Ageing of wheat grains : Two (or more) ways to die?

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INTRODUCTION :

During storage of seeds, ageing leads to a progressive decrease in their vigour, and then in a loss of their viability. Seed deterioration is associated with numerous cellular and biochemical alterations including loss of membrane integrity, impairment of RNA and protein synthesis, and DNA degradation. However, reactive oxygen species accumulation and lipid peroxidation are often invoked to be the major causes of seed deterioration (Priestley, 1986). Soluble sugars might also be involved in seed storability (Obendorf, 1997). Soluble sugar contents and antioxidant enzyme activities were measured in the embryo of wheat grains throughout their storage in 2 conditions (45°C and 100% RH and 30°C and 75% RH) in order to investigate whether grain deterioration during ageing was related to lipid peroxidation resulting in a decrease in the efficiency of the antioxidant defence system, and in changes in sugar metabolism.

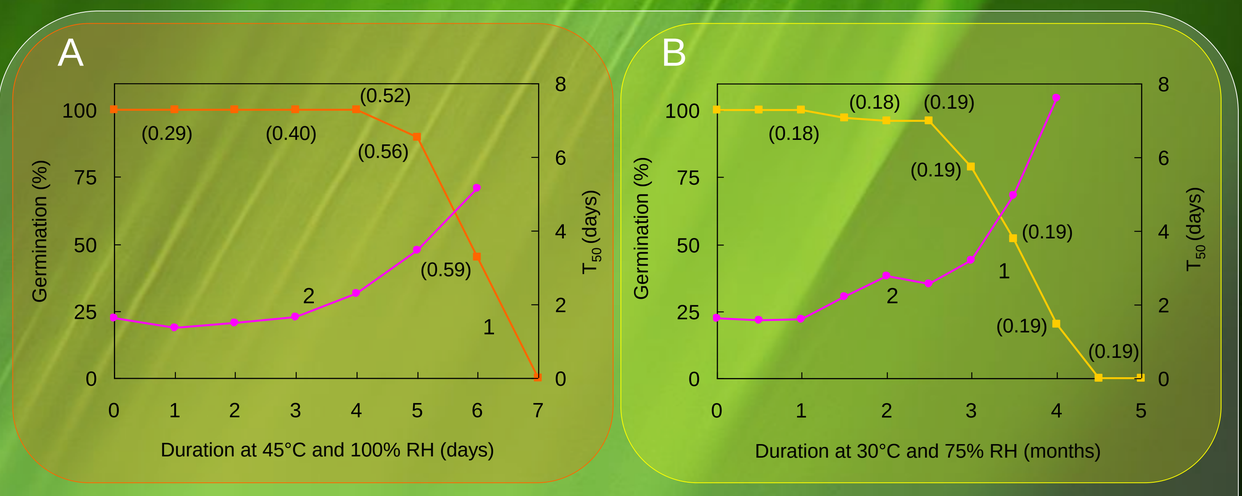

Fig. 1. Changes, during accelerated ageing at 45ºC and 100% RH (A) and at 30ºC and 75% RH (B), in grain viability (1) and the time to reach 50% of the germination percentages obtained after 14 days (T_{50}) (2). Numbers in brackets represent the water content (g H_2O g^{-1}DW) of the embryo during ageing. Means of 4 replicates (germination) or 3 measurements (water content).

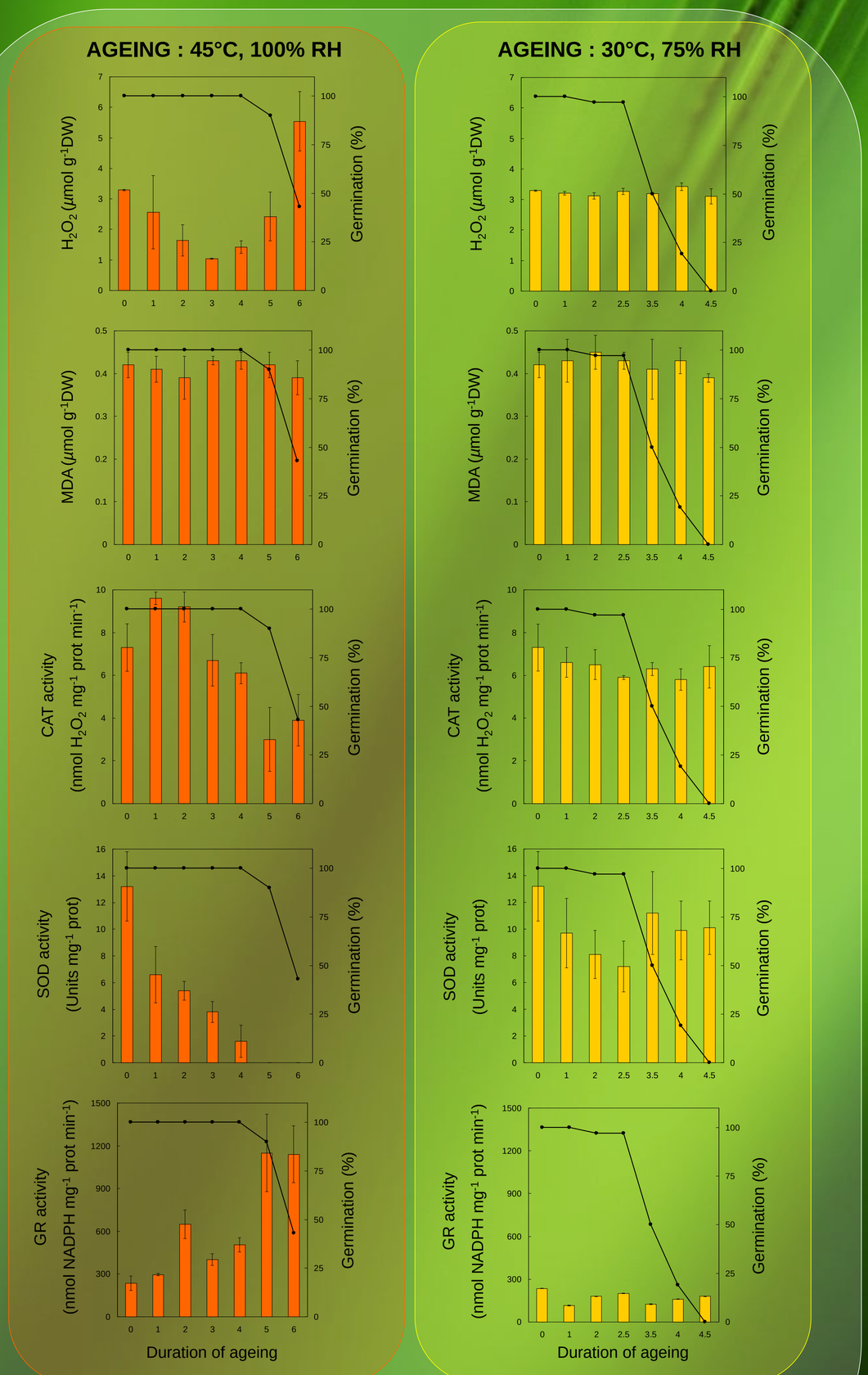

Fig. 2. Changes in H_2O_2 and MDA contents and in CAT, SOD and GR activities in embryos isolated from grains aged for various durations at 45°C and 100% RH (left hand panel) or at 30°C and 75% RH (right hand panel). Black curves represent seed viability. Means of 3 measurements ± SD.

RESULTS:

❶ Effect of ageing treatments on seed germination

Ageing at 45°C and 100% RH (Fig. 1A) and at 30°C and 75% RH (Fig. 1B) was associated with a decrease in seed vigour, evaluated by T_{50} (Fig. 1, curve 1) and then in seed viability (Fig. 1, curve 2). The longer the treatment and the higher the temperature and the RH, the stronger the reduction of seed viability was. The half viability period was only 6 days at 45°C and 100% RH (Fig. 1A), as against about 3.75 months at 30°C and 75% RH (Fig. 1B).

❷ H_2O_2 content and lipid peroxidation

H_2O_2 content remained close to 3.1-3.4 µmol g^{-1} DW during ageing at 30°C and 75% RH. In contrast, at 45°C and 100% RH, H_2O_2 content decreased during the first 3 days reaching 1.04 µmol g^{-1} DW and then increased regularly up to 5.54 µmol g^{-1} DW after 6 days of ageing (Fig. 2). MDA content of the embryo did not change significantly during both ageing treatment (Fig. 2).

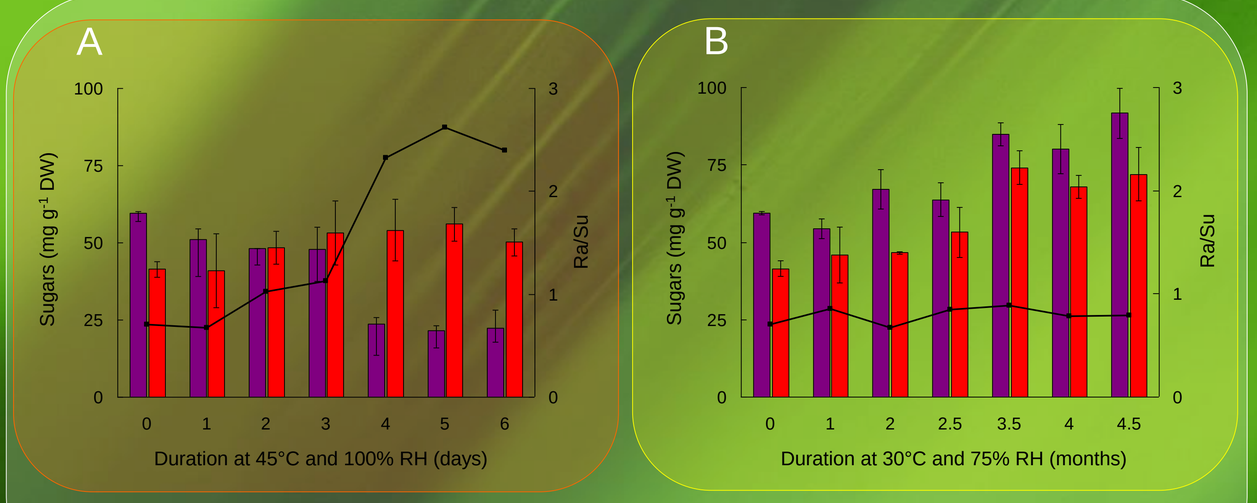

Fig. 3. Changes in sucrose (■) and raffinose (■) contents and in the Ra/Su ratio (black curve) in embryos isolated from grains after various durations at 45°C and 100% RH (A) and at 30ºC and 75% RH (B). Means of 3 measurements ± SD.

❸ Antioxidant enzyme activities

When seed are aged at 30°C and 75% RH, there is no clear change in CAT, SOD and GR activities (Fig. 2, right hand panel). In contrast, at 45°C and 100% RH (Fig. 2, left hand panel), loss of viability was associated with a strong decrease in CAT and SOD activities, whereas after 6 days of treatment GR activity increased more than 4 fold compared to the control.

❹ Sucrose and raffinose contents

At 30°C and 75% RH, loss of viability was associated with an increase in both sucrose and raffinose. Consequently the Ra/Su ratio remained constant at about 0.6-0.9 while seeds were dying (Fig. 3B). When ageing was performed at 45°C and 100% RH, loss of viability was associated with a strong decline in sucrose content, when raffinose content remained constant (Fig. 3A). The Ra/Su ratio increased then progressively to reach 2.5 after 6 days of treatment as against 0.7 for the non-aged control seeds (Fig. 3A).

CONCLUSION :

➔ As expected, wheat seeds were more sensitive to accelerated ageing performed at 45°C and 100% RH (i.e. when the water content of the embryo was 50-60% DW) rather than when ageing was performed at 30°C and 75% RH (i.e. When the water content of the embryo was around 18% RH).

➔ Loss of grain viability did not seem to be associated with lipid peroxidation. At 45°C and 100% RH, loss of viability might also be attributed to H_2O_2 production without MDA accumulation.

➔ Loss of viability was concomitant to a reduction of the potential efficiency of the antioxidant defence system during ageing at 45°C and 100% RH.

➔ Changes in soluble sugars contents were not good markers of loss of seed vigour.

➔ Our results suggested that many different biochemical mechanisms are involved in grain death and that using one type of ageing is not enough to understand and to predict seed storability.

Material and Methods :

Plant material : Wheat (*Triticum aestivum* L., cv Charger) plants were grown in the experimental fields of Syngenta-Agro located in Gaillon (Eure, France) in 2004. Ears were collected at the end of the maturation drying phase. Experiments were carried out with non dormant grains and no sprouting injury was observed. Germination tests were performed with whole non dormant caryopses. Biochemical measurements were carried out on isolated embryos cut off the seeds with as little endosperm as possible, though still covered dorsally by the seed coat.

Germination assays and seed viability determination : Germination assays were performed by placing whole caryopses (4 replicates of 25 grains each) on a layer of cotton wool moistened with deionised water, in darkness at 20°C. A grain was regarded as germinated when the radicle had pierced the seed coat. Germination counts were made daily up to 14 d. The results presented are the means of the germination percentages obtained in 4 replicates ± SD. They are expressed as the germination percentages obtained after 14 d, or as the time to reach 50% of the germination percentages obtained after 14 d (T_{50}) estimated graphically from the cumulative germination curves. Topographical tetrazolium testing (Moore, 1973) was also used to verify that seeds which did not germinate at 20°C were dead.

Accelerated ageing treatment : Ageing treatment were carried out by placing seeds for 1 to 5 days at 45°C in tightly-closed boxes with free water at the bottom in order to obtain 100% RH and for 1 to 4.5 months at 30°C in tightly-closed boxes with saturated NaCl solution at the bottom in order to obtain 75% RH. After ageing, seeds were dried back to their original water content by placing them for 1 week at 20°C and 70% RH, and germination was tested at 20°C for 14 days.

Enzyme extraction and assays : Superoxide dismutase (SOD, EC 1.15.11), catalase (CAT, EC 1.11.16), Glutathione reductase (GR , EC 1.6.4.2) activities were determined at 25°C according to Lehner *et al*. (2006).

Hydrogen peroxide content and malondialdehyde measurements : H_2O_2 contents of isolated embryos were determined according to O'Kane *et al*. (1996) using a modified protocol from Lehner *et al*. 2006. Lipid peroxidation was evaluated by measuring malondialdehyde (MDA) content from 0.5 g FW of isolated embryos, according to Heath and Parker (1968).

Soluble sugar measurements : Soluble sugars were extracted according to Black *et al*. (1996).

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