *et al* (2006), Rani *et al* (2006). Sunandakumari *et al* (2004), Wali and Siddiqui (2003), Iyer and Pai (2000) and Rady and Saker (2000) found that multiplied shoots produced roots in the presence of 0.2 mg /l IBA. Cuenca and Amo-Marco (2000) achieved rooting of shoots on MS medium without growth regulators. The addition of the auxins IAA or IBA did not improve the rooting .While NAA had an inhibitory effect. The rooted plantlets were acclimatized to *ex vitro* conditions with a survival percentage > 70 % under greenhouse conditions. Zahariev and Slavova (2003) reported that the cloned plants were rooted in a medium containing 1.0 mg /l NAA and 3 or 4% sucrose. Ali *et al* (2004) transferred regenerated shoots of *Mentha arvensis* for rooting on MS medium containing various concentrations of NAA and IBA (0.5: 2.0 mg/l). Arikat *et al* (2004) found that micro shoots were rooted on MS medium supplemented with IBA, IAA, or NAA. Echeverrigaray *et al* (2005) reported that the best condition for rooting was MS medium supplemented with 2.5 uM NAA. The aim of this study is to propagate *Capparis spinosa* by the rapid clonal propagation of this wild population and conservation of elite germplasm as genetic resource.

MATERIALS AND METHODS

This work was carried out in the laboratories of Tissue Culture Unit, Genetic Resources Dept, DRC, Mataria, Cairo, Egypt, throughout the years from 2004 to 2006. *Capparis spinosa* explants were collected from the naturally growing plants at Ras-Sedr in South Sinai at different seasons through two years (2004 and 2005). Plant materials were put in an ice box until culture. Small segments (3-4 cm long) from new flushes of *Capparis spinosa* contained two buds were used in the starting culture *in vitro.* Surface sterilization was accomplished by soaking explants in Ethanol 70% for 3 min, then in 20% Sodium hypochlorite (5.2% active ingredient) for 20

min. These were thoroughly washed with sterile water for five times .The surface sterilized explants were placed vertically in culture jars of Murashige and Skooge (MS) medium (1962) supplemented with sucrose (30gm /l), vitamins at 0.5mg /l of (Thiamin- HCl, Pyridoxine-HCl, Nicotinic acid) and 100 mg / l Myo- inositol . Concentrations of BA and 2ip (0.0, 0.5 and 1.0 mg/l) were applied in the establishment and multiplication stages. The pH value was adjusted at 5.6 – 5.7 by adding HCl (0.1M) or NaOH (0.1M). Medium was gelled with 2.5g/l phytagel and then was autoclaved at 121□C for 20 mins. For rooting experiments, MS medium was used at full and half strength and supplemented with NAA and IBA at three concentrations (0.0, 0.5 and1.0 mg/l) of each. Small clusters of 3-4 shoots were soaked in 2000 ppm IBA solution for 3, 6 h and overnight before culture on rooting media. Moreover, BA and NAA were applied in combinations of two concentrations (0.5 and 0.1 mg) of each.

RESULTS AND DISCUSSION

Stem segments of *Capparis spinosa* were cultured on media after they were sterilized by using 20% v/v sodium hypochlorite solution and then rinsed in distilled sterilized water. This was the best concentration to produce low contamination percent. Data in Table (1) showed that the highest survival percent was in autumn (92.3%) followed by 91.2% in winter and the lowest survival percent was obtained in summer 83.2% and in spring 84.4% . Low numbers of the survival explants produced shoots, which were divided into small segment and cultured on multiplication medium to produce more shoots and subsequent whole plants. Fig. 1 shows the proliferation of shoots on the stem segments of *Capparis spinosa* on establishment culture medium. the proliferated shoots were transferred to multiplication stage after they were 5 cm length at lest and had bud nodes and good shot tip.The stem segment explants were used here in and by many investigators such as Ali *et al* (2004), Neeti and Kothari (2005), Skala and Wysokinska (2004) and Husain and Mohammad (2006). Data in Table (2) and Fig. (2) showed the effect of medium on the multiplication rate of *Capparis spinosa* shoots. Explants were cultured on media for multiplication in the combinations of BA and 2ip at 0.0, 0.5 and 1.0 mg/l of each. Highest growth response of explants was 100% on medium containing 0.5 mg 2ip/l, followed by 83.3% in the presence of 1.0 mg BA+0.5 mg 2ip/l, and 75% with 0.5 mg /l BA+1.0mg /l 2ip or with 1.0 mg /l BA.

Table 1. The effect of culture season on explants survival of *Capparis spinosa*

Season	Number of explants	Survival		Contamination		Shoot proliferation	
		No	%	No	%	No	%
Winter	180	165	91.9	25	8.1	26	8.4
Spring	140	118	84.4	37	15.6	3	1.5
Summer	140	117	83.5	38	16.5	6	3.2
Autumn	182	169	92.3	13	7.7	46	25.3

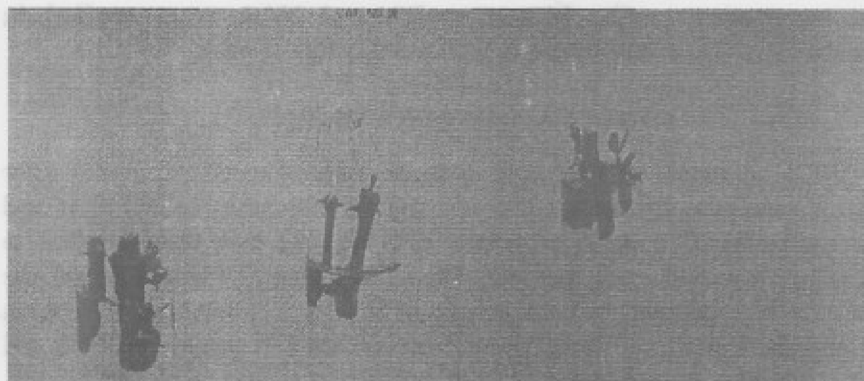

Fig. (1): Establishment stage of *capparis spinosa* explants

The lowest response was 50% observed with 1.0 mg /l BA+1.0 mg /l 2ip. Best effect of BA on shoot number was (7.3 shoots /explant) in the presence of 0.5 mg /l BA, followed by 6.0 shoots /explant with 1.0 mg /l BA and 5.43 shoots /explant with the control medium. The effect of 2ip on the shoot number was the best with 0.5mg /l 2ip which was 7 shoots/explant followed by 5.4 shoots / explant with the control and 1.0 mg/l 2ip. The mean shoot length in the presence of BA (1.0mg/l) was 5.87cm/shoot followed by the control (4.61cm/shoot).

On the other hand, the shoot length was 4.86 cm/shoot with 0.5mg /l 2ip followed 4.61 cm in the control. It seems that 1.0mg/l BA was more effective than 2ip in the medium at the concentration 0.5 or 1.0 mg/l. The combination between BA and 2iP produced the highest rate of shoot proliferation. The total shoot number was the highest (88 shoots) and the mean number of shoots/explant (8 shoots/explant) in the presence of 0.5 mg/l BA+0.5mg/l 2ip in medium, and the mean shoot length was 6.4cm, followed by 1.0 mg BA/l+0.5mg/l 2ip which produced total shoot number 54 (mean 5.4) and the mean shoot length was 6.22 cm/shoot. When medium contained 0.5mg/l BA+1.0 mg/l 2ip the shoot number was 45, the mean number was 5 and the mean shoot length/explant was 5.56 cm.

Table 2. The effect of growth regulators on the multiplication of *Capparis spinosa in vitro*

Hormones concentration		Multiplication responce		shoot/ explant		Shoot length cm	
BA mg/l	2ip mg/l	Total No	%	Total	Mean	Total	Mean
0.0	0.0	12	58.7	38	5.43	175	4.61
0.0	0.5	6	100	42	7	204	4.86
0.0	1.0	15	66.6	54	5.4	198	3.67
0.5	0.0	15	66.6	73	7.3	328	4.49
0.5	0.5	15	73.3	88	8	564	6.41
0.5	1.0	12	75	45	5	250	5.56
1.0	0.0	12	75	54	6	317	5.87
1.0	0.5	12	83.3	54	5.4	336	6.22
1.0	1.0	12	50	36	6	160	4.44

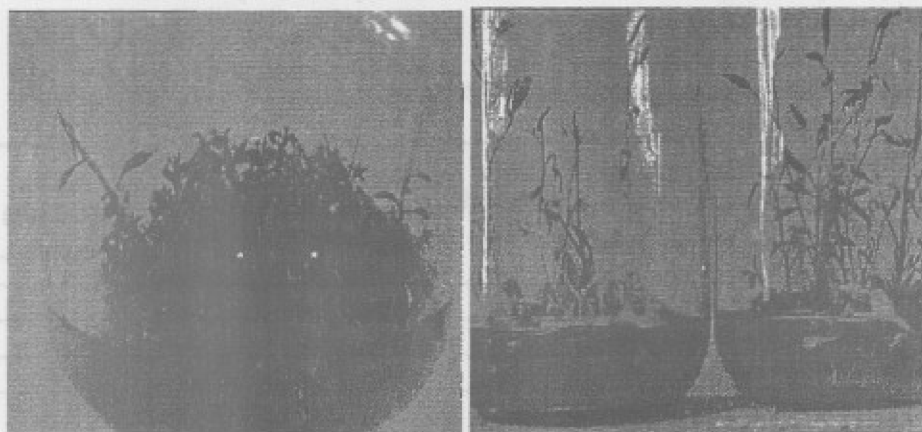

Fig. (2): Multiplication and rooting of *Capparis spinosa* shoots .

The presence of 1.0mg/l BA+1.0 mg/l 2ip produced 36 shoots (mean 6 shoots/explant) and the mean shoot length was 4.44 cm /explant. These results indicated that BA was more effective in media than 2iP with respect to shoot length and number. But the combination of 0.5mg/l BA+0.5mg/l 2iP produced the highest shoot number and shoot length. These results agreed with those obtained by Singh *et al* (1999), Ali *et al* (2004) and Neeti and Kothari (2005) who multiplied shoots from shoot tips on MS medium supplemented with 0.5-1mg/l BAP. Elangomathavan *et al* (2003), Lee and Chan (2004), Skala and Wysokinska (2004) and Husain and Mohammad (2006) found that the best shoot proliferation from shoot tips

was obtained on MS medium supplemented with BAP and 2.9μM IAA and the highest shoot regeneration frequency as well as the maximum number of shoots were recorded on MS medium supplemented with BA. As it clear in Fig. (2), that there were high number of the multiplied shoots grown on the cultured proliferated explants. The proliferated shoots were transferred to rooting stage. Shoot clusters were separated into small groups each one contained 3 or 4 shoots (Fig 2) and then transferred to rooting media which contained auxins to enhance root formation. Different concentrations and combinations between NAA and IBA in the presence of BA are shown in Table (3). Explants with the three treatments (0.5mg/l BA+0.5mg/l NAA, 0.5 mg/l BA+1.0mg/l NAA and 1.0 mg/l BA+0.5mg/l NAA) formed hairy roots; the first combination was the best one (Fig. 2). The rooting response was about 60-80% of the cultured explants. The mean number of roots / explant was ranging from 4 to 5 and the mean root length was ranging between 8.5 and 10.25cm (Figure 2)in the media contained (1.0 mg/l BA+0.5mg/l NAA), (0.5mg/l BA+1.0 mg/l NAA) and (0.5mg/l BA+0.5mg/l NAA), respectively. The formed roots were hairy and very thin that reduced acclimatization rate.

Table 3. The effect of growth regulators on rooting of *Capparis spinosa* shoots

MS medium +			Number of explant	Rooting %	Total root no	Total root length (cm)	Mean root length (cm)	Mean no roots /explant.
BA mg/l	NAA mg/l	IBA mg/l						
0.5	0.5	--	10	80	40	340	8.5	5
0.5	1.0	-	10	60	30	279	9.3	5
1.0	0.5	-	10	60	24	246	10.25	4

Despite the presence of NAA and IBA at different concentrations in media incubated for long period (90 days), there was no effect on forming roots on shoot clusters which disagree with that of Rodriguez *et al* (1990) who mentioned that higher rooting responses of *Capparis spinosa* were obtained after a 20 day incubation period in darkness on solid half-strength MS (MS mineral salts + 0.5 μM Myo-inositol +1μM thiamine) medium plus IAA (30μM), followed by a subsequent 20-day culture period on half – strength MS basal medium. Andrade *et al* (1999) and Ndoye *et al* (2003) reported that roots were induced on shoots transferred to MS medium individually supplemented with IBA or NAA (٤-٠mg/litre). Micro propagated plants were successfully transferred to soil. Singh and Sehgal (1999) and Cuenca and Amo-Marco (2000) found that the *in vitro* regenerated shoots rooted on MS growth regulator-free medium and the

addition of the auxins IAA or IBA did not improve the rooting. Iyer and Pai (2000), Rady and Saker (2000), Wali and Siddiqui (٢٠٠٣) Hembrom *et al* (2006), and Rani *et al* (2006) found that shoots produced roots in the presence of IBA. Zahariev and Slavova (2003) reported that the cloned plants were rooted in a medium containing NAA and 3 or 4% sucrose. Ali *et al* (2004) reported that micro cuttings were transferred for rooting on MS medium containing various concentrations of NAA and IBA. Arikat *et al* (2004) found that micro shoots were rooted on MS medium supplemented with indole-3-butyric acid (IBA), indole-3-acetic acid or alpha–naphthalene acetic acid. Echeverrigaray *et al* (2005) reported that the best condition for rooting was MS medium plus NAA.

REFERANCES

Ali, A., H. Afrasiab, M. Saeed and J. Iqbal (2004). An *in vitro* study of regeneration and micropropagation of *Mentha arvensis*. International J. of Biology and Biotechnology 1(4): 519-528.

Andrade, L. B., S. Echeverrigaray, F. Fracaro, G.F. Pauletti and L. Rota (1999). The effect of growth regulators on shoot propagation and rooting of common lavender (*Lavandula vera*) .Plant Cell Tiss. and Org. Cult., 56(2): 79-83.

Arikat, N. A., F.M. Jawad, N.S. Karam and R.A. Shibli (2004). Micropropagation and accumulation of essential oils in wild sage (*Salvia fruticosa* Mill). Sci. Hort., 100(1/4): 193-202.

Cuenca, S. And J.B. Amo-Marco (2000). *In vitro* propagation of two Spanish endemic species of *Salvia* through bud proliferation. *In Vitro* Cellular and Developmental Biology.36(3): 225-229.

Das, R., A.C. Deka, S. Sarma and M.C. Kalita (1999). *In vitro* propagation of *Leucas lavandulifolia* Rees: a potent herbal medicinal plant . Neo Botanica.1999; 7(1): 39-42.

Echeverrigaray, S.; R. Basso and L.B. Andrade (2005). Micropropagation of *Lavandula dentata* from axillary buds of field-grown adult plants. Biologia Plantarum.49(3): 439-442.

Elangomathavan, R., S. Prakash, K.Kathiravan, S. Seshadri and S. Ignacimuthu (2003) .High frequency *in vitro* propagation of Kidney Tea Plant. Plant Cell, Tiss.and Org. Cult. 72(1): 83-86.

Husain, M.K and A. Mohammad (2006) . Rapid *in vitro* propagation of *Eclipta alba* (L.) Hassk by shoot tip culture. J.of Plant Biochemistry and Biotechnology. 15(2): 147-149.

Hembrom, M.E, K.P. Martin, S.K. Patchathundikandi and J. Madassery (2006). Rapid *in vitro* production of true to type plants of *Pogostemon heyneanus* through differentiated axillary buds. In Vitro Cellular and Developmental Biology.42 (3): 283-286.

Iyer, P.V. and J.S. Pai (2000). *In vitro* regeneration of *Majorana hortensis* (Moench) from callus and nodal stem segments. J.of Spices and Aromatic Crops. 9 (1): 47-50

Kulkarni,V.M. and P.S. Rao (1999). *In vitro* propagation of sweet flag (*Acorus calamus,* Araceae) . J.of Medicinal and Aromatic Plant Sciences. 21: (2), 325-330

Lee-WaiLeng and Chan-LaiKeng (2004). Plant regeneration from stem nodal segments of *Orthosiphon stamineus* Benth., a medicinal plant with diuretic activity *In Vitro* Cellular and Developmental Biology.40(1): 115-118

Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15: 473-479.

Ndoye, M.,I. Diallo and Y. K. Gassama (2003). *In vitro* multiplication of the semi arid forestry tree, *Balanites aegyptiaca* (i.)Del. African J. of Bio. Vol. 2 (11): 421-424.

Neeti,Dhaka and S.L ,Kothari (2005). Micropropagation of *Eclipta alba* (L.) Hassk - an important medicinal plant *.In Vitro* Cellular and Developmental Biology, 41(5): 658-661.

Rady, M.R. and M.M. Saker (2000). Micropropagation of *Lavandula officinalis* L. through shoot tip culture of mature plants . Egyptian J. of Hort., 27(3): 305-314.

Rani, G., D. Talwar, A. Nagpal and G.S. Virk (2006). Micropropagation of *Coleus blumei* from nodal segments and shoot tips. Biologia Plantarum 50(4): 496-500.

Rodriguez, R., M. Rey, L. Cuozzo and G. Ancora (1990). *In vitro* propagation of caper (*Capparis spinosa* L.). *In vitro* Cellular and Developmental Biology 26 (5): 531-536.

Singh, N.K. and C.B.Sehgal (1999). Micropropagation of 'holy basil' (*Ocimum sanctum* Linn.) from young inflorescences of mature plants .Plant Growth Regulation. 29 (3): 161-166

Singh,S., B.K. Ray, S. Mathew, P. Buragohain, J. Gogoi, S. Gogoi, B.K. Sharma and P.C. Deka (1999). Micropropagation of a few important medicinal plants. Phytomorphology 49: 1, 43-47.

Skala, E. and H. Wysokinska (2004). *In vitro* regeneration of *Salvia nemorosa* L. from shoot tips and leaf explants . *In Vitro* Cellular and Developmental Biology. 40 (6): 596-602.

Sudha, C.G., P.N. Krishnan and P. Pushpangadan (1998). *In vitro* propagation of *Holostemma annulare* (Roxb.) K. Schum., a rare medicinal plant *.In Vitro* Cellular and Developmental Biology 1998; 34(1): 57-63

Sumana K.R., K.M. Kaveriappa and H.K. Bhat (1999). *In vitro* micropropagation of *Holarrhena pubescens* .J.of Medicinal and Aromatic Plant Sciences. 21: 2, 299-303 .

Sunandakumari, C., K.P.Martin, M.Chithra, S. Sini, and P.V. Madhusoodanan (2004). Rapid axillary bud proliferation and *ex vitro*

rooting of herbal spice, *Mentha piperita* L. Indian J. of Biotechnology. 3(1): 108-112.

Wali, S.A. and B.A. Siddiqui (2003). High frequency of multiple shoot regeneration in *Mentha piperita* L., a multipurpose herb .Physiology and Molecular Biology of Plants, 9 (1):153-156.

Zahariev, D. and Y. Slavova (2003). Vegetative propagation of wood betony (*Stachys officinalis* (Trev.) L.) cultured *in vitro*. Bulgarian J. of Agric.Sci. 9(2): 197-202.

الاكثار المعملى لنبات اللصيف الطبى

محمد محمد عبد الله –مهدية فريد جبر- محمد رضا عبدالمجيد أحمد

مركز بحوث الصحراء –قسم الأصول الوراثية –وحدة زراعة الانسجة

نبات الكبارس (اللصيف) احد النباتات البرية الاقتصادية التي يصعب اكثارها في الطبيعة بجنوب سيناء. كانت افضل المنفصلات المستخدمة هي القمة النامية والعقل الساقية في اكثاره معمليا. كانت بيئة موراشيج وسكوج المحتوية على ٠,٥ مجم بنزيل ادينين و ٠,٥ مجم ايزوبنتيل ادينين افضل البيئات اعطت عدد افرع عالي / منفصل نباتى واكبر طول للفرع .بينما البيئة المحتوية على تركيز مشترك من البنزيل ادينين والايزوبنتيل ادينين (٠,٥ مجم/لتر من كلا منهما)اعطى اعلى عدد افرع/منفصل. تكونت جذور مع وجود البنزيل ادينين ونفثالين حمض الخليك . نقلت تلك النباتات المستولدة بنجاح الى الصوبة للاقلمة