QUALITY AND SELECTED METALS CONTENT OF SPRING WHEAT (*TRITICUM AESTIVUM* L.) GRAIN AFTER THE TREATMENT WITH BRASSINOSTEROIDS DURING CULTIVATION*

M. Kroutil[1], D. Hradecká[2], L. Kohout[3], J. Lachman[1]

Czech University of Life Sciences, Faculty of Agrobiology, Food and Natural Resources,
[1]Department of Chemistry, [2]Department of Plant Production, Prague, Czech Republic
[3]Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Department of Steroid Chemistry, Prague, Czech Republic

Spring wheat (*Triticum aestivum* L.) variety Vánek was cultivated on 10 m^2 trial field plots on brown loamy soil during period 2005–2007. Plants were treated with eight different types of brassinosteroids (24-epibrassinolide; 24-epicastasterone; compound 4154 and five androstane and pregnane analogues) at growth phase 49–59 DC (from visible awns to complete inflorescence emergence). The content of metals (Ca, Cu, Fe, K, Mg, Mn and Zn), yield and quality parameters of wheat grain were determined. Our three-year results showed that after the BRs treatment of spring wheat some changes of the metals content were determined. However, these changes differed among the experimental years. Grain quality, grain yield per hectare and TGW were not affected by the treatment with brassinosteroids in the investigated years.

brassinosteroids; spring wheat; metals; Falling number; protein content; gluten content; Zeleny sedimentation test

INTRODUCTION

Brassinosteroids (BRs) are natural plant polyhydroxysteroids supporting plant growth; their structure resembles animal steroid hormones. Brassinosteroids were classified as essential plant hormones nearly 20 years after the discovery of brassinolide, the first brassinosteroid (Grove et al., 1979) in the rape (*Brassica napus* L.) pollen. Presence of brassinosteroids was demonstrated in many species including higher and lower plants in various parts, e.g. pollen, seeds, leaves, stems, roots and flowers (Sakurai et al., 1999). In 2003, 70 compounds of the class of brassinosteroids have been characterized, among them 65 in free form and 5 conjugated (Bajguz, Tretyn, 2003). Brassinosteroids are phytohormones with pleiotropic effects. They influence growth, seed germination, cell elongation, morphogenesis and senescence (Upreti, Murti, 2004). In relation to the growth and growth regulators, the typical effect of BRs is coincidental elicitation of cell prolongation and division (Worley, Mitchell, 1971). Investigations confirm the ability of brassinosteroids to affect quantitatively plant morphogenesis which leads to yield increase by the enhancement of number and growth of productive lateral shoots and branches and thereby also to the enhancement of number of spikes, pods etc. (Sakurai et al., 1999). An increase of the metals content in aerial plant biomass was demonstrated following the treatment with BRs (Pirogovskaya et al., 1996; Nafie, El-Khallal, 2000; Ageeva et al., 2001). Brassinosteroids can affect quality of plant products. Treatment with brassinosteroids during anthesis increased starch content in rice kernels (Fujii, Saka, 2001); it increased the content of fatty acids in barley ectoplasts and the change of their rate at tillering stage application (Khripach et al., 1999).

BRs also help to overcome stresses provoked by low (Jiang, Wang, 1996) and high (Kulaeva et al., 1991) temperature, drought (Schilling et al., 1991), salt (Hathout, 1996; Anuradha, Rao, 2001; Ali, Abdel-Fattah, 2006; Ali et al., 2007), infection (Krishna, 2003), pesticides (Cutler, 1991) and heavy metals (Volynets et al., 1997; Bajguz, 2000; Janeczko et al., 2005; Cao et al., 2005; Sharma, Bhardwaj, 2007a, b; Kagale et al., 2007; Ali et al., 2008a; Bajguz, Hayat, 2009).

Results of many laboratory and model experiments with BRs treatment indicate their ability to affect quality and yield of plant production. The aim of this work was to evaluate the ability of BRs to affect the quality parameters of spring wheat grain (bulk density, falling number, protein content, gluten content and Zeleny sedimentation index), change of the content of metals in grain and the yield increase of spring wheat cultivated under real field conditions after the treatment with BRs.

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

MATERIAL AND METHODS

Plant material and conditions of cultivation

In the three-year period 2005–2007 spring wheat (*Triticum aestivum* L.) variety Vánek (maintenance of variety: Lochow-Petkus, GmbH, Germany, producer: Selekta, Inc., Czech Republic) was cultivated at 10 m^2 trial field plots (50°2’0”N, 14°36’54”E) on brown loamy soil. There were sowed 217 kg of seeds per hectare. Broad bean was cultivated as a foregoing crop on the trial field before wheat plants every year. Before wheat sowing, the field was fertilized with dose 60 kg N ha^{-1}, 45.9 kg P ha^{-1} and 45.9 kg K ha^{-1} with a combined nitrogen-phosphorus-potassium fertilizer. Average content of metals in trial field soil is given in Table 1.

Brassinosteroids (BRs) and their treatment pattern

Plants were treated with eight different brassinosteroids (24-epibrassinolide; 24-epicastasterone; compound 4154 and five androstane and pregnane analogues of brassinosteroids marked KR1, KR2, KR3, KR4 and KR5*)* at 49–59 DC (from visible awns to complete inflorescence emergence) growth phase referred to a decimal code for the growth stages of cereals (Z a d o k s et al., 1974). All brassinosteroids were applied in the form of 10^{-6} mol L^{-1} of efficient compound in the solution by spraying on all aerial biomass. Each of the tested brassinosteroids (see below) 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 synthesised 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 in the EU (Nr. 1401278, conferred on 28 Sept. 2005). Compounds KR1–KR5 are synthetic BRs, permanently studied androstane and pregnane analogues, which do not occur naturally in plants.

Harvest and grain sampling

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 the methodology ISO 13690:1999 by quartering of cleaned grain. There were prepared one common (average) analytical sample of four trial plots grain.

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) was used. Muffle oven (LM 112.10, MLW, Germany), heating plate ALTEC JRT 350 with temperature graduation per 10 °C and ultrasonic bath

Table 1. Average content of minerals in field trial soil (mg kg^{-1} DM)

Depth of mould	N (NO_3^-)	N (NH_4^+)	N (total)	K	Mg	Ca	P	pH_{KCl}
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 ± 0.10
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 ± 0.10

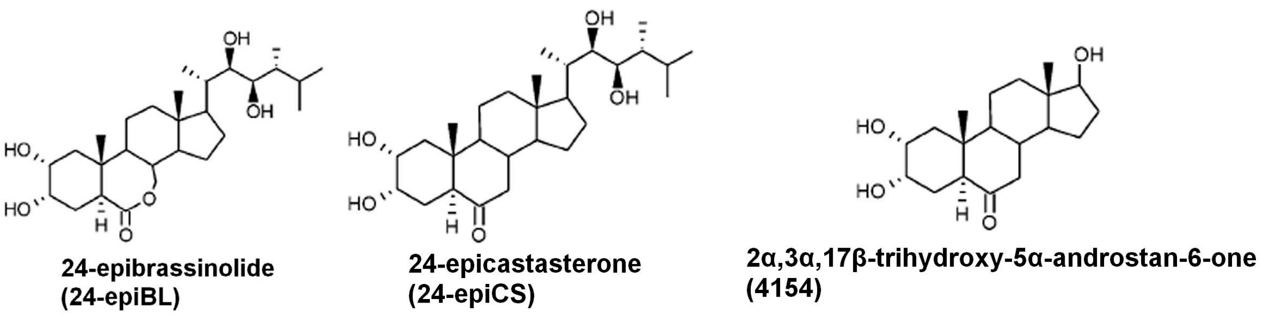

Fig. 1. Chemical structure of brassinosteroids used for wheat treatment

Elma Transonic T660/H were used for the dry decomposition of samples. Analyses of the metals were performed with atomic spectrometer VARIAN SpectrAA 110 (VARIAN A.G., Australia) with the possibility to measure emission spectra.

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).

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 (M e h l i c h , 1984; Z b í r a l , 2000) and organic nitrogen by the Kjeldahl method (B r e m n e r , 1960).

Dry decomposition procedure

Samples of grain were before analysis thermally decomposed. For the dry decomposition, SOP-3C (Standard Operation Procedure for Dry Decomposition of Higher Plants and Green Algae) was used (M a d e r et al., 1998). Samples of grain were prior to the dry decomposition coarsely ground in the IKA A11 Basic mill equipped with stainless steel working parts. Weight of homogenized sample was about 1 g and each sample was analyzed in three replicates. Initial temperature of thermal decomposition on the heater plate was 150 °C, final temperature 350 °C. After coaling the samples were combusted in a muffle oven at 480 °C. Resulting ash was dissolved in 1.5 mL conc. HNO_3 (65% p.p.) and then repeatedly combusted at 480 °C. After the combustion, the samples (decomposed to white ash) were dissolved in 5 mL 1.5% HNO_3 after addition of 1 mL conc. HNO_3 (65% p.p.).

F-AAS determination (flame atomic absorption spectrometry) of metals

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. LOD and LOQ of the metals determination are given in Table 6.

Determination of wheat grain dry weight

Dry matter of grain samples was determined in a laboratory oven by drying at 130 °C to constant weight (methodology ISO 712:1998).

Determination of wheat grain quality

Protein (N x 5.70) content was determined by Kjeldahl method by an 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 to ISO 5529:1992. Determination of bulk density, called "mass per hectoliter" was performed according to ISO 7971-2:1995.

Statistical analysis and replicates

Each brassinosteroid was applied in four replicates on field plots. All results obtained by the analyses of grain harvested from different plots were statistically evaluated 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.

RESULTS AND DISCUSSION

Content of Ca, Cu, Fe, K, Mg, Mn and Zn in wheat grain after BRs treatment

Values of metals content in wheat grain in individual years of cultivation are given in Table 2. 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

Table 2. Content of Ca, Cu, Fe, K, Mg, Mn and Zn (mg kg^{-1} DM) in spring wheat grain (analytical samples were made by quartering of cleaned grain, there were prepared one common-average analytical sample of four trial plots grain)

Variant	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*

* statistically significant difference (at the level of significance $p < 0.05$) between treated and untreated plants
Abbreviations of the applied brassinosteroids: 24-epiBL – 24-epibrassinolide, 24-epiCS – 24-epicastasterone, 4154 – compound 4154, KR1–KR5 – pregnane and androstane analogues of brassinosteroids

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 5) in whole three-year period. Mean precipitation in 2005 and 2006 was lower as 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.

Quality of wheat grain after BRs treatment

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; a 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 BRs or untreated. Average values of qualitative parameters of wheat grains in individual years are reported in Table 3.

Yield of wheat grain after BRs treatment

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 deter-

Table 3. Quality parameters of harvested grain after spring wheat treatment with brassinosteroids

Variant	Year	Bulk density	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

No statistically significant differences (at the level of significance $p < 0.05$) between treated and untreated plants were found
For abbreviations of the applied brassinosteroids see Table 2

mined 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 4.

Changes of metal composition of plants treated with BRs were reported in quite a few experiments. Most of these experiments are related to the ability of BRs 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 (B a j g u z , 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 (A l i et al., 2008b). It has been proved that the pre-sowing treatment of spring barley kernels had increased calcium content in roots and aerial parts of plants (A g e e v a et al., 2001). Different distribution of calcium between various parts of the plant has also been shown: at the complete head emergence phase, calcium content was higher in the stem of treated plants and lesser in the leaves as compared to the control. On the other hand, potassium content was higher in leaves as compared to stems. 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 (N a f i e , E l - K h a l l a l , 2000). Our three-year results showed that after the BRs treatment of spring wheat some changes of the metals content were determined. However, these changes differed among the experimental years. BRs application primarily affected content of K, Mg, Zn and Fe in grain. However, it did not affect Cu content.

BRs 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 (S a k u r a i et al., 1999). Yield increase depends on variety, climatic conditions, soil, application of fertilizers and also on frequency and dates of BRs application (Khripach et al., 2000, 2003). 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. The relative effects of BRs 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 (Vildflush et al., 2001). However, our experiments, where NPK fertilization at a dose of 60 kg N ha^{-1} was also used, no significant increase of grain yield per hectare was 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). 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 confirmed the results of experiments with barley (Vildflush et al., 2001) and buckwheat (Prusakova et al., 1999), where as well no difference was determined between control and treated plants after BRs treatment. 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.

Table 4. Yield of grain and thousand-grain weight after spring wheat treatment with brassinosteroids

Variant	Year	Yield (corrected on moisture 14%)	Thousand-grain 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

* statistically significant difference (at the level of significance $p < 0.05$) between treated and untreated plants
For abbreviations of the applied brassinosteroids see Table 2

CONCLUSION

The contents of seven metals in grains of spring wheat (*Triticum aestivum* L.) variety Vánek were determined after the treatment of plants with eight brassinosteroids. Total content of the metals differed between individual years (except for Cu). Changes in the metals content differed according to the used brassinosteroid and investigated year. Thus, unambiguous tendencies of changes or effects were not recorded. The results achieved in the three-year period 2005–2007 showed a possible effect of

Table 5. Long-term values of air temperature and precipitation at the cultivation site (50°2'0"N, 14°36'54"E)

	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 6. LOD and LOQ of the metals determination

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

the year of cultivation on metal content of grain, affected probably by the precipitation level.

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 statistically different. Similar results were obtained for the sedimentation index and bulk density. Falling number values differed depending on the date of harvest and year of cultivation; however, in comparison with control plants no difference was recorded.

As well as in grain quality, no differences in grain yield per hectare and TGW of treated plants related to control were determined (except the variant with 24-epiBL in the year 2005).

The achieved results showed that utilization of brassinosteroids for plant treatment in the methods of real agricultural management with presented level of agricultural technology is not effective.

Abbreviations

24-epiBL – 24-epibrassinolide; 24-epiCS – 24-epicastasterone; ANOVA – Analysis of Variance; BRs – Brassinosteroids; DM – dry matter; F-AAS – Flame Atomic Absorption Spectrometry; RM – Reference Material; TGW – thousand-grain weight

REFERENCES

AGEEVA, L. F. – PRUSAKOVA, L. D. – CHIZHOVA, S. I.: Effects of brassinosteroids on stalk formation and content of calcium and potassium ions in spring barley plants. Agrokhimiya, 2001(6): 49–55.

ALI, B. – ABDEL-FATTAH, R. I.: Osmolytes-antioxidant behaviour in *Phaseolus vulgaris* and *Hordeum vulgare* with brassinosteroid under salt stress. J. Agronom., *5*, 2006: 167–174.

ALI, B. – HASAN, S. A. – HAYAT, S. – HAYAT, Q. – YADAV, S. – FARIDUDDIN, Q. – AHMAD, A.: A role for brassinosteroids in the amelioration of aluminium stress through antioxidant system in mung bean (*Vigna radiata* L. Wilczek). Environ. Exp. Bot., *62*, 2008a: 153–159.

ALI, B. – HAYAT, S. – AHMAD, A.: 28-Homobrassinolide ameliorates the saline stress in chickpea (*Cicer arietinum* L.). Env. Exp. Bot., *59*, 2007: 33–41.

ALI, B. – HAYAT, S. – FARIDUDDIN, Q. – AHMAD, A.: 24-Epibrassinolide protects against the stress generated by salinity and nickel in *Brassica juncea*. Chemosphere, *72*, 2008b: 1387–1392.

ANURADHA, S. – RAO, S. S. R.: Effect of brassinosteroids on salinity stress induced inhibition of seed germination and seedling growth of rice (*Oryza sativa* L.). J. Plant Growth Regul., *33*, 2001: 151–153.

BAJGUZ, A.: Blockage of heavy metals accumulation in *Chlorella vulgaris* cells by 24-epibrassinolide. Plant Physiol. Biochem., *38*, 2000: 797–801.

BAJGUZ, A. – HAYAT, S.: Effects of brassinosteroids on the plant responses to environmental stresses. Plant Physiol. Biochem., *47*, 2009: 1–8.

BAJGUZ, A. – TRETYN, A.: The chemical structures and occurrence of brassinosteroids in plants. In: HAYAT, S., AHMAD, A. (eds.): Brassinosteroids, pp. 1–44. Dordrecht, Kluwer Acad. Publ. 2003.

BREMNER, J. M.: Determination of nitrogen in soil by the Kjeldahl method. J. Agr. Sci., *55*, 1960: 11–33.

CAO, S. Q. – XU, Q. T. – CAO, Y. J. – QIAN, K. – AN, K. – ZHU, Y. – HU, B. Z. – ZHAO, H. F. – KUAI, B. K.: Loss-of-function mutation in DET2 gene lead to an enhanced resistance to oxidative stress in Arabidopsis. Physiol. Plant., *123*, 2005: 57–66.

CUTLER, G. C.: Brassinosteroids through the looking glass: An appraisal. In: CUTLER, H. G. – YOKOTA, T. – ADAM, G. (eds.): Brassinosteroids. Chemistry, Bioactivity, and Application. ACS Symp. Series No. 474, Washington, DC, Am. Chem. Soc., 1991, pp. 334–345.

FUJII, S. – SAKA, H.: Distribution of assimilates to each organ in rice plants exposed to low temperature at the ripening stage and effect of brassinolide on the distribution. Plant Prod. Sci., *4*, 2001: 210–214.

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.: Brassinolide, a plant growth-promoting steroid isolated from *Brassica napus* pollen. Nature, *281*, 1979: 216–217.

HATHOUT, T. A.: Salinity stress and its counteraction by the growth regulator "brassinolide" in wheat plants (*Triticum aestivum* L. cultivar Giza 157). Egypt. J. Physiol. Sci., *20*, 1996: 127–152.

HNILIČKA, F. – HNILIČKOVÁ, H. – MARTÍNKOVÁ, J. – BLÁHA, L.: The influence of drought and the application of 24-epibrassinolide on the formation of dry matter and yield in wheat. Cer. Res. Commun., *35*, 2007: 457–460.

JANECZKO, A. – KOSCIELNIAK, J. – PILIPOWICZ, M. – SZAREK-LUKASZEWSA, G. – SKOCZOWSKI, A.: Protection of winter rape photosystem 2 by 24-epibrassinolide under cadmium stress. Photosynthetica, *43*, 2005: 293–298.

JIANG, Y. W. – WANG, G. J.: The effects of Ca^{2+} and BR on chilling tolerance in maize respiratory. Acta Agric. Bor. Sin., *11*, 1996: 73–76.

KAGALE, S. – DIVI, U. K. – KROCHKO, J. E. – KELLER, W. A. – KRISHNA, P.: Brassinosteroids confers tolerance in *Arabidopsis thaliana* and *Brassica napus* to a range of abiotic stresses. Planta, *225*, 2007: 353–364.

KHRIPACH, V. A. – ZHABINSKII, V. N. – DE GROOT, A. E.: Brassinosteroids: A New Class of Plant Hormones. San Diego, Acad. Press 1999.

KHRIPACH, V. A. – ZHABINSKII, V. N. – DE GROOT, A. E.: Twenty years of brassinosteroids: steroidal plant hormones warrant better crops for the XXI century. Ann. Bot. Fenn., *86*, 2000: 441–447.

KHRIPACH, V. A. – ZHABINSKII, V. N. – KHRIPACH, N. B.: New practical aspects of brassinosteroids and results of their ten-year agricultural use in Russia and Belarus. In: HAYAT, S. – AHMAD, A. (eds.): Brassinosteroids, pp. 189–230. Dordrecht, Kluwer Acad. Publ. 2003.

KRISHNA, P.: Brassinosteroid-mediated stress responses. J. Plant Growth Regul., *22*, 2003: 289–297.

KULAEVA, O. N. – BURKHANOVA, E. A. – FEDINA, A. B., KHOKHLOVA, V. A. – BOKEBAYEVA, G. A. – VORBRODT, H. M. – ADAM, G.: Effect of brassinosteroid on protein synthesis and plant-cell ultrastructure under stress condition. In: CUTLER, H. G. – YOKOTA, T. – ADAM, G. (eds.): Brassinosteroids. Chemistry, Bioactivity, and Ap-

plication. ACS Symp. Series No. 474, Washington, DC, Am. Chem. Soc., 1991, pp. 155–163.
MADER, P. – SZÁKOVÁ, J. – MIHOLOVÁ, D.: Classical dry ashing of biological and agricultural materials. Part II. Loses of analytes due to their retention in an insoluble residue. Analusis, *26*, 1998: 121–129.
MEHLICH, A.: Mehlich 3 soil test extraction: a modification of Mehlich 2 extractant. Commun. Soil Sci. Plant Anal., *15*, 1984: 1409–1416.
NAFIE, E. M. – EL-KHALLAL, S. M.: Effect of brassinolide application on growth certain metabolic activities and yield of tomato. Egypt. J. Physiol. Sci., *24*, 2000: 103–117.
PIROGOVSKAYA, G. V. – BOGDEVICH, I. M. – NAUMOVA, G. V. – KHRIPACH, V. A. – AZIZBEKYAN, S. G. – KRUL, L. P.: New forms of mineral fertilizers with additives of plant growth regulators. Proc. Plant Growth Regul., Soc. Am., *23*, 1996: 146–151.
PRUSAKOVA, L. D. – EZHOV, M. N. – SAĽNIKOV, A. I.: Application of emistim, epibrassinolide and uniconazol for overcoming heterogeneity of buckwheat crop. Agrar. Rossiya, *1*, 1999: 41–44.
SAKURAI, A. – YOKOTA, T. – CLOUSE, S. D.: Brassinosteroids: Steroidal Plant Hormones. Tokyo, Springer-Verlag 1999.
SCHILLING, G. – SCHILLER, C. – OTTO, S.: Influence of brassinosteroids on organ relations and enzyme activities of sugar beet plants. In: CUTLER, H. G. – YOKOTA, T. – ADAM, G. (eds.): Brassinosteroids. Chemistry, Bioactivity, and Application. ACS Symp. Series No. 474, Washington, DC, Am. Chem. Soc., 1991, pp. 208–219.
SHARMA, P. – BHARDWAJ, R.: Effects of 24-epibrassinolide on growth and metal uptake in *Brassica juncea* L. under copper metal stress. Acta Physiol. Plant., *29*, 2007a: 259–263.
SHARMA, P. – BHARDWAJ, R.: Effect of 24-epibrassinolide on seed germination, seedling growth and heavy metal uptake in *Brassica juncea* L. Gen. Appl. Plant Physiol., *33*, 2007b: 59–73.
UPRETI, K. K. – MURTI, G. S. R.: Effects of brassinosteroids on growth, nodulation, phytohormone content and nitrogenase activity in French bean under water stress. Biol. Plant., *48*, 2004: 407–411.
VILDFLUSH, I. P. – DEEVA, V. P. – GURBAN, K. A.: Influence of biologically active compounds on barley plants (*Hordeum vulgare* L.) on sward-podzolic light-loamy soils. Vestsy Natsional. Akad. Nauk Belarusi Ser. Biol. Nauk, *1*, 2001: 23–26.
VOLYNETS, A. P. – PSHENICHNAYA, L. A. – KHRIPACH, V. A.: The nature of protective action of 24-epibrassinolide on barley plants. Plant Growth Regul. Soc. Am., *24*, 1997: 133–137.
WORLEY, J. F. – MITCHELL, J. W.: Growth responses induced by brassins (fatty plant hormones) in bean plants. J. Am. Soc. Hortic. Sci., *96*, 1971: 270–273.
ZADOKS, J. C. – CHANG, T. T. – KONZAK, C. F.: A decimal code for the growth stages of cereals. Weed Res., *14*, 1974: 415–421.
ZBÍRAL, J.: Determination of phosphorus in calcareous soils by Mehlich 3, Mehlich 2, CAL, and Egner extractants. Commun. Soil Sci. Plant Anal., *31*, 2003: 3037–3048.

Received for publication on December 9, 2009
Accepted for publication on March 3, 2010

KROUTIL, M. – HRADECKÁ, D. – KOHOUT, L. – LACHMAN, J. (Česká zemědělská univerzita, Fakulta agrobiologie, přírodních a potravinových zdrojů, katedra chemie, katedra rostlinné výroby, Praha; Ústav organické chemie a biochemie Akademie věd České republiky, Oddělení steroidní chemie, Praha, Česká republika):

Kvalita zrna a obsah vybraných kovů v obilkách jarní pšenice (*Triticum aestivum* L.) po ošetření brassinosteroidy v průběhu pěstování.

Scientia Agric. Bohem., *41*, 2010: 65–72.

V letech 2005–2007 byla v polním pokusu na parcelách o výměře 10 m^2 pěstována jarní pšenice odrůdy Vánek. Rostliny byly ve vývojové fázi 49–59 DC (viditelné osiny klasu až plné metání rostlin) ošetřeny osmi různými typy brassinosteroidů: 24-epibrassinolidem, 24-epikastasteronem, látkou s označením 4154 a pěti syntetickými androstanovými a pregnanovými analogy brassinosteroidů. Cílem práce bylo zjistit, jakým způsobem se mění minerální složení (resp. obsah Ca, Cu, Fe, K, Mg, Mn, Zn), kvalitativní parametry a výnos zrna po ošetření rostlin brassinosteroidy. Výsledky tříletého experimentu prokázaly změny minerálního složení zrna. Z hlediska absolutního obsahu sledovaných minerálních látek byly (kromě obsahu Cu) shledány rozdíly mezi jednotlivými ročníky. Ovlivnění kvality zrna, hektarového výnosu a hmotnosti tisíce zrn po ošetření rostlin brassinosteroidy nebylo ve sledovaných ročnících prokázáno.

brassinosteroidy; jarní pšenice; kovy; číslo poklesu; bílkoviny; lepek; Zeleny test

Contact Address:

Ing. Milan K r o u t i l , Josefa Němce 28, 190 11 Praha 9-Běchovice, Česká republika, tel.: +420 732 718 127, e-mail: milan.kroutil@seznam.cz