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The effect of heavy metals and thidiazuron on winter wheat (*Triticum aestivum* L.) seedlings

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Abstract

We studied the influence of a synthetic cytokinin-like growth regulator thidiazuron (TDZ) and ions of heavy metals (HMs) – Pb^{2+}, Cu^{2+}, Zn^{2+}, and Ni^{2+} on the growth, generation of superoxide anion, concentration of total peroxides, lipid peroxidation, and catalase activity in the leaves of 7-day-old winter wheat plants (*Triticum aestivum* L. cv. 'Mironovskaya 808'). It was found that 1 mM solution of HMs inhibited the growth of roots in the following sequence: $Zn^{2+} < Pb^{2+} < Cu^{2+} < Ni^{2+}$. HMs inhibited the growth of aboveground parts of young wheat plants, too. HMs stimulated superoxide production by a factor of 1.3–4.9. The content of total peroxides in wheat leaves increased in the presence of HMs in the growth medium. Our analyses showed that malonic dialdehyde (MDA) content in leaves increased with increasing Cu^{2+} or Ni^{2+} concentrations in the medium and hardly changed with increasing Pb^{2+} or Zn^{2+} concentrations. HMs enhanced catalase activity in wheat plants 1.1–2.8-fold at all concentrations studied. All these regularities are indications of HMs-induced oxidative stress in wheat plants. In most treatments, TDZ alleviated the HMs-induced oxidative stress and promoted an increase in Wilkinson tolerance index (WTI). This suggests that the wheat seedlings treated with TDZ were more HMs-resistant than the untreated ones.

Key words: *Triticum aestivum*, heavy metals, thidiazuron, peroxides, catalase activity, oxidative stress.

Introduction

Heavy metals (HMs) pollution is a very important problem because of increasing anthropogenic interference with the environment (Башмаков, Лукаткин, 2009). Many plant species including agricultural crops can accumulate higher amounts of heavy metals than those present in the soil. As a result HMs enter the food chain (Seregin, Kozhevnikova, 2008). Many HMs are indispensable microelements for plants, since they participate in a wide range of enzymatic redox reactions. The essential elements are a group of HMs that are needed in trace amounts for plant metabolism, growth, and development but are toxic at high concentrations (Ivanova et al., 2010). Among the metals examined here, copper, zinc and nickel belong to this group, because they combine a high biological activity and toxicity (Seregin, Kozhevnikova, 2006; Wang et al., 2009). Other metals, Pb in particular, are called nonessential elements. They are actively involved in cell metabolism but are not necessary or toxic to plants. Heavy metals are known to interact with proteins, inhibit plant growth, water regime, metabolism, etc. (Seregin, Ivanov, 2001; Dukhovskis et al., 2003; Panda et al., 2003; Duchovskis et al., 2006; Singh et al., 2007; Gajewska, Sklodowska, 2008). But the cause of all the destructive functional changes in plants under HMs stress is the alteration in balance of activated oxygen species (AOS) and antioxidants in plant cells. HMs can initiate generation of AOS such as superoxide anion (O_2^-), hydroxyl radical (OH) and hydrogen peroxide (H_2O_2) (Devi, Prasad, 2005; Pradedova et al., 2011). Oxidative processes occur in cells in normal conditions too, but the amount of AOS is held at a stable level by the antioxidative system. The last one includes low-molecular antioxidants (some amino acids, carotenoids, polyamines, glutathione, ascorbate, tocopherols, etc.) and antioxidative enzymes (mono- and dehydroaskorbate reductases, guaiacol peroxidase, glutation peroxidase, glutation reductase, ascorbate peroxidase, catalase and superoxide dismutase) (Arora et al., 2002; Panda et al., 2003; Gajewska, Sklodowska, 2008; Pradedova et al., 2011). The interrelation of prooxidants and antioxidants varies according to stressful environmental factors. It is supposed, that the shift of this interrelation triggers reactions of plant cell damage. AOS are capable of initiating reactions of proteins and lipids peroxidation (LPO). These processes invoke an increase of plasma membrane permeability, breaking of DNA, proteins as well as a whole metabolism and lead to cell destruction.

Enzymatic and non-enzymatic defence system is being actively studied in the field of action of different biotic and abiotic stress factors (Полесская, 2007). Different solutions, including biologically active substances such as plant growth regulators, are extensively used in the modern agriculture to neutralize the harmful effects of environmental stresses on plants (Lukatkin et al., 2003; Серегина, 2004; Лукаткин и др., 2005; Lukatkin et al., 2007).

It is known that biologically active substances (BAS) are capable of modifying plant response to abiotic stressors (Lukatkin et al., 2003; Лукаткин и др., 2005). Therefore it is possible to expect enhanced plant resistance to HMs by treatment of seeds and/or seedlings with plant growth regulators.

Over the last years, numerous articles have been published discussing a possibility of BAS application to modify the negative effect of HMs on cultivated plants (Серегина, 2004; Drazic, Mihailovic, 2005; Janeczko et al., 2005).

Several studies have been done on the application of cytokinin-like growth regulators as an agent raising resistance of plants to HMs. Treatment of maize seeds with kinetin resulted in relief of negative symptoms of HMs (zinc and nickel): it improved the growth of roots and shoots, decreased the leakage of electrolytes from leaf discs, and increased the activity of ascorbate peroxidase (Lukatkin et al., 2007). A preliminary treatment of cucumber (*Cucumis sativus* L., cv. 'Monastyrskii') seedlings with a synthetic growth regulator thidiazuron (TDZ) fully or partly prevented the Pb and Cu-induced stimulation of electrolyte leakage from cotyledon segments (Lukatkin et al., 2003).

We researched the physiological and biochemical parameters of young wheat plants affected by HMs in relation to thidiazuron. It has been assumed that seed treatment with TDZ will lead to a decrease in the oxidative damage induced by HMs. Thus, the aims of our study were: 1) to detect the influence of equimolar solutions of ions Zn^{2+}, Cu^{2+}, Ni^{2+} or Pb^{2+} on growth, generation of superoxide anion (O_2^-), LPO intensity and catalase activity in wheat leaves and 2) to identify the effect of TDZ on growth and oxidative events in wheat plants affected by these HMs.

Material and methods

The study was carried out in 2010–2011 at the Mordovian State University, Department of Biology.

Plant material. Commercial seeds of the winter wheat (*Triticum aestivum* L.) subspecies lutescens cv. 'Mironovskaya 808' were used in the study.

Experimental design. Wheat seeds were treated with 0.5% $KMnO_4$ for 5 min to surface sterilize and with 10 nM TDZ (3-(1,2,3-thiadiazoline-5)-1-phenylurea) for 8 h (control seeds were treated with distilled water), and then germinated in plastic pots (50 seeds per pot) in water (50 ml per pot) supplemented with 10 μM or 1 mM $Pb(NO_3)_2$, or $CuSO_4 \times 5H_2O$, or $NiSO_4 \times 7H_2O$ or $ZnSO_4 \times 7H_2O$, at temperature 22–24°C, photoperiod 16/8 h (day/night), PFD 80 μmol m^{-2} s^{-1} for 7 days. In 7-day seedlings we measured superoxide anion generation, lipid peroxidation (LPO) intensity, catalase activity, and length of axial organs.

Biometric measurments. The length of axial organs of 30 selected wheat seedlings per treatment was measured. To assess the tolerance of seedlings to HMs we applied a Wilkinson tolerance index (WTI): $I_t = (l_{me}/l_c) \times 100\%$, where l_{me} indicates the increase in root growth in a metal ion solution and l_c is the increase in root growth in the control (Koornneef et al., 1997).

Determination of superoxide anion generation. Superoxide anions in leaf disks were detected using a method that is based on the capacity of superoxide radical to oxidize adrenaline to adrenochrome (Lukatkin, 2002). 300 mg of leaves was homogenized with 15 ml of distilled water. The solution was centrifuged for 15 min at 3000 rpm. 0.1 ml of adrenaline solution (0.01%) was added to 3 ml of homogenate and incubated for 45 min at ambient temperature and 200 μM m^{-2} s^{-1}. Immediately after incubation, the optical density of the adrenochrome that formed was measured against homogenate in water on a spectrophotometer UV-mini 1240 ("Shimadzu", Japan) at $\lambda = 480$ nm. The generation of superoxide anions was calculated by adopting a molar extinction coefficient ($\varepsilon = 4020$ M^{-1} cm^{-1}) in μM g^{-1} min^{-1}.

Detection of lipid peroxidation (LPO) intensity. LPO intensity in leaf disks was detected using a method that is based on a storage of malonic dialdehyde (MDA) (Лукаткин, Голованова, 1988). 300 mg of leaves was homogenized with 10 ml of isolation medium containing 0.1 M Tris-HCl buffer, pH 7.6, with 0.35 M NaCl. 2 ml of thiobarbituric acid (TBA) in 20% trichloroacetic acid (TCA) was added to 3 ml of homogenate, heated in a boiling water bath for 30 min and filtered. The optical density of the solution was measured against isolation medium on a spectrophotometer UV-mini 1240 ("Shimadzu") at $\lambda = 532$ nm. The optical density was recorded on a spectrophotometer at 532 nm. The concentration of MDA was calculated by adopting a molar extinction coefficient ($\varepsilon = 1.56 \cdot 10^{-5}$ M^{-1} cm^{-1}) in μM g^{-1} of fresh leaf weight.

Detection of catalase activity. 1 g of leaf disks was homogenized with 10 ml of 50 mM phosphate buffer (pH 7.0). The homogenate was filtered and centrifuged for 10 min at 8000 g. 2.9 ml of phosphate buffer (pH 7.0) was added to 25 ml of enzyme extract. Directly before the measurement, 90 μl of 3% hydrogen peroxide was added to the solution. The optical density decrease during 1 min was measured on a spectrophotometer UV-mini 1240 ("Shimadzu") at $\lambda = 240$ nm. The activity of catalase was calculated by adopting a molar extinction coefficient ($\varepsilon = 39.4$ mM^{-1} cm^{-1}) in mM g^{-1} min^{-1} (Patterson et al., 1984).

Statistical analysis. All experiments were conducted in triplicate, and each experiment consisted of 150 seeds or seedlings. For all measurements averages and standard errors were calculated in *Microsoft Excel 2007*. Differences between means were assessed by the *Student's t*-test at $P = 0.05$.

Results and discussion

The influence of metal ions and thidiazuron on wheat seedling growth. In this experiment, we demonstrated the influence of the TDZ treatment on the growth parameters of wheat plants exposed to HMs ions (Table 1).

Table 1. The influence of thidiazuron (TDZ) on root and shoot length (mm) of wheat plants exposed to heavy metals (HMs) ions

Treatment	Control	Ni		Pb		Zn		Cu	
		10 μM	1 mM	10 μM	1 mM	10 μM	1 mM	10 μM	1 mM
				Roots					
Without TDZ	33 ± 2	50 ± 2	3 ± 0	42 ± 3	20 ± 1	36 ± 3	31± 3	45 ± 4	10 ± 2
10 nM TDZ	75 ± 3	41 ± 3	4 ± 0	76 ± 3	38 ± 2	73 ± 3	46 ± 2	76 ± 2	10 ± 1
				Shoots					
Without TDZ	89 ± 6	60 ± 4	21 ± 2	104 ± 6	73 ± 4	105 ± 5	89 ± 8	109 ± 3	60 ± 3
10 nM TDZ	115 ± 3	107 ± 3	25 ± 2	113 ± 3	97 ± 3	114 ± 4	114 ± 2	126 ± 2	74 ± 2

Note. The seeds were treated with TDZ or water (8 h) and then grown on solutions of HMs or water for 7 days (in all Tables).

HMs significantly influence wheat growth. As a result, root growth in the solution of 10 μM concentration of HMs was stimulated, except for Zn^{2+}. But 1 mM solution of HMs inhibited root growth. 1 mM solutions of ions Ni^{2+} and Cu^{2+} were the most toxic. The root length decreased in the following sequence: $Zn^{2+} < Pb^{2+} < Cu^{2+} < Ni^{2+}$ and the reduction from the water control was 6%, 39%, 70% and 91%, respectively.

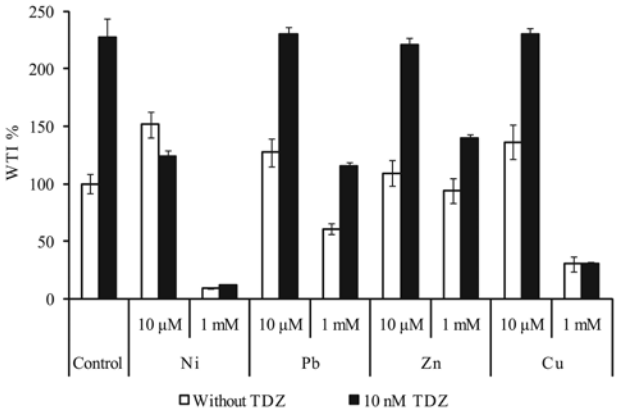
The shoot length of wheat seedlings was affected by HMs treatment too (Table 1). Like root growth, HMs ions inhibited the growth of above-ground parts of young wheat plants. The strongest inhibition of shoot growth was observed at nickel ions, the least at zinc ions. The decrease in shoot growth by 76%, 33% and 18% was observed at 1 mM of Ni^{2+}, Cu^{2+} and Pb^{2+}, respectively, as compared to the water control.

The addition of TDZ increased the length of wheat roots and shoots significantly. Root growth in water (control seedlings) was enhanced by 2.3 times. When TDZ was applied in combination with most HMs, the root length increased (with the exception of 10 μM Ni^{2+} and Cu^{2+}). A maximal increase was noted at suboptimum concentration of Zn^{2+} and at 1 mM of Pb^{2+} (203% and 190% for the treatment without TDZ, respectively).

Seed treatment with TDZ stimulated growth of above-ground parts in all experimental treatments. The treatment with TDZ increased shoot length by 109–178% and by 109–133% (for 1 mM and 10 μM of HM, respectively), as compared to HMs treatments without growth regulator. As a result, TDZ treatment led to reduced negative effect of HMs on shoot growth. But in some cases there was only partial effect of TDZ treatment, e.g., at 1 mM of Ni^{2+} and Cu^{2+} there were significant decreases in shoot length by 72% and 17%, respectively, as compared with water control.

Stifun, a natural plant growth regulator based on graminoid metabolites, modified toxic effect of Cd through changes of synthesis and transport of plant defence agents. Under cadmium stress Stifun prevented a decrease in the concentration of phytohormones (zeatin, zeatin riboside, dihydrozeatin riboside, isopentenyladenosine, abscisic acid (ABA), indoleacetic acid). Activation of plant organs growth was associated with the enhancement of cell division and elongation (Лубянов, 2009).

To assess the tolerance of seedlings to HMs we applied a WTI which indicates the ratio of root growth in HMs solution to root growth in the control. Analysis of WTI in wheat seedlings showed that this index increases at low doses of HMs, and decreases at high concentrations of HMs in the medium, except for Zn^{2+} and 10 μM Pb^{2+} (insignificant difference from water control) (Fig.). TDZ promoted an increase in WTI in most treatments, especially at 10 μM concentrations of all HMs researched and at 1 mM concentration of Zn^{2+} (as compared to water control).



Note. White columns – without TDZ (water or metal), black columns – metal + TDZ.

Figure. The effect of thidiazuron (TDZ) on Wilkinson tolerance index (WTI) in wheat seedlings treated with heavy metals

Grishko and Demura investigated the influence of growth regulators on tolerance, LPO intensity and ascorbic acid content in the seedlings of *Zea mays* hybrids 'Blitz 160 MV', 'Premia 190 MV', 'Euro 190 MV', 'Euro 401 SV' and 'Ton 320 VS' under combined stress of Cd and Ni (Гришко, Демура, 2009). Protective action of the growth regulator Emistim was found only for 'Ton 320 VS' seedlings. Zeastimulin provided a more essential decrease or even full removal of negative influence of Cd and Ni on the growth processes in 'Blitz 60 MV' and 'Euro 401 SV'. Seed treatment with Zeastimulin promoted root uptake of HMs ions. But this growth regulator significantly reduced LPO intensity (15% for Ni and twice for Cd). An increase in HMs-tolerance of plants was closely related to production of ascorbic acid as antioxidant.

The influence of metal ions and TDZ on O_2^- generation. Superoxide anion is the most readily generated AOS in plant cells (Барсукова, 1997; Башмаков, Лукаткин, 2009). It is not stable and its level in plants is continually changing. In normal environment, the O_2^- level is low, but under the influence of biotic or abiotic stressors it rises dramatically.

HMs ions stimulated superoxide production by a factor of 1.3–4.9 at all concentrations studied, but the extent of stimulation varied for different metals applied at equimolar concentrations (Table 2). The highest level of superoxide anion radical generation was noted in the

presence of Ni^{2+}, whereas the lowest level of O_2^- was observed in the presence of Zn^{2+}.

In most treatments, except for Zn^{2+}, the rate of O_2^- generation increased with HMs concentration. Generally, the rate of superoxide anion radical production increased in the following sequence: $Zn^{2+} < Pb^{2+} < Cu^{2+} < Ni^{2+}$.

Table 2. The effect of thidiazuron (TDZ) and heavy metals (HMs) ions on superoxide anion generation (μM g^{-1} min^{-1}) in wheat leaves

Treatment	Control	Cu		Ni		Pb		Zn	
		10 μM	1 mM	10 μM	1 mM	10 μM	1 mM	10 μM	1 mM
Without TDZ	2.1 ± 0.3	4.4 ± 1.4	4.7 ± 1.1	5.6 ± 0.3	10.5 ± 1.9	1.5 ± 0.5	2.7 ± 0.3	0.8 ± 0.2	1.1 ± 0.7
10 nM TDZ	3.6 ± 0.3	8.9 ± 0.8	11.3 ± 0.6	9.5 ± 0.1	10.2 ± 0.5	10.7 ± 1.0	5.3 ± 0.3	0.5 ± 0.2	5.0 ± 1.5

The addition of TDZ elevated the level of superoxide anion radical in the absence of HMs as well as in combination with most HMs. It can be noted that this growth regulator had variable effects on the O_2^- level in the presence of various HMs. The strongest effect of TDZ was observed at 1 mM of Ni^{2+} and 10 μM of Zn^{2+} (the decrease in O_2^- level by 3 and 36%, respectively, as compared to HMs treatments without growth regulator). However, in other experimental treatments TDZ increased the O_2^- levels in wheat seedlings.

The influence of HMs ions and TDZ on lipid peroxidation. It is known that AOS react with many cell components, including lipids, proteins, and nucleic acids, thus damaging their structure and functions (Mittler, 2002; Apel, Hirt, 2004). The extent of oxidative stress in cells is usually assessed from lipid peroxidation rate. During oxidation of polyunsaturated fatty acids by $^1O_2$, OH, and HO_2, lipid peroxides are formed (Mittler, 2002; Temple et al., 2005). Oxidized fatty acids are unstable and degrade in secondary reactions. Thus, after the primary generation of peroxides, further damage increases considerably since radical chain reactions are initiated (Mittler, 2002). The content of TBA-reactive substances, particularly MDA, is often used as an indicator of cell membrane degradation (Лукаткин, Голованова, 1988). In many works, an increase in MDA content was observed in response to HMs treatment (Prasad et al., 2005; Gajewska, Sklodowska, 2007). Thus, lipid peroxidation can be considered as one of the primary phytotoxic effects of HMs on the integrity of plant membranes.

Analysis of lipid peroxidation rate in wheat leaves showed that Cu and Ni strongly elevated the level of TBA-reactive substances (Table 3). The largest increase in MDA content was observed in Cu^{2+}-treated seedlings (more than 3-fold increase as compared to MDA content in leaves of water-grown plants). Pb^{2+} and Zn^{2+} ions did not change the content of TBA-reactive substances in wheat leaves. Our analyses showed that MDA content in leaves increased with concentration of Cu^{2+} and Ni^{2+} in the medium, but not with concentrations of Pb^{2+} or Zn^{2+}. As a result, HMs enhanced LPO in leaves of wheat plants in the following sequence: $Pb^{2+} < Zn^{2+} < Ni^{2+} < Cu^{2+}$. The MDA accumulation upon HMs treatment indicates the appearance of oxidative stress in leaf cells and the disturbance of pro-/antioxidant balance (Garmash, Golovko, 2009).

Table 3. The effect of thidiazuron (TDZ) on malonic dialdehyde (MDA) content (μM g^{-1}) in the leaves of wheat seedlings treated with heavy metals (HMs)

Treatment	Control	Cu		Ni		Pb		Zn	
		10 μM	1 mM	10 μM	1 mM	10 μM	1mM	10 μM	1 mM
Without TDZ	15.9 ± 1.2	28.8 ± 1.2	49.7 ± 11.0	21.7 ± 5.5	27.0 ± 4.1	14.5 ± 1.5	15.3 ± 2.5	15.9 ± 2.8	17.2 ± 1.5
10 nM TDZ	14.8 ± 1.9	11.6 ± 1.4	36.0 ± 2.5	31.5 ± 12.0	14.8 ± 1.9	12.2 ± 1.0	14.3 ± 2.4	22.0 ± 3.4	13.5 ± 0.5

TDZ insignificantly decreased the lipid peroxidation rate in the leaves of water-grown seedlings but significantly decreased MDA content in seedlings treated with HMs (except for 10 μM Ni^{2+} and Zn^{2+}). The largest positive effect of TDZ treatments was noted in the treatments with $Cu^{2+}$and high dose of Ni^{2+} (decrease in MDA level by 40–72%). In plants grown on Pb^{2+}-containing media, the effect of TDZ treatment on lipid peroxidation in leaves was weak or statistically insignificant.

So, TDZ significantly decreased the lipid peroxidation rate in seedlings treated with most of the HMs concentrations except for 10 μM concentrations of nickel and zinc.

The influence of HMs ions and TDZ on catalase activity. Plants are able to regulate AOS level and avoid oxidative damage owing to a well-developed complex of antioxidants, consisting of low-molecular-weight compounds and oxidative scavenging enzymes. The antioxidants prevent accumulation of AOS in amounts toxic to plant cells, thus decreasing the risk of oxidative stress (Pitzschke et al., 2006). Stressed cells subjected to HMs maintain homeostasis through activation of superoxide dismutase, which serves as the first line of defence, converting the O_2^- into H_2O_2. Hydrogen peroxide is decomposed to water by oxidative scavenging enzymes like catalase and various peroxidases (Liu et al., 2008).

The activity of catalase is one of the most important indicators of oxidative status and oxidative stress in plants. Therefore, we determined the TDZ influence not only on the rate of superoxide generation, but also on the activity of antioxidant enzyme catalase (Table 4).

HMs ions raised catalase activity in wheat plants by a factor of 1.1–2.8 at all concentrations studied. The maximum catalase activity was noted in the presence of 1 mM Ni^{2+}, whereas the lowest one was observed at 10 μM Ni^{2+}. In most treatments, except for Cu^{2+}, the activity of catalase increased with HMs concentration increase in the medium.

Table 4. The influence of thidiazuron (TDZ) on catalase activity (mM g^{-1} min^{-1}) in wheat plants treated with heavy metals (HMs) ions

Treatment	Control	Ni		Pb		Zn		Cu	
		10 μM	1 mM	10 μM	1 mM	10 μM	1 mM	10 μM	1 mM
Without TDZ	0.39 ± 0.01	0.44 ± 0.06	1.10 ± 0.03	0.51 ± 0.04	0.73 ± 0.03	0.57 ± 0.10	0.89 ± 0.01	0.76 ± 0.06	0.75 ± 0.02
10 nM TDZ	0.54 ± 0.02	0.94 ± 0.06	1.63 ± 0.01	0.90 ± 0.15	1.02 ± 0.05	1.07 ± 0.13	0.93 ± 0.09	1.41 ± 0.16	1.04 ± 0.06

The addition of TDZ elevated catalase activity in the absence of HMs as well as in combination of TDZ with all HMs researched. The increase of catalase activity can be a defence mechanism of plant cells to oxidative stress induced by HMs ions.

Conclusions

1. Thidiazuron (TDZ) significantly enhanced seedling growth indices, decreased the lipid peroxidation rate in the seedlings treated with heavy metals (HMs), except for 10 μM of Ni and Zn, and elevated catalase activity when applied in combination with all HMs. However, TDZ treatment did not reduce the elevated O_2^- generation induced by HMs. So, in most treatments, TDZ alleviated oxidative stress caused by HMs.

2. TDZ promoted the increase in Wilkinson tolerance index (WTI) in most treatments. This indicates that the wheat seedlings treated with TDZ developed more resistance to HMs than the untreated ones.

3. The protective properties of TDZ increased in the sequence $Ni^{2+} > Zn^{2+} \geq Cu^{2+} > Pb^{2+}$. Synthetic cytokinin-like plant growth regulator TDZ almost completely eliminated the negative impact of Cu^{2+} and Pb^{2+}.

Considering the protective effects of TDZ, this growth regulator can be effectively applied to improve condition of plants growing in the media containing HMs. The mechanisms underlying the protective action of TDZ, especially in the presence of HMs, require further investigation.

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Sunkiųjų metalų ir tidiazurono poveikis žieminių kviečių daigams

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Santrauka

Tirta sintetinio augimo reguliatoriaus citokinino (thidiazuron, TDZ) ir sunkiųjų metalų (SM) jonų – Pb^{2+}, Cu^{2+}, Zn^{2+} bei Ni^{2+} – įtaka 7 dienų žieminio kviečio (*Triticum aestivum* L.) veislės 'Mironovskaya 808' augimui, superoksido anijonų gamybai, bendrai peroksidų koncentracijai, lipidų peroksidacijai ir katalazės aktyvumui. Nustatyta, kad 1 mM SM tirpalas šaknų augimą slopino taip: $Zn^{2+} < Pb^{2+} < Cu^{2+} < Ni^{2+}$. SM taip pat slopino jaunų augalų antžeminės dalies augimą ir didino superoksido anijonų kiekį. Augimo terpėje esant sunkiųjų metalų, bendras peroksidų kiekis padidėjo. Malono dialdehido (MDA) kiekis lapuose padidėjo, terpėje didėjant Cu^{2+} arba Ni^{2+} koncentracijai, tačiau liko beveik nepakitęs, didėjant Pb^{2+} arba Zn^{2+} koncentracijai. Visos tirtos SM koncentracijos katalazės aktyvumą žieminiuose kviečiuose padidino 1,1–2,8 karto. Šie dėsningumai rodo SM sukelto augalų oksidacinio streso būklę. Daugeliu atvejų TDZ mažino sunkiųjų metalų sukeltą oksidacinį stresą, taip pat skatino Wilkinsono tolerancijos indekso (WTI) padidėjimą. Tai rodo, kad sunkiųjų metalų poveikiui TDZ apdoroti žieminių kviečių daigai buvo atsparesni nei neapdoroti.

Reikšminiai žodžiai: *Triticum aestivum*, sunkieji metalai, tidiazuronas, peroksidai, katalazės aktyvumas, oksidacinis stresas.