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A Comparison of Different Propagation Methods of Common Caper-bush (*Capparis spinosa* L.) as a New Horticultural Crop

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ABSTRACT

Erosion along with degraded soil and scarcity of water are serious threat to agriculture in Iran. Plants with the ability to complete their life cycle under these conditions could contribute in stemming erosion by establishing a good green conservative cover. Finding these plants may be among the priorities of eco-agriculture. Rehabilitation of these areas with these plants would require efficient propagation and successful establishment. *Capparis spinosa* L. (caper-bush) is commonly distributed in such areas. Therefore two experiments were conducted in Shiraz University, Iran, during 2005-2007 to investigate its sexual and vegetative propagation. Matured seeds were collected from natural vegetation and were treated with concentrated sulfuric acid and different concentrations of gibberellic acid (GA_3). For vegetative propagation tests, leafy and leafless semi-hardwood cuttings, with a length of 15 cm, were treated with various concentrations of indole-3-butyric acid (IBA) and/or naphthaleneacetic acid (NAA). Acid scarification was a necessary factor for water imbibition, but not for seed germination *per se*. The highest germination percentage (60.2%) was obtained by soaking seeds for 30 min in concentrated sulfuric acid followed by 90 min in a 200 mg l^{-1} solution of GA_3. The highest rooting equal to 67.1 and 61.4% were obtained with 6000 and 9000 mg l^{-1} IBA in leafy semi-hardwood cuttings. Our results suggest that stimulation of rooting in cuttings is a rapid and successful method for propagation of caper bush.

Keywords: concentrated sulfuric acid, germination, gibberellic acid, indolebutyric acid, naphthaleneacetic acid, rhizogenesis, rooting

INTRODUCTION

Many parts of Iran have an arid and semi arid climate, with low annual precipitation. The large area of its lands consisted of calcareous, saline, and sodic soils, subjected to erosion. Thus, existence of tolerant plants to these harsh conditions is very important in respect to success and sustainability of agriculture as well as water economy. Furthermore each year, high volume of valuable soil is degrading due to various kinds of erosion and large volume of rainfall water is losing without any beneficial usage. Accordingly, those plants that can persist under these adverse climates and prevent soil erosion are very valuable to conserve water and soil resources. Caper-bush (*Capparis spinosa* L.) is a saline- and drought-tolerant plant that can be used for rehabilitation of rangelands and desert lands, prevention of soil erosion and dune stabilization. Concerning of many characters such as proper growth in poor nutrient soils, adaptability to low input systems, its low input (water and fertilizer) requirements for establishment, resistance to pests and diseases, salt- and drought-tolerance, and alleviating weeds growth due to dense rooting system and extended canopy, it can be considered as a useful crop in low-input agriculture (Rhizopoulou and Psaras 2003; Trewartha and Trewartha 2005).

Caper is a perennial, deciduous, spiny bush with deeply and large extensive root system which its branches are more often seen hanging, draped and sprawling (Barbera and Di Lorenzo 1984). Caper cultivation as a special crop for its edible buds has been drawn interests in some European countries (Barbera 1991). *C. spinosa* is said to be native to the Mediterranean basin, but its dispersal range stretches from the Atlantic coasts of the Canary Islands and Morocco to the Black Sea to the Crimea and Armenia, and eastward to the Caspian Sea and into Iran (Negbi *et al*. 1966; Barbera and Di Lorenzo 1984), and in Iran it has spread throughout the country (Zargary 1992; Sabety 2003).

The most common and simplest method of caper propagation is by seed. Because propagation by seed leads to segregation, there is a chance to obtain new varieties which predominate over existent ones. If a superior variety could simultaneously be obtained, or there be a well-known variety adapted to a given area, it can propagate via vegetative propagation; still it should be noticed that many varieties show poor rhizogenesis in vegetative propagation (Hamilton and Carpenter 1975). Caper plants which grow from new seeds do not develop perfectly and in general less than 30% of new seeds germinate (Sozzi and Chiesa 1995). It has been observed that only 5% of seeds germinate during two to three months after sowing of caper seeds (Barbera and Di Lorenzo 1984). Bond (1990) reported that during 10 days, only 10% of new caper seeds sown in pots germinated. It has been determined that by applying concentrated sulfuric acid, gibberellic acid (GA_3) and KNO_3 caper seed germination could be improved (Orphanos 1983; Sozzi and Chiesa 1995). Caglar *et al*. (2005) reported that utilization of indole-3-butyric acid (IBA) increases rhizogenesis of caper cuttings.

In order to promote cultivation of ground cover crops in rangelands and deserts, the constraints against their propagation and establishment should be eliminated. Herein, overcoming seed dormancy and determination of the most rapid and efficient method for their propagation is the main priority. The present study was aimed to assess caper seed dormancy, finding the most proper approach to eliminate seed dormancy using chemicals and plant growth regulators,

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investigate proper cuttings for vegetative propagation and determine the effects of growth regulators on rhizogenesis of caper cuttings.

MATERIALS AND METHODS

This study was conducted as two separate experiments on vegetative and sexual propagation of caper in the greenhouse and laboratory at the Faculty of Agriculture, Shiraz University, Iran, during 2005-2007. The caper seeds and cuttings required for this study were provided from stock plants in Farashband, Fars Province (Southeast of Iran, 28° 87′ N, 52° 08′ E). The sexual propagation test was arranged as a factorial experiment based on a completely randomized design with four and seven replications in sexual and vegetative material, respectively. Each treatment included 100 seeds (total of 400 for four replications) which were scarified using concentrated sulfuric acid (CSA) for 20, 30, and 40 min, along with a non-scarified treatment as control. After acid scarification, seeds were soaked for 90 min in GA_3 solutions prepared as 0, 100, 200, 300, and 400 mgL^{-1}. Then, seeds were disinfected superficially using Clorox (sodium hypochlorite, 0.5%) for 15 min followed by treating with a benomyl solution (2 gL^{-1}) for 25 min. Finally, seeds were put between two layers of filter paper in two Petri dishes (50 seeds each) to germinate in a germinator in the dark at 25 ± 2°C (Sozzi and Chiesa 1995). Germination properties were recorded at 48 h intervals for 32 days. Seeds with a radicle equal to 2 mm were scored as having germinated and the length of radicle and plumule, and seedling dry weight was determined at the end of experiment. Germination Percentage (GP) was calculated as sum of germinated seeds during experiment and germination rate (GR), mean time of germination (MTG), and germination index (GI) were determined using the following equations (Agrawal and Dadlani 1992):

$$GR = \sum \frac{n_i}{t_i} \quad [1]$$

$$MTG = \sum (n_i \times t_i) \Big/ \sum n \quad [2]$$

$$GI = \sum (n_i \times t_i) \Big/ S \quad [3]$$

where t_i, n_i, Σn and S indicate days after the beginning of the experiment, number of germinated seeds at t_i, total germinated seeds, and total seeds number, respectively. To measure radicle and plumule length, 13 seedlings were selected randomly from each treatment and after measuring, were put in oven at 70°C for 48 h and then weighed (Agrawal and Dadlani 1992; ISTA 1995, 2002).

In the vegetative propagation test, two plant growth regulators (auxins), namely IBA and α-naphthaleneacetic acid (NAA) at 3000, 6000, and 9000 mgL^{-1} were used to stimulate rhizogenesis in cuttings using a quick-dip method (Moallemi and Chehrazi 2004). All growth regulators used in both experiments had 99% purity (Sigma Chemical Co.). Each treatment was replicated seven times with 10 cuttings in each replication. Leafy and leafless semi-hardwood cuttings 15 cm in length were prepared in April 2006. The cuttings' rooting bed, a mixture of one third moss peat and two-thirds sand, was equipped with intermittent mist and bottom heat systems (Humax Co., UK). The measured traits were percentage rhizogenesis and root number, length, diameter and dry weight, all determined 27 days after the beginning of the experiment.

Data were analyzed statistically using SAS Package (V8) and the mean comparisons were done with Duncan's multiple range test at P = 0.01. n = 60 for sexual propagation study: 15 treatments (3 CSA × 5GA) × 4 replications; n = 42 for vegetative propagation study: 6 treatments (IBA and NAA hormones × 3 concentrations for each) × 7 replications.

Table 1 Properties of caper seed germination as affected by main and interaction effects of different subjecting times to concentrated sulphuric acid (CSA) and different concentrations of gibberellic acid (GA_3).

Scarifying time with CSA (min)	GA_3 concentration ($mg\ L^{-1}$)	Germination percentage (%)	Germination rate (germinated seeds/day)	Mean germination time (days)	Germination index	Seedling dry weight (mg)
0	0	5.5 h	0.3 j	19 a	1.0 g	33.7 fg
	100	6.0 h	0.4 j	17 ab	1.0 g	33.5 fg
	200	6.3 h	0.4 j	19 a	1.2 g	33.3 g
	300	6.3 h	0.4 j	18 ab	1.1 g	34.5 efg
	400	6.0 h	0.4 j	18 ab	1.1 g	31.9 g
20	0	23.8 g	1.4 i	19 a	4.5 f	36.2 def
	100	29.8 f	1.9 gh	18 ab	5.3 ef	34.6 efg
	200	40.3 cd	2.5 ef	18 ab	7.3 c	37.0 de
	300	38.5 de	2.3 efg	18 ab	7.1 c	36.7 de
	400	36.8 de	2.2 fg	18 ab	6.7 cd	36.9 de
30	0	43.3 c	2.7 de	18 ab	6.7 c	40.3 bc
	100	50.5 b	3.2 c	18 ab	7.7 b	42.5 ab
	200	60.2 a	3.8 ab	18 ab	9.1 a	40.7 bc
	300	57.5 a	3.6 b	18 ab	10.9 a	43.9 a
	400	59.3 a	4.1 a	17 ab	10.4 a	41.8 ab
40	0	27.3 fg	1.7 hi	18 ab	4.9 ef	40.8 bc
	100	35.0 e	2.5 ef	16 b	5.7 de	41.0 bc
	200	44.0 c	2.9 cd	18 ab	7.7 c	40.8 bc
	300	44.0 c	2.9 cd	18 ab	7.7 c	39.7 bc
	400	44.0 c	2.9 cd	17 ab	7.5 c	38.3 cd
	0	24.9 c	1.5 c	18.5 a	4.6 c	37.8 ab
	100	30.3 b	2.0 b	17.4 b	5.3 b	37.9 ab
	200	37.7 a	2.4 a	18.2 ab	6.8 a	38.0 ab
	300	36.6 a	2.3 a	18 ab	6.6 a	38.7 a
	400	36.5 a	2.4 a	17.7 ab	6.3 a	37.2 b
0		6.0 d	0.4 d	18.2 ab	1.1 d	33.4 d
20		33.8 c	2.1 c	18.4 a	6.2 c	36.3 c
30		54.2 a	3.5 a	17.8 ab	9.6 a	41.9 a
40		38.9 b	2.6 b	17.3 b	6.7 b	40.1 b

For each main and/or interaction effects, there is a significant difference between means with similar letter in each column at P = 0.01.

Table 2 Main and interaction effects of different GA_3 concentrations and time of subjecting to concentrated sulphuric acid (CSA) on length of radicle and plumule of caper seedlings.

Organ	Scarifying time with CSA (min)	Gibberellic acid (GA_3) (mgL^{-1}) 0	100	200	300	400	Mean
Radicle	0	6 ef	5 fg	6 ef	7 de	4 g	6.0 C
	20	7 de	5 fg	6 ef	5 fg	5 fg	6.0 C
	30	8 cd	10 ab	8 cd	11 a	9 bc	9.0 A
	40	7 de	8 cd	8 cd	8 cd	6 ef	7.4 B
Plumule	0	34 hi	35 hi	35 ghi	36 ghi	32 i	34.4 D
	20	41efg	39 fgh	44 def	45 de	44 def	42.6 C
	30	54 bc	55 ab	54 ab	60 a	56 ab	56.0 A
	40	55 ab	55 ab	54 ab	51 bc	48 cd	53.0 B
	Radicle	46 AB	46 AB	47 AB	48 A	45 B	
	Plumule	7 A	7 A	7 A	7 A	6 B	

For each main and interaction effect, means with common letters are not different significantly at P = 0.01.

RESULTS

Sexual propagation

Results showed that eliminating physical seed dormancy by different CSA concentrations enabled caper seeds to germinate, and GA_3 had an enhancing role in germination of this species. It seems that treating seeds with CSA for more than 30 min caused seeds to be damaged and GA_3 concentrations more than 200 mgL^{-1} did not improve caper germination significantly (**Table 1**).

Evaluation of caper seed germination under interaction of different CSA and GA_3 concentrations indicated that treating seeds with CSA for 30 min and then dunking them in GA_3 solutions with 200, 300 or 400 mgL^{-1} led to the highest germination. Also, the maximum GR was obtained with 30 min CSA plus 200 and 400 mgL^{-1} GA_3, the lowest MTG (16 days) with 40 min CSA plus 100 mgL^{-1} GA_3, and the best GI with 30 min CSA plus 200, 300 or 400 mgL^{-1} GA_3 (**Table 1**). According to these results, while subjecting seeds to CSA for 30 min in combination with some GA_3 improved all germination properties, but applying GA_3 alone had no effect on the germination progress if seeds were not pre-treated with CSA.

Similar to germination, the highest seedling dry matter was obtained from interaction of 30 min CSA plus 400 mgL^{-1} GA_3. The main effect of these two levels of treatments also resulted in the highest seedling dry matter (**Table 1**). Plumule and radicle also showed the highest length after scarifying seeds for 30 min with CSA and then treating with GA_3 at 300 mgL^{-1}. Increase of GA_3 concentration to 400 mgL^{-1}, especially when seeds were not treated with CSA previously, had an inhibitory effect and led to the shortest plumule and radicle (**Table 2**).

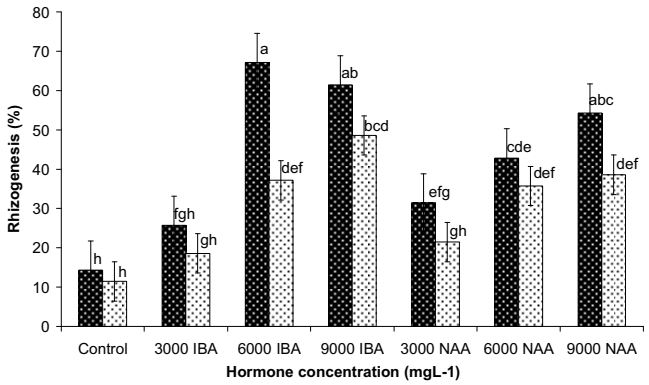

Fig. 1 Effect of different concentrations of indole-3-butyric acid (IBA) and α-naphthaleneacetic acid (NAA) on rhizogenesis percentage of leafy (dark bars) and leafless (light bars) cuttings of caper. Non-similar letters on bars show significant differences at P = 0.01.

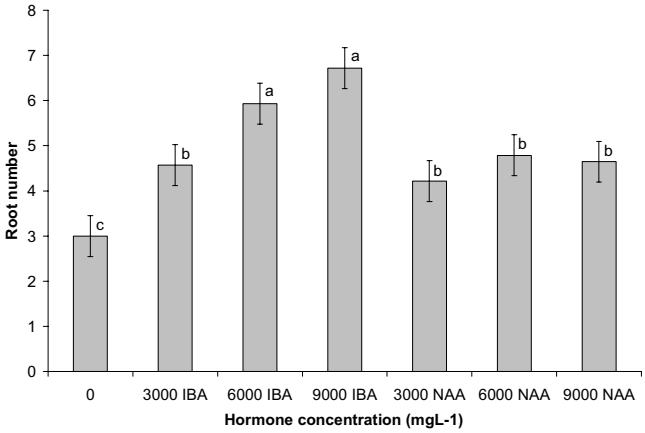

Fig. 2 Effect of different concentrations of indole-3-butyric acid (IBA) and α-naphthaleneacetic acid (NAA) on roots number of caper cuttings. Non-similar letters on bars show significant difference at P = 0.01.

Vegetative propagation

We observed significant difference between leafy and leafless cuttings in rhizogenesis percentage among different treatments (results not shown). **Fig. 1** illustrates the effects of different hormone concentrations on rhizogenesis. Leafy cuttings which were treated with 6000 and 9000 mg IBA L^{-1} showed the highest rhizogenesis percentage. The percentage of rooted cuttings did not differ significantly between leafy and leafless cuttings in control (**Fig. 1**).

Number of produced roots did not differ significantly between leafy and leafless cuttings, and also in response to interaction between cuttings types and different concentrations of auxins. Effect of different auxin concentrations on number of produced roots indicates that the highest root numbers formed in cuttings which were treated with 6000 and 9000 mg IBA L^{-1}, significantly different to control cuttings (**Fig. 2**).

There was no significant difference in root length between leafy- and leafless cuttings. Treating cuttings with 3000, 6000, and 9000 mg L^{-1} IBA or 600 mg/L NAA resulted in significantly increased length of produced roots over control ones (**Fig. 3**). A significant interaction was observed between type of cuttings and different concentrations of auxins, as leafy cuttings treated with 6000 and 9000 mg L^{-1} IBA produced the longest radicles (**Fig. 4**).

There was no significant difference between leafy and leafless cuttings, nor in interactions between type of cuttings and different auxin concentrations with respect to root number. **Fig. 5** illustrates the effects of various auxin concentrations on root diameter of caper cuttings. It is obvious that all auxin concentrations had a similar effect on root diameter, significantly different from the control ($p<0.01$).

There was a significant difference between leafy and leafless cuttings in root dry matter. Effects of different IBA

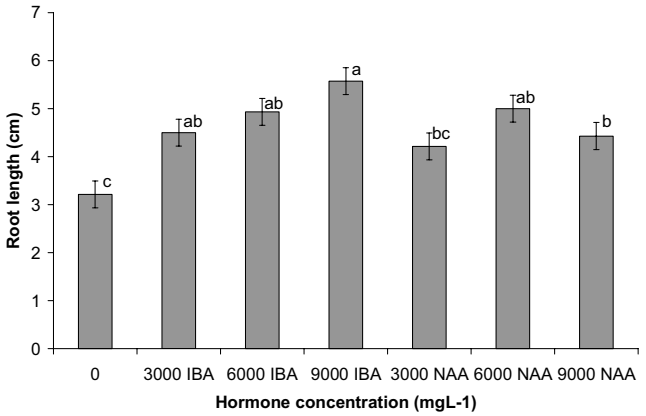

Fig. 3 Response of root length of caper cuttings to different concentrations of indole-3-butyric acid (IBA) and α-naphthaleneacetic acid (NAA) on roots number. Non-similar letters on bars show significant difference at P = 0.01.

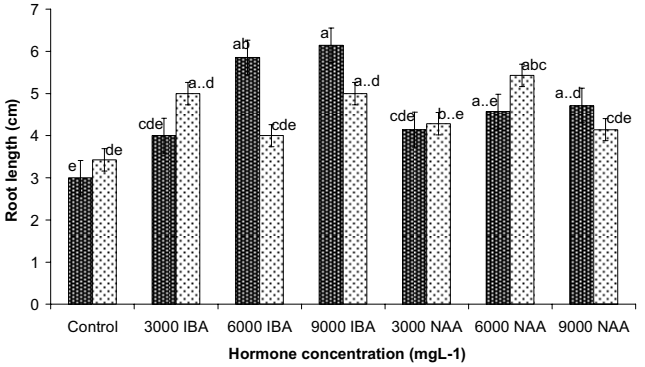

Fig. 4 Effect of different concentrations of indole-3-butyric acid (IBA) and α-naphthaleneacetic acid (NAA) on root length of leafy (dark bars) and leafless (light bars) cuttings of caper. Non-similar letters on bars show significant difference at P = 0.01.

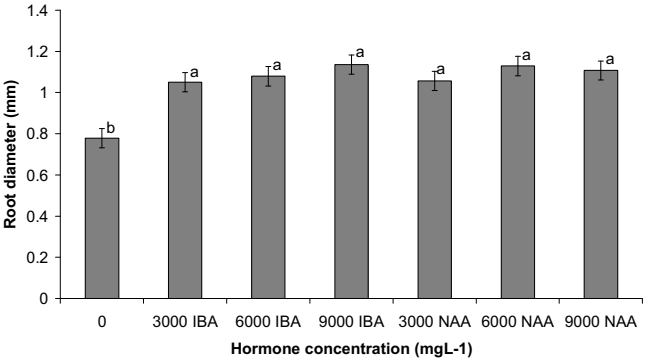

Fig. 5 Response of root diameter of caper cuttings to different concentrations of indole-3-butyric acid (IBA) and α-naphthaleneacetic acid (NAA). Non-similar letters on bars show significant difference at P = 0.01.

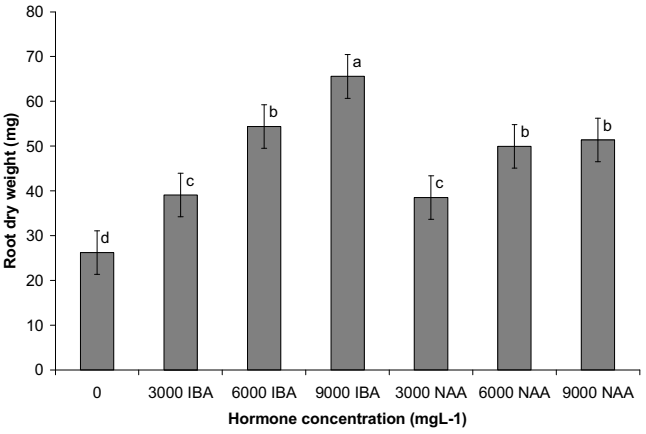

Fig. 6 Effect of different concentrations of indole-3-butyric acid (IBA) and α-naphthaleneacetic acid (NAA) on root dry weight of caper cuttings. Non-similar letters on bars show significant difference at P = 0.01.

and NAA concentrations on root dry matter of caper cuttings are illustrated in **Fig. 6**. The highest root weight was produced by cuttings that were treated by 9000 mg L^{-1} IBA, which was significantly different to other hormonal treatments as well as control. However, interactions between different hormone concentrations and type of cuttings had no significant effect on root dry weight.

DISCUSSION

In general, there are two primary types of dormancy: embryo dormancy and that induced by the seed coat (testa). It is unlikely that the inability of caper seeds to germinate arose from embryo dormancy (Bewley and Black 1982, 1985). It has been proved that removing some parts of the testa leads to the highest germination stimulation in caper seed by effective treatments (Sozzi and Chiesa 1995).

According to results of this study, scarification is the most crucial step to eliminate seed dormancy in caper species. It should be noted, however, that applying this treatment is essential, but not sufficient. The influence of scarification with CSA on seed germination can be regarded in relation to seed coat impenetrability. Some studies (e.g. Orphanos 1983; Sozzi and Chiesa 1995) have shown that caper seeds have impenetrable seed coat, which is in agreement with our findings. Although caper seeds need GA_3 to stimulate germination, their embryo is not dormant. Dormancy in these seeds is induced preliminary by an impenetrable seed coat and next by endosperm (Orphanos 1983). The seeds of *Capparis* spp. are bi-layered and their cell walls are lignified (Sozzi and Chiesa 1995), and only the mesophyll settles between the inner and outer parts of the tegument, which is not lignified (Corner 1976). Because the integrity of the seed coat performs an important role in persistence of dormancy in caper seeds, it has been supposed that seed coverings (with consecutive lignified structures) are the main reason for hindrances in caper seed germination (Sozzi and Chiesa 1995).

According to the above, there is a close correlation between caper seed germination and seed covering structure. There is evidence that GA_3 in some other species improves germination by affecting microscopic apertures of the endosperm (Bewley *et al.* 1983; Groot and Karssen 1987; Groot *et al*. 1988). In our study, caper seeds became sensitive to GA_3 only after treating with CSA. Therefore, it seems that preliminary control of germination in caper seed is induced by the seed coat and that GA_3 acts as a stimulator only after breaking seed coat. GA_3 treatments are required to promote germination, because generating of mucilage on seed coat, especially when there is plentiful water in medium, has been regarded as a restraint for germination enhancement, because it acts as an oxygen barrier (Witztum *et al.* 1969). The structure of the seed and development of mucilage when seeds are placed in water can restrict oxygen diffusion to the embryo. In this case, scarification of the seed coat is not sufficient to supply the embryo with adequate oxygen and the role of GA_3 is to provide the embryo with oxygen under this condition (Groot *et al*. 1988). GA_3 elicits reduction of oxygen requirements for seed germination (Negbi *et al*. 1966). Our work describes the response of caper seeds to physical and chemical factors and indicates the profitability of using CSA followed by applying GA_3 to break seed dormancy and stimulate its germination in caper plants. However, much attention should be paid to the concentration of applied GA_3 and the time of floating in CSA to avoid seed damage and to obtain the best results. It seems likely that under natural conditions, the seed coat is scarifying by microbial activity and climatic factors during winter until seeds

could germinate in spring (Clark *et al*. 1997). For example, it has been shown that seeds of *Drosera* species are dormant at maturity and require cold stratification for dormancy break. Seeds of *D. rotundifolia* mostly germinate in May (spring) in Sweden (Redbo-Torstensson 1994), indicating that dormancy break occurs during winter. Dormancy break in seeds of *D. anglica* also occurs during winter when environmental conditions in habitats that are not favorable for seedling establishment (Baskin *et al*. 2001).

The treatments which were used to stimulate rhizogenesis in cuttings showed the higher ability of auxins in generating adventitious roots in cuttings of this species. Results of this study indicated that hormone usage enhanced rhizogenesis up to 55%, which is in agreement with the results of Caglar *et al*. (2005). Leafy and leafless cuttings responded differently with respect to rhizogenesis percentage, as leafy cuttings treated with 6000 and 9000 mg L^{-1} IBA showed highest rhizogenesis, 67.1 and 61.4%, respectively. The study of Moalemi and Chahrazi (2004) on *Bougainvillea spectabilis* also proved that there is a positive correlation between rhizogenesis percentage of cuttings and the number of cutting's leaves. It is known in pears (*Pyrus communis* var. Home-old) that when leafy cuttings were treated by 4000 mg L^{-1} IBA, production of adventitious roots was more than non-treated leafy cuttings (Khoshkhui 1996).

As we observed, IBA affected root number per cutting more than NAA, and the highest root number was produced by cuttings which were treated by 6000 and 9000 mg L^{-1} IBA. Also we found that application of IBA and NAA leads to a significant increase of root length of cuttings than the control with no hormonal application. Al-Saqri and Alderson (1996) demonstrated that the effect of IBA on root length in *Rosa centifolia* is dependent on the type of rooting media.

In general, results of this study indicated that overcoming caper seed dormancy is possible by using CSA to make seeds penetrable and then applying GA_3 to stimulate more germination. In the case of vegetative propagation, if leafy caper cuttings are used and IBA is applied to the base of cuttings, not only rhizogenesis percentage, but also some other traits such as root length and dry weight can increase more than the control, leading to successful, optimal establishment of caper plants. It is obvious that in the case where caper is used in rangeland rehabilitation and construction of a green cover against erosion, exploiting vegetative propagation will lead to more rapid establishment with higher efficiency. However, in the context of potential use of this species as an option for renovation of degraded lands and construction of hand-planted ranges, and also to restrain erosion intrusion of agricultural lands and to conserve valuable agricultural soil resources, there is a need to investigate more about the ecology and physiology of caper seed and its optimal establishment in natural areas.

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