A comparison of macro- and microelement concentrations in the whole grain of four *Triticum* species

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ABSTRACT

The aim of this study was to compare the concentrations of five macro- and fifteen microelements in the whole grain of spring lines of emmer, einkorn, spelt and two common wheat cultivars, all grown under identical environmental conditions. All elements were determined by ICP-SFMS analysis. The studied *Triticum* species differed significantly with respect to the concentrations of P, Mg, Zn, Fe, Mn, Na, Cu, Sr, Rb, and Mo. The grain of all hulled wheats, compared with common wheat, contained significantly more Zn (from 34% to 54%), Fe (from 31% to 33%) and Cu (from 3% to 28%). In the majority of cases, there were no relationships between the concentrations of the analyzed elements, except for significant positive correlations between the levels of Fe, Zn and Mn, in particular in *T. monococcum* and *T. dicoccum.* The classical linear discriminant analysis enabled to distinguish between the three studied *Triticum* species with regard to the concentrations of all analyzed elements in their grain. A significant discrimination indicates that the concentrations of the investigated elements are a species-specific character. A strong correlation between Zn, Fe and Mn could have important implications for wheat quality breeding.

Keywords: hulled wheats; elements; discriminant analysis; ICP-SFMS

The grain of modern high-yielding common wheat (*Triticum aestivum* L.) varieties is generally characterized by a decreasing content of essential nutrients and microelements (Fan et al. 2008). The concentrations of trace elements and vitamins that are valuable for human nutrition steadily decrease with an increase in yield, which has adverse consequences, including 'hidden hunger' (Cakmak et al. 2010). *Triticum* species other than common wheat have attracted a growing scientific interest due to the nutritive and dietary value and unique taste attributes of their grain (Abd-el-Aal et al. 1995, Stallknecht et al. 1996). Besides, the growing trend of organic farming and an increase in the consumption of healthy food have led to an interest in hulled wheats among producers and consumers (De Vita et al. 2006). *Triticum dicoccum* L., *Triticum spelta* L. and *Triticum monococcum* L. also known as emmer, spelt and einkorn, respectively, had been widely used until the late Roman Empire. These ancient wheats have hardly been exposed to modern breeding techniques. Thus, they are believed to be a more 'natural' product and are grown by many organic farmers. Their suitability for low-input agriculture, adaptation to marginal lands, excellent competition with weeds and high potential for the growing of healthy food, make emmer, einkorn and spelt attractive crops (D'Antuono and Pavoni 1993, Stallknecht et al. 1996). Consumer demand for healthy food and heritage wheat varieties, combined with the awareness of adverse reactions to food, has led spelt, emmer and einkorn to appear in our diet. However, little information is available on the differences in the chemical composition, nutritive value and baking quality of the cultivars under

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production (Stallknecht et al. 1996, Abd-el-Aal et al. 1997). Ekholm et al. (2007) reported that the trace element concentrations in plants are known to be affected by the cultivar, soil conditions during growing, the use of fertilizers and the state of maturity at harvest. The knowledge of the microelement content of food products is very important because of their fundamental nutritional role in the human diet.

Scientific literature provides scant information on the differences in microelement concentrations in various wheat species (Abd-el-Aal et al. 1995, Ranhotra et al. 1995). Piergiovanni et al. (1997) found that emmer and spelt accessions differed from wheat cultivars mostly with regard to their higher concentrations of Li, Mg, P, Se, and Zn. Ruibal-Mendieta et al. (2005) reported 30% to 60% higher concentrations of Fe, Zn, Cu, Mg, and P in spelt than in common wheat. Lachman et al. (2011) found that higher selenium content in grain is related to emmer (58.9–68.4 μg/kg DM) and einkorn (50.0–54.8 μg/kg DM) accessions and varieties; in spring varieties selenium content was lower and ranged from 29.8 to 39.9 μg/kg DM. Selenium content is important because some soils are poor in selenium that is a trace mineral essential to human with beneficial impact on health and possess also antioxidant activity (Lachman et al. 2012). Contrary, some heavy metals are very toxic (Cd, Pb) and there is an effort to decrease their content in wheat by treatment with brassinosteroids (Kroutil et al. 2010).

The aim of this study was to compare the concentrations of macro- and microelements (K, P, S, Mg, Ca, Zn, Fe, Mn, Na, Cu, Al, Ba, Sr, B, Rb, Mo, Ni, Cr, Cd, Pb) in the whole grain of spring accessions of emmer, einkorn and spelt and two common spring wheat cultivars, all grown under identical environmental conditions.

MATERIAL AND METHODS

Field experiment. The experimental material consisted of 28 pure spring lines of three *Triticum* species obtained from the National Centre for Plant Genetic Resources Radzików, Poland, the Leibniz Institute of Plant Genetics and Crop Plant Research in Gatersleben, Germany, and the National Germplasm Resources Laboratory, USA (Table 1). The above genotypes were reproduced at the Department of Plant Breeding and Seed Production, University of Warmia and Mazury in Olsztyn, Poland. Additionally, four spring varieties were examined: *T. dicoccum* Emmer 1 (a local variety from a Polish organic farm), *T. monococcum* Terzino (a cultivar registered in Germany) from Cereal Breeding Research Darzau, Germany and *T. aestivum* cv. Torka and Parabola (registered Polish cultivars with high flour quality (List of Agricultural Cultivars 2008).

A field experiment was performed at the Experimental Station in Bałcyny (53°36'N latitude, 19°51'E longitude), in 2007, in three replications. Plot area was 10 m^2. The preceding plant was winter rape. N/P/K fertilizer was applied at 20/25/80 kg/ha once, prior to sowing. After harvest at the overripe stage BBCH 92 (Witzenberger et al. 1989), the grains of all hulled lines and cultivars were dehulled by hand and were stored in polypropylene tubes at −20°C in a freezing chamber (York International) until analysis.

Chemicals and reagents. Water was purified successively by reverse osmosis and a Milli-Q plus system from Millipore (Molsheim, France). All chemicals used for inductively coupled plasma sector field mass spectrometry (ICP-SFMS, Thermo Electron Corporation, Bremen, Germany), sample preparation and the preparation of calibration standards, were of pro analysi (p.a.) or supra-pure (s.p.) quality. Nitric acid s.p. (69%), a multi-element atomic spectroscopy standard solution (containing 10 mg/L Ag, Al, Ba, Be, Bi, Cd, Co, Cr, Cs, Cu, Ga, In, Li, Mg, Mn, Mo, Ni, Pb, Rb, Sr, Tl, V, and Zn each, as well as 100 mg/L Ca, Fe, K and Na each in 5 mol/L HNO_3) and the rhodium internal ICP-MS standard (containing 10 mg/kg $Rh(NO_3)_3$ in 0.5 mol/L HNO_3) were purchased from Fluka, Sigma-Aldrich, (Steinheim, Germany). All solutions were prepared and stored in 100 mL or 500 mL polyethylene terephthalate serum bottles (purchased from Greiner packaging GmbH, Kremsmünster, Austria), or in 50 mL polypropylene tubes (Sarstedt, Nümbrecht, Germany). For ICP-SFMS analysis, the sample solutions were transferred into 14 mL polystyrene tubes with low-density polyethylene push caps (Sarstedt). Certified standard reference material NIST 8436 (durum wheat flour) was purchased from the National Institute of Standards and Technology, Gaithersburg, MD, USA. The internal standard solution (IS) for ICP-SFMS analysis contained Ge and Tl at 1 mg/L each in 0.5% HNO_3 (v/v).

Sample preparation. An Anton Paar High Pressure Asher (HPA) equipped with seven quartz vessels of 30 mL volume was used throughout. 500 mg ± 5 mg three-replicate bulk samples of whole grain were weighed into the quartz vessels.

Table 1. Genotypes of *Triticum* sp. examined in the experiment

T. monococcum L.			*T. dicoccum* Schrank			*T. spelta* L.			*T. aestivum* L.		
No.	line/cultivar	source	No.	line	source	No.	line	source	No.	cultivar	source
1	PL 020790	NCPGR[1]	13	Cltr 9258	NGRL	26	PL 021981	NCPGR	31	Torka	PBS[5)]
2	PL 021985	NCPGR	14	PI 164582	NGRL	27	TRI 17506	IPK	32	Parabola	PBS
3	PL 024068	NCPGR	15	PI 244341	NGRL	28	TRI 17513	IPK			
4	PL 020751	NCPGR	16	PL 020758	NCPGR	29	TRI 3419	IPK			
5	PI 290511	NGRL[2]	17	PL 021606	NCPGR	30	TRI 982	IPK			
6	PI 326317	NGRL	18	PL 021984	NCPGR						
7	PI 330551	NGRL	19	PL 022863	NCPGR						
8	PI 352479	NGRL	20	PL 024063	NCPGR						
9	PI 418587	NGRL	21	TRI 17029	IPK[4)]						
10	PI 584654	NGRL	22	TRI 18219	IPK						
11	PI 428171	NGRL	23	TRI 2246	IPK						
12	cv Terzino	CBRD[3]	24	TRI 8310	IPK						
			25	Emmer 1	own						

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After the addition of 2 mL HNO_3, the vessels were sealed with Teflon tape and quartz glass disks, they were placed into the digestion unit and pressurized with nitrogen at 100 bar. The sample digestion procedure was described previously (Wiwart et al. 2009).

ICP-SFMS analysis. ICP-SFMS measurements were performed on a double-focusing ICP-sector field MS model Finnigan ELEMENT 2 (Software 2.42, Thermo Electron Corporation, Bremen, Germany) equipped with a CETAC ASX-520 autosampler (CETAC Technologies, Omaha, USA). The instrument was equipped with a cyclonic spray chamber (Jacketed Cinnabar Cyclonic, 20 mL, from Glass Expansion, West Melbourne, Australia) and a micro-flow nebulizer made of PFA (MicroFlow Nebuliser PFA-ST from Elemental Scientific Inc., Omaha, USA) connected to a 700 µL/min self-aspiration capillary (0.5 mm inner diameter) (both from AHF Analysentechnik, Tübingen, Germany). Argon (Ar 4.6, 99.996% from Messer Austria GmbH) cool gas flow was 16 L/min, auxiliary (plasma) gas and sample (nebulizer) gas flows were optimized daily before each measurement series to obtain the maximum signal intensity, the former typically between 0.75 L/min and 0.90 L/min, the latter in the range of 0.85 L/min to 0.95 L/min. Radio frequency (RF) power was between 1185 W and 1195 W. The following isotopes were measured in low-resolution mode, R_s = 300, 10% valley definition: ^{11}B, ^{85}Rb, ^{88}Sr, ^{97}Mo and ^{208}Pb. ^{23}Na, ^{24}Mg, ^{27}Al, ^{31}P, ^{32}S, ^{44}Ca, ^{55}Mn, ^{56}Fe, ^{63}Cu, ^{66}Zn, ^{138}Ba and ^{111}Cd, were determined in medium-resolution mode, R_s = 4000 whereas ^{39}K was measured in high-resolution mode, R_s = 10 000. In order to compensate for drifts in signal intensity during measurement sequences, ^{72}Ge and ^{205}Tl were used as internal standards. Generally, 25 scans with 0.02 s per peak were measured. A quantitative analysis of the samples was performed by external calibration. For that purpose, multi-element standard solutions in 0.5% HNO_3 (v/v) were prepared at four concentration levels for all elements.

Statistical analyses. The results were processed statistically using STATISTICA data analysis software system version 8.0 (StatSoft, Inc. 2008, www.statsoft.com). The ANOVA procedure was applied, and the significance of differences between mean values was estimated by the multiple Student Newman-Keuls test. The Pearson correlation analysis test was conducted for all studied elements, and the multivariate analysis performed was a classical linear discriminant analysis.

RESULTS AND DISCUSSION

The analyzed *Triticum* species differed significantly with respect to the concentrations of phosphorus, magnesium, zinc, iron, manganese, sodium, copper, strontium, rubidium, and molybdenum in their grain (Table 2). It should be stressed that in none of the samples the levels of cadmium and lead exceeded 0.2 mg/kg, which is the EU maximum permissible level of those contaminants in cereals and cereal products intended for human consumption (European Commission Regulation 2006).

The grain of *T. aestivum* contained significantly less phosphorus (4.18 g/kg) than the grain of the other three wheat species, and the noted difference ranged from 12% in spelt to 24% in einkorn. The grain of common wheat contained also significantly less magnesium (1.44 g/kg), compared with *T. dicoccum* (by 16%), *T. monococcum* (by 13%) and *T. spelta* (by slightly more than 4%). Potassium levels were insignificantly higher in common wheat than in the remaining three *Triticum* species. Piergiovanni et al. (1997) and Grela (1996) noted significantly higher potassium concentrations in the grain of spelt and emmer than in common wheat, whereas Ruibal-Mendieta et al. (2005) did not find significant differences between spelt and common wheat in this respect. In contrast to the cited authors, we investigated spring accessions whose mineral content had been generally less researched than that of winter cultivars. According to Erdman and Moul (1982), hard red spring (HRS) wheat contained more Ca, K, Mg, Na, P, S, Sr, and Zn than hard red winter (HRW) wheat. The lower phosphorus, sulfur and magnesium content of *T. aestivum* grain, in comparison with the studied hulled wheats, corresponds with the findings of the above authors, and it validates the results of our previous studies investigating *T. spelta* (Wiwart et al. 2009).

The concentrations of zinc and iron were lowest in common wheat, while the highest copper concentration (5.0 mg/kg) was noted in spelt grain. The grain of *T. monococcum* contained 1.2 mg/kg molybdenum, i.e. nearly 85% more than the grain of common wheat. Rubidium was present at very low concentrations in wheat grain. The highest rubidium content was observed in common wheat (1.45 mg/kg), and the lowest – in einkorn and emmer (0.8 mg/kg). Rubidium and potassium are metabolic antagonists, and both elements are

Table 2. Concentrations of macro- and microelements in the whole grain of the studied *Triticum* species. Within a column, mean values followed by the same letter do not differ significantly at $P < 0.01$

	Macroelements (g/kg)					Microelements and trace elements (mg/kg)										
	K	P	S	Mg	Ca	Zn	Fe	Mn	Na	Cu	Al	Ba	Sr	B	Rb	Mo
Triticum monococcum (n = 12)																
Mean	4.29	5.20^{a}	1.93	1.63^{a}	0.42	53^{a}	49^{a}	28^{a}	7^{b}	4.0^{ab}	2.5	2.6	5.4^{a}	0.8	0.8^{c}	1.2^{a}
Max	4.66	5.92	2.45	1.80	0.49	68	62	36	8	5.1	8.8	4.2	9.7	1.3	0.8	1.6
Min	3.90	4.54	1.18	1.17	0.32	33	32	14	5	2.7	1.4	1.3	2.1	0.6	0.7	0.6
RSD (%)	5.4	6.9	17	10	11	20	16	21	14	25	80	30	38	25	12	25
Triticum dicoccum (n = 13)																
Mean	4.39	5.12^{a}	1.88	1.67^{a}	0.36	54^{a}	49^{a}	24^{b}	12^{a}	4.1^{ab}	3.8	2.0	2.6^{b}	0.6	0.8^{c}	1.0^{ab}
Max	4.78	5.87	2.14	2.02	0.41	69	55	34	15	5.2	5.6	3.1	3.1	0.8	1.1	1.4
Min	3.83	4.78	1.63	1.48	0.32	38	43	16	7	2.3	2.2	1.3	2.1	0.5	0.6	0.4
RSD (%)	6.6	6.4	9.0	8.4	8.3	14	6.1	20	16	22	28	35	11	16	25	30
Triticum spelta (n = 5)																
Mean	4.17	4.70^{a}	1.80	1.50^{ab}	0.39	47^{a}	50^{a}	27^{a}	10^{ab}	5.0^{a}	4.4	3.5	3.6^{ab}	0.7	1.1^{b}	0.7^{ab}
Max	4.38	5.17	1.91	1.68	0.50	61	62	33	12	6.0	8.8	4.1	4.9	0.8	1.4	0.9
Min	3.72	4.26	1.63	1.31	0.27	41	42	22	8	4.5	2.5	2.6	2.4	0.6	0.7	0.4
RSD (%)	6.5	7.4	5.6	10	23	17	16	14	10	20	56	20	25	14	27	28
Triticum aestivum (n = 2)																
Mean	5.00	4.18^{b}	1.40	1.44^{b}	0.43	35^{b}	37.5^{b}	26^{b}	10^{ab}	3.9^{b}	1.7	2.95	3.0^{ab}	0.75	1.45^{a}	0.65^{b}
Max	5.19	4.24	1.49	1.45	0.43	35	41	28	11	4.4	2.1	3.7	3.7	0.8	1.7	0.8
Min	4.81	4.12	1.31	1.42	0.42	35	34	24	9	3.5	1.3	2.2	2.3	0.7	1.2	0.5

The concentrations of Ni, Cr, Cd, Pb were lower than 0.02 mg/kg; RSD – relative standard deviation (%)

alkali metals marked by mutual chemical affinity in plants and animals (Palavinskas et al. 1984).

According to Ruel and Buis (1998), human diets in various regions of the world are characterized by zinc deficiencies owing to low concentrations of this element in the soil. In the above authors' opinion, this problem could be solved through the breeding of cultivars characterized by higher effectiveness of zinc absorption from the soil. Emmer could constitute highly valuable input material for that purpose (Genc and McDonald 2008). The grain of the three hulled wheats analyzed in this study was characterized by insignificantly higher iron levels than the grain investigated by Zhao et al. (2009), while zinc levels were more than two-fold higher in comparison with the results noted by the above authors.

In the majority of cases, there were no clear relationships between the concentrations of pairs of the investigated elements in wheat grain, except for significant positive correlations between the levels of iron, zinc and manganese, in particular in *T. monococcum* (Table 3) which implies that the selection of genotypes abundant in one of those elements allows to obtain forms where the remaining elements are also present in high quantities. The results reported in this study for *T. aestivum* and *T. spelta* show that in the group of microelements iron was found in the highest concentrations, while copper was the least predominant. It should also be noted that the grain of *T. monococcum* and *T. dicoccum* contained more zinc than iron. The presence of positive correlations between the concentrations of zinc and iron in *T. monococcum* and *T. dicoccum* could have important implications for the breeding of new varieties containing high levels of both elements.

One of the most valuable research studies investigating mineral concentrations in the grain of winter wheat in the past 160 years was carried out by Fan et al. (2008) who relied on archive grain samples, harvested annually and preserved at a research station in Rothamsted, UK. The above authors observed a significant drop in zinc, copper, magnesium and iron levels in the studied grain upon the introduction of high-yielding, semi-dwarf varieties characterized by high harvest index values. The cited authors are of the opinion that there exists sufficient variation among the germplasm of the key crops and their wild relatives for the initiation of breeding programs aimed at developing high-yielding varieties of high nutritive value.

A classical discriminant analysis enabled to distinguish between the three hulled *Triticum* species with regard to the concentrations of all analyzed elements in their grain (Figure 1). A high discrimination was confirmed by a low value of Wilks' lambda (0.014) and a high value of approximated F (5.084, significant at $P = 0.0001$), as well as by F values significant at $P < 0.05$ between groups: $F = 0.748$ for *T. monococcum* and *T. dicoccum*, $F = 0.759$ for *T. monococcum* and *T. spelta*, $F = 0.283$ for *T. dicoccum* and *T. spelta*. The obtained results suggest that both the concentrations of the analyzed elements and the relationships between them are species-specific characters. A significant discrimination of three *Triticum* species based on

Table 3. The Pearson's correlation coefficient (r) for microelement concentrations in the grain of the studied *T. monococcum*, *T. dicoccum* and *T. spelta* genotypes. Only the treatments in which r values were higher than the critical value at $P < 0.05$ are presented

Elements	Pearson's correlation coefficient r	Significance level of r
Triticum monococcum		
Fe:Zn/Zn:Fe	0.775	0.003
Mn:Zn/Zn:Mn	0.841	<0.001
Rb:Zn Zn:Rb	–0.777	0.003
Mo:Zn/Zn:Mo	0.849	<0.001
Mn:Fe/Fe:Mn	0.776	0.003
Na:Fe Fe:Na	–0.582	0.047
B:Fe/Fe:B	0.656	0.021
Rb:Fe/Fe:Rb	–0.595	0.041
Mo:Fe/Fe:Mo	0.656	0.021
Rb:Mn/Mn:Rb	–0.666	0.018
Mo:Mn/Mn:Mo	0.864	<0.001
Sr:Ba/Ba:Sr	0.916	<0.001
Zn:B/B:Zn	0.628	0.029
B:Rb/Rb:B	–0.591	0.043
Mo:Rb Rb:Mo	–0.656	0.021
T. dicoccum		
Fe:Zn/Zn:Fe	0.773	0.002
Mn:Fe/Fe:Mn	0.570	0.042
Sr:Ba/Ba:Sr	0.697	0.008
B:Sr/Sr:B	0.562	0.046
T. spelta		
Mn:Zn/Zn:Mn	0.949	0.014
Al:Fe/Fe:Al	0.876	0.050
Ba:Na Na:Ba	–0.888	0.044
Rb:Sr/Sr:Rb	–0.905	0.035

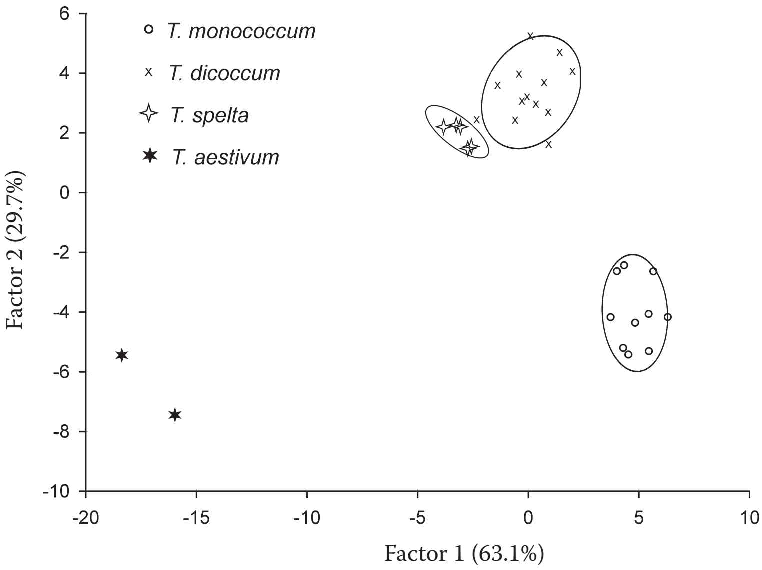

Figure 1. Graph presenting the results of a discriminant analysis including all of the studied elements in three *Triticum* species. The Gaussian confidence ellipses were drawn for a probability of 0.669. The eigenvalues of factor 1 and factor 2 are equal to 33.61 and 15.83, respectively. The proportions of the total variation for both factors are given in brackets

the concentrations of the determined elements in their grain points to species-specific variations. It should be noted that the discrimination between *T. monococcum* and *T. dicoccum* was affected mainly by factor 1 (in particular the concentrations of K, Ba, Zn, Sr, and Na), whereas factor 2 (Rb, Mo, Mn, and Na) enabled the discrimination of the *T. spelta* group. The results of previous studies analyzing the concentrations of 11 microelements in the grain of ten spelt and common wheat varieties testify to the usefulness of multidimensional analysis in research of the type (Wiwart 2009). In a study by Škrbić and Onjia (2007), a principal component analysis (PCA) applied to eight microelements supported a strong discrimination between samples of wheat grain of different origin.

Mineral concentrations in wheat grain are largely a varietal feature, although they are also determined by soil type, climate and cultivation practices, which explains the significant differences in the results noted by various authors (Anglani 1998). Regardless of the above, continued research efforts are required to find new genetic sources of essential minerals in plants of the genus *Triticum.* In the near future, such sources may be used directly as cultivated forms or indirectly as input material for breeding new, more nutritionally rich varieties of common wheat.

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