

Loss of Viability in Lettuce Seeds and the Accumulation of Chromosome Damage under Different Storage Conditions

N. K. RAO*, E. H. ROBERTS and R. H. ELLIS

Department of Agriculture, University of Reading, Earley Gate, P.O. Box 236, Reading RG6 2AT, UK

Accepted 23 February, 1987

ABSTRACT

Loss of seed viability in lettuce (*Lactuca sativa* L.) during storage is associated with an increase in the frequency of cells in the surviving seeds showing chromosome damage during first mitoses. The relation is linear when probit of the frequency of aberrant cells is plotted as a function of probit percentage normal germination. The slope of the relation, however, varies according to moisture content so that the proportion of aberrant cells for any given loss of germination increases with decrease in moisture content over the range 13.0-5.5 per cent. At 3.3 per cent moisture content, however, the proportion of aberrations was no greater than at 5.5 per cent moisture content; and at 18.1 per cent moisture content the proportion was no less than at 13.0 per cent moisture content. Despite these differences, the increase in chromosomal aberrations per unit time for a given temperature was always less the lower the moisture content. Diplontic selection markedly reduced the frequency of chromosomal aberrations and eliminated the differences in these frequencies between the different storage treatments. But even after five weeks' growth, root tips from aged seed still contained about twice as many aberrant cells as compared with similar root tips derived from the original seed stock. Studies on the frequency of recessive mutations indicated that excessive amounts of heritable mutations were not present in the progenies of aged seed, even when stored at moisture contents as low as 5.5 per cent. All this and other evidence reinforces the view that orthodox seeds for genetic conservation should be stored at not more than about 5 per cent moisture content, and that even lower moisture contents are worth considering. The results also emphasise the need for maintaining a high regeneration standard, i.e. the percentage to which seed viability is allowed to fall during storage before the seed stock is regenerated.

Key words: *Lactuca sativa*, lettuce, seed storage, seed viability, chromosomal aberrations, phenotypic mutations.

INTRODUCTION

Several phases have been recognized in the development of our understanding of the accumulation of chromosome damage during seed storage (Roberts, 1978, 1987). In the early 1930's several independent laboratories showed that chromosomal aberrations and gene mutations, or the events which give rise to them, are induced during seed storage (e.g. Navashin, 1933*a, b*; Peto, 1933; Cartledge and Blakeslee, 1934; Avery and Blakeslee, 1936). Later that decade it became

evident that chromosome damage is a function not only of the time but also of temperature and moisture content (e.g. Navashin and Gerassimova, 1936*a, b*; Cartledge, Barton and Blakeslee, 1936). The fact that increase in both temperature and moisture content also decrease longevity then led to the examination of the relation between seed viability and chromosome damage. Over a certain range of temperatures (25-45 °C) and moisture contents (12-18 per cent), the amount of chromosome damage which accumulates in seeds can be simply related to the percentage viability of the seed lot, and the relation is unaffected by storage conditions or the rate of loss of viability (Roberts, Abdalla and Owen, 1967; Abdalla and Roberts, 1968; Murata, Roos and Tsuchiya, 1979, 1981; Dourado and Roberts, 1984*a*). In contrast it has

* Present address: Genetic Resources Unit, International Crops Research Institute for the Semi-Arid Tropics, Patancheru P.O., Andhra Pradesh 502 324, India.

recently become evident that when seeds are fully imbibed (at about 50 per cent moisture content) and can be prevented from germinating, their longevity is considerably greater than when they are stored at somewhat lower moisture contents (Villiers, 1975; Villiers and Edgcumbe, 1975) providing oxygen is present (Ibrahim and Roberts, 1983; Ibrahim, Roberts and Murdoch, 1983). Under such fully hydrated conditions little chromosome aberration was observed (Villiers, 1974, 1975). However, in these cytological experiments on fully imbibed seeds there was no loss of viability, and it was still not clear at this stage whether the relationship between loss of viability and the amount of chromosome aberration induced would have been any different from drier seeds had the imbibed seeds been maintained until some loss of viability had occurred.

Recently when data from Harrison (1966) and Villiers (1975) on lettuce were replotted as percentage of seeds or cells containing aberrant chromosomes as a function of percentage seed viability (Roberts and Ellis, 1984) the results suggested the possibility that, for a given loss of viability, the accumulation of chromosome damage may be greater at moisture contents less than about 7 per cent. Indirect arguments also suggested that there may be less chromosome damage at very high moisture contents as a result of the postulated operation of repair processes which are not possible in dry seeds (Villiers, 1975). This possibility was first suggested for fully imbibed lettuce seeds at about 50 per cent moisture content; although subsequent experiments indicate that such mechanisms are most active in fully hydrated seeds they also suggest that at least some repair is possible down to a critical seed moisture content which is about 15 per cent in lettuce and 18 per cent in onion (Ibrahim, 1981; Ibrahim and Roberts, 1983); in contrast to these oily seeds, in non-oily seeds the evidence suggests that the critical moisture content occurs at a much higher moisture content—26–28 per cent (Roberts, 1986) or even 28–30 per cent (Petruzzelli, 1986)—but in all species probably at a similar water activity of about 0.95 (Roberts, 1986) or water potential of about -14.5 MPa (Roberts, 1987).

The earlier concept that the amount of chromosome damage could be considered solely as a function of percentage viability irrespective of moisture content was therefore put in doubt. This suspicion was confirmed by investigations on onion seed in this laboratory which showed that although storage at moisture contents between 12.2 and 17.4 per cent did not affect the relationship between percentage seed viability and the fre-

quency of chromosomal aberrations, at 36 per cent moisture content the proportion of aberrant anaphases for a given loss of viability was significantly less (Sirikwanchai, 1985; Roberts, 1987).

In this paper the question has been investigated much more comprehensively in lettuce seeds in which particular attention has also been paid to very dry seeds. Very dry seeds were included, not only because the indications were that chromosome damage may be more severe for a given loss of viability under such conditions, but also because there is now considerable interest in the use of ultra-dry conditions as a potentially inexpensive method of long-term storage of seeds for genetic conservation which avoids refrigeration (IBPGR, 1985a). For example, in *Sesamum indicum* L. a reduction in seed moisture content for storage from 5 to 2 per cent increased seed longevity by a factor of 40 (Ellis, Hong and Roberts, 1986). This result suggests that storage at 20 °C and 2 per cent moisture content, for example, may result in similar longevity to that at -20 °C and 5 per cent moisture content—thus removing the need for refrigeration in some circumstances.

Clearly in the context of genetic conservation it is important to know the consequences for chromosome stability of using the low moisture content already recommended, viz. 5 ± 2 per cent (IBPGR, 1985b), as well as the even lower moisture content of 2.5–3.0 per cent now contemplated. The induction of chromosomal aberrations themselves are not worrying, since they tend to be lost through diploic selection (Gaul, 1961; Abdalla and Roberts, 1969b; Murata, Tsuchiya and Roos, 1984). Nevertheless they give some indication of the extent to which heritable point mutations have been induced which are much more difficult to quantify directly but which have significant consequences for genetic conservation and maintenance of elite seed stocks (Roberts and Ellis, 1984; Dourado and Roberts, 1984b; Roberts, 1987). Accordingly in this study we also investigated whether storage of very dry seeds influenced the frequency of chromosomal aberrations in later mitoses, and whether it had any major influence on the incidence of heritable point mutations which are expressed in subsequent generations.

MATERIALS AND METHODS

Untreated achenes (described here as seeds) of lettuce (*Lactuca sativa* L. cv. Trocadero Improved) of 5.5 per cent moisture content (fresh weight basis) and showing 98 per cent normal germination

were used in this study. These had been purchased in 1978 and stored subsequently at -20°C until the work started in 1984. Seed moisture contents were adjusted by placing the seeds on moist paper for a short time to increase moisture, or by placing in a desiccator over silica gel at 20°C to reduce moisture. After adjustment the seeds were left to equilibrate for 24 h at $3\pm 1^{\circ}\text{C}$ within sealed containers, before moisture contents were determined by the high constant temperature oven method of the International Seed Testing Association, viz. 1 h at $130\text{--}133^{\circ}\text{C}$ in a forced draught oven (ISTA, 1985*a, b*) and expressed as a percentage of their initial weight. Seeds at each moisture content were then divided into sub-samples of between 500 and 750 seeds and each sub-sample sealed within a laminated aluminum foil packet ($50\times 100\text{ mm}$). The seeds were then stored for different periods at various combinations of moisture and temperature. Sub-sample size was varied according to the severity of the treatment in order to provide sufficient viable seeds for the cytological investigations described below.

Following storage the seeds were set to germinate on top of moist filter papers (Whatman 181) at 20°C under continuous white fluorescent light for 7 d in two distinct groups. The first group comprised 400 seeds for a standard germination test in which the seedlings were evaluated according to ISTA rules (ISTA, 1985*a, b*); that is only seedlings which produced morphologically normal roots and shoots were considered to have germinated (normal germination). The remaining seeds were also set to germinate under these conditions, in this case to provide roots for the cytological investigations. Germinating seeds were collected on several occasions during each test (to ensure a random selection of both rapid and slow germinating seeds). Since the occurrence of the first mitotic divisions is known to be delayed in relation to root extension in aged seeds (e.g. Abdalla and Roberts, 1968; Dourado and Roberts, 1984*a*), seeds were selected when their radicles were between 1.0 and 1.5 mm, 1.3 and 1.8 mm, or 1.5 and 2.0 mm depending upon whether normal germination was anticipated to be greater than 85 per cent, between 50 and 85 per cent, or below 50 per cent, respectively. These lengths were determined from the results of a preliminary study of this seed lot.

The germinating seeds were fixed in a 33 per cent solution of acetic acid in ethanol for 24 h. They were then rinsed in distilled water and stored temporarily at 3°C in a 70 per cent solution of ethanol in water. The germinated seeds were later

immersed in 1 N hydrochloric acid at 60°C for 10 min to hydrolyse the radicles, then immersed in Schiff's reagent (BDH Chemicals) for 45–60 min at laboratory temperature. The root meristems were then excised from the radicle tips, one drop of 1 per cent aceto-carmin was added, and the root squashes were prepared using a separate slide for each meristem.

Chromosome damage was assessed by counting all cells at anaphase and recording the number in which aberrations (fragments and/or bridges) were observed. At each sampling time approximately 500 cells at anaphase were scored. This required examination of 15–35 root tip squashes.

The data were subjected to probit analysis using the package GLIM. Since lifespans are typically distributed normally, many symptoms of deterioration relate more simply to the probit of percentage viability rather than to untransformed values (Roberts, 1986). Accordingly, percentage normal germination values were transformed to probits and treated as the dose (unweighted and independent values on the x axis). The frequency of aberrant cells (those containing one or more chromosome aberrations) were also transformed to probits and treated as the dependent variable. Thus the central, more reliable, values of the frequency distribution of accumulated aberrant cells were weighted according to the principles of probit analysis. The linear relations derived justified this method of analysis and allowed an iterative routine to be developed within GLIM in order to provide the best estimate of the original status of the seed lot with respect to the incidence of aberrant cells and normal germination.

The influence of seed storage environment on the frequency of chromosome damage which appeared in later mitoses was studied using seeds from five storage regimes of the main experiment. Root tip squashes from five-week old plants grown from stored seeds were compared with roots taken from control plants of the same age grown from the original stock of seeds. In each treatment 15 plants were grown in potting compost (Levington Universal) at 15°C for five weeks, and three root tips were taken from each plant for cytological studies.

Two experiments were undertaken to study the influence of seed storage conditions on the induction of phenotypic mutations. Seeds were stored under various conditions in sealed packets. The aged seeds were designated A_1 and were sown in Seed Compost (Levington) in a glasshouse at 15°C . After three to four weeks the seedlings were transplanted into peat blocks and then into field plots under a glass shutter (first experiment)

or into pots containing compost in glasshouses (second experiment). At maturity the seeds from each plant (designated A_2) were collected separately, dried to 6 per cent moisture content in a forced draught at 20 °C and 25–30 per cent r.h., cleaned in a seed blower and stored in moisture-proof plastic bags at 3 °C. These A_2 seeds were sown in compost in the glasshouse at 15 °C. After three to four weeks each line was screened for putative mutant phenotypes. Then at maturity A_3 seeds were collected from lines containing mutations and subsequently grown in the glasshouse to determine segregation ratios.

RESULTS AND DISCUSSION

Effect of seed storage environment on induction of chromosomal aberrations

Lettuce seeds were stored in sealed packets in 12 different combinations of temperature and moisture content. They included six moisture contents from 3.3 to 18.1 per cent and five temperatures from 30 to 60 °C (Fig. 1). An orthogonal design was not used since seed longevity would have been very prolonged (at least several decades) in several combinations of the lower temperatures and moisture contents used; but at each moisture content seeds were stored at two different but appropriate temperatures in order to confirm whether any conclusion with respect to moisture content was temperature-dependent.

Within each treatment viability was lost, as would be expected, at a rate which was dependent on temperature and moisture content: decreasing either increased longevity (Fig. 1). At the same time within each treatment the percentage of anaphase cells at first mitosis which showed chromosome abnormalities (bridges or fragments) increased with period of storage (Fig. 2). The increase in aberrant cells per unit time differed markedly between the treatments as did the maximum number of aberrant cells accumulated. In general it is clear that the higher the temperature and moisture content, the more rapidly seeds lost viability and the more rapidly chromosomal aberrations were induced. Furthermore it is clear that the maximum number of chromosomal aberrations that can accumulate is greater at lower moisture contents.

In order to clarify the effect of moisture content on the induction of aberrations, percentage aberrations were plotted against the percentage normal germination of the seed samples. The curves showed concavities with respect to the origin at low moisture contents and convexities at

higher moisture contents. But when probability scales are used for both coordinates then the curves are linearized (Fig. 3). Clearly the slope of the linear relation between probit of percentage aberrant cells and probit percentage normal germination differed between treatments. Temperature had no effect on any of the relationships but probit analysis showed that, so far as moisture content is concerned, four discrete relationships could be discerned: division of the data from the 12 separate storage environments into four groups (30–40 °C with 13.0–18.1 per cent moisture content; 35–40 °C with 9.8 per cent moisture content; 35–40 °C with 8.1 per cent moisture content; and 30–60 °C with 3.3–5.5 per cent moisture content) did not result in any significant increase in error ($P > 0.25$). Furthermore, constraining these four discrete relationships to a common point of intersection, where percentage aberrant cells and percentage normal germination were 0.94 and 99.2 respectively (Fig. 3), did not result in a significant increase in error ($P > 0.25$). But the difference in probit percentage aberrant cells with loss in probit percentage normal germination (the slopes of the curves) did differ significantly between the four moisture content ranges mentioned above ($P < 0.001$). Thus differences in seed moisture content within the range 5.5 to 13.0 per cent had a profound effect on the relationship between percentage normal germination and the accumulation of chromosomal aberrations: the lower the moisture content the greater the proportion of aberrant cells for a given loss of germination. However, Fig. 3 also shows that the frequency of aberrant cells as a function of percentage normal germination was not decreased by increasing seed moisture content from 13.0 to 18.1 per cent; nor was it increased if the moisture content was decreased from 5.5 to 3.3 per cent.

When the results of Villiers (1975) on lettuce seeds stored at 30 °C were plotted as percentage of aberrant anaphase cells as a function of percentage seed viability, the relationship appeared to be similar for storage at either 7 or 10 per cent moisture content (Roberts and Ellis, 1984), and similar to the results reported here for seeds at 9.8 per cent moisture content. The results reported by Villiers on seed stored at 5 per cent moisture content and on seeds he obtained from commercial seed stores were similar to those reported here for seeds stored at 3.3 to 5.5 per cent moisture content. Thus there is now no doubt that considerably more chromosomal aberrations can arise for a given loss of germination ability as a result of storage at the low moisture contents used for genetic conservation and in some commercial seed

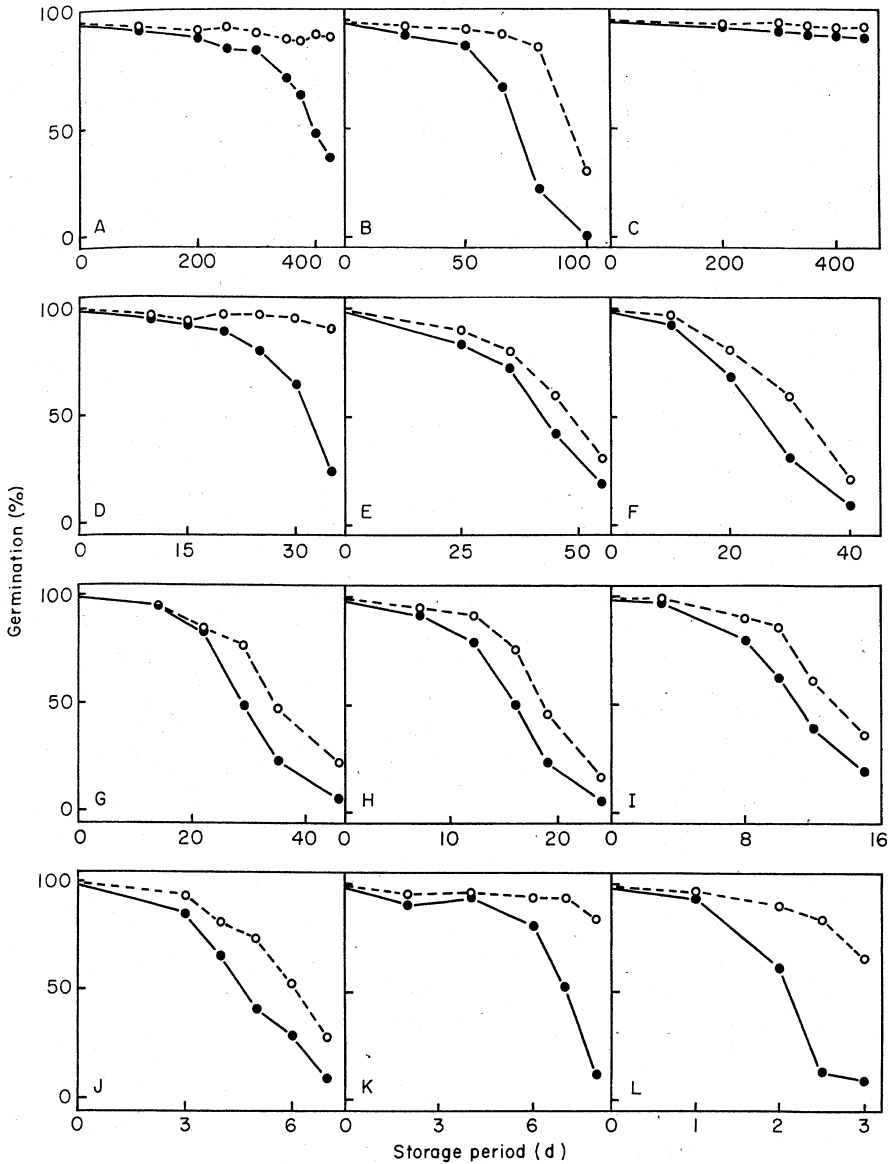


FIG. 1. Loss of germination (●, normal; ○, total) of lettuce seeds stored hermetically for various periods at the following temperatures and moisture contents: A, 50 °C, 3.3 per cent; B, 60 °C, 3.3 per cent; C, 30 °C, 5.5 per cent; D, 50 °C, 5.5 per cent; E, 35 °C, 8.1 per cent; F, 40 °C, 8.1 per cent; G, 35 °C, 9.8 per cent; H, 40 °C, 9.8 per cent; I, 35 °C, 13.0 per cent; J, 40 °C, 13.0 per cent; K, 30 °C, 18.1 per cent; and L, 40 °C, 18.1 per cent. Note that for clarity, the scale of the x axis in each case is different. Initial germination was 98 (normal) or 99 per cent (total).

stores as compared with storage at higher moisture contents.

It is not clear why increasing seed moisture content from 5.5 to 13.0 per cent should decrease the frequency of chromosome aberrations for any

given loss of seed viability. Although it is probable that macromolecular repair mechanisms are active in fully hydrated seeds (Villiers, 1975) and that some repair is possible at lower moisture contents, the evidence suggests that no repair is likely below

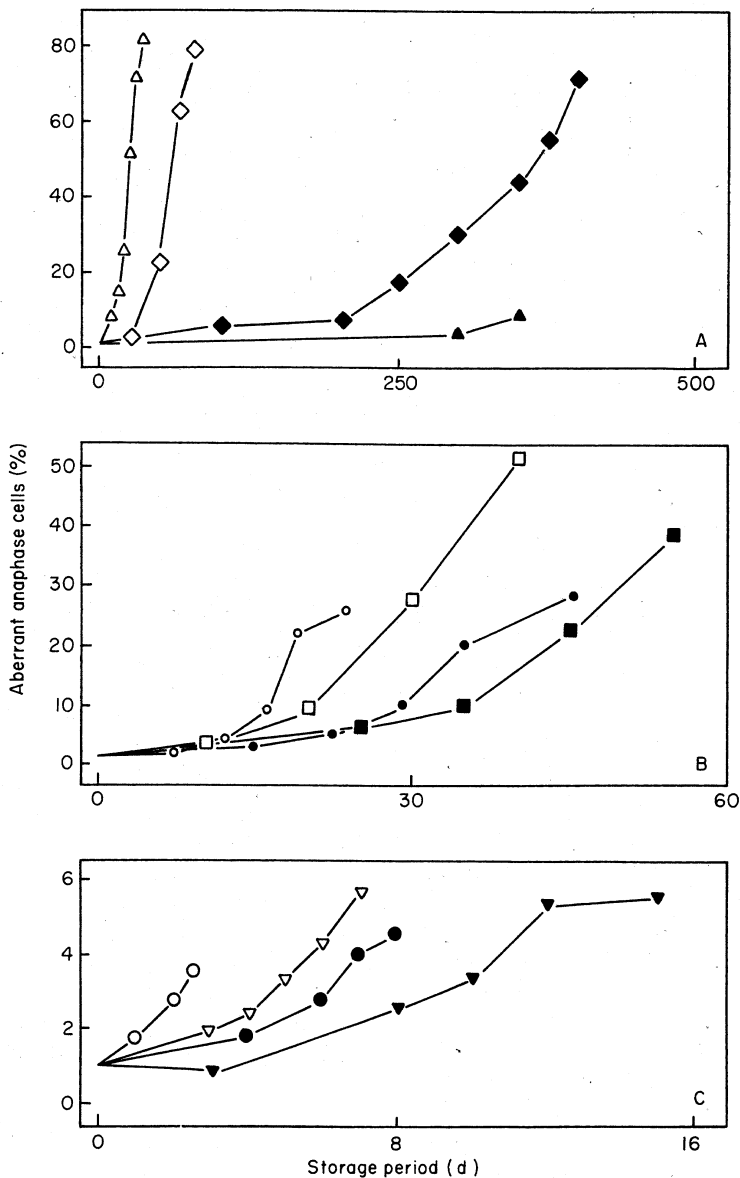


FIG. 2. Increase in the frequency of aberrant anaphase cells in surviving seeds of lettuce with period of storage at various temperatures and moisture contents: A, 50 °C, 3.3 per cent (◆), 60 °C, 3.3 per cent (◇), 30 °C, 5.5 per cent (▲), 50 °C, 5.5 per cent (△); B, 35 °C, 8.1 per cent (■), 40 °C, 8.1 per cent (□), 35 °C, 9.8 per cent (●), 40 °C, 9.8 per cent (○); and C, 35 °C, 13.0 per cent (▼), 40 °C, 13.0 per cent (▽), 30 °C, 18.1 per cent (●), 40 °C, 18.1 per cent (○). Note that, for clarity, the scales of both axes vary between A, B and C.

15 per cent moisture content in lettuce (Ibrahim and Roberts, 1983; Ibrahim *et al.*, 1983). Accordingly it is unlikely that the differences observed in this paper are due to repair. Indeed in those treatments in which some repair activity might

have been feasible, i.e. at 18.1 per cent moisture content, there was no decrease, as compared with seeds stored at 13.0 per cent moisture content, in the frequency of aberrant cells as a function of loss of seed viability. Accordingly it would seem that

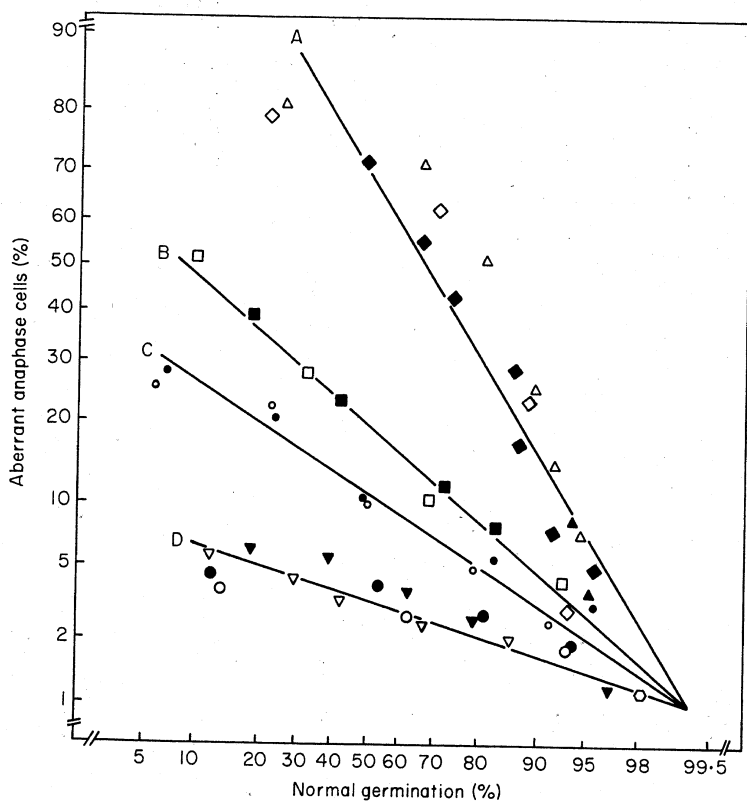


Fig. 3. Relations between the frequency of aberrant anaphase cells in surviving seeds of lettuce (probability scale) and the percentage of seeds producing morphologically normal seedlings (probability scale) following storage at various moisture contents: A, 3.3–5.5 per cent; B, 8.1 per cent; C, 9.8 per cent; D, 13.0–18.1 per cent. The caption to Fig. 2 provides the details of the seed storage environment for each observation, except for the control value (O).

the variation with moisture content in the relationship between frequency of chromosomal aberrations and percentage germination arises because, between 13.0 and 5.5 moisture content, a decrease in hydration decreases the rate of loss in germination by more than it decreases the rate of accumulation of chromosomal aberrations.

The frequency of chromosomal aberrations in roots of five-week old plants derived from aged seeds

Seeds from five of the ageing treatments used in the previous section were used to produce plants from which root tips were sampled for cytological studies when they were five weeks old. The results were compared with those obtained from control plants derived from untreated seeds from the same stock (Table 1). In all treatments, as would be expected as a result of diplontic selection, a marked reduction in chromosomal aberrations

had occurred within the five-week period of root growth. Nevertheless, although there were now no significant differences amongst the ageing treatments, all these treatments showed a significantly greater frequency of aberrant cells than the control roots.

The ability to produce a morphologically normal seedling (ISTA, 1985*a, b*) was the criterion of germination used to indicate viability in Fig. 3, since it is only these seedlings which would probably survive in the field. Indeed, most of the abnormal seedlings observed in the present studies were characterized by stunted and distorted radicles, many of which ceased growth during the germination test. The radicle tips for cytological investigation had to be sampled very early (see Materials and Methods) in order to capture first mitoses. At this stage it was impossible to distinguish which roots would have become normal or abnormal. It is possible, however, that

TABLE 1. Frequency of aberrant anaphases in lettuce root tips after various seed storage treatments, determined at radicle emergence and after five weeks growth.

Storage treatments				Aberrant anaphases (%)	
Temperature (°C)	Moisture content (% f. wt.)	Duration (d)	Normal germination (%)	In young radicle tips	In root tips from five week old plants
	Control	0	98	1.1	0.9
40	9.8	12	79	4.8	2.4*
40	9.8	24	6	26.3	2.6*
35	8.1	25	84	7.3	2.2*
35	8.1	45	43	23.1	2.1*
60	3.3	80	23	79.8	1.9*

* Significantly greater frequency of aberrant cells than the control ($P < 0.05$).

there may have been some functional relation between frequency of aberrant cells and tendency to morphological abnormality since a high frequency of non-functional daughter cells would probably have been a disruptive influence in the meristem. Certainly numbers of aberrant cells and morphological abnormalities were both so great in the drier storage treatments that most of the aberrations observed must have been in root tips which, had they not been sampled, would have become morphologically abnormal. However a general relationship between aberrant cells and morphologically abnormal roots, though plausible, cannot be established from the present studies. If there were such a relation, however, then in addition to diplontic selection within tissues, there would also be a more radical selection pressure for removing gross chromosome damage through failure of abnormal seedlings to survive. Both factors therefore may have led to the large reduction in chromosome aberrations observed in root tips after five weeks growth.

The frequency of recessive mutation in aged seeds

Although most chromosomal aberrations induced by seed ageing are removed by diplontic selection (as we have confirmed here in lettuce plants), point mutations, most of which are recessive, may be transmitted to successive generations. Several types of mutation have been described, but the most obvious are those which cause chlorophyll deficiencies of various types. In several other species such mutations are typically induced in 1 to 3 per cent of surviving seeds when ageing has reduced viability to about 50 per cent (Abdalla and Roberts, 1969a) or sometimes when

even smaller losses of viability have occurred (Dourado and Roberts, 1984b).

In the investigations described here it was not thought necessary to demonstrate that chlorophyll-deficiency or other obvious morphological phenotypic mutations are also induced in lettuce seeds by ageing. This is because the principle is now well-established in other species (Roberts and Ellis, 1984; Roberts, 1987) and, furthermore, the amount of work required to demonstrate an increase in mutation of 1 or 2 per cent is considerable. The work load is large because the recessive mutations induced in the aged seeds (i.e. in the A_1 generation) do not segregate until the A_2 generation. However, since the A_1 plants are genetic chimaeras, it is not until the A_2 generation that Mendelian segregation occurs. Thus, if the genetic nature of a mutation is to be confirmed unequivocally, lines containing putative mutations need to be followed through to the A_3 generation.

No attempt was made here then simply to establish that phenotypic mutations result from seed ageing. Nevertheless, a genetical investigation was carried out to establish whether large amounts of mutation are transmitted to progeny after loss of seed viability under relatively dry conditions. The reasons were as follows. A relation has often been observed between chromosomal aberrations and gene mutation (Roberts, Abdalla and Owen, 1967). The frequency of chromosomal aberrations observed here in dry lettuce seeds was much greater than has previously been recorded as a result of seed ageing. This was obviously partly a function of dryness but it is also probably a function of species since it is clear that some species show more aberration than others under similar circumstances (Roberts and Ellis, 1984).

TABLE 2. Frequency of A_1 plants containing recessive mutations confirmed by segregation in A_2 and A_3 following seed storage under various conditions

Experiment no.	Storage treatment			Normal germination (%)	Total no. of A_2 lines observed, each derived from a single A_1 plant	No of A_2 lines in which a mutant phenotype* occurred	A_1 plants containing mutants (%)
	Temperature (°C)	Moisture content (%)	Duration				
1	Control		0	98	486	2 luteo-maculata 1 narrow-leaved	0.62
	40	9.8	12 d	79	514	3 luteo-maculata 1 lutescens 1 chlorotica 1 dwarf	1.17 ^{ns}
	40	9.8	19 d	23	527	3 luteo-maculata 1 viridalbo-maculata 1 chlorina virescens	0.95 ^{ns}
2	Control		0	98	169	0	0
	50	9.9	54 h	67	208	1 luteo-maculata	0.32 ^{ns}
	50	5.5	30 d	68	198	1 luteo-maculata 1 chlorina-virescens 1 thick-leaved dwarf 2 thin-leaved dwarf	2.65 ^{ns}

* Photographs and complete descriptions of phenotypes are given in Rao (1986); the terminology follows that derived for pea by Blixt (1961).
ns, Not significantly different from control ($P > 0.05$).

But, whatever the reason, the possibility existed that large amounts of gene mutation might be induced in lettuce under conditions used in commercial and long-term seed storage. From the standpoint of seed storage practice for genetic conservation the visible chromosomal aberrations themselves are of little consequence because of their removal by diplontic selection. However, if the large amounts of aberration observed here were correlated with very large amounts of heritable gene mutation, this would be worrying. Accordingly an investigation was undertaken to check whether large amounts of heritable mutation result when dry seeds lose viability during storage.

In order to have a 90 per cent chance of showing an increase in the frequency of mutations from a base level of 1 per cent to a level after storage of 2 per cent, with a probability of being wrong of 0.05 or less, for example, the number of A_1 plants examined would need to be 3098. But in order to demonstrate an increase per treatment in mutation from a base level of 1 per cent to a level after storage of 5 per cent with otherwise similar assumptions, the number of A_1 plants in each treatment would need to be only 377. Two experiments were therefore carried out in which the number of A_1 plants was intended to be similar to the latter value, since this should be ample to demonstrate any increase in mutation which would be disconcertingly larger than those which have been observed in previous studies on other species using seed at higher moisture contents.

In the first of these experiments sufficient seeds were stored to enable approximately 500 A_1 plants per treatment to be available after allowance had been made for loss of viability and poor emergence. Seeds were aged at 40 °C and 9.8 per cent moisture content for 12 d, which led to a reduction in normal germination from 98 to 79 per cent; another batch was stored for a further 7 d under these conditions so that normal germination fell to 23 per cent. In neither case was the observed increase in the number of A_1 plants segregating phenotypic mutations in subsequent generations sufficient to achieve significance (Table 2).

In a second experiment seeds were stored at 50 °C at 9.9 per cent moisture content for 54 h, or 5.5 per cent moisture content for 30 d so that normal seed germination was reduced from 98 to 67 or 68 per cent, respectively. In this case approximately 170 to 200 A_1 plants were established per treatment, less than originally intended owing to poorer emergence than expected, but nevertheless sufficient to give a 90 per cent probability of showing an increase in the frequency

of mutation in the progeny from zero to 5 per cent, or from 1 to 8 per cent. Again the increases in mutation observed following storage at either moisture content were not sufficient to achieve significance (Table 2).

Despite the failure to achieve significance in these studies, the maximum frequency of A_1 plants detected was within the range observed in other species at higher moisture contents (Abdalla and Roberts, 1969*a*; Dourado and Roberts, 1984*b*). We conclude that, if loss of viability of lettuce seeds under dry conditions is associated with the induction of recessive mutations, the number transmitted to the progeny is probably little different from what has been reported in seeds of other species stored under more moist conditions.

The removal of gross chromosome damage by (i) failure of abnormal seedlings to survive and (ii) diplontic selection would not directly affect transmission to subsequent generations of any recessive point mutations which may be induced as a result of seed ageing. However, if there were a greater tendency for point mutations to arise in those cells suffering major aberrations then of course there would be a greater tendency for indirect selection against some of these point mutations before they were expressed, particularly in those seeds which contained such large numbers of aberrations that the seedlings failed to survive. It may be that it is due to such factors that large numbers of point mutations, which otherwise might have been expected, were not observed in progeny of those treatments which showed very large amounts of chromosomal aberration.

Consequences for genetic resources conservation by long-term storage

As already mentioned, current preferred practice in seed storage for genetic conservation involves storage at about 5 per cent moisture content, and there is now considerable interest in even lower moisture contents. In order to consider the implications of the work described here for current recommendations and possible future practice five main points should be considered. (1) There is no doubt in lettuce (Fig. 1) and in many other species of orthodox seeds (unpublished work in this laboratory) that improvement in seed longevity increases with decrease in seed moisture content down to 3 per cent (or even lower in some cases). (2) At any given temperature, the lower the moisture content the slower the increase in accumulation of chromosomal aberrations, at least down to 3.3 per cent (Fig. 2). (3) Even though more chromosomal aberrations arise for a given

loss of viability in very dry seeds (Fig. 3), most of the damage is removed by diploptic selection (compare Fig. 3 with Table 1), and possibly through seedling selection. (4) Moreover the relationship between seed viability and chromosomal aberrations is no more deleterious at 3.3 per cent moisture content than it is at 5.5 per cent (Fig. 3). (5) There is no evidence of inheritance of excessively large numbers of point mutations from aged dry seeds (Table 2). Accordingly current practices appear to be justified and the further exploration of even drier seed storage for genetic conservation would seem worthwhile.

In addition to providing these reassurances, the results also confirm that it is prudent to adopt a high regeneration standard in seed storage for genetic conservation as currently recommended (IBPGR, 1985*b*), and this advice is especially apposite for very dry seeds. The regeneration standard is that percentage viability to which a seed accession is allowed to fall but when reached the accession is regenerated, i.e. seed is grown to produce a fresh stock for further storage. For most species a value of 85 per cent viability is currently suggested (IBPGR, 1985*b*); but the possibility of improving this standard in some cases might now be considered.

Our investigations were carried out at high temperatures in order to achieve some loss of seed viability within a reasonable experimental time. They showed no effect of temperature between 30 and 60 °C on the relation between chromosomal aberrations and seed viability. Furthermore, the results obtained by Villiers (1975) with commercial seed stocks at slightly lower temperatures (we presume these would have been stored at 5–15 °C with 6–8 per cent moisture content before Villiers received them) tally with those presented here. We assume, for the present, then, that a similar relationship would be observed in seeds stored at cooler temperatures. But, of course, under such conditions seed deterioration will be very much less rapid. Such evidence as exists from conditions comparable to those employed in long-term storage suggest, as expected, no detectable damage to chromosomes occurs when seeds are stored very dry at sub-zero temperatures for relatively long periods during which there has been no detectable loss of seed viability. For example the seed used in present investigations had been stored since receipt at –20 °C and 5.5 per cent moisture content for six years and yet they contained less than 1 per cent aberrant cells (see intercept value in Fig. 3). Similarly Harrison and Carpenter (1977) found no increase in the frequency of chromosomal aberrations of onion seeds stored for 3.7 years at

–20 °C with 2.6 per cent moisture content, or at –196 °C with 4.0 per cent moisture content.

ACKNOWLEDGEMENTS

This work was undertaken while N. K. Rao was on leave from the International Crops Research Institute for the Semi-Arid Tropics, Hyderabad (ICRISAT) under the sponsorship of the Association of Commonwealth Universities. It complements work at Reading being supported by the International Board for Plant Genetic Resources, Rome (IBPGR). We are most grateful to these organizations for their cooperation and support. We also thank Dr P. Hadley for providing some growing facilities.

LITERATURE CITED

- ABDALLA, F. H. and ROBERTS, E. H., 1968. Effects of temperature, moisture and oxygen on the induction of chromosome damage in seeds of barley, broad beans and peas during storage. *Annals of Botany* **32**, 119–36.
- 1969*a*. The effects of temperature and moisture on the induction of genetic changes in seeds of barley, broad beans and peas during storage. *Annals of Botany* **33**, 153–67.
- 1969*b*. The effect of seed storage conditions on the growth and yield of barley, broad beans, and peas. *Annals of Botany* **33**, 169–84.
- AVERY, A. G. and BLAKESLEE, A. F., 1936. Visible mutations from aged seeds. *American Naturalist* **70**, 36–37.
- BLIXT, S., 1961. Quantitative studies of induced mutations on peas. V. Chlorophyll mutations. *Agri Hortique Genetica* **19**, 402–47.
- CARTLEDGE, J. L. and BLAKESLEE, A. F., 1934. Mutation rate increased by ageing seeds as shown by pollen abortion. *Proceedings of the National Academy of Science* **20**, 103–10.
- BARTON, L. V. and BLAKESLEE, A. F., 1936. Heat and moisture as factors in the increased mutation rate from *Datura* seeds. *Proceedings of the American Philosophical Society* **76**, 663–85.
- DOURADO, A. M. and ROBERTS, E. H., 1984*a*. Chromosome aberrations induced during storage in barley and pea seeds. *Annals of Botany* **54**, 767–79.
- 1984*b*. Phenotypic mutations induced during storage in barley and pea seeds. *Annals of Botany* **54**, 781–90.
- ELLIS, R. H., HONG, T. D. and ROBERTS, E. H., 1986. Logarithmic relationship between moisture content and longevity in sesame seeds. *Annals of Botany* **57**, 499–503.
- GAUL, H., 1961. Studies on diploptic selection after X-irradiation of barley seeds, pp. 117–38. In *Effects of Ionizing Radiations on Seeds*, 655 pp. International Atomic Energy Agency, Vienna.

- HARRISON, B. J., 1966. Seed deterioration in relation to storage conditions and its influence upon germination, chromosomal damage and plant performance. *Journal of the National Institute of Agricultural Botany* 10, 644–63.
- and CARPENTER, R., 1977. Storage of *Allium cepa* seed at low temperatures. *Seed Science and Technology* 5, 699–702.
- IBRAHIM, A. E. and ROBERTS, E. H., 1983. Viability of lettuce seeds. I. Survival in hermetic storage. *Journal of Experimental Botany* 34, 620–30.
- and MURDOCH, A. J., 1983. Viability of lettuce seeds. II. Survival and oxygen uptake in osmotically controlled storage. *Journal of Experimental Botany* 34, 631–40.
- INTERNATIONAL BOARD FOR PLANT GENETIC RESOURCES, 1985a. *Cost-effective Long-term Seed Stores*, 38 pp. International Board for Plant Genetic Resources, Rome.
- 1985b. *IBPGR Advisory committee on Seed Storage. Report of the Third Meeting*, 22 pp. International Board for Plant Genetic Resources, Rome.
- INTERNATIONAL SEED TESTING ASSOCIATION, 1985a. International rules for seed testing. Rules 1985. *Seed Science and Technology* 13, 299–355.
- 1985b. International rules for seed testing. Annexes 1985. *Seed Science and Technology* 13, 356–513.
- MURATA, M., ROOS, E. E. and TSUCHIYA, E. T., 1979. Relationship between loss of germinability and the occurrence of chromosomal aberrations in artificially aged seed in barley. *Barley Genetics Newsletter* 9, 65–7.
- 1981. Chromosome damage induced by artificial seed ageing in barley. I. Germinability and frequency of aberrant anaphases at first mitosis. *Canadian Journal of Genetics and Cytology* 23, 267–80.
- TSUCHIYA, E. T. and ROOS, E. E., 1984. Chromosome damage induced by artificial seed ageing in barley. *Theoretical and Applied Genetics* 67, 161–70.
- NAVASHIN, M., 1933a. Origin of spontaneous mutations. *Nature* 131, 436.
- 1933b. Ageing of seeds is a cause of chromosome mutations. *Planta* 20, 233–43.
- and GERASSIMOVA, H., 1936a. Natur und Ursachen der Mutationen. I. Das Verhalten und die Zytologie der Pflanzen, die aus infolge Alterns mutierten Kernen stammen. *Cytologia* 7, 324–62.
- 1936b. Natur und Ursachen der Mutationen. III. Über die Chromosomenmutationen, die in den Zellen von ruhenden Pflanzenkeimen bei deren Altern auftreten. *Cytologia* 7, 437–65.
- PETO, F. H., 1933. The effect of ageing and heat on the chromosomal mutation rates in maize and barley. *Canadian Journal of Research* 9, 261–64.
- PETRUZZELLI, L. 1986. Wheat viability at high moisture content under hermetic and aerobic storage conditions. *Annals of Botany* 58, 259–65.
- RAO, N. K., 1986. Chromosomal aberrations and gene mutations induced in lettuce (*Lactuca sativa* L.) seeds during storage, 214 pp. Ph.D. Thesis, Reading University.
- ROBERTS, E. H., 1978. Mutations during seed storage. *Acta Horticulturae* 83, 279–82.
- 1986. Quantifying seed deterioration, pp. 101–122. In *Physiology of Seed Deterioration*, eds M. B. McDonald Jr and C. J. Nelson, 123 pp. Crop Science Society of America, Madison, Wisconsin.
- 1987. Seed ageing – the genome and its expression. In *Senescence and Ageing in Plants*, eds L. D. Nooden and A. C. Leopold. Academic Press, New York (in press).
- and ELLIS, R. H., 1984. The implications of the deterioration of orthodox seeds during storage for genetic resources conservation, pp. 18–37. In *Crop Genetic Resources: Conservation and Evaluation*, eds J. H. W. Holden and J. T. Williams, 296 pp. George Allen & Unwin, London.
- ABDALLA, F. H. and OWEN, R. J., 1967. Nuclear damage and the ageing of seeds, pp. 65–100. In *Aspects of the Biology of Ageing*. S.E.B. Symposium XXI, ed. H. W. Woolhouse, 634 pp. Cambridge University Press, Cambridge.
- SIRIKWANCHAI, S., 1985. The effect of moisture and oxygen on the accumulation of chromosome damage in relation to loss of viability in stored onion (*Allium cepa* L.) seed, 190 pp. Ph.D. Thesis, Reading University.
- VILLIERS, T. A., 1974. Seed ageing: chromosome stability and extended viability of seeds stored fully imbibed. *Plant Physiology* 53, 875–78.
- 1975. Genetic maintenance of seeds in imbibed storage, pp. 297–316. In *Crop Genetic Resources for Today and Tomorrow*, eds O. H. Frankel and J. G. Hawkes, 492 pp. Cambridge University Press, Cambridge.
- and EDGCUMBE, D. J., 1975. On the cause of seed deterioration in dry storage. *Seed Science and Technology* 3, 761–74.