Environmental Science and Pollution Research (2022) 29:25965–25969
https://doi.org/10.1007/s11356-022-19067-4

SHORT RESEARCH AND DISCUSSION ARTICLE

Cell selection for increasing resistance of ornamental plants to copper

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Received: 9 September 2021 / Accepted: 1 February 2022 / Published online: 14 February 2022

Abstract
Cell selection was used to obtain copper-resistant plants. Developed technologies for obtaining copper-resistant plants *Agrostis stolonifera* and *Chrysanthemum carinatum* can be applied to other plant species. We obtained copper-resistant plants *Festuca rubra*, *Brachycome iberidifolia* and *Linum perenne*. The concept of obtaining plants resistant to copper has been developed. This concept consists of two methods. The first method is applicable when calli is highly sensitive to copper. The second method is applicable when calli are moderately sensitive to copper.

Keywords Cell selection · *Agrostis stolonifera* · *Chrysanthemum carinatum* · *Festuca rubra* · *Brachycome iberidifolia* · *Linum perenne* · Copper

Heavy metals are among the priority pollutants (Sakson et al. 2018). Cu is one of the most enriched elements in soil. Especially high concentrations of copper in the soil are observed in areas located near copper smelter (Radojevic et al. 2017).

Copper is among the contaminant of agricultural soil (Sacristan and Carbo 2016; Napoli et al. 2019; Htwe et al. 2022). Copper is one of the urban soil pollutants. For example, in Copenhagen soils, total Cu concentrations were significantly higher compared to the reference agricultural soil (Li et al. 2014). Copper in urban soils is being accumulated mainly due to anthropogenic activities under rapid urbanisation (Li et al. 2018). Copper limits the spread of urban plants (Gladkov and Gladkova 2021b).

Cell selection has been used to increase plant resistance to copper. Technologies have been developed to obtain copper-resistant *Agrostis stolonifera* and *Chrysanthemum carinatum* plants. The detailed description of these technologies is given in Gladkov et al. (2021a). Can the developed technologies be used for other plant species? These technologies have been applied to other plant species.

The objects of our study were ornamental plants: *Festuca rubra* L., *Brachycome iberidifolia* Benth. and *Linum perenne* L.

Festuca rubra (red fescue) is used as a lawn grass in ornamental gardening. *Linum perenne* is a perennial plant with blue flowers.
Linum perenne is very effective in joint plantings with other flowering plants.
Brachycome iberidifolia features attractive foliage and blooms blue, purple, lilac, pink and white flowers (Fig. 1). *Brachycome iberidifolia* can be planted in rock gardens and flower beds.

Different modified mediums were used for each plant species. However, Murashige-Skoog modified medium was the same for two lawn grass species (*Festuca rubra* and *Agrostis stolonifera*) (Gladkov and Gladkova 2021a). Sucrose concentration was 30 g/L, casein hydrolysate 500 mg/L for *Festuca rubra* and *Agrostis stolonifera* in modified Murashige-Skoog medium. Seeds were used to obtain lawn grass callus.

Callus of *Agrostis stolonifera* was obtained on Murashige-Skoog modified medium with 3 mg/L 2,4-dichlorophenoxyacetic acid. Callus of *Festuca rubra* was obtained on

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Fig. 1 Brachycome iberidifolia

Murashige-Skoog modified medium with 3 mg/L 2,4-dichlorophenoxyacetic acid.

Calli of *Agrostis stolonifera* and *Festuca rubra* were cultured on Murashige-Skoog medium supplement with 1 mg/L 2,4-dichlorophenoxyacetic acid.

The composition of the mediums for *Chrysanthemum carinatum*, *Brachycome iberidifolia* and *Linum perenne* differed.

Gamborg modified medium was modified to obtain callus of *Brachycome iberidifolia* and *Linum perenne*. Callus of *Linum perenne* was obtained on Gamborg medium with 6 mg/L 2,4-dichlorophenoxyacetic acid. The regeneration of *Linum perenne* plants was on Gamborg modified medium with 1 mg/L 6-benzylaminopurine and 0.1 mg/L α-naphthaleneacetic acid. Calli (Fig. 2) were cultivated on Gamborg modified medium until shoots were formed. The duration of cultivation was 2–3 passages.

Rooting of *Linum perenne* plants was on Murashige-Skoog modified medium with the addition of naphthaleneacetic acid. Root development on liquid medium was faster than on agar medium. Seedlings were transplanted to liquid medium and mounted on filter paper bridges.

The regeneration of chrysanthemum plants was on Murashige-Skoog modified medium with 0.5 mg/L 6-benzylaminopurine.

To prevent the death of callus *Brachycome iberidifolia*, the growing cycle was no more than 21–26 days. The regeneration of *Brachycome iberidifolia* plants was on

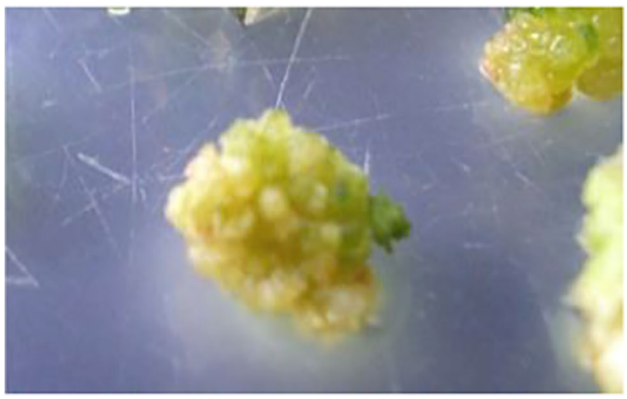

Fig. 2 Calli of *Linum perenne*

Murashige-Skoog modified medium with 2 mg/L 6-benzylaminopurine and 0.1 mg/L α-naphthaleneacetic acid.

Calli of *Agrostis stolonifera* and *Chrysanthemum carinatum* had different resistance to copper (Gladkov et al. 2021a). Therefore, we used different cell selection schemes.

Copper negatively affects morphogenic potential (Yemets et al. 2013; Ahmad et al. 2015).

However, small concentrations of copper can increase the regenerative capacity of calli (Kintzios et al. 2000; Malik et al. 2021). High copper concentrations always lead to a decrease in morphogenic potential (Gladkov et al. 2019, 2021a, b). Therefore, the use of copper at the stage of

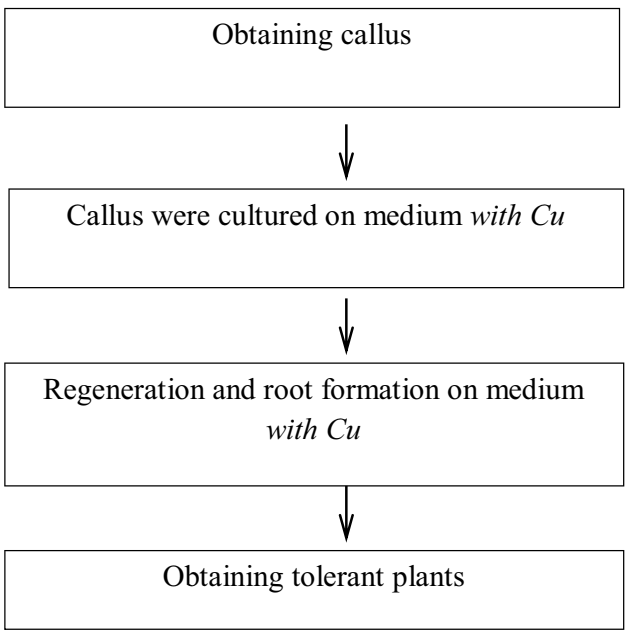

Fig. 3 Cell selection scheme for obtaining lawn grasses tolerant to copper

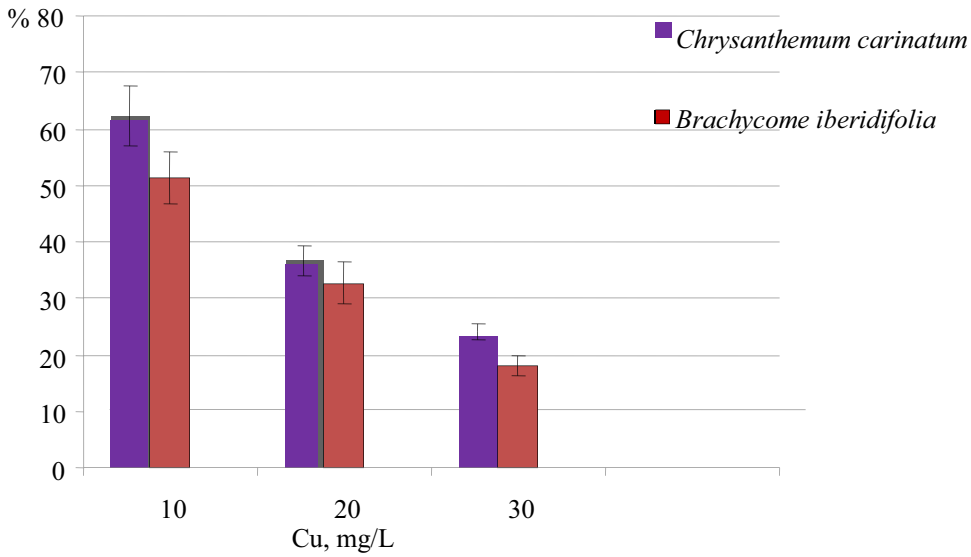

Fig. 4 Effect of copper concentration (mg/L) on callus survival in the second passage (%)

regeneration is impractical in the case of very high sensitivity of calli.

Calli of *Festuca rubra* and *Agrostis stolonifera* had comparable sensitivity to copper (Gladkov et al. 2021a, b). The selective copper concentration for calli of *Festuca rubra* and *Agrostis stolonifera* was 150 mg/L. Therefore, a cell selection scheme for *Agrostis stolonifera* was used for *Festuca rubra* (Gladkov et al. 2019, 2021a, b). Calli of *Agrostis stolonifera* and *Festuca rubra* showed moderate sensitivity to copper. Therefore, copper was used at all the stages of cultivation. We recommend using the developed scheme of cell selection with an average degree of resistance of calli to copper (Fig. 3).

The sensitivity of *Brachycome iberidifolia* calli was comparable to that of *Chrysanthemum carinatum* (Fig. 4). Calli of *Brachycome iberidifolia* was dead at the selective copper concentration for lawn grass.

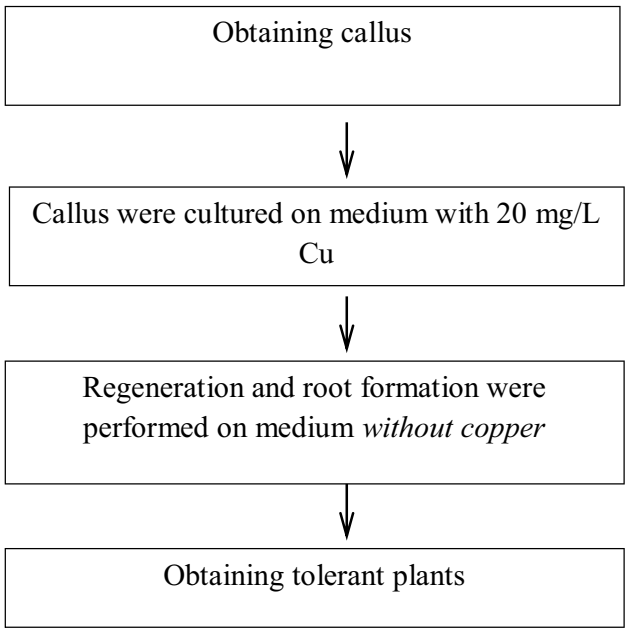

Fig. 5 Cell selection scheme for obtaining *Brachycome iberidifolia* and *Linum perenne* tolerant to copper

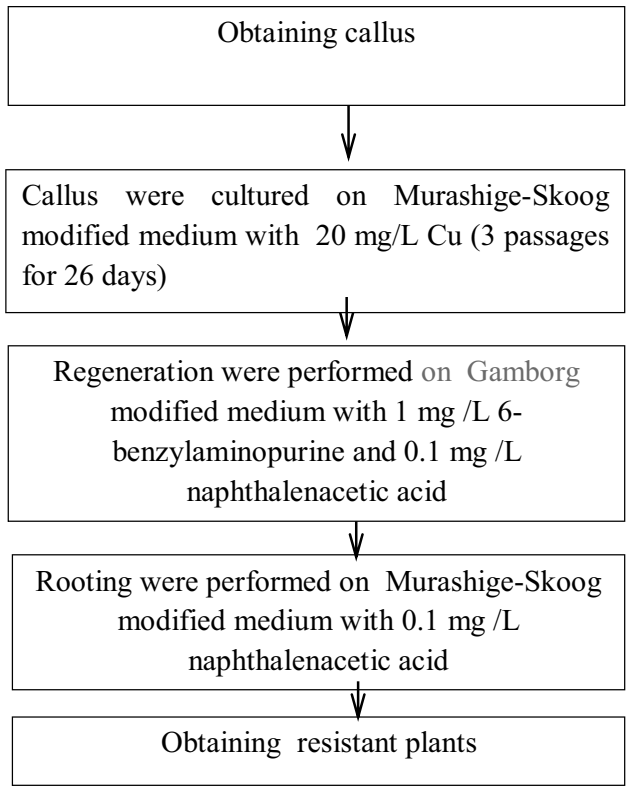

Fig. 6 Cell selection scheme for obtaining *Linum perenne* tolerant to copper

The selective copper concentration for calli of *Chrysanthemum carinatum*, *Brachycome iberidifolia* and *Linum perenne* was 20 mg/L. Calli of *Chrysanthemum carinatum*, *Brachycome iberidifolia* and *Linum perenne* were less resistant to copper than calli of lawn grass.

A cell selection scheme for *Chrysanthemum carinatum* was used for *Brachycome iberidifolia* and *Linum perenne* (Fig. 5). The duration of cultivation depended on the species.

Copper-resistant *Linum perenne* plants were particularly difficult to obtain. Various modified Gamborg and Murashige-Skoog media were used to obtain resistant plants (Fig. 6).

The concept of obtaining copper-resistant plants has been developed. This concept consists of two methods. The first method is applicable when calli is highly sensitive to copper. Copper is not used in the stages of regeneration and rooting. The second method is applicable when calli are moderately sensitive to copper. Copper is used at all the stages (cultivation, regeneration and rooting).

We obtained copper-resistant plants *Festuca rubra*, *Brachycome iberidifolia* and *Linum perenne*. Therefore, developed technologies for obtaining copper-resistant plants *Agrostis stolonifera* and *Chrysanthemum carinatum* can be applied to other plant species.

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Evgeny Aleksandrovich Gladkov—40%, Ilina Igorevna Tashlieva—30%, Olga Victorovna Gladkova—30%.

Funding The research was carried out within the state assignment of Ministry of Science and Higher Education of the Russian Federation (theme 121050500047–5).

Data availability All data generated or analysed during this study are included in this published article.

Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

Conflict of interest The authors declare no competing interests.

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