PERIODICA POLYTECHNICA SER. CHEM. ENG. VOL. 46, NO. 1–2, PP. 65–71 (2002)

EFFECT OF CADMIUM STRESS ON AMINO ACID AND POLYAMINE CONTENT OF WHEAT SEEDLINGS

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Received: March 30, 2003

Abstract

The effect of cadmium and combined cadmium and titanium-ascorbate or magnesium was examined on wheat seedlings (*Triticum aestivum* L. cv. Alföld-90). The one-week-old hydroponically-grown seedlings of wheat were exposed to Cd^{2+}(10^{-7} M, 10^{-3} M), Cd^{2+}(10^{-7} M, 10^{-3} M) + titanium-ascorbate (5μg L^{-1}) and Cd^{2+}(10^{-7} M, 10^{-3} M) + 1 % $MgCl_2$ stresses. The stress effects were followed by changes in free amino acid and polyamine contents in roots and shoots. The results showed significant differences between the two organs of the plant (root and shoot) with respect to the biochemical response to the applied stress. The cadmium stress caused the accumulation of proline in both shoots and roots. Both titanium-ascorbate and magnesium treatments seemed to reduce the negative effects of cadmium damage in wheat seedlings.

Keywords: wheat, cadmium, stress, magnesium, titanium, amino acid, polyamine.

1. Introduction

One of the most serious environmental stresses is the harmful effect of heavy metals. Toxic metal stress affects various physiological and biochemical processes through modifying many metabolic pathways [1, 2]. Cadmium is considered to be one of the most dangerous heavy metals, its toxic effect on plants and animals is well known [3, 4]. Plants often accumulate a huge quantity of cadmium without poisoning symptoms, which entering the food chain endangers human health as well.

Numerous biologically active molecules can indicate stress. The components examined most often are stress enzymes (e.g. superoxide dismutase, catalase, peroxidases), heat shock proteins, amino acids, polyamines, phytoalexines and carbohydrates.

The beneficial physiological role of titanium was proved in plants [5]. Titanium-ascorbate supports absorption of important nutrient elements, this fact can explain its favourable physiological effect. Latest researches suggest that first of all in physiological processes of plants the stress effects resulted in the presence of different toxic heavy metals (Pb, Cd, Hg) can be reduced by titanium-ascorbate treatment [6].

Magnesium plays an important role in many biological processes, e.g. photosynthesis. Usually this element increases the activity of enzymes. Magnesium stimulates protein synthesis through its effect on the enzymes [7].

Among amino acids, proline responds most sensitively to stress conditions [8]. Proline accumulation in plants can serve as biomarker of heavy metal stress. Polyamine accumulation was investigated in a wide variety of stress conditions, e.g. lack of K, Ca and Mg [9], low pH stress, osmotic stress, salt stress, cadmium toxicity [10], aluminium toxicity and ozone stress.

During the experiments the effect of cadmium was studied in wheat shoots and roots and the modifying effects of titanium-ascorbate and magnesium were investigated in the changes caused by cadmium in biochemical parameters (amino acid and polyamine content).

2. Materials and Methods

2.1. Plant Samples and Treatment

Seeds of wheat (*Triticum aestivum* L.) were swollen in distilled water for 24 hours. Then the seeds were placed into incomplete Knopp solution [11] for a week. 300–300 seedlings were grown for each treatment. The one-week-old hydroponically grown seedlings were exposed to the following treatments for 24 hours, at 25°C [12, 13]:

- 10^{-7} M Cd^{2+}
- 10^{-3} M Cd^{2+}
- 10^{-7} M Cd^{2+} + 5 μg L^{-1} titanium (IV)-ascorbate
- 10^{-3} M Cd^{2+} + 5 μg L^{-1} titanium (IV)-ascorbate
- 10^{-7} M Cd^{2+} + 1 % magnesium (II)-chloride
- 10^{-3} M Cd^{2+} + 1 % magnesium (II)-chloride

During this time the control seedlings were grown in incomplete Knopp solution.

After the treatment the nine-days-old seedlings were rinsed with distilled water and dried immediately with paper filter. 300 plants for each treatment were separated into roots and shoots, then the plant organs were homogenised with liquid nitrogen. The average samples were taken from the homogeneous plant samples for the analyses.

2.2. Free Amino Acid Analysis

200 mg homogeneous fresh weight samples were shaken in 2 cm^3 7% trichloroacetic acid for one hour, then they were filtrated by paper filter and membrane filter (0.45 μm). The analysis was carried out using BIOTRONIK LC 3000 amino acid analyser [14].

2.3. Polyamine Identification by Overpressured Layer Chromatography

Dansylated derivatives of polyamines were identified on HPTLC silica gel 60 F_{254} (Merck Co.) [15]. Dansyl polyamines were analysed by overpressured layer chromatographic separation (OPLC Chromatograph, OPLC-NIT Co. Ltd., Budapest, Hungary) with stepwise gradient elution. Quantitative evaluation of the dansyl amines was accomplished at $\lambda = 313$ nm by means of a Shimadzu CS-930 TLC/HPTLC scanner (Shimadzu Co., Kyoto, Japan) [16].

3. Results and Discussion

3.1. Free Amino Acid Content

Total free amino acid content ranged from 1302 μg g^{-1} to 2528 μg g^{-1} in root and from 2277 μg g^{-1} to 3422 μg g^{-1} in shoot samples. It was found that the total free amino acid content of the shoots was higher than that of the roots in all samples (*Fig. 1*). The ratio of total amino acid content in shoot samples showed 1.5 times increase at the higher Cd^{2+} concentration (10^{-3} M) compared to the control samples. Cadmium treatment at the higher concentration caused the highest accumulation of total amino acid content in both the shoots and roots.

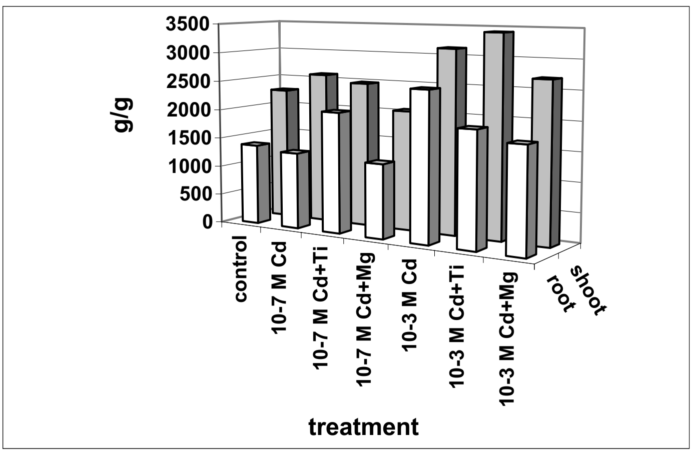

Fig. 1. Total amino acid content of wheat seedlings

Among the free amino acids the threonine, glutamic acid, proline, alanine, valine, isoleucine, leucine, histidine, lysine, arginine, serine, asparagine and glutamine concentrations showed the most significant changes. Based on the typical accumulations of free amino acids in the two organs of wheat seedlings the different

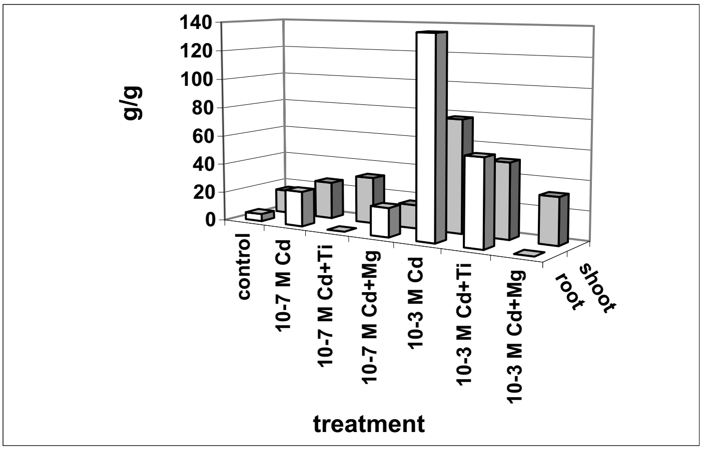

Fig. 2. Proline content of wheat seedlings

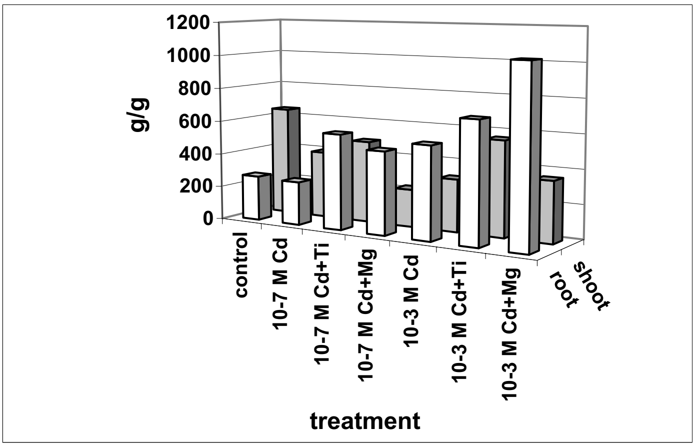

Fig. 3. Total polyamine content of wheat seedlings

effects of the cadmium and the combined cadmium + titanium-ascorbate treatment can be characterised.

Proline (Pro) content showed the highest increase in root at the 10^{-3}M Cd^{2+} treatment (27.5 times), while in shoot Pro content increased 4.8 times. In the case of 10^{-7}M Cd^{2+} + titanium-ascorbate treatment Pro could not be detected in root and Pro content in shoot showed a slight increase (2 times) compared to the control (*Fig. 2*). Thus Pro indicated the possible protective effect of titanium-ascorbate.

The applied 1% Mg^{2+} concentration decreased the well known stress marker proline accumulation in both parts of the plant. This trend was most effective at the

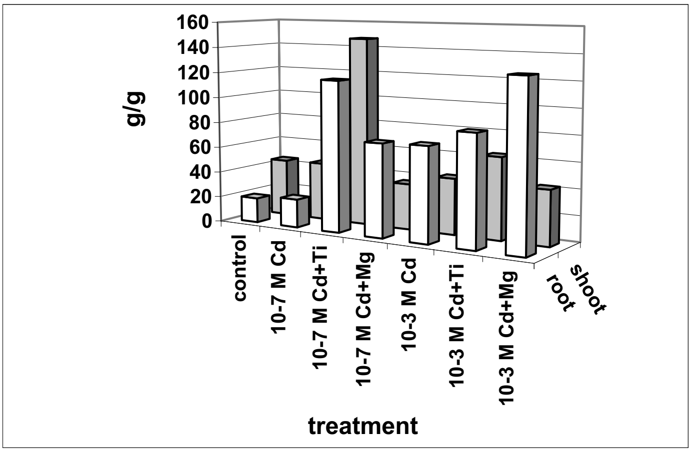

Fig. 4. Putrescine content of wheat seedlings

higher Cd^{2+} concentration combined with 1% Mg^{2+}. Toxic effects of Cd^{2+} were decreased by Mg^{2+}. Proline concentration significantly decreased and glutamic acid concentration increased after combined Cd^{2+} and Mg^{2+} (1%) stresses. In contrast to proline, glutamic acid content increased in shoots in the presence of magnesium.

3.2. Polyamine Content

Total polyamine content was 2.4 times higher in the control shoot than in the control root sample (*Fig.* 3).

In root only the cadmium treatment at the higher concentration (10^{-3} M) increased the total polyamine content (2.1 times) compared to the control.

Agmatine (Agm), putrescine (Put), spermine (Spm), spermidine (Spd), cadaverine (Cad) and tyramine (Tym) polyamines were found in wheat samples. Agm was detected in the highest concentration. Spd showed the most considerable change of concentration.

Among polyamines Put is considered as a stress marker in plants (*Fig.*4). According to our results Agm seems to play an important role in the formation of Put in wheat seedlings.

Spd concentration showed the greatest change in both organs of wheat. After the 10^{-7}M Cd^{2+} treatment Spd concentration in shoot highly increased (9.1 times). In the case of 10^{-3}M Cd^{2+} + Ti-ascorbate treatment, in root (9.8 times) and in shoot (9.9 times) as well, very high values were measured compared to the control.

Based on the typical accumulation of polyamines the different effect of cadmium and the combined cadmium and titanium-ascorbate treatments can be characterised in both organs of wheat seedlings.

The major component was agmatine in the control shoot while that was putrescine in the shoot sample treated with Cd^{2+} (10^{-7} M) combined with titanium-ascorbate. In the control shoot samples beside the increase of Agm concentration, Put concentration decreased, while in contrast to that, in the case of the shoot treated with Cd^{2+} (10^{-7} M) combined with titanium-ascorbate agmatine concentration decreased and Put concentration increased.

Magnesium treatment caused remarkable increases both in 10^{-3} M Cd^{2+} (3.9 times) and in 10^{-7} M Cd^{2+} (1.8 times) treated root samples in total polyamine content. In contrast, magnesium reduced the total polyamine content in shoot samples.

Both cadmium treatments combined with magnesium increased agmatine concentration in root samples. 1.6 times and 2.9 times increases were detected in samples 10^{-7} M Cd^{2+} + Mg^{2+} and 10^{-3} M Cd^{2+} + Mg^{2+}, respectively, compared to the control sample.

Agmatine content decreased in all samples of shoot. Considerable increases were detected in root samples in the case of putrescine (10^{-3} M Cd^{2+} 3.8 times; 10^{-7} M Cd^{2+} + Mg^{2+} 3.7 times; 10^{-3} M Cd^{2+} + Mg^{2+} 6.6 times) and spermidine (10^{-7} M Cd^{2+} 8.1 times; 10^{-3} M Cd^{2+} + Mg^{2+} 35.2 times).

In shoot samples spermidine concentration increased 9.1 times in 10^{-7} M Cd^{2+} treatment and 6.7 times after the 10^{-3} M Cd^{2+} + Mg^{2+} treatment compared to the control.

4. Conclusion

Summarising our results it can be concluded that the heavy metal stress caused typical biochemical changes in wheat seedlings concerning contents of amino acids and polyamines. The differences between the two organs (shoot and root) are due to the different transport processes and their different biological pathway. Titanium-ascorbate treatment seemed to reduce the negative effects of cadmium pollution in wheat seedlings. Further research is needed to find out the relationship between the cadmium stress and these biochemical changes in wheat seedlings and also to prove the beneficial role of titanium-ascorbate.

Acknowledgement

This research was supported by a grant from Hungarian National Scientific Research Fund (OTKA T 030 165).

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