

ISSN 1392-3196 / e-ISSN 2335-8947

Zemdirbyste-Agriculture, vol. 108, No. 2 (2021), p. 125–132

DOI 10.13080/z-a.2021.108.016

The efficiency of pre-treatment of maize seeds with plant growth regulators for resistance of maize seedlings to zinc ions

Dmitry I. BASHMAKOV¹, Alexandr S. LUKATKIN¹, Jurga MILIAUSKIENĖ²,
Laisvūnė DUCHOVSKIENĖ², Pavelas DUCHOVSKIS²

¹National Research Mordovia State University, Department of Botany, Physiology and Ecology of Plants
Bolshevistskaja 68, Saransk 430005, Russia
E-mail: aslukatkin@yandex.ru

²Lithuanian Research Centre for Agriculture and Forestry
Kauno 30, Babtai, Kaunas distr., Lithuania

Abstract

In this research, the effects of maize seed pre-treatment with synthetic (Thidiazuron, Cytodef, Kinetin and Epin-extra) and natural (Ribav-extra) plant growth regulators (PGRs) on axial growth of seedlings of maize (*Zea mays* L.) cultivar 'Tzaritza' exposed to zinc ions (Zn^{2+}) were investigated. Also, the influence of applied PGRs on Zn^{2+} allocation in maize root tissues was studied. The seeds of maize were treated with various PGRs and grown for 7 days on 1 μ M, 10 μ M, 0.1 mM and 1 mM Zn^{2+} concentrations. After that the growth of shoot and root, and generation of catalase in maize leaves were recorded. In order to detect the effect of PGRs on Zn^{2+} allocation in maize root tissues, the seeds were treated with solutions containing different PGRs then 5-day old seedlings were treated with 1 mM Zn^{2+} for 3 days. The transverse root slices were stained with dithizone and observed under a microscope. It was found that pre-treatment of maize seeds with solutions containing different PGRs modified the allocation of Zn^{2+} in root tissues: Thidiazuron enhanced, while Epin-extra impaired the absorption of Zn^{2+} in root tissues. The effect of pre-sowing treatment of seeds with each PGR on seedling growth depended on the concentration of Zn^{2+} in the growth medium. Pre-sowing treatment of seeds with Epin-extra and Ribav-extra was the most effective under higher (0.1 and 1 mM) Zn^{2+} concentration by inducing both axial growth and the activity of catalase in maize, whereas pre-sowing treatment of seeds with Kinetin was effective for maize root growth under all applied Zn^{2+} concentrations.

The research data suggests that the maize plants, whose seeds had been treated with PGRs, were more Zn^{2+} -resistant than the untreated ones.

Key words: axial organs, catalase, growth, pre-treatment, root, *Zea mays*.

Introduction

Heavy metal pollution is a very important problem because of the increasing anthropogenic interference on the environment (Turhan et al., 2020). Many plant species, including agricultural crops, are able to accumulate larger amounts of heavy metals, which results in their accumulation in the food chain (Ali et al., 2019). Many heavy metals are indispensable microelements for plants, since they participate in a wide range of enzymatic redox reactions. Zinc ions (Zn^{2+}) are essential elements for plants and are actively involved in cell metabolism. Violation of intracellular Zn^{2+} homeostasis leads to serious changes in metabolic processes, disturbs physiological functions in plants. Zn^{2+} is associated with more than 2300 proteins in 181 gene families, including all six classes of enzymes. These proteins include many transcription factors, Zn-finger

proteins, oxidoreductases and hydrolytic enzymes such as protein kinases, metalloproteases and phosphatases (Broadley et al., 2007). Zn^{2+} provides proteins' structural stability, regulates enzyme transcription and translation; it also activates or participates in the activation of the antioxidant enzymes (White, 2012). Moreover, Zn^{2+} is involved in abiotic stress signalling (Davletova et al., 2005). Both excess and deficiency of Zn^{2+} can initiate generation of reactive oxygen species (ROS). In plants, enzymatic and non-enzymatic defence system due to response to Zn^{2+} is being actively studied (Vuletić et al., 2014). As well as other micronutrients, Zn^{2+} can lead to physiological and biochemical disturbances in plants and limit their growth at excessive concentrations (Glińska et al., 2016).

High concentrations of Zn^{2+} in soils usually arise from various industrial and agricultural sources

Please use the following format when citing the article:

Bashmakov D. I., Lukatkin A. S., Miliauskienė J., Duchovskienė L., Duchovskis P. 2021. The efficiency of pre-treatment of maize seeds with plant growth regulators for resistance of maize seedlings to zinc ions. Zemdirbyste-Agriculture, 108 (2): 125–132. DOI 10.13080/z-a.2021.108.016

of pollution. Global Zn mine production exceeds 13.5 million tons year⁻¹, industrial Zn²⁺ emissions exceed natural levels by almost 40 times (1.62 million tons year⁻¹), resulting in an increase in Zn²⁺ emission to the environment. Increased anthropogenic emissions to the environment make this metal one of the most dangerous pollutants (Wei et al., 2020). Therefore, in many regions of the world fodder and food plants are grown under deficiency or excess of Zn²⁺ in soils. Symptoms of Zn²⁺ toxicity in plants usually occur, when Zn²⁺ concentration in the leaves exceeds 300 mg kg⁻¹ dry weight, although the toxicity thresholds can be highly variable even within the same species (Broadley et al., 2007).

Nowadays there are known several methods how to improve deleterious environment. Various solutions, including biologically active substances such as plant growth regulators (PGRs), are extensively used in modern agriculture to neutralize the harmful effects of environmental stresses on plants (Gruznova et al., 2018; Yan et al., 2020). Biologically active substances are able to modify the response of plant to abiotic stressors. There are numerous studies investigating the modification of heavy metals absorption using biologically active substances in order to improve phytoremediation of contaminated soils (Chen et al., 2020). With these objectives, EDTA and other chelating agents (Chen et al., 2019), plant hormones (Sun et al., 2020), natural regulators in rhizobacteria (Wang et al., 2014) or synthetic PGRs (Gruznova et al., 2017) were applied. Therefore, it is possible to expect enhanced plant resistance to heavy metals by treatment of seeds and/or seedlings with PGRs.

In recent years, numerous articles have been published discussing the possibility of using biologically active substances to modify the adverse effects of heavy metals on cultivated plants (Asgher et al., 2015; Sytar et al., 2019). There are several studies involving cytokinin-like PGRs (Bashmakov et al., 2012; Sazanova et al., 2012), melatonin (Kholodova et al., 2018), jasmonic (Bali et al., 2018) or salicylic (Ahmad et al., 2018; Sharma et al., 2020) acids as agents that increase plant resistance to heavy metals.

Maize (*Zeamays* L.) is one of the oldest cultivated plants in the world and ranks second after wheat and rice. Also, it is one of the most popular foods in the world. In industry, maize is used to produce synthetic fibres, the production of nylon, plastics and other synthetic substances, pharmaceuticals, glue, paper, agents for leather and material dressing, paints, substitutes for soap and rubber, fertilizers, furfural, etc. Maize plants are often exposed to adverse environmental conditions and,

to reduce the harmful effects of the external environment, the plants are treated with various PGRs. These effects were revealed in maize plants affected by drought (Talaat et al., 2015), waterlogging (Ren et al., 2019), chilling (Yan et al., 2020), etc. In addition to other unfavourable factors affecting the cultivation of maize plants, soil contamination with heavy metals often has deleterious effect (Jain et al., 2020; Wei et al., 2020). In this regard, treatment of seeds or plants with PGRs can reduce the toxic effects of heavy metals on maize plants. Jain et al. (2020) have documented the positive effects of PGRs on maize plants exposed to excessive concentration of Zn²⁺ in the soil. However, a comprehensive evaluation of the efficacy of PGRs against Zn²⁺ and an assessment of the mechanisms involved in its detoxification in maize plants have not been performed previously.

This study aimed to evaluate the significance of synthetic and natural plant growth regulators on the growth of maize plants under Zn²⁺ exposure. Hence, this research investigated the efficacy of pre-sowing seed treatment with plant growth regulators on Zn²⁺ allocation in root tissues.

Materials and methods

The experiment was conducted in 2019 at the Laboratory of Cytophysiology and Cell Engineering, Department of Plants Botany, Physiology and Ecology, Mordovia State University in Saransk, Russia and in the Laboratory of Plant Physiology, Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry, Babtai, Lithuania.

Experimental design. Maize (*Zea mays* L.) cultivar ‘Tzaritza’ was used for this experiment. It is a middle-late cultivar, 190–210 cm in height, inferior corn cob height 70 cm, 1000 grains mass 180–200 g, and crop capacity 17 t ha⁻¹. Maize seeds were treated with 0.5% KMnO₄ for 5 min to sterilize the surface, thereafter with solutions, containing PGRs for 4–8 h (the optimum treatment time and concentration of PGR were matched in preliminary experiments) (Table). In control treatment, seeds were rinsed with distilled water. Seeds germinated and grew in plastic pots (50 seeds per pot) in water (50 ml per pot), supplemented with ZnSO₄ × 7H₂O (from 1 μM to 1 mM), at temperature 22–24°C, photoperiod 16/8 h (day/night), photosynthetic photon flux density 80 μmol m⁻² s⁻¹ for 7 days. On the 7th day, the length of axial organs of seedlings and catalase (CAT) activity in leaves were measured.

Table. Plant growth regulators used for maize

Biologically active substances	Chemical formula	Effective concentration	Treatment time
Synthetic plant growth regulators			
Thidiazuron (TDZ)	<i>N</i> -phenyl- <i>N'</i> -1,2,3-thiadiazol-5-ylurea	10 nM	8 h
Cytodef (CTD)	<i>N</i> -(1,2,4-triazol-4-yl)- <i>N'</i> -phenylurea	0.1 μM	8 h
Kinetin	<i>N</i> -(2-furanylmethyl)-1H-purin-6-amine	1 μM	6 h
Epin-extra (24-EB)	(1S,2R,4R,5S,7S,11S,12S,15R,16S)-15-[(2S,3R,4R,5R)-3,4-dihydroxy-5,6-dimethylheptan-2-yl]-4,5-dihydroxy-2,16-dimethyl-9-oxatetracyclo[9.7.0.02,7.012,16]octadecan-8-one	1 μM	6 h
Natural plant growth regulator			
Ribav-extra	metabolic product of ginseng roots mycorrhizal fungus	10 ppm	4 h

Histochemistry. To prepare the dithizone (diphenylthiocarbazone) solution, just prior to slicing, 3 mg of dithizone was dissolved in 6 ml of acetone adding 2 ml of the distilled water and 0.2 ml of ice-cold acetic acid (Seregin, Ivanov, 1997). Cross-slices of maize roots in different zones were prepared manually using a razor blade. Cross-slices were maintained in dithizone solution, then rinsed well in distilled water and observed under the microscope LUMAM R8 (LOMO, Russia) at $\times 300$ magnification. Some slices were photographed with a digital camera connected to the microscope. Localization of Zn ions (Zn^{2+}) was identified by red-coloured tissues. The stronger colour intensity shows the more intense accumulation of Zn. Sensitivity of the method is 10 μM of Zn^{2+} .

Detection of catalase (CAT) activity. Leaf disks (1 g) were homogenized in 10 ml of 50 mM phosphate buffer (pH 7.0). The homogenate was filtered and centrifuged for 10 min at 8000 g, and then 25 μl of enzyme extract was added to 2.9 ml of phosphate buffer (pH 7.0). Shortly before the measurement, 90 μl of 3% hydrogen peroxide was added to the solution. The optical density decrease during 1 min was measured at $\lambda = 240$ nm using a spectrophotometer UV-mini 1240 (Shimadzu, Japan). The activity of CAT was calculated by adopting a molar extinction coefficient ($\epsilon = 39.4 \text{ mM}^{-1} \text{ cm}^{-1}$) in $\mu M \text{ g}^{-1} \text{ min}^{-1}$ (Lukatkin, 2002).

Effectiveness of plant growth regulators (PGRs). To find out the most efficient PGR, it was applied the previously developed universal index of effectiveness (Gruznova et al., 2017). Index of effectiveness (IE) was calculated employing the formula:

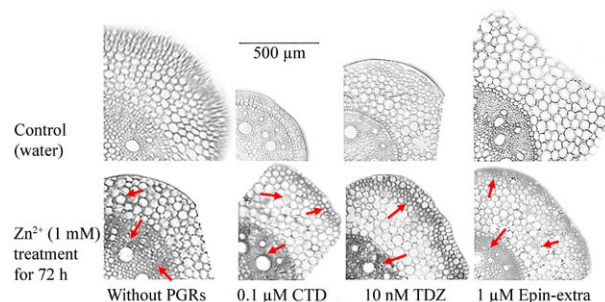
$$IE = (\pm |P1 - 100| \pm |P2 - 100| \pm \dots \pm |Pn - 100|) / n,$$

where $P1$, $P2$, etc., are the measured parameters (% relative to the untreated plants, which was taken as 100%); n is the amount of parameters considered in the IE calculation. We took the difference with the sign “+” if the effect of the PGR was positive, and the sign “-” if the last one was negative. To indicate the efficiency of the PGRs, the following gradation was used: 1) $0 < IE < 20$ – very low (vl), 2) $21 < IE < 40$ – low (l), 3) $41 < IE < 60$ – moderate (m), 4) $61 < IE < 80$ – above moderate (am), 5) $81 < IE < 100$ – high (h), 6) $IE > 100$ – very high (vh).

Statistical analysis. All experiments were conducted in triplicate, and each experiment consisted of 150 seeds or seedlings. All biochemical measurements had three analytical replications. For all measurements, the averages and standard errors were calculated in the *MS Excel* (Microsoft Inc., USA). Differences between means were assessed by the Duncan’s multiple range test or Student’s *t*-test at $P \leq 0.05$ using the *MS Excel* and software *Statistica*, version 12 (StatSoft Inc., USA).

Results

Allocation of Zn^{2+} in root tissues. In this experiment, the effects of plant growth regulators (PGRs) on the Zn^{2+} distribution in root tissues of maize seedlings exposed to very high (1 mM) Zn^{2+} concentration for 72 h were observed. After 3 days exposure to 1 mM Zn^{2+} , Zn ions deposited into the mesoderm and endoderm (both cell wall and protoplasm). Also, the cells of pericycle, phloem, xylem and stele parenchyma coloured in red indicating the presence of significant amounts of Zn^{2+} (Figure 1).



Note. By red arrows are indicated Zn-accumulating cells and tissues; CTD – Cytodef, TDZ – Thidiazuron.

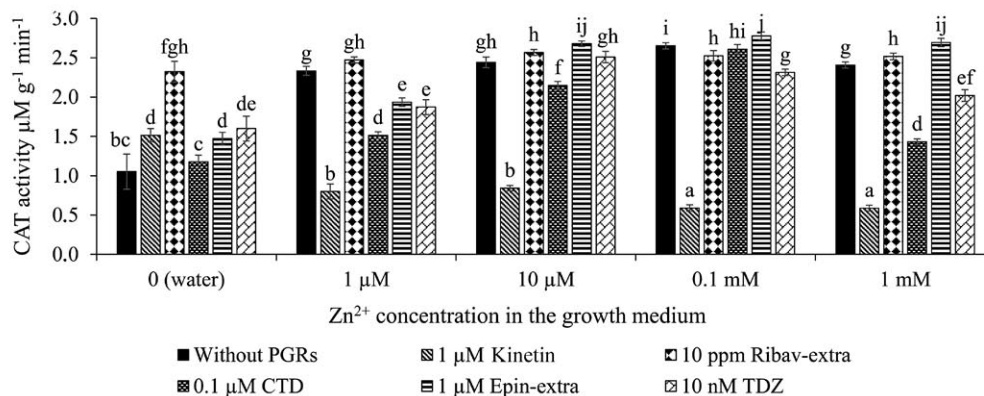
Figure 1. Allocation of zinc ions (Zn^{2+}) in maize root tissues in relation to plant growth regulators (PGRs)

Pre-treatment of maize seeds with solutions containing various PGRs caused significant modifications in the allocation of Zn^{2+} in root tissues. In the plants whose seeds had been treated with 0.1 μM CTD, Zn^{2+} deposited into the 1–2 layers of ectoderm and in some cells of mesoderm (into cell wall as a rule and rare in protoplasm) (Figure 1). Moreover, a certain amount of Zn^{2+} was observed in the parenchyma cells of the stele. It was observed by changing colour of the conducting bundles that indicated the accumulation of Zn^{2+} and its long-distance transport through the plant. In plants whose seeds had been treated with 10 nM TDZ, the colouring of slices was much more intense compared to the control plants, except for mezo- and endoderm cells. Abundance of Zn^{2+} was found in the cells of stele and pericycle. In root cortex, Zn^{2+} accumulated within 3–4 layers of exoderm cells and cells of rhizodermis. The strong colouring of cell walls and the whole protoplasm were detected. Finally, the pre-treatment of maize seeds with 1 μM Epin-extra caused strong accumulation of Zn^{2+} in cells of pericycle, stele parenchyma and 2–3 layers of ectoderm cells (Figure 1). Cells of stele parenchyma accumulated rather less Zn^{2+} compared to the cells in slices of untreated plants.

Effects of Zn^{2+} and PGRs on catalase (CAT) activity. CAT activity increased, when Zn^{2+} was added to the growth medium of cultivated maize seedlings (Figure 2). As compared to Zn^{2+} untreated plants, Zn^{2+} induced CAT activity in maize leaves by a factor of 2.2–2.5 under all applied Zn^{2+} concentrations. The highest CAT activity was detected at 0.1 mM Zn^{2+} . However, the activity of CAT changed insignificantly as Zn^{2+} concentration increased in the growth medium.

The effects of synthetic and natural PGRs on CAT activity in leaves of maize seedlings exposed to various concentrations of Zn^{2+} were assessed. Almost all analysed PGRs changed the activity of CAT in maize seedlings (Figure 2).

The effect of each PGR depended on the concentration of Zn^{2+} in the growth medium. Against weak (10 μM) Zn^{2+} concentration, in maize plants whose seeds were pre-treated with Ribav-extra, Epin-extra or TDZ there was detected the positive effect (from 2.8% to 9.7%) on CAT activity compared to plants non-pre-treated with PGRs. Against 0.1 mM Zn^{2+} , the most effective (4.8%) for CAT activity in maize plants was the seed pre-treatment with Epin-extra. Finally, against the highest (1 mM) Zn^{2+} concentration, the most effective was the pre-treatment of seeds with Epin-extra or Ribav-extra, as the CAT activity in maize plants was significantly higher (11.9% and 4.6%,



Note. Different letters indicate differences between means assessed by Duncan's multiple range test at $P \leq 0.05$; CTD – Cytodef, TDZ – Thidiazuron.

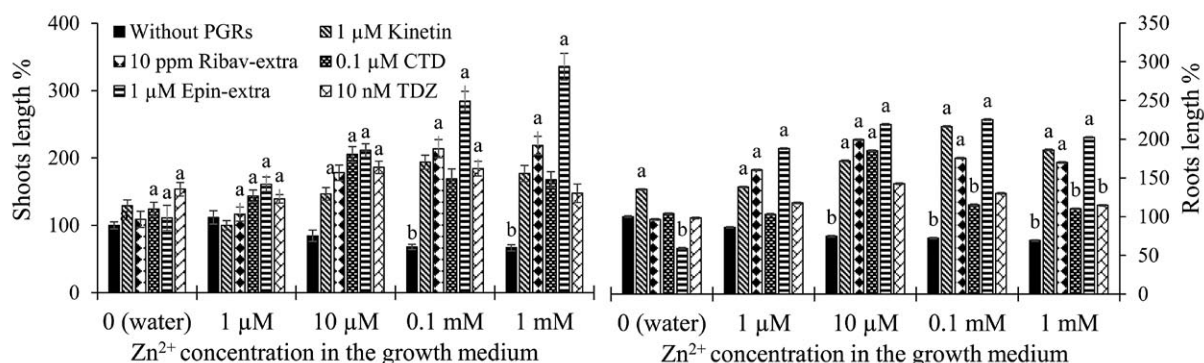
Figure 2. The effects of different concentrations of zinc ions (Zn^{2+}) on catalase (CAT) activity in maize leaves in relation to plant growth regulators (PGRs)

respectively) in comparison to non-pre-treated plants. Kinetin had no positive effect on CAT activity at any Zn^{2+} concentration used in the growth medium.

Effects of Zn^{2+} and PGRs on the growth of axial organs. The effects of seed pre-treatment with PGRs on the growth of maize seedlings exposed to Zn^{2+} were evaluated by root and shoot growth rate of pre-treated and non-pre-treated plants (Figure 3).

The pre-treatment of seeds with PGRs modified the elongation of shoots and roots of maize seedlings.

Depending on Zn^{2+} concentration, Kinetin stimulated the growth of roots (but not shoots) by 1.4–2.2 times. Ribav-extra enhanced root and shoot growth by 1.6–1.8 and 1.2–2.2 times, respectively. The CTD stimulated shoot growth by 1.4–2.1 times at Zn^{2+} concentration range from 1 to 10 μM , and root growth by 1.9 times against 10 μM Zn^{2+} only. In all cases, Epin-extra accelerated shoot growth by 1.6–3.4 and root growth by 1.9–2.3 times. TDZ significantly stimulated only shoot growth by 1.4–1.9 times at Zn^{2+} from 1 to 100 μM .



Root/shoot length significantly: a – higher than control at $P \leq 0.05$, b – lower than control at $P \leq 0.05$; CTD – Cytodef, TDZ – Thidiazuron

Figure 3. The effects of different concentrations of zinc ions (Zn^{2+}) on the growth of maize seedlings in relation to plant growth regulators (PGRs)

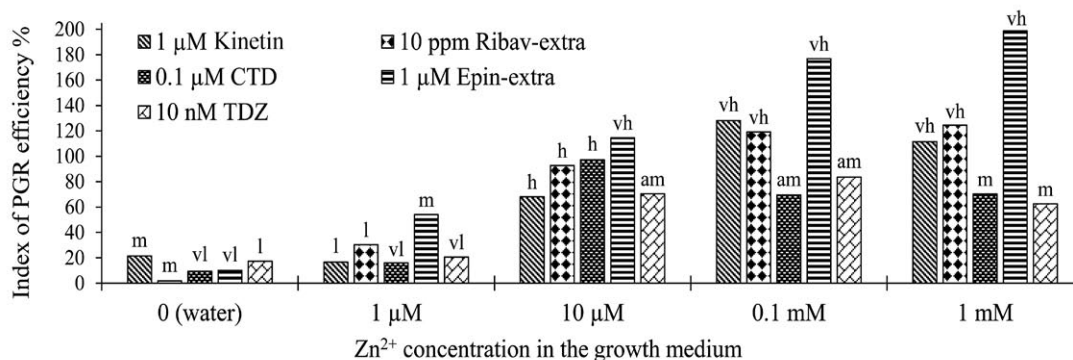
The efficiency of all PGRs tested was low and very low (1 μM) against the Zn^{2+} deficiency in the medium (Figure 4). At physiological (10 μM) Zn^{2+} concentration, CTD, Ribav-extra and Epin-extra showed high and very high efficiency; at moderate (0.1 mM) or high (1 mM) Zn^{2+} concentrations, Kinetin, Ribav-extra and Epin-extra showed very high efficiency.

Discussion

The metal-specific mechanisms of heavy metal deficiency or excess on plant growth characteristics have been investigated in several studies. In general, for plant growth 0.5 to 5 μM Zn^{2+} is necessary (Asadi et al., 2012). The lack of Zn^{2+} inhibits the shoot growth of wheat (Kaznina, Titov, 2017) and rice (Asadi et al., 2012), while excess of

it can inhibit the plant growth and development (Glińska et al., 2016; Kaznina, Titov, 2017), biomass accumulation (Zhao, Wu, 2017), induce aging, oxidative damage and other phytotoxicity symptoms (Asati et al., 2016), disturb many metabolic functions (Cherif et al., 2011).

In our experiment, Zn^{2+} inhibited the growth of axial organs of maize seedlings by increasing Zn^{2+} concentration in the growth medium (Figure 3). This effect was more pronounced in the roots than in the shoots and occurred in the roots at a much lower Zn^{2+} concentration in the growth medium. Inhibition of plant growth is explained by the negative effect of Zn^{2+} on cell division and elongation (cell division is blocked due to cytokinesis disorder), impaired water metabolism, mineral nutrition and photosynthesis in plants as well as over-accumulation of Zn^{2+} in the root cells and tissues and



Note. Different letters indicate efficiency of the PGRs: vl – very low ($0 < IE < 20$), l – low ($21 < IE < 40$), m – moderate ($41 < IE < 60$), am – above moderate ($61 < IE < 80$), h – high ($81 < IE < 100$), vh – very high ($IE > 100$); CTD – Cytodef, TDZ – Thidiazuron.

Figure 4. The efficiency of seed pre-treatment with plant growth regulators (PGRs) against zinc ions (Zn^{2+}) negative effect on the maize seedlings

altered hormonal balance (Asadi et al., 2012; Kaznina, Titov, 2017). Differences in the growth inhibition strength of roots and shoots are related to the barrier function of root tissues, which prevents heavy metals translocation to shoots (Asadi et al., 2012).

The obtained results show that the distribution of Zn^{2+} in root tissues and root cell was relatively uniform (Figure 1). Zn^{2+} was found in all root tissues and was particularly intensively deposited in the mesoderm and endoderm and in the stele parenchyma. Xylem colour saturation indicates significant translocation of metal to overground organs. Similar data were obtained in other studies (Bashmakov et al., 2015), adding that the transition of heavy metals to xylem sap is strongly determined by the pool of free L-histidine (Seregin et al., 2019).

Since maize plants survived under the chronic exposure to sublethal (1 mM) Zn^{2+} concentration, it was supposed that plants have a strong mechanism to reduce the toxicity of this metal. ROS generation is considered to be a major factor in initiating a negative plant response to high levels of essential and non-essential heavy metals (Anjum et al., 2015 a). Non-scavenged ROS inhibits cellular metabolism (Anjum et al., 2012) and disrupts cell redox homeostasis, oxidizes lipids and proteins (Anjum et al., 2015 b). Plant exposure to redox-inactive heavy metals, e.g., Zn^{2+} , can also cause oxidative stress through indirect mechanisms, e.g., disruption of the electron transport chain, induction of lipid peroxidation due to heavy metal-induced increases in lipoxygenase activity or interactions with the antioxidant defence system (Sunitha et al., 2013). Usually, plants counteract ROS using an antioxidant scavenging system, including antioxidant enzymes: CAT, superoxide dismutase (SOD), ascorbate peroxidase, glutathione reductase, etc., and non-enzymatic antioxidants: ascorbate acid, glutathione, tocopherol, carotenoids, flavonoids, etc. (Anjum et al., 2012). CAT is an inducible enzyme, and its activity is a good indicator of plant resistance to heavy metals. In current experiment, any concentration of Zn^{2+} induced a multiple increase in enzyme activity, particularly pronounced at 0.1 mM Zn^{2+} , indicating a successful resistance of plants' antioxidant system to oxidative stress caused by both deficiency (1 µM) and excess (1 mM) of Zn^{2+} in the growth medium (Figure 2).

Exogenous PGRs increase the activity of stress-reducing enzymes (CAT and SOD) leading to a reduction

in MDA (malondialdehyde) concentration (Sharma et al., 2020; Sun et al., 2020). Also, PGRs improve photosynthesis by increasing Rubisco and carbonic anhydrase activity. They have the property of minimizing oxidative stress by reducing free radical production and maintaining free radical scavenging enzymes and reduced glutathione concentration (Ahmad et al., 2018). PGRs enhance the concentrations of anthocyanins, proline and total phenols (Kholodova et al., 2018). So PGRs improve the growth and productivity of plants affected by various heavy metals, including Zn^{2+} (Qiao et al., 2019). In our experiment, all PGRs (except Kinetin) applied to maize seeds increased CAT activity in maize leaves indicating stimulation of plant antioxidant system activity (Figure 2).

Results of the experiment showed that pre-treatment of seeds with Epin-extra and Ribav-extra had the highest positive effect against physiological (10 µM), increased (0.1 mM) or sublethal (1 mM) Zn^{2+} concentrations. Scientific literature describes various mechanisms to explain the protective effect of PGRs such as: (1) their effect on cellular regulatory mechanisms at the genetic and metabolic levels, (2) the phytohormonal effect of PGRs by enhancing or disrupting regulatory functions of phytohormones, (3) synthetic PGRs influence changes in endogenous levels of natural plant hormones, which allows to shift plant growth and development, and (4) the presence of phytohormones or PGRs signalling pathways is triggered by a metal, that initiates the expression of genes encoding protective proteins that regulate the heavy metal uptake, translocation or binding in cell compartments (Bücker-Neto et al., 2017) and others.

In our experiment, it was found that CTD and Epin-extra slowed radial Zn^{2+} transport in maize root tissues as well as Epin-extra inhibited metal xylem translocation, and TDZ increased metal accumulation in root tissues (Figure 1). In addition, in plants pre-treated with any PGR, Zn^{2+} was bound in the nodules that are visible on the cell surface (cell walls) of cortex, especially endoderms and mesoderms. All these changes led to modifications in plant growth parameters. Thus, in most of the cases studied, PGRs stimulated the elongation of the axial organs of pre-treated plants. The pre-treatment of seeds with Epin-extra mostly stimulated the growth of seedlings exposed to the weakest (1 µM) Zn^{2+} concentration. Against 10 µM Zn^{2+} , the pre-treatment

of seeds with Epin-extra, Ribav-extra and CTD was the most effective for the growth of shoots and roots of maize plants. With increasing Zn^{2+} concentrations up to 0.1 or 1 mM in the growth medium, the most effective PGRs for seed pre-treatment were Epin-extra and Ribav-extra for shoot growth and Epin-extra and Kinetin for root growth. Thus, the pre-treatment of maize seeds with different PGRs had ambiguous effects on the plants. Maize seedlings exposed to various concentrations of Zn^{2+} showed different responses of physiological and biochemical processes.

Conclusion

The pre-treatment of maize seeds with the solutions containing different plant growth regulators (PGRs) modified the allocation of zinc ions (Zn^{2+}) in root tissues. Thidiazuron (TDZ) enhanced, while Epin-extra impaired the absorption of Zn^{2+} in root tissues. The effect of pre-treatment of maize seeds with each plant growth regulator on seedling growth depended on the concentration of Zn^{2+} in the growth medium. Against high Zn^{2+} concentrations, only the pre-treatment of seeds with Epin-extra and in addition with Ribav-extra was effective.

Under the lack of Zn^{2+} in the growth medium, the most effective for young maize plants was the pre-treatment of maize seeds with Epin-extra. The efficiency of seed pre-treatment with PGRs against moderate or high Zn^{2+} concentration decreased as follows: Epin-extra > Ribav-extra > Kinetin > TDZ > CTD.

Received 26 06 2020
Accepted 28 01 2021

References

- Ahmad B., Jaleel H., Sadiq Y., Khan M. M., Shabbir A. 2018. Response of exogenous salicylic acid on cadmium induced photosynthetic damage, antioxidant metabolism and essential oil production in peppermint. *Journal of Plant Growth Regulation*, 86 (2): 273–286. <https://doi.org/10.1007/s10725-018-0427-z>
- Ali H., Khan E., Ilahi I. 2019. Environmental chemistry and ecotoxicology of hazardous heavy metals: environmental persistence, toxicity, and bioaccumulation. *Journal of Chemistry*, 2019: 6730305. <https://doi.org/10.1155/2019/6730305>
- Anjum N. A., Ahmad I., Mohmood I., Pacheco M., Duarte A. C., Pereira E., Umar S., Ahmad A., Khan N. A., Iqbal M., Prasad M. N. V. 2012. Modulation of glutathione and its related enzymes in plants' responses to toxic metals and metalloids – a review. *Environmental and Experimental Botany*, 75: 307–324. <https://doi.org/10.1016/j.envexpbot.2011.07.002>
- Anjum N. A., Duarte A. C., Pereira E., Ahmad I. 2015 (a). *Juncus maritimus* root-biochemical assessment for its mercury-stabilization potential in Ria de Aveiro coastal lagoon (Portugal). *Environmental Science and Pollution Research International*, 22 (3): 2231–2238. <https://doi.org/10.1007/s11356-014-3455-x>
- Anjum N. A., Singh H. P., Khan M. I., Masood A., Per T., Negi A., Batish D., Khan N. A., Duarte A. C., Pereira E., Ahmad I. 2015 (b). Too much is bad – an appraisal of phytotoxicity of elevated plant-beneficial heavy metal ions. *Environmental Science and Pollution Research*, 22: 3361–3382. <https://doi.org/10.1007/s11356-014-3849-9>
- Asadi M., Saadatmand S., Khavari-Nejad R. A., Ghasem-Nejad M., Fotokian M. H. 2012. Effect of zinc (Zn) on some physiological characteristics of rice seedling. *Biomedical and Pharmacology Journal*, 5 (2): 203–210. <https://doi.org/10.13005/bpj/345>
- Asati A., Pichhode M., Nikhil K. 2016. Effect of heavy metals on plants: an overview. *International Journal of Application or Innovation in Engineering and Management*, 5 (3): 56–66.
- Asgher M., Khan M. I. R., Anjum N. A., Khan N. A. 2015. Minimising toxicity of cadmium in plants – role of plant growth regulators. *Protoplasma*, 252: 399–413. <https://doi.org/10.1007/s00709-014-0710-4>
- Bali S., Kaur P., Kaur Kohli S. K., Ohri P., Thukral A. K., Bhardwaj R., Wijaya L., Alyemeni M. N., Ahmad P. 2018. Jasmonic acid induced changes in physio-biochemical attributes and ascorbate-glutathione pathway in *Lycopersicon esculentum* under lead stress at different growth stages. *Science of the Total Environment*, 645: 1344–1360. <https://doi.org/10.1016/j.scitotenv.2018.07.164>
- Bashmakov D. I., Pynenkova N. A., Sazanova K. A., Lukatkin A.S. 2012. Effect of the synthetic growth regulator cytoDEF and heavy metals on oxidative status in cucumber plants. *Russian Journal of Plant Physiology*, 59 (1): 59–64. <https://doi.org/10.1134/S1021443712010049>
- Bashmakov D. I., Lukatkin A. S., Anjum N. A., Ahmad I., Pereira E. 2015. Evaluation of zinc accumulation, allocation, and tolerance in *Zea mays* L. seedlings: implication for zinc phytoextraction. *Environmental Science and Pollution Research International*, 22 (20): 15443–15448. <https://doi.org/10.1007/s11356-015-4698-x>
- Broadley M. R., White P. J., Hammond J. P., Zelko I., Lux A. 2007. Zinc in plants. *New Phytologist*, 173 (4): 677–702. <https://doi.org/10.1111/j.1469-8137.2007.01996.x>
- Bücker-Neto L., Paiva A. L. S., Machado R. D., Arenhart R. A., Margis-Pinheiro M. 2017. Interactions between plant hormones and heavy metals responses. *Genetics and Molecular Biology*, 40 (1): 373–386. <https://doi.org/10.1590/1678-4685-gmb-2016-0087>
- Chen L., Wang D., Long C., Cui Z.-X. 2019. Effect of biodegradable chelators on induced phytoextraction of uranium- and cadmium-contaminated soil by *Zebrina pendula* Schnizl. *Scientific Reports*, 9: 19817. <https://doi.org/10.1038/s41598-019-56262-9>
- Chen L., Long C., Wang D., Yang J. 2020. Phytoremediation of cadmium (Cd) and uranium (U) contaminated soils by *Brassica juncea* L. enhanced with exogenous application of plant growth regulators. *Chemosphere*, 42: 125112. <https://doi.org/10.1016/j.chemosphere.2019.125112>
- Cherif J., Mediouni C., Ben Ammar W. B., Jemal F. 2011. Interactions of zinc and cadmium toxicity in their effects on growth and in antioxidative systems in tomato plants (*Solanum lycopersicum*). *Journal of Environmental Sciences (China)*, 23 (5): 837–844. [https://doi.org/10.1016/S1001-0742\(10\)60415-9](https://doi.org/10.1016/S1001-0742(10)60415-9)
- Davletova S., Schlauch K., Coutu J., Mittler R. 2005. The zinc-finger protein Zat12 plays a central role in reactive oxygen and abiotic stress signaling in Arabidopsis. *Plant Physiology*, 139 (2): 847–856. <https://doi.org/10.1104/pp.105.068254>
- Glińska S., Gapińska M., Michlewska S., Skiba E., Kubicki J. 2016. Analysis of *Triticum aestivum* seedling response to the excess of zinc. *Protoplasma*, 253 (2): 367–377. <https://doi.org/10.1007/s00709-015-0816-3>
- Gruznova K. A., Bashmakov D. I., Brazaitytė A., Duchovskis P., Lukatkin A. S. 2017. Efficiency index as the integral indicator of *Triticum aestivum* response to growth regulators. *Zemdirbyste-Agriculture*, 104 (4): 299–304. <https://doi.org/10.13080/z-a.2017.104.0838>

- Gruznova K. A., Bashmakov D. I., Miliauskienė J., Vaštakaitė V., Duchovskis P., Lukatkin A. S. 2018. The effect of a growth regulator Ribav-Extra on winter wheat seedlings exposed to heavy metals. *Zemdirbyste-Agriculture*, 105 (3): 227–234. <https://doi.org/10.13080/z-a.2018.105.9029>
- Jain D., Kour R., Bhojjiya A. A., Meena R. H., Singh A., Mohanty S. R., Rajpurohit D., Ameta K. D. 2020. Zinc tolerant plant growth promoting bacteria alleviates phytotoxic effects of zinc on maize through zinc immobilization. *Scientific Reports*, 10: 13865. <https://doi.org/10.1038/s41598-020-70846-w>
- Kaznina N. M., Titov A. F. 2017. Effect of zinc deficiency and excess on the growth and photosynthesis of winter wheat. *Journal of Stress Physiology and Biochemistry*, 13 (4): 88–94 (in Russian).
- Kholodova V. P., Vasil'ev S. V., Efimova M. V., Voronin P. Yu., Rakhmankulova Z. F., Danilova E. Yu., Kuznetsov V. V. 2018. Exogenous melatonin protects canola plants from toxicity of excessive copper. *Russian Journal of Plant Physiology*, 65: 882–889. <https://doi.org/10.1134/S1021443718060080>
- Lukatkin A. S. 2002. Contribution of oxidative stress to the development of cold-induced damage to leaves of chilling-sensitive plants: 2. The activity of antioxidant enzymes during plant chilling. *Russian Journal of Plant Physiology*, 49 (6): 782–788. <https://doi.org/10.1023/A:1020965629243>
- Qiao K., Wang F., Liang Sh., Wang H., Hu Zh., Chai T. 2019. Improved Cd, Zn and Mn tolerance and reduced Cd accumulation in grains with wheat-based cell number regulator TaCNR2. *Scientific Reports*, 9: 870. <https://doi.org/10.1038/s41598-018-37352-6>
- Ren B., Hu J., Zhang J., Dong S., Liu P., Zhao B. 2019. Spraying exogenous synthetic cytokinin 6-benzyladenine following the waterlogging improves grain growth of waterlogged maize in the field. *Journal of Agronomy and Crop Science*, 205 (6): 616–624. <https://doi.org/10.1111/jac.12355>
- Sazanova K. A., Bashmakov D. I., Lukatkin A. S., Brazaityte A., Bobinas C., Duchovskis P. 2012. The effect of heavy metals and thidiazuron on winter wheat (*Triticum aestivum* L.) seedlings. *Zemdirbyste-Agriculture*, 99 (3): 273–278.
- Seregin I. V., Ivanov V. B. 1997. Histochemical investigation of cadmium and lead distribution in plants. *Russian Journal of Plant Physiology*, 44 (6): 915–921.
- Seregin I. V., Kozhevnikova A. D., Schat H. 2019. Comparison of L-histidine effects on nickel translocation into the shoots of different species of the genus *Abyssum*. *Russian Journal of Plant Physiology*, 66 (2): 340–344. <https://doi.org/10.1134/S1021443719020122>
- Sharma A., Sidhu G. P. S., Araniti F., Bali A. S., Shahzad B., Tripathi D. K., Skalicky M., Landi M. 2020. The role of salicylic acid in plants exposed to heavy metals. *Molecules*, 25 (3): 540. <https://doi.org/10.3390/molecules25030540>
- Sun S., Zhou X., Cui X., Liu C., Fan Y., McBride M. B., Li Y., Li Z., Zhuang P. 2020. Exogenous plant growth regulators improved phytoextraction efficiency by *Amaranthus hypochondriacus* L. in cadmium contaminated soil. *Journal of Plant Growth Regulation*, 90: 29–40. <https://doi.org/10.1007/s10725-019-00548-5>
- Sunitha M. S. L., Prashant S., Kumar S. A., Rao S., Narasu M. L., Kishor P. B. K. 2013. Cellular and molecular mechanisms of heavy metal tolerance in plants: a brief overview of transgenic plants over-expressing phytochelatin synthase and metallothionein genes. *Plant Cell Biotechnology and Molecular Biology*, 14 (1–2): 33–48. <https://www.ikprress.org/index.php/PCBMB/article/view/1204>
- Sytar O., Kumari P., Yadav S., Brestic M., Rastogi A. 2019. Phytohormone priming: regulator for heavy metal stress in plants. *Journal of Plant Growth Regulation*, 38: 739–752. <https://doi.org/10.1007/s00344-018-9886-8>
- Talaat N. B., Shawky B. T., Ibrahim A. S. 2015. Alleviation of drought-induced oxidative stress in maize (*Zea mays* L.) plants by dual application of 24-epibrassinolide and spermine. *Environmental and Experimental Botany*, 113: 47–58. <https://doi.org/10.1016/j.envexpbot.2015.01.006>
- Turhan Ş., Garad A. M. K., Hançerlioğulları A., Kurnaz A., Gören E., Duran C., Karataşlı M., Altıkulaç A., Savacı G., Aydın A. 2020. Ecological assessment of heavy metals in soil around a coal-fired thermal power plant in Turkey. *Environmental Earth Sciences*, 79 (6): 134. <https://doi.org/10.1007/s12665-020-8864-1>
- Vuletić M., Marković K., Kravić N., Hadži-Tašković Šukalović V., Vučinić Z., Maksimović V. 2014. Differential response of antioxidative systems of maize (*Zea mays* L.) roots cell walls to osmotic and heavy metal stress. *Plant Biology*, 16 (1): 88–96. <https://doi.org/10.1111/plb.12017>
- Wang Y., Yang X., Zhang X., Dong L., Zhang J., Wei Y., Feng Y., Lu L. 2014. Improved plant growth and Zn accumulation in grains of rice (*Oryza sativa* L.) by inoculation of endophytic microbes isolated from a Zn hyperaccumulator, *Sedum alfredii* H. *Journal of Agricultural and Food Chemistry*, 62 (8): 1783–1791. <https://doi.org/10.1021/jf404152u>
- Wei X., Zhou Y., Jiang Y., Tsang D. C. W., Zhang C., Liu J., Zhou Y., Yin M., Wang J., Shen N., Xiao T., Chen Y. 2020. Health risks of metal(loid)s in maize (*Zea mays* L.) in an artisanal zinc smelting zone and source fingerprinting by lead isotope. *Science of the Total Environment*, 742: 140321. <https://doi.org/10.1016/j.scitotenv.2020.140321>
- White P. J. 2012. Heavy metal toxicity in plants. Shabala S. (ed.). *Plant Stress Physiology*. CABI, p. 210–237. <https://doi.org/10.1079/9781845939953.02100>
- Yan P., Chen C.-X., Xu T.-J., Dong Z.-Q. 2020. A novel plant growth regulator ameliorates chilling tolerance for spring maize in Northeast China. *Plant Growth Regulation*, 91: 249–261. <https://doi.org/10.1007/s10725-020-00603-6>
- Zhao K., Wu Y. 2017. Effects of Zn deficiency and bicarbonate on the growth and photosynthetic characteristics of four plant species. *PLoS ONE*, 12 (1): e0169812. <https://doi.org/10.1371/journal.pone.0169812>

Sėklų apdorojimo augalų augimo reguliatoriais efektyvumas kukurūzų daigų atsparumui cinko jonų poveikiui

D. I. Bashmakov¹, A. S. Lukatkin¹, J. Miliauskienė², L. Duchovskienė², P. Duchovskis²

¹Nacionalinis Mordovijos valstybinis universitetas, Rusija

²Lietuvos agrarinių ir miškų mokslų centras

Santrauka

Tyrimo metu nustatytas kukurūzų sėklų apdorojimo sintetiniiais (Tidiazurono (TDZ), Cytodefo (CTD), Kinetino bei Epin-extra) ir natūraliu (Ribav-extra) augalų augimo reguliatoriais poveikis paprastojo kukurūzo (*Zea mays* L.) veislės 'Tzaritza' daigų, paveiktų cinku (Zn^{2+}), augimui. Taip pat tirta augalų augimo reguliatorių įtaka Zn^{2+} paskirstymui kukurūzų šaknų audiniuose. Kukurūzų sėklos buvo apdorotos skirtingais augalų augimo reguliatoriais ir 7 dienas augintos terpėse su 1 μM , 10 μM , 0,1 mM ir 1 mM Zn^{2+} koncentracijomis. Po to stebėtas ūglių bei šaknų augimas ir katalazės kaupimas kukurūzų lapuose. Siekiant nustatyti augalų augimo reguliatorių poveikį Zn^{2+} paskirstymui kukurūzų šaknų audiniuose, sėklos buvo apdorotos tirpalais su skirtingais augalų augimo reguliatoriais. Tada penkių dienų daigai tris dienas buvo auginti terpėje su 1 mM Zn^{2+} . Skersinės šaknies skiltelės nudažytos ditizonu ir stebėtos mikroskopu. Kukurūzų sėklų apdorojimas tirpalais su skirtingais augalų augimo reguliatoriais pakeitė Zn^{2+} pasiskirstymą šaknies audiniuose. Augalų augimo reguliatorių poveikis priklausė nuo Zn^{2+} koncentracijos augimo terpėje. Tidiazuronas pagerino, o Epin-extra pablogino Zn^{2+} absorbciją šaknies audiniuose. Sėklų apdorojimas Epin-extra ir Ribav-extra buvo veiksmingiausias augimo terpėje su didesne (0,1 mM ir 1 mM) Zn^{2+} koncentracija, nes sukėlė kukurūzų ašinį augimą ir katalazės aktyvumą augalų lapuose. Kukurūzų sėklų apdorojimas Kinetinu buvo veiksmingas augalų šaknų augimui terpėse su visomis tirtomis Zn^{2+} koncentracijomis.

Tyrimo duomenys rodo, kad kukurūzų augalai, kurių sėklos prieš sėją buvo apdorotos augalų augimo reguliatoriais, buvo atsparesni Zn^{2+} poveikiui nei neapdoroti.

Reikšminiai žodžiai: ašiniai organai, augimas, išankstinis apdorojimas, katalazė, šaknys, *Zea mays*.