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ORIGINAL PAPERS

PHYSIOLOGICAL RESPONSE OF PLANTS AND CADMIUM ACCUMULATION IN HEADS OF TWO CULTIVARS OF WHITE CABBAGE*

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Abstract

Plants of the *Brassicaceae* family are considered useful for phytoremediation owing to their tolerance to high concentrations of heavy metals, which may be hyperaccumulated in the tissues. Cabbage seems to be a useful phytoextractor because of the high biomass concentrated in the head and cabbage cultivation technologies which ensure high yield. In a pot experiment, the response of plantlets of two cabbage cultivars: Ditmarska Najwcześniejsza (DN) and Kamienna Głowa (KG) to 10 and 40 mg Cd kg^{-1} DM of soil (Cd10 and Cd40, respectively) was studied. In addition, the content of Cd in the heads after the growing season was assayed. It was established that the Cd-induced stress was temporary and did not prevent head formation by cabbage plants. Higher leaf membrane leakiness and lower chlorophyll content, which were noticed during the 3^{rd} week of vegetation, disappeared by the 8^{th} week. Cd stimulated production of glucosinolates by plants. The

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higher dose of cadmium (40 mg Cd kg^{-1} DW of soil) diminished the fresh weight of heads of DN by ca 33%, and KG by 15%. However, the Cd accumulation recalculated to dry weight was high, and increased with the Cd dose. DN heads harvested from Cd40 contained up to 260-fold more Cd than control heads; for KG heads, the Cd content was over 560--fold higher. Hence, white cabbage can be used for phytoextraction of cadmium polluted soil. Higher resistance of KG than DN cultivar to stress caused by heavy metals may be attributable to a more efficient biosynthesis of organic sulphur compounds in the former cultivar, as reflected by the GLS content in both control and Cd-exposed KG specimens.

Key words: cadmium, chlorophyll, cell membrane, cabbage, phytoextraction.

REAKCJA FIZJOLOGICZNA ROŚLIN I AKUMULACJA KADMU W GŁÓWKACH DWÓCH ODMIAN KAPUSTY BIAŁEJ

Abstrakt

Rośliny z rodziny *Brassicaceae* są uważane za przydatne do celów fitoremediacyjnych w związku z tolerancją wysokich stężeń metali ciężkich, które mogą ulec hiperakumulacji w tkankach. Kapusta wydaje się rośliną, która może być wykorzystana jako fitoekstraktor w związku z dużą biomasą skoncentrowaną w główce i techniką uprawy tego warzywa umożliwiającą uzyskanie wysokiej wydajności. W doświadczeniu wazonowym badano reakcję sadzonek dwóch odmian kapusty białej głowiastej: Ditmarska Najwcześniejsza (DN) i Kamienna Głowa (KG) na kadm zastosowany w ilości 10 i 40 mg Cd kg^{-1} s.m. gleby (Cd10 i Cd40) oraz zawartość Cd w główkach po zakończeniu okresu wegetacji. Stwierdzono, że stres wywołany Cd był przejściowy, co umożliwiło roślinom wytworzenie główek. Zwiększenie przepuszczalności błon komórkowych liści oraz zmniejszenie w nich zawartości chlorofilu stwierdzone w 3. tygodniu wegetacji nie pojawiło się już w 8. tygodniu. Cd stymulował w roślinach produkcję glukozynolanów (GLS). Większa z zastosowanych dawek kadmu (40 mg Cd kg^{-1} s.m. gleby) wpłynęła na zmniejszenie świeżej masy główek DN o ok. 33%, a KG o 15%. Akumulacja Cd w jednostce suchej masy główek była jednak bardzo wysoka i wzrastała wraz ze wzrostem dawki. Główki DN pochodzące z uprawy z zastosowaniem Cd40 zawierały do 260 razy więcej Cd niż główki pochodzące z kontroli, analogiczne główki KG zakumulowały nawet ponad 560 razy więcej Cd w stosunku do kontroli. Zatem kapustę głowiastą białą można wykorzystać do fitoremediacji gleby zanieczyszczonej kadmem. Większa odporność odmiany KG niż DN na stres wywołany metalami ciężkimi może wynikać z bardziej wydajnej biosyntezy związków siarkoorganicznych, co znalazło odbicie w zawartości GLS, wyższej zarówno u roślin kontrolnych, jak i eksponowanej na Cd odmiany KG.

Słowa kluczowe: kadm, chlorofil, błony komórkowe, kapusta, fitoekstrakcja.

INTRODUCTION

Phytoextraction is a phytoremediation technique which employs metal-accumulating plants, able to translocate chemical elements from the soil and accumulate them in harvestable parts. The species belonging to the botanical family *Brassicaceae* are often considered as usable for phytoextraction of heavy metal contaminated soils. So far, Indian mustard *Brassica juncea* (L.) Czern., *Cardaminopsis halleri* Hayek and *Thlaspi caerulescens* J.

Presl & C. Presl have been taken into consideration (Kumar et al. 1995, Cunningham, Ow 1996). Here, we are discussing a possible use of white cabbage for the soil cleanup of cadmium by phytoextraction. White cabbage has already been studied in this context, but the analyzed plants were harvested from fields with a relatively low Cd content (Ciura et al. 2005, Sękara et al. 2005). The advantages of using cabbage are as follows: high biomass concentrated in a relatively small volume of the head, the plant structure which prevents big loss of plant parts, and well-known methods of cabbage cultivation.

In the present study, relatively high doses of Cd were introduced to soil (according to Antonkiewicz et al. 2006). These doses exceeded the permissible levels set by the Ordinance of the Minister for the Environment of 9.09.2002 on quality standards of soils and ground (Journal of Law, 2002, no. 165, item 1359.) The study evaluated the degree of stress to young plants transplanted to the contaminated soil and, should they survive under such chemical exposure, their ability to accumulate Cd in the heads. Analyses of the cell membrane status, chlorophyll content and F_v/F_m parameter of chlorophyll *a*, which were carried out, are often used in environmental studies performed on plants subjected to heavy metals, where they serve as a measure of the physiological stress (Chaoui et al. 1997, Jasiewicz et al. 1997, 2004, Sikora et al. 2009). Another interesting goal of this research has been to elucidate the effect of Cd on the biosynthesis of glucosinolates (GLS). GLS are secondary metabolites specific to the *Brassicaceae* family, and their synthesis is often stimulated by environmental stress (chemical or biological one) (Jensen et al. 1996, Kusznierewicz et al. 2008). As the cabbage species embraces numerous cultivars growing in different seasons, two have been selected for this experiment: Ditmarska Najwcześniejsza (DN; early), and Kamienna Głowa (KG; late). These cultivars are popular in Poland because they enable farmers to produce high yield and are resistant to changeable weather conditions.

MATERIAL AND METHODS

The experiment was performed in 2009-2010. Cabbage plants were grown in a phytotron at the University of Agriculture in Krakow, in 10 dm^3 pots filled with local soil (clay silt, 35% silt and clay, pH 7.0) containing 0.38 mg Cd dm^{-3} DM of soil. Two cabbage (*Brassica oleracea subsp. capitata f. alba*) cultivars Ditmarska Najwcześniejsza (DN; early, vegetation period 65-75 days), and Kamienna Głowa (KG; late, 150-160 days), were used. Plantlets were produced from seeds and transplanted into soil at the stage of 6-8 leaves.

The soil and the plants were fertilized according to the agricultural standards, but at the minimal level ensuring proper growth and development of the plants: N (NO_3-N + NH_4-N) – 105 (early cultivar) and 120 mg dm^{-3} DM of soil (late cultivar), P ($Ca(HPO_4)_2$ –50 and 60 mg dm^{-3}, K (KCl, 60% potassium salt) – 160 and 180 mg dm^{-3}, respectively. Cadmium was added to the soil 10 days before the planting, as $CdSO_4 \cdot 8H_2O$, in the amount of 10 and 40 mg Cd kg^{-1} DM of soil (according to ANTONKIEWICZ et al. 2006). Control pots contained the same soil but without added Cd. The level of the stress to plants was estimated during the 3rd and 8th week of plantlets growing in the pots. The cell membrane disturbance was assayed conductometrically and expressed as the ion leakage index (MARKOWSKI, SKRUDLIK 1995, BĄCZEK-KWINTA, KOŚCIELNIAK 2009). The chlorophyll content was measured photometrically with a SPAD chlorophyllometer (Konica Minolta, Japan). Additionally, in the 8th week, the photosynthetic apparatus was assayed by analyzing the parameters of chlorophyll *a* fluorescence (FMS-2, Hansatech, UK). Moreover, the maximal photochemical efficiency of photosystem II (PSII), F_v/F_m, was taken into consideration. The measurements and analyses were conducted on non-senescent, fully developed leaves (3rd to 5th, counting from the developing head).

The plants were grown in pots for 113 days (DN) and 134 (KG). After harvesting, the heads were dried and mineralized at 450°C. The content of Cd in plants was assayed by inductively coupled plasma atomic emission spectroscopy ICP-AES JY-238 Ultrace. The content of glucosinolates (GLS) in plants was determined according to the EU official method (ISO 9167-1). From freeze-dried plant material, GLS were extracted with boiling MeOH. The extract was purified on anion-exchange resin with concomitant enzymatic hydrolysis of sulfate. Desulfo-GLS were analyzed using an Agilent Model 1100 HPLC system on a Lichrosphere RP-18e column. The separation was performed by linear gradient elution (A – H_2O, B – 20% acetonitrile; 0-25 min 0-100% B). The identification and quantification of desulfo-GLS relied on glucotropaeolin used as an internal standard and reference material (BCR-367 R, rapeseed). The detailed protocol of GLS determination has been described elsewhere (KUSZNIEREWICZ et al. 2008).

Statistical analysis was carried out by one-way Anova and Duncan test on the data for each cultivar.

RESULTS AND DISCUSSION

In the 3rd week of cultivation, the plantlets were stressed by Cd presence in the soil. Leaf cell membranes were injured, as the index of ion leakage was generally higher in the case of Cd-treated plants than in the control ones. However, DN plants were more severely affected by Cd than those of KG, because their ion leakage was raised by just as little as 10 mg Cd kg^{-1} DM of soil. There was no such increase in KG plantlets. Additionally, the higher dose of Cd (40 mg Cd kg^{-1} DM of soil) resulted in higher electrolyte leakage values in DN that in KG compared to the control plants (Figure 1). Degradation of the cell membrane due to lipid peroxidation is a typical effect produced by heavy metals on plants (OURITI et al. 1997). The reason is the overproduction of reactive oxygen species, which cause membrane lipid peroxidation, leading to increased membrane leakiness to ions (CHAOUI et al. 1997). Interestingly, in the 8th week, plants exhibited no symptoms of membrane injury. All the values determined were similar for each treatment and the cultivar (Figure 1). This suggests immobilization of the metal in vacuoles and cell walls, mainly in the older parts of the plant, or in the stem, so that younger parts revealed no injury symptoms.

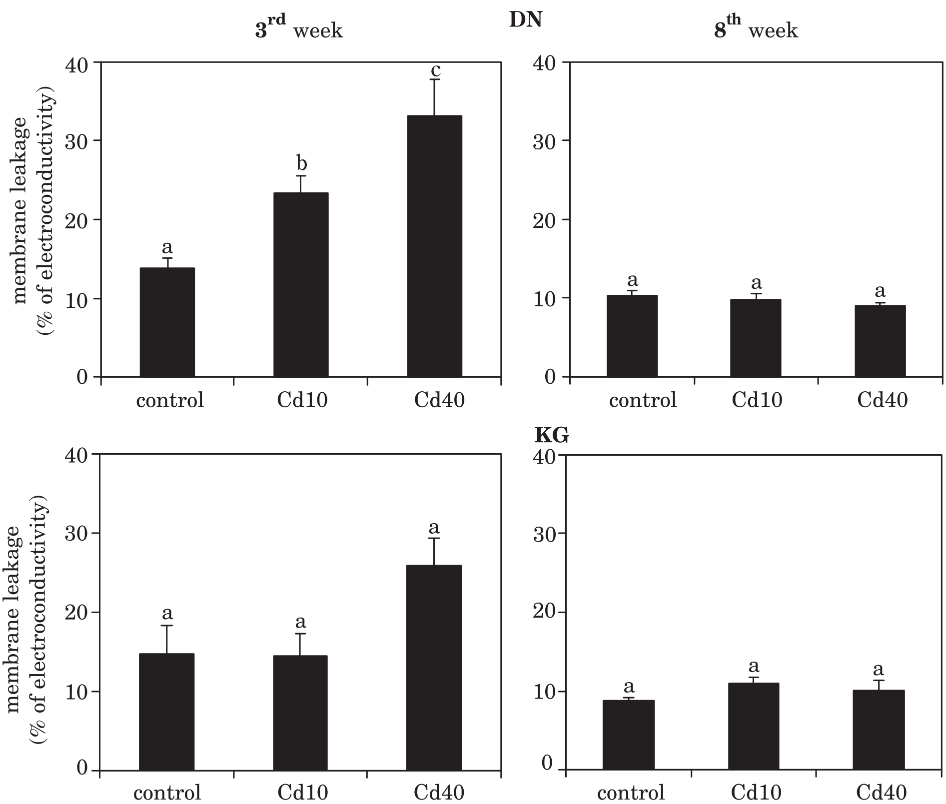

Fig. 1. The influence of Cd on the leaf cell membrane status, assayed conductometrically as ion leakage. Mean ± SE was given (n=5). Means denoted with the same letters do not differ significantly among the cultivar and the stage (Duncan's multiple range test; p=0.05)

In the 3rd week, the chlorophyll content was depressed in leaves, but the response of plants of both cultivars was not identical (Figure 2). In leaves of DN, even the smaller dose of cadmium (Cd10) depressed the SPAD values, which was similar to the effect produced by Cd40. In KG plants, the decline was caused only by Cd40, and it was smaller versus the control than that established for DN. The chlorophyll content as well as the chlorophyll *a* fluorescence parameter F_v/F_m are important markers providing information on the photosynthetic ability of plants grown on media containing a heavy metal (JASIEWICZ et al. 2004, EBBS, UCHIL 2008). Chlorophyll may be destroyed by the substitution of Mg in its central part with Cd (KÜPPER et al. 1998) or else the synthesis of the pigment may be disturbed at the level of its precursor, i.e. aminolevulinic acid (ALA, STOBART et al. 1985, NIKOLIĆ et al. 2008).

In DN plants, the inhibitory effect of Cd40 on SPAD persisted until the 8th week (Figure 2). However, at this stage, the difference in the chlorophyll content between the control and Cd40 plants was smaller than ob-

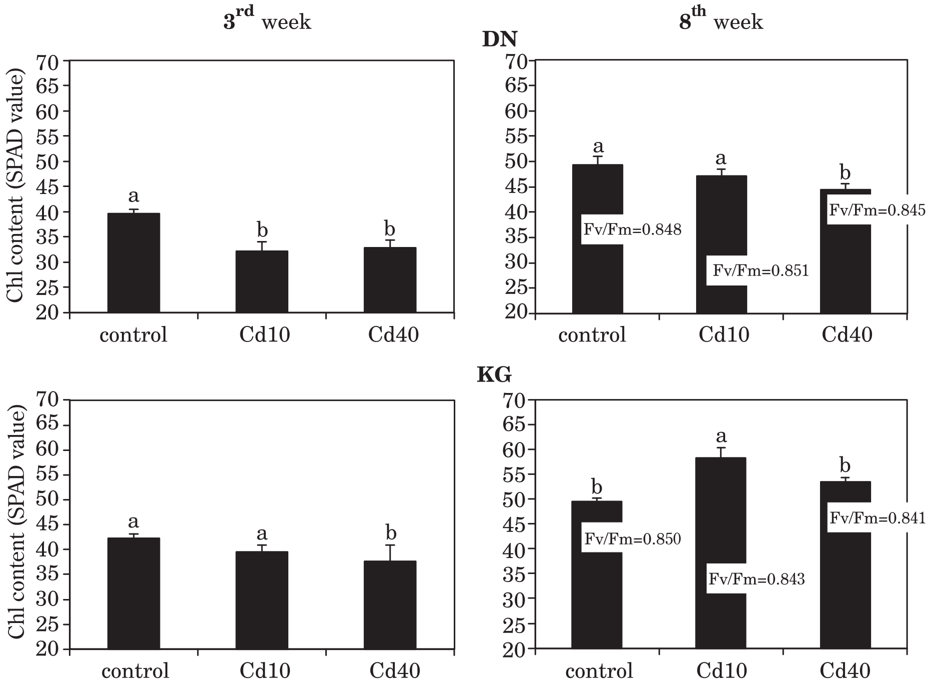

Fig. 2. The influence of Cd on the leaf chlorophyll content, assayed photometrically as the SPAD index. Data in frames: values of the fluorescence parameter F_v/F_m, measured parallel on the same leaves. Mean ± SE was given (n=5). Means denoted with the same letters do not differ significantly among the cultivar and the stage (Duncan's multiple range test; p=0.05)

served before (in the 3rd week). In KG plants, no such decline was observable in the 8th week (Figure 2), and in Cd10 plants an increase in chlorophyll was even noticed. The analysis of the chlorophyll *a* fluorescence parameter F_v/F_m revealed that in the 8th week the photochemical efficiency of photosystem II of all plants grown on Cd-contaminated soil was at the control level. Additionally, all SPAD values were higher in the 8th than in the 3rd week (Figure 2). Such an effect was also observed by SCHEPERS et al. (1992) or COSTA et al. (2003). This may suggest that Cd was incorporated into older leaves and the stem of contaminated plants, thus not affecting chlorophyll synthesis in younger plant parts. Thereby, plants were able to maintain an adequate level of photosynthesis, the source of biomass production.

However, the initial chemical stress to which the plants grown on Cd40 contaminated substrate were subjected, had a residual effect on the yield of heads. Cd40 diminished the fresh mass of DN by *ca* 30%, and that of KG by 15% in relation to the respective controls (Table 1).

Table 1

The influence of Cd on the yield and Cd content of heads of two cabbage cultivars. Mean ± SE was given (n=5). Means denoted with the same letters do not differ significantly (Duncan's multiple range test; p=0.05)

Treatment	DN		KG	
	yield of the head FW (% of ontrol)	Cd content in the head (mg kg^{-1} DW)	yield of the head FW (% of ontrol)	Cd content in the head (mg kg^{-1} DW)
Control	100 +/- 6.66 *a*	0.35 +/- 0.04 *a*	100 +/- 6.49 *a*	0.38 +/- 0.09 *a*
Cd10	95.7 +/- 17.5 *a*	5.01 +/- 0.25 *b*	99 +/- 21.9 *a*	4.62 +/- 0.53 *b*
Cd40	66.3 +/- 12.1 *b*	8.45 +/- 0.61 *c*	84 +/- 14.9 *b*	18.84 +/- 2.27 *c*

The accumulation of Cd in the dry mass of heads was high, as could have been anticipated, and tended to rise with the increasing Cd content in the soil. The highest Cd concentration was noticed in KG plants treated with Cd40. Their heads accumulated *ca* 19 mg kg^{-1} DM of biomass, which was about 560-fold more than accumulated in the control plants.

The post-harvest content of glucosinolates (GLS) is presented in Table 2. As can be seen, plants of the cultivar KG are characterized by more efficient biosynthesis (over 30%, in untreated and Cd exposed KG cabbage) of GLS when compared to DN plants. In both cultivars, the exposure to Cd stimulated GLS production. Importantly, the GLS synthesis capacity was parallel to the plants' resistance to Cd, which was tolerated better by KG plants. In sum, the data presented in Table 2 suggest that the synthesis of these secondary metabolites may help to select the most appropriate cultivar for phytoremediation.

Table 2

The influence of Cd on the glucosinalate (GLS) content in two cabbage cultivars. Mean ± SE was given (n=5). Means denoted with the same letters do not differ significantly (Duncan's multiple range test; p=0.05)

Treatment	DN	KG
	GLS content (nmoles kg^{-1} DW)	GLS content (nmoles kg^{-1} DW)
Control	3.60 +/- 0.239 *a*	6.17 +/- 0.038 *a*
Cd10	4.12 +/- 0.058 *a*	8.56 +/- 0.807 *b*
Cd40	8.10 +/- 0.071 *b*	12.0 +/- 0.595 *c*

All these results suggest that cabbage has a potential to be exploited for ion phytoextraction of Cd-polluted soil. *Thlaspi caerulescens*, well-known as a Cd hyperaccumulator tested in field trials, may accumulate up to 3,600 mg kg^{-1} DW (Robinson et al. 1998). However, the shoot biomass of *Thlaspi* is up to 3.89 t ha^{-1} (Yanai et al. 2006), whereas cabbage biomass is 60-90 t ha^{-1} (Jabłońska-Ceglarek, Rosa 2002). Therefore, the capacity of cabbage to remove cadmium from soil is 8–fold lower than that of *Thlaspi caerulescens*. However, technologies for cultivation and harvesting cabbage have been well designed and tested, in contrast to *Thlaspi*. Additionally, mineral fertilization of the soil and the plants, which was implemented to optimize vegetation, might have restrained the phytoextractive potential of cabbage (Gorlach, Gambuś 1991). The use of synthetic chelators, e.g. EDDS ((S,S-ethylenediaminedisuccinic acid), increases Cd accumulation in plants used for phytoextracion (Luo et al. 2002), so it would be worthwhile elucidating the benefits of EDDS addition in further experiments.

CONCLUSIONS

1. Cabbage plants are able to survive cadmium stress (40 mg Cd kg^{-1} DM of soil) and form the heads, which accumulate Cd.

2. The late cultivar Kamienna Głowa accumulates more Cd and reveals less severe phytotoxic symptoms than cv. Ditmarska Najwcześniejsza, thereby it has a better potential for phytoextraction.

3. The higher resistance to chemical stress, observed for the plants of cv. Kamienna Głowa, may result from their more efficient biosynthesis of organic sulphur compounds, as reflected by the GLS content, which both in untreated and Cd-exposed KG cabbage is about 30% higher than in the cultivar Ditmarska Najwcześniejsza.

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