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RESEARCH ARTICLE

Ornamental plants adapted to urban ecosystem pollution: lawn grasses and painted daisy tolerating copper

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Abstract

Lawns and flower are major aesthetical and environmental elements of the urban ecosystem. However, harsh urban conditions such as pollution by heavy metals are unfavorable for plants. For example, copper is toxic for ornamental plants, limiting the spread of lawn grass and flowering plants. Therefore, here we hypothesized that plants could be adapted to urban conditions by breeding. We first showed the possibility of using environmental biotechnology in urban greening to obtain, tolerating copper flowering plants and lawn grasses. We tested the adaptation of creeping bentgrass (*Agrostis stolonifera* L.) and painted daisy (*Chrysanthemum carinatum* Schousb.) to copper. We measured Cu resistance in the next generations of those plant species. Results show that some next generations of plant regenerants have increased resistance up to 100 mg/kg Cu for *Agrostis stolonifera*, and up to 30 mg/kg for *Chrysanthemum carinatum*. Our findings thus imply that city plants may be adapted and improved by cell selection. Our approach thus represents a novel biotechnology consisting of adapting plants to pollution by cell selection.

Keywords Cell selection · Lawn grass · *Agrostis stolonifera* · *Chrysanthemum carinatum* · Pollutant · Copper · Urban ecosystem

Introduction

Lawns and flower are major aesthetical and environmental elements of cities. Objectives and environmental conditions determine the choice of plants for lawns. For example, *Agrostis stolonifera* L. forms the highest quality lawn. Meadow grasses are popular too; they contain annual flowering grasses, for example, *Chrysanthemum carinatum*, *Linum perenne*, *Brachycome Iberidifolia*, *Bellis perennis*, *Centaurea cyanus*, and *Calendula officinalis*. *Chrysanthemum carinatum* is also used to create beautiful flower gardens in urban gardening.

Urban conditions are unfavorable for plants (Tsvetkova 2003). For example, green spaces in Moscow are degraded by 70–80% (Tsvetkova 2003). According to reports on the state of the environment in the city of Moscow, only 32% of the lawns were solid grass (Report 2013 2014; Report 2011 2012; Report 2017 2018).

Soils in urban environments can be highly compacted and anoxic and therefore are not well adapted to plant growth and development. Heavy metals are priority pollutants soil of Russian cities (Overview of the state and environmental pollution in the Russian Federation for 2015 2016; State report 2017 2018). The geoaccumulation index shows that the contamination of Cu, Cr, Ni, Pb, Zn, and Cd is widespread in urban soils of China and urban road dusts of the cities (Wei and Yang 2010). Mineral oil and heavy metals are the main contaminants contributing around 60% to soil contamination of the European Union (Panagos et al. 2013).

Metals have been classified according to their degree of toxicity for plants in the following sequence: Cu > Ni > Cd > Pb > Hg > Fe > Mo > Mn (Alekseeva-Popova 1991; Guralchuk,

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1994). The negative impact of metals on plants depends on many factors, including plant species and variety. For example, there is a significant difference in sensitivity among strawberry varieties (Abyzov 2008). Among the most toxic pollutants for plants are cadmium and copper, but copper is more distributed in the environment (Report on the state of the environment in the city of Moscow in 2017 2018). At low concentration, Cu is a trace element essential as a micronutrient for higher plants at a low concentration. Copper plays key roles in photosynthetic and respiratory electron transport chains, in ethylene sensing, cell wall metabolism, oxidative stress protection, and biogenesis of the molybdenum cofactor (Yruela 2009).

Copper is a priority soil pollutant in major cities of Russia (State report 2017, 2018). There is a decrease in the concentration of copper in the soil (Report 2017, 2018). It was previously reported that the average copper contents in Moscow were 33.5 mg/kg (Report 2011, 2012), but the official data on soil pollution in Moscow are controversial due to the mosaic of soils. In some areas, there is an excess 1.03–1.34 times of approximate permissible concentration, which is 132 mg/kg (Report 2013, 2014).

Copper is used in agriculture to control oomycetes, fungi, and bacteria. Copper based-pesticides, in particular fungicides, bacteriocides, and herbicides, are widely used in agricultural practice throughout the world (Husak 2015). The use of Cu has long-term consequences due to its accumulation in the soil (Torre et al. 2018). High concentrations of copper are very toxic for plants, such as inhibition of photosynthesis. Cu is thus limiting the spread of lawn grass and flowering plants.

Cell selection has been used to produce plants that are resistant to adverse environmental factors. For example, previous reports show the production of salinity-resistant lawn grass (Gladkov et al. 2014), drought-resistant maize plants (Al'-Kholani et al., 2010), and other agricultural crops (Lestari 2006). Yet, there is less knowledge on breeding to produce metal-resistant plants resistant to heavy metals is rare (Gori et al. 1998); specifically, the objective of previous reports was limited to obtaining resistant cells (Jackson et al. 1984) and to study the effect of metals on cells (Gomes-Junior et al. 2006). This method has not been used to produce urban plants resistant to toxic metals. Therefore, here we breed *Agrostis stolonifera* and *Chrysanthemum carinatum* to test the Cu resistance of plant next generations.

Experimental

Plants

Agrostis stolonifera L. is a perennial grass species in the family Poaceae. *Agrostis stolonifera* has vegetative reproduction, unlike other lawn grasses. This allows creating various lawn for different purposes. The advantage of *Agrostis stolonifera* is that it does not need to be cut often; it withstands shadowing and is relatively resistant to gases.

Chrysanthemum carinatum Schousb. is an annual plant in the family Asteraceae. It has a height up to 60 cm and forms small bushes. This plant is widely used in urban landscaping in prefabricated and single plantings.

Callus induction and culture

We used seeds for getting callus of *Agrostis stolonifera* and *Chrysanthemum carinatum*. Callus of *Agrostis stolonifera* was obtained on Murashige-Skoog (MS)–modified medium. The medium was selected in our previous study (Gladkov et al. 2014).

Callus of *Chrysanthemum carinatum* was obtained on MS-modified medium supplemented with 1 mg/l 6-benzylaminopurine and 0.1 mg/l indole-3-acetic acid. This combination of cytokinin and auxin induced callus initiation from explants of *Chrysanthemum carinatum* (Litvinova and Gladkov 2012; Litvinova et al. 2017).

Selection of Cu-resistant species

To select tolerant clones callus were cultivated on Petri dishes on filter paper containing the various growth media and solutions of $CuSO_4.5H_2O$. The cultures were maintained in a growth room at 26 °C and exposed to a 16-h photoperiod in the light and humidity 70% (Gladkov et al. 2019). $CuSO_4.5H_2O$ was used in all experiments. In all experiments, data are calculated in terms of copper content.

To select tolerant clones, *Agrostis stolonifera* callus was cultivated on MS-modified medium supplemented with 1 mg/l 2,4-dichlorophenoxyacetic acid and 150 mg/l (Cu2+).

Callus tissue of *Chrysanthemum carinatum* was cultivated on modified MS medium supplemented with 0.5 mg/l 6-benzylaminopurine and 20 mg/l $CuSO_4.5H_2O$. Twice with an interval of 18 days, calluses, with an increase in biomass and without necrosis, were selected and re-transferred to a fresh solution medium. Thereafter, calluses were cultivated on MS-modified mediums without $CuSO_4.5H_2O$ for induction of shoots and roots.

Results and discussion

Cell selection *Agrostis stolonifera*

The aim of the work was the development of technology for obtaining resistant plants of *Agrostis stolonifera* to copper. To select tolerant plants, we developed methods of introduction into the culture *in vitro*, culturing, and plant regeneration. Callus tissue was cultivated on MS medium with copper in various combinations (100–450 mg/l). At 200 mg/l copper, only a few calluses were capable of morphogenesis. A concentration of 150 mg/l copper was selected as selective. The scheme selection included subcultivations on the medium for callus growth, one passage on the medium for shoot regeneration, and one passage on the medium for rooting (Fig. 1). All media contained 150 mg/l copper. The duration of cell selection of *Agrostis stolonifera* was 4–5 months.

The regenerants after cell selection were checked for the stability of copper resistance. Most of the tested plants produced from copper-resistant cells were more tolerant to copper than original plants. Most obtained plants possessed increased resistance to 150 mg/kg of copper. The copper resistance of the descendants of four generations of one of the most productive regenerants (descendants of clone "Margarita") was evaluated in water solution. The growth of control plants in an aqueous solution with 100 mg/l of copper was only 40 %, the growth of best plants obtained after the selection was more than 70% (Fig. 2). Descendants of four generations possessed increased resistance to 100 mg/kg of copper (Table 1). The tolerance has remained in the next four generations.

Selective plants were planted in open ground. The test plants fully retain high decorative quality (Fig. 3a).

Cell selection *Chrysanthemum carinatum*

The aim of the work was the development of technology for obtaining resistant plants of *Chrysanthemum carinatum* to copper. For cell selection, it was necessary to determine the sensitivity of callus cultures to copper (Fig. 4) and selective concentration. Calluses were placed on a medium MS with the addition of copper. For the second subculturing, we selected viable calluses with an increase in biomass, without necrosis, light green color. There was a high sensitivity of calluses in the second subculturing. This is probably due to the accumulation

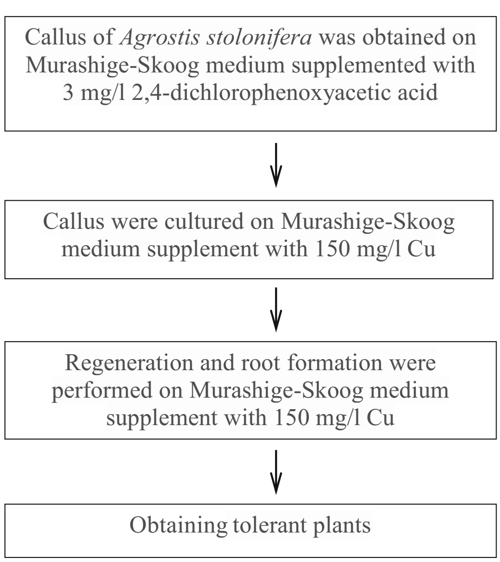

Fig. 1 Technology for obtaining resistant plants of *Agrostis stolonifera* to copper

Table 1 Influence of copper (100 mg/kg) on the growth of shoots of initial and selected *Agrostis stolonifera* in soil

Tested plants	Shoot growth, % of control
Original plants	51.0 ± 5.2
R 2	78.0 ± 8.6
R 3	75.0 ± 5.6
R 4	75.0 ± 10.0

Original plants, plants grown from the seeds without cell selection; R_1, first generation of regenerants; R_2, second generation of regenerants; R_3, third generation of regenerants; R_4, fourth generation of regenerants

of copper ions in the cultivated tissues or the exhaustion of the capabilities of the defense mechanisms. After 7 days of cultivation, necrosis appeared on calluses and part of the calluses died. A high level of inhibition was observed already at a copper concentration of 20 mg/l. Only some tissues retained embryogenic capacity and biomass growth. Increasing the concentration to 40 mg/l caused death in 86% of calluses.

Only some tissues retained embryogenic capacity and biomass growth. For the selection of resistant clones was chosen a selective concentration of 20 mg/l.

It was chosen scheme selection consists of culturing callus on MS medium with a concentration of copper 20 mg/l within thee subculturing, regeneration, and rooting without toxicant (Fig. 5). The duration of cultivation of shoots of *Chrysanthemum carinatum* was 3–4 subculturing.

As a result of the cell selection were received 21 plants of *Chrysanthemum carinatum* cv. Eldorado, 10 plants have rooted. Regenerants of *Chrysanthemum carinatum* retained their

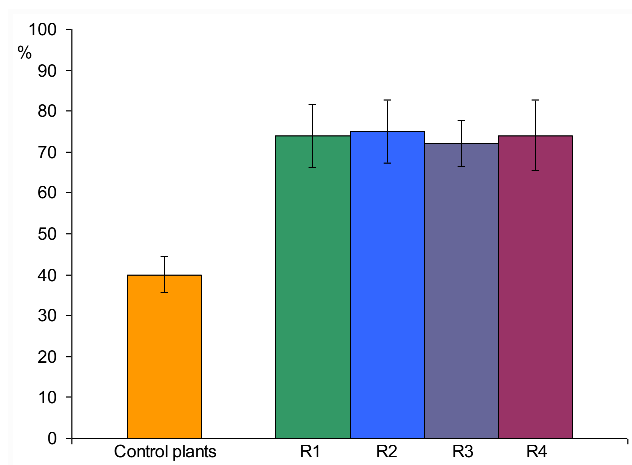

Fig. 2 Influence of copper (100 mg/l) on the growth of shoots of control and selected *Agrostis stolonifera* plants in water solution. Descendants of four generations possessed increased resistance to 100 mg/kg of copper compared to the control plants. The tolerance has remained in the next four generations. In the experiments was used copper sulfate ($CuSO_4.5H_2O$) with a final Cu^{2+} content of 100 mg/l. Control plants, plants grown from the seeds without cell selection; R_1, first generation of regenerants; R_2, second generation of regenerants; R_3, third generation of regenerants; R_4, fourth generation of regenerants

Fig. 3 Regenerants after cell selection. **a** Regenerant of *Agrostis stolonifera* was planted in the soil after cell selection. **b** Regenerant of *Chrysanthemum carinatum* was planted in the soil after cell selection. **c** The regenerant after cell selection formed large inflorescences comparable to control plants

decorative qualities; the diameter of the flowers was about 4 cm (Fig. 3b, c).

Plants of two descendants of the regenerants (no. 10, 18) of *Chrysanthemum carinatum* were checked for maintaining stability in the plant. Regenerants were planted in vessels with soil and copper was added at a concentration of 20 and 30 mg/kg. The regenerants showed increased resistance to this concentration, unlike the original plants (Table 2).

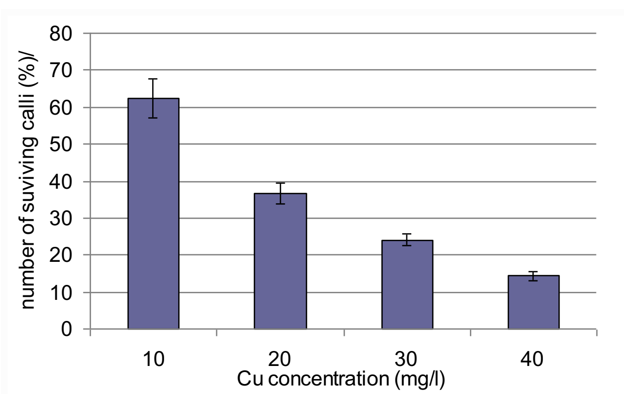

Fig. 4 Determination of selective copper concentration for a callus of *Chrysanthemum carinatum*. Influence of copper in various concentrations on callus of *Chrysanthemum carinatum* during the second subculturing on MS medium. In the second subculturing, the number of necrosis calluses increases. For the selection of resistant clones was chosen a selective concentration of 20 mg/l. In all experiments, data are calculated in terms of copper content

In the studied clones at 20 mg/kg of copper in the soil, the flowering was the same as in the plants in the soil without copper. At 30 mg/kg, the number of flowering plants decreased, but flowering plants fully maintained decorative qualities. In control plants, flowering was not observed at a concentration of 20 and 30 mg/kg. Thus, the developed environmental technologies have been effective in obtaining

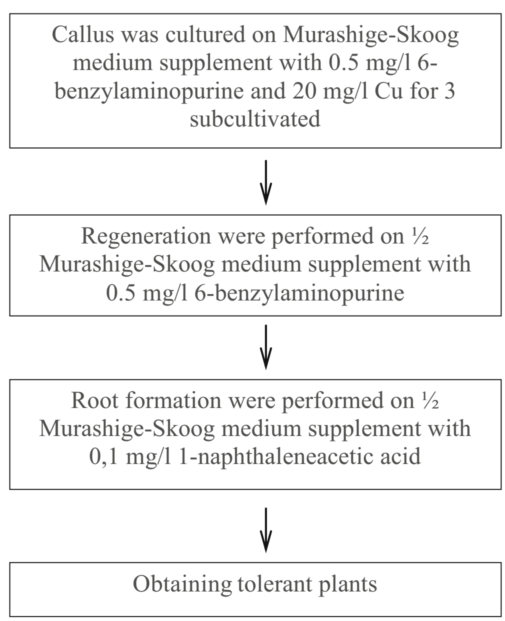

Fig. 5 Technology for obtaining resistant plants of *Chrysanthemum carinatum* to copper

Table 2 Effect of copper ions on the height of shoots of *Chrysanthemum carinatum*

The descendants of the regenerants	Copper concentration in soil (mg/kg)		
	0	20	30
No. 18	26.0 ± 1.1	30.0 ± 2.4	17.7 ± 0.5
No. 10	28.0 ± 1.2	24.3 ± 1.2	18.6 ± 0.4
Original plants	25.6 ± 0.9	15.2 ± 0.8	11.6 ± 1.0

resistance to the copper lawn grass *Agrostis stolonifera* and flowering plants *Chrysanthemum carinatum*.

Conclusion

Thus, the achievement and novelty of the work is the development of technologies for obtaining urban plants resistant to copper. The developed technologies have significant differences. Copper was used at all stages of the technology of obtaining resistant plants *Agrostis stolonifera*, as it has a greater resistance of callus and plants to copper, unlike in *Chrysanthemum carinatum*. Achievement and novelty of the work are to obtain not only resistant cells, as in similar works on agricultural work, but also to obtain plants with preservation of the sign of resistance to copper. For *Agrostis stolonifera*, for the first time, it is shown that 4 generations remain stable. It was taken more than 4 years to develop a technology for producing resistant plants *Agrostis stolonifera* and *Chrysanthemum carinatum*. For each plant species, environmental technology is unique.

Due to the high level of pollution of urban soils with copper, the introduction of this technology in the selection process will significantly reduce the adverse effects of toxicants on *Agrostis stolonifera* and *Chrysanthemum carinatum*. Since the average concentration of copper in the soil of Moscow is about 30 mg/kg, the resulting *Chrysanthemum* plants can be used in urban gardening.

The steady line *Agrostis stolonifera* can also be used in urban gardening with a high level of copper fouling (100 mg/kg). We first showed the possibility of using environmental biotechnology in urban greening to obtain, tolerating copper flowering plants and lawn grasses.

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Ilina Igorevna Tashlieva—30%
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Data availability All data generated or analyzed during this study are included in this published article

Compliance with ethical standards

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

Conflict of interest The authors declare that they have no competing interests.

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