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Activity of phosphofructokinase and phosphoenolopyruvate carboxylase in Norway maple (*Acer platanoides* L.) seeds during dormancy breaking

Abstract: We analysed changes in the activity of phosphofructokinase (PFK) and phosphoenolopyruvate carboxylase (PEPC), and in the glucose and sucrose contents of Norway maple seeds stratified at 3°C (dormancy broken) or treated at temperature 15°C (dormancy not broken).
We found that changes in the activity of enzymes are not linear, and 2–3 stages may be distinguished. Dormancy breaking and seed germination is associated with a high activity of PFK and PEPC, and a high glucose level in embryo axes.

Additional key words: glucose, sucrose

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Introduction

Dormant seeds are not always characterised by a metabolic deficit, sometimes this applies to respiration as well (Roberts 1973). After ripening, seeds of Norway maple (*Acer platanoides* L.) are in a state of deep dormancy of embryo axes, to break the dormancy, they must undergo cold stratification.

According to one hypothesis (Roberts 1973 and Roberts and Smith 1977) seed dormancy is controlled by the activity of pentose phosphate pathway (PPP) relative to that of glycolysis and Krebs cycle.

Results of our earlier study of the physiology of dormancy breaking in Norway maple (Szczotka and Tomaszewska 1979) suggest that the rate of respiration in dry dormant seeds is very low before stratification. During cold stratification at 3°, under conditions of a high moisture, the rate of respiration increases.

A significant role in dormancy breaking is ascribed to the activity of the pentose phosphate pathway (Roberts 1973, 1977, Swanny 1986). However, in Norway maple seeds, during our investigation, the ratio of glycolysis, as compared with the pentose phosphate pathway, grow during dormancy breaking (Szczotka and Żymańczyk 1994).

The aim of the this study was to analise the dynamics of the activity of two crucial respiratory enzymes: phosphofructokinase (PFK) and phosphoenolopyruvate carboxylase (PEPC) during dormancy breaking in Norway maple seeds. This enzymes play very important role in respiration (Podesta and Plaxton 1994, Bettey and Finch-Savage 1996). PFK is enzyme of glycolysis, while PEPC is an anaplerotic enzyme, whose function is to replenish oxaloacetate in the Krebs cycle by carboxylation of phosphoenolopyruvate (PEP), which is a product of glycolysis during stratification at 3° (dormancy broken) or warm stratification at 15° (dormancy not broken). Changes in the concentration of glucose and sucrose were analysed, during seeds stratification. We expected that the concentration of respiratory substrates would change depending on dormancy.

Material and methods

Maple tree seeds were collected in the autumn of 1998 in the Kórnik Arboretum. After collection, the seeds were dried at room temperature until they reach approximately 10% water content. Such dried seeds were then stored at –3°C. After imbibition (48 hours at room temperature) they were subjected to cold (3°C) or warm (15°C) stratification.

Germination characteristics

The four seed lots, each with 50 seeds, had quite similar germination curves in a standard germination test during 3°C. No germination occured during 15°C incubation. The seeds subjected to cold stratification (3°C) started germination between 8 and week 14 and germinate at 87%. The seeds stratified at 15°C failed to germinate and remained in a state of deep dormancy until the end of experiment.

Preparation of enzyme extracts

Ten embryo axes or ten cotyledons were homogenized using a chilled pestle and mortar. For embryo axes 1 cm^3 and for cotyledons 2 cm^3 of extract medium used: 50 mM Hepes pH 8.0, containing 1 mM ethylenediaminetetraacetic acid (EDTA), 2.5 mM $MgCl_2$, 10 mM dithiothreitol (DTT), with 20 mg insoluble polyvinyl pyrollidone. The extract was spun in a centrifuge 12 000 g for 5 min, at 4°C and the supernatant stored on ice.

Assays were carried out spectrophotometrically at 25°C and 340 nm in final volume of 1 ml, using 3 replicate extracts for each seed lot. Assay methods were taken from Podesta and Plaxton (1994), modified by Bettey and Finch-Savage (1996)

Protein assay

Samples of extracts used for enzyme assay were taken for protein assay (Bradford, 1976), using bovine serum albumin as standard.

Enzyme assays

All enzymes were assayed at room temperature (20°C) in 1 ml reaction mixtures by monitoring NADH oxidation at 340 nm using a Specord recording spectrophotometer: All assays were: – optimized for pH and substrate/cofactor concentration, – corrected for NADH oxidase activity, – linear with respect to time and concentration of enzyme assayed. Reported enzymatic activities represent the mean of at least three independent determinations and are reproducible to within ± 10% SE.

Phosphofructokinase (PFK) EC 2.7.1.11

50 mM Hepes pH 7.4, 8 mM fructose-6-phosphate, 4 mM $MgCl_2$, 0.15 mM beta nicotinamide adenine dinucleotide, reduced form (NADH), 1U aldose, 10 U triose phosphate isomerase, 1 U glycerophosphate dehydrogenase, 50 μl enzyme extract, 1.2 mM ATP. The PFK reactions were started by the addition of ATP.

Phosphoenolopyruvate carboxylase (PEPC) EC 4.1.1.31

50 mM Hepes pH 7.8, 8 mM $KHCO_3$, 8 mM $MgCl_2$, 0.15 mM NADH, 10 U malate dehydrogenase, 50 μl extract, 4 mM phosphoenolopyruvate (PEP). The PEPC activity was recorded for approximately 3 min. Assays of PEPC were initiated by the addition of PEP.

Sugars content

Embryo axes or cotyledons were homogenized using 80% methanol. After evaporation of methanol water extract was used for sugar content analysis. Reducing sugars (glucose) were determined by the Somogyi (1952) and Nelson (1957) methods. Sucrose was determined using invertase. Concentration was calculated as mg per gram of dry weigh. Three replicates for each determination were used. All data were analysed statistically.

Results and discussion

The activity of both enzymes is significantly higher in embryo axes than in cotyledons (Figs. 1 and 2). The enzymes activity were different at cold and warm stratification.

PFK activity (Fig. 1) in embryo axes at 3°C was the highest from week 1 till week 4, and from week 8 till the end of the experiment, i.e. till 90% of seeds had germinated. In comparison, PFK activity in embryo axes of the seeds stratified at 15°C was generally lower. It reached a maximum from week 5 till week 9, and later it fell to a level characteristic of the few non-germinated seeds. In cotyledons there were no marked differences in PFK activity between cold-stratified and warm-stratified seeds (Fig. 1). In both cases it was at a low level throughout the experiment, with small peaks in weeks 1 and 6.

PEPC activity (Fig. 2) in embryo axes was clearly higher till week 7 of stratification at 15°C than at 3°C. Later on the activity fell below the level observed at 3°C. Under conditions of cold stratification PEPC activity was initially low, but from week 5 till week 8 it was growing, and later remained at a relatively high level. In cotyledons throughout the experiment PEPC activity was higher at 15°C than at 3°C.

These results show that a high metabolic activity of PFK and PEPC in dormant seeds is not always associated with dormancy breaking. Moreover, the studied enzymes react to cold and warm stratification differently. PFK activity is markedly higher at the low temperature that leads to dormancy breaking, while PEPC activity is the highest at the beginning of warm

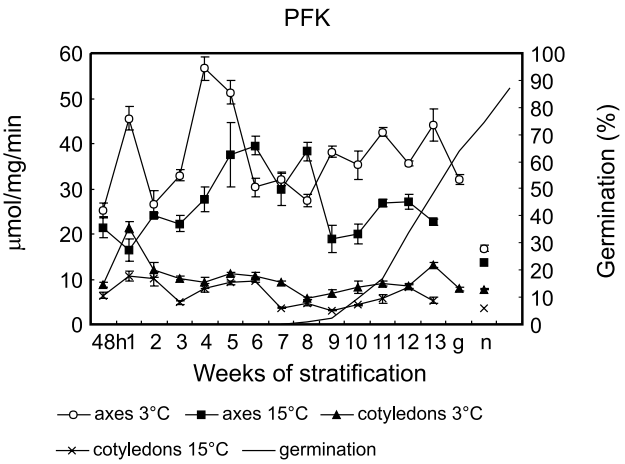

Fig. 1. Phosphofructokinase (PFK) activity during cold (3°C) and warm (15°C) stratification of *Acer platanoides* seeds (g – germinated, n – no germinated seeds)

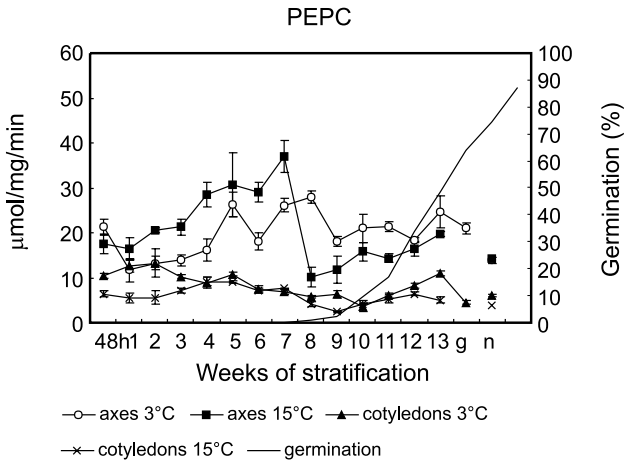

Fig. 2. Phosphoenolopyruvate carboxylase (PEPC) during cold (3°C) and warm (15°C) stratification of *Acer platanoides* seeds (g – germinated, n – no germinated seeds)

stratification. This difference is much clearer in embryo axes than in cotyledons, as the activity of both enzymes is generally lower in cotyledons. However, it is important that when dormancy is broken and seeds start to germinate, the activity of both enzymes is higher under conditions of cold stratification.

A small percentage of seeds stratified at 3°C did not germinate and their viability decreased. The activity of both enzymes declined substantially in these seeds, like in the viable non-germinated seeds stratified at 15°C.

It is noteworthy that changes in the activity of the studied enzymes are not linear, as periods of high activity alternate with periods of low activity. During warm stratification two stages may be distinguished for both enzymes: high till week 8 and lower till the end of the experiment. During cold stratification there are three stages, particularly in the case of PFK, The third stage of a continuously high activity is the period from week 9 till the germination of the majority of seeds (3°C).

Similar changes in the activity of respiratory enzymes during dormancy breaking in Norway maple seeds at 3°C have been reported for glutamate dehydrogenase, malate dehydrogenase and alcohol dehydrogenase (Kieliszewska 1985). A clear three-stage activity of glucose-6-phosphate dehydrogenase, a crucial enzyme of the pentose phosphate pathway, was observed by Szczotka and Żymańczyk (1994) at 3°C and 15°C, but in the last stage the activity during warm stratification was much lower.

The glucose content of embryo axes in seeds stratified at 3°C increased in the course of the experiment and since week 5 it was higher than at 15°C (Fig. 3). At 15°C the glucose content decreased till week 6, but later slightly increased but stayed at a low level till the end of the experiment. Changes in the glucose con-

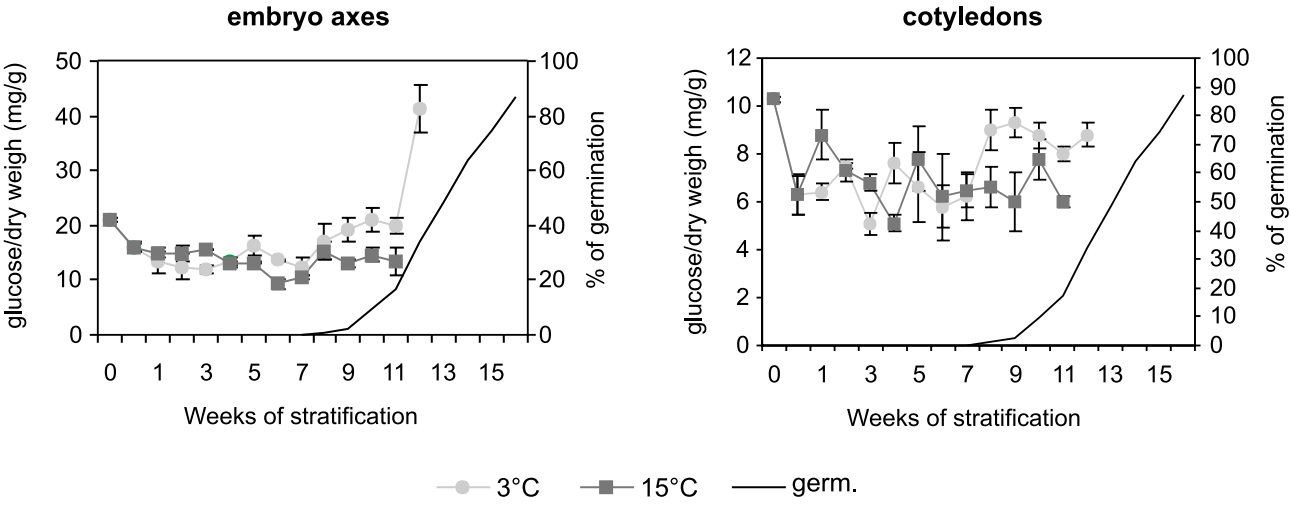

Fig. 3. Glucose content in *Acer platanoides* seeds during cold (3°C) and warm (15°C) stratification (g – germinated seeds)

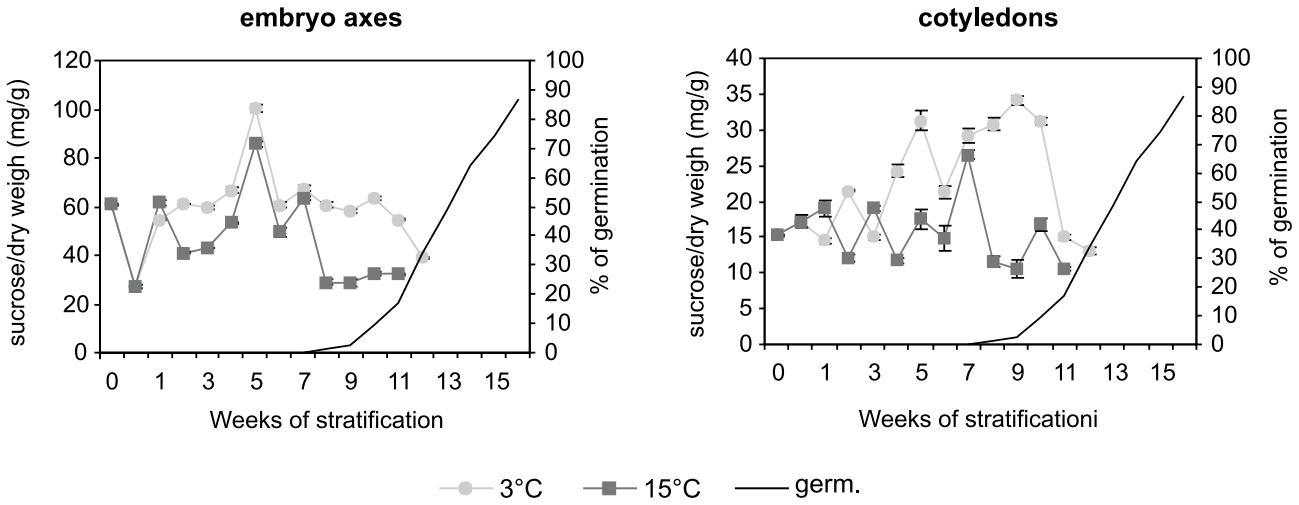

Fig. 4. Sucrose content in *Acer platanoides* seeds during cold (3°C) and warm (15°C) stratification (g – germinated seeds)

tent of cotyledons in seeds stratified at 3°C and 15°C were similar till week 7 (begining of germination). Since then till the begining of the experiment the glucose content was higher in cold-stratified seeds.

The sucrose content of embryo axes changed in both temperatures of stratification in a similar rhythm, although it was higher at 3°C than at 15°C throughout the experiment (Fig. 4). It was characteristic, however, that the level of glucose at 3°C was constantly growing, while the level of sucrose increased only till week 5, and later decreased till the end of the experiment.

The results suggest that sucrose hydrolysis is initiated during dormancy breaking, which results in a higher glucose level. The same conclusion was drawn by Pukacka (1995), who studied the activity of acid and alkaline invertase in Norway maple seeds. In cotyledons this relationship is less clear, but after week 5 of cold stratification also their glucose content was found to increase. Glucose and probably other monosugars, the natural products of metabolism, play an important role in the regulation of germination and growth of seedlings (Krawiarz 1988).

Conclusions

Dormancy breaking and germination of seeds is correlated with high activity of PFK and PEPC, and a high level of glucose in seed tissues, particularly in embryo axes.

The higher activity of both the analysed enzymes around the middle of the experiment at 15°C, followed by a dramatic decline, may be associated with the subsequent acceleration of seed ageing caused by warm stratification, and the resultant reduction in their vitality. The high metabolic activity during warm stratification did not lead to dormancy breaking, but to a rapid loss of respiratory substrates (glucose and sucrose).

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