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PLANT PHYSIOLOGY

The Resistance of *Phleum pratense* and *Elytrigia repens* to High Concentrations of Zinc

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Abstract—The influence of increased zinc concentrations on seed germination, growth activity, photosynthetic apparatus, and water metabolism in two perennial grasses (*Phleum pratense* L. and *Elytrigia repens* (L.) Nevski) was studied in laboratory and vegetation experiments to assess plant metal tolerance. In laboratory conditions it was established that seeds of both species may germinate in a wide range of zinc concentrations. In vegetation experiments, the possibility of successful growth and accumulation of biomass of both grasses in the presence of high zinc concentration in the root medium was revealed. At the same time, high water contents in root and shoot tissues were maintained, as well as the necessary intensity of photosynthesis (due to maintenance of the efficiency of photosystem II and the amount of carotenoids). It was noted that the established high resistance of both species of grasses to zinc, as well as their ability to accumulate significant amounts of metal ions in the roots, indicates that *P. pratense* and *E. repens* may be used for phytoremediation of soils contaminated with zinc.

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INTRODUCTION

At present, in many countries, the influence of heavy metals on cultivated plant species of the Poaceae family is actively being studied, which is associated with their economic importance. Wild-growing grasses have been studied much less, although they play the leading cenotic role in various plant communities (Tsvelev, 1976), where they occupy a dominant or co-dominant position (meadows, steppes, savannas). Many of them are involved in the natural overgrowing of areas with plant cover disturbed due to anthropogenic impact, which is associated with their high resistance to various stress factors, including heavy metals (Bezel and Zhuikova, 2007; Majugina et al., 2008; Laidinen et al., 2011).

Zinc is one of the most common heavy metals, which comes in large quantities into the environment with industrial and domestic waste, and also when high doses of mineral fertilizers are applied to the soil. In some areas, the content of this metal in the soil can be tens or even hundreds of times higher than the established MAC (maximum allowable concentration) (Il'in, 1991; Plekhanova and Obukhov, 1992). Although zinc is a necessary microelement for growing plants, it has a strong negative impact on their vital activity in high concentrations, leading to a decrease in the productivity of both individual plants and whole phytocenoses, and in some cases to complete degradation of the vegetation cover. Note that the natural overgrowth of areas contaminated with heavy metals is extremely slow (Chernenkova, 2002; Stavrova, 2005). Therefore, the search for and study of metal-resistant plant species of wild flora, including those from the Poaceae family, are very relevant as they could be used to restore such areas.

Our goal was to study the resistance of two species of perennial grasses, *Phleum pratense* and *Elytrigia repens*, to high zinc concentrations. These plants are widely distributed in different climatic zones, and an assessment of their possible role for phytoremediation of soils contaminated with this metal may be useful.

MATERIALS AND METHODS

Two species of perennial grasses, *Phleum pratense* L. (timothy grass) and *Elytrigia repens* (L.) Nevski (quack grass), were the objects of our study. To study the effect of zinc on the germination of seeds under laboratory conditions, they were germinated in Petri dishes in the dark at 22°C. In the control variants, distilled water was used; in experimental conditions we used solutions of the zinc salt in concentrations (by element) of 10^{-5}, 10^{-4}, 10^{-3}, and 10^{-2} M. Germination of *P. pratense* seeds was assessed on the 8th day, and *E. repens* seeds were germinated on the 14th day (GOST no. 12038-84).

Plant resistance to zinc was studied under vegetation experiments in sand culture. The germinated seeds were planted in vegetation vessels (volume 4 L),

Table 1. Effect of zinc on seed germination of *Phleum pratense* and *Elytrigia repens* (%)

Species	Zinc concentration, M				
	0	10^{-5}	10^{-4}	10^{-3}	10^{-2}
P. pratense	96 ± 2.3	97 ± 2.5	98 ± 1.0	98 ± 0.0	65 ± 0.5*
E. repens	54 ± 0.1	69 ± 0.6*	77 ± 0.6*	73 ± 0.5*	70 ± 2.3*

* The differences with the control are reliable at $P < 0.05$ (for Tables 1–5).

12 pieces per vessel. Zinc (as sulfuric acid salt) at a concentration of 160 mg/kg substrate was applied in a dry form once during the filling of the vessels with sand. This concentration of the metal was selected on the basis of preliminary experiments in which a similar inhibitory effect on the height of the shoots of the studied grasses species in the early phases of their development was found. The plants were watered with a Knop solution supplying micronutrients.

Forty days after sowing, the plants were analyzed. The resistance of grasses to zinc excess was judged on the basis of the change (in relation to the control) in a number of growth parameters (length of the most developed root, height of the shoot, area of the fifth (the youngest) leaf, biomass of the roots and shoots), photosynthetic apparatus (content of photosynthetic pigments, maximum quantum efficiency of photosystem II (PS II), photosynthesis intensity), and water exchange (transpiration, root and shoot water content). In addition, the zinc content was determined in the roots and shoots of plants.

The dry weight of roots and shoots was determined after sample desiccation at 105°C. The area of the leaf blade was calculated by the formula $S = 0.66ld$, where l is the length and d is the width of the leaf blade (Anikiev and Kutuzov, 1961). The contents of chlorophylls *a*, *b* and carotenoids were determined on an SF-2000 spectrophotometer (Spectrum, Russia), after extraction from the leaves with 80% acetone (Shlyk, 1971). The maximum quantum efficiency of PS II (F_v/F_m, where F_v is a variable and F_m is the maximum fluorescence of chlorophyll) was measured in leaves adapted to darkness (Maxwell and Johnson, 2000) by a MINI-PAM fluorimeter (Walz, Germany). Photosynthesis and transpiration intensity was assessed by the gas analyzer (HCM-1000, Walz). The water contents of root and shoot tissues were calculated according to the generally accepted formula. The zinc contents in the root and shoot were determined by the method of inversion voltammetry on the polarograph ABS-1.1 (Volta, Russia).

In laboratory experiments, the repetition within each variant was 100 seeds, and in the vegetation experiments, it varied from 6 to 20 plants. Analytical repetition during chemical analyses was 3- to 5-fold. All experiments were repeated twice. The tables show the average values for two independent experiments and their standard errors. The reliability of the differences between the variants of the experiments was determined with Student's test ($P < 0.05$).

The research was carried out on the scientific equipment of the Center for Collective Use of the Federal Research Center "Karelian Research Center of the Russian Academy of Sciences."

RESULTS

It was shown that the seeds of both species of grasses germinated successfully in the range of zinc concentrations (Table 1). In particular, the metal in concentrations of 10^{-5}–10^{-3} M did not influence the germination of *P. pratense* seeds, and only when the concentration was increased to 10^{-2} M did the number of seedlings significantly decrease. All zinc concentrations (10^{-5}–10^{-2} M) stimulated the germination of *E. repens*, so the number of germinated seeds in the experimental variants was 27–42% more than in the control (depending on the metal concentration).

When studying the effect of zinc on plant growth, a stronger inhibitory effect on root growth was found. In particular, in the presence of Zn at a concentration of 160 mg/kg substrate, the length of the most developed root in *P. pratense* and *E. repens* plants decreased in compare to the control by 44 and 37%, respectively (Table 2). However, a significant reduction in the accumulation of root biomass, as well as a marked decrease in the height of the shoot, the dimensions of the leaf blade, and the accumulation of shoot biomass was noted only in *P. pratense*. In *E. repens* plants, on the contrary, these parameters influenced by zinc even increased.

It is known that, among the physiological processes determining the growth and productivity of plants, photosynthesis is the most important one. An estimate of the effect of zinc on photosynthesis intensity showed a significant (more than twofold) slowing-down of photosynthesis in *P. pratense* and the absence of a similar effect in *E. repens* (Table 3). Under the influence of zinc, the content of chlorophylls, especially chlorophyll *b*, also decreased appreciably. In *E. repens*, the content of chlorophylls increased mainly due to chlorophyll *a*. In both species, the presence of zinc in the substrate did not affect the carotenoid content and the F_v/F_m rate.

Note also that zinc in the studied concentrations in both *P. pratense* and *E. repens* plants caused a slight

Table 2. Effect of zinc (160 mg/kg substrate) on some growth characteristics in *Phleum pratense* and *Elytrigia repens*

Parameter	*P. pratense*		*E. repens*	
	control	Zn^{2+}	control	Zn^{2+}
Root length, cm	19.6 ± 0.7	10.9 ± 1*	17.7 ± 1.2	11.2 ± 0.7*
Root dry weight, mg	65.4 ± 6.4	38.3 ± 8.8*	71.8 ± 11.9	82.9 ± 9.5
Shoot height, cm	30.1 ± 0.7	24.0 ± 1.4*	30.6 ± 2	36.7 ± 1.8*
Leaf area, cm^2	7.5 ± 0.4	5.7 ± 0.4*	5.1 ± 0.6	7.2 ± 0.5*
Shoot dry weight, mg	135.2 ± 12.1	86.0 ± 11.5*	109.4 ± 13.3	145.7 ± 17.6

Table 3. Effect of zinc (160 mg/kg substrate) on photosynthetic apparatus of *Phleum pratense* and *Elytrigia repens*

Parameter	*P. pratense*		*E. repens*	
	control	Zn^{2+}	control	Zn^{2+}
Chlorophyll *a* content, mg/g fr wt	1.23 ± 0.06	1.08 ± 0.004*	1.49 ± 0.04	1.83 ± 0.02*
Chlorophyll *b* content, mg/g fr wt	0.68 ± 0.03	0.36 ± 0.01*	0.64 ± 0.01	0.64 ± 0.003
Carotenoids content, mg/g fr wt	0.53 ± 0.03	0.48 ± 0.01	0.63 ± 0.03	0.62 ± 0.03
F_v/F_m	0.77 ± 0.003	0.78 ± 0.005	0.73 ± 0.011	0.76 ± 0.009*
Photosynthesis, μmol $CO_2/(m^2\ s)$	26.1 ± 1.5	11.3 ± 1.2*	28.8 ± 0.4	25.8 ± 1.3

increase in the intensity of transpiration (Fig. 1). However, the water content of root and shoot tissues was maintained at a level close to the control (Table 4).

Analysis of the metal content in the roots and shoots showed that *P. pratense* and *E. repens* were able to accumulate sufficiently high zinc concentrations (Table 5). At the same time, in the roots of both species, much more metal was accumulated than that in the shoots (2.7- and 3.2-fold, respectively).

DISCUSSION

It is generally recognized that phytoremediation is one of the most promising and economically viable technologies for the recovery of strongly polluted soils. One of the methods of phytoremediation (phytostabilization) involves the use of higher plants to strengthen the soil cover, as well as to reduce the mobility of toxic ions in the soil due to their immobilization in the roots. For successful application of this method, on the one hand, plants are needed that possess high resistance to heavy metals, and on the other hand, the plants must be capable of accumulate metals mainly in the roots by creating a barrier for metal movement to the aerial organs (Berti and Cunningham, 2000; Padmavathiamma and Li, 2007; Bolan et al., 2011). Therefore, Poaceae species are of interest, among which zinc-resistant populations are found, in particular *Festuca rubra* L., *Dactylis glomerata* L., and *Phragmites australis* (Cav.) Trin. ex Steud., accumulating metal to a greater extent in the roots than in the shoots of plants (Symeonidis, 1990; Atabaeva, 2007; Caldelas et al., 2011). We have studied the resistance of two widely distributed species of wild-growing grasses from many climatic zones (*P. pratense* and *E. repens*) to high concentrations of zinc, as well as their ability to accumulate this metal in the roots and shoots.

It is known that one of the important criteria of metal resistance is seed ability to germinate under a high content of heavy metals in the environment. In the experiments carried out, the seeds successfully ger-

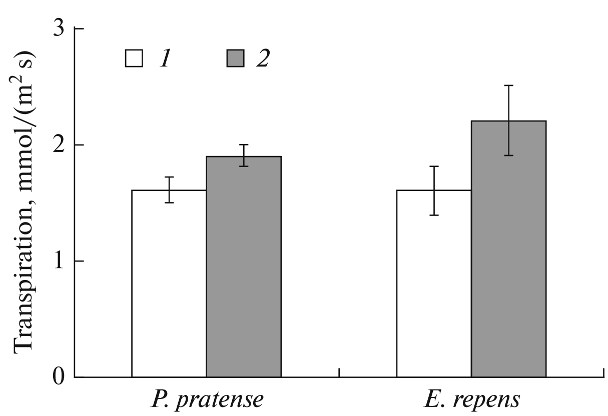

Fig. 1. Effects of zinc (160 mg/kg substrate) on transpiration in *Phleum pratense* and *Elytrigia repens*. (*1*) Control, (*2*) Zn^{2+}.

Table 4. Effect of zinc (160 mg/kg substrate) on water content (%) in root and shoot tissues of *Phleum pratense* and *Elytrigia repens*

Organ	*P. pratense*		*E. repens*	
	control	Zn^{2+}	control	Zn^{2+}
Root	89.3 ± 0.5	91.1 ± 0.5	85.2 ± 1.4	84.2 ± 1.0
Shoot	82.6 ± 0.6	85.5 ± 0.5*	77.6 ± 0.5	79.0 ± 1.1

Table 5. Content of zinc (mg/kg dry wt) in the roots and shoots of *Phleum pratense* and *Elytrigia repens* after 40 days growth in the presence of metal (160 mg/kg substrate)

Variant	*P. pratense*		*E. repens*	
	root	shoot	root	shoot
Control	76.9 ± 3.6	26.6 ± 2.1	56.4 ± 6.4	27.7 ± 1.1
Zn^{2+}	2842 ± 450*	1067 ± 35*	1158 ± 150*	364 ± 43*

minated in a wide range of zinc concentrations (10^{-5}–10^{-2} M), which agrees with the results of our earlier studies (Batova et al., 2008, Kaznina et al., 2009), and with the data of other authors (Majugina et al., 2008; Drab et al., 2011; Rasafi et al., 2016). Moreover, in *E. repens* plants, all zinc concentrations studied exerted even a stimulating effect on seed germination and only in *P. pratense* plants did seed germination in the presence of the highest concentration of metal turn out to be less than in the control. The stimulating effect of some heavy metals, including zinc, on seed germination has repeatedly been noted in other reports (Kaznina et al., 2005; Batova et al., 2008; Jadia and Fulekar, 2008; Statu and Costică, 2015). The reasons for this are still not completely clear. Perhaps this effect is associated with some increase in the generation of reactive oxygen species, which is necessary to activate the germination process and stimulate the cell cycle and antioxidant system activity (Bailly et al., 2008; Lefevre et al., 2009; Kranner and Colville, 2011). It is also possible that the positive effect of zinc on the initial growth of seedlings is associated with its important functions as a necessary micronutrient, which participates in many processes of plant metabolism.

The results of our experiments carried out under vegetation conditions indicate also a high resistance of the studied grasses species to an excess of zinc. In particular, both *P. pratense* and *E. repens* plants have successfully grown for a long time (40 days) in the presence of a Zn concentration in the substrate of 160 mg/kg, which is three times higher than the value of the roughly permissible concentration (RPC) for sod-podzolic sandy loam soils of Russia (GN 2.1.7.2511-09). At the same time, in the plant response to the effect of excess zinc, certain specific differences were found. Thus, in *P. pratense*, root and shoot growth in the presence of the metal was slowed down, the dimensions of the leaf blade decreased, and the content of photosynthetic pigments decreased, which adversely affected the rate of photosynthesis and the accumulation of biomass by plants. In *E. repens*, the excess of zinc led only to a certain inhibition of root growth, whereas growth of shoots, on the contrary, increased. The leaf blade dimensions and the content of chlorophyll *a* also increased. At the same time, the photosynthesis intensity and the biomass of roots and shoots remained at the control level.

The negative effects of high zinc concentrations on plants are fairly well known, but in many cases, lower metal concentrations than in our experiments were responsible for the inhibitory effect. For example, in plants of *Hordeum vulgare* L., *Saccharum officinarum* L., *Holcus lanatus* L., and *Setaria viridis* (L.) Beauv., a decrease in the biomass of the root and/or shoot was observed even in the presence of zinc in a concentration of 40–80 mg/kg substrate (Rengel, 2000; Kaznina et al., 2009, 2010; Jain et al., 2010). Increasing the metal concentration to 80–130 mg/kg substrate resulted in a marked decrease in the amount of chlorophyll in the leaves and a slowing down of the rate of photosynthesis in *Zea mays* L., *Hordeum vulgare* L., and *Phragmites australis* (Cav.) Trin. ex Steud. plants (Kaznina et al., 2010; Caldelas et al., 2011; Paula et al., 2015).

As you know, the photosynthetic apparatus (PSA) of plants is very sensitive to the action of heavy metal, so the metal resistance of plants depends largely on the stability of their PSA. In our experiments, perennial wild-growing grasses were able to maintain the required level of carotenoids in the presence of high zinc concentrations. Carotenoids perform a number of important functions in photosynthesis, in particular the antenna, acting as additional "light gatherers" (Lichtenthaler, 1987; Mokronosov et al., 2006), and are also an important component of the antioxidant system. The maintaining of a high level of these pigments is considered one of the features promoting plant adaptation to various unfavorable factors (Vasilev et al., 1995; Khudsar et al., 2001; Talanova et al., 2001). In experimental plants of both species studied, there were no changes in such an important PSA index as the maximum quantum efficiency of PS II, which indicated the maintenance of the photochemical activity of PS II. As we showed earlier, the mechanisms that support the necessary carotenoid concentration and the activity of PS II in Poaceae plants are important for ensuring their resistance to heavy metals (Kaznina, 2016).

For successful growth and development of plants under unfavorable conditions, it is also extremely important to maintain the necessary level of water content in their cells and tissues (Barceló and Poschenrieder, 1990). Many studies have shown that under the influence of heavy metals, including zinc, the rate of transpiration in plants slows down, which contributes to the maintenance of a high water content of tissues with a decrease in the size of the root system under similar conditions (Khudsar et al., 2004; Kaznina et al., 2010; Caldelas et al., 2011). In some studies, in such cases there were no changes in the water regime, which was also found in perennial grasses, for example in *Dactylis glomerata* L. and *Festuca pratensis* L. (Polovnikova, 2007), in *Cenchrus ciliaris* L., and *Cynodon dactylon* (L.) Pers. (Mukhtar et al., 2013). In our studies, the transpiration in both species of grasses increased, despite a slight slowdown in root growth. At the same time, the water content of root and shoot tissues remained at a high level. It is assumed that under stressful conditions this effect may be associated with an increase in the hydraulic conductivity of roots and is one of the mechanisms that allow plants to set off transpiration losses and ensure the maintenance of the necessary water content of tissues (Kudoyarova et al., 2001). In addition, the increase in hydraulic conductivity allows us to leave the stomata in the open state, which is extremely important for maintaining a high level of photosynthesis. Since the degree of water content of tissues affects many aspects of plant life, as well as their ability to adapt to unfavorable environmental factors, the maintenance of a high water level in root and shoot tissues in *E. repens* and *P. pratense* in the presence of zinc indicates the protection of the balance of the basic physiological processes in plants under such conditions.

As was noted above, to determine the possibility of using different plant species for phytoremediation of soils polluted with heavy metals, it is important to know how many of these elements the plants may accumulate in the roots and shoots. According to the literature, wild species of the Poaceae family may accumulate a fairly large amount of zinc in the roots. Thus, in *Calamagrostis epigeios* (L.) Roth., *Festuca arundinaceae* Schreb., and *Lolium perenne* L. in the presence of high zinc concentrations in the root zone, the metal content in the roots exceeded 1000 mg/kg dry wt, which was significantly higher than in the shoots (Rizzi et al., 2004; Bashmakov and Lukatkin, 2009; Bidar et al., 2009). In our studies, the metal concentrations in *P. pratense* and *E. repens* roots turned out also to be very high and amounted to 2842 and 1158 mg/kg dry wt, respectively, but in plant shoots, importantly, the metal content was much lower.

Thus, our study showed that, in general, the species of wild-growing grasses are characterized by high resistance to zinc excess in the substrate and are able to accumulate a large amount of this metal in the roots, which meets the requirements for plants that are intended to be used for phytoremediation of soils polluted with an increased content of this metal. Moreover, the resistance of *E. repens* to zinc was higher than that of *P. pratense*.

In conclusion, it should also be noted that our studies carried out in the laboratory and in vegetation experiments are the first stage of the program aimed at identifying and selecting wild-growing grasses that are promising for their use in phytoremediation of soils contaminated with zinc. In the subsequent stages, not only will a more detailed (in-depth) assessment of each object be required, but so will, most importantly, field trials, which, among other things, will allow for maximum consideration of the biological characteristics of each species. For example, *E. repens* is characterized primarily by vegetative renewal (rhizomes), which allows it to compete successfully with other species, including *P. pratense*, and participate actively in the formation of continuous vegetative cover. At the same time, *P. pratense* possesses good seed productivity and high seed germination, which provide plants of this species effective seed renewal in natural conditions. In general, the results of this stage of research indicate that both species of perennial grasses are of undoubted interest in their possible use for soil phytoremediation. But it should also be borne in mind that the final choice of a species will also depend on the specific conditions (climatic, edaphic, etc.) that are characteristics of the area that requires restoration.

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