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Amelioration of the effects of ageing in onion seeds by osmotic priming and associated changes in oxidative metabolism

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Abstract

Osmotic priming of aged onion seeds with 25 % polyethylene glycol-8000 for 5 d resulted in a marked increase in the rate of germination and early seedling growth. Priming reduced electrolyte leakage as well as lipid peroxidation in seeds implying the activation of membrane repair processes. Priming was also associated with increased levels of antioxidants, *i.e.* ascorbic acid and tocopherols particularly the latter and the activities of catalase and peroxidase involved in the mitigation of oxidative damage. In comparison with the priming of unaged seeds, the aged seeds experienced a diminution of response in terms of changes in the levels of antioxidants and scavenging enzymes.

Introduction

Even at relatively good storage conditions, onion seeds lose viability and vigour at a faster rate than seeds of most other crops (Sijbring 1963, Mackay and Tonkin 1967). They undergo less deterioration during storage at high seed moisture content, suggesting activation of repair processes (Ellis and Roberts 1977).

Although seed osmotic priming has been used to improve vigour of many species (Heydecker and Coolbear 1977), its effects in relation to seed storage and ageing are still not well defined. Priming permits water uptake into the seed to an extent which allows metabolism to proceed (including presumably repair processes in both low and high-vigour seeds), except for radicle emergence. Dearman *et al.* (1986) investigated the effects of ageing and storage on primed onion seeds. Priming before and after accelerated ageing improved the rate of germination compared with untreated seeds. In addition, priming before ageing delayed the loss of viability due to ageing, but priming after ageing had no effect on viability. The mechanisms are, however, little understood (Ellis and Butcher 1988).

Several studies have suggested the involvement of membrane lipid peroxidation in seed ageing (Wilson and McDonald 1986). Harman and Mattick (1976) and Stewart

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and Bewley (1980) demonstrated that oxidation damage was occurring in pea and soybean subjected to accelerated ageing, while Buchvarov and Gantchev (1984) directly demonstrated large increase in free radicle levels in the axes of both naturally and artificially aged soybean seeds. However, contradictory reports also exist (Priestley and Leopold 1979, Francis and Coolbear 1988). Attempts have also been made to correlate declining seed vigour with a decline in the level of antioxidants such as tocopherols (Fielding and Goldsworthy 1980), which have the ability to quench free radicals. The present study was undertaken to understand the priming-induced invigoration of naturally-aged and unaged onion seeds.

Materials and methods

Freshly harvested (unaged) and one year old (aged) seeds of onion (*Allium cepa* L. cv. Punjab Red-48) were used in this study. Both types of seeds were primed on filter paper in Petri dishes using 25 % or 35 % polyethylene glycol-8000 (PEG) and kept in the dark at 20 ± 1 °C for 3 or 5 d. After priming, the adhering PEG was washed-off followed by surface-drying or drying-back of the seeds to original moisture content at 20 ± 1 °C for 2 d. The surface-dried and dried-back seeds were then tested for germination in distilled water in comparison with untreated controls. For germination, four replicates of 50 seeds each were placed in Petri-dishes (Ø 9.0 cm) and allowed to germinate in water at 28 ± 1 °C. Germination counts were made daily for 7 d. The mean time to germination was then calculated (Basra *et al.* 1988). Primary root length and shoot length were measured after 7 d.

Electrolyte leakage and lipid peroxidation were monitored as a measure of membrane deterioration. For electrolyte leakage, 100 mg samples of seeds were immersed in 10.0 cm^3 deionized water and stirred continuously at 28 ± 1 °C. Electrical conductivity of the leachate was recorded using a conductivity meter after 5 h. The conductivity was recorded again after boiling of the samples for 30 min. Per cent leakage was then calculated as (conductivity before boiling/conductivity after boiling) × 100.

Lipid peroxide formation was studied by the thiobarbituric acid colour reaction according to Bernheim *et al.* (1948). Ascorbic acid was analyzed according to the method of Roe and Oesterling (1943). Total lipids were extracted by the cold extraction method of Folch *et al.* (1957), and the extract used to determine tocopherol content according to Jayaraman (1981). Activities of catalase and peroxidase were determined after Chance and Maehly (1955) and Shannon *et al.* (1966), respectively.

Results and discussion

PEG priming did not improve the germination percentage but the rate of germination was markedly improved in both aged and unaged seeds (Table 1). In general, a longer duration of priming, *i.e.* 5 d instead of 3 d, was required to achieve a better response

in aged seeds. Invigoration response of primed seeds dried-back to their original moisture content was less in comparison primed and surface-dried seeds, but their performance was still better in comparison with the untreated control (Table 1).

Priming with 25 % PEG for 5 d followed by surface-drying or drying-back was used as the standard treatment for further investigation.

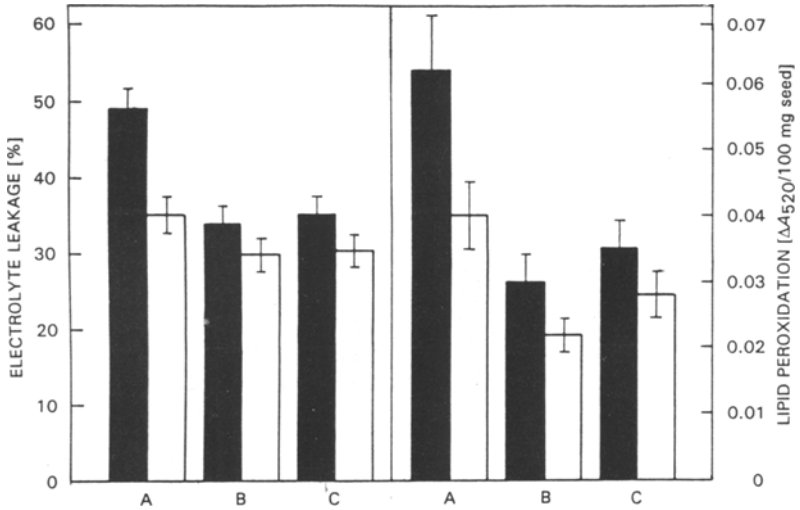

Fig. 1. Priming-induced changes in electrolyte leakage and lipid peroxidation of aged (*closed columns*) and unaged (*open columns*) seeds of onion. *A* - untreated seeds, *B* - primed and surface-dried seeds, *C* - primed and dried-back seeds.

Aged onion seeds showed greater electrolyte leakage compared with unaged seeds (Fig. 1). The electrolyte leakage from a tissue can be considered as an index of the effectiveness of the membrane as barrier to solute diffusion (Simon 1974, Parrish and Leopold 1978). The imbibition of healthy seeds initiates a rapid return to biochemical and structural integrity of membranes, whereas its loss in deteriorated seeds leads to an increased electrolyte leakage (Parrish and Leopold 1978, Prietley 1986, Pukacka 1991). In the present study, the priming of aged seeds reduced the electrolyte leakage to a greater extent compared with unaged primed seeds, and the repair effect was largely retained on drying-back (Fig. 1). Thus, the predisposition of aged seeds to increased electrolyte leakage in imbibed seeds was reversed by priming. Evidence for repair processes in the invigoration of other crop seeds has also been suggested (Burgass and Powell 1984, Tilden and West 1985, Pandey 1988). Similarly, lipid peroxidation in aged seeds was higher compared with unaged seeds, suggesting a positive relationship with increased electrolyte leakage (Fig. 1). These are in turn inversely correlated with the viability and vigour of seeds (Table 1). Osmotic priming caused a noticeable decline in lipid peroxidation of both aged and unaged seeds (Fig. 1). Choudhuri and Basu (1988) also recorded decreased lipid peroxide levels in onion seeds due to hydration and dehydration treatments, which improved their storability. Therefore, it is likely that during the priming process an effective repair of vital seed membranes and/or a counteraction of lipid peroxidation

were operative to cause invigoration. Primed and surface-dried seeds also recorded a marked increase in activities of catalase and peroxidase but a diminution of response was observed during the drying-back process (Fig. 2).

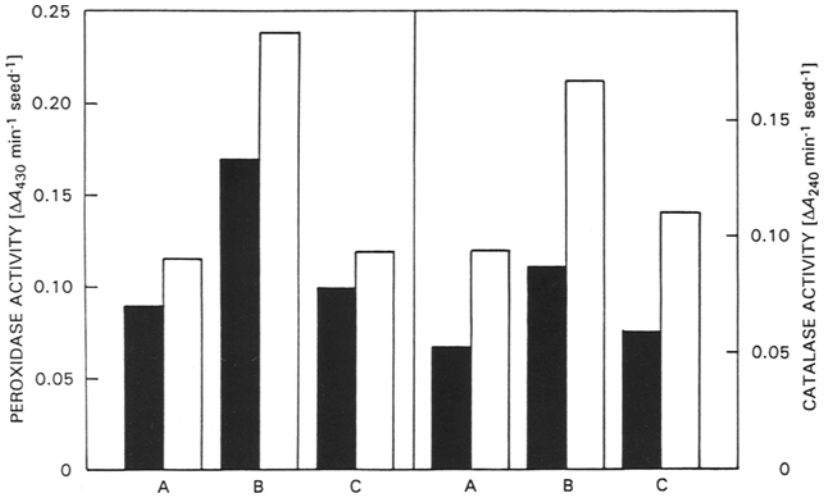

Fig. 2. Priming induced changes in catalase and peroxidase activities of aged and unaged seeds of onion. Otherwise as in Fig. 1.

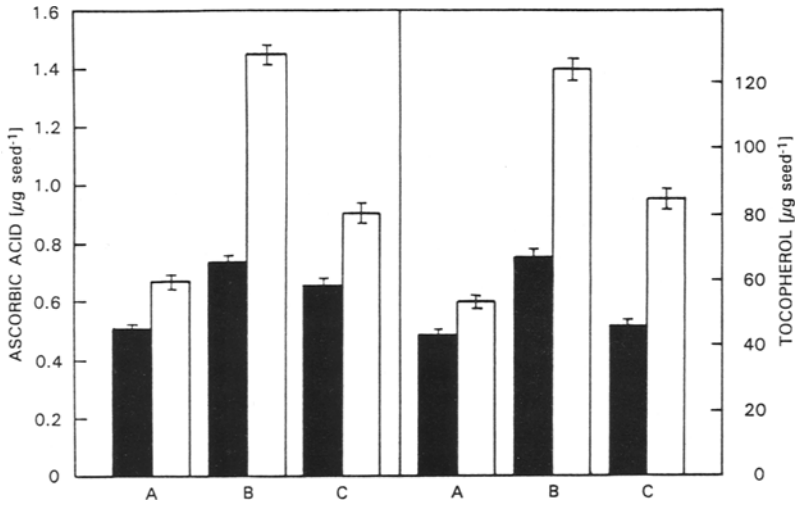

Fig. 3. Priming-induced changes in ascorbic acid and tocopherol levels of aged and unaged seeds of onion. Otherwise as in Fig. 1.

Vitamin C (ascorbic acid) and E (tocopherols) were detectable in both aged and unaged seeds, but the latter was present in greater amounts (Fig. 3). The levels of both the antioxidants were lower in the aged seeds compared with the unaged seeds.

Table 1. Germination and seedling growth of aged and unaged onion seeds when tested either unprimed or following a priming treatment with polyethylene glycol (PEG) and surface drying (SD) or drying-back (DB). Priming was carried out in Petri-dishes at 20 ± 1 °C in the dark. Mean ± standard error.

Priming treatment	Priming duration [d]	Germination [%]		Mean germination time [h]		Primary root length [cm]		Shoot length [cm]	
		SD	DB	SD	DB	SD	DB	SD	DB
Aged									
Control		46.00 ± 2.80		85.99 ± 3.55		1.47 ± 0.25		1.79 ± 0.37	
25% PEG	3	50.00 ± 2.51	50.00 ± 2.49	67.01 ± 2.51	72.98 ± 2.79	1.71 ± 0.16	1.57 ± 0.21	2.97 ± 0.25	2.28 ± 0.31
25% PEG	5	48.50 ± 2.47	46.00 ± 2.15	61.01 ±2.42	60.00 ± 2.59	1.77 ± 0.11	1.65 ± 0.18	3.05 ± 0.20	2.43 ± 0.27
30% PEG	3	48.50 ± 2.38	50.50 ± 2.40	72.00 ± 2.62	77.88 ± 2.85	1.62 ± 0.19	1.59 ± 0.25	2.83 ± 0.29	2.20 ± 0.34
30% PEG	5	52.50 ± 2.52	48.00 ± 2.25	67.22 ± 2.56	73.92 ± 2.71	1.67 ± 0.15	1.64 ± 0.71	2.95 ± 0.26	2.37 ± 0.30
Unaged									
Control		82.10 ± 3.25		79.06 ± 2.71		1.89 ± 0.28		2.50 ± 0.39	
25% PEG	3	85.50 ± 3.01	84.00 ± 3.15	56.23 ± 2.25	61.42 ± 2.40	2.13 ± 0.18	1.99 ± 0.24	3.50 ± 0.27	3.13 ± 0.34
25% PEG	5	89.50 ± 3.25	85.50 ± 3.00	52.80 ± 2.16	57.89 ± 2.31	2.25 ± 0.14	2.12 ± 0.19	3.71 ± 0.21	3.31 ± 0.28
30% PEG	3	79.00 ± 2.90	75.00 ± 2.95	59.64 ± 2.37	68.57 ± 2.55	2.06 ± 0.20	2.04 ± 0.26	3.41 ± 0.29	3.02 ± 0.35
30% PEG	5	82.00 ± 3.31	82.50 ± 3.16	56.23 ± 2.25	67.90 ± 2.36	2.21 ± 0.17	2.10 ± 0.22	3.69 ± 0.26	3.27 ± 0.30

Seeds in particular contain large amount of vitamin E, which components are very effective antioxidants for plant membranes (Kunert and Ederer 1985, Rise *et al.*, 1989, Franzen and Haab 1991). α-tocopherol effectively reduces lipid peroxidation even at low concentration (Wu *et al.* 1979, Gorecki and Harman 1987) and has the ability to quench free radicals (Leibovitz and Siegel 1980). Pertinently, priming of onion seeds resulted in marked increase of tocopherol and ascorbic acid levels in aged seeds (Fig. 3). Drying-back treatment, however, had a suppressing effect. Woodstock *et al.* (1983) demonstrated that impregnation of onion seeds with vitamin E reduced ageing and markedly improved their storability.

It is worth mentioning that the extent of priming-induced increase in both antioxidant levels (Fig. 3) and activities of scavenging enzymes (Fig. 2) was higher in case of unaged seeds compared with aged seeds implying thereby that the ageing seeds experienced a loss in ability to affect the above-mentioned changes. Overall, it appears that osmotic priming helps in invigoration of aged seeds by causing enhanced synthesis of antioxidants, particularly tocopherols and activities of catalase and peroxidase resulting in reduced lipid peroxidation and membrane damage.

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