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THE EFFECTS of HEAVY METAL APPLICATIONS on ANTIOXIDANT DEFENCE ACTIVATION in BARLEY and WHEAT VARIETIES

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ABSTRACT

Heavy metals are known as agents for oxidative stress by formation of reactive oxygen species and accumulated on the earth. This accumulation can than be transported via food chain to humans and causes some more serious health problems. As a multicellular higher organisms, plants are the first stop for heavy metal accumulation during this traffic. Therefore, plants are not only the vehicle of this transportation, but also another affected organisms together with animals and humans, due to their lowered self productivity. However, as antioxidant defence systems play a crucial defence against oxidative stress, these responses could be used as early biomarkers of heavy metal toxicity in plants. Based on this, we have examined whether antioxidant defence responses are reliable indicators for the toxicity of heavy metals cadmium and lead in different crop plants within this study. By using the seeds of *Hordeum vulgare* cv. Çıldır and *Triticum aestivum* cv. Gerek, different single and combined concentrations of $CdCl_2$ and $PbCl_2$ treatments were applied to investigate glutathione (GSH), protein contents and glutathione *S*-transferase (GST) activities in the roots and shoots of these mentioned varieties. Our results shown that, heavy metals had an effect on the tested parameters and variability in results reflect the differences in the rate of metabolism with regard to heavy metals between varieties. On the other hand, due to the high GSH and GST values observed in the studied plants, it should be mentioned that they are generally adaptable to stress conditions with regard to applied heavy metals in the study.

Keywords: Antioxidant mechanisms, Barley, Heavy metals, Oxidative stress, Wheat

1. INTRODUCTION

Due to sessile characters, plants always experience different types of stress. Heavy metals (HM), especially cadmium (Cd) and lead (Pb) attract much attention because of their wide range of distribution, as one of the main causes of this stress for plants [1]. In addition to other stress factors, Cd and Pb increase the formation of reactive oxygen species (ROS), which triggers the oxidative stress. Therefore, not only proteins and lipids, but also DNA are one of the sites of ROS affected locations in the plant cells [2].

Plants protect themselves from the harmfull effects of ROS and oxidative stress by using some detoxification systems. These systems include non-enzymatic antioxidants or antioxidant enzymes [3]. Among non-enzymatic antioxidants, glutathione (GSH) show high affinity for increasing concentrations to toxic metals. Therefore, its level is considered as a significant bio-indicator of oxidative stress [4]. GSH also works as part of the glutathione reductase (GR) and glutathione peroxidase (GPOX) enzymes for antioxidative defence. However, most importantly, it is used by cells for detoxification reactions through glutathione *S*-transferases (GSTs) as a co-substrate [5].

As multifunctional enzymes, GSTs (EC.2.5.1.18) catalyse the conjugation of the GSH thiol group to different electrophilic centers on the lipophilic molecules. Generally, the new glutathione *S*-conjugates are less toxic than their parental compounds and ready to be transported into the vacuole or through ABC transporters to cell wall [6].

GSTs found in all organisms, however their discovery in plants depends on GSH-conjugating activities by using herbicide (atrazine) in maize [7]. Today, 14 classes of GSTs in plants organisms have been

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identified according to the latest study in 2014. Of those, some of them belong to Ser-GSTs [containing serine amino acid as active site residue; like Tau, Phi, Zeta, Theta and tetrachloro-hydroquinone dehalogenase (TCHQD) classes], some to Cys-GSTs [containing cysteine amino acid; such as Iota GSTs (GSTIs), Hemerythrin GSTs (GSTHs), Dehydroascorbate reductases (DHARs), Lambda GSTs (GSTLs), GHRs, mPGES-2s and metaxins classes], while the other two classes have less clear catalytic residues (EF1Bγ and Ure2p classes). Again, some of these classes are specific to plant kingdom (Lambda and DHAR) and some are found among different kingdoms (Zeta and Theta) [8].

In this study, we aimed to examine the relationship between oxidative stress and antioxidant defense systems triggered by HM and investigate some biochemical responses in commonly cultivated plant varieties exposed to Pb and Cd regimens singly and in combination.

2. MATERIALS AND METHODS

2.1. Plant Materials and Growth Conditions

The seeds of plants (*Triticum aestivum* L. cv. Gerek and *Hordeum vulgare* L. cv. Çıldır) were obtained from Transitional Zone Agricultural Research Institute (Eskisehir, Turkey) and surface sterilized according to Riaz et al. [9] with 1% (v/v) NaOCl.

Germination was carried out with dH_2O in a growth chamber (22°C ± 1°C / dark / 3 days and 16 h photoperiod / 7 days). Seedlings were transferred to beakers containing 250 ml of Hoagland solution [10] and pH of the nutrient solution was adjusted to 6.5 ± 0.1 with 0.1 M NaOH. At the end of the incubation (3 days) in the growth chamber, different concentrations of single $CdCl_2$ or $PbCl_2$ (0, 50 and 100 µM) and combined $CdCl_2$+$PbCl_2$ (0, 50 + 50 and 100 + 100 µM) solutions were added into the nutrient medium. Each treatment was in triplicate. Plants were exposed to a 16 h photoperiod (7 days). Seedlings were harvested 10 days after the application of HM [11], roots and shoots were separated and pulverized in liquid N_2 for further analysis. Pulverized materials were stored at - 80 °C for other measurements.

2.2. GSH Determination

GSH content was determined using the DTNB procedure [12] for pulverized roots and shoots, separately. The absorbance of the reaction mixture was determined at 412 nm and the content of GSH was calculated from a standard curve using reduced GSH.

2.3. Preparation of Cytosolic Extracts from Plant Materials

Pulverized root and shoot materials extracted separately in a ratio of 1:3 w/v, with 100 mM pH 7.0 phosphate buffer at 4 °C. Obtained mixture was homogenized for 2 min. by Ultra-Turrax at on ice. After centrifugation (15,000 rpm for 30 min. at 4 °C), the supernatant fraction was immediately assayed for protein and GST activities [13].

2.3.1. Total Protein Determination

Lowry et al. [14] method was used for protein content determinations.

2.3.2. Determination of GST Activities

GST activities against the substrate 1-chloro-2,4 dinitrobenzene (CDNB) were determined spectrophotometrically according to the method of Habig et al. [15]. The reactions were followed for 5 min and reaction rate was calculated using the ε values of CDNB as 0.0096 μM^{-1} cm^{-1}.

3. RESULTS

3.1. GSH Contents in the Roots and Shoots of Crop Varieties

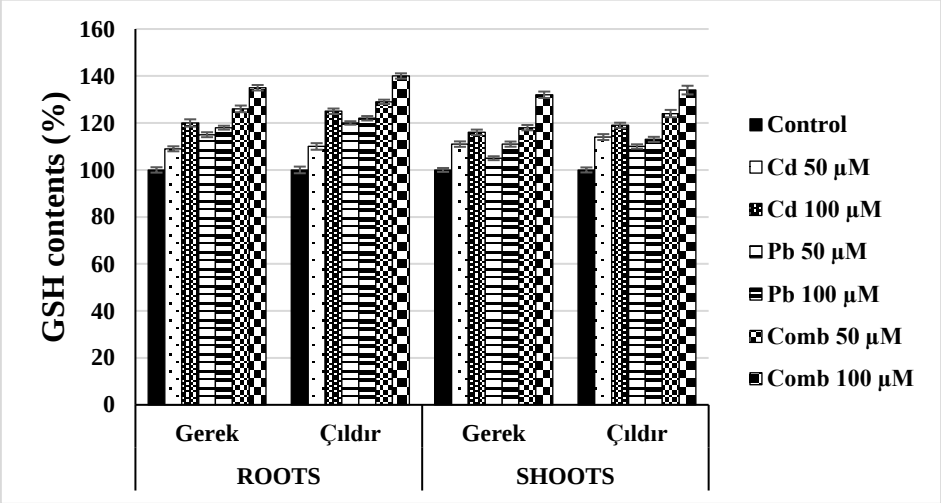

Figure 1. Effects of heavy metal applications on plant GSH contents. Absolute values can be seen in the text. Changes in values are shown as percentage of their controls.

The absolute values of GSH contents of the control samples belonging to the Gerek and Çıldır varieties were measured as 8.00 and 11.00 µg mg^{-1} in the shoots and 17.00 and 21.00 µg mg^{-1} in the roots, respectively. Figure 1 shows the effects of different metal applications on the GSH contents of the Çıldır and Gerek varieties. It was observed that there was a dose-dependent increase in GSH contents with increasing HM doses in the plant parts of both varieties, after all single and combined HM applications. Single Pb applications were found to be less effective than single Cd applications, especially in plant shoots. Our results show that, while the measured GSH content in the roots was higher than the shoots, the highest GSH content was belong to the roots of Çıldır variety with the application of 100 µM $CdCl_2$+$PbCl_2$.

3.2. Protein Contents in the Roots and Shoots of Crop Varieties

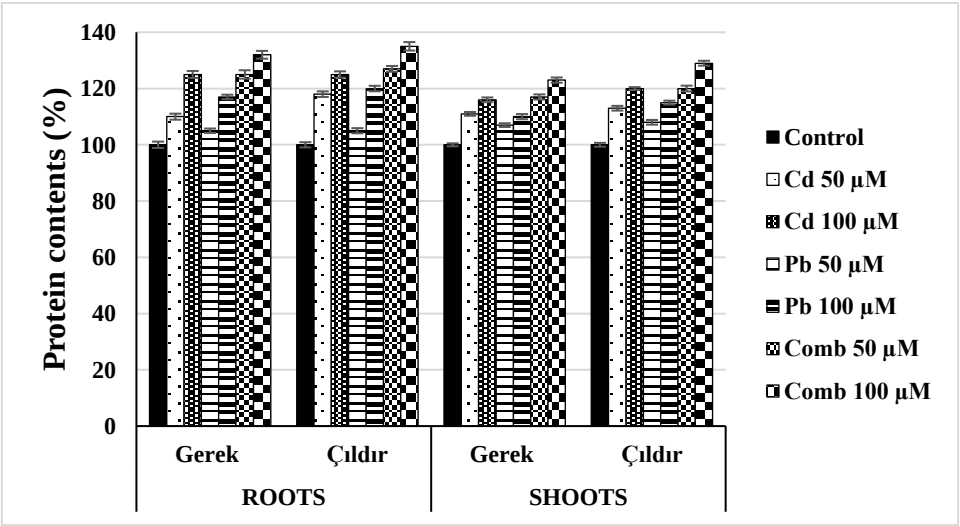

Figure 2. Effects of heavy metal applications on plant protein contents. Absolute values can be seen in the text. Changes in values are shown as percentage of their controls.

The absolute values of protein content of the control samples belonging to Gerek and Çıldır varieties were measured as 1.40 and 1.80 mg ml^{-1} in shoots and 2.00 and 2.40 mg ml^{-1} in roots, respectively. Figure 2 shows the effects of different metal applications on the protein content of Çıldır and Gerek varieties. As a result of all single and combined HM applications, it was observed that there was a dose-related increase in protein content with increasing HM doses in the plant parts of both varieties. The situation that we observed only in shoots, that single Cd applications are more effective in GSH contents than single Pb applications, is also present in roots in terms of protein content. According to our results, the protein content in the roots was higher than the shoots, and the highest protein content was measured in the roots of Çıldır variety with the application of 100 µM $CdCl_2$+$PbCl_2$.

3.3. GST Activities in the Roots and Shoots of Crop Varieties

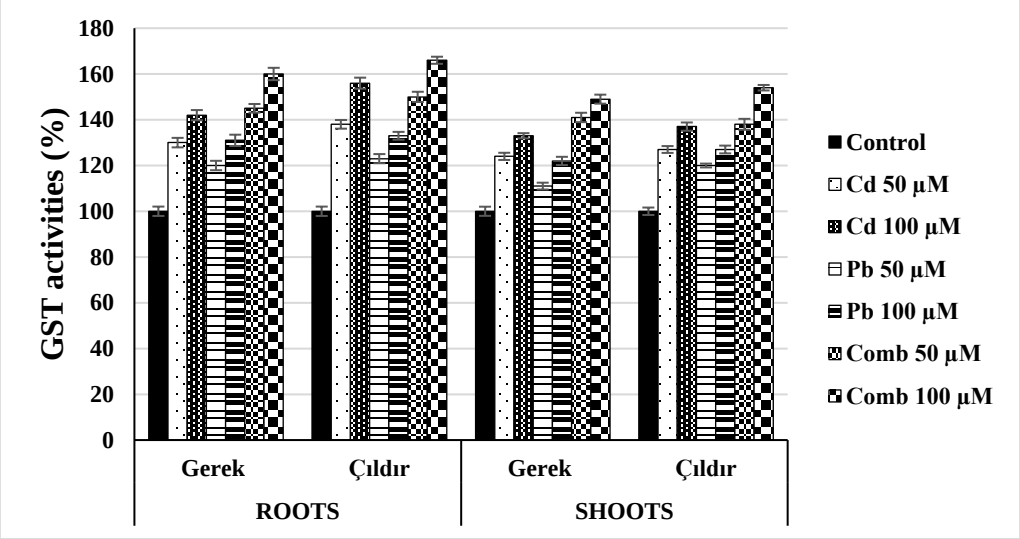

Figure 3. Effects of heavy metal applications on plant GST activities. Absolute values can be seen in the text. Changes in values are shown as percentage of their controls.

Absolute values of GST activities of the control samples belonging to Gerek and Çıldır varieties were measured as 350 and 390 nmol min^{-1} mg protein $^{-1}$ in shoots, 430 and 500 nmol min^{-1} mg $protein^{-1}$ in roots, respectively. Figure 3 shows the effects of different metal applications on the GST activities of Gerek and Çıldır varieties. As mentioned in previous parameters, dose-depended increases in GST activities were observed with increasing HM doses in the plant parts of both varieties, as a result of all single and combined HM treatments. Again, similar to protein contents, all single Cd applications are more effective than single Pb applications. Detailed examinations show that the highest GST activity was measured in the roots of Çıldır variety with $CdCl_2$+$PbCl_2$ application at 100 µM and the enzyme activity in the roots was observed higher than the shoots of examined varieties.

4. DISCUSSION

4.1. GSH Contents in the Roots and Shoots of Crop Varieties

Usage of antioxidant molecules to protect themselves against ROS is a common process in plants. As an antioxidant molecule, GSH takes part in detoxification reactions as a co-substrate of GST and other related enzymes [16], in addition to its work against oxidative stress which induced by HM toxicity [17]. Due to its different functions, the concentration of GSH decreases as stated in some articles [18]. However, some reports state the increase in GSH concentrations [11, 19], which are supporting our current results. In this study, it was observed that increasing Cd and Pb concentrations caused a dose-

dependent increase in the amount of GSH in both plants and their examined parts. While the GSH contents in the shoots were found to be lower than in the roots, the highest amount of GSH was determined in the roots of Çıldır by comparing to Gerek variety.

4.2. Protein Contents in the Roots and Shoots of Crop Varieties

Protein degradation through HM triggered oxidation is accepted as an indicator of oxidative stress in cells, due to an increase in protein conversion rate and a decrease in plant development [20]. Nevertheless, different results were reported regarding either inhibitory or stimulating effect of HMs on cell protein content by different groups [21-24]. In the present study, a dose-dependent increase in protein content was observed for the roots and shoots of both varieties with increasing Cd and Pb applications. Similar to observations in GSH contents, single Pb treatments were found to be less effective by comparing to single Cd treatments in both varieties, especially on the shoots. This result could be interpreted as direct evidence for GSH level control, at least in terms of shoots. Our results indicate that, protein content in the roots is higher than the shoots and the highest protein content was observed in Çıldır roots. The relevant increase in protein amounts shows that as a result of HM stress, the increased level of gene expression of proteins which have a defensive function in plants.

4.3. GST Activities in the Roots and Shoots of Crop Varieties

HMs show their effect in plants in three different routes. In of them, specific metal ion displacement from binding site causes malfunction in normal function of enzymes. This situation is also important for GST, as they are catalyzing the thiol group of GSH. Depending on this information, when different enzymes are treated with different HM applications, either decreases or increases in their activities were reported [25]. In the current study, it has been found that increasing Cd and Pb concentrations cause dose-dependent increase in GST activities with the highest values belonged to Çıldır variety roots. While the reason for an increase in enzyme activity is explained in literature as the changes in enzyme synthesis or enzyme inhibitor immobilizations due to HM applications [26], our results compatible with previous studies [27, 28].

5. CONCLUSIONS

During this study, we have searched for some biomarkers of HM action and found that the biochemical parameters tested under HM stress are increased. Due to this increase, which is an indicator of a general adaptation to stress conditions in plants, the biochemical parameters we studied can be used as biomarkers for the early determination of oxidative stress. In addition, our findings show that Çıldır variety, which is more resistant to HM stress and shows rapid biochemical changes, can also be used for improvement of cultivation areas.

CONFLICT OF INTEREST

The author stated that there are no conflicts of interest regarding the publication of this article.

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