

The Influence of Pre and Post-storage Hydration Treatments on Chromosomal Aberrations, Seedling Abnormalities, and Viability of Lettuce Seeds

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ABSTRACT

A 2 h soaking treatment in distilled water, or in aqueous solutions of cysteine, potassium iodide, or sodium thiosulphate, had no significant effect ($P > 0.25$) on the subsequent longevity of lettuce seeds (*Lactuca sativa* L.) in two different storage environments. Neither did these treatments influence relations between loss in germination and the frequency of chromosomal aberrations observed during first mitoses after storage. In contrast partial hydration of lettuce seeds after storage by exposure to moist air (humidification) or to an osmoticum (priming) reversed some of the damage which resulted from ageing. Most of the benefits occurred during the first 3 d of humidification during which seed moisture content rose to 34 per cent, or during the first 7 d of priming when seed moisture content increased to 44 per cent. Both post-storage hydration treatments reduced the frequency of chromosomal aberrations, increased the rate of root growth, and decreased the frequency of morphologically abnormal seedlings. Either treatment could be of practical use, but it is suggested that humidification is more convenient. Consideration should be given to adopting a humidification treatment as standard practice following long-term seed storage for genetic conservation.

Key words: *Lactuca sativa*, lettuce, seed storage, seed viability, chemical pre-treatment, seed longevity, seed humidification (conditioning), seed priming, chromosome repair, seedling abnormalities.

INTRODUCTION

We have shown that in lettuce (*Lactuca sativa* L.) seed moisture content during storage markedly influences the relation between percentage seed viability and the number of chromosomal aberrations which accumulate: with decrease in seed moisture between 13.0 and 5.5 per cent moisture content there is a marked increase in the percentage of aberrant anaphase configurations for any given loss in percentage normal germination (Rao, Roberts and Ellis, 1987). In that paper we argued that the effects observed were probably not due to differences in repair. This conclusion was based on other indirect evidence which suggests that repair of sub-cellular components is not feasible in lettuce seeds at moisture contents below 15 per cent. Providing oxygen is present, some repair may be possible at this moisture content, and further improvements in the rate of repair are possible as

moisture content increases up to full hydration at 47-50 per cent moisture content (Ibrahim and Roberts, 1983; Ibrahim, Roberts and Murdoch, 1983).

Villiers and Edgcumbe (1975) showed that the frequency of anaphase cells containing chromosomal aberrations in lettuce seeds which had been stored at 30 °C and 7 per cent moisture content for five months was progressively reduced from 8 to 6 per cent when seeds were subsequently stored fully imbibed (i.e. at about 50 per cent moisture content) at 30 °C for a further seven months. However after more severe deterioration, during which the frequency of aberrant cells had increased to 15 per cent, they reported that improvement was not possible because such seeds were subject to microbial attack in imbibed storage (Villiers and Edgcumbe, 1975). They also showed that imbibed storage of lettuce seeds following γ -irradiation also reduced the frequency of aberrant cells and increased hypocotyl lengths.

A similar recovery from damage caused by chemical mutagens has been extensively investigated in barley (*Hordeum vulgare* L.). Gichner,

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Veleminský and Pokorný (1974) showed that seeds stored at 30 per cent moisture content for up to six weeks after various mutagen treatments were capable of some recovery as indicated by increased seedling height, decreased frequency of chromosomal aberrations in radicle tips and by a decrease in frequency of chlorophyll-deficiency mutations appearing in the next generation. Storage at 15 per cent moisture content after mutagen treatment was not sufficient to support repair activity and indeed it exacerbated the problem (Švachulová, Gichner and Veleminský, 1973; Soyfer *et al.*, 1977; Gichner and Veleminský, 1979). It has been argued elsewhere that water potentials in excess of -14.5 MPa are probably necessary for repair and this means a greater percentage moisture content, say 28–30 per cent, in barley as compared with approx. 15 per cent in an oily seed like lettuce (Roberts, 1987). Some recovery from damage probably occurs automatically during the imbibition which is a prelude to germination, e.g. Osborne (1982) has cited evidence of unscheduled DNA repair activity during the initial hydration of rye (*Secale cereale* L.) seeds when they are set to germinate.

In addition to this evidence of repair of DNA and chromosomes, there is also considerable evidence that short periods of hydration can lead to a considerable improvement in other aspects of seed quality. For example seed priming – in which seeds are held before germination in an osmoticum at a water potential which prevents germination – can reduce the time taken for seeds to germinate, and some of this effect is maintained even if the seeds are dried back before germination (Heydecker, Higgins and Gulliver, 1973; Heydecker, Higgins and Turner, 1975; Heydecker and Gibbins, 1978). Short periods of imbibition in water, which are too short to allow germination, followed by drying can also increase germination rate and extend longevity. Work on this subject, pioneered by R. N. Basu and his colleagues on a number of species, is extensive and has been reviewed elsewhere (Roberts, 1983). It has been supported by Goldsworthy, Fielding and Dover (1982) and Kuo and Chin (1986) in wheat and rice seeds respectively. Work in this laboratory on lettuce seed (Ibrahim, 1981) has shown that at 25°C the optimum period of a single hydration treatment for extending longevity was 2–4 h and typically such treatments increased longevity by 10–20 per cent. Successive short periods of hydration, however, were capable of extending longevity by a factor of two to three.

Slow hydration treatments following seed storage may therefore be beneficial in repairing some

of the nuclear and cytoplasmic damage that has occurred. Some repair will automatically occur during initial imbibition which is an integral part of the germination process but, in order for repair to chromosomes to be maximized, it may be best if the period for repair could be prolonged before semi-conservative DNA replication during the normal cell cycle consolidates errors beyond the possibility of repair. This approach implies hydration treatments involving water potentials below that required for germination, but well above the critical moisture content (15 per cent in lettuce) postulated to be the minimum necessary for the repair of cellular components.

There was a further reason for exploring alternative hydration treatments following dry storage. It is now well established that when very dry seeds, such as those stored for long-term genetic conservation, are tested for germination, the rapid uptake of water which ensues on contact with liquid water can lead to imbibition injury and decreased germination (e.g. Ellis, Osei-Bonsu and Roberts, 1982). Moreover, there is also some evidence to suggest that very rapid hydration increases mutation (Blixt, 1965). Consequently genebanks have been recommended to condition seeds in an atmosphere of high relative humidity for, say, 24 h at 20°C before germinating them to ensure slow initial uptake of water (Ellis, Hong and Roberts, 1985).

Instead of trying to repair damage after it has occurred, an alternative is to attempt to protect seeds from damage by treating them before storage. This has been attempted by either hydrating and then dehydrating seeds before storage (Basu, 1976; Savino, Haigh and de Leo, 1979) or by treating the seeds with various chemicals in aqueous solution and then dehydrating the seeds (Basu, 1976; Basu and Dasgupta, 1978). There is also some suggestion that certain chemical pre-treatments can improve longevity independently of hydration (Basu, Pan and Punjabi, 1979; Woodstock *et al.*, 1983). However, Barnes and Berjak (1978) have suggested that the main benefits derive from hydration and dehydration rather than the presence of the chemicals. The major technical problem with investigations of this type is that seed longevity is extremely sensitive to slight differences in seed moisture content (see, particularly, Ellis and Roberts, 1981; Ellis, Hong and Roberts, 1986). It is difficult to adjust the moisture content of different batches of seed after hydration treatments to precisely the same value, and this may explain some of the differences reported in the literature. Of the many diverse chemicals investigated by Basu (1976) the most effective, apparently, in

reducing subsequent seed deterioration were anti-oxidants and those chemicals that are known to control free radical chain reactions in radiobiological studies. Examples of these types of chemicals were chosen for the study reported here on the basis of their apparent efficacy in the earlier reports (particularly Basu, 1976; Basu and Dasgupta, 1978).

Consequently in this paper the effect of various pre-storage and post-storage treatments of seeds were investigated. The pre-storage hydration treatments included conventional radiation protectants, potassium iodide and cysteine, and an anti-oxidant, sodium thiosulphate. The post-storage hydration treatments were humidification in air, and priming in a polyethyleneglycol solution.

MATERIALS AND METHODS

Achenes (hereafter described as seeds) of the lettuce cv. Trocadero Improved were used in this study. Before any of the various treatments were applied these seeds had an initial moisture content of 5.5 per cent, showed 99.8 per cent total germination (radicle protrusion), 97.8 per cent normal germination (morphologically normal seedlings), and 1.101 per cent of the root tip cells at anaphase in the first mitotic division showed abnormal configurations. The seed lot and general methods of hermetic storage were the same as those reported in a previous paper (Rao *et al.*, 1987).

Treatment of the seeds prior to storage was carried out by soaking samples of seeds in double their own volume of distilled water, or aqueous solutions of cysteine (10^{-5} M), potassium iodide (10^{-4} M), or sodium thiosulphate (10^{-5} M) for 2 h at 20 °C. The seeds were then surface dried, weighed, and then dried at 20 °C and 25–30 per cent r.h. until their weight declined to that anticipated for 6 or 8 per cent moisture content. The seeds were then sealed in laminated aluminium foil packets at 3 °C for 24 h to enable moisture to equilibrate throughout the seeds. Moisture contents were then determined using the high constant temperature oven method (ISTA, 1985*a, b*). Additional unsoaked control treatments were provided by storing seeds at either 40 or 50 °C combined with approx. 8 or 6 per cent moisture content, respectively.

Humidification of the seeds following storage was carried out by placing the seeds in a water-saturated atmosphere at 20 °C for one or more days. The change in weight following humidification was determined so that seed moisture content could be estimated. Priming of the seeds following storage was carried out by placing 400

seeds on top of a filter paper (Whatman 181) moistened with 5 ml of Polyethyleneglycol 6000 solution at an osmotic potential of -0.5 MPa (212 g kg water $^{-1}$) at 30 °C for various periods up to 28 d. Following this treatment the seeds were washed in running water for 5 min, rinsed in distilled water and then dried at 20 °C and 40–45 per cent r.h. for about 2 h.

Germination tests were carried out as before using four replicates per test of 50 or 100 seeds per replicate in Petri dishes (Rao *et al.*, 1987), except that primed seeds were pre-chilled at 3 ± 1 °C for 7 d to remove secondary dormancy induced during the priming treatment. Accordingly the control samples which were compared with these treatments were also pre-chilled at 3 ± 1 °C for 7 d. After 7 d in test at 20 °C the seedlings produced during the germination tests were evaluated for morphological abnormalities and seedling root lengths were recorded.

The cytological methods were as described previously (Rao *et al.*, 1987), except that following either the humidification or priming treatments radicles of only 1.5 mm were selected for cytological examination in order to coincide with the peak of first mitotic divisions which occurred earlier in these samples. The aberrations observed within each cell were classified as chromatid or chromosome-types, mixed, multiple or others. Chromatid-type aberrations comprised single fragments, two or more fragments of unequal size, or a ring fragment with or without an accentric fragment. Chromosome-type aberrations comprised one or more double fragments, one or more double bridges with or without double fragments, or two ring fragments. Where both chromosome and chromatid-type aberrations were observed within a cell the damage was classified as mixed. In some cells the damage was extensive and difficult to categorize; in such cases the damage was classified as multiple. A small category, other damage, of lagging chromosomes was also recorded.

RESULTS

Hydration and chemical treatments before storage

Despite slight differences in moisture content, none of the treatments with aqueous solutions of cysteine, potassium iodide or sodium thiosulphate applied before storage significantly influenced subsequent longevity at either 50 °C with 5.8–6.1 per cent moisture content or 40 °C with 7.7–8.0 per cent moisture content ($P > 0.25$); single survival curves for each environment are therefore shown (Fig. 1) which explain 83 per cent of the variation

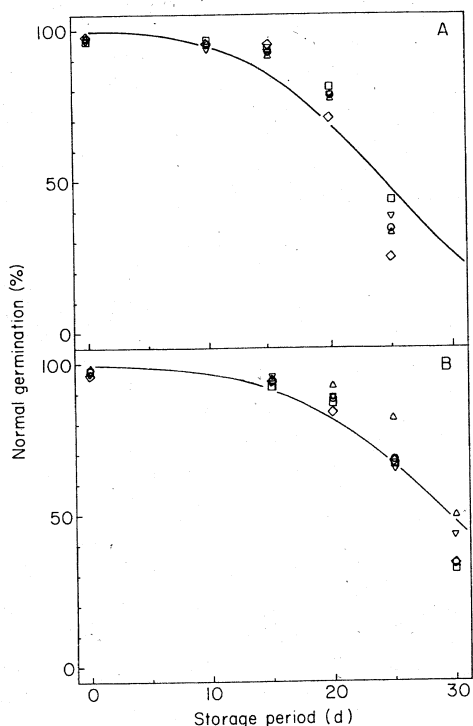


FIG. 1 The influence of pre-storage hydration/dehydration treatments in distilled water (○), or aqueous solutions of 10⁻⁵ M cysteine (□), 10⁻⁴ M potassium iodide (▽), or 10⁻⁵ M sodium thiosulphate (△) on the subsequent reduction in percentage normal germination with storage period for lettuce seeds stored at A, 50 °C with approx. 6 per cent moisture content, and B, 40 °C with approx. 8 per cent moisture content. Unsoaked seeds (◇) were also stored as a control treatment. The two survival curves shown are negative cumulative normal distributions with a common intercept of 2.75 and standard deviations of A, 8.9 d, and B, 10.7 d. A common curve for all treatments in each environment is provided as no significant treatment effect was detected ($P > 0.25$). Precise seed storage moisture contents for the two environments were: 6.0 and 7.8 per cent (control); 6.1 and 8.0 per cent (distilled water); 5.8 and 7.9 per cent (cysteine); 6.0 and 7.7 per cent (potassium iodide); and 6.1 and 7.8 per cent (sodium thiosulphate).

observed. The frequency of chromosomal aberrations after 20 d storage at 50 °C with 5.8–6.1 per cent moisture content was not significantly influenced by pre-treatment ($P > 0.2$). After 25 d storage at 40 °C with 7.7–8.0 per cent moisture content a significant effect on the frequency of chromosomal aberrations ($P < 0.01$) of pre-treatment with sodium thiosulphate (subsequent seed moisture content 7.8 per cent) only, was detected. However, Fig. 2 shows that all the results following

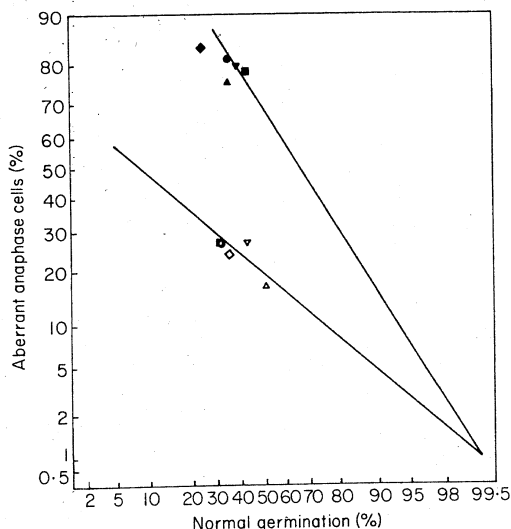


FIG. 2. A comparison of the relation between the percentage of aberrant cells at first mitosis (probability scale) and percentage normal germination (probability scale) of lettuce after 25 d storage at 50 °C with approx. 6 per cent moisture (solid symbols) and after 30 d storage at 40 °C with approx. 8 per cent moisture content (open symbols), following the various pre-storage hydration/dehydration treatments described in Fig. 1, with those relationships (solid lines) defined at these moisture contents in an earlier study (Rao *et al.*, 1987).

pre-treatment and storage conformed to the relations between loss in germination and frequency of chromosomal aberrations at the two moisture contents quantified by Rao *et al.* (1987) previously. Thus there is no evidence that any of the pre-treatments influenced either loss in normal germination or the accumulation of chromosomal aberrations with loss in germination during storage.

Hydration treatments after storage: (i) humidification

The results following humidification of the seeds at 20 °C for 24 h after storage in several different regimes (Table 1) show that when seed moisture content after storage was raised to values between 25 and 32 per cent over a period of 24 h, total germination was not altered but normal germination was increased by 7–28 per cent and the production of morphologically abnormal seedlings was correspondingly reduced by 5–25 per cent. It follows that many of the seeds which otherwise would have produced ageing-induced morphologically abnormal seedlings were altered by the

TABLE 1. The influence of post-storage humidification of lettuce seeds for 24 h on normal germination, abnormal germination, and the frequency of aberrant anaphase cells at the first mitotic division

Storage environment			Humidification	Subsequent moisture content (% f.wt)	Normal germination (% mean \pm s.e.)	Abnormal germination (% mean \pm s.e.)	Aberrant cells (%)
Moisture (% f.wt)	Temperature (°C)	Period (d)					
3.3	50	350	—	(3.3)	74 (± 2.1)	19 (± 1.5)	44.5
			+				
		400	—	27.4	87 (± 2.2)	8 (± 1.2)	35.6
			+	(3.3) 30.7	48 (± 1.9) 60 (± 2.9)	46 (± 0.7) 33 (± 1.1)	72.2 54.5
5.5	50	25	—	(5.5)	81 (± 1.6)	17 (± 1.1)	50.9
			+				
		30	—	25.2	91 (± 2.3)	8 (± 1.6)	36.1
			+	(5.5) 31.5	65 (± 1.1) 82 (± 2.2)	31 (± 1.8) 17 (± 2.6)	71.2 53.4
8.1	30	35	—	(5.5)	26 (± 2.2)	63 (± 1.5)	80.9
			+	30.3	38 (± 1.3)	51 (± 2.7)	72.1
		50	—	(8.1)	82 (± 1.9)	12 (± 1.8)	9.3
			+				
		70	—	26.8	89 (± 1.6)	7 (± 0.4)	7.5
			+	(8.1)	55 (± 4.0)	36 (± 2.3)	23.0
			—				
			+	26.3	83 (± 1.3)	11 (± 0.8)	20.7

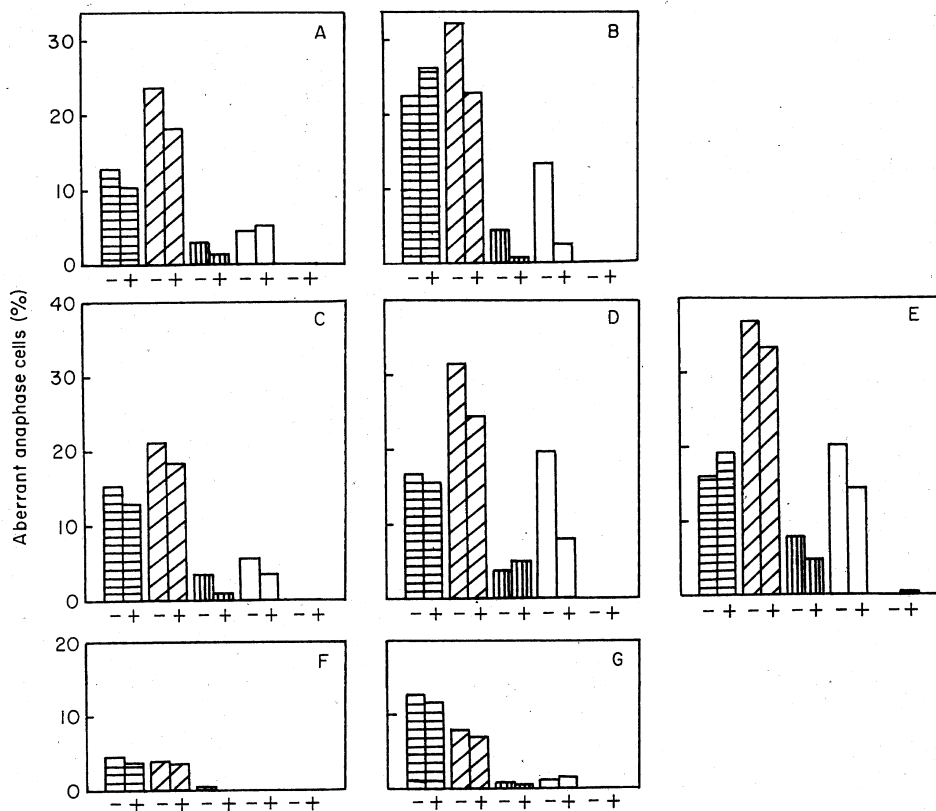


FIG. 3. Comparison of the frequencies of the major types of chromosomal aberrations (chromatin-type, horizontal lines; chromosome-type, oblique lines; mixed-type, vertical lines; multiple, open; others, solid) for lettuce seeds not humidified (—) or humidified for 24 h in a water-saturated atmosphere at 20 °C (+) prior to the germination test following storage at 50 °C with 3.3 per cent moisture content for 350 d (A) or 400 d (B); 50 °C with 5.5 per cent moisture content for 25 d (C), 30 d (D), or 35 d (E); or 30 °C with 8.1 per cent moisture content for 50 d (F) or 70 d (G). The proportions of seeds which produced morphologically normal or abnormal seedlings are given in Table 1.

post-storage partial-hydration treatment so that they produced morphologically normal seedlings. In addition the humidification treatment decreased the frequency of anaphase cells which showed chromosomal aberrations by between 1.8 and 18 per cent. The reduction in frequency was less when seeds had been stored at the highest moisture content, 8.1 per cent. No doubt this was related to the fact that these seeds had accumulated less potential chromosomal aberrations during storage (Table 1): as shown previously (Rao *et al.*, 1987), seeds stored at higher moisture contents develop fewer chromosomal aberrations for a given loss of seed viability. In all cases there was generally some reduction in all categories of chromosomal aberrations. However, seed stored at the lower moisture contents contained relatively large num-

bers of cells showing multiple aberrations and the frequency of these was more reduced by humidification than were the other categories of aberration (Fig. 3). The greater reduction in cells containing multiple aberrations may be because enough of the damage was repaired in these cells for them to be transferred to the other categories which contained less damage; while some aberrations in the categories showing less damage were sufficiently repaired to be classified as normal. Consequently overall there would have been a greater reduction in the category showing multiple aberrations.

Figure 4 shows the effect of the period of humidification on seeds previously stored at 50 °C and 5.9 per cent moisture content for 25 d. During the first 3 d of humidification, seed moisture

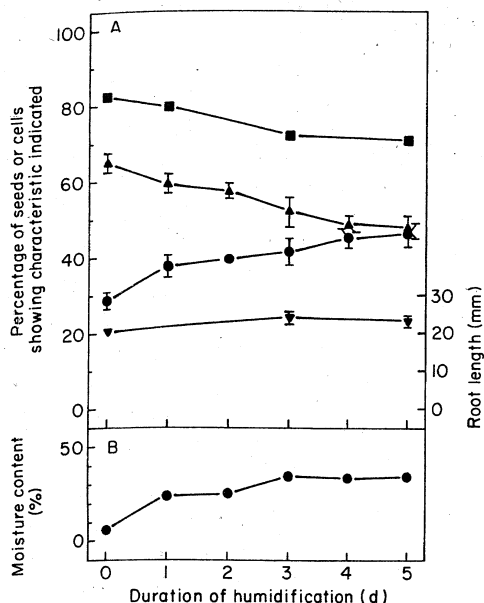


FIG. 4. The influence of the period of humidification in a water-saturated atmosphere at 20 °C on (A), normal germination, % (●), abnormal germination, % (▲), mean root length of normal seedlings after 7 d, mm (▼), and the frequency of aberrant cells in the first mitotic divisions of root tip cells, % (■), and B, moisture content, % f.wt (●). The lettuce seeds had previously been stored at 50 °C with 5.9 per cent moisture content for 25 d. The vertical bars indicate s.e.

content rose to a maximum of 34 per cent and, at the same time, there was a continuous fall in the percentage of anaphase cells containing chromosomal aberration, and an increase in the rate of root growth. Also during this period the proportion of morphologically abnormal seedlings decreased, whilst the proportion of normal seedlings (normal germination) showed a concomitant increase. There was some indication that this trend in decreasing seedling abnormalities may have continued longer but, in general, most of the benefits of humidification in respect of all types of damage were achieved within 3 d (Fig. 4). The investigation on the effect of period of humidification was not continued beyond 5 d because thereafter the seeds succumbed to fungal attack. During the period a large proportion of the cells containing chromosome-type aberrations or multiple aberrations decreased while, at the same time, the number of chromatid-type aberrations increased – presumably because, as a result of repair, some cells which otherwise would have shown

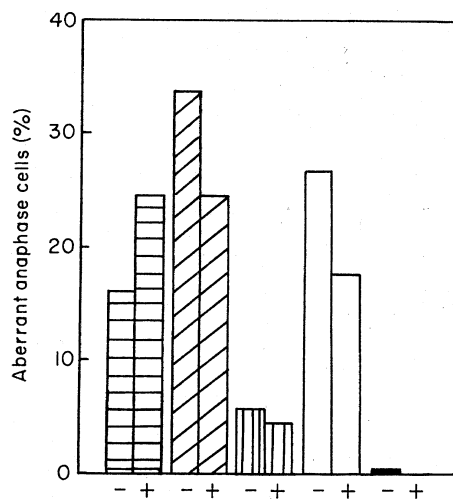


FIG. 5. Comparison of the frequencies of the major types of chromosomal aberrations (chromatid-type, horizontal lines; chromosome-type, oblique lines; mixed-type, vertical lines; multiple, open; others, solid) for lettuce seeds not humidified (-) or humidified for 5 d in a water-saturated atmosphere at 20 °C (+) following 25 d storage at 50 °C with 5.9 per cent moisture content.

multiple aberrations were transferred to this category (Fig. 5).

Hydration treatments after storage: (ii) priming

The moisture content of seeds previously stored at 50 °C with 6.3 per cent moisture content for 20 d rose to 44.1 per cent within 3 d of imbibing at 30 °C on filter papers moistened with a poly-ethyleneglycol solution of -0.5 MPa osmotic potential (Fig. 6). This level of hydration was then maintained until the experiment ended. Most of the benefits of the priming treatment were achieved within 7 d (Fig. 6). During this period the proportion of anaphase cells showing chromosomal abnormalities decreased, the proportion of seeds capable of germinating without developing morphological abnormalities increased, and the rate of root growth also increased. Prolonged priming treatments of three to four weeks slightly reduced some of the benefits already achieved for, while they did not reverse the benefits of reduced chromosomal aberrations, the rate of seedling root growth began to decline a little and the proportion of morphologically abnormal seedlings began to increase.

The overall reduction in the frequency of aberrant anaphase cells which resulted from

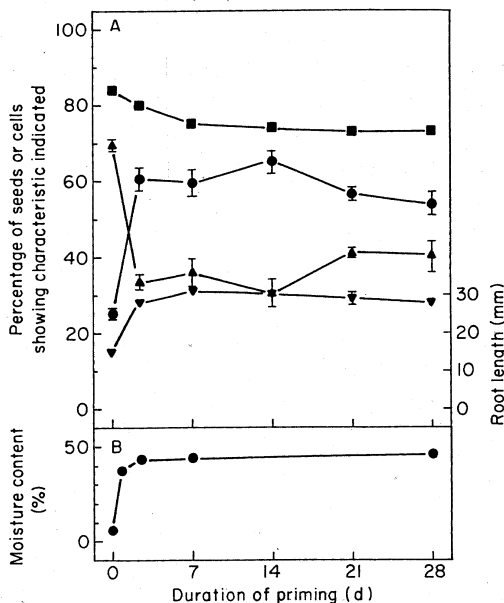


FIG. 6. The influence of the period of priming in a polyethyleneglycol solution at -0.5 MPa on A, normal germination, % (●), abnormal germination, % (▲), mean root length of normal seedlings after 7 d, mm (▼), and the frequency of aberrant anaphase cells in the first mitotic divisions of root tip cells, % (■), and B, moisture content, % f.wt (●). The lettuce seeds had previously been stored at 50°C with 6.3 per cent moisture content for 20 d. The vertical bars indicate s.e.

priming was mainly achieved by a decrease in the frequency of those cells which showed multiple aberrations (Fig. 7). The number of cells showing simple chromatid-type or chromosome-type aberrations increased presumably, as explained before, by partial repair resulting in transfer of cells from the multiple-aberration category.

DISCUSSION

Clearly none of the pre-storage hydration treatments either in water or in solutions of 'protective' chemicals was beneficial in reducing the subsequent deterioration of the seeds when stored in either of the environments used, whether deterioration is considered in terms of longevity (Fig. 1) or the frequency of cells showing aberrant chromosomes (Fig. 2). Although there was an apparent beneficial effect on longevity of pre-treatment with 10^{-5} M sodium thiosulphate when the seeds were stored at 40°C with 7.8 per cent moisture content, this was not significant. Moreover, no effect was apparent in the other storage

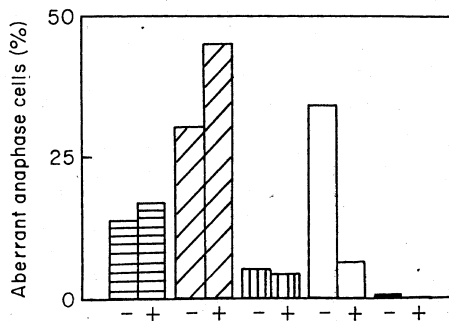


FIG. 7. Comparison of the frequencies of the major types of chromosomal aberrations (chromatid-type, horizontal lines; chromosome-type, oblique lines; mixed-type, vertical lines; multiple, open; others, solid) for lettuce seeds not treated (-) or primed for 28 d in a polyethyleneglycol solution at -0.5 MPa (+) following 20 d storage at 50°C with 6.3 per cent moisture content.

regime. We conclude therefore, that these pre-storage hydration treatments of this high quality seed lot were of little or no benefit in preventing damage. These results contrast with previous work carried out in this laboratory on the same seed lot in which it was shown that, if the seeds were allowed to deteriorate a little before treatment, then significant increases in longevity were observed during subsequent storage at 25°C and various moisture contents between 16 and 40 per cent (Ibrahim, 1981). All this is compatible with the work reported by Basu and Pal (1980) and Kuo and Chin (1986) on rice (*Oryza sativa* L.). Both groups noted no improvement in subsequent longevity resulting from temporary hydration of freshly harvested high-quality rice seeds, but a significant improvement was observed after similar temporary hydration treatments on the same seed lots when the treatments were applied after some deterioration had taken place. It may be that temporary hydration treatments cannot extend longevity in fresh seed since in such cases insufficient damage has accumulated for repair to have a significant effect. The remaining part of this discussion therefore concentrates on the immediate benefits of the partial hydration treatments investigated in this paper which were applied after storage and therefore on seeds which had already undergone some deterioration.

The main purpose of *restraining* the rate of hydration of dry and very dry seeds after storage is to avoid imbibition injury which otherwise might occur when the seeds are set to germinate (Ellis *et al.*, 1985). The main purpose of *maintaining* seeds for a time at a relatively high water potential,

but less than that necessary for germination, is to provide an increased opportunity before germination for the repair of the ageing-induced sub-cellular damage which, to a greater or lesser extent, is an inevitable consequence of storage. Both objectives can be achieved by a single treatment, and two alternative treatments are available for this purpose, viz. either placing seeds in a humid atmosphere which allows them to adsorb water slowly towards an equilibrium state of partial hydration, or placing seeds in contact with an inert osmoticum which has a similar effect. (Note that although a brief soaking treatment in water would achieve the required state of partial hydration, it would also increase the potential damage from imbibition injury because of initial rapid water uptake). The work reported here shows that qualitatively both humidification and priming have similar benefits in terms of reduction in number of chromosomal aberrations, reducing the number of morphological abnormalities which occur during seedling development, and increasing the rate of seedling growth. At first sight it may appear that, according to the latter two criteria, the priming treatment was superior; but in fact this is not necessarily the case since the two experiments are not strictly comparable. The two treatments provided different levels of hydration; the humidified seeds reached only 34 per cent moisture content, whereas the primed seeds reached 44 per cent (Figs 4 and 6).

Provisionally we conclude that the main criterion affecting the choice of which technique to use is one of practical convenience. From this point-of-view there is little doubt that humidification in a saturated atmosphere is the simplest technique for handling seeds in quantity. This treatment has already been recommended for avoiding imbibition injury following the storage of seeds for genetic conservation (Ellis *et al.*, 1985); it is now clear that, because such treatments also raise moisture contents above the critical values for repair, this recommendation carries with it the additional benefit of decreasing chromosome damage. While chromosomal aberration is not of major concern in its own right, owing to subsequent removal of diploic selection, it is typically associated with heritable mutations of major and minor genes (Róberts and Ellis, 1984; Roberts, 1987).

The storage damage manifest as morphological abnormalities and slow rates of root growth in seedlings may well be due to deterioration of several sub-cellular systems, all of which do not necessarily involve DNA. In this paper, however, we are more specifically concerned with damage to

the genome and its repair because of the implication of these processes for genetic conservation. A previous paper dealt mainly with the effect of moisture content during storage on the induction of damage to chromosomes (Rao *et al.*, 1987); and so the remainder of this discussion will focus on their repair.

The results of the cytological investigations reported in this paper show close parallels with earlier work on the partial hydration of seeds following their treatment with chemical mutagens. For example when barley seed after treatment with mutagens is subsequently held at 30 per cent moisture content – but not at 20 per cent moisture content or less – then the mutagenic damage is ameliorated. This has been shown by an increase of seedling growth rate and a decrease in the frequency of chromosomal aberrations and also by a decrease in the frequency of chlorophyll-deficiency mutants in the following (M_2) generation (see the extensive work reviewed by Gichner *et al.*, 1974). The recovery from damage involves repair enzymes, is encouraged by aerobic conditions (Švachulová, Gichner and Velemínský, 1973), and is connected with repair of DNA (Velemínský and Gichner, 1976).

It has previously been shown in lettuce seeds that extension of seed longevity with increase in moisture content becomes possible above 15 per cent moisture content, but only in the presence of oxygen (Ibrahim and Roberts, 1983; Ibrahim *et al.*, 1983). At 20 °C (the temperature adopted for the humidification treatment in the investigations reported here) it has previously been shown (Ibrahim *et al.*, 1983) that lettuce seeds develop relatively high rates of respiration after 2 d which gradually settle down to a lower steady state after about a week at moisture contents covering the range used here; similar effects were shown at 30 °C (the temperature adopted for the priming treatment used here) although, as one would expect, the peak rate of oxygen uptake occurs earlier at 30 °C and the steady-state rate is about twice that at 20 °C (Table 2). Clearly, significant amounts of respiration can be supported over the range of moisture contents used in the hydration treatments in the experiments reported here (24–34 per cent in the humidification treatments and 44 per cent in the priming treatment). Khan *et al.* (1978) showed that osmotically conditioned lettuce seeds are capable of RNA and protein synthesis; they also found that the activity of acid phosphatase and esterase was sustained in seeds treated in this way. Studies on other species have shown evidence of considerable metabolic activity in seeds exposed to high relative humidity, e.g. protein

TABLE 2. Rate of oxygen uptake in lettuce seeds (*cv. Trocadero Improved*) imbibed in air on polyethyleneglycol (PEG) solutions of different osmotic potentials at 20 and 30 °C (from Ibrahim, Roberts and Murdoch, 1983)

Water potential of PEG solution (MPa)	Moisture content of seeds (per cent f. wt)	Peak oxygen uptake ($\mu\text{l g}^{-1} \text{ d. wt h}^{-1}$, achieved after the time shown in parentheses)		Steady-state oxygen uptake (approx.) measured after 14–20 d ($\mu\text{l g}^{-1} \text{ d. w h}^{-1}$)	
		20 °C	30 °C	20 °C	30 °C
0 (water)	47	1080(1 d)	—	*	—
–0.5	44	—	680(12 h)	—	170
–2.5	32	170(2 d)	400(12 h)	70	150
–5.0	24	100(2 d)	300(12 h)	50	100
–7.5	19.5	80(2 d)	280(12 h)	40	70
–10.0	17	60(6 d)	200(12 h)	30	50

* Not measured owing to onset of germination.

turnover in soyabeans (*Glycine max* [L.] Merrill) (Knypl, Janas and Radziwonowska-Joswiak, 1980) and nucleic acid metabolism in tomato (*Lycopersicon esculentum* Mill.) (Coolbear and Grierson, 1979).

It has been argued elsewhere (Dourado and Roberts, 1984; Roberts, 1987) that most of the chromosome damage that arises as a result of seed storage is most easily interpreted on the basis of the Exchange Theory of chromosome breakage originally suggested by Revell (1959) and further developed by Evans (1977). According to this interpretation the primary event of ageing which occurs during seed storage is an unstable lesion which only gives rise to an aberration in one of the resulting chromatids during semi-conservative replication when the whole genome is reproduced during interphase.

Repair of lesions therefore needs to take place before they are consolidated as chromatid breaks (or possibly as gene mutations) during semi-conservative DNA replication which is scheduled to occur when the entire genome is replicated during the S phase following G_1 . In rye embryos there is evidence that a certain amount of unscheduled DNA synthesis occurs during the initial phase of imbibition which initiates the onset of germination, i.e. during the lag phase which precedes semi-conservative DNA replication during germination (Osborne, 1982). Under humidification conditions similar to those used in this paper, the evidence suggests that very little or no semi-conservative DNA synthesis occurs (e.g. Gichner and Velemínský, 1979; Coolbear and Grierson, 1979; Hecker and Köhler, 1979). But

such conditions, as we have documented, allow a considerable amount of varied metabolism to take place and, in effect, may be considered as a method of prolonging the lag phase during which some DNA repair may be feasible.

It may be possible that further manipulation of the precise conditions of partial hydration before germination might improve further the amount of repair that is facilitated. In the meantime the humidification technique described by Ellis *et al.* (1985) for avoiding imbibition injury is now also recommended to minimize damage which will have occurred in dry storage, especially when there has been considerable loss of seed viability. In view of the additional benefit in avoiding imbibition injury after storage at very low moisture contents (Ellis *et al.*, 1985), it is suggested that post-storage humidification treatments should become routine in genebanks not only for germination tests used to monitor viability, but also when sowing seeds to produce plants for regeneration or utilization in breeding programmes.

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