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Effect of Increasing Concentrations of Heavy Metals on the Growth of Barley and Wheat Seedlings

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Abstract—The tolerance to increasing doses of lead and cadmium salts on the growth and survival of barley (*Hordeum vulgare* L.) and wheat (*Triticum aestivum* L.) seedlings were studied. Seedlings grown under controlled conditions were treated with dilute (0.001–0.005 mM) solutions of either lead nitrate or cadmium bromide for 1, 4, or 7 days. Subsequently, they were incubated for 7 days in solutions of the same compounds, but at sublethal or lethal concentrations (0.05–10 mM). Plant pretreatment with low concentrations of heavy metals induced an increase in their tolerance to the metals, because pretreated plants could tolerate heavy metals at high concentrations. It is concluded that plant tolerance to increasing concentrations of heavy metals is related to the activation of protective and adaptive processes in their tissues.

Key words: Hordeum vulgare - Triticum aestivum - lead - cadmium - growth - tolerance

INTRODUCTION

All other things being equal, the sudden strong influence of a specific stress factor or a slow, gradual increase in its action differently affect plant tolerance. For example, it was established that, during a gradual increase or decrease in temperature from hardening to injuring ranges, plants had time to adapt to this factor. Subsequently, such plants were able to tolerate extreme temperatures that would injure unhardened plants [1, 2]. There is similar evidence as regards salt tolerance. Pretreatment of sorghum plants with low concentrations of NaCl increased their tolerance to higher salt concentrations that would be lethal for nonpretreated plants [3]. Thus, a gradual increase in the dose of a stress factor (high and low temperature, salt) results in the development of tolerance that is considerably higher than that formed by a factor of constant intensity.

It is still unknown whether this conclusion can be applied to other stress factors, in particular, to heavy metals, because this problem remains little investigated [4, 5]. Therefore, taking into account a continuous increase in the industrial heavy-metal pollution of the environment, we have studied the responses of plants to increasing concentrations of lead and cadmium salts.

MATERIALS AND METHODS

Barley (*Hordeum vulgare* L., cv. Otra) and wheat (*Triticum vulgare* L., cv. Mironovskaya 808) seedlings were used in the experiments. The seedlings were grown for 3 days in filter-paper rolls on a half-strength Knop solution, pH 6.2–6.4, without air bubbling, at a relative humidity of 60–70%, an illuminance of 10 klx, a 14-h photoperiod, and 22–25°C.

The seedlings were incubated for 1, 4, or 7 days on dilute (0.001–0.005 mM} solutions of lead nitrate or cadmium bromide or on distilled water as a control. This was followed by 7-day-long treatments with more concentrated (0.05–10 mM) solutions of the same salts. These high concentrations of lead and cadmium salts were shown earlier to inhibit plant growth, and, after more prolonged treatment, to induce plant injury and death [6]. Throughout the experiment, some of the seedlings were treated with solutions of lead or cadmium salts of constant (low or high) concentrations. Other experimental conditions were kept constant. Reagent grade salts of heavy metals produced in Russia were used.

The responses of seedlings to the treatments were evaluated by the alterations in fresh and dry weights and the survival rate.

Three experiments were performed. Each treatment included five groups of four seedlings. Arithmetic means from three independent experiments and their standard errors are presented in the figures and the table.

RESULTS

Treatment of barley seedlings with 1 mM lead nitrate for 7 days resulted in a significant inhibition of fresh and dry matter accumulation (Figs. 1a and 1c). Drastic retardation of growth to the point of total inhibition was observed regardless of the time of the onset

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Effect of pretreatment of barley seedlings with 0.001 mM lead nitrate or cadmium bromide on their dry weight measured after the subsequent treatment with more concentrated solutions

Duration of seedling pretreatment with salt solutions or water, days	Increment of dry wt for 7 days, mg			
	seedlings treated with 1 mM lead nitrate		seedlings treated with 0.05 mM cadmium bromide	
	after pretreatment with water	after pretreatment with 0.001 mM lead nitrate	after pretreatment with water	after pretreatment with 0.001 mM cadmium bromide
0	1.7 ± 0.6	–	2.5 ± 0.5	–
1	1.4 ± 0.5	7.3 ± 0.8	0.9 ± 0.3	7.0 ± 1.3
4	2.3 ± 0.3	3.8 ± 0.5	1.2 ± 0.4	5.1 ± 1.1
7	1.8 ± 0.5	4.1 ± 0.6	1.5 ± 0.5	5.0 ± 0.8

Note: The values in columns 2–5 correspond to curves *3* in Figs. 1c, 1d, 4c, and 4d, respectively.

of lead-ion treatment, viz., before the experiment or in 1, 4, or 7 days after its onset.

When seedlings were preimposed to a low concentration (0.001 mM) of lead nitrate for 1, 4, or 7 days and then to the 1 mM lead nitrate, the fresh weight of seedlings was inhibited to a lesser degree than that of the nonpretreated samples (Figs. 1a and 1b, curves *3*). It should be emphasized that the pretreated seedlings virtually did not differ from those treated with 0.001 mM lead nitrate in their dry weight (Fig. 1d, curves *2* and *3*). The dry weight increment in seedlings pretreated for 1, 4, and 7 days with 0.001 mM lead nitrate and subsequently with a 1 M concentration was higher than that in the seedlings imposed to a high lead nitrate concentration only (table, columns 2 and 3). The survival rate of seedlings pretreated with 0.001 mM lead nitrate did not differ from that of seedlings treated with 1 mM lead nitrate without pretreatment (Fig. 2a).

Treatment of barley seedlings with 10 mM lead nitrate resulted in a still higher inhibition of the fresh matter accumulation. It even decreased as compared to that before treatment (treatment 4 and 7 days after the onset of the experiment, Fig. 3a). The application of this injuring dose of lead nitrate after the pretreatment of seedlings with 0.001 mM lead nitrate also resulted in an inhibition of seedling fresh-matter accumulation regardless of the duration (1, 4, or 7 days) of the pretreatment with a low concentration of lead nitrate (Fig. 3b, curve *3*).

The alteration in the barley seedling dry weight under the influence of lead ions in this experiment was somewhat different. Thus, the treatment with 10 mM lead nitrate resulted in the complete inhibition of dry matter accumulation. This was observed after both the direct treatment of seedlings with this metal concentration and their pretreatment with 0.001 mM lead nitrate (Figs. 3c and 3d). Thus, the 10 mM lead nitrate induced a disturbance in water relations, which resulted in the lack of the seedling fresh weight increase.

Treatment of barley seedlings with a 0.05 mM cadmium bromide stopped the accumulation of both fresh and dry matter (Figs. 4a and 4c, curves *3*). As in the case of lead nitrate, pretreatment of seedlings with 0.001 mM cadmium bromide for 1, 4, or 7 days decreased the inhibitory effect of 0.05 mM cadmium bromide as compared to that without pretreatment (Figs. 4a and 4c). In this case, the dry-weight increment was higher than that in nonpretreated seedlings (table, columns 4 and 5). In addition, as compared with nonpretreated samples, pretreatment of seedlings at a low concentration of cadmium bromide improved the survival of seedlings after subsequent treatment at a high

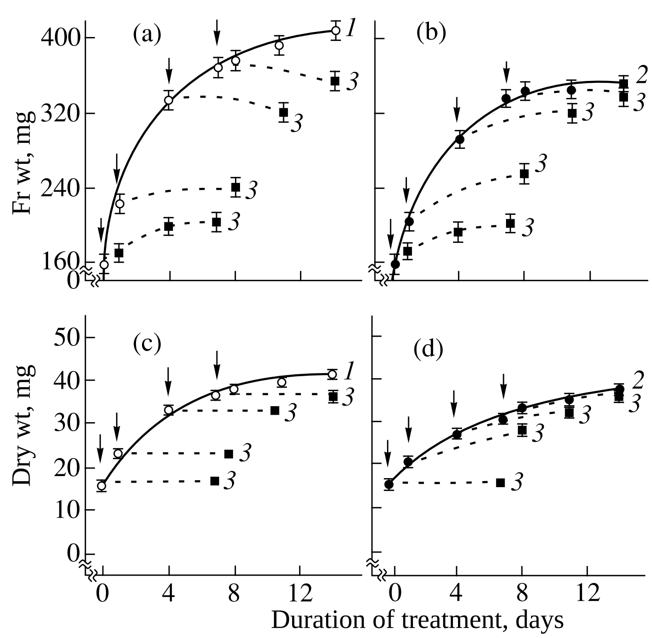

Fig. 1. The effect of increasing concentrations of lead nitrate on the accumulation of fresh and dry matter in barley seedlings.

(*1*) Water; (*2*) 0.001 mM lead nitrate; (*3*) 1 mM lead nitrate; arrows indicate the onset of the action of 1 mM lead nitrate on (a, c) control seedlings and (b, d) 0.001 mM lead nitrate-treated seedlings.

concentration of cadmium bromide (Fig. 2b, curves *3* and *4*).

Similar results were obtained in experiments with wheat seedlings. After pretreatment with 0.001 mM cadmium bromide, the survival of seedlings incubated subsequently at a high concentration (0.05 mM) of cadmium bromide was higher than that of nonpretreated ones (Fig. 2d, curves *3* and *4*). In contrast, after their pretreatment with 0.005 mM lead nitrate for 1, 4, or 7 days followed by the treatment with 1 mM lead nitrate, the survival of seedlings did not differ from that without pretreatment (Fig. 2c, curves *1* and *5*).

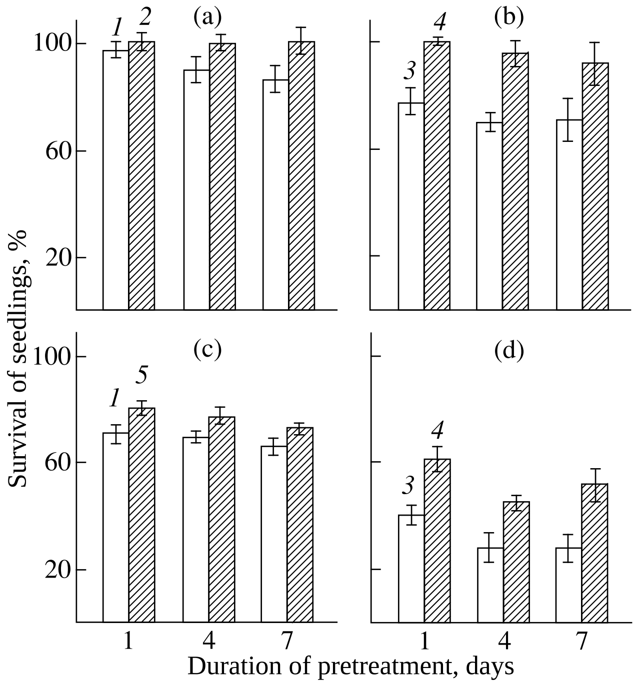

Fig. 2. The effect of pretreatment at low concentrations of (a, c) lead nitrate and (b, d) cadmium bromide on the survival of (a, b) barley and (c, d) wheat seedlings after the subsequent action of high concentrations of these salts.

(*1*) Water (1–7 days) + 1 mM lead nitrate (7 days); (*2*) 0.001 mM lead nitrate (1–7 days) + 1 mM lead nitrate (7 days); (*3*) water (1–7 days) + 0.05 mM cadmium bromide (7 days); (*4*) 0.001 mM cadmium bromide (1–7 days) + 0.05 mM cadmium bromide (7 days); (*5*) 0.005 mM lead nitrate (1–7 days) + 1 mM lead nitrate (7 days).

DISCUSSION

As a whole, the results obtained showed that the pretreatment of seedlings at low concentrations of lead and cadmium salts induced an increase in the tolerance of barley and wheat seedlings to the heavy-metal ions and made it possible for them to tolerate without injury and with less inhibition of growth, the subsequent action of sublethal doses of these chemical agents. At the same time, the level of tolerance to the increasing doses of a stress factor depends to some extent on both the plant species specificity and the conditions of their pretreatment, such as metal-ion concentration and duration of incubation.

Plant tolerance to the heavy-metal ions was shown to be related to the activation of protective and adaptive

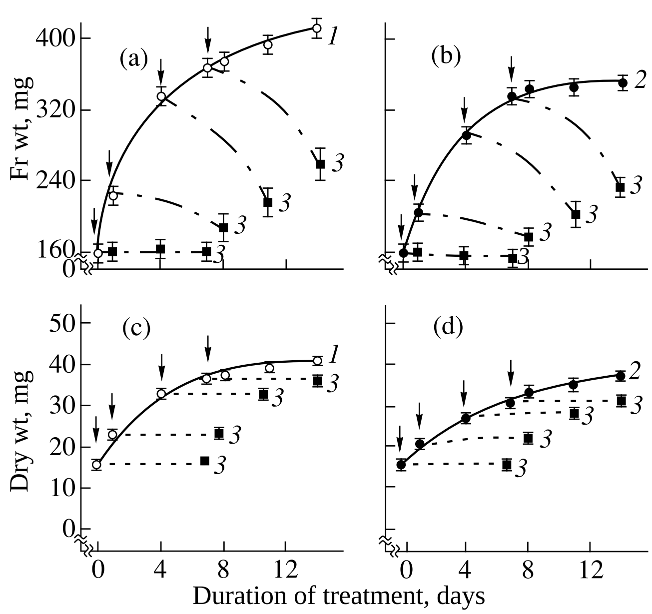

Fig. 3. The effect of lead nitrate at increasing concentrations on the accumulation of fresh and dry matter in barley seedlings.

(*1*) Control seedlings; (*2*) 0.001 mM lead nitrate; (*3*) 10 mM lead nitrate; arrows indicate the onset of action of 10 mM lead nitrate on (a, c) control seedlings and (b, d) seedlings pretreated with 0.001 mM lead nitrate.

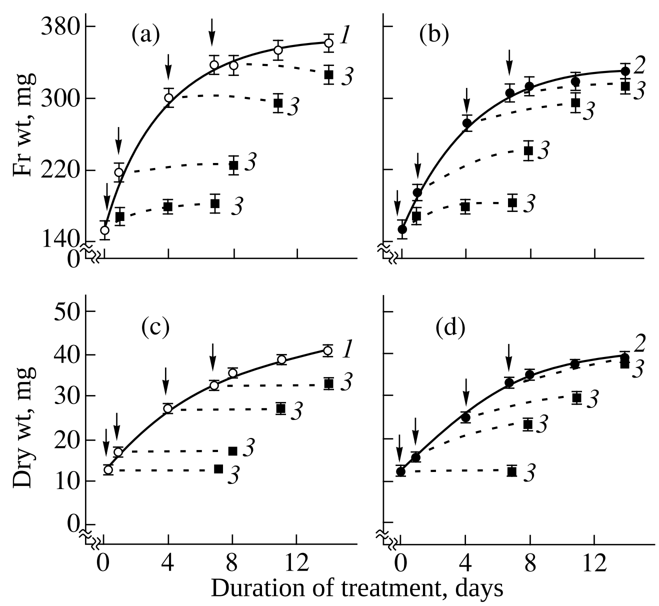

Fig. 4. The effect of cadmium bromide at increasing concentrations on the accumulation of fresh and dry matter in barley seedlings.

(*1*) Control seedlings; (*2*) 0.001 mM cadmium bromide; (*3*) 0.05 mM cadmium bromide; arrows indicate the onset of action of 0.05 mM cadmium bromide on (a, c) control seedlings and (b, d) seedlings pretreated with 0.001 mM cadmium bromide.

responses in plants. In particular, it was found that heavy-metal ions affected the activity of a number of enzymes [7], stimulated the synthesis of metallothioneins [8] and phytochelatins [9–11], enhanced the accumulation of stress proteins [11] and free amino acids [11, 12], etc. However, the nature of changes in physiological and biochemical processes in plants caused by the action of increasing doses of heavy-metal ions and the specific adaptive mechanisms had thus far not been established. The results of this work demonstrate that treatment with increasing doses of heavy-metal ions results not only in growth retardation, but also in a disturbance of water relations. This disturbance is caused by a decrease either in the hydraulic conductivity of roots, in the water-holding capacity of leaves, or in the formation of lateral roots and root hairs [12].

Evidence on the effect of increasing doses of heavy-metal ions on plants is very limited thus far. In particular, experiments with peas demonstrate the ability of plants to adapt themselves to the toxic action of cadmium. Indeed, soaking pea seeds for 7 h in 3.8 μM $CdCl_2$ decreased the toxic effect of the subsequent 30-min-long treatment of its 48-h-old seedlings with 25 μM $CdCl_2$ [5]. Other researchers showed that the pretreatment of velvet-grass plants with 0.2 μg/ml Cd_2SO_4 increased the tolerance of roots to the subsequent toxic action of a higher concentration of this salt [4]. It was established that yeast cultivation in a low cadmium medium enables them to grow subsequently in the presence of much higher concentration of the metal, which otherwise would be lethal for the cells [13]. In addition, very slow accumulation of a metal in fish and zooplankters, even up to quite significant concentrations, exerts no toxic action due to the binding of the metal in the cell to metallothioneins [14].

Similar results were obtained in the studies on the action of increasing unfavorable temperatures [1, 2, 15, 16] and salinity [3, 17] on plants.

Thus, the results obtained in this work along with previously published results demonstrate that in the response to increasing doses of heavy-metal ions and of some other stressors, such as extremely high and low temperatures and salinity, activate protective and adaptive reactions in plant tissues that result in the developing ability to tolerate without injury higher doses of metal ions.

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REFERENCES

1. Al'tergot, V.F., *Deistvie povyshennoi temperatury na rastenie v eksperimente i prirode* (The Effect of High Temperature on Plants in Experiments and Nature), Moscow: Nauka, 1981.
2. Ignat'ev, L.A. and Kalimullina, F.R., Ultrastructure of Chloroplasts from Leaves of Spring Wheat Adapted to Heating, *Fiziol. Rast.* (Moscow), 1986, vol. 33, pp. 581–587 (*Sov. Plant Physiol.*, Engl. Transl.).
3. Amzallag, G.N., Lerner, H.R., and Poljakoff-Mayber, A., Induction of Increased Salt Tolerance in *Sorghum bicolor* by NaCl Pretreatment, *J. Exp. Bot.*, 1990, vol. 41, pp. 29–34.
4. Brown, H. and Martin, M.H., Pretreatment Effect of Cadmium on the Root Growth of *Holcus lanatus* L., *New Phytol.*, 1981, vol. 89, pp. 621–629.
5. Sobolev, A.S., Mel'nichuk, Yu.P., and Kalinin, F.L., Plant Adaptation to the Inhibiting Effect of Cadmium, *Fiziol. Biokhim. Kul't. Rast.*, 1982, vol. 14, pp. 84–88.
6. Titov, A.F., Talanova, V.V., and Boyeva, N.P., Growth Responses of Barley and Wheat Seedlings to Lead and Cadmium, *Biol. Plant.*, 1996, vol. 38, pp. 431–436.
7. Foy, C.D., Chaney, R.L., and White, M.C., The Physiology of Metal Toxicity in Plants, *Annu. Rev. Plant Physiol.*, 1978, vol. 29, pp. 511–566.
8. Murphy, A., Zhou, J., Goldsbrough, P.B., and Taiz, L., Purification and Immunological Identification of Metallothioneins 1 and 2 from *Arabidopsis thaliana*, *Plant Physiol.*, 1997, vol. 113, pp. 1293–1301.
9. Steffens, J.C., The Heavy Metal-Binding Peptides of Plants, *Annu. Rev. Plant Physiol.*, 1990, vol. 41, pp. 553–575.
10. Rauser, W.E., Phytochelatins, *Annu. Rev. Biochem.*, 1990, vol. 59, pp. 61–86.
11. Sanitá di Toppi, L. and Gabrielli, R., Response to Cadmium in Higher Plants, *Environ. Exp. Bot.*, 1999, vol. 41, pp. 105–130.
12. Gural'chuk, Zh.Z., Mechanisms of Plant Resistance to Heavy Metals, *Fiziol. Biokhim. Kul't. Rast.*, 1994, vol. 26, pp. 107–117.
13. Magara, J., Accommodation of Yeast to Toxic Levels of Cadmium Ions, *J. Gen. Microbiol.*, 1978, vol. 104, pp. 321–324.
14. Burdin, K.S. and Polyakova, E.E., Metallothioneins, Their Structures, and Functions, *Usp. Sovrem. Biol.*, 1987, vol. 103, pp. 390–400.
15. Drozdov, S.N., Kurets, V.K., and Titov, A.F., *Termorezistentnost' aktivno vegetiruyushchikh rastenii* (Thermoresistance of Actively Growing Plants), Leningrad: Nauka, 1984.
16. Titov, A.F., Sherudilo, E.G., and Boeva, N.P., Development of Plant Heat Tolerance under Increasing Temperature, *Adaptatsiya, rost i razvitie rastenii* (Adaptation, Growth, and Development of Plants), Drozdov, S.N. and Titov, A.F., Eds., Petrozavodsk: Karel. Nauch. Tsentr Ross. Akad. Nauk, 1994, pp. 46–55.
17. Harrington, H.M. and Alm, P.M., Interaction of Heat and Salt Shock in Cultured Tobacco Cells, *Plant Physiol.*, 1988, vol. 88, pp. 618–625.