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High viability recorded in ultra-dry seeds of 37 species of Brassicaceae after almost 40 years of storage

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Summary

Final germination percentages of 25 accessions of Brassicaceae from the UPM (Universidad Politécnica de Madrid) seed bank were evaluated after 38-40 years of storage. The seeds were preserved at temperatures between -5°C and -10°C in flame sealed vials containing dehydrated (blue) silica gel. Seed moisture content ranged between 0.3 and 3% (f.w.b) after storage. Most accessions (ten out of twelve) with high initial (before storage) germination rate (low initial dormancy) maintained these high values almost intact after storage (91-100% germination). In two accessions, seeds had developed a secondary dormancy, which was successfully overcome by scarification. A second set of 13 accessions had low initial germination rates (0-20%, i.e. high initial dormancy). In one accession, dormancy had been broken during storage (92% germination) and in nine accessions germination was significantly enhanced by GA_3 and or scarification. Seed dormancy most often decreases during storage but it may also increase or remain unchanged. The consideration of these dormancy variations is very important when evaluating seed longevity in wild species. The preservation method based on silica gel and low temperature (-5°C and -10°C) has proved highly efficient at least for Brassicaceae. Vials with seeds of 12 additional accesssions had remained at room temperature during 34-39 years and those seeds showed germination percentages that were similar to those preserved in the cold room. This result suggests that temperature might not be as important as expected – at least for medium-term preservation – and supports the possibility of using ultra-dry methods.

Introduction

The long-term storage of orthodox seeds is the most widely used method for *ex situ* preservation of plant genetic resources. The technology used in the UPM seed bank since 1966 consists of storing the seeds at low temperature (between -5°C and -10°C) and with low moisture content (approximately between 1.5% and 3% f.w.b.), achieved by desiccation with silica gel and placing some dehydrated silica gel together with the seeds within flame-sealed glass vials (Gómez-Campo, 1972, 1985). For suggestions to adapt this method to larger size crop seeds see Gómez-Campo (2002).

As of 2006 the UPM bank is 40 years old. The bank holds approximately 750 taxa of the Brassicaceae family and was designated base bank for wild members of this family by the IPGRI (International Plant Genetic Resources Institute, formerly IBPGR) in 1983. Furthermore, a large collection of threatened species of the Iberian Peninsula and the

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Macaronesian region is also preserved. Although routine periodical germination tests of stored seeds are not carried out in our bank (see later), we have only rarely encountered germination problems during this time when many accessions were sown almost every year under greenhouse conditions for different types of studies. Neither have we received significant negative reactions regarding germination from external users of our material. On several occasions in the past, germination tests on our seed collection have yielded positive results (Ellis *et al.,* 1993; Ramiro *et al.,* 1995; Maselli *et al.,* 1999).

Contradictory opinions exist regarding the effect of seed over-drying on seed longevity for low temperature storage in general and on the use of silica gel in particular (i.e. Ellis, 1998; Hu *et al.,* 1998; Walters and Engels, 1998). According to Ellis (1998) maximum longevity is acquired when desiccation takes places at 10-12% RH at 20°C. Desiccation to 2-3.7% moisture content (10% RH and 20°C) did not improve seed viability of several species after 5 and 10 years storage when it was carried out at -20°C, although it improved at 20°C (Ellis *et al.,* 1996; Hong *et al.,* 2005). For other authors, optimal seed moisture for conservation depends on storage temperature (Vertucci and Roos, 1993), for example for pea optimal water content for storage at -20°C would be 0.12 g water/g dry weight, which could be acquired with equilibration at 57% RH at 25°C.

In a recent article by Walters *et al.* (2005), the storage behaviour of 276 species held by the National Center of Genetic Resources Preservation (NCGRP, USA) was characterized from periodical germination data of those accessions. The time at which germination would decline to 50% was calculated (P50). Seed longevity of the Brassicaceae family (14 species were studied) showed P50 values averaging 54 years and ranging from 19 years (*Lesquerella palmeri*) to 164 years (*Lesquerella grandiflora*). These findings were of interest for our bank as many of the accessions belong to this family.

The main objective of this work was to evaluate the germination response of seeds from a number of Brassicaceae stored in our UPM bank for 38-40 years in the conditions defined in the first paragraph of this introduction. As a side study, some additional accessions from some odd situations (either being stored at room temperature or having their moisture content accidentally raised) were also evaluated.

Materials and methods

Four sets of Brassicaceae accessions from our seed bank were selected:

Set number 1. Accessions with high initial germination rate (i. e. with low interference from any initial dormancy), which had been kept for 38-39 years with blue (dehydrated) silica gel in the cold room. The aim was to test to what extent their high germination percentages were maintained after that long period.

Set number 2. Accessions with low or very low initial germination rate (expected to have a strong initial dormancy), which had been kept for 38-40 years with blue (dehydrated) silica gel in the cold room. The goal was to test to what extent their dormancy was present after that long period.

These accessions were chosen among the ones stored during the first two years of the seed bank functioning from which initial germination tests (before storage) had been carried out.

Set number 3. Accessions with different initial germination rates (often not even recorded) kept for 34-39 years with blue silica gel in a closet in our laboratory at room temperature. The goal was to test how their germination ability had been affected by these temperature conditions.

Set number 4. Accessions extracted from sets 1, 2 and 3 by selecting vials where the gel had accidentally turned pink i.e. failing to exclude moisture. The goal was to test their general behavior under these conditions of non-controlled moisture.

The accessions had been collected in 1966 and 1967. Initial germination percentages were obtained from samples of 60 seeds with 23°-25°C incubation (with no previous treatment). Accessions were divided among several flame-sealed glass vials. Each vial contained seeds and silica gel separated by a filter paper divider (for more details see Gómez-Campo 1972, 1985; Ellis *et al.* (1993). These vials had been stored at -5°C from 1996 to 1981 and subsequently at -10°C (except for the vials kept at room temperature, set number 3). In 2004 our cold room was tuned again to -5°C.

In several accessions, seeds from one to four vials were used for determination of seed moisture content using the air oven method (103°C for 17h, ISTA, 2003). Vials were opened to obtain the seeds when they had reached room temperature. Moisture content was determined from seeds which were in vials with blue silica gel and also with pink color. The last situation indicated that the ampoule had failed to exclude moisture because a route for water vapor intake (probably on the sealed tip) had been produced.

The seeds were tested for germination on top of two filter papers (previously moistened with 3.5 ml distilled water) in 7cm glass Petri dishes. Four replicates of 25 seeds each were used in each trial. The Petri dishes were incubated at 25°C and 25/15°C under a 16-h light/8-h dark photoperiod. Insufficient amount of seeds in some accessions prevented that all the germination tests could be conducted for the two selected incubation temperature regimes. Distilled water was replaced regularly. Seeds with an emerged radicle were counted every two days and removed from the Petri dishes. The final germination percentage was scored after a 7 week incubation period.

The application of gibberellic acid (GA_3) was used in some cases to rescue possible dormant but viable seeds. The treatment was performed by soaking the seeds in a GA_3 water solution (1000 $mg \cdot l^{-1}$) for 24 h prior to sowing. Seeds were germinated at 25/15°C under a 16 h light photoperiod. When the germination of the control seeds was higher than 85%, a GA_3 trial was not conducted. Seeds of several species were soaked for 48 h in distilled water and then their coat was completely removed with a single-edge razor blade prior to sowing (scarified seeds). In some instances GA_3 and scarification treatments were combined. At the end of the germination period, the final germination percentage (mean value ± standard error) was calculated.

Results

The seed moisture content ranged from 0.3 to 3% for the seeds stored in vials with deep blue silica gel (see tables 1, 2 and 3) and from 5 to 6.0% for the seeds stored in vials where the silica gel color had turned to pink (table 4).

Most accessions from set number 1 (table 1) maintained their high initial germination percentages during the studied period. Ten out of twelve were above or around 95% of the initial value, with seven in the range of 98-100% and five in the range of 99-100%. Three cases with an initial germination rate of 100% yielded again 100% after almost forty years. In *Alyssoides utriculata* and *Matthiola sinuata* the germination rate apparently dropped to very low values but a scarification treatment helped to overcome an obvious secondary dormancy which had developed during storage. The use of alternate temperatures contributed sometimes to higher final germination percentages, thus providing a more accurate approach to the real percentage of viable seeds.

Table 1. Final germination percentages (mean value ± standard error) of 12 Brassicaceae accessions with high initial germination percentage (low dormancy) after 38-39 years of storage. Storage conditions: temperature ranged between –5°C and -10°C; blue silica gel (low moisture content). In bold: maximum germination values.

Accession number[1]	Taxon (MC % fwb)[2]	Years of storage	Germination (% ± SE)			
			Initial (before storage)	Final 25°C	Final 25/15°C	Scarified seeds[3]
0588	*Alyssoides utriculata* (2.0)	38	100	5 ± 2.61	0	**95** ± 2.71
0303	*Alyssum saxatile* (2.5)	38	100	89 ± 3.28	**96** ± 1.41	--
1261	*Barbarea intermedia*	38	95	96 ± 1.41	**99** ± 0.87	--
1280	*Brassica napus*	38	100	**100**	99 ± 0.87	--
1166	*Coincya rupestris*	38	92	91 ± 1.66	**98** ± 1.00	--
0430	*Erucastrum abyssinicum* (1.9)	39	100	**100**	97 ± 1.66	--
0238	*Erysimum cheiri* (1.7)	38	100	**97** ± 2.38	96 ± 1.43	--
0205	*Erysimum odoratum* (1.2)	38	100	95 ± 0.87	**98** ± 1.08	--
1163	*Erysimum repandum* (1.7)	38	100	76 ± 5.83	**100**	--
0946	*Isatis tinctoria* (2.7)	38	100	**91** ± 2.60	79 ± 6.33	--
0016	*Matthiola incana*	38	95	**99** ± 0.87	94 ± 2.24	--
1248	*Matthiola sinuata*	39	100	4 ± 1.50	12 ± 8.20	**99** ± 1.08

(1) Accession number in the seed bank of the Universidad Politécnica de Madrid (Spain).
(2) Moisture content (%) determined only in some accessions due to shortage of seeds.
(3) --: scarification not carried out when final germination at any of the incubation conditions studied was higher than 85%.

Table 2. Final germination percentages (mean value ± standard error) of 13 Brassicaceae accessions with low initial germination percentage after 38-40 years of seed storage. Storage conditions: temperature ranged between -5°C and -10°C; blue silica gel (low moisture content). Last column: GA_3 treatment and/or scarification were performed to remove dormancy (incubation at 25/15°C, except 25°C for *Arabis turrita*). In bold: maximum germination values.

Accesion number[1]	Taxon (MC % fwb)[2]	Years of storage	Germination (% ± SE)			
			Initial (before storage)	Final 25°C	Final 25/15°C	Final[3] + GA_3
1129	*Alyssum granatense* (1.5)	38	3	4 ± 1.41	48 ± 3.74	**92** ± 2.00
1130	*Alyssum scutigerum* (1.5)	38	2	85 ± 4.77	**92** ± 2.45	--
1216	*Alliaria petiolata*	38	0	0	1 ± 0.87	**3** ± 2.88
1251	*Arabis turrita*	39	0	2 ± 1.73	4 ± 1.41	**91** ± 4.71
1060	*Capsella bursa-pastoris*	38	11	18 ± 4.12	18 ± 4.12	**94** ± 1.00
1175	*Coincya longirostra*	38	0	37 ± 9.20	79 ± 3.57	**87** ± 4.97
0934	*Conringia orientalis*	40	0	0	0	**90** ± 3.00
0886	*Descurainia sophia*	40	2	1 ± 0.87	8 ± 2.83	**94** ± 1.63
1173	*Draba hispanica*	39	0	5 ± 1.66	10 ± 3.80	**97** ± 3.03
1278	*Lepidium latifolium*	39	0	27 ± 3.28	54 ± 4.12	**92** ± 2.49
0863	*Moricandia arvensis*	39	20	37 ± 2.60	31 ± 4.55	**96** ± 1.41
1213	*Sinapis arvensis*	38	3	5 ± 2.18	46 ± 3.60	**99** ± 0.87
1148	*Sisymbrium orientale* (1.5)	39	3	30 ± 3.60	61 ± 9.63	**100**

(1) Accession number in the seed bank of the Universidad Politécnica de Madrid (Spain).
(2) Moisture content (%) determined only in some accessions due to shortage of seeds.
(3) --: GA_3 treatment not carried out when final germination at any of the incubation conditions studied was higher than 85%.

Dormancy problems strongly interact with the evaluation itself in set number 2 (table 2) where the initial germination percentages were low or very low. In most cases, the final germination at 25°C or 25/15°C increased significantly, a sign that dormancy often decreases spontaneously during storage. A gibberellic acid (GA_3) treatment strongly enhanced germination in all the tested species. Table 2 illustrates the importance of considering dormancy whenever seeds of wild species are being preserved and their longevity evaluated in a seed bank. Other anti-dormancy methods might increase these figures even further.

It is confirmed that seed dormancy normally decreases during storage as already shown by Ellis *et al.* (1993). As a consequence, final germination might some times be higher than the initial one. However, both tables 1 and 2 include cases showing that it may also be maintained during long periods (*Conringia orientalis* and *Sinapis arvensis,* table 2) or may be developed during storage (*Alyssoides utriculata* and *Matthiola sinuata,* table 1).

Table 3. Final germination percentages (mean value ± standard error) of 12 Brassicaceae accessions after 34-39 years of seed storage. Storage conditions: room temperature; blue silica gel (low moisture content). In some cases a GA_3 treatment was also performed (incubation at 25/15°C). In bold: maximum germination values.

Accession number[1]	Taxon (MC % fwb)[2]	Years of storage	Germination rate (% ± SE)			
			Initial (before storage)	Final 25°C	Final 25/15°C	Final[3] + GA_3
1599	*Biscutella intermedia*	37	---	**65** ± 1.82	NO	NO
1136	*Brassica fruticulosa* (0.8)	38	---	82 ± 5.38	83 ± 4.97	**99** ± 0.87
1958	*B. fruticulosa glaberrima* (1.7)	34	---	**86** ± 1.00	70 ± 3.32	--
0879	*Diplotaxis catholica* (1.7)	39	72	68 ± 5.83	**88** ± 2.45	--
0846	*Diplotaxis harra* (0.8)	39	90	39 ± 5.54	20 ± 2.45	**96** ± 2.00
1126	*Erucastrum leucanthum* (0.3)	38	---	**96** ± 1.41	95 ± 3.28	--
0735	*Erysimum odoratum* (1.7)	39	---	**97** ± 0.87	95 ± 1.66	--
0855	*Erysimum scoparium*	39	7	8 ± 2.16	**21** ± 7.40	5 ± 4.33
0959	*Lepidium heterophyllum* (2.1)	39	---	37 ± 3.84	56 ± 7.87	**98** ± 1.00
1547	*Sinapis flexuosa* (2.2)	37	---	83 ± 2.18	**97** ± 1.66	--
1186	*Sisymbrium arundanum* (1.9)	38	---	77 ± 7.26	**86** ± 3.00	--
1757	*Thelipodium lasiophyllum* (1.6)	36	---	**94** ± 2.24	64 ± 8.12	--

(1) Accession number in the seed bank of the Universidad Politécnica de Madrid (Spain).
(2) Moisture content (%) determined only in some accessions due to shortage of seeds.
(3) --: GA_3 treatment not carried out when final germination at any of the incubation conditions studied was higher than 85%.
NO: no trial conducted due to shortage of seeds.

The accessions from set number 3 (table 3) were set aside originally for different reasons and were never stored at low temperature but kept in a laboratory closet instead. Their initial germination percentages are unknown in most cases. However, their final maximum germination rates are high, matching very often to those shown in table 1 for cold preserved accessions. Only two out of twelve were lower than 80%. Not shown in the tables is the case of *Lepidium hirtum* (collected in 1973), where a group of vials stored in the cold room showed a 95% germination rate, another group stored in the closet showed a 95% germination rate and their germination percentage prior to storage (estimated on fresh seeds from a regenerated accession) was also 95%. The coincidence of the three figures might be casual but is in line with the results shown in table 3. The case of *Erysimum odoratum* (included in both tables 1 and 3) is very similar - the vials kept in the cold room and those stored at room temperature showed an almost identical germination rate.

Set number 4 (table 4) has a heterogeneous origin and includes seeds from vials belonging to sets 1, 2 and 3. For individual comparisons it is necessary to look at tables 1, 2

Table 4. Final germination percentages (mean value ± standard error) of seeds from vials where humidity had accidentally entered, thus rising the seed moisture content and rendering the silica gel pink. The date when this occurred is unknown. Data of their counterparts with blue silica gel can be found in tables 1, 2, 3.

Accession number[1]	Taxon (MC % fwb)[2]	Years of storage	Germination rate (% ± SE)			
			Initial (before storage)	Final 25°C	Final 25/15°C	Treated seeds
0588	*Alyssoides utriculata*	38	100	52 ± 4.33	NO	89 ± 5.99[3]
0303	*Alyssum saxatile*	38	100	0	NO	NO
1251	*Arabis turrita*	38	0	NO	0	NO
1136	*Brassica fruticulosa* (6.0)	38	---	59 ± 6.84	51 ± 3.8	47 ± 6.54[4]
0934	*Conringia orientalis*	39	0	NO	0	77 ± 5.30[3]
1173	*Draba hispanica*	38	0	0	0	27 ± 10.50[4]
0430	*Erucastrum abyssinicum*	39	100	95 ± 3.27	NO	NO
1126	*Erucastrum leucanthum* (4.9)	38	---	0	0	NO
0238	*Erysimum cheiri*	38	100	94 ± 3.51	NO	NO
0016	*Matthiola incana*	38	95	0	NO	NO
1248	*Matthiola sinuata*	38	100	2 ± 1.95	NO	86 ± 3.90[3]
1547	*Sinapis flexuosa*	37	---	0	0	NO

(1) Accession number in the seed bank of the Universidad Politécnica de Madrid (Spain).
(2) Moisture content (%) determined only in some accessions due to shortage of seeds.
(3) Scarified seeds.
(4) GA_3 treatment.
NO: no trial conducted due to shortage of seeds.

or 3. In all of the vials of this set, moisture had entered, turning the silica gel pink. However, since the exact date when this occurred is unknown, there was some heterogeneity to be expected in the results. Five accessions showed a rather high germination rate, three are intermediate and five are completely dead. Compared with their blue gel counterparts, it is most significant that the seeds from *Alyssum saxatile*, *Erucastrum leucanthum*, *Matthiola incana* and *Sinapis flexuosa* died. Cases showing acceptable figures probably derived from a rather recent moisture intake. Taken as a whole, the picture here is significantly more negative, showing that any increase in moisture content is clearly deleterious.

From a physiological point of view the already recognized ability of GA_3 (tables 2, 3) or scarification (table 1) to counteract dormancy and to enhance germination is confirmed. Both treatments (and perhaps others) are useful to recover dormant seeds. It is obvious that without them our data would have been much less meaningful. For most samples, no significant differences in germination rates were found between both incubation temperatures assayed. However, seeds generally germinated faster at 25/15°C than at 25°C (data not shown).

Discussion

Studies to determine the optimal water content for seed storage have been performed at higher temperatures than the ones used for seed storage, at several moisture contents to provide information on the ageing of seeds under extreme dry conditions (Ellis *et al.,* 1988, 1990; Ellis *et al.,* 1990; Vertucci and Leopold, 1987; Vertucci and Roos, 1990). Information on seed behavior after long-term low-temperature seed storage is scarce.

The moisture content of the seeds in this study is lower than that recommended by FAO/IPGRI (FAO/IPGRI, 1994) of 5 ± 2%. Furthermore, it has been shown in short-term experiments that the storage at low temperature (< 45°C) of seeds with water contents lower than 2-3% reduced their longevity (Walters and Engels, 1998; Vertucci and Roos, 1990; Vertucci *et al.,* 1994). Therefore, we could have expected very low survival of the Brassicaceae species studied in the present work. However, most of them showed a very high final germination percentage (higher than 87% in 18 accessions out of 25, see tables 1, 2) after almost 40 years storage at low temperature (-5° to -10°C). The very low water content could be due to high seed lipid content, which is quite general among the Brassicaceae. However, in previous studies carried out with seeds from our seed bank the equilibrium relative humidity at 20°C was 6-7% for most vials after storage (Ellis *et al.,* 1993), much lower than the recommended 10-12% (FAO/IPGRI, 1994).

Few seed banks have already completed four decades of existence. Comparing our results to those obtained by Walters *et al.* (2005) for similar storage periods, we could conclude that the Brassicaceae seeds studied in the present work have generally shown much higher longevity (average germination 98.4% of the initial value against only 54.8%). We cannot estimate the P50 values for our accessions because these obviously stay in the initial quasi-linear stage of any sigmoidal approach. Nevertheless, it is possible to compare the longevity of two species that are represented in both studies. From data of 12 accessions of *Brassica napus*, a P50 value of 25 years was estimated (Walters *et al.,* 2005). The only one accession of *Isatis tinctoria* had a P50 value of 27 years. In our seed bank, after 38 years of storage, the accessions of these two species had shown a final germination of 100 and 91% respectively (both with initial germination of 100%). In our opinion, the reason for this sharp difference depends on the way accessions were stored (as all the accessions in both studies had high initial germination percentages). In NCGRP "seed water content was adjusted to 4-8% and seeds placed in screw-cap metal cans, but transferred years later to foil-laminate bags" (Walters *et al.,* 2005). Storage temperature was 5°C for the first 20 years approximately and then -18°C. However, seed water content at the end of the storage period was not stated. The poorer results of the NCGRP seed bank could have been due to the non-hermetical condition of the seed containers currently used (Gómez-Campo, 2002). Their affirmation that seeds of the Brassicaceae are usually short-lived cannot be maintained since they are clearly long-lived when properly preserved.

Many other seed banks with accessions stored for 2-3 decades are often unhappy with their germination results and deeply worried about the premature need to regenerate their material. Our results strongly support the practical efficiency of the silica gel method in spite of criticisms based on theoretical correlations and/or artificial ageing. Silica gel could be used to desiccate seeds and as an indicator of accidental leakage of the seed container.

It is obvious that at least for seeds of Brassicaceae – and probably for many other species – the advantages of silica gel largely exceed its claimed inconveniences. Even for starchy seeds, long term storage at equivalent moisture contents (Steiner and Ruckenbauer, 1995) seems possible.

The longevity of seed bank accessions is usually estimated through periodical germination tests every 5 or 10 years according to recommendations by FAO/IPGRI (FAO/IPGRI, 1994). However, when an efficient preservation procedure is used such tests obviously become largely meaningless. Our UPM bank has performed tests only on a very limited basis thus saving enormous amounts of time, labor, effort and, most of all, seed material. When dealing with wild species, simple germination tests are also meaningless if they are not accompanied by parallel studies on dormancy. In germination tests where only germination is directly scored, many seeds which are alive but dormant remain ignored while they are important for the final longevity figures to be accurate (final germination could be even higher than the initial one). The presence of dormancy may be very high in wild species as shown in table 2 where much of the dormancy probably remains hidden.

Low temperature showed to play only a minor role in our set of accessions which remained 38-39 years in a laboratory closet (table 3). At least in the medium term, ultra-dry methods might be a good alternative for seed preservation - provided that low moisture is effectively maintained. It should not be recommended to unplug all the cold chambers holding seeds, but it would be perhaps wise to avoid very low temperatures (as -20°C, so widely used), and save electricity by using more moderate ones. On theoretical grounds, Walters and Engels (1998) and Buitink *et al.* (2000) conclude that too low temperatures combined with too low moisture contents make a "sub-optimal" combination which reduces seed longevity. If this is ever confirmed in the practice, perhaps the best strategy for the dilemma would be to abandon very low temperatures while maintaining the low moisture content obtained with silica gel.

Low moisture is certainly the key factor for the long-term preservation of orthodox seeds. We regret that our data of table 4 can only be taken as an orientation because the moment when the silica gel became pink was unknown. However, the presence of several dead accessions provides direct evidence on the dramatic effects of failures in moisture control.

For the first time, the longevity of seeds preserved for a period close to 40 years in equilibrium with dehydrated silica gel has been evaluated. Our results suggest the following main conclusions:

a) At least for Brassicaceae (and probably for many other species), preservation methods based on dehydrated silica gel are highly efficient.
b) Dormancy usually decreases during storage but instances can be found where it increases or is maintained over long periods. Consideration of dormancy variations and the eventual application of anti-dormancy treatments are very important when evaluating the seed longevity of wild species.
c) Comparatively, low moisture seems to be much more important for seed preservation than low temperature. Ultra-dry preservation at room temperature might be an efficient method at least in the medium and probably in the long term.

Further research is needed to fully understand this spectacular high germination rates of wild Brassicaceae species after almost 40 years storage at very low water content and low temperatures. Factors such as the "atmosphere" composition inside the vials (how it is affected by the presence of silica gel and the flame enclosure) or the possible relationships between dormancy and longevity in wild species (Debeaujon *et al.,* 2000) should be explored.

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