

# Storage of scarified carob seeds: influence of container, temperature and duration on seed quality

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## Storage of scarified carob seeds: influence of container, temperature and duration on seed quality.

### ABSTRACT

Carob seeds, mechanically scarified or not, were stored for 0, 6, 12 or 18 months, at + 3 °C or - 3 °C, in sealed containers or in vacuum packing. Thirty-two different treatments, studied through a multifactor design, were experienced. Whatever the seed storage duration, scarified seeds gave the most rapid and complete germination processes. As no loss in germination was observed, it seems reasonable to assume that storage for at least 18 months is highly safe for scarified seeds viability. Considering the effect of single factors on germination, no influence was recorded for storage temperature and duration. While mechanically scarified seeds behaved much better than non-scarified ones, seeds stored in hermetically sealed containers germinated more than those vacuum packed.

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### KEYWORDS

*Ceratonia siliqua*,  
scarification, seed treatment,  
seed storage, seed longevity,  
temperature.

## Conservation de graines de caroube scarifiées : effets du récipient, de la température et de la durée de stockage sur la qualité des graines.

### RÉSUMÉ

Des graines de caroube, mécaniquement scarifiées ou non, ont été conservées pendant 0, 6, 12 ou 18 mois, à + 3 °C ou - 3 °C, dans des récipients scellés ou dans des emballages sous vide. Trente-deux traitements différents, étudiés selon un plan plurifactoriel, ont été expérimentés. Quelle qu'ait été leur durée de stockage, les graines scarifiées ont germé de façon plus complète et plus rapide que les graines non scarifiées. Comme aucune perte de germination n'a été observée, un stockage d'au moins 18 mois s'avère sans risque pour la viabilité des graines scarifiées. L'étude des effets individuels a montré que les facteurs température et durée de stockage n'avaient pas d'influence sur la conservation de la qualité germinative de la graine. Alors que les graines scarifiées mécaniquement se sont comportées beaucoup mieux que celles non scarifiées, les graines placées en boîtes hermétiquement fermées ont mieux germé que celles qui avaient été mises en emballages sous vide.

### MOTS CLÉS

*Ceratonia siliqua*,  
scarification, traitement des  
semences, stockage des  
semences, longévité des  
semences, température.

## Conservación de semillas de algarrobo escarificadas : efectos del envase, de la temperatura y de la duración del almacenamiento sobre la calidad de las semillas.

### RESUMEN

En el presente estudio, semillas de algarrobo, escarificadas mecánicamente o no tratadas, se conservaron durante 0, 6, 12 o 18 meses, a + 3 °C o a - 3 °C, en envases herméticos o al vacío. Los 32 tratamientos experimentales resultantes fueron estudiados en base a un diseño multifactorial. Las semillas escarificadas, conservadas por 0, 6, 12 o 18 meses, exhibieron los procesos germinativos más rápidos y completos. Parecería factible la posibilidad de almacenar semilla escarificada durante 18 meses ya que no fueron observadas pérdidas de facultad germinativa al cabo de dicho período. Considerando independientemente el efecto de cada uno de los factores estudiados sobre la germinación, ninguna influencia ejercieron la temperatura y la duración de la conservación, mientras que el tratamiento de escarificación produjo mejor germinación respecto al tratamiento testigo (no escarificación) así como las semillas conservadas en envases herméticos dieron mejores resultados que las almacenadas al vacío.

### PALABRAS CLAVES

*Ceratonia siliqua*,  
escarificación, tratamiento de  
semillas, almacenamiento de  
semillas, viabilidad de la  
semilla, temperatura.

## ● introduction

### growth habit

Carob is an evergreen, generally dioecious, tree (*Ceratonia siliqua* L.) belonging to the *Leguminosae* *Caesalpinioideae*. It has paripinnate leaves, with leaflets (2.4 x 3-6 cm) in two to five pairs, elliptical or obovate to suborbicular, coriaceous, dark green and shining above, pale green beneath. The flowers, borne in small, lateral, red racemes, bloom from the end of summer all through autumn. The fruit is a coriaceous, indehiscent pod 10 to 30 cm long, 0.6 to 2 cm thick, filled with a sweet, pulpy substance bearing five to 15 obovate, transverse, brown, bony seeds about 0.6 cm wide. Fruits ripen, turn dark brown and begin to fall in autumn, while the seed dispersal date depends on the variety and weather conditions.

Carob has a heliophilous, thermophilous and xerophilous habit (GAMBI, 1984). The survival strategy in the Mediterranean climate is based on avoiding water stress (CATARINO, 1993): for instance, orientation of leaves in favour of limited exposure to sun, leaf abscission in the case of heavy drought, etc. It survives in a wide variety of soils, sometimes clinging to hill sides that seem to be almost pure rock, and grows in stony areas unsuited to other crops provided its roots can penetrate crevices. It cannot withstand waterlogged soils, nor can it be grown in wet ground or in hard clay with poor drainage (ADVISORY COMMITTEE ON TECHNOLOGY INNOVATION, 1979).

### occurrence

Carob is native to the eastern Mediterranean, from the southern coast of Asia Minor to Syria. Since historical times this small to medium (3 to 15 m high) evergreen tree, tolerant and long living, has long been cultivated as a forage crop in Asia, Europe and North Africa (YOUNG and YOUNG, 1992). It has been introduced to North and South America and to Australia as well. It is a basic component of the vegetation of coastal areas of the Mediterranean, but, above all, it characterizes the *Oleo-Ceratonia* alliance within the thermophilous belt.

### establishment

Carobs are usually started from seed, but can also be propagated by cuttings, suckers, layers and air-layering. Since the long tap root is easily injured, seeds should be sown in containers, avoiding bare-root growing. Direct sowing is also practised. For fast growing and high yielding trees, buds from good varieties are grafted to 3-4 year old seedlings in the field. Plantations usually have a low density, ranging from 25 to 100 plants per ha (FANELLI, 1954; CATALAN BACHILLER, 1991), and yields of 1.5-4 t of pods per ha are achieved if the plants are grown under good conditions and are well tended. Much higher yields (12 t per ha) have been reported for irrigated plantations (ADVISORY COMMITTEE ON TECHNOLOGY INNOVATION, 1979). Female plants are interspaced with 5% well-placed male ones, providing the necessary pollen; in some cases a male branch is grafted to a female tree to supply pollen *in situ*. Severe prunings affect fruiting negatively as flowers are born on the old wood including the trunk (FANELLI, 1954).

### importance and uses

Annual world yield of dried pods reaches about 400,000 t, mostly from Spain, Italy and Portugal (CATARINO, 1993). Plants bear fruit when 6 to 8 years old and abundant crops occur every second year. Average annual yield of fruit per tree is 90 to 110 kg (YOUNG and YOUNG, 1992). The main consuming nations are Western Europe, the United Kingdom and the United States. Cultivation and preparation of industrial products is quickly increasing in North Africa, especially in Morocco (CATARINO, 1993). Carob pods make excellent fodder. In food value, they are almost equal to many cereal grains (ADVISORY COMMITTEE ON TECHNOLOGY INNOVATION, 1979). About half the weight of the pulp is sugar, and today it is sometimes sold as a dried confection called St John's bread. Although usually eaten like a candy, it can be converted to syrup and fermented to liquor. It is also widely used as a tasty chocolate substitute.

Carob seeds, at present the most valuable product of the plant, represent 10% of the weight of the pod, and contain up to 21% protein. By weight, about one-third of the seed consists of a mucilaginous gum, a neutral galactomanan that is widely

used as an emulsion stabilizer and thickener in ice cream, cheese, salad dressings, cosmetics, textiles, paper, pharmaceutical and other industrial products. Carob gum, variously known as locust bean gum, tragasol, etc, is commercially valuable because it is especially viscous, low in starch and other impurities, and can be used in place of some expensive gums (ADVISORY COMMITTEE ON TECHNOLOGY INNOVATION, 1979).

The carob tree offers a way to control erosion, conserve soil and reclaim land at the same time it is producing crop. It is also recommended for wind and fire breaks (MARES, 1971). It is chiefly valuable as an ornamental evergreen, but has also been used to some extent in environmental planting (TOTH, 1965). In urban forestry, generally only female trees are used, as male flowers have an unpleasant smell. The tree coppices well and the wood makes reasonable fuel. Research based on biotechnologies could improve separation and obtention of tannins, as well as xanthorrea resin production through specialized microorganisms (CATARINO, 1993).

### factors affecting germination

A large number of tree seed species do not germinate due to hard seed-coats hindering the uptake of water (physical dormancy) and the seed will not germinate unless the seed-coat is scarified<sup>1</sup>. Soaking seed in hot or boiling water or in sulphuric acid are common methods used in the nurseries to scarify the seed and improve germination. However, some of these procedures are either too risky or not specific enough to give consistent results (DUGUMA et al, 1988). Critical parameters are the duration of soaking and temperature during soaking which depends partly on the volume of water with respect to the volume of seed. It may also prove difficult to control the temperature of a large amount of seeds during treatment. Health hazards involved in working with acid are also special problems to be considered (POULSEN and STUBSGAARD, 1995). Furthermore, the extent of injury to embryos from soaking seeds in concentrated sulphuric acid or boiling water is usually directly proportional to the percentage of permeable seed-coats (CHAPMAN, 1936), thus provoking an unavoidable selection in favour of hard seeds. Hard seeds occur

in many *Leguminosae*, in which water impermeability may or may not be combined with other dormancy mechanisms (ROLSTON, 1978). Carob seeds sown from recently ripened pods germinate well without pretreatment, but if the seeds are allowed to dry, over time they develop very hard seed-coats (YOUNG and YOUNG, 1992) and do not imbibe water readily (COIT, 1951). The low germination percentage of untreated seeds makes it difficult to achieve uniform establishment either in the nursery or in the field.

Mechanical scarification, causing fine cracks on the seed-coats, can be accomplished by various hand tools or by motor-driven scarifiers such as the Denbigh Disk scarifier, the modified Ames scarifier or the Forsberg scarifier (BONNER et al, 1974). The Danida Forest Seed Centre undertook the development of mechanical scarifying methods as alternatives to manual ones. Three mechanical methods described by POULSEN and STUBSGAARD (1995) were developed: the glow burner, the seed gun and the mechanical burner. Manual scarification with hand tools such as files, knives and sandpaper are also effective in allowing entry of water (WANG and PITEL, 1991); it is performed to some extent, but, although simple, this method is excessively time-consuming for modern nurseries. Even if easy and not hazardous, the use of mechanical scarifiers requires some skill. Moreover, such equipment is not always available in nurseries, especially in developing countries. LAURIDSEN and STUBSGAARD (1987) stored scarified seeds of two tropical tree legumes for two months to investigate damages in seed quality after pretreatment. They concluded that seed could be scarified at a seed centre or central nursery and then packed and shipped without any special precaution. Similar practices are reported for pretreated seeds of *Acacia mangium* (hot water) in Malaysia and *Acacia albida* (sulphuric acid) in France (LAURIDSEN and STUBSGAARD, 1987). However, storage of *Leguminosae* pretreated tree seeds has not been studied intensively.

The study reported in this paper was designed to evaluate the influence of important storage conditions, such as type of container, temperature and length of conservation, on the quality of *C. siliqua* scarified seeds.

<sup>1</sup> Any treatment which destroys or reduces seedcoat impermeability is commonly known as 'scarification' [WILLAN, 1990].

## ● materials and methods

The experiment was carried out with *C siliqua* seeds collected in Sperlonga (Latina) in 1992; seeds were stored in hermetically sealed containers, at + 3 °C, until trials began in August 1993. The moisture content, 10.9%, was determined upon receipt following the International Seed Testing Association rules for seed testing (ISTA, 1985). The average weight of 1,000 seeds was 198 g (5,050 seeds/kg).

Following the seed scarification, and after 6, 12 and 18 months' storage, germination tests using

six replications of 50 seeds each were carried out. The same procedure was applied for non-scarified seeds. Regarding germination test conditions, *C siliqua* is not one of the species listed in the ISTA (1993) rules. The germination tests were therefore conducted, as prescribed by ISTA (1993) rules for *Acacia* spp, at alternating temperatures: 8 hours at 30 °C in light plus 16 hours at 20 °C in dark. A germination event was recorded if the radicle emerged at least 10 mm. Each germination box was censused for germination every two days until the 45th (final count). Germination percentage (G%) on the 45th day was calcu-

Table I

Analysis of variance for the effects of scarification (factor A), storage container (factor B), storage temperature (factor C) and storage length (factor D) on germination percentage and on Djavanshir and Pourbeik's germination value of *Ceratonia siliqua* L seeds.

Source of variation	DF	Germination percentage <sup>1</sup>			Germination value		
		MS	F	SL	MS	F	SL
<i>main effects</i>							
A: scarification	1	24 638.7	1 711.0	0.0000	5 161.6	1290.4	0.0000
B: storage container	1	106.5	7.4	0.0073	54.3	13.6	0.0003
C: storage temperature	1	26.3	1.8	0.1792	16.7	4.2	0.0428
D: storage length	3	36.9	2.6	0.0571	4.2	1.0	0.3753
E: replicates	5	18.7	1.3	0.2680	4.6	1.1	0.3397
<i>Interactions</i>							
AB	1	57.4	4.0	0.0478	47.7	11.9	0.0007
AC	1	12.5	0.9	0.3632	22.9	5.7	0.0181
AD	3	56.5	3.9	0.0099	15.3	3.8	0.0114
BC	1	57.4	4.0	0.0478	14.4	3.6	0.0604
BD	3	46.0	3.2	0.0254	11.4	2.8	0.0401
CD	3	25.5	1.8	0.1553	10.9	2.7	0.0465
ABC	1	0.3	0.0	0.8958	3.8	0.9	0.3444
ABD	3	10.7	0.7	0.5281	6.0	1.5	0.2202
ACD	3	47.0	3.3	0.0232	14.5	3.6	0.0148
BCD	3	32.5	2.3	0.0847	8.1	2.0	0.1147
ABCD	3	34.1	2.4	0.0733	9.8	2.4	0.0657
Residual	155	14.4			4.0		
Total	191						

<sup>1</sup>Data were analyzed on transformed arcsin square root of germination percentage. DF: degrees of freedom; MS: mean square; F: F ratio; SL: significance level.

lated, DJAVANSHIR and POURBEIK'S (1976) germination value (GV) being measured as well. GV combines speed and completeness of germination into a single index: the higher the GV, the more rapid and complete the germination. The latter parameter is obtained by the formula:

$$GV = (\Sigma DGS / N) \times G\% \times 10$$

where DGS is the daily germination speed, which is obtained by dividing the cumulative G% by the number of days since the beginning of the test, G% is the germination percentage at the end of the test and N is the number of counts.

Before beginning the present experiment, small-scale trials were conducted to identify the most appropriate length of scarification (5", 15", 30", 45", 60") and sandpaper type (Fiar Corindone no 40, 60, 80). Scheduled seeds were therefore rubbed for 60" in an ad hoc modified Forsberg mechanical scarifier with a capacity of 2.30 l, lined with sandpaper Fiar Corindone type no 60, and operated electrically. The speed of the internal blades was set at 1,425 rpm.

The experiment employed a multifactor design with five factors. Factor A was seed scarification with two levels: mechanically scarified seeds and non-scarified seeds. Factor B, with two levels, was storage container type: hermetically sealed container and vacuum packing. Factor C, with two levels, was storage temperature: + 3 °C and - 3 °C. Factor D, storage length, had four levels: 0, 6, 12 and 18 months. The last factor (E) was represented by six replications. Data were analyzed by multifactor ANOVA (STATGRAPHICS, 1991) on transformed arcsin square root of G% (table I); tabulated values are the untransformed data (table II). GVs were also subjected to ANOVA (table I). Means were compared by the Tukey's test (STEEL and TORRIE, 1960; STATGRAPHICS, 1991).

For a better comprehension of the influence of the various factors, all possible combinations ( $2 \times 2 \times 2 \times 4$ ) were also compared: data were analyzed as for a randomized complete block design with six blocks. Experimental treatments, described in table IV, were therefore 32. The best ones were identified by the Tukey's test.

A germination test employing manually chipped seed, as prescribed for *Acacia* spp (ISTA, 1993), was carried out under the conditions described

Table II

Influence of scarification (factor A), storage container (factor B), storage temperature (factor C) and storage length (factor D) on germination percentage and Djavanshir and Pourbeik's germination value of *Ceratonia siliqua* L seeds.

Factors	Germination percentage	Germination value
<i>Factor A: scarification</i>		
Mechanically scarified seeds (s)	65.1 b	12.4 b
Non-scarified seeds (ns)	26.7 a	2.0 a
<i>Factor B: storage container</i>		
Hermetically sealed container	46.9 b	7.7 b
Vacuum packed	44.3 a	6.7 a
<i>Factor C: storage temperature</i>		
+ 3 °C	46.2 a	7.5 a
- 3 °C	44.9 a	6.9 a
<i>Factor D: storage length</i>		
0 months	44.7 a	7.6 a
6 months	45.5 a	7.0 a
12 months	47.7 a	7.3 a
18 months	44.4 a	6.9 a
<i>Factor A ÷ factor D</i>		
s / 0 months	66.8 b	13.5 b
s / 6 months	64.6 b	12.1 b
s / 12 months	66.6 b	12.3 b
s / 18 months	62.4 b	11.5 b
ns / 0 months	23.6 a	1.6 a
ns / 6 months	27.2 a	2.0 a
ns / 12 months	29.2 a	2.2 a
ns / 18 months	27.1 a	2.3 a

According to the Tukey's test, means followed by the same letter, within each factor, are not significantly different at  $P < 0.01$ . s: mechanically scarified seeds. ns: non scarified seeds.

earlier; six replications of 50 seeds each were used. G% on the 45th day was 67.5%.

## ● results and discussion

For G% and GV, highly significant differences ( $P < 0.01$ ) were observed for scarification (factor A) and storage containers (factor B). As far as interactions are concerned, the highest significance level was observed for G% ( $P = 0.0099$ ) in A x D and for GV ( $P = 0.0007$ ) in A x B (table I). Factorial analysis allowed a direct view of the effects attributable to the single factors as well as to the relevant interactions (table I). Parameters under study gave an identical response to the influence of the different levels of the factors. No significant differences at  $P < 0.01$  were recorded for G% or GV within levels of factors C, D and E (table II). While mechanically scarified seeds

Table III

Analysis of variance for the effects of 32 experimental treatments on germination percentage and on Djavanshir and Pourbeik's germination value of *Ceratonia siliqua* L seeds.

Source of variation	DF	Germination percentage <sup>1</sup>				Germination value		
		MS	F	SL	MS	F	SL	
<i>Main effects</i>								
Treatments	31	831.2	57.7	0.0000	179.4	44.8	0.0000	
Replicates	5	18.7	1.3	0.2680	4.6	1.1	0.3497	
Residual	155	14.4			4.0			
Total	191							

<sup>1</sup> Data were analyzed on transformed arcsin square root of germination percentage. DF: degrees of freedom; MS: mean square; F: F ratio; SL: significance level.

Table IV

Germination percentage and Djavanshir and Pourbeik's germination value for 32 experimental treatments resulting from the combination of the different levels of factors tested on *Ceratonia siliqua* L seeds.

Experimental treatment	Germination percentage	Germination value
s / hsc / + 3 °C / 0 m	66.0 bcd	13.5 cd
s / hsc / + 3 °C / 6 m	66.3 bcd	13.2 cd
s / hsc / + 3 °C / 12 m	73.3 d	15.8 d
s / hsc / + 3 °C / 18 m	71.0 cd	15.3 d
s / hsc / - 3 °C / 0 m	66.3 bcd	13.5 cd
s / hsc / - 3 °C / 6 m	66.0 bcd	12.6 bcd
s / hsc / - 3 °C / 12 m	65.7 bcd	11.8 bcd
s / hsc / - 3 °C / 18 m	62.0 bcd	11.5 bcd
s / vp / + 3 °C / 0 m	67.0 bcd	13.5 cd
s / vp / + 3 °C / 6 m	62.7 bcd	11.3 bcd
s / vp / + 3 °C / 12 m	69.0 cd	13.3 cd
s / vp / + 3 °C / 18 m	52.0 b	8.2 b
s / vp / - 3 °C / 0 m	67.7 bcd	13.5 cd
s / vp / - 3 °C / 6 m	62.7 bcd	11.3 bcd
s / vp / - 3 °C / 12 m	56.7 bc	8.6 bc
s / vp / - 3 °C / 18 m	62.7 bcd	11.1 bcd
ns / hsc / + 3 °C / 0 m	24.0 a	1.6 a
ns / hsc / + 3 °C / 6 m	27.3 a	2.0 a
ns / hsc / + 3 °C / 12 m	31.3 a	2.4 a
ns / hsc / + 3 °C / 18 m	28.3 a	2.5 a
ns / hsc / - 3 °C / 0 m	23.0 a	1.6 a
ns / hsc / - 3 °C / 6 m	23.7 a	1.4 a
ns / hsc / - 3 °C / 12 m	30.0 a	2.3 a
ns / hsc / - 3 °C / 18 m	28.7 a	2.6 a
ns / vp / + 3 °C / 0 m	24.0 a	1.6 a
ns / vp / + 3 °C / 6 m	29.3 a	2.1 a
ns / vp / + 3 °C / 12 m	25.7 a	1.6 a
ns / vp / + 3 °C / 18 m	24.3 a	1.8 a
ns / vp / - 3 °C / 0 m	24.0 a	1.6 a
ns / vp / - 3 °C / 6 m	27.3 a	2.4 a
ns / vp / - 3 °C / 12 m	31.3 a	2.4 a
ns / vp / - 3 °C / 18 m	27.3 a	2.2 a

Means followed by the same letter are not significantly different at  $P < 0.01$ , according to the Tukey's test. s: mechanically scarified seeds; ns: non scarified seeds; hsc: hermetically sealed containers; vp: vacuum packed; + 3°C: storage temperature + 3°C; - 3°C: storage temperature - 3°C; 0m: 0 month's storage length; 6m: 6 month's storage length; 12m: 12 month's storage length; 18m: 18 month's storage length.

behaved better than non-scarified ones (factor A), seeds stored vacuum packed (table II) germinated less than those in hermetically sealed containers (factor B). It is interesting to emphasize that the latter containers allow an extremely easy handling if compared to vacuum packing.

However, even if differences between the two levels of factor B were statistically relevant ( $P = 0.0073$  for G%), in nursery terms such differences should not be considered very important. Factor C, storage temperature (+ 3 °C or - 3 °C), did not influence seed viability probably because of the seed peculiarity. In fact, most legume seeds can be easily stored at room temperature without loss of quality. This is useful from the nurseryman's point of view, principally in developing countries, because temperature requirements to storage safely either treated or untreated seeds can be fulfilled by normal refrigerators. Length of storage (factor D) did not adversely affect germination parameters, regardless of the other factors. No loss in germination was observed during the trial. Such a result is extremely positive for carob seed storage in general and carob scarified seed in particular. Therefore, it is useful to examine factor A × factor D interaction, that is, the influence of duration of storage on scarified and non-scarified seeds (table II). G% and GV% were far better for scarified seeds with respect to non-scarified ones, but within each of these two groups no differences were observed with relation to storage length (0, 6, 12, 18 months). For instance, germination of scarified seeds stored for 0 months (66.8%) was not significantly different from those stored for 18 months (62.4%).

These results, having practical applications for commercial seedsmen or nurserymen, express a good utilization of simple, modern seed technology. In this way carob seeds could be scarified properly and without risks by means of a mechanical scarifier, operated electrically, and then subjected to storage at + 3 °C, in sealed containers, until delivery. For at least 18 months, the quality of scarified seeds should not be affected. Moreover, seed damage due to the scarifier seems to be negligible as germinative capacity of mechanically scarified seeds (65.1%) is similar to that of seeds subjected to germination tests as prescribed by ISTA (1993) rules (67.5%).

Results of comparison of all possible combinations (table III) point out two large groups of 16 means each (table IV): the first one, quite homogeneous, with high G%<sub>s</sub> and GV<sub>s</sub>, was reached by experimental treatments including scarification, whereas the second, completely homogeneous and characterized by poor germination, regarded experimental treatments excluding scarification. Consequently, it seems reasonable to assume that long-term storage is highly safe for viability of scarified carob seeds regardless of container type and temperature during storage.

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