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Original Contribution

IMPROVE GERMINATION OF CAPER (*CAPPARIS SPINOSA* L.) SEEDS BY DIFFERENT INDUCTION TREATMENTS OF SEED DORMANCY BREAKING

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ABSTRACT

Seed dormancy is a common phase of the plant life cycle; different treatments can be used for breaking dormancy. The aim of this study was to find the best treatment for breaking dormancy and improve germination of caper (*Capparis spinosa* L.). This study based on the completely randomized design (CRD) with 3 replications was done at research Laboratory of Medicinal Plants Institute, ACECR, Karaj, Iran. After different induction treatments containing different levels of potassium nitrate (KNO_3), GA_3, soaking and soaking with runner water, sulfuric acid, thidiazuron, and benzyl amino-purine, seeds were placed in Petri-dishes and incubated in two temperature regimes, first at fixed 20℃, and second, alternate between 20 and 30℃. The result showed that the germination percentage and germination rate of caper increased up to 75% and 1.35 respectively when the seeds treated with sulfuric acid for 15 min, and 2000 ppm GA_3 under alternate 20-30℃ temperatures. Caper seed dormancy is mainly due to the inhibitors and hard seed coat that it prevents seed germination.

Keywords: Germination rate, Germination percentage, Soaking, GA_3, Potassium nitrate.

INTRODUCTION

Caper (*Capparis spinosa* L.) belongs to family of Capparidaceae and is said to be native to the Mediterranean region, but it is also found and cultivated on the Atlantic coasts of the Canary Islands, Morocco, Spain (Almeria, Grenada & Balearic Islands), France (Provence) and Italy, Greece, Cyprus, Turkey, and Iran both under cultivated and rainfed conditions (1-2). Caper has been using as a medicinal and aromatic plant for a long time. Capers reduce flatulence and to be anti-rheumatic in effect. Capparis are recorded as hepatic protectors, improving liver function, arteriosclerosis, as diuretics, kidney disinfectants, and tonics, dropsy, anemia, arthritis and gout (3).

Caper plants can be propagated from seeds or stem cuttings, however, both methods present serious problems and restrictions to the commercial expansion of this crop (4). Seed dormancy is a common phase of the plant life cycle, and several parts of the seed can contribute to dormancy (5). There are basically two types of dormancy involving different mechanisms (6-7): embryo dormancy and coat-imposed dormancy. The inability to germinate in caper seeds is unlikely to be due to embryo dormancy: a partial removal of the seed covering structures, as well as cuts or punctures through them, have been the most effective treatments in stimulating caper germination (8). Fresh caper seeds germinate readily but in low percentages (1– 2%), whereas drying of seeds induces severe dormancy, which is difficult to overcome naturally (9). As the dormancy in caper 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 (2).

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 (10-11), pre-treatment with Sulphuric acid (H_2SO_4) (12), H_2SO_4+GA_3 (8) and warm water+chilling (13).

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The purpose of this study was to found methods for breaking the dormancy of caper (*Capparis spinosa* L.) seeds.

MATERIAL AND METHODS

Seed Source and Preparing

Caper seeds (SB-MPI-1225) were obtained from National Gene Bank and Seed Collection of Institute of Medicinal Plants (IMP) in Iran and all studies were conducted at this place. During germination experiments, the seeds were surface-sterilized by 5 min exposure to 3% calcium hypochlorite, thoroughly rinsed with sterile distilled water and transferred to plates (14). Fifty seeds were placed on filter paper (Whatman # 2) in 11 cm diameter Petri-dishes with four replicates per treatment in each experiment. The filter paper was moistened with 7 ml of distilled water or test solution. After moistening, the Petri-dishes were sealed to prevent desiccation with a plastic film (Parafilm, American National Can, 101 Merntt, Greenwich, CT 06836). Each treatment was applied to three dishes. The seeds were considered germinated when the radicle was 5 mm or longer (15). Germination tests were conducted in controlled environment chambers (Plant growth chamber, Model GC-300 LT/H, JEIO Tech Co., Ltd., Seoul, South Korea).

Treatments

+ Potassium nitrate (KNO_3): seeds were soaked in 0.1 and 0.2 % PN.
+ GA_3: seeds were soaked in 500, 1000 and 2000 ppm GA_3 for 72 h and then used to the germination test process.
+ Soaking in running water: seeds were soaked in running water for 24 h.
+ Sulfuric acid (H_2SO_4): used sulfuric acid 96% for 15 and 30 minute.
+ Soaking: seeds were soaked in water for 72 h and then transferred to the germination test process.
+ Soaking under different temperature: seeds were put into flasks contained water and then flask put into Ben Murray (40, 50, 60 and 70 c) for 15, 30 and 60 minute. After that germination test process occurred.
+ Thidiazuron (TDZ): is a plant growth regulator used 20 ppm in this experiment.
+ Benzyl Amino-Purine (BAP): is a synthetic cytokinin used 20 ppm in this experiment.

Design and Statistical Analysis:

Germination percentage was calculated as [(the number of germinated seeds)/the number of sampled seeds] ×100 (16):
The data analyzed by a one-way analysis of variance (ANOVA) using SAS version 9.1. The data were analyzed using a randomized complete design and LSD test was used to determine if there were significant ($p < 0.05$) differences among treatment means and used Excel 2010 for drawing the figure.

RESULT AND DISCUSSION

The result of this study showed that the using different treatments (concentration of potassium nitrate, GA_3, soaking in running water, soaking, sulfuric acid, thidiazuron and benzyl amino-purine) had the significant effect ($p \leq 0.01$) on seed germination of caper **(Table 1)**.

Table 1. *Analyses of Variances of Germination Percentage of Capparis Spinosa L. Under Different Treatments*

S.o.v	df	Germination percentage	Germination rate
Treatment	34	13.42**	0.058**
Error	70	157.05	0.012
CV	-	28.03	12.25

**= significant at 1%

Sulfuric acid increased germination percentage, if it is used with other germination promote, germination will be better. Caper germination and Germination rate increased up to 75% and 1.35 respectively, when seeds treated with sulfuric acid for 15 min and 2000 ppm GA_3 under alternate 20, 30 ◦C temperatures. (**Table 2**).

Researchers (17) investigated effects of dormancy-breaking treatments on germination in caper (*Capparis spinosa* L.). Their result showed that leaching and 1000 ppm GA_3 had the most germination. Similar to our result (18), revealed that caper seeds treated with sulfuric acid for 30 min and 200-400 ppm GA_3, had the most significant percentage germination. Reaching or using sulfuric acid washes Mucilage, ABA and other germination inhibitors in pericarp and testa of the seeds (19). The time of using sulfuric acid is very important because increasing acid time damage to the embryo and decrease percentage germination (20-21).

Our results matched to Bhoyar *et al*. (4), they declared that seed treatment with sulfuric acid for 40 min and GA_3 400 ppm showed the best germination. 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 researchers (2). 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 O2, thus preventing germination (4).

Table 2. *Seed Germination Percentage and Germination Rate with Dormancy Breaking Treatments for Capparis Spinosa L.*

Dormancy breaking treatments	**Temperature**	**Germination percentage**		**Germination rate**	
Control	T20	23.33	d-i	0.380	b-k
0.1% PN	T20	10.26	g-m	0.343	b-l
0.2% PN	T20	4	l-n	0.162	f-l
500ppm GA_3	T20	25	c-h	0.528	b-f
1000ppm GA_3	T20	20.59	e-j	0.426	b-j
soaking in running water (24 h)+ 1000ppm GA_3	T20	16.66	f-l	0.533	b-g
Sulfuric acid (30 min) + 500 ppm GA_3	T20	11.66	f-m	0.206	e-l
Soaking (24 h)+ 1000 ppm GA_3	T20	15	f-m	0.502	b-h
Sulfuric acid(30 min)+soaking in runner water (24 h)+ 1000ppm GA_3	T20	18.33	e-k	0.342	b-l
Sulfuric acid (30 min)+ 1000ppm GA_3 (90min)	T20	8.33	i-n	0.265	c-l
Sulfuric acid (30 min)	T20	6.66	h-n	0.127	g-l
Sulfuric acid (60 min)	T20	8.33	h-n	0.205	e-l
Sulfuric acid (15 min)+ 1000ppm GA_3	T20,T30	50	a-d	0.701	b
Sulfuric acid (15 min)+Soaking (40 c for 30 min)+ 1000ppm GA_3	T20,T30	53.33	a-c	0.607	b-d
Sulfuric acid (15 min)+Soaking (40 c for 60 min)+ 1000ppm GA_3	T20,T30	50	a-d	0.499	g-g
Sulfuric acid (15 min)+Soaking (50 c for 30 min)+ 1000ppm GA_3	T20,T30	50.33	a-c	0.429	b-g
Sulfuric acid (15 min)+Soaking (60 c for 30 min)+ 1000ppm GA_3	T20,T30	30	b-g	0.351	b-l
Sulfuric acid (15 min)+Soaking (70 c for 15 min)+ 1000ppm GA_3	T20,T30	0	n	0.000	l
Sulfuric acid (15 min)+ 2000ppm GA_3	T20,T30	75.92	a	1.348	a
Sulfuric acid (15 min)+Soaking (40 c for 30 min)+ 2000ppm GA_3	T20,T30	40	b-e	0.654	b-d
Sulfuric acid (15 min)+Soaking (40 c for 60 min)+ 2000ppm GA_3	T20,T30	53.33	a-c	0.658	bc
Sulfuric acid (15 min)+Soaking (50 c for 30 min)+ 2000ppm GA_3	T20,T30	60	ab	0.611	b-d
Sulfuric acid (15 min)+Soaking (60 c for 30 min)+ 2000ppm GA_3	T20,T30	50	a-d	0.388	b-k
Sulfuric acid (15 min)+Soaking (70 c for 15 min)+ 2000ppm GA_3	T20,T30	6.66	k-n	0.049	j-l
Control	T20,T30	5	j-n	0.044	j-l
soaking (24 h)	T20,T30	5	i-n	0.044	j-l
soaking (48 h)	T20,T30	13.33	f-m	0.116	g-l
Sulfuric acid (15 min)+ soaking (24 h)	T20,T30	1.66	mn	0.015	kl
Sulfuric acid (15 min)+ soaking (48 h)	T20,T30	5	j-n	0.044	j-l
soaking (24 h)+ 500 ppm GA_3	T20,T30	20	e-j	0.174	f-l
soaking (24 h)+ 1000 ppm GA_3	T20,T30	25	c-h	0.217	d-l
Sulfuric acid (15 min)+ soaking (24 h)+ 1000ppm GA_3	T20,T30	33.33	b-f	0.290	b-l
Sulfuric acid (15 min)+ soaking (48 h)+ 1000ppm GA_3	T20,T30	33.33	b-f	0.290	b-l
Soaking (48 h)+ 20ppm TDZ	T20,T30	8.33	i-n	0.073	i-l
Soaking (48 h)+ 20ppm BAP	T20,T30	10	h-n	0.087	i-l

The different letters showed significantly by LSD test at $p < 0.05$
GA_3; Gibberellic acid: ‹PN: Potassium nitrate (KNO_3) ‹BAD ; Benzyl amino-purine TDZ: Thidiazuron

Results of Sozzi and Chiesa (1995) obtained caper seeds treated with H_2S0_4 and H_2S0_4 + GA_3 had the highest germination. This was in agreement with other work on this species (22).

In others' research, sulfuric acid had the original effect on caper germination (8) and GA_3 used for Complementary effect. In this study GA_3 2000 ppm had the main effect and sulfuric acid alone no performance on seeds germination. This result in this study may due to GA_3 concentration (2000 ppm) is 20 times more than they result.

CONCLUSION

Sulfuric acid for 15 min and 2000 ppm GA_3 under alternate 20, 30 ◦C temperatures were the best method of breaking seed dormancy of caper. Results obtained by treatment clearly suggest that the dormancy of the caper seed is imposed by its covering structures.

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