The efficiency of hydration - dehydration seed treatments for improving the storage of wheat seeds

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Abstract

Three pre-sowing hydration-dehydration treatments have been evaluated for their capacity to protect or repair the germination performance of wheat (*Triticum aestivum* L.) seeds in simulated storage (either severe conditions of 100% R.H., 40°C for up to 6 days, or slower ageing in sealed packages for up to 60 days at 35°C, 15% seed moisture content).

Applied before storage, a short soaking treatment (2h at 25°C followed by drying) allowed some small protection of seed germinability, but was of no advantage when used after storage. In contrast, two longer hydration treatments (either 24 hours at 15°C in water, or 20 hours at 20°C in -0.37 MPa PEG solution, followed by drying) offered considerable potential for improving the vigour of deteriorated but still viable seed after storage. Any differences in effectiveness between these two methods could be accounted for by duration of treatment expressed on a thermal time basis.

However, when the two longer hydration treatments were applied to seed before storage, they greatly increased the grains' susceptibility to deterioration. We have started to investigate the possibility that some of these deleterious effects are due to changes in the activity of hydrolytic enzymes associated with seed germination, by looking at α-amylase activity in PEG treated and aged seeds. Treatment of unaged seeds results in marked increases in α-amylase activity which are localised in both the embryo and the portion of the endosperm adjacent to the embryo. These high levels are maintained during ageing and are associated with considerably increased leakage of soluble sugars from the seeds.

Additional key words: *α-amylase, germination, germination rate, leakage, seed deterioration, sprouting damage*

Introduction

Over the past two decades a small number of papers have been published reporting attempts to improve the germination performance of cereal grains using pre-sowing hydration treatments (e.g., Abdul-Baki and Anderson, 1970; Hanson, 1973). A particular focus has been on the potential of these treatments to improve seed storage, either as protective treatments applied prior to ageing (e.g., Rudrapal and Basu, 1982) or as repair treatments applied to aged seeds (Goldsworthy *et al.*, 1982; Dell'Aquila *et al.*, 1984).

Little coherent information has emerged, however, due to varying responses between cultivars and seed lots, different treatments used, whether or not seeds were dried-back after treatment and, not least, the wide variety of evaluation conditions used. In many cases the improvements noted are relatively small in absolute terms (e.g., Goldsworthy *et al.*, 1982) and tend to disappear when the grains are germinated under unstressed conditions (e.g., Hanson, 1973); but even here, conflicting results have been recorded. For example, Goldsworthy *et al.* (1982) reported a response to their treatments only under low temperature, but not under osmotic stress at higher temperatures. In their paper they misreported Hanson (1973) who found conflicting results with the wheat cv. Cappelle Desprez where clearer advantages were found under osmotic stress than at 10°C. In this latter study germination advancement effects disappeared almost entirely at higher temperatures, a problem not reported by Basu (1976).

Very little information is available on the possible mechanisms of these hydration treatments in cereals although Rudrapal and Basu (1982) demonstrated that treated seeds showed reduced leaching on subsequent ageing and less peroxidation damage. Apart from this, the only available evidence in wheat comes from the work of Hanson (1973) who found that treated grains showed increased respiratory activity and increased ^{14}C-leucine incorporation into aleurone tissue. He also demonstrated that hydration treatment advanced α-amylase synthesis on subsequent germination.

It was therefore decided to carry out a detailed comparison of appropriate modifications of three of the most promising treatments reported in the literature (Hanson, 1973; Goldsworthy *et al.*, 1982; Dell'Aquila *et al.*, 1984) and, using a single high quality seed lot of cv. Karamu, examine the interactions between these seed treatments and seed ageing. The opportunity was also taken to develop Hanson's preliminary findings on α-amylase in an attempt to further our understanding of the mechanisms of germination advancement and deterioration of wheat grains.

Materials and Methods

Seed material

A freshly harvested (1988) seed lot of wheat (*Triticum aestivum* L. cv. Karamu) was obtained from Hodder and Tolley, Ltd., New Zealand. As purchased, the seed stock had a normal germination percentage of 96% and an initial seed moisture content (SMC) of 12.5% (fresh weight basis). Seeds were stored in sealed containers at 5°C until required.

Storage treatments

Two artificial ageing regimes were chosen, a very harsh accelerated ageing (AA) treatment where seeds were held for up to 6d at 40°C, 100% RH (Delouche and Baskin, 1973) and a much less severe controlled deterioration method where seeds were stored at a constant 15% SMC for up to 60 days at 35°C, using a modification of the approach proposed by Matthews and Powell (1987). After 6 days under the severe conditions the decrease in final percentage radicle emergence was very similar to that found after 50d in the less severe controlled deterioration conditions.

Hydration treatments

Three pre-sowing treatments were used in this study. The first of these (after Goldsworthy *et al.*, 1982) was a short soaking treatment where seeds were held for 2h in twice their own weight of distilled water at 25°C. Two longer treatments were also evaluated. In one, seeds were imbibed in distilled water at 15°C (cf. Hanson, 1973). Preliminary work showed that a 24h treatment was the most appropriate for this cultivar to obtain maximum germination advancement without radicle emergence during the treatment (data not shown). The other longer hydration treatment was similar, except that seeds were soaked for 20h at 20°C using a -0.37 MPa PEG 6000 solution to prevent radicle emergence during the treatment period. At the end of this time the seeds were washed in distilled water to remove any adhering PEG. This approach was previously used by Dell'Aquila *et al.* (1984) for the cultivar Appulo where treatments for up to 48h were used. Here, preliminary studies indicated 20h was the optimum time for cv. Karamu.

After all treatments, grains were dried for 48h at 25°C, returning them to moisture contents (determined by the oven method, ISTA, 1985) within the range 11.3 - 13.7% fresh weight. Both Hanson (1973) and Goldsworthy *et al.* (1982) dried their seeds before evaluating treatment effects, but this was not done in the previous PEG studies (Dell'Aquila *et al.*, 1984).

Evaluation of germination performance

Radicle emergence was assessed by germinating samples of 50 seeds on filter paper in petri dishes at 10°C and counting at very frequent intervals (less than 6h at times of peak activity) so that times to 50% radicle emergence (T_{50}) could be accurately assessed using the method described by Coolbear *et al.* (1984). After preliminary comparisons with assessment of germination at 20°C, the lower temperature was chosen to emphasise differences in germination performances (cf. Hanson, 1973 and Goldsworthy *et al.*, 1982, discussed in the introduction).

α-amylase activity

Enzyme activity was determined using a dye conjugated β-limit dextrose as substrate (Phadebas blue starch powder, Pharmacia Diagnostics Ltd.). Either ten whole seeds or four embryos or sections of endosperm were ground using a pestle and mortar and extracted as described by Cornford *et al.* (1987), adjusting the volume of extraction buffer as necessary. One ml. of enzyme extract was added to 10mg substrate in a total reaction volume of 5ml and incubated for 20 mins at 37°C. The reaction was terminated by adding 1 ml 0.5M NaOH and the mixture centrifuged at 2000g for 10 min and the absorbency of the supernatant at 620mm measured. Assays were standardised against pure barley malt

α-amylase (Sigma Chemical Co.), one enzyme unit being defined as the amount of enzyme that will hydrolyse 1.0mg maltose from starch at pH 6.9 in 3 min at 20°C.

Soluable sugars

The total soluble sugar content of seeds was determined using the anthrone method (McCready *et al.*, 1950) to assay hot 80% (v/v) ethanolic extracts of 200mg seed powder (Adams *et al.*, 1980). For the leachate studies, 25 seeds were soaked for 24h in 50 ml de-ionised water at 25°C and an aliquot of leachate assayed by the same method. Assays were standardised against glucose.

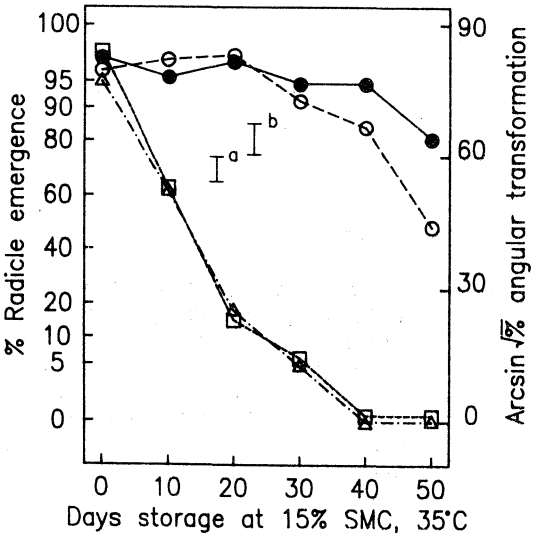

Figure 1: The effect of pre-storage hydration treatments on percentage radicle emergence of wheat seeds, cv. Karamu, stored at 15% SMC, 35°C: O, untreated controls; ●, imbibed for 2h in distilled water at 25°C; Δ, imbibed for 24h in distilled water at 15°C; and □, imbibed for 20h in 0.37 MPa PEG at 20°C. All seeds were dried-back after treatment. Each data point is the mean of four replication. a, LSD for means within ageing times and b, LSD for means between ageing times (p= 0.05). Data were subject to arcsin transformation before analysis.

Results

Germination performance

None of the three pre-sowing hydration treatments examined caused a significant change in germination capacity of the seed stock (Fig. 1), but all caused an increase in radicle emergence rates (Fig. 2). The effects of the 2h hydration treatment on T_{50} radicle emergence were very marginal (a decrease of only 7% at 10°C) compared to the two longer treatments where decreases T_{50} of around 35% were achieved. When held at 35°C and 15% SMC, untreated seeds began to lose germination capacity after 20 days, less than 50% of the seeds being capable of producing radicles after 50 days storage. Applying the 2h hydration treatment offered significant protection to the grains (Fig. 1). In marked contrast, the longer hydration treatments applied before storage, dramatically increased the rate of deterioration, resulting in total loss of germinability after 40 days at 35°C. Very similar responses were found when the treated seeds were exposed to the more severe accelerated ageing conditions (data not shown).

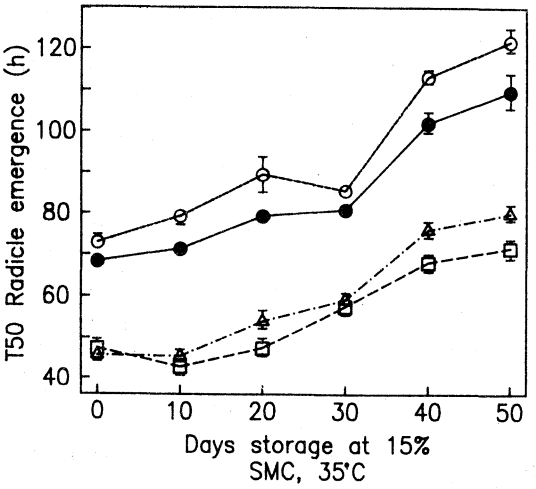

Figure 2: The effect of different post-storage hydration treatments on the median radicle emergence time after seed storage at 15% SMC, 35°C. Symbols as for Figure 1. Each data point is the mean of four replications. Individual SEM's are shown where larger than the symbols used.

When seeds were treated after storage, none of the techniques had any effect on the germination capacity of the aged seeds. However, all treatments resulted in an improvement of the radicle emergence rates of the remaining viable seeds, the two longer treatments being much more effective, resulting in decreases in T_{50} radicle emergence by up to 50% (Fig. 2). Seeds imbibed for 20h in -0.37 MPa PEG at 20°C generally performed slightly better than those seeds held for 24h in distilled water at 15°C. The effects of the treatments on seeds after accelerated ageing were very similar, except that no advantage in radicle emergence rates was obtained by the two hour hydration treatment on seeds after 4 or 6 days AA (data not shown).

α-amylase activity

In all subsequent experiments only the effects of imbibing the seeds for 20h in -0.37 MPa PEG followed by drying back were investigated as part of a preliminary attempt to understand the changes induced by treatment and ageing on hydrolytic enzyme activity and its relationship (if any) to germination performance. This treatment reduced the T_{50} of unaged seeds by 26% (Fig. 2), but resulted in a several-fold increase in α-amylase activity. Analysis of this in dissected grains identified the location of increased activity (Fig. 3). There was a significant increase of activity in the endosperm region adjacent to the embryo and the appearance of activity in the embryo itself. α-amylase activity in more distal parts of the endosperm were unaffected by the seed treatment.

During storage at 15% SMC, 35°C, previously treated seeds retained their high levels of α-amylase activity with no significant losses over 60d (data not shown). However, soluble sugar levels within seeds remained unchanged at around 1.7-1.8 mg/seed, irrespective of treatment and/or storage for 40d. On the other hand, while untreated seeds showed a small but significant increase in sugar leakage during soaking after ageing, the leakage from treated seeds after storage increased by more than an order of magnitude (Table 1).

Despite the enhanced rate of radicle emergence gained by aged seed if treated after storage at 15% SMC, 35°C (Fig. 2), there was only a marginal increase (if any) in α-amylase activity in these seeds prior to germination (Fig. 4). It is interesting to note that, while seeds stored for 20d showed germination responses (either before or after treatment) which were very similar to those of unaged seeds (Figs. 1 and 2), changes in α-amylase activity were closer to those found in 40d aged material (Fig. 4).

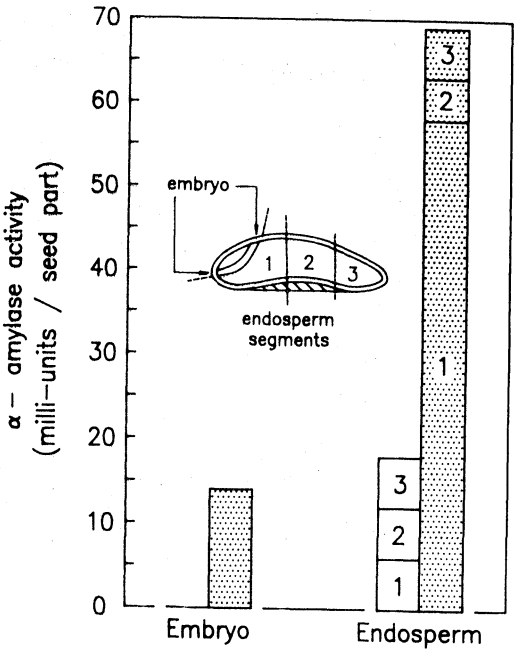

Figure 3: **α-amylase activity from different parts of the seed before or after (open or shaded histogram bars, respectively) pre-sowing treatment (20h in -0.37 MPa PEG solution at 20°C, followed by drying back). Data are means of two replicate assays. The inset diagram shows how the grains were dissected.**

TABLE 1: **Amount of soluble sugars leached from grains after 24h soaking at 25°C. Seeds were subjected to a pre-sowing treatment (20h imbibition in -0.37 MPa PEG solution at 20°C, followed by drying back) and an ageing treatment (40d at 35°C, 15% SMC).**

	Soluable sugars in leachate (μg/seed)[1]	
	Untreated	PEG treated
Unaged	13.7 (±1.03)	12.3 (±2.23)
Aged	38.3 (±8.27)	188.5 (±10.66)

[1] Values are means of three replicates (±SEM's).

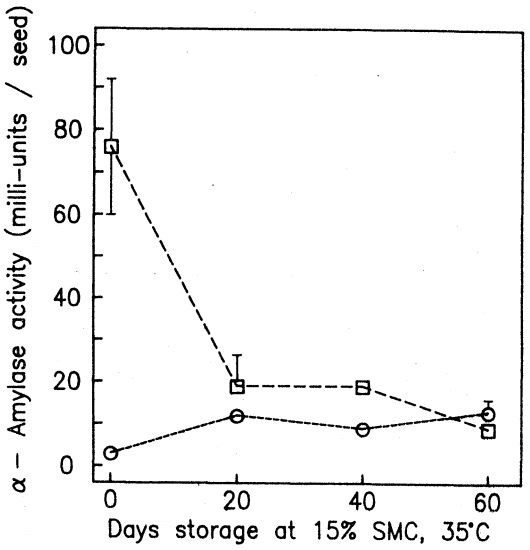

Figure 4: Changes in α-amylase activity in untreated seeds during storage at 15% SMC, 35°C (O) and in seeds given a pre-sowing treatment of (20h in -0.37 MPa PEG solution at 20°C followed by drying-back) after storage (□). Data are means of three replications; individual SEM's are shown when larger than the symbols used.

Discussion

Germination performance

The effects of the short hydration treatment on unaged seeds are marginal, but it has a clear protective effect if applied prior to ageing (Fig. 1). This is a confirmation of earlier findings by Basu (1976) and Rudrapal and Basu (1982) although, in the former paper at least, germination percentages presented were interim rather than final counts. The effect of the two longer hydration treatments is in marked contrast to this (Fig. 1). Applied before storage, both treatments cause a severe acceleration of seed deterioration. Although the results of Lush *et al.* (1981) suggested that wheat seeds, given a hydration treatment, were more susceptible to deterioration when exposed to 50°C for up to 3 days, the results reported here were unexpected since the storage conditions were less severe and in view of work on pre-storage treatments of vegetable seeds (Coolbear *et al.*, 1984; Dearman *et al.*, 1986). However, recent work on tomato seed treatments by Alvarado and Bradford (1988) and Argerich *et al.* (1989) has suggested that osmotically primed seeds store less well than untreated ones.

The beneficial effects of the two longer hydration treatments on times to fifty percent radicle emergence in aged and unaged seeds (Fig. 2) confirm earlier findings on rates of germination of unaged wheat (Hanson, 1973) and in deteriorated vegetable seeds (e.g., Coolbear *et al.*, 1984; Dearman *et al.*, 1986). It is, however, the first demonstration that such treatments are effective on aged wheat seeds when the grains are subsequently dried back after treatment.

The additional effectiveness of the PEG treatment at 20°C over the effects of distilled water treatment at 15°C can be explained on a thermal time basis if we disregard any water stress imposed by this concentration of PEG and take the minimum germination temperature for wheat grains as 4°C (Mayer and Poljakoff-Mayber, 1982). On this basis PEG treated grains receive 2.3 day-degrees more treatment than those treated in water at 15°C.

It is very possible that the advantages of the short hydration treatments simply reflect the leaching of oxidisable toxic compounds from the seeds. Fielding and Goldsworthy (1982) demonstrated that germination of naturally aged seed lots of wheat was inhibited by heating to temperatures above 50°C and that this inhibition was associated with the evolution of unidentified volatiles. The levels of emission of these compounds and the inhibition of germination were both decreased by applying the heat treatment in a non-oxidising environment. While we know that both oxidative reactions and accumulation of toxins are both likely mechanisms of seed deterioration (Priestley, 1986), Fielding and Goldsworthy did not make any explicit connection between their two papers. Nevertheless we do have some evidence that the leachate derived from the 2h soak treatment is indeed inhibitory to the rate of wheat germination (Nath, unpublished data).

Changes in α-amylase activity

During ageing there is a small but significant increase in enzyme activity in untreated seeds (Fig. 4). These results are in agreement with those of Warchalewski *et al.* (1985) who observed a 68% increase in α-amylase activity in wheat stored for 4 years. It is possible that these small increases are due to the release of enzyme held in the bound form

during ageing, because inclusion of papain during extraction has been shown to increase the yield of α-amylase from ungerminated wheat seed extracts (Kneen *et al.*, 1942). In contrast, Perl *et al.* (1978) reported that α-amylase activity in sorghum showed an initial small increase followed by a rapid decline to much lower levels during ageing even though germination capacity was decreased by less than 10% under the ageing conditions used (Gelmond *et al.*, 1978).

It is doubtful, however, whether there is a direct relationship between treatment effects on α-amylase activity and changes in the seeds' responses to ageing. Although high α-amylase levels are maintained in treated seeds on subsequent storage, soluble sugar analysis of intact grains provides no evidence that there is significant breakdown of starchy endosperm. However, very large increases in leachate sugar levels from treated, aged seeds (Table 1) are evidence that other hydrolytic damage (particularly that affecting membrane permeability) is occurring in these rapidly deteriorating seeds. Similarly, changes in α-amylase levels in grains treated after ageing show no relationship between advancement of radicle emergence and enzyme activity in the ungerminated seed (compare Figs. 2 and 4).

It is possible that short hydration treatments may have potential commercial value for protecting wheat seed held in poor conditions. This would need careful evaluation over several storage environments with a range of cultivars and seed lots. Although the longer treatments applied post-storage show clear benefits, it is unlikely that the reduction in germination rate obtained would be of economic significance. However, the system provides a useful tool for understanding the control of hydrolytic enzyme activity in the grain. Further work is currently being undertaken on both changes in proteolytic enzymes in these seeds and on the effects of the PEG hydration treatment and ageing conditions on the gibberellic acid control of α-amylase activity during subsequent germination. The latter, in particular, constitutes a major area of interest for the physiology of sprouting damage, where low *in vivo* α-amylase activity during late development is a key factor in the maintenance of flour quality (cf. Kruger, 1980; Halverson and Zeleny, 1988).

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