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Effect of Copper on Cadmium-Resistant Plants of *Agrostis stolonifera*

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ABSTRACT

Environmental pollution with heavy metals has a negative impact on lawn grasses. Heavy metals are one of the priority pollutants of anthropogenic ecosystems. Earlier, plants Agrostis stolonifera, resistant to cadmium, were obtained using biotechnological method. Plants that are resistant to one heavy metal may be cross-resistant to another. The assessment of the resistance of plants obtained by biotechnological methods to other heavy metals is of practical value. The object of our study was to lawn grass - *Agrostis stolonifera* L. The aim of this work was to assess the tolerance of the next generation descendants of the regenerant *Agrostis stolonifera*, resistant to cadmium, to one of the most phytotoxic heavy metals - copper. Cadmium -tolerant plants were more resistant to copper. The tolerance of cadmium – resistant plants to copper is associated with nonspecific mechanisms. However, the increase in plant resistance was not very significant. Therefore, it is more expedient to obtain plants that are resistant to copper.

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INTRODUCTION

Heavy metals are one of the priority pollutants of anthropogenic ecosystems. Copper has a very high phytotoxicity. Cu pollution also exerts a potential risk to soil fertility (Keiblingeret al.,2018). Some crops accumulate certain heavy metals. For example, oats showed higher absorption of copper than lead and zinc (Bazdyrev, 2001). Plant species and cultivars show varying degrees of tolerance to various metals. A significant difference in sensitivity to metals can be observed between cultivars. Some strawberry cultivars had varying degrees of metal resistance (Abyzov, 2008). A nearly two-fold variation range of the heavy metal concentrations (i.e., Cd, Cu et al) was observed among the cultivars of water spinach (He et al., 2015).

Environmental pollution with heavy metals negatively affects plants. When soils are contaminated with heavy metals, plants have a slowdown in growth, a decrease in the number of internodes, and a decrease in biomass. There is a decrease in the ornamental qualities of plants and the range of plants used is significantly reduced. Lawn grasses are highly sensitive to heavy metals. Copper and zinc are among the priority pollutants among heavy metals. However, copper is considerably more toxic to plants than zinc. Phytotoxic effects of Zn were found to be less severe than those of Cu (Pillay et al., 1994).

Earlier, plants *Agrostis stolonifera*, resistant to cadmium, were obtained using biotechnological

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method (cell selection) (Gladkova et al., 2021). However, plants resistant to one environmental factor may be cross-resistant to another. The assessment of the resistance of plants obtained by biotechnological methods to other heavy metals is of practical value (Gladkov, Gladkova, 2020). The aim of this work was to assess the tolerance of the next generation descendants of the regenerant *Agrostis stolonifera*, resistant to cadmium, to one of the most phytotoxic heavy metals - copper.

MATERIALS AND METHODS

The object of our study was to lawn grass - *Agrostis stolonifera* L. The advantage of *Agrostis stolonifera* is the possibility of creating lawns using only this lawn grass. Cadmium - resistant plants were obtained using cell selection (Gladkova et al., 2021). Primary callus was obtained from seeds on a modified Murashige-Skoog medium (Gladkov, Gladkova, 2022; Gladkov, 2023). For the growth of callus, the Murashige-Skoog medium was used with the addition of 1 mg/L 2,4- dichlorophenoxyacetic acid (2,4-D) (Gladkov et al., 2022). To obtain plants resistant to cadmium, $CdCl_2$ was added to the modified Murashige-Skoog medium. Callus was planted on a selective Murashige-Skoog medium containing 10 mg/L of cadmium.

After cultivation for one month, calli were transplanted for further cultivation in Murashige-Skoog medium with cadmium. The concentration of cadmium was increased at the stage of cultivation and regeneration, or regeneration and rooting (Gladkova et al., 2021).

The obtained regenerants were resistant to 50- 100 mg/kg cadmium.

The descendants of cadmium-tolerant regenerants were used to test for copper resistance.

To assess the resistance to copper, 10 seeds of the next generation descendants of the regenerant or the original plants were placed in each Petri dish on filter paper moistened with a solution of the toxicant. The seeds were germinated on Petri dishes with filter paper with $CuSO_4 \cdot 5H_2O$. Water was used as a control. The growth of the shoots was assessed.

Each variant of the experiment was carried out in 4 times. Mean values with their standard deviations were computed with Microsoft Excel.

RESULTS AND DISCUSSION

The adaptation of plants to the toxic effect of copper is associated with the functioning of both specialized and general mechanisms of resistance. The earlier the defense mechanisms are triggered, the less the plant is exposed to toxic effects (Zhuikova, Zinnatova 2014).

High copper concentration leads to stunted growth of roots and shoots (Amin et al., 2021). The inhibitory effect of copper on shoot growth was at 50 mg/L (Fig. 1).

Plants tolerant to cadmium showed greater resistance to copper compared to the original plants at all studied concentrations. There are plants with complex resistance to copper and cadmium. For example,T. *qataranse* is tolerant of, Cu, and Cd (Usman et al., 2019).

Cadmium and copper are among the most toxic metals to plants. Cd and Cu synergistic toxicity was noted on plant growth and oxidative stress (Mwamba et al., 2016). Excess of Cd or Cu induced higher accumulation of tartrate and malate and, additionally, copper increased the citrate content (Dresler et al., 2014).

Cu transport plays an important role in the resistance of plants to cadmium stress (Chen et al., 2022). .Suggest that oxidative stress triggers an NADPH oxidase-mediated signaling pathway, which contributes to cadmium translocation and basal plant resistance (Seguí et al., 2015)

The relationship of Cd and Cu in plants is shown. Cd stimulates Cu accumulation in roots of *A. thaliana* and increases mRNA expression of three plasma membrane-localized Cu uptake transporters, COPT1, COPT2 and COPT6 (Gayomba et al., 2013). OsZIP1 is a transporter that is required for detoxification of excess Cu and Cd in rice (Liu et al., 2019). Thus, Metal

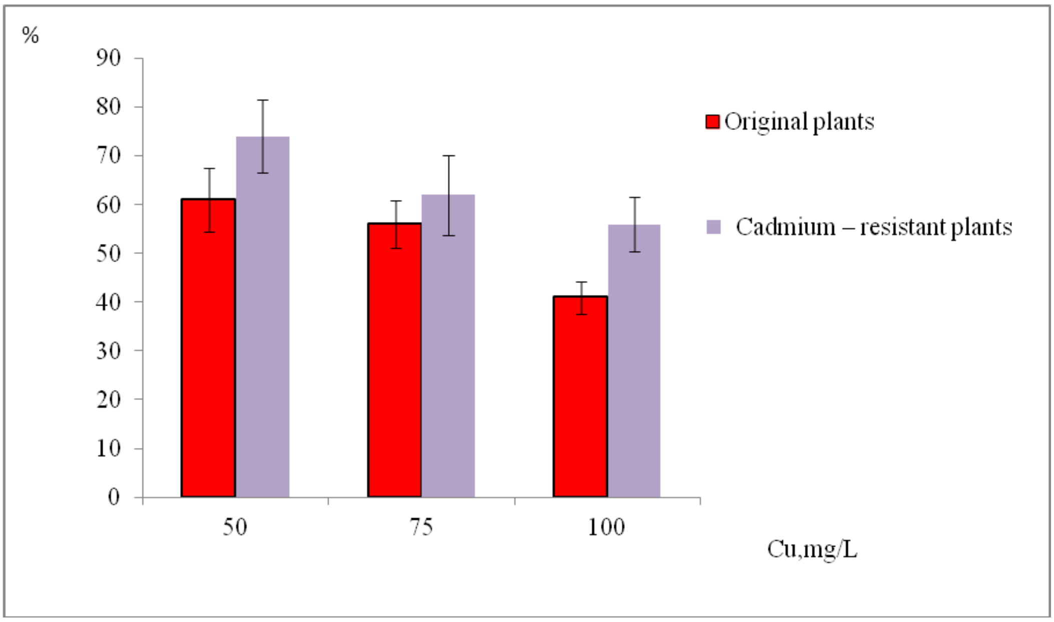

Fig. 1. Effect of copper on cadmium-tolerant lawn grasses (% relative to control)

homeostasis is important in plant adaptation and is governed to some extent by a variety of metal transporters.

The tolerance of cadmium – resistant plants to copper is associated with nonspecific mechanisms. Copper resistance may also be related to possible synthesis of metallothioneins (Quan et al., 2007).

However, the increase in plant resistance was not very significant. The absence of high resistance to copper is associated with the manifestation of specific resistance mechanisms.

CONCLUSIONS

Thus, plants resistant to cadmium obtained by cell selection had an increased resistance to copper, but this resistance is insufficient at a high level of pollution. Therefore, it is more expedient to obtain plants that are resistant to copper.

GRANT SUPPORT DETAILS

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CONFLICTS OF INTEREST

The authors declare that there is not any conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/ or falsification, double publication and/or submission, and redundancy has been completely observed by the authors.

LIFE SCIENCE REPORTING

No life science threat was practiced in this research.

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AUTHORS CONTRIBUTIONS

Conceptualization: Evgeny A. Gladkov; Olga V. Gladkova.
Methodology: Evgeny A.Gladkov; Olga N. Gladkova; Olga V. Gladkova.
Experimental work: Evgeny A. Gladkov; Olga N. Gladkova.
Resources (seeds of cadmium-tolerant regenerants): Evgeny A. Gladkov;Olga V. Gladkova.
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