Pérez-García, F., González-Benito, M.E. and Gómez-Campo, C. (2008), *Seed Sci. & Technol.,* **36**, 407-422

Germination of fourteen endemic species from the Iberian Peninsula, Canary and Balearic Islands after 32-34 years of storage at low temperature and very low water content

F. PÉREZ-GARCÍA, M.E. GONZÁLEZ-BENITO AND C. GÓMEZ-CAMPO

Departamento Biología Vegetal, Escuela Universitaria de Ingeniería Técnica Agrícola, Universidad Politécnica de Madrid, Ciudad Universitaria s/n, 28040 Madrid, Spain (E-mail: felix.perez@upm.es)

(Accepted February 2008)

Summary

Final germination percentage and mean germination time of fourteen accessions from the Universidad Politécnica de Madrid (Spain) seed bank were evaluated after 32-34 years of storage. All the accessions chosen, belonging to fourteen genera and twelve different plant families, are of species endemic to the Iberian Peninsula, Canary or Balearic Islands. Three of these taxa are classified as "vulnerable" and one as "rare" according to the IUCN (International Union for the Conservation of Nature) criteria. The seeds had been stored at temperatures between -5ºC and -10ºC in flame sealed vials containing dehydrated (blue) silica gel. Seed water content was in general lower than 3% after storage. The germination trials were carried out under controlled conditions of light and temperature. In half of the accessions, the highest germination percentage was reached by seeds without any pre-sowing treatment (untreated seeds). In the other seven accessions, the germination was significantly enhanced by soaking in gibberellic acid (GA_3) or mechanical scarification. In ten of the fourteen accessions the final germination after storage was equal to or higher than 90%; in two accessions it was over 70% and in the remaining two accessions where germination was lower than 55%, tetrazolium test showed that viability was much higher. Therefore, the seed preservation method based on silica gel and relatively low temperature (-5ºC and -10ºC) has proven to be highly efficient in the endemic species assayed. The results obtained in this work support the possibility of using ultra-dry methods for long-term storage of orthodox seeds from a range of plant families.

Introduction

For plant species with orthodox seeds, seed banks provide the most practical method for preserving large amounts of genetic material in a small space (Gómez-Campo, 2006). The technology used in the Universidad Politécnica de Madrid seed bank since 1966 comprises storing seeds at low temperature (between -5ºC and -10ºC) and with very low water content (ultra-dry conditions, normally between 1% and 3%), achieved by desiccation with silica gel and placing some dehydrated silica gel together with the seeds within flame-sealed glass vials (Gómez-Campo, 1985, 2002, 2006).

Our bank holds approximately 750 taxa of the *Brassicaceae* family and a large collection of threatened and endemic species of the Iberian Peninsula, Balearic Islands and the Macaronesian region.

The germination tests carried out with different accessions of our seed bank have shown positive results (Ellis *et al*., 1993; Pérez-García *et al*., 1995; Ramiro *et al*., 1995; Masselli *et al*., 1999; Pérez-García *et al*., 2007). In a recent study (Perez-García *et al*., 2007), we have proven the high viability of seeds of 37 species of the *Brassicaceae* family after 40 years of ultra-dry storage. In that work, seed moisture content ranged from 0.3 to 3% f.w.b. (fresh weight basis) after storage, and the germination percentages obtained remained close to 100%. It should be indicated that seed desiccation to moisture contents lower than 3% (ultra-drying of orthodox seeds) has rarely been used in seed banking (Hong *et al*., 2005; Gómez-Campo, 2006). Furthermore, the results obtained in that work suggested that temperature might not be as important factor as expected for seed preservation and supported the possibility of using ultra-dry methods, at least for orthodox seeds of the *Brassicaceae* family.

Recently, Demir and Ozcoban (2007) have shown that ultra-dry moisture content storage (in equilibrium with around 13% RH at 20ºC) results in greater germination than seed stored at 5.1-6.3% moisture content in seeds of cultivated species of several families, although the storage period considered by those authors was only just over five years.

Contradictory opinions exist regarding the effect of ultra-drying on seed longevity for low temperature storage (e.g. Ellis, 1998; Hu *et al*., 1998; Walters and Engels, 1998; Walters *et al*., 2005; Ellis and Hong, 2006). According to Ellis (1998) and Hong *et al*. (2005) maximum longevity is acquired when desiccation takes places at 10-12% RH at 20ºC. However, Vertucci and Roos (1993) maintained that optimal seed moisture content for conservation depends on storage temperature.

There are relatively few previous reports on the seed germination behaviour of the fourteen endemic species studied in the present work (Pita, 1988; Thanos *et al*., 1992; Reghunath *et al*., 1993; Nogales *et al*., 1995). *Rumex lunaria*, an endemic shrub to Canary Islands, is used as a dietary supplement for ruminants and is of special interest due to its adaptation to semi-arid conditions (Méndez *et al*., 2003). *Chamaecytisus proliferus* is a fodder legume shrub from the Canary Islands with a high potential in agricultural systems of subtropical zones. It is a highly productive fodder crop and its nutritional value and palatability is similar to that of other forages (Reghunath *et al*., 1993).

Chamaecytisus proliferus and *Echium auberianum*, endemics to the Canary Islands, and *Ononis crispa*, endemic to the Balearic Islands, have been classified as "vulnerable" according to the IUCN criteria (VV. AA., 2000). *Digitalis dubia*, also endemic to the Balearic Islands, has been classified as "rare" (Walter and Gillett, 1998).

In this paper we have chosen fourteen taxa, belonging to fourteen different genera and a wide range of plant families, to prove that orthodox seeds from families other than *Brassicaceae* can also be preserved for the long term at very low moisture content, and low temperature. The main objective of this work was to evaluate the germination response of seeds from these endemic species stored in our seed bank for 32-34 years. For each one of the species studied, the effect of incubation temperature upon the germination response was evaluated and, for the species with low germination percentages, the most adequate presowing treatment was determined.

Materials and methods

Plant material

Table 1 includes the period of seed storage, distribution and ecological preference of the species studied. The fourteen accessions (14 genera, 12 families) were selected because they were endemics to the Iberian Peninsula, Canary or Balearic Islands belonging to different families: *Boraginaceae, Caryophyllaceae, Cistaceae, Lamiaceae, Poaceae, Polygonaceae, Resedaceae, Rubiaceae, Scrophulariaceae, Zygophyllaceae, Asteraceae* and *Fabaceae*.

The accessions were collected from the wild from 1972 to 1974, desiccated with silica gel and sealed in glass vials which were flame-sealed. Each vial contained seeds and silica gel separated by a filter paper (for more details see Gómez-Campo, 1985; Ellis *et al.*, 1993; Pérez-García *et al.*, 2007). These vials were stored in a cold room at -5ºC from 1972-1974 to 1981 and subsequently at -10ºC. In 2004 the temperature of the cold room was adjusted again to -5ºC.

In four accessions (*Cistus symphytifolius*, *Digitalis dubia*, *Gypsophylla tomentosa* and *Reseda virgata*) seed moisture content was determined using the air oven method (103ºC for 17 h, ISTA 2003; for more details see Pérez-García *et al.*, 2007), and in two of them also total lipid content (MAPA, 1994). The seed moisture content was not determined for all species because of the low number of seeds of most accessions. In two accessions (*Echium auberianum* and *Zygophyllum fontanesii*) viability percentages were determined in two replications of 25 seeds by staining with triphenyltetrazolium chloride (1% w/v) after removing the seed coat (ISTA, 2003).

Seed germination trials

The seeds were tested for germination on top of two sheets of filter paper (previously moistened with 3.5 ml distilled water, which was periodically added) in 7-cm diameter glass Petri dishes. Four replicates of 25 seeds each were used in all trials, except for *Chamaecytisus proliferus*, for which two or one replicates of 10 seeds each were used due to the insufficient amount of seeds from this accession. Seed incubation took place at 16 h light/8 h dark photoperiod (the light was provided by cool white fluorescent tubes with an irradiance of 35 $\mu mol \cdot m^{-2} \cdot s^{-1}$) under constant temperature regimes (15ºC, 20ºC, 25ºC) or alternating temperature of 25ºC (light)/15ºC (darkness). The constant temperature 6ºC was also assayed for *Ptilostemon hispanicus* and *Trisetum hispidum*. Germinating seeds were counted and removed every two days over a 56 day incubation period (70 days for *Cistus symphytifolius*). The criterion for germination was emergence of the radicle through the seed coat (minimum 1 mm long). For *Trisetum hispidum* (*Poaceae*), caryopses were used in the germination trials and seeds were considered germinated when the coleoptile had emerged 1 mm beyond the surrounding seed structure. Similarly, for the germination trials of *Onopordum corymbosum,* cypselas were used.

Presowing treatments

Depending on the species, different presowing treatments were applied in order to enhance seed germination: (1) mechanical scarification by abrasion of seeds between two

sheets of fine-grained sandpaper; (2) mechanical scarification removing 2-3 mm^2 of the seed coat, at the area opposite the hilum, using small pliers (only for *Chamaecytisus proliferus* seeds); (3) the previous procedure followed by 24 h soaking in distilled water and then removing the seed coat with a single-edge razor blade (only for *Chamaecytisus proliferus* seeds); (4) seeds soaked for 24 or 48 h in distilled water; (5) seeds immersed in distilled water heated to 100ºC and then left to cool to room temperature (ca. 23ºC) for 24 hours; (6) seeds soaked in distilled water heated to 100ºC for 6 or 9 minutes (only for *Chamaecytisus proliferus* and *Ononis crispa* seeds); (7) seeds immersed in distilled water at 80ºC for 5 minutes; (8) seeds placed in an oven at 100ºC from 5 to 90 min; (9) seeds soaked in gibberellic acid (GA_3) water solution (1000 $mg \cdot L^{-1}$) for 24 or 48 h. After these presowing treatments, seeds were set to germinate at different temperatures depending on the species. Control seeds (untreated) were sown in the same incubation conditions.

Statistical analysis

At the end of the germination period, the final germination percentage (mean value ± standard error) and the mean germination time (MGT, mean value in days ± standard error) were calculated. The latter was determined according to the following formula (Ellis and Roberts, 1981): $MGT = \Sigma DN / \Sigma N$, 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 MGT was not calculated when the final germination was equal to or less than 5%. The values of final germination percentages were arc-sine transformed and then subjected to analysis of variance using the computing package SPSS. One-way factorial ANOVA was used to test the effects of temperature regimes and presowing treatments. Means that exhibited significant differences were compared using the least significant difference test (LSD) at the 5% significance level. In the same way, the statistical analysis of MGT was also carried out using one-way ANOVA.

Results

Because of the reduced quantity of seeds available for most accessions, seed moisture content could only be determined in four of the fourteen accessions. The seed moisture content values obtained after storage were lower than 5%: 1.9% for *Digitalis dubia*, 2.0% for *Reseda virgata*, 3.0% for *Cistus symphytifolius* and 4.7% for *Gypsophylla tomentosa* (all of them f.w.b). The lipid content of *G. tomentosa* and *Reseda virgata* was also determined (5.4 and 30.6% f.w.b., respectively).

Table 1 provides the highest germination percentage reached in this work for each species whilst Table 2 shows the effect of incubation temperature (15ºC, 20ºC, 25ºC and 25/15ºC) on the germination percentage and MGT of the untreated seeds (control) of the studied species. High germination percentages (higher than 85%) were reached at the four temperatures assayed for *Digitalis dubia*, *Gypsophylla tomentosa* and *Rumex lunaria*. In these species, no significant differences were found between the four germination percentages, neither temperature affected the germination rate of *Digitalis dubia.* However, temperature influenced the germination rate of *Gypsophylla tomentosa* and *Rumex lunaria*;

Table 1. Years of storage, highest germination percentage obtained in this study, distribution and ecological preference for seeds from fourteen endemic species of Iberian Peninsula, Canary and Balearic Islands studied.

Accesion number [1]	Taxa	Years of storage	Highest germination (%)[2]	Distribution and ecological preference (categories IUCN Red List)
3170	*Chamaecytisus proliferus* (L. fil.) Link (Fabaceae)	32	100	Endemic species of Canary Islands. Fodder tall shrub legume (Vulnerable)
3230	*Cistus symphytifolius* Lam. (Cistaceae)	32	97	Endemic of Canary Islands. Low shrub widely distributed in the Canarian pine forest. 800-1800 m
3398	*Digitalis dubia* Rodr. (Scrophulariaceae)	32	94	Endemic low shrub of Balearic Islands. Rocky slopes of woods and shrubs, it preferably grows on calcareous soils (Rare)
3173	*Echium auberianum* Webb & Berth. (Boraginaceae)	32	53 (91*)	Endemic of Cañadas del Teide (Tenerife, Canary Islands). Subalpine semi-arid species found in volcanic pumice and fine cinder. 2100 m. Perennial hemycrptophyte (Vulnerable)
2699	*Gypsophylla tomentosa* L. (Caryophyllaceae)	33	99	Endemic low shrub of Central and Southeast Iberian Peninsula. Path borders, depressions and saline soils. 450-900 m
3380	*Ononis crispa* L. (Fabaceae)	32	98	Endemic shrub of Balearic Islands (Mallorca and Menorca). Stony and sandy soils (Vulnerable)
2707	*Onopordum corymbosum* Willk. (Asteraceae)	33	94	Endemic biennial species of half East Spain. Edges of paths and crops
3284	*Phlomis lychnitis* L. (Lamiaceae)	32	90	Endemic species widely distributed by Iberian Peninsula (except Northwest). This low shrub preferably grows on dry calcareous stony soils. 0-1500 m
3215	*Plocama pendula* Ait. (Rubiaceae)	32	74	Endemic of Canary islands. Shrub up to 2m of low xerophytic areas. Dry slopes and coastal areas
3268	*Ptilostemon hispanicus* (Lam.) Greuter (Asteraceae)	32	95	Endemic species of Sierras Béticas (Andalucía, South Spain). Stony and sunny soils. 400-1700 m
2554	*Reseda virgata* Boiss. & Reut. (Resedaceae)	34	98	Endemic low shrub of Central and Northwest Iberian Peninsula. Slimy and sandy soils, edges of paths and crops. 400-1400 m
2575	*Rumex lunaria* L. (Polygonaceae)	33	98	Endemic of Canary Islands. Low shrub commonly occurring in low nitrified sites and coastal zones
2739	*Trisetum hispidum* Lange (Poaceae)	33	76	Endemic perennial grass of Central and Northwest Iberian Peninsula. Cracks of rocky slopes
3200	*Zygophyllum fontanesii* Webb & Berth. (Zygophyllaceae)	32	36 (77*)	Endemic succulent low shrub of Canary Islands. Halophyte in dunes and coastal rocks

(1) Accession number in the seed bank of the UPM (Universidad Politécnica de Madrid, Spain).
(2) Highest final germination percentage reached by each accession in this work (* Viability percentage reached after tetrazolium test).

in the first species it was improved by warmer temperatures while in the latter by cooler. There were also no significant differences among the germination percentages reached by *Reseda virgata* seeds (43-63%), although slightly higher percentages and higher rates were observed with the highest temperature tested (25ºC) or with the alternating regimen (table 2).

In the rest of the species temperature influenced final germination percentage (Table 2). Two species showed the highest germination percentage at the highest temperature tested (25ºC): *Onopordum corymbosum* and *Plocama pendula*. For the first of these species germination was quicker at 25ºC and 25/15ºC than at the two lower temperatures, while no differences were found for the latter.

Ptilostemon hispanicus and *Trisetum hispidum* showed the highest germination percentage at 15ºC, 79% and 55%, respectively (Table 2). This low temperature improved germination rate (5.27 days) in *Phlomis lychnitis*, but there were not significant differences with the final germination obtained with the other temperatures (90-70%).

The remaining five species showed low germination percentages (0-21%) when only incubation temperature was taken into account. From the data obtained from the non-treated seeds it could be observed that only *Echium auberianum* germinated better at alternating temperatures, *Ononis crispa* either at low (15ºC) or alternating, and *Zygophyllum fontanesii* at lower temperatures. *Chamaecitysus proliferus* and *Cistus symphytifolius* did not germinate or showed very low germination (0-3%, table 2).

Several treatments (and one more germination temperature, 6ºC) were studied in most of the species in order to improve germination percentage and rate, to determine the actual viability and, when relevant, the best procedures to break dormancy.

Germination percentage of *Digitalis dubia* and *Rumex lunaria*, whose untreated seeds had shown high germination percentages (94 and 98%, respectively), was not affected by immersion in GA_3 (table 3). However, germination rate (as expressed by MGT values) improved in both species and, at least for *D. dubia*, this effect was not due just to a quicker imbibition, as seeds treated just with distilled water did not germinate faster. Germination of *Reseda virgata* and *Onopordum corymbosum* seeds, which had shown an intermediate germination (63% and 59%, respectively) at alternating or high temperatures, was improved by GA_3, reaching 98-94%. GA_3 also improved germination rate in *R. virgata*.

Both *P. hispanicus* and *T. hispidum* had shown the highest germination percentage at 15ºC (79% and 55%, respectively). Seed imbibition in GA_3 or water for 24 h did not improve germination (table 3). However, final germination was improved in *T. hispidum* when seeds were left to soak for 48 h in water (77%). In these two species incubation at a lower temperature (6ºC) significantly increased final germination compared with incubation at 15ºC (95 ± 2.50, $P<0.01$ for *P. hispanicus*; and 76 ± 3.16, $P<0.05$ for *T. hispidum*). On the other hand, germination rate was in both cases significantly decreased ($P<0.001$).

Untreated seeds of *Chamaecytisus proliferus* did not germinate at any of the temperatures assayed. However, 100% germination was reached when a slight cut was made in the seed coat using pliers and, after seed imbibition for 24 h, the seed coat was completely removed (table 4). Furthermore, with this pre-sowing treatment germination

Table 2. Effect of different temperature incubation regimes on the final germination percentage (mean value ± standard error) and mean germination time (MGT, mean value in days ± standard error, in parenthesis) of seeds from the studied species. Results obtained after 56 days of incubation (70 days for *C. symphytifolius*). When germination was ≤ 5%, MGT was not calculated (NC). For each species, mean germination percentages and mean germination times in a row followed by the same letter are not significantly different at the 5% level of probability as determined by the least significant difference test.

Species	Germination (% ± SE) and MGT (days ± SE, in parenthesis)			
	15ºC	20ºC	25ºC	25/15ºC
Chamaecitysus proliferus	NO	NO	0 (NC)	0 (NC)
Cistus symphytifolius	3 ± 2.60 a (NC)	0 a (NC)	3 ± 0.87 a (NC)	0 a (NC)
Digitalis dubia	89 ± 2.29 a (22.75 ± 2.29 a)	87 ± 4.09 a (21.72 ± 2.34 a)	91 ± 3.77 a (27.00 ± 1.95 a)	94 ± 1.58 a (29.82 ± 1.37 a)
Echium auberianum	NO	NO	4 ± 3.40 a (NC)	21 ± 7.97 a (36.33 ± 4.84)
Gypsophylla tomentosa	99 ± 0.87 a (7.97 ± 0.14 b)	97 ± 1.66 a (3.52 ± 0.06 a)	97 ± 1.66 a (3.45 ± 0.22 a)	98 ± 1.73 a (3.12 ± 0.06 a)
Ononis crispa	17 ± 3.19 b (7.52 ± 0.70 a)	NO	1 ± 0.94 a (NC)	18 ± 2.97 b (8.35 ± 0.57 a)
Onopordum corymbosum	30 ± 1.73 a (9.25 ± 0.80 b)	26 ± 4.12 a (6.90 ± 0.72 ab)	59 ± 3.19 b (5.15 ± 0.13 a)	33 ± 5.17 ab (4.87 ± 0.29 a)
Phlomis lychnitis	90 ± 3.53 a (5.27 ± 0.41 a)	77 ± 4.14 a (6.35 ± 0.40 b)	70 ± 8.46 a (8.30 ± 0.39 c)	76 ± 7.97 a (9.07 ± 1.10 c)
Plocama pendula	56 ± 1.41 a (20.27 ± 1.19 a)	43 ± 8.29 a (16.90 ± 1.85 a)	74 ± 9.54 b (18.85 ± 0.41 a)	44 ± 3.74 a (18.25 ± 1.63 a)
Ptilostemon hispanicus	79 ± 3.70 b (6.37 ± 0.24 a)	54 ± 5.10 a (9.12 ± 1.05 a)	NO	NO
Reseda virgata	43 ± 2.16 a (9.20 ± 0.14 c)	38 ± 4.85 a (7.47 ± 0.38 bc)	50 ± 3.70 a (5.12 ± 0.35 a)	63 ± 4.39 a (6.12 ± 0.20 ab)
Rumex lunaria	96 ± 2.45 a (5.50 ± 0.18 a)	92 ± 3.16 a (6.12 ± 0.10 ab)	93 ± 1.66 a (10.15 ± 0.29 bc)	98 ± 1.00 a (12.52 ± 1.26 c)
Trisetum hispidum	55 ± 2.18 b (12.82 ± 0.59 a)	34 ± 2.24 ab (24.00 ± 3.25 ab)	9 ± 3.57 a (26.20 ± 0.94 ab)	27 ± 2.18 ab (38.77 ± 3.78 b)
Zygophyllum fontanesii	15 ± 3.84 a (16.42 ± 1.14 b)	14 ± 4.58 a (11.35 ± 0.32 a)	10 ± 2.19 a (11.50 ± 1.30 a)	5 ± 2.18 a (NC)

NO: trial not carried out because of the low number of seeds.

Table 3. Final germination percentage (mean value ± standard error) and mean germination time (MGT, mean value in days ± standard error) of seeds from *Digitalis dubia, Rumex lunaria, Reseda virgata, Onopordum corymbosum, Ptilostemon hispanicus,* and *Trisetum hispidum* for two presowing treatments (immersion in distilled water or GA_3). Results were obtained after 56 days of incubation at the temperatures stated. For each temperature, mean values in a row followed by the same letter are not significantly different at the 5% level of probability as determined by the least significant difference test.

Species	Incubation temperature	Immersion time (h)	Germination (% ± SE)			MGT (days ± SE)		
			Control	Distilled water	GA_3	Control	Distilled water	GA_3
D. dubia	15ºC	24	89 ± 2.29 a	82 ± 4.55 a	88 ± 0.71 a	22.75 ± 2.29 b	21.37 ± 0.61 b	13.42 ± 0.81 a
	20ºC	24	87 ± 4.09 a	83 ± 2.06 a	84 ± 0.71 a	21.72 ± 2.34 b	19.62 ± 1.67 b	14.02 ± 0.59 a
	25ºC	24	91 ± 3.77 a	77 ± 2.19 a	85 ± 4.97 a	27.00 ± 1.95 b	27.30 ± 1.00 b	18.10 ± 0.62 a
	25/15ºC	24	94 ± 1.58 b	86 ± 2.49 b	72 ± 4.26 a	29.82 ± 1.37 b	25.80 ± 1.53 b	19.90 ± 1.11 a
R. lunaria	25ºC	24	98 ± 1.00 a	97 ± 2.60 a	94 ± 3.32 a	12.52 ± 1.26 b	7.75 ± 0.57 a	6.60 ± 0.52 a
R. virgata	25/15ºC	24	63 ± 4.39 a	66 ± 3.53 a	98 ± 0.71 b	6.12 ± 0.20 c	5.07 ± 0.27 b	4.12 ± 0.02 a
O. corymbosum	25ºC	24	59 ± 3.19 a	55 ± 2.96 a	94 ± 3.05 b	5.15 ± 0.13 a	5.05 ± 0.25 a	4.75 ± 0.30 a
	25/15ºC	24	33 ± 5.17 a	30 ± 5.74 a	94 ± 1.73 b	4.87 ± 0.29 a	4.89 ± 0.13 a	4.17 ± 0.07 a
P. hispanicus	15ºC	24	79 ± 3.70 a	85 ± 3.02 a	88 ± 3.03 a	6.37 ± 0.24 a	6.61 ± 0.16 a	6.22 ± 0.19 a
T. hispidum	15ºC	24	55 ± 2.18 a	52 ± 4.47 a	63 ± 5.69 a	12.82 ± 0.59 b	12.51 ± 1.28 b	7.97 ± 0.24 a
	15ºC	48	---	70 ± 3.43 b	51 ± 2.18 a	---	9.42 ± 0.58 a	10.55 ± 0.46 b

Control: untreated seeds.
Distilled water: seeds were soaked for 24 h or 48 h in distilled water.
GA_3: seeds were soaked for 24 h or 48 h in a gibberellic acid water solution of 1000 $mg \cdot L^{-1}$.

Table 4. Final germination percentages (mean value ± standard error) and mean germination time (MGT, mean value in days ± standard error) of seeds of the two *Fabaceae* species assayed: *Chamaecytisus proliferus* and *Ononis crispa* after different presowing treatments. For *O. crispa* and for each temperature, the significance level between results from each treatment and the control (untreated seeds germinated at the same incubation temperature, see values in Table 2) are showed. When germination was ≤ 5%, MGT was not calculated (NC). Results were obtained after 56 days of incubation.

Treatment	Duration of treatment	Incubation temperature	Germination (% ± SE)	MGT (days ± SE)
Chamaecytisus proliferus				
Mechanical scarification[1]				
MS1	---	25/15ºC	11 ± 0.62 (A)	3.00 ± 0.00
MS2	---	25/15ºC	100 (B)	3.00
Boiling water (100ºC)	6 min	25ºC	0 (B)	---
	9 min	25ºC	20 (B)	---
	9 min + MS2	25ºC	56 (B)	---
Ononis crispa				
Mechanical scarification[2]	---	15ºC	96 ± 2.26 ***	4.42 ± 0.39 *
		25ºC	96 ± 1.75 ***	4.60 ± 0.07
		25/15ºC	98 ± 1.51 ***	4.17 ± 0.08 ***
Boiling water (100ºC)	---	25/15ºC	65 ± 7.40 *	7.95 ± 0.31 ns
	6 min	25/15ºC	3 ± 1.03 **	NC
	9 min	25/15ºC	1 ± 1.08 **	NC

(1) MS1, a slight cut (2-3 mm^2) was made using small pliers at the area of the seed coat opposite to hilum; MS2, same as above followed by soaking for 24 h in distilled water and then the seed coat was completely removed using a single-edge razor blade.
(2) Mechanical scarification of seeds was achieved by abrasion between two pieces of sandpaper.
(A) Two replicates of 10 seed each one.
(B) only one replicate of 10 seeds.
*** Significantly different from control at the same temperature at $P<0.001$; **$P<0.01$; *$P<0.05$; ns, not significant

was very quick (the value of MGT was 3.00 days). With the other pre-sowing treatments assayed (immersion in boiling water or just a small cut) the final germination percentage ranged from 11% to 56% (table 4). Similarly, scarification treatments were applied to seeds of *Ononis crispa*, the other *Fabaceae* species of this study, to improve germination. The mechanical scarification of the seed coat with sand paper significantly increased the final germination percentage at all temperatures assayed (96-98%, Table 4). The value of MGT (4.17-4.60 days) was significantly lower than the value reached by untreated seeds (7.52-8.35 days). Soaking seeds in boiling water also increased germination significantly (65%) compared with control seeds (Table 4); however, longer exposures to hot water damaged the seeds.

Scarification between two sheets of sand paper increased germination of *Cistus symphytifolius* dramatically (from 3% for untreated seeds to 97%) (table 5). Other presowing treatments (boiling water and dry heat) were also assayed but the final germination and MGT were always significantly more negative than with the sand paper treatment.

Table 5. Final germination percentages (mean value ± standard error) and mean germination time (MGT, mean value in days ± standard error) of seeds of *Cistus symphytifolius* for five presowing treatments and various application times. Results were obtained after 70 days of incubation at 15ºC. When germination was ≤ 5%, MGT was not calculated (NC). Mean values in a column followed by the same letter are not significantly different at the 5% level of probability as determined by the least significant difference test.

Treatment	Duration of treatment	Germination (% ± SE)	MGT (days ± SE)
Mechanical scarification[1]	---	97 ± 1.44 e	5.10 ± 0.09 a
Boiling water (100ºC)	---	61 ± 5.73 d	19.17 ± 1.00 b
Dry heat (100ºC)	5 min	14 ± 2.41 ab	30.90 ± 3.25 bc
	10 min	45 ± 4.49 cd	19,65 ± 2.73 b
	20 min	43 ± 1.29 cd	28.40 ± 2.21 bc
	30 min	45 ± 3.72 cd	22.95 ± 0.71 bc
	40 min	42 ± 5.21 cd	24.40 ± 1.24 bc
	50 min	34 ± 1.54 bc	21.90 ± 1.80 bc
	60 min	36 ± 3.94 bc	33.52 ± 3.37 c
	90 min	26 ± 1.00 bc	32.92 ± 1.70 c
Soaking in distilled water	24 h	1 ± 0.65 a	NC
Soaking in GA_3 (1000 $mg \cdot L^{-1}$)	24 h	3 ± 1.66 a	NC

(1) Mechanical scarification of seeds was achieved by abrasion between two pieces of sandpaper.

For the different pre-sowing treatments tested with *Echium auberianum*, the highest germination percentage (53%) was obtained by soaking seeds for 24 h in GA_3, and this was significantly higher than the result obtained in control seeds (Table 6). In general, all the presowing treatments with GA_3 significantly accelerated germination. The MGT values of seeds soaked in GA_3 ranged from 5.10 to 8.25 days (Table 6). To a lesser extent, soaking seeds in distilled water also improved the rate of germination (19.75 days vs. 36.33 for control seeds).

None of the pre-sowing treatments assayed for *Plocama* pendula increased germination compared with control seeds (table 6). On the contrary, all were detrimental to the seeds (soaking in distilled or hot water, and dry heat).

Germination was improved in *Zygophyllum fontanesii* by mechanical scarification of the seed coat with sand paper (36% vs. 15% for untreated seeds) (table 6). However, none of the pre-sowing treatments assayed for this species enhanced significantly the final germination percentage (table 6).

Viability was determined by the tetrazolium test for these two last species (*Echium auberianum* and *Zygophyllum fontanesii*), and, in both cases, the value was higher than the maximum germination percentage obtained (91 ± 9% and 77 ± 4%, respectively), thus indicating that some unremoved dormancy had persisted.

Table 6. Final germination percentages (mean value ± standard error) and mean germination time (MGT, mean value in days ± standard error) of seeds of *Echium auberianum*, *Plocama pendula* and *Zygophyllum fontanesii* after different presowing treatments. For each species and temperature, the significance levels between results from each treatment and the control (untreated seeds, see values in Table 2) are showed. Results were obtained after 56 days of incubation.

Treatment	Duration of treatment	Incubation temperature	Germination (% ± SE)	MGT (days ± SE)
Echium auberianum	40 min	25/15ºC	17 ± 10.23 ns	27.00 ± 2.53 ns
Dry heat (100ºC)	60 min	25/15ºC	14 ± 5.98 ns	27.67 ± 0.27 ns
	24 h	25/15ºC	53 ± 9.54 *	5.10 ± 0.78 ***
Soaking in GA_3 (1000 mg·L^{-1})	48 h	25/15ºC	35 ± 10.61 ns	8.25 ± 0.18 ***
	48 h	15ºC	46 ± 2.47 ns	7.60 ± 1.13 ***
Soaking in distilled water	48 h	25/15ºC	29 ± 3.57 ns	19.75 ± 2.57 **
Plocama pendula	10 min	25ºC	31 ± 3.15 *	11.40 ± 1.58 **
Dry heat (100ºC)				
Soaking in hot water (80ºC)	5 min	25ºC	6 ± 2.38 ***	28.17 ± 5.12 ns
Soaking in distilled water	24 h	25ºC	34 ± 4.92 *	11.40 ± 2.84 ns
	48 h	25ºC	37 ± 7.46 ns	13.22 ± 1.26 ns
Soaking in GA_3 (1000 mg·L^{-1})	24 h	25ºC	41 ± 5.99 ns	12.27 ± 2.34 ns
Zygophyllum fontanesii	----	20ºC	36 ± 5.96 ns	10.97 ± 1.48 ns
Mechanical scarification[1]				
Dry heat (100ºC)	10 min	20ºC	15 ± 4.87 ns	13.40 ± 1.84 ns
	30 min	20ºC	8 ± 3.61 ns	16.57 ± 0.84 **
Soaking in hot water (80ºC)	5 min	20ºC	17 ± 3.28 ns	15.02 ± 0.48 *
Soaking in distilled water	24 h	25ºC	15 ± 2.48 ns	13.10 ± 2.55 ns
Soaking in GA_3 (1000 mg·L^{-1})	24 h	20ºC	18 ± 2.38 ns	12.42 ± 0.81 ns
	24 h	25ºC	18 ± 4.12 ns	11.20 ± 2.01 ns

(1) Mechanical scarification of seeds was achieved by abrasion between two pieces of sandpaper.
*** Significantly different from control at the same temperature at $P<0.001$; ** $P<0.01$; * $P<0.05$; ns, not significant.

Discussion

In the present work, the highest germination percentage reached after storage for 32-34 years in ten out of the fourteen accessions was equal to or higher than 90%. In two accessions, *Plocama pendula* and *Trisetum hispidum*, the highest germination was 74% and 76%, respectively. Only in two accessions, *Echium auberianum* and *Zygophyllum fontanesii*, the highest germination obtained was lower than 55% (53% and 36%, respectively). However, the viability percentages determined by the tetrazolium test of these two accessions became 91% and 77%, respectively.

Collectively these results show that the seed preservation method based on silica gel desiccation and low temperature (between -5ºC and -10ºC) was highly efficient in the accessions assayed. In two previous studies carried out with *Brassicaceae* seeds from our seed bank (Ellis *et al*., 1993; Pérez-García *et al*., 2007), the high viability recorded in ultra-dry orthodox seeds from 37 crucifer species after almost 40 years of storage was proven. The *Brassicaeae* seeds were preserved under the same conditions as the accessions studied in this work: that is very low seed moisture content and at temperatures between -5ºC and -10ºC. It was shown that the equilibrium relative humidity at 20ºC for most of those vials after storage was 6-7% (Ellis *et al*., 1993), much lower than the recommended 10-12% (FAO/IPGRI, 1994). Therefore, the results obtained in our work support and extend the possibility of using ultra-dry methods for long-term storage of orthodox seeds of several different families.

For seven of the fourteen species assayed (*Digitalis dubia*, *Gypsophylla tomentosa, Phlomis lychnitis, Plocama pendula, Ptilostemom hispanicus, Rumex lunaria* and *Trisetum hispidum*), the highest germination percentage was reached without any pre-sowing treatment.

The germination values obtained for *Gypsophylla tomentosa* at the four temperatures assayed, were very similar to those obtained by Escudero *et al.* (1997) for *G. struthium* (species widely distributed on gypsum outcrops of the Iberian Peninsula) at the same temperatures. Different gypsophyllous species germinate efficiently under a broad range of temperatures (Escudero *et al.*, 1997). This germination behaviour seems inappropriate for survival in semi-arid Mediterranean areas. Thus, cyclic seasonal changes in seed dormancy or exogenous factors (i.e. light, salinity, etc.) might modulate seed response to temperature, thereby narrowing the effective range of germination (Kigel, 1995).

The fruits of *Plocama pendula* are eaten by birds, lizards and rabbits. The role of these frugivores selective animals as seed dispersers of this Canarian endemic shrub is well known (Nogales *et al*,. 1999). Valido *et al*., (2003) proved that the majority of seeds found in droppings of the lizard *Gallotia galloti*, a Canary Islands endemic, corresponded to *Plocama pendula*. Nogales *et al*. (1995) proved that the percentage of damaged seeds of *Plocama pendula* found in droppings of rabbits was only 16.5%. However, seeds collected directly from plants reached a higher germination percentage than seeds found in the droppings of rabbit: 66.5% vs. 34.5%. These percentages of germination are very similar to the values obtained in our work: 74% for untreated seeds and always less (6 to 41%) for pretreated seeds. For three of the fourteen species studied (*Echium auberianum*, *Onopordum corymbosum* and *Reseda virgata*), seeds soaked in gibberellic acid (GA_3)

reached final germination percentages significantly higher than untreated seeds (control seeds). The behaviour of *Onopordum corymbosum* seeds is similar to other species of the genus *Onopordum* in which gibberellic acid clearly promotes germination of seeds (Pérez-García and Durán, 1990; Pérez-García, 1993; Qaderi and Cavers, 2000; Fernández *et al*., 2002). Therefore, the seeds of all these species could show a physiological dormancy (according to the classification system for seed dormancy proposed by Baskin and Baskin, 2004). Besides, the presowing treatment with GA_3 significantly decreased the MGT values in five species (*Digitalis dubia*, *Echium auberianum*, *Reseda virgata*, *Rumex lunaria* and *Trisetum hispidum*).

For four of the fourteen species studied (*Chamaecytisus proliferus*, *Cistus symphytifolius*, *Ononis crispa* and *Zygophyllum fontanessi*) the highest germination percentages were reached by mechanical scarification. For these four species this was the most appropriate presowing treatment for the seed coat to become permeable to water with no adverse effects on the embryo. Therefore, the seeds of these species showed physical dormancy (according to Baskin and Baskin, 2004). Physical dormancy is currently known in the *Cistaceae* and *Fabaceae* families (Baskin and Baskin, 1998, 2004). Physical dormancy is an adaptive seed trait because it allows seed germination over time and space, thus increasing the probability of resulting in an adult plant (Nikolaeva, 1999; Baskin and Baskin, 2000).

As occurs in other species of the genus *Cistus* (i.e. Corral *et al.*, 1990; Thanos *et al*., 1992; Valbuena *et al*., 1992; Trabaud, 1995; Pérez-García, 1997; Pérez-García and Escudero, 1997), mechanical scarification of seed coat significantly improved germination in seeds of *Cistus symphytifolius*, suggesting that physical dormancy associated with these seeds might be due to the impermeability of the seed coat. Mechanical scarification improves seed germination by making the seed coat more permeable to water, allowing imbibition. In these species, under natural conditions, a number of factors (e.g. diurnal fluctuations in temperature, rainfall wash, wetting and drying cycles, mechanical abrasion by soil particles, microbial action, passage through the digestive tract of vertebrates, etc.) can alter seed coats in a degree similar to mechanical scarification (Baskin and Baskin, 1998, 2000).

In the same way, and as occur in many *Fabaceae* taxa (i.e. González-Melero *et al*., 1997; Baskin and Baskin, 1998; Rincón-Rosales *et al*., 2003; Zeng *et al*., 2005a,b; Zida *et al*., 2005; Eisvand *et al*., 2006; Finch-Savage and Leubner-Metzger, 2006; Silveira and Fernandes, 2006; Gresta *et al*., 2007), the impermeable seed coat is the cause of the physical dormancy of *Chamaecitysus proliferus* and *Ononis crispa* seeds. The positive results obtained from this study with the scarification of seed coat of *Chamaecytisus proliferus* seeds are in accordance with the results reached by Reghunath *et al*. (1993). The seeds of many *Fabaceae* species show a high longevity (Baskin and Baskin, 1998).

The germination behaviour of untreated seeds from *Digitalis dubia*, *Gypsophylla tomentosa* and *Rumex lunaria* was very similar. Thus, the seed of these three species reached high final germination percentages (higher than 85%) at all incubation temperatures assayed. Therefore, these species seem to show an opportunistic strategy for germination (Albert *et al*., 2002). The untreated seeds of five species (*Ononis crispa, Phlomis lychnitis, Ptilostemom hispanicus, Reseda virgata* and *Trisetum hispidum*) reached

their highest percentage of germination at intermediate constant temperatures (15ºC and 20ºC) or alternating temperatures (25/15ºC). All these five accessions are endemic species from the Iberian Peninsula or the Balearic Islands. This germination behaviour is a typical strategy of Mediterranean plants with optimal germination temperature ranging between 15 and 20ºC (Bell *et al*., 1993; Thanos *et al*., 1992, 1995; Baskin and Baskin, 1998). For these plants, soil moisture conditions would be the most decisive factor for germination and even for seedling establishment in late winter or early spring, when winter plus spring rainfall would meet moisture requirements. Untreated seeds of *Ptilostemom hispanicus* and *Trisetum hispidum* reached their highest germination percentages at 6ºC, which could indicate that these two species could germinate in winter. For *Trisetum flavescens*, Dixon (1995) recorded 15ºC as the temperature that resulted in a high germination percentage.

On the other hand, untreated seeds of biennial *Onopordum corymbosum* and *Plocama pendula* reached the highest germination at 25ºC. As occurs in *Onopordum acanthium* (Pérez-García, 1993) and *O. nervosum* (Pérez-García and Durán, 1990), the seeds of *O. corymbosum* reached higher germination percentages at 25ºC than at 15ºC or 20ºC.

The results obtained in this study suggest the following main conclusions:

a) Seed preservation methods based on dehydrated silica gel were highly efficient for all endemic species studied (14 species of 12 plant families). The germination results obtained provide a solid hope for the possibility of highly efficient preservation of orthodox seeds over long periods.
b) For several accessions, seed dormancy (physical and physiological dormancy) was maintained over the 32-34 years of preservation. Therefore, applications of pre-sowing treatments for breaking seed dormancy are very important when evaluating the seed longevity of wild species.
c) The germination behaviour of some of the accessions studied was in accordance with the climatic conditions of the natural habitat in which the species grows.

Acknowledgements

This study was partially supported by the Project CGL2006-10536/BOS of the Ministerio de Educación y Ciencia, Spain. We wish to thank Marta Huertas for her kindly cooperation in the laboratory.

References

Albert, M.J., Iriondo, J.M. and Pérez-García, F. (2002). Effects of temperature and pretreatments on seed germination of nine semiarid species from NE Spain. *Israel Journal of Plant Sciences*, **50**, 103-112.

Baskin, C.C. and Baskin, J.M. (1998). *Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination*. Academic Press, San Diego.

Baskin, J.M. and Baskin, C.C. (2000). Evolutionary considerations of claims for physical dormancy-break by microbial action and abrasion by soil particles. *Seed Science Research*, **10**, 409-413.

Baskin, J.M. and Baskin, C.C. (2004). A classification system for seed dormancy. *Seed Science Research*, **14**, 1-16.

Bell, D.T., Plummer, J.A. and Taylor, S.K. (1993). Seed germination ecology in southwestern Australia. *Botanical Review*, **59**, 24-73.

Corral, R., Pita, J.M. and Pérez-García, F. (1990). Some aspects of seed germination in four species of *Cistus* L. *Seed Science and Technology*, **18**, 321-325.

Demir, I. and Ozcoban, M. (2007). Dry and ultra-dry storage of pepper, aubergine, winter squash, summer squash, bean, cowpea, okra, onion, leek, cabbage, radish, lettuce and melon seeds at -20ºC and 20ºC over five years. *Seed Science and Technology*, **35**, 165-175.

Dixon, J.M. (1995). Biological Flora of the British Isles. *Trisetum flavescens* (L.) Beauv. (*T. pratense* Pers., *Avena flavescens* L.). *Journal of Ecology*, **83**, 895-909.

Eisvand, H.R., Arefi, H.M. and Tavakol-Afshari, R. (2006). Effects of various treatments on breaking seed dormancy of *Astragalus siliquosus*. *Seed Science and Technology*, **34**, 747-752.

Ellis, R.H. (1998). Longevity of seeds stored hermetically at low moisture contents. *Seed Science Research*, **8**, 9-10.

Ellis, R.H. and Hong, T.D. (2006). Temperature sensitivity of the low-moisture content limit to negative seed longevity-moisture content relationships in hermetic storage. *Annals of Botany*, **97**, 785-791.

Ellis, R.H., Hong, T.D., Martin, M.C., Pérez-García, F. and Gómez-Campo, C. (1993). The long-term storage of seeds of seventeen crucifers at very low moisture contents. *Plant Varieties and Seeds*, **6**, 75-81.

Ellis, R.H. and Roberts, E.H. (1981). The quantification of ageing and survival in orthodox seeds. *Seed Science and Technology*, **9**, 373-409.

Escudero, A., Carnes, L.F. and Pérez-García, F. (1997). Seed germination of gypsophytes and gypsovags in semi-arid central Spain. *Journal of Arid Environment*, **36**, 487-497.

FAO/IPGRI (1994). *Genebank Standards*. Food and Agriculture Organization of the United Nations/International Plant Genetic Resources Institute, Rome.

Fernández, H., Pérez, C., Revilla, M.A. and Pérez-García, F. (2002). The levels of GA_3 and GA_{20} may be associated with dormancy release in *Onopordum nervosum* seeds. *Plant Growth Regulation*, **38**, 141-143.

Finch-Savage, W.E. and Leubner-Metzger, G. (2006). Seed dormancy and the control of germination. *New Phytologist*, **171**, 501-523.

Gómez-Campo, C. (1985). Seed banks as an emergency conservation strategy. In *Plant Conservation in the Mediterranean Area*, (ed. C. Gómez-Campo), pp. 237-247, Dr. W. Junk Publishers, Dordrecht.

Gómez-Campo, C. (2002). Long term seed preservation: the risk of using inadequate containers is very high. *Monografías ETSIA, Universidad Politécnica de Madrid*, **163**, 1-10 (www.seedcontainers.net).

Gómez-Campo, C. (2006). Erosion of genetic resources within seed genebanks: the role of seed containers. *Seed Science Research*, **16**, 291-294.

González-Melero, J.A., Pérez-García, F. and Martínez-Laborde, J.B. (1997). Effect of temperature, scarification and gibberellic acid on the seed germination of three shrubby species of *Coronilla* L. (Leguminosae). *Seed Science and Technology*, **25**, 167-175.

Gresta, F., Avola, G. and Abbate, V. (2007). Germination ecology of *Scorpiurus subvillosus* L. seeds: the role of temperature and storage time. *Plant Ecology*, **190**, 123-130.

Hong, T.D., Ellis, R.H., Astley, D., Pinnegar, A.E., Groot, S.P.C. and Kraak, H.L. (2005). Survival and vigour of ultra-dry seeds after ten years of hermetic storage. *Seed Science and Technology*, **33**, 449-460.

Hu, X., Zhang, Y., Hu, Ch., Tao, M. and Chen, S. (1998). A comparison of methods for drying seeds: vacuum freeze-drier versus silica gel. *Seed Science Research*, **8**, 29-33.

ISTA (2003). *International Rules for Seed Testing*. Basserdorf, Switzerland.

Kigel, J. (1995). Seed germination in arid and semiarid regions. In *Seed Development and Germination*, (eds. J. Kigel and G. Galili), pp. 645-699, Marcel Dekker, New York.

MAPA (1994). *Métodos Oficiales de Análisis*. Ministerio de Agricultura, Pesca y Alimentación, Madrid.

Maselli, S., Pérez-García, F. and Aguinagalde, I. (1999). Evaluation of seed storage conditions and genetic diversity of four crucifers endemic to Spain. *Annals of Botany*, **84**, 207-212.

Méndez, P., Álvarez, S., Díaz, A., Díaz, C. and Fresno, M. (2003). Nutritive value of vinagra (*Rumex lunaria*), and endemic forage shrub of Canary Islands. Proceedings of the 12th Symposium of the European Grassland Federation, Pleven, Bulgaria.

Nikolaeva, M.G. (1999). Patterns of seed dormancy and germination as related to plant phylogeny and ecological and geographical conditions of their habitats. *Russian Journal of Plant Physiology*, **46**, 369-373.

Nogales, M., Valido, A. and Medina, F.A. (1995). Frugivory of *Plocama pendula* (Rubiaceae) by the rabbit (*Oryctolagus cuniculus*) in xerophyte zones of Tenerife (Canary Islands). *Acta Oecologica*, **16**, 585-591.

Pérez-García, F. (1993). Effect of the origin of the cypsela on germination of *Onopordum acanthium* L. (Asteraceae). *Seed Science and Technology*, **21**, 187-195.

Pérez-García, F. (1997). Germination of *Cistus ladanifer* seed in relation to parent material. *Plant Ecology*, **133**, 57-62.

Pérez-García, F. and Durán, J.M. (1990). The effect of gibberellic acid on germination of *Onopordum nervosum* Boiss. Seeds. *Seed Science and Technology*, **18**, 83-88.

Pérez-García, F. and Escudero, A. (1997). Role of the seed coat in germination of *Cistus populifolius* L. *Israel Journal of Plant Sciences*, **45**, 329-331.

Pérez-García, F., González-Benito, M.E. and Gómez-Campo, C. (2007). High viability recorded in ultra-dry seeds of 37 species of Brassicaceae after almost 40 years of storage. *Seed Science and Technology*, **35**, 143-153.

Pérez-García, F., Iriondo, J.M., González-Benito, M.E., Carnes, L.F., Tapia, J., Prieto, C., Plaza, R. and Pérez, C. (1995). Germination studies in endemic plant species of the Iberian Peninsula. *Israel Journal of Plant Sciences*, **43,** 239-247.

Pita, J.M. (1988). Germinación en especies endémicas de las islas Canarias. *Investigación Agraria: Producción y Protección Vegetales*, **3***,* 39-43.

Qaderi, M.M. and Cavers, P.B. (2000). Interpopulation variation in germination responses of Scotch thistle, *Onopordum acanthium* L., to various concentrations of GA_3, KNO_3, and $NaHCO_3$. *Canadian Journal of Botany*, **78**, 1156-1163.

Ramiro, M.C., Pérez-García, F. and Aguinagalde, I. (1995). Effect of different seed storage conditions on germination and isozyme activity in some *Brassica* species. *Annals of Botany*, **75**, 570-585.

Reghunath, B.R., Francisco-Ortega, H., Newbury, J., Ford-Lloyd, B.V. (1993). Methods for increasing the efficiency of seed germination in the fodder legumes tagasaste and escobon (*Chamaecytisus proliferus* (L.fil.) Link *sensu lato*. *Seed Science and Technology*, **21**, 225-235.

Rincón-Rosales, R., Culebro-Espinosa, N.R. and Gutierrez-Miceli, F.A. (2003). Scarification of seeds of *Acacia angustissima* (Mill.) Kuntze and its effect on germination. *Seed Science and Technology*, **31**, 301-307.

Silveira, F.A.O. and Fernandes, G.W. (2006). Effect of light, temperature and scarification on the germination of *Mimosa foliosa* (Leguminosae) seeds. *Seed Science and Technology*, **34**, 585-592.

Thanos, C.A., Georghiou, K., Kadis, C. and Pantazi, C. (1992). Cistaceae: A plant family with hard seeds. *Israel Journal of Botany*, **41**, 251-263.

Thanos, C.A., Kadis, C.C., Skarou, F. (1995). Ecophysiology of germination in the aromatic plants thyme, savory and oregano (Labiatae). *Seed Science Research*, **5**, 161-170.

Trabaud, L. (1995). Modalités de germination des cistes et des pins méditerranéens et colonisation des sites perturbés. *Revue Ecologie (Terre Vie)*, **50**, 3-14

Valbuena, M.I., Tarrega, R. and Luis, E. (1992). Influence of heat on seed germination of *Cistus laurifolius* and *C. ladanifer*. *International Journal Wildland Fire*, **2**, 15-20.

Valido, A., Nogales, M. and Medina, F. (2003). Fleshy fruits in the diet of Canarian lizards *Gallotia gallotia* (Lacertidae) in a xeric habitat of the Island of Tenerife. *Journal of Herpetology*, **37**, 741-747.

Vertucci, C.W. and Ross, E.E. (1993). Theorical basis of protocols for seed storage. II. The influence of temperature on optimal moisture levels. *Seed Science Research*, **3**, 201-213.

VV. AA. (2000). Lista roja de la flora vascular española (valoración según categorías IUCN). *Conservación Vegetal*, **6**, 11-38.

Walter, K.S. and Gillett, H.J. (eds.) (1998). *1997 IUCN Red List of Threatened Plants*. Compiled by the World Conservation Monitoring Centre. IUCN-The World Conservation Union, Gland, Switzerland and Cambridge.

Walters, C. and Engels, J. (1998). the effects of storing seeds under extremely dry conditions. *Seed Science Research*, **8**, 3-8.

Walters, C., Wheeler, L.M. and Grotenhuis, J.M. (2005). Longevity of seeds stored in a genebank: species characteristics. *Seed Science Research*, **15**, 1-20.

Zeng, L.W., Cocks, P.S. and Kailis, S.G. (2005a). Softening of impermeable seeds of six Mediterranean annual legumes on the soil surface and buried beneath the soil surface. *Seed Science and Technology*, **33**, 551-561.

Zeng, L.W., Cocks, P.S., Kailis, S.G. and Kuo, J. (2005b). Structure of the seed coat and its relationship to seed softening in Mediterraneam annual legumes. *Seed Science and Technology*, **33**, 351-362.

Zida, D., Tigabu, M., Sawadogo, L. and Oden, P.C. (2005). Germination requirements of seeds of four woody species from the Sudanian savanna in Burkina Fasso, West Africa. *Seed Science and Technology*, **33**, 581-593.