Pak. J. Bot., 44: 225-230, Special Issue May 2012.

ROLES OF DURATION AND CONCENTRATION OF PRIMING AGENTS ON DORMANCY BREAKING AND GERMINATION OF CAPER (*CAPPARIS SPINOSA* L.) FOR THE PROTECTION OF ARID DEGRADED AREAS

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Abstract

Caper *Capparis spinosa* has deep roots, a drought tolerant species, and produces a satisfactory vegetative cover which protects soils from erosion; it can be highly useful for the prevention of land degradation. It is endangered species in its natural habitats in Iran and many other countries due to climate change and overutilization. Domestication of capers as medicinal, vegetable or soil surface coverage plant is complicated by limited and variable seed germination under artificial conditions. In order to examine the role of different levels of KNO_3 (0, 500, 1000, 2000, 4000 and 8000 ppm), gibberellic acid (GA_3) (0, 50,100, 250, 500, 1000 and 2000 ppm), durations (3, 12, 24 and 48 h) and concentrated sulphuric acid (0.5, 1, 5, 10, 20 and 30 min), on germination of Iranian caper seeds, these experiments were conducted at Physiological Research Laboratory, Ferdowsi University of Mashhad, Iran, in 2010. There was not any symptom of germination of caper seeds in distilled water. Sulphuric acid could not improve the germination properties of caper and some seeds became demolished before the end of the experiment. In general 2000 mg/l gibberellic acid treatment resulted in more vigorous seed germination (42%) at any duration compared to any other concentration of the gibberellic acid. The highest seed germination of 26% was achieved when the seeds were treated 24 hour with 4000 ppm KNO_3 solution but it was decreased in 8000 mg/l. The highest germination percentage (72%) was observed in seeds placed in filter papers wetted with in 250 ppm gibberellic acid after treatment with 8000 mg/l KNO_3 for 24 h (this duration was the best time span in two previous experiments). It seems that GA_3 and KNO_3 can replace partly to improve seed germination of caper. The highest radicle length (148 mm) and seedling dry weight was achieved as seeds were treated in 100 ppm gibberellic acid plus 1000 ppm potassium nitrate. Therefore, it can be concluded that for best germination percentage of caper seeds, 250 ppm GA_3 and 8000 ppm KNO_3 and for the strongest seedling 100 ppm GA_3 plus 1000 ppm KNO_3 can be used.

Introduction

Caper (*Capparis spinosa* L.) plant is typical of the tropical Mediterranean areas as well as central Asia, Europe (Spain, Italy, Greece and Turkey) as well as North Africa and Middle East countries including Iran (Olmez *et al.*, 2004; Movafeghi *et al.*, 2008). It is a perennial shrub, covering soil surface widely, and produces one of the deepest root systems (Olmez *et al*., 2006; Sakcali *et al*., 2008). The long roots and wide ecological amplitude allow it to withstand harsh environments. The species thus appears to be a suitable candidate for the protection of degraded areas in arid areas (Olmez *et al*., 2006; Sakcali *et al*., 2008).

Apart from its roles in soil conservation, different parts of the caper plant is used in Iranian traditional medicine as a liver provoking agent, treatment of vessel clogs, anti-rheumatism and a diuretic agent (Ramazani *et al*., 2009). Recent studies have proved its uses to treat hysteria and nervousness (Jangju-Borzelabad and Tavakkoli, 2008). The fresh aerial parts of *Capparis spinosa*, including the fruit and the flower buds (capers) are stored in vinegar or brined and eaten pickled. Different parts of this plant contain chemical compound rich with beneficial compounds (Sakcali *et al.,* 2008). Suleiman *et al.,* (2009) reported that caper is a highly potential candidate for survival, adaptation and acclimatization in Kuwait for greenery and culinary values because of its ability to grow under conditions of aridity.

Caper species is endangered in its natural habitats in Iran and many other countries due to climate change and overutilization. Domestication of capers as medicinal, vegetable or soil surface coverage plant is complicated by limited and variable seed germination under artificial conditions. According to some researchers, there are germination obstacles in the caper seeds and; thus, there are propagation difficulties of caper seedlings (Orphanos, 1983; Olmez *et al.,* 2004). To ensure high plantation and viability, a reliable and high germination percentage is required. Ramazani *et al.,* (2009) showed that drought and salinity had a significant adverse effect on germination percentage, germination rate, shoot and root length, vigor index and seedling dry weight of *C. spinosa*. They reported that caper is more sensitive to salinity than drought. The caper's vegetative canopy covers soil surfaces which help to conserve soil water reserves (Rhizopoulou & Psaras, 2003).

Dormancy is defined by the lack of visible growth that confers advantages to the plant from developmental synchronization to survival in the presence of environmental stresses (Lang, 1996). Seed dormancy is an adaptive mechanism in many species particularly wild species to protect seedlings from freeze damage during the winter, or from drought stress in water shortage conditions (Shirazi, 2003). Plant growth regulators such as gibberellic acid (GA) (Hilhorst & Karssen, 1992; Iglesias & Babiano, 1997) and chemicals such as KNO_3 (Kevserog lu, 1993; Hartmann *et al*., 1997) have been recommended to break seed dormancy and enhance germination. Gibberellins promote growth by increasing extendibility of the cell wall followed by the hydrolysis of starch to sugars which reduces the potential in the cell, resulting in the entry of water into the cell causing elongation (Arteca, 1996). KNO_3 is the most widely used chemical for germination promoting (Olmez *et al.,* 2004). Solutions of 0.1 to 0.2% KNO_3 are common in routine germination testing and are recommended by the

Association of Official Seed Analysts and the International Seed Testing Association for germination tests of many species (Copeland & McDonald, 1995; Basra, 1994).

Information on seed germination of capers is still limited. Therefore, it was thought that treatment of the seeds with plant growth regulators may influence rapid germination and root formation. The present study was conducted to examine the role of H_2SO_4, KNO_3 and GA_3 that might affect germination of Iranian capers seeds and study the possible advantages of in vitro germination over direct sowing of seeds in the soil.

Materials and Methods

In order to evaluate germination properties and effect of different treatments on dormancy breaking of *caparis spinosa,* these experiments were conducted at Physiological Research Laboratory, Ferdowsi University of Mashhad, Iran, in 2010. The seeds used in this study were collected from Shothern Khorasan province, Iran, where capers grows abundantly. The seeds were separated from the fruit material, rinsed in tap water, dried in shade and kept at room temperature in linen sacks until sowing.

Viability test: Seed lots viability was examined using tetrazolium test. Twenty five seeds soaked for 18 h in a container with 1% tetrazolium solution, which entirely covered with the solution. Then the samples were completely washed with distilled water to stop the reaction. Viable seeds were counted based on pigmentation. An optical microscope was used to determine the pigmentation and viability of embryo and storage tissues.

Germination tests: The germination test conducted to find out germination properties of caper seeds in H_2O as control, concentrated sulfuric acid, gibberellic acid, potassium nitrate, gibberellic acid + potassium nitrate. A sample of 50 randomly selected seeds was used with four replications. Experiments were carried out in 90 mm diameter Petri dishes using Whatman No. 1 filter papers in the bottom and top of the petri dishes to cover the seeds. Experiment was continued for 21 days and every day the procedure of germination was monitored.

Sulphuric acid seed treatment was carried out to assess the effect of duration of sulphuric acid exposure on germination performance by placing seeds in concentrated sulphuric acid for 0.5, 1, 5, 10, 20 and 30 min. Following acid treatment seeds washed in running water until the pH was neutral, and then rinsed in sterile water. After rinsing, the seeds were placed in Petri dishes for germination.

Similar to previous experiment, samples of twenty five randomly selected seeds with four replications were soaked in different doses (0, 50,100, 250, 500, 1000 and 2000 ppm) and durations (3, 12, 24 and 48 h) of gibberellic acid solution. No further water was added during the experiment period. For control treatment, Filter papers were moistened with distilled water.

Another 21 days experiment was conducted to find out the effect of potassium nitrate solution on germination. Seeds soaked in different doses (0, 500, 1000, 2000, 4000 and 8000 ppm) (Amri, 2010) and durations (3, 12, 24 and 48 h) of potassium nitrate. Samples and germinated seeds were counted every day until the end of the experiment.

The seeds were placed in filter papers were soaked in 0, 50,100, 250, 500, 1000 and 2000 ppm gibberellic acid solution after applying different doses (0, 500, 1000, 2000, 4000 and 8000 ppm) of potassium nitrate solution for 24 h. For control treatment, filter papers were moistened with distilled water. This experiment also continued for 21 days and seed germination was monitored every day.

The combined experiment of gibberellic acid and potassium nitrate was arranged in the polyethylene pots filled with growing medium composed of clay, sand and manure (1:1:1). Pots were kept in open air conditions after sowing. Seven different gibberellic acid treatment (0, 50, 100, 250, 500, 1000 and 2000 ppm) and six different potassium nitrate (0, 500, 1000, 2000, 4000 and 8000 ppm) treatment were applied on the seeds in the spring (March) of 2010. The experimental design was a factorial based on randomized complete block with three replications (35 pots for each block) for every treatment.

Statistical analysis: Analysis of variance performed using SAS 9.1 (SAS Institute, Cary, NC) and general linear models (PROC GLM) procedure. Multiple comparisons were conducted for significant effects with the least significant difference (LSD) test at $\alpha = 0.05$ to determine the rate of seed germination for each patch and seed treatment

Results and Discussions

Germination test: There was no any symptom of germination of caper seeds in distilled water, Olmez *et al.,* (2004) reported only 3.67% germination in this plant using distilled water. The seed infection was started form the 4^{th} day of the experiment and after 21 days, there were some damaged seeds in each petri dish. This result proposed two hypothesizes; first one is that seeds had deep dormancy and different kind of priming may be needed for germination. Another theory offered low viability of the seeds and assumed the non-germinated seeds as dead seeds. Thus, a series of experiments was conducted to evaluate these hypothesizes. Tetrazolium test of caper seeds showed that the viability is not the problem of germination. The results showed that seed viability was in a suitable condition with highest viability percentage of 94%.

Sulphuric acid treatment: Sulphuric acid could not improve the germination properties of caper and some seeds became demolish before the end of the experiment (Rehman *et al.,* 1999). Other researchers have described different effects of acid scarification and reported positive effects of H_2SO_4 in germination (Orphanos, 1983; Olmez *et al.,* 2004); in some cases sulfuric acid did not increase seed germination because it negatively affects the seeds viability (Rehman *et al.,* 1999). The lower effectiveness of some chemical scarification treatments compared with mechanical scarification can be explained by two factors:

(i) the endocarp or seed coat did not erode enough to break dormancy after a short time of exposure to sulfuric acid; or (ii) the acid penetrated enough to kill or damage the embryo (Upreti and Dhar, 1997; Habib *et al.*, 2010).

Gibberellic acid treatment: The highest seed germination of 42% was achieved after 24 h of soaking in 2000 mg/l GA3 (Table 1). However, 38% seed germination was achieved in the same duration at 1000 mg/l GA3 (table 1). In general 2000 mg/l GA3 treatment resulted in more vigorous seed germination at any duration compared to any other concentration of the Gibberellic acid. This indicates that the regulation of endogenous GA levels after seed imbibitions is a crucial factor in determining seed germination. Duration of exposure of seeds to GA3 is also important; for instance the germination percentage at 1000 mg/l of GA3 for 6 hours was only 8% while at the same concentration of GA3 it was 38% after 24 hours of GA3 treatment. Germination percentage was reduced beyond 24 hours of soaking in gibberellic acid at all concentrations. Gibberellic acid is known to play an essential role in seed germination, leaf expansion, stem elongation, flowering and flower development (Yanmaguchi and Kamiya, 2002). Our results are in agreement with that of Negbi *et al*., (1966) for *Hirschfeldia incana* and Orphanos (1983) and Olmez *et al.,* 2004 for *Capparis spinosa*, who found that the seed dormancy is mainly due to the seed coat that prevents germination and GA3 has a positive effect on germination. They observed that when the seeds get in touch with sole water, mucilage accumulated on the coat and hinders embryo to take O_2 and consequently preventing germination. However, the relationship between GA3 and O_2 is not known. Mayer and Shahin (1974) observed that the gibberellins reduces oxygen requirement for germination. The caper germination percentage obtained in this experiment is higher than previously reported e.i. 27.4 % in Olmez *et al.,* (2004) experiment. Gibberellic acid may also increases synthesis of hydrolytic enzymes located under aleuron layer. Synthesized enzymes are transported to endosperm and are used for decomposing of stored food to supply energy required for germination (chen *et al.,* 2008).

Table 1. Effect of different duration and concentration of GA_3 on seed germination percentage of *C. spinosa*.

Soaking time (hours)	GA_3 dose (mg/l)					
	50	**100**	**250**	**500**	**1000**	**2000**
6	6j*	6j	6j	10hi	8i	10hi
12	8i	8i	10hi	12gh	12gh	13fgh
24	24d	30c	30c	32c	38b	42a
48	14fg	16f	16f	24d	20e	22de

*Values followed by different letters are significantly different at 0.05 level using LSD. There was no germination in distilled water (zero GA_3)

Potassium nitrate treatment: Treatment with exogenous KNO_3 stimulated of germination percentage of caper seeds. The highest seed germination of 26% was achieved when the seeds were treated 24 hour with 4000 mg/l KNO_3 solution but it was decreased in 8000 mg/l. Moreover, germination percentage of caper seeds were dependence to concentrations up to 4000 mg/l and duration dependence up to 24 hours (Table 2). Several workers have reported that KNO_3 improved the seed germination of many plants seed (Cirak *et al.,* 2004; Olmez *et al.,* 2004). Potassium nitrate was found to be effective in breaking dormancy of many species (Agrawal and Dadlani, 1995). Use of KNO_3 has been an important seed treatment in seed-testing laboratories for many years without a good explanation for its action (Hartmann *et al.,* 1997).

Table 2. Effect of different duration and concentration of KNO_3 on seed germination percentage of *C. spinosa*.

Soaking time	KNO_3 dose (mg/l)				
	500	**1000**	**2000**	**4000**	**8000**
6 hour	6c*	6c	8b	12c	10d
12 hour	12b	14fg	20cd	24ab	14fg
24 hour	16ef	18de	20cd	26a	22bc
48 hour	18de	16ef	18de	22bc	18de

*Values followed by different letters are significantly different at 0.05 level using LSD. There was no germination in distilled water (zero KNO_3)

Potassium nitrate + gibberellic acid treatment: The highest germination percentage (72%) was observed in seeds placed in filter papers wetted with in 250 ppm gibberellic acid after treatment with 8000 mg/l KNO_3 for 24 hour (this duration was the best time span in two previous experiment), however there was no any significant difference between this germination percentage and that of 1000 ppm gibberellic acid after treatment with 4000 mg/lit KNO_3 (Table 3). It seems that GA_3 and KNO_3 can replace partly to improve seed germination of caper. No germination in case of control was possibly due to the seed coat of the capers that forms mucilage on soaking in water (Orphanos, 1983). The mucilage surrounding the seed is supposed to inhibit diffusion of oxygen to the embryos and prevent germination. Khan and Ungar (1985) believed that vegetative hormones can break embryo dormancy and neutralize prevention role of Abscissas acid (ABA) directly or indirectly. But we could not find the synergistic effects of GA_3 and KNO_3 on germination of caper and other species in the literature.

According to the results shown in Table 4, radicle length changed significantly at different combination and levels of gibberellic acid and potassium nitrate. The highest radicle length (148 mm) was achieved as seeds were treated in 100 ppm gibberellic acid + 1000 mg/l potassium nitrate. The lowest radicle length (30 mm) was observed in treatment with 2000 ppm gibberellic acid treatment + 8000 mg/l potassium nitrate. Therefore, gibberellic acid concentration beyond 100 ppm and potassium nitrate beyond 1000 ppm did not impose any significant increase in caper radicle length particularly when both treatments applied in high concentration, shorter radicles were produced.

Table 3. Effect of different concentration of KNO3 + GA_3 on seed germination percentage of *C. spinosa*.

GA_3 dose (mg/l)	0	KNO_3 dose (mg/l)				
		500	1000	2000	4000	8000
0	0	16u*	18tu	20st	22rs	22rs
50	24qr	22rs	26q	36lm	36lm	44hi
100	30op	30op	32no	42ij	42ij	64bc
250	30op	40jk	34mn	48fg	52e	72a
500	32no	44hi	42ij	50ef	66b	46gh
1000	38kl	46gh	44hi	62c	70a	48fg
2000	42ij	46gh	56d	46gh	50ef	46gh

*Values with in a column followed by different letters are significantly different at 0.05 level using LSD

Table 4. Effect of different concentration of KNO3 + GA_3 on radicle length (mm) of *C. spinosa*.

GA_3 dose (mg/l)	0	KNO3 dose (mg/l)				
		500	1000	2000	4000	8000
0	0	86no*	94lmn	88mno	80op	78op
50	98jklm	107hijk	132bcd	125def	125def	136bc
100	104ijkl	117efgh	148a	140a	115fgh	108hij
250	114ghi	132bcd	133bcd	127cde	109hi	97klm
500	112hi	114ghi	117efgh	123defg	74pq	62rs
1000	60s	66qrs	73pq	72pqr	47tu	49t
2000	38uvw	41tuv	49t	45tu	30w	34vw

*Values followed by different letters are significantly different at 0.05 level using LSD

Seedling establishment: Significant effect of seed priming treatment was observed in the final seeding establishment. The highest seedling dry weight of 25 mg/plant was achieved when the seeds were treated with 100 ppm Gibberellic acid treatment + 1000 ppm Potassium Nitrate (Table 5). The germination phase of planted seeds is critical because it directly determines the density of a crop stand especially under arid conditions where dry soil may impair imbibitions of water and high temperature may affect seed viability and eventual density of a crop stand (Hadas and Russo, 1974). Hadas and Russo (1974) further observed that a good stand can be ensured by a complete and fast germination and if germination seed is slow in taking up water, emergence is impaired and consequently the final stand is reduced.

Table 5. Effect of KNO3 + GA3 on seedling dry weight (mg) of *C. spinosa*.

GA_3 dose (mg/l)	0	KNO3 dose (mg/l)				
		500	1000	2000	4000	8000
0		17ghi	18fgh	19efg	19efg	18fgh
50	18fgh*	19efg	19efg	20def	20def	19efg
100	20def	21cde	22bcd	23abc	23abc	22bcd
250	20def	23abc	25a	24ab	23abc	22bcd
500	19efg	20def	22bcd	21cde	20def	18fgh
1000	16hij	18fgh	18fgh	19efg	17ghi	15ijk
2000	12l	13kl	14jkl	14jkl	13kl	12l

*Values followed by different letters are significantly different at 0.05 level using LSD.

Conclusion

In general to improve caper seed germination, 2000 mg/l Gibberellic acid treatment resulted in more vigorous seed germination (42%) at any duration compared to other concentrations. While, the highest seed germination of 26% was achieved when the seeds were treated 24 hour with 4000 ppm KNO_3 solution but it was decreased in 8000 mg/l. The highest germination percentage (72%) was observed in seeds placed in filter papers wetted with in 250 ppm gibberellic acid after treatment with 8000 mg/l KNO_3 for 24 hour (this duration was the best time span in two previous experiments). It seems that GA_3 and KNO_3 have a synergist effect and can replace partly to improve seed germination of caper. The highest radicle length (148 mm) and seedling dry weight was achieved as seeds were treated in 100 ppm gibberellic acid + 1000 ppm potassium nitrate. Therefore, it can be concluded that for best germination percentage of caper seeds, 250 ppm GA_3 and 8000 ppm KNO_3 and for the strongest caper seedling 100 ppm GA_3 + 1000 ppm KNO_3 could be recommended for dormancy breaking of caper seeds.

Acknowledgements

We would like to thank Vice President of Research and Technology, Ferdowsi University of Mashhad for the financial support of this project (Code 2/16404). We also thank Research department (Dr Shahriari and Mrs Abeddan) of Faculty of Agriculture for their help.

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(Received for publication 16 April 2012)