Indian Journal of Agricultural Sciences **80** (7): 621–5, July 2010

Effects of various dormancy breaking treatments on the germination of wild caper (*Capparis spinosa*) seeds from the cold arid desert of trans-Himalayas

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Received: 19 October 2009; Accepted: 29 April 2010

ABSTRACT

A study was conducted during 2008–09 to examine the role of various dormancy breaking treatments, viz hot water treatment, scarification, stratification, concentrated acids (H_2SO_4, HNO_3 and HCl), gibberellic acid, potassium nitrate, alcohol, acetone and gamma-rays irradiation on the germination of caper (*Capparis spinosa* L.) seeds. Dried seeds were placed in the germination chambers for 20–28 days at constant temperature of 25±2°C under continuous light (20 hr) photoperiod after its treatments. Highest germination of 62% was obtained when seeds were pretreated with H_2SO_4 (40 min.), followed by 400 ppm of gibberellic acid soaking (2 hr). The results revealed that the seeds were epitomized by both physical and physiological type of dormancy that should be overcome to have maximum germination percentage.

Key words: Acid treatment, Caper seed germination, *Capparis spinosa*, Dormancy, Gibberellic acid, Nitric acid

Caper *(Capparis spinosa* L.) (Capparidaceae), locally known as *kabra* is one of the oldest known medicinal plants in *Amchi system* (local medicinal system) which is occasionally used by local people of Ladakh as a leafy vegetable and forage. It is an under-utilized wild plant which grows in roadside, dry rocky slopes and stony soils, can withstand extreme temperature (–40p C to +40p C) of Ladakh and is highly drought-tolerant (Mishra *et al*. 2009). In India, it is found in inner valleys of trans-Himalayas between 3 020 and 3 890 m which includes Indus, Nubra and Suru valleys of Ladakh region, Spiti valley of Himachal Pradesh and Deccan peninsula.

This plant has multiple uses in cuisine as salad, leafy vegetable, pickle and condiments. Besides these qualities it brings many environmental benefits, including soil and water conservation, desertification control and land reclamation in fragile cold ecosystem of Ladakh (Mishra *et al*. 2009). Bio-chemical studies have reported the presence of alkaloids, lipids, flavenoids and glucosinolates, cancer preventing agents and biopesticides in *C. spinosa*. Capers contain considerable amount of the antioxidant bio–flavenoids *rutin* (Germano *et al*. 2002).

Caper plants can be propagated through seeds or stem cuttings, however, both methods present serious problems and restrictions to the commercial expansion of this crop. Capers are widely grown on dry and poor soil, where environmental conditions are not easy for the cultivation of other crops. It is difficult to propagate through seedlings because caper seed have both physical (seed coat) and physiological dormancy which is difficult to germinate. Fresh caper seeds germinate readily but in low percentages (1–2%), whereas drying of seeds induces severe dormancy, which is difficult to overcome naturally (Olmez *et al*. 2004). As the dormancy in this crop is due to the hard seed coat, external treatments are necessary to overcome the prevailing dormancy. The structure of the seed and the mucilage which develops when the seed is placed in contact with water could impose an effective barrier against the diffusion of oxygen to the embryo (Soyler and Khawar 2007).

Pre-chilling, scarification, and treatments with gibberellic acid (GA_3) or nitric acid (KNO_3) are the standard procedures used to enhance seed germination of dormant seeds. To obtain higher germination (%) in *Capparis* various treatments were reported, viz gibberellic acid+KNO_3 (Fernandez *et al*. 2002, Pupalla and Fowler 2002), pre-treatment with Sulphuric acid (H_2SO_4) (Kara *et al*. 1996), H_2SO_4+GA_3 (Sozzi and Chiesa 1995) and warm water+chilling (Kontaxis 1997).

Fruit weight, position on the mother plant and maturation stage also affects caper seed germination, besides an efficient method has been standardized for ensuring satisfactory seed germination by breaking the physical and/or physiological dormancy (Pascual *et al*. 2004). Pascual *et al*. (2006) reported

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that freshly harvested caper seeds showed highest germination rate and the shortest time to reach 50% of the final germination percentage. The effect of seed soaking treatments and soaking time individually or in combination was studied with the addition of gibberellic acid to maximize the seed germination percentage (Pascual *et al*. 2009). The effects of temperature, light, pre-soaking treatment and removal of seed coat have been reported to effect germination of various crops (Travlos *et al*. 2007, Shaik *et al*. 2008).

Research studies on the seed germination of caper are scanty and insufficient across the world. Moreover, till date there is no report on the method to enhance the caper seed germination from cold arid desert of Ladakh. Considering the potential of caper for rural livelihood, as erosion control agent, as new crop and new income sources, present investigation was conducted to examine the importance of various dormancy breaking treatments that might affect germination of wild caper naturally occurred in Ladakh. The present investigation revealed the outcome aimed for maximizing caper seed germination under cold arid desert of Ladakh.

MATERIALS AND METHODS

The seeds of *Capparis* used in the present investigation were collected from the population of wild plants in Ladakh region, where it grows abundantly. Ladakh is part of Indian Himalaya which is located at the latitude of 31° 44′ 57″–32° 59′ 57″ N and longitude of 76° 46′ 29″ – 78° 41′ 34″ E and at an altitude of 975–2220 m above mean sea level, is characterized by diverse and complex land formations. The temperature ranges between +40°C in summer and –40°C during winter and annual precipitation is around 20–30 mm (mostly as snow) along with low relative humidity (20–30%).

The dehisced fruits were collected in October 2008 from different parts of Ladakh. The seeds were separated form the fruits, rinsed in tap water thoroughly, dried in shade and kept at room temperature in polyethylene bags. The average seed moisture content and 1 000-seed weight were determined by moisture meter and digital weighing balance, respectively. The seed viability was determined by Tetrazolium test using 500 seeds in 5 replications of 100 seed each (ISTA 2002).

The viable parts of the seed showed red or pink colour and non-viable parts remained white. The seed germination experiment was conducted as a completely randomized design with 4 replications. Each experimental unit was a petri-dish with 100 seeds. Prior to various treatment seed surface was sterilized by washing with 0.01% mercuric chloride, rinsed with distilled water, wash with 70% alcohol and finally rinsed with 3 washes of MilliQ water. The surface sterilized seeds were used for the following treatments:

T_1, Seeds were dipped in concentrated acids, ie H_2SO_4, HCl and HNO_3 for 10–60 min at 10 min. interval; T_2, gamma rays irradiation of seeds at different doses (ie 10–50 KR at 10 KR interval) using the ^{60}Co gamma cell irradiator facility at the Botany Department, RTM, Nagpur University, Nagpur followed by dipping in concentrated H_2SO_4 for 10–60 min. at 10 min. interval; T_3, seeds were first pretreated in concentrated H_2SO_4 for 20–60 min. at 10 min. interval, further dipped in GA_3 solutions (ie 100–500 ppm at 100 ppm interval) for a period of 1, 2 and 3 hr; T_4, seeds were soaked at 3 different doses of KNO_3 (ie 0.1, 0.2 and 0.3%) for a period of 6, 8 and 12 hr after pre-soaking in concentrated H_2SO_4 for 20, 30 and 40 min; T_5, scarification of seeds by P320A sandpaper (sand grain/cm^2) then dipped in GA_3 solutions (ie 200–600 ppm at 100 ppm interval) for a period of 1, 2, and 3 hr; T_6, seeds were stratified at –20°C for 1–30 days at 5 day interval; T_7, seeds were dipped in the hot water at 50, 60 and 70°C for 30–120 min at 30 min interval and T_8, seeds were first soaked in absolute alcohol and acetone for 1, 2, 3, 4 and 5 days and then dipped in GA_3 solutions (100–600 ppm at 100 ppm interval) for a period of 1, 2, and 3 hr.

All the treated seeds were placed in closed 9 cm Petri-dishes (Ø 9 cm) which were lined with 2 sheets of filter papers Whatman No.1 and moistened with sterilized MilliQ water to which 250 ppm Carbendazim was added to prevent any fungal infection. Treated seeds were placed on the moist paper for germination for 20–28 days and light was provided by Philips daylight lamps (324 µmol/m^2/s). A clear labeled lid was placed on top of each Petri-dish denoting the treatment, temperature and replication. All these Petri-dish were then kept in seed germinator at 25±2°C with relative humidity of 80% and 20 hr of light. Petri-dishes were checked daily for germinated seeds and filter paper was moistened with sterilized MilliQ water as needed. Germination was determined by observing a visible radical or shoot. The number of seeds used for the germination tests were 4 replications × 100 seeds/replication for each treatment and was repeated twice. Data given in percentages were subjected to arcsine ($\sqrt{X}$) transformation before statistical analysis. Means were compared by one-way ANOVA using SPSS for win-dows (Version 12.0) and differences between the means were compared by Duncan's multiple range test (DMRT). A probability of ≤0.05 was considered significant.

RESULTS AND DISCUSSION

The average seed moisture content and 1000-seed weight were found 7% and 7.81 g, respectively. The seed viability was found 89% as determined by Tetrazolium test (ISTA 2002).

Concentrated acid treatment

A comparison to observe the effect of various durations of concentrated H_2SO_4 and HCl showed a positive effect of H_2SO_4 on seed germination, while no significant germination was observed with concentrated HCl. In-case of concentrated HNO_3 treatment there was no seed germination at all. Maximum germination of 33.0±1.73% was observed with 40 min. of soaking in concentrated H_2SO_4 (Table 1).

Table 1 Effect of soaking durations of concentrated acids (H_2SO_4 and HCl) on germination (%)

Soaking duration (min.)	Concentrated acids	
	H_2SO_4	HCl
10	$20.67^{ab} \pm 1.76$	4.33 ± 0.67
20	$23.33^{b} \pm 1.20$	4.67 ± 0.33
30	$28.67^{c} \pm 0.88$	6.00 ± 1.00
40	$33.00^{d} \pm 1.73$	6.33 ± 1.20
50	$20.67^{ab} \pm 0.88$	7.67 ± 0.88
60	$19.00^{a} \pm 0.58$	5.33 ± 0.33
Mean germination (%) SEm ±	24.22 ± 1.29	5.72 ± 0.39

[a, b, ab, c, d] means bearing different superscripts in a column differ significantly ($P < 0.01$)

However, any increase or decrease in acid soaking time significantly reduced the seed germination which can be attributed to embryo damage. Poor germination or no germination in case of concentrated HCl and HNO_3, respectively might be due to the inability of these treatments to break the physical dormancy.

Pre-treatment

Pre-treatment with H_2SO_4 for 15–30 min. was found to be an effective method to increase germination (Kara *et al*. 1996). While seed treatments with KNO_3 or GA_3 is known to enhance the germination percentage (Fernandez *et al*. 2002). When H_2SO_4 pre-treated seeds were treated with KNO_3 in different combinations the highest germination of 61% (Table 2) was obtained in case of pre-soaking in concentrated H_2SO_4 (for 20 min.), followed by dipping in KNO_3 (0.2% for 8 hr). Any increase or decrease in the concentration of KNO_3 or soaking duration along with further increase in the presoaking time showed negative effect on the overall germination (%).

Germination percentage 44.3 was also good in seed soaked in 0.3% KNO_3 for 8 hr along with 20 min. H_2SO_4 pre-soaking. In present study average of germination percentage of 35.33±6.70 in 0.2% KNO_3 was higher than the 0.1 and 0.3% KNO_3, beside this in the application of KNO_3 for 8 hr increased the germination percentage of the seeds over the treatment of 6 and 12 hr (Table 2).

Seed treatment

Gibberellic acid is known to play an essential role in seed germination, stem elongation and flower development (Pupalla and Fowler 2002). The highest seed germination (62%) was obtained for pre-soaking in sulfuric acid (40 min.), followed by dipping in 400 ppm GA_3 for 2 hr (Table 3). Lesser or longer treatment time was inhibitory in each case. However, 300 and 500 ppm GA_3 treatment showed the highest germination of 47 and 45% after 2 hr soaking in GA_3 alongwith 40 min. of H_2SO_4 pre-soaking. This means that the regulation of endogenous gibberellic levels after seed imbibitions along with specific H_2SO_4 presoaking time of 40 min. is crucial factor in determining the seed germination. Sozzi and Chiesa (1995) obtained 68% germination using concentrated H_2SO_4, followed by a 90 min. soaking in 0.01% GA_3 solution.

It is supposed that disruption of seed coat allowed diffusion of oxygen in interaction with both growth regulators positively removed seed dormancy. Our results are in agreement with that Soyler and Khawar (2007). They reported that the seed dormancy is mainly due to the hard seed coat that prevents germination. They observed that when the seeds get in touch with water, mucilage comes into existence on the seed coat and hinders embryo to take O_2, thus preventing germination. They further pointed out that GA_3 has positive effect on germination. However, the relationship between GA_3 and O_2 is not known. Probably GA_3 decreases the O_2 need for the germination.

Gamma rays irradiation

Positive effect of gamma rays irradiation on seed germination is already known in many agricultural crops. Therefore, caper seeds were also treated with different doses

Table 2 Effect of pre-soaking treatments of concentrated H_2SO_4 (durations) in combination with KNO_3 (concentrations and durations) on germination (%)

H_2SO_4 pre-soaking duration (min.)	KNO_3 concentration (%)									Mean germination (%) SEm±
	0.1			0.2			0.3			
	Soaking duration (hr)			Soaking duration (hr)			Soaking duration (hr)			
	6	8	12	6	8	12	6	8	12	
20	38.0	12.0	12.7	27.0	61.0	13.0	34.0	44.3	13.0	28.3^{b}±3.25
30	32.0	36.0	18.0	36.0	29.0	23.0	38.0	30.0	16.0	28.7^{b}±1.51
40	8.0	11.0	13.0	21.0	16.0	11.0	21.0	9.0	12.0	13.6^{a}±0.96
Mean germination (%)	26.00^{xyz}	19.67^{wxy}	14.56^{wx}	28.00^{yz}	35.33^{z}	15.67^{wx}	31.00^{yz}	27.78^{yz}	13.67^{w}	
SEm±	±4.62	±4.13	±1.09	±2.29	±6.70	±1.93	±2.61	±5.20	±0.94	

[a, b, c] different superscripts in a column differ significantly ($P < 0.01$)
[w, x, y, z] different superscripts in a row differ significantly ($P < 0.01$)

Table 3 Effect of concentrated H_2SO_4 pre-soaking (durations) along with GA_3 (concentrations and durations) on germination (%)

GA_3 (ppm)	Duration (hr)	H_2SO_4 pre-soaking duration (min.)					Mean germination (%) SEm±
		20	30	40	50	60	
100	1.0	8.0	31.0	33.0	29.0	5.0	21.2±3.25
	2.0	10.0	34.0	39.0	32.0	7.0	24.4±3.54
	3.0	7.0	27.0	31.0	24.0	6.0	19.0±2.85
200	1.0	11.0	33.0	38.0	32.0	11.0	25.0±3.13
	2.0	12.0	37.0	41.0	37.0	13.0	28.0±3.46
	3.0	17.0	28.0	33.0	27.0	9.0	22.8±2.35
300	1.0	12.0	39.0	42.0	34.0	15.0	28.4±3.36
	2.0	14.0	43.0	47.0	39.0	16.0	31.8±3.78
	3.0	8.0	35.0	39.0	31.0	11.0	24.8±3.44
400	1.0	16.0	42.0	48.0	39.0	15.0	32.0±3.71
	2.0	7.0	47.0	62.0	42.0	17.0	35.0±5.42
	3.0	11.0	37.0	44.0	36.0	12.0	28.0±3.72
500	1.0	14.0	34.0	39.0	35.0	13.0	27.0±3.04
	2.0	15.0	39.0	45.0	38.0	14.0	30.2±3.53
	3.0	10.0	29.0	36.0	32.0	11.0	23.6±2.94
Mean germination (%)		11.47	35.67	41.13	33.80	11.67	
SEm±		±0.55	±0.88	±1.15	±0.77	±0.58	

Pre-soaking with H_2SO_4 (P<0.01); GA_3 (P<0.01); Duration (P<0.01); Pre-soaking with H_2SO_4 × GA_3 (P<0.01); Pre-soaking with H_2SO_4 × Duration (P<0.01); GA_3 × Duration (P=0.642); Pre-soaking with H_2SO_4 × GA_3 × duration (P<0.01)

of gamma rays along with concentrated H_2SO_4 treatment for different durations. Maximum germination of 26% was observed when seeds were treated with 30 Kr gamma rays, followed by 40 min. concentrated H_2SO_4 treatment. Obviously, any further increase or decrease dose of gamma rays or H_2SO_4 duration showed negative effect on the overall germination (%) (Table 4).

Other treatment

Scarification, stratification and hot water treatments were found ineffective to break seed coat dormancy. Application of acetone, alcohol and HNO_3 although broke the seed coat but there is no further germination.

Table 4 Effect of gamma rays (doses) in combination with concentrated H_2SO_4 (durations) on germination (%)

Gamma rays	H_2SO_4 soaking duration (min.)			Mean germination (%) SEm±
(KR)	20	40	60	
10	7.0	18.0	10.0	11.67^{a}±1.76
20	9.0	19.0	13.0	13.67^{b}±1.57
30	17.0	26.0	19.3	20.78^{d}±1.57
40	14.0	23.0	17.0	18.00^{c}±1.38
50	11.0	21.0	13.0	15.00^{b}±1.57
Mean germination (%) SEm ±	11.60^{x} ±1.06	21.40^{z} ±0.91	14.47^{y} ±0.95	

[a, b, c] different superscripts in a column differ significantly (P < 0.01)

[x, y, z] different superscripts in a row differ significantly (P < 0.01)

The control showed low germination percentage (0–2%) was possibly due to the seed coat of the capers that forms mucilage and release leachate on soaking in water. The mucilage surrounding the seed is supposed to inhibit diffusion of oxygen to the embryos and prevent germination (Soyler and Khawar 2007). Sulfuric acid treatment to remove mucilage and soaking in either of KNO_3, GA_3 or gamma ray treatment was found effective to allow penetration of oxygen from the surroundings to the embryos and increased germination of seeds. Germination in each case was superior over the control (0–2%).

When a comparison is made between GA_3, KNO_3 and gamma ray treatments along with H_2SO_4 pre-treatment, results showed that GA_3 is in general superior to KNO_3 and also superior over gamma ray treatment in breaking seed dormancy in caper. However, high germination values were almost similar in GA_3 and KNO_3.

There are possibilities that hard seed coat was not the only cause for poor germination in caper and dormancy was partly due to the physiological restrictions as well (Pascual *et al*. 2004). As germination in caper is controlled by both physical and physiological factors, therefore, pre-sowing treatments are necessary to overcome the dormancy. Sozzi and Chiesa (1995) reported that caper seeds were only sensitive to GA when they had been H_2SO_4 pre-treated which means that the primary control of germination in caper seeds resides in the seed coats, and that GA action is conditioned by seed coat disruption. In the present investigation too it was observed that acid scarification, followed by addition

of GA_3 or KNO_3 solution to the germination substrate is a simple, efficient and cost-effective method for ensuring satisfactory seed germination. This procedure could help to improve the germination of caper seeds in pots or nurseries before transplant the seedlings to the desired places in the field.

It is recommended to germinate the caper seeds in the Petri-dishes under specified treatments and then transfer these seedlings first in the greenhouse for 2–3 months and then to the open field conditions during spring–summer season (May–August) of Ladakh. The described method will ultimately help in the widespread cultivation of this wonder plant at the farmers' field of Ladakh. Further experiments will continue to study the effect of time between fruit harvest and seed germination, especially under cold arid desert of Ladakh.

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