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BARIUM CONCENTRATION IN GRAIN OF *AEGILOPS* AND *TRITICUM* SPECIES

ABSTRACT: The aim of this study was to evaluate the concentration of barium in grain of various *Aegilops* and *Triticum* species with different genomes. The studied species differed significantly with respect to the concentration of barium. The grain of wild diploid *Aegilops speltoides,* the donor of B genome, contained significantly higher Ba concentration than all other analyzed genotypes. Wild and cultivated tetraploid wheats (*Triticum diciccoides, Triticum dicoccon, Triticum turgidum* and *Triticum durum)* had the lowest Ba concentration in grain. The modern cultivated hexaploid varieties presented substantial variation in grain concentration of barium. The highest Ba concentration (3.42 mg/kg) occurred in Serbian winter wheat variety Panonnia.

KEYWORDS: barium concentration, grain, wheat, genotypes, diploid, tetraploid, hexaploid

INTRODUCTION

Barium (Ba) is a silvery-white alkaline earth metal that occurs naturally in different compounds. Ba is relatively abundant in the earth's crust, with mean values ranging between 265 and 835 μg/g dry weight (DW) depending on the soil type [Lide 2005]. Ba is not a very mobile element in most soil systems due to the formation of water-insoluble salts and their partition into soils and sediments [WHO 2001].

Ba has not been reported as an essential trace element for plants, and it was included in a list of elements that pose a risk to human health and are most com-

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monly found in cases of soil contamination [USEPA 2009; CETESB 2001]. Ba absorption by plant species grown in polluted areas has been observed by Abreu *et al.* [2012]. Pais *et al.* [1998] found that Ba contents of 200 mg/kg could be moderately toxic and that 500 mg/kg could be considered toxic for plants. Therefore, there is an increasing concern regarding Ba in plants, especially in edible plants, because Ba can cause damage in the human body. The ingestion of Ba can result in several human health problems such as: high blood pressure, muscular paralysis, gastrointestinal disturbances, kidney damage, respiratory failure, and, in some cases, even death [Jacobs *et al.*, 2003; Lenntech 2005].

The land is enriched in Ba from the atmosphere with a range of 40–80 g/ha/year. In atmosphere Ba comes from the production of barium compounds, combustion of coal and oil, and in arable land primarily by using mineral fertilizers. Manure contains Ba from 20 to 100 mg/kg, compost from 35 to 100 mg/kg, slaughterhouses meat meal from 4 to 16 mg/kg, sludge wastewater from 110 to 200 mg/kg [Kádár *et al.*, 2012; Kádár 2013], phosphatic fertilizers 200 mg/kg, and the means of calcification 150 to 250 mg/kg [Kabata-Pendias and Pendias 1984].

The effects of Ba on plant growth are in general toxic as small concentrations can retard growth [Robinson *et al.*, 1938]. The various harmful effects of Ba in cereals, such as reduction in germination, root-shoot length, changes in the activity of various enzymes and grain yield, are reported [Debnath and Mukerija 1982; Iqbal and Naz 1989; Suwa *et al.*, 2008].

Ba concentration in the dry matter of plants varies within a wide range from 1 to 200 mg/kg. Kőrös [1980] reported the plants of the family Myrtaceae characterized by particularly great accumulation of Ba. The legumes are also characterized by larger accumulation of Ba. According to Kádár [2013], Ba concentration in dry matter in monocots, dicots and legume grown on lime chernozem was 23, 41 and 150 mg/kg (respectively), and on serpentine soil 9, 14 and 29 mg/kg (respectively).

The wheat group has evolved through allopolyploidization, namely, through hybridization among species from the plant genera *Aegilops* and *Triticum* followed by genome doubling. Bread wheat (*Triticum aestivum*) is an allohexaploid species (2n = 6x = 42, genome BBAADD) that originated from hybridization events involving three different diploid progenitors: (i) *Triticum urartu*, the donor of the A genome [Dvorak 1976], (ii) a yet undiscovered extant (or extinct) *Aegilops* species closely related to *Aegilops speltoides,* the donor of the B genome, and (iii) *Aegilops tauschii*, the donor of the D genome [Kihara 1944].

This paper focuses on evaluation of barium concentration in whole grain of diploid and tatraploid wheat progenitors and ancestors of common wheat as well as in hexaploid commercial cultivars all grown at the same location for three years.

MATERIAL AND METHODS

Six diploid genotypes with different genome formula BB, AA, and DD, five tetraploids (BBAA) and nine hexaploids (BBAADD) wheat were used in the experiment. Among the diploid wheats, four were wild and one (*Triticum monococcum subsp. monococcum*) was primitive cultivated wheat. Among the tetraploid wheats used, three genotypes were wild einkorn while two were cultivated. All hexapolids were cultivated cultivars (Table 1).

The wheat genotypes were planted in a randomized complete block design with three replicates at the experiment field of the Institute of Field and Vegetable Crops, Novi Sad (45.2° N, 19.5° E, 80 m elevation), in 2011, 2012 and 2013, on weakly calcareous chernozem. Meteorological data for the experimental years show that the year 2013 was characterized by higher average temperatures as well as precipitation in comparison to years 2011 and 2012 (Table 2).

Field plots of 2.5 m^2 with 10 rows spaced 10 cm apart were planted with 400 seed per m^2. In the beginning of October before planting, the experimental area was fertilized with 50 kg N ha^{-1}, and 50 kg P_2O_5 ha^{-1}. The soil at the experimental site was well provided with potassium. The genotypes were planted in mid-October which is the optimal time for winter wheat. There was one top-dressing in early February with 50 kg N ha^{-1}. During the spring the field plots were protected against weeds once without fungicides application. The genotypes were harvested at crop maturity and all hulled genotypes were dehulled by hand. All grain samples selected for this study were visibly intact without any sign of degradation.

Table 1. *Genotypes of* Aegilops *and* Triticum *species (after Van Slageren, 1994) examined in the experiments*

Species and subspecies	Genome	Name	Source/Origin
Aegilops speltoides ssp. *speltoids 1*	BB		IPK*
Aegilops speltoides ssp. *speltoids 2*	BB		IPK
Triticum urartu	AA		IPK
Triticum monococcum ssp. *aegilopoides*	AA	Wild einkorn	IFVC, SRB**
Triticum monococcum ssp. *monococcum*	AA	Cultivated einkorn	IFVC, SRB
Aegilops tauschii ssp. *tauschii*	DD	Goat grass	IPK
Triticum turgidum ssp. *dicoccoides* (IPK)	BBAA	Wild emmer	IPK
Triticum turgidum ssp. *dicoccoides* (IFVC)	BBAA	Wild emmer	IFVC, SRB
Triticum turgidum ssp. *dicoccon*	BBAA	Cultivated emmer	IFVC, SRB
Triticum turgidum ssp. *turgidum*	BBAA	Rivet wheat	IPK
Triticum turgidum ssp. *durum* (cv. Durumko)	BBAA	Durum wheat	IFVC, SRB
Triticum aestivum ssp. *spelta* (cv. Nirvana)	BBAADD	Spelt wheat	IFVC, SRB
Triticum aestivum (cv. Panonnia)	BBAADD	Common wheat	IFVC, SRB
Triticum aestivum (cv. Bankut 1205)	BBAADD	Common wheat	HUN
Triticum aestivum (cv. Bezostaja 1)	BBAADD	Common wheat	RUS
Triticum aestivum (cv. Siete Cerros)	BBAADD	Common wheat	MEX
Triticum aestivum (cv. Florida)	BBAADD	Common wheat	USA
Triticum aestivum (cv. Renan)	BBAADD	Common wheat	FRA
Triticum aestivum (cv. Condor)	BBAADD	Common wheat	AUT
Triticum aestivum (cv. Bolal)	BBAADD	Common wheat	TUR

* IPK – Genebank Gatersleben of the Leibniz Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany; ** IFVC, SRB – Institute of Field and Vegetable Crops, Novi Sad, Serbia; HUN – Hungary; RUS – Russia; MEX – Mexico; USA – United State of America; FRA – France; AUT – Australia; TUR – Turkey.

Table 2. *Monthly mean temperature and precipitation during three years*

Month	Mean air temperature (ºC)			Precipitation (mm/m^2)		
	2010/11.	2011/12.	2012/13.	2010/11.	2011/12.	2012/13.
October	9.2	10.7	13.7	67	35	48
November	9.5	2.8	10.1	46	2	36
December	0.8	4.3	0.7	64	50	55
January	0.2	2.0	2.8	25	43	60
February	-0.3	-5.0	4.3	37	67	50
March	5.8	8.3	6.0	26	4	68
April	13.2	13.0	13.5	23	83	30
May	16.7	17.2	18.2	63	51	118
June	21.0	22.6	20.2	37	34	126
Average/Sum	8.5	8.4	9.9	388	366	591
Average yield*	4.88	4.48	5.50			

*average wheat yield in the province of Vojvodina

Milling was carried out using a Perten Laboratory Mill 3100 to produce wholemeal. After digestion of grain wholemeal in a mixture of HNO_3 (65%) and H_2O_2 (30%) total concentrations of Ba were determined by an ICP-OES (Varian Vista-Pro) in Research Institut for Soil Science and Agricultural Chemistry, Budapest. All statistical analyses were done with program XLSTAT-Pro (demo version, Version 3.02.2009).

RESULTS AND DISCUSSION

Like other similar alkaline earth metals barium is poorly mobile in plants. Kastori *et al.* [2007] emphasized the low index of Ba translocation from vegetative organs to grain in wheat. Barium mostly accumulates in the vegetative parts of the plants, in older leaves and stem, and to a much lesser extent in the generative organs (grain). This is confirmed by the results of Kádár and Szemes [1994] in triticale, where Ba concentration in grain was 1.7 mg/kg while in the stem it was 31 mg/kg.

The analyzed *Aegilops* and *Triticum* species differed significantly with respect to the concentration of barium (Tab. 3). The grain of both genotypes of *Aegilops speltoides* with B genome contained significantly higher concentration of barium (9.02 mg/kg and 10.19 mg/kg respectively) than the grain of all others species including related species (*Aegilops tauschii*). This is an indication that species which belong to the same genera can be quite genetically different. Among the diploid wheats the lowest Ba concentration occurred in the wild encorn (*Triticum monococcum subsp. aegilopoides*) (Tab. 3). In the set of tetraploids, wild emmer wheat (from IFVC) contained significantly higher Ba concentration than all other tetraploids including the modern cultivated durum variety. The primitive cultivated emmer (*Triticum turgidum* subsp. *dicoccon*) had the lowest Ba concentration when compared to all analyzed wheat genotypes (Tab. 3).

Table 3. *Concentration of barium (mg/kg, DW) in the whole grain of* Aegilops *and* Triticum *species*

Species and subspecies	Year			Average
	2011	2012	2013	
Aegilops speltoides ssp. *speltoids 1*	6.75	8.38	11.93	9.02[b]
Aegilops speltoides ssp. *speltoids 2*	9.67	8.98	11.93	10.19[a]
Triticum urartu	1.94	2.38	2.23	2.18[efg]
Triticum monococcum ssp. *aegilopoides*	2.29	1.92	1.55	1.92[efgh]
Triticum monococcum ssp. *monococcum*	2.28	2.17	2.07	2.17[efg]
Aegilops tauschii ssp. *Tauschii*	1.92	2.04	2.15	2.04[efg]
Triticum turgidum ssp. *dicoccoides* (IPK)	1.62	1.65	1.62	1.63[fgh]
Triticum turgidum ssp. *dicoccoides* (IFVC)	2.12	2.14	2.16	2.14[efg]
Triticum turgidum ssp. *dicoccon*	0.86	0.98	1.10	0.98[h]
Triticum turgidum ssp. *turgidum*	2.52	0.99	1.46	1.66[fgh]
Triticum turgidum ssp. *durum* (cv. Durumko)	2.02	1.01	1.32	1.45[gh]
Triticum aestivum subsp. *spelta* (cv. Nirvana)	1.70	1.61	1.51	1.61[gh]
Triticum aestivum (cv. Panonnia)	3.63	2.89	3.74	3.42[c]
Triticum aestivum (cv. Bankut 1205)	2.65	2.73	3.22	2.87[cde]
Triticum aestivum (cv. Bezostaja 1)	1.71	0.87	1.55	1.38[gh]
Triticum aestivum (cv. Siete Cerros)	4.08	2.45	3.38	3.30[cd]
Triticum aestivum (cv. Florida)	3.34	1.25	3.30	2.63[cdef]
Triticum aestivum (cv. Renan)	3.20	0.87	2.54	2.20[efg]
Triticum aestivum (cv. Condor)	2.22	0.93	2.01	1.72[fgh]
Triticum aestivum (cv. Bolal)	2.92	1.92	2.28	2.37[defg]
Average	2.97[a]	2.41[a]	3.15[b]	

Different letters indicate significant difference at $P < 0.05$ level.

The modern cultivated hexaploid varieties presented substantial variation in grain concentration of barium. The highest Ba concentration (3.42 mg/kg) occurred in Serbian winter wheat variety Panonnia, which is still being produced in Serbia, Bulgaria and Czech Republic. Contrary the well known variety Bezostaja 1 had 2.5 times lower concentration of barium in grain (Tab. 3). Among hexaploid wheats, cultivated spelt variety (cv. Nirvana) had the second lowest level of Ba concentration in grain. The existence of a large variation in concentration of barium in grain of modern cultivated wheat varieties indicates that the concentrations of Ba in hexaploid wheats are genetically controlled. The similar results in tetraploids for Zn concentration reported Cakmak *et al.* [2000].

It is quite clear that two accessions of *Aegilops speltoides* with B genome had exceptionally large concentration of barium in grain, many times larger than all others analyzed genotypes. The main reason for this is that there is a large genetic distance between these genotypes and all other genotypes. Second, high grain Ba concentration in the wild *Aegilops* species is partly related to the lowest grain weights, indicating a role of "concentration effects". The species from other two diploids with A and D genomes did not differ significantly in barium concentration in the grain (Tab. 4).

Table 4. *Concentration of barium (mg/kg DW) in the species with five different genomes*

Genome	Year			Average
	2011	2012	2013	
BB	8.21	8.68	11.93	9.61^{a}
AA	2.17	2.16	1.95	2.09^{bc}
DD	1.92	2.04	2.15	2.04^{bc}
BBAA	1.83	1.35	1.53	1.57^{c}
BBAADD	2.83	1.72	2.61	2.39^{b}
Average	3.39^{a}	3.19^{a}	4.04^{b}	

Different letters indicate significant difference at P < 0.05 level.

Table 5. *Analysis of variance of concentration of Ba concentration in the whole grain of* Aegilops *and* Triticum *species*

Source of variation	df	SS	MS	F value
Genotyp (G)	19	986.32	51.91**	147.02
Year (Y)	2	18.14	9.07**	25.68
G *Y	38	73.67	1.94**	5.49
Pooled error	120	42.37	0.35	

In average, the tetraploids (wild and cultivated emmer) with BA genome had the least concentration of barium comparing to all diploid and hexaploid analyzed genotypes (Tab. 4). It was unexpected that diploids with A and B genome did not significantly differ from cultivated hexaploid modern varieties (Tab. 4). It confirms that plumper grain which occurred in modern hexaploid varieties does not necessarily contain a smaller concentration of barium. This finding is also consistent with studies on various trace elements concentrations of McDonald *et al.* [2008] and Zhao *et al.* [2009].

It is quite obvious that concentration of barium in grain of various wheats is influenced by genotype to a great extent. Results of ANOVA (Tab. 5) show that conditions over the year also had significant effect to Ba concentration in grain, which can be seen in Table 4 as well. The concentration of Ba in the grain of tested wheat genotypes was significantly lower in dry years (2011 and 2012) than in humid year 2013 (Tab. 4).

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КОНЦЕНТРАЦИЈА БАРИЈУМА У ЗРНУ ВРСТА *AEGILOPS* И *TRITICUM*

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РЕЗИМЕ: Циљ овог истраживања био је да се процене концентрације баријума у зрну различитих генотипова *Aegilops* и *Triticum* врста. Испитиване врсте су се значајно разликовале у односу на концентрацију баријума. Зрно дивљег диплоида *Aegilops speltoides,* донатор Б генома садржи знатно већу концентрацију баријума него сви остали испитивани генотипови. Дивља и питома тетраплоидна пшеница (*Triticum diciccoides, Triticum dicoccon, Triticum turgidum* и *Triticum durum*) имала је најнижу концентрацију баријума у зрну. Модерне гајене хексаплоидне сорте показале су значајне варијације у концентрацији баријума у зрну. Највеће концентрације (3,42 mg/kg) установљене су код „Пононије", озиме сорте пшенице произведене у Србији.

КЉУЧНЕ РЕЧИ: концентрација баријума, зрна, пшеница, генотип, диплоиди, тетраплоиди, хексаплоиди