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BRIEF COMMUNICATION

Phytochelatin synthesis in maize seedlings in response to excess zinc

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Abstract

Buthionine sulfoximine (BSO) specifically inhibits γ-glutamylcysteine synthetase and decreases a cellular level of glutathione (GSH) in maize seedling roots. Exogenous GSH restores Zn-phytochelatins synthesis in BSO-treated maize plants.

Additional key words: Glutathione, *Zea mays*, zinc stress.

In higher plants, tripeptide glutathione (γ-ECG) is involved in storage and distribution of reduced sulfur in the regulation of sulfur nutrition and is also an essential component of the plant defence system against environmental stresses (heavy metal stress, xenobiotic stress, oxidative stress, drought and temperature stress and pathogen attack).

Higher plants, algae and some fungi, when exposed to heavy metals, also synthesize γ-glutamyl-cysteinyl-glycines $(\gamma\text{-EC})_n$Gs which were firstly identified by Grill *et al.* (1985). Other γ-EC isopeptides [$(\gamma\text{-EC})_n$, $(\gamma\text{-EC})_n$S, $(\gamma\text{-EC})_n$-β-A, $(\gamma\text{-EC})_n$E] are also involved in metal chelation and metal tolerance in the plant kingdom (Grill *et al.* 1986, Klapheck *et al.* 1992, Meuwly *et al.* 1993).

In higher plant cells glutathione is synthesized in two steps by the action of γ-EC synthetase (EC 6.3.2.2) which binds cysteine with glutamic acid, followed by γ-ECG synthetase (EC 6.3.2.3) which adds glycine to γ-EC and forms γ-ECG (Rennenberg 1982).

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Abbreviations: BSO - buthionine sulfoximine, (γ-ECG) - glutathione or GSH, $(\gamma\text{-EC})_n$G - (γ-glutamyl-cysteinyl)-glycines or phytochelatins or PCs, $(\gamma\text{-EC})_n$ - (γ-glutamyl-cysteines), $(\gamma\text{-EC})_n$S - (γ-glutamyl-cysteinyl)-serines, $(\gamma\text{-EC})_n$-βA - (γ-glutamyl-cysteinyl)-β-alanines, $(\gamma\text{-EC})_n$E - (γ-glutamyl-cysteinyl)-glutamic acid.

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A specific inhibitor of the enzyme catalyzing the first step of glutathione biosynthesis is buthionine sulfoximine (BSO). In plant cells, BSO has been shown to reduce glutathione levels, although the precise mode of action of this inhibitor in plants has not been determined. This inhibition can be partially overcome by supplying exogenous glutathione (Scheller *et al.* 1987, Mendum *et al.* 1990).

The purpose of the experiments described in the present work was to examine the role of glutathione in $(\gamma\text{-EC})_n$Gs synthesis in maize seedling roots.

Maize seeds (*Zea mays* L. cv. Hidosil) were germinated in a moist paper towel for 3 d. The seedlings were transferred into pots containing aerated Hoagland nutrient solution and cultivated for 7 d at temperature of 23 °C , air humidity of 95 % and 16 h photoperiod (irradiance 100 μmol m^{-2} s^{-1}). The following groups of seedlings were analyzed: 7 d control nutrient solution, 5 d control and 2 d nutrient solution with 500 μM Zn^{2+} as $ZnSO_4$, 7 d nutrient solution with 1 mM BSO, 5 d with 1 mM BSO and 2 d with 500 μM Zn^{2+}, 5 d with 1 mM BSO and 2 d with 500 μM Zn^{2+} + 1 mM GSH.

10 root apical segments of intact seedlings (length 1 cm) were cut off and homogenized in ice with 5 % sulfosalicylic acid. Homogenates were centrifuged at 10 000 *g* for 5 min at 4 °C, filtered over 0,45-μm organic filter (*Cole-Parmer*) and used for GSH and PCs separation by HPLC method.

A liquid chromatograph (*Beckman model 126/166*) with *Nucleosil C-18*, 5 μm (4.6 × 250 mm) column was used for analysis of samples. The flow rate through the column was 16.66 mm^3 s^{-1}. The column was equilibrated with 0.1 % trifluoroacetic acid (TFA) in water and after injecting a sample the column was developed with a linear gradient from 0 to 20 % acetonitrile in 0.1 % TFA during 36 min. The column effluent was derivatized with 200 μM Ellman's reagent (DTNB) which was added at a flow rate of 8.33 mm^3 s^{-1} to a 10 mm^3 mixing chamber. The absorbance of derivatized material was measured at 405 nm.

The root growth of maize seedlings exposed to 1 mM BSO for 7 d was not significant different from control seedlings. The presence of 500 μM Zn^{2+} in nutrient solution did not inhibit growth of roots, but growth of roots was inhibited by 20 % when 1 mM BSO and 500 μM Zn^{2+} were added simultaneously.

Root extracts of maize control seedlings contained 320 μmol kg^{-1} (f.m.) of glutathione (Fig. 1A), but when treated with 1 mM BSO for 7 d their glutathione level decreased to 35 μmol kg^{-1} (f.m.) (Fig. 1C). During 48-h growth of seedlings in the presence of 500 μM Zn^{2+} new thiol peaks appeared (Fig. 1B). They were eluted at the same retention time as those induced by Cd^{2+} ions in maize seedling roots, which were identified earlier as phytochelatins (Tukendorf 1993). The level of glutathione decreased by 57 % in comparison with control. Earlier treatment of seedlings with 1 mM BSO and then with 500 μM Zn inhibited almost completely the synthesis of phytochelatins (Fig. 1D). This experiment demonstrated that glutathione is an essential factor in Zn-phytochelatin synthesis. However, Zn-PCs were found present in roots of maize seedling previously treated with 1 mM BSO if 500 μM Zn was simultaneously introduced with 1 mM GSH into their nutrient solution (Fig. 1E).

Exogenous glutathione is taken up by roots of maize seedlings and restores the ability of plants treated with BSO to synthesize phytochelatins under zinc excess stress.

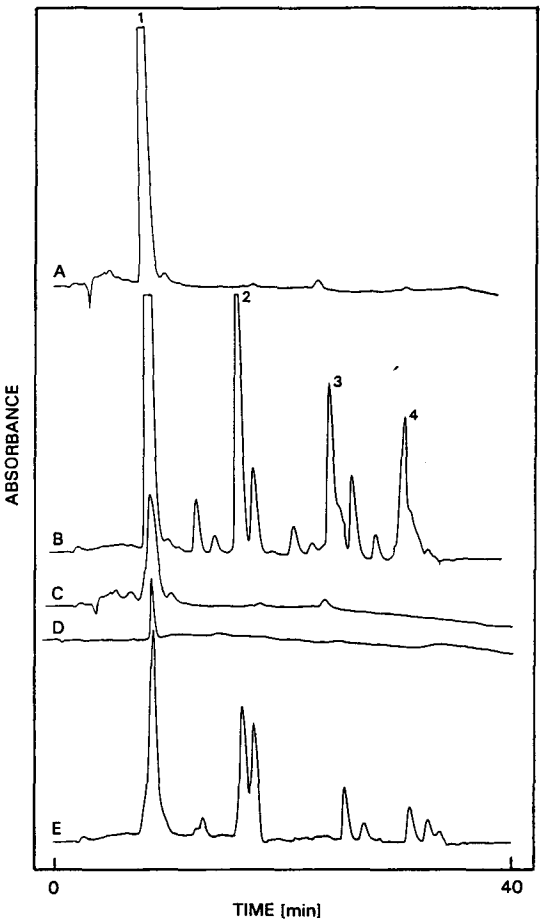

Fig. 1. HPLC profiles of thiols in crude extracts of maize roots exposed to: *A* - control, *B* - 500 μM Zn, *C* - 1 mM BSO, *D* - 1 mM BSO + 500 μM Zn, *E* - 1 mM BSO + 500 μM Zn + 1 mM GSH. The identified peaks are: *1* - GSH, *2* - PC_2, *3* - PC_3, *4* - PC_4.

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