Increasing Tolerance *Agrostis Stolonifera*, *Festuca Rubra*, *Brachycome Iberidifolia*, *Chrysanthemum Carinatum* to Copper

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Abstract There were used biotechnological methods for stress-tolerant lines through tissue culture based in vitro selection. The authors developed technologies for obtaining plants *Agrostis stolonifera* L., *Festuca rubra rubra*, *Brachycome iberidifolia Benth., Chrysanthemum carinatum Schousb*. resistant to copper. It was carried on a previously investigation that these plants are very sensitive to copper. For each object of the study was determined a selective concentration of copper and developed selection scheme. The scheme selection of *A. stolonifera* and *F. rubra rubra* included two–three subcultivations on the medium for callus growth, one passage on the medium for shoot regeneration, and one passage on the medium for rooting. All media contained 150 mg/l copper. The scheme selection of *B. iberidifolia* and *C. carinatum* consists of culturing callus on medium with addition a concentration of copper 20 mg/l during two passages, regeneration, and rooting on mediums without toxicant.

Keywords *Agrostis stolonifera* · *Brachycome iberidifolia* · *Chrysanthemum carinatum* · Copper · Lawn grasses · Biotechnology

1 Introduction

The plants are an important part of the urban ecosystem. Lawn grasses are a basis of a grassy cover of Moscow, their role in the conditions of an adverse ecological situation continuously grows. Purpose and environmental conditions determine the choice of plants for the lawn. For example, *Agrostis stolonifera* L. forms the highest quality lawn. In addition to traditional lawns, meadow grasses are popular too. Meadow grasses consist of annual flowering grasses, for example, *Brachycome*

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R. Cárdenas et al. (eds.), *Proceedings of the 2nd International Conference on BioGeoSciences*, https://doi.org/10.1007/978-3-030-04233-2_15

iberidifolia Benth., *Chrysanthemum carinatum* L. Unfortunately, these plants as well as most ornamental plants are very sensitive to pollution of urban soil.

Among the most dangerous pollutants for plants are copper ions [1]. Copper is one of the main pollutants of urban soil, and it can lead to inhibition of plant grown, dark leaves, root elongation, height shoots reduction [2]. In direct contact with high concentrations of copper in the above-ground plant organs observed twisting and falling of the leaves. Young tissues and organs are particularly susceptible to excess copper. Damaged landscape can be restored with the help of biotechnology. Biotechnological methods allow keeping the biodiversity of grass cover.

Cellular selection is environmentally safe technology to create sustainable forms of plants. For example, cell selection was used to produce resistant plants and cell lines to heavy metals and chloride salinity [3–5]. However, there is almost no work of improving the sustainability of urban plants to copper.

2 Objects and Methods

2.1 Description of research objects

The objects of our study were grasses and flowering plants. *A. stolonifera* L. is a perennial grass, it has an advantage over many other lawn grasses. The lawn from *A. stolonifera* does not need to be cut often, and it withstands a shadowing and relatively resistant to gases (Fig. 1).

Fig. 1 *A. stolonifera*

B. iberidifolia Benth. is an annual herb, Asteraceae. The leaves of *B. iberidifolia* are fully divided, each having long and narrow segments from the midrib. The ray florets are varied in color, white through pink and blue to violet (Fig. 2).

B. iberidifolia used for decoration of the meadow grasses, flower beds, curbs, rocky hills.

Festuca rubra rubra is a perennial grass and is widely used in all types of lawns. Dark green glossy leaves fescue remain even during drought (Fig. 3). *F. rubra* persists in herbage 10 and more years.

C. carinatum Schousb. is annual plant, Asteraceae, height up to 60 cm. This plant is widely used in urban landscaping for growing in groups, mixborders, near shrubs (Fig. 4).

Fig. 2 *B. iberidifolia*

Fig. 3 *F. rubra*

Fig. 4 *C. carinatum*

2.2 In Vitro Selection

For getting callus it was used seeds. The methods for obtaining calluses, regeneration, and rooting of all plants were developed previously [6, 7]. Cultures were incubated in petri dishes on filter paper moistened with a solution of copper at 26 °C, exposed to a 16-h photoperiod in the light and humidity 70%. Callus cultures of *A. stolonifera*, *F. rubra*, *C. carinatum* were inoculated on Murashige and Skoog (MS) medium [8] supplemented with 2, 4-diclorophenoxiacetic acid (2, 4-D), 3% sucrose, 0.05% mg/l caseinhydrolysat, 0.7% agar-agar. Then callus was transferred to MS medium for regeneration and rooting. Callus of *B. iberidifolia* was cultured on Gambourg medium [9] supplement with 3% sucrose, 2 mg/l 2, 4-D, and 2 mg/l kinetin. Then embryo callus was transferred to ½MC medium supplement with 1 mg/l 6-Benzylaminopurine (BAP) and 0.1 mg/l 1-Naphthaleneacetic acid (NAA). Callus of *C. carinatum* was cultured on MS medium supplement with 3% sucrose, 1 mg/l BAP, and 0.1 mg/l Indole-3-acetic acid. For regeneration, callus was transferred to ½MC supplement with 0.5 mg/l BAP and for rooting −½MC supplement with 1% sucrose, 0.1 mg/l NAA.

Concentration of selecting agents for *A. stolonifera and F. rubra* was 150 mg/l $CuSO_4 * 5H_2O$. Callus of this plants was cultured on MS medium with addition 150 mg/l $CuSO_4 * 5H_2O$. After culturing for 1-month light callus with increased in size was collected. Then calli were transplanted on regeneration and rooting medium supplemented with copper at a concentration 150 mg/l.

Concentration of selecting agents for *B. iberidifolia* and *C. carinatum* was 20 mg/l $CuSO_4 * 5H_2O$. Calli were cultured during 26 days and then induction shoot and root on the mediums without copper.

3 Results and Discussion

For in vitro selection, it was chosen shock treatment, in which cultures are directly subjected to a shock of high concentration and only those which would tolerate that level will survive [10] (Fig. 5). The selective factor was present in the mediums at all stages of selection, including regeneration and rooting of shoots, in order to increase the probability of producing of resistant plants.

The authors developed technology for obtaining plants *A. stolonifera*, resistant to copper. The selection scheme included two–three subcultivations on the medium for callus growth, one passage on the medium for shoot regeneration and one passage on the medium for rooting (Fig. 6). All media contained 150 mg/l copper.

An increase of culturing time up to four months results in decrease of regenerative capacity. 78 out of 180 regenerated *A. stolonifera* plants were successfully rooted in the soil. To test the resistance to high concentrations of copper, thirty regenerants after cell selection were planted in soil containing 150 mg/kg copper (Fig. 7). The most of the tested plants produced from copper-resistant cells were more tolerant to copper than original plants.

The developed approaches for obtaining plants resistant to copper were used for *F. rubra.*

Calluses of *B. iberidifolia and C. carinatum* were much more sensitive to copper then cell tissue of *A. stolonifera* and *F. rubra*. We have been shown previously that

Fig. 5 Plant regeneration of *A. stolonifera* on the medium with copper

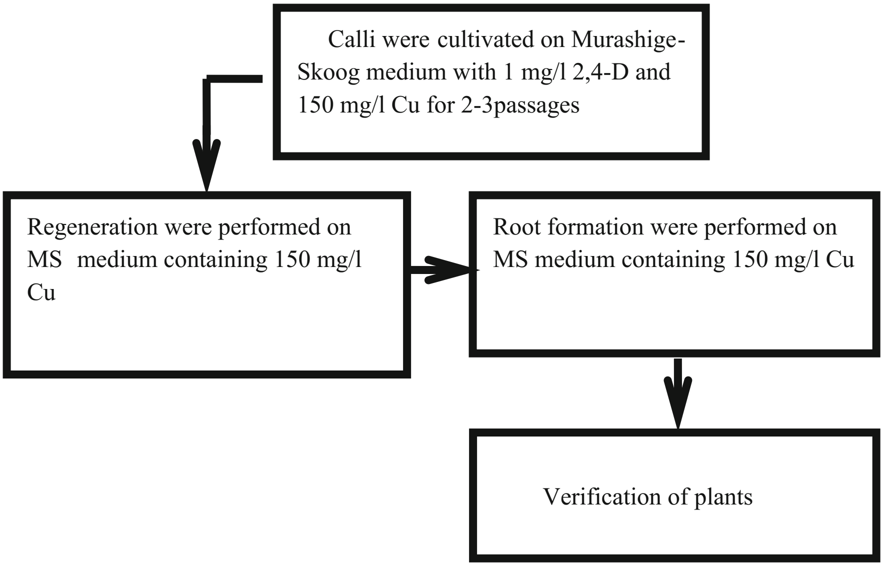

Fig. 6 Cell selection scheme of *A. stolonifera* for producing plants tolerant to copper

Fig. 7 Reentrants of *A. stolonifera* after cell selection

callus cultures of *B. iberidifolia* and *C. carinatum* were tolerant to 10 mg/l of copper, but at the concentration of 20–30 mg/l copper toxic effect is greatly enhanced. Therefore, it was necessary to substantially modify the approach for obtaining plants resistant to copper. It was chosen scheme selection consists of culturing callus on MS medium with a concentration of copper 20 mg/l within two passages, regeneration for 3–4 passages and rooting without toxicant (Fig. 8).

As a result of the selection were received 27 plants of *B. iberidifolia*. Most obtained regenerants possessed increased resistance to 20 mg/l of copper.

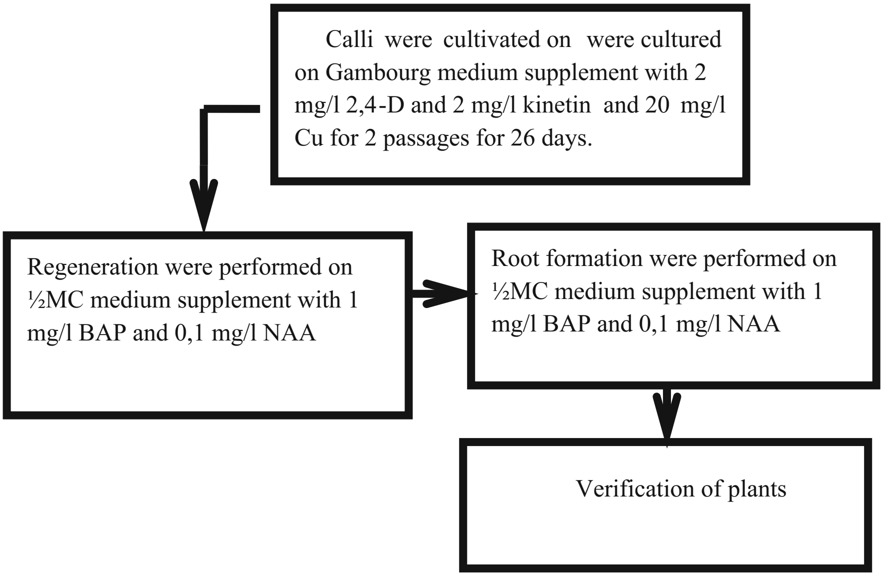

Fig. 8 Cell selection scheme of *B. iberidifolia* for producing plants tolerant to copper

The developed approaches for obtaining plants resistant to copper were used for *C. carinatum*. As a result of the selection was received 21 plants of *C. carinatum*, 10 plants have rooted.

Thus, there were 2 concepts for obtaining resistant plants with in vitro selection. In the first case, the method was suitable for more resistant plants (*A. stolonifera*, *F. rubra)* to copper. Cell selection and regeneration were carried out on media with a toxicant. On the other hand, *B. iberidifolia* and *C. carinatum* were very sensitive to copper ions, so the cultivation of calli was carried out only in the first stage, then regeneration and rooting were made without copper.

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