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Collection Guidelines to Achieve a Viable Caper Commercial Propagation

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Abstract: The caper (*Capparis spinosa* L.) is a perennial plant characteristic of the Mediterranean region that presents difficulties in its propagation, both vegetatively and by seeds. The main aim of this study is to provide collection guidelines to achieve a viable caper commercial propagation, for which three experiments were undertaken to determine the viability and germination in different seed lots. In the first experiment, commercial and own produced seeds (collected with the same criteria as commercial seeds) were analysed; the commercial seeds presented the lowest viability and germination. The second experiment analysed the effect of the fruit (from which the seeds were extracted) at its maturation stage, obtaining the lowest seed viability and germination in the seeds extracted from the dry fruits. In the third experiment, seed viability and germination were analysed immediately after collection, following a short drying period (3 d), and after six storage months. Viability and germination decreased with seed storage. Overall, it can be stated that caper seeds are sensitive to desiccation; consequently, a general rule of thumb is to collect the fruits once a week, to extract the seeds, and to plant them immediately for germination.

Keywords: *Capparis spinosa* L.; seed viability; germination percentage; seed moisture content; gibberellic acid

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1. Introduction

The caper (*Capparis spinosa* L.) is a perennial plant characteristic of the Mediterranean region, which can grow spontaneously or cultivate in arid or semi-arid climates. It is a creeping shrub that can reach a height of up to 0.5 m with branches up to 3 m long. It has a high agricultural potential and a great demand for exploitation [1] due to its various uses, highlighting food (flower buds and immature fruits, pickled in brine; [2]), pharmaceutical industry [1,3,4], and in xerogardening and landscaping for its ornamental value [5], its resistance to drought, and its ability to reduce soil erosion [1,6–8].

Traditionally, caper has been propagated both vegetatively, using stem cuttings, and by seeds, presenting important difficulties in both cases. Recently, Sottile et al. [9] reported the state-of-the-art of the main techniques of sexual and vegetative propagation, including in vitro propagation, used in capers.

In vegetative propagation, our working group [10,11] obtained results that can be considered acceptable: up to 41% of cuttings rooted and sprouted with cuttings obtained in October from the basal section of the branches, and up to 83% of cuttings rooted in July from apical section of the branches. This led the group to conclude that the presence of active buds in each cutting is essential, so that in addition to rooting, sprouting occurs. The group obtained positive results using in vitro propagation by means of nodal shoot explants (data not published).

Regarding the propagation of caper by seed, caper fruits have many seeds, which could facilitate sexual propagation; however, these seeds present low germination percentages,

and the germination is a slow process [12,13], making it difficult to be used in commercial propagation. This low seed germination has been related to the presence of dormancy. Baskin and Baskin [14] pointed out the presence of physiological dormancy by reporting six references from different research groups, including three references from our research group. Pascual et al. [15] stated that physical and physiological dormancy occurs by obtaining high germination percentages (90%) with sulphuric acid scarification, which is followed by the addition of a gibberellic acid solution (GA_3) to the germination substrate. Later, it was also obtained that sulphuric acid and a GA_3 solution application under alternate temperatures was the best method for breaking the caper seed dormancy [16]. Žutić et al. [17] stated that the GA_3 addition could partly eliminate the dormancy of non-scarified caper seed. In the last few years, our work team has achieved germination percentages up to 99% with the unique application of a GA_3 solution to the germination substrate [5]; furthermore, we have verified that imbibition takes place through the hilar region [18], thus, considering both studies, we stated that dormancy caused by a water-impermeable seed coat should not be considered.

This research team found great variability in the germination percentage obtained in caper seeds from different lots [13]. Foschi et al. [18] stated the low germination of some lots of commercial seeds ($\leq$5%), while using mature dark brown seeds extracted from ripe fruits collected both at dehiscence and immediately before or after it led to germination percentages up to 99% (with the application of a GA_3 solution to the germination substrate [5]). Collecting the fruits immediately before or after dehiscence requires substantial labour; although feasible in carrying out research, it may be unfeasible in commercial seed production. Generally, fruits with different degrees of maturity are collected in each collecting pass, containing apparently ripe fruits before or after its dehiscence, including both fruits with fresh and dry pulp after its dehiscence. In prior studies (unpublished data), we stated a higher and speedy germination of recently collected caper seeds than those obtained with seeds collected a few weeks before starting the germination tests. In this sense, a short period of drying (2–3 days) may increase the ability of seeds to respond to dormancy-breaking treatments [14].

The main aim of this study is to provide collection guidelines to achieve a viable caper commercial propagation by considering the results obtained in three experiments. In the first experiment, the viability and germination of caper seeds from various lots, both commercial and of their own production, are analysed. In the latter, a distinction was made between seeds (and fruits) collected, with the same criteria as commercial seeds, and seeds obtained from dry fruits; in all three cases, the seeds were produced in 2019 and 2020. The second experiment was carried out to analyse the effect of the fruit (from which the seeds were extracted) at the maturation stage on the viability and germination of seeds of their own production. In the third experiment, seed viability and germination were analysed immediately after collection, following a short drying period (3 days), and after six storage months. Given that in previous studies, the application of GA_3 to the substrate significantly increased the caper seeds germination, the substrate was moistened both with water or a GA_3 solution in the germination test.

2. Materials and Methods

2.1. Experiment 1

In the first experiment, two standard category commercial lots (CS, commercial seeds) were used; these were purchased from a private company (Cantueso Natural Seeds). According to the information provided by the company, they were produced in 2019 and 2020 (each lot corresponding to one year) in national parks in the province of Córdoba (Spain). Seeds were extracted without any chemical treatment and came mainly from apparently ripe fruits before dehiscence, although some seeds could also come from open fruits but prior to the fruits deteriorating by pulp degradation (personal communication).

The own produced seeds (OS) were produced in the same years in Llíria (Valencia, Spain). They were collected from adult plants in September, following the criterion indi-

cated by the private company for the CS, including seeds extracted from ripe fruits collected on the day of dehiscence and from fruits located in the position before and after it. In addition, seeds extracted from deteriorated fruits with dry pulp (type IV) were considered separately. Thus, three different seed lots were used each year: CS, OS, and type IV.

All of our own produced seeds were extracted from the fruits, disinfected with sodium hypochlorite for 10 min, rinsed twice in tap water, and dried in the shade at room temperature (23–25 °C, 20–50% relative humidity) for two weeks, after which they were stored in closed airtight plastic containers at 7 ± 0.5 °C until the tests were conducted. Before drying, mature seeds were selected by rejecting the light seeds by flotation in tap water. Flotation is a common method for separating viable from non-viable seeds; it involves placing seeds in water so that heavy, sound seeds sink to the bottom and the lightweight and unfilled seeds float to the top [19].

The seed moisture content for each lot was determined by the constant temperature oven method; samples of 50 seeds were dried in quadruplicate for 24 h at 103 °C [20] in a forced-air oven (Selecta 297; Selecta, Barcelona, Spain). The calculation was determined on a fresh mass basis [20]:

$$\text{Seed moisture (\%)} = 100 \times (\text{Fresh mass} - \text{Dry mass})/\text{Fresh mass}. \quad (1)$$

Seed viability was determined by the tetrazolium test [21]. Preconditioning of the seeds was applied by soaking them in water at 20 °C during 18 h, after which they were cut longitudinally off at the widest Feret diameter and soaked in a 1% tetrazolium solution at 30 °C during 18 h. Seeds were observed with a photomicroscope (U500X Digital Microscope; Cooling Tech, Guangdong, China). For the evaluation, the maximum area of unstained tissue permitted to consider a seed as viable was the radicle tip. Following [22], three tissue categories were considered according to their characteristics: sound (S. Staining proceeds gradually and uniformly from the exposed surfaces inward. Changes in the colour intensity are gradual without distinct boundaries); weak but viable (WV. Stain greyish red or brighter red than normal); and weak, not viable (WNV. Tissue colour can be mottled. Cut surfaces may appear white, while the inner tissues may appear dark red. The unstained dead tissues look flaccid, liquid-logged, blurred, chalky white, and lack-lustre). Samples consisted of 200 seeds (four replications of 50 seeds each).

The germination tests were carried out by the Between Paper method (BP) as described in the International Rules for Seed Testing [20], in Petri dishes with a diameter of 9 cm. The substrate was moistened with pure water (Wasserlab G.R type II analytical grade water system, hereinafter referred to as water) or 500 mg L^{-1} GA_3 solution (Berelex L.; herein referred to as GA_3). To prevent fungal problems, in both cases, 2 g L^{-1} captan (CAPTAN 50 BAYER) was added. Petri dishes were placed in a growth chamber (model Climatronic, Barcelona, Spain) under an alternating temperature and light regime: 12 h at 20 ± l °C in the dark and 12 h at 30 ± l °C under a photosynthetic photon flux density of 324 µmol m^{-2} s^{-1} for a maximum of 150 days. The seeds were considered germinated when the radicle protruded from the seed coat [20] and germinated seeds were eliminated from the Petri dish; evaluation was carried out every three days. Four replicates were used, consisting of 100 seeds. For each replicate, the germination data were fitted to the logistic function [12,23], defined as a special case of Richards' function [24]:

$$G = A/1 + e(\beta - kt) \quad (2)$$

where G is the cumulative germination (%); A represents the final germination percentage; t is the germination time (d; days); and β and k are function parameters. These parameters were used to determine the time (in d) required to reach 50% of G ($Gt_{50} = \beta/k$) and the mean relative cumulative germination rate ($k/2$, d^{-1}).

Moisture content, viability, and germination tests started on March 2020 and 2021, lasting 24 h, 48 h, and 150 d, respectively.

2.2. Experiment 2

In the second experiment, seeds were classified depending on the fruits from which they were extracted according to their maturation stage: Types I, II, III, and IV (Figure 1). Type II included the seeds extracted from fruits collected the day of their dehiscence, while the seeds from fruits in the anterior and posterior position (with dehiscence up to 3 days after and before, respectively) were classified as Type I and III, respectively. Type IV, as in Experiment 1, were seeds obtained from deteriorated fruits with dry pulp. This experiment was carried out in 2019 and 2020, using in each year four seed lots.

Figure 1. Fruit types from which the seeds were extracted according to their maturation stage. From left to right: ripe fruits collected before dehiscence (type I; 42 days after anthesis (daa)), ripe fruits immediately after dehiscence (type II; 45 daa), overripe dehiscent fruits before they deteriorate due to pulp degradation (type III; 48 daa), and deteriorated fruits with dry pulp (type IV; 55 daa).

Moisture content, viability, and germination tests were carried out as indicated in Experiment 1, starting after seed collection in September 2019 and 2020.

2.3. Experiment 3

In the third experiment, seed moisture content, viability, and germination tests were carried out immediately after collection (fresh seeds; FS), after a short (3 d) drying period (dried seeds, DS), and after 6 storage months (stored seeds; SS). All these seeds belonged to a seed lot (type II) collected in September 2020.

Seed moisture content, viability, and germination tests were carried out as indicated in Experiment 1 after the storage period (0, 3, and 180 d).

2.4. Statistical Analysis

The differences between the maximum and minimum values of the four replicates obtained in all the tests met the tolerance levels of the International Seed Testing Association [20]. In all three experiments, results were analysed by multi-way analyses of variance (ANOVA [25]). The percentage data were arcsin transformed before analysis. The viability and seed moisture analysis were analysed by two-way ANOVA, considering the factors type of seed and year of production (except in Experiment 3), while for the germination data analysis, the AG_3 addition to the germination substrate was also considered. A probability of $\leq$0.05% was considered significant. Mean separations were performed when appropriate, using the Fisher's least significance difference (LSD test) at $p \leq 0.05$.

3. Results and Discussion

3.1. Experiment 1

Neither the type of seed nor the year of production significantly affected ($p \leq 0.05$) the moisture content of the seed at the beginning of the viability and germination tests, ranging, on average, from 9.35% and 9.51% (Table 1).

Table 1. Effect of the type of seed (own produced (OS), type IV, and commercial (CS)) and the year of production on the seed moisture content (%) at starting the viability and germination tests and on the viability of the seeds (%), considering both the sound seeds (S) and the sound and weak but viable tissues (S + WV) criteria; mean values.

	Seed Moisture Content	**Viability (S)**	**Viability (S + WV)**
Type of seed (TS)			
OS	9.51	52.50 a	73.75 a
IV	9.36	31.25 b	43.75 b
CS	9.40	6.25 c	17.50 c
Year of production (Y)			
2019	9.48	27.50	44.17
2020	9.35	32.50	45.83
Analysis of variance			
Source (degrees of freedom)	% sum of squares		
TS (2)	6.06 NS	72.67 **	74.56 **
Y (1)	10.82 NS	1.27 NS	0.10 NS
TS × Y (2)	3.75 NS	1.48 NS	1.52 NS
Residual (18)	79.37	24.58	23.82
Standard deviation	0.20	12.69	15.00

Mean values followed by different lower-case letters in each column indicate significant differences at $p \leq 0.05$ using the Fisher's least significance difference (LSD) test. NS indicates not significant differences. ** Indicates significant differences at $p \leq 0.01$.

The type of seed was the only factor that significantly ($p \leq 0.05$) influenced the seed viability, considering both S and S + WV (Table 2), corresponding to the highest value to OS, which is followed by type IV seeds, and the lowest viability corresponded to CS. The low viability of type IV seeds (31.3% for S) and particularly that of CS (6.3% for S) is notable, since they should not present viability nor germination restrictions given their age (6 months) [13].

Although the viability obtained for CS was extremely low, it doubles that obtained by [18] (on average 3.15% for S) in a commercial seed lot produced in 2018 and that was provided by the same company. Using a stereoscopic microscope, based on the integrity of the seed coat, these authors classified the seeds of that lot into four groups: intact, scraped, cracked, and broken seeds. In the 2018 seed lot, the deterioration of the seed coat considerably contributed to the low viability of the seeds, since cracked and broken seeds supposed 31% and 16%, respectively, of the total seeds, their respective viability being low (7.5% and 0%, respectively, for S criterion). This suggested that in the 2019 and 2020 lots, the deterioration of the seed coat could have contributed considerably to the low viability of the seeds; nevertheless, cracked and broken seeds were considerably reduced in these lots, the cracked seeds accounting for 1.3% and the 1.8% and the broken seeds accounting for 0.5% and 1.0% of the total seeds in 2019 and 2020, respectively. It seems that, although slowly, this company has been improving the quality of their caper seeds.

The low viability of both type IV and CS seeds agrees with [26], who stated that some studies confirmed that the seed quality (i.e., viability, germination rate) in a number of species, such as Arabidopsis, beans, melon, rape, pepper, and tomato, continues to increase after physiological maturity, but once the seeds have dried below about 20% moisture content, their metabolism has ceased, and deterioration may begin. Probably for this reason, according to [26], the seeds of some crops are harvested with relatively high moisture

contents and then carefully dried to avoid deterioration in the field and to preserve the highest quality. Seeds of type IV and probably also part of CS were quickly dried in the field, which could lead to a reduced viability.

Table 2. Effects of the type of seeds (own produced (OS), type IV, and commercial (CS) seeds), the year of production, and the substrate saturation solution on the germination parameters: cumulative germination (G, %), final germination percentage (A, %), time (d) required to reach 50% of final germination (Gt_{50}), and average germination rate ($k/2$; d^{-1}); mean values.

	G	A	Gt_{50}	$k/2$
Type of seed (ST)				
OS	39.31 a	39.42 a	50.74	0.058 b
IV	10.69 b	10.90 b	58.98	0.096 b
CS	1.56 c	1.59 c	50.37	0.148 a
Year of production (Y)				
2019	16.79	16.77	55.26	0.086
2020	17.58	17.84	51.46	0.116
Saturation solution (S)				
Water	5.33 b	5.65 b	74.37 a	0.087
GA_3	29.04 a	28.96 a	32.35 b	0.115
Analysis of variance				
Source (degrees of freedom)	% sum of squares			
ST (2)	44.91 **	45.63 **	2.02 NS	22.71 **
Y (1)	0.03 NS	0.05 NS	0.46 NS	3.74 NS
S (1)	24.40 **	23.94 **	56.34 **	3.25 NS
ST × Y (2)	0.03 NS	0.08 NS	2.21 NS	2.39 NS
ST × S (2)	29.11 **	28.77 **	0.94 NS	4.48 NS
Y × S (1)	0.07 NS	0.06 NS	5.11 **	1.85 NS
ST × Y × S (2)	0.43 **	0.34 **	11.85 **	12.83 *
Residual (36)	1.02	1.13	21.07	48.75
Standard deviation	2.80	2.93	14.84	0.06

Mean values followed by different lower-case letters in each column indicate significant differences at $p \leq 0.05$ using the Fisher's least significance difference (LSD) test. NS indicates not significant differences. ** (*) Indicates significant differences at $p \leq 0.01$ ($p \leq 0.05$).

As expected, given the low viability, the accumulated germination (G) values were low for OS and very low for IV and CS. The coefficient of determination (R^2) for 48 curves (four replicates from three combinations of variation sources: three types of seeds, GA_3 or water addition to the germination substrate and both years of production) ranged from 0.891 to 0.995, with F ratio values of the model statistically significant ($p \leq 0.01$; data not shown). This indicates that the use of the logistic function is suitable for analysing caper seed germination as it was in similar studies carried out both in caper [12,18] as in other crops [23,27], weeds [28], and fungi [29].

Figure 2 presents the cumulative germination curves fitted to the logistic model obtained for the average values of each type of seed and saturation solution combination.

The cumulative germination (G) and the final germination percentage (A) values are similar (both in this experiment, Table 2, and in experiments 2 and 3), being both significantly affected by the same factors; thus, only A values will be discussed.

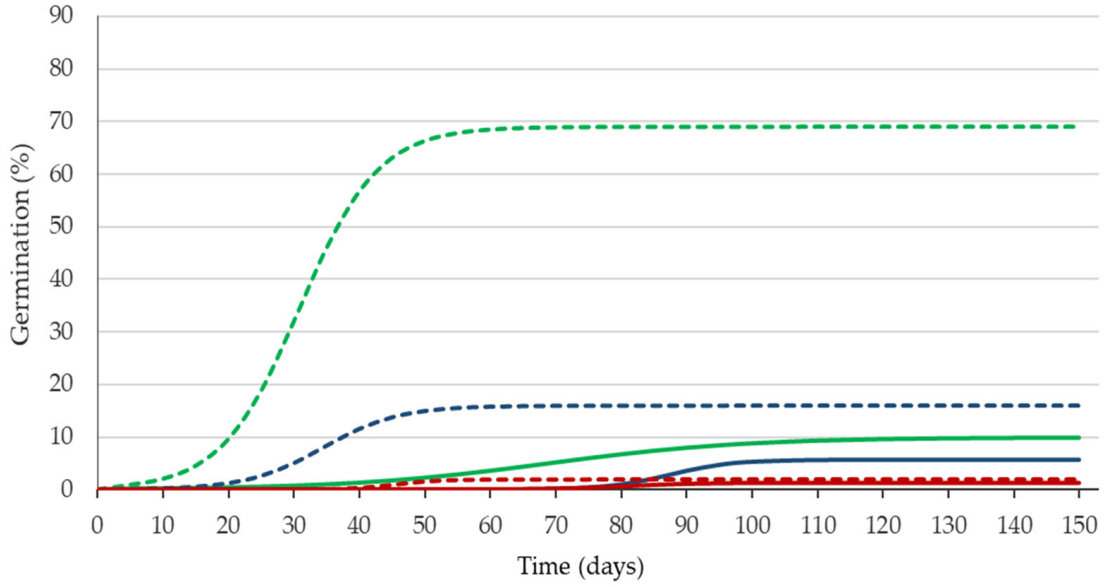

Figure 2. Logistic model fitted to the cumulative germination curves of caper seeds. Mean values for the combination of the type of seed (own produced (OS), type IV, and commercial (CS) seeds in green, blue and red, respectively) and substrate saturation solution (water in continuous lines and GA_3 solution in dashed lines).

Both the type of seed and the substrate saturation solution as well as their interaction influenced ($p \leq 0.05$) in A, the highest value corresponding to OS and the lowest value corresponding to CS. The GA_3 addition to the substrate increased the value of A. When analysing their interaction (Figure 2), it is observed that GA_3 considerably increased germination in OS and, to a lesser extent, in the type IV seeds, but it did not increase germination in the CS. The germination obtained with the GA_3 addition can be considered acceptable (69.3%) in OS, very low in the type IV seeds (16%), and practically negligible in CS (1.9%). These values agree with the viability obtained (S + WV, 73.8% for OS). It seems that as previously stated and according to [26], deterioration begins with the seed drying after reaching its physiological maturity.

As can be seen in Figure 2 and Table 2, the caper seeds germination is very slow, so that on average, the time required to reach 50% of G has exceeded 50 d, as has been obtained in previous studies [15,18]. The GA_3 application reduced the Gt_{50} (on average from 74.4 to 32.4 d; $p \leq 0.01$), and the significant interaction of this factor with the year of production ($p \leq 0.01$) indicates that this decrease was greater in seeds produced in 2019 than in those produced in 2020.

The $k/2$ was affected ($p \leq 0.01$) by the type of seeds, the highest value corresponding to CS, but it is necessary to emphasise that only less than 2% of these seeds germinated. As in viability, it can be stated that overall, the values of the germination parameters obtained with the CS agree with those obtained in previous studies [18].

3.2. Experiment 2

There were significant differences ($p \leq 0.01$) in the moisture content depending on the type of seeds, decreasing with increasing fruit maturity (Table 3). The moisture content of type IV seeds (14.7%) was clearly lower than that of other types of seeds (on average 27.8%). Logically, this seed moisture content was much higher than that presented in Experiment 1 (on average 9.4%), since results were obtained after drying and storing for six months.

Table 3. Effects of the type of seeds and the year of their production on the seed moisture content (%) and viability (%), considering only the sound seeds (S) and both the sound and viable but with weak tissues (S + WV); mean values.

	Seed Moisture Content	Viability (S)	Viability (S + WV)
Type of seed (ST)			
I	28.45 a	80.00 a	93.75 a
II	27.55 b	90.00 a	95.00 a
III	26.82 c	58.75 b	68.75 b
IV	14.65 d	33.75 c	37.50 c
Year of production (Y)			
2019	24.27	61.89	69.38
2020	24.46	69.38	78.13
Analysis of variance			
Source (degrees of freedom)	% sum of squares		
ST (3)	98.95 **	62.17 **	75.70 **
Y (1)	0.03 NS	1.88 NS	2.65 NS
ST × Y (3)	0.28 NS	6.98 NS	4.81 NS
Residual (24)	0.75	25.97	16.85
Standard deviation	0.57	17.02	12.75

Mean values followed by different lower-case letters in each column indicate significant differences at $p \leq 0.05$ using the Fisher's least significance difference (LSD) test. NS indicates not significant differences. ** Indicates significant differences at $p \leq 0.01$.

In the case of fruits that are kept in the field after dehiscence, the seeds dry out before being collected, reducing their weight and, consequently, their density. These seeds can be both mature and immature. When the seeds have a density below a threshold, they float in the water; thus, it is not feasible to separate the mature seeds from the immature seeds by flotation method [19]. On average, the percentage (on weight basis) of mature seeds in seed types I, II, and III (not floating in tap water) represented 61.5% and 67.0% of the total seeds of 2019 and 2020, respectively. In the type IV seeds, the desiccation led to the flotation of all the seeds. This fact agrees with the lower seed moisture content of the type IV seeds (14.7%) in relation to the other seed types (on average 27.6%). According to the results obtained for the flotation method, the mature seeds (those that did not float) of types I, II, and III, and all seeds of the type IV, were used in the viability and germination tests.

The viability of type IV seeds, considering both the seeds of category S and those of category S + WV, was lower ($p \leq 0.05$) than that of type III, which in turn was lower ($p \leq 0.05$) than of types I and II, with no differences ($p \leq 0.05$) between them (Table 3). Although the difference between the viability of the seeds of types I and II was not significant ($p \leq 0.05$), the proportion of WV was lower in seeds of type II (5%) than in those of type I (13.8%). As previously cited, ref. [26] reported that the quality of the seed is maximum during and shortly after its physiological maturity.

The viability of the type IV seeds was very low (<38%, considering S + WV), although it must be considered that these lots include both mature and immature seeds (because all of them floated in tap water); thus, these figures could underestimate (although in a small percentage) the viability of the mature seeds, as mentioned afterwards. In addition, it is common for ants, wasps, and birds to take seeds, especially the best quality seeds, when the fruits dry in the field.

The coefficients of determination (R^2) for 64 curves (four replicates from three combinations of variation sources: four types of seeds, GA_3 or water addition to the germination substrate, and two years of production) ranged from 0.931 to 0.996, with F ratio values of the model statistically significant ($p \leq 0.01$; data not shown), meaning that the use of the logistic function is suitable to analyse the germination of caper seeds in this experiment, as shown in Experiment 1 (Figure 3).

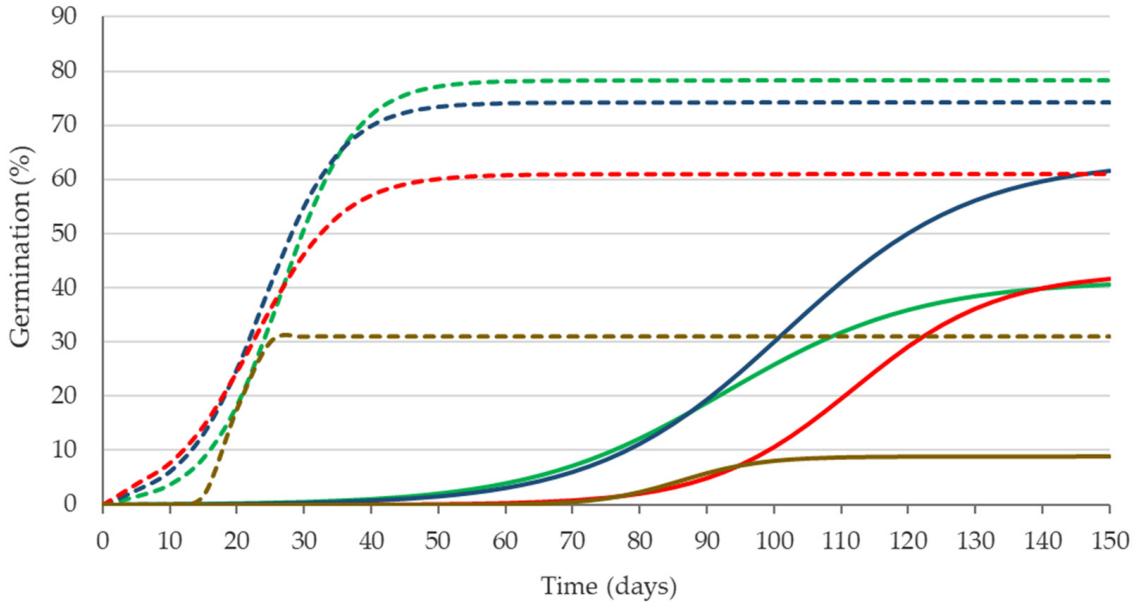

Figure 3. Logistic model fitted to the cumulative germination curves of caper seeds. Mean values for the combination of the type of seed (I, II, III, and IV in green, blue, red, and brown, respectively) and substrate saturation solution (water in continuous lines and GA_3 solution in dashed lines).

The type of seed influenced ($p \leq 0.05$) its germination, so that the type II seeds presented the highest A values ($p \leq 0.05$), while the lowest value was obtained for the type IV seeds. The year of seed production did not affect A ($p \leq 0.05$). As mentioned, the GA_3 application increased ($p \leq 0.05$) the A values, as well as the $k/2$, decreasing Gt_{50} ($p \leq 0.05$).

The interaction between the type of seed and the GA_3 application significantly affected A ($p \leq 0.01$; Table 4). It can be observed in Figure 3 that while with the GA_3 application, there were no differences ($p \leq 0.05$) between the germination obtained in seeds of type I (78.3%) and type II (74.3%), being high in both cases, when only water was applied to the substrate, the germination obtained by the type II seeds (63.5%) was higher ($p \leq 0.05$) than that obtained by those of type I (41.4%). On the other hand, the A value was higher ($p \leq 0.05$) with the GA_3 application in type I seeds (78.3%) than in type III seeds (61.0%), while there were no differences when only water was applied (41.4% and 42.8% for I and III, respectively; $p \leq 0.05$). It is worth noting the high germination obtained with type II seeds, both with water and GA_3 application, which agrees with that stated by [26], in the sense that the ideal state for the seed collection is just in the fruit dehiscence, since the seed quality is maximum during and shortly after the physiological maturity of the seeds.

Germination of the type IV seeds may be considered as very low (30.9% with GA_3 and 9.0% with water; on average $A \approx 20\%$), although it must be considered that these lots included both mature and immature seeds (because all of them floated in tap water, as mentioned above). Thus, these figures could underestimate, by a small percentage, the germination of the mature seeds. Specifically, considering for the seed types I, II, and III that on average, 35.75% (on weight basis) of the seeds floated, and that 13.15% of these seeds germinated (parallel studies; data not shown), the cited underestimation could be around 5% (specifically 4.7%). Therefore, the germination of the mature seeds in type IV could reach percentages up to 36% with the AG_3 application.

The interaction between the type of seed and the GA_3 application also significantly affected ($p \leq 0.01$) the Gt_{50} (Table 4), in the sense that Gt_{50} values obtained in the four types of seeds with the GA_3 application did not differ between them, requiring, on average, 23.3 d to reach the 50% of the corresponding final germination. However, when only water was applied to the germination substrate, the highest time ($p \leq 0.05$) was required by type III seeds (113.2 d) and the lowest time ($p \leq 0.01$) was required by type IV (80.6 d), but this shorter time of the latter is related with its low germination percentage (Figure 3).

Table 4. Effects of the type of seeds, the year of production, and the substrate saturation solution on the germination parameters: cumulative germination (*G*, %), final germination percentage (*A*, %), time (d) required to reach 50% of final germination (Gt_{50}), and average germination rate ($k/2$; d^{-1}); mean value.

	G	***A***	***Gt*$_{50}$**	***k/2***
Type of seeds (ST)				
I	59.06 b	59.85 b	61.73 a	0.06
II	67.19 a	68.87 a	63.09 a	0.06
III	50.69 c	51.86 c	68.19 a	0.06
IV	20.06 d	19.95 d	52.55 b	0.07
Year of production (Y)				
2020	49.06	49.96	60.99	0.06
2019	49.44	50.30	61.78	0.06
Saturation solution (S)				
Water	37.09 b	39.13 b	97.96 a	0.04 b
GA_3	61.41 a	61.13 a	24.82 b	0.09 a
Analysis of variance				
Source (degrees of freedom)	% sum of squares			
ST (3)	57.58 **	60.92 **	2.14 **	2.83 NS
Y (1)	0.01 NS	0.01 NS	0.01 NS	0.03 NS
S (1)	26.76 **	21.69 **	89.88 **	65.46 **
ST × Y (3)	1.35 NS	1.29 S	0.17 NS	2.72 NS
ST × S (3)	3.32 **	4.08 **	2.46 **	2.56 NS
Y × S (1)	0.06 NS	0.11 NS	0.00 NS	0.07 NS
ST × H × S (3)	0.59 NS	0.64 NS	0.22 NS	10.01 **
Residual (48)	10.33	11.27	5.12	16.33
Standard deviation	8.72	9.16	10.08	0.01

Mean values followed by different lower-case letters in each column indicate significant differences at $p \leq 0.05$ using the Fisher's least significance difference (LSD) test. NS indicates not significant differences. ** Indicates significant differences at $p \leq 0.01$.

3.3. Experiment 3

As expected, the state of the seed at the start of the viability and germination test (consequence of the period between the extraction of the seeds from the fruits, which was carried out immediately after the collection, and the start of the tests) significantly affected ($p \leq 0.01$) the moisture content of the seed (Table 5). The moisture content of the FS was higher ($p \leq 0.05$) than that of DS, which in turn was higher ($p \leq 0.05$) than that of SS, although the difference between the last two are small in absolute value (about 1%), which indicates that the desiccation of the seed occurs mainly in the first days of drying.

The state of the seed significantly affected ($p \leq 0.01$) its viability, considering both the S and S + WV criteria (Table 5). The lowest values corresponded to the SS without differences between the FS and DS ($p \leq 0.05$), which means that seed viability decreases with storage, decreasing the proportion of S and increasing those of WV (0%, 10%, and 30% in FS, DS, and SS, respectively).

Both the state of the seeds and substrate saturation solution, as well as their interaction, influenced the final germination (Figure 4 and Table 6), the highest *A* value corresponding to FS and the lowest corresponding to SS ($p \leq 0.05$; Table 6). As occurred in experiment 2, the GA_3 application to the substrate increased the value of *A*. When analysing the interaction of both factors (Figure 4), the final germination percentage obtained with the GA_3 addition can be considered as very high in FS (89.2%) and high in DS and SS (72.1% and 72.6%, respectively). These *A* values were expected considering the viability obtained with the S criterion for FS and DS (97.5% and 82.5%, respectively) and with that obtained with the S + WV criterion for SS (77.5%). It can be observed in Figure 4 that GA_3 considerably increased germination percentages compared to those germinated with water, in SS (64.2%),

to a lesser extent in DS (35.6%), and even less in FS (25.8%). This seems to be related to a weakening of a part of the seed coat or to the low embryo growth potential, so that the increase in germination obtained with the GA_3 addition (compared to water) increases with storage, that is, the GA_3 effect was more important when the seeds had been stored and their viability had decreased. The high germination percentage obtained with seeds stored for one month (with the GA_3 application), which allows the distribution and sow of the seeds during this period, not being necessary to apply techniques such as priming. Priming is a practice used by the seed industry to increase the performance of commercial seed lots, improving the germination rate and uniformity [19,30]. Nevertheless, priming tends to shorten seed life in storage, and the benefits of priming can be lost during storage [19]. Recently harvested seeds (FS and DS) presented higher viability and germination values than those harvested six months before (SS), thereby agreeing with [13], who recommended a storage period for caper seeds no longer than two years because during this period, its viability does not significantly decrease, and high germination percentages can be obtained, although Gt_{50} increased for the seeds stored for one year compared to those stored for just one month. This highest viability and germination obtained in FS differs from seeds of other common families such as Asteraceae and Poaceae, among others [31], which present nondeep physiological dormancy and undergo after-ripening, that is, dormancy break during dry storage. The herein presented results, as well as those reported in [13,18], indicate that caper seed viability and germination not only do not increase with dry storage, but they decrease.

The GA_3 addition to the substrate reduced Gt_{50} ($p \leq 0.01$), obtaining similar values for the three periods (23.6 d on average, with no differences ($p \leq 0.05$) between them), while without the GA_3 application, the highest value ($p \leq 0.05$) corresponded to FS (103.7 d; with the highest final germination) and the lowest value ($p \leq 0.05$) corresponded to SS (56 d; related to its low germination value). The GA_3 addition to the germination substrate increased $k/2$ ($p \leq 0.01$).

Table 5. Effect of the state of the seed (fresh seeds (FS), dried seeds (DS), and stored seeds (SS)) on the seed moisture content (%) at starting the viability and germination tests and on the viability of the seeds (%), considering both the sound seeds (S) and the sound and weak but viable tissues (S + WV) criteria; mean values.

	Seed Moisture	**Viability (S)**	**Viability (S + WV)**
State of the seed			
FS	27.80 a	97.5 a	97.5 a
DS	9.95 b	82.5 a	92.5 a
SS	8.86 c	47.5 b	77.5 b
Analysis of variance			
Source (degrees of freedom)	% sum of squares		
State (2)	99.55 **	72.2 **	79.4 **
Residual (9)	0.45	27.8	20.6
Standard deviation	0.68	15.0	5.0

Mean values followed by different lower-case letters in each column indicate significant differences at $p \leq 0.05$ using Fisher's least significance difference (LSD) test. ** Indicates significant differences at $p \leq 0.01$.

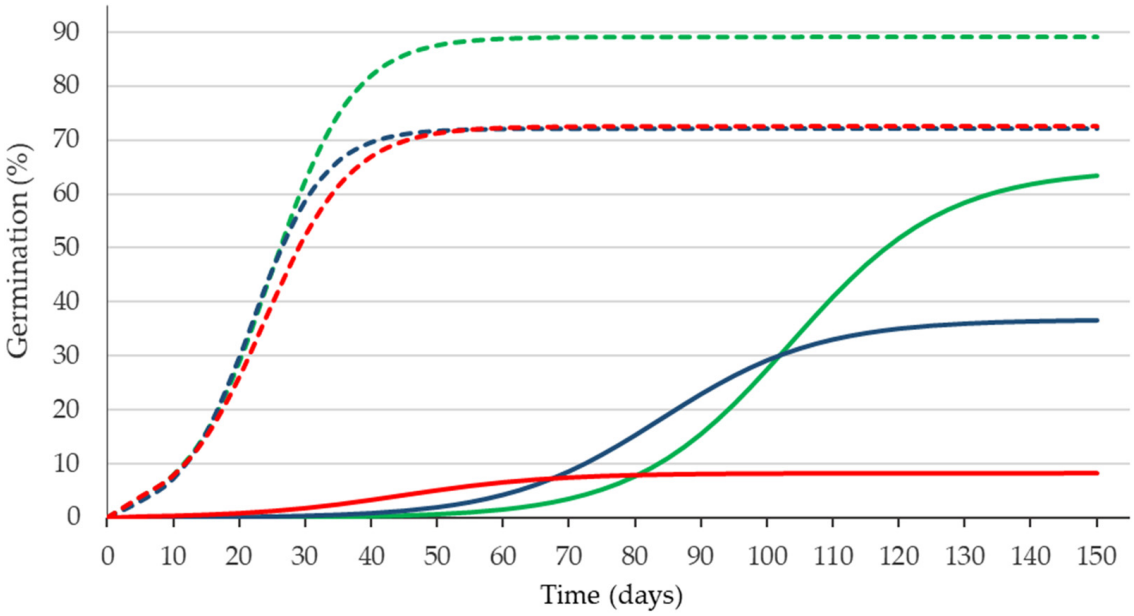

Figure 4. Logistic model fitted to the cumulative germination curves of caper seeds. Mean values for the combination of the state of the seeds (fresh seeds (FS), dried seeds (DS), and stored seeds (SS) in green, blue, and red, respectively) and substrate saturation solution (water in continuous lines and GA_3 solution in dashed lines).

Table 6. Effect of the state of the seed (fresh seeds (FS), dried seeds (DS), and stored seeds (SS)) and the substrate saturation solution on the germination parameters: cumulative germination (G, %), final germination percentage (A, %), time (d) required to reach 50% of final germination (Gt_{50}), and average germination rate ($k/2$; d^{-1}); mean values.

	G	A	Gt_{50}	$k/2$
State of the seed				
FS	75.37 a	76.89 a	64.39 a	0.06
DS	54.62 b	54.38 b	53.04 a	0.07
SS	40.50 c	40.48 c	39.92 b	0.06
Saturation solution				
Water	34.92 b	36.54 b	81.28 a	0.04 b
GA_3	78.75 a	77.96 a	23.63 b	0.08 a
Analysis of variance				
Source (degrees of freedom)	% sum of squares			
State (2)	26.33 **	29.48 **	8.94 **	2.11 NS
Solution (1)	61.66 **	56.19 **	74.32 **	61.87 **
State × Solution (2)	7.45 **	9.18 **	8.25 **	1.64 NS
Residual (18)	4.56	5.15	8.49	34.39
Standard deviation	6.88	7.24	11.25	0.02

Mean values followed by different lower-case letters in each column indicate significant differences at $p \leq 0.05$ using the Fisher's least significance difference (LSD) test. NS indicates not significant differences. ** Indicates significant differences at $p \leq 0.01$.

4. Conclusions

In view of the obtained results, it is advisable to collect the caper fruits on the day of dehiscence; however, if the required labour is considered excessive, the fruits located immediately before and after the dehiscent one could also be collected. In practice, this is the equivalent of carrying out one pass per week. Caper seeds are sensitive to desiccation; accordingly, a general rule of thumb is to collect the fruits once a week, to extract the

seeds, and to plant them immediately for germination by using a GA_3 solution to moisten the substrate.

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