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Phyton (Horn, Austria) Special issue: "Sulfur-Metabolism"	**Vol. 32**	Fasc. 3	(19)-(24)	18. 12. 1992

Phytochelatins and Glucosinolates Accumulation in Seedlings of *Brassica napus* treated with Cadmium Chloride

By

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Key words: *Brassica napus*, cadmium, phytochelatins, glucosinolates.

Summary

CLOSSAIS BESNARD N. & LARHER F. 1992. Phytochelatins and glucosinolates accumulation in seedlings of *Brassica napus* treated with cadmium chloride. - Phyton (Horn, Austria) 32 (3): (19)-(24).

Thirty days old seedlings of *Brassica napus* var. oleifera were submitted to short term treatments with $CdCl_2$ in order to investigate the effect of this heavy metal on sulfur channelling for both phytochelatins (PC) and glucosinolates (GLS) synthesis and accumulation. PC peptides, from the roots, containing 2 to 5 γ-glutamyl-cysteinyl units were characterized and their individual content determined in relation to both treatment durations and $CdCl_2$ concentrations. PC synthesis was detectable 1 hour after the onset of the treatment, being then maintained at a constant rate until reaching a level related to $CdCl_2$ concentration in the external medium. A maximum PC level of 1.28 μmol SH g fresh weight^{-1} was obtained after a 48 h treatment with 150 μM $CdCl_2$. The relative levels of various PCs remained constant except for low concentrations (below 10 μM) and in treatments shorter than 15 h. In these cases, the relative abundance of PC2 and PC3 are increased. PC accumulation in rapeseed was prevented when $CdCl_2$ and buthionine sulfoximine were applied together. This inhibitory effect was abolished with the glutathione addition. Thus, it is concluded that the processes involved in PC synthesis and accumulation by rapeseed roots are quite similar to that occurring in a number of other species. Preliminary results obtained for GLS stored in vegetative parts of young rape plants treated with $CdCl_2$ suggest that sulfur consumption for PC synthesis did not occur at the expense of the sulfur available for GLS synthesis since the effects induced by Cd were not significant.

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Introduction

Higher plants which are grown in the presence of heavy metals accumulate phytochelatins (PCs), γ-glutamyl-cysteinyl peptides which are structurally related to glutathione. Whether PCs play a role in the detoxification of heavy metals is not clearly understood (GRILL & al. 1989).

In the present study, seedlings of *Brassica napus* were exposed to short-term exposure to $CdCl_2$. It was demonstrated that rape roots accumulated a range of PCs differing in the number of γ-glutamyl-cysteinyl units (PC2 to PC7) depending on either the concentration of $CdCl_2$ or the duration of the exposure. Rape plants contain substantial amounts of glucosinolates (GLS) (CLOSSAIS BESNARD & LARHER 1991). The effect of $CdCl_2$ on sulfur channelling towards this toxic compounds while the tissue are accumulating PCs was also investigated.

Materials and Methods

Seedlings of *Brassica napus* L. cv. Bienvenu, were grown under controlled environmental conditions with a 14 h photo period (22 W m^{-2}) and 25° C and 20° C day and night temperatures respectively, on vermiculite watered with a half strength Hoagland's nutrient solution. $CdCl_2$ exposures with or without buthionine sulfoximine (BSO) were applied to plants which were transferred to a water culture.

Glucosinolates were extracted with hot methanol and separated by isocratic HPLC as described elsewhere (CLOSSAIS BESNARD & al. 1990) with 0.01 M phosphate buffer (pH 7.0) containing tetraheptylammonium bromide (1.5 mM) and modified by 38 % acetonitrile. Detection was performed at 235 nm.

Phytochelatins were extracted with 100 mM HCl and separated by HPLC as described by TUKENDORF & RAUSER 1990 with a linear gradient of 0 to 20 % acetonitrile in 0.1 % trifluoracetic acid. Detection was performed at 405 nm by post column derivatization with 1.8 mM 5,5'-dithiobis 2-nitrobenzoic acid in 300 mM potassium phosphate buffer (pH 7.8).

Results and Discussion

Exposure of rape to various concentrations of $CdCl_2$ resulted in an accumulation of PCs in the roots, its content increased with the $CdCl_2$ concentration and it reached a maximum level at 150 μM after a 48 h exposure (Fig 1). Accumulation of PCs upon $CdCl_2$ exposure occurred rapidly. It was already possible to detect PCs in the root after 1 h of exposure. The PCs content increased almost linear with time during the 53 h exposure at both 1 and 150 μM $CdCl_2$, however, the rate of accumulation was much higher at 150 than 1 μM $CdCl_2$ (Fig. 2). A whole range of PCs were synthesized in rape roots upon $CdCl_2$ exposure (Fig. 3). At low $CdCl_2$ concentrations (0.5 μM) mainly PCs with a low number of γ-glutamyl-cysteinyl units (PC2 to PC4) were synthesized (Fig. 3a). At higher concentrations also PC5 and PC6 became more abundant. At 150 μM $CdCl_2$, a 15 h exposure was needed to obtain a steady state in the composition of PCs pool (Fig. 3b). As expected, the

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first compound which was synthesized was PC2. PC3, PC4, PC5 and PC6 were detected after 1, 3, 5, and 15 h after the onset of the exposure, respectively (Fig. 3b). The changes in PCs composition as related to exposure duration were in good agreement with the ability of PC synthase to utilize either glutathione or shorter phytochelatins for PCs synthesis (GRILL & al. 1989). However, the system seemed to reach an equilibrium when the total thiols stored in phytochelatins reached 0.5 µmol g dry weight^{-1}. At this time, the composition of the PCs pool remains constant, PC3 and PC4 being the main compounds involved.

As found in a number of other species, the PCs synthesis depended on the availability of glutathione. Since, the addition of BSO, a well known inhibitor of glutathione synthesis (RENNENBERG & FILNER 1982), strongly reduced the $CdCl_2$-induced accumulation of PCs (Fig. 4).

The level of PCs in rape roots upon $CdCl_2$ exposure strongly depended on the age of the plant. The potential to synthesize PCs decreased drastically with age. The PCs content of 20 and 60 days old rape after 43 h of exposure at 150 µM $CdCl_2$ was 2.5 and 0.7 µmol g dry weight^{-1}, respectively (data not shown).

The following four glucosinolates were detected in the roots: phenethylglucosinolate, 2-hydroxybut-3-enylglucosinolate, indol-3-ylmethylglucosinolate and N-methoxyindol-3-ylmethylglucosinolate (Table 1). Exposure of rape to the various concentrations of $CdCl_2$ did not affect the GLS content and composition. As demonstrated elsewhere (NUSSBAUM & al. 1988) when PCs are being synthesized in response to cadmium, sulfate reduction is stimulated. As a consequence, sulfur is not a limiting factor for glucosinolate synthesis in rape roots during $CdCl_2$ exposure. However, glucosinolates and phytochelatins pathways could be expected to compete for sulfur when sulfate availability in the medium is restricted. Alternatively, PCs and GLS synthesis might depend on the fluxes through the different pools. This is currently under study in our group.

Table 1. Glucosinolate levels in roots of rape plants upon exposure to various concentrations of $CdCl_2$ for 48 hours. Data represent the mean of two measurements with 6 plants each.

$CdCl_2$ (µM)	Glucosinolates (µmol g DW^{-1})				
	2-hydroxy but-3-enyl	phenethyl	indol-3-yl methyl	N-methoxy indol-3-ylmethyl	total
0	13.1	24.6	5.2	0.7	43.6
5	13.6	20.1	5.0	0.9	39.6
10	14.6	22.2	5.6	1.0	43.4
100	12.9	21.7	5.8	0.7	41.2
175	16.7	21.7	5.8	0.7	44.9

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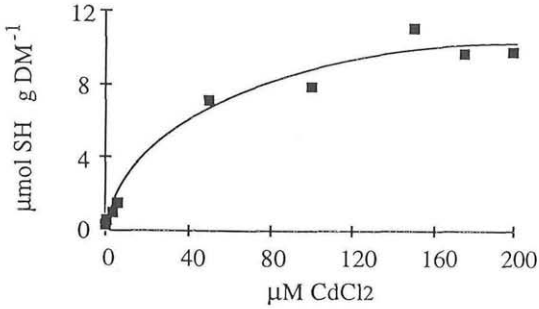

Fig. 1. Accumulation of phytochelatins in roots of rape plants upon exposure to various concentrations of $CdCl_2$ for 48 hours. Data represent the mean of two measurements with 6 plants each.

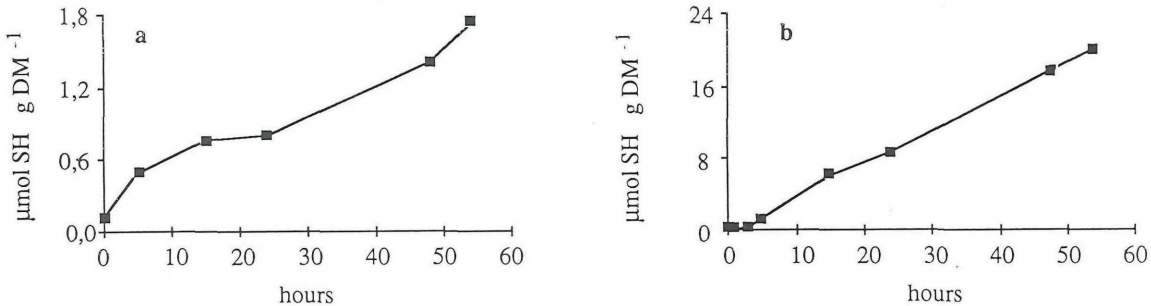

Fig. 2. Time course of phytochelatins accumulation in roots of rape plants upon exposure to 1 µM (a) and 150 µM (b) $CdCl_2$.

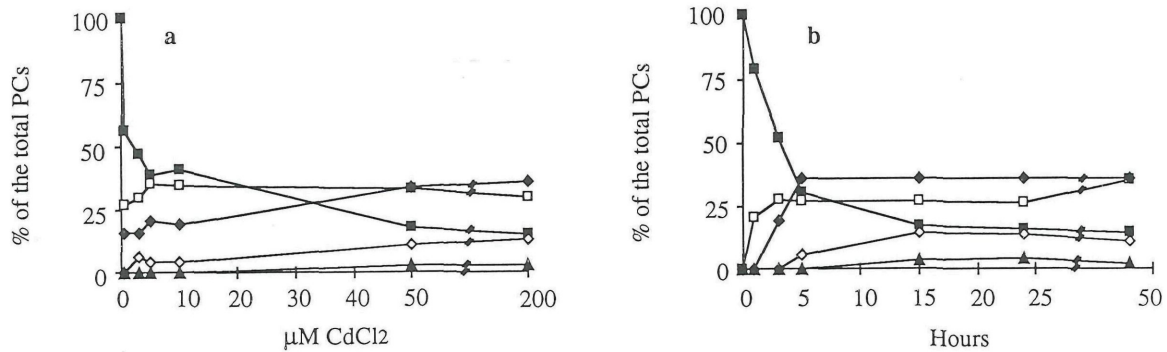

Fig. 3. Composition of the phytochelatin pool as affected by the $CdCl_2$ concentration (48 h; **a**) and the duration of exposure (150 µM $CdCl_2$; **b**). PC2 (■), PC3 (□), PC4 (♦), PC5 (◊), PC6 (Δ). The content of the PCs is expressed as % of the total and represents the mean of two measurements with 6 plants each.

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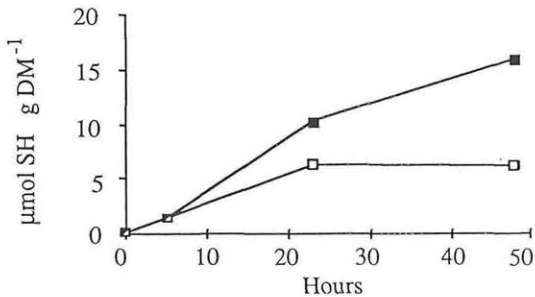

Fig. 4. The effect of BSO on phytochelatin accumulation in rape roots upon exposure to 150 µM BSO. 0 mM BSO (■), 0.5 mM BSO (□). Data represent the mean of three measurements with 5 plants each.

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Zeitschrift/Journal: Phyton, Annales Rei Botanicae, Horn

Jahr/Year: 1992

Band/Volume: 32_3

Autor(en)/Author(s): Clossais Besnard N., Larher F.

Artikel/Article: Phytochelatins and Glucosinolates Accumulation in Seedlings of Brassica napus treated with Cadmium Chloride. 19-24