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REGULAR ARTICLE

Genotypic difference in cadmium effect on agronomic traits and grain zinc and iron concentration in winter wheat

Andrijana Rebekić[1]*, Zdenko Lončarić[2]

[1]*Chair for Plant Genetics, Plant Improvement and Seed Science, Department for Plant Production, Faculty of Agriculture in Osijek, J. J. Strossmayer University of Osijek, Osijek, Croatia,* [2] *Chair for Plant Physiology and Plant Nutrition, Department for Agroecology, Faculty of Agriculture in Osijek, J. J. Strossmayer University of Osijek, Osijek, Croatia*

ABSTRACT

A pot experiment was conducted to study the effect of soil cadmium contamination on agronomic traits and grain zinc and iron concentration in a set of 51 winter wheat varieties of different origin and year of release. An experiment was set up according to completely randomized design with two treatments (0 and 20 mg Cd kg^{-1} soil) and four replicates. Results showed that soil cadmium contamination increased grain cadmium concentration for 70 fold in average. All agronomic traits were reduced on cadmium contaminated in comparision to values obtained on an uncontaminated soil. The greatest reduction was observed in grain weight per spike and spike weight (27%) followed by plant weight (25%) and a number of grains per spike (23%). Furthermore, on uncontaminated soil, cadmium concentration in grain was in negative correlation to all agronomic traits (except for 1000 kernel weight) and cultivars year of release. Grain zinc concentration was not affected by soil Cd contamination while grain iron concentration was higher on a Cd contaminated soil in comparison to values obtained on uncontaminated soil.

Keywords: Cadmium toxicity; Grain yield; Zinc accumulation

INTRODUCTION

Cadmium (Cd) is a toxic, cancerogenic and teratogenic element that doesn't have any biological role in living tissue. In non-smokers, diet is the main source of Cd and about 80% of the food-cadmium comes from cereals, vegetables and potato (Olsson et al., 2002). In human organism Cd can damage kidneys, inhibit mineralization, vitamin D activation and calcium uptake increasing the risk of osteoporosis (Järup and Åkesson, 2009). Besides, Cd accumulates in the human body and the long term consummation of foods with high Cd concentration can lead to chronic toxicity.

In past decades Cd concentration in agricultural fields has been increased mostly due to anthropogenic processes such as: industrial emission, use of phosphate fertilizers containing Cd in low concentrations, usage of sewage sludge amendments, municipal waste disposal and leaching from roadways (Kubo et al., 2008; Wu et al., 2007). In cereal crops Cd accumulation is a function of the interaction of soil, plant, and environmental factors that influences it's phytoavailability (Gao et al., 2011, Grant et al., 1998). The Cd affects many physiological and biochemical processes in plants that can disturb growth and development (Wahid et al., 2000, Yourtchi and Bayat, 2013) and lead to reduction in yield and lower grain quality even at very low concentrations. Adoption and accumulation of cadmium in the grain is frequently studied due to its toxicity and for now, not sufficiently explained relationship with zinc and iron. It was assumed that because of their similar chemical properties they are adopted by plant through the same mechanisms (Liu et al., 2003; Chen et al., 2008), but there are also reports on Cd role in changes of plasma membrane permeability and interaction with other elements during transportation across the membrane (Obata and Umebayashi, 1997). Plant species and different varieties of the same species differs in uptake and accumulation of Cd in different tissues (Greger and Löfstedt, 2004, Grant et al., 2008). Traditional plant breeding has been aimed on high

Corresponding author:
Andrijana Rebekić, Chair for Plant Genetics, Plant Improvement and Seed Science, Department for Plant Production, Faculty of Agriculture in Osijek, J. J. Strossmayer University of Osijek, Osijek, Croatia. E-mail: arebekic@pfos.hr

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yielding cultivars that resulted in decreasing micronutrients in grain (Fan et al., 2008) and Gao et al. (2011) found that grain Cd was in negative correlation to grain yield. On the other hand, according to Arduini et al. (2014) older varieties of Italian durum wheat had lower concentration of Cd in grain than modern varieties.

Accordingly, the objectives of this study were: (i) to investigate how soil Cd contamination affects agronomic traits, grain Zn and Fe concentration (ii) to point out most and least affected agronomic trait, as well as to point out most and least sensitive cultivars in a term of Cd influence on agronomic traits, and (iii) to examine relationship between grain Cd concentration, cultivars year of release and agronomic traits in a set of 51 winter wheat cultivars. The obtained results can be used in a selection of cultivars for further research on Cd toxicity and interaction of Cd, Zn and Fe in winter wheat.

MATERIALS AND METHODS

Winter wheat cultivars selected for this research are shown in Table 1. Out of 51 wheat cultivars tested in this experiment, 33 are originally from Croatia (HR), 7 is Austrian (AT), 5 Hungarian (HU), 3 French (FR), and one German (DE), Italian (IT) and Russian (RU) cultivar. Cultivars included in this study differed in the year of release, ranging from year 1936 to 2008.

Pot experiment

Experiment was carried out at location near Osijek, Croatia (45° 36' 57" N. 18° 44' 36"). Soil used in the experiment was collected from 0 – 30 cm depth at Brestovac site located approximately 20 km from Osijek (45° 19' 47" N. 17° 35' 44"). The experimental soil had 5.00 pH_{H2O}, 3.83 pH_{KCl} (ISO 10390; 1994), 2.71 % humus (ISO 14235; 1998), 162 mg ammonium-lactate extractable phosphorus kg^{-1} soil, 336 ammonium-lactate extractable potassium kg^{-1} soil (Egner et al., 1960), 84.5 mg ZnARkg^{-1} soil (ISO 1146; 1995) and 0.188 mg EDTA extractable Cd kg^{-1} soil (Trierweiler and Lindsay, 1969).

Winter wheat cultivars were grown in the field in plastic pots of 11 kg capacity. Two Cd treatments were applied: uncontaminated (0 mg Cd kg^{-1} soil) and Cd contaminated (20 mg Cd kg^{-1} soil). Soil Cd contamination was conducted prior sowing. Sowing rate corresponding to 850 grains/m^2.

Sample collection and chemical analysis

At a full maturity ten plants were cut from each pot. Stem length (cm), spike length (cm), plant weight (g) and spike weight (g) were measured. Heads were removed from plants and after threshing, number of grains per spike, grain weight per spike (g) and 1000 kernel weight (g) were determined. Grain samples (1 g) were wet digested with 9 mL 65% (v/v) HNO_3 and 2 mL 30% (v/v) H_2O_2 in microwave vessels (Kingstone and Lassie, 1986). Concentrations of Cd, Zn and Fe were determined by inductively coupled plasma-optical emission spectrometry (ICP-OES) technique.

Statistical analysis

The experiment was set up according to completely randomized design with two treatments (Cd) and four replicates. The descriptive statistics, unequal variance t-test and Welch ANOVA were generated using Enterprise Guide 5.1. of the SAS System for Windows (Copyright© 2012 by SAS Institute Inc., Cary, NC, USD, All Rights Reserved). The JMP® 9.0.2 statistical package (Copyright© 2012 by SAS Institute Inc., Cary, NC, USD, All Rights Reserved) was employed for the Spearman's and Pearson's correlation analysis and cluster analysis.

RESULTS AND DISCUSSION

The effect of Cd on agronomic traits in winter wheat cultivars

Winter wheat cultivars examined in this experiment differed significantly in their stem length (cm), spike length (cm), plant weight (g), spike weight (g), number of grains per spike, grain weight per spike (g), and 1000 kernel weight (g) on uncontaminated (0 mg Cd kg^{-1} soil) and Cd contaminated soil (20 mg Cd kg^{-1} soil) (Table 2).

Most variable agronomic trait on uncontaminated and Cd contaminated soil was grain weight per spike, followed by the number of grains per spike and spike weight on uncontaminated and spike weight and plant weight on Cd contaminated soil (Table 2).

Examined wheat cultivars were released between year 1936 and 2008 and they were created in different breeding programs in different countries. During that period many breeding programs, with different breeding goals, were established around the world. Generally, the main objective of majority of breeding programs was to increase grain yield. Besides, adoptability to different environmental, climatic and stress conditions were also important objectives that were sought to achieve. Consequently, differences between cultivars in agronomic traits on uncontaminated soil are primarily due to their genotypic specificity which is in agreement with Erkul et al., 2010 and Yao et al., 2014. Furthermore, with stem length as exception, average values of all agronomic traits are considerably reduced on Cd contaminated soil (Table 2). Given that the Cd concentration in arable soil varies between 0.01-2.0 mg Cd kg^{-1} soil (Xu and Yang, 1995), soil contamination with 20 mg Cd kg^{-1} created a toxic soil environment for plants. In such conditions, plant growth

Table 1: Country of origin, pedigree and year of release of cultivars included in experiment. Cultivars are arranged in alphabetical order

Num.	Cultivar	Country of origin	Pedigree	Year of release
1	Adriana	HR	ZG 1758-70/TpR-349	1998
2	Aida	HR	Srpanjka/Rialto	2006
3	Alka	HR	Osk. 5.140-22-91/Sana	2003
4	Ana	HR	Osk-4.216-2-76/ZG-2877-74	1988
5	Andelka	HR	Srpanjka/Demetra	2008
6	Antonius	AT	Pokal/Karat//Ekspert/Severin	2003
7	Barbara	HR	GO 3135/Žitarka	1997
8	Bastide	FR	Fertil/Arche	2002
9	BC Elvira	HR	Bc 2377-79/MV-C2-33//Irena	2002
10	Bezostaja 1	RU	Skorospelka 2/Lutescens 17	1963
11	Dekan	DE		1999
12	Demetra	HR	Osk. 4.216-2-76/Zg 2877-74	1991
13	Divana	HR	Favorit/5/Cipriz/4/J.Kwang/2/Atlas66/Comanc./3/Velvet	1995
14	Edison	AT	Agron/Regent/Capo	2001
15	Eurofit	AT	Pegassos/Kontrasst	2004
16	Eurojet	AT		2006
17	Felix	HR	Srpanjka/K160/86	2008
18	GK Kalasz	HU	GK-Gobe//Lovrin-24/GK-Korany	1996
19	Golubica	HR	Slavonija/Gemini	1998
20	Ilirija	HR	Osk. 14.294-16-95/Soissons	2008
21	Janica	HR	Osk. 5.36-9-91/Srpanjka	2003
22	Katarina	HR	Osk. 5.B.4-1-94/Osk. 5.140-22-91	2006
23	Lela	HR	Srpanjaka/Osk. 5.136-8-90	2006
24	Libellula	IT	Tevere/Giuliari/San Pastore	1965
25	Lucija	HR	Srpanjka/Kutjevčanka	2001
26	Ludwig	AT	Ares/Farmer	1997
27	Mihaela	HR	Srpanjka/Osk. 5.136-11-90	2008
28	MW Emesse	HU	MV-MA/MV-12//F-2098-W-2-21	2000
29	MW Magdalena	HU	Mironovskaya-Yubilenaya-50/Fundulea-29/MV-MA	1996
30	MV Magvas	HU	F-26-70/Macvanka/2//MV-MA	1998
31	MW Mambo	HU	GK-Kalaka/MV-16//F-2076	2001
32	Njivka	HR	Slavonka/Osk. 5.123-2-74	1987
33	Osjecka crvenka	HR	Libellula/Bezostaja	1976
34	Panonija	HR	Heines-VII/Zagreb-129	1964
35	Patria	HR	Odesskaya-51 x ZG IPK 82 10 x GK-32-82	1994
36	Pipi	HR	Soissons/Osk. 6.83-5-91	2006
37	Renan	FR	Mironovs. 808/M.Huntsm/3/VPM/Moisson 1.5//Courtot	1989
38	Renata	HR	Žitarka/2/Osk. 7.5-4-82/KB160-86/3/Srpanjka	2006
39	Ruzica	HR	Osk. 5.36-9-91/Srpanjka//Brea	2008
40	Sana	HR	Mura x CI 14123 x Bc-2413/72	1983
41	Seka	HR	Srpanjaka/Demetra	2006
42	Slavonija	HR	Osjecka 20/Osk. 4.216-2-76	1984
43	Soissons	FR	Lena/HN 35	1987
44	Srpanjka	HR	Osk. 4.50-1-77/Zg 2696	1989
45	Super Zitarka	HR	GO 3135/Žitarka	1997
46	SW Maxi	AT		2002
47	U1	HR	Carlotta Strampeli/Marquis	1936
48	Valerius	AT	Carolus//Monopol/Karat//Ekspert/Severin	2003
49	Zlata	HR	Srpanjka/Demetra	2008
50	Zlatna Dolina	HR	Zg 414-57/Leonardo	1971
51	Zitarka	HR	Osk-6.30/20 x Slavonka x H-68 x Osk-154/19 x Kavkaz	1985

and development are disturbed, Cd causes root defection and interferes with many enzymes enabling normal plant metabolism (Fediuc and Erdei, 2002). Accordingly, probable cause of significant decrement in average values of all agronomic traits is Cd toxicity. The greatest reduction on Cd contaminated in comparison to uncontaminated soil

Table 2: Mean values, coefficient of variation (CV, %), equal variance t-test for differences between Cd treatments (t-test) and Welch ANOVA for differences between cultivars in grain Cd, Zn and Fe concentrations (mg kg^{-1}), stem length (cm), spike length (cm), plant weight (g), spike weight (g), number of spikelets per spike, number of grains per spike, grain weight per spike (g), and 1000 kernel weight (g)

	Cd treatment (mg kg^{-1} soil)	Mean±SD[(a)]	Welch ANOVA	CV (%)	t-test
Grain Cd (mg kg^{-1})	0	0.05±0.02	F=8.4; P<0.01[(d)]	51	df=203.2[(b)]; P<0.01
	20	3.5±1.1	F=50.4; P<0.01[(e)]	35	
Grain Zn (mg kg^{-1})	0	34.5±4.8	F=9.3; P<0.01[(d)]	22	df=400.3[(b)]; P=0.56
	20	34.1±5.6	F=11.4; P<0.01[(e)]	20	
Grain Fe (mg kg^{-1})	0	41.3±5.9	F=8.26; P<0.01[(d)]	23	df=376.1[(b)]; P<0.01
	20	48.7±6.0	F=84.3; P<0.01[(e)]	15	
Stem length (cm)	0	50.2±7.0	F=28.9; P<0.01[(d)]	15	df=406[(c)]; P=0.90
	20	50.1±6.5	F=25.5; P<0.01[(e)]	14	
Spike length (cm)	0	7.4±1.0	F=11.9; P<0.01[(d)]	16	df=406[(c)]; P<0.01
	20	6.8±1.20	F=15.7; P<0.01[(e)]	19	
Plant weight (g)	0	3.0±0.7	F=15.3; P<0.01[(d)]	31	df=406[(c)]; P<0.01
	20	2.2±0.7	F=13.3; P<0.01[(e)]	42	
Spike weight (g)	0	1.9±0.5	F=16.9; P<0.01[(d)]	35	df=406[(c)]; P<0.01
	20	1.4±0.4	F=45.5; P<0.01[(e)]	44	
Number of spikelets per spike	0	17.7±1.3	F=6.3; P<0.01[(d)]	10	df=392.2[(b)]; P<0.01
	20	16.3±1.3	F=4.5; P<0.01[(e)]	13	
Number of grains per spike	0	34.0±8.6	F=13.1; P<0.01[(d)]	35	df=406[(c)]; P<0.01
	20	26.1±8.2	F=12.2; P<0.01[(e)]	41	
Grain weight per spike (g)	0	1.4±0.4	F=11.0; P<0.01[(d)]	36	df=406[(c)]; P<0.01
	20	1.0±0.3	F=9.3; P<0.01[(e)]	44	
1000 kernel weight (g)	0	42.3±4.1	F=6.7; P<0.01[(d)]	12	df=406[(c)]; P<0.01
	20	40.1±4.5	F=11.4; P<0.01[(e)]	14	

[(a)]Standard deviation; [(b)]Unequal variance t-test (Satterthwaite); [(c)]Equal variance t-test (Pooled); [(d)]Differences between cultivars on uncontaminated soil (Welch ANOVA, df = 50); [(e)]Differences between cultivars on Cd contaminated soil (Welch ANOVA, df=50)

was observed in grain weight per spike (28%), spike weight (27%), plant weight (25%) and number of grains per spike (23%), while spike length (8.1%), number of spikelets per spike (7.7%), 1000 kernel weight (5.2%) and stem length (0.22%) were less affected by Cd toxicity. Grain weight per spike, spike weight and number of grains per spike are important factors of grain yield so this result implying on negative effect of soil Cd contamination on agronomic traits and consequently, grain yield.

Effect of Cd on grain Zn and Fe concentration in winter wheat cultivars

Obtained results on grain Cd accumulation, showed great variability in cultivars potential to accumulate Cd in grain on uncontaminated (0.01 – 0.11 mg kg^{-1}) and Cd contaminated (1.09 – 6.15 mg kg^{-1}) soil. In spite of significant difference between cultivars (Table 2) and high variability of grain Cd concentration on uncontaminated soil (CV = 51%) obtained mean values for all cultivars were below maximal permitted value of 0.2 mg kg^{-1} (Codex Alimentarius, 2005). In addition, there was also a difference between cultivars in their reaction to soil Cd contamination. For example, cultivar Golubica had 27 fold while cultivar Sana had 304 fold higher grain Cd concentration on Cd contaminated than on uncontaminated soil. In average grain Cd concentration increased for about 70 fold on Cd contaminated compared to uncontaminated soil. Grain Zn concentrations were in range between 24.6 – 47.1 mg kg^{-1} on uncontaminated and 24.9 – 54.2 mg kg^{-1} on Cd contaminated soil. Unlike grain Zn concentration that, in average, was not affected by soil Cd contamination (Table 2), grain Fe concentration increased about 18% on average on Cd contaminated in comparison to uncontaminated soil, although range of concentrations was similar on uncontaminated (31.7 – 55.1 mg kg^{-1}) and Cd contaminated (34.3 – 59.6 mg kg^{-1}) soil.

Relationship between agronomic traits, grain Cd, Zn and Fe concentration and year of release

Since 1960s with introduction of semi-dwarf and high yielding cultivars grain mineral density have decreasing trend. Furthermore, increase in yield resulted in a decrease in grain mineral concentration (Zn, Fe, Cu, Mg) indicating segregation of genes affecting grain mineral concentration and yield (Fan et al., 2008). On the contrary modern cultivars have tendency to accumulate more Cd in grain in compare to older cultivars (Arduini et al., 2014). In a present study relationship between cultivars year of release is in stronger negative relationship to grain Cd concentration on uncontaminated (r_s = -0.41; P < 0.01; N=204), than on Cd contaminated (r_s = -0.17; P < 0.01; N=204) soil. Furthermore, there is no evidence of significant relationship between year of release and grain Zn and Fe concentrations on uncontaminated soil, while on Cd contaminated soil grain Zn concentration is in significant negative relationship (r_s = -0.21; P < 0.01; N=204) to year of release. Besides, year of release was in very weak and weak but statistically significant positive correlation to spike length (r_s = 0.19; P < 0.01), plant weight (r_s = 0.25; P < 0.01); spike weight (r_s = 0.30; P < 0.01), number of grains per spike (r_s = 0.32; P < 0.01) and grain weight per spike (r_s = 0.29; P < 0.01) on uncontaminated soil, while on Cd contaminated soil there is no evidence of statistically significant correlation between cultivars year of release and agronomic traits. According to that, wheat cultivars examined in this research, showed that older cultivars have better ability to accumulate more Cd and Zn in grain in comparison to newer cultivars, while yield related traits (number of grains per spike and grain weight per spike) are lower in older than in newer cultivars.

Relationships betweeen agronomic traits an grain Cd, Zn and Fe concentration are shown in Table 3. On uncontaminated soil significant negative relationship is found between grain Cd concentration and all examined agronomic traits (Table 3) (only exceptions are number of spikelets per spike and 1000 kernel weight), while on Cd contaminated soil only agronomic trait that is not related to grain Cd concentration is number of spikelets per spike (Table 3).

Reports concerning Zn and Cd accumulation in the grain are contradictory, but both elements are in negative relationship to grain yield (Chen at al., 2007, Gao et al., 2011, Hart et al., 2002). In this research there was no evidence of significant relationship between grain Cd and Zn concentration (Table 3).

Using the dendrogram in Fig. 1A we can distinguish six clusters. Cultivar U1 is separated as single cluster because of its above average height and grain Cd concentration, while all other agronomic traits as well as grain Zn and Fe concentrations are below average. On contrary, cultivar Ilirija is separated due to its above average value of all agornomic traits, grain Zn and Fe concentration and low grain Cd concentration. Cultivars grouped in cluster 6 (Fig. 1A) are group together primarily due to their above average grain Cd concentration, but they also have average grain Zn and Fe concentration and 1000 kernel weight, while all other agronomic traits are below average.

Cultivars grouped in cluster 3 Fig. 1A have below average grain Cd and above average grain Zn and Fe concentration, while cultivars in cluster 2 have below average grain Cd, Zn and Fe concentration and above average values of all agronomic traits. Examined cultivars had different reaction to soil Cd contamination that resulted in different clustering under contaminated (Fig. 1B) in comparison to uncontaminated condition. For exmaple, cultivars Ludwig, Eurojet, Antonius and MV Magvas are grouped together both on uncontaminated and Cd contaminated soil due to similarity between them in values of examined traits. But on Cd contaminated soil cultivars MV Magdalena, Edison, SW Maxi and Eurofit are group together with them creating cluster 2, while on uncontaminated soil, these cultivars were in different clusters (Fig. 1B). Cultivars in cluster 2 are characterized by above average values of agronomic traits and low grain Cd, Zn and Fe concentrations. As cultivars with high values of agronomic traits, these cultivars can be pointed out as cultivars with low sensitivity to soil Cd contamination, as well as cultivar MV Mambo (cluster 1) that is separated as cultivar with highest 1000 kernel weight and below average grain Cd, Zn and Fe concentration on Cd contaminated soil. Cultivars grouped in cluster 2 (Fig. 1B) are all release betwen year 1996 and 2006 and originate from Austria and Hungary and most probable reason of resemblance between them is similar genetic background. These cultivars could be used for future research of Cd toxicity and as genetic material for breeding on low sensitivity to Cd toxicity. Furthermore, cultivars Golubica, Slavonija and Ana that share common ancestors (Table 1) and Bezostaja 1 (frequently used as parent material in Croatian breeding programs) are group

Table 3: Correlation among agronomic traits and grain Fe, Zn and Cd concentration in 51 winter wheat cultivars grown on uncontaminated and Cd contaminated soil (n = 204)

Uncontaminated soil (0 mg Cd kg^{-1} soil)											
Traits	SL	SpL	PW	SW	NSS	NGS	GWS	TKW	Fe	Zn	Cd
Fe	0.14*	n.s	0.42**	0.36**	n.s	0.31**	0.36**	0.17*	1		
Zn	0.20**	n.s	0.36**	0.30**	n.s	0.20**	0.31**	0.32**	0.60**	1	
Cd	-0.23**	-0.32**	-0.53**	-0.55**	n.s	-0.58**	-0.54**	n.s	-0.15*	n.s	1
Contaminated soil (20 mg Cd kg^{-1} soil)											
Fe	n.s	-0.19	-0.19**	-0.23**	-0.22**	-0.25**	-0.24**	n.s	1		
Zn	n.s	n.s	0.16*	0.17*	n.s	0.16	0.18**	n.s	0.39**	1	
Cd	-0.37	-0.41	-0.45	-0.42**	n.s	-0.36**	-0.40**	-0.20**	0.16*	n.s	1

*,**Pearson's correlation is significant at the 0.05 and 0.01 level respectively SL: Stem length, SpL: Spike length, PW: Plant weight, SW: Spike weight; NSS: Number of spikelets per spike, NGS: Number of grains per spike, GWS: Grain weight per spike, TKW: Thousand kerenel weight, Fe: Grain iron concentration, Zn: Grain zinc concentration, Cd: Grain cadmium concentration

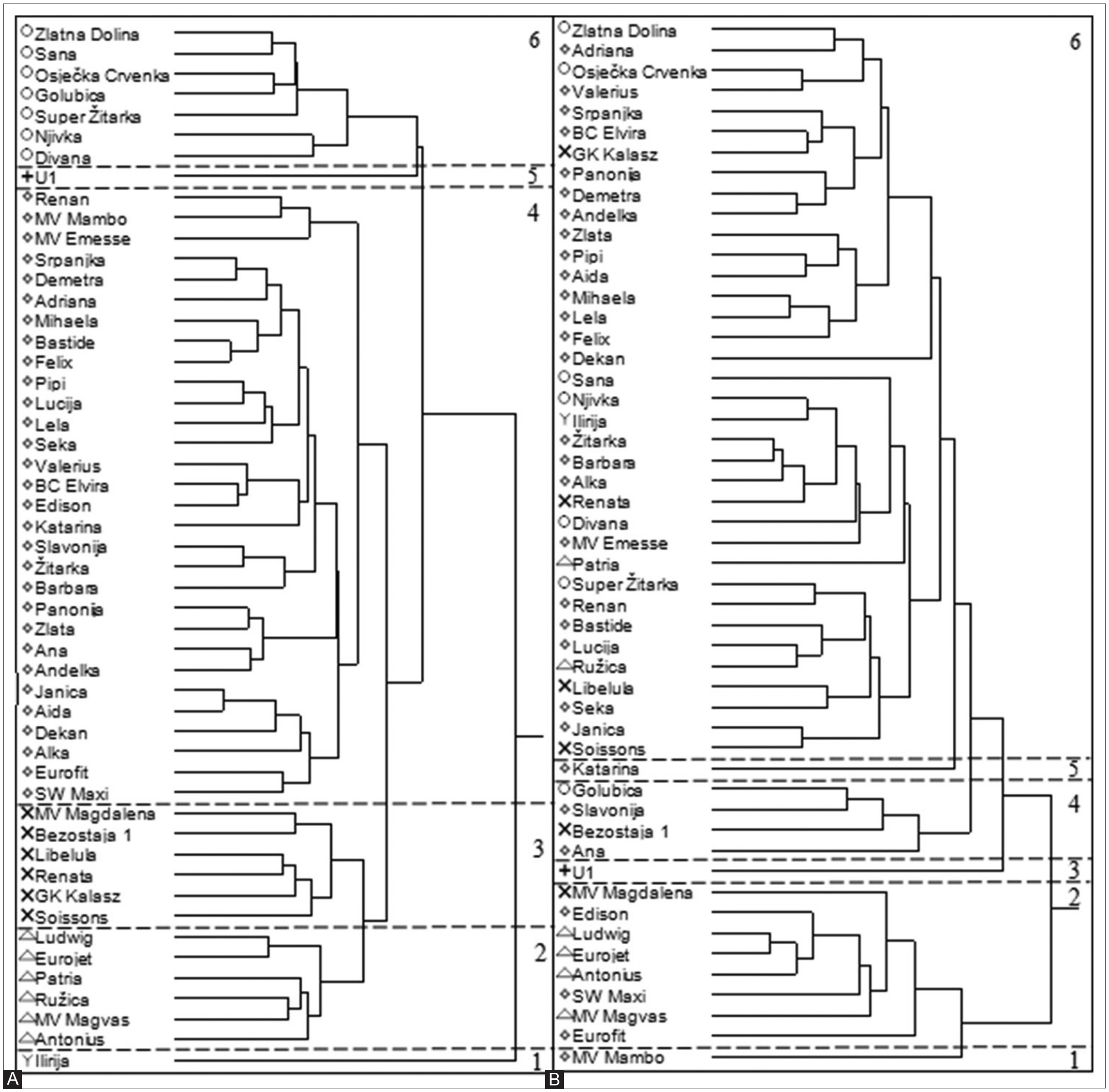

Fig 1. Hierarchical cluster diagram based on agronomic traits, grain Cd, Zn and Fe concentration on uncontaminated (A) and Cd contaminated (B) soil using average linkage method (on uncontaminated soil (A), cultivars belonging to the same cluster are mark with the same symbol and separated by dashed line; on Cd contaminated soil (B), cultivars belonging to the same cluster are separated by dashed line, symbols in front of the cultivars name showing cultivars cluster belonging on the uncontaminated soil)

in cluster 4 (Fig. 1B). These cultivars are good material for further research on relationship between grain Zn, Fe and Cd, since they have low Cd and above average grain Zn and Fe on Cd contaminated soil.

CONCLUSION

High soil Cd concentration causes a decrease in all agronomic traits. Most pronounced decline is observed in yield related traits such as grain weight per spike, spike weight and number of grains per spike. On agricultural fields where soil Cd concentration is within the permitted limits, Cd concentration in grain will not exceed 0.2 mg kg^{-1} (Codex Alimentarius Commission, 2005), but negative effect of Cd will be visible in decrement of agronomic traits. Furthermore, some cultivars are more sensitive to soil Cd toxicity than others, so such cultivars could be used to exploit genotypic specificity of cultivars in terms of Cd influence on agronomic traits. Hierarchical clustering of cultivars under Cd contaminated conditions yielded, among others, a group of cultivars with low Cd and high grain Zn and Fe concentration. These cultivars could be used for further research on interaction beween Cd, Zn and Fe, with an aim to select genotype with high grain Zn and Fe and low Cd concentration.

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Author's contribution

Authors have contributed equally.

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