EFFECTS OF CADMIUM ON ISOPEROXIDASE PATTERN AND ANTIOXIDANT ENZYME ACTIVITIES IN GERMINATING WHEAT SEEDS

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Key words: isoperoxidase pattern, wheat, enzymes, cadmium

Abstract: The objective of the study was to investigate the changes in growth parameters and in enzyme activities during wheat seed germination, in laboratory conditions, in presence of different amounts of cadmium excess. Two varieties of wheat were studied (Triticum aestivum L.cv. Boema and cv. Simnic 30) in order to select plants which are tolerant to cadmium stress. The activity of ascorbate peroxidase, soluble peroxidase, isoperoxidase and catalase, enzymes involved in the scavenging of reactive oxygen species, were measured. Obtained results show cadmium induced changes in investigated enzyme activities and illustrate that cadmium uptake was positively correlated with some enzyme activities and negatively correlated with other ones. It is reasonable to think that enzyme stimulation plays a role in the cellular protection against metal toxicity, but it cannot be excluded that in some cases the increased enzyme activity might represent a toxic effect. Measurement of oxidoreductase activity, especially peroxidase and specific change in the isoperoxidase pattern, might be used as biomarkers to asses the phytotoxicity for wheat grown on cadmium contaminated media.

INTRODUCTION

One of the major environmental problems caused by industrialization is the increment in the concentration of heavy metals in the air, land and water. The increasing contamination and consequent accumulation of heavy metals in the soil can have serious implications on agriculture, since the concentration of such metals can reach unacceptably high contents in plant tissues. (Kevrešan et al. 2003, Ozkutlu et al. 2007). Amongst the heavy metals, cadmium, which is not a nutrient for plant, is particularly toxic and can also accumulate in different human tissues and organs (Šottníková et al. 2003) as well as in several tissues of higher plants (Vitoria et al. 2001, Arduini et al. 2003, Arduini et al. 2004). One of the clearest phytotoxic symptoms induced by heavy metals is a diminution in plant growth which is associated with disturbance of several metabolic processes. Some plant species have developed heavy metal adaptation which enables them to survive and reproduce in such unfavorable conditions. Heavy metal tolerance may results from different strategies including the formation of complexes with phytochelatins, cysteine-rich peptides (Rauser et al. 2000). One possible mechanism by which excess heavy metals may damage plant tissues is the stimulation of free radical production, by imposing oxidative stress (Foyer et al. 2000). Plant cells are equipped with several free radical detoxifying enzymes to protect them against oxidative damage. These enzymes include superoxide dismutase, ascorbate peroxidase, glutathione reductase, catalase and peroxidase (Foyer et al. 1997, Wu et al., 2003).
The objective of the study was to investigate the changes in growth parameters and in enzyme activities during wheat seed germination, in laboratory conditions, in presence of different amounts of cadmium.

MATERIAL AND METHOD

Two varieties of wheat were studied (Triticum aestivum L.cv. Boema and cv. Simnic 30) in order to select plants which are tolerant to cadmium stress. The activity of ascorbate peroxidase, total soluble peroxidase, isoperoxidase and catalase, enzymes involved in the scavenging of reactive oxygen species, were measured.

Wheat (Triticum aestivum L. cv. Boema and cv. Simnic 30) seeds were obtained from University of Craiova, Faculty of Agronomy. Dry seeds sterilized with 5% sodium hypochlorite were rinsed twice with distilled water and sown in Petri dishes with Hoagland solution containing 0.5% agar and supplied with cadmium (as CdCl$_2$) at three concentrations 0 (control), 100 µM and 150 µM. Distilled water was added when necessary, in order to maintain a constant degree of humidity. Seven days old seedlings was homogenated with 0.1M phosphate buffer (pH 7.5) containing 0.1mM EDTA. Homogenated were centrifuged for 20 min at 6000 r.p.m. and the supernatants were used for enzyme assays.

The activity of ascorbate peroxidase was measures by determining the oxidative rate of ascorbic acid. Total soluble peroxidase (guaiacol-type E.C.1.11.1.7) were assayed by measuring the increase in $A_{436}$ due to the guaiacol oxidation and their activity was expressed as $\Delta A$/min/1g fresh weight (Putter, 1974). Catalase (E.C.1.11.1.6) was assayed through the colorimetric method of Sinha (1972) and expressed as mmoles H$_2$O$_2$/min/g at 25°C.

Separation of soluble isoperoxidases was done by agarose gel electrophoresis at pH 8.6, performed in a horizontal gel system Stratton 301 E, at 6V/cm for 4h. Gels were stained in 1% benzidine reagent and quantitative evaluation of zymograms was performed with a Karl Zeiss K1 40 densitometer. The isoperoxidase fractions were numbered from the start to the anode for the anionic ones (A1, A2) and to the cathode for the cationic ones (C1-C3).

Protein concentration was evaluated by the method of Bradford using bovine albumin as a standard.

RESULTS AND DISCUSSION

The obtained data concerning the content of total soluble protein are presented in figure 1. For both studied varieties it can be observed an increase of the protein content according to the applied dose. Higher values of the protein content can be noticed in the Simnic 30 variety. In presence of cadmium, plants have developed an ability to synthesize proteins and peptide that can tightly bind and sequester this metal.

The catalase activity (figure 1), for both varieties of wheat is increasing with the cadmium dose, in accordance with its role of decomposing the hydrogen peroxide formed in excess. The great increase of the catalase activity is indicating an oxidative stress state generated by the dose of the applied heavy metal. The results show higher values of the catalase activity on the Simnic 30 variety.

The activity of total soluble peroxidases (figure 1) is also increasing in case of both varieties of wheat comparing to the untreated control and this increase is proportional to the applied dose. The activity of the ascorbat peroxidase is decreasing, comparing to the control for both varieties of wheat and in the case of both applied dozes.
Figure 1 Total soluble protein content and enzymatic activity in germinated wheat seeds

Analysis of the isoperoxidases pattern (Figure 2) has shown the same number of fractions either in the untreated control or the treated variants: two anionic and three cationic isoforms. One can notice an increase in each isoperoxidase fraction at each studied variant in comparison to the control. In the case of the Boema variety the total peroxidase activity notably increases along with the applied dose, in the case of the V3 variant (150 µM) the total isoperoxidase activity tripling in comparison to the control.

Figure 2 Isoperoxidases pattern in germinated wheat seeds
CONCLUSIONS

Obtained results show cadmium induced changes in investigated enzyme activities and illustrate that cadmium uptake was positively correlated with some enzyme activities and negatively correlated with other ones. It is reasonable to think that enzyme stimulation plays a role in the cellular protection against metal toxicity, but it cannot be excluded that in some cases the increased enzyme activity might represent a toxic effect. Heavy metal toxicity is considered to induce the production of reactive oxygen species and may result in significant damage to cellular constituents. Perhaps a constitutively high antioxidant capacity or increase in the levels of one or more antioxidants could prevent the oxidative damage and improve resistance to oxidative stress. The increased activities of catalase and peroxidase suggest that the plant depend on these antioxidative enzymes for elimination of H$_2$O$_2$ under stress.

Measurement of oxidoreductase activity, especially peroxidase and change in the isoperoxidase pattern, might be used as biomarkers to asses the phytotoxicity for wheat grown on cadmium contaminated media.

BIBLIOGRAPHY