

Germination characteristics and intrapopulation variation in carob (*Ceratonía siliqua* L.) seeds

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Abstract

The aims of this work were to study the germination characteristics (under controlled conditions of light and temperature and using different pretreatments for promoting germination) and variability of *Ceratonía siliqua* seeds. Seed collected from different individual trees were tested. Constant (10°C, 15°C, 20°C, 25°C) and alternating 25/15°C temperature regimes and 16/8 h light/dark photoperiod conditions were used. Mechanical scarification, dry heat, boiling water, sulphuric acid, soaking in distilled water and soaking in gibberellic acid solution were used as presowing treatments applied for enhancing germination. The untreated seeds showed a deep dormancy at all temperature regimes assayed (final germination percentages ranged from 23 to 28%). Mechanical scarification, sulphuric acid and boiling water drastically improved final germination percentages (99, 88 and 80%, respectively). Therefore, the impermeability to water of the seed coat (physical dormancy) seems to be the most important causes of the seed dormancy present in this species. Great variability in seed weight, seed water content and germination parameters were found among seeds belonging to different individual trees. Significant differences between different individual trees under the same incubation temperature were detected for seed germination (final germination percentage ranged from 7 to 50%). However, germination rate (as expressed by mean germination time) was relatively similar among seeds from different trees. A negative significant relationship between seed weight and final germination percentage was found: the lightest seeds reached the highest germination percentages. Moreover, seed weight showed a positive significant correlation with seed water content.

Additional key words: final germination percentage, germination rate, intrapopulation variability, physical dormancy, presowing treatments, seed weight, water content.

Resumen

Características germinativas y variación intrapoblacional en semillas de algarrobo (*Ceratonía siliqua* L.)

Se han estudiado la germinación (bajo condiciones controladas de luz y temperatura y usando diferentes pretratamientos para promover la germinación) y la variabilidad de semillas de *Ceratonía siliqua*. Se analizaron semillas recolectadas de diferentes árboles. Se utilizaron temperaturas constantes (10°C, 15°C, 20°C, 25°C) y alternas (25/15°C) y unas condiciones de fotoperiodo de 16/8 horas de luz/oscuridad. Se emplearon como tratamientos de presiembra: escarificación mecánica, calor seco, agua hirviendo, ácido sulfúrico, imbibición en agua destilada y en ácido giberélico. Las semillas no pretratadas presentaron una dormición profunda para todas las temperatura ensayadas (la germinación varió del 23 al 28%). La escarificación mecánica, el ácido sulfúrico y el agua hirviendo incrementaron los porcentajes de germinación (99, 88 y 80%, respectivamente). La impermeabilidad al agua de la cubierta seminal (dormición física) parece ser la causa más importante de la dormición presente en estas semillas. Se encontró una gran variabilidad en el peso, el contenido de humedad y la germinación entre semillas pertenecientes a diferentes árboles. Se detectaron diferencias significativas en la germinación de semillas de diferentes árboles bajo la misma temperatura de incubación (los porcentajes de germinación variaron del 7 al 50%). La velocidad de germinación (expresada mediante el tiempo medio de germinación) fue relativamente similar entre las semillas de diferentes árboles. Se encontró una relación significativa negativa entre el peso y el porcentaje de germinación: las semillas más ligeras alcanzaron los mayores porcentajes. El peso mostró una correlación significativa positiva con el contenido de humedad de la semilla.

Palabras clave adicionales: contenido de humedad, dormición física, peso, porcentaje final de germinación, tratamientos de presiembra, variabilidad intrapoblacional, velocidad de germinación.

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Introduction

Carob tree (*Ceratonia siliqua* L.) is a long living tree native to the eastern Mediterranean basin of the family *Fabaceae* (subfamily *Cesalpinioideae*). This species is normally dioecious and it is a sclerophyllous evergreen tree up to 10 m high (Ortiz *et al.*, 1995). Carob tree has been grown since antiquity in most countries of the Mediterranean basin and it is one of the most useful native trees (Ciccarese *et al.*, 1988; Batlle and Tous, 1997). Carob legumes (pods) have traditionally been used as animal food and they have an almost equal food value to many cereal grains. Annual world yield of dried pods reaches 400,000 tons, mostly from Spain, Italy and Portugal (Catarino, 1993). Carob is an economically important tree and it can be used in many tree-planting activities: charcoal, wood production, soil erosion control, land reclamation, ornamental evergreen tree. Carob gum is especially viscous and can be used instead of some gums (Piotto and Piccini, 1996; Batlle and Tous, 1997). Carob is now being used in xerogardening in Mediterranean countries and it is recommended for forestation of degraded coastal areas threatened by soil erosion and desertification (Batlle and Tous, 1997).

Carob has seeds that are difficult to germinate. They are extremely hard and not readily imbibe water (Coit, 1951). Carob seeds have physical dormancy afforded by their hard seed coat, forming a persistent seed bank in the soil (Ortiz *et al.*, 1995). Like other legume seeds, carob shows seed coat impermeability to water so that the seed will not germinate unless the coat is scarified (Mitrakos, 1981; De Michele *et al.*, 1988; Frutos, 1988; Piotto and Piccini, 1996; Batlle and Tous, 1997; Piotto and Ciccarese, 1999; Piotto and Di Noi, 2003). Martins-Louçao *et al.* (1996) and Piotto and Di Noi (2003) showed that the best presowing treatments for enhancing carob seeds germination were chemical scarification with acids, treatment with warm water and scarification with the appropriate machines (mechanical scarification).

The main aims of this study were: (1) to determine germination characteristics (final germination percentage and mean germination time) of carob seeds to different temperature regimes; (2) to investigate the effects of several presowing treatments on the germination of carob seeds and use this information to determine the germination behaviour of seeds of this species; (3) to compare the germination responses of seeds belonging to different individual trees of the population studied; (4) to evaluate the possible correlations among seed weight, seed water content, final germination percentage and germination rate.

Material and methods

Seed material

Ripe fruits (pods) containing mature seeds of *Ceratonia siliqua* were collected in August 2007 from a wild growing population in the vicinity of the village of Sopalmo, Almería province, south east Spain (37°05' N; 1°87' E), near coastal area. Mean annual rainfall is 160–200 mm and average temperature is 19.5°C. The criterion followed to select the pods was they complete ripeness. The seeds from all selected pods, likewise, showed a similar degree of ripeness, as observed from their colour and hardness. Germination experiments were carried out with seeds belonging to 20 individual trees chosen at random. All the seeds from ripe pods randomly collected were bulked, cleaned manually, placed in paper bags and stored dry under laboratory conditions at about 25°C until the start of the trials in November 2007. At the same time, seeds belonging to 17 different individual trees chosen at random were collected and kept separately (without bulking). For each tree, the seeds from all pods harvested were bulked.

Seed germination tests

Germination of seeds bulked from 20 trees were tested under four different constant temperatures (10°C, 15°C, 20°C and 25°C) with a 16-h light photoperiod (provided by cool white fluorescent tubes with an irradiance of 35 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) and the alternate temperatures of 25/15°C (25°C for 16 h in light and 15°C for 8 h in dark). In all trials, four replicates of 25 seeds each were tested for germination on top of two sheets of filter paper (previously moistened with 4.5 mL distilled water) in 9-cm diameter glass Petri dishes. Filter papers were rewetted regularly with distilled water as required. Dishes were checked three times a week over a period of 56 days and germinated seeds (radicle longer than 2 mm) were counted and removed.

Presowing treatments

Seeds belonging to pods from 20 trees were bulked and six different presowing treatments were applied in order to enhance seed germination:

– *Mechanical scarification*. Chipping was accomplished using a sharp scalpel to carefully remove the

seed coat, without damaging the radicle, at the radicle end of the seed.

– *Soaking in distilled water.* Seeds were soaked in distilled water (3 volumes of water for each volume of seeds), at room temperature (approximately 25°C) for 24, 48, or 72 h. When treatment was applied longer than 24 h, the distilled water was renewed daily.

– *Boiling water.* Seeds were immersed in boiling distilled water (3 volumes of water for each volume of seeds) and then left to cool at room temperature (approximately 25°C) for 24 h.

– *Dry heat.* Seeds, spread on glass dishes, were placed in an oven at 100°C for various lengths of time, between 30 s and 30 min.

– *Sulphuric acid.* Concentrated sulphuric acid (96% H₂SO₄) was used to soak seeds for 1, 2 or 30 min (3 volumes of acid for each volume of seeds). The seeds were then washed in running water for 1 hour to remove any trace of acid, before being tested for germination.

– *Gibberellic acid.* Seeds were immersed in a gibberellic acid solution (250 mg·L⁻¹ GA₃) for 24 h (3 volumes of acid solution for each volume of seeds).

After these presowing treatments, seeds were set to germinate at alternating temperatures of 25°C (16-h light)/15°C (8-h darkness). Control seeds (untreated seeds) were sown in the same incubation conditions.

Germination of seeds from different trees

Seed lots from each one of 17 different individual trees were collected and kept separately (without bulk-ing) and then were set to germinate after pretreatment with boiling water or soaking in sulphuric acid for two min. Control (untreated seeds) and pretreated seeds were set to germinate at 25/15°C under a 16-h light photoperiod.

Seed water content and seed weight

Seed water content was determined for seeds belonging to 17 different individual trees (2 replicates of 50 seeds each) using the high temperature oven method (130°C for 1 h; ISTA 2003). Water content was expressed as percentage of fresh weight.

Seed weight (mg) was determined for seeds belonging to the same 17 individual trees (two replicates of 50 seeds each).

Statistical analysis

At the end of the germination period, the final germination percentage (mean value $\pm$ standard error) and the mean germination time (MGT, mean value in days $\pm$ standard error) were calculated. The latter was determined according to the following formula (Ellis and Roberts, 1981): $MGT = \sum DN / \sum N$; where D is the number of days counted from the date of sowing and N is the number of seeds germinated on day D. The values of final germination percentages were arcsine transformed and then subjected to analysis of variance (untransformed data appear in tables) using the computing package SPSS. One-way factorial ANOVA was used to test the effects of the different treatments and temperature regimes on seed germination capacity. In the same way, to determine differences among pretreatments and seeds belonging to different individual trees, data were analysed by means of one-way ANOVA. Where ANOVA indicated a significant effect, a comparison of mean values was carried out through the least significant difference test (LSD). The statistical analysis of MGT was also carried out using one-way factorial ANOVA.

Results

The effect of incubation temperature on the germination of carob seeds bulked from 20 different individual trees of the population studied is shown in Table 1. No significant differences were found among temperature regimes assayed for the final germination percentages (from 23 to 28%). However, the MGT values shown significant differences ($P < 0.05$). The seeds germinated faster at 20°C, 25°C and 25/15°C (Table 1) than at 10°C and 15°C.

The effect of six presowing treatments on the germination of carob seeds is shown in Table 2. The seeds which were set to germinate without any pretreatment (control) showed only 25% of germination. Seed germination was significantly ($P < 0.001$) increased by mechanical scarification. In the same way, boiling water and sulphuric acid treatments significantly improved ($P < 0.01$) the germinability of seeds compared to control seeds. The highest germination percentage (99%) was recorded in seeds scarified at the radicle end, indicating that mechanical scarification is the most effective method for improvement of germination in these seeds. When the seeds were boiled, the germination percentage reached 80%. In seeds soaked in concentrated sulphuric

Table 1. Effect of different temperature regimes on the final germination percentages (mean values $\pm$ standard error) and mean germination time (MGT, mean values in days $\pm$ standard error) of *Ceratonia siliqua* seeds. Results after 56 days of incubation under a 16-h light photoperiod. Seeds from 17 individual trees were bulked. Mean values within a column followed by the same letters are not significantly different at the 5% level of probability as determined by the least significant difference test (LSD)

Temperature	Germination (% $\pm$ SE)	MGT (days $\pm$ SE)
10°C	23 $\pm$ 2.9 a	26.9 $\pm$ 1.3 bc
15°C	23 $\pm$ 4.6 a	29.9 $\pm$ 5.7 c
20°C	22 $\pm$ 2.2 a	17.6 $\pm$ 2.6 ab
25°C	28 $\pm$ 2.4 a	10.8 $\pm$ 1.8 a
25/15°C	25 $\pm$ 4.8 a	14.5 $\pm$ 1.2 a

acid for 1, 2 or 30 min, the final germination percentages were 88, 80 and 86%, respectively. However, exposure to dry heat at 100°C for different periods, soaking in distilled water (24, 48 and 72 h) and in gibberellic acid solution did not significantly increase the final germination percentages compared to control seeds, varying within the range of 6 to 30%. Seeds subjected to dry heat for 30 min showed the lowest germination (6%), but differences with control seeds were not significant.

Only two out of six presowing treatments assayed (mechanical scarification and soaking in sulphuric acid for 30 min) significantly decreased ($P < 0.05$) the MGT values (Table 2).

The germination parameters (final germination percentages and MGT values) of seeds belonging to 17 different individual trees for untreated seeds (control seeds) and two presowing treatments (boiling water and soaking in sulphuric acid for two minutes) was further studied (Table 3). Final germination percentages of control seeds ranged from 7 to 50% depending on the tree showing very highly significant differences ($P < 0.001$) among seeds from individual trees. Germination percentages were lower than 30% for 11 out of 17 trees, and only in four they were higher than 40%. Similarly, the germination of boiled seeds was extremely variable, ranging from 9 to 92%; while the germination of seeds soaked in sulphuric acid ranged from 17 to 64%. On the other hand, the boiling water treatment only significantly increased germination ($P < 0.05$) compared to control seeds in five out of 17 individual trees, while sulphuric acid treatment did it in four out of 15 individual trees.

No significant differences were found among the MGT values reached by control seeds and among those

Table 2. Final germination percentages (mean values $\pm$ standard error) and mean germination time (MGT, mean values in days $\pm$ standard error) of *Ceratonia siliqua* seeds after different presowing treatments. The significance level (S) between results from each treatment and the control (untreated seeds) is showed. Seeds belonging to pods from 17 different trees were bulked. Results after 56 days of incubation at 25/15°C under a 16-h light photoperiod

Treatment	Duration of treatment	Germination (% $\pm$ SE)	S ¹	MGT (days $\pm$ SE)	S ¹
Untreated seeds (control)	---	25 $\pm$ 7.5	---	10.5 $\pm$ 1.3	---
Mechanical scarification	---	99 $\pm$ 0.9	***	5.5 $\pm$ 0.3	*
Boiling water (100°C)	---	80 $\pm$ 0.2	**	8.4 $\pm$ 0.2	ns
Dry heat (100°C)	0.5 min	19 $\pm$ 4.2	ns	9.4 $\pm$ 1.0	ns
	1 min	30 $\pm$ 5.6	ns	10.2 $\pm$ 0.9	ns
	5 min	30 $\pm$ 8.4	ns	10.2 $\pm$ 1.4	ns
	10 min	27 $\pm$ 2.1	ns	14.7 $\pm$ 1.5	ns
	15 min	23 $\pm$ 2.8	ns	11.6 $\pm$ 1.1	ns
	30 min	6 $\pm$ 1.1	ns	18.1 $\pm$ 3.7	ns
Soaking in sulphuric acid (H ₂ SO ₄)	1 min	88 $\pm$ 6.0	**	9.2 $\pm$ 0.3	ns
	2 min	80 $\pm$ 5.5	**	10.8 $\pm$ 0.4	ns
	30 min	86 $\pm$ 3.2	**	5.6 $\pm$ 0.3	*
Soaking in distilled water	24 h	23 $\pm$ 2.8	ns	14.5 $\pm$ 2.4	ns
	48 h	29 $\pm$ 4.4	ns	11.4 $\pm$ 2.0	ns
	72 h	27 $\pm$ 1.4	ns	16.1 $\pm$ 2.4	ns
Soaking in GA ₃ (250 mg·L ⁻¹)	24 h	20 $\pm$ 2.1	ns	11.7 $\pm$ 2.0	ns

¹ S: *** Significantly different to control at $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; ns, not significant.

Table 3. Seed water content, seed weight and effect of soaking in boiling water or in sulphuric acid (2 minutes) in the final germination percentage (mean values $\pm$ standard error) and mean germination time (MGT, mean values in days $\pm$ standard error) of *Ceratonía siliqua* seeds belonging to 17 individual trees. For each individual tree, germination and MGT mean values followed by different letters are significantly different ($P < 0.05$) according to the least significant difference test (LSD). Seeds were germinated for 56 days at 25/15°C under a 16-h light photoperiod.

Individual tree	Seed water content (% $\pm$ SE)	Seed weight (mg $\pm$ SE)	Germination (% $\pm$ SE)			MGT (days $\pm$ SE)		
			Control ¹	Boiling water	H ₂ SO ₄	Control	Boiling water	H ₂ SO ₄
1	6.35 $\pm$ 0.00	187.74 $\pm$ 9.70	7 $\pm$ 1.66 a	9 $\pm$ 2.18 a	17 $\pm$ 2.68 a	14.6 $\pm$ 2.7 a	29.7 $\pm$ 6.5 a	22.0 $\pm$ 2.9 a
2	5.17 $\pm$ 0.13	185.24 $\pm$ 3.38	11 $\pm$ 2.60 a	53 $\pm$ 3.28 c	32 $\pm$ 4.61 b	16.6 $\pm$ 4.2 a	23.1 $\pm$ 1.5 a	18.2 $\pm$ 0.4 a
3	6.38 $\pm$ 0.06	184.92 $\pm$ 3.67	12 $\pm$ 2.00 a	11 $\pm$ 2.96 a	28 $\pm$ 5.20 a	22.8 $\pm$ 5.5 a	13.8 $\pm$ 3.2 a	15.8 $\pm$ 2.5 a
4	6.41 $\pm$ 0.00	202.98 $\pm$ 1.76	14 $\pm$ 3.00 a	13 $\pm$ 2.60 a	18 $\pm$ 4.28 a	14.6 $\pm$ 2.1 a	16.7 $\pm$ 7.0 a	25.6 $\pm$ 4.6 a
5	5.98 $\pm$ 0.17	185.49 $\pm$ 0.41	15 $\pm$ 1.66 a	45 $\pm$ 5.36 b	30 $\pm$ 6.65 ab	18.9 $\pm$ 4.1 a	26.0 $\pm$ 2.9 a	20.2 $\pm$ 2.6 a
6	5.65 $\pm$ 0.03	181.26 $\pm$ 0.90	19 $\pm$ 2.96 a	35 $\pm$ 4.66 a	28 $\pm$ 5.72 a	19.0 $\pm$ 2.4 ab	28.7 $\pm$ 3.7 b	14.7 $\pm$ 1.2 a
7	3.61 $\pm$ 0.31	167.82 $\pm$ 1.76	20 $\pm$ 3.75 a	38 $\pm$ 5.00 b	NC ²	20.3 $\pm$ 2.1 a	19.6 $\pm$ 1.0 a	NC
8	6.04 $\pm$ 0.10	177.66 $\pm$ 2.00	22 $\pm$ 5.38 a	24 $\pm$ 4.24 a	NC	14.3 $\pm$ 1.4 a	12.6 $\pm$ 2.6 a	NC
9	5.30 $\pm$ 0.10	191.44 $\pm$ 4.69	24 $\pm$ 4.69 a	31 $\pm$ 2.96 a	29 $\pm$ 1.70 a	27.2 $\pm$ 3.8 a	20.3 $\pm$ 3.4 a	19.7 $\pm$ 1.8 a
10	5.14 $\pm$ 0.17	146.61 $\pm$ 2.49	27 $\pm$ 1.66 a	35 $\pm$ 2.60 a	55 $\pm$ 5.09 b	18.7 $\pm$ 2.3 a	22.1 $\pm$ 2.7 a	14.1 $\pm$ 0.9 a
11	4.24 $\pm$ 0.21	168.13 $\pm$ 4.89	28 $\pm$ 3.16 a	57 $\pm$ 1.66 c	46 $\pm$ 1.70 b	20.0 $\pm$ 3.5 a	20.0 $\pm$ 3.5 a	18.3 $\pm$ 1.4 a
12	5.82 $\pm$ 0.18	176.26 $\pm$ 9.35	33 $\pm$ 5.36 a	36 $\pm$ 2.83 a	44 $\pm$ 5.47 a	15.8 $\pm$ 1.0 a	19.4 $\pm$ 2.3 a	19.9 $\pm$ 1.5 a
13	4.05 $\pm$ 0.17	171.40 $\pm$ 3.51	33 $\pm$ 3.84 a	40 $\pm$ 1.41 a	64 $\pm$ 7.27 b	15.7 $\pm$ 2.3 a	14.1 $\pm$ 1.4 a	15.3 $\pm$ 1.3 a
14	4.77 $\pm$ 0.02	181.06 $\pm$ 8.02	42 $\pm$ 5.38 a	34 $\pm$ 5.38 a	43 $\pm$ 2.94 a	20.4 $\pm$ 1.8 a	19.0 $\pm$ 2.1 a	18.5 $\pm$ 0.7 a
15	7.15 $\pm$ 0.36	200.96 $\pm$ 0.61	47 $\pm$ 5.36 a	52 $\pm$ 7.48 a	71 $\pm$ 4.91 a	17.4 $\pm$ 0.7 a	20.0 $\pm$ 1.3 a	19.7 $\pm$ 0.9 a
16	3.83 $\pm$ 0.11	134.09 $\pm$ 7.14	48 $\pm$ 5.10 a	92 $\pm$ 2.45 b	60 $\pm$ 5.09 a	15.1 $\pm$ 1.7 b	10.6 $\pm$ 0.5 a	16.4 $\pm$ 0.9 b
17	3.98 $\pm$ 0.20	145.05 $\pm$ 2.11	50 $\pm$ 3.32 a	47 $\pm$ 3.84 a	53 $\pm$ 0.00 a	19.6 $\pm$ 1.4 b	14.1 $\pm$ 3.0 a	18.7 $\pm$ 0.6 b
Mean $\pm$ SE	5.29 $\pm$ 0.25	175.77 $\pm$ 4.47	26.59 $\pm$ 3.22	38.35 $\pm$ 4.67	41.20 $\pm$ 4.19	18.29 $\pm$ 0.80	19.40 $\pm$ 1.28	18.47 $\pm$ 0.70
S ³	***	***	***	***	***	ns	*	ns

¹ Control: untreated seeds. ² NC: trial not carried-out due to shortage of seeds. ³ S, significance level of each parameter: *** $P < 0.001$; * $P < 0.05$; ns, not significant.

values reached by seeds soaked in sulphuric acid from individual trees (Table 3). In contrast, the MGT values of boiled seeds showed significant differences ($P < 0.05$) among individual trees. Only in two out of the 17 individual trees, control seeds germinated significantly ($P < 0.05$) slower than boiled seeds. No significant differences were found between MGT values reached by control seeds and seeds soaked in sulphuric acid.

Seed water content, expressed as percentage of fresh weight, varied significantly ($P < 0.001$) among individual trees, ranging from 3.61 to 7.15% (Table 3). Similarly, the mean seed weight varied significantly ($P < 0.001$) from 134.09 to 202.98 mg depending on the individual tree considered (Table 3).

A significant positive correlation was observed between the seed water content and the seed weight

($r^2 = 0.547$, $P = 0.0007$). Naturally, the heavier the seeds, the higher seed water content. Moreover, final germination percentage of control seeds showed a significant negative relationship with seed weight ($r^2 = 0.243$, $P = 0.0446$). Thus, the seeds belonging to individual trees with heavier seeds reached germination percentages that were lower than those of trees with smaller seeds. However, seed water content did not affect germination ($r^2 = 0.149$, $P = 0.1256$). Final germination percentage was not correlated with MGT ($r^2 = 0.000$, $P = 0.982$). There was no significant correlation between seed weight and MGT values ($r^2 = 0.005$, $P = 0.7864$). In the same way, no significant relationship was found between seed moisture content and MGT ($r^2 = 0.021$, $P = 0.5813$).

Discussion

Optimum temperature for carob seed germination was found to be 25°C by De Michele *et al.* (1988) and 27.5°C by Mitrakos (1981). However, in this work no significant differences were found among different temperature regimes assayed. The seeds of the population studied reached similar final germination percentages at all incubation temperatures. Therefore, this species seems to show an opportunistic strategy for seed germination. In the natural habitat in which this species grows, soil moisture conditions would be the most decisive factor for germination and even for seedling establishment.

Most of the seeds from the population studied are impermeable to water at all five temperature regimes assayed. Therefore, carob seeds showed physical dormancy (according to Baskin and Baskin, 2004). Only a small fraction (approximately 25%) of carob seeds from the population studied could germinate without any pre-sowing treatment. These results of untreated seeds germination were higher than those previously reported. Thus, several authors (Spyropoulos and Lambiris, 1980; Piotto and Di Noi, 2003) have showed that the germination percentage of untreated seeds rarely exceeds 10%. Under natural conditions, only a reduced percentage of seeds are able to germinate. These seeds are probably the ones that would germinate shortly after they are dispersed. In contrast, most seeds (approximately 75%) did not germinate, even though they remained viable. Physical dormancy is an adaptative trait because it allows seed germination over time and space, thus increasing the probability of resulting in an adult plant (Baskin and Baskin, 2000). In this species, under natural conditions, a number of factors (mechanical friction with soil particles, microbial action, passage through the digestive tract of mammals that feed on them, etc.) can alter seed coat.

The results have shown that germination of control seeds was drastically lower than that of mechanically scarified ones (25% and 99%, respectively). These results are in accordance with those obtained by Piotto and Di Noi (2003). The impermeable seed coat, as occur in many *Fabaceae* taxa (Baskin and Baskin, 1998, 2004; Eisvand *et al.*, 2006; Finch-Savage and Leubner-Metzger, 2006; Silveira and Fernandes, 2006; Gresta *et al.*, 2007; Pérez-García, 2008; Pérez-García *et al.*, 2008), is the cause of the physical dormancy of carob seeds. Most carob seeds do not germinate due seed coat impermeability hindering the uptake of water and they will not

germinate unless the seed coat is scarified (Piotto and Piccini, 1996).

Exposure to dry heat at 100°C for different periods did not significantly increase the final germination percentages over the untreated seeds. These results could indicate that carob seeds are not dependent on fire for seed germination. These results are in accordance with those obtained by Ortiz *et al.* (1995). These authors showed that experiments simulating fire effect resulted in a decreased germination. In this work, the germination percentage reached by untreated seeds and preheated seeds were very similar.

In the same way, the gibberellic acid solution applied (250 mg·L⁻¹) was not effective in promoting carob seed germination. This result is in accordance with those obtained by De Michele *et al.* (1988) using different concentrations of this growth regulator.

Acid scarification or heat treatments are often used when it is desirable to break physical dormancy in large quantities of seeds. However, seed coat scarification with boiling water and sulphuric acid is not only hazardous, but also the embryos can get hurt and, besides, it also acts as an unavoidable selection process in favour of hard seeds by killing off seeds with thin coats (Piotto and Piccini, 1996). On the contrary, mechanical scarification only cause fine cracks on the seed coat and can be accomplished by several hand tools such as scalpels, knives, files and sandpaper. In conclusion, mechanical scarification of carob seeds is a suitable procedure to improve germination. It could replace other risky pre-sowing treatments (e.g. boiling water or sulphuric acid soaking) that tend to damage embryos in those seeds exhibiting thin seed coat.

The results of this study indicate that there is a considerable variability in seed weight, seed water content and germination characteristics within the population studied. Final germination percentages of control seeds belonging to 17 different individual trees ranged from 7 to 50%. These results show that there is a high degree of variability among seeds as far as the toughness of the seed coats is concerned. The production of seeds with different germinability is one of the most important survival strategies for species growing under variable and unpredictable environmental conditions (Koller and Hadas, 1982; Kigel, 1995; Baskin and Baskin, 1998; Cruz *et al.*, 2003; Qaderi and Cavers, 2000; Qaderi *et al.*, 2005). The ability to produce seeds with different degree of dormancy is a mechanism by which *C. siliqua*, as other species (Pérez-García, 1993, 1997; Kigel, 1995), adapts to new environmental situations and

ensures its survival by facilitating the germination of seeds over time. It is probable that this variation, as occur in several others species (Pérez-García, 1993; Bewley and Black, 1994; Qaderi and Cavers, 2002), can be attributed to genetic differences among individual parent plants, even within a small geographic area.

In some species there is a narrow correlation between seed weight and germination characteristics (Stanton, 1984; Milberg *et al.*, 1996; Vera, 1997; Baloch *et al.*, 2001; Delgado *et al.*, 2008). Most studies have reported higher germination percentages for heavier seeds when compared to lighter seeds of the same species (Khan, 2004; Moles *et al.*, 2005; Chiu *et al.*, 2006). However, several authors have reported that higher germination could not be clearly linked to heavier seeds (Pérez-García *et al.*, 1995; Delgado *et al.*, 2008). In this study a significant negative correlation between final germination percentage and seed weight was found. Thus, the trees with heavier seeds generally reached lower final germination percentages than did trees with lighter seeds. On the other hand, a correlation between seed weight and seed germination rate has been reported for some species (Baloch *et al.*, 2001; Daws *et al.*, 2005; Delgado *et al.*, 2008). Nevertheless, in this work no significant correlation was found between carob seed weight and germination rate (as expressed by MGT values). This last result is in accordance with those obtained by Jurado and Westoby (1992) with arid species of Central Australia. Although several authors have reported a positive correlation between germination rate and final germination percentage (Simmons and Johnston, 2000; Baloch *et al.*, 2001), final germination percentages of carob seeds were not correlated with MGT values.

The above results highlight that the source (origin) of seed samples should always be taken into account when defining models of germination behaviour, especially in wild species with a high degree of morphological and physiological variability. The variability of the germinative response within seeds of one population is relatively frequent in wild plants, and should be considered when extrapolating to whole species data obtained from genotypically limited material. *Ceratonia siliqua* produces seeds with different degrees of physical dormancy and the degree of dormancy varies among individual trees. Therefore, seeds are released from dormancy at different times and germinate intermittently over a determinate period.

In conclusion, mechanical scarification drastically improved germination in carob seeds of the population studied, showing that dormancy in these seeds is due to

the impermeability of the seed coat. In addition, this physical dormancy was broken also by boiling water and sulphuric acid treatments. Besides, the results obtained in this study show a high intrapopulation variation in relation to seed weight, seed water content and germination pattern of seeds from different individual trees. This could be due to the existence of a considerable variability in the toughness of the seed coat. Seed weight was negatively correlated with final germination percentage.

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