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Priming-induced changes in polyamine levels in relation to vigor of aged onion seeds

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Abstract. Seed ageing leads to decreased polyamine levels. Osmotic priming with polyethylene glycol resulted in marked invigoration of aged onion seeds, *Allium cepa* L. cv. Punjab Red-48. Priming-induced increases in the levels of polyamines, especially putrescine and spermidine, were related to the improved seed vigor. The role of polyamines is discussed in relation to membrane protection and/or repair.

Keywords: Ageing; *Allium cepa*; Onion; Osmotic priming; Polyamines; Seed vigor.

Introduction

Even under relatively good storage conditions, onion (*Allium cepa* L.) seeds lose vigor and viability faster than do seeds of most other crops, exhibiting signs of physiological deterioration in less than a year (Toole et al., 1948; Sijbring, 1963; Mackay and Tonkin, 1967). Physiological deterioration is associated with the alteration of metabolic processes in ageing seeds (Priestley, 1986).

Contrary to the response of orthodox seeds, onion seeds deteriorate less when stored with a high seed moisture content (Roberts, 1973), possibly due to the activation of repair mechanisms (Ellis and Roberts, 1977; Ward and Powell, 1983).

Similarly, seed priming, a technique of treating seeds in osmotic solutions, has been used to improve seed vigor (Heydecker and Coolbear, 1977), but little is known about priming-induced invigoration of aged seeds. Dearman et al. (1986) investigated the affects of ageing and storage on primed onion seeds. Priming, both before and after accelerated ageing, improved the rate of germination compared with that of untreated seeds. Priming before ageing delayed the loss of viability, but priming after ageing could not restore lost viability. The increase in longevity and the improved rate of germination has been interpreted as an indication that accumulated damage is repaired during seed priming (Ellis and Butcher, 1988), but information about the mechanism of priming-induced repair in naturally aged seeds is scarce.

Oxidation damage of membranes and of other cellular constituents has been invoked as a mechanism of seed ageing (Wilson and McDonald, 1986). Buchvarov and Gantcheff (1984) directly demonstrated a large increase in free radical levels in the embryonic axes of naturally and of artificially aged soybeans. Attempts have also been made to correlate declining seed vigor with a decline in the levels of anti-oxidants, such as tocopherols (Fielding and Goldsworthy, 1980), which have the ability to quench free radicals. Furthermore, changes in polyamine levels have been linked with seed viability in wheat (Anguilessi et al., 1974) and rice (Mukhopadhyay et al., 1983; Mukhopadhyay and Ghosh, 1986). Studies of priming-induced changes in the polyamine levels of aged seeds, however, are not available. In view of the relationship between polyamines and the alleviation of deteriorative senescence (Galston and Kaur-Sawhney, 1987), such an investigation is warranted. The objective of our research, therefore, was to investigate this relationship during priming-induced invigoration of aged onion seeds.

Materials and Methods

Aged (one-year old) seeds of onion (*Allium cepa* L. cv. Punjab Red-48) were obtained from the Department of Vegetable Crops, Punjab Agricultural University, Ludhiana. The seeds were primed with polyethylene glycol-8000 (PEG) on filter paper in Petri dishes and incubated at 20 ± 1 °C in the dark. PEG was used at 25% and 30% concentration. After priming for 3 or 5 days, the adhering osmoticum was washed off and the treated seeds were either surface dried or dried back to original moisture content at 20 ± 1 °C for 2 days. The primed seeds and unprimed controls were then tested for germination in distilled water. The affects of 100 μmole each of exogenous putrescine, spermidine, and spermine were

also investigated by application either alone or in combination with PEG priming.

For the germination test, four replicates of 50 seeds each were placed on filter paper in Petri-dishes (9.0 cm) containing 5.0 ml of distilled water and incubated at 28 ± 1 °C. Germination counts were taken daily for 7 days. The mean germination time was then calculated (Basra et al., 1988). Determinations of primary root length and shoot length were made after 7 days. For analysis of polyamines, whole seeds (unprimed controls as well as those primed for 5 days with 25% PEG at 20 ± 1 °C) were used. Primed seeds were either surface dried or dried

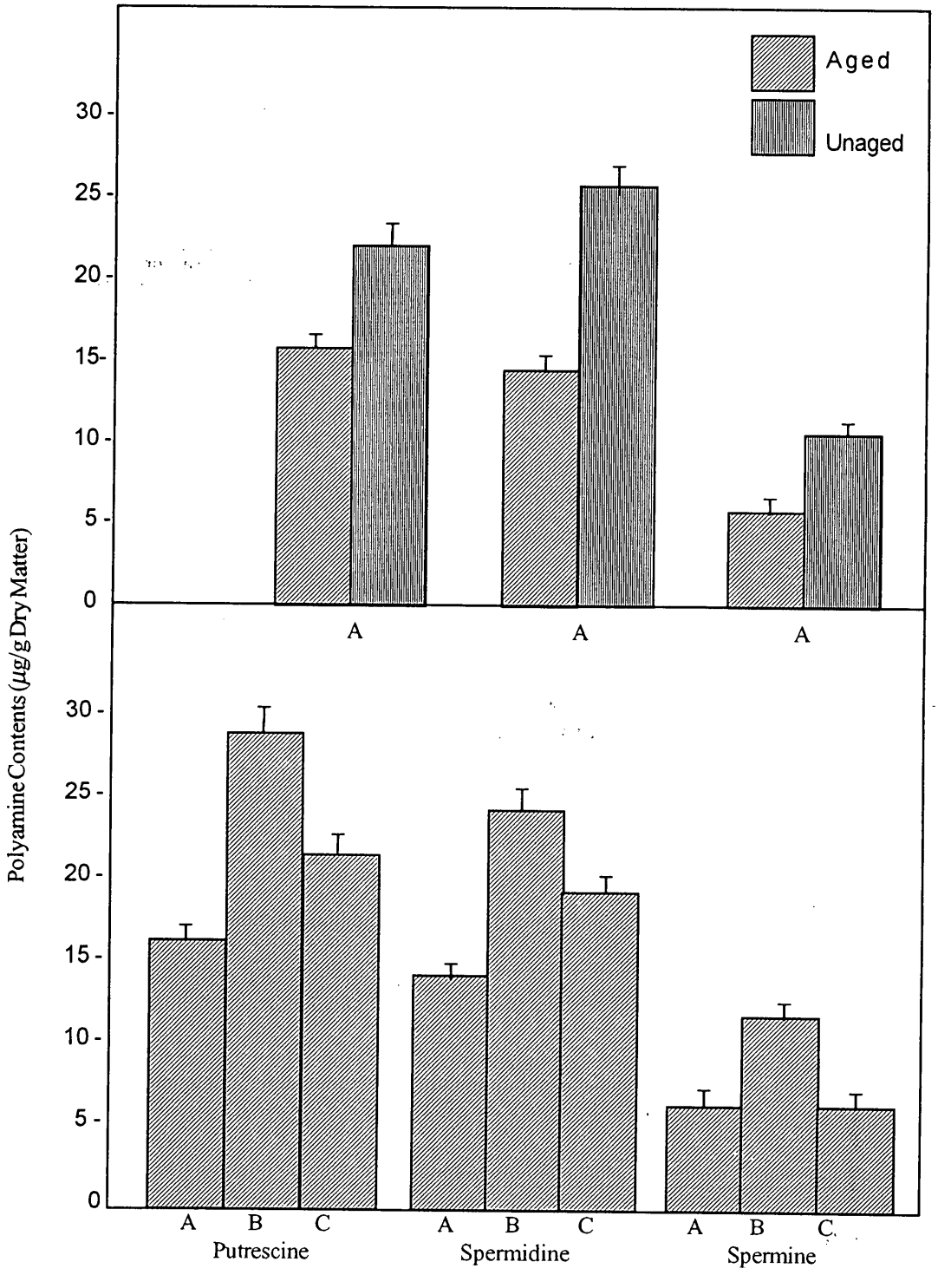

Fig. 1. Priming-induced changes in polyamine levels of onion seeds. Aged seeds were primed with 25% polyethylene glycol for 5 days, followed by either surface drying or drying-back. A=Dry seeds. B=Primed and surface-dried seeds. C=Primed and dried-back seeds.

Table 1. Germination and early seedling growth of aged onion seeds. Unprimed, or primed with polyethylene glycol (PEG) and surface-dried (SD) or dried-back (DB). Priming was done in Petri-dishes at 20 °C in the dark. ± denotes the standard error.

Priming treatment	Duration	Germination (%)		Mean germination time (h)		Primary root length (mm)		Shoot length (mm)	
		SD	DB	SD	DB	SD	DB	SD	DB
Untreated control		-46.0 ± 2.8		85.9 ± 3.5		10.5 ± 2.5		17.9 ± 3.7	
25% PEG	3	50.0 ± 2.5	50.0 ± 2.5	67.0 ± 2.5	72.9 ± 2.8	17.1 ± 1.6	15.7 ± 2.1	29.7 ± 2.3	22.8 ± 3.1
25% PEG	5	48.5 ± 2.5	46.0 ± 2.1	61.0 ± 2.4	69.0 ± 2.6	17.7 ± 1.1	16.5 ± 1.8	30.5 ± 2.0	24.3 ± 2.7
30% PEG	3	48.5 ± 2.4	50.5 ± 2.4	72.0 ± 2.6	77.9 ± 2.8	16.2 ± 1.9	15.9 ± 2.5	28.3 ± 2.9	22.0 ± 3.4
30% PEG	5	52.5 ± 2.5	48.0 ± 2.2	67.2 ± 2.6	73.9 ± 2.7	16.7 ± 1.5	16.4 ± 2.1	29.5 ± 2.6	23.7 ± 3.0

Table 2. Germination and early seedling growth of aged onion seeds. Influence of polyamine treatment (100 μmole) applied alone, or in combination with polyethylene glycol (PEG) priming (25%, 5 days) and surface drying (SD) or drying back (DB). Priming was done in Petri-dishes at 20 °C in the dark. ± denotes the standard error.

Priming treatment	Germination (%)		Mean germination time (h)		Primary root length (mm)		Shoot length (mm)	
Untreated control	46.0 ± 2.8		85.90 ± 3.5		10.50 ± 2.5		17.90 ± 3.7	
Putrescine	44.5 ± 1.5		75.10 ± 3.2		13.65 ± 2.8		24.74 ± 3.1	
Spermidine	48.0 ± 2.0		77.36 ± 3.6		14.28 ± 2.2		22.58 ± 3.5	
Spermine	46.0 ± 3.0		75.90 ± 2.8		12.18 ± 2.5		21.40 ± 3.0	
	SD	DB	SD	DB	SD	DB	SD	DB
PEG	48.5 ± 2.5	46.0 ± 2.1	61.0 ± 2.4	69.0 ± 2.6	17.7 ± 1.1	16.5 ± 1.8	30.5 ± 2.0	24.3 ± 2.7
PEG + putrescine	50.5 ± 2.6	49.0 ± 2.6	62.4 ± 2.7	67.0 ± 2.9	17.1 ± 2.1	15.2 ± 2.4	32.1 ± 2.9	30.2 ± 3.5
PEG + spermidine	54.0 ± 2.6	53.5 ± 2.5	60.5 ± 2.7	67.2 ± 2.8	17.5 ± 2.0	16.3 ± 2.5	29.1 ± 2.9	27.2 ± 3.6
PEG + spermine	49.0 ± 2.5	46.5 ± 2.4	62.4 ± 2.6	69.2 ± 2.8	18.1 ± 1.8	16.9 ± 2.2	31.0 ± 3.1	25.0 ± 3.59

back to original moisture content at 20 ± 1 °C for 2 days. Extraction and analysis of polyamines was performed according to Szczotka (1984), as detailed earlier (Dhillon-Grewal et al., 1992).

Results and Discussion

Osmotic priming with PEG did not affect the germination percentage, but the rate of germination was markedly improved along with root and shoot growth (Table 1). Priming with 25% PEG for 5 days proved to be better than with 30% PEG for a similar duration. Primed seeds dried back to their original moisture content retained the beneficial effect of priming, but the response was best with primed and surface-dried seeds.

In view of the priming-induced improvement of germination rate and seedling growth, the seeds were monitored to determine if the levels of endogenous polyamines could be correlated with the observed response. Putrescine, spermidine, and spermine were detectable both in freshly harvested and in one-year old seeds, but the aged seeds had decreased levels (Fig. 1). This observation is in marked contrast to other studies, which have reported increased polyamine levels during the loss of seed viability (Anguilessi et al., 1974; Mukhopadhyay et al., 1983). A correlation between higher polyamine contents and superior seed storage performance in maize has, however, been established (Lonzavo et al., 1989).

Interestingly, the priming-induced invigoration of aged seeds related to enhanced accumulation of polyamines, but the increase of putrescine and spermidine was more pronounced than that of spermine (Fig. 1). Drying-back treatment reduced the extent of this accumulation.

Much of the polyamine activity may be membrane related. Since osmotic priming also results in reduced lipid peroxidation (our unpublished data), it is probable that polyamines act to reverse seed-ageing effects in onion by a mechanism involving membrane protection and/or repair. Their protection of membranes from lipid peroxidation (Kramer and Wang, 1989) could involve both their ability to interact with phospholipids (Roberts, 1986) and their radical-scavenging properties (Drolet et al., 1986). This conclusion is supported by the fact that exogenous application of polyamines could partly replace the priming treatment. Incorporation of 100 μmole of putrescine, spermidine, or spermine in the

priming medium, however, did not enhance the response further (Table 2), suggesting that the response was maximized by a priming-induced increase of endogenous polyamine levels.

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多元胺量的改變誘導老化之洋蔥種子活力的變化

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種子老化引起多元胺含量降低，而 PEG 的滲透壓處理可使老化的洋蔥 (*Allium cepa* L. cv. Punjab Red-48) 種子活力明顯恢復，滲透壓處理所造成多元胺，特別是 putrescine 及spermidine，含量的提昇與種子活力之促進有關。本文並討論多元胺對膜之保護和維修所扮演的角色。

關鍵詞：老化；洋蔥；滲透激發；多元胺；種子活力；*Allium cepa.*