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Aspects of Regeneration *Chrysanthemum carinatum, Brachyscome iberidifolia, Linum grandiflorum, Linum perenne*, Biotechnology and Pollution

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ABSTRACT

Beautiful flowering plants can be used not only in ornamental gardening, but also in urban greening. However, ornamental plants are not tolerant to soil pollution in urban environments. Environmental biotechnological methods can be used to increase tolerance to urban environments. However, for environmental biotechnology, the development of technology for plant regeneration from calli is needed. For many ornamental plants, technologies for cell culture and plant regeneration have not been developed. The aim of this work was to regeneration *Chrysanthemum carinatum, Brachycome iberidifolia, Linum grandiflorum, Linum perenne* using in vitro methods. Technologies for regeneration of *Chrysanthemum carinatum, Brachycome iberidifolia, Linum grandiflorum, Linum perenne* plants have been developed.
Murashige-Skoog modified media were used for regeneration of *Chrysanthemum carinatum, Brachycome iberidifolia*. For example, for regeneration of *Chrysanthemum carinatum* plants, Murashige-Skoog modified medium with different contents of 6-benzylaminopurine were used. The highest shoot formation was on ½ Murashige-Skoog modified medium with 0.5 mg/L 6-benzylaminopurine. Gamborg modified medium were used for regeneration of *Linum grandiflorum and Linum perenne.*

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INTRODUCTION

Beautifully flowering plants are of interest in ornamental horticulture and floriculture.

Chrysanthemum is one of the most important and popular ornamental plants over the world (Zhao *et al*. 2009). Chrysanthemums are the second most important flowering crop after roses (Spaargaren, Geest, 2018; Eisa *et al*. 2022). Chrysanthemum has a cultivation history of more than 3000 years (Zhang, 2017; Gao *et al*. 2023). Among the ornamental traits of chrysanthemums include floral color, flower type, shape, floral scent, flowering time, and others (Mekapogu *et al*. 2022). Annual chrysanthemum is one of the most important flower crops (Nagdeve *et al*. 2021). Annual chrysanthemum is generally tall growing (Hawa *et al.* 2021)

Chrysanthemum carinatum belongs to the annual chrysanthemums.

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Chrysanthemum carinatum can be used as a cut flower. (Banerjee, & Ali 2000). *Chrysanthemum carinatum (Glebionis carinata, Ismelia carinata)* can be used extensively in ornamental horticulture.

Brachyscome iberidifolia as an ornamental plant is used in different countries of the world (Andrade *et al*. 2021). *Brachycome iberidifolia* has high ornamental qualities and high adaptive potential (Sarlaeva, Vasilyeva 2021)

Many members of the genus *Linum* are highly ornamental plants. For example, *Linum grandiflorum* is grown as a colorful ornamental annual in many parts of the world (Lyakh, Belova. 2016)

Biotechnological research on ornamental plants is of great interest.

For many ornamental plants, technologies for cell culture and plant regeneration have not been developed. Most biotechnological research is related to perennial chrysanthemum (Dendrodema). An efficient and rapid *in vitro* regeneration protocol was developed for chrysanthemum (*Chrysanthemum morifolium* Ramat) (Chowdhury *et al*. 2022). Increased flower diameter in regenerated plantlets via somatic embryogenesis has been shown (Ali *et al*. 2023).

A protocol for in vitro culture of *Linum grandiflorum* has been developed (Asad *et al*. 2021).

These beautiful flowering plants can be used not only in ornamental gardening, but also in urban greening. However, ornamental plants are not tolerant to soil pollution in urban environments.

Soil pollution with heavy metals is a global issue (Ahsan *et al*. 2021).

Heavy metals and deicing reagents are among the priority pollutants of urban soils. Urban plants are particularly sensitive to soil pollution by copper, cadmium and sodium chloride.

Environmental biotechnological methods can be used to increase tolerance to urban environments (Gladkov, Gladkova, 2022; Gladkov *et al*. 2021; Gladkov *et al*. 2022; Gladkov, Gladkova, 2023). However, to use environmental biotechnology, plant regeneration technology needs to be developed. The aim of this work was to regeneration *Chrysanthemum carinatum, Brachyscome iberidifolia, Linum grandiflorum, Linum perenne* under *in vitro* conditions..

MATERIALS AND METHODS

Chrysanthemum carinatum Schousb. (*Glebionis carinata, Ismelia carinata*), *Brachyscome iberidifolia* Benth. *(Brachycome iberidifolia), Linum grandiflorum* Desf., *Linum perenne* L. (Figure 1-3) were used as research objects.

Fig. 1. *Chrysanthemum carinatum (Glebionis carinata)*

Fig. 2. *Brachyscome iberidifolia (Brachycome iberidifolia)*

Fig. 3. *Linum perenne*

Various modified Murashige-Skoog and Gamborg media with 30 mg/L sucrose were used for callus formation and regeneration. 2,4-dichlorophenoxyacetic acid, α-naphthylacetic acid, Indole-3-acetic acid, 6-benzylaminopurine, and kinetin in various concentrations were added to the media.

Callus of *Chrysanthemum carinatum* was obtained on Murashige-Skoog modified medium with Indole-3-acetic acid and 6-benzylaminopurine.

Callus of *Brachycome iberidifolia* was obtained on Gamborg modified medium with 2,4-dichlorophenoxyacetic acid and kinetin.

Callus of *Linum grandiflorum* and *Linum perenne* were obtained on Gamborg modified medium and Murashige-Skoog modified medium with 2,4-dichlorophenoxyacetic acid.

Agarised nutrient media were used for callus formation and shoot production. Mean values were computed with Microsoft Excel.

RESULTS AND DISCUSSION

The wide use of annual chrysanthemum varieties in ornamental horticulture causes interest in the study and production of new forms by biotechnological methods. But the plant regeneration technologies developed for perennial chrysanthemum is not suitable for cell culture of *Chrysanthemum carinatum*.

For regeneration of *Chrysanthemum carinatum* plants (variety Eldorado), Murashige-Skoog modified medium with different contents of 6-benzylaminopurine were used. The calli were cultured on medium with 6-benzylaminopurine until seedling formation within 2-3 passages

(Figure 4). The highest shoot formation was on ½ Murashige-Skoog modified medium with 0.5 mg/L 6-benzylaminopurine (Figure 5). This medium was also used to obtain *Chrysanthemum carinatum* regenerants (variety Joy).

Thus the medium for regeneration of *Chrysanthemum carinatum* was different from that for *Chrysanthemum morifolium (*Yesmin *et al.* 2014).The calli of *Brachycome iberidifolia* were transplanted onto Murashige-Skoog modified medium with different contents of 6-benzylaminopurine and naphthalenacetic acid.

We used Murashige-Skoog modified medium with half the content of all mineral components and a sucrose content of 15 mg/L. The duration of cultivation was 2-3 passages.

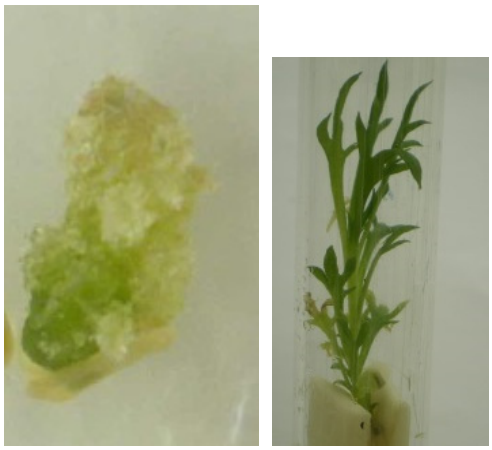

Fig. 4. Callus and Regenerant of *Chrysanthemum carinatum*

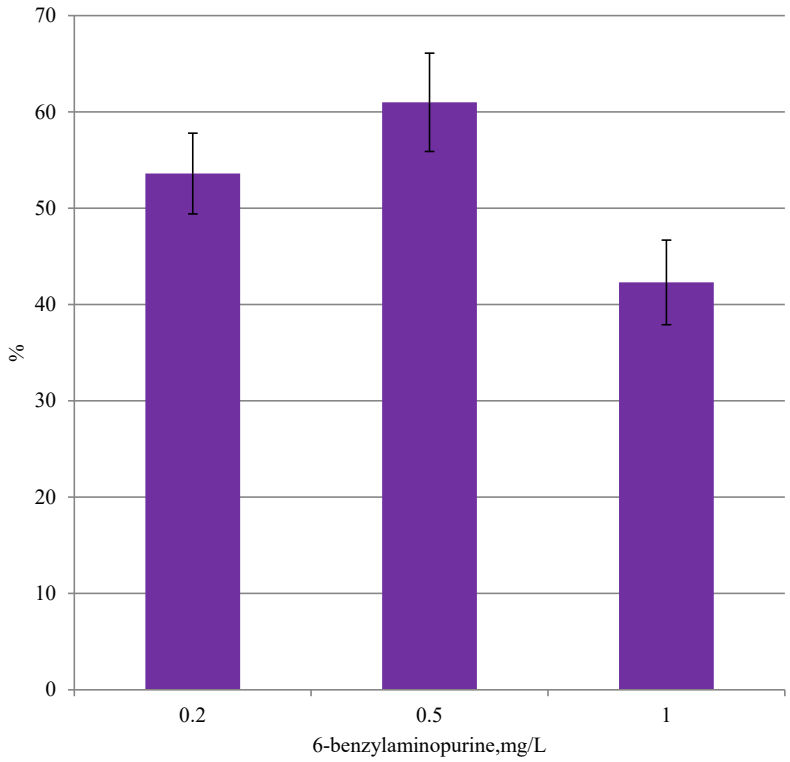

Fig. 5. Effect of 6-benzylaminopurine on the frequency of shoot formation of *Chrysanthemum carinatum* (variety Eldorado)

Table 1. Effect of 6-benzylaminopurine and naphthalenacetic acid on *Brachycome iberidifolia* regeneration Murashige-Skoog modified medium .

6-benzylaminopurine, mg/L	naphthalenacetic acid, mg/L	Frequency shoot formation, %
1	0,5	21 ± 1,7
2	0,1	62 ± 4,3
2	0,5	47 ± 3,0
2	1	43 ± 3,6

The highest percentage of shoot formation of *Brachycome iberidifolia* was observed on medium with 2 mg/L 6-benzylaminopurine and 0.1 mg/L naphthalenacetic acid (62%).

A large number of combinations of growth regulators in combination with amino acids have been tried to stimulate morphogenesis of *Linum grandiflorum* (variety Blue) and *Linum perenne*.

The biotechnological aspects of regeneration of *Linum grandiflorum* and *Linum perenne* were similar. The formation of the largest number of shoots was observed on Gamborg modified medium at concentrations of 6-benzylaminopurine 1 mg/L and 0.1 mg/L naphthalenacetic acid during in 2 passages. A high percentage of regenerants (more than 50%) was obtained when naphthalenacetic acid were added up to 0.5 mg/L. Gamborg modified medium supplemented with 1 mg/L 6-benzylaminopurine and 0.1 mg/L naphthalenacetic acid was also used for the formation of shoots of *Linum grandiflorum* (varieties Rubrum and Sharm).

However, the biotechnological aspects of callus formation of different varieties of *Linum grandiflorum* had differences. The mediums on which calli were previously obtained differed. To obtain callus of *Linum grandiflorum* (variety Blue), Murashige-Skoog modified medium with 8 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D) was used. Gamborg modified medium with 4 mg/L 2,4-D was used to obtain callus of *Linum grandiflorum* (variety Rubrum), for variety Charm with 6 mg/L 2,4-D. To obtain calli of *Linum perenne* (variety Blue Silk), we used Gamborg modified medium with 6 mg/L 2,4-D.

Thus technologies for regeneration of *Chrysanthemum carinatum, Brachycome iberidifolia Linum grandiflorum, Linum perenne* plants have been developed.

The selected modified media for plant regeneration can be used in the development of various environmental biotechnology (Gladkov et al. 2021; Gladkov et al. 2022). In the development of biotechnologies for obtaining plants resistant to environmental pollution, an important step is plant regeneration. This is because plants resistant to toxicant pollution are of greatest interest, not callus. As media for regeneration of *Chrysanthemum carinatum, Brachycome iberidifolia Linum grandiflorum, Linum perenne* plants resistant to soil pollution (for example, soil pollution to copper) it is possible to use selected media for the species under research. The most interesting is the production of the studied plant species resistant to soil contamination with copper and sodium chloride. Toxicant (e.g. copper) may or may not be added to the selected medium during the regeneration step, depending on the cell selection scheme. It depends on the duration of cultivation of calli with toxicant(e.g. copper), the resistance of calli to the toxicant and other factors. Prolonged cultivation of callus on medium supplemented with toxicant can significantly reduce regeneration ability. Therefore, the duration of callus cultivation should not exceed 2-3 passages.

CONCLUSION

Technologies have been developed for 4 species of ornamental plants.

Regenerants of *Chrysanthemum carinatum, Brachycome iberidifolia, Linum grandiflorum, Linum perenne* were obtained. The biotechnological aspects of regeneration of the studied

plant species differed. Murashige-Skoog modified medium were used for regeneration of *Chrysanthemum carinatum, Brachycome iberidifolia*. Gamborg modified medium were used for regeneration of *Linum grandiflorum* and *Linum perenne.* 6-benzylaminopurine was used for regeneration in all species tested. α-naphthylacetic acid was used to regenerate *Brachycome iberidifolia, Linum grandiflorum* and *Linum perenne.*

Thus the data obtained are of interest for general and environmental biotechnology.

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.

AUTHORS CONTRIBUTIONS

Conceptualization: Evgeny A. Gladkov; Ilina I.Tashlieva; Olga V.Gladkova
Methodology of the article: Evgeny A.Gladkov; Ilina I. Tashlieva; Olga V. Gladkova
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