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Aerobic conditions and antioxidative system of *Azolla caroliniana* Willd. in the presence of Hg in water solution

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A b s t r a c t. Heavy metals are among the toxic substances for plants. Their presence causes a state called "heavy metals stress" which manifests itself by many physiological changes in plants. One of them is the formation of reactive oxygen species (ROS) which are scavenged by enzymatic and non enzymatic defending systems. The physiological responses of aquatic fern *Azolla caroliniana* Willd. at different mercury concentrations was investigated. During 9 days of the experiment under laboratory conditions, *Azolla caroliniana* was cultivated on medium enriched in 1, 20 and 30 mg Hg(II) dm^{-3}. In this time the oxygen diffusion rate (ODR) and the superoxide dismutase activity (SOD) were determined. A strong release of oxygen by the fern roots (ODR up to 50 µg $m^{-2}s^{-1}$) was recorded. It was related with an increase in SOD activities (up to 4000 units) above control values, which testifies to the existence of a defense reaction of *A. caroliniana* to the heavy metal stress.

K e y w o r d s: *Azolla*, Hg, ODR, oxygen availability, superoxide dismutase

INTRODUCTION

High concentration of heavy metals, like other stress conditions in plants, affects a number of physiological and biochemical reactions and induces oxidative stress in the plant cells (Noctor and Foyer, 1998; Blokhina *et al.*, 1999; Hunter *et al*., 1983; Yan *et al.*, 1996).

The formation of reactive oxygen species (ROS) takes place in the cells of all the plants and is a consequence of normal aerobic metabolism. Under optimal conditions, the content of ROS is maintained, with the help of antioxidative defense system, at a level which is safe for the organism (Larson, 1988). Thus, superoxide dismutase scavenges the superoxide anion-radical (O_2^-) in the cytoplasm, chloroplast and mitochondria (Bowler *et al.*, 1992). Under stress conditions, the formation of ROS can exceed the antioxidative potential of the cell and cause oxidative damage (Halliwell, 1984).

The plant capability to activate the defense system against oxidative destruction may be a key link in the mechanism of plant tolerance to unfavorable conditions. Changes in the activity level of one or more antioxidative enzymes are connected with the plant resistance to stressor action (Allen, 1995).

Previously, we found (Bennicelli *et al*., 1998) that maize response to soil aeration conditions (as evaluated by biomass production and stomatal diffusive resistance) and the advancement of destructive processes (as assessed by malondialdehyde content) as well as the antioxidant system status (expressed by SOD activity) were related to soil oxygen availability as measured by oxygen diffusion rate (ODR). Similar dependences of growth and oxidative processes to soil aeration parameters were also shown for pea known as a flood intolerant plant (Zakrzhevsky *et al*., 1995).

The aim of the paper was to investigate the response of *Azolla caroliniana* Willd. (*Azollaceae*) defense system in the presence of different concentration of Hg and availability of oxygen in water solution.

MATERIALS AND METHODS

The studies were performed under laboratory conditions with the use of aquatic fern *A. caroliniana*. The air temperature was kept at 25±2°C and an 18/6 photoperiod was applied. The relative air humidity was on the level of 70±5%. As the indicator of aeration conditions, oxygen diffusion rate (ODR) was used as described by Gliński and Stępniewski (1985).

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Plant defense system was characterized by superoxide dismutase (SOD) measurements by the spectrophotometric method according to Paoletti *et al.* (1986), based on oxidation of NADH by superoxide anion radicals. The enzyme extract was diluted 20-fold before the analysis. As a unit of SOD activity, 50% inhibition of NADH oxidation rate was used. Analyses were performed in control and treatment plants from the crude homogenates and enzyme extracts. All the results were calculated per one gram of leaf dry mass.

Three replications of each experimental treatment were made. The variability of treatments was analyzed by ANOVA test. The relationship between ODR or SOD activity and time (days) of prolonged experiment was expressed by correlation coefficient (R^2) and by correlation equation.

RESULTS AND DISCUSSION

The availability of oxygen to roots of *A. caroliniana* plants differentiated in a wide range what was indicated by significant (P<0.05) changes of the control ODR values 20-30 to 50 $\mu g\ m^{-2}\ s^{-1}$ under higher Hg concentration (Fig. 1). In the presence of 30 mg Hg(II) dm^{-3}, adaptation time (1-4 days) for *A. caroliniana* plants was needed. After this time, increasing values of ODR were observed. The level of the availability of oxygen (ODR) indicated the release oxygen from roots *A. caroliniana* to the water medium. Release of oxygen from the roots of *Typha latifolia* at the level of 0.12-0.2 mmol O_2 d.w. h^{-1} was described by Jespersen *et al.* (1998).

The SOD activity (Fig. 2) in the leaves of the control plants was, during the experimental period, within the normal physiological range of 1800-3000 units. In the presence of Hg(II) in the water solution, a significant increase of SOD activity in plant tissue was observed up to 4000 units (P<0.05), which indicates the sensitive response of the defense system of *A. caroliniana* to environmental stress.

This reaction is comparable to the adaptive response of plant under oxygen deficiency conditions experienced by the plant roots during the period of flooding the soil, and takes place in the leaves which are in the normal aerobic conditions (Zakrzhevsky *et al.*, 1995; Yan *et al.*, 1996).

Under these conditions, limitation of the amount of final electron acceptor in electron transport chain – $NADP^+$, favours functioning of oxygen as an alternative acceptor of electrons. Induction of such reactive oxygen species as superoxide radical (O_2^-) and hydrogen peroxide (H_2O_2) initiates processes of lipid peroxidation and leads to serious damage of cells and of the entire organism (Egneus *et al.*, 1975). Increase of the rate of O_2^- and H_2O_2 generation in leaves of plant under soil hypoxia was correlated with lipid peroxidation (Yan *et al.*, 1996).

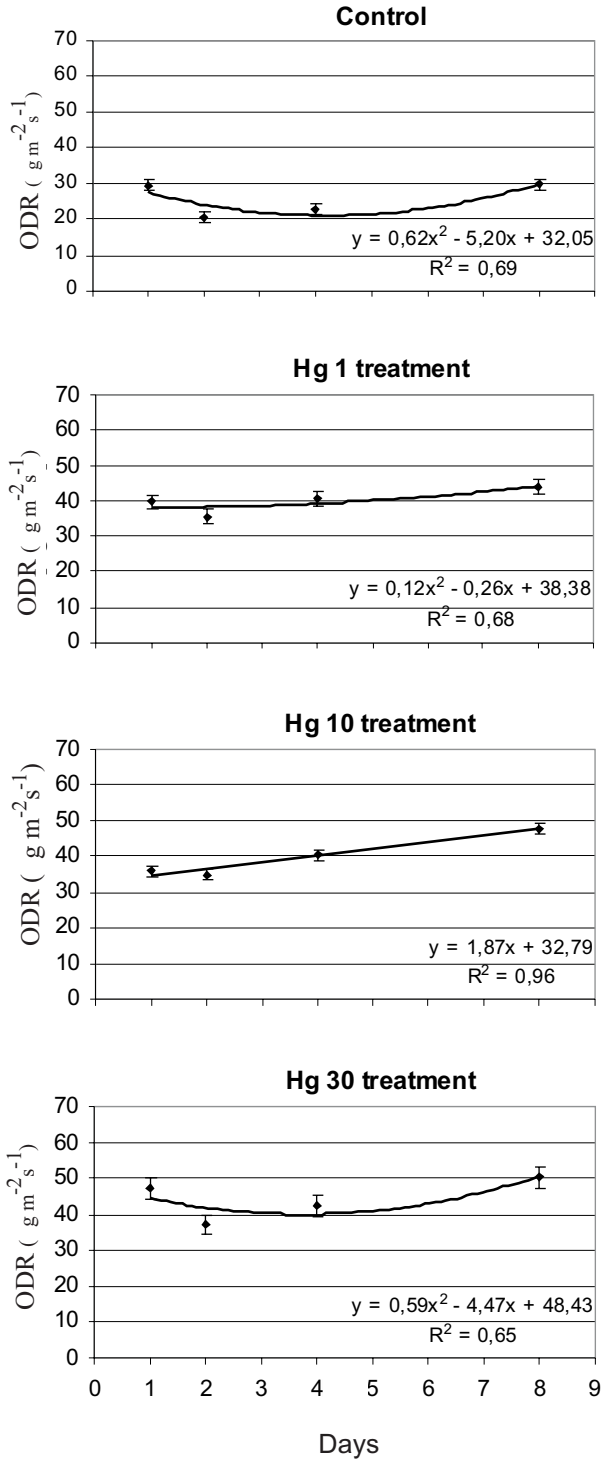

Fig. 1. ODR (mean values with 95% Fisher's LSD Intervals) in subsequent days of experiment with *Azolla* at various Hg treatments.

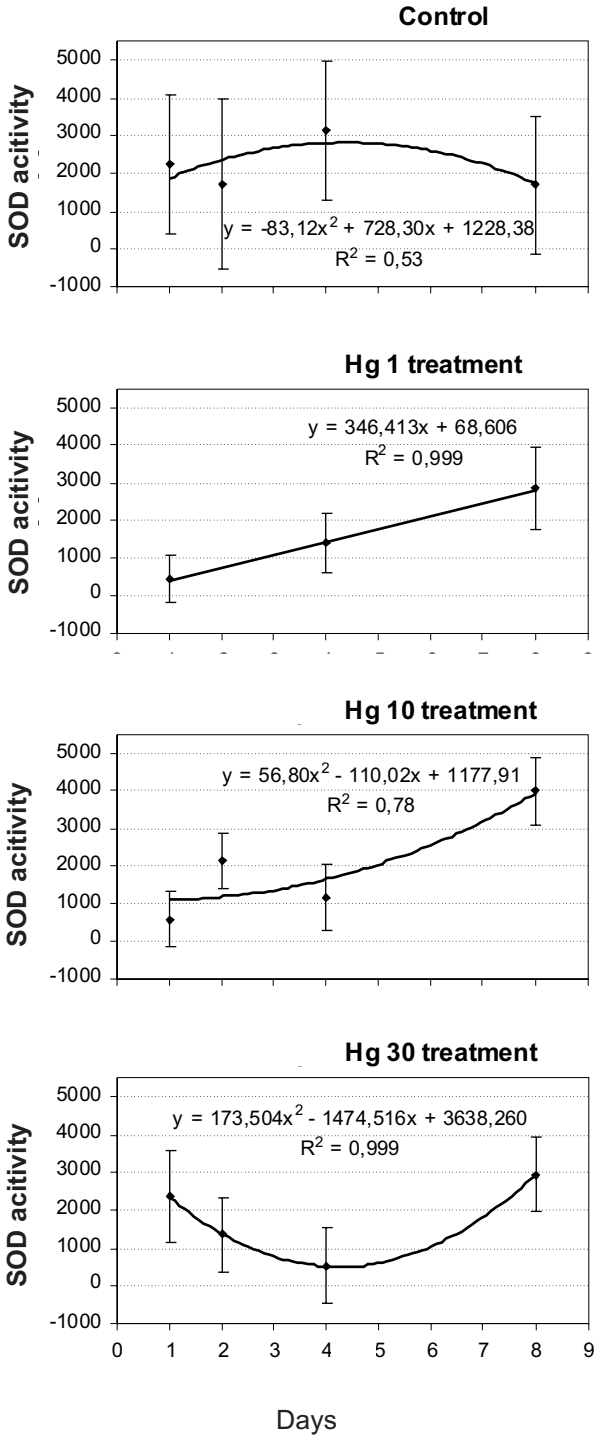

Fig. 2. SOD activity (mean values with 95% Fisher's LSD Intervals) in subsequent days of experiment with *Azolla* at various Hg treatments.

Protection of plants from oxidative destruction is associated with active functioning of SOD activity in roots and leaves under waterlogging conditions (Zakrzhevsky *et al*., 1995).

CONCLUSIONS

1. This experiment showed that *A. caroliniana* survived, despite the presence in the medium of such a toxic element as Hg(II) in the range of 1-30 mg dm^{-3}, thanks to its effective defense system involving a significant increase of SOD activity.

2. Simultaneously, 2-3 times increase of oxygen diffusion rate from plant roots to water solution was noted.

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