Phyton (Horn, Austria) Special issue: "Sulfur-Metabolism"	**Vol. 32**	Fasc. 3	(109)-(112)	18. 12. 1992

Effect of Cadmium and/or Removal of Kernels or Shoots on the Levels of Cysteine, γ-Glutamyl-cysteine, Glutathione, and TCA-soluble Thiols in Maize Seedlings

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K e y w o r d s : *Zea mays*, cadmium, cysteine, γ-glutamyl-cysteine, glutathione, phytochelatins.

S u m m a r y

RÜEGSEGGER A. & BRUNOLD CH. 1992. Effect of cadmium and/or removal of kernels or shoots on the levels of cysteine, γ-glutamyl-cysteine, glutathione, and TCA-soluble thiols in maize seedlings. - Phyton (Horn, Austria) 32 (3): (109)-(112).

Six day old maize seedlings (*Zea mays* L.) were exposed as intact plants (A), after the removal of kernels (B) or shoots (C) or after the removal of kernels and transfer into the dark (D) to 0 or 50 micromolar cadmium for 2 days. The roots were analyzed for fresh weight, total TCA-soluble thiols (including phytochelatins), glutathione, and its precursor compounds cysteine and γ-glutamyl-cysteine. With all treatments, cadmium caused an increase in the contents of cysteine, γ-glutamyl-cysteine and total TCA-soluble thiols and a decrease in glutathione content. Our data indicate that the roots are at least in part autonomous to provide the thiols required for phytochelatin synthesis.

Introduction

In plants exposed to heavy metals, the formation of metal-binding polypeptides with the general structure $(\gamma\text{-glutamyl-cysteine})_n$-glycine, n = 2 to 11, known as phytochelatins is induced (RAUSER 1990, STEFFENS 1990). An enzyme that synthesizes phytochelatins has been found in *Silene cucubalus* cell cultures (GRILL & al. 1989). Phytochelatin synthase or γ-glutamyl-cysteine dipeptidyl

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transpeptidase forms the peptide chain by the addition of γ-glutamyl-cysteine moieties from glutathione to an acceptor glutathione molecule. Little is known about the source of glutathione additionally required for phytochelatin synthesis in the root, the organ which is most affected by heavy metal treatments. In the present study, we examined the alterations of root thiol levels caused by Cd and/or the removal of plant parts which possibly export glutathione to the seedling roots.

Materials and Methods

Maize kernels (*Zea mays* L. cv LG 9, Limagrain, Ennezat, France) were soaked for 1 day in aerated water at room temperature, germinated between several layers of damp paper in the dark at 24 to 26° C for 2 days, and eight seedlings were placed in pots with 160 ml of nutrient solution (NUSSBAUM & al. 1988). The plants were cultivated in continuous light (70 μmol m^{-2} s^{-1}) at 24 to 26° C and 60 to 65 % relative humidity. Three days after the plants were transferred to the cultivation pots, Cd was added to the nutrient solution as $CdCl_2$ and the following additional treatments were performed: none (intact plants, A), removal of the kernels (B), removal of the shoots (C) and removal of the kernels and transfer into the dark (D).

After 2 days of treatment, roots were harvested and rinsed extensively with tap water before extraction. Plant material was homogenized in a ratio 1 : 10 (w/v) in 0.1 N HCl containing 1 mM Na_2EDTA. The homogenates were filtrated through one layer of 100 % viscose fleece and further centrifuged for 30 min at 30,000 g and 4 °C. Total TCA-soluble thiols in the supernatants were determined photometrically after the reaction of the sulfhydryl groups with 2,2`-dithiodipyridine (GRASSETTI & MURRAY 1967). Cysteine, γ-glutamyl-cysteine and glutathione were separated and quantified by reverse-phase HPLC after reduction with $NaBH_4$ and fluorescent labelling with monobromobimane (SCHUPP & RENNENBERG 1988) as previously described (RÜEGSEGGER & BRUNOLD 1992).

Abbreviation: TCA, trichloroacetic acid.

Results and Discussion

Varying with additional treatments, Cd caused increases of 100 to 420 % for total TCA-soluble thiols, 50 to 100 % for cysteine, 570 to 1500 % for γ-glutamyl-cysteine and a decrease of 50 to 70 % for glutathione (Fig. 1). The removal of kernels did not change any thiol content in the roots of plants grown without Cd, whereas with Cd, kernel removal caused a 20 % lower content in total TCA-soluble thiols but no effect with cysteine, γ-glutamyl-cysteine or glutathione. The removal of the shoot caused higher levels of all thiols in the roots of plants grown without Cd, showing the largest increase (by 90 %) with glutathione, whereas with Cd-treatment the contents in glutathione and total TCA-soluble thiols did not change. This may be explained by the fact, that in absence of Cd the portion of glutathione which is translocated from the scutellum to the shoot in intact seedlings (RAUSER & al. 1991) may be moved to the roots after shoot removal. With Cd, this portion of glutathione may be used for phytochelatin synthesis. Surprisingly, the removal of the kernel did not provoke substantial changes in any thiol contents of the roots, only the Cd-related accumulation of total TCA-soluble thiols was

somewhat lower than in intact seedlings. Probably, the roots may compensate the loss of the kernel by enhancing their own glutathione synthesis. In the roots of the seedlings cultivated in the dark after the kernel has been removed, the absence of the kernel as a thiol source and the simultaneous failure of the shoot as a source for energy equivalents or possibly for thiols caused a comparable small accumulation of total TCA-soluble thiols as a result of Cd treatment. Our data suggest that the roots are at least in part autonomous to provide the additional thiols required for phytochelatin synthesis and that the storage reserve or the thiol synthesizing capacity of the kernels may predominantly act as a thiol source for the shoots under normal conditions. This is consistent with our previous findings, which demonstrate an increased *in vivo* incorporation of ^{35}S-radiolabel from ^{35}S-sulfate into cysteine, γ-glutamyl-cysteine and glutathione in isolated roots from Cd-exposed maize seedlings (RÜEGSEGGER & BRUNOLD 1992).

References

GRASSETTI D. R. & MURRAY J. F. 1967. - Arch. Biochem. Biophys. 119: 41-49.

GRILL E., LÖFFLER S., WINNACKER E.-L. & ZENK M. H. 1989. - Proc. Natl. Acad. Sci. USA 86: 6838-6842.

NUSSBAUM S., SCHMUTZ D. & BRUNOLD C. 1988. - Plant Physiol. 88: 1407-1410.

RAUSER W. E. 1990. - Annu. Rev. Biochem. 59: 61-86.

— , SCHUPP R. & RENNENBERG H. 1991. - Plant Physiol. 97: 128-138.

RÜEGSEGGER A. & BRUNOLD C. 1992. - Plant Physiol. 99: 428-433.

SCHUPP R. & RENNENBERG H. 1988. - Plant Sci. 57: 113-117.

STEFFENS J. C. 1990. - Annu. Rev. Plant. Physiol. Plant. Mol. Biol. 41: 553-575.

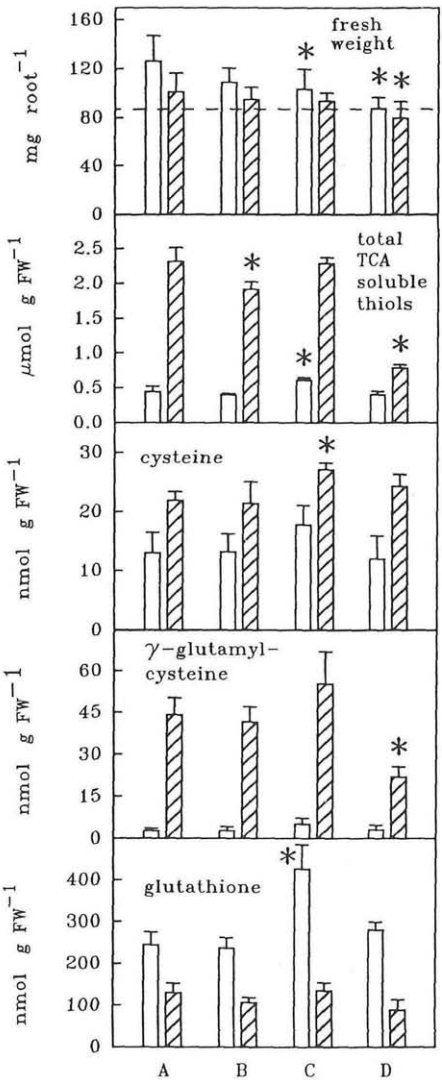

Fig. 1. Fresh weight and contents of total TCA-soluble thiols, cysteine, γ-glutamyl-cysteine and glutathione in roots of 8 day old maize seedlings. Control plants(□)and plants exposed to 50 µM Cd^{2+} (▨)for the last 2 days had no additional treatment (A), kernels removed (B), shoots removed (C), or they were transferred into the dark after kernel removal (D). All additional treatments were performed at the time of Cd application. Data represent mean of five independent experiments (± SD), except for total TCA-soluble thiols (three experiments). Asterisks indicate a significant difference due to the additional treatments (Wilcoxon $\alpha \leq 5$ %).