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INFLUENCE OF TEMPERATURE AND ABSCISIC AND GIBBERELLIC ACIDS ON POLYAMINE BIOSYNTHESIS IN EUROPEAN BEECH (*FAGUS SYLVATICA* L.) SEEDS DURING DORMANCY BREAKING

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The effects of the exogenous growth regulators abscisic and gibberellic acids (ABA and GA_3) on the activity of arginine (ADC) and ornithine decarboxylases (ODC) during dormancy breaking were studied in beech seed. During cold-stratification at 3°C, ADC and ODC activity increased rapidly starting from week 7 in embryo axes and week 8 in cotyledons. At 15°C, ADC activity was higher than ODC activity in embryo axes until week 7 and in cotyledons until week 8. Exogenous growth regulators clearly affected ADC and ODC activity. In embryo axes, ADC activity reached its maximum under the influence of GA_3 between weeks 4 and 8. In the control variant (water temp. 3°C), enzymatic activity was moderately high, peaking in week 9 when a high proportion of seeds already showed germinability. In cotyledons the influence of GA_3 on ADC activity was noticeable particularly during the first and last weeks. In the control variant the pattern of changes in the activity of this enzyme was similar but at a much lower level. ABA in both organs clearly inhibited ADC activity, but particularly at the end of the experiment. ODC activity in all variants of the experiment was higher in embryo axes than in cotyledons. The dynamics of change in ODC activity were similar to the changes in ADC activity in embryo axes and in cotyledons.

Key words: European beech seeds, abscisic acid, gibberellic acid, arginine decarboxylase, ornithine decarboxylase, dormancy breaking.

INTRODUCTION

Seed dormancy, dormancy breaking and germination are complex phenomena controlled by a large number of factors which are the results of gene expression. Gene expression is affected by growth regulators, the most important of which are gibberellic acid (GA_3), abscisic acid (ABA) and polyamines (Nicolás et al., 1996, 1997, 1998; Brady and McCourt, 2003; Finkelstein, 2004; Kucera et al., 2005). Polyamines are a new group of regulators involved in seed development (Minocha et al., 2004; Swamy et al., 2004). Polyamines play many roles in plant growth and development. They regulate cell division and the synthesis of macromolecules such as proteins and nucleic acids, and stabilize plant cell membranes (Evans and Malmberg, 1989; Berta et al., 1997). Some researchers suggest that polyamines play a role in seed development and dormancy breaking (Szczotka and Lewandowska, 1989; Grzywacz et al., 1991; Pukacka et al., 1991; Hilhorst and Karssen, 1992; Hilhorst, 1995; Szczotka et al., 2003).

Polyamines (putrescine, spermidine and spermine) are synthesized via decarboxylation of arginine by arginine decarboxylase (ADC, EC 4.1.1.19) and of ornithine by ornithine decarboxylase (ODC, EC 4.1.1.17). ADC and ODC activity varies in plants in different stages of development. ADC and ODC activity is very good marker of polyamine metabolism in seeds (Pukacka et al., 1991).

Generally ABA is a positive regulator of dormancy induction and most likely also of its maintenance, while it is a negative regulator of germination. GA_3 releases dormancy, promotes germination, and counteracts the effects of ABA (Kucera et al., 2005). Literature and investigations concerning the hormones' role in regulation of tree seed dormancy breaking are scant.

This study is a continuation of our earlier research on mechanisms of dormancy breaking in seeds of European beech and Norway maple in regard to polyamine metabolism (Pukacka et al., 1991; Szczotka et al., 2003). We have already stud-

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Abbreviations: ABA – abscisic acid; ADC – arginine decarboxylases; GA – gibberellic acid; ODC – ornithine decarboxylases.

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ied the relation between polyamine content and protein synthesis expressed as ADC and ODC activity during dormancy breaking in *Acer platanoides* L. seeds; we found ADC and ODC activity to be correlated with dormancy breaking. ODC activity was very high in dry dormant Norway maple seeds, while ADC activity was high during dormancy breaking.

In this investigation we assess the influence of exogenous GA_3 and ABA on the activity of ADC and ODC during dormancy breaking in *Fagus sylvatica* seeds.

MATERIAL AND METHODS

Seeds were collected in 2000, dried to 10% moisture content and stored in tightly sealed plastic containers at -3°C. After storage, in the first treatment variant, seeds were gradually rehydrated (48 h) to 30% moisture content and then either stratified at 3°C (control), which leads to dormancy breaking, or kept at 15°C, which did not lead to dormancy breaking. In experiments to assess the influence of exogenous phytohormones on the metabolism and concentration of polyamines, we used only cold stratification at 3°C and 30% moisture content.

In the second variant, before stratification the seeds were soaked for 48 h in aqueous solutions of physiological concentrations of GA_3 (100 μM) or ABA (50 μM). Then the seeds were stratified at 3°C, which breaks dormancy. In such conditions, seeds started to germinate after 7 weeks. Germination tests (4×50 seeds) were performed weekly and seed samples were taken for analyses at the same time. Each treatment was done in four replicates.

The activity of arginine and ornithine decarboxylases (ADC and ODC) was assessed by the method of Dumortier et al. (1983). Samples of 20 embryo axes or 200 mg of cotyledons were ground in liquid nitrogen. Enzymes were extracted with 2 ml 100 mM Na-phosphate buffer (pH 7.6). The homogenate was centrifuged for 20 min at 24,000 g at 0°C. The supernatant was used for assessment of enzyme activity. The reaction mixture contained 200 μl enzyme extract, 0.8 cm^3 10 mM unlabelled arginine or ornithine in Na-phosphate buffer (pH 7.6), and 25 μl radiolabelled precursors L-(l-^{14}C) arginine or ornithine (Amersham) (1.85 MBq cm^3).

The mixture was incubated in a water bath at 37°C for 45 min. Next, 0.2 ml 10% TCA (trichloroacetic acid) was added and incubation was continued for another 45 min. The carbon dioxide released during the enzymatic reaction was absorbed with KOH-soaked filter paper (Whatman 3, diameter 10 mm) suspended above the mixture.

After incubation, the filter paper was transferred to scintillation vessels, covered with PPO-POPOP (2,5-diphenyloxazole – 1,4-nos[5-phenyl–2-oxazolyl]benzene) cocktail in toluene, and the radioactive impulses were counted. Enzymatic activity was expressed as the number of impulses cpm per 100 μg protein.

The protein content of the samples was assayed by Bradford's (1976) method.

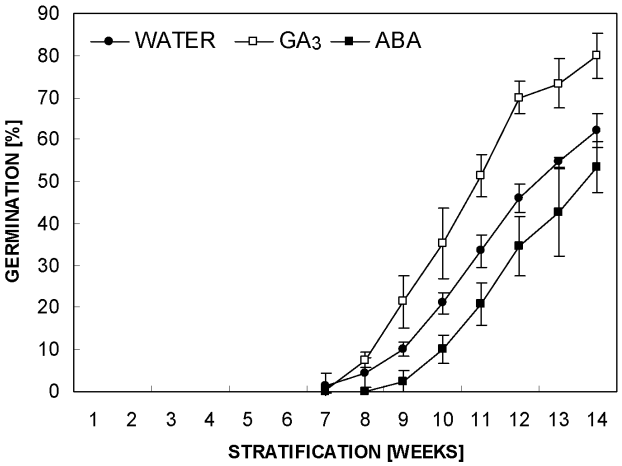

Fig. 1. Influence of GA_3 and ABA applied for 48 h before stratification on germination of beech seeds at 3°C. Germination was checked each week. Means and standard deviations of four replicates.

RESULTS

EFFECTS OF TEMPERATURE, GA_3 AND ABA ON SEED GERMINATION

At 3°C germination started at about week 7, but at 15°C no seeds germinated (Fig. 1). After GA_3 treatment, germination started at the same time, but from week 8 it was much more dynamic, so a higher proportion of seeds germinated. By contrast, germination was delayed after ABA treatment; the proportion of germinated seeds was the lowest in this variant. After 14 weeks, 80% of the seeds germinated in the GA_3 treatment, 50% with ABA, and more than 60% in the control.

EFFECT OF STRATIFICATION ON ADC AND ODC ACTIVITY

Changes in the enzymatic activity of the studied enzymes during dormancy breaking and in germinating seeds stratified at 3°C or kept at 15°C (the latter temperature inhibiting dormancy breaking) are presented in Figure 2.

ADC activity was much higher in embryo axes than in cotyledons. Up to week 6 of stratification, changes in ADC activity were similar at the two temperatures. In week 1 its activity was relatively high

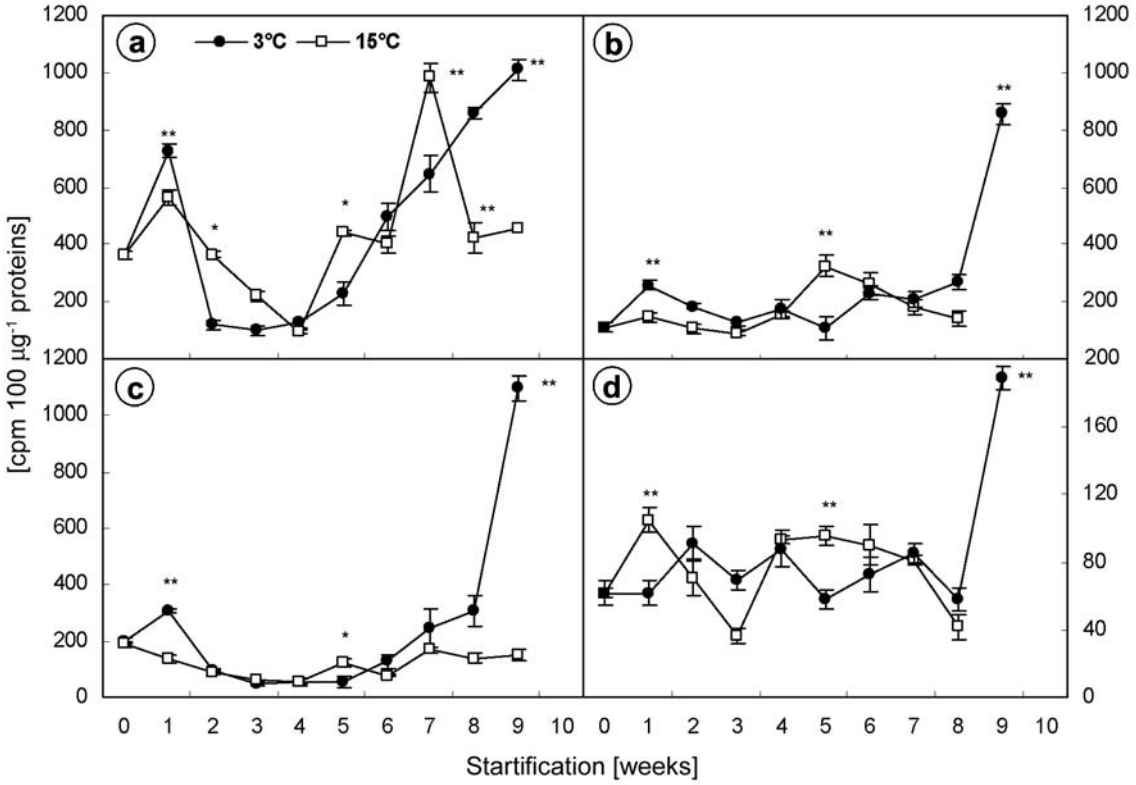

Fig. 2. Activity of arginine decarboxylase (**a, c**) and ornithine decarboxylase (**b, d**) during stratification at 3°C and 15°C. a, b – embryo axes. c, d – cotyledons of beech seeds. Enzyme activity was expressed as cpm ^{14}C per µg proteins. Means and standard deviations of three replicates. * – difference significant at $p < 0.05$; ** – difference significant at $p < 0.01$.

but declined by week 2, and later gradually increased. At 3°C it increased to the end of the experiment, but in organs of already germinated embryo axes it was much lower. At 15°C, ADC activity peaked in week 7 and later declined to minimum in week 9, while at 3°C it peaked later, after dormancy breaking, when the seeds were ready for germination.

In cotyledons, ADC activity during cold stratification successively increased and in week 9 reached a high level similar to that observed in embryo axes. By contrast, its activity at 15°C was generally low, and increased slightly only between weeks 5 and 6.

Changes in ODC activity in embryo axes of seeds subjected to cold stratification were like those of ADC activity. It increased gradually from week 2 and reached maximum in week 9. At 15°C, ODC activity in embryo axes was stabilized at a low level.

The profile of ODC activity in cotyledons was similar in both temperature variants until week 8, after which it markedly decreased at 15°C while at 3°C it rose dramatically, reaching maximum in week 9.

EFFECT OF ABA AND GA_3 ON ADC AND ODC ACTIVITY DURING COLD STRATIFICATION

Germination tests of stratified seeds (Fig. 1) showed that in embryo axes the germination rate was increased by exogenous GA_3, but ABA retarded germination and decreased the proportion of germinated seeds versus the control.

The influence of exogenous hormones on ADC and ODC activity is also apparent. ADC activity in embryo axes (Fig. 3) was highest in the GA_3 treatment between weeks 4 and 6. In the control, enzymatic activity was moderate, and highest in week 9 when the seeds were able to germinate. ABA clearly limited ADC activity. In cotyledons (Fig. 3) the stimulating effect of GA_3 on ADC activity was evident nearly throughout the period of stratification, but particularly at the beginning and end of the experiment. The profile of enzymatic activity was similar in the control, but at a much lower level. ABA clearly inhibited ADC activity in cotyledons throughout the experiment.

In all treatments, ODC activity (Fig. 3) was higher in embryo axes than in cotyledons. The dynamic

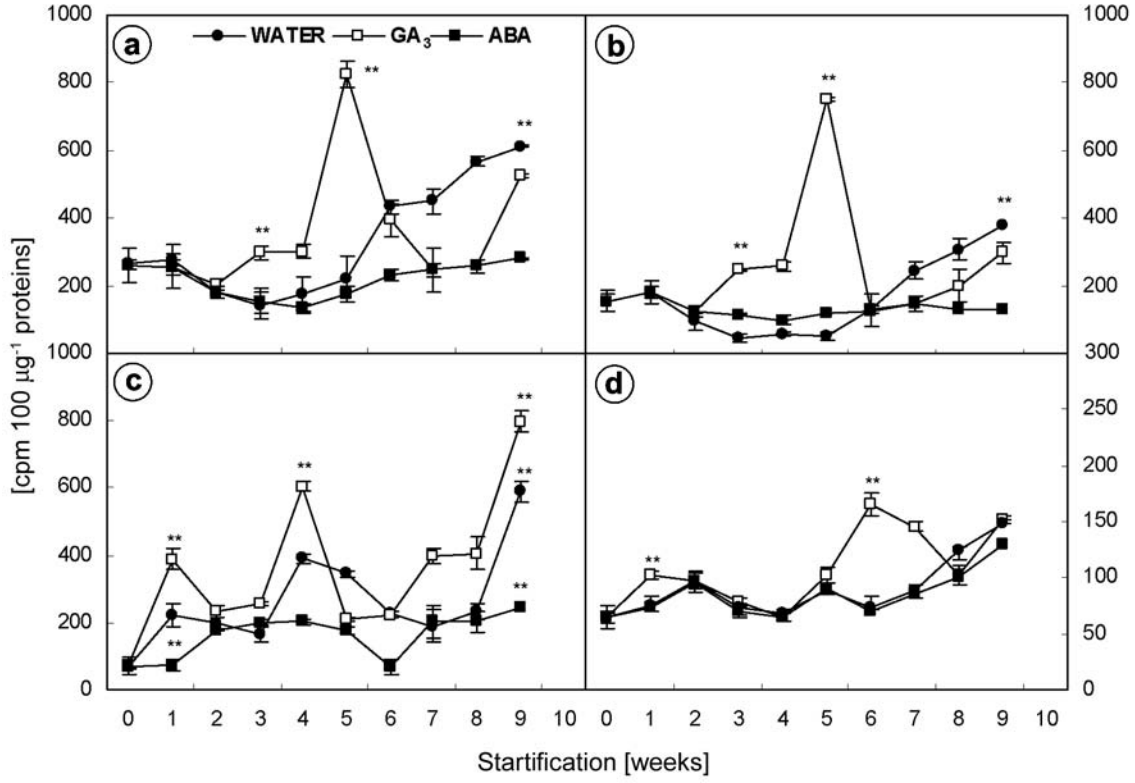

Fig. 3. Influence of GA_3 and ABA on arginine decarboxylase (**a, c**) and ornithine decarboxylase (**b, d**) activity during stratification of beech seeds at 3°C. Enzyme activity was expressed as cpm ^{14}C per µg proteins. Means and standard deviations of three replicates. * – difference significant at $p < 0.05$; ** – difference significant at $p < 0.01$.

of its activity was similar to that of ADC activity both in embryo axes and in cotyledons.

DISCUSSION

In our study, dormancy breaking in beech seeds was determined mainly by temperature and the influence of exogenous hormones (GA_3 and ABA).

Generally, ABA inhibits dormancy breaking and seed germination, while gibberellins stimulate these processes (Bewley and Black, 1994; Szczotka, 1999; Kucera et al., 2005). GA regulates putrescine and spermidine content and ADC and ODC activity (Galston et al., 1983).

Exogenous GA_3 supplied at the beginning of stratification accelerated dormancy breaking and increased the number of germinated seeds. ABA retarded seed germination, lowering the number of germinated seeds at the end of the experiment. Polyamines generally are regulators of many processes during seed development. They also influence dormancy breaking and the hasten germination of seeds (Szczotka and Lewandowska, 1989; Grzywacz et al., 1991; Szczotka et al., 2003). Temperature and the applied hormones clearly affected the activity of ADC and ODC, which are responsible for the beginning of polyamine biosynthesis. At 15°C, although dormancy was not broken and seeds were not germinated, the activity profiles of both enzymes were similar to those at 3°C until weeks 7 or 8. When the seeds started to germinate, the activity of both ADC and ODC increased rapidly at 3°C but declined at 15°C. This indicates that polyamine synthesis in beech seed is associated with the final stage of *Fagus sylvatica* seed dormancy breaking and germination. The changes in the activity of both enzymes were similar for this species.

In our earlier studies on Norway maple seeds we found that ADC activity increased during cold stratification already in the first few weeks, but ODC activity started to increase later, just before germination (Pukacka et al., 1991).

Thus it can be suggested that in the various tree species whose seeds are characterized by deep dormancy which must be broken by cold stratification, the intensity of polyamine metabolism differs and is related to the stage of dormancy release, so their role in individual stages of this process is likewise species-specific.

Santanen and Simola (1999) found that vernalization of spruce embryos is associated with increased activity of ADC but not of ODC.

The results presented here and in earlier studies show that dormancy release in beech seeds is more clearly correlated with changes in the activity of enzymes responsible for polyamine synthesis than with changes in polyamine content (Szczotka et al., 2003). This may be due to the fast metabolism of polyamines and to their occurrence in free or bound forms, as well as their association with protein synthesis. This observation applies to changes in polyamine content during dormancy release in beech seeds (Szczotka et al., 2003) and also in maple and ash seeds (Szczotka and Lewandowska, 1989). Minocha et al., (2004) found that changes in the ratios of various polyamines and ADC and ODC activity were clearly correlated with the developmental stage of red spruce embryos. Changes in polyamine content and arginine decarboxylase activity were associated with elongation of hypocotyls in *Rhizophora apiculata* (Swamy et al., 2004).

GA_3 or ABA treatment of beech seeds caused simultaneous changes in germination dynamics and in the activity of both ADC and ODC. GA_3 increased the levels of those parameters, while ABA decreased them. The similar influence of exogenous GA_3 at 3°C and at 15°C on ADC and ODC activity in embryo axes can be explained by their generally increased metabolic activity. It is apparently the result of the stimulating effect of GA_3, which either accelerates dormancy release at 3°C or only temporarily activates metabolism at 15°C, causing accelerated seed aging, and dormancy is not broken in such conditions. This is confirmed by the results of our research on concentrations of nucleotides (ATP, ADP and AMP) and energy charge in maple and beech seeds (Krawiarz and Szczotka, 2005), as well as on respiration-related enzymes (Krawiarz and Szczotka, 2002). For dormant beech seeds, 15°C temperature is a stress factor, which leads to accelerated seed aging. This may be associated with the increased activity of ADC and ODC responsible for polyamine synthesis. Phelps and McDonald (1990) reported higher accumulation of polyamines in somatic embryos of *Picea rubens* under thermal stress.

In addition to the experiment presented here, we also made observations of the influence of GA_3 and ABA on changes in protein content during dormancy breaking in beech seed (Pawłowski, 2007). A comparison of those observations with the data on ADC and ODC activity presented here and changes in energy charge (Krawiarz and Szczotka, 2005) helps to explain the mechanisms associated with dormancy breaking in beech seeds. GA_3 and low temperature cause beech seed embryo axes to accumulate proteins related to energy metabolism, while ABA causes accumulation of protective proteins (Pawłowski and Szczotka, 2006; Pawłowski, 2007).

Changes in polyamine content and ADC and ODC activity in plant tissues are also associated with the concentration and metabolism of nucleic acids (Twardowski and Szczotka, 1989; Szczotka, 1999) and protein pattern changes (Szczotka et al., 2003; Pawłowski, 2007; Pawłowski and Szczotka, 1997). In many cases no relationship has been found between the levels of hormones (ABA) and seed dormancy (Suszka and Tomaszewska, 1971; Tomaszewska, 1976; Le Page-Degivry and Garello, 1996).

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