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Selenium in colour-grained winter wheat and spring tritordeum

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ABSTRACT

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Eighteen winter wheat cultivars with different grain colour (purple-, blue-, yellow- and red-grained) and three spring tritordeum yellow-grained cultivars and breeding lines were assessed for grain selenium (Se) content from the crop season 2014/2015 on the experimental field Agrotest fyto, Ltd., Kroměříž (Czech Republic). Se content has shown to be genotype dependent, with the highest contents in control red-grained cv. Bohemia (0.235 mg/kg dry matter (DM)) and yellow-grained cv. Bona Vita (0.229 mg/kg DM), and breeding lines V2 10–16 (blue-grained), KM 53–14 (blue-grained) and V2 15–16 (yellow-grained) winter wheats. In new spring tritordeums, average Se content was comparable (0.039 mg/kg DM) with purple pericarp wheats (0.042 mg/kg DM); in wheats with blue aleurone and yellow endosperm it was higher (0.057 mg/kg DM and 0.069 mg/kg DM). Although in most cultivars the Se contents were not significantly different, statistically significant differences were determined between the cvs. Bohemia and Bona Vita with the highest Se content and breeding line V2 31–16 with the lowest Se content as well as between the cv. Bohemia and breeding line KM 178–14. Grain colour of wheat cultivars and breeding lines affected Se content, so possible wheat genetic resources for use in the breeding process can be assessed. Diversity in certain wheat accessions offers genetic potential for developing cultivars with better ability to accumulate beneficial Se micronutrient in grains.

Keywords: cereal; deficiency; antioxidant; *Triticum aestivum*; × *Tritordeum martinii* A. Pujadas nothosp. nov.

Selenium (Se) occurs in two distinctly different forms – inorganic and organic (Whanger 2002). Inorganic forms – selenites (IV) and selenates (VI) occur only in soils. These forms are assimilated by plants and converted to L-selenomethionine (Pyrzyńska 2009). While selenate is taken up in plant roots by sulphate transporters (Sors et al. 2005), selenite is believed to be taken up into plants passively and/or by phosphate transporters (Li et al. 2008). Li et al. (2008) also found that selenate is the major species in neutral to alkaline soils and selenite is the major inorganic species in acidic to neutral ones. Selenite is less bioavailable than selenate in soils because iron oxides and/or hydroxides strongly absorbe selenite.

Se has a crucial antioxidant role as a part of the enzyme glutathione peroxidase also known as selenoproteins, which are a family of antioxidant enzymes that speed the reaction between glutathione and toxic free radicals. Because the organic forms of selenium act as antioxidants, these help to prevent DNA damage and heavy metal toxicity. Thereby, selenium organic forms can prevent cancer and degenerative diseases (Finley et al.

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2001). Under oxidative stress-related conditions, a low dietary selenium intake leads to immune dysfunctions, senility, and the development of Alzheimer's diseases (Fordyce 2013). According to the World Health Organization (1996), the narrowest range of selenium between dietary deficiencies is lesser than 40 μg/day and toxicity is greater than 400 μg/day.

Plants are readily taken up selenium in the form of selenite and selenate (Hawrylak-Nowak 2013). In soils, there are frequently low amounts of available selenium; hence wheat is an important dietary source for this element (Rayman 2002). In wheat grain, the concentration of selenium is highly variable. The crustal abundance of Se is 0.050 mg/kg. Selenium-rich soils or crop produced selenium are the main sources to provide selenium. In addition, the genetic breeding of new cultivars which can accumulate more selenium in grain is also other source (Ducsay et al. 2007). The direct source of selenium to crops is probable atmospheric deposition of selenium on crops. To enrich the Se status of plants, foliar application of selenite or selenate is a good way. In low Se soil, soil applications of commercial fertilizer which are enriched with Se are a safe method. In the research of Curtin et al. (2006), foliar application of Se was found more effective than soil fertilization in increasing growth and yield of wheat plants. Among field crops, wheat is the most important accumulator of Se (De Temmerman et al. 2014).

The aim of this study was to assess Se levels in coloured grains of selected winter wheat cultivars and new breeding lines (purple- and blue-grained containing anthocyanins and yellow-grained containing carotenoids) and compare them with the standard cv. Bohemia and yellow-grained tritordeum spring lines and to select the cultivars and breeding lines that may be useful Se genetic resources for further breeding and crossing.

MATERIAL AND METHODS

Wheat and tritordeum materials. The study was carried out in 2016 at the Czech University of Life Science Prague (Department of Chemistry). A total of eighteen wheat species and three tritordeum cultivars were grown in crop season 2014/15 at the Agricultural Research Institute in Kroměříž, Czech Republic (49.2851172N, 17.3646269E). Their characteristics are described in Table 1. The experimental field is located 235 m a.s.l., has Luvic Chernozem (Loamic), an average annual temperature 9.2°C, mild winters and precipitations averaging 576 mm. Se average content in soil was 1.179 ± 0.077 mg/kg dry matter (DM) and pH_{KCl} 5.75 (acidic soil).

Determination of Se with HGAAS. The content of selenium was determined in digested samples of the cereals by atomic absorption spectrometry (AAS) with hydride generation technique (HGAAS). Grain samples were ground finely and microwave digested in an acid solution using MWS-3+ (Berghof Products + Instruments, Eningen, Germany). 400 mg of the sample was weighted into the Teflon digestion vessel DAP-60S and 2 mL of nitric acid 65% Suprapur, p.a. ISO (EMD Millipore Merck, KGaA, Darmstadt, Germany) and 3 mL H_2O_2 30%, TraceSELECT Ultra (Sigma-Aldrich, Pty. Ltd., Castle Hill, Australia) were added. The mixture was shaken carefully and the vessel was closed after half an hour of waiting and heated in the microwave oven. The decomposition proceeded within 1 h in the temperature range 100–190°C.

The digest obtained was transferred into the 50 mL silica beaker and evaporated to wet residue, then diluted with minimum of 10% hydrochloric acid prepared from HCl 37%, p.a. + (Analytika, Co., Ltd., Prague, Czech Republic) and deionised water (Barnstead, Dubuque, USA). Formic acid 98%, puriss. p.a. (Sigma-Aldrich, St. Louis, USA) in the volume of 1 mL was added for the reduction of nitrogen oxides from the reaction mixture. To reduce all selenium compounds in the digest to Se^{4+} 5 mL of hydrochloric acid diluted with deionised water 1:1 (v/v) was added and the solution was heated at 90°C for half of hour. Then digests were transferred to probes and adjusted with 10% HCl to 10 mL.

The concentration of selenium in the digests of cereals were measured by the HGAAS technique using Varian AA 280Z (Varian, Mulgrave, Victoria, Australia) with vapour generation accessory VGA-76 and sample preparation system Varian SPS3. Standard solution Astasol (Analytika, Prague, Czech Republic) of selenium was used in the preparation of a calibration curve for the measurement. Samples of the cereals were analysed in three replicates.

The quality of analytical data was assessed by simultaneous analysis of certified reference mate-

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Table 1. Analysed wheat and tritordeum samples and their characteristics

Field No. 2016	Official name	Grain colour	Origin	State of origin	Cultivar status
Wheat (*Triticum aestivum* L.), winter forms					
V2 3–16		Ba	BAUB 2786.2 × Skorpion	CZE	breeding line
V2 9–16	KM 53-14*	Ba	Skorpion × Ludwig	CZE	breeding line
V2 10–16		Ba	Skorpion × Magister	CZE	breeding line
V2 13–16	Skorpion**	Ba	Line 5 × Versailles**	CZE	released var.
V2 14–16		Ba	KM 824-1-01 × RU 440-5	CZE	breeding line
V2 15–16		Ye	Citrus × Bona Dea	CZE	breeding line
V2 16–16	Bona Vita	Ye	(SO-690 × Arida) × Arida	SVK	released var.
V2 17–16	Citrus	Ye	(Sunnan × Monopol) × Stamm GI 912	GER	released var.
V2 18–16		Pp	purple grain line from Slovakia × Akteur	CZE	breeding line
V2 22–16	KM 178-14*	Pp	Meritto × ANK-28A	CZE	breeding line
V2 28–16	PS Karkulka	Pp	ANK-28A × PS 11	SVK	released var.
V2 31–16		Pp	(Indigo × Akteur) × (Skorpion × Bohemia)	CZE	breeding line
V2 32–16		Pp	Blaucorn × Zappa	CZE	breeding line
V2 33–16		Pp	purple grain line from Slovakia × Akteur	CZE	breeding line
SU 5–16	Bohemia	R	(540i × U6192) × (540i × Kontrast)	CZE	released var.
V1 47–16		Ba	(Skorpion × V1-702) × (Citrus × Bona Dea)	CZE	breeding line
V1 48–16		Ba	Skorpion × UC 66049	CZE	breeding line
V1 50–16		Pp	Indigo × Mironovskaya 808	CZE	breeding line
Tritordeum (× *Tritordeum* Ascherson et Graebner), spring forms					
1 m^2–81–16	HT 439*	Ye	*Triticum* sp. × *Hordeum chilense*	ESP	breeding line
1 m^2–88–16	JB 1	Ye	*Triticum* sp. × *Hordeum chilense*	ESP	released var.
1 m^2–89–16	JB 3	Ye	*Triticum* sp. × *Hordeum chilense*	ESP	released var.

Ba – blue aleurone; Pp – purple pericarp; Ye – yellow endosperm; R – standard red grain; *Breeding lines (KM-lines are tested in the Official State Tests in the Czech Republic); **Origin of cv. Line 5: (Barevná 5 × Brigand) × </ (Brimstone × Židlochovická osinatka) × Hana/ × Hana>; cv. Barevná 5 is a donor of blue aleurone from heritage of Erich von Tschermak-Seysenegg. Lines without official name are breeding lines or genetic resources. **Cv. Skorpion was developed in the Czech Republic and in 2011 registered in Austria. Countries of origin: CZE – Czech Republic; ESP – Spain; GER – Germany; SVK – Slovak Republic

rial BCR 281 (rye grass) (4% of all the samples). The accuracy for selenium with respect to the reference material was 96.5%. The background of the trace element laboratory was monitored by analysis of 17.5% blanks prepared under the same conditions, but without samples, and experimental data was corrected by mean concentration of analyte in blanks, and compared with detection limit (mean ± 3 SD (standard deviation) of blanks) (0.002 mg Se/kg).

Statistical analysis. All experiments were conducted in triplicates. For all measurements, averages and standard errors were calculated in Microsoft Excel 2007. The data were processed by Chromeleon (Thermo Fisher Scientific, Inc., Waltham, USA) and Excel (Microsoft, Redmond, USA). Statistical evaluation was performed using the Statistica software (ver. 12; StatSoft, Inc., Tulsa, USA). Genotype differences in Se contents were evaluated by one-way ANOVA ($P \leq 0.05$).

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The Tukey's Post Hoc *HSD* (honest significant difference) test was used for detailed evaluation and non-parametric Kruskal-Wallis *H*-test.

RESULTS AND DISCUSSION

Se content in wheat and tritordeum grains. Se concentration measurements were based on the dry mater basis (mg/kg DM). The results were the average of three replicated samples, expressed to one standard deviation. The reliability of our methods was shown by the low standard deviation.

Large variations were observed in investigated grain Se concentrations for some wheat species (Table 2). The grain Se concentrations ranged from 0.022 to 0.235 mg/kg DM, with an average of 0.067 mg/kg DM. The cultivars with the highest grain Se concentrations were the control red-grained wheat cv. Bohemia (0.235 mg/kg DM) and yellow-grained cv. Bona Vita (0.229 mg/kg DM). Average Se content in wheat cultivars with blue aleurone, purple pericarp and yellow grain was 0.057, 0.042 and 0.069 mg/kg DM, respectively (except cv. Bona Vita). Se content in blue-, purple- and yellow-grained wheats ranged between 0.042–0.083, 0.022–0.053 and 0.058–0.079 mg/kg DM, respectively. According to the study of Lachman et al. (2011) comparable data were also determined in einkorn (0.050–0.055 mg/kg DM), emmer wheat (0.059–0.065 mg/kg DM) and spring wheat (0.030–0.068 mg/kg DM). In colour-grained wheat, statistically significant differences were determined between the cv. Bohemia (standard red grain) and cv. Bona Vita (yellow-grained) cultivars with the highest Se content and breeding line V2 31–16 (purple-grained) with the lowest Se content

Table 2. Total content of selenium (Se) in wheat and tritordeum grain (mg Se/kg dry matter (DM) ± standard deviation (SD)) and selenium yield in grain (g/ha)

Cereal type	Field Nos. 2016	Official name	Grain colour	Total Se content (mg Se/kg DM ± SD)	Yield (t/ha)	Se in grain (g/ha)
Winter wheat	V2 3–16		blue aleurone	0.049 ± 0.0011^{bc}	9.92	0.486
	V2 9–16	KM 53-14	blue aleurone	0.073 ± 0.0214^{de}	10.93	0.798
	V2 10–16		blue aleurone	0.083 ± 0.0113^{e}	7.97	0.661
	V2 13–16	Skorpion	blue aleurone	0.042 ± 0.0023^{bc}	9.99	0.419
	V2 14–16		blue aleurone	0.047 ± 0.0072^{bc}	7.21	0.339
	V2 15–16		yellow endosperm	0.079 ± 0.0041^{e}	10.31	0.815
	V2 16–16	Bona Vita	yellow endosperm	0.229 ± 0.0415^{f}	9.27	2.123
	V2 17–16	Citrus	yellow endosperm	0.058 ± 0.0057^{cd}	9.66	0.560
	V2 18–16		purple pericarp	0.045 ± 0.0054^{bc}	9.02	0.406
	V2 22–16	KM 178-14	purple pericarp	0.032 ± 0.0026^{ab}	11.49	0.368
	V2 28–16	PS Karkulka	purple pericarp	0.050 ± 0.0009^{bc}	9.75	0.488
	V2 31–16		purple pericarp	0.022 ± 0.0002^{a}	9.60	0.211
	V2 32–16		purple pericarp	0.052 ± 0.0020^{cd}	8.56	0.445
	V2 33–16		purple pericarp	0.038 ± 0.0033^{abc}	9.27	0.352
	SU 5–16	Bohemia	standard red grain	0.235 ± 0.0209^{f}	10.87	2.224
	V1 47–16		blue aleurone	0.055 ± 0.0023^{cd}	8.58	0.472
	V1 48–16		blue aleurone	0.048 ± 0.0024^{bc}	6.76	0.324
	V1 50–16		purple pericarp	0.053 ± 0.0071^{cd}	10.68	0.566
Spring tritordeum	1 m^2–81–16	HT 439	yellow endosperm	0.037 ± 0.0012^{abc}	2.26	0.084
	1 m^2–88–16	JB 1	yellow endosperm	0.041 ± 0.0021^{abc}	2.03	0.083
	1 m^2–89–16	JB 3	yellow endosperm	0.040 ± 0.0049^{abc}	2.38	0.095

Different letters in the Se concentration column indicate a significant difference ($P \leq 0.05$)

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and likewise between cv. Bohemia and breeding line KM 178–14 (purple-grained).

Effect of different factors on Se content. Humans and animals commonly obtain selenium from cereals, grains, and vegetables grown on seleniferous soils and from animal products such as meat, milk, fish, and eggs (Fairweather-Tait et al. 2010). This element enters the food chain through plants and, consequently, it is highly dependent upon its bioavailability in soils (Ducsay and Ložek 2006). Se foliar application effectively increases its content in cereal grain, as was reported in barley (Ducsay et al. 2009) and winter wheat (Ducsay et al. 2007).

The uptake of Se from soils into plants depends on several parameters such as bio-available Se concentration, soil characteristics, Se speciation, plant species and concentration of competing ions (Hegedüsová et al. 2016). Soil pH can influence on the selenium content of the plants. It has been proven that chemical oxidation in alkaline soils produces selenate which is available for plants, but pH value of the soil in our experimental field was 5.75 (acidic soil). The decrease of selenium from plants can only occur through the volatilization (Whanger 2004). The soil in the experimental location of our study had pseudototal (*aqua regia* soluble) Se average content 1.179 ± 0.077 mg/kg DM; this corresponds to the range of Se levels between 0.2–1.4 mg/kg in the Czech soils (Száková et al. 2015). Selenium is a rare element on our planet, with the average concentration in igneous bedrock being only 0.05 mg/kg, which is less than for any other nutrient element. Selenium is unevenly distributed over the surface of the Earth, ranging from near zero to 1250 mg/kg. In many parts of Europe, soil Se concentrations are relatively high because of high deposition either naturally from the sea (e.g. Ireland, England, Scotland and the Netherlands) or from polluted rains (e.g. Germany, the Czech Republic, Slovakia and Poland) (Haug et al. 2007). Our results indicate that only a little portion of selenium is accumulated in cereal grain (from 1.87% in V2 31–16 to 19.93% of Se content in soil in cv. Bohemia) and that there are significant differences between cultivars and breeding lines. Therefore, exploiting the genetic variability in crop plants for micronutrient density may be an effective method to improve Se intake in human nutrition, and use of plants that naturally contain more Se than others, or breeding plant and crop cultivars with enhanced Se-accumulation characteristics, may be plausible approaches to increase the Se concentration of the human diet.

Cereals were reported poor in bioavailability and concentration of microelements such as Zn, Fe, and Se in the seeds (Cakmak 2008). However, cereals play an essential and invaluable role in human diet of which wheat is the third most produced staple cereal on earth. Currently around 758 million tons of wheat are produced in the world and its global consumption is 67 kg/capita/year (FAO 2017). Zn and Se concentrations in grains exhibit 2- and 1.5-fold difference between wheat accessions (Souza et al. 2014). Se income in Slovakia from cereals was estimated as 14% of total (Tóth et al. 2012). Grain Se concentrations in divers wheat germplasm may be found in the range 0.005–0.720 mg/kg, but much of this variation is associated with spatial variation in soil selenium. When they are grown in microelement deficient soils, this situation is more serious. In wheat meal, white bread and raw bread in Slovakia average Se content has been evaluated as 0.0251, 0.0176 and 0.0165 mg/kg with ranges 0.015–0.0323, 0.0134–0.0215 and 0.0155–0.0185 mg/kg, respectively (Tóth et al. 2012). However, some wheat species like the diploid wheat *Aegilops tauschii* was 42% higher in grain Se concentration than commercial bread and durum wheat (Lyons et al. 2005). One of the promising solutions for reducing malnutrition is developing cereals that are genetically enriched in micronutrients and proteins (Lyons 2010).

The normative requirement estimates of dietary Se are 0.04 and 0.03 mg/day for man and woman, respectively (World Health Organization 1996). European and USA recommended dietary allowance for selenium 0.055 mg/day (Hawkesford and Zhao 2007), while in New Zealand and Australia 0.07 and 0.06 mg/day are recommended for men and women, respectively (National Health and Medical Research Council 2005). Because of its high consumption, wheat is one of the primary sources of dietary selenium, with the major available form found in grains being selenomethionine. Comparing to fish, selenium from wheat grain is highly bioavailable – 81.0 ± 3.0% (Fox et al. 2005). This is the reason in recent years, why more and more researchers have focused on exploiting the genetic resources and developing of genetically selenium-enriched and protein-Se-enriched wheat using genomics tools (Lyons et al. 2005).

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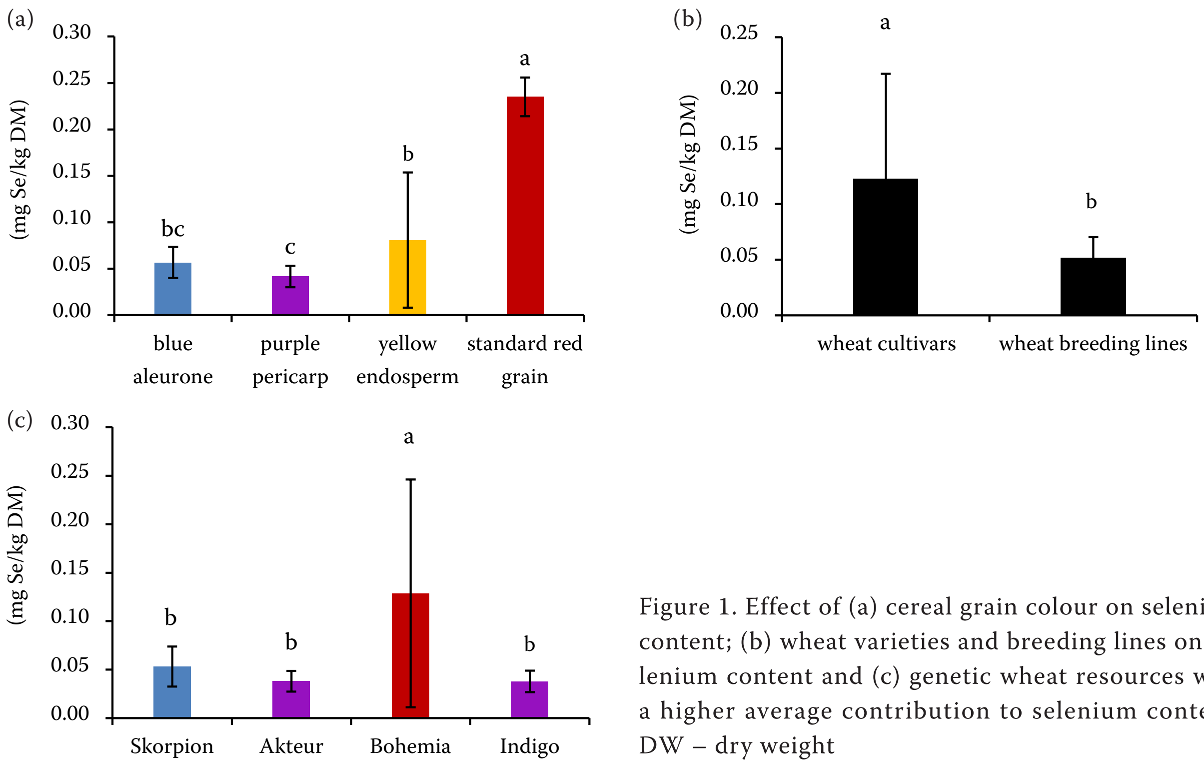

Figure 1. Effect of (a) cereal grain colour on selenium content; (b) wheat varieties and breeding lines on selenium content and (c) genetic wheat resources with a higher average contribution to selenium content. DW – dry weight

A relationship between colour grain and Se content was statistically evaluated by one way ANOVA (Figure 1a). The highest Se content was found in standard bread red grain cv. Bohemia, which differed from other wheats with coloured grain. Wheats with yellow endosperm differed significantly from wheats with purple pericarp. Comparison of wheat with coloured grain revealed that Se content decreases in order yellow endosperm > blue aleurone > purple pericarp. The effect of wheat cultivars on selenium content was higher as compared with breeding lines and cultivars differed significantly from breeding lines (Figure 1b). Accordingly, for Se crossing better genetic resources appear with a higher average contribution to Se content in some wheat cultivars (Figure 1c). Between analysed wheats, cvs. Bohemia, Skorpion, Indigo and Akteur may be recommended as suitable genetic resources. Cv. Bohemia differed significantly from other cultivars with higher Se content.

In conclusion, comparison of Se contents in wheat and tritordeum grains revealed differences between some cultivars and genotypes. The highest levels were determined in red-grained cv. Bohemia, yellow-grained cv. Bona Vita, blue-grained breeding line V2 10–16 (Skorpion × Magister), KM 53–14 (Skorpion × Ludwig) and yellow-grained V2 15–16 (Citrus × Bona Dea). Diversity in certain wheat accessions offers genetic potential for developing cultivars with better ability to accumulate important micronutrients in grains. Selenium in wheat grain in the form of selenoproteins glutathione peroxidases could also contribute to antioxidant activity of wheat and tritordeum grain containing anthocyanins especially in blue and purple grain and carotenoids with antioxidant properties in yellow grain.

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