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Variation of Zinc Accumulation in the Grain of Spring Wheat Cultivars Depending on Foliar or Soil Application

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Abstract

The research aimed at analysis of variation in zinc accumulation in the grain of spring wheat cultivars depending on the degree of environmental contamination. The experiment was carried out in Wagner type pots containing up to 6 kg soil. The experimental plants were five spring wheat cultivars. Zinc in the form of zinc sulphate solution was applied into the soil or sprayed on leaves in the following doses: a) 2.05 g Zn/pot, b) 0.68 g Zn/pot, c) 0.03 g Zn/pot, d) foliar dose – 0.5 g Zn SO_4) liter. Discriminant and cluster analyses revealed that in Jasna and Banti the variation of zinc cumulation in grain was higher than in the other cultivars. At the highest contamination of soil (2.05 g Zn/pot) Jasna and Banti showed significantly higher accumulation of zinc in comparison with the other treatments. Cultivar Henika stood out by the smallest acumulation of zinc in its grain with the contamination doses, while Banti with lower Zn content in the environment.

Keywords: zinc doses, cultivars, multivariate analysis, Zn cumulation in grain

Introduction

Zinc belongs to the group of microelements which are indispensable to plants and animals for their proper growth and development. The element is easily accessible in most soils, though its availability to plants may be abated by liming, phosphatic fertilizers or sulphur compounds [1, 2]. Among the soil properties influencing the bio-availability of zinc the major role is ascribed to the soil's reaction, sorptive capacity and content of organic matter [3, 4]. Antoniadis and Alloway [5] proved that increase in soil organic matter was followed by increased availability of zinc to plants. However, Impellitteri *et al.* [6] found out that organic matter caused increased solubility of zinc only under the conditions of high pH of the soil. It has been empirically demonstrated that fixation of Zn^{+2} ions by organic matter under soil pH up to 8.4 exerted significant influence on solubility of zinc in soil [7]. Higher soil pH resulted in precipitation of the element in soil minerals. Both excess or deficiency of zinc in soil may influence plants unfavourably due to disturbances in their physiological functions and metabolic processes [8, 9].

In the European Union countries the limits concerning zinc phytotoxicity show considerable differences [10]. The admissible limit for zinc in England, Sweden or Germany has been fixed for its available or total forms. In those countries different reagents are used for for extraction of the element, which renders any comparison of the results difficult [11, 12]. In England and Sweden as well as in Germany, the limits are independent of soil conditions, while in Poland they depend on the sorptive capacity, pH and agronomic category of soil [13]. The degree of zinc cumulation in plants depends not only on a plant species, but also on cultivars [14]. The research on differentiated uptake of zinc by cultivars usually comprises doses in the form of intraedaphic or foliar fertilization. There are no reports concerning complex response of cultivars to zinc doses applied in the form of fertilizers or in phytotoxic amounts. Therefore, this research has aimed at analysis of the variation of zinc accumulation in grain of several spring wheat cultivars depending on foliar or soil application.

Experimental Procedures

The experiment was carried out at the IUNG Tillage Department, Jelcz-Laskowice, in Wagner type pots containing up to 6 kg soil. The substrate was brown soil humic layer of weakly loamy sand granulometric composition, containing 5.52 mg P and 8.3 mg K available (acc. to Egner-Riehm) in conversion to 100 g soil. The mean content of clay fractions was 20%. The soil was characterized by low $C_{org.}$ content – 0.90%. In all the treatments soil reaction was brought to pH_{KCl} = 6.0 through addition of calcium carbonate. The test plants were five spring wheat cultivars: Henika, Ismena, Banti, Jasna, Eta. Zinc was applied into the soil or leaves in form of zinc sulphate solution ($ZnSO_4 \cdot 7\ H_2O$) in following doses: a) 2.05 g Zn/pot, b) 0.68 g Zn/pot, c) 0.03 g Zn/pot; the intrafoliar spray was made with solution cotaining 0.5 g $ZnSO_4$) liter in the dose of 10 mL/m^2, corresponding to fertilization with that microelement at 50 g Zn/ha with use of 100 L water. The response of the cultivars to those doses was compared with control treatments. Basic fertilization as well as that with microelements (except Zn) was applied according to the methods generally accepted. Spraying with zinc sulphate solution was done in the stage of early shooting. Soil moisture in the pots was kept at 60% water capacity. During vegetation differences between the plants in the treatments were observed in successive stages of their development. The plants were harvested at the full maturity stage, in each treatment separately. Zinc content in wheat grain was analyzed using the AAS method, and in soils through extraction with aqua regia. After the harvest the zinc content in air-dry soil matter was determined (Table 1). No significant differences among the Zn contents in soil were found in control treatments or in those with intrafoliar fertilization.

To asses the influence of different zinc doses on variation of the element cumulation in grain of the cultivars tested we used the method of discriminant analysis as described in the papers by Krzyśko [15], Caliński and Chudzik [16] and Mądry [17]. The analysis makes it possible to assess a particular cultivar in pentadimension space defined by four zinc doses and control. To compare the cultivars in respect of the degree of zinc accumulation in their grain, analysis of concentrations was made with the method of farthest proximity. The calculations were carried out with the Statistica software programme.

Results

Analysis of variance for the zinc content in the grain of spring wheat cultivars showed significant differences among the treatments tested (Table 2).

Interaction between the zinc doses and the cultivars was significant at p=0.01 level. This would testify variable response of wheat cultivars to different Zn doses. With the highest contamination of soil (2.05 g Zn/plot) accumulation of zinc in grain of Jasna and Banti cultivars was significantly higher as compared with the other treatments. The lowest concentration of zinc was found in grain of Henika cultivar. Jasna and Banti cultivars were characterized by considerably shorter plants, lower grain weight and number of grains per ear than Ismena, Eta and Henika. Contamination of soil with zinc at 0.68 g per pot brought about greatest cumulation of the element in Ismena, and significantly lower in Eta. With the doses 2.05 g and 0.68 g per pot the plants were significantly shorter and the weight and number of grains per ear smaller as

Table 1. Mean Zn content in mg·kg^{-1} (ppm) air dry soil matter depending on the dose.

Zinc doses per pot	2.05 g	0.68 g	0.007 g	Control
Zinc content in soil (mg·kg^{-1})	1260.0	423.0	88.3	71.3

Table 2. Mean Zn contents in mg·kg^{-1} (ppm) grain of wheat cultivars depending on the degree of environmental contamination.

Cultivars	Ismena	Eta	Henika	Jasna	Banti
2.05 gram	188	184	172	241	233
0.68 gram	98	84	89	90	92
0.007 gram	42	41	41	41	32
Intrafoliar dose	42	39	38	37	31
Control	38	35	38	35	28
Mean	82	77	76	89	83

NIR of cultivars = 6.32; NIR of cultivar x zinc doses = 10.17

compared with the control treatments and fertilizing doses. To verify the multidimensional zero hypothesis concerning lack of differences between the above zinc doses for five wheat cultivars we used the MANOVA variance analysis, which is a discriminant analysis. Wilks' lambda statistics of total discrimination, calculated as the ratio of intragroup variance and covariance matrix determinant to total variance and covariance matrix determinant, showed the variation of zinc cumulation in wheat cultivars in the environments to be significant (Table 3).

Particularly high influence on differentiated uptake of zinc in the wheat cultivars was exerted by the toxic dose 2.05 g. On the other hand, the doses applied in the form of foliar or soil fertilization resulted in smaller variation of zinc accumulation in wheat grain. Considerable differentiation of zinc cumulation in the grain of wheat cultivars in control treatments resulted probably from lower availability of the element in the soil. In contrast, readily available form of zinc applied with foliar or soil fertilization clearly diminished differences in the uptake of that element by the cultivars tested. Further statistical analysis allowed us to obtain four linear-independent functions in the form of characteristic roots, presenting multivariant differentiation in the uptake of zinc by the cultivars in canonical variables space (Table 4). The values of the roots – canonical variables – were assessed with *chi*-square test. The real dimension of the discriminant space is determined by the first two canonical roots, which differ significantly from zero at significance level = 0.05. The first two canonical variables in 91% explain mutual distances between the cultivars tested. For interpretation of the meaning of canonical variables standardized coefficients of correlation between the variants of zinc doses and the canonical roots were used.

High absolute values of canonical coefficients, as well as significant correlations between the zinc and canonical variables, point to marked contribution of particular loading or fertilizing doses for discrimination of the cultivars tested. The greatest influence on formation of the first canonical variable, which in 79% ensured multivariant variation of the cultivars, was exerted by the dose 2.05 g Zn per pot and control treatment. Those experimental treatments were also characterized by higher coefficients of correlation with that canonical element.

The dose 2.05 g per pot had the greatest contribution to formation of the second canonical variable. That dose also showed stronger correlation with the second canonical root as well as the highest relative discriminant strength in differentiation of zinc uptake by the spring wheat cultivars. Table 5 presents squared Mahalanobis distances, which constitute the measure of distances between pairs of cultivars in the space formed by four zinc doses and control. Banti and Jasna cultivars were distinguished by their response to changes in the zinc content in soil, which was different from that of other cultivars. In those cultivars particularly high cumulation of zinc

Table 3. Discriminant function analysis results.

Wilks' lambda 0.254; F=3.07; p<0.0001				
Doses of Zn/pot	Lambda Wilks'	Partial Wilks'lambda	Statistics F	Level p.
2.05 gram	0.397	0.640	5.05	0.002
0.68 gram	0.312	0.814	2.04	0.058
0.007 gram	0263	0.967	0.30	0.872
Intrafoliar dose	0.284	0.895	1.04	0.396
Control	0.330	0.769	2.69	0.043

Table 4. Standardized coefficients for canonical variables.

Doses of Zn/pot	Root 1	Root 2	Root 3	Root 4
2.05 gram	-0.74	0.65	0.34	0.01
0.68 gram	0.40	0.04	-0.91	0.45
0.007 gram	-0.13	0.45	-0.03	0.11
Intrafoliar dose	0.36	-0.17	0.42	0.92
Control	0.72	0.34	-0.007	-069
Eigenvalues	1.67	0.23	0.17	0.01
% Cumulated	0.79	0.91	0.99	1.00

was found in treatments with the dose 2.05 g Zn per pot. Big Mahalanobis distances between those cultivars and the other treatments point to a different response of the genotypes to differentiated zinc contents. Variability of zinc contents in the grain of cultivars Ismena, Eta and Henika was to some extent similar, as shown by insignificant Mahalanobis distances between those treatments. To compare the variability of zinc accumulation in grain of the cultivars, analysis of concentrations was made with the method of farthest proximity. The dendrogram presented determines the Euclidean distances between the analyzed genotypes in pentadimensional space (Fig. 1). The closer to each other is the position of the cultivars, the greater intergroup similarity of the treatments being compared. A considerable Euclidean distance was found between the group of Banti and Jasna cultivars and the other treatments. It may be stated that a similar response to differentiation in the zinc doses was revealed by Henika, Eta and Ismena, though the latter was distinguishable by its somewhat greater Euclidean distance in relation to the genotypes mentioned (Fig. 1). The fact that the groups of cultivars formed in the dendrogram were slightly different (as compared with the results shown in Table 5) comes from the assumption of no correlation between dependent variables which are taken into account in Mahalanobis distances. The dendrogram shows, however, much convergence of the results obtained on the basis of Mahalanobis distances, thus confirming significant variability of spring wheat cultivars' response to differentiated zinc doses used in the experiment. On the basis of the analysis it may be stated that the highest influence on variation of zinc accumulation in the cultivars was exerted by the dose 2.05 g Zn per pot as well as control treatment, while the fertilizing doses differentiated the uptake of zinc to a lesser degree. The discriminant and cluster analyses proved that Jasna and Banti distinguished themselves by increased variation of zinc accumulation in the grain as compared with the other cultivars. Varied response of cultivars to differentiated fertilization with macro- as well microelements has been also implied in other reports [18]. Jaśkiewicz [19] reported that wheat cultivars typically take up varied amounts of Cu, Zn, Mn and Fe in the initial stages of their development depending on the soil microelements availability. Also Mikos and Styk [20] as well as Kulczycki and Grochowski [21] found that concentration of microelements in the grain of cultivars was differentiated.

Duscussion

Differentiated uptake of zinc by spring wheat cultivars as tested in the control treatments was found to result from their considerable genetic differences. Wheat, as a natural hexaploid, distinguishes itself by significantly greater variability than other cultivated species. Small content of organic matter in the soil was probably the cause of lower availability of zinc in the control samples. On the other hand, readily available form of zinc supplied with fertilizing doses ($ZnSO_4$) caused less variation in zinc accumulation in wheat grain. Significant interaction between zinc doses and wheat cultivars produced different response of the cultivars to the loading doses of zinc in comparison to the control treatments or fertilizing doses. In literature no reports can be found concerning the response of wheat cultivars to high environmental contamination with zinc. Most often the papers describe variation of zinc accumulation in wheat cultivars at deficiency of the microelement in the soil. Sought for are wheat cultivars with increased assimila-

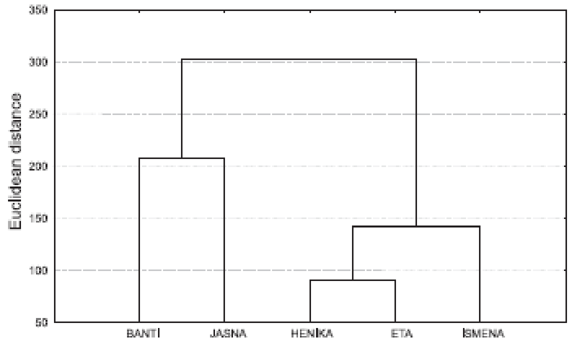

Fig. 1. Dendrogram of the cluster analysis of zinc accumulation in grain of spring wheat cultivars depending on zinc doses applied in the experiment.

Table 5. Squared Mahalanobis distances.

Cultivars	Ismena	Eta	Henika	Jasna	Banti
Ismena	0.00	2.47	0.69	5.15**	12.34**
Eta	2.47	0.00	1.00	3.06*	7.76**
Henika	0.69	1.00	0.00	4.88**	11.07**
Jasna	5.15**	3.06*	4.88**	0.00	3.92*
Banti	12.34**	7.76**	11.07**	3.92*	0.00

*significant at $\alpha = 0.05$; **significant at $\alpha = 0.01$

bility of zinc [22-24]. The zinc content in grain is not a reliable parameter for diagnosing deficiencies of that element in wheat cultivars [25-27]. Cultivars showing improved utilization of zinc are not always characterized by increased concentration of the element in leaves or grain. Such cultivars usually have longer culms and roots or higher dry matter content in aerial parts. Our results have suggested that the effect of fertilizing doses was not due to a simple uptake mechanism, but to physiological and morphological characters or genotype of the plants [27, 28]. The differentiated response of genotypes should then be considered at the cell level through analysis of the activity of dismutase or carbonic anhydrase enzymes [29, 30]. So far there has been scarce information available on inheriting improved zinc uptake efficiency by cultivars. Graham and Welch [23] report about one major gene with dominant action. On the other hand, Majumder *et al.* [31] point to polygenic inheritance with additive action and incomplete dominance. Proved genotypic variation at the level of loading and fertilizing doses would also point to differentiated response of Polish cultivars to variable zinc content in the environment.

Conclusions

1. The greatest influence on variation of zinc accumulation in cultivars was exerted by the dose 2.05 g Zn/pot as well as the control, while the fertilizing doses differentiated zinc uptake by the cultivars tested to a lesser degree.
2. Cultivar Henika distinguished itself by the lowest accumulation of zinc under the zinc contamination doses, while the same was true for Banti at a lower zinc content in the soil.
3. Discriminant and cluster analyses showed that the response of Jasna and Banti to the contents of zinc in the environment was different from that of the other cultivars. The cultivars accumulated more zinc in its grain when soil Zn doses were high.

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