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Research Note

Seed treatments for improved germination of caper (*Capparis spinosa*)

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Summary

This study analyses the effects of seven treatments for removing hardseededness and four for breaking physiological dormancy in caper seeds. Seeds were germinated in a growth chamber and the maximum germination percentage, the time to reach 50% of final germination and the mean relative cumulative rate were calculated. The logistic function was suitable for analysing caper seed germination. Acid scarification followed by the addition of a GA_3 solution to the germination substrate was the best**,** efficient and cost effective method for ensuring satisfactory seed germination. Acid scarification can be substituted by mechanical scarification with ultrasound, hot water scarification or soaking, but these procedures require longer germination periods to reach satisfactory germination levels. The soaking method proved useful enough to remove hardseededness and it is also the most simple among the assayed treatments.

Experimental and discussion

Caper (*Capparis spinosa* L.) is a typical suffruticose deciduous plant considered as ruderal of Mediterranean climates; it is well adapted and profited in countries such as Greece, Cyprus and Turkey and it is cultivated commercially in Morocco, Spain and Italy. Valued for its medicinal qualities, the caper is used in the food and cosmetic industries, in afforestation projects and landscape control of erosion and it is also appreciated from an ornamental point of view. In the food industry, the caper is used principally for its young flower buds pickled in vinegar. A growing worldwide demand for this food product has been observed in recent years and thus an increased interest in the cultivation of the caper as a commercial crop has been noted. However, both methods of propagation, by cutting and by seed, present serious problems, being this one of the greatest restrictions to the commercial expansion of this crop. The poor germination capacity of caper seeds in field conditions has been cited in different countries as Spain (Imbernón, 2000), Italy (Barbera and Di Lorenzo, 1984), the United States of America (Bond, 1990), Cyprus

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(Orphanus, 1983) and Turkey (Tansi, 1999; Soyler and Arslan, 1999). Sozzi and Chiesa (1995) attributed the poor germination of caper to seed dormancy, imposed probably by the seed coat. Imbernón (2000) reported a positive response to single gibberellic acid (GA_3) applications when soaked or added to the test substrate. Yildirim (1998) and Tansi (1999) obtained the highest germination percentages by means of chemical scarification followed by soaking in GA_3.

The main objective of the present research was to analyze germination curves and to seek a simple, efficient and cost effective method for ensuring satisfactory seed germination.

Ripe fruits were collected in September of 2000 from four ten-year-old plants of the cv. *Común*, grown in the province of Valencia (Spain). The seeds were extracted from the fruit, rinsed in tap water and dried in the shade at room temperature for two days. Mature dark brown seeds were selected and stored in closed plastic cans at 7 ± 0.5°C until needed. Seed viability was determined by a tetrazolium test (Perry, 1987) using 400 control seeds, placed in 4 separate replications of 100 seeds. The same test was applied to seeds that failed to germinate.

To enhance seed germination, treatments of two types, individually or in combination, were assayed: 1) for removing hardseededness (physical dormancy), scarifying or softening the coat; 2) for breaking physiological dormancy. Products, concentrations, times and temperatures were selected on the basis of our previous research (Imbernón, 2000) and this in turn on the International Rules for Seed Testing (ISTA, 1999), Hartman *et al*. (1990), Yambe and Takeno (1992) and the previously cited studies dealing with caper seed dormancy.

1) Treatments for removing hardseededness (T-I):
   Mechanical scarification with sandpaper (MSS):
   using a seed scarifier with a cylinder (∅ 7.5 cm) lined with sandpaper (AS 4 Debray) rotating at 60 rpm for 90 min. For each sample, the used sandpaper was removed and replaced with new sandpaper.
   Mechanical scarification with ultrasound (MSU):
   ultrasonic treatment in water, initially at room temperature, for 30 min, using an ultrasonic apparatus (P. Selecta Ultrasons, model 513, 150 w, 40 kHz).
   Acid scarification (AS): soaking the seeds in 100 ml concentrated sulphuric acid at room temperature for 60 min.
   Hot water scarification (HWS): soaking the seeds in 100 ml hot water (50°C) for 30 min.
   Enzymatic scarification (ES): soaking the seeds in 100 ml 0.5% Driselase (from Basidiomycetes, Sigma) solution for 36 h.
   Soaking (S): soaking the seeds in 100 ml water at room temperature for 24 h.
   Control (C-I): no treatment.

2) Treatments for breaking physiological dormancy (T-II):
   Potassium nitrate (KN): adding 0.2% KNO_3 solution to saturate the test substrate. The substrate was moistened thereafter using distilled water.

GA_3 (GA): adding 500 ppm *GA_3* solution to saturate the test substrate.
Soaking in GA_3 (GAS): soaking the seeds in 100 ml 500 ppm *GA_3* solution for 12 h.
Control (C-II): no treatment.

To prevent fungal diseases, the seeds were surface sterilised for 10 min in sodium hypochlorite, rinsed twice in tap water and once more in distilled water. Germination tests, conducted at the *Universidad Politécnica de Valencia* (Valencia, Spain), were performed in closed 9-cm Petri dishes (∅ 9 cm) using the BP (between paper) method with two layers of filter paper Whatman No.1 (ISTA, 1999) moistened with distilled water, GA_3 or potassium nitrate solutions, to which 250 ppm carbendazim was added to prevent fungal development. The sample test consisted of 400 seeds taken at random, placed in 4 separated replications of 100 seeds. Petri dishes were placed in a growth chamber (model Climatronic) at 30 ± 1/20 ± 1°C, 85 ± 1% r.h., a photoperiod of 12 h and a photosynthetic photon flux density of 324 μmol m^{-2} s^{-1} for 100 days. A seed was considered germinated when the radicle protruded from the seed coat. Each assay was considered satisfactory when the difference between the maximum and the minimum germination percentages of the 4 replications was no higher than the tolerance indicated in the International Rules for Seed Testing (ISTA, 1999). If the opposite occurred, the assay was repeated. Germination tests started at the beginning of October 2000.

Germination data of each replicate were fitted to the logistic function $G = A[1 \pm \exp(\beta - kt)]^{-1}$ (Torres and Frutos, 1990; Cheng and Gordon, 2000). Derived quantities with biological significance were also calculated, as time to reach 50% of final germination percentage ($Gt\ 50 = \beta/k$, days) and mean relative cumulative germination rate ($k/2$, $days^{-1}$). The design was a 7 × 4 factorial (seven T-I and four T-II treatments), replicated into four blocks. Variables (A, β, k, β/k and $k/2$) and non-viable seeds after test germination were analysed by SAS analysis of variance (SAS Institute, 1989). Percentage data were arcsin transformed before analysis. A probability of ≤ 5% was considered significant.

The tetrazolium staining pattern revealed a high viability of untreated seeds, 94.25%, similar those cited in the literature (93% Sozzi and Chiesa, 1995; 97% Tansi, 1999; 96% Rinaldelli, 2000).

The coefficients of determination (R^2) for 112 curves (4 replicates from 28 combinations of variation sources) ranged from 0.9126 to 0.9974 (data not indicated), with F ratio values of the model statistically significant ($P < 0.01$). This indicates that the use of the logistic function is suitable for analysing caper seed germination as done in similar studies of other crops (Torres and Frutos, 1990; Tei and Ciriciofolo, 1997; Hara, 1999; Cheng and Gordon, 2000). Figures 1 and 2 show the fitted curves corresponding to the average values of each treatment.

Final germination percentages (A), β and β/k were affected by treatments of each type, whilst function parameter k only by T-I treatments; no interaction resulted significant. Mean values of the function parameters A and β, the derived quantities *Gt 50* and *k/2* are shown in table 1. High values of A were found, as those obtained with S, MSU and HWS treatments, to be over 70% (with differences in regard to the other T-I treatments) and especially high were those obtained with the GA treatment, with average values over 90% (with differences in regard to the other T-II treatments), much higher than the maximum

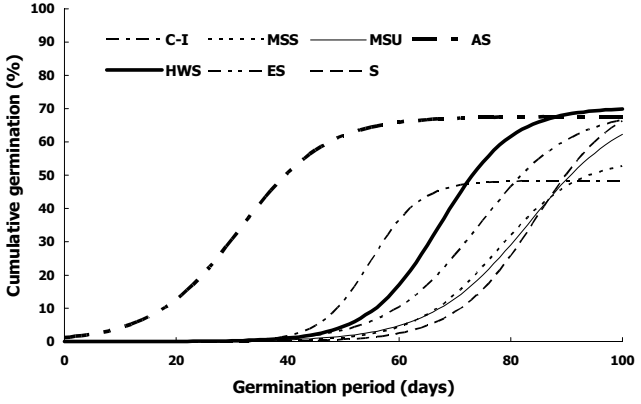

Figure 1. Logistic model to cumulative germination curves of caper seed. Mean values for treatments to remove hardseededness (T-I): C-I, control; MSS, mechanical scarification with sandpaper; MSU, mechanical scarification with ultrasound; AS, chemical scarification; HWS, hot water scarification; ES, enzymatic scarification; S, soaking.

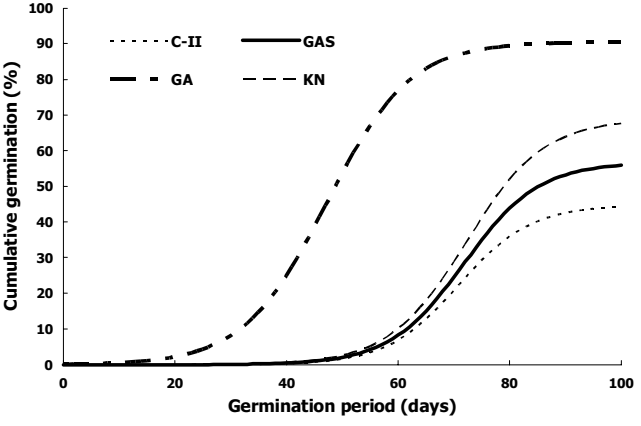

Figure 2. Logistic model to cumulative germination curves of caper seed. Mean values for treatments to break physiological dormancy (T-II): C-II, control; GAS, soaking in GA_3; GA, adding GA_3 to the test substrate; KN adding KNO_3 to the test substrate.

values cited in the literature (38% Macchia and Casano, 1993; 48% Yildirim, 1998; 53% Tansi, 1999; 68% Sozzi and Chiesa, 1995; above 50% Rinaldelli, 2000) and higher than the 80% obtained by Orphanos (1983). Both β and *Gt 50* were affected by both treatment types, in the sense that the lower average values in both cases were obtained with GA among T-II treatments (without any more significant difference) and AS among T-I. By contrast, with the high values of *A*, the seeds showed slow germination rates, requiring a long test period (in this case up to 100 days) and also a long period to reach 50% of final germination (a figure on the order of 62-83 days), higher than the total

Table 1. Mean values of the variables *A*, β, *Gt 50* and *k/2* of germination caper seeds, as related to treatments for remove hardseededness (T-I) and to break physiological dormancy (T-II).

		A (%)	β	*Gt 50 (days)*	*k/2 (days^{-1})*
T-I	C-I	48.33 d	12.24 a	61.89 c	0.223 a
	MSS	55.19 c	10.72 abc	78.30 ab	0.138 bc
	MSU	71.74 a	9.42 c	83.05 a	0.113 c
	AS	67.55 b	4.02 d	33.28 d	0.128 bc
	HWS	70.19 a	10.43 bc	69.27 bc	0.155 b
	ES	69.36 b	9.05 c	73.86 ab	0.122 c
	S	74.25 a	11.59 ab	81.26 a	0.137 bc
T-II	C-II	44.76 d	11.00 a	74.28 a	0.155
	GAS	56.71 c	10.77 a	75.72 a	0.150
	GA	90.48 a	6.37 b	49.72 b	0.135
	KN	68.97 b	10.41 a	75.77 a	0.144

T-I: C-I, control; MSS, mechanical scarification with sandpaper; MSU, mechanical scarification with ultrasound; AS, acid scarification; HWS, hot water scarification; ES, enzymatic scarification; S, soaking.
T-II: C-II, control; GAS, soaking in GA_3; GA, adding GA_3 to the test substrate; KN, adding KNO_3 to the test substrate; Means in columns followed by different letter differ significantly at $P < 0.05$ using LSD test.

time of incubation normally used in the previously cited studies (30 days Sozzi and Chiesa, 1995; 2 months Tansi, 1999; 60 days Rinaldelli, 2000). On the other hand, long test periods are usually required for species in which dormancy is common (Thompson, 1979). Parameter *k* and then *k/2* (i.e. the mean relative cumulative germination rate) was only affected by T-I treatments, the lower values corresponding to MSU and ES and the higher values to C-I treatments.

Results of the tetrazolium test applied to seeds that failed to germinate (expressed as percentage of number of non-viable seeds regarding the 100 seeds initially used in each replication of germination test) revealed that seed viability was affected by T-I but not by T-II treatments and no interaction resulted significant. AS was the only treatment that reduced the seed viability (mean value being 91.81%) in terms of the C-I (mean value being 94.75%) and despite the fact that this difference was statistically significant, it was observed the high viability of acid scarified seeds.

The fact that differences in the results of germination tests did emerge among treatments to overcome the physiological dormancy and the appreciable average values of *A* corresponding to C-I treatments (28.4, 41.3, 74.5 and 49.0% corresponding respectively to C-II, GAS, GA and NK), proves that caper seeds analysed not only were sensitive to gibberellins when they have been previously scarified, but also without previous scarification, contradicting what has been reported by Sozzi and Chiesa (1995). Thus, the control of germination in the caper seeds analysed not only resides in the seed coat (physical dormancy), but it is likely to be due as well to a physiological dormancy. The lack of significance in the interaction between the two groups of treatments allows us to assume, as concluded by Rinaldelli (2000), that the two types of treatments exert an effect which is more additive than synergetic.

Acid scarification followed by the addition of a GA_3 solution to the germination substrate is a simple, efficient and cost effective method for ensuring satisfactory seed

germination. Acid scarification can be substituted for a mechanical scarification with ultrasound, a hot water scarification or a soaking, but these procedures require longer germination periods to reach satisfactory levels of germination. The soaking method has proved useful enough to remove hardseededness and it is also the most simple among the assayed treatments.

Taking into account the large differences observed in the results obtained by diverse authors using the same treatments with different plant material, further experiments should be conducted to study the role played by plant materials and their origins in the response to these treatments.

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