17

Quality and Selected Metals Content of Spring Wheat (*Triticum aestivum* L.) Grain and Biomass After the Treatment with Brassinosteroids During Cultivation

Jaromír Lachman[1], Milan Kroutil[1] and Ladislav Kohout[2]
[1]*Department of Chemistry , Faculty of Agrobiology, Food and Natural Resources, University of Life Sciences in Prague,*
[2]*Department of Steroid Chemistry, Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Prague*
Czech Republic

1. Introduction

Brassinosteroids (BRs) are plant natural polyhydroxysteroids supporting the plant growth; their structure resembles animal steroid hormones (Bajguz, 2010). In plants, steroid hormones serve as endogenous signaling molecules. Brassinosteroids act as positive growth regulators or as compounds responsible for plant stress tolerance. Phytoecdysteroids probably show an antifeedant activity (Kamlar et al., 2010). Brassinosteroids were classified as essential plant hormones nearly thirty years after the discovery of brassinolide (the first brassinosteroid) by Groove et al. (1979) in the rape (*Brassica napus* L.) pollen. Presence of brassinosteroids was demonstrated in many plant species including higher and lower plants and at the same time they were detected in parts of plants, e.g. pollen, seeds, leaves, stems, roots and flowers (Sakurai et al., 1999). Up to date it was characterized 70 compounds belonging to the class of brassinosteroids, among them 65 in free form and 5 conjugated (Zulo & Adam, 2002, Bajguz & Tretyn, 2003).
Brassinosteroids are phytohormones with pleiotropic effects. They influence growth, seed germination, cell elongation, photomorphogenesis and senescence (Upreti & Murti, 2004). In relation to the growth and growth regulators, the typical effect of brassinosteroids is coincidental elicitation of cell prolongation and division (Worley & Mitchell, 1971). Investigations confirm the ability of brassinosteroids quantitatively affect plant morphogenesis; this leads to the enhancement of number and growth productive lateral shoots and branches and thereby also to the enhancement of number of spikes, pods etc. (Sakurai et al., 1999). Brassinosteroids help to overcome stresses provoked by low and high temperature, drought, salt, infection, pesticides and heavy metals (Takematsu et al., 1986; Cutler, 1991; Kulaeva et al., 1991; Schilling et al., 1991; Hathout, 1996; Bajguz 2000; Anuradha & Rao, 2001; Krishna, 2003; Janeczko et al., 2005; Cao et al., 2005; Sharma & Bhardwaj, 2007 a, b; Kagale et al., 2007; Ali & Abdel-Fattah, 2006, Ali et al., 2007, 2008, Kroutil et al., 2010 a,b). Heavy metals give rise to antioxidant stress and brassinosteroids can

it effectively reduce and induce enhancing of antioxidants under heavy metal stress (Hayat et al., 2007a). In term of the affecting of the uptake of minerals after treatment with brassinosteroids an increase of the content of minerals in aerial plant biomass was demonstrated (Nafie & El-Khallal, 2000) as well as the BRs ability to decrease uptake of heavy metals and accumulation of radioactive elements (Cs, Sr) by plants (Bajguz, 2000; Khripach et al., 1999).
In term of the affecting of the uptake of minerals after treatment with brassinosteroids an increase of the content of minerals in aerial plant biomass was demonstrated (Nafie & El-Khallal, 2000). Brassinosteroids can affect quality of plant products. Treatment with brassinosteroids at anthesis increased the starch content in rice kernels (Fujii & Saka, 2001); at tillering it increased the content of fatty acids in barley ectoplasts and the change of their rate (Khripach et al., 1999).
The aim of this work was to evaluate the ability of brassinosteroids to affect the quality parameters of spring wheat grain: change of the content of minerals in grain and the yield increase of spring wheat cultivated in rational-intensity conditions after brassinosteroids treatment. Another goal of this study was to evaluate the ability of brassinosteroids to lessen the uptake and accumulation of heavy metals (Cd, Pb, Zn, Cu) in spring wheat plants cultivated on contaminated soil of a polluted burdened region in the Czech Republic. Content of heavy metals was investigated in biomass, grains and straw of treated and control plants.

2. Material and methods

2.1 Plant material and conditions of cultivation

In the three-year period 2005-2007 spring wheat (*Triticum aestivum* L.) "Vánek" variety (maintenance of variety: Lochow-Petkus, GmbH, Germany, producer: Selekta, Inc., Czech Republic) was cultivated at 10 m^2 trial field plots outside environment (50°2'0"N, 14°36'54"E) on brown loamy soil. Every tested compound was applied in four replicates (10 m^2 field plots). There were sowed 217 kg of seeds per hectare. As foregoing crop broad bean was cultivated on the trial field before wheat plants every year and before wheat sowing the field was fertilized with the dose 60 kg N ha^{-1} with nitrogen-phosphorus-potassium fertilizer. Average content of minerals in trial field soil is described in Table 1.
In another experiment spring wheat, Vánek variety (maintenance of variety: Lochow-Petkus, GmbH, Germany, producer: Selekta, Inc., Czech Rep.) was cultivated for two years (2006, 2007) in pots in the outside environment. Plants were cultivated in the soil anthropogenic contaminated with heavy metals from the location Příbram, Central Bohemia, historically polluted from metal ores mining and smelting activities. Average content of minerals in contaminated soil are given in Table 2. Sowing was performed into the pots of 5 L volume filled with 5 kg of homogenized soil. Each pot was fertilized with the same dose of NPK (1.43 g N in the NH_4NO_3 form, 0.16 g P and 0.40 g K in the K_2HPO_4 form). The final number of plants in a pot was twenty. Plants were irrigated with demineralised water.
Weather conditions in cultivation period (from April to July) were similar in both years. Mean air temperature in both years was higher compared with the long-term normal. Mean precipitation in 2006 was higher in April, May and June, lower in July compared with the long-term normal. In April and May 2007 mean precipitation was by 25 per cent lower than normal and in June and July was higher compared with the long-term normal.

Depth of mould	N (NO_3^-)	N (NH_4^+)	N (total)	K	Mg	Ca	P	pH_{KCl}
	mg kg^{-1} DM							-
30 cm	21.1±2.1	0.4±0.04	21.5±2.2	264±13.2	132±6.6	3380±169	134±6.7	6.70±0.1
60 cm	4.8±0.5	0.4±0.04	5.2±0.5	185±9.3	141±7.1	2763±138	44±2.2	6.39±0.1

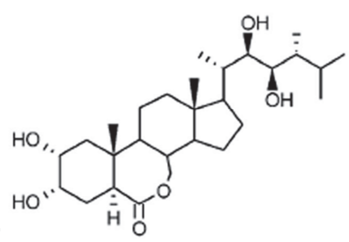
Table 1. Average content of minerals in the soil in field experiment

Soil „Příbram“	Cation H^+ exchange capacity	pH_{KCl}	Cox	Zn	Cu	Cd	Pb
Unit	1 mmol kg^{-1}	-	%	mg kg^{-1} DM			
Value	123	4.52±0.02	1.91±0.006	187±8.0	42.7±2.0	3.60±0.17	1321±71

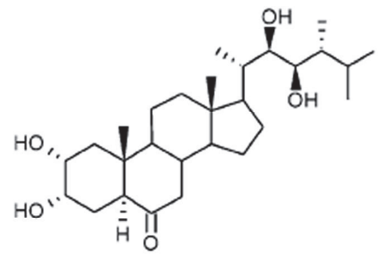
Table 2. Content of selected metals and characteristics of used soil contaminated with heavy metals from the district of Příbram, Czech Republic in outside environment pot experiment

2.1.1 Brasssinosteroids and their treatment pattern

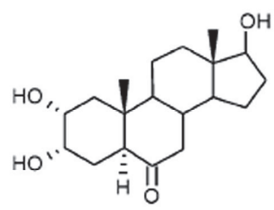
Plants were treated with eight different brassinosteroids (24-epibrassinolide; 24-epicastasterone; 4154 compound and five androstane and pregnane analogues of brassinosteroids marked KR1, KR2, KR3, KR4 and KR5) in 49-59 DC (growth phase referred to a *decimal code* for the growth stages of cereals - from visible awns to complete inflorescence emergence) (Zadoks et al., 1974). All brassinosteroids were applied in the form of 1 nmol L^{-1} of efficient compound in the water solution by spraying on all aerial biomass. Each of the tested brassinosterids was applied in four parallel replicates (4 x 10 m^2 field plots). Untreated plants were cultivated as well in tetraplicates as the control variant. Applied brassinosteroids (Fig. 1) were synthesized by the Institute of Organic Chemistry and Biochemistry of the Academy of Sciences of the Czech Republic. 24-epibrassinolide (24-epiBL) and 24-epicastasterone (24-epiCS) are naturally occurring plant phytohormones, compound 4154 is a synthetic brassinosteroid registered in the Czech Republic (Registration Nr. 294343, conferred on 4 Oct 2004) and the EU (Nr. 1401278, conferred on 28 Sep. 2005). Compounds KR1 - KR5 are synthetic permanently studied brassinolide analogues, which do not occur naturally in plants and will be published after finishing the synthesis of similar structures and protecting of these compounds by a patent (Vlašánková et al., 2009).
In pot experiment plants were treated with three brassinosteroids (24-epibrassinolide, 24-epicasterone, and 4154) in two different growth stages in four parallel replicates in each brassinosteroid. Plants or experimental pots were divided before the application of brassinosteroids into four groups that differed with growth stage in the date of treatment and number of brassinosteroids applications (Table 3).



24-epibrassinolide (24-epiBL)



24-epicastasterone (24-epiCS)



2α,3α,17β-trihydroxy-5α-androstan-6-one (4154)

Fig. 1. Chemical structure of brassinosteroids used for wheat treatment

Treatment*	Stage of brassinosteroids application DC	
	29-31	59-60
A-I	+	-
B-I	+	-
C-I	+	-
A-II	+	+
B-II	+	+
C-II	+	+
A-III	-	+
B-III	-	+
C-III	-	+
D-control	-	-

Table 3. Variants of analyzed spring wheat plants treated with brassinosteroids at different growth stages; *1st group of plants (pots A-I, B-I, C-I) was treated with brassinosteroids A (24-epibrassinolide), B (24-epicastasterone) and C (4154) once at the growth plant stage according to Zadoks growth scale 29-31 DC (off shooting); 2nd group (pots A-II, B-II, C-II) was treated with brassinosteroids two times, firstly in the plant growth stage 29-31 DC and again in the plant growth stage 59-60 DC (beginning of flowering); 3rd group (pots A-III, B-III, C-III) was treated once in the plant growth stage 59-60 DC (beginning of flowering); 4th group (D) consisted of untreated control plants

2.1.2 Harvest and sampling of plant material and grain

Plants of experimental field plots were harvested at physiological maturity (growth stage 90 DC) by a harvester thresher HEGE 140 (Hans-Ulrich Hege GmbH & Co, Germany). After the harvest, the grains were cleaned on the sieves by flow of air and then yield and thousand-grain weight were determined. Analytical samples were made according to methodology ISO 13690:1999 by quartering of cleaned grain. A common (average) analytical sample of four trial plots grain was prepared.

Sampling from the experimental pots was performed three weeks after the application of brassinosteroids in plant growth stages referred to a decimal code for the growth stages of cereals. The first sampling was performed in the plant growth phase 47-49 DC (visible awns), the second sampling in the growth stage 73-75 DC (30-50 % of final grain size). Grain and straw samples were taken in the growth phase Z90-92 (full ripeness). Green plants were taken from experimental pots cleaned up with distilled water and subsequently freeze-dried.

2.2 Methods of determination

2.2.1 Chemical and laboratory material and equipments

For dry decomposition there were used: nitric acid (65%, p.p., Lachema Neratovice CZ and Suprapur Merck, Germany), demineralised water (quality degree 1 according to EN ISO 3696 for the calibration of ICP-OES). Water calibration solutions with one element (Analytika, Ltd., CZ) were used for the calibration of F-AAS: Ca (1.000 ± 0.002 g L^{-1}) in 2% HCl, Cu (1.000 ± 0.002 g L^{-1}) in 2% HNO_3, Fe (1.000 ± 0.002 g L^{-1}) in 2% HNO_3, K (1.000 ± 0.002 g L^{-1}) in 2% HNO_3, Mg (1.000 ± 0.002 g L^{-1}) in 2% HCl, Mn (1.000 ± 0.002 g L^{-1}) in 2% HNO_3 and Zn (1.000 ± 0.002 g L^{-1}) in 2% HNO_3. For the testing of the dry decomposition method, a certified reference material NIST 8436 (Durum Wheat Flour) and internal reference material (IRM) from International Plant Analytical Exchange (IPE), RM Sample 3, Wheat 684, Quarterly Report 2000.3 were used. For the calibration of AAS and testing of the method of modified dry decomposition the following materials were used: calibration solutions with one element (Analytika, Ltd., CZ) 1.000 ± 0.002 g L^{-1} in 2% HNO_3 for elements Cu, Pb and Zn, while cadmium was dissolved in 2% HCl. Muffle oven (LM 112.10, MLW, Germany), heating plate ALTEC JRT 350 with temperature graduation per 10 °C and ultrasonic bath Elma Transonic T660/H were used for the dry decomposition of samples. Analyses of the metals were performed by atomic spectrometer VARIAN SpectrAA 110 (VARIAN A.G., Australia) with the possibility emission spectra and Varian SpectrAA 280Z atomic absorption spectrometer furnished with GTA 120 electrothermic atomizer. Laboratory hammer mills LM3100 and LM120, falling number bath FN 1500, Glutomatic 2200 and Gluten Index centrifuge 2015 made by Perten Instruments AB (Sweden) were used for the determination of Falling number, gluten content and gluten index. For protein determination there were used: nitric acid (HNO_3, 65%, p.a., Lachema Neratovice, CZ), automatic nitrogen analyzer Kjeltec system. A laboratory mill LM3100, lactic acid and bromphenol blue were used for the determination of sedimentation index (Zeleny sedimentation test).

2.2.2 Soil analysis

Soil pH was measured in suspension using 1:2.5 (w/v) ratio of soil and 0.2 M KCl at 20 ± 1°C by WTW pH 340i set. Available forms of nutrients (Ca, K, Mg and P) were determined using the Mehlich 3 soil extraction procedure (Mehlich, 1984; Zbíral, 2000) and organic nitrogen by the Kjeldahl method (Bremner, 1960).

2.2.3 Dry decomposition procedure

Samples of grain, straw and freeze-dried green plants were mineralized before analyses by dry thermal decomposition using SOP-3C (Standard Operation Procedure for Dry

Decomposition of Higher Plants and Green Algae) (Mader et al., 1998). Before dry decomposition, samples of freeze-dried plants, straw and grains were roughly ground in an IKA A11 Basic mill equipped with stainless steel working parts. Weight of the homogenized sample was about 1 g and each sample was analysed in two replicates. Initial temperature of the heater plate was 150 °C, final temperature was 350 °C. After cooling, the samples were combusted in a muffle oven at 480 °C; the ash was dissolved in 1.5 mL conc. HNO_3 (65% p.a.) and then repeatedly combusted at 480 °C. After the combustion, the samples (white ash) were dissolved in 5 mL of 1.5% HNO_3 after the addition of 1 mL conc. HNO_3 (65% p.a.).

2.2.4 FAAS determination (flame atomic absorption spectrometry) and ET-AAS (atomic absorption spectrometry with electrothermic atomization)

Determination of metals (Ca, Cu, Fe, K, Mg, Mn and Zn) content was performed with flame atomic absorption spectrometry (F-AAS) by calibration curve method. Atomization of samples proceeded in the flame acetylene/air; rate of injection of samples into the flame was 4.5 mL min^{-1}. Wavelengths used for the metals determination were 422.7, 285.2, 766.5, 213.9, 324.8, 279.5 and 248.3 nm for Ca, Mg, K, Zn, Cu, Mn and Fe, respectively. Determination of all metals content was performed with atomic spectrometer VARIAN SpectrAA 110. Limits of detection (LOD) and limits of quantification (LOQ) of the metals determination are given in Table 4. Determination of Cd, Zn and Cu was performed with flame atomic absorption spectrometry in samples prepared with dry decomposition. Atomization of samples was proceeded in the flame acetylene/air; rate of injection of samples into the flame was 4.5 mL min^{-1}. Wavelengths used for the metals determination were 228.8, 324.8 and 213.9 nm for Cd, Cu and Zn, respectively. Determination of Pb was performed with atomic absorption spectrometry with electrothermic atomization by Varian SpectrAA 280Z atomic absorption spectrometer furnished with GTA 120 electrothermic atomizer at wavelength 283.3 nm.

Parameter	Metal						
	Ca	Mg	K	Zn	Cu	Mn	Fe
LOD (mg kg^{-1})	1.0	0.03	0.08	0.09	0.01	0.15	0.18
LOQ (mg kg^{-1})	3.3	0.11	0.28	0.31	0.04	0.51	0.59

Table 4. Limits of detection (LOD) and limits of quantification (LOQ) of the metals determination

2.2.5 Determination of dry weight

Dry matter of straw samples was determined by drying at 105 ^{0}C in a laboratory oven and of that grain at 130 ^{0}C to constant weight (ISO 612).

2.2.6 Determination of wheat grain quality

Protein (N x 5.70) content was determined by Kjeldahl method by automatic nitrogen analyzer (methodology ISO 1871:1975). Falling number was determined according to ISO 3093:2004. Gluten content was determined by Glutomatic according to ISO 7495:1990. Sedimentation index of wheat flour (Zeleny sedimentation test) was determined according ISO 5529:1992. Determination of bulk density, called "mass per hectoliter" was performed according to ISO 7971-2:1995.

2.2.6 Replicates and statistical analysis

All variants were cultivated and treated in four replicates. Statistical evaluation was performed with ANOVA. Post-hoc analyses were performed by Tukey's HSD (Honestly Significant Difference) test ($p < 0.05$) for metals content and by Fisher's LSD (Least Significant Difference) test ($p < 0.05$) for grain quality parameters, thousand-grain weight and yield of grain.

3. Results

3.1 Content of Cd, Cu, Pb and Zn in aerial plant biomass (cultivation in experimental pots)

Experimental plants of the first group (A-I, B-I and C-I) and the second one (A-II, B-II and C-II) treated with BRs in the plant growth stage 29–31 DC did not differ in the growth stage 47 – 49 DC from untreated control and the plants of the third group (not treated in the stage 29 – 31 DC). The first differences in the content of investigated metals were shown in the plant growth stage 73 – 75 DC (Table 5). A distinct trend in copper content was not observed in the plant biomass. Content of lead decreased in all variants of treated plants. A decreased lead content was determined in the plants of the second group (as a whole treated two times) and the third (A-III, B-III and C-III) group (as a whole treated ones), in which the last BRs application was performed in growth stage 59–60 DC. In the first group that was treatedonly once in the stage 29–31 DC, lead content was higher than those in the other two groups. Similarly to lead content in the plants of the second group and the third group, lower cadmium and zinc contents were determined as related to the contents of the first group and in control plants (with the exception of plants treated with 4154 in the third group, where the lower Zn content was not determined). After the harvest of plants in the growth stage 90–92 DC (Table 5), a lower copper content in the first group and the third group (with the exception of plants treated with 4154 in the third group) was determined in plant straw. Likewise in the growth stage 73–75 DC, lower zinc content was determined in all plants of the second group and in the plants of the third group treated with 24-epiBL and 24-epiCS.

	Growth stage 73 - 75 DC				Growth stage 90 – 92 DC			
	Cu	Zn	Cd	Pb	Cu	Zn	Cd	Pb
D (control)	2.71	123	6.48	5.28	3.07	55.2	26.1	5.30
A-I	3.59*	130	5.91	3.59*	2.17*	47.4	29.3	4.70
B-I	3.35	129	5.42	2.37*	2.30*	46.8	30.6	4.80
C-I	1.60*	128	5.06*	3.39*	2.28*	46.0	31.7*	4.54
A-II	3.02	96.8*	4.01*	2.33*	2.48	33.7*	19.9*	3.56*
B-II	3.71*	100*	5.04*	1.70*	2.98	41.8*	26.5	3.65*
C-II	1.63*	108*	4.10*	2.95*	2.64	34.2*	25.6	4.45
A-III	2.52	92.0*	3.14*	1.36*	2.08*	26.3*	22.4	3.48*
B-III	1.94	93.5*	3.01*	1.88*	1.75*	26.2*	22.7	2.57*
C-III	3.56*	112	5.08*	2.68*	2.36	47.0	26.6	4.54

Table 5. Content of copper, zinc, cadmium and lead in plants (aerial biomass) treated with brassinosteroids and in untreated control in the growth stage 73 – 75 DC and 90 – 92 DC (mg kg^{-1} DM)

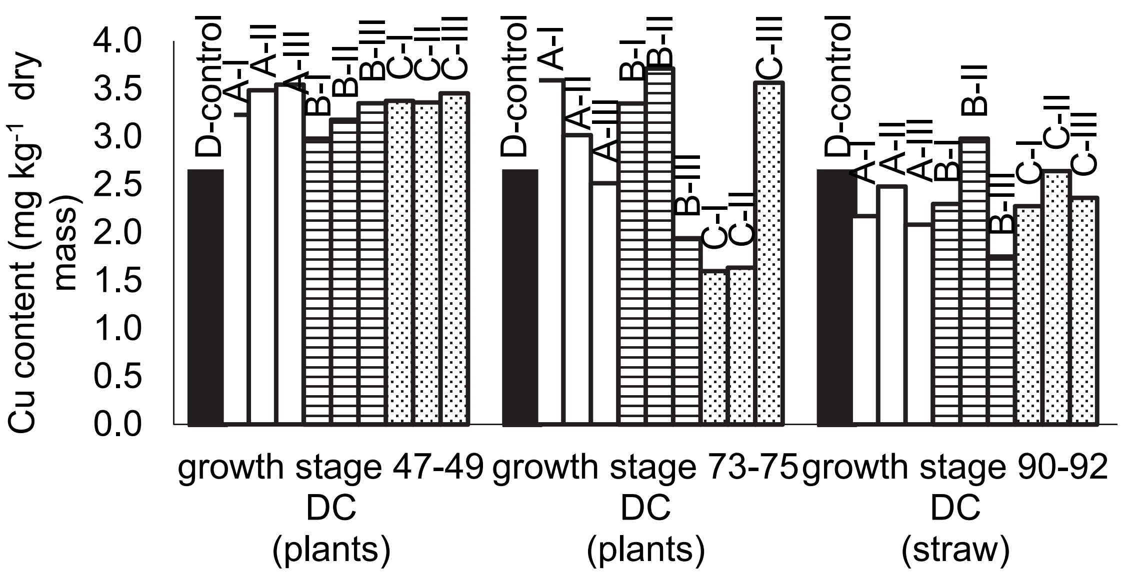


Fig. 2. Content of copper in aerial biomass of plants (mg kg^{-1} DM)

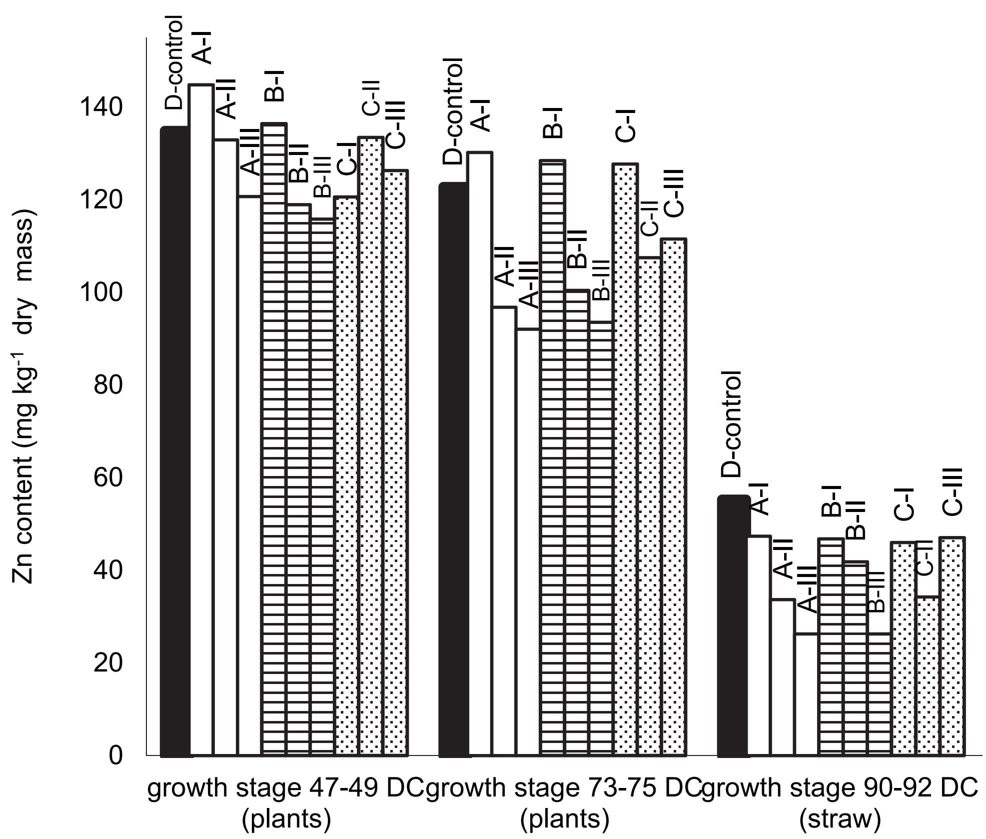


Fig. 3. Content of zinc in aerial biomass of plants (mg kg^{-1} DM)

No significant difference of the cadmium content in the growth stage 90–92 DC was found, with the exception of plants treated with 4154 in the first group and 24-epiBL in the second group. Lower lead content was determined in the plants of the second group and the third group treated with 24-epiBL and 24-epiCS. Copper content was affected more likely according to the actual and individual status of plants, however, in some cases these physiological processes could be affected by brassinosteroids treatment (Fig. 2). Zinc content in aerial biomass decreased during plant growth (Fig. 3). A significant decrease of cadmium content was determined after the applications of brassinosteroids in the growth stage 73–75 DC in the plants of the second group and the third group (Fig. 4). Lead was accumulated in the plant biomass of the control group during the all vegetation period (Fig. 5). Lower lead

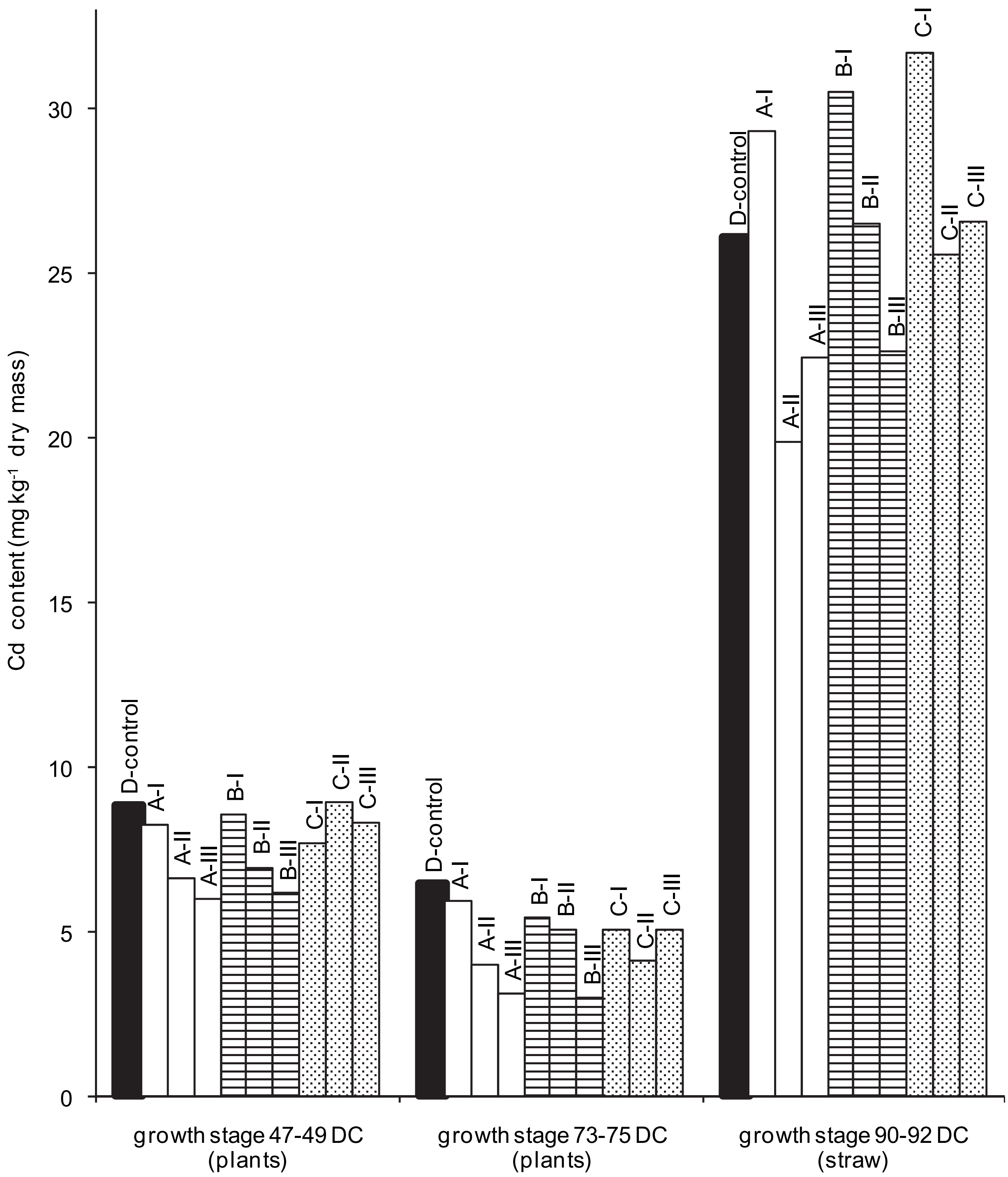


Fig. 4. Content of cadmium in aerial biomass of plants (mg kg^{-1} DM)

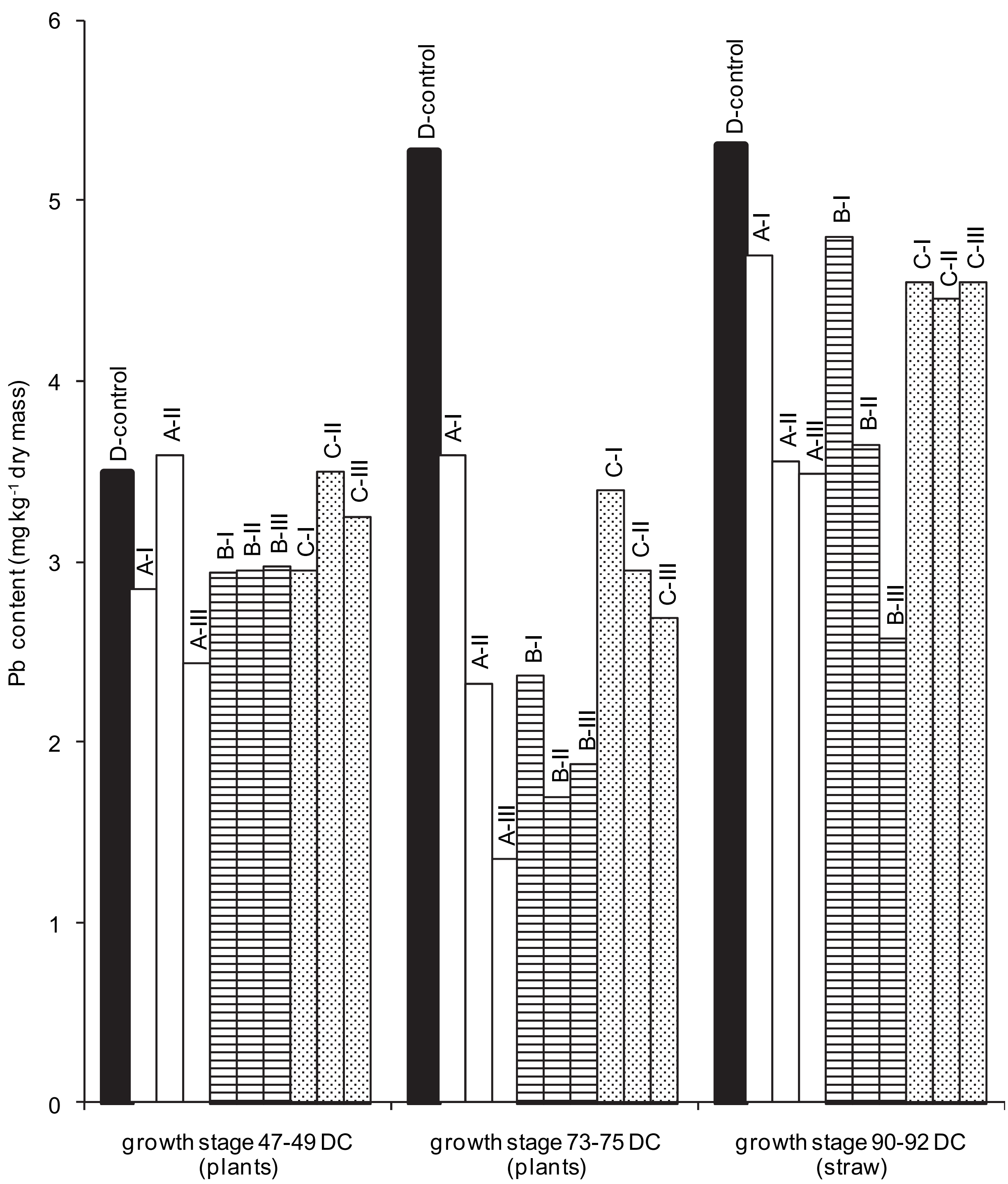


Fig. 5. Content of lead in aerial biomass of plants (mg kg^{-1} DM)

content in the stage 90–92 DC was found only in the plants of the second group and the third group that were treated with 24-epiBL and 24-epiCS. No significant difference was found in the plants of the first group that were treated in the growth stage 29–31 DC and in the treatment with brassinosteroid 4154.

3.2 Content of Cd, Cu, Pb and Zn in grains (cultivation in experimental pots)

After the application of BRs, lead content in grains decreased in the second and the third group (Table 5). While copper content significantly decreased in the plants of the third group following 24-epiCS treatment, the decrease of copper content was not statistically significant in other variants. Effect of brassinosteroids on the content of metals in grains of control plants is shown in Fig. 6.

	Copper	Zinc	Cadmium	Lead
D-control	4.88	15.6	11.7	1.87
A-I	4.15	15.9	12.9	1.33
B-I	3.90*	10.6	13.2	1.21
C-I	4.24	14.2	14.2*	1.76
A-II	4.49	14.7	14.2*	0.57*
B-II	3.95*	10.7	12.5	0.74*
C-II	4.72	14.9	11.5	0.49*
A-III	3.98*	12.3	12.1	0.97*
B-III	3.81*	16.3	12.0	0.57*
C-III	4.10*	17.0	12.6	0.83*

Table 5. Content of Cu, Zn, Cd and Pb in grain of plants treated with brassinosteroids and in untreated control (mg kg^{-1} DM); *statistically significant difference related to untreated control; for the used symbols of experimental variants see Table 2

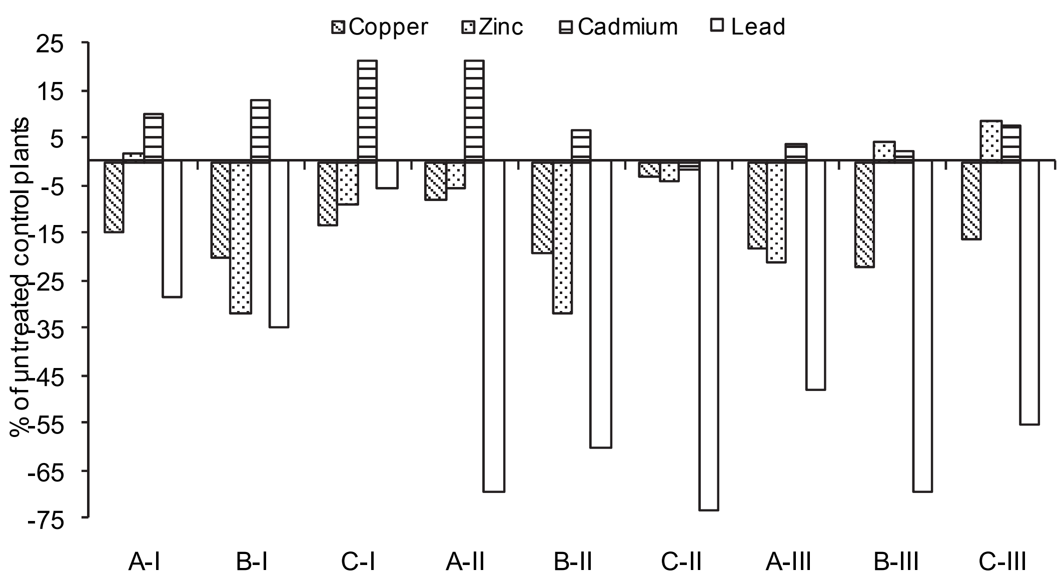


Fig. 6. Content of Cu, Zn, Cd and Pb in the grains of treated plants (in % of the content in untreated plants)

3.3 Content of Ca, Cu, Fe, K, Mg, Mn and Zn in wheat grain after BRs treatment (field experiment)

Values of metals content in wheat grain in individual years of cultivation are given in Table 6. The changes of the metals content in spring wheat grain were observed during the field experiments. Statistically significant differences in the total content of metals between years were found in the Ca, Mg, Mn and Fe contents. In potassium content, year 2007 differed from 2005 and 2006, while no difference was found between 2005 and 2006. Likewise, zinc content in 2005 differed from years 2006 and 2007. No statistically significant difference between 2005, 2006 and 2007 was found in copper content. In 2005, no differences in the selected metals content between untreated control plants and plants treated with BRs were determined. In 2006, potassium content increased in plants treated with 24-epiBL (by 22.2%), 4151 (by 31.2%) and KR1 (by 24.5%), while zinc content decreased in variants treated with 24-epiCS (by 14.5%) and KR1 (by 12.4%) as compared to the control variant. 2007, Mg, Mn and Fe contents decreased. In comparison with untreated control plants, there was lower magnesium content (by 11%) and manganese content (at least 7.5%) in variants treated with 24-epiCS, 4154 and with KR2–KR5. Different iron content was determined in variants treated with 24-epiBL, 24-epiCS and with KR1–KR5. Weather conditions were similar in all three years. Mean air temperature was higher as compared with the long-term normal value(Table 7) in whole three-year period. Mean precipitation in 2005 and 2006 was lower as

Variety	Year	Calcium	Magnesium	Potassium	Zinc	Copper	Manganese	Iron
untreated plants	2005	190.4	1337	3073	34.2	4.87	38.1	43.2
24-epiBL		187.3	1350	3089	36.6	4.93	37.9	44.0
24-epiCS		192.8	1353	3382	35.4	4.77	38.0	44.8
4154		190.8	1334	3330	37.8	4.77	36.9	44.6
KR1		191.5	1379	3097	37.4	4.82	37.5	45.8
KR2		188.4	1338	3062	34.9	4.80	38.2	46.0
KR3		186.7	1311	3146	35.2	4.77	36.8	44.3
KR4		189.6	1335	3445	35.7	4.90	38.4	46.4*
KR5		187.1	1351	3394	34.9	5.06	37.3	44.9
untreated plants	2006	304.5	1407	2591	37.3	4.76	44.6	46.1
24-epiBL		282.0	1439	3168*	33.4	4.77	46.4	44.8
24-epiCS		291.4	1428	3106	31.8*	4.91	46.2	47.2
4154		312.9	1456	3399*	33.5	4.92	45.2	47.2
KR1		287.7	1420	3226*	32.6*	4.75	43.5	46.1
KR2		301.3	1459	3174	35.2	4.82	44.2	47.8
KR3		294.3	1450	3111	35.2	4.70	42.4	48.4
KR4		315.1	1468	3117	33.2	4.84	46.7	47.7
KR5		306.7	1421	2898	34.4	4.78	45.7	48.1
untreated plants	2007	315.3	1262	3718	34.8	4.92	35.2	57.7
24-epiBL		313.9	1178	3533	34.4	4.83	34.0	49.4*
24-epiCS		320.5	1112*	3462	34.8	5.00	32.5*	49.6*
4154		327.7	1056*	3341	32.5*	4.77	32.3*	59.0
KR1		310.8	1145	3393	33.9	4.62	34.1	48.7*
KR2		314.7	976*	3314	33.1	4.80	29.3*	52.9*
KR3		318.2	1015*	3334	34.2	4.92	30.7*	65.7*
KR4		319.5	977*	3295	33.3	4.77	30.5*	48.6*
KR5		307.4	996*	3584	33.4	5.00	30.2*	48.5*

Table 6. Content of Ca, Cu, Fe, K, Mg, Mn and Zn (mg kg^{-1} DM) in spring wheat grain; *statistically significant difference (at the level of significance $p < 0.05$) between treated and untreated plants

compared with the long-term normal value, while close to long-term level in 2007. The results achieved in three-year period 2005–2007 indicate a possible effect of the year on metal content of grain affected probably by precipitation. In 2007, with usual precipitation level, contents of Fe, K, Mg and Mn were decreased. In 2005 and 2006 with below average precipitation, total content of metals were comparable or higher than content of metals in untreated control plants grain. Nevertheless, such hypothesis needs to be tested in further experiments.

	unit	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Temperature	°C	-2.1	-0.8	3.4	8.2	13.4	16.3	18.2	17.5	14.0	8.6	3.2	-0.5
Precipitation	mm	28	27	31	46	65	74	74	72	49	41	34	34

Table 7. Long-term normal of mean air temperature and mean precipitation of cultivation area (50°2'0"N, 14°36'54"E)

Variety	Year	Bulk density (mass per hectolitre)	Falling number	Protein	Gluten	Sedimentation index (Zeleny test)
		kg hL^{-1}	sec	%	%	mL
untreated plants	2005	79.3	153.8	13.8	34.0	61.0
24-epiBL		79.4	138.8	13.9	34.4	58.3
24-epiCS		79.4	145.3	13.9	34.4	61.0
4154		79.6	146.7	13.8	34.2	58.8
KR1		79.3	156.3	13.9	34.4	61.8
KR2		79.4	151.3	14.0	34.6	58.3
KR3		79.5	160.8	13.7	33.9	57.3
KR4		79.4	153.5	13.5	33.2	58.0
KR5		79.4	152.5	13.6	33.5	55.5
untreated plants	2006	80.1	346.5	12.9	29.9	46.5
24-epiBL		79.9	335.8	12.2	28.1	42.8
24-epiCS		79.9	344.8	12.6	28.7	43.0
4154		80.1	346.8	12.9	29.4	45.5
KR1		80.2	350.3	12.6	28.7	42.5
KR2		80.2	348.8	12.8	29.1	45.8
KR3		79.9	353.8	12.8	29.5	46.3
KR4		79.9	344.0	12.5	28.7	43.8
KR5		80.0	332.5	12.9	30.6	46.3
untreated plants	2007	80.4	274.3	15.6	44.0	68.5
24-epiBL		80.6	290.5	15.5	44.1	70.5
24-epiCS		80.7	275.8	15.5	44.1	68.8
4154		80.5	273.0	15.4	43.4	68.8
KR1		80.6	282.8	15.3	44.4	69.0
KR2		80.3	274.3	15.3	43.7	68.8
KR3		80.5	286.0	15.3	43.5	68.8
KR4		80.6	269.0	15.4	43.6	69.3
KR5		80.6	269.0	15.3	43.8	68.3

Table 8. Quality parameters of grain after spring wheat treatment with brassinosteroids

Variety	Year	Yield (corrected on moisture 14 %)	1000 kernels weight
		t ha^{-1}	g
untreated plants	2005	6.54	53.24
24-epiBL		6.09*	56.99*
24-epiCS		6.19	55.77*
4154		6.36	56.61*
KR1		6.20	54.99*
KR2		6.12	54.77*
KR3		6.41	54.91*
KR4		6.37	55.59*
KR5		6.33	55.38*
untreated plants	2006	7.10	45.04
24-epiBL		6.66	45.10
24-epiCS		7.12	45.10
4154		7.19	44.46
KR1		7.04	45.22
KR2		7.12	44.62
KR3		7.13	44.17
KR4		6.86	44.62
KR5		7.09	44.38
untreated plants	2007	4.57	45.60
24-epiBL		4.32	44.71
24-epiCS		4.60	45.74
4154		4.53	45.44
KR1		4.53	45.05
KR2		4.52	45.10
KR3		4.45	45.33
KR4		4.48	46.00
KR5		4.65	45.87

Table 9. Yield of grain and 1000 kernels weight after spring wheat treatment with brassinosteroids; *statistically significant difference (at the level of significance $p < 0.05$) between treated and untreated plants

3.4 Quality of wheat grain after BRs treatment (field experiment)

Values of determined qualitative parameters of food wheat grains (bulk density, falling number, protein content, gluten content and sedimentation index) were different according to the cultivation years; statistically significant difference has been proved between years. No statistically significant difference was observed between values of the grain qualitative parameters of plants treated with brassinosteroids or untreated. Average values of qualitative parameters of wheat grains in individual years are reported in Table 8.

3.5 Yield of wheat grain after BRs treatment (field experiment)

Yield of grain and values of thousand-grain weight (TGW) were different during the investigated period; a statistically significant difference between years was demonstrated. In 2005, an increase of TGW was determined in all treated variants. The yield per hectare decreased by 6.9% in the variant treated with 24-epiBL. In 2006 and 2007, no difference between grain yields of control and treated plants was observed. No difference was found also between TGW values. Average grain yields and TGW values are given in Table 9.

4. Discussion

Changes of metal composition of plants treated with brassinosteroids were reported in quite a few experiments. Most of these experiments are related to the ability of brassinosteroids to decrease the intake of heavy metals with plants. 24-epiBL at the concentration of 10^{-8} mol L^{-1} in combination with heavy metals blocked metal accumulation in algal cells (Bajguz, 2000) and treatment of *Brassica juncea* plants with 24-epiBL detoxified the stress generated by NaCl and/or $NiCl_2$ and significantly improved growth, the level of pigments and photosynthetic parameters (Ali et al., 2008b). After foliar application of brassinolide on tomato plants an increase in metals (P, K, Ca and Mg) in aerial parts of plants has been recorded (Nafie & El-Khallal, 2000). Our three-year results showed that after the brassinosteroids treatment of spring wheat some changes of the metals content were determined. However, these changes differed among the experimental years. Brassinosteroids application primarily affected content of K, Mg, Zn and Fe in grain. However, it did not affect Cu content. Brassinosteroids stimulate morphogenesis of plants which causes an increase in leaf area, number of leaves, dry and fresh mass of stems and roots and number of tillers and productive branches. Due to these effects on physiological processes in plants, an increase in the yield and quality of crops production has been observed (Sakurai et al., 1999). Yield increase depends on variety, climatic conditions, soil, application of fertilizers and also on frequency and dates of brassinosteroids application (Khripach et al., 2000, 2003; Janeczko et al., 2010). Different preparations (mixtures of natural 24-epibrassinolide and its synthetic isomers) especially used under unfavourable cultivation conditions cause an increase in yield of crops such as rice, maize, wheat, cotton, tobacco, vegetables and fruit. Exogenous brassinosteroids such as 24-epibrassinolide influences brassinosteroid balance in seedlings of wheat after soaking seeds, drenching or spraying plants and content of endogenous brassinosteroids brassinolide and castasterone varies with leaf insertion and plant age (Janeczko & Swaczynowá, 2010). The relative effects of brassinosteroids may be low, when the conditions under which plants are growing are generally favourable (Khripach et al., 2000). Treatment of barley cultivated in light-textured clay podzolic soil with brassinosteroids in a combination with nitrogen-phosphorus-potassium fertilizer (dose 60 kg N ha^{-1}) increased grain yield by 360 kg ha^{-1}; content of total protein in grain was not affected. However, in our experiments, where NPK fertilization at a dose of 60 kg N ha^{-1} was applied, no significant increase of grain yield per hectare has been proved. However, the application of brassinosteroids could reduce the negative effect of the stress factors on the yield and dry matter in wheat (Hnilička et al., 2007, Bajguz, 2009). In a greenhouse experiment with exogenously applied 24-epibrassinolide on two hexaploid wheat (*Triticum aestivum* L.) cultivars, S-24 (salt tolerant) and MH-97 (moderately salt sensitive), the application of 24-epibrassinolide increased plant biomass and leaf areal per plant of both cultivars under non-saline conditions. However, under saline conditions improvement in growth due to foliar application of 24-epibrassinolide was observed only in salt tolerant cultivar (Shahbaz et al., 2008). Drought stress and high temperature were found to have a negative effect on the amount of dry matter in the above-ground wheat biomass and the yield of grain and straw. Our results regarding the total protein content are in agreement with the results of experiments with wheat after exogenous plant treatment with 24-epibrassinolide, where as well no difference in soluble protein content between control and treated plants after brassinosteroid treatment was determined (Janeczko et al., 2010). In our experiments, no difference was recorded between treated plants and control plants in other qualitative parameters such as gluten content, sedimentation index and bulk density, which are affected more likely by varietals properties, or in Falling number, which is dependent on the harvest date and weather course during the harvest period.

Enhanced resistance of brassinosteroid-treated plants to extreme temperature, salt, pathogens and environmental stresses (heavy metals) was reported by Krishna (2003). The present study revealed the effect of brassinossteroids treatment on the accumulation of Cd, Cu, Pb and Zn contents in aerial wheat biomass or grains. The obtained results are in agreement with the results of Bajguz (2000), who observed that 24-epiBL at the concentration of 10^{-8} mol L^{-1} in combination with heavy metals blocked metal accumulation in algal cells. At metal concentrations of 10^{-6} – 10^{-4} mol L^{-1}, a combination with 24-epiBL appeared to have a stronger stimulatory effect on a number of cells than a single metal (a stronger inhibitory effect). The inhibitory effect on metal accumulation of 24-epiBL mixed with different heavy metals was arranged in the following order: zinc > cadmium > lead > copper. Our results obtained for spring wheat as an important crop confirm and are complementary to the results of Sharma & Bhardwaj (2007a, b), which describe the effects of 24-epiBL on plant growth, heavy metals uptake in the plants of *Brassica juncea* L. under heavy metal (Zn, Cu, Mn, Co and Ni) stress. 24-epiBL after the pre-germination treatment blocked copper metal uptake and accumulation in the plants. Likewise results of Anuradha & Rao (2007), obtained in a study on radish (*Raphanus sativus* L.) after the treatment with 24-epiBL and 28-homobrassinolide clearly indicated the inhibitory influence of brassinosteroids on the cadmium toxicity. Brassinosteroids supplementation alleviated the toxic effect of cadmium and increased the percentage of seed germination and seedling growth. Treatment with brassinosteroids regulates and enhances the activities of antioxidant enzymes ascorbate peroxidase, glutathione reductase, catalase, peroxidase and superoxide dismutase (Sharma, I. et al., 2010) and in drought stressed plants proline and protein content (Behnamnia et al., 2009). The application of brassinosteroids at low concentrations at a certain stage of development reduced significantly the metal absorption in barley, tomatoes and sugar beet. Our results indicate that for the decrease of heavy metals content in plants after the brassinosteroids application the growth stage of spring wheat is very important (Figs. 7 and 8).

The present study shows that the content of heavy metals in wheat plants is reduced variously in different growth stages. The plants of the second group and the third group contained in biomass at the growth stage 73–75 DC lower Pb content as compared to control

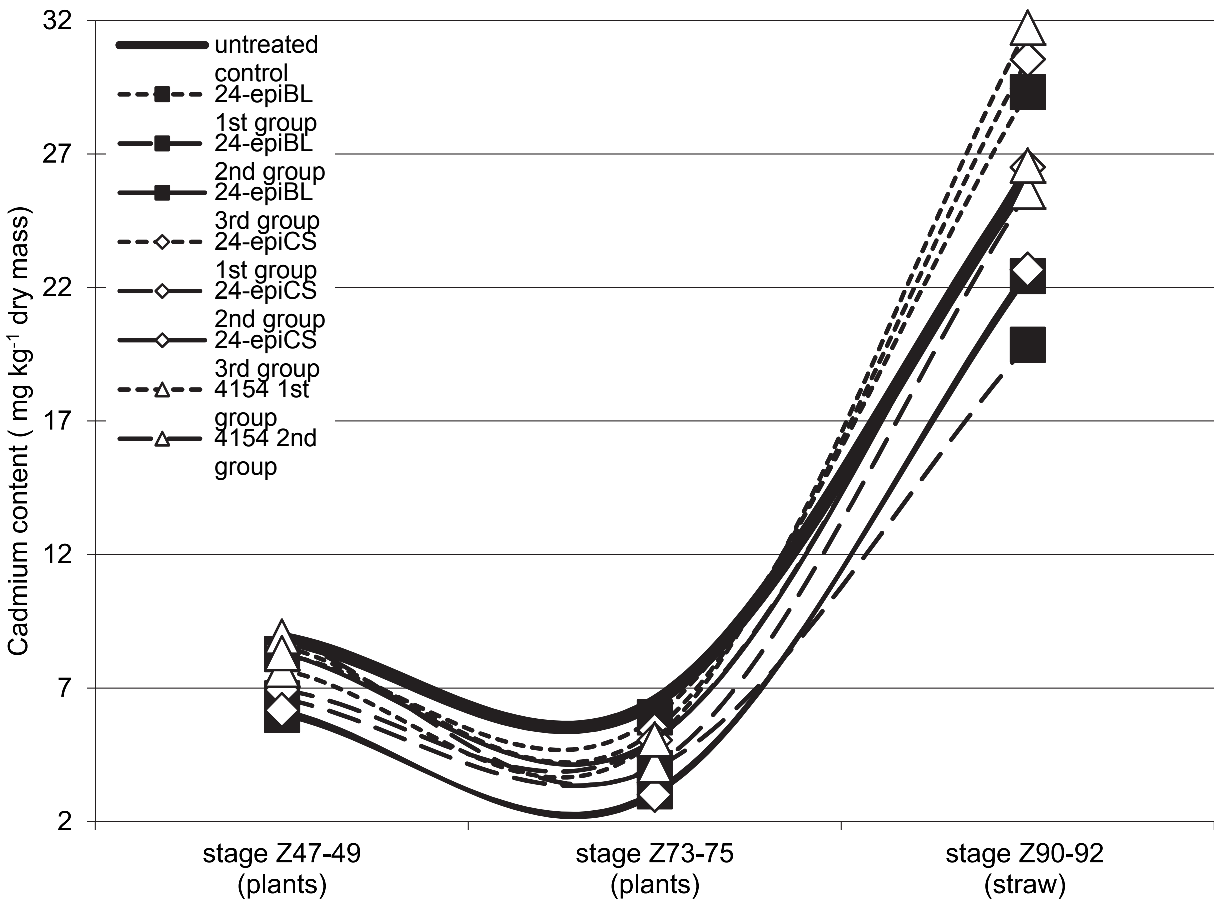


Fig. 7. Cd content in above ground biomass in untreated control and with BRs treated wheat variants; *1st group of plants (pots A-I, B-I, C-I) was treated with brassinosteroids A (24-epibrassinolide), B (24-epicastasterone) and C (4154) once in the growth plant stage according to Zadoks growth scale 29-31 DC (off shooting); 2nd group (pots A-II, B-II, C-II) was treated with brassinosteroids two times, firstly in the plant growth stage 29-31 DC and again in the plant growth stage 59-60 DC (beginning of flowering); 3rd group (pots A-III, B-III, C-III) was treated once in the plant growth stage 59-60 DC (beginning of flowering)

plants and the plants of the first group, which was treated with brassinosteroids last at the growth stage 29 – 31 DC. Also in the plants of the second group and the third group at the growth stage 73 – 75 DC lower Cd and Zn contents were determined (with the exception of brassinosteroid 4154 in the third group). The treatment of wheat plants with brassinosteroids 24-epiBL, 24-epiCS and 4154 at the plant growth stage 29–31 DC did not significantly influence content of the heavy metals in aerial plant biomass at the growth stage 47 – 49 DC. In the straw at the growth stage 90–92 DC, lower Pb and Zn contents were subsequently determined only in the plants treated with 24-epiBL and 24-epiCS (Zn also with the application of 4154 in the second group). Lower Cd content was determined only in the variant treated two times with 24-epiBL, which was considered as a highly active brassinosteroid. Lower Pb content was found in the grains of plants of the second group (treated two times in the stages 29–31 DC and 59–60 DC) and the third group (treated once in the stages 59–60 DC).

In terms of the content of heavy metals related to the number and growth stage of brassinosteroids applications, the most effective variants of treatment leading to decrease of metal content proved either double treatments in the growth stages 29 – 31 DC and 59 – 60 DC (plants of the second group) or one treatment only in the stage 59 – 60 DC (plants of the third group).

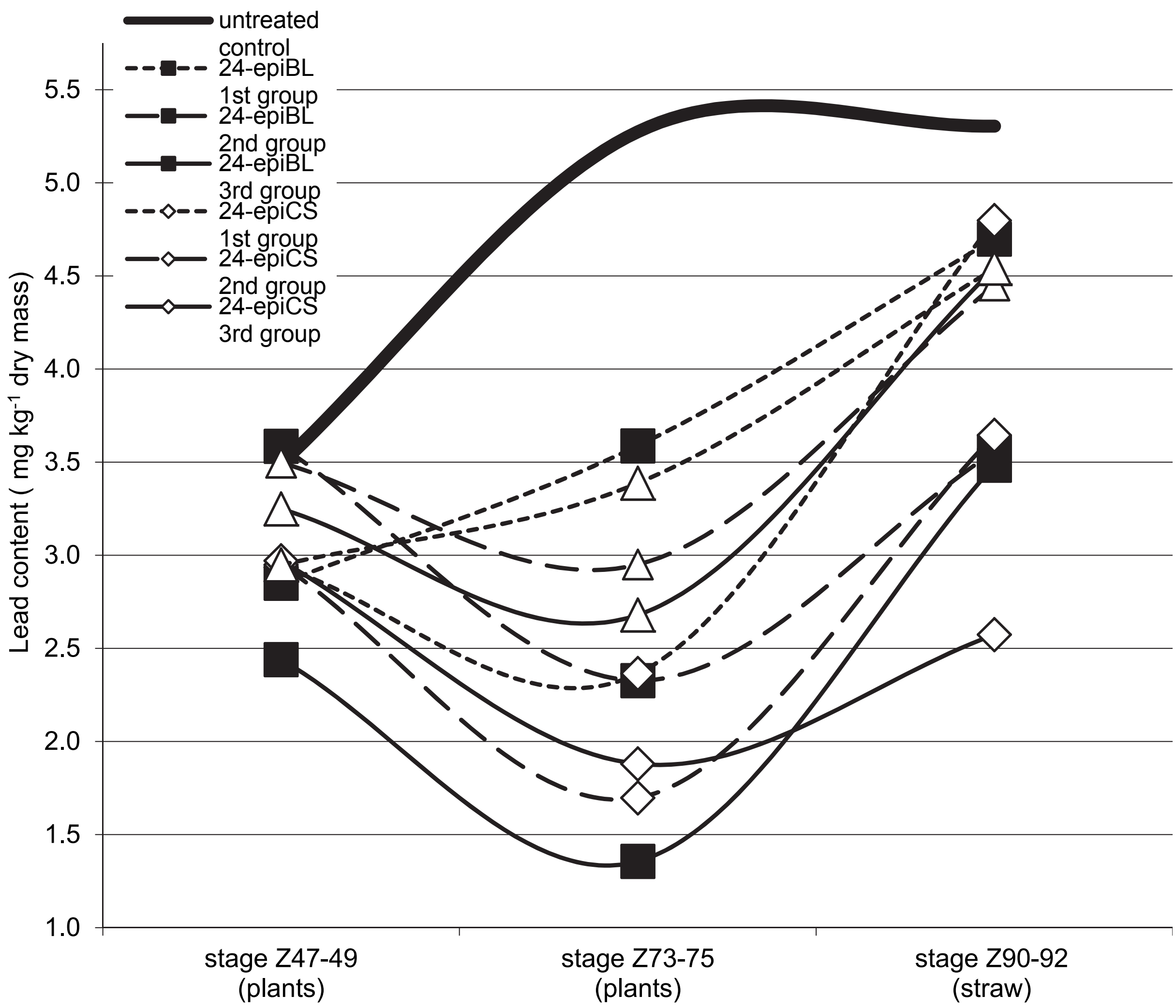


Fig. 8. Pb content in above ground biomass in untreated control and with BRs treated wheat variants (described in Fig. 7)

Brassinosteroids are able to manage plant water economy during a drought period by decreasing plant activity with a simultaneous conservation of the whole plant for more favourable conditions. Brassinosteroid-treated plants are then able to overcome the drought period in a much better condition than non-treated plants (Sasse, 1999). Their increase in net photosynthetic rate due to brassinosteroids application has already been observed in wheat, tomato and cucumber under normal condition and environment stresses (Ogweno et al., 2008; Shabaz et al., 2008; Xia et al., 2009; Yuan et al., 2010, Holá, 2010). Nowadays biological effects not only naturally occurring brassinosteroids, but also their androstane and pregnane analogues are widely synthesised and their biological effects studied (Hniličková et al., 2010) as well as their miscellaneous metabolic pathways in plants involving dehydrogenation, demethylation, epimerization, esterification, glycosylation, hydroxylation, side-chain cleavage and sulfonation (Bajguz, 2007). Because brassinosteroids control several important agronomic traits (Kang & Guo, 2010) such as flowering time, plant architecture, seed yield and stress tolerance, the genetic manipulation of brassinosteroids biosynthesis, conversion or perception offers a unique possibility of both changing plant metabolism and protecting plants from environmental stresses confirming the value of further research on brassinosteroids to improve productivity and quality of agricultural crops (Divi & Krishna, 2009) or their possible use for phytoremediation application (Barbafieri & Tassi, 2010).

5. Conclusion

From the perspective of minimal heavy metals content in biomass and grains related to the number of treatments and growth stage the most effective options of application of brassinolide treatment are those, which lead to a reduction in heavy metals in biomass: either dual treatment in growth stages DC 29-31 and DC 59-60 or single treatment only in the DC 59-60. Favourable is effective reduction of the content of heavy metals in the biomass of plants in grain milk stage (DC 73-75). After treatment of plants with brassinosteroids, when the plants are harvested for ensilage, the content of toxic metals was effectively reduced. Thus, treatment of plants with brassinosteroids can effectively reduce the content of heavy metals in plants (Cd and Pb) or harvested grain (Pb) of wheat and reduce the input of these contaminants into the food chain either cereal or meat products from the food industry. From the point of view of final effect on the content of the heavy metals in plant biomass and grains, the most suitable variant appears to be the single treatment in the growth stage 59–60 DC, which is economically preferable and its final effect does not differ remarkably from double treatments. Likewise lead content in grains decreased in the plants of the second group by 70–74% and of the third group by 48–70%. Thus, treatment of plants with brassinosteroids effectively decreased content of cadmium and lead in wheat plants (biomass) and content of lead in harvested grain and diminished in such way the input of these contaminants into the food chain.

Changes in the minerals content differed according to used brassinosteroid (variant) and investigated year; however unambiguous tendencies of changes or effects were not recorded. In comparison with control plants in the year 2005 the content of minerals in grain of treated plants did not differed significantly. In the year 2006 an increase of K after treatment with 24-epiBL, 4154 and KR1 compounds and a decrease of Zn content after treatment with 24-epiCS and KR1 compounds were recorded. In the year 2007 a decrease of Mg, Mn and Fe content was determined.

Similarly grain quality was not affected by the treatment with brassinosteroids in the investigated years. Content of proteins and gluten in the grains of treated and untreated plants was not significantly different. Similar results were obtained in the sedimentation index and bulk density. Falling number values differed depending on the date of harvest and year of cultivation; in comparison with control plants no difference was recorded. The hypothesis presented is that utilisation of brassinosteroids for plant treatment in the methods of agricultural management with a normal (rational) level of agricultural engineering is not effective. However, by contrast, their application could represent a high economic gain in all cases where the conditions for the cultivation of cereals are not quite ideal, e.g. under conditions of action of different environmental plant stressors, especially with cultivation on soils contaminated with heavy toxic metals or in different arrangements of agricultural engineering. The brassinosteroids-induced enhancement of photosynthetic capacity and regulation of antioxidant enzymes or growth could be under stress factors such saline conditions cultivar specific.

6. Acknowledgment

This study was supported by a grant project of the Ministry of Education, Youth and Sport *MSM 6046070901* of the Czech Republic and the Ministry of Agriculture of the Czech Republic *NAZV QH92111.*

7. References

Ali, A.A. & Abdel-Fattah, R.I. (2006). Osmolytes-antioxidant behaviour in *Phaseolus vulgaris* and *Hordeum vulgare* with brassinosteroid under salt stress. *Journal of Agronomy,* Vol. 5, No.1, (March 2006), pp. 167-174, ISSN 1812-5379

Ali, B., Hayat, S., Aiman-Hasan, S. & Ahmad, A. (2006). Effect of root applied 28-homobrassinolide on the performance of *Lycopersicon esculentum*. *Scientia Horticulturae,* Vol.110, No.3, (November 2006), pp. 267-273, ISSN 0304-4238

Ali, B., Hasan, S.A., Hayat, S., Hayat, Q., Yadav, S., Fariduddin, Q. & Ahmad, A. (2008). A role for brassinosteroids in the amelioration of aluminium stress through antioxidant system in mung bean (*Vigna radiata* L. Wilczek). *Environmental and Experimental Botany,* Vo.62, No.2, (March 2008), pp. 153-159, ISSN 0098-8472

Ali, B., Hayat, S., Ahmad, A., 2007. 28-Homobrassinolide ameliorates the saline stress in chickpea (*Cicer arietinum* L.). *Environmental and Experimental Botany,* Vol.59, No.2, (March 2007), pp. 217–223, ISSN 0098-8472

Anuradha, S. & Rao, S.S.R. (2001). Effect of brassinosteroids on salinity stress induced inhibition of seed germination and seedling growth of rice (*Oryza sativa* L.). *Plant Growth Regulation,* Vol. 33, No.2, (February 2001), pp. 151–153, ISSN 0167-6903

Anuradha, S. & Rao, S.S.R. (2007). The effect of brassinosteroids on radish (*Raphanus sativus* L.) seedlings growing under cadmium stress. *Plant, Soil and Environment,* Vol.53, No.11, (November 2007), pp. 465-472, ISSN 1214-1178

Bajguz, A. (2000). Blockage of heavy metals accumulation in *Chlorella vulgaris* cells by 24-epibrassinolide. *Plant Physiology and Biochemistry,* Vol.38, No.10, (October 2000), pp. 797-801, ISSN 0981-9428

Bajguz, A. (2007). Metabolism of brassinosteroids in plants. *Plant Physiology and Biochemistry,* Vol. 45, No.2, (February 2007), pp. 95-107, ISSN 0981-9428

Bajguz, A. (2009). Effect of brassinosteroids on the plant responses to environmental stresses. *Plant Physiology and Biochemistry,* Vol.47, No.1, (January 2009), pp. 1-8, ISSN 0981-9428

Bajguz, A. (2010). Brassinosteroids – occurrence and chemical structures in plants. In: *Brassinosteroids: A Class of Plant Hormone,* S. Hayat & A. Ahmad, (Eds.), 1-28, Springer Science + Business Media B.V., ISBN 978-007-0188-5, Heidelberg, Germany

Bajguz, A. & Godlewska-Zylkiewicz, B. (2004). Protective role of 20-hydroxyecdysone against lead stress in *Chlorella vulgaris* cultures. *Phytochemistry,* Vol.65, No.6, (March 2004), pp. 711-720, ISSN 0031-9422

Bajguz, A. & Tretyn A. (2003). The chemical structures and occurrence of brassinosteroids in plants. In: *Brassinosteroids. Bioactivity and Crop Productivity*, S. Hayat & A. Ahmad, (Eds.), 1-44, Kluwer Academic Publishers, ISBN 1-4020-1710-3, Dordrecht, The Netherlands

Barbafieri, M. & Tassi, E. (2010). Brassinosteroids for phytoremediation application. In: *Brassinosteroids: A Class of Plant Hormone,* S. Hayat & A. Ahmad, (Eds.), 403-438, Springer Science + Business Media B.V., ISBN 978-007-0188-5, Heidelberg, Germany

Behnamnia, M., Kalantari, K.M. & Ziaie, J. (2009). The effects of brassinosteroid on the induction of biochemical changes in *Lycopersicon esculentum* under drought stress. *Turkish Journal of Botany,* Vol.33, No.6, pp. 417-428, ISSN 1300-008X

Cao, S.Q., Xu, Q.T., Cao, Y.J., Qian, K., An, K., Zhu, Y., Hu, B.Z, Zhao, H.F. & Kuai, B.K. (2005). Loss-of- function mutation in DET2 gene lead to an enhanced resistance to oxidative stress in *Arabidopsis*. *Physiologia Plantarum,* Vol.123, No.1, (January 2005), pp. 57-66, ISSN 0031-9317

Cutler, G.C. (1991). Brassinosteroids through the looking glass: An appraisal. In: *Brassinosteroids: Chemistry, Bioactivity and Application,* H.G., Cutler, T., Yokota, G., Adam (Eds.), 155-163, ACS Symposium Ser., Vol. 474, American Chemical Society, ISBN 0-8412-2126-X, Washington, DC, USA

Divi, U.K. & Krishna, P. (2009). Brassinosteroid: a biotechnological target for enhancing crop yield and stress tolerance. *New Biotechnology,*Vol.26, No.3-4, Sp.Iss.2, (October 31, 2009), pp. 131-136, ISSN 1871-6784

Fujii, S. & Saka, H. (2001). Distribution of assimilates to each organ in rice plants exposed to low temperature at the ripening stage and the effect of brassinolide on the distribution. *Plant Production Science,* Vol.4, No.2 (June 2001), pp. 136-144, ISSN 1343-943X

Grove, M.D., Spencer, G.F., Rohwedder, W.K., Mandava, N., Worley, J.F., Warthen, J.D., Steffens, G.L., Flippen-Anderson, J.L. & Cook, J.C. (1979). Brassinolide, a plant growth-promoting steroid isolated from *Brassica napus* pollen. *Nature,* Vol.281, No.5728, pp. 216-217, ISSN 0028-0836

Hathout, T.A. (1996). Salinity stress and its counteraction by the growth regulator „brassinolide"in wheat plants (*Triticum aestivum* L. cultivar Giza 157). *Egyptian Journal of Physiological Sciences,* Vol.20, No.1-2, (December 1996), pp. 127-152, ISSN 0301-8660

Hayat, S., Ali, B. & Ahmad, A. (2006). Response of *Brassica juncea* to 28-homobrassinolide grown from the seeds exposed to salt stress. *Journal of Plant Biology,* Vol.33, No.1&2, (April 2006), pp.169–174, ISSN 1226-9239

Hayat, S., Ali, B., Hasan, S.A. & Ahmad, A. (2007a). Brassinosteroid enhanced the level of antioxidants under cadmium stress in *Brassica juncea*. *Environmental and Experimental Botany,* Vol.60, No.1, (May 2007), pp. 33-41, ISSN 0098-8472

Hayat, S., Ali, B., Hasan, S.A., Ahmad, A. (2007b). Effect of 28-homobrassinolide on salinity-induced changes in *Brassica juncea*. *Turkish Journal of Biology,* Vol.31, No.3, (August 2007), pp. 141-146, ISSN 1010-7576

Hnilička, F., Hniličková, H., Martínková, J. & Bláha, L. (2007). The influence of drought and the application of 24-epibrassinolide on the formation of dry matter and yield in wheat. *Cereal Research Communications,* Vol.35, No.2, (April 30-May 05, 2007), pp. 457-460, ISSN 0133-3720

Hniličková, J., Kohout, L., Capdevila, E., Esteve, A., Vilaplana, M., Molist, M., Brosa, C., Swaczynowá-Oklestková, J. & Slavíková, B. (2010). The synthesis of androstane analogues with α-azido acid ester groups in position 17β. *Steroids,* Vol. 75, No.12, (December 2010), pp. 1005-1010, ISSN 0039-128X

Holá, D. (2010). Brassinosteroids and photosynthesis. In: *Brassinosteroids: A Class of Plant Hormone,* S. Hayat & A. Ahmad, (Eds.), 143-192, Springer Science + Business Media B.V., ISBN 978-007-0188-5, Heidelberg, Germany

Janeczko, A. & Swaczynowá, J. (2010). Endogenous brassinosteroids in wheat treated with 24-epibrassinolide. *Biologia Plantarum,* Vol.54, No.3, (September 2010), pp. 477-482 ISSN 0006-3134

Janeczko, A., Biesaga-Koscielniak, J., Oklesťkova, J., Filek, M., Dziurka, M., Szarek-Lukaszewska, G. & Koscielniak, J. (2010). Role of 24-epibrassinolide in wheat production: physiological effects and uptake. *Journal of Agronomy and Crop Science,* Vol. 196, No.4, (August 2010), pp. 311-321, ISSN 0931-2250

Janeczko, A., Koscielniak, J., Pilipowicz, M., Szarek-Lukaszewsa & G., Skoczowski, A. (2005). Protection of winter rape photosystem 2 by 24-epibrassinolide under cadmium stress. *Photosynthetica,* Vol.43, No.2, pp. 293-298, ISSN 0300-3604

Kagale, S., Divi, U.K., Krochko, J.E., Keller, W.A. & Krishna, P. (2007). Brassinosteroids confers tolerance in *Arabidopsis thaliana* and *Brassica napus* to a range of abiotic stresses. *Planta,* Vol.225, No.2, (January 2007), pp. 353-364, ISSN 0032-0935

Kamlar, M., Uhlík, O., Kohout, L., Harmatha, J & Macek, T. (2010). Steroid Phytohormones: Function, Mechanism of Action, Significance. *Chemické listy,* Vol.104, No.2, (February 2010), pp. 93-99, ISSN 0009-2770

Kang, Y.Y. & Guo, S.R. (2010). Role of brassinosteroids on horticultural crops. In: *Brassinosteroids: A Class of Plant Hormone,* S. Hayat & A. Ahmad, (Eds.), 269-288, Springer Science + Business Media B.V., ISBN 978-007-0188-5, Heidelberg, Germany

Khripach, V.A., Zhabinskii, V.N. & de Groot, A.E. (1999). *Brassinosteroids - A New Class of Plant Hormones,* Academic Press, ISBN 0-12-406360-8, San Diego, USA

Khripach, V.A., Zhabinskii, V.N. & de Groot, A.E. (2000). Twenty years of brassinosteroids: steroidal plant hormones warrant better crops for the XXI century. *Annals of Botany,* Vol.86, No.3, (June 2000), pp. 441-447, ISSN 0305-7364

Khripach, V.A., Zhabinskii, V.N & Khripach, N.B. (2003). New practical aspects of brassinosteroids and results of their ten-year agricultural use in Russia and Belarus. In: *Brassinosteroids, Bioactivity and Crop Productivity,* S., Hayat & A. Ahmad, (Eds.), 189-230, Kluwer Academic Publishers, ISBN 1-4020-1710-3, Dordrecht, The Netherlands

Krishna, P. (2003). Brassinosteroid-mediated stress responses. *Journal of Plant Growth Regulation,* Vol.22, No.4, (December 2003), pp. 289-297, ISSN 0721-7595

Kroutil, M., Hejtmánková, A. & Lachman, J. (2010a). Effect of spring wheat (*Triticum aestivum* L.) treatment with brassinosteroids on the content of cadmium and lead in plant aerial biomass and grain. *Plant, Soil and Environment,* Vol.56, No.1, (January 2010) pp. 43-50, ISSN 1214-1178

Kroutil, M., Hradecká, D., Kohout, L. & Lachman, J. (2010b). Quality and selected metals content of spring wheat (*Triticum aestivum* L.) grain after the treatment with brassinosteroids during cultivation. *Scientia Agriculturae Bohemica,* Vol.41, No.2, (June 2010), pp. 65-72, ISSN 0582-2343

Kulaeva, O.N., Burkhanova, E.A., Fedina, A.B., Khokhlova, V.A., Bokebayeva, G.A., Vorbrodt, H.M. & Adam, G. (1991). Effect of brassinosteroid on protein synthesis and plant-cell ultrastructure under stress condition. In: *Brassinosteroids: Chemistry, Bioactivity and Application,* H.G., Cutler, T., Yokota, G., Adam (Eds.), 155-163, ACS Symposium Ser., Vol. 474, American Chemical Society, ISBN 0-8412-2126-X, Washington, DC, USA

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,* Vol.26, No.3, (April 1998), pp. 121-129, ISSN 0365-4877

Nafie, E.M. & El-Khallal, S.M. (2000). Effect of brassinolide application on growth certain metabolic activities and yield of tomato. *Egyptian Journal of Physiological Sciences,* Vol.24, No.1, (January 2000), pp. 103-117, ISSN 0301-8660

Ogweno, J.O., Song, X.S., Shi, K., Hu, W.H., Mao, W.H., Zhou, Y.H., Yu, J.Q. & Nogues, S. (2008). Brassinosteroids alleviate heat-induced inhibition of photosynthesis by increasing carboxylation efficiency and enhancing antioxidant systems in *Lycopersicon esculentum*, *Journal of Plant Growth Regulation,* Vol.27, No.1, (March 2008), pp. 49–57, ISSN 0721-7595

Sakurai, A., Yokota, T. & Clouse, S.D. (1999). *Brassinosteroids - Steroidal Plant Hormones.* Springer-Verlag, Springer-Verlag, ISBN 4-431-70214-8, Tokyo, Japan

Sasse, J.M. (1999). Physiological actions of brassinosteroids. In: *Brassinosteroids – Steroidal Plant Hormones,* A., Sakurai, T., Yokota & S.D., Clouse (Eds.), 137-155, Springer-Verlag, ISBN 4-431-70214-8, Tokyo, Japan

Schilling, G., Schiller, C. & Otto, S. (1991). Influence of brassinosteroids on organ relations and enzyme activities of sugar beet plants, In: *Brassinosteroids. Chemistry, Bioactivity, and Application*, H.G., Cutler, T., Yokota, G. & Adam, (Eds.), 208-219, ACS Symposium Ser., Vol. 474, American Chemical Society, ISBN 0-8412-2126-X, Washington, DC, USA

Shahbaz, M. & Ashraf, M. (2007). Influence of exogenous application of brassinosteroid on growth and mineral nutrients of wheat (*Triticum aestivum* L.) under saline conditions. *Pakistan Journal of Botany,* Vol.39, No.2, (April 2007), pp. 513-522, ISSN 0556-3321

Shahbaz, M., Ashraf, M. & Athar, H.R. (2008). Does exogenous application of 24-epibrassinolide ameliorate salt induced growth inhibition in wheat (*Triticum aestivum* L.)? *Plant Growth Regulation,* Vol. 55, No.1, (January 2008), pp. 51-64 ISSN 0167-6903

Sharma, I., Pati, P.K. & Bhardwaj, R. (2010). Regulation of growth and antioxidant enzyme activities by 28-homobrassinolide in seedlings of Raphanus sativus L. under cadmium stress. *Indian Journal of Biochemistry & Biophysics,* Vol.47, No.3, (June 2010), pp. 172-177, ISSN 0301-1208

Sharma, P. & Bhardwaj, R. (2007a). Effects of 24-epibrassinolide on growth and metal uptake in *Brassica juncea* L. under copper metal stress. *Acta Physiologiae Plantarum,* Vol.29, No.3, (June 2007), pp. 259-263, ISSN 0137-5881

Sharma, P. & Bhardwaj, R. (2007b). Effect of 24-epibrassinolide on seed germination, seedling growth and heavy metal uptake in *Brassica juncea* L. *General and Applied Plant Physiology,* Vol.33, No.1-2, (June 2007), pp. 59-73, ISSN 1312-8183

Sharma, P., Bhardwaj, R., Arora, N. & Arora, H.K. (2007c). Effect of 28-homobrassinolide on growth, zinc metal uptake and antioxidative enzyme activities in *Brassica juncea* L. seedlings. *Brazilian Journal of Plant Physiology,* Vol.19, No.3, (July-September 2007), pp. 203-210, ISSN 1677-0420

Takematsu, T., Takeuchi, Y. & Choi, C.D. (1986). Effects of brassinosteroids on growth and yields of crops. In: *Brassinosteroids: Steroidal Plant Hormones,* A., Sakurai, T., Yokota & S.D., Clouse (Eds.), 137-161, Springer-Verlag, ISBN 4-431-70214-8, Tokyo, Japan

Upreti, K.K. & Murti, G.S.R. (2004). Effects of brassinosteroids on growth, nodulation, phytohormone content and nitrogenase activity in French bean under water stress. *Biologia Plantarum,* Vol.48, No.3, (September 2004), pp. 407-411, ISSN 0006-3134

Vardhini, B.V. & Rao, S.S.R. (2002). Acceleration of ripening of tomato pericarp discs by brassinosteroids. *Phytochemistry,* Vol.61, No.7, (December 2002), pp. 843-847, ISSN 0031-9422

Vlašánková, E., Kohout, L., Klemš, M., Eder, J., Reinöhl, V. & Hradilík, J. (2009). Evaluation of biological activity of new synthetic brassinolide analogs. *Acta Physiologiae Plantarum,* Vol. 31, No.5, (September 2009), pp. 987-993 ISSN 0137-5881

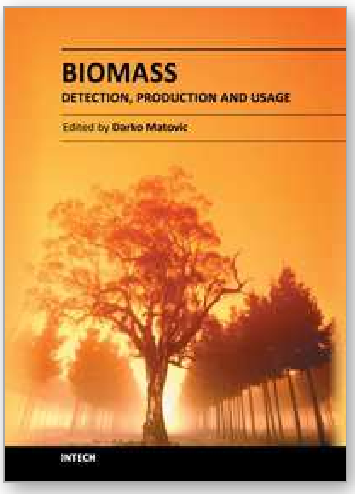
Worley, J.F. & Mitchell, J.W. (1971). Growth responses induced by brassins (fatty plant hormones) in bean plants. *Journal of the American Society for Horticultural Science,* Vol.96, No.3, pp. 270-273, ISSN 0003-1062

Xia, X.J., Huang, L.F., Zhou, Y.H., Mao, W.H., Shi, K., Wu, J.X., Asamim, T., Chen, Z.X. & Yu, J.Q. (2009). Brassinosteroids promote photosynthesis and growth by enhancing activation of Rubisco and expression of photosynthetic genes in *Cucumis sativus, Planta,* Vol.230, No.6, (November 2009), pp. 1185–1196, ISSN 0032-0935

Yuan, G,F., Jia, C.G., Li, Z., Sun, B., Zhang, L.P., Liu, N. & Wang, Q.M. (2010). Effect of brassinosteroids on drought resistance and abscisic acid concentration in tomato under water stress. *Scientia Horticulturae,* Vol.126, No.2, (September 2010), pp. 103-108, ISSN 0304-4238

Zadoks, J.C., Chang, T.T. & Konzak, C.F. (1974). A decimal code for the growth stages of cereals. *Weed Research,* Vol.14, No.6, pp. 415-421, ISSN 0043-1737

Zulo, M.A.T. & Adam, Q. (2002). Brassinosteroid phytohormones – structure, bioactivity and applications. *Brazilian Journal of Plant Physiology,* Vol.14, No.3, (September-December 2002), pp. 143-181, ISSN 1677-0420



Biomass - Detection, Production and Usage

Edited by Dr. Darko Matovic

ISBN 978-953-307-492-4

Hard cover, 496 pages

Publisher InTech

Published online 09, September, 2011

Published in print edition September, 2011

Biomass has been an intimate companion of humans from the dawn of civilization to the present. Its use as food, energy source, body cover and as construction material established the key areas of biomass usage that extend to this day. Given the complexities of biomass as a source of multiple end products, this volume sheds new light to the whole spectrum of biomass related topics by highlighting the new and reviewing the existing methods of its detection, production and usage. We hope that the readers will find valuable information and exciting new material in its chapters.

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821