Studies on the physiology of deterioration of wheat (*Triticum aestivum* L.) seeds

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Introduction

In cereals where a small embryo is accompanied by a very large insoluble food reserve, enzymes involved in the mobilisation of that reserve play a crucial role in the establishment of a healthy seedling. The activity of these enzymes is closely controlled during germination and subsequent seedling growth and this control has been the subject of much research (e.g., Fincher, 1989). Relatively little attention, however, has been paid to any changes in activity of these enzymes during seed deterioration and their importance in the loss of seed viability and vigour.

This poster reports preliminary attempts to determine changes in protease and α-amylase activity in a single seed lot of wheat (cv. Karamu). In an attempt to determine the importance of any of the changes observed, seed quality was also manipulated by a pre-sowing seed treatment which we have already shown to enhance the germination performance of wheat seeds, but which accelerates seed deterioration on subsequent storage (Nath *et al.*, 1991).

Materials and Methods

A freshly harvested (1988) seed lot of wheat (cv. Karamu) was used throughout these studies. In order to simulate ageing, seeds were stored at a constant seed moisture content of 15% at 35°C for up to 60 d. Pre-sowing treatment was carried out by imbibing the seeds for 20 h at 20°C in a -0.37 MPa polythylene glycol (PEG) solution followed by drying the seeds back to near original seed moisture contents. Subsequent germination testing of the seeds was carried out in Petri dishes at 10°C, monitoring percentage and rates of radicle emergence. Further details of these procedures are given in Nath *et al.* (1990).

α-Amylase activity, extracted from both whole grains and embryoless half grains incubated in $2x10^{-6}$ M GA_3, was assayed using a dye-conjugated β-limit dextran as a substrate as per Cornford *et al.* (1987). Soluble sugars were determined on 80% ethanolic extracts of seed powder using the anthrone method (McCready *et al.*, 1950) and general protease levels using azocoll as a substrate, following the method of Ragster and Chrispeels (1979).

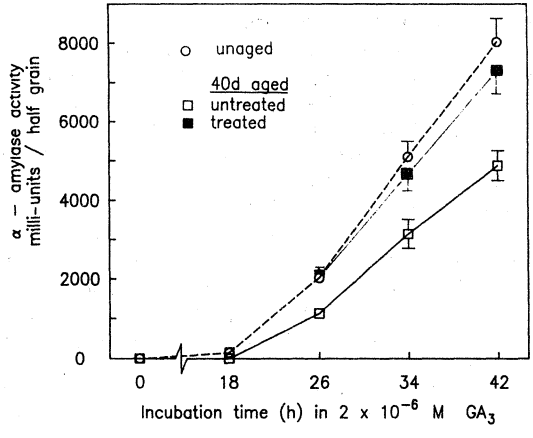

Figure 1. α-Amylase production by embryo-less half grains of cv. Karamu in response to gibberellic acid. Each data point is the mean of three replications, individual standard errors are shown when larger than the symbols used.

Results and Discussion

Viability and α-amylase production

During storage, seed viability (measured as final percentage radicle emergence) began to decrease after 20 d, while rates of radicle emergence (a measure of seed vigour) decreased within 10 d. PEG treatment after ageing increased rates of radicle emergence, but did not improve seed viability. The endosperm tissue of wheat grains stored for 40 d lost sensitivity to exogenously applied GA_3, but if grains were subsequently primed with PEG after storage, α-amylase production in response to GA_3 was restored to its original level (Fig. 1).

Seeds treated before ageing showed elevated α-amylase levels which were maintained during storage, but total soluble sugar levels were unaffected. In addition, scanning electron microscopy (SEM) examination of the endosperm in the scutellar-crease region provided no evidence of any starch breakdown in ungerminated aged seeds. Accordingly, it is unlikely that raised α-amylase activity is involved in the accelerated loss of viability of stored, treated seeds. However, loss of vigour of the remaining viable seeds in a deteriorated population may be intimately related to aleurone responsiveness to gibberellic acid.

Changes in proteins and protease activity

Scanning electron microscopy of the endosperm in the scutellar-crease region indicated that PEG treatment resulted in the breakdown of matrix protein in this area. Typically, untreated seeds showed a decrease in protease levels during storage, but activity of this group of enzymes tended to remain high in seeds treated before ageing. Previous studies on total protein extracts (Nath, 1991) had shown that a high molecular weight (270 kD) protein fraction appeared to be selectively deleted from seeds treated before ageing. This missing fraction is thought to be a glutelin aggregate, but HPLC protein analyses of whole seeds provided no supporting evidence of quantitative changes in these grains.

From these preliminary results we conclude that protease and possibly localised protein changes may be contributory factors to the accelerated rate of deterioration of wheat seeds resulting from pre-sowing treatment prior to storage. This is the subject of further work being undertaken at present.

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