EFFECT OF PLANT GROWTH REGULATORS ON CONTENTS OF CD AND RNA IN VEGETATIVE ORGANS OF RED BEET

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Abstract

Cadmium is one of the most dangerous for people's health pollutants with a high degree of phytotoxicity. The aim of this work is to study the possibility of modification of cadmium accumulation as well as its redistribution in vegetative organs of red beet by plant growth regulators (emistim C, polystimulin K). The organ specificity of emistim C and polystimulin K effect on both level and dynamics of Cd accumulation in red beet (*Beta vulgaris* L.) roots and leaves under cadmium pollution has been established. Foliar treatments by emistim C decreased the phytotoxicity of cadmium in leaves where RNA synthesis was restored practically to control level, with a negligible change of cadmium accumulation. In roots, on the contrary, a stable level of RNA and smaller Cd contents were observed. Different influences of plant growth regulators on Cd and RNA contents and a possibility of regulation of this element by emistim C and polystimulin K in roots have been established. The perspective of application of plant growth regulators for modification of cadmium accumulation and its phytotoxicity is under discussion.

Introduction

Cadmium is one of the most toxic heavy metals that are usually found in the polluted environment. This heavy metal occurs in nature mainly as a trace impurity in zinc, lead and copper ores. Cd can be accumulated due to soil genesis from Cd-containing parent materials and as a consequence of human activities (mining, melting and engine combustion). In agricultural soils the main sources of Cd are pesticides and fertilisers or organic (especially sewage sludge) amendments, atmospheric deposition from urban and from industrial activities (1).

Cadmium is easily taken up by plant roots and can have a toxic effect on plant growth, metabolism and gene expression (2). This element is very dangerous for the human health. Cadmium toxicity is considered to be caused owing to a high affinity to SH-containing ligands. The main targets of cadmium in plants are the metalloenzymes, especially Zn-containing, such as RNA- and DNA polymerases, carboanhydrase, alcohol dehydrogenase, and membrane phospholipids (3). The ability of the plants to accumulate Cd is different. It is known that the beet plants can take up relatively high amounts of this element (4).

Under conditions of high environmental pollution by heavy metals it is necessary to decrease their levels in edible parts of plants as well as to increase the accumulation of heavy metals in the shoots to clean up of contaminated soils. In this connection we studied the effect of plant growth regulators which cause the

influence on the plant metabolism at very small concentrations. It is known that both natural and synthetic plant growth regulators as well as cadmium can affect the functioning of plant genome.
The aim of this work is to study the influence of cadmium on the accumulation of this heavy metal and RNA contents in organs of red beet plants, and the possibility of its regulation by growth regulators. The influence of polystimulin K (6-BAP), emistim C (substance obtained from the culture of rhizosphere microorganisms of ginseng and other plants) was evaluated.

Methods

Greenhouse pot culture experiments were conducted, where cadmium was added in the soil as sulphate (10 mg/kg). The plants were sprayed by the solutions of polystimulin K (10^{-4} M) and emistim C (1:30000) twice during a vegetation on the 43^{rd} and 83^{rd} days after their germination. Cd content was determinated in the ashes of roots and leaves by atomic absorption spectrophotometry (Saturn, Ukraine) (5).
RNA contents in organs of red beet plants was determinated as described by Nagy et al. (6). Total RNA was prepared from plant tissue by grinding 1 g of frozen tissue in liquid nitrogen using a mortar and pestle and extracting with 5 mL of buffer consisting of 300 mM NaCl, 50 mM Tris-HCl (pH 8,0), 5 mM EDTA (pH 8,0), 2 % (w/v) SDS, 10 mM β-mercaptoethanol, 1 mM ATA. After the incubation of the tube at 50 ^{o}C water bath, 0,7 mL 3 M KCl was added, the slurry was diluted with 2,5 ml of 8 M LiCl, and RNA was precipitated. The sample was centrifuged for 15 min at 6000 g, the pellet was dissolved in 1.0 mL of sterile, distilled water. Residual proteins were extracted from the aqueous phase, and RNA was precipitated with ethanol after NaCl addition. The pellet was washed twice with 70 % ethanol, was dried and RNA was dissolved in sterile water. OD was measured at 260 nm on Specord M 40 spectrophotometer (Carl Zeiss Jena, Germany) to determine RNA concentration. The data obtained were analysed statistically.

Results

Data in fig. 1 and 2 indicate a high Cd content in vegetative organs of red beet plants on soil with 10 mg/kg Cd. In the long-term experiments cadmium accumulation in roots and leaves of *Beta vulgaris* L. plants was different. This heavy metal was accumulated mainly in the leaves (5-25 times more than in the roots). Furthermore, there is the difference in dynamics of the cadmium accumulation between these organs: the increase of Cd quantity in leaves and the decrease in roots during ontogenesis. The results demonstrate the variability of Cd accumulation in the red beet plants.
Cadmium caused the decrease of the RNA contents in leaves of red beet plants by 25-30 % (Fig. 3). It has been established that the foliar treatment by the above mentioned plant growth regulators, especially by emistim C restored the RNA contents almost to control level. The quantity of RNA under the combined polystimulin K and Cd treatment was higher than that of only Cd treatment. Nevertheless, the effect of this plant growth regulator was less than that of emistim C.

Figure 1: Influence of plant growth regulators on Cd contents in leaves of red beet

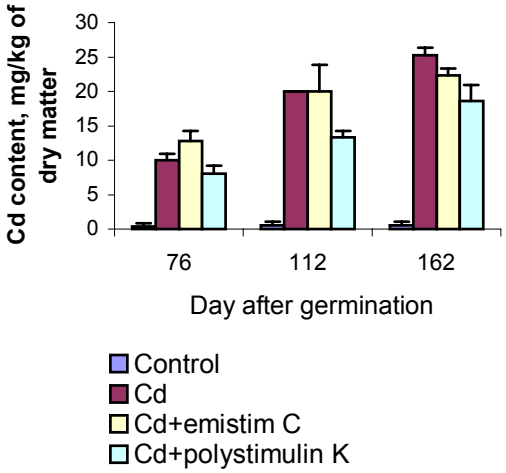
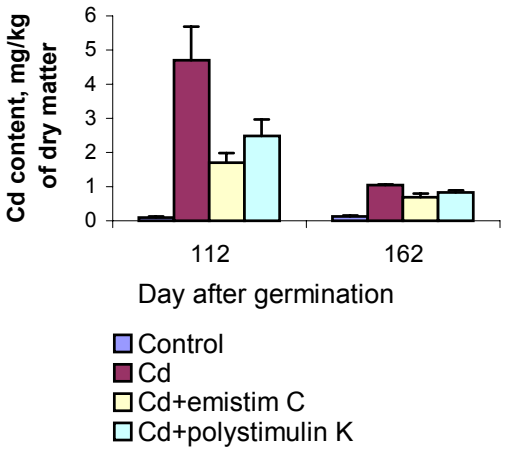


Figure 2: Influence of plant growth regulators on Cd content in roots of red beet



RNA level decreased under cadmium application not only in the leaves, but also in the roots of the red beet plants (Fig. 4). At the end of vegetative period the level of RNA in the roots of red beet was lower by 18 % than in the control plants. In this case the foliar treatment of plants by emistim C did not affect the RNA level.

Figure 3: Effect of Cd and plant growth regulators on the RNA content in the leaves of red beet

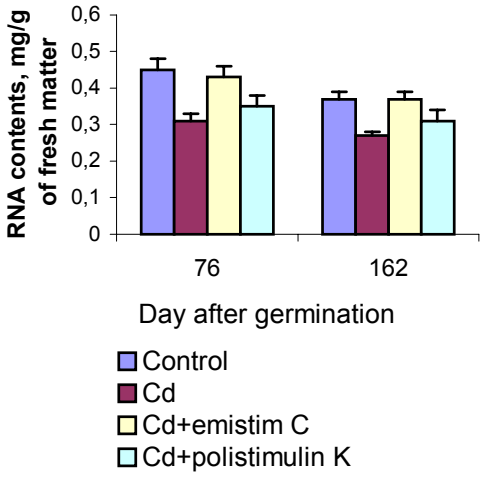
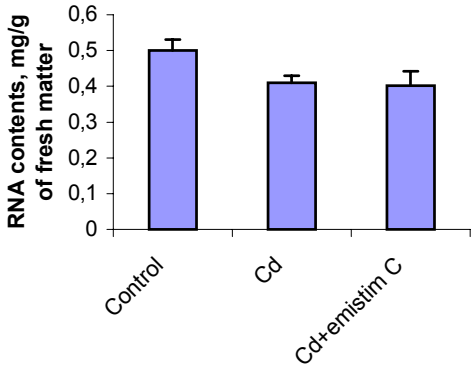


Figure 4: Effect of Cd and emistim C on RNA contents in the red beet roots at the end of growth period



Discussion

In this paper the effects of Cd application to soil on Cd accumulation and RNA content were examined. In the course of this investigation we have established that Cd is easily absorbed by plant roots taking into account a sufficiently high level of this element in vegetative organs of *Beta vulgaris* L. There was a significant difference between accumulation of Cd in the roots and the leaves, the level of Cd in the above ground organs of red beet was greater than that in the roots. The concentration of Cd in the leaves exceeded 20 mg/kg of dry matter, it allows us to consider the red beet to be a plant with a high ability to accumulate Cd (7). Literature data evidence that concentrations of both Pb and Cd in non-edible parts of various vegetable plants (e.g. leaves of cauliflower, cabbage, turnip, carrot and corn) were almost always higher than those in corresponding edible parts (8). This type of the distribution within plants are reported in a number of studies (9, 10), although some studies show that these metals can be distributed differently, often in response to different environmental or management conditions (11,12).

The foliar treatments of plants by emistim C had a different effect on Cd content in the vegetative organs of red beet plants. Its influence on Cd accumulation in the leaves was smaller than in the roots. The decreasing of Cd content in the roots has been found, moreover, this effect was more pronounced on the 112^{th} day after germination, than on the 162^{nd} day. The decreasing of Cd accumulation in roots under growth regulator treatment, especially under application of emistim C, could be a consequence of a “dilution” effect due to increasing the root masses, but it is more likely that the changes of Cd levels were caused by the restriction of translocation of this element to storage organs.

The quantitative and qualitative changes of gene expression of different plants as a consequence of a gene regulation at the transcriptional and post-transcriptional levels under Cd application have been established (13, 14). To study the effect of plant growth substances on the functioning of genome of red beet plants it was expedient to determinate such general indices as RNA content in cells. Cd caused the decreasing of RNA content in the leaves by 25-30 %. Foliar treatment by plant growth regulator emistim C restored it practically to control level. Polystimulin K increased RNA content too, however its effect was less pronounced in comparison with emistim C. In the roots, like in the leaves, Cd decreased the RNA content also, but in this case foliar treatment by emistim C didn’t affect the quantity of RNA. This may be caused by the manner of growth regulator application during vegetation.

In general, the increasing of RNA level as affected by plant growth regulators evidences a decreasing of Cd phytotoxicity, probably, as a consequence of its sequestration from cytoplasm and translocation to vacuole. Another possibility of Cd detoxification is its binding by phytochelatins (15, 16).

It should be noticed that the RNA level is a general index of genome expression, and its reducing as affected by Cd doesn’t contradict to the literature data concerning an increasing level of expression of individual genes such as glutathion-S-transferase, enzymes of phytochelatin synthesis (14). Furthermore, the effective Cd detoxification under the influence of plant growth regulators may be a consequence of the shift in phytohormonal status which lead to modification of gene expression taking part in detoxification process, and ultimately to a decrease of Cd accumulation in vegetative organs of red beet plants.

Conclusions

The organ-specificity of plant growth regulators (emistim C and polystimulin K) effect on Cd accumulation as well as RNA content in red beet (*Beta vulgaris* L.) roots and leaves is shown. Foliar treatments by these plant growth regulators decreased the Cd phytotoxicity in leaves, however only polystimulin K reduced Cd content in this organ. At the same time emistim C is more effective for reducing of Cd accumulation in roots. The possibility of application of plant growth substances for modification of cadmium accumulation and its phytotoxicity is shown.

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