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Effect of magnesium on free amino acid and polyamine content in wheat seedling exposed to cadmium stress

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ABSTRACT The effect of cadmium and combined cadmium and magnesium was examined on wheat seedlings (*Triticum aestivum* L. cv. Alföld-90). The one-week-old hydroponically-grown wheat seedlings were exposed to Cd^{2+} (10^{-7} M, 10^{-3} M) and Cd^{2+} (10^{-7} M, 10^{-3} M) + 1 % $MgCl_2$ stresses. Free amino acid content was determined by ion-exchange liquid chromatography. Polyamine content was identified by OPLC (overpressured layer chromatography). The results showed significant differences between the two organs of the plant (root and shoot). The cadmium stress caused typical accumulation of proline in both shoots and roots. Whereas in the samples treated with combined cadmium and magnesium proline content decreased. The magnesium treatment seemed to reduce the negative effects of cadmium in wheat seedlings. With respect to the total free polyamine content, considerable decrease in shoots and increase in roots were found under stress conditions. The major components were agmatine, putrescine and spermidine. Organ-specific changes were found in the case of agmatine.
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KEY WORDS
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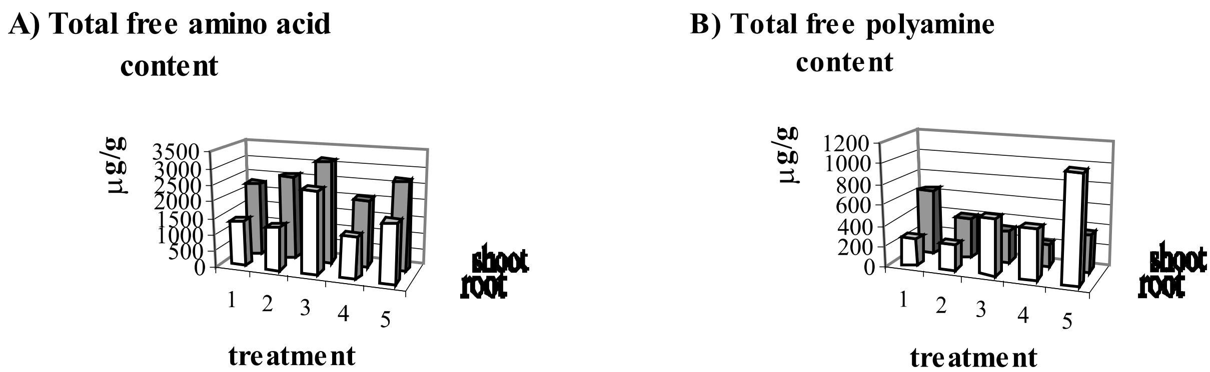
Heavy metal stress affects many physiological and biochemical processes in plants resulting in the alteration of some metabolic pathways (Van Asschee and Clijster 1990). Cadmium is one of the most dangerous heavy metals. Cadmium from various sources of pollution accumulates in soil and it is taken up by plants (Ernst 1980) and indicates abiotic stress in the plants. Magnesium plays 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 (Balla and A. Kiss 1996).

The aim of our study was to characterise the adaptive processes of wheat seedlings under Cd^{2+} stress. Furthermore, some effects of Mg^{2+} on Cd^{2+} treatments were also investigated.

Materials and Methods

Plant samples and treatment

The seeds of wheat (*Triticum aestivum* L. cv. Alföld-90) were swollen in distilled water for 24 hours. Then the seeds were placed into Knopp-solution (Suba, 1978) for one week. The one-week-old seedlings were exposed to Cd^{2+} (10^{-7} M; 10^{-3} M) and Cd^{2+} (10^{-7} M; 10^{-3} M) + Mg^{2+} (1%) stresses for 24 h. The control was in Knopp-solution for 24 hours without any treatment. The 24 h treatment was carried out according to our previous experiments (Stefanovits-Bányai et al., 2001, Leskó et al. 2001). 300 plants for each treatment were separated into shoots and roots, then the plant organs were homogenised with liquid nitrogen. The average samples were taken from the homogenous plant samples for the analyses.



1: control **2: 10^{-7} M Cd^{2+}** **3: 10^{-3} M Cd^{2+}** **4: 10^{-7} M Cd^{2+} + 1 % Mg^{2+}** **5: 10^{-3} M Cd^{2+} + 1 % Mg^{2+}**

Figure 1. Free amino acid and polyamine content in treated wheat samples.

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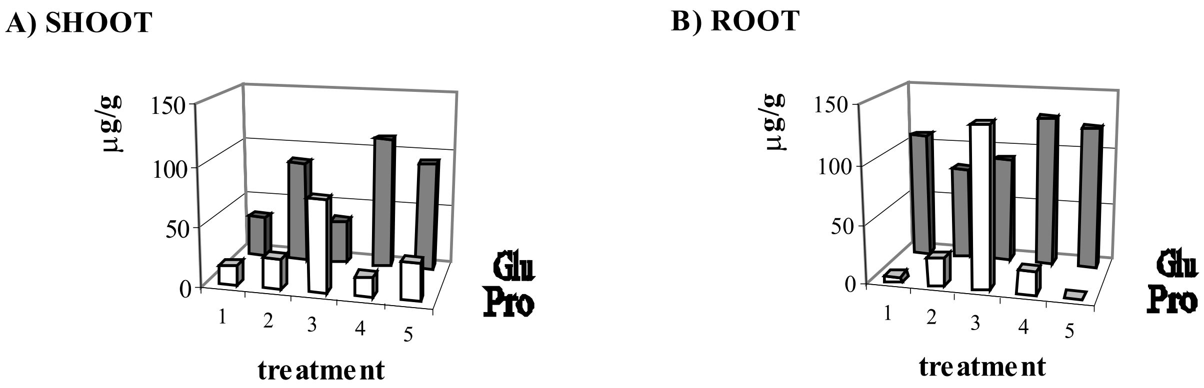

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Figure 2. Glutamic acid and proline content in treated wheat samples.

Only one chromatographic analysis for each sample was possible to perform. The standard deviation of the methods was 5%.

Free amino acid analysis

200 mg fresh weight samples were shaken in 2 cm^3 7% trichloroacetic acid for one hour, then they were filtrate by paper filter and membrane filter (0.45 µm). The analysis was carried out using BIOTRONIK LC 3000 amino acid analyser (Galiba et al. 1992).

Polyamine analysis

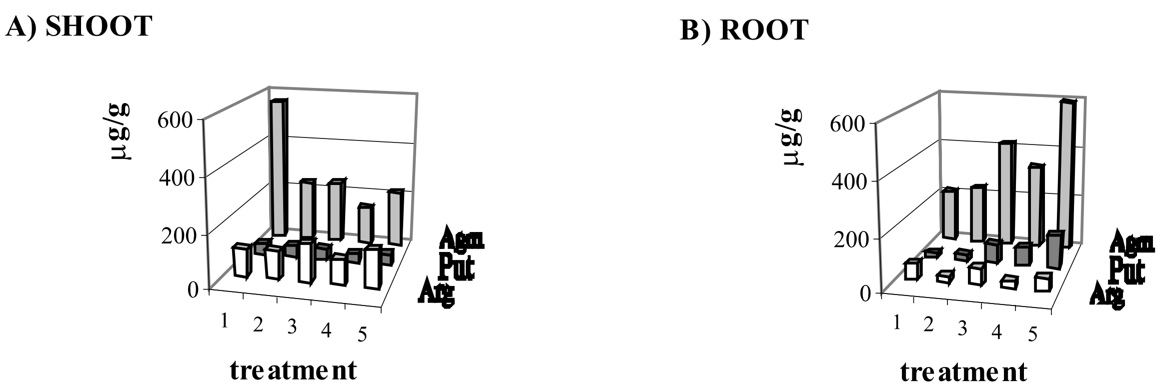
Dansylated derivatives of polyamines were identified on HPTLC silica gel 60 F_{254} (Merck Co.) (Simon-Sarkadi and Galiba, 1988). Dansyl polyamines were analysed by over-pressured layer chromatographic separation (OPLC Chromatograph, OPLC-NIT Co., Ltd., Budapest, Hungary) with stepwise gradient elution. Quantitative evaluation of the dansyl amines was accomplished at $\lambda = 313$ nm by mean of a Shimadzu CS-930 TLC/HPTLC scanner (Shimadzu Co., Kyoto, Japan) (Kovács et al. 1998).

Results and Discussion

Amino acid content

All applied concentrations of cadmium caused considerable changes in free amino acid content in wheat seedlings. The cadmium treatment at higher concentration (10^{-3} M Cd^{2+}) caused the highest accumulation in total free amino acid content (Fig. 1/A).

The total free amino acid content ranged from 1260 µg/g to 2530 µg/g in root and from 2050 µg/g to 3140 µg/g in shoot samples. The major amino acids were aspartic acid, alanine, valine, lysine, histidine, arginine in control samples. After Cd^{2+} treatment the highest accumulation were observed in the well known stress marker proline in root (at 10^{-3} M Cd^{2+} 28 times) and in shoot (at 10^{-7} M Cd^{2+} 5 times) samples (Fig. 2). While glutamic acid concentration slightly decreased in roots. The applied 1% Mg^{2+} concentration



1: control **2: 10^{-7} M Cd^{2+}** **3: 10^{-3} M Cd^{2+}** **4: 10^{-7} M Cd^{2+} + 1 % Mg^{2+}** **5: 10^{-3} M Cd^{2+} + 1 % Mg^{2+}**

Figure 3. Agmatine, putrescine and arginine content in treated wheat samples.

decreased proline accumulation in both parts of the plant. This trend was most effective at the highest 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.

Polyamine content

Total free polyamine content was 2.4 times higher in control shoot than in control root sample (Fig. 1/B).

In shoot 10^{-7} M Cd^{2+} and 10^{-3} M Cd^{2+} treatments reduced total free polyamine content 0.6 and 0.5 times, respectively, compared to the control.

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

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 free polyamine content. In contrast magnesium reduced polyamine content in shoot samples.

The major polyamines found in wheat seedlings were agmatine (Agm), putrescine (Put) and spermidine (Spd).

Agmatine content decreased in all samples of shoot (Fig. 3/A). Considerably increases were detected in root (Fig. 3/B) 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).

Both cadmium treatments combined with magnesium increased agmatine concentration in root samples (Fig. 3/B). 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.

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.

Putrescine and the precursor arginine accumulated at the higher cadmium concentration in both organs. Organ-specific changes were found in the case of agmatine. Agmatine increased at the higher cadmium concentration in root, while considerably decrease was observed in shoots.

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