Journal of Elementology ISSN 1644-2296

Wiwart M., Suchowilska E., Wachowska U., Krska R., Kandler W. 2016. *The elemental composition of seedlings of selected Triticum* sp. *genotypes and a commercial dietary supplement – a comparative analysis*. J. Elem., 21(3): 937-945. DOI: 10.5601/jelem.2015.20.4.974

THE ELEMENTAL COMPOSITION OF SEEDLINGS OF SELECTED *TRITICUM* SP. GENOTYPES AND A COMMERCIAL DIETARY SUPPLEMENT – A COMPARATIVE ANALYSIS*

Marian Wiwart[1], Elżbieta Suchowilska[1], Urszula Wachowska[2], Rudolf Krska[3], Wolfgang Kandler[3]

[1]Chair of Plant Breeding and Seed Production
[2]Chair of Phytopathology and Entomology
University of Warmia and Mazury in Olsztyn
[3]Center for Analytical Chemistry, Department for Agrobiotechnology
University of Natural Resources and Life Sciences in Vienna, Austria

Abstract

Cereal seedlings are quite broadly used in the dietary supplement industry. The elemental composition of seedlings of five species of the genus *Triticum* characterized by different ploidy levels (*Triticum spelta* line UWM10*, T. aestivum* cv. Torka 2n=6x*, T dicoccon* cv. Lamela*, T. polonicum* line Pol 5*,* and *T. turanicum* cv. Kamut 2n=4x) was determined by the Inductively Coupled Plasma Sector Field Mass (ICP-SFMS) method to analyze their suitability for use in the manufacture of dietary supplements as compared with a dietary supplement product made from *T. spelta* seedlings, which is commercially available in the European Union. Seedlings of five *Triticum* genotypes had similar concentrations of the analyzed elements, and contained essential microelements and only trace amounts of heavy metals. The commercially available dietary supplement had a very high content of aluminum (421.1 μg g^{-1} i.e. nearly 74- to 150-fold higher in comparison with wheat seedlings) and lanthanides (1.074 μg g^{-1} i.e. 44- to 87-fold higher in comparison with wheat seedlings). Fe, Cr, and V occurred in large quantities in the analyzed supplement (262.7, 11.3, and 0.080 μg g^{-1} i.e. 4.0, 3.7 and 101.4- fold higher in comparison with wheat seedlings). The principal component analysis (PCA) revealed that the supplement and the analyzed wheats had completely different elemental profiles. Similarities in the elemental profiles were noted between the wheats Kamut and cv. Lamela (both wheats are tetraploid) and between cv. Torka (hexaploid) and Polish wheat line Pol5 (tetraploid). Spelt line UWM10 significantly differed from the four remaining genotypes in the elemental profile of seedlings.

Keywords: wheat, seedlings, macroelements/microelements, principal component analysis, ICP-SFMS.

prof. dr hab. Marian Wiwart, Chair of Plant Breeding and Seed Production, University of Warmia and Mazury in Olsztyn, pl. Łódzki 3/401, 10-724 Olsztyn, Poland, e-mail: marian.wiwart@uwm.edu.pl, phone: +48895234842, fax: +4889523488

* This work has been partially supported by the Polish Ministry of Science and Higher Education, Project No. 0882/B/P01/2009/37.

INTRODUCTION

Cereal seedlings are broadly used in the dietary supplement industry (http://www.swansonvitamins.com). The health-promoting properties of dietary supplement products originate from the presence of nutraceuticals (mostly vitamins, microelements and phenolic compounds) in their composition and their antioxidant activity (Březinová et al. 2010). The growing conditions of cereal crops must be adequate to ensure the quality and safety of commercial preparations (Kulkarni et al. 2006). Biofortification is seen as a strategy for preventing essential micronutrient deficiencies around the world (White, Broadley 2009). Soil and plants differ in nutrient concentrations due to the selective ion uptake by plants and differences in ion availability determined by the pH of soil and antagonistic interactions between ions. When different crop species/varieties are grown under identical environmental conditions, differences in the nutrient content of plant tissues are caused only by the genotype, which allows for effective selection. Ancient cereal species, in particular members of the genus *Triticum*, attract considerable attention owing to their high nutritional value and the health benefits provided by consumption of grain and grain products (Stallknecht et al. 1996, Hammed, Simsek 2014, Benincasa et al. 2015). Ancient wheats have also attracted the interest of manufacturers of dietary supplements produced from seedlings. It should be noted, however, that there is general scarcity of reliable information about the quality of raw material used in the production process of such supplements.

The aim of this study was to compare the concentrations of macroelements and microelements in dried seedlings of spring varieties of emmer, spelt, Polish wheat, common wheat and Kamut wheat with those determined in a dietary supplement made from spelt grass, commercially available in the EU in the form of tablets.

MATERIAL AND METHODS

The experimental material comprised seedlings of five lines/varieties of wheat (*Triticum aestivum* – cv. Torka, *T. spelta* – line UWM10, *T. dicoccum* – cv. Lamela, *T. polonicum* – line Pol5 and *T. turanicum* – cv. Kamut) and a dietary supplement commercially available in the EU, which consists of 99% dried seedlings of *T. spelta* and 1% silicic acid (according to the manufacturer). Seeds were sown in an unheated greenhouse, in soil suitable for wheat cultivation, with moderate levels of essential macroelements and microelements (Table 1). Seedlings were harvested when they reached an average height of 15 cm, and were subsequently air-dried in a thermostat at 40°C.

ICP-SFMS analysis was performed according to the method proposed by

Table 1

Concentrations of elements (μg g^{-1}) in the soil in which the plants were grown in greenhouse conditions

Al $x10^4$	Fe $x10^3$	K $x10^3$	Ca $x10^3$	Mg $x10^3$	P $x10^2$	Mn $x10^2$	Na $x10^2$	S $x10^2$	Ba $x10^2$	Cr $x10^2$	Rb $x10^2$	V $x10^2$	Sr $x10^2$	ΣPb* $x10^2$	Zn x10
1.23	9.68	3.44	2.58	2.26	7.13	3.77	1.68	1.34	62.2	27.7	25.4	18.6	17.8	12.5	3.90

B x10	Li	Ni	Y	Cu	Ga	Co	As	Cs	W $x10^{-1}$	Se $x10^{-1}$	Cd $x10^{-1}$	Mo $x10^{-2}$	Ge $x10^{-2}$	ΣLAs** $x10^1$
1.28	9.49	7.31	7.09	4.30	3.79	3.37	2.65	2.58	2.84	1.20	1.77	8.84	8.76	5.60

*sum of isotopes ^{206}Pb, ^{207}Pb and ^{208}Pb; ** sum of detected lanthanides (La, Ce, Nd, and Pr)

WIWART et al. (2013). The sample digestion procedure is described elsewhere (WIWART et al. 2009).

ICP-SFMS analysis

Chemicals and reagents

Water was purified successively by reverse osmosis and in a Milli-Q plus system from Millipore (Molsheim, France). All chemicals used for inductively coupled plasma sector field mass spectrometry (ICP-SFMS), sample preparation and the preparation of calibration standards, were of pro analysis (p.a.) or supra-pure (s.p.) quality. Nitric acid s.p. (69%), a multi-element atomic spectroscopy standard solution (containing 10 mg l^{-1} 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^{-1} Ca, Fe, K and Na each in 5 mol^{-1} HNO_3) and the rhodium internal ICP-MS standard (containing 10 mg kg^{-1} $Rh(NO_3)_3$ in 0.5 mol l^{-1} 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.

Analysis of the concentrations of elements

An Anton Paar High Pressure Asher (HPA) equipped with seven quartz vessels of 30 ml volume was used throughout. 500 mg ± 5 mg wheat samples (whole grain) were weighed into the quartz vessels. After the addition of 2 ml HNO_3, the vessels were sealed with Teflon tape and quartz glass disks. Then, they were placed into a digestion unit and pressurized with nitrogen at 100 bar. The vessels were heated up to 110°C within 15 min, after which the temperature was rapidly increased to 260°C and maintained for 95 min. After cooling to approximately 30°C, clear digestion solutions were obtained. The digestion solutions were transferred into pre-weighed 100 ml bottles and were diluted with ultra-pure water to ~75 ml. After the addition of 1 ml internal standard (IS) solution containing Ge and Tl at 1 mg l^{-1} each in 0.5% HNO_3 (v/v), the samples were replenished with ultra-pure water to 100 g ± 0.1 g. For determination of the major elements, P, S, Mg and K, additionally 1:250 dilution of the samples was prepared by diluting 200 µl sample solution spiked with 0.5 ml of the above IS solution with 0.5% HNO_3 (v/v) to 50 g. All sample solutions were stored at 6°C until ICP-SFMS analysis.

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, NE, 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, NE, USA) connected to a 700 µl min^{-1} 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^{-1}, 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^{-1} and 0.90 l min^{-1}, the latter in the range of 0.85 l min^{-1} to 0.95 l min^{-1}. RF power was between 1185 W and 1195 W. The following isotopes were measured in the low-resolution mode, Rs = 300, 10% valley definition (LRM), ^{7}Li, ^{11}B, ^{23}Na, ^{24}Mg, ^{85}Rb, ^{88}Sr, ^{89}Y, ^{93}Nb, ^{97}Mo, ^{101}Ru, ^{105}Pd, ^{139}La, ^{140}Ce, ^{141}Pr, ^{143}Nd, ^{147}Sm, ^{151}Eu, ^{157}Gd, ^{161}Dy, ^{165}Ho, ^{166}Er, ^{169}Tm, ^{172}Yb, ^{184}W, ^{187}Re, ^{200}Hg, ^{205}Tl, ^{206}Pb, ^{207}Pb, ^{208}Pb. ^{23}Na, ^{27}Al, ^{31}P, ^{32}S, ^{135}C, ^{44}Ca, ^{48}Ti, ^{51}V, ^{52}Cr, ^{55}Mn, ^{56}Fe, ^{59}Co, ^{60}Ni, ^{63}Cu, ^{66}Zn, ^{111}Cd, ^{138}Ba, and ^{205}Tl were determined in the medium-resolution mode, Rs= 4 000 (MRM), whereas ^{39}K, ^{69}Ga, ^{72}Ge, ^{75}As, ^{77}Se, and ^{133}Cs were measured in the high-resolution mode Rs = 10 000 (HRM). 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.

The data were subjected to an analysis of variance and the mean values were compared by post-hoc t-testing. The data were also analyzed by principal component analysis (PCA). All analyses were conducted using Statistica v. 10.0 software (www.statsoft.com.)

RESULTS AND DISCUSSION

The analyzed wheats did not differ significantly in the concentrations of macroelements, except for sodium and magnesium (Table 2). Spelt seedlings had the highest magnesium content (over 65% and 3-fold higher in comparison with Kamut wheat and the dietary supplement, respectively). The sodium content of Kamut wheat seedlings was over 23-fold and nearly 800-fold higher compared with spelt seedlings and the dietary supplement, respectively. The commercially available dietary supplement had a very high content of aluminum (nearly 74- to 150-fold higher in comparison with wheat se-

Table 2

Concentrations of elements (μg g^{-1}) in the seedlings of the studied *Triticum* genotypes and in the dietary supplement (Suppl)

	K ($x10^4$)	Na ($x10^3$)	Ca ($x10^3$)	S ($x10^3$)	P ($x10^3$)	Mg ($x10^3$)	Cl ($x10^2$)	Fe	Mn	Al	Zn	Ti	Ba
Mean	4.85	6.15	6.65	5.17	2.19	1.99	3.46	66.4	106.5	4.26	40.8	29.6	21.3
Min - Max	3.76 - 5.88	0.84 – 17.90	5.84 - 7.20	4.26 – 6.30	1.88 - 2.35	1.75 - 2.24	2.53 - 4.88	53.0 - 75.5	86.5 - 117.6	2.79 - 5.73	29.9 - 51.7	25.2 - 34.7	9.14 - 36.9
Suppl	2.43**	0.03*	3.21**	1.48**	0.53**	0.77**	1.89	262.7**	32.2**	421.1**	18.27**	17.15**	9.70

	Rb	Cu	Sr	B	Mo	Li ($x10^{-1}$)	Cr ($x10^{-1}$)	Se ($x10^{-1}$)	Ni ($x10^{-1}$)	Cs ($x10^{-1}$)	As ($x10^{-1}$)	Cd ($x10^{-1}$)	Σ Pb† ($x10^{-1}$)
Mean	20.4	9.58	7.77	3.69	1.18	9.57	3.03	4.87	2.98	3.62	1.67	1.54	1.12
Min - Max	17.9 - 22.8	8.17 - 10.1	6.76 - 8.57	3.38 - 4.65	0.84 - 1.42	6.47 - 12.2	1.45 - 5.24	2.53 - 9.80	1.95 - 4.14	1.33 - 10.0	1.10 - 2.44	1.09 - 2.12	0.78 - 1.50
Suppl	3.47**	5.11**	7.38	3.83	1.56	4.13**	11.3**	0.17	7.11**	0.53	0.14	0.40*	8.01**

	Co ($x10^{-2}$)	Ga ($x10^{-2}$)	Tl ($x10^{-2}$)	Ge ($x10^{-2}$)	Σ LAs‡ ($x10^{-2}$)	V ($x10^{-3}$)	Pd ($x10^{-3}$)	W ($x10^{-3}$)	Hg ($x10^{-3}$)	Ru ($x10^{-3}$)	Y ($x10^{-3}$)	Re ($x10^{-3}$)	Nb ($x10^{-4}$)
Mean	2.02	1.21	2.79	2.19	1.93	0.79	7.82	4.30	4.20	1.28	2.04	1.24	6.32
Min - Max	1.82 - 2.45	0.97 - 1.47	1.21 - 5.68	1.57 - 2.89	1.23 - 2.42	0.60 - 1.17	7.40 - 8.50	3.20 - 6.60	3.50 - 2.40	0.90 - 1.80	1.50 - 2.70	0.80 - 1.60	4.90 - 8.41
Suppl	11.0**	0.14	0.63***	3.24*	107.4**	80.1**	34.9**	14.4**	3.13	0.55*	0.18**	0.09**	129.1**

† – sum of isotopes ^{206}Pb, ^{207}Pb and ^{208}Pb, ‡ - sum of detected lanthanides (Ce, Nd, La, Gd, Pr, Sm, Dy, Eu, Er, Yb, and Tm),
*, ** – difference between mean values for studied *Triticun* genotypes and dietary supplement significant at $p < 0.05$ and $p < 0.01$, respectively

edlings) and lanthanides (LAs) (44- to 87-fold higher in comparison with wheat seedlings). PCA revealed that the supplement and the analyzed wheats had completely different elemental profiles (Figure 1). Similarities in the

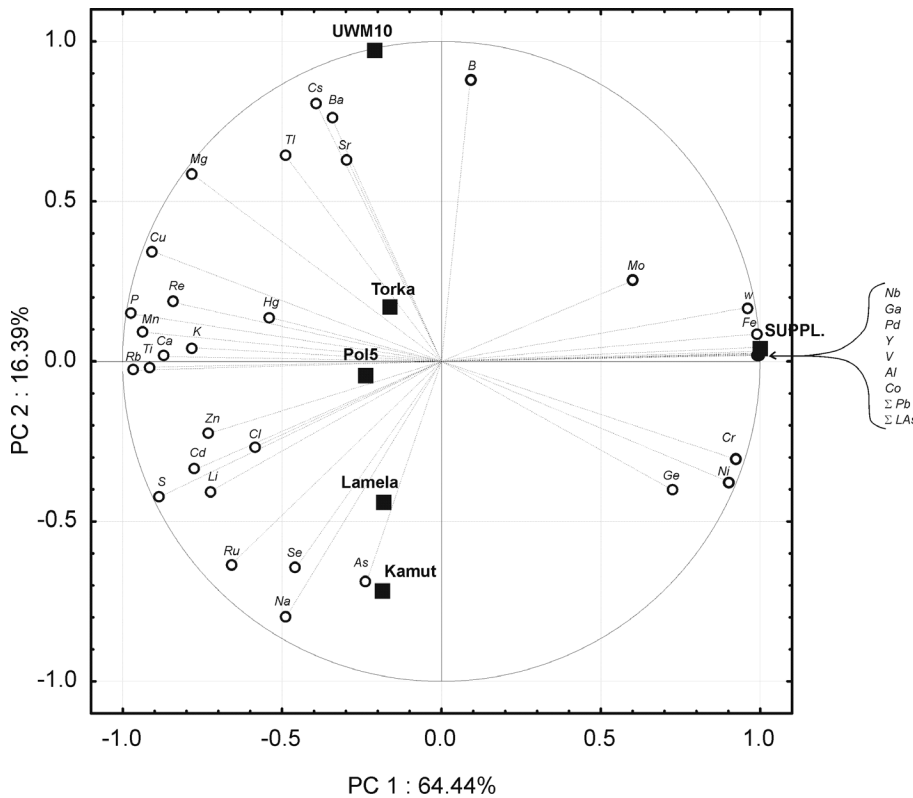

Fig. 1. Biplot presenting the results of PCA including all elements (marked as small circles) detected in the studied *Triticum* genotypes and in the dietary supplement (marked as black squares). Σ Pb – sum of isotopes ^{206}Pb, ^{207}Pb and ^{208}Pb, Σ LAs – sum of detected lanthanides (Ce, Nd, La, Gd, Pr, Sm, Dy, Eu, Er, Yb, and Tm)

elemental profiles were noted between Kamut wheat and emmer cv. Lamela (both wheats are tetraploid) and between wheat cv. Torka (hexaploid) and Polish wheat line Pol5 (tetraploid). Spelt line UWM10 significantly differed from the four remaining genotypes in the elemental profile of seedlings. For easier interpretation, the results of PCA are presented in a biplot, where points representing elements and types of experimental material (genotypes and the supplement) can be displayed in a single chart. The first two principal components (PC1 and PC2) explained 80.33% of variance, which indicates that PCA strongly discriminated the analyzed materials. It should also be noted that the coefficient of the factor-variable correlation was close to 1 for the supplement and spelt line UWM10, hence in this case discrimination was particularly strong.

The concentrations of microelements in the grain of intensively cultivated wheat varieties have decreased over the past few decades, which gives rise to concern because wheat is the most widely consumed cereal grain (Fan et al. 2008). Supplementation options include natural products with a high content of microelements in the right proportions. In plant tissues, elements (in particular metals) form complexes with organic compounds, which makes them more bioavailable than inorganic compounds (e.g. salts) used as ingredients of medications and dietary supplements. Therefore, the use of cereal seedlings in dietary supplements seems fully justified (Kulkarni et al. 2007). Effective processing technologies should be developed to maintain the quality and nutritional value of plant materials during the manufacturing of dietary supplements. Fe, Cr, V and Nb, present in large quantities in the analyzed supplement, are constituents of alloy steels. Based on our past experience, the reason for the surprisingly high concentrations of the analyzed elements in plant materials (leaves, kernels, seeds) could be some contamination from the processing equipment and machines, whose main components are made of steel. The technological process might also be responsible for accumulation of Al (considered undesirable in human nutrition) in the tested supplement. Aluminum trays are sometimes used in hydroponically grown wheat (http://www.grandeurafrica.com/how-to-make-hydroponically-grown-barleywheat-fodder-to-grow-evenly/), which could contribute to higher Al concentrations in seedlings. Small amounts of LAs can be added to pigs' diets as alternative growth promoters (He, Rambeck 2000). They also have a stimulating effect on seed germination, chlorophyll synthesis, root growth, nutrient uptake and photosynthesis. The uptake of LAs by plants varies depending on their abundance in soil, but generally it is not very high (Kabata-Pendias 2010). However, LAs are unlikely to exert adverse effects on human health if a dietary supplement is used as recommended by the manufacturer (Li et al. 2013). The results of our study indicate that the microelement profile of the dietary supplement made of spelt grass is completely different from the elemental profiles of wheat seedlings, including spelt. Despite the relatively small number of samples, our findings provide preliminary grounds for hypothesizing that the analyzed supplement or grown crops could have been enriched with LAs or that the plants used as ingredients in the manufacture of this product had been grown in the substrate or soil with an extremely high LA content. Since the information on growing conditions has not been provided by the manufacturer, it is possible that LAs could be contained in fertilizers used in hydroponic or aeroponic culture. This is a speculative conclusion, but it seems to be the only explanation for the high LAs content of the analyzed supplement.

REFERENCES

Benincasa P., Galieni A., Manetta A.C., Pace R., Guiducci M., Pisante M., Stagnari F. 2015. *Phenolic compounds in grains, sprouts and wheatgrass of hulled and non-hulled wheat species.* J. Sci. Food Agric., 95: 1795-1803.

Březinová Belcredi N., Ehrenbergerová J., Fiedlerová V., Běláková S., Vaculová K. 2010. *Antioxidant vitamins in barley green biomass.* J. Agric. Food Chem., 58: 11755-11761.

Fan M.S., Zhao F.J., Fairweather-Tait S.J., Poulton P.R., Dunham S.J., McGrath S.P. 2008. *Evidence of decreasing mineral density in wheat grain over the last 160 years.* J. Trace Elem. Med. Bio., 22: 315-324.

Hammed A.M., Simsek S. 2014. *Hulled Wheats: A Review of Nutritional Properties and Processing Methods.* Cereal Chem., 91: 97-104

He M.L., Rambeck W.A. 2000. *Rare earth elements – a new generation of growth promoters for pigs?* Arch. Tierernahr., 53: 323-334.

Kabata-Pendias A. 2010. *Trace elements in soils and plants.* 4th ed. Boca Raton, London, New York: CRC PressTaylor & Francis Group.

Kulkarni S.D., Acharya R., Rajurkar N.S., Reddy A.V.R. 2007. *Evaluation of bioaccessibility of some essential elements from wheatgrass (Triticum aestivum* L.*) by in vitro digestion method.* Food Chem., 103: 681-688.

Kulkarni S.D., Tilak J.C., Acharya R., Rajurkar N.S., Devasagayam T.P.A., Reddy A.V.R. 2006. *Evaluation of the antioxidant activity of wheatgrass (Triticum aestivum* L.*) as a function of growth under different conditions.* Phytotherap. Res., 20: 218-227.

Li X., Chen Z., Chen Z., Zhang Y. 2013. *A human health risk assessment of rare earth elements in soil and vegetables from a mining area in Fujian Province, Southeast China.* Chemosphere, 93: 1240-1246.

Stallknecht G.F., Gilbertson K.M., Ranney J.E. 1996. *Alternative wheat cereals as food grains: Einkorn, emmer, spelt, kamut, and triticale.* In: *Progress in new crops* (Ed. by Janick J.). ASHS Press, Alexandria, VA, pp. 156-170.

White P.J., Broadley M.R. 2009. *Biofortification of crops with seven mineral elements often lacking in human diets – iron, zinc, copper, calcium, magnesium, selenium and iodine.* New Phytol., 182: 49-84.

Wiwart M., Kandler W., Perkowski J., Berthiller F., Preinerstorfer B., Suchowilska E., Buśko M., Laskowska M., Krska R. 2009. *Concentrations of some metabolites produced by fungi of the genus Fusarium and selected elements in spring spelt grain.* Cereal Chem., 86: 52-60.

Wiwart M., Suchowilska E., Kandler W., Sulyok M., Groenwald P., Krska R. 2013. *Can Polish wheat (Triticum polonicum* L.*) be an interesting gene source for breeding wheat cultivars with increased resistance to Fusarium head blight?* Gen. Res. Crop Evol., 60: 2359-2373.