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In vito micro propagation of two native *Capparis spinosa* L. cultivars from Iran

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ABSTRACT: *Capparis spinosa* L. (Caper) is a multipurpose crop that can be used for ornamental, culinary, cosmetics, pharmaceutical and medicinal purposes. As ornamental shrub, caper plant is used for the prevention of soil erosion and help to conserve soil water reserves. Every part of caper has medicinal uses. Due to the great population diversity and difficulties in conventional propagation methods, for the protection of *C. spinosa* from genetic erosion; a tissue culture method would be a good way for mass propagation of this plant. In vitro propagation of caper was begun with seeds. The highest shoots proliferation was obtained on MS medium supplemented with 3 mgl^{-1} cytokinin. Varzegan cultivar had the highest shoot and nod number and shoot length. The results showed that Zeatin was quite effective in the proliferation of capper explants. More Shoot multiplication was obtained at the same medium by sub-culturing shoot segment with 2-3 nodes every seven weeks (for two times). Auxin type (IBA and NAA), levels (0, 1 and 2 mgl^{-1}) and media (½MS, MS) as well as cultivar (Varzegan and Miyaneh) had profound effects on root number. The result showed that the highest root number was obtained from Varzegan cultivar. With increasing auxins concentration rooting percentage was increased. The greatest rooting response shoots was acquired from MS media supplemented with 2 mgL^{-1} of NAA.

Keywords: *Capparis spinosa*, BAP, IBA, Propagation.

INTRODUCTION

Capparis spinosa L. (Caper) from; Capparidaceae is an indigenous perennial of most Mediterranean countries (Abu-khalaf and Arafeh, 2015). 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 (Vidaeus, 2002). It usually thrives in rocky and anhydrous habitats fully exposed to the sunrays, and is able to withstand high temperatures above 40°C (Al-Mahmood *et al.,* 2012). Caper has never been exploited before because of local culinary customs and the lack of home market for its products. Caper also is a medicinal plant can be used in Folk medicine and pharmaceutical industry (Abou Hadid *et al.,* 2004). Capers are said to reduce flatulence and have anti-rheumatic properties, uses for arteriosclerosis, as diuretics, kidney disinfectants, vermifuges and in folk therapy. Moreover, infusions and decoctions from caper root bark have been traditionally used for dropsy, anemia, arthritis and gout. Capers contain considerable amounts of the anti-oxidant bio-flavinoid rutin (Soyler and Khawar, 2007). Today's, with increasing worldwide demand for flower buds, cultivation of the caper crop plays an important role in the village society. Due to the great variability of the population (because of cross pollination behaviors of the plant that result in high degree of heterozygosity in progenies) (Musallam *et al.,* 2011) and also difficulties conventional propagation methods (due to problems associated with its lignified cutting, and rooting problems; and very low germination rates of seeds) (Rodriguez *et al.,* 1999), (Chalaka and Elbitar, 2006), and for utilization of its products as well as for better natural exploitation and to prevent genetic erosion of native population , in vitro culture methods have been experienced to overcome these problems and to improve caper mass clonal propagation production. Ma *et al.,* 2010 showed that, MS medium supplemented with 6-BAP(0.6 mgl^{-1})+ 2,4,D (1mgl^{-1}) was suitable for proliferation and MS supplemented with IBA (0.8mgl^{-1}) and activated carbon (300 mgl^{-1}) was suitable for rooting of *C. spinosa*.

Musallam *et al.,* (2011) showed that 1/2 MS medium supplemented with 5 mgl^{-1} IBA was the best for the rooting behavior of proliferated in vitro derived shoots. The present study was conducted to evaluate the in vitro cultural responses of two Iranian caper clones for the possible future exploitation of the secondary metabolites and to advise to the extension section.

MATERIALS AND METHODS

The experiment was conducted at the Biotchnology Laboratory of Azarbijan Shahid Madani University in Tabriz, Iran. The seeds were collected from the Varzegan and Miyaneh (from Northwest of Azerbaijan province), in September 2015.

Table 1: Geographical coordinates and climates condition of Varzegan and Mieaneh cityes.

City	Geographical coordinates	Rainfall and relative humidity	Elevation above sea level (m)	Maximum and minimum temperature	The number of frost month
Varzegan	Latitude: 38°42′N	Rainfall. 350mm	1607	+33 oc	6 month
	Longitude: 47°17′ E	RH. 52-85%		-22oc	
Miyaneh	Latitude: 37°42′ N	Rainfall. 320mm	750	+37 oc	4 month
	Longitude: 47°42′E	RH. 68%		-12oc	

Seeds were soaked in warm water (40°C) over night and treated with sulfuric acid (H2SO4; 95%) for 1 hour and immediately we rinsed several times with distilled water were used for in vitro culture. Seeds were surface sterilized with 40% sodium hypochlorite for 20 minutes and then rinsed with sterile-water for 3 times. Seeds were germinated in hormone free medium. Single nodes were taken from the plants. Single nod cutting were transferred in to MS medium containing 0.1 mgl^{-1} GA3, 0.1 mgl^{-1} IBA and supplemented cytokine (BAP and Zeatin) with different levels (0,1.5 and 3 mgl^{-1}). Ten explants were cultured in each tube in 3 replications. Cultures were incubated at 25-270c under fluorescent light for 16 h daily. Shoot multiplication was conducted on the same medium with shoot segment having 2-3 nodes. Every seven-week explants were sub cultured at the same medium (samples were sub cultured for two times). For rooting, two media types (½MS and MS) and two auxine types (NAA and IBA) with three levels (0, 1 and 2 mgl^{-1}) were employed. For each treatment, 10 cuttings were as experimental units with 3 replications were employed. Data for rooting traits were collected 34 days after culture. Data were analyzed based on Factorial Experiment based on RCBD by SPSS and MSTATC software. Mean comparison was performed using the Least Significant Difference (LSD) methods. Rooted plantlet (2.5-4cm) were hardened in growth chamber and then transferred to the pot containing sterilized organic compost.

Chemicals: PGRs: Inole-3- butric acid (IBA), Gibbrellic acid (GA3), ?-naphthaleneacitic acid (NAA), 6- Benzylaminopurin (BAP) and Zatin purchsed from duchefa (Netherlands).

RESULTS AND DISCUSSION

A. In vitro propagation of C. spinosa L.

Cytokine type (BAP and Zeatin) had significant effect on shoot number and length as well as node number (Table 2 and 3). The results showed that Zeatin had more significant effect on the above mentioned traits (Table 2). The results showed that adventitious shoot formation in plantlets was highly influenced by the Cytokine levels (Table 2 and 4). Cytokine amounts had positive effects on shoot multiplication rate, shoot number, shoot length and node number (Table 4).

Table 2: ANOVA for the effects of cultivar and different concentrations of BAP and Zeatin on proliferation rate, shoot number, shoot length and nods number of in vitro grown *C. spinosa* L.

Source of variation	df	proliferation rate	Shoot number	Shoot length	Nods number
cultivar	1	0.002^{ns}	0.52^{*}	1.57^{**}	0.82^{*}
Cytokine type	1	0.230^{**}	2.73^{**}	1.09^{**}	2.62^{**}
cultivar× Cytokine type	1	0.009^{ns}	0.0001^{ns}	0.10^{ns}	0.09^{ns}
Cytokine concentration	2	1.003^{**}	12.29^{**}	6.66^{**}	5.47^{**}
cultivar× Cytokine concentration	2	0.013^{ns}	0.22^{ns}	0.195^{ns}	0.46^{ns}
Cytokine type ×Cytokine concentration	2	0.52^{ns}	0.36^{*}	0.052^{ns}	0.22^{ns}
cultivar ×Cytokine type ×Cytokine concentration	2	0.032^{ns}	0.092^{ns}	0.22^{ns}	0.11^{ns}
Error	24	0.024	0.071	0.72	0.154
C.V.%		9.46	11.19	12.11	16.02

ns,* and ** show non significant and significancy at P?0.05 and P?0.01, respectively.

Table 3: Mean comparison for the effects of BAP and Zeatin on shoot number, shoot length and nods number of in vitro grown *C. spinosa* L.

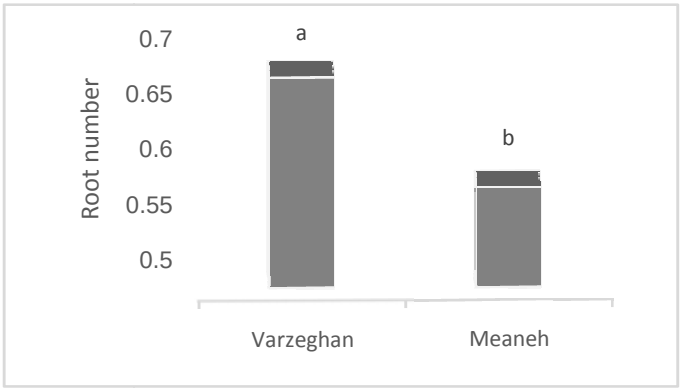
Cytokine type	Shoot number	Shoot length (mm)	Nods number
BAP	1.22[b]	1.2[b]	1.38[b]
Zeatin	1.77[a]	1.66[a]	1.82[a]
LSD%	0.06	0.09	0.08

Similar letters in the column show non-significancy based on LSD test.

Table 4: Mean for the effects of cytokine concentration on proliferation rate, shoot number, shoot length and nods number in vitro grown *C. spinosa* L. in vitro

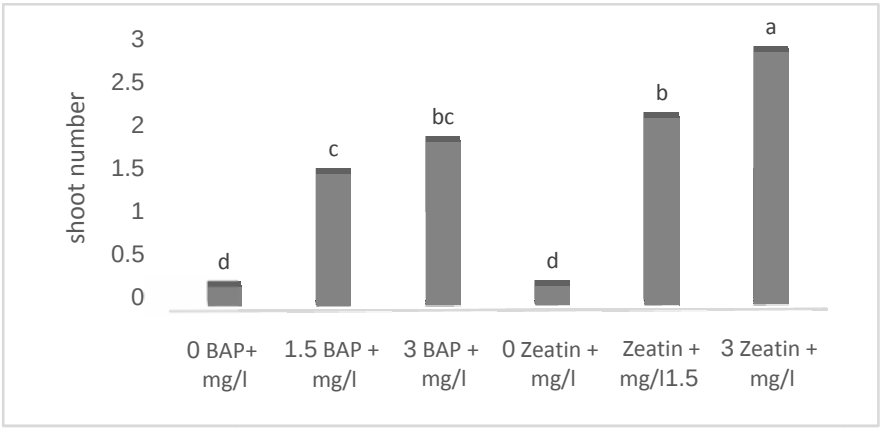
Cytokine concentration	Proliferation rate (%)	Shoot number	Shoot length (mm)	Nods number
0	0.35[b]	0.22[c]	0.52[c]	1.03[b]
1.5	0.79[a]	1.85[b]	1.55[b]	1.48[b]
3	0.82[a]	2.42[a]	2.22[a]	2.30[a]
LSD%	0.053	0.068	0.11	0.101

Similar letters in the column show non-significant based on LSD test.



Letters in the column show significant difference based on LSD test.

Fig 1. Mean comparison for the effects of cultivars (Varzegan and Miyaneh) on shoot number in vitro grown *C. spinosa* L.



Similar letters on the column show non-significant based on LSD (1%) test.

Fig. 2. Mean comparison for the effects of BAP, Zeatin on shoots and nods number of in vitro grown *C. spinosa* L.

There was significant difference between two cultivars considering proliferation rates and Varzegan in nearly all the traits was superior to Miyaneh (Fig. 1). Overall, there was meaningful difference in proliferation rate using BAP and Zeatin (Fig 2). Zeatine was the compound of choice shoot number (Fig. 2). In vitro growth and development of *C. Spinosa* L. plants were highly influenced by the concentration of growth regulators that were supplemented to the culture medium (Fig. 2). The plant multiplication rate (shoot and node number) in the control plant were the last (Table 4). The greatest shoot and node per explant number was obtained on medium supplemented with 3 mgL^{-1} of cytokinin (Table 4). Shoot proliferation was responded positively and the highest number was recorded at 3 mgL^{-1} Zeatin (Fig. 2). Al-Mahmood (2012) reported that successful in vitro multiplication of *C. spinos* was achieved on MS medium supplemented with BAP at 0.8 mgL^{-1}. Musallam *et al*., (2011) note that WPM was the best medium for the establishment of mother plant and the multiplication rates were obtained on WPM supplemented with 0.8 mgL^{-1} Kinetin in combination with 0.05 mgL^{-1} IBA and 0.1 mgl^{-1} GA3.

B. In vitro root formation

Root initiation was accrued from the base of the shoot, on auxin containing medium. Rooting was derived without the calli formation. The results showed significant difference between two cultivars on roots number, and Varzegan had the highest root numbers (Fig. 3).

The effect of treatment (auxin type and levels) and media had positive effects on root number (Table 5) and (Table 6) but it didn't have significant effect on rooting percentage and roots length. a very low root initiation was observed in auxin deprived medium (Table 6). The MS medium with 2 mgl^{-1} NAA increased root number (Table 6). Medium type (MS and ½ MS) had significant effect on root formation. Eventually In vitro root formation of *C. spinosa* L. CV. Varzegan was successful on MS medium (Table 7). Medium supplemented with 2 mgL^{-1} of NAA was the best for rooting (Table 8). The result also indicated that with increasing auxin concentrations, rooting percentage was increased (Table 8). The results supports similar idea reported by Aubo-khalaf and Arafeh (2015) where there was evidence of root induction on MS medium containing 1mgl^{-1} NAA for *C. Spinosa* L.

Musallam *et al*., (2011) showed that high rooting frequency (80%) was obtained on ½ MS medium supplemented with 5 mgL^{-1} IAA. There was no meaningful difference in root number between IBA and NAA. NAA and IBA commercially have been tried for the enhancement of cuttings rooting potential and for to increase rooting percentage. Besides, IBA at different concentrations gave lower rooting initiation rate (Table 8). This could be could be attributed to the factors like genetic variation between caper, and to the exogenous factor employed with invitro cultures and culture manipulations (Abu-khalaf and Arafeh, 2015). Adventitious roots formation and emergence at the bottom of cuttings are vital developmental phenomen in the survival of the young plants. The main developmental stages in the adventitious roots formation in plants are induction, initiation and the roots growth. The timing for the mentioned stages is quite different with diverse plant taxon's ranging from some hours (in mung beans) up to several weeks in Camelia (Kollarova, 2005). Adventitious roots formation is stimulated by auxins and the response to auxin and its role in controlling the roots formation and their length and number are quite interesting (Yan *et al*., 2014). Roots primordia formation in cutting is dependent upon the internal auxin content and some synergistic compound such as diphenyls. These compounds stimulate the related RNAs biosynthesis and hence improve the roots primordia initiation (Henrique *et al*., 2006).

Table 5: ANOVA for the effects of cultivar, media and treatment auxin concentration on rooting traits of *Capparis spinosa* L.

Source of variation	df	Rooting (%)	Root number	Root length (mm)
Cultivar	1	0.035^{ns}	0.143^{*}	0.04^{ns}
Medium	1	0.077^{*}	0.674^{**}	0.58^{**}
Cultivar× Medium	1	0.0009^{ns}	0.032^{ns}	0.005^{ns}
Treatment (NAA and IBA, concentration)	4	0.905^{**}	0.543^{**}	0.77^{**}
Cultivar× Treatment	4	0.005^{ns}	0.026^{ns}	0.01^{ns}
Medium× Treatment	4	0.003^{ns}	0.288^{**}	0.162^{ns}
Cultivar ×Medium× Treatment	4	0.007^{ns}	0.02^{ns}	0.008^{ns}
Error	40	0.014	0.025	0.051
C.V.%		7.94	9.56	15.01

ns,* and ** show non-significant and significant at P?0.05 and P?0.01, respectively.

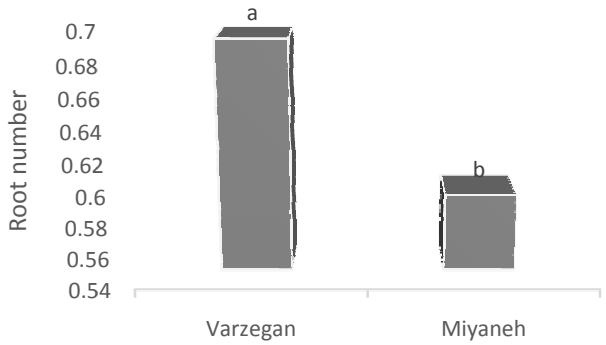


Fig. 3. Mean comparison for the cultivars effects on root number of *Capparis spinosa* L. Similar letters on the bars show non-significant based on LSD (1%) test.

Table 6: Mean comparison for the effects of media and Auxin type × concentration on root number of *Capparis spinosa* L.

Medium	Treatment	Root number
1/2MS	0	0.1^{e}
1/2MS	IBA+1mg/l	0.46^{de}
1/2MS	IBA+2mg/l	0.99^{b}
1/2MS	NAA+1mg/l	0.58^{cd}
1/2MS	NAA+ 2mg/l	0.67^{bcd}
MS	0	0.17^{e}
MS	IBA+1mg/l	0.69^{bcd}
MS	IBA+2mg/l	0.92^{bc}
MS	NAA+1mg/l	0.73^{bcd}
MS	NAA+ 2mg/l	1.57^{a}
LSD 1%		0.348

Similar letters in the column show non-significant based on LSD test.

Table 7: Mean comparison for the effects of basal media type on rooting traits of *Capparis spinosa* L.

Medium	Rooting (%)	Root number	Root length (mm)
1/2MS	0.46^{b}	0.56^{b}	0.42^{b}
MS	0.53^{a}	0.81^{a}	0.63^{a}
LSD%	0.0584	0.0941	0.146

Similar letters in the column show non-significant based on LSD test.

Table 8: Mean comparison for the effects of different Auxin type and concentrations on rooting traits of *Capparis spinosa* L.

Treatment	Rooting (%)	Root number	Root length (mm)
control	0.05^{d}	0.13^{c}	0.13^{b}
IBA+1mg/l	0.46^{c}	0.58^{b}	0.45^{ab}
IBA+2mg/l	0.62^{b}	0.95^{a}	0.73^{a}
NAA+1mg/l	0.52^{bc}	0.66^{b}	0.52^{a}
NAA+ 2mg/l	0.81^{a}	1.12^{a}	0.80^{a}
LSD%	0.139	0.246	0.336

C. Acclimatization

Rooted plant, when moved to acclimatization condition showed 60% survival rate. Plant resumed normal growth in the greenhouse.

Transfer of the sterile rooted plantlets to the greenhouse condition is a critical step. Rooted plantlets first were transferred in a plastic pot and for 10 days were kept in growth chamber.

Fig. 1. Micropropagation of *C. spinosa* L. Vazegan. A: Seeds on MS medium, B: multiplication of shoot on MS medium supplemented with Zeatin C: Root formation on MS medium supplemented with auxin.

To maintain high relative humidity, the pots covered with plastic bags, Relative humidity was reduced gradually and complete removal of plastic bag took place after 10 days. At the end of second week, the pots were transferred to greenhouse.

CONCLUSION

Due to the mechanical and possibly physiological dormancy, germination percentage and rate in *Capparis spinosa* is very low. Using cuttings for the propagation bears many more problems due to hard rooting nature of the plant. Tissue culture methods would be accessible propagation were for this high value crop. The tissue culture protocol employed in the present experiment gave us promising results. The overall results revealed that in between the clones studied, there was a meaningful differences considering proliferation and rooting rate. The highest shoots number and length as well as root number was belonged to ?Varzegan¸ cultivar. BAP had mild effect on the proliferation rate, In contrast; Zeatin was a good choice for the proliferation of Capparis spinosa. The highest rooting percent was recorded in MS medium containing 2 mgl^{-1} NAA. The data obtained clearly emphasize that, tissue culture and using the right hormonal combination can be tried for the multiplication and possibly mass propagation of this precious native medicinal plant.

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