BIO Web of Conferences **43**, 02011 (2022) https://doi.org/10.1051/bioconf/20224302011
VVRD 2021

The influence of barley genotype and growing conditions on zinc and cadmium accumulation

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Abstract. Zinc and cadmium accumulation of in plant tissues of barley of the line 999-93 and of its regenerant forms resulting from cell selection was assessed. The scheme of the experiment: 1) control; 2) acidic; 3) cadmium. We used the method of inversion voltammetry to assess the share of zinc and cadmium in plant samples. To assess the share of active and total forms of zinc and cadmium in soil samples collected from root rhizosphere we used the method of atomic absorption spectroscopy. Barley plants accumulated zinc from 0.2 to 79.7 mg·kg^{-1} of dry phytomass. The genotype of plants has little influence on zinc accumulation, and plant organs participate in equal measure in zinc accumulation. As for the soil background with cadmium excess, there is a tendency to lessening zinc absorption in all the plant organs, and it is not connected with the plant's genotype. Unlike zinc, cadmium gets accumulated mostly in the root system. In the control background the share of cadmium in roots was 0.2–0.4 mg·kg^{-1}; in the acidic one – 0.2–3.6 mg·kg^{-1}; in the cadmium one – 5.5–9.5 mg·kg^{-1}. In barley grain grown on soil with excess of cadmium we did not find any IPC excess of cadmium. On backgrounds of the same acidity, the more cadmium concentration grew, the less zinc concentration in grain was, mostly it concerns the original genotype, to a smaller degree it concerns the regenerant line on the selective medium with cadmium and aluminum. Coupling accumulation of zinc and cadmium took place mostly on acidic background, it was characteristic of barley with the original genotype and the regenerant selected in vitro as cadmium-resistant; on control background coupling accumulation is characteristic of aluminum-resistant regenerant. These regenerant genotypes had a tendency to eliminating cadmium and absorbing zinc.

1 Introduction

Nowadays soil is mostly contaminated with heavy metals, so that screening and breeding of cereals, in particular, of barley with a low level of cadmium accumulation is quite topical [1, 2]. The mechanisms of plants' tolerance to cadmium accumulation still need to be researched [3]. As a rule, identification of heavy-metals-resistant genotypes is

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based on the cytogenetic analysis and on the analysis of morphological changes in plants [4, 5]. As for cereals, one should pay special attention to cadmium accumulation in grain. Methods of Molecule Biology contribute to successful work in this sphere, so that gene-modified crops can appear. Microbe inoculants play a great role; they stimulate plants' growth in conditions of contamination with cadmium [6–9]. It is known that zinc in soil lessens cadmium phyto-toxicity. Still many factors restrict the effect of zinc, including concentration and long-term impact of cadmium in the environment, as well as the species and genotype of the plant, and its growth conditions [10]. Zinc and cadmium ion mobility depends on acidity of the soil solution, and it reaches its maximum within the interval of pH=4.5–5.5 [11–13].

The aim of the paper is to research zinc and cadmium accumulation in barley in conditions of different soil background.

2 Methods

The research objects were barley plants of the line 999-93 and its regenerant forms which were induced by the original genotype in the callus culture in the process of cell selection in the media: 1) with 40 mg·dm^{-3} Al^{3+} (line RA_{Al}); 2) with 15% Polyethylene glycol – PEG as osmoticum (line RA_{PEG}); 3) with 15 mg·dm^{-3} Cd^{2+} (line RA_{Cd}) [14, 15]. The plant-regenerants got in vitro were planted in vegetation vessels with sod-podzolic soil. The experiment scheme included three soil backgrounds: 1) control (pH_{KCl}=5.8); 2) acidic (pH_{KCl}=4.5); 3) cadmium (5 mg·kg^{-1} Cd^{2+} at pH_{KCl}=4.5). the challenge background was created by means of introducing $Cd(CH_3COO)_2$ in soil with its further regular moistening (to 80% maximum water-holding capacity) and its mixing during a month in order to fix cadmium in the soil absorbing complex. The seeds of the original and regenerant genotypes were planted in soil so that there were three plants per vessel, and three vessels in each variant. The plants grew in natural conditions. As soon as plants grew ripe, they were extracted from soil, their roots were washed, the plants were dried and divided into structural components (root, straw, grain). We used dry mineralization for preliminary preparation of plant samples. We assessed the share of zinc and cadmium in the plant samples by the method of inversion voltammetry [16]. To assess the share of active and total (acid-soluble) forms of zinc and cadmium in soil samples collected from root rhizosphere we used the method of atomic absorption spectroscopy with the help of the device "SPEKTR-5-4". Air-dry soil samples were preliminary extracted with ammonium acetate buffer (AAB pH=4.8) and nitric acid at boiling accordingly [17].

The influence of such factors as genotype (factor gradations: the original genotype; the regenerant line RA_{Al}, RA_{PEG}, RA_{Cd}) and soil background (factor gradations: control, acidic, cadmium) on cadmium and zinc accumulation in barley tissues was assessed with the help of variance analysis.

In order to characterize the uptake properties of barley roots we calculated the hindrance factor or Acropetal coefficient (AC) according to the formula:

$$AC = C_r / C_c,$$

with C_r and C_c – the share of zinc and cadmium in barley roots and grain accordingly, mg·kg^{-1}.

We statistically processed the experimental data using the method variance analysis, for analyzing the results we calculated Pearson coefficient of correlation (r) (Excel MS Office 2007).

3 Results

During the research we assessed the share of total and active zinc forms in soil samples from the rhizosphere of barley plants on different soil backgrounds (Table 1).

Table 1. The share of zinc in soil samples from the rhizosphere of barley grown on different soil backgrounds.

Experiment variant		Concentration, mg·kg^{-1}		
№	**Plant genotype**	**Control**	**Acidic background**	**Cadmium background**
1	Original	6.06±1.99 / 15.7±5.2	1.27±0.45 / 77.5±25.6	1.08±0.36 / 56.5±18.6
2	RA_{Al}	5.06±1.67 / 13.5±5.8	0.8±0.3 / 11.5±4.9	1.6±0.5 / 48.0±15.8
3	RA_{PEG}	5.06±1.67 / 17.0±7.31	2.6±0.9 / 39.5±16.9	2.19±0.72 / 51.8±17.1
4	RA_{Cd}	5.7±1.9 / 21.0±9.0	1.5±0.5 / 63.0±27.1	2.4±0.8 / 48.5±16.0
Initial concentration		4.7±0.4 / 26.8±2.2	5.7±1.9 / 61.0±9.0	5.7±0.5 / 49.9±13.0
MPC / IPC		23.0 / 110.0		

Note to Table 1 and 2: in the numerator – active connections, in the denominator – total forms.

As compared with the original level (before planting the seeds), the concentration of zinc total forms in soils of different backgrounds was changing in different ways and varying, depending on the genotype (in mg·kg^{-1} of soil): from13.5 to 21.0 in control; from 11.5 to 77.5 in acidic and from 48.0 to 56.5 in cadmium (with excess of cadmium) background. The concentration of active connections mostly was by an order lower than that of total forms and was, accordingly: 5.06–6.06; 0.8–2.6 and 1.08–2.4 mg·kg^{-1} soil. Zinc is easily absorbed both by mineral and organic components, so in most types of soil it is accumulated in surface horizons. It is important to note that the concentration of active zinc forms extracted by AAB from agricultural soil should be not less than 4 mg·kg^{-1}. In soil of the most backgrounds under research zinc concentration was considerably less than the standardized share of MPC and IPC and it was attributed to the class of low or very low (less than 4 and 4–6 mg·kg^{-1} accordingly).

The amount of cadmium in soil depends on the same soil-formation factors, as the amount of zinc. Natural share of active cadmium in soil is lower than that of zinc by two orders [18]. We followed this regulation in the experiment, excluding artificially made cadmium layer with concentration of total and active forms exceeding its IPC (2 mg·kg^{-1}), it varied within one order – 2.13–3.9 and 1.7–3.02 mg·kg^{-1} of soil accordingly (Table 2).

Then we assessed metal ion accumulation in barley tissues. Different character accumulating zinc and cadmium is connected with their role in plants: zinc is an essential metal, while cadmium is its toxic counterpart. Zinc actively drifted from plants' roots to their aboveground organs.

Table 2. The share of cadmium in soil samples from plant rhizosphere on different soil backgrounds.

Experiment variant		Concentration, mg·kg^{-1}		
№	**Plant genotype**	**Control**	**Acidic background**	**Cadmium background**
1	Original	0.11±0.03 / 0.27±0.07	0.15±0.04 / 0.34±0.09	1.9±0.6 / 2.41±0.60
2	RA_{Al}	0.14±0.03 / 0.27±0.07	0.11±0.03 / 0.22±0.04	1.7±0.5 / 2.13±0.53

3	RA_{PEG}	0.12±0.05 0.28±0.07	0.12±0.05 0.27±0.07	3.02±0.75 3.19±0.79
4	RA_{Cd}	0.12±0.04 0.22±0.06	0.14±0.05 0.26±0.06	2.24±0.56 3.9±0.9
Initial concentration		0.18±0.03 0.44±0.03	5.7±1.9 21.0±9.0	4.2±0.4 5.9±0.6
MPC IPC		– 2.0		

Depending on the genotype and the plant organ, as well as on growth conditions, its amount varied from 0.22 to 79.7 mg·kg^{-1} of dry biomass (Fig. a-f).

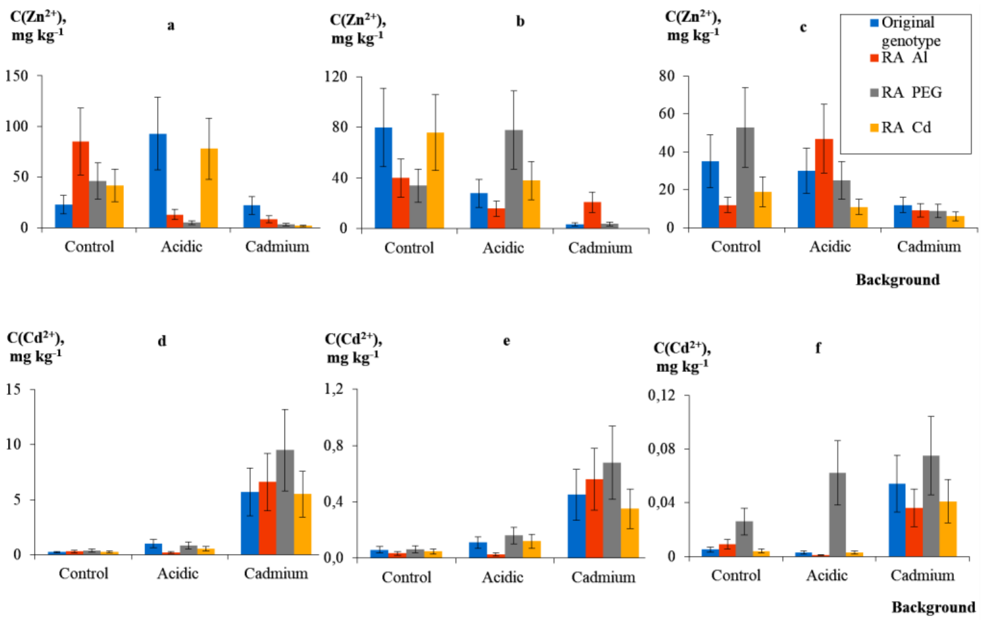

Fig. Zinc and cadmium share in vegetative organs (a, d – roots; b, e – straw) and in reproductive organs (c, f – grain) of barley, mg·kg^{-1}.

Assessing metal accumulation in grain was of special practical interest. Grain of the original genotype on control and acidic background, as well as of the regenerant line RA_{Al} and RA_{PEG}, RA_{Cd} on acidic and control background accordingly, had a high level of zinc accumulation. In case of cadmium excess in soil zinc concentration in barley organs was lower, as compared with other backgrounds, even with the ones with similar acid-alkali conditions.

Unlike zinc, the degree of cadmium accumulated in plant tissues was lower (excluding cadmium challenge background). Prevailing cadmium concentration took place on the level of the root system, which is probably connected with the root barrier in plants. Cadmium share in roots of all the genotypes under research was one order higher than that in aboveground organs. In control background cadmium amount was 0.21–0.38; in acidic and cadmium ones – 0.2–3.6 and 5.5–9.5 mg·kg^{-1} accordingly. In straw of the most genotypes the amount of cadmium was lower, as compared with roots, and in grain it was lower in all the cases, without any exception.

For control background cadmium concentration in grain was 0.004–0.026; for acidic and cadmium ones – 0.001–0.062 and 0.036–0.075 mg·kg^{-1} accordingly. The standardized cadmium MPC (0.1 mg·kg^{-1} of air-dry mass) in grain was not stated. It was so even when the cadmium amount in soil of the challenge background was higher than the level of IPC.

On comparing backgrounds with similar acidity level (acidic and cadmium ones), we noticed that the more cadmium concentration in soil grew, the less zinc concentration in grain got. It was most clearly seen in the original genotype and it was less noticeable in the regenerant barley line (RA_{Cd}) induced in the selective medium with Cd^{2+}.

It is noteworthy that, in case growing in cadmium background soil, the average amount of zinc concentration in tissues of barley of different genotypes was considerably less, as compared with that in control and acidic backgrounds. Two-factor variance analysis showed that zinc accumulation in plant tissues scarcely depends on the genotype and the organ of barley. This process is more dependable on growth conditions, i. e. the type of soil background (Table 3).

Table 3. Assessment of how much the factors influence zinc and cadmium accumulation in barley vegetative and reproductive organs according to variance analysis.

Variation source	df	SS	F	P
Zinc accumulation in grain				
Genotype	3	310.5	1.34	0.3474
Soil background	2	3.0	0.01	0.9872
General variation	6	232.1		
Cadmium accumulation in grain				
Genotype	3	0.0010	8.19	0.0153
Soil background	2	0.0019	14.92	0.0047
General variation	6	0.000128		
Zinc accumulation in the straw				
Genotype	3	114.4	0.18	0.9056
Soil background	2	2613.1	4.13	0.0745
General variation	6	632.6		
Cadmium accumulation in the straw				
Genotype	3	0.009	1.30	0.3589
Soil background	2	0.3	35.33	0.0005
General variation	6	0.007		
Zinc accumulation in the root				
Genotype	3	437.5	0.38	0.7731
Soil background	2	2053.1	1.77	0.2487
General variation	6	1159.7		
Cadmium accumulation in the root				
Genotype	3	1.3	1.22	0.3823
Soil background	2	54.2	49.24	0.0002
General variation	6	1.1		

For all the genotypes, the greatest difference in zinc accumulation in plant tissues took place in acidic background. It could be caused by different activity of zinc and cadmium in conditions of different soil acidity. Zinc is more active in acidic soils, while cadmium – in subacidic.

Cadmium accumulation in barley depended on all the factors considered. Growth conditions had the most reliably relevant influence on accumulation of ions of the toxic metal in different barley organs (factor – soil background). Despite decrease of cadmium concentration in the line ‘root > straw > grain’ in all the backgrounds, it was most clearly seen in soil with cadmium excess, which proves that the root system is the main barrier on the way of translocation of heavy metal ions within the system ‘soil – plant’. Calculation of acropetal coefficients proves the research results, they show that cadmium mostly accumulates in roots, cadmium background (according to the maximum values) (Table 4).

Table 4. Acropetal coefficient.

Plant genotype	Soil background					
	Control		Acidic		Cadmium	
	Zn	Cd	Zn	Cd	Zn	Cd
Original genotype	0.66	44	3.1	133	1.83	106
RA_{Al}	7.08	31	0.28	200	0.92	183
RA_{PEG}	0.87	15	0.20	13	0.37	127
RA_{Cd}	2.21	60	7.09	177	0.31	134

On the average, cadmium concentration in grain for different barley genotypes grown under the conditions reached 0.05 mg·kg^{-1}. At the same time in control and acidic backgrounds it was about 0.02 mg·kg^{-1}. Besides, cadmium accumulation in plant organs, including grain, was considerably dependent on barley genotype. The maximum acropetal coefficient values were fixed on both stress backgrounds for the genotypes RA_{Al} and RA_{Cd}, which were got by means of cell selection on the media *in vitro* with aluminum and cadmium.

In course of assessing zinc and cadmium accumulation in barley organs on different soil backgrounds peculiar features of definite barley genotypes were found out (Table 5).

Table 5. Coupling accumulation of zinc and cadmium in barley in different soil backgrounds.

Soil background	Correlation coefficient for plants' genotypes			
	Original	RA_{Al}	RA_{PEG}	RA_{Cd}
Control	-0.47	0.95	0.05	0.06
Acidic	0.99	-0.66	0.62	0.98
Cadmium	0.16	-0.79	-0.57	-0.42

Accumulation of the metals in tissues of plants with the original genotype took place in the acidic background; in plant tissues of the regenerant line RA_{Al} and RA_{Cd} – in the control and acidic ones accordingly. Accumulation of both zinc and cadmium in cadmium background did not occur in plant tissues of any genotype considered. Still, it is notable that the design value of correlation coefficient for the original genotype was positive, while for its renegant forms it was negative. It suggests that renegant forms of barley have a stronger mechanism of protection from toxicants due to absorbing preferentially essential microelements and eliminating their toxic counterparts.

4 Discussion

Cd is a heavy metal with no apparent beneficial role in plant metabolism [19]. Cultivation of low-Cd cultivars has been of particular interest and is one of the most cost-effective and promising approaches to minimize human dietary intake of Cd. Several low-Cd cereal cultivars and genotypes have been developed worldwide through cultivar screening and conventional breeding [20, 21]. Since cultivars are cultivated under the same ecological and pedological conditions and are exposed to the same Cd concentration, the variability of Cd accumulation can be determined by intraspecific variations in the activity of genes responsible for the absorption and transport of Cd in the plant [22]. Roots accumulated more Cd than the stems or leaves . This possibly could be connected to the fact that the root is the first organ coming into contact with the Cd ions in soil [23].

5 Conclusion

Thus, our research proved that the ways of accumulating essential and toxic metal ions by barley are different. As for zinc, the essential element, we did not find out either that barley genotype influences its accumulation or that any plant organ plays the leading role in the process. The only exception was zinc accumulation in grain. Still soil background with excess of cadmium contributed to decreasing the intensity of zinc absorption by plants, as compared with control and acidic backgrounds.

The research of cadmium content in plant tissues showed that the root system has a barrier function. It means that the toxicant accumulates mostly in roots, and its migration to the aboveground organs of plants is decreases. The degree of such barriers considerably depends on barley genotype, it was the most in the genotypes RA_{Al} and RA_{Cd} which are resistant to ions of toxic metals. At this stage the regulations in coupling accumulation of zinc and cadmium by some barley regenerants in different soil conditions require further research. The importance of feather research consists in the fact that excessive accumulation of cadmium can be dangerous for people’s health in case of their using grain production as food. Still, the research shows that regenetrant lines of barley got on selective media in the culture in vitro differ from the original genotype: they are not inclined to coupling accumulation of cadmium in all the tissues in case of cadmium excess in soil and they replace it by zinc.

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