



## *In vitro* germination and seedling development of tunisian caper (*Capparis spinosa* L.)

Rhimi Awatef<sup>1\*</sup>, Hannachi Hédia<sup>1</sup>, Hjaoujia Sonia<sup>1</sup>, Yousfi Haifa<sup>1,2</sup>, Bousaid Mohamed<sup>3</sup>

<sup>1</sup>Laboratory of Plant Biotechnology, National Gene Bank of Tunisia (NGBT). Tunis Cedex, Tunisia

<sup>2</sup>Department of Biology, National Institute of Agronomy of Tunis (INAT), Tunis- Mahrajène, Tunisia

<sup>3</sup>Department of Biology, National Institute of Applied Sciences and Technologies (INSAT). Tunis Cedex, Tunisia

Article published on January 25, 2017

**Key words:** Micropropagation. *In vitro* pretreatments, Caper populations.

### Abstract

*Capparis spinosa* L. (Capparaceae) is a perennial tropical and subtropical shrub plant known worldwide. Despite the increasing demand and economic importance of capers, little information is available regarding the propagation of this shrub. In fact, it is usually propagated by seed, but its percentage of germination is very low. In this report, *in vitro* seed germination and seedling development of four caper populations were studied and several treatments were evaluated to determine the ability of several factors to increase the percentage of germination. The seeds were treated with sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) for 30 min. These seeds were left out in two different germination media (MS and H<sub>2</sub>O), for 30 days by soaking them in 1000 or 2000 mg.l<sup>-1</sup> of GA<sub>3</sub> each for 6, 12, 24 or 48 h. High variability was observed among the germination percentages of the different treatments varying from 4.16 to 75%. The variability was due to the Gibberellic acid level (GA<sub>3</sub>), to the genotype and to the media culture. The highest germination rate of 75% was obtained from the Nahli site seeds treated with 2000 mg.l<sup>-1</sup> GA<sub>3</sub> for 48 hours.

For all populations, regenerated plants were transplanted in pots on a sterile substrate. The rate of survival plants after acclimatization was 100%.

\* Corresponding Author: Rhimi Awatef ✉ [rhimiawatef74@gmail.com](mailto:rhimiawatef74@gmail.com)

## Introduction

Caper (*Capparis spinosa* L.) belongs to the Capparaceae family native to the Mediterranean region. It is cultivated on a large scale in Spain, Italy, France, Greece, and North Africa (Zohary, 1960; Rhizopoulou *et al.*, 2006). *C. spinosa* is a perennial crop representing one of the most common aromatic plants that grow along the roadside, on the slopes, rocky and stony area and generally well adapted to dry areas. The plant is not cultivated and grows wild and has been known for centuries in traditional phytomedicine, which exploited its properties for several purposes. The aqueous extract from total aerial parts of the plant has been used for its antifungal, anti-inflammatory, antidiabetic and antihyperlipidemic (Eddouks *et al.*, 2005) activities and is among the constituents of polyherbal formulations to treat liver ailments, diuretic, antihypertensive and poultice. Various parts of caper plant can be used as drugs, cosmetics and foods, and are also used in different areas for landscaping, control of erosion or animal feeding (Baytop, 1999; Sakcali *et al.*, 2008).

Propagation of caper through seed is difficult due to the embryos dormancy as seed coat contains inhibitors, and consequently the germination rates of this species are very lower. Breeding of capers is complicated by limited and variable seed germination under natural conditions. The problem becomes serious when the plantations of capers are required for greater production. To ensure high plantation and viability, a high germination percentage is required. Germination using plant growth regulators has been reported previously for many plants (Crunkilton *et al.*, 1994; Swaminathan and Srinivasan, 1996). The effects of temperature, light, pre soaking treatment and removal of seed coat have been reported to effect germination of various crops (Shankarraja and Sulikeri, 1993; Kyauk *et al.*, 1995). Information on seed germination of capers is still limited. Therefore, it was thought that treatment of the seeds with plant growth regulators may influence root formation and rapid germination. The present study reports the outcome of preliminary investigations aimed at

maximizing seed germination and obtaining seedlings of capers under controlled conditions using gibberellic acid and MS or H<sub>2</sub>O media.

## Material and methods

### *Plant materials*

Seeds used in this study were obtained from the National Gene Bank of Tunisia (NGBT). These seeds were collected in 2009 from capers grows abundantly in the north of Tunisia. The collection areas included Nahli, Fahs, Mateur and Hammam Lif are located between 9.66E and 10.33E, and between 36.36N and 37.04N. The altitude of the collection areas ranges between 1100 and 1200 m above sea level and these areas receive an average amount of annual rain between 400 and 600 mm.

### *Sterilization procedure*

Seeds were cleaned manually of all foreign materials. They were washed under running water for 1h, and then soaked in calcium hypochlorite (6%) for 20 min. Under sterile conditions, the seeds were rinsed with sterile distilled water five times with continuous agitation. They were then treated with sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) for 30 min.

The seeds were transferred to 70% alcohol for 5 min and then transferred to 6% sodium hypochlorite for 20 min with continuous agitation. Finally, the samples were washed with sterile distilled water five times for 5 min each before their culture. These seeds were incubated in the germination chamber for 30 days by soaking them in 1000 or 2000 mg.l<sup>-1</sup> of GA<sub>3</sub> for 6, 12, 24 and 48h (Soyler and Khawar, 2007).

### *Germination medium and culture conditions*

Seeds germination potentialities were tested on the MS basal medium (Murashige and Skoog, 1962) and on sterile distilled water to determine the optimal germination medium.

The MS medium containing inorganic salts, myo-inositol (100 mg.l<sup>-1</sup>), thiamine-HCl (0.10 mg.l<sup>-1</sup>), nicotinic acid (0.50 mg.l<sup>-1</sup>) and pyridoxine-HCl (0.50 mg.l<sup>-1</sup>). After adjusting the pH to 5.8, media were

autoclaved at 121°C, 100 KPa for 20 min. seeds were placed in petri dishes (100 × 15 mm<sup>2</sup>) containing each 25 ml of liquid medium. Cultures were incubated at 22 ± 1°C under 16h photoperiod with 42 μMol photons m<sup>-2</sup>s<sup>-1</sup> by fluorescent light. Various treatments were tested to break dormancy of the seed and promote growth of the shoots. Two replicates with 12 seeds per replicate were used for each treatment.

*Acclimatization*

Germination seedlings fifteen days old were transferred to pots containing a sterile soil (2/3 peat and 1/3 sand), initially placed under high humidity (80%) in a growth chamber and subsequently grown in a greenhouse.

*Statistical analysis*

The germination percentages (mean values ± standard error) were calculated for each treatment.

The data at different soaking times were subjected to multifactorial analysis of variance (MANOVA) to evaluate the population, the media and GA3 concentrations effects and their interactions on germination percentage. The analysis was performed using Statistica software (version 8).

**Results**

*Germination induction*

The H<sub>2</sub>O and MS media and the GA3 levels (1000 and 2000 mg.l<sup>-1</sup>) were tested for their ability to induce germination for the considered populations (Table 1).

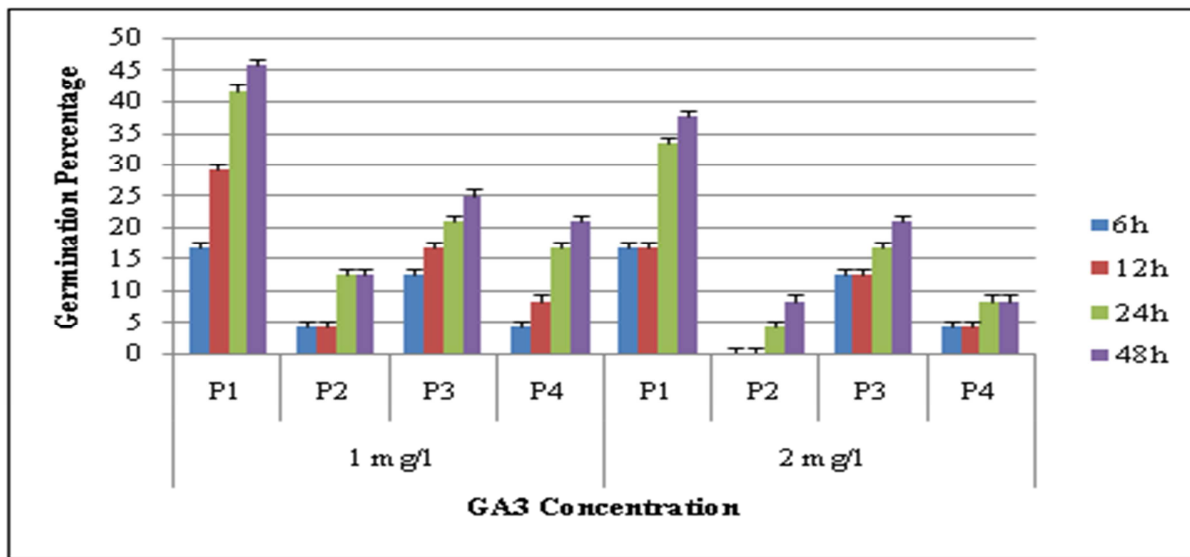
**Table 1.** Effect of Media (H<sub>2</sub>O, MS) and GA3 levels on seed germination percentages of *C. spinosa*.

Population	Media	Treatment dose (GA3 mg.l <sup>-1</sup> )	Soaking time			
			6h	12h	24h	48h
P1	MS	1000	16.66 ± 4.945	29.16±5.036	41.66 ± 5.098	45.83 ± 4.704
P2			4.16 ± 3.378	4.16 ± 3.378	12.50 ± 3.806	12.50 ± 4.148
P3			12.50 ± 4.489	16.66 ± 4.945	20.83 ± 5.110	25.00 ± 5.107
P4			4.16 ± 2.823	8.33 ± 3.388	16.66 ± 3.807	20.83 ± 4.643
P1	H <sub>2</sub> O	1000	37.50 ± 3.807	41.66 ± 4.643	54.16 ± 5.063	66.66 ± 5.089
P2			12.50 ± 2.041	12.50 ± 2.041	16.66 ± 3.378	20.83 ± 3.378
P3			29.16 ± 3.378	37.50 ± 3.817	45.83 ± 4.150	50.00 ± 4.423
P4			8.33 ± 2.041	12.5 ± 2.821	25.00 ± 4.423	29.16 ± 4.148
P1	MS	2000	16.66 ± 5.089	16.66 ± 5.036	33.33 ± 4.634	37.5 ± 4.423
P2			0.00	0.00	4.16 ± 4.422	8.33 ± 4.643
P3			12.50 ± 4.815	12.50 ± 5.041	16.66 ± 5.100	20.83 ± 4.869
P4			4.16 ± 1.12	4.16 ± 1.041	8.33 ± 2.823	8.33 ± 2.036
P1	H <sub>2</sub> O	2000	45.83 ± 3.807	58.33 ± 3.816	70.83 ± 4.815	75.00 ± 4.945
P2			12.50 ± 3.378	16.66 ± 3.807	25 ± 2.204	29.16 ± 2.823
P3			33.33 ± 3.382	41.66 ± 3.338	54.16 ± 3.809	62.50 ± 4.148
P4			20.83 ± 2.041	33.33 ± 4.815	37.5 ± 4.990	41.66 ± 2.823
Source of variation			Test F			
Population(P)			8.122***	10.195***	12.609***	14.493***
Media (M)			19.595***	26.506***	25.048***	29.912***
P x M			0.945*	0.761*	0.852*	0.880*
Population (P)			7.689***	9.497***	11.757***	13.347***
GA3 levels (GA3)			0.483*	0.406*	0.212*	0.112*
P x GA3			0.225*	0.233*	0.035*	0.046*
Media (M)			18.537***	24.969***	23.127***	27.187***
GA3 levels (GA3)			0.482*	0.410*	0.210*	0.111*
M x GA3			0.945**	4.744***	4.248***	3.557***

Each value is the mean of 2 replicates each with 12 seeds; F: test F applied to evaluate the effect of the media on the final germination percentage using AMOVA procedure; Cont.: control: without GA3 in MS and H<sub>2</sub>O media; \*\*\* significantly different at p<0.001; \*\* significantly different at p<0.01; \* not significant.

The two media (H<sub>2</sub>O and MS) differed by the basal salt composition, the MS containing micro, macro elements and vitamins. The results obtained with the different combinations (1000 or 2000 mg.l<sup>-1</sup> of GA<sub>3</sub> for 6, 12, 24 and 48h) for the four studied caper populations are shown in Table 1. *C. spinosa* showed great of variability in germination rate. Seeds developed on MS or H<sub>2</sub>O control medium (H<sub>2</sub>O and MS without GA<sub>3</sub>) did not exhibit any germination before 17 days (not shown in the table). The maximum germination rates were 12.50, 8.33 and

4.16%, respectively for the populations coded P1, P3 and P4 on H<sub>2</sub>O media. In contrast on the other media where GA<sub>3</sub> was added, the seeds germinated after one week of cultivation. All of seedlings were green and phenotypically normal. These plantlets, when transferred to fresh media, continued to grow and did not exhibit any transformation. All germination stages have been observed on the different populations. Statistically, the level of the germination varied according to the media, GA<sub>3</sub> dose and genotype.



**Fig. 1.** Seeds germination percentage of *C. spinosa* populations (P1, P2, P3 and P4) using MS media under different GA<sub>3</sub> levels according to the soaking times.

The population (P1) exhibits the best abilities to germination. The highest seed germination (75%) was achieved when the seeds were treated for 48h with 2000 mg.l<sup>-1</sup> GA<sub>3</sub> solution. The roots observed from the seedlings were longer and vigorous compared to those obtained from the control. The highest germination in each case was obtained from seeds soaked for 48 h in GA<sub>3</sub>. Low germination percentage in case of control was possibly due to dormancy and hard seeds.

*Genotype effect on germination percentage of caper seeds*

The data of germination percentages and the results of AMOVA were reported in the Table 1.

Highly significant differences ( $p < 0.001$ ) in

germination percentage at different soaking times were obtained among the different studied populations suggesting that the genotype has an effect on the seed germination.

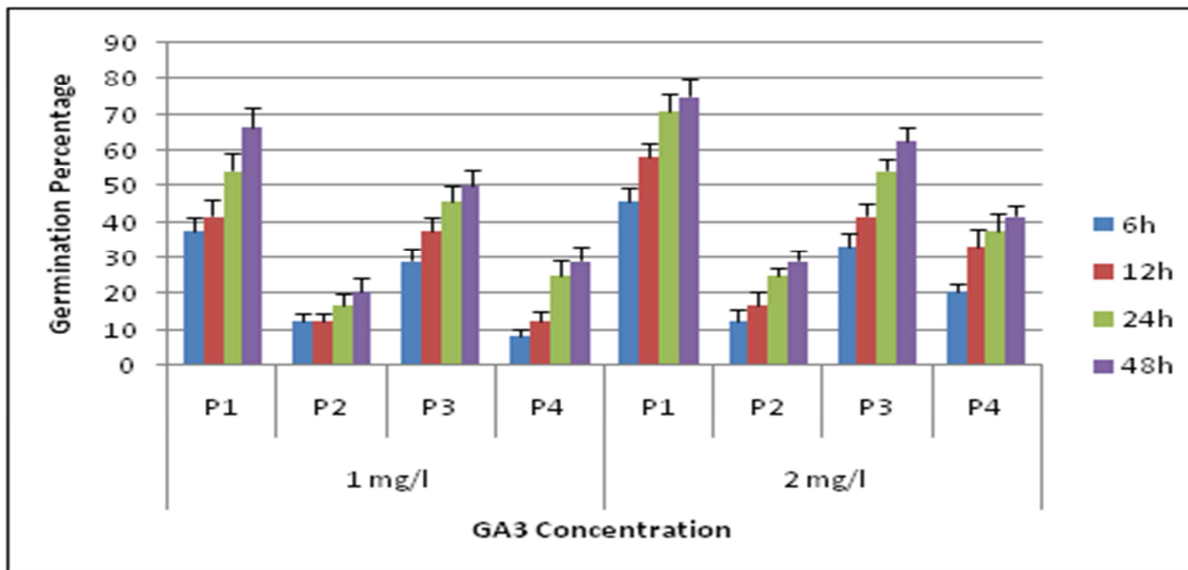
Therefore, independently to the tested conditions, the genotype seemed to be a significant factor influencing germination induction. The responses of P1 were better than those observed for P2, P3 and P4 populations (Table 1). Statistically, there was significant difference among the populations (P1, P2, P3 and P4) for MS an H<sub>2</sub>O medium at various treatments concerning the percentages of germination (Table 1). On other hand, the population-media interaction has not any significant difference. In control condition, the germination percentage varied from 4.16 to 12.50%. The population P2 can't

germinate at control condition. However, placed in control condition caper seeds have germination difficulties which would be explained by the seed dormancy.

*Media effect on germination percentage of caper seeds*

The two growth media MS and H<sub>2</sub>O were used for the experiment. The results have shown significant differences ( $p < 0.001$ ) between the two tested media

pertaining to their effect on the germination induction of caper growing *in vitro*. For all populations, H<sub>2</sub>O medium performed the better germination induction than MS medium (Figure 1). The highest seed germination was 75% obtained by using the H<sub>2</sub>O medium compared to 37.5% using MS medium. Under different GA<sub>3</sub> levels, the use of H<sub>2</sub>O media seemed to be better for germination of *C. spinosa* populations (P1, P2, P3 and P4) compared to the MS media.



**Fig. 2.** Seeds germination percentage of *C. spinosa* populations (P1, P2, P3 and P4) using H<sub>2</sub>O media under different GA<sub>3</sub> levels according to the soaking times.

*GA3 level effect on germination percentage of caper seeds*

The increase of GA<sub>3</sub> from 1000 to 2000 mg.l<sup>-1</sup> in MS media showed a negative response of seed germination of P1, P2 and P4 populations. In fact, the use of the MS media and 2000 mg.l<sup>-1</sup> GA<sub>3</sub> decreased the germination percentages of all studied populations (Figure 2). In the MS medium with 2000 mg l<sup>-1</sup> GA<sub>3</sub>, the soaking time 6 and 12 h appeared to be inhibiting germination of the population P2. However, the population P4, at the same condition, showed lower germination percentage of 4.16%. At the control conditions (H<sub>2</sub>O and MS without GA<sub>3</sub>) the P2 and P4 populations were unable to germinate.

The germination percentage was just 4.16 (P1 and P3) in MS media. For H<sub>2</sub>O medium, the effect of various

concentrations of GA<sub>3</sub> for different treatment times showed positive responses on seed germination for the all populations. The highest germination in each case was obtained from seeds soaked for 48 h in GA<sub>3</sub> at level of 2000 mg.l<sup>-1</sup>. The drop in germination was very clear in case of 2000 mg.l<sup>-1</sup> at 6, 12, 24 and 48 h of GA<sub>3</sub> soaking. We speculate that the increase of soaking duration, independently to the GA<sub>3</sub> concentration, was stimulatory showing improvement of germination percentage. The increase of germination percentage varied according the studied populations and the GA<sub>3</sub> levels.

*Survival of acclimatized caper plants*

Plantlets issued from seeds germination were acclimatized. Around 95% of the plants have survived. They were later transferred to pots containing a

sterile soil (2/3 peat and 1/3 sand), initially placed under high humidity (80%) in a growth chamber and subsequently grown in a greenhouse. All of the plants reached maturity (Figure 3).

### Discussion

The conservation of genetic resources of medicinal plants has been of increasing interest in worldwide.

The ex situ conservation of plant genetic resources by seed storage, when feasible, is the simplest and most economical method. Therefore, the seed germination behavior is an integral part of *ex situ* conservation, especially for developing standard viability monitoring protocols and to ensure sufficient populations for germplasm regeneration (Bewley and Black, 1994).



**Fig. 3.** Acclimated caper plants growing in pots in the greenhouse 3 months after transfer from disks.

Germination is known to be a complex trait that is affected by interactions between genetic determinants and environmental factors. From the literature it appears that the germination performance of caper seed is poor (Sozzi and Chiesa, 1995). Sozzi and Chiesa (1995) reported that caper seed dormancy is induced by coat structure. Pre-treatments are used to help break this dormancy, often simulating environmental processes.

The caper seed is known by its weakness to germinate under natural condition. To ensure high germination, temperature, light, pre soaking treatment and scarification of seed coat have been applied (Shirazi, 2003; Arefi *et al.*, 2012; Soyler and Arslan, 1999). Soyler and Khawar (2007) reported role of NAA and GA<sub>3</sub> in breaking seed dormancy and determine the extent of their effectivity in seed germination of *Capparis ovata* var. *herbacea*. Also, Saifi *et al.* (2013) show that the *capparis* seeds were characterized by a

combined (physical, light, thermal and physiological) type of dormancy, with the seed coat involved in the maintenance of physical dormancy. In this study, *in vitro* seed germination and seedling development of *Capparis spinosa* L. were studied and several treatments were evaluated to determine the ability of several factors to increase the percentage of germination. High variability was observed among the germination percentages of the different treatments (4.16-75%) due to their different efficiency to break dormancy.

Our research showed that the germination rate was affected by genotype, gibberellic acid (GA<sub>3</sub>) and the germination medium. These factors are determinant to improve germination percentage. Large numbers of seedlings can be raised in a relatively short time using *in vitro* germination which is an important part of the conservation program of the caper seeds at Tunisian National Gene Bank.



Variation in germination behaviour that occurs among different populations within the same species has been widely reported (Andersson and Milberg, 1998; Baskin and Baskin, 1998). The use of H<sub>2</sub>O as media and a GA<sub>3</sub> level of 2000 mg/l showed an improvement of the germination percentage of caper seeds. Therefore, the use of these conditions is required to favorite the caper seed germination.

At the control condition the caper seeds showed lower germination percentage. This incapacity to germinate would be explained by a dormancy of embryos. It has been reported that the gibberellic acid (GA<sub>3</sub>) enhances seed germination in species exhibiting physiological or morphophysiological dormancy (Bewley and Black, 1994; Baskin and Baskin, 1998; Saifi *et al.* 2013). The most important factor of germination percentage appears to be the genotype reflecting by the significant differences within studied populations.

### Conclusion

For achieving re-establishment of the natural vegetation which is on the verge of extinction, restoration practices like artificial establishment of the same type of vegetation is essential. Utilizing native plants in the urban landscape promotes their sustainability in that region, facilitates conservation of natural plant diversity and imparts a fully natural appeal to the landscape. This is the initial step for the commercial production of these plants in Tunisia's nurseries. Mass propagation of native plants like *Capparis spinosa*, a rare perennial in Tunisia, can be standardized for further commercial use and also to ensure sufficient populations for germplasm regeneration for conservation purpose. The germination process is influenced by several factors. The genotype, media and GA<sub>3</sub> concentration, influenced caper seed germination. In the present study we noted that the optimum conditions for germination percentage 75% were: i) the use of H<sub>2</sub>O as media for *in vitro* germination and ii) pre treating the seeds with 2000 mg l<sup>-1</sup> GA<sub>3</sub> for 48 hours . The results indicate the high potential of capers that could be used in propagation. The technical implementation

of the protocols used in seed germination should allow the conservation of the Tunisian caper and also make possible the cultivation of this species as a new crop of promising market.

### Acknowledgement

The authors thank the Tunisian Ministry of Environment, the National Gene Bank of Tunisia for financial support and the group of medicinal and aromatic plants of the National Gene Bank.

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