RESEARCH ARTICLE | NOVEMBER 19 2021

Response reactions of *Zinnia elegans* seedlings to the impact of different copper ions concentrations

D. S. Plotnikov; A. S. Tugbaeva ✉; A. A. Ermoshin; I. S. Kiseleva

AIP Conf. Proc. 2388, 030034 (2021)
https://doi.org/10.1063/5.0068392

Response Reactions of *Zinnia elegans* Seedlings to the Impact of Different Copper Ions Concentrations

D. S. Plotnikov[b)], A. S. Tugbaeva[a)], A. A. Ermoshin[c)] and I. S. Kiseleva[d)]

Ural Federal University named after the first President of Russia B.N. Yeltsin,
51, Lenina ave., Ekaterinburg, 620000, Russia

[a)]Corresponding author: anastasia.tugbaeva@urfu.ru
[b)]dmitry.plotnikov@urfu.ru
[c)]alexander.ermoshin@urfu.ru
[d)]irina.kiseleva@urfu.ru

Abstract. The present work aims to study the responses (seed germination, root and hypocotyl size, hydrogen peroxide concentration, lipid peroxidation products, and peroxidase activity) of *Zinnia elegans* Jacq. seedlings to different concentrations of Cu^{2+} (25, 50, 100 and 200 μM). At low Cu^{2+} content (25–50 μM) seed germination, biomass of seedlings, and hypocotyl length did not change, but root length decreased. Under 100–200 μM Cu^{2+}, a decrease was revealed in seed germination percentage, root and hypocotyl length, and the biomass in 8-days plants. An increase in hydrogen peroxide concentration and lipid peroxidation products, induction of apoplastic and cytosolic guaiacol peroxidase (GPOX) activity were observed in the root tissues under 50–200 μM Cu^{2+}. The negative effects were less pronounced in the hypocotyl tissues: increase of lipid peroxidation was noted under 200 μM Cu^{2+}, and hydrogen peroxide under 100 and 200 μM Cu^{2+}; the activity of peroxidases did not change compared to control. Thus, the root was more sensitive to the excess of copper ions. A specific increase in the activity of apoplastic and cytosolic GPOX in root tissues can be considered as a biochemical marker for the selection of resistant forms of plants at the early stages of development.
Keywords: *Zinnia elegans* seedling, copper ions, hydrogen peroxide, guaiacol peroxidases, oxidative stress.

INTRODUCTION

Both human activity and natural erosion cause the problem of soil pollution by heavy metals (HM), including copper. HM in high concentrations prevent seed germination, reduce the growth rate of roots and shoots, and decrease plant biomass [1]. Their toxic effects, among others, induce the development of oxidative stress that leads to violations in membrane permeability, conformational changes of proteins, etc. [2, 3].

Copper ions are involved in several processes in plants, e.g. chlorophyll synthesis, photosynthesis, electron transport chains in chloroplasts and mitochondria [2]. High copper concentrations cause stress in plants as it stimulates hyperproduction of reactive oxygen species (ROS) that induce oxidative damage of lipids, proteins, and nucleic acids [1, 2]. Also, the high toxicity of copper ions is associated with their ability to interact with SH-groups of proteins, which leads to changes in their conformation and functions [4].

Negative effects of copper stress can be reduced by antioxidant enzymes, e.g. class III peroxidases (EC 1.11.1.7). Guaiacol peroxidase (GPOX) could be localized in the protoplast and cell walls. It oxidizes a wide variety of phenolic substrates using hydrogen peroxide. Induction of its activity is a nonspecific plant response to stress [5]. Since copper ions are transported through the apoplast of the primary root cortex [6], we assume that the increase in the amount of hydrogen peroxide in the cell walls, mediated by stressors, should increase the activity of apoplastic GPOX. It is known that plants are most sensitive to stressors at the early stages of development [7]. Therefore, seedlings are the convenient object for the study of early markers of stress-resistance in plants.

The aim of this work was to study the response reactions of *Z. elegans* seedlings to different Cu^{2+} concentrations.

Modern Synthetic Methodologies for Creating Drugs and Functional Materials (MOSM2020)
AIP Conf. Proc. 2388, 030034-1–030034-4; https://doi.org/10.1063/5.0068392
Published by AIP Publishing. 978-0-7354-4142-2/$30.00

MATERIALS AND METHODS

Plants (*Zinnia elegans* Jacq.) were grown in petri dishes with 25, 50, 100 and 200 μM $CuSO_4$, under 24 °C and 16/8 photoperiod. Control – water. The experiments were repeated three times with fifteen replicates for each treatment. Seed germination was checked on the 5th day; biomass of seedlings, length of roots and hypocotyl – on the 8th day. Biochemical characteristics were also assessed in 8-day-old seedlings. Hydrogen peroxide amount (μM per g^{-1} dry weight) was measured spectrophotometrically [8]. The lipid peroxidation (LPO) level was estimated as the accumulation of malondialdehyde (MDA) and expressed in μM per g^{-1} dry weight [9].

Enzyme activity was estimated in crude extracts. Fresh roots and hypocotyl were homogenized in 0.05 M Tris-HCl buffer (pH 7.0), centrifuged at 4 °C. Extraction procedure was repeated twice. The supernatant was used for cytosolic enzyme activity assay. The pellet was resuspended in 0.05 M Tris-HCl buffer (pH 7.0) with the addition of 1 M KCl, incubated 30 min, then centrifuge at 4 °C [10]. This extract was used for the assay of cell wall-bound (apoplastic) enzyme activity. Guaiacol peroxidase (GPOX, EC 1.11.1.7) activity was measured according to Chance and Maehly [11] and expressed as μM of oxidized tetraguaiacol per $mg^{-1}$of protein per min^{-1}. Protein content was estimated according to Bradford method [12], using BSA as a standard. All measurements were done in three biological and five analytical replications. The optical density was measured using "Tecan Infinity 200" (Austria); or "Shimadzu UV-1800" (Japan) for GPOX.

Mean values ± standard deviation (SD) are presented in figures and tables. The Student's *t* test was used for morphological, and Mann – Whitney *U*-test for biochemical characteristics at $P < 0.05$. Statistical analysis was completed in STATISTICA 13 for Windows 10.

RESULTS

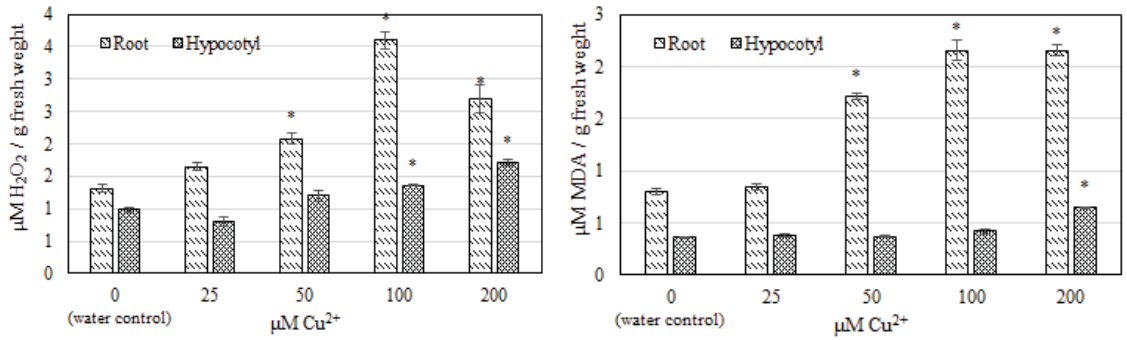
Plants treated with 25 and 50 μM Cu^{2+} had the same germination percentage as the control, whereas under 100 and 200 μM it was decreased by 11% (Table 1). The biomass of seedlings under low concentration of Cu^{2+} had no significant changes compared to the control. It, however, decreased under 100 and 200 μM Cu^{2+} by 14 – 15.4%. The root was more sensitive to the impact of copper ions. Its length reduced by 32 and 41%, respectively, under 25 and 50 μM Cu^{2+}, while under 100 – 200 μM Cu^{2+} by 82 – 84%. The browning of the roots was also noted under high concentrations of Cu^{2+}. The hypocotyl length did not change under low copper concentration, whereas at 100 – 200 Cu^{2+}μM it decreased by 36 – 38%.

TABLE 1. Germination percentage on the 5th day; total dry biomass and length of roots and hypocotyl in 8-days *Z. elegans* seedling; *significantly different from control at $P < 0.05$ level (*t*-test).

Cu^{2+} concentration, μM	Germination percentage, %	Total dry biomass, mg	Root length, mm	Hypocotyl length, mm
Water control	84.0 ± 5.7	18.8 ± 0.9	27.3 ± 2.7	42.3 ± 1.8
25	80.4 ± 4.1	17.8 ± 0.6	19.3 ± 1.6*	40.3 ± 2.2
50	79.5 ± 3.7	16.6 ± 0.7	16.8 ± 1.5*	41.3 ± 1.9
100	72.0 ± 4.3	15.9 ± 0.5*	5.3 ± 0.4*	33.2 ± 2.2*
200	71.3 ± 4.8	15.7 ± 0.6*	4.9 ± 0.5*	23.3 ± 3.5*

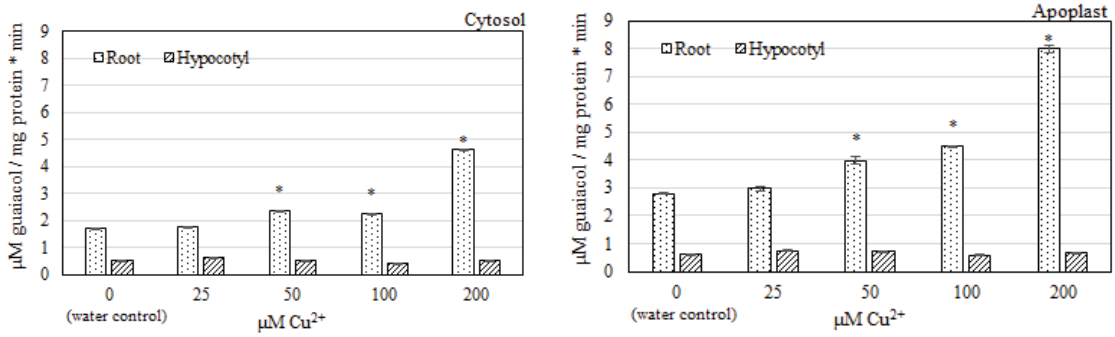
The concentration of hydrogen peroxide and LPO rate were higher in roots than in hypocotyl of stressed plants (Fig. 1). MDA content in roots increased under 50, 100 and 200 μM Cu^{2+} by 88% − 170% compared to the control. Concentration of H_2O_2 was 1.6, 2.7 and 2.1 times higher in roots treated with 50, 100 and 200 μM Cu^{2+}. In hypocotyl, LPO increased under 200 μM by 78% compared to the control: the level of hydrogen peroxide significantly increased under 100 and 200 μM – 38% and 74%, respectively.

The enzyme activity of cell wall-bound and cytosolic GPOX in roots was higher than in hypocotyl in all groups of plants (Fig. 2). The induction of enzyme activity under copper stress was more pronounced in roots than in hypocotyl. Cytosolic GPOX activity increased under 50 and 100 μM Cu^{2+} by 30 – 36%, and under 200 μM – by 167% compared to untreated plants. The same effect was noted for cell wall-bound peroxidase: its activity increased by 43 and 61% under 50 and 100 μM respectively; and by 186% under 200 μM Cu^{2+}. The activity of apoplastic and cytosolic GPOX did not reveal significant difference in hypocotyl.



(a) (b)

FIGURE 1. The hydrogen peroxide (a) and MDA (b) content in roots and hypocotyl of *Z. elegans* seedlings.
*Significantly different from control at $P < 0.05$ level (*U*-test)



(a) (b)

FIGURE 2. Activity of cytosolic (a) and cell wall-bound (b) GPOX in roots and hypocotyl tissues of *Z. elegans* seedlings.
*Significantly different from control at $P < 0.05$ level (*U*-test)

DISCUSSION AND CONCLUSION

It was shown [13, 14] that the absorption of copper ions on the seed surface could limit their translocation into the embryo tissue. In our study low concentrations of copper ions did not affect seed germination, probably for this reason. Under high concentrations of Cu^{2+} (100–200 μM) the germination decreased. It was likely caused by the toxic and/or osmotic effects of copper solutions. They could worsen water absorption by seeds, which is an important factor for germination [15]. Other possible reasons for the decrease in seed germination are: the changes in the selective permeability of cell membranes, accelerated breakdown of storage substances, redistribution of energy for the synthesis of antioxidant defense enzymes, decrease in the activity of hydrolytic enzymes, violation of redox homeostasis, hormonal imbalance due to a low rate of abscisic acid destruction [16, 17]. At the same time, it was shown that the toxic effect of Cu^{2+} was less pronounced at the early stages of seed germination, but more characteristic at the stage of seedlings [14]. In our work, we noted visible symptoms of copper stress at 100–200 μM Cu^{2+}concentrations as the browning of the tips of the main and lateral roots, as well as the decrease in biomass and linear size of roots and hypocotyl. Similar effects were described for *Medicago sativa* L., *Arabidopsis thaliana* L., *Triticum aestivum* L., *Pisum sativum* L., *Solanum lycopersicum* L., etc. [14, 18, 19].

It is known that root performs a barrier function, limiting the absorption and transport of Cu^{2+} into the shoot [2, 19]. Copper ions can be accumulated in the apoplast, which prevents absorption into cytosol and transport by xylem vessels [20]. When absorbed in cytosol copper may cause the generation of hydroxyl radicals via Fenton and Haber–Weiss reactions and participate in LPO leading to damage of membranes [7]. We also observed the hyperproduction of hydrogen peroxide and the development of oxidative stress, as evidenced by the increase in the MDA content in

roots. LPO-products were one of the factors that led to the decrease of seedling size under the impact of Cu^{2+} in *Hordeum vulgare* L., *Daucus carota* L., and others [7, 21]. It is suggested that oxidative stress could indirectly change the distribution of phytohormones, suppress cell division and cell growth [13]. In our study lower accumulation of LPO-products in hypocotyl tissues compared to roots evidenced to a barrier function of the root in seedlings of *Z. elegans*.

The induction of peroxidase activity is considered as a nonspecific response to stress factors [5]. It is likely that in *Z. elegans* the increase of hydrogen peroxide and MDA in roots stimulated the activity of both cytosolic and apoplastic peroxidases. Under copper stress, the activity of apoplastic GPOX was 1.5–1.8 times higher than cytosolic. It is known that Cu^{2+} can bind to pectin and lignin in cell walls, and thus could not be translocated into cells. The induction of oxidative processes was shown in apoplast under excess copper ions [10]. In our study, the activity of apoplastic peroxidase in roots changed to a greater extent than cytosolic. This could be due to the accumulation of hydrogen peroxide in the apoplast under stress. In hypocotyl, the activity of cytosolic and apoplastic GPOX did not change in treated plants as the level of MDA products and hydrogen peroxide were not high.

Thus, in *Z. elegans* seedlings roots were more sensitive to excess copper ions (50–200 μM), than hypocotyl as evidenced by the high level of H_2O_2 and LPO products, and higher activity of cytosolic and apoplastic GPOX. GPOX activity in roots could be considered as a marker for early diagnostics of plant resistance to copper stress.

ACKNOWLEDGMENTS

The work was supported by the Ural Federal University Competitiveness Enhancement Program, Act 211 Government of the Russian Federation (contract № 02.A03.21.0006).

REFERENCES

1. R. Fryzova, M. Pohanka, P. Martinkova, H. Cihlarova, M. Brtnicky, J. Hladky, and J. Kynicky, Rev. Environ. Contam. Toxicol. **245**, 129–156 (2017).
2. I. Yruela, Funct. Plant Biol. **36(5)**, 409–430 (2009).
3. S. K. Sethy and S. Ghosh, J. Nat. Sci. Biol. Med. **4(2)**, 272–275 (2013).
4. M. W. Paschke and E. F. Redente, Environ. Toxicol Chem. **21**, 2692–2697 (2002).
5. H. Jouili and E. E. Ferjani, Acta Physiol. Plant. **26(1)**, 29–35 (2004).
6. B. Printz, S. Lutts, J.-F. Hausman, and K. Sergeant, Front. Plant Sci. **7**, 601 (2016).
7. W. Ke, Z. Xiong and M. Xie, Plant Soil. **292**, 291–304 (2007).
8. D. Bellincampi, N. Dipierro, G. Salvi, F. Cervone, and G. De Lorenzo, Plant Physiol. **122**, 1379–1385 (2000).
9. M. Uchiyama and M. Mihara, Anal. Biochem. **86**, 287–297 (1978).
10. E. Jamet, H. Canut, G. Boudart, and R.F. Pont-Lezica, Trends in Plant Sci. **11(1)**, 33–39 (2009).
11. A Maehly and B Chance, Methods Biochem. Anal. **2**, 764–775 (1954).
12. M. M. Bradford, Anal. Biochem. **72**, 248–254 (1976).
13. S. Liu, C. Yang, W. Xie, C. Xia, and P. Fan, Procedia Environ. Sci. **16**, 293–298 (2012).
14. W. Li, M. A. Khan, S. Yamaguchi, and Y. Kamiya, Plant Growth Regul. **46**, 45–50 (2005).
15. E. Bezini, A. Abdelguerfi, B. Nedjimi, M. Touati, B. Adli, and B. Yabrir, Agric. Conspec. Sci. **84(4)**, 357–364 (2019)
16. O. Atici, G. Ağar, and P. Battal, Biol. Plant **49(2)**, 215–222 (2005).
17. M. Shafiq, I. M. Zafar, and M. Athar, J. Appl. Sci. Environ. Manag. **12(2)**, 61–66 (2008).
18. C. Aydinalp and S. Marinova, Bulgarian J. of Agric. Sci. **15(4)**, 347–350 (2009).
19. N. Baruah, S. C. Mondal, M. Farooq, and N. Gogoi, Water Air Soil Pollut. **230**, 273 (2019).
20. M. Wójcik and A. Tukiendorf, Biologia Plantarum **46**, 79-84 (2003).
21. R. Juknys, G. Vitkauskaitė, M. Račaitė, and J. Venclovienė, Cent. Eur. J. Biol. **7(2)**, 299–306 (2012).