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EFFECT OF CADMIUM IONS ON LIPID PEROXIDATION AND ACTIVITIES OF ANTIOXIDANT ENZYMES OF GROWING WHEAT (*Triticum aestivum L.*) SHOOTS

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The investigation of the effect of $CdCl_2$ different concentrations (20, 50, and 100 μM Cd) on growth of germs of winter wheat (*Triticum aestivum L.)* has been carried out for 12 days. The influence of Cd^{2+} ions on lipid peroxidation and antioxidant system function of wheat shoots cells has been also studied. It has been shown that increasing Cd supply decreased shoots growth (fresh weight) and induced oxidative stress as was indicated by the enhancement of lipid peroxidation rate (MDA-level). Activities of antioxidant enzymes –catalase (CAT) and guiacol peroxidase (GPX) also have been changed under Cd-stress; CAT activity was less affected and even lowered, in case when GPX activity was markedly increased. The role of plant age on formation of biosystem reaction was also discussed in present work. The very high peroxidase activity in wheat shoots, probably may be an important part of Cd-resistance machinery of wheat. The obtained results indicated that Cd-tolerance of wheat may be related with the activation of the antioxidant system to avoiding the toxicity of heavy metal.

Triticum aestivum L. – Cadmium – Antioxidant system – Shoots weight – Catalase – Peroxidase – Plant age

Ուսումնասիրվել է $CdCl_2$ տարբեր կոնցենտրացիաների (20, 50, և 100 մկՄ Cd) ազդեցությունը ցորենի (*Triticum aestivum* L.) ծիլերի աճի, դրանցում լիպիդների պերօքսիդային օքսիդացման և հակաօքսիդանտային ֆերմենտների ակտիվության վրա: Ցույց է տրվել, որ Cd^{2+} առկայությամբ աճեցված բույսերի ծիլերի զանգվածը նվազում է, և աճում է լիպիդների պերօքսիդացման վերջնական արգասիքներից մեկի՝ մալոնային երկալդեհիդի (ՄԵՄ) քանակը: Cd^{2+}-ինդուկցված սթրեսը արտահայտվում է նաև կատալազի ակտիվության նվազմամբ և պերօքսիդազի ակտիվության նշանակալի աճով: Քննարկվում է բույսի տարիքային գործոնի հնարավոր դերը կենսահամակարգի պատասխան ռեակցիայի ձևավորման մեջ: Պերօքսիդազի ակտիվության աճը՝ ի պատասխան ծանր մետաղի ազդեցության, հավանաբար, վերջինիս նկատմամբ բույսի դիմադրության (Cd-ռեզիստենտության) մեխանիզմի կարևոր բաղադրիչը կարող է հանդիսանալ:

Triticum aestivum L. – կադմիում – հակաօքսիդանտային համակարգ – ծիլերի զանգված – կատալազ – պերօքսիդազ – բույսի տարիք

Изучено влияние разных концентраций $CdCl_2$ (20, 50, и 100 мкМ) на рост, интенсивность процессов перекисного окисления липидов и функционирование антиоксидантной системы проростков пшеницы (*Triticum aestivum L.)*. Установлено, что использованные концентрации кадмия ингибируют увеличение биомассы проростков, а также приводят к накоплению малонового диальдегида (МДА) в них. При этом активность

каталазы в проростках в присутствии кадмия снижается, а активность пероксидазы значительно возрастает. Обсуждается вопрос возможной возрастной зависимости ответной реакции растения на воздействие ионов тяжелого металла. Высокая пероксидазная активность, вероятно, может быть частью механизма Cd-толерантности пшеницы.

Triticum aestivum L. – кадмиум – антиоксидантная система – вес проростков – каталаза – пероксидаза – возраст растения

Abiotic stress is the main factor negatively affecting crop growth and productivity world-wide. Plants are continuously confronted with the harsh environmental conditions such as soil salinity, drought, heat, cold, flooding and heavy metal contamination [5, 9, 18, 25].

Cadmium (Cd) is one of the most toxic heavy metals with no biological function, which is commonly released into the arable soil from industrial processes and farming practices [9,20]. Cadmium is well known for its phytotoxicity, which is associated with number of morphological, physiological and biochemical events [2,4, 6, 20]. Being readily taken up by roots, probably in competition with other divalent ions, Cd restricts plant growth and development. It arrests the plant growth and thus affects the biomass [2,10,11].

Heavy metals cause damage to plant growth in many ways. One of the possible mechanisms is that heavy metals lead to the production of free radicals- reactive oxygen species (ROS) in plants [5,10]. Although the mechanism of metal damaging action is not clearly understood, there is increasing evidence suggesting that, at least in part, metal toxicity is due to the oxidative damage [5, 6, 7,10, 11].

Although a lot of reports regarding influence of Cd are available, to our knowledge the mechanisms high plants tolerance to heavy metal-stress remained yet not clearly understood.

Wheat **(***Triticum aestivum L.*) is a crop plant of the Poaceae family. Increased cadmium uptake from contaminated soils leads to altered plant metabolism and limits the crop productivity. Keeping in view the importance of wheat as an important yield crop and the Cd-stress being faced by the crop, the present study was designed to test effects of Cd ions different concentrations on growth (weight), lipid peroxidation (MDA accumulation) and the activity of antioxidant enzymes CAT and guaiacol peroxidase GPX in growing wheat seedlings.

Materials and methods. **Plant Culture and Treatment -**The seeds of winter wheat **(***Triticum aestivum L.*) of "Bezostaya" sort were surface sterilized with 0,03 % potassium permanganate ($KMnO_4$) solution, then moistened by water during 12 hours. These seeds were germinated on wet filter paper in Petri dishes at 25^0 C in thermostat in the dark for 3 to 12 days. $CdCl_2$ treatment was performed in Petri dishes by once adding $CdCl_2$ solution at the concentrations 25, 50 and 100 μM. The etiolated shoots of 3-, 6- and 12 days-old plants both- control and Cd-treated were harvested and then subjected to biochemical analysis.

Extract preparation- The shoots (500mg) of both control and Cd-treated plants were harvested at respective time period and were homogenized in 5ml 25 mM cold phosphate buffer [pH 7,0, containing EDTA (1 mM), Triton X-100 (0,5 %)] in a mortar and pestle. All procedures were performed in cold conditions. The homogenate was centrifuged at 12.000g for 10 min at 4^0 C to remove plant debris. The supernatant was used for assessing the protein content and GPX and CAT activities using UV-visible Spectrophotometer (model SF-46, USSR). All enzymatic activities were measured at 25^0 C.

The method of Lowry [12] was followed to estimate protein content in the shoots using bovine serum albumin as a standard.

Analyses of lipid peroxidation- Lipid peroxidation in shoots was determined by estimation of the MDA content following the method of Costa H. [3] with slight modification. Shoots fresh samples (500 mg) were homogenized in 5 ml of 0,1% trichloroacetic acid (TCA). The homogenate was centrifuged at 10000g for 5 min. To every 1 ml of aliquot, 4 ml of 17% TCA containing 0,5 % thiobarbituric acid (TBA) was added. The mixture was heated at 95^0 C for 20 min and then cooled quickly on ice bath. The resulting mixture was centrifuged at 10000 g for 15 min, and the absorbance of the supernatant was taken at 532 and 600 nm. The nonspecific absorbance at 600 nm was subtracted from the absorbance at 532 nm. The concentration of MDA was calculated using the extinction coefficient 156 mM^{-1} cm^{-1} and expressed as nmol/mg protein.

Catalase CAT (EC 1.11.1.6) activity – The method based on the reaction of the H_2O_2 in a mixture with ammonium molibdate ($(NH_3)_2$ .MoO_4) proposed by Korolyuk M.. [24] was used to estimate CAT activity. The change in optical density due to the emergence of complex H_2O_2 – ammonium molibdate is measured spectrophotometrically at 1-st and 10-th min at 410 nm. The assay mixture contained 1ml Tris-HCl (0,02M, pH 7,4) 2ml H_2O_2 (0,03 %), and 0,1 ml enzyme sample. This was incubated for 10 min at 25^0C in dark, after which the reaction was stopped by adding 1ml of ammonium molibdate (4 %).To the blank 0,1 ml distillated water was added of the zero time of the same assay mixture. A decrease in the absorbance of H_2O_2 within 10 min at 410 nm ($E=22,2 .10^3$ $mM^{-1}cm^{-1}$) was recorded.

The CAT activity was expressed in unit activity (UA). mg^{-1} protein. UA is defined as the change in absorbance by 1 min^{-1} mg^{-1} protein.

Guaiacol peroxidase GPX (EC 1.11.1.7) activity was measured using the method of Hemeda H. [8]. Peroxidase activity was determined in guaiacol oxidation reaction by optical density changing measured of assay mixture every 20 s for 2 min at 25^0C and 450 nm. The reaction started since the moment of injection of 1,0 ml 0,3% H_2O_2 solution into reaction mixture. The reaction mixture contained 1,5 ml of 50 mM potassium phosphate buffer [pH 6,6, containing 0,1mM EDTA], 1ml 1% guaiacol, and 1,0 ml of 0,3 % H_2O_2 and the 0,2 ml enzyme aliquot. Instead of enzyme aliquot 0,2 ml distillated water was add into control sample. The enzyme activity was measured by the increase of absorbance at 450 nm caused by guaiacol oxidation ($E =26,6.10^3$ $mM^{-1}\cdot cm^{-1}$).

All the experiments were performed in triplicates and values presented here are the mean of three values ± standard error.

Results and Discussion. *Triticum aestivum* L. plants response reaction to Cd-stress was determined based on lipid peroxidation system activity changes in germ cells and seedlings biomass. It has been shown that Cd-treatment effects on mass change of germinated seed shoots during their growth.

Effects of $CdCl_2$ supplement on growth of wheat's shoots were carried on samples of 3-, 6-, and 12-days-old plants. Cadmium treatment did not cause visible leaf chlorosis in wheat shoots and also did not cause significant changes in amount of shoots mass (fig. 1) for all – 3, 6- and 12-day-old plants.

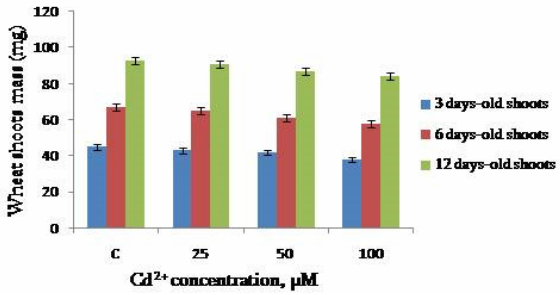

Fig. 1. The mass change of wheat germ shoots treated by $CdCl_2$ solution during their growth

As it is obvious from represented data (fig. 1), among experimental 3-days-old seedlings mass maximum inhibition- 16% was noticed at 100 µM Cd, when 50µM concentration results by 4,5% mass decrease as compared with untreated plants. Similar was the case with 6-days –old plants, grown under Cd metal stress. Their shoots mass was decreased by 9 % and 15 % respectively for supplemented Cd concentrations as compared with control plants of the same age. Besides, the inhibition of seedlings growth intensity shows clear concentration dependence on the amount of the metal ions existing in the environment (fig. 1). According to the obtained data Cd toxicity induces

decreased shoot mass in all seedlings, but in the course of time (6-12 days-old shoots) the growth intensity showed trend for slight increasing.

Reduced shoots length and weight in winter wheat (*Triticum aestivum L.*) and canola *(Brassica napus L.)* plants subjected to cadmium metal toxicity have been reported [10,11,13, 16, 23]. Similar was the observation in lead-stressed wheat seedlings, in which metal application led to an increase in activities of SOD, CAT, APX and MDA in seedling extracts at different concentrations of metall [10, 11].

Malondialdehyde (MDA) level is widely used as the indicator of lipid peroxidation. From the data represented in fig. 2 it is obvious that in untreated seedlings MDA amount changes according to the age of shoots. Thus, approximately the same MDA value was recorded in 3- and 6-days-old shoots. In the case of 12-days-old shoots, MDA level was decreased at 35 % compared to the same parameter in 3-days-old plants.

Obtained data shown, that Cd-treatment results by increase lipid peroxidation process activity, which expressed by MDA content increasing.

So, in cells of 3-days-old shoots growing under 25 μM. and 50 μM. Cd-treatment the MDA amount increases by 1,75- and 1,5 times as compared to control plants. At the same time, in the shoots of 3-days-old plants subjected to 100 μM Cd increasing of MDA amount is not observed, which witnessed that lipid peroxidation intensity does not exceed the appropriate control (fig. 2).

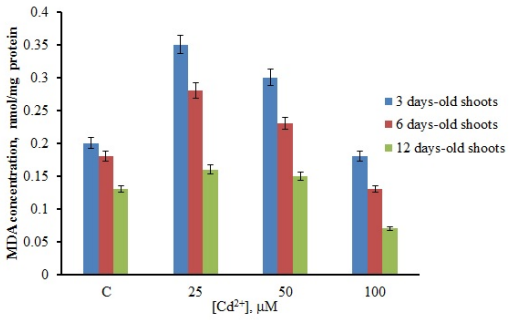

Fig. 2. Effect of Cd treatment on the level of MDA in the shoots of wheat

As it is presented from fig. 2 Cd- treated 6-days-old shoots also demonstrate high level of MDA, but which was lower, than that of 3-days-old shoots. Thus, at presence of 25 μM. and 50 μM $CdCl_2$ in growing environment the MDA level in these shoots increased by 1, 55- and 1, 27- times respectively as compared with control. In the same age shoots grown under 100μM Cd stress MDA amount was 0,13 ± 0,021nmol/mg protein, which was lower than the rate of the respective control.

Comparing obtained results of MDA level and catalase activity in Cd-stressed plant shoots, we can notice that the comparatively high level of MDA in the shoots being in early phase of their growth is correlated to the suppression of catalase activity in the shoots of the same age. In the course of time (6-12 days), data about the increase of the catalase activity correspond well to slowdown of MDA level in the shoots of the same age.

Our obtained data on the increase of lipid peroxidation measued by enhanced Cd supply is in good correlation with data [2, 13, 16].

It was found, that in untreated plants catalase activity was grown according to their age, and in 12-days-old shoots it was for 1,72- times higher than in 3-days-old shoots (fig. 3). The presence of 25μM of cadmium in the environment causes 28% decrease of catalase activity in 3-days-old wheat shoots compared to the control. The further increase of cadmium amount in the environment of growth up to 100μM, causes

decrease in enzyme activity for more than three times compared to the control shoots of the same age (fig. 3). Parallel to the increase in supplemented cadmium concentration, enzyme activity decreases in the tested wheat shoots of the same age. At the same time, the catalase activity showed trend for increasing for each Cd concentration, according to the age of the shoots.

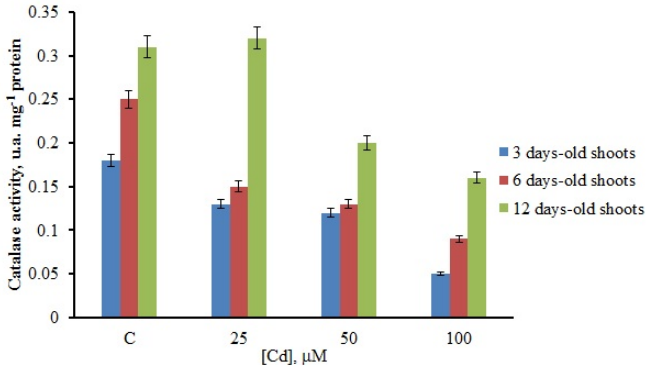

Fig. 3. Effect of Cd treatment on the catalase activity of wheat shoots

GPX activity was found to increase at all Cd concentrations and exposure durations.

As shown in fig. 4, upon Cd expose, there were significant changes in GPX activity in examined seedlings, compare with control. Activity of GPX in 3- and 12-days-old shoots was found to increase at 25 µM Cd, which was by 1,43- and 2,53- times higher than in control, respectively. In the wheat shoots grown in the presence of 50µM and 100 µM of cadmium, too, GPX activity increases compared to control, however, the highest activity was registered in 6-days-old shoots. As a result of analyzing the given data, we can conclude that with the growing age of the wheat shoots the activity of GXP increases. Moreover, with the increase of shoots age, the maximum amount of enzyme transfers to the sector of the lower concentrations of cadmium. The high activities of GPX appear to been involved in effective scavenging ROS generated by Cd treatments.

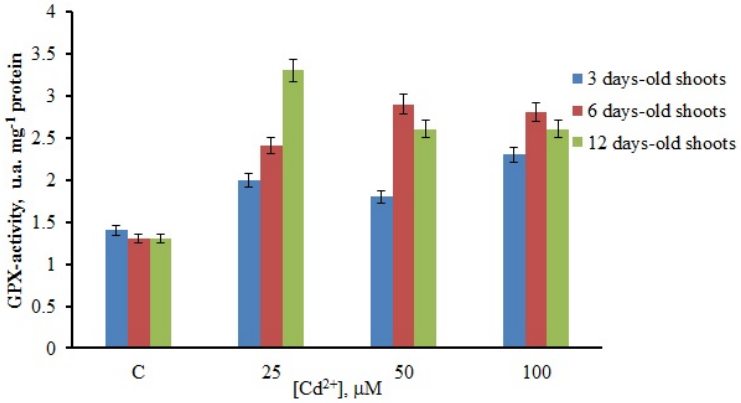

Fig. 4. Effect of Cd-treatment on the GPX guaicol peroxidase activity of wheat.

In plants submitted to metal stress, CAT and GPX act as a defense mechanism which gets activated [2, 13]. The response of antioxidant enzymes differed to Cd-stress in wheat seedlings. Thus GPX activity was distinctly high at all Cd concentrations, whereas CAT activity was decreased (fig. 3, 4). Increase in activities of antioxidant enzymes *viz.* catalase and guaicol-peroxidase under Cd-stress in present study is consistent with the studies [2, 10, 16] in which seeds *Triticum aestivum L*. were grown under $CdCl_2$ stress and antioxidative enzymes activities were found to increase in 3-7-days old shoots.

Similar results are registered in work by Kolesnichenko V. [23] in etiolated wheat and barley 7-14-days-old shoots at high Cd concentrations.

There is little information in literature concerning the relationship between plant's age and its Cd- tolerance. Now it is known that the capability of plants to accumulate cadmium can be changed during the ontogenesis [19, 21]. Particularly, the examination of age effects of cadmium accumulation and its distribution according to plant organs made on barley shoots has made it clear that in more mature plants, the amount of cadmium passing from the root to the stem decreases on account of the intensification of barrier function [21, 22]. Probably, this can explain the obvious decrease of MDA accumulation in wheat shoots related to the plant age.

The decrease of the wheat seedlings biomass, increase in MDA amount and in CAT and GPX activity under cadmium stress in present investigation signifies the toxic effects of this heavy metal. Differences of wheat shoots response to the influence of cadmium can be related to the ontogenetic difference of resistance to the heavy metal, as well as to non-uniform effectiveness of adaptation mechanisms of plants being in different phases of development.

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