ACTIVITY OF ATPases DURING DORMANCY BREAKING IN NORWAY MAPLE (*ACER PLATANOIDES* L.) SEEDS

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ABSTRACT

The activity of ATPases was studied in embryo axes and cotyledons of Norway maple seeds stratified at 3°C (dormancy broken) and 15°C (dormancy not broken). The activity of mitochondrial (F. III) and activity connected with all cellular membranes (F. II) and soluble fraction (F. I) ATPases was investigated.

It was found that mitochondrial ATPases are the most active. The activity of all types of ATPases is greater in seeds stratified at 3°C. It increases during seed dormancy breaking, particularly in the case of mitochondrial ATPases.

The rise in the activity of mitochondrial and others ATPases in embryo axes of seeds stratified at 3°C is stepwise. In seeds stratified at 15°C ATPase activity generally decreases.

KEY WORDS: *Acer platanoides*, seeds dormancy, ATPase.

INTRODUCTION

The system generating an electrochemical H^+ gradient is probably composed of a complex of enzymes (H^+-ATPase and NAD(P)H oxidoreductase), and is the major factor of electron transfer across membranes (Alberts et al. 1999; Kopcewicz and Lewak 1998)

There are several forms of ATPases, controlling turgor, intracellular pH, transport of ions and organic compounds into vacuoles, and processes of internal secretion (Taiz and Zeiger 1991). Also the level of ATP in mitochondria and chloroplasts is associated with and dependent on ATPase activity (Taiz and Zeiger 1991; Kopcewicz and Lewak 1998).

Although ATPases play such an important role in the energy metabolism of plant cells, there are no reports on changes in the activity of those enzymes in various physiological processes in plants during their life cycle. This applies also to tree-seed dormancy and its breaking. In an earlier study (Szczotka and Tomaszewska 1981) we found that significant changes in ATP level and acid phosphatase activity take place during dormancy breaking in Norway maple seeds. In reference to those findings, the aim of this study was to investigate the relationship between the activity of mitochondrial, membranous and soluble ATPases and dormancy breaking in Norway maple seeds.

MATERIAL AND METHODS

After ripening, Norway maple seeds are in a state of deep dormancy of embryo axes and are not capable of germination. Before germination they must undergo cold stratification for at least 12 weeks.

The seeds used in our experiments were stored at -3°C, with a moisture content below of 10%. After removal from storage, the seeds were soaked in water for 48 h and then stratified for 14 weeks at 3°C (which led to dormancy breaking), or at 15°C (which did not lead to dormancy breaking but the seeds remained viable).

Enzymatic activity was assessed once a week. Each time the enzyme extract was prepared from 10 embryo axes or 10 cotyledons. Extraction was carried out at three replications.

Preparation of enzyme extracts

The methods described by Buczek et al. (1981) were used for assessment of enzymatic activity. The enzyme extracts were prepared from embryo axes and cotyledons of stratified seeds. The fresh tissue was ground in a medium containing 250 mM sucrose, 1 mM EDTA and 50 mM tris-maleate at pH 7.0. For 10 embryo axes or 10 cotyledons 2 cm^3 of the medium were used. The homogenate was later subjected to successive centrifugation (Typ K 24). The supernatant from centrifugation at 10 000 g for 10 min was referred to as Fraction I (mixture of all fragments, organelles and cellular membranes) and was used for the next centrifugation. The supernatant at 18 000 g for 15 min was referred to as Fraction II (soluble fraction of ATPases). The 18 000 g pellet was referred to as Fraction III (mitochondria enriched fraction). The pellet was washed in the extraction medium, sedimented and finally resuspended in a fresh extraction medium. All the preparation described above was carried out at 0°C.

Enzyme assays

ATPase activity was measured at 35°C in 2 cm^3 of a mixture of 3 mM ATP (tris salt), 100 mM tris-maleate (pH 6.5),

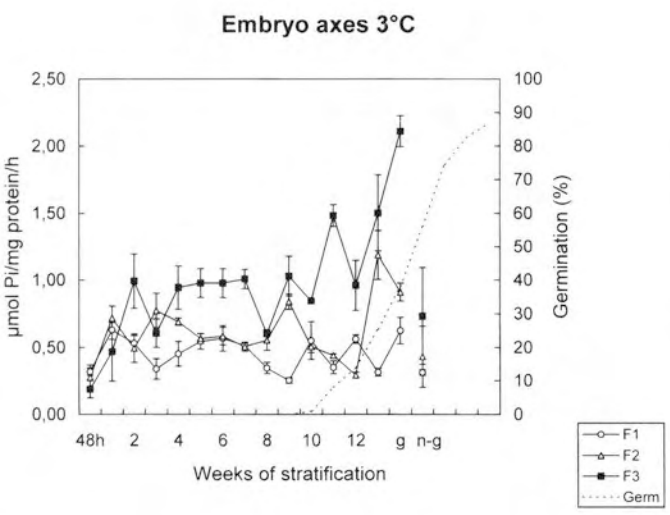

Fig. 1. ATP-ases activity in embryo axes at 3°C during cold stratification (g – germinated, n-g – not germinated seeds).

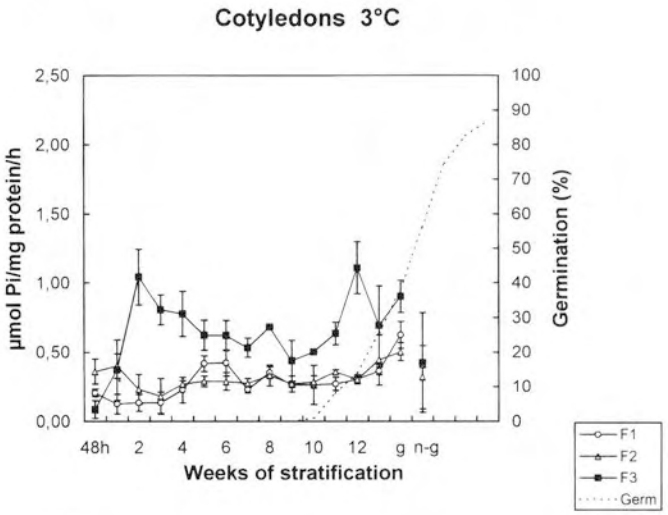

Fig. 2. ATP-ases activity in cotyleodones at 3°C during cold stratification (g – germinated, n-g – not germinated seeds).

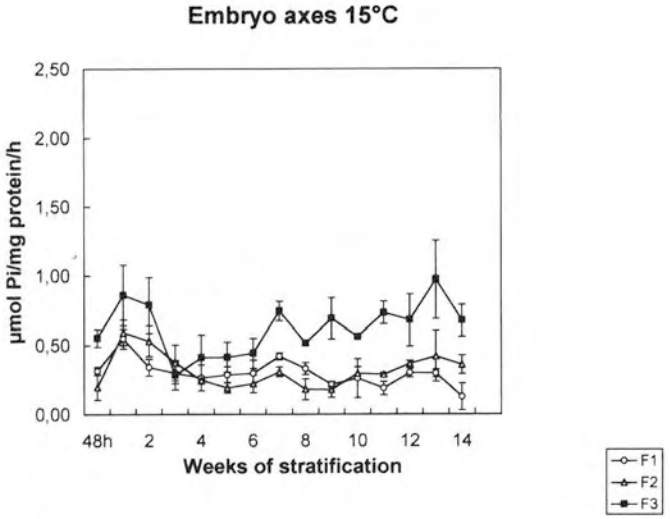

Fig. 3. ATP-ases activity in embryo axes at 15°C during warm stratification.

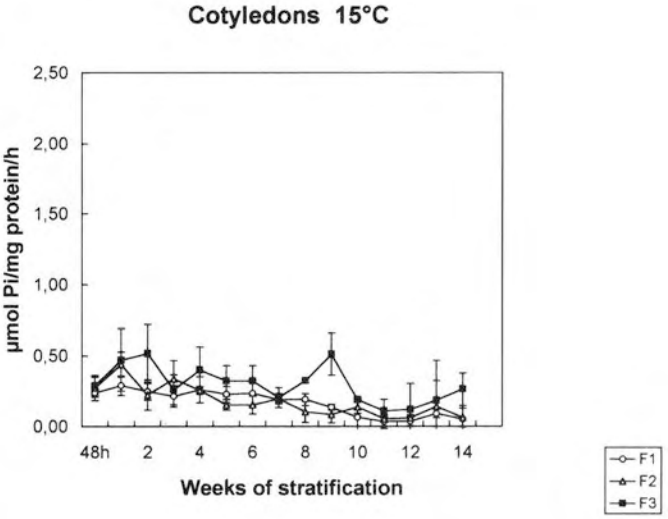

Fig. 4. ATP-ases activity in cotyledones at 15°C during warm stratification.

25 mM $MgSO_4$, 250 mM sucrose and 0.2 cm^3 of enzyme extract containing approximately 0.1 mg protein.

After removal of the protein, the inorganic phosphate released from ATP was assessed by the method of Fiske and Subbarow (1925). The activity of ATPase was expressed as micromoles of P liberated per 1 mg of protein per hour. Soluble protein was determined according to Bradfords (1976) method.

RESULTS AND DISCUSSION

Seed germination is presented in Figs 1 and 2. The seeds stratified at 3°C started to germinate in week 9, whereas after 16 weeks 90% of them germinated. None of the seeds stratified at 15°C germinated (Figs 3 and 4).

Changes in the activity of the ATPases studied are presented in Figs 1-4. It was found that in both variants of the experiment the activity of all ATPases was variable. Moreover, in both variants the activity of those enzymes in embryo axes was higher than in cotyledons. During the warm and cold stratification, both in embryo axes and in cotyledons, the activity of mitochondrial ATPases was the highest.

The activity of all types of ATPases in embryo axes and cotyledons was higher under conditions of cold stratification (Figs 1-4).

At the end of stratification at 3°C, particularly in the case of mitochondrial ATPases, their activity increased, whereas at 15°C it decreased. In comparison with others, the activity of mitochondrial ATPases exhibited the greatest dynamics of changes. In embryo axes of seeds stratified at 3°C their activity increased stepwise, several times in the final effect (Fig. 1). At 15°C the activity of mitochondrial ATPases did not change significantly and remained close to values observed after two weeks of stratification (Fig. 3). The highest values of activity of mitochondrial ATPases in cotyledons of seeds stratified at 3°C were recorded in week 2 and 12 of stratification (Fig. 2). Under conditions of warm stratification the activity of those enzymes was relatively low throughout the experiment and decreased markedly since week 9 (Fig. 4).

The activity of solubles ATPases (fraction II) in embryo axes was higher than fraction I ATPase activity, sometimes

even several times higher, particularly at the end of stratification (Figs 1 and 2).

In embryo axes and cotyledons of seeds stratified at 15°C the activity of fraction I and II ATPases was low, showed little variation and had a similar course, with a clear tendency to decrease during the experiment (Figs 3 and 4).

Several more or less conspicuous maxima of activity of the studied ATPases could be noticed during stratification, particularly in the case of cold stratification of embryo axes (Fig. 1). During the first three weeks of warm and cold stratification of embryo axes the activity of all the ATPases increased. Later on, under conditions of cold stratification, another maximum of activity was recorded between week 3 and 8, which was not observed during warm stratification. Since week 8 in embryo axes of seeds stratified at 3°C, the highest activity of mitochondrial and soluble fraction II ATPases was detected. This did not apply to fraction I ATPases.

The obtained data suggest that under conditions of cold stratification a rise in ATP activity is associated with maple seed dormancy breaking. This applies to mitochondrial ATPases F III to the greatest degree, to soluble FII ATPases to a lesser degree, and to fraction I ATPases to the least degree. The rise in activity of mitochondrial and soluble ATPases in embryo axes of seeds stratified at 3°C was stepwise. Three phases of this process can be distinguished: the first at the beginning of stratification, the second in the middle period and the third during seed germination. Similar phases in analogous periods of stratification were observed in an earlier study of changes in ATP concentration and acid phosphatase activity during dormancy breaking in Norway maple seeds (Szczotka and Tomaszewska 1981). We suppose that this similarity results from a relationship between ATPase activity and ATP level in plant tissues. Stepwise changes observed during dormancy breaking in Norway maple seeds apply also to other metabolic processes (Szczotka and Tomaszewska 1980; Pawłowski et al. 1997).

CONCLUSIONS

Results of our studies suggest that the first phase of increase in ATPase activity is associated with seed imbibition, the second with the actual breaking of dormancy, and the third with seed germination.

It is characteristic that under conditions of warm stratification, which does not lead to dormancy breaking, no phase of ATPase activity rise, considered to be associated with actual breaking of dormancy, was observed. At the end of stratification opposite changes in ATPase activity were recorded in the two variants of the experiment: it increased at 3°C and decreased at 15°C. This does not result from a decline in seed vitality under conditions of warm stratification, as the seeds remain viable for many weeks, but from the fact that they remain dormant.

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AKTYWNOŚĆ ATPaz W CZASIE USTĘPOWANIA SPOCZYNKU NASION KLONU ZWYCZAJNEGO (*ACER PLATANOIDES* L.)

STRESZCZENIE

Aktywność ATPaz badano w osiach zarodkowych i liścieniach nasion klonu zwyczajnego, stratyfikowanych w temp. 3°C (spoczynek ustępuje) i 15°C (spoczynek nie ustępuje). Badano aktywność ATPaz mitochondrialnych oraz związanych z błonami cytoplazmatycznymi i frakcją rozpuszczalną.

Stwierdzono, że najbardziej aktywne są ATPazy mitochondrialne (FIII). Aktywność wszystkich rodzajów ATPaz jest większa w nasionach poddanych stratyfikacji chłodnej, a szczególnie w przypadku ATPaz mitochondrialnych rośnie w miarę ustępowania spoczynku nasion.

Wzrost aktywności ATPaz mitochondrialnych i związanych z membranami (FII) w osiach zarodkowych nasion stratyfikowanych w 3°C ma charakter etapowy (fazowy). W nasionach stratyfikowanych w 15°C aktywnośćć ATPaz generalnie obniża się.

SŁOWA KLUCZOWE: Acer platanoides, spoczynek nasion, ATPazy.