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RNases and nucleases in embryos and endosperms from naturally aged wheat seeds stored in different conditions

Carmelina Spanò*, Riccardo Buselli, Monica Ruffini Castiglione, Stefania Bottega, Isa Grilli

Dipartimento di Biologia, Università di Pisa, Via Luca Ghini 5, 56126 Pisa, Italy

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Summary

Temperature and moisture content are particularly important factors influencing the longevity of seeds, and therefore the ageing of seeds is closely tied to storage conditions. The ageing process is characterised by many physiological and biochemical changes: membranes tend to leak, enzymes lose catalytic activity, and chromosomes accumulate mutations. Since viability loss is also associated with the breakdown of nucleic acids, the aim of the study was to determine whether the damage induced by ageing could be associated with changes in the activity of RNases and nucleases in embryos and endosperms of differently stored wheat seeds. In order to better characterise seed conditions, the damage to membranes during seed ageing was evaluated by measuring the conductivity of the soaking solution during imbibition, and by using the Evans Blue colorant; lipid peroxidation was also recorded. RNases and nucleases were studied by SDS—PAGE and activity staining. Ageing of seeds stored in a dry state involved a progressive loss of membrane integrity, which increased with the degree of ageing, while lipid peroxidation remained unchanged. Changes in nucleolytic enzyme activity were recorded in embryos: a decrease in RNases and an increase in nucleases. In the endosperm compartment there were no significant differences in ribonuclease and nuclease patterns during seed ageing. Moreover, neutral RNases were absent in endosperms of dry seeds and were activated following imbibition. Present studies reveal that embryos and endosperms have different enzymatic patterns, thus highlighting that the two seed compartments age independently. A different nucleolytic pattern was

Abbreviations: GE, germination energy; MV, middle viable; NV, non-viable; rt, room temperature; SSPN, single-strand-preferring nuclease; TBA, thiobarbituric acid; TBA-rm, thiobarbituric acid-reactive material; V, viable.

*Corresponding author. Tel.: +39 0502215368; fax: +39 0502215380.
E-mail address: cspano@biologia.unipi.it (C. Spanò).

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present in seeds of comparable viability and membrane damage, which were stored differently, and nuclease metabolism was subject to regulation according to both ageing and the length of the storage period.

Introduction

Orthodox seeds, i.e. seeds that can be stored with a low moisture content (Bewley and Black, 1994), retain viability for varying periods and with time they succumb and die. There is generally a gradual decline in germinability and the subsequent vigour of the resultant seedling (Leopold, 1980; Anguillesi et al., 1990; Alsadon et al., 1995; Rice and Deyer, 2001), associated with a higher sensitivity to stress upon germination, and eventually a loss of the ability to germinate.

Several factors affecting seed viability should be considered to determine the optimal storage conditions. In particular, seed survival is not only genetically but also environmentally controlled, and it is generally accepted that storage conditions, above all temperature and seed moisture content, are determinant factors for seed longevity (Walters, 1998). High temperatures and high moisture content reduce longevity and these two parameters are factors in the equations that have been developed to predict the life spans of seeds (Roberts, 1972; Ellis, 1991). In fact, it has been hypothesised that the reactions involved in seed ageing are controlled by the thermodynamic status of water (Walters, 1998). Low moisture content and low temperature could induce a decrease in the enzymatic reactions involved in seed ageing (Priestley, 1986).

Low temperatures have been found to play a protective role in *Triticum durum*: caryopses stored at +10 °C just after harvesting, with a constant moisture content (12%), maintain unchanged germinability for at least 23 years, while the period of relative stability lasts for 5 years when stored under laboratory conditions, and the germination capacity is lost after 7 years (Stefani et al., 2000).

Ageing in seeds stored in the dry state, involves the gradual loss of integrity of membranes, which are barriers that play an essential role in biochemical or physiological events. The deterioration of membranes, which probably involves lipid peroxidation and associated free radical oxidative stresses (Murthy and Sun, 2000), leads to membrane leakage (Priestley, 1986). The leakage of ions, amino acids and sugars is a clear sign of membrane deterioration that results in greatly increased permeability (Bewley and Black, 1994; Priestley, 1986). In the present study, loss of membrane integrity was evaluated by electrolyte leakage assay and by Evans Blue staining in naturally aged seeds of *Triticum durum* cv. Cappelli stored at room or at low temperature.

In addition to membrane deterioration, many biochemical and physiological changes have been identified in ageing seeds. Since the physiological expression of ageing suggests low rates of synthesis, many attempts have been made to assess the involvement of DNA, RNA, and protein synthesis in the deteriorative processes that characterise an ageing seed (Smith and Berjak, 1995). Progressive deterioration of 18S rRNA has been observed in dry non-viable (NV) rye embryos and in wheat embryos (Dell'Aquila et al., 1976); lesions become amplified with progressive imbibition, either as a result of the intrinsic properties of the rRNA, or due to an increase in activity of other components such as nucleases (Osborne, 1980; Smith and Berjak, 1995). In *Triticum durum* a progressive rRNA deterioration has been recorded in embryos of ageing seeds. Embryos of cold stored old seeds maintain the structural stability of RNA and the whole amount of poly(A)$^+$ RNA, and this state persists during imbibition (Grilli et al., 1982).

In the present study, RNases and nucleases were examined using SDS–PAGE and activity staining (Spanò et al., 1999), to verify whether they are subject to regulation during ageing. Both embryos and endosperms were used, as it is known from the literature that the two seed compartments age independently (Floris, 1970). One specific aim was to determine whether damage induced by ageing is associated with changes in the activity of RNases and nucleases. Various forms of RNases were considered: acidic, neutral salt-stimulated and neutral salt-inhibited RNases, while single-strand-preferring nucleases (SSPN) were also studied.

Materials and methods

Plant material

Caryopses (referred to in this paper as seeds) of *Triticum durum* Desf. cv. Cappelli were obtained from plants cultivated in fields specifically used for