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Control of Age- and Irradiation-Induced Seed Deterioration in Lettuce (*Lactuca sativa* L.) by Hydration-Dehydration Treatments

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Hydration-dehydration treatment of stored lettuce seed (1-year-old, medium-vigour, greatly slowed down their deterioration during subsequent storage under accelerated and natural ageing conditions. Hydration-dehydration of seeds, before or soon after X- and γ-irradiation, considerably minimized the adverse effect of irradiation on the development of biological after-effects responsible for the fall in germinability, especially the large reduction of root growth of seedlings. Pre- and post-irradiation treatments gave broadly similar effects. The results have been discussed in terms of a possible involvement of a cellular (biochemical) repair mechanism in the hydration phase and also on the basis of a physico-chemical control of free radical pathology in the aged and irradiated seed.

Key Words: Lettuce, Seed deterioration, Irradiation, Ageing, Hydration-dehydration

Introduction

Hydration-dehydration pretreatment of stored seeds greatly reduces their physiological deterioration under accelerated and natural ageing conditions (Basu et al. 1974 and Basu 1976). These treatments have shown highly significant radioprotective action in wheat and jute seeds (Dasgupta et al. 1977 and Basu & Dasgupta 1978).

In lettuce, viability could be very well maintained in fully imbibed seeds on moist filter papers at 30°C (a dormancy-maintaining temperature for lettuce) whereas dry seeds, when stored in a humid atmosphere, showed a rapid loss of viability (Toole & Toole 1954). According to Villiers (1975) and Villiers and Edgcumbe (1975), the capacity of the fully imbibed seed to retain viability is due to the effective operation of a biochemical repair mechanism as against an accumulation of damage to cellular organelles in the air-dry state (5–20% moisture). Villiers and Edgcumbe (1975) further showed that post-irradiation damage to chromosomes was much less in seeds kept fully imbibed after γ-irradiation.

Studies in our laboratory have shown great efficacy of soaking-drying and moisture equilibration-drying treatments in the maintenance of lettuce seed viability (Basu et al. 1979). The data presented in this paper would show that a fully imbibed condition is not necessary for the radioprotective action. Ageing damage of lettuce seeds as well as X- and γ-ray induced deterioration of seeds could be very effectively counteracted by short-term soaking-drying or by moisture equilibration of the seed at 100% RH followed by drying back.

Materials and Methods

Harvest-fresh seeds of lettuce cv. Sutton's A 1 were obtained from Messers Sutton and Sons, Calcutta and stored in a rubber-stoppered bottle for one year before use. This cultivar requires a wet pre-chilling treatment before it can germinate. At temperatures over 25°C the non-chilled soaked seeds would remain dormant. One-year-old seeds (medium-vigour) were given the hydration-dehydration treatments (HD) before and after subjecting them to X- or γ- irradiation of 5 kR. In the soaking-drying treatment (SD), seeds were kept immersed in water for 2 hr after which they were taken out and dried back to the original weight in an air current at 35 ± 1°C. In the dipping-drying treatment (DD), the seeds were dipped in water for 2 minutes and then dried back as above. Seed hydration by moisture equilibration (ME) was done by keeping seeds in a humidity cabinet at 100% RH for 24 hr. As a result of this, moisture content of the seeds increased from the original 7% to 21.8% (on wet weight basis) after equilibration. The seeds were then dried back to their original weight (MED). Seeds of all the treatments including respective controls were stabilized for moisture by keeping them over fused calcium chloride to avoid possible treatment differences due to variations in seed moisture contents.

In the pre-irradiation hydration-dehydration set (HD + R), the seeds were subjected to X- and γ-irradiation after SD, DD, and MED treatments; the untreated (no HD treatment) seeds served as the control. In case of post-irradiation set (R + HD), the aforesaid HD treatments were given immediately after X- and γ-irradiation. The radiation dose was 5 kR for both the sources. The dose rate for X-ray was 1 kR per minute and for γ-irradiation the rate was 220 R/min from a hospital cesium source (2500 Ci ^{137}Cs of Picker Research Centre, USA). A non-irradiated set, in which the seeds were given only the HD treatments were subjected to accelerated ageing at 95% RH and 40°C for 8 days to evaluate the effect on age-induced damage. Non-irradiated as well as pre- and post-irradiated seeds were otherwise stored under ambient conditions for different periods to determine their storability. Germination tests were carried out on moist blotters spread on glass plates which were then placed in polythene packets according to the method of Dasgupta et al. (1976) with some modifications as described by Punjabi and Basu (1980). During the germination tests seeds were first given a chilling pretreatment at 2°C for 24 hr after which they were placed in a germinator at 22±1°C for 8 days. As the data on germination percentages cannot be analyzed statistically without transformation, the values were converted to respective degrees (Fisher & Yates 1963). The transformed data were then subjected to analysis of variance for evaluation of treatment effects. Biological after-effects for germination percentage, root and shoot lengths have been calculated as the ratios of before storage and after storage values for the respective parameters.

Results

Hydration-dehydration treatments very effectively slowed down the loss of vigour and viability of lettuce seeds. The treatments did not show any significant effect on immediate germinability (before ageing) but when stored under conditions favouring seed deterioration SD, DD and MED treatments showed great anti-ageing effects. The ageing effect in lettuce was initially apparent on root length; germination percentage and shoot length were affected only after prolonged storage. Of the three methods of hydration, DD and SD proved a little better than MED under accelerated (table 1) as well as natural ageing conditions (figure 1). The relative efficacy of the different methods of hydration, however, depends on the physiological age of the seed. Our unpublished results with lettuce have shown that MED is better in high-vigour seed but SD is superior to the former in low-vigour seed.

HD treatments given either before or after irradiation of the seeds with X- and γ-rays considerably reduced the adverse

Table 1 *Effect of hydration-dehydration treatments on germinability of lettuce seeds subjected to accelerated ageing at 95% RH and 40°C for 8 days*

Hydration-dehydration treatments	Germination %	Arc-sin value	Mean root Length(mm)	Mean shoot Length(mm)
Control (CON)	39.8	39.1	30.0	26.5
Soaking-drying (SD)	74.3	59.5	46.0	29.5
Dipping-drying (DD)	73.0	58.7	47.3	32.0
Moisture equilibration-drying (MED)	69.8	56.7	44.8	32.0
L.S.D. at 0.05 P		5.7	0.8	1.3
,, ,, 0.01 P		10.4	1.4	2.4

Treatments were given to 1-year-old seeds. Data on root and shoot lengths were recorded after germination for 8 days. Mean root and shoot lengths were calculated on the basis of germinated seedlings only.

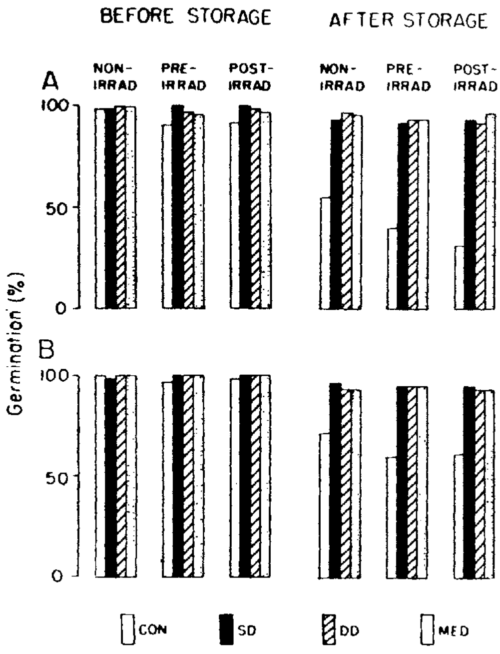

Figure 1 A–B Effect of pre- and post-irradiation hydration-dehydration (HD) treatments on germination of lettuce seeds before and after storage, for the X-irradiation set (A) and γ-irradiation set (B) CON, control; **SD**, soaking-drying; **DD**, dipping-drying; **MED**, Moisture equilibration-drying; **Non-irrad**, no irradiation; **Pre-irrad** (pre-irradiation HD treatment; HD + 5 kR); **Post-irrad** (post-irradiation HD treatment; 5 kR + HD).

effect of irradiation on germinability (tables 2 & 3). DD and SD were more effective than MED. Irradiation effect was very drastic on root growth and it was that character which was benefited most by the HD treatments. As expected, X- and γ-irradiation gave similar response so far as the effect of HD treatment was concerned. Pre-irradiation and post-irradiation HD treatments also gave broadly similar results. The development of biological after-effects (as measured by ratios of before and after storage values for different germination parameters) was considerably minimized by all the HD treatments (table 4). SD, DD and MED were more or less equally effective. The after-effect was most pronounced on root growth of seedlings of X- and γ-irradiated seeds which did not receive any HD before or after irradiation. It was less in germination percentage and still lesser so far as shoot growth was concerned. Ageing alone (in

Table 2 *Effect of hydration-dehydration treatment on germinability of X-irradiated lettuce seeds immediately after treatment (before storage) and after 75 days of storage under ambient laboratory conditions (average RH 81%, temp. 29°C)*

Hydration-dehydration treatment	Before storage			After storage		
	Non-irrad (OkR)	*Pre-irrad* (HD+5kR)	*Post-irrad* (5kR+HD)	*Non-irrad* (OkR)	*Pre-irrad* (HD+5kR)	*Post-irrad* (5kR+HD)
(i) *Germination angles** (arc-sin)						
CON	81.9	71.6	73.6	47.9	39.2	34.5
SD	81.9	90.0	90.0	74.7	73.6	75.8
DD	90.0	80.0	81.9	78.5	75.8	73.6
MED	90.0	77.1	71.5	77.1	74.7	78.5
LSD at 0.05 P	NS	2.3	5.9	9.1	7.6	7.8
,, ,, 0.01 P	NS	3.9	NS	15.7	13.0	13.3
(ii) *Mean root length* (mm)						
CON	42	31	29	30	7	5
SD	40	36	39	41	31	27
DD	45	35	44	45	34	26
MED	41	34	40	40	26	25
LSD at 0.05 P	NS	2.1	2.6	2.2	2.9	0.6
,, ,, 0.01 P	NS	NS	4.4	3.9	1.1	5.1
(iii) *Mean shoot length* (mm)						
CON	37	31	29	35	22	23
SD	33	33	31	34	34	29
DD	37	36	30	35	35	30
MED	35	33	31	35	34	31
LSD at 0.05 P	NS	NS	NS	NS	2.6	4.7
,, ,, 0.01 P	NS	NS	NS	NS	4.5	NS

* Data on germination percentage have been transformed to respective arc-sin angles for statistical analysis. Original germination percentages shown in figure 1. Other details same as in table 1.

NS, Non-significant

non-irradiated set) showed an increase in damage but except for shoot growth irradiation greatly aggravated the situation.

Discussion

An important observation of this study is the broad parallelism between the effects of HD treatments in the counteraction of age- and irradiation-induced seed deterioration. The development of biological after-effect of radiation was greatly minimized by the HD treatments which maintained a high level of vigour and viability of seeds in storage.

The beneficial effect of HD treatment in the maintenance of seed viability is still being elucidated in this laboratory. Advancement of germination, leaching of toxic metabolites from the seed and differential absorption/desorption of moisture by treated and untreated seed have been ruled out as possible causes of viability maintenance (Basu 1976, Basu & Dasgupta 1978 and Rudrapal & Basu 1979). Two important views on seed deterioration need critical consideration in the present context. These are the hypotheses of failure of cellular repair mechanism in the dry-stored seed and the involvement of free radical and

Table 3 *Effect of hydration-dehydration treatment on germinability of γ-irradiated lettuce seeds immediately after treatment (before storage) and after 75 days of storage under ambient laboratory conditions (average RH 81%, temp. 29°C)*

Hydration-dehydration treatments	Before storage			After storage		
	Non-irrad (OkR)	*Pre-irrad* (HD+5kR)	*Post-irrad* (5kR+HD)	*Non-irrad* (OkR)	*Pre-irrad* (HD+5kR)	*Post-irrad* (5kR+HD)
(i) *Germination angles* (arc-sin)						
CON	90.0	80.0	81.9	57.4	50.8	51.4
SD	81.9	90.0	90.0	81.9	77.1	77.1
DD	90.0	90.0	90.0	75.8	77.1	75.8
MED	90.0	90.0	90.0	74.7	77.1	75.8
LSD at 0.05 P	NS	NS	NS	8.9	5.6	5.3
,, ,, 0.01 P	NS	NS	NS	NS	12.9	9.2
(ii) *Mean root length* (mm)						
CON	40	36	36	25	8	9
SD	40	43	42	35	31	36
DD	43	41	43	37	32	33
MED	40	41	38	37	33	32
LSD at 0.05 P	NS	1.7	2.3	2.5	1.7	3.3
,, ,, 0.01 P	NS	2.9	3.9	4.2	3.0	5.6
(iii) *Mean shoot length* (mm)						
CON	32	33	31	31	25	25
SD	35	34	33	32	32	33
DD	34	34	34	32	32	34
MED	33	36	37	34	34	34
LSD at 0.05 P	NS	NS	NS	NS	1.5	2.2
,, ,, 0.01 P	NS	NS	NS	NS	2.7	3.8

Details same as in table 2

Table 4 *Effect of pre- and post-irradiation hydration-dehydration treatments on the development of biological after-effects* in lettuce seeds stored after treatment for 75 days under ambient conditions (average RH 81%, temp. 29°C)*

Hydration-dehydration treatment	X-irradiation set			Y-irradiation set		
	Non-irrad (OkR)	*Pre-irrad* (HD+5kR)	*Post-irrad* (5kR+HD)	*Non-irrad* (OkR)	*Pre-irrad* (HD+5kR)	*Post-irrad* (5kR+HD)
(i) *Germination*						
CON	1.78	2.25	2.88	1.41	1.62	1.61
SD	1.05	1.09	1.06	1.00	1.05	1.05
DD	1.04	1.03	1.07	1.06	1.05	1.06
MED	1.05	1.02	1.00	1.08	1.05	1.06
(ii) *Root length*						
CON	1.40	4.43	5.80	1.60	4.50	4.00
SD	0.98	1.16	1.44	1.14	1.39	1.17
DD	1.00	1.03	1.69	1.16	1.28	1.30
MED	1.03	1.31	1.60	1.08	1.24	1.19
(iii) *Shoot length*						
CON	1.06	1.41	1.26	1.03	1.32	1.24
SD	0.97	0.97	1.07	1.09	1.06	1.00
DD	1.06	1.03	1.00	1.06	1.06	1.00
MED	1.00	0.97	1.00	0.97	1.06	1.09

* Biological after-effects have been expressed as the ratios of before storage and after storage values for germination percentage, root length and shoot length of respective treatments (vide tables 2 & 3)

lipid peroxidation reactions in seed deterioration.

According to Villiers (1975), the ageing damage in the dry-stored seed is due to a lack of functioning of the cellular repair system in the air-dry condition. Villiers and Edgcumbe (1975) showed that imbibed storage of dormant lettuce seeds checked the occurrence of chromosomal aberrations in irradiated seeds; there was even some reversal of damage in the moist storage. These observations clearly suggested that viability maintenance and radioprotection in the imbibed seed is attributable to the operation of an efficient biochemical repair mechanism in a water milieu. The possibility of a similar repair phenomenon occurring in the hydration phase of the present HD treatments requires critical consideration.

All the three methods of hydration (followed by drying) employed in this study, namely soaking, dipping and moisture equilibration were quite effective in viability maintenance and radioprotection. In the dipping treatment, hydration was done for two minutes only and in the subsequent drying process the seed moisture content gradually fell and in about an hour it came down to 12–13% (on fresh weight basis). Whether the time available for effective repair of damage would be sufficient should be experimentally verified. Villiers and Edgcumbe (1975) observed that in a water-saturated atmosphere, the moisture attained by the seed may not be sufficient for repair and membrane synthesis. It should, however, be pointed out that in the above study, the seed moisture content was not more than 14% but in our experiments the moisture content in the equilibrated seeds (24 hr equilibration) rose to over 21%. Improvement in post-storage germinability has, however, been noted in this laboratory in moisture-equilibrated-dried lettuce seed in which the moisture content was raised to 13–14% only (before drying back) from an initial 6% but the effect was more apparent at higher moisture levels (Basu et al. 1979 and Pan & Basu unpublished). Nevertheless, in view of observations of Villiers and Edgcumbe (1975), we had been inclined to suggest that the beneficial effect of moisture equilibration-drying treatment might not be attributable to cellular repairs (Basu & Dasgupta 1978, Basu & Pal 1980 and Rudrapal & Basu 1979). More recently Roberts (1981) has indicated that the trend of viability loss with rise in moisture content ceases to operate at certain critical moisture levels and in lettuce an improvement in germination is noted at moisture content of about 16% in the presence of oxygen. Recent studies in this laboratory have also shown that moisture-equilibrated lettuce seeds (24 hr equilibration), if kept in air without allowing loss of moisture, would remain viable for a long period, while in sealed bottles they rapidly lose viability. It is possible that the beneficial effects are due to a continuous repair of damages to bioorganelles. But, besides the practical problem of keeping such hydrated seeds free from microbial attack, the question remains as to how long the energy source (respiratory energy from nutritional reserves) needed for biosynthetic reactions would be available. Osborne (1981) has shown that the dormant seed may carry out all the reactions (obviously at the expense of stored reserves) normally expected in a non-dormant seed except the replicative DNA synthesis. The subject definitely needs a critical consideration.

It therefore appears that the beneficial effects of treatments in viability maintenance and radioprotection can be explained on the basis of a biochemical repair mechanism in the hydration phase before drying. But an interpretation of the treatment effects can also be made in terms of a counteraction of free radical pathology.

A significant role of free radicals and lipid peroxidation reactions in ageing and senescence in general has been proposed by Tappel (1973) and a strong support for a similar mechanism in dry-stored seed has been given by Pammenter et al. (1974) who could greatly extend the longevity of maize seeds through provision of a source of free electrons. The question of involvement of lipid peroxidation is still debatable and supporting (Harman & Mattick 1976, Stewart & Bewley 1980 and Flood & Sinclair 1981) as well as opposing (Priestley & Leopold 1979, Priestley et al. 1980 and Pearce & Abdul-Samad 1980) views have been put forward on the subject. Neverthless, if free radicals initiate ageing, their quenching would delay the process. That seed hydration can bring about radical quenching has been shown in radiobiological experiments (Ehrenberg 1961, Cook 1964, Haber & Randolph 1967). According to Ehrenberg (1961), the enhanced mobility of free radicals upon hydration may facilitate their recombination into harmless non-radical products. Haber and Randolph (1967) showed that 92% of the radiation-induced ESR signals could be eliminated by HD treatments. Whether a similar quenching mechanism would cut off endogenous radical chain propagation reactions in the naturally ageing seed needs to be studied.

A concept of physico-chemical mechanism for the development and modification of after-effects is supported by a number of reports on post-irradiation hydration of barley seeds. Kesavan and coworkers (Kesavan 1973, Kesavan et al. 1978 and Afzal & Kesavan 1979) have criticallystudied the oxid and anoxic post-irradiation damages and their modifications by various treatments. Kesavan et al. (1978) favoured a physico-chemical mechanism (Palcic & Skarsgard 1975) for the chemical modification of radiation damage. Afzal and Kesavan (1979), in interpreting the differential effects of seed moisture content on the development of oxygen-dependant damages, also pointed out that the oxic effect was certainly of a physico-chemical nature. The broad parallelism in the effects of HD treatment in ageing and irradiation would suggest that similar sites were affected by the treatment implying thereby some common physico-chemical mechanism in the two phenomena.

Germination tests conducted immediately after HD and irradiation did not show any major effect on the vigour and viability of seeds. The reason is an obvious one; germination of seed requires full imbibition which would check the development of biological after-effects.

The similarity between pre- and post-irradiation HD treatments is difficult to interpret with our present observations. We have, however, noted that the pre-irradiation treatment is ineffective in harvest-fresh seeds. If free radicals are involved, post-irradiation hydration of stored seeds should quench age- as well as X- and γ-irradiation-induced radicals and should therefore be more effective than pre-irradiation HD which would remove only age-induced radicals. Perhaps, our irradiation and hydration techniques did not bring out the full potential of post-irradiation hydration. If those were done in oxygen-free environment (irradiation in vacuum and hydration in deoxygenated water) to prevent oxic damage, post-irradiation treatment would prove better. Studies are presently being conducted with seeds of different vigour status under more controlled conditions to further elucidate the situation.

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References

Afzal S M J and Kesavan P C 1979 Influence of seed moisture content and post-irradiation hydration temperature on the kinetics of reactivity towards oxygen or decay of oxygen sensitive sites; *Int. J. Radiat. Biol.* **36** 161–176

Basu R N 1976 Physico-chemical control of seed deterioration; *Seed Res.* **4** 15–23

——, Chattopadhyay K and Pal P 1974 Maintenance of seed viability in rice (*Oryza sativa* L.) and jute (*Corchorus capsularis* L. and *C. olitorius* L.); *Indian Agric.* **18** 76–79

—— and Dasgupta M 1978 Control of seed deterioration by free radical controlling agents; *Indian J. exp. Biol.* **16** 1070–1073

—— and Pal P 1980 Control of rice seed deterioration by hydration-dehydration pretreatments; *Seed Sci. Technol.* **8** 151–160

—— Pan, Dolly and Punjabi, Bina 1979 Control of lettuce seed deterioration, *Indian J. Pl. Physiol.* **22** 247–253

Cook R F 1964 The effect of water and a protective agent on gamma-ray-induced free radicals in mustard seeds; *Int. J. Radiat. Biol.* **7** 497–504

Dasgupta M, Basu P and Basu R N 1976 Seed treatment for vigour, viability and productivity of wheat (*Triticum aestivum* L.); *Indian Agric.* **20** 265–273

——, Chattopadhyay K, Basak S L and Basu R N 1977 Radioprotective action of seed invigoration treatments; *Seed Res.* **5** 104–118

Ehrenberg A 1961 in *Free Radicals in Biological Systems* 337–350 pp. eds. M S Blois (Jr.), H W Brown, R M Lemmon, R O Lindblom and M Weisbluth (New York: Academic Press)

Fisher R A and Yates F 1963 in *Statistical Tables for Biological, Agricultural and Medical Research* 6th Edn. pp 15–16 and 74–75 (London: Oliver and Boyd)

Flood R G and Sinclair A 1981 Fatty acid analysis of aged permeable and impermeable seeds, of *Trifolium subterraneum* (subterranean clover); *Seed Sci. Technol.* **9** 475–477

Haber A H and Randolph M L 1967 Gamma-ray-induced ESR signals in lettuce. Evidence for seed-hydration-resistant and -sensitive free radicals; *Radiat. Bot.* **7** 17–28

Harman G E and Mattick L R 1976 Association of lipid oxidation with seed ageing and death; *Nature* **260** 323–324

Kesavan P C 1973 Effect of caffeine and cysteine applied during post-irradiation hydration on an oxygen-independent component of damage in barley seed; *Radiat. Bot.* **13** 355–359

——, Sharma G J and Afzal S M 1978 Differential modification of oxic and anoxic radiation damage by chemicals I. Simulation of the action of caffeine by certain inorganic radical scavengers; *Radiat. Res.* **75** 18–30

Osborne D J 1981 Dormancy as a survival stratagem; *Ann. appl. Biol.* **98** 525–531

Palcic B and Skarsgard L D 1975 Absence of ultrafast processes of repair of single-strand breaks in mammalian DNA; *Int. J. Radiat. Biol.* **27** 121–133

Pammenter N W, Adamson J H and Berjak P 1974 Viability of stored seed. Extension by cathodic protection; *Science* **186** 1123–1124

Pearce R S and Abdul-Samad I M 1980 Changes in fatty acid contents of polar lipids during ageing of seeds of peanut (*Arachis hypogea* L.); *J. exp. Bot.* **31** 1283–1290

Priestley D A and Leopold A C 1979 Absence of lipid oxidation during accelerated ageing of soybean seeds; *Pl. physiol.* **63** 726–729

——, McBride M B and Leopold A C 1980 Tocopherol and organic free radical levels in soybean seeds during natural and accelerated ageing; *Pl. Physiol.* **66** 715-719

Punjabi, Bina and Basu R N 1980 A simple method for testing germination and seedling vigour; *ISTA Congress*, Vienna; Preprint No. 77/III, 1–6

Roberts E H 1981 Physiology of ageing and its application to drying and storage; *Seed Sci. Technol.* **9** 359–372

Rudrapal A B and Basu R N 1979 Physiology of hydration-dehydration treatment in the maintenance of seed viability in wheat (*Triticum aestivum* L); *Indian J. exp. Biol.* **17** 768–771

Stewart R R C and Bewley J D 1980 Lipid peroxidation associated with accelerated ageing of soybean axis; *Pl. Physiol.* **65** 245–248

Tappel A L 1973 Lipid peroxidation damage to cell components; *Fedn. Proc.* **32** 1870–1874

Toole V K and Toole E H 1954 Seed dormancy in relation to seed longevity; *Proc. Int. Seed Test. Assoc.* **18** 325–328

Villiers T A 1975 Genetic maintenance of seeds in imbibed storage; in *Crop Genetic Resources for Today and Tomorrow* pp 297–315 eds O H Frankel and J G Hawkes (Cambridge University Press)

——and Edgcumbe D J 1975 On the cause of seed deterioration in dry storage; *Seed Sci. Technol.* **3** 761–774