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Genetic differences in aluminium accumulation in the grains of field grown *Aegilops* and *Triticum*

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Abstract: Plant species and genotypes differ considerably with respect to the accumulation of mineral elements. This study examined the accumulation of aluminium (Al) in *Aegilops* and *Triticum* species with different genomes (AA, BB, BBAA, BBAADD and DD) and correlations between concentration of Al in the grain and features of the spike. Twenty different genotypes were included in three-year field experiments. The examined species and genomes differed significantly in their Al concentration in grain. The highest concentrations of Al were found in the grains of wild diploid *Aegilops speltoides* (BB genome), and the lowest in tetraploids (BBAA genome). A significant positive correlation was found between the concentration of Al in the grain and spike length, while negative correlations were found between concentration of Al in the grain and the number of grains per spike, grain weight per spike and thousand grains weight. The presence of higher Al content in the individual grains of tetraploid and hexaploid wheat with respect to diploid ancestors suggests that during the increase in ploidity the capacity of plants to uptake Al from soil increased concomitantly with the increase of grain capacity to serve as Al sink.

Keywords: essential element; toxicity; stress factor; tolerance; ploidy level

Aluminium (Al) is widely present in the biosphere and it constitutes about 7% of the Earth's crust. It is not essential element for plants. Nevertheless, there are data suggesting that low concentrations of Al may have stimulating effect on plant growth (Ghanati et al. 2005). A favourable effect of low Al concentrations can be expected at the first place in plant species and genotypes that better tolerate high Al concentrations (Marschner 2012). Aluminium toxicity exists only in acidic soils because low pH (below 5.5) leads to its solubilisation, resulting in toxicity of aluminium to plants; with pH values higher than 6.0 Al is not available to plants. Therefore, aluminium is globally studied mainly as one of stress factors present in acidic soils, where it confines plant growth. Acidic soils limit plant production at about 30% to 40% of arable land. Excessive soil acidity limits plant production often indirectly, by affecting the availability of other elements in the soil solution (Matsumoto 2000, Zhao et al. 2014). Nevertheless, at acidic soils, the most limiting factor for plant production is indeed high concentration of soluble Al (Kochian 1995). Moreover, the Al^{3+} ion exhibits a toxic effect. With an increase in pH, its concentration in the soil solution however declines. At soil solution with pH > 6, Al is present in the form of an anion $Al(OH)_4^-$ but its phytotoxic features were not studied in detail (Ma et al. 2003). Since small grains are very important crops, a lot of research has been done with the aim to understand and alter mechanisms of tolerance towards high concentrations of Al and the results show that significant differences exist between different genotypes (Tang et al. 2002, Darkó et al. 2012).

Plant species differ in their ability to take up, accumulate, translocate and use mineral elements (Mengel 1982), but differences exist also between genotypes,

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lines and individual parts of the whole plants within a species or variety (Sarić 1981, Darkó et al. 2004, 2012). Accumulation of mineral elements in grain depends at the first place on the rate of their uptake by the root system and subsequent translocation into generative parts. Mineral elements undesirable in plants, such as Al, may thus affect the quality of food obtained from such plant parts, enter the food chain and contribute to the overall intake of Al through the diet.

The aim of this experiment was to assess the influence of the ploidy level and features of the spike on the accumulation of Al in the wheat grain, deriving from plants grown in the field, on the soil not prone to excessive concentrations of soluble Al.

MATERIAL AND METHODS

Plant material. Six diploid genotypes of wheat with different genome formulas (BB, AA or DD), five tetraploids (BBAA) and nine hexaploids (BBAADD) were used in the experiment (Table 1). Among the diploid wheats, four were wild and one (*Triticum monococcum* var. *monococcum*) was primitive cultivated wheat. Among the tetraploid wheats included in the experiment, two genotypes were wild emmer, one was rivet wheat, and two were cultivated genotypes. All hexaploids were cultivated genotypes (Table 1).

Experimental site and setup of the experiment. The experiments were set up at the experimental fields of the Institute of Field and Vegetable Crops, Novi Sad (45.2°N, 19.5°E, 80 m a.s.l.), in three consecutive years. The soil of the experimental field was classified as a calcic gleyic chernozem (FAO 2006), which is characterised by pH 7.2 and 8.0 in KCl and H_2O, respectively (Kastori et al. 2017). On such type of soil average concentrations of total Al varies typically between 42 mg/kg in the 0–30 cm soil layer to 21 mg/kg in the 125–170 cm layer (Altermann et al. 2005). Solubility of Al in the soil arouses as a problem for plant growth at pH below 5 (Darkó et al. 2012). Therefore, under the present conditions, the toxicity of Al was not an issue. Consequently, differences with

Table 1. Genotypes of *Aegilops* and *Triticum* species (classified according to van Slageren 1994) examined in the experiments

No.	Species and subtaxa	Name	Genome(s)	Accession name	Source
1	*Aegilops speltoides* TAUSCH var. *speltoides*	–	BB	–	D
2	*Aegilops speltoides* TAUSCH var. *speltoides*	–	BB	–	D
3	*Triticum urartu* THUM. ex GANDIL.	red wild einkorn	AA	–	RS
4	*Triticum monococcum* L.	wild einkorn	AA	Tr. Monococcum	RS
5	*Triticum monococcum* L. var. *monococcum*	cultivated einkorn	AA	Krupnik	RS
6	*Aegilops tauschii* COSS.	Tausch's goatgrass	DD	–	D
7	*Triticum dicoccoides* (KOERN. ex ASCHERS. et GRAEBN.) SCHWEINF.	wild emmer	BBAA	–	D
8	*Triticum turgidum* L. var. *rubralbum* FLAKSB.	wild emmer	BBAA	–	RS
9	*Triticum turgidum* L. var. *turgidum*	cultivaed emmer	BBAA	–	RS
10	*Triticum turgidum* L.	rivet wheat	BBAA	Berkners Rauhweizen	D
11	*Triticum durum* DESF. var. *pseudosalomonis* PAPAD.	durum wheat	BBAA	Durumko 1	RS
12	*Triticum spelta* L. var. *duhamelianum* (MAZZ.) KOERN.	spelt wheat	BBAADD	–	RS
13	*Triticum aestivum* L. var. *lutescens* (ALEF.) MANSF.	common wheat	BBAADD	Panonia	RS
14	*Triticum aestivum* L. var. *lutescens* (ALEF.) MANSF.	common wheat	BBAADD	Bankut 1205	H
15	*Triticum aestivum* L.	common wheat	BBAADD	Besostaya 1 1	RUS
16	*Triticum aestivum* L.	common wheat	BBAADD	Italian	MEX
17	*Triticum aestivum* L.	common wheat	BBAADD	Florida	D
18	*Triticum aestivum* L. var. *aestivum*	common wheat	BBAADD	Renan	F
19	*Triticum aestivum* L.	common wheat	BBAADD	Condor	AUS
20	*Triticum aestivum* L.	common wheat	BBAADD	Bolal	TR

D – Germany; RS – Serbia; H – Hungary; RUS – Russia; MEX – Mexico; F – France; AUS – Australia; TR – Turkey. For details comprising accession numbers of studied genotypes please refer to the paper Kastori et al. (2017)

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respect to genotypic specificity to accumulate Al in plant tissues, and specifically in the grain, allowed detecting concentrations typically found in wheat grains and affect quality of agricultural products containing wholegrain flour. Agricultural management practices were standard for wheat production in the region and soil type.

Wheat genotypes were sown in a randomised complete block design, in three replications. The surface of field plots was 2.5 m^2, each of which contained 10 rows, with row spacing that amounted to 10 cm; 400 seeds were sown per m^2. Genotypes included in the experiment were harvested at crop maturity and all hulled genotypes were manually de-hulled. All grain samples used for the analysis in this study were visibly intact without any sign of damage. Grains were milled to produce whole meal, which was used in further analyses. Details on soil and climate data, experimental setup, management practices during vegetation and analyses of samples are available in the paper of Kastori et al. (2017). After digestion of grain whole meal in a mixture of 10 mL HNO_3 (65%) and 2 mL of H_2O_2 (30%) using the microwave technique, the concentrations of total Al were determined by inductive coupled plasma emission spectrometer (ICP-OES Varian Vista-Pro, Palo Alto, USA).

Statistical procedures. Studied data were subjected to a combined analysis of variance, treating environment and the genotypes as experimental factors, and the significance of differences among means were determined using the Tukey's test. Standard deviations, analysis of variance and Pearson's linear correlation coefficients among all the traits were obtained by Infostat (Di Rienzo et al. 2016). The relationship between the total Al in grain and grain mass was performed by linear regression using the Infostat (Di Rienzo et al. 2016). The association of Al concentration with the other features of analysed genotypes (thousand grain weight and spike characteristics: grain mass per spike, spike length, number of spikelets and the number of grain per spike) were analysed by the principle component analysis (PCA).

To assess the degree of stability of wheat and *Aegilops* genotypes with respect to the concentration of Al in their grains, coefficient of variation, regression coefficient and deviation from regression,

Table 2. Average concentrations of aluminium in the whole grain (mg/kg dry matter), stability parameters and their ranks in *Aegilops* and *Triticum* species over three experimental years

Genot. No.*	Average	Rank	*CV* (%)	Rank	b_i	Rank	S^2d_i	Rank	σ_i^2	Rank	Wi	Rank
1	6.98	1	35.64	17	4.78	20	3.112	20	4.93	20	8.94	20
2	5.52	2	31.87	16	3.88	19	0.042	7	1.88	19	3.45	19
3	2.98	8	13.37	9	0.76	1	0.022	5	0.02	2	0.11	2
4	2.16	16	6.96	2	–0.06	8	–0.021	4	0.24	8	0.50	8
5	3.38	5	19.00	11	1.28	2	0.094	14	0.07	4	0.19	4
6	2.53	12	50.10	20	2.81	17	–0.032	6	0.71	16	1.35	16
7	2.84	10	12.37	7	–0.11	9	0.177	15	0.37	12	0.74	12
8	2.08	17	21.33	13	–0.92	18	–0.015	3	0.82	17	1.54	17
9	2.21	15	28.33	14	1.39	4	–0.062	11	0.00	1	0.06	1
10	2.74	11	20.03	12	–0.20	11	0.521	18	0.61	15	1.17	15
11	1.33	18	11.65	6	0.28	7	–0.048	8	0.09	5	0.23	5
12	1.28	19	7.62	3	–0.11	10	–0.051	9	0.25	9	0.51	9
13	0.99	20	16.38	10	–0.31	13	–0.051	10	0.35	11	0.70	11
14	2.89	9	11.38	5	–0.62	16	–0.003	1	0.58	14	1.12	14
15	3.49	4	13.15	8	0.63	3	0.197	17	0.14	6	0.32	6
16	2.47	13	28.71	15	1.52	6	0.012	2	0.06	3	0.18	3
17	2.35	14	45.62	18	2.32	14	0.062	12	0.43	13	0.83	13
18	3.13	7	3.33	1	–0.23	12	–0.065	13	0.30	10	0.61	10
19	3.81	3	11.27	4	0.55	5	0.183	16	0.15	7	0.33	7
20	3.34	6	47.09	19	2.38	15	2.615	19	1.88	18	3.44	18

*Genotypes of *Aegilops* and *Triticum* species examined in the experiments are given in Table 1. *CV* – coefficient of variation (Francis and Kannenberg 1978); bi – regression coefficient; S^2d_i – deviation from regression (Eberhart and Russell 1966); σ_i^2 – Shukla stability variance (Shukla 1972); Wi – ecovalence (Wricke 1962)

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Shukla stability variance and ecovalence were calculated and the genotypes were ranked accordingly.

Principal coordinates analysis was used to find the eigenvalues and eigenvectors of a matrix containing the distances between all data points (Davis 1986) applying the Euclidean correlation.

RESULTS AND DISCUSSION

Significant differences were found in Al accumulation in different wheat and aegilops genotypes (Table 2), where the lowest average found was 0.99 mg/kg dry weight (DW) in *Triticum aestivum* L. var *lutescens*, cv. Panonia and 1.28 in *Triticum spelta* L. var. *duhamelianum*. The highest Al concentration was found in the grains of two accessions of *Aegilops speltoides* (6.98 and 5.52 mg/kg DW, respectively) that bare exclusively the BB genome and it was significantly larger than the concentration found in all the other genotypes included in the analyses. Coefficient of variation with respect to the Al concentration, which reflects the genotypic features, had overall the highest rank in DD genotype followed with BB genotypes, and the lowest in AA genotypes (Table 2). The calculated parameters of stability (regression coefficient, deviation from regression and Shukla stability variance) gave very similar ranks of the examined genotypes, regarding Al in the grains (Table 2). Overall, genomes aligned by decreasing stability (starting by the most stable) have the following order: AA > BBAA > BBAADD > DD > BB, suggesting that those baring AA genome have higher stability.

This distinction between *A. speltoides* and the other *Aegilops* and *Triticum* species with respect to the concentration of Al was consistent in all years of the study. The lowest concentrations of Al were found in genotypes baring BBAA genome and it was overall significantly lower than in BB and AA genomes (Table 3). There were significant differences between years, genomes and their interactions. Principal component analysis was used to establish the association of different traits, important in wheat breeding, with the concentration of Al in grains, namely spike length (SL), thousand grain weight (TGW), number of spikelets per spike (NSP), number of grains per spike (NG) and grains weight per spike (GWS). It was found that Al concentration is associated only with the spike length, whereas NG, GWS and TGW formed the second group of trait and NSP was not found to be associated with any of the other analysed traits (Figure 1). Analyses of correlation coefficients showed that there is a significant positive correlation between Al concentration in the whole grain and spike length, whereas highly significant negative correlations were found between Al concentration in the whole grain and NG, GWS and TGW (Figure 2). The results of PCA and correlation coefficients are well in line with one another.

There is substantial amount of data available on the differences in tolerance of different *Triticum* species to Al present in the substrate (Moustakas et al. 1992, De Souza 1998, Darkó et al. 2012). Published data give evidence on the effects of Al on plant growth, changes in enzymatic status and susceptibility to elevated Al concentrations. However, there are no data on the Al concentration in the grains and its

Table 3. Concentration of aluminium in the whole grain (mg/kg dry matter) of five genomes of *Aegilops* and *Triticum* species over 3 years

Genome	Genotype No.	Year			Average
		2011	2012	2013	
BB	1	7.43	4.30	9.22	6.98^{a}
	2	4.53	4.47	7.55	5.52^{b}
Average		5.98^{b}	4.39^{c}	8.38^{a}	6.25^{a}
AA	3	2.61	2.92	3.40	$2.98^{c–g}$
	4	2.01	2.31	2.15	2.16^{fg}
	5	3.44	2.71	3.99	3.38^{cde}
Average		2.69^{e}	2.65^{ef}	3.18^{de}	2.84^{b}
DD	6	1.86^{f}	1.74^{f}	3.99^{cd}	2.53^{bc}
BBAA	7	3.23	2.56	2.72	$2.84^{c–g}$
	8	2.08	2.52	1.63	2.08^{g}
	9	1.91	1.79	2.93	2.21^{fg}
	10	3.36	2.32	2.54	$2.74^{d–g}$
	11	1.38	1.16	1.46	1.33^{hij}
Average		2.39^{ef}	2.07^{ef}	2.26^{ef}	2.24^{c}
BBAADD	12	1.39	1.24	1.21	1.28^{ij}
	13	1.13	1.01	0.81	0.99^{j}
	14	2.82	3.25	2.60	$2.89^{c–g}$
	15	3.76	2.96	3.75	3.49^{cd}
	16	1.97	2.16	3.28	$2.47^{d–g}$
	17	1.64	1.83	3.59	$2.35^{f–h}$
	18	3.16	3.21	3.01	$3.13^{c–f}$
	19	3.33	3.95	4.15	3.81^{c}
	20	1.65	3.62	4.76	3.34^{cde}
Average		2.32^{ef}	2.58^{ef}	3.02^{de}	2.64^{bc}
Average		2.74^{b}	2.60^{b}	3.44^{a}	

Different letters indicate significant difference among average values at $P \leq 0.05$

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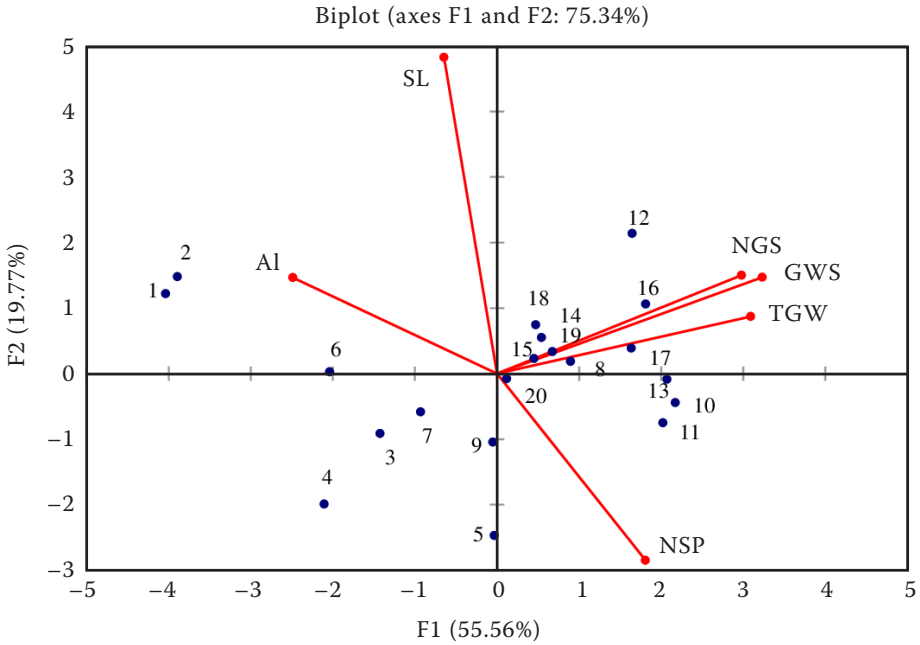

Figure 1. Principal component analysis (PCA) of trait association in *Aegilops* and *Triticum* species over three experimental years. *$P < 0.05$; **$P < 0.01$; ns – not significant; SL – spike length; TGW – thousand grain weight; NSP – number of spikelets per spike; NGS – number of grains per spike; GWS – grains weight per spike

dependence on genetic background. As it is evident from the present results, such influence may be substantial; in genotypes bearing DD, AA or BBAADD genomes, the Al concentration in the grains is between 40% and 45%, whereas it is only 35% in genotypes bearing BBAA or that found in *Aegilops*, which bares only BB genome.

The ancestors of common wheat, baring BB genome, had lower grain weight and higher grain Al concentration. It is not surprising that species and cultivars with significantly lower TGW have higher Al concentration. In larger grains, the proportion of bran is lower and a similar tendency was found both for essential and non-essential elements (Zhao et al. 2009, Kastori et al. 2017). However, diploid genotypes baring DD and AA genome that had lower Al concentration had higher Al content per one individual grain. On average, tetraploid and hexaploid genotypes had larger grains in comparison to diploid genotypes and they were more clustered around larger Al content in the individual grains (Figure 3). The exhibited differences in the concentration and content of Al between wheat ancestors baring only BB genome, characterised also by lower grain weight with respect to tetraploid and hexaploid genotypes, suggest that during the development of contemporary cultivars not only the size of grain (due to an increase in the content of carbohydrates and other organic compounds) has increased, but also the amount of Al in individual grains. Moreover, the presence of higher Al content in the individual grains of tetraploid and hexaploid wheat suggests that during the increase in ploidity, the capacity of plants to uptake Al from soil increased concomitantly with the increase of grain capacity to serve as Al sink.

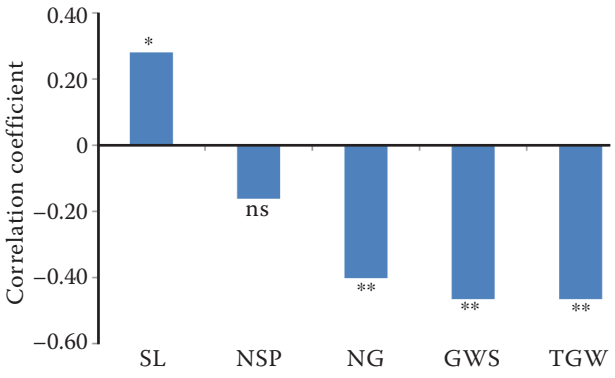

Figure 2. Correlation coefficients between aluminium concentration in the grain and spike length (SL), number of spikelets per spike (NSP), number of grains per spike (NG), grains weight per spike (GWS) and thousand grains weight (TGW); *$P < 0.05$; **$P < 0.01$; ns – not significant

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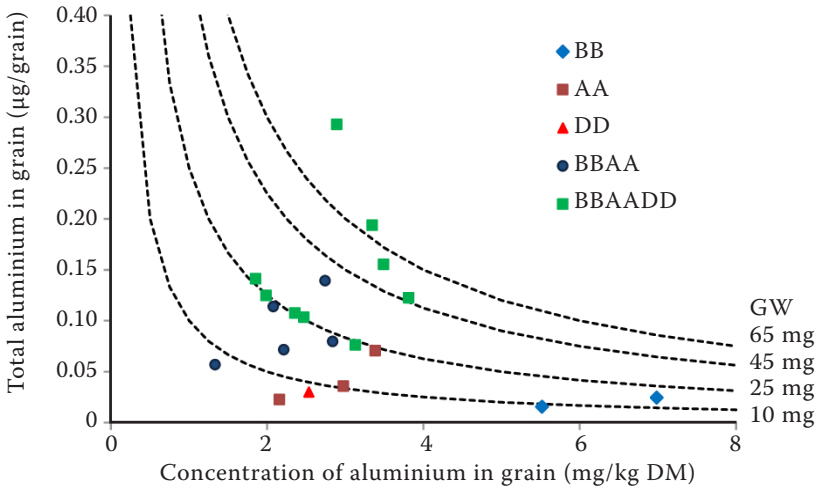

Figure 3. Relationship between aluminium concentration and total aluminium in the grains of *Aegilops* and *Triticum* genomes (BB, AA, DD, BBAA and BBAADD) studied in three growing seasons. The dotted lines represent lines for iso-grain weight. GW – grain weight; DM – dry matter

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