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Germination behaviour after storage of caper seeds

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Summary

This five-year study (1999-2003) was designed to analyse the effects of the duration of the storage period on the viability and germination of caper seeds. Seeds were stored at 7°C until the tests were conducted. Germination and viability were tested using seeds of the same lot, both recently-harvested and after storage for one, two, three and four years. Germination tests were performed in closed Petri dishes in a growth chamber. Seed viability was determined by tetrazolium testing. A treatment to enhance seed germination was assayed, consisting in acid scarification to remove hardseededness, followed by the addition of a gibberellic acid solution to saturate the test substrate to break physiological dormancy. Recently-harvested caper seeds present the highest germination rate and the shortest time to reach 50% of the final germination percentage. The longevity of caper seeds stored at 7°C is 3.85 years, but a storage period no longer than two years is recommended. During this period the viability does not decrease and high germination percentages can be obtained with scarification followed by the addition of gibberellic acid to the germination substrate. Individual treatment with gibberellic acid is preferable for longer periods of seed storage.

Introduction

The poor germination of caper seeds in field conditions is one of the greatest restrictions to the expansion of this crop. A number of studies (Orphanos, 1983; Bond, 1990; Sozzi and Chiesa, 1995; Yildirim, 1998; Soyler and Arslan, 1999; Rinaldelli, 2000) have been carried out in order to obtain high germination percentages (*G*). Pascual *et al.* (2004) concluded that acid scarification followed by the addition of a gibberellic acid (GA) solution to the germination substrate was the best, most efficient and cost-effective method for ensuring satisfactory seed germination, over 90%.

On the other hand, a large variation in the percentages cited for caper germination between and within seed lots has been identified (Imbernón, 2000; Ferreros, 2001). Pascual *et al.* (2003) analysed the germination of caper seeds as influenced by the position of fruit on the mother plant, fruit maturation stage and fruit weight, concluding that the final germination percentage was affected by fruit position and unit fruit weight, but not by the stage of fruit maturation. The best results (over 90% germination) were obtained with seeds proceeding from large or medium-sized fruits and with fruits set in the central or apical region of the branches, chemically scarified and then applying a GA solution to the germination substrate.

Occasionally, there are lots in which no seed germinated, leading to the generalized conclusion that seed longevity is reduced, although there are no published studies on this subject. Nevertheless, Orphanos (1983) noted that *G* declined when the seed was stored for more than 12 months at room temperature.

The objective of this research was to study the germination behaviour of seeds of caper (*Capparis spinosa* L.) as related to the duration of the storage period. In particular, we analysed the viability (*V*) and germination curves to determinate the precise storage period of the seeds maintained at a low temperature (7ºC).

Material and methods

Ripe fruits were collected in September 1999 from four nine-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 indoors at room temperature for two days. Mature, dark-brown seeds were selected, rejecting both the light brown seeds by flotation in tap water, and the small ones (< 2mm). The selected seeds were stored in closed plastic containers at 7 ± 0.5°C until the tests were conducted. Seed moisture content (ranging from 7.1% to 7.3%) was determined by drying triplicate samples of 30 seeds for 24 hours at 103°C. Five germination tests were carried out, each one initiated at the beginning of October 1999, 2000, 2001, 2002 and 2003 respectively.

In addition to the testing control seeds, we assayed the treatment that gave the best results for enhancing seed germination in previous studies (Pascual *et al.,* 2004). This treatment consisted first of acid scarification (AS) to remove hardseededness by soaking the seeds in 0.1 L concentrated sulphuric acid at room temperature for 1h, and second, to break physiological dormancy, a 500 mg L^{-1} GA (GA_3; AG-100 L.S.) solution was added to saturate the test substrate. Germination tests were conducted in agreement with the International Rules for Seed Testing (ISTA, 1999) and the conditions were the described in Pascual *et al.* (2003). The viability (*V*) of seeds that failed to germinate (both control and treated seeds) was determined by a tetrazolium test (Perry, 1987) using a Petri dish for each replication and also considering as viable the seeds germinated in germination tests.

The longevity period was determined as the time taken for 50% of the seeds to die (half-viability period [p_{50}]), because it is generally considered as the most accurately determined longevity period (Roberts, 1972; Ellis *et al.,* 1990; Tang *et al.,* 2000; Nayal *et al.,* 2002).

The design was a 5 × 2 factorial (five years and two treatments), replicated into four blocks. Germination data of each replicate were fitted to the logistic function $G = A[1 + \exp(\beta\text{-}kt)]^{-1}$, which is a special case of the Richards function, when $\nu = 1$ [$G = A[1 \pm \exp(\beta\text{-}kt)]^{-1/\nu}$], where ν estimates the inflexion point of the curve (Causton and Venus, 1981; Torres and Frutos, 1990; Cheng and Gordon, 2000). Derived quantities with biological significance were also calculated, as time in days to reach 50% of final germination percentage ($Gt\ 50 = \beta/k$) and mean relative cumulative germination rate ($k/2$, $days^{-1}$). *V*, *G* and variables (*A*, β, $k/2$, and *Gt 50*) were analysed by SAS analysis of variance (SAS Institute, 1989). Percentage data were arcsin transformed before analysis. A probability of

≤ 0.05% was considered significant. Mean separations were performed when appropriate using the least significant difference (LSD) at $P \leq 0.05$.

In light of the results obtained with the combined treatments on seeds stored for three years, with the seeds stored for four years, separate treatments were conducted 1) with acid scarification (AS) and 2) the addition of gibberellic acid to the germination substrate (GA). The design was a 2 × 2 factorial replicated into four blocks, allowing for the independent analysis of the effect of the treatments assayed T I) for removing hardseededness (AS) and T II) for breaking physiological dormancy (GA) with seeds stored for four years.

Results

Parameters *V* and *G* were affected ($P < 0.0001$) by the storage period and treatment as well as their interaction ($P < 0.0001$) (table 1). Untreated seeds showed a high *V* until three years of storage, ranging from 84 to 93%, decreasing significantly to 41% after four years of storage. From the fitted curve corresponding to *V* of the control seeds, p_{50} was obtained, the result being 3.85 years (figure 1). With treated seeds, *V* decreased significantly to 57% after three years and to 36% after four years of storage (figure 1).

With treated seeds, the evolution of *G* as related to the storage period (figure 1) was very similar to that of *V*, but with untreated seeds there were marked differences between the *G* and *V* values from the same storage period. The lower values corresponded to *G*, and never reached the value of 40%. The seed treatment significantly enhanced the germination of caper seeds.

In relation to the logistic function, the coefficients of determination (R^2) for 40 curves (4 replicates from 10 combinations of variation sources) were higher than 0.965, with F ratio values of the model statistically significant (P 0.01). Thus, the use of the logistic function is suitable for analysing caper seed germination, permitting the use of the variable *A* (final germination percentage) instead of *G* as well as other variables such as β and *k*.

The figure 2 shows the fitted curves corresponding to the average values for each year and treatment. There are marked differences between the curves of untreated and treated seeds (scarification followed by the addition of GA) with higher *A* values for the latter. There is also a clear displacement to the left of curves corresponding to treated seeds, that is to say, a more rapid germination.

Table 1. Analysis of variance of germination percentage (*G*) and viability (*V*) obtained for seeds stored up to four years and treated (acid scarification followed by GA_3 addition to the test substrate) to ensure satisfactory seed germination.

		G		*V*	
Source	d.f.	F ratio	*P*	F ratio	*P*
Storage period	4	56.225	< 0.0001	102.764	< 0.0001
Treatment	1	464.372	< 0.0001	25.485	< 0.0001
Storage period × Treatment	4	26.868	< 0.0001	10.083	< 0.0001

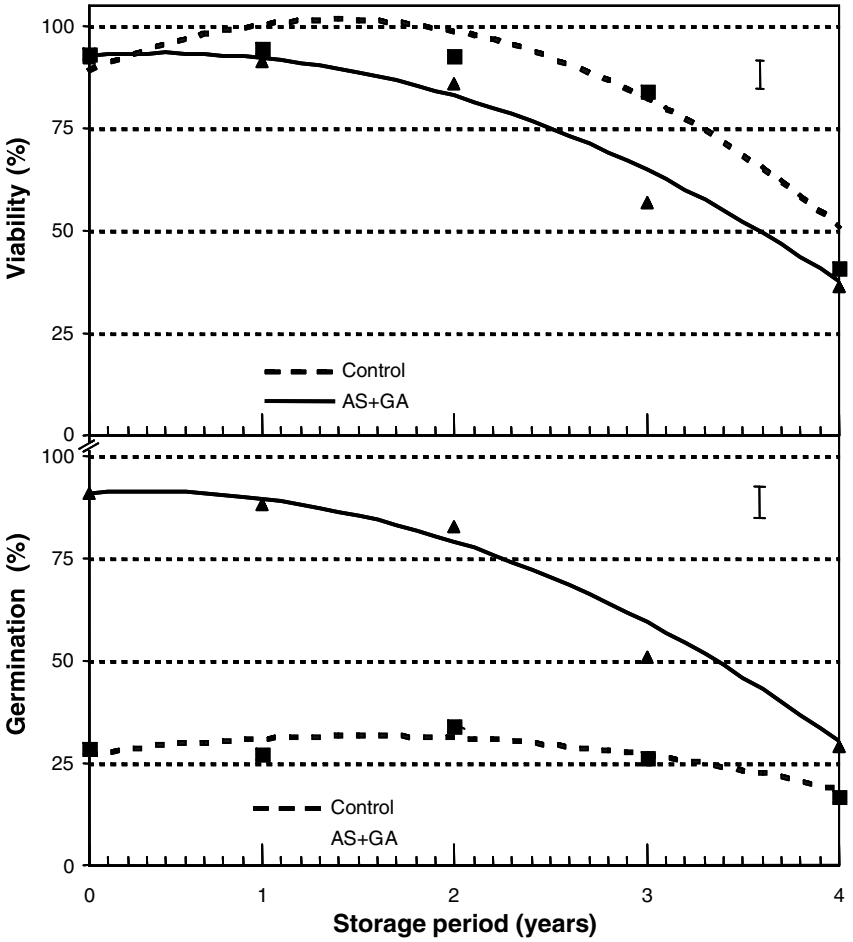

Figure 1. Evolution of viability and germination percentage of caper seeds, as related to storage period and treatment to ensure satisfactory seed germination (AS+GA, acid scarification followed by GA_3 addition to the test substrate). Values are means for the four replications. Vertical bars show LSD ($P \leq 0.05$). The curves drawn are the best-fit, second order polynomials. The equations of these curves are as follows (being Y the number of years of storage):

V (control seeds) = 90.30 + 16.03 Y – 6.88 Y2, R2 = 0.95, $P = 0.04$
V (treated seeds) = 92.79 + 3.35 Y – 4.48 Y2, R2 = 0.98, $P = 0.02$
G (control seeds) = 27.23 + 6.22 Y – 2.16 Y2, R2 = 0.82, $P = 0.18$
G (treated seeds) = 91.11 + 2.52 Y – 4.64 Y2, R2 = 0.98, $P = 0.02$

The variable *A* was affected by both storage period and treatment, resulting significant the respective interaction ($P < 0.01$) (table 2). The *A* value of recently-harvested control seeds was very low, a figure on the order of 30%, and similar values were obtained with seeds stored up to three years, dropping to 18% after the fourth storage year. These values increased with scarification followed by the addition of GA, ranging from 80 to 89% in seeds stored up to two years, but the values dropped to 51% after three years, and to 35% after four years of storage, as did the corresponding viability.

The function parameter β, which places the curve in relation to the time axis, without any biological significance, was only affected by seed treatment (the interaction resulted not significant), the higher values corresponding to the control seeds (table 2).

The function parameter *k* (a "rate parameter") and thus *k/2* (the mean relative cumulative germination rate) were only affected by the duration of the storage period, the higher values corresponding to recently-harvested seeds; the interaction did not result significant (table 2).

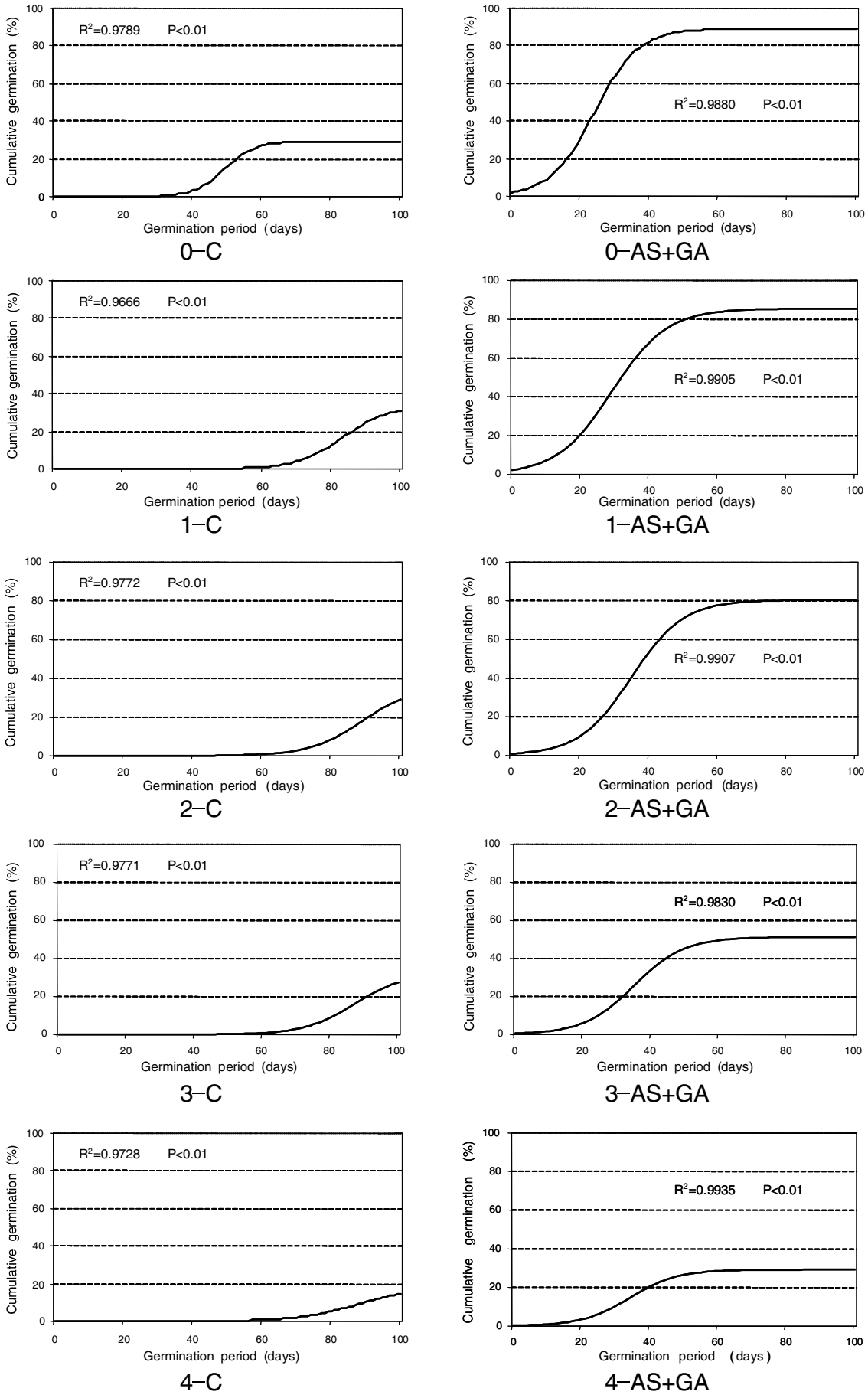

Figure 2. Logistic model fitted to cumulative germination curves of caper seeds, as related to storage period (0, recently harvested seeds; 1, 2, 3, 4, years of storage) and treatment to ensure satisfactory seed germination (C, control; AS+GA, acid scarification followed by GA_3 addition to the test substrate).

Table 2. Influence of storage period (0, recently harvested seeds; 1, 2, 3, 4, years of storage) and treatment to ensure satisfactory seed germination (AS+AG: acid scarification followed by GA_3 addition to the test substrate) on values of variables *A*, β, *k/2* and *Gt 50* of germination caper seeds.

		A	β	*k*/2	*Gt 50*
Storage period					
	0	59.22a	6.96-	0.0969a	33.83c
	1	59.29a	8.19-	0.0696b	53.19b
	2	58.16a	8.19-	0.0662b	61.97a
	3	41.65b	8.22-	0.0674b	60.97a
	4	23.66c	7.62-	0.0654b	61.09a
Treatment					
	Control	29.53b	11.33a	0.0767-	78.03a
	AS+GA	67.07a	4.34b	0.0695-	31.59b
Storage period × Treatment		**	NS	NS	**
	0 Control	29.33	10.13	0.1150	42.74
	0 AS+GA	89.11	3.79	0.0788	24.92
	1 Control	33.12	12.73	0.0765	83.25
	1 AS+GA	85.47	3.65	0.0628	29.12
	2 Control	35.89	11.77	0.0662	89.00
	2 AS+GA	80.44	4.61	0.0663	34.93
	3 Control	32.11	11.72	0.0673	87.08
	3 AS+GA	51.20	4.72	0.0675	34.85
	4 Control	18.19	10.3	0.0586	88.06
	4 AS+GA	29.14	4.93	0.0723	34.13
	LSD	10.82	----	----	6.55
Analysis of variance					
Source	(d.f.)		F ratio		
Storage period	(4)	35.209**	0.701^{NS}	3.829*	55.437**
Treatment	(1)	248.385**	141.401**	1.386^{NS}	1047.701**
Storage period × Treatment	(4)	16.113**	1.079^{NS}	1.909^{NS}	24.928**

Means in columns followed by different letters differ significantly at $P \leq 0.05$ using LSD test.
NS, * and ** indicates not significant or significant at $P \leq 0.05$ and $P \leq 0.01$, respectively.

The variable *Gt 50* (time to reach 50% of the final germination percentage) was affected by both seed treatment and storage period, the lowest values corresponding respectively to treated seeds and to recently-harvested seeds, followed in the latter case by seeds stored for one year (table 2). On analysing the interaction, it was observed that the recently-harvested control seeds differed from all the other older seeds while for the treated seeds, the ones stored for one year did not differ from either the recently-harvested or the older seeds.

The results obtained in the assays carried out with seeds stored for four years (table 3) showed that the individual treatment with acid scarification reduced both *V* and *G*, whilst adding GA increased *G* without affecting *V*, and the interaction was not significant.

Table 3. Influence of individual treatments to ensure satisfactory seed germination (T I: acid scarification; T II: GA_3 addition to the test substrate) on germination percentage (*G*) and viability (*V*) of caper seeds, stored for four years.

Treatment		*G* (%)	*V* (%)
T I	Control	26.00 a	45.25 a
	Acid scarification	20.50 b	32.38 b
T II	Control	15.00 b	37.50 -
	GA_3 addition to the test substrate	31.50 a	40.13 -
Analysis of variance			
Source	(d.f.)	F ratio	
T I	(1)	4.973*	9.718**
T II	(1)	44.753**	0.404^{NS}
T I × T II	(1)	0.370^{NS}	1.540^{NS}

Means in columns followed by different letter differ significantly at $P \leq 0.05$ using LSD test.
NS, * and ** indicates not significant or significant at $P \leq 0.05$ and $P \leq 0.01$, respectively.

Discussion

The use of the logistic function is suitable for analysing caper seed germination, as demonstrate in previous studies (Pascual *et al.,* 2003; Pascual *et al.,* 2004). *A* value of recently-harvested control seeds was very low, agreeing with values cited in the literature (Orphanos, 1983; Bond, 1990; Yildirim, 1998; Rinaldelli, 2000); this value increased with acid scarification followed by the addition of GA as reported by Pascual *et al.* (2004).

Seeds cannot retain their viability indefinitely and after a period of time, depending on the species, the seeds deteriorate. Although the occurrence of hardseededness, as in the caper, extends seed longevity (Copeland and McDonald, 1995), it has been reported that caper seed germination declined when the seed was kept at room temperature for more than 12 months (Orphanos, 1983). However, in the present experiment, it has been found that the viability of control seeds was maintained over 84% in storage for a three -year period and the corresponding *A* value, although very low, was maintained at values similar to the recently-harvested seeds, a figure on the order of 30%. The *V* and *A* values of control seeds stored for four years fell to 41% and 17% respectively. When, after a given period of storage (in the conditions of this experiment) the seeds were chemically scarified and GA was added to the germination substrate, the values of *V* and *A* remained very high after a period of up to two years, but both values dropped to about 50% after three years and 30% after four years of storage. Since the germination percentage of seeds stored for four years is far lower than the germination standards for seeds of different species in international commerce (Maynard and Hochmuth, 1997), it is not necessary to continue storing the seeds for a longer period.

Several theories have been suggested as basic causes of seed deterioration during storage that operate in combination (Copeland and McDonald, 1995). There are enzymatic and metabolic reactions whose rates are enhanced by high temperature, causing a more rapid deterioration (Copeland and McDonald, 1995) so that a reduction in storage temperature improves longevity (Ellis, 1991). This may explain the earlier declining germination obtained by Orphanos (1983) in caper seeds stored at room temperature, although it could also be due to a lower reserve content of these seeds, since this author does not indicate his criteria for seed selection.

The longevity of caper seeds, analysed in the conditions of this experiment, can be considered as 3.85 years, but evidently seed deterioration had started earlier. Recently-harvested caper seeds presented the highest germination rate, and the time to reach 50% of the final germination percentage presented significant differences in relation to ageing, from the third year of storage in control seeds and from the second year in treated ones. Probably the seed coat disruption, which increases with ageing, makes seeds more sensitive to sulphuric acid, damaging the embryo; this is confirmed by the results obtained from the assays carried out with seeds stored for four years.

In conclusion, recently-harvested caper seeds present the highest germination rate. The longevity of caper seeds of the assayed lot stored at 7°C is 3.85 years, but a storage period of no longer than two years is recommended, because during this period the viability does not decrease and high germination percentages are obtained with scarification with sulphuric acid followed by the addition of a solution of GA to the substrate. For seeds stored for longer periods, the individual treatment with GA is preferable. Further experiments should be conducted to study the vigour in relation to deterioration under controlled-deterioration storage conditions.

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