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ACID PHOSPHATASE ACTIVITY IN SUN-EXPOSED AND SHADE BEECH (*FAGUS SYLVATICA* L.) LEAVES

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Summary. Acid phosphatase activity along with photosynthetic rate was studied in sun-exposed and shade leaves of European beech (*Fagus sylvatica* L.) in July and September 2009. In both periods, the acid phosphatase activity was higher in the shade compared to the sun-exposed leaves while in July the acid phosphatase activity was three-fold higher. Simultaneously in July and September the rate of photosynthesis was higher in sun-exposed leaves than in shade leaves. In July the acid phosphatase activity was higher in the shade beech leaves where the intensity of photosynthesis was lower. The present results suggest a negative correlation between the activities of acid phosphatase and the rate of photosynthesis.

Key words: acid phosphatase; beech; *Fagus sylvatica* L.; shade and sun-exposed leaves.

Abbreviations: FW – fresh weight; *p*-NP – *p*-nitrophenylphosphate.

INTRODUCTION

Plant phosphatases (EC 3.1.3) are suggested to be involved in the production, transport, and recycling of phosphate. Acid phosphatases (E.C. 3.1.3.2) are enzymes highly expressed in plants and especially in plant tissues such as seeds, bulbs, roots, tubers, coleoptiles and leaves. Leaf phosphatases from beans (*Phaseolus vulgaris*) (Tejera Garcia et al., 2004; Yan et al., 2001), soybean (*Glycine max*) (Staswick et al., 1994) and rice (*Oryza sativa*) (Shih & Kao, 1997) have been characterized. In some plants the increased acid phosphatase activity in leaves is associated with phosphorus deficiency symptoms (Duff et al., 1994). Changes in the seasonal activity of acid phosphatase from European beech (*Fagus sylvatica* L.)

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leaves were recently shown (Hadjiivanova and Tzvetkova, 2008). In spite of the intensive studies, the precise physiological function of plant leaf phosphatases is still not elucidated. For this purpose, the activity of acid phosphatase as well as the photosynthetic rate were studied in sun-exposed and shade beech leaves.

MATERIALS AND METHODS

Sample collection

Sun-exposed and shade leaves of a forty-year-old beech tree (*Fagus sylvatica* L.) grown as part of a mixed stand in the dendrarium of the University of Forestry in Sofia were used in the experiments. Leaves were taken and measurements were performed between 11:00 and 12:00 a.m. in clear sunny days in the middle (7th July 2009) and the end (2nd September 2009) of the vegetation period. Shade leaves were taken from the inner parts of the crown not directly exposed to sun. Leaves used in the study were fully developed and were not affected by herbivores and diseases.

Gas-exchange measurements

Measurements of CO_2 exchange of beech leaves were performed using a portable infrared gas analyzer Li-6400 (Li-Cor Inc., Lincoln, NE, USA). Flow rates through the chamber were maintained at 500 mmol min^{-1}. CO_2 concentrations in the air were adjusted to 330-350 μmol min^{-1}. Relative humidity in the chamber was about 50% and leaf temperature was 20°C. In the experiments artificial light was used, produced by built-in controllable LED (Light Emitting Diode) light source with intensity 1000 μmol m^{-2} s^{-1} PAR. At least 20 readings per leaf were used for data proceeding.

Acid phosphatase extraction and estimation

Plant tissues were weighed, frozen in liquid nitrogen and grounded (1:10 w/v) in 0.1 M acetate buffer pH 5.0. After centrifugation at 10000 x *g* for 10 min, the supernatant was used to assay the enzyme activity. The assay mixture containing 0.02 ml of the enzyme extract in 1.0 ml 0.6 mM *p*-nitrophenylphosphate in 0.05 M acetate buffer, pH 5.0, was incubated at 37°C (Kolari and Sarjala, 1995). After 30 min the reaction was stopped with 1 ml 1 N NaOH. Blank samples had the same content, but 1 ml 1 N NaOH was added before the addition of the enzyme extract. The produced *p*-nitrophenol (*p*-NP) was estimated by the absorbance measured at 410 nm, using a molar extinction coefficient of 16200 M^{-1} cm^{-1}. Acid phosphatase activities have been calculated on a unit fresh weight (FW) as nmol *p*-NP g FW^{-1} min^{-1}. In each experiment 3 different samples were obtained from both sun-exposed and shade leaves, assayed in either duplicate of triplicate.

Values are given as means ±SD or ±SE and differences were regarded significant at $P< 0.05$ by Student's *t*-test, using Systat computer program (SPSS Inc., Chicago, USA, 1996).

RESULTS AND DISCUSSION

The acid phosphatase activities in sun-exposed and shade beech leaves at different stages of vegetation, i.e. in July and September are shown in Table 1. In both experiments the enzyme activity

Table 1. Acid phosphatase activities in sun-exposed and shade beech leaves.

Acid phosphatase activity [nmol *p*-NP g FW^{-1} min^{-1}]		
Month	Sun-exposed leaves	Shade leaves
July	521.6±81.2	1608.4±24.2*
September	332.4±76.9	446.8±116.8

Values are means ±SE, *p*-nitrophenol (*p*-NP). * – significant difference between sun-exposed and shade leaves at $P < 0.05$.

was higher in the shade than in the sunny leaves, but the differences were significant and more pronounced in July when the acid phosphatase activity was 3-fold higher in the shade than in the sun-exposed leaves. In addition, the acid phosphatase activities were much higher in July than the enzyme activities in September confirming the previously observed seasonal changes in beech leaves acid phosphatase activity (Hadjiivanova and Tzvetkova, 2008). The physiological role of leaf acid phosphatases is not clear. The present study is part of an investigation aimed to find a correlation between changes in leaf acid phosphatase activities and changes in leaf physiological activities. Therefore, the intensity of photosynthesis was also studied. As expected, during the measurements performed in July and September the intensity of photosynthesis was significantly higher in sun-exposed than in shade leaves (Table 2). The lower photosynthetic activity in both sun-exposed and shade leaves in July was probably due to the very hot and dry weather in this period. In addition, the present results showed that during both measurements the acid phosphatase activity was higher in the shade beech leaves where photosynthetic rate was lower. This trend was much clearer in July, i.e. in the middle of the vegetation period. The present finding might reflect the role of acid phosphatase in salt and osmotic stress (Duff et al., 1994) to which plants are exposed during hot and dry weather. The significance of the higher acid phosphatase activity in the shade than in the sun-exposed beech leaves is not clear.

Table 1. Acid phosphatase activities in sun-exposed and shade beech leaves.

Net photosynthetic rate [μmol CO_2 m^{-2} s^{-1}]		
Month	Sun-exposed leaves	Shade leaves
July	5.71±1.08*	4.86±0.70
September	9.65±1.84*	7.90±1.34

Values are means ±SD, *p*-nitrophenol (*p*-NP). * – significant difference between sun-exposed and shade leaves at $P < 0.05$.

It might be assumed that high leaf acid phosphatase activities are needed for the observed enhancement of light-harvesting efficiency of shade leaves exposed to low light (Niinemets, 2007). In conclusion, the present results suggest a negative correlation between the activities of acid phosphatase and photosynthetic rate, especially in the middle of vegetation.

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