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Role of antioxidants in the protection against the combined effect of Cd, Zn, and Ni in wheat

(Presented by Academician of the NAS of Ukraine D. M. Grodzynsky)

The effect of the combined treatment with Cd, Zn, *and* Ni *on the accumulation of these metals, growth, and contents of antioxidants in wheat roots is studied. The metals were added in two concentrations to the nutrient solution for seedlings, and the higher one had a stronger effect on the studied parameters. The heavy-metal tolerances of 8 wheat varieties are compared in order to select the most tolerant (Kuial'nik) and most sensitive one (Sonechko) for biochemical studies. The heavy-metal uptake and, subsequently, the lipid peroxidation are smaller in the tolerant genotype, than in the sensitive one. The higher glutathione concentration in the tolerant genotype under control conditions could contribute to the improved heavy-metal tolerance. The metal-induced decrease in the glutathione content can be an indicator of the increased phytochelatin synthesis. The great tolerance of Kuial'nik can be explained by its decreased heavy-metal uptake and the greater glutathione-based antioxidant capacity.*

Keywords: *Triticum aestivum* L., cadmium, nickel, zinc, lipid peroxidation, ascorbic acid, glutathione.

Heavy metal pollution is one of the major environmental problems that should be solved throughout the world. The decontamination of soil requires a higher attention, because the high level of heavy metals threatens the life of plants and animals. Analysis of the available literature data indicates that most of the studies examined the effect of metals separately and analyzed only one or a few physiological processes in plants. However, the combined pollution with several metals occurs in the nature. Therefore, the study of their simultaneous influence is very important.

Cadmium is one of the most toxic heavy metals and has a real danger to the public health. By chemical properties, zinc is similar to cadmium, but these elements are greatly different in the biological relevance and in the toxicity level. The similarity in chemical structures results in a similar transport of zinc and cadmium in plants and may lead to a positive correlation between their accumulations [1, 2]. In contrast with this hypothesis, certain researchers found the negative correlation between the zinc and cadmium accumulations, and other ones suggested that there is no correlation between the absorption and translocation of cadmium and zinc [3]. Because of these contradictions, we would like to clarify the possible similarities or differences in the accumulation of cadmium, nickel, and zinc in wheat.

The antioxidant system has a crucial role in the plant adaptation to adverse environmental factors, such as the combined effect of heavy metals. This system is induced by a heavy-metal stress. Ascorbic acid (AsA) and glutathione play a leading role in the elimination of oxygen radicals and the termination of free radical reactions [4].

Based on the fact that the displacement of the prooxidant-antioxidant balance can act as a primary factor in the rapid response of plant cells to heavy metals, the aim of this study was to compare the combined effect of Cd, Zn, and Ni on changes in the lipid peroxidation and the contents of ascorbic acid and glutathione in wheat varieties with different heavy metal tolerances.

The heavy metal tolerances of 8 wheat (*Triticum aestivum* L.) varieties, Uzhynok, Epokha, Yuviliar, Favorytka, Antonivka, Sonechko, Misiia, and Kuial'nik, were compared at the seedling stage. Cadmium, zinc, and nickel were added in the form of sulfuric acid salts in the following concentrations: 3 and 9 mg Cd^{2+}/l; 4 and 12 mg Ni^{2+}/l; 23 and 69 mg Zn^{2+}/l. The previously surface-sterilized seeds were germinated in Petri dishes for 48 h in heavy-metal solutions. The heavy metal tolerance was estimated by the measurement of a main root length and the calculation of the root index (RI), as described by Wilkins [5]. Based on these results, Sonechko and Kuial'nik were chosen for further studies as heavy-metal sensitive and tolerant varieties, respectively. Seeds were germinated in distilled water for 5 days. The seedlings were grown for 10 days on the Hoagland nutrient medium in a 12-h photoperiod at 260 umol/m2/s, d 20/20 °C, 70/75% RH. Then the nutrient solution was supplemented with the heavy metals in the above-described concentrations for 2 days.

Cadmium, zinc, and nickel contents were measured in the whole root system. Plant tissues were dried at 80 °C and used for the determination of heavy metals according to Angelova et al. [6]. The concentration of heavy metals was determined on an atomic adsorption spectrophotometer S-115 (Ukraine) and was given on a fresh weight basis [7]. The intensity of lipid peroxidation was evaluated by the measurement of TBA-active products [8]. The ascorbic and dehydroascorbic acid contents were determined according to Kampfenkel et al. [9] with modifications by de Pinto et al. [10].

The qualitative and quantitative determinations of GSH were performed by a reverse-phase HPLC (Waters, Milford, MA, USA) connected to a fluorescence scanning detector (W474, Waters), as described earlier by Kranner and Grill and Kocsy et al. [11, 12].

The statistical analysis was done by the accepted methods of parametric statistics at the 95% confidence level. The analytical repetition was 4 times, and the repetition of each biological experiment was 3-fold.

Cadmium, zinc, and nickel in low concentrations suppressed the root development of seedlings only in the sorts Antonivka, Favorytka, and Sonechko, in which the length of roots was by 11–15% smaller compared to control (Table 1). The RI values were between 1.00–1.05 in these three sorts. These data indicate that that the sorts Antonivka, Favorytka, and Sonechko are sensitive to cadmium, nickel, and zinc compounds.

After the addition of the metals in higher concentrations, their negative effect was stronger, as shown by a greater inhibition of the root growth. After this treatment, the genotypes Uzhynok, Yuviliar, Favorytka, and Sonechko, in which the main root length decreased by 62–66% compared with control, proved to be more sensitive to metals. In contrast, the effect of heavy metals was much weaker in the sorts Epokha, Misiia, and Antonivka (decrease by 44, 52 and 50%).

Kuial'nik was the most resistant to the heavy-metal stress, since the inhibition of the root growth was not observed at low concentrations, and the root growth was reduced only by 37% at high concentrations. The tolerance of this genotype is also confirmed by the highest value of RI (0.63). The most sensitive variety was Sonechko in view of the results obtained both at lower and higher metal concentrations.

The heavy metals were taken up in a high amount even at their application in lower concentrations (Table 2). The contents of cadmium, zinc, and nickel in control roots were similar in both sorts. The intensities of nickel ion accumulation by roots tissues at low and high concentrations of metals were the same for both sorts. Thus, the endogenous Ni concentration increased 3.5–3.8-fold at low metal concentrations and 8.1–8.3-fold at high metal concentrations in comparison with control.

The two genotypes accumulated cadmium and zinc on different levels. Thus, in the resistant wheat sort Kuial'nik at low concentrations of metals, the cadmium content in roots was 4.5-fold greater than in control, while it was 7.1-fold greater in Sonechko. At the high concentrations of metals in roots, tissues of the sort Kuial'nik seedlings showed an increase in the quantity of the cadmium accumulation amounted to 7.7, and those of Sonechko — 8.7. A similar effect was observed in work [13] by Fontes et al., while studying two lettuce (*Lactuca sativa* L.) cultivars. Specifically, a significantly higher level of cadmium and zinc accumulation was observed for the cultivar CRV compared with the cultivar CMM.

An analogous trend of greater Zn absorption was observed in the sensitive sort, whose difference was greater at higher metal concentrations in a solution. At low metal concentrations, the accumulation of zinc ions by roots increased 20-fold relative to control plants in Kuial'nik, while 29-fold in the sort Sonechko. The resistant sort accumulated the twice smaller quantity of Zn compared with seedlings of the sort Sonechko. Thus, it is possible that the higher metal tolerance of the sort Kuial'nik was probably due to the more efficient functioning of barrier mechanisms leading to the limited accumulation of toxicants in tissues.

Table 1. Length of the primary roots (mm) and root indices (RI) in different sorts of wheat treated with heavy metals

Sort	Control	3 mg Cd^{2+}/l + 4 mg Ni^{2+}/l + + 23 mg Zn^{2+}/l		9 mg Cd^{2+}/l + 12 mg Ni^{2+}/l + + 69 mg Zn^{2+}/l	
	$M \pm m$	$M \pm m$	RI	$M \pm m$	RI
Uzhynok	37.1 ± 0.88	37.2 ± 0.91	1.00	$14.3 \pm 0.33^*$	0.38
Epokha	30.3 ± 0.57	31.7 ± 0.63	1.05	$17.0 \pm 0.39^*$	0.56
Yuviliar	32.9 ± 0.75	33.5 ± 0.84	1.02	$12.9 \pm 0.31^*$	0.39
Favorytka	34.8 ± 0.70	$30.9 \pm 0.65^*$	0.89	$11.9 \pm 0.30^*$	0.34
Antonivka	37.3 ± 0.56	$31.5 \pm 0.57^*$	0.85	$18.4 \pm 0.34^*$	0.49
Sonechko	39.1 ± 0.58	$33.4 \pm 0.56^*$	0.85	$13.7 \pm 0.27^*$	0.35
Misiia	38.4 ± 0.58	38.4 ± 0.56	1.00	$16.3 \pm 0.31^*$	0.42
Kuial'nik	27.4 ± 0.60	27.4 ± 0.43	1.00	$17.2 \pm 0.35^*$	0.63

*Statistically significant differences ($p < 0.05$) from control.

Table 2. Cd, Zn, and Ni contents (μg/g FW) in wheat seedling roots exposed to heavy metals for 72 h

Variant	Cd (μg/g FW)	Ni (μg/g FW)	Zn (μg/g FW)
	Kuial'nik		
Control	0.42 ± 0.01	0.47 ± 0.01	0.07 ± 0.01
3 mg Cd^{2+}/l + 4 mg Ni^{2+}/l + 23 mg Zn^{2+}/l	$1.92 \pm 0.03^*$	$1.77 \pm 0.05^*$	$1.34 \pm 0.06^*$
9 mg Cd^{2+}/l + 12 mg Ni^{2+}/l + 69 mg Zn^{2+}/l	$3.23 \pm 0.16^*$	$3.91 \pm 0.19^*$	$3.86 \pm 0.19^*$
	Sonechko		
Control	0.58 ± 0.01	0.56 ± 0.02	0.08 ± 0.01
3 mg Cd^{2+}/l + 4 mg Ni^{2+}/l + 23 mg Zn^{2+}/l	$4.15 \pm 0.13^*$	$1.99 \pm 0.09^*$	$2.48 \pm 0.10^*$
9 mg Cd^{2+}/l + 12 mg Ni^{2+}/l + 69 mg Zn^{2+}/l	$5.09 \pm 0.33^*$	$4.56 \pm 0.09^*$	$7.78 \pm 0.38^*$

*Statistically significant differences ($p < 0.05$) from control.

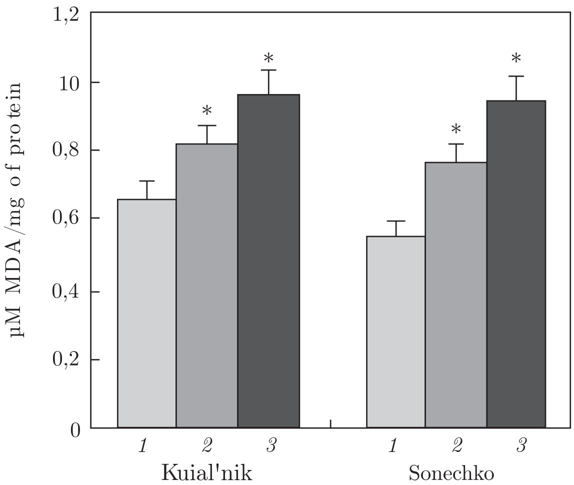

Fig. 1. The content of TBA-active products in roots of wheat seedlings. *1* — control; *2* — 3 mg Cd^{2+}/l+4 mg Ni^{2+}/l+23 mg Zn^{2+}/l; *3* — 9 mg Cd^{2+}/l+12 mg Ni^{2+}/l+69 mg Zn^{2+}/l; * — statistically significant difference relative to control at $p < 0.05$

Cadmium and zinc have similar geochemical and ecological properties, ionic structures, and electronegativities; however, they have different ionic radii (0.074 nm for Zn, and 0.097 nm for Cd). Its chemical similarities can cause the interaction in the accumulation by plant tissues. According to some researchers, the reduced cadmium accumulation due to the impact of zinc ions could be a result of the competition in the transport of these two ions [1]. In this regard, Nan and coauthors found that the addition of zinc to the growth medium inhibits the adsorption of cadmium by roots and, thus, leads to a reduction of the latter in plants [2].

It is of importance to investigate such biochemical and physiological parameters, whose changes can indicate the later appearance of stress-induced damages. It is possible that, under stressful actions, the activation of initial stages of lipid peroxidation may be associated, for example, with the release of iron ions, which can catalyze the formation of reactive oxygen species [14].

The lipid peroxidation increased in the roots of different wheat sorts to different levels, by following the treatment with cadmium, nickel, and zinc in low concentrations (Fig. 1). In the tolerant sort Kuial'nik, this increase was 25%, while it was 37% in the sensitive sort Sonechko compared to control. High concentrations of heavy metals resulted in even greater differences between the varieties. Particularly in the sensitive Sonechko, the 22-% increase in TBA-active products in the roots exceeded the change observed in Kuial'nik. In our opinion, this could be due to a considerably greater quantity of absorbed cadmium and zinc ions in Sonechko, which could lead to more damages compared to Kuial'nik. However, the more efficient functioning of the antioxidant defense in the tolerant sort Kuial'nik cannot be excluded.

Cadmium, nickel, and zinc caused the different directions of changes in the ascorbic acid content of the two varieties (Fig. 2, *a*). Thus, in the resistant sort Kuial'nik, low concentrations of metals did not change significantly the AsA content. However, the high concentration of metals resulted in a decrease in the ascorbate concentration by 20%. However, in the sensitive sort Sonechko, the opposite trend was observed (Fig. 2, *a*). Low concentrations of cadmium, nickel, and zinc increased the AsA content in the roots by 20%, and the high concentration of metals — 2.5-fold.

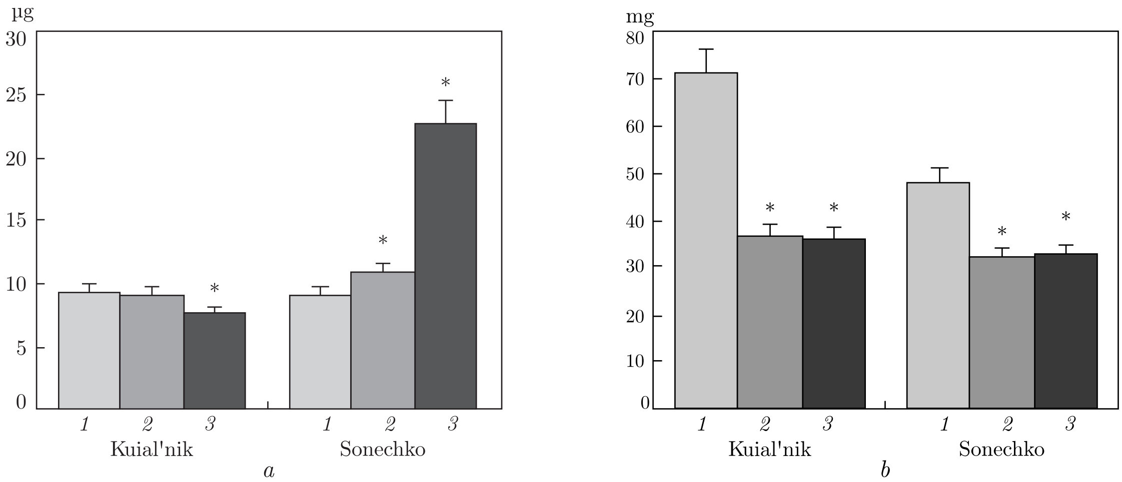

Fig. 2. The content of ascorbic acid (*a*) and glutathione (*b*) in roots of wheat seedlings. *1* — control; *2* — 3 mg Cd^{2+}/l+4 mg Ni^{2+}/l+23 mg Zn^{2+}/l; *3* — 9 mg Cd^{2+}/l+12 mg Ni^{2+}/l+69 mg Zn^{2+}/l; * — statistically significant difference relative to the control at $p < 0.05$

Interestingly, the increased quantity of ascorbate in the sort Sonechko did not cause a decrease in the lipid peroxidation, which can be explained by the reduced activity of ascorbate peroxidase.

The glutathione content was greater under control conditions in the tolerant sort Kuial'nik compared to Sonechko, which can contribute to its greater heavy metal tolerance (Fig. 2). The decrease in GSH after the heavy metal treatment can be explained by its use in the the phytochelatin synthesis, as observed in aquatic plants [15]. Phytochelatin can form complexes with heavy metals and transfer them into vacuoles, and their toxic effects are prevented in this way.

The greater heavy metal tolerance of Kuial'nik compared to Sonechko can be explained by the smaller uptake of heavy metals. In addition, its greater glutathione content under control conditions allows the formation of phytochelatins in higher concentrations, making a more efficient chelating of heavy metals possible. Consequently, the less reactive oxygen species will be formed, as indicated by the smaller lipid peroxidation.

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Роль антиоксидантів у захисті проти сумісного ефекту Cd, Zn і Ni у пшениці

Наведено результати вивчення комбінованого впливу Cd, Zn *і* Ni *на накопичення цих металів, ріст і вміст антиоксидантів у коренях пшениці. Метали додавали в двох концентраціях у поживне середовище проростків, вища концентрація справляла сильніший вплив на досліджувані показники. Вивчали толерантність 8 сортів пшениці до важких металів з метою обрати найбільш толерантний (Куяльник) і найбільш чутливий (Сонечко) для біохімічних досліджень. Поглинання важких металів та інтенсивність пероксидного окиснення ліпідів були меншими у толерантного генотипу порівняно з чутливим. Висока концентрація глутатіону у толерантного генотипу за контрольних умов може бути пов'язана з підвищеною толерантністю до важких металів. Металіндуковане зменшення вмісту глутатіону може бути індикатором підвищеного синтезу фітохелатинів. Більшу толерантність сорту Куяльник можна пояснити зниженим поглинанням важких металів і більш інтенсивним функціонуванням глутатіонзалежної ланки антиоксидантного захисту.*

Ключові слова: *Triticum aestivum* L., кадмій, нікель, цинк, пероксидне окиснення ліпідів, аскорбінова кислота, глутатіон.

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Роль антиоксидантов в защите против совместного эффекта Cd, Zn и Ni у пшеницы

Приведены результаты изучения комбинированного воздействия Cd, Zn *и* Ni *на накопление этих металлов, рост и содержание антиоксидантов в корнях пшеницы. Металлы добавляли в двух концентрациях в питательную среду проростков, высокая концентрация оказывала более сильное влияние на исследуемые показатели. Изучали толерантность 8 сортов пшеницы к тяжелым металлам с целью выбрать наиболее толерантный (Куяльник) и наиболее чувствительный (Солнышко) для биохимических исследований. Поглощение* *тяжелых металлов и интенсивность пероксидного окисления липидов были меньше у толерантного генотипа по сравнению с чувствительным. Высокая концентрация глутатиона у толерантного генотипа в контрольных условиях может быть связана с повышенной толерантностью к тяжелым металлам. Индуцированное металлами снижение содержания глутатиона может быть индикатором повышенного синтеза фитохелатинов. Большую толерантность сорта Куяльник можно объяснить пониженным поглощением тяжелых металлов и более интенсивным функционированием глутатионзависимого звена антиоксидантной защиты.*

Ключевые слова: *Triticum aestivum* L., кадмий, никель, цинк, пероксидное окисление липидов, аскорбиновая кислота, глутатион.