Annals of Botany **75**: 579–585, 1995

Effect of Different Seed Storage Conditions on Germination and Isozyme Activity in Some *Brassica* Species

M. C. RAMIRO, F. PÉREZ-GARCÍA and I. AGUINAGALDE

Departamento de Biología Vegetal, Escuela Técnica Superior de Ingenieros Agrónomos, Universidad Politécnica de Madrid, Ciudad Universitaria, 28040 Madrid, Spain

Received: 29 October 1993 Accepted: 4 November 1994

Sixteen seed accessions of four *Brassica* species (*B. cretica*, *B. incana*, *B. montana* and *B. oleracea*) were examined following 5–22 years' storage in the germplasm bank. Germinability and isozyme activity of seeds stored under long-term (−10 °C and 3% moisture content) and short-term (5 °C and 8% moisture content) storage conditions were compared. Long-term storage produced no deterioration and the ability to germinate was satisfactorily maintained over 8–22 years. Short-term storage conditions maintained germination ability up to 10–12 years in all accessions of *B. cretica* and *B. montana*. However, seed multiplication might be essential every 10 years for some accessions of *B. oleracea* stored in this way. In the 8 to 9-year-old accessions of *B. cretica*, *B. incana* and *B. montana*, no significant differences were detected between conservation systems for germinability and frequency of seeds showing isozyme activity for seven enzyme systems (ACO, IDH, MDH, ME, PGI, PGM and 6-PGD). However, significant differences were found for the ADH enzyme system. Moreover, in the 5–22-year-old *B. oleracea* accessions, significant differences were found between storage conditions with respect to isozyme activity for all enzyme systems studied.

Key words: *Brassica cretica*, *Brassica incana*, *Brassica montana*, *Brassica oleracea*, accessions, isozymes, germination, seed storage.

INTRODUCTION

Simple techniques are available to conserve *ex situ* the viability of seeds over long periods of time. These techniques have been used widely for both cultivated and wild species (Roberts, 1973, 1975; Hanson, Williams and Freund, 1984; Gómez-Campo, 1985; Vertucci and Roos, 1990, 1991; Ellis, Hong and Roberts, 1991, 1992).

The technology of *ex situ* conservation used in the crucifer seed bank of the Universidad Politécnica de Madrid (Spain) is based on long-term storage of seeds at low temperature and very low moisture content (Gómez-Campo, 1990). Recently, the results obtained by Ellis *et al.* (1993) have confirmed that the storage of seeds of several crucifer species under such conditions is a satisfactory procedure.

Electrophoresis techniques are relevant tools for population genetic studies in order to detect genetical differences among individuals and describe populations precisely. They may therefore, be useful for genebanks (Yndgaard and Iloskuldsson, 1985) as a method of indicating the genetic consequences of seed deterioration.

The family Brassicaceae contains many economically useful and botanically interesting species. The crucifer seed bank established in the laboratory of the authors contains nearly 600 accessions, with major emphasis in the genus *Brassica* (70 accessions). This study was undertaken to: (1) document any relationship between storage conditions and germinability or changes in isozyme activity (which could be used as an indicator of seed deterioration) in the selected accessions of four *Brassica* species and (2) examine levels of genetic variation in the accessions, by electrophoretic analysis of eight enzyme systems to find out how much of the original diversity was retained. The second objective, which requires the knowledge about the genetic diversity of the same species collected in the natural environment, is still in progress, because only fresh seeds from a *Brassica montana* natural population were available. This material was used as a reference point for the isozyme studies.

MATERIALS AND METHODS

Plant material

The *Brassica* seeds used in the experiments were of the following species: *B. cretica* Lam., *B. incana* Ten., *B. montana* Pourret and *B. oleracea* L. The 16 accessions (Table 1) were directly collected from their natural habitats, mostly in the S.W. Mediterranean region. Seed samples were conserved in sealed glass vials with silica gel (Gómez-Campo, 1990). Encapsulation in vials was done 4–6 months after harvesting. The vials were stored at +5 to −10 °C in the crucifer germplasm collection of the Universidad Politécnica de Madrid, Spain. The duration of the cold storage before the experiments ranged from 5 to 22 years. *Brassica* seeds were stored under two conditions: −10 °C and 3% moisture content for long-term (LT) storage and 5 °C and 8% moisture content for short-term (ST) storage (Table 1). Seeds of accessions 10 and 11 of *B. oleracea* were

0305-7364/95/060579+07 $08.00/0

TABLE 1. *Geographical origin, storage conditions and germination percentages (mean ± s.e.) of 16 accessions of* Brassica *species after 8–22 years at 5 °C and 8% moisture content (ST = short-term storage) or −10 °C and 3% moisture content (LT = long-term storage)*

Accession number	Species	Reference number*	Geographical origin	Storage time (years)	Storage conditions	Germination percentages†			
						15 °C	20 °C	25 °C	15/25 °C
1	*B. cretica*	6010-84	Charakas (Greece)	9	ST	76 ± 4^{ab}	82 ± 4^{a}	66 ± 6^{b}	85 ± 6^{a}
2		6010-84	Charakas (Greece)	9	LT	68 ± 3^{a}	47 ± 6	31 ± 2	67 ± 4^{a}
3	*B. incana*	6560-84	Capri (Italy)	9	LT[A]	92 ± 2^{a}	89 ± 4^{a}	92 ± 2^{a}	—
4		6560-84	Capri (Italy)	9	LT	80 ± 1^{a}	90 ± 1	81 ± 6^{a}	—
5		5974-81	Svalof (Sweden)	12	ST	—	—	—	—
6	*B. montana*	6807-85	Savonna (Italy)	8	ST	92 ± 1^{a}	—	91 ± 3^{a}	92 ± 1^{a}
7		6801-85	Palmaiola (Italy)	8	LT	96 ± 1^{a}	—	92 ± 2^{a}	92 ± 1^{a}
8		6813-85	Ile Ste.Margarite Toulon (France)	8	LT	90 ± 2^{a}	—	88 ± 1^{a}	92 ± 2^{a}
9	*B. oleracea*	2191-71	Swanage (U.K.)	22	ST	—	—	0	0
10		2191-71	Swanage (U.K.)	5	ST[B]	—	—	20 ± 3	—
11		2191-71	Swanage (U.K.)	11	ST[C]	—	—	72 ± 12	—
12		2191-71	Swanage (U.K.)	22	LT	70 ± 2^{a}	62 ± 6^{a}	65 ± 7^{a}	69 ± 3^{a}
13		2192-74	Glamorgan (U.K.)	19	ST[D]	—	—	2 ± 1	—
14		2192-74	Glamorgan (U.K.)	19	LT[E]	—	—	56 ± 5	—
15		6606-84	Salerno (Italy) (N)	9	ST	99 ± 1^{a}	99 ± 1^{a}	97 ± 1^{a}	—
16		6606-84	Salerno (Italy) (N)	9	LT[A]	98 ± 1^{a}	99 ± 1^{a}	98 ± 1^{a}	—

* Four first numbers correspond to the No. of accession of the crucifer germplasm collection of the Universidad Politécnica de Madrid (Spain). The last two numbers correspond to the year the seeds were collected and stored.

N, Naturalized.

[A] −10 °C and 8% moisture content.

[B] Seeds obtained by multiplication of accession 9 in 1988.

[C] Seeds obtained by multiplication of accession 9 in 1972.

[D] Room temperature from 1974 to 1982 and subsequently under ST conditions.

[E] 5 °C from 1974 to 1982 and subsequently under LT conditions.

† Mean germination percentages with the same superscript letter in each accession are not significantly different at 5% level.

—, No germination tests.

obtained by multiplication here of accessions 9 after 17 and 11 years storage, respectively. Fresh seeds from *B. montana* used as reference (sample R) were collected in 1993 from a natural population (Gerona, Spain).

Seed germination tests

One hundred seeds for each treatment (four replicates of 25 seeds each) were placed in Petri dishes (7 cm inner diameter) on two cut sheets of Whatman paper moistened with 3 ml distilled water. The distilled water was replaced regularly. Germinated seeds (radicles visible) were counted every second day and removed from the Petri dishes. Incubation of seeds was carried out in a germination chamber at different temperatures (15, 20, 25 or 15/25 °C) and 16 h light photoperiod. When the temperature regime was 15/25 °C the lower temperature was linked to the dark period. Insufficient amounts of seeds in some accessions prevented germination tests being carried out for all four selected temperatures. The final germination percentage was recorded after 24 d.

Electrophoretic analysis

Twenty to 60 seeds were surveyed from each of the 17 samples (16 accessions and sample R). Crude extracts for electrophoresis were obtained from seeds as separate individuals. Each seed was crushed in the presence of 45 μl distilled water. Eight enzyme systems were determined in each extract: aconitase (ACO), alcohol dehydrogenase (ADH), isocitrate dehydrogenase (IDH), malate dehydrogenase (MDH), malic enzyme (ME), phosphoglucose isomerase (PGI), phosphoglucomutase (PGM) and 6-phosphogluconate dehydrogenase (6-PGD). Horizontal electrophoresis (SGE) was conducted on 11·5% starch gels following standard methods (Murphy *et al.*, 1990). A Tris/citric acid buffer system (pH 6·2) was used (2% gel and 25% electrode buffers). Enzyme assay conditions followed standard methods (Murphy *et al.*, 1990). Number of loci and number of alleles detected at each locus were studied in the selected enzymes (Table 2). The locus specifying the isozyme with the most anodal migration was called 1, the next 2, etc. (Table 2). Because the number of isozymes and their genetics in *Brassica* were previously reported for ACO, ADH, 6-PGD, PGI, PGM and IDH systems using SGE (Arus and Shields, 1983; Da Silva Dias, 1992), they were chosen for study, together with ME and MDH. Only tentative interpretations of the electrophoretic patterns of MDH could be made, because of its complexity.

Statistical analysis

Variance analysis of the seed germination percentages were conducted. Allelic frequencies for 19 isozymes (19 loci)

TABLE 2. *Allelic frequencies observed at 19 isozyme loci in* Brassica *species conserved under different storage conditions. For each species all ST or LT accessions were considered as one pool in order to obtain the allelic frequencies*

		B. oleracea		*B. cretica*		*B. incana*		*B. montana*	
Loci	Alleles	ST	LT	ST	LT	ST	LT	ST	LT
Aco-1	*1*	0·083	0·406	0·031	0·105	0·0	0·045	0·250	0·036
	2	0·125	0·250	0·031	0·105	0·0	0·045	0·0	0·0
	3	0·0	0·0	0·0	0·0	0·0	0·0	0·150	0·036
Aco-2	*1*	0·188	0·281	0·469	0·316	0·042	0·0	0·200	0·250
	2	0·118	0·610	0·281	0·368	0·542	0·068	0·350	0·286
	3	0·0	0·016	0·062	0·053	0·416	0·159	0·250	0·250
Idh-1	*1*	0·139	0·344	0·250	0·182	1·0	0·455	0·600	0·179
	2	0·069	0·125	0·125	0·182	0·0	0·0	0·200	0·393
Idh-2	*1*	0·563	0·672	1·0	1·0	0·500	0·545	0·550	0·821
	2	0·132	0·297	0·0	0·0	0·500	0·455	0·250	0·179
6Pgd-1	*1*	0·507	0·875	0·875	1·0	1·0	0·910	0·800	1·0
	2	0·028	0·094	0·0	0·0	0·0	0·0	0·0	0·0
6Pgd-2	*1*	0·394	0·938	0·875	0·895	0·917	0·910	0·800	0·643
	2	0·183	0·031	0·063	0·053	0·083	0·0	0·0	0·357
Mdh-3	*1*	0·127	0·125	0·344	0·0	0·0	0·023	0·0	0·0
	2	0·268	0·875	0·594	1·0	1·0	0·932	0·900	1·0
Mdh-5	*1*	0·394	1·0	0·938	1·0	1·0	0·955	0·900	1·0
Mdh-4	*1*	0·394	1·0	0·938	1·0	1·0	0·955	0·900	1·0
Mdh-2	*1*	0·070	0·188	0·625	0·368	0·0	0·023	0·0	0·0
	2	0·085	0·281	0·0	0·526	0·0	0·023	0·0	0·0
Mdh-1	*1*	0·0	0·0	0·0	0·105	0·0	0·0	0·0	0·0
	2	0·0	0·219	0·0	0·0	0·0	0·0	0·0	0·0
Me-1	*1*	0·091	0·173	0·0	0·0	0·0	0·0	0·0	0·0
	2	0·045	0·327	0·938	1·0	0·0	0·0	0·0	0·0
Me-2	*1*	0·076	0·0	0·0	0·0	0·0	0·045	0·500	0·0
	2	0·227	0·115	0·688	0·578	1·0	0·455	0·0	0·125
	3	0·129	0·346	0·250	0·211	0·0	0·455	0·0	0·0
	4	0·038	0·462	0·0	0·211	0·0	0·0	0·0	0·0
Pgi-1	*1*	0·227	0·423	0·0	0·0	1·0	0·727	0·800	1·0
Pgi-2	*1*	0·182	0·019	0·0	0·0	0·083	0·045	0·0	0·0
	2	0·152	0·173	0·875	0·0	0·375	0·205	0·250	0·036
	3	0·341	0·308	0·125	0·0	0·417	0·636	0·450	0·179
	4	0·053	0·404	0·0	0·714	0·125	0·0	0·200	0·785
	5	0·0	0·096	0·0	0·286	0·0	0·068	0·0	0·0
Pgm-1	*1*	0·189	0·300	0·0	0·0	0·333	0·023	0·250	0·357
	2	0·242	0·450	1·0	1·0	0·542	0·0	0·300	0·107
	3	0·167	0·250	0·0	0·0	0·042	0·159	0·150	0·214
	4	0·068	0·0	0·0	0·0	0·0	0·773	0·100	0·322
Pgm-2	*1*	0·197	0·050	0·0	0·0	0·500	0·500	0·0	0·250
	2	0·182	0·200	0·125	0·286	0·042	0·182	0·250	0·286
	3	0·136	0·350	0·625	0·174	0·458	0·0	0·550	0·464
	4	0·0	0·400	0·0	0·0	0·0	0·0	0·0	0·0
Adh-1	*1*	0·333	0·063	0·375	0·0	0·333	0·0	0·200	0·143
Adh-2	*1*	0·222	0·094	0·938	0·263	0·500	0·0	0·300	0·143
	2	0·139	0·096	0·0	0·0	0·083	0·0	0·100	0·0

were calculated for ST as well as for LT storage accessions (i.e. number of times each allele appears divided by the total number of alleles present in each locus for the ST and for the LT storage accessions). Chi-square (χ^2) values were used to compare the obtained frequencies of seeds showing isozyme activity in both storage conditions (Table 2).

RESULTS AND DISCUSSION

Seed viability

Table 1 shows the final germination percentages, for the *Brassica* accessions studied. In general, there were no significant differences in the germination percentages for each accession at the temperatures used. Only in the accessions 1 and 2 of *B. cretica* and 4 of *B. incana* were significant differences ($P < 0·05$) found.

For each temperature regime, no significant differences ($P < 0·05$) were detected between storage conditions for the germination percentages of the accessions of *B. montana* (Table 1). For all temperatures assayed, the germination percentages of ST accession 1 of *B. cretica* were significantly greater ($P < 0·05$) than those of LT accession 2 (Table 1). ST conditions maintained viability at $\geqslant 66\%$ in *B. cretica* (Acc. 1) and *B. montana* (Acc. 6) and in two accessions of *B.*

TABLE 3. *Frequencies of seeds showing distinguishable phenotypes on the zymograms at 19 enzyme loci (significant level of χ^2 test for comparison ratios in ST and LT conditions; * = P < 0·01, ** = P < 0·05). These results were calculated for each locus as the sum of the allelic frequencies reported in Table 2*

	B. oleracea			*B. cretica*			*B. incana*			*B. montana*		
Isozyme	ST	LT	χ^2	ST	LT	χ^2	ST	LT	χ^2	ST	LT	χ^2
Aco-1	0·208	0·656	**	0·062	0·210		0·0	0·090		0·400	0·072	**
Aco-2	0·306	0·907	**	0·812	0·737		1·0	0·227	**	0·800	0·786	
Idh-1	0·208	0·469	**	0·375	0·364		1·0	0·455	**	0·800	0·572	
Idh-2	0·695	0·969	**	1·0	1·0		1·0	1·0		0·800	1·0	
6Pgd-1	0·535	0·969	**	0·875	1·0		1·0	0·910		0·800	1·0	
6Pgd-2	0·577	0·969	**	0·938	0·948		1·0	0·910		0·800	1·0	
Mdh-3	0·395	1·0	**	0·938	1·0		1·0	0·955		0·900	1·0	
Mdh-5	0·394	1·0	**	0·938	1·0		1·0	0·955		0·900	1·0	
Mdh-4	0·394	1·0	**	0·938	1·0		1·0	0·955		0·900	1·0	
Mdh-2	0·155	0·469	**	0·625	0·894	*	0·0	0·046		0·0	0·0	
Mdh-1	0·0	0·219	**	0·0	0·150		0·0	0·0		0·0	0·0	
Me-1	0·136	0·500	**	0·938	1·0		0·0	0·0		0·0	0·0	
Me-2	0·470	0·923		0·938	1·0		1·0	0·955		0·500	0·125	
Pgi-1	0·227	0·423	**	0·0	0·0		1·0	0·727		0·800	1·0	
Pgi-2	0·728	1·0	**	1·0	1·0		1·0	0·954		0·900	1·0	
Pgm-1	0·666	1·0	**	1·0	1·0		0·917	0·955		0·800	1·0	
Pgm-2	0·515	1·0	**	0·750	1·0		1·0	0·682		0·800	1·0	
Adh-1	0·333	0·063	**	0·375	0·0	**	0·333	0·0	*	0·200	0·143	
Adh-2	0·361	0·094	**	0·938	0·263	**	0·583	0·0	**	0·400	0·143	

oleracea (11 and 15) over 8 to 11 years, while in three ST accessions of *B. oleracea* (9, 10 and 13) ⩽ 20% germination was detected. Germination percentages at 25 °C were between 65% and 72% for accessions 11 (seeds after 11 years of ST) and 12 (seeds after 22 years of LT) of *B. oleracea*. However, the germination percentages of two other accessions of this species, 9 (seeds kept for 22 years of ST conditions and with no regeneration) and 10 (seeds after 5 years of ST), were significantly lower ($P < 0·05$) compared to accessions 11 and 12 (Table 1). No germination was obtained in accession 9 after 22 years storage. Moreover, seeds of accessions 10 and 11, which were obtained by multiplication of accession 9 after 17 and 11 years of ST respectively, showed lower viability the longer the time interval to multiplication. Finally, the germination percentage of LT accession 14 of *B. oleracea* was significantly higher ($P < 0·05$) than seeds of accession 13 which were collected at the same time but stored under ST conditions (Table 1).

In general, therefore, LT storage conditions maintained germination ability over 8 to 22 years in all *Brassica* species studied. ST storage conditions maintained germination ability up to 8–9 years in all accessions of *B. cretica* and *B. montana*. However, the results obtained support the idea that seed regeneration is essential every 10 years in accessions of *B. oleracea* under ST storage conditions.

Seeds of *B. cretica* stored under LT conditions exhibited high levels of germination over a broader range of temperatures compared to those stored under ST conditions. This suggests that the additional desiccation and/or cooling associated with LT storage may have increased the dormancy level of the seeds.

In two accessions of *B. oleracea* (13 and 14), the germination percentage was significantly higher ($P < 0·05$) in seeds following storage at LT (Acc. 14) compared to ST conditions. However, seeds of the accession 13 were kept for 8 years at room temperature before being stored under ST conditions, which could be the cause of the loss of viability.

The natural population of *B. montana* from Gerona (Spain) showed very high germination percentages (88%) at 25 °C (data not shown), which are usually associated with freshly harvested seeds of $n = 9$ Mediterranean *Brassica* species.

Isozyme studies

Allelic frequencies for the ST and LT stored *Brassica* accessions and for fresh seeds of *B. montana* (sample R) were calculated for the eight selected enzyme systems: ACO, ADH, IDH, MDH, ME, PGI, PGM and 6-PGD. All enzyme systems were adequately resolved. Nineteen isozymes were detected: two for each system, except for MDH which had five variants. For each species all ST or LT accessions were considered as one pool in order to obtain the allelic frequencies. Table 2 shows the allelic frequencies observed in each locus for seeds showing well resolved patterns of isozyme bands. These results, which allowed the four *Brassica* species to be distinguished, were used to estimate the frequencies of seeds showing distinguishable phenotypes on the zymograms (Table 3). Statistical analysis (χ^2) was also conducted to detect significant differences in these frequencies between ST and LT storage conditions (Table 3). Frequency of bad samples, i.e. seeds with loss of enzymatic activity, smeared zymogram bands or any sign of seed deterioration, which could result from inadequate storage conditions, can also be deduced for each isozyme.

Allelic frequencies observed at the nineteen loci in sample R (Fig. 1, F, G, H) were no different from LT accessions of *B. montana* and as a consequence these are not shown in Table 2.

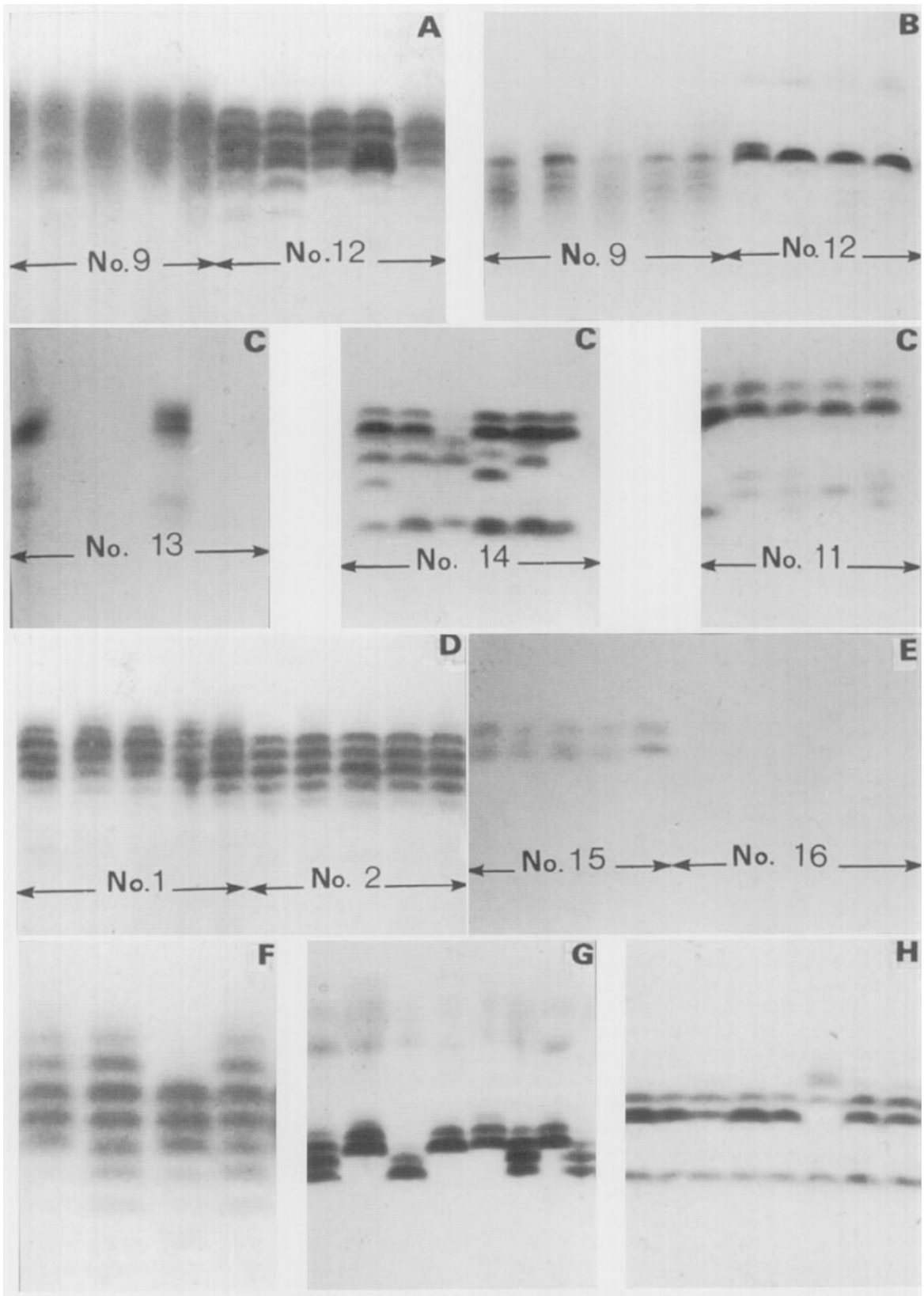

FIG. 1. Zymograms of different enzyme systems: A, MDH and B, IDH of *Brassica oleracea* ST accession 9 (22 years) and LT 12 (22 years); C, PGM of *B. oleracea* ST accession 13 (room temperature 1974–82, and subsequently ST), 14 (ST 1974–82 and subsequently LT) and 11 (obtained from multiplication of 9 in 1982); D, MDH of *B. cretica* ST accession 1 (9 years) and LT 2 (9 years); E, ADH of *B. oleracea* ST accession 15 (9 years) and LT 16 (9 years); F, MDH, G, PGI and H, PGM of *B. montana* sample R.

Activity of enzyme systems was well conserved in the dry seeds; seven were active after the hydration/extraction procedure, showing their respective isozyme patterns. An exception was the ADH system which showed activity mainly in the ST accessions. Loss of activity, lower intensity or diffusion of zymogram bands, among the other enzyme systems could also be detected in ancient ST accessions (Fig. 1A, B) and rarely some diffusion on 9-year-old ST accessions (ADH in accession 15; Fig. 1E). *Brassica oleracea* LT accessions (12, 14 and 16) showed a significantly ($P < 0{\cdot}01$) higher number of seeds showing distinguishable phenotypes on the zymograms when compared with ST ones (9, 10, 11, 13 and 15) for ACO, IDH, MDH, ME, PGI, PGH and 6-PGD systems (Table 2 and Fig. 1C). While accession 9 showed deterioration (Fig. 1A, B), accession 11 obtained from accession 9 in 1982 showed no deterioration (Fig. 1C). This result emphasizes again the need to regenerate *Brassica oleracea* species after 10 years ST storage and indicates that regeneration has the desired effect of improving seed quality. It is of utmost importance to regenerate before seed ageing has progressed too far as Stoyanova (1992) has shown in wheat seeds that the genetic shifts which result from regeneration are not as great as those induced by seed ageing.

In *B. cretica*, *B. incana* and *B. montana* significant differences were, only sporadically, detected in the frequency of seeds showing well resolved isozyme patterns for ACO, IDH, MDH, ME, PGI, PGM and 6-PGD between LT and ST accessions (Table 2 and Fig. 1D). These ST accessions were 8–9 years old, showing again that storage of *Brassica* seeds under ST conditions is acceptable at least up to, but perhaps for not longer than, 10 years.

In all studied species, except *B. montana*, ST accessions showed a significantly higher frequency of seeds with ADH activity (isozymes ADH-1 and ADH-2) than LT accessions ($P < 0{\cdot}05$ or $0{\cdot}01$) (Table 2 and Fig. 1E). Increase of ADH activity as a result of stress (anaerobic induction) has been shown previously (Good and Muench, 1993).

Implications for seed banking

Seed storage may influence seed viability and reduces seed vigour depending on the time span and storage conditions (Dell'Aquila, 1987). Many studies on seed viability loss have been reported (Perl, 1988), but less information is available about change caused by long-term storage. The exact biochemical changes involved in seed deterioration have not been very well studied, however they have been attributed to various biochemical processes, namely denaturation of biomolecules, accumulation of toxic substances and loss of membrane integrity (Basavarajappa, Shetty and Prakash, 1991). These biochemical changes, which were also related to a decrease in variability (Cheng, Zheng and Tao, 1991), might cause the altered isozyme patterns. Biochemical parameters are increasingly used as indicators of seed viability and vigour (Perl and Kretschemer, 1988; Sánchez de Jiménez *et al.*, 1991). Besides, attempts have been made to use enzymatic activity as a measure of seed viability, although the conflicting reports supported the need for further research (Justice and Bass, 1979). The present study provides evidence that changes in enzyme activity can be used as an indicator of seed deterioration.

The International Seed Testing Association (ISTA) has commented on the necessity of developing new methods of seed testing (Bekendam, 1984). Our results suggest that isozyme analysis (SGE) can serve as a fast and economical tool for genebank personnel interested in studying the effect of different conservation conditions on the viability of stored seeds. The ADH enzyme system which shows an increment in activity, induced under ST conditions, seems to be related to seed ageing and its use as a genetic marker for seed deterioration could be further explored in future. Similarly, the MDH enzyme system could be taken as a genetic marker for seed viability and vigour as it is affected considerably by storage time. While LT *Brassica* species and 8–9-year-old ST *B. cretica*, *B. incana* and *B. montana* accessions showed almost 100% activity for *Mdh-3*, *Mdh-5* and *Mdh-4*, *B. oleracea* ST accessions (which include the most ancient ones: 5–22 years of storage) showed approximately 40% activity for these isozymes (Table 2).

In summary, the results presented here show that many enzymatic systems are not affected by LT storage conditions over a period of 8–22 years and demonstrate that changes in their activity under ST storage conditions can be used as an indicator of seed deterioration.

ACKNOWLEDGEMENTS

We thank Professor C. Gómez-Campo for providing seeds and for skilful guidance and the DGICYT (Direccion General de Investigación Científica y Técnica) for financial support. We thank as well Dr Ockendon for providing *Brassica oleracea* from natural populations of the United Kingdom and the I.B.P.G.R. for financial assistance to collect wild *Brassica* seeds in the Mediterranean area.

Communication presented at the Society for Low Temperature Biology's symposium *Low Temperature Aspects of Seed Conservation* (14–15 September 1993), organised by H. W. Pritchard and S. H. Linington (Royal Botanic Gardens, Kew, Wakehurst Place).

LITERATURE CITED

Arus P, Shields CR. 1983. Cole crops (*Brassica oleracea* L.). In: Tanksley SD, Orton TJ, eds. *Isozymes in plants genetics and breeding. Part B.* Amsterdam: Elsevier Science Publishers, 339–351.

Basavarajappa BS, Shetty HS, Prakash HS. 1991. Membrane deterioration and other biochemical changes, associated with accelerated ageing of maize seeds. *Seed Science and Technology* **19**: 279–286.

Bekendam J. 1984. Practical viability testing. In: Dickie JB, Linnington S, Williams JT, eds. *Seed management techniques for genebanks.* Rome: International Board for Plant Genetic Resources, 131–142.

Cheng H, Zheng G, Tao KL. 1991. Effects of ultradying on ageing, cell ultrastructure and vigour of Chinese cabbage seed. *FAO/IBPGR Plant Genetic Resources Newsletter* **83/84**: 9–14.

Da Silva Dias JC. 1992. *Taxonomia das couves galaico-portuguesas utilizando caracteres morfologicos, isoenzimas e RFLPs.* Lisboa: PhD Thesis. Universidade Técnica de Lisboa, Instituto Superior de Agronomía.

Dell'Aquila A. 1987. Mean germination time as a monitor of the seed ageing. *Plant Physiology and Biochemistry* **25**: 761–768.

Ellis RH, Hong TD, Roberts EH. 1991. Seed moisture content, storage, viability and vigour. *Seed Science Research* **1**: 275–277.

Ellis RH, Hong TD, Roberts EH. 1992. The low-moisture-content limit to the negative logarithmic relation between seed longevity and moisture content in three subspecies of rice. *Annals of Botany* **69**: 53–58.

Ellis RH, Hong TD, Martín MC, Pérez-García F, 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.

Gómez-Campo C. 1985. Seed banks an emergency conservation strategy. In: Gómez-Campo C, ed. *Plant conservation in the mediterranean area.* Dordrecht: Dr. W. Junk Publishers, 237–247.

Gómez-Campo C. 1990. *A germplasm collection of Crucifers.* INIA Catálogos Num. 22. Madrid: Instituto Nacional de Investigaciones Agrarias.

Good AG, Muench DG. 1993. Long-term anaerobic metabolism in root tissue. Metabolic products of pyruvate metabolism. *Plant Physiology* **101**: 1163–1175.

Hanson J, Williams JT, Freund R. 1984. *Institutes conserving crop germplasm: the IBPGR global network of genebanks.* Rome: International Board for Plant Genetic Resources.

Justice OL, Bass LN. 1979. *Principles and practices of seed storage.* Beccles and London: Castle House Publications Ltd.

Murphy RW, Sites JW, Buth DG, Haufler, CH. 1990. Proteins: isozyme electrophoresis. In: Hillis DM, Moritz C, eds. *Molecular systematics.* Sunderland: Sinauer Associates Publishers, 45–127.

Perl M. 1988. Recent development in seed physiology and biochemistry. *Seed Science and Technology* **16**: 135–138.

Perl M, Kretschemer M. 1988. Biochemical activities and compounds in seeds: possible tools for seed quality evaluation. *Annals of Botany* **62**: 61–68.

Roberts EH. 1973. Predicting the storage life of seeds. *Seed Science and Technology* **1**: 499–514.

Roberts EH. 1975. Problems of long-term storage of seed and pollen for genetic resources conservation. In: Frankel OH, Hawkes JG, eds. *Crop genetic resources for today and tomorrow.* Cambridge: Cambridge University Press, 269–298.

Sánchez de Jiménez E, Sepúlveda G, Reynoso E, Molina-Galán J, Albores M. 1991. Long-term maize seed storage and loss of viability: polyamines and auxin contents during germination. *Seed Science and Technology* **19**: 83–92.

Stoyanova SD. 1992. Effect of seed ageing and regeneration on the genetic composition of wheat. *Seed Science and Technology* **20**: 489–496.

Vertucci CW, Roos EE. 1990. Theoretical basis of protocols for seed storage. *Plant Physiology* **94**: 1019–1023.

Vertucci CW, Roos EE. 1991. Seed moisture content, storage, viability and vigour. *Seed Science Research* **1**: 277–279.

Yndgaard F, Iloskuldsson A. 1985. Electrophoresis: A tool for genebanks. *FAO/IBPGR Plant Genetic Resources Newsletter* **63**: 34–40.