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Antioxidant status of *Acer platanoides* seeds during accelerated ageing

Abstract

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The antioxidant status of Norway maple (*Acer platanoides* L.) was investigated in relation to loss of seed viability during accelerated ageing treatment at 30°C and 100% relative humidity, in the dry and imbibed state. Activity of superoxide dismutase (SOD) and the antioxidant potential in the lipid fraction, as well as level of -SH groups decreased during treatment. A marked increase of free fatty acid (FFA) content was observed. The results indicate that the ageing process is associated with exposure to an oxidative stress.

Additional key words: Norway maple, superoxide dismutase, free fatty acids.

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INTRODUCTION

During prolonged storage seeds deteriorate and loose their germinability. Among many theories attempting to explain seed ageing many recognise membrane deterioration as the main factor causing loss of seed viability (Berjak and Villiers, 1972). One widely promoted hypothesis explaining membrane deterioration is lipid peroxidation (Harrington, 1973; Stewart and Bewley 1980). *In vivo* lipid peroxidation is the major source of free radicals in tissues (Harman, 1969) which are belived to be damaging factors, leading to a decline of vigour and viability of seeds (Wilson and McDonald, 1986). Free radical damage to cellular membranes could be exerted by deesterification of phospholipids and could result in the accumulation of free fatty acids (Niehaus, 1978, Senaratna et al., 1987). An increased level of free radical has been found in naturally aged soybean seeds (Buchvarov and Gantcheff, 1984) and in artificially aged pollen (Priestley et al., 1985). In

normal cells free radicals are kept under control by a series of scavengers which may be enzymic in nature or not. The enzymic scavengers include superoxide dismutase (SOD) and catalase. Nonenzymic endogenous scavengers include α-tocopherol, ascorbic acid, glutathione. In ageing tissues the balance between free radical producing and scavenging reactions might be disturbed.

In the present studies the level of endogenous antioxidants as well as the free fatty acid content were assayed in *Acer platanoides* seeds submitted to accelerated ageing conditions in the dry and in the imbibed state. Their probable role in the loss of seed viability is discussed.

Material and methods

Seeds of Norway maple were collected form trees growing in the Kórnik Arboretum. They were dried at room temperature for a few days until they reached ca 10% water content, and were then stored in tightly sealed foil bags at −3°C.

Accelerated ageing treatment

The seeds were artificially aged in the dry and imbibed condition by exposing them to 30°C and 100% relative humidity on a plastic net in a covered water bath, placed in a thermostat. The seeds were never in direct contact with water. Before imbibition the seeds were preliminarily sterilized by dipping for several minutes into 0.5% mercury chloride solution, followed by thorough washing in distilled water. The experiments lasted 4–5 weeks. Seed viability was dtermined every week by the tetrazolium test, according to ISTA rules (Anonymous, 1976), taking five samples of 10 seeds each.

SOD assay

SOD was assyed on the basis of its ability to inhibit the photochemical reduction of nitro blue tetrazolium (Beauchamp and Fridovich, 1971). Samples of 10 seeds were homogenized in a cold 50 mM phosphate buffer, pH 7.0. The homogenates were centrifuged at 20 000g for 30 min. The supernatants were used as enzyme extracts. The reaction mixture contained: 50 mM phosphate buffer pH 7.8, 1 mM DTE. 75 μM NBT, 2μM riboflavine, 100 μM EDTA and 0–200 μl of the enzyme extract. The reaction was started by switching light on (30 W fluorescent tube) and run 10 min., then it was stopped by swithing the light off and tubes were covered with black cloth. Absorbance was measured at 560 nm. A non irradiated reaction mixture had A of zero at 560 nm. Log A was plotted as a function of the μg protein of enzyme extract in the reaction mixture. Simmultaneously the samples of seeds incubated in optimal conditions at 3°C were taken for analysis. Protein was estimated according to Lowry et al. (1951).

Antioxidant assay

Lipid-soluble antioxidant activities were dtermined according to Senaratna et al. (1985), by monitoring the inhibition of linoleic acid oxidation, catalyzed by Fe^{+2}, by an aliquot of the lipid extract from maple seeds. The oxidation of linoleic acid in the presence of Fe^{+2} is a linear function of time and absorbance was measured at 232 nm.

The levels of -SH groups were determined in deproteinized extracts from seeds according to de Kok et al. (1981). A standard curve was made with glutathione (GSH). Absorbance was measured at 415 nm and content of -SH groups was expressed as GSH equivalents.

Ascorbic acid concentrations were measured in samples of seeds without seed coats, according to Arakawa et al. (1981). The absorbance was measured at 534 nm and concentrations were determined by comparison with a standard curve. Free fatty acids were determined in lipid extracts according to Kendall and McKersie (1989).

Results

SOD activity was measured in seeds during accelerated ageing in the imbibed state. In dry seeds the enzyme was not active and was hardly of any importance as a natural scavenger. In hydrated seeds, during the first weeks of incubation at 30°C, SOD activity increased in embryo axes and cotyledons and then rapidly decreased (Fig. 1). This decrease appeared before the viability decline took place (Fig. 2).

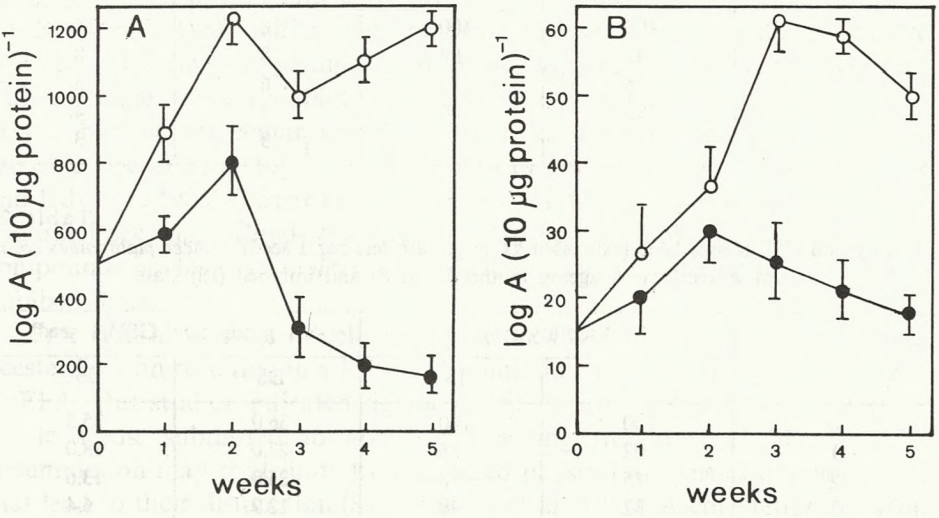

Fig. 1. Superoxide dismutase activity in embryo axes (A) and cotyledons (B) of *Acer platanoides* seeds during accelerated ageing at 30°C (●) and in optimal conditions at 3°C (○).

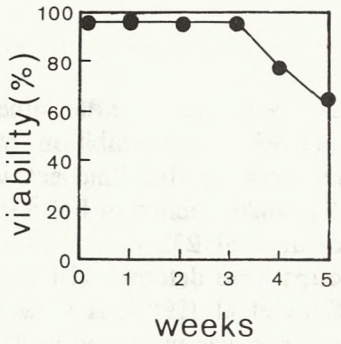

Fig. 2. Viability of *Acer platanoides* seeds during accelerated ageing at 30°C and 100% relative humidity

During accelerated ageing of dry and imbibed *A. platanoides* seeds a visible decrease was found in antioxidant activity of lipid extracts consistent with the decrease of seed viability (Table 1). Similarly the leael of -SH groups significatly decreased during incubation of seeds at 30°C (Table 2). Ascorbic acid occured only in trace quantities in dry and imbibed seeds. A significant increase in FFA content was observed during accelerated ageing of dry and imbibed seeds (Table 3).

Table 1.

Viability and lipid- soulble antioxidant level (expressed as μg α-tocopherol per mg lipid) of *Acer platanoides* seeds during accelerated ageing in the dry (DS) and imbibed (IS) state

Weeks at 30°C	Viability (%)		μg α-toc/mg lip.	
	DS	IS	DS	IS
0	100	100	18	16
1	92	88	8	8
2	75	78	6	5
3	32	40	5	4
4	22	31	3	3

Table 2.

Viability and -SH groups level (expressed as μg glutathione per 1 seed) of *Acer platanoides* seeds during accelerated ageing in the dry (DS) and imbibed (IS) state

Weeks at 30°C	Viability (%)		-SH groups (μg GSH/1 seed)	
	DS	IS	DS	IS
0	100	100	36.0	35.5
1	92	88	27.0	18.0
2	75	78	25.0	13.6
3	32	40	12.9	6.4
4	22	31	6.6	3.8

Table 3.
Viability and free fatty acid (FFA) content of *Acer platanoides* seeds during accelerated ageing in the dry (DS) and imbibed (IS) state

Weeks at 30°C	Viability (%)		FFA (μg/mg lipid)	
	DS	IS	DS	IS
0	100	100	9.0	9.0
1	94	78	9.4	24.2
2	68	44	9.0	64.9
3	34	30	107.9	76.7
4	21	15	93.3	185.5

DISCUSSION

Previously it was reported that in *Acer platanoides* seeds submitted to accelerated ageing treatment in a dry and imbibed state, membrane deterioration was the cause of loss of seed viability (Pukacka, 1983; Pukacka and Kuiper, 1988). It indicated phospholipid degradation and unsaturated fatty acid peroxidation during treatment. This suggested that the mode of cell destruction involved reactions with free radicals (Buchvarov and Gantcheff, 1984; Senaratna et al., 1988). For this reason the antioxidant status in seed tissues during accelerated ageing was investigated, as a possible scavenger system against free radical action. Activity of SOD in seeds at 30°C was markedly lower than that at 3°C and decreased coincidentally with seed viability (Figs. 1 and 2). Our early experiments (Pukacka and Kieliszewska-Rokicka, 1988) indicated a marked decrease in the activity of catalase and peroxidase in *Acer platanoides* seeds during accelerated ageing, consistent with a decline od seed viability. A defensive role against free radical action is played by low molecular scavengers such as α-tocopherol, glutathione or ascorbic acid (Priestley et al. 1989; Pauls and Thompson, 1984; Kunert and Ederer, 1985; Gorecki and Harman, 1987). The present data indicate a marked decrease of lipid-soluble antioxidant levels (Table 1) as well as of -SH compounds in *Acer platanoides* seeds during accelerated ageing in the dry and imbibed state.

Free radical damage to cellular membranes is manifested as phospholipid deesterification resulting in a loss of phosholipid content and an accumulation of FFA. Our studies indicated significant increase of FFA content in Norway maple seeds, submitted to accelerated ageing treatment (Table 3). FFA accumulation may contribute to the altered physical properties of membranes that lead to their disfunction (Senaratna et al., 1988). A correlation between membrane alterations and increase of FFA content has been reported in

a number of stress-injured plant tissues (Senaratna et al., 1984; Kendall et all., 1985; Borochov et al., 1987). The above data raises the possibility that a lowered antioxidant status in *Acer platanoides* seeds during accelerated ageing in the dry and imbibed state is one of the factors leading to memberane deterioration and loss of seed viability.

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Abbreviations. SOD – superoxide dismutase. DTE – 1.4- dithioerythritol. NBT-nitro blue tetrazolium, EDTA-ethylendinitrilotetraacetic acid. FFA-free fatty acid.

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Poziom antyutleniaczy w nasionach *Acer platanoides* podczas przyspieszonego starzenia

STRESZCZENIE

W nasionach klonu zwyczajnego (*Acer platanoides* L.) badano poziom antyutleniaczy w relacji do spadku żywotności, podczas przyspieszonego starzenia w temperaturze 30°C i 100% wilgotności, w stanie suchym i napęczniałym. Aktywność dysmutazy ponadtlenkowej i potencjał antyutleniający frakcji lipidowej, jak również poziom grup -SH, spadał podczas starzenia. Zaobserwowano znaczny wzrost zawartości wolnych kwasów tłuszczowych. Wyniki wykazują, że proces starzenia się nasion może być związany z działaniem wolnych rodników.

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