Effect of cadmium on flavonoid content in young barley (*Hordeum sativum* L.) plants

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ABSTRACT

The effect of an abiotic stress caused by cadmium on the total flavonoid content in roots, shoots and leaf blades of spring barley variety Kompakt grown in a climate-control room for 28 days was investigated. Total flavonoid content (F) was determined spectrophotometrically with sodium nitrite, Cd content by atomic absorption spectrometry. Treatment of barley plants with Cd (1.10^{-6} mol/l) in nutrition solution caused the decrease of F in the all parts of the plant. The relatively highest decrease was found in the roots (from 20.0 to 3.05 g/kg dry matter), lesser decrease in the shoots (from 24.2 to 9.33 g/kg dry matter) and the leaf blades (from 58.3 to 27.3 g/kg dry matter). Statistically significant decrease (at least $P < 0.05$) of F and increase of Cd contents in all the investigated parts of the plant was found. Statistically significant differences of F and Cd contents among barley roots, shoots, and leaf blades were found.

Keywords: barley; roots; shoots; leaf blades; flavonoids; Cd-stress effect; Cd-flavonoid complexes

Polyphenols in barley

The outer layer coats of plant seeds contain different polyphenolic compounds, e.g. flavonoids, phenolic acids, coumarins and anthocyanins. Their content and composition is strongly influenced by different factors (Weidner and Paprocka 1996). *O*- and *C*-glycosides of flavones are characteristic for cereals, especially those derived from apigenin and luteolin (Lachman et al. 1998). Glycosides of quercetin were also described in rye and triticale. Markham and Mitchell (2003) found saponarin, lutonarin and 2´´-*O*-glucosylvitexin as the major flavonoids in young green barley leaf blades. Polyphenols are involved in the defence mechanism of plants and their levels are enhanced as a response to biotic and abiotic stresses (Weidner and Paprocka 1996, Dudjak et al. 2004).

The average contents of total polyphenols (TP) and especially those of catechol, resorcinol and phloroglucinol type compounds (CRP) in barley caryopses differ from other cereals, e.g. triticale. TP and CRP contents in barley caryopses varied from 857 to 1690 and from 95 to 448 mg/kg dry matter, respectively, while corresponding values in triticale were 607–4400 mg/kg dry matter and 5.7–10.5 mg/kg dry matter, respectively (Lachman et al. 1998). It shows considerable antioxidant activity of barley grains (Lee et al. 2003, Wu et al. 2003). TP content in barley caryopses depends significantly on the variety and is influenced by the site, specific weather features of a year and the conditions of ageing.

Wu et al. (2003) investigated the effects of four Cd levels on lipid peroxidation and activities of antioxidant enzymes in barley plants during ontogenesis. This investigation showed that Cd-stress induced a concentration- and gene type-dependent oxidative stress response in barley leaf blades, characterised by an accumulation of malondialdehyde and the alteration pattern of antioxidative enzymes, regarding the genotypic difference in Cd tolerance. It has already been reported that the antioxidant mechanism of flavonoids may also come from the interaction between transition-metal ions and flavonoids to produce complexes that keep the metal ions from their participation in free-radical generation (Miller et al. 1996). At the same time, as natural metal chelators, flavonoids show a significant function on the bio-utilization of metal and antimetal-toxicosis (Chen et al. 1990). They form complexes with metals (Viswanathan et al. 2000), as was shown for rutin and transitions metals complexes (Bai et al. 2004).

The aim of this work was to determine the effects of the abiotic stress caused by cadmium on the flavonoid levels in young barley plants.

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MATERIAL AND METHODS

Cultivation of spring barley. The experiment was carried out under controlled conditions in a climate-controlled room at the Department of Plant Botany and Physiology at the Czech University of Agriculture in Prague. Spring barley variety Kompakt was selected as the experimental material. Caryopses germinated in redistilled water at laboratory temperature (25°C) during 4 days. Then the seedlings were replanted into special boxes with nutrients medium. Two variants of the cultivation experiment were carried out. The control variant without added cadmium and the experimental variant with the addition of cadmium as $CdCl_2.2\ H_2O$ into nutrient medium in cadmium concentration 1×10^{-6} mol/l. Each variant was cultivated in 8 boxes. The number of cultivated plants varied from approx. 100 to 125 according to dimensions of the used boxes. Plants were cultivated in the conditioning plastic boxes for 28 days after their replantation and nutrient medium was exchanged one time per week. Young plants were grown at stable light fluency rate (300 μmol photon/m^2/s) with day period 16 hours, at a temperature of 22°C and air humidity 60–80%. Nutrient mediums for the both variants differed only in cadmium occurrence. Basic nutrients were supplied in the form of Knop's nutrient solution diluted into half concentration, which is useful for spring barley, and microelements were supplied in the form of Shive's solution diluted into half concentration. After cultivation, the plants were separated into roots, leaf blades and shoots and plant material was subsequently freeze-dried using a Lyovac GT 2 (Leybold-Heraeus, Germany) freeze-drier.

Determination of total flavonoid content. A modified spectrophotometric method of El-Kommos and Maksiutina (1979) and Spilková et al. (1996) with sodium nitrite was used. Freeze-dried and homogenised plant samples (approx. 0.3 g) were extracted in a Soxhlet apparatus with ethanol-water mixture (80:20 v/v) for 20 hours and the extract was quantitatively transferred into a volumetric flask and adjusted to 100 ml. For the determination 5 ml aliquots were pipetted into 25 ml volumetric flasks, 3 ml of H_2SO_4 (0.2 mol/l), 3 ml of $NaNO_2$ (3 mol/l), 3 ml of NaOH (10%) were added. Then the volume was adjusted with distilled water to 25 ml. Absorbance values were measured after 15 min using a Helios γ (Spectronic Unicam, GB) spectrophotometer against the blank (methanol) at λ = 395 nm and expressed as mg of quercetin in 1 kg dry matter of a sample. The relative standard deviation of the method determined from parallel determinations within repeatability scope was 1.96%.

Determination of Cd content by a method of atomic absorption spectrometry (AAS). Freeze-dried samples (250–300 mg of leaf blades, 150–250 mg of shoots and 50–100 mg of roots) were decomposed according to the standard operational procedure described by Mader et al. (1998) and then analysed by the procedure described by Dudjak et al. (2004).

Statistical evaluation. The results (mean values from three parallel determinations) were statistically evaluated with Statgraphics program by the analysis of variance with multiple grouping. More detail evaluation was performed by Scheffe test.

RESULTS AND DISCUSSION

The average values were calculated from three parallel determinations. The highest flavonoid content was found in leaves (58.3 g/kg dry matter), the lowest in roots (20.0 g/kg dry matter), thus descending in the order leaves > shoots > roots (Table 1). In all these plant organs Cd-treatment caused a decrease of flavonoid content (Figure 1). As compared with the control variant, the relatively most considerable decrease could be observed in roots (by 84.7%) > shoots (by 61.4%) > roots (by 53.2%). Incomparably increased cadmium contents were found in treated plants of barley. Mean cad-

Table 1. Decrease of total flavonoid content (mg/kg dry matter) caused by cadmium treatment

Variant	Part of plant	Parallel determinations	Average content	Decrease (%)	Standard deviation	Mean average error	–95%	95%
Control	roots	6	20.0		1.02	0.42	18.9	21.1
	shoots	6	24.2		0.95	0.39	23.2	25.2
	leaf blades	5	58.3		8.81	3.94	47.3	69.2
Cd 1.10^{-6} mol/l	roots	6	3.05	84.74	0.71	0.32	2.17	3.93
	shoots	5	9.33	61.38	2.41	1.08	6.34	12.3
	leaf blades	5	27.3	53.19	6.28	2.56	20.7	33.9

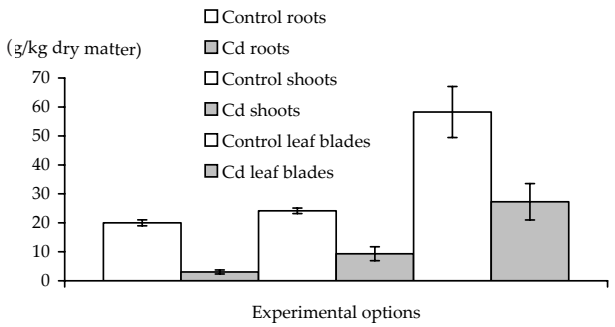

Figure 1. Total flavonoid content (g/kg dry matter) in different parts of barley plant

Table 2. Cd content (mg/kg dry matter) in barley in the experimental variants

Variant	Part of plant	Average content	Standard deviation
Control	roots	2.29	4.02
	shoots	0.32	0.277
	leaf blades	0.22	0.132
Cd 1×10^{-6} mol/l	roots	240.0	106.3
	shoots	16.3	4.50
	leaf blades	5.78	1.33

mium contents in the control plants were 2.29, 0.32 and 0.22 mg/kg dry matter, while in the treated plants 240, 16.3 and 5.78 mg/kg dry matter in roots, shoot and leaf blades, respectively (Table 2). The relative increase of cadmium content in the individual parts of the treated plants decreased in order roots > shoots > leaf blades. It is evident that Cd-increase and flavonoid decrease in plant parts are reciprocal. Because flavonoids are efficient metal chelators (Chen et al. 1990, Miller et al. 1996, Bai et al. 2004), we believe that many flavonoid structures are bound with transition metals in chelate complexes, which structures were recently elucidated on rutin-Cu and Zn complexes (Figure 2) by Bai et al. (2004) and this is a reason of a decrease of free flavonoids in Cd-treated variants. Thus plants use apparently the formation of chelate complexes with flavonoids for the reduction of stress caused by heavy metals.

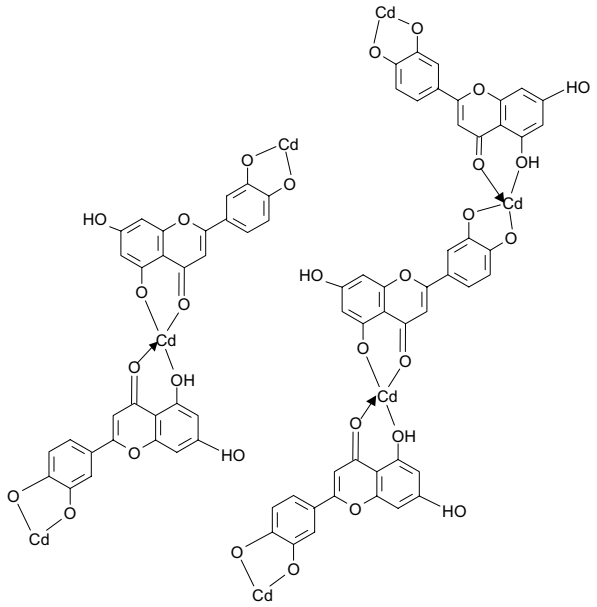

Figure 2. Proposed structures of luteolin-Cd complexes after Bai et al. (2004)

REFERENCES

Bai Y., Song F., Chen M., Xing J., Liu Z., Liu S. (2004): Characterization of the rutin-metal complex by electrospray ionisation tandem mass spectrometry. Analytical Sciences, *20*: 1147–1151.

Chen Y.T., Zheng R.L., Jia Z.J., Ju Y. (1990): Flavonoids as superoxide scavengers and antioxidants. Free Radical Biology and Medicine, *9*: 19–21.

Dudjak J., Lachman J., Miholová D., Kolihová D., Pivec V. (2004): Effect of cadmium on polyphenol content in young barley plants (*Hordeum vulgare* L.). Plant, Soil and Environment, *50*: 471–477.

El-Kommos M.E., Maksiutina N.P. (1979): Spectrophotometric determination of flavonoids using nitrous acid. Farmatsia (Moscow), *28*: 23–26.

Lachman J., Hosnedl V., Pivec V., Orsák M. (1998): Polyphenols in cereals and their positive and negative role in human and animal nutrition. In: Vaculová K., Ehrenbergerová J. (eds.): In: Proceedings of Cereals for human health and preventive nutrition, Brno: 118–125.

Lee S.H., Jew S.S., Chang P.S., Hong I.J., Hwang E.S., Kim K.S., Kim K.T. (2003): Free radical scavenging effect and antioxidant activities of barley leaf blades. Food Science and Biotechnology, *12*: 268–273.

Mader P., Száková J., Miholová D. (1998). Classical dry ashing of biological and agricultural materials. Part II. Loses of analytes due to their retention in an insoluble residue. Analusis, *26*: 121–129.

Markham K.R., Mitchell K.A. (2003): The mis-identification of the major antioxidant flavonoids in young barley (*Hordeum vulgare*) leaf blades. Zeitschrift für Naturforschung – Section C: Biosciences, *58*: 53–56.

Miller N.J., Castellucio C., Tijburg L., Rice-Evans C. (1996): The antioxidant properties of theaflavins and their gallate esters – radical scavengers or metal chelators? FEBS Letters, *392*: 40–44.

Spilková J., Nováková P., Žaludová L. (1996): Stanovení obsahu flavonoidů v nati jehlice rolní – *Onosis arvensis* L. Česká a Slovenská Farmacie, *45*: 79–82.

Viswanathan P., Sriram V., Yogeeswaran G. (2000): Sensitive spectrophotometric assay for 3-hydroxy-substituted flavonoids, based on their binding with molybdenum, antiomony, or bismuth. Journal of Agricultural and Food Chemistry, *48*: 2802–2806.

Weidner S., Paprocka J. (1996): Phenolic acids and dormancy in oat (*Avena sativa* L.) and rye (*Secale cereale* L.) caryopses. Acta Physiologiae Plantarum, *18*: 277–286.

Wu F.B., Zhang G.P., Dominy P. (2003): Four barley genotypes respond differently to cadmium. Lipid peroxidation and activities of antioxidant capacity. Environmental and Experimental Botany, *50*: 67–78.

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ABSTRAKT

Vliv kadmia na obsah flavonoidů v ječmeni (*Hordeum sativum* L.)

V modelových pokusech byl sledován vliv abiotického stresu způsobeného kadmiem na obsah celkových flavonoidů v kořenech, nadzemních částech a čepelích listů rostlin ječmene starých 28 dnů. Obsah celkových flavonoidů (F) byl stanoven spektrofotometricky po reakci s dusitanem sodným, obsah kadmia atomovou absorpční spektrometrií. Přídavek kadmia (1.10^{-6} mol/l) do živného média způsobil pokles F ve všech částech rostliny. Relativně nejvyšší pokles byl nalezen v kořenech (o 84,7 % z 20,0 na 3,05 g/kg sušiny), menší pokles pak v nadzemní části (o 61,4 % z 24,2 na 9,33 g/kg sušiny) a listových čepelích (o 53,2 % z 58,3 na 27,3 g/kg sušiny). Byl zjištěn statisticky významný pokles ($P < 0,05$) obsahů flavonoidů a zvýšení obsahu kadmia ve všech zkoumaných částech rostlin ječmene. Byly zaznamenány statisticky významné rozdíly ($P < 0,05$) v obsahu celkových flavonoidů a kadmia v kořenech, nadzemních částech a čepelích listů ječmene.

Klíčová slova: ječmen; kořeny; nadzemní části; čepele listů; flavonoidy; vliv Cd-stresu; komplexy flavonoid-Cd

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