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Peroxidase and catalase activities during the accelerated ageing of Norway maple (*Acer platanoides* L.) seeds *

Abstract

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Changes of enzyme activity of peroxidase and catalase in imbibed Norway maple seeds during 5 weeks of storage under various temperature conditions were studied. It was found that the enzymes were most active in seeds stored at 3°C. At the higher temperatures (20°C and 30°C) total enzyme activity of peroxidase and catalase and the activity of particular isoperoxidases gradually decreased. The reduction of catalase activity in cotyledons corresponded in time with the decrease of the seed viability.

Additional key words: Seeds viability, isoenzyme patterns.

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INTRODUCTION

Seeds of Norway maple (*Acer platanoides* L.) belong to the mesobiotic group of seeds, which under favourable conditions (sufficiently low humidity and temperature) can be stored over several years without loss of viability. After maturation and dispersal seeds of this species are in a state of deep dormancy and require for their germination several weeks of cold stratification. However, at an increased temperature the seeds do not germinate and loose progressively their viability.

High temperature, moisture content and oxygen pressure are ageing factors for many seeds (Roberts, 1981). According to the hypothesis of Harrington (1964) the main expression of seed ageing is the loss of the ability to synthesize protein de novo (Kulka, 1971, 1973, Roberts et al., 1973, Szczotka, 1975). Moreover denaturation of the protein part of enzymes and decrease of their activities takes place (Roberts, 1972). The loss of activity of

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certain enzymes in seeds seems to be an early event in seed deterioration. Abdul-Baki (1969) has pointed out that greatest interest of authors has centered on respiratory and associated enzymes e.g. catalase, peroxidase, cytochrom oxidase and glutamic acid oxidase, whose activities tend to decline with the loss of seed viability. Gosling and Ross (1981), however, found an increase of peroxidase activity during ageing of hazelnut seeds and Szczotka (unpublished data) observed different tendencies of peroxidase activity in various oak species. Enzyme activities of peroxidase and catalase are considered to be indicators of leaf senescence (Parish, 1968, Lazar and Farkas, 1970, Patra et al., 1978, Braber, 1980).

This paper reports changes of peroxidase and catalase activities as well as isoperoxidase patterns in Norway maple seeds stored in conditions of high humidity at temperatures 20° and 30°C. These results are compared with those of cold stratification at 3°C.

MATERIAL AND METHODS

Plant material and conditions of the experiments were similar as in a previous study work (Pukacka, 1983).

Seeds of Norway maple with a water content of 10% after collection were stored in tightly sealed polyethylene bags at −3°C. For the experiments the seeds were surface sterilized by dipping for several minutes into 0.5% $HgCl_2$ solution and washed several times with distilled water. After imbibition the seeds were placed in conditions of high humidity on plates covered with filter paper permanently connected with a water source, at temperatures 20° and 30°C. A part of the seeds was placed at temperature of 3°C, that is in conditions of cold stratification. The experiment lasted for 5 weeks. Material for analysis was taken at weekly intervals.

Seed viability was determined by the tetrazolium test according to ISTA rules (Anonym, 1976), taking four samples of 10 seeds each for each analysis.

Enzyme preparations. Crude enzyme preparations from embryo axes and cotyledons were made. Batches of 50 embryo axes were homogenized in a porcelain mortar with 4 ml of 0.01 M phosphate buffer pH 7, containing 1% polyclar AT. The homogenate was centrifuged at 15 000 × *g* for 10 minutes. The supernatant was used as an enzyme preparation. Also 50 cotyledons were ground in a porcelain mortar in the presence of fluid nitrogen. The powdered tissues were transfered into glass filter G4 and washed several times with cold acetone — first 80%, then 100%. The protein fraction was extracted for half an hour with 0.01 M phosphate buffer pH 7, containing 1% polyclar AT. The homogenate was centrifuged at 15 000 × *g* for 10 minutes. The supernatant was used as the enzyme preparation.

Determination of protein content. The method of Potty (1969) was used with bovine serum albumin as the standard.

Enzyme assays. Peroxidase and catalase activities were determined according to Chance and Maehly (1955). The reaction mixture for peroxidase contained 0.5 ml of 0.1 M phospate buffer pH 6, 0.5 ml of 0.2 M H_2O_2, 0.5 ml of 1% guaiacol and 50—100 μl of the enzyme preparation. Peroxidase activity was defined as $\Delta OD_{400\ nm}$ per 10 μg of protein per 1 minute. The reaction mixture for catalase activity determination contained 1 ml of 0.01 M phosphate buffer pH 7, 1 ml of 0.001 M H_2O_2 and 50—100 μl of the enzyme preparation. In the analytical controls H_2O_2 was omitted. The decrease of optical density was measured using Specord spectrophotometer at $\lambda = 230$ nm. Catalase activity was defined as $\Delta OD_{230\ nm}$ per 100 μg of protein per 1 minute.

Isoenzyme separation. Isoperoxidases were separated by the polyacrylamide disc electrophoresis method (Davis, 1964). Comparable amounts of protein were applied onto gel columns.

Isoenzyme staining. Peroxidase isoenzymes were stained by the method described by Schrauwen (1966) with benzidine and guaiacol as substrates. The reaction was performed for 15 minutes at room temperatures. After staining the gel columns were scanned in the Vitatron MPS densitometer.

RESULTS AND DISCUSSION

Fig. 1 presents the viability of Norway maple seeds during storage under various temperature conditions. At 3°C and high humidity, when natural dormancy breaking takes place, the seeds did not lose their viability. At 20° and 30°C viability of the seeds gradually decreased, particularly at 30°C.

The level of peroxidase activity during storage of Norway maple seeds under various temperature conditions changed in a different way in embryos (Fig. 2A) and in the cotyledons (Fig. 2B). Nevertheless the highest proxidase activity was found in seeds stored at 3°C, which is optimal for their viability. Higher temperatures decreased peroxidase activity, most drastically in cotyledons, particularly at 30°C.

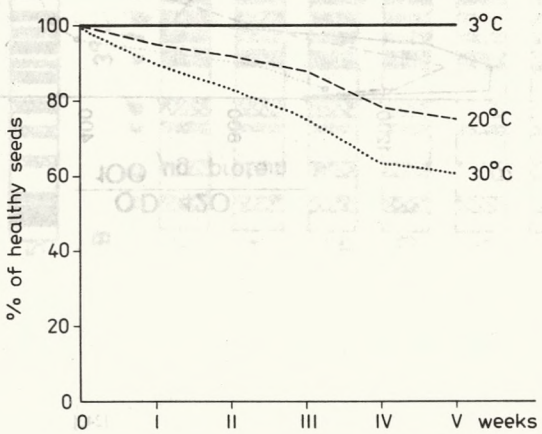

Fig. 1. Viability of *Acer platanoides* seeds stored after imbibition at 3°, 20° and 30°C and at high humidity

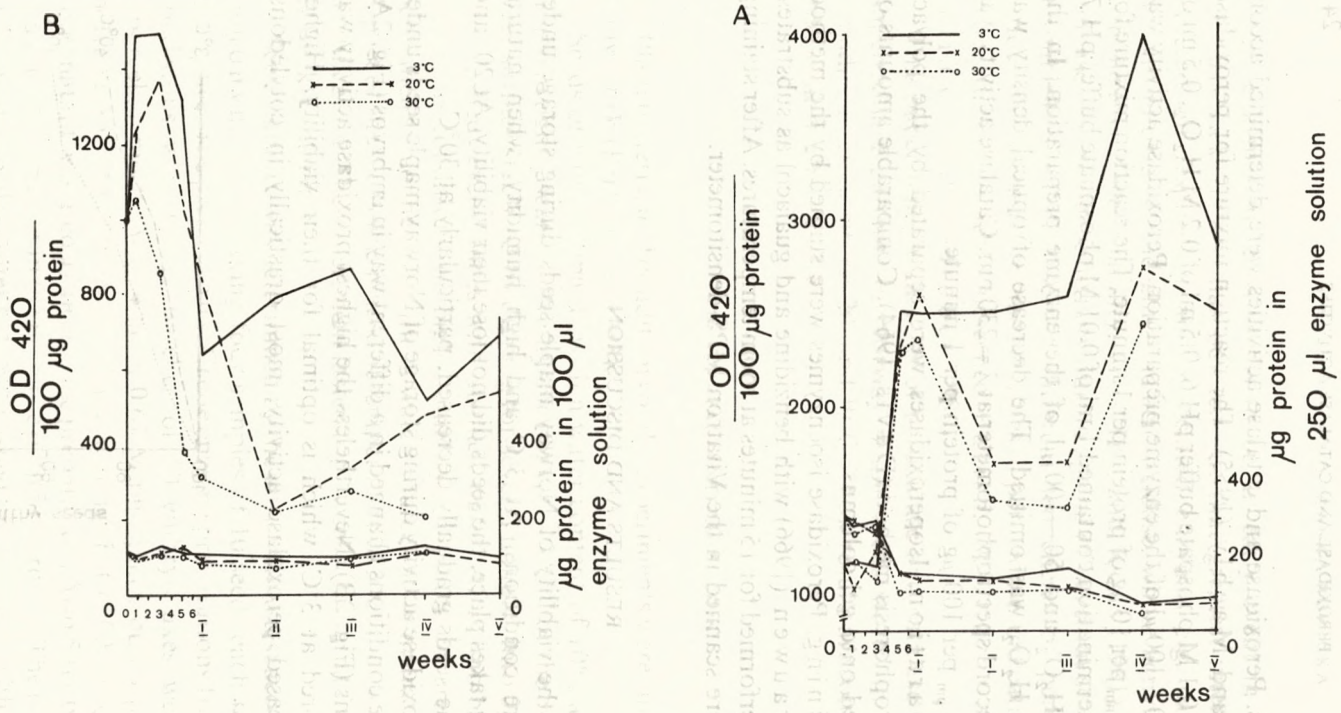

Fig. 2. Changes of peroxidase activity in embryo axes (A) and cotyledons (B) of *Acer platanoides* seeds stored after imbibition at 3°, 20° and 30° C and humidity. At the lower part of the graphs values for protein content are given

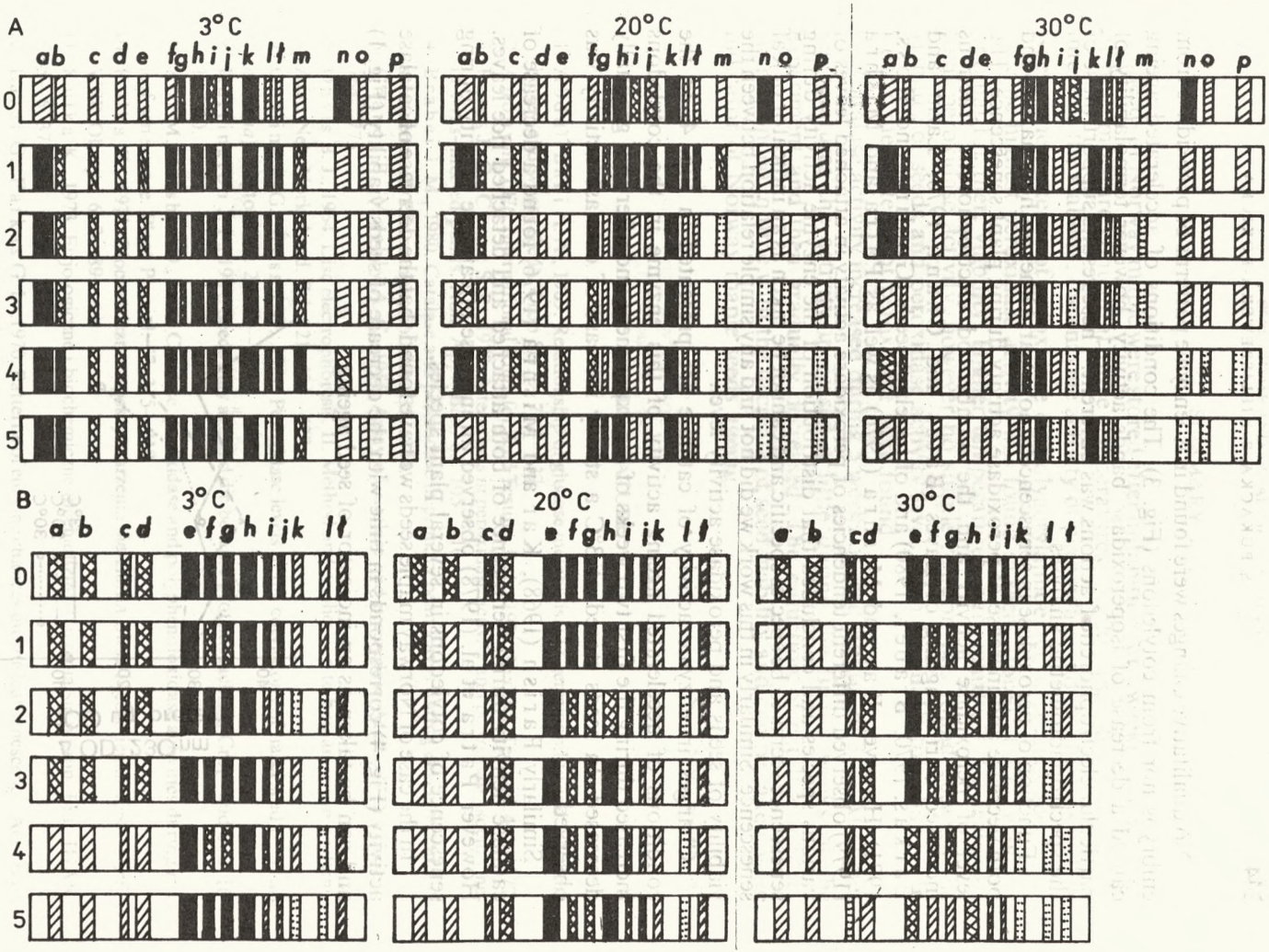

Fig. 3. Isoenzyme patterns of peroxidase in embryo axes (A) and cotyledons (B) of *Acer platanoides* seeds stored after imbibition at 3°, 20° and 30° C and at high humidity. Polyacrylamide gel electrophoresis was carried out in an anionic system at pH 8.3

No qualitative changes were found in isoenzyme patterns of peroxidase from embryos nor from cotyledons (Fig. 3). The conditions of accelerated ageing caused a decrease of isoperoxidase bands activity, however termolability of particular electrophoretic fractions was different. These results support those of the spectrophotometric analyses.

Function of peroxidase in senescence is not clear. Other authors have found both decrease and increase of peroxidase activity during plant senescence. The level of peroxidase activity and the number od electrophoretic fractions increased during ageing of leaves (Bates and Chant, 1970, Lazar and Farkas, 1970, Braber, 1980) and of hazelnut seeds (Gosling and Ross, 1981). However, Kar and Mishra (1976) as well as Patra and Mishra (1979) observed different tendencies of peroxidase activity in attached leaves of various species and concluded that distribution of the enzyme activity during senescence seems to be species specific and cannot be taken as an indicator of leaf senescence. Similarly in this work we did not find any simple relation between the viability of seeds and peroxidase activity level.

Changes in enzyme activity of catalase are presented in Fig. 4. In the conditions of accelerated ageing activity of this enzyme in the cotyledons increased during the first two weeks of the experiment and after that gradually decreased. In seeds stored at 3°C a steady increase of catalase activity was observed.

Similarly Parish (1968), Kar and Mishra (1976) found a decrease of catalase activity during senescence of both attached and detached rice leaves. However Patra et al. (1978) observed an increase of catalase activity during senescence of cotyledons in several plant species.

In the case of Norway maple seeds we have found that the decrease of catalase activity (Fig. 4) corresponds in time with the decrease of seeds viability (Fig. 1) and can be taken as an indicator of seed ageing.

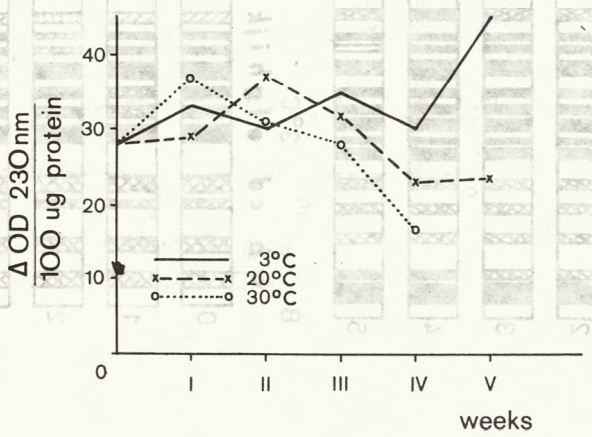

Fig. 4. Changes of catalase activity in cotyledons of *Acer platanoides* seeds stored after imbibition at 3°, 20° and 30°C and at high humidity

SUMMARY

Seeds of Norway maple were stored after imbibition for 5 weeks in conditions of high moisture content at three temperatures: 3°, 20° and 30°C. At weekly intervals the viability, the enzyme activity of peroxidase and catalase, as well as isoenzyme patterns of peroxidase on polyacrylamide gel, were analysed.

The highest peroxidase activity was found in seeds stored at 3°C, which is optimal for their viability. Higher temperatures, particularly 30°C, decreased total peroxidase activity, however no simple relation was found between the viability of seeds and peroxidase activity level. Also no qualitative changes in isoenzyme patterns of peroxidase were found.

Catalase activity increased during 5 weeks of seed storage at 3°C. At higher temperatures the activity increased a little during the first 2 weeks of the experiment and then gradually decreased. The reduction of catalase activity corresponded in time with the decrease of seed viability and can be taken as an indicator of Norway maple seeds ageing.

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Aktywność enzymatyczna peroksydazy i katalazy podczas przyspieszonego starzenia nasion klonu zwyczajnego (*Acer platanoides* L.)

Streszczenie

Nasiona klonu zwyczajnego były przechowywane po spęcznieniu przez 5 tygodni w warunkach dużej wilgotności w trzech temperaturach: 3°, 20° i 30°C. W tygodniowych odstępach analizowano żywotność nasion, całkowitą aktywność peroksydazy i katalazy oraz, elektroforetycznie, wzory izoenzymowe peroksydazy na akrylamidzie.

Najwyższą aktywność peroksydazy stwierdzono w nasionach przechowywanych w temperaturze 3°C, która była optymalna dla ich żywotności. W wyższych temperaturach aktywność peroksydazy ulegała obniżeniu, zwłaszcza w temperaturze 30°C, ale nie obserwowano prostej zależności między zmniejszaniem się aktywności tego enzymu a spadkiem żywotności nasion. Nie stwierdzono także zmian jakościowych w obrazie pasm elektroforetycznych peroksydazy.

Aktywność katalazy wzrastała w czasie 5 tygodni przechowywania nasion w temperaturze 3°C, natomiast w wyższych temperaturach poziom aktywności tego enzymu podnosił się nieco w ciągu dwóch tygodni doświadczenia, a następnie stopniowo obniżał się. Redukcja aktywności katalazy odpowiadała w czasie spadkowi żywotności nasion i enzym ten może być uważany za wskaźnik starzenia się nasion klonu zwyczajnego.

Энзиматическая активность пероксидазы и каталазы во время ускоренного старения семян клена остролистного (*Acer platanoides* L.)*

Резюме

Семена клена обыкновенного хранились после набухания в течение 5 недель в условиях большой влажности в трех температурах: 3°, 20° и 30°C. После каждой недели анализировали жизнеспособность семян, полную активность пероксидазы и каталазы, а также электрофоретически ферментные спектры пероксидазы на акриламиде.

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Самую большую активность пероксидазы найдено в семенах хранимых в температуре 3°C, которая была оптимальной для их жизнеспособности. В более высоких температурах активность пероксидазы понижалась, особенно в 30°C, но не наблюдалось простой зависимости между уменьшением активности этого фермента и понижением жизнеспособности семян. Не найдено также качественных изменений в электрофоретических спектрах пероксидазы.

Активность каталазы возрастала в течение 5 недель хранения семян в температуре 3°C, в свою очередь в более высоких температурах уровень активности этого фермента повышался немного в течение двух недель опыта, а потом постепенно понижался. Уменьшение активности каталазы отвечало по времени понижению жизнеспособности семян и этот фермент может считаться показателем старения семян клена обыкновенного.