

Optimization of clonal micropropagation of *Chrysanthemum*

I. Borodulina¹, A. Trufanova¹, G. Shevchenko¹, G. Sokolova¹, T. Plaksina²

¹Altai State University pr. Lenina 61, Barnaul, 656049, Russia. E-mail: borodulina.irina@gmail.com

²Federal State Budget Scientific Institution Federal Altai Scientific Centre of Agro-BioTechnologies Nauchnyy gorodok, 35, Barnaul, 656910, Russia E-mail: niilisavenko1@yandex.ru

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Micropropagation of *Chrysanthemum* is an alternative to the traditional method of reproduction. Thanks to this method, the *Chrysanthemum* reproduction time is reduced to 3-4 months. For clonal micropropagation, sterile microshoots of *Chrysanthemums* of the varieties Snow White, Stranger, and Baltica White were used. At the stage of the micropropagation, the Murashige-Skoog (MS) medium with the full and half composition of mineral salts and growth regulators (kinetin, 6-benzylaminopurine, β -indolylacetic acid) were used. A universal culture medium for clonal micropropagation of all varieties of *Chrysanthemum* and optimal mediums, taking into account variety-specificity were established. It was noted that under *in vitro* conditions, high proliferative activity was observed in Neznakomka variety.

Key words: Clonal micropropagation; *Chrysanthemum*; Reproduction; Microshoots

Introduction

The floriculture industry has become one of the most important branches of agriculture. The largest producers of flower and decorative crops are currently the Netherlands, Poland, USA, Japan, Italy, Germany, Belgium and Canada, and the main consumers are Germany, USA, France and the UK (Getu, 2009). In Russia, the demand for new plants is growing in order to use them for internal and external gardening. Therefore, the problem of mass reproduction of annual and perennial ornamental and flower crops becomes relevant (Timofeeva, Nevmerzhitskaya, 2012). Decorative *Chrysanthemums* are one of the first flower crops on the world market (Teixeira da Silva, Kulus, 2014). They have been cultivated for over 2000 years. Currently, in Japan alone, there are more than 15 000 varieties of *Chrysanthemums* (Datta, 2013). Traditional methods of propagation of *Chrysanthemums* do not satisfy the needs of the market. Biotechnological methods are widely used in the propagation of *Chrysanthemums* in an *in vitro* culture, as evidenced by numerous publications (Datta, 2012; Deen et al., 2013a, b; Kolomiets, Malyarovskaya, 2013; Kulus et al., 2013; Kuritskaya et al., 2016; Lim et al., 2012; Shinoyama et al., 2006; Teixeira da Silva et al., 2013; Tymoszyk A., Zalewska M., 2014; Zhou et al., 2014). Biotechnological methods of clonal micropropagation of *Chrysanthemums* are quite expensive. Therefore, reducing the time of growing *Chrysanthemums* to a product standard is an urgent problem. An important aspect is to reduce the cost of production of planting material without changing its quality. The purpose of our research is to increase the efficiency of clonal micropropagation of *Chrysanthemum*.

Material and Methods

Research was conducted at the Altai Center for Applied Biotechnology of Altai State University. For propagation, three varieties of *Chrysanthemums* from the *in vitro* collection were used: Snow White and Neznakomka (Korean *Chrysanthemum*), Baltica White (Indian *Chrysanthemum*). For clonal micropropagation of *Chrysanthemum*, the induction process of adventitious and axillary shoot formation was used. The explants were microcranes with a leaf and axillary kidney. Explants were grown in flasks on eight variants of culture mediums (Table 1).

Table 1. Variants of culture medium for clonal micropropagation of *Chrysanthemum*.

№	The composition of the culture medium	n
1	MS (the control)	15
2	MS + Kn 1.0 μ M + IBA 0.2 μ M	15
3	MS + BA 1.0 μ M + IBA 0.2 μ M	15
4	MS + Kn 1.0 μ M + IBA 0.2 μ M + agar 4.0 g L ⁻¹ + starch 20.0 g L ⁻¹	15
5	0.5MS (the control)	15
6	0.5MS + Kn 1.0 μ M + IBA 0.2 μ M	15
7	0.5MS + BA 1.0 μ M + IBA 0.2 μ M	15
8	0.5MS + Kn 1.0 μ M + IBA 0.2 μ M + agar 4.0 g L ⁻¹ + starch 20.0 g L ⁻¹	15

The cultivation of *Chrysanthemum* microshoots was carried out under the following conditions: light mode – 16/8, illumination – 3000 lux, temperature – 24 \pm 1°C. The growth and development of regenerants was evaluated on day 30 of cultivation. Statistical data processing was performed using the Microsoft Office Excel 2010 software. The arithmetic mean, arithmetic mean error, confidence interval, and Student t-test values were calculated at $P \leq 0.05$.

Results and Discussion

In our experiments, active proliferation of adventitious and axillary shoot formation was observed in the fourth and eighth variants (Figure 1). The reproduction coefficient in the control was 4.3 ± 0.6 , in the fourth and eighth variants were 3.8 ± 1.1 and 4.2 ± 0.9 respectively.

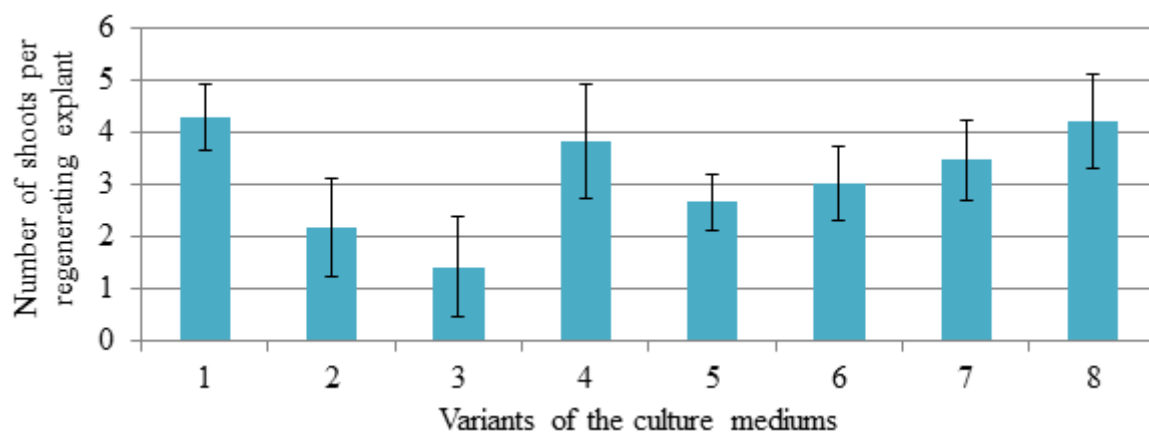


Figure 1. The reproduction rate of *Chrysanthemum* *in vitro* culture.

In general, a decrease in the mineral composition of culture mediums (variants 5–8) had a positive effect on the regeneration of *Chrysanthemum*. milar results were obtained, E.V. Kuritskaya et al. (2016) during the propagation of microprobes of the East Asian species of *Chrysanthemum Chrysanthemum leiophyllum*. Optimal for this variety was the MS medium with half the content of mineral salts. The maximum reproduction rate (5.4 ± 1.3) was recorded in the variety Snow White on the fourth variant of a culture medium with a complete composition of mineral salts. It was 4.5 times higher than the same indicator in other variants. Varieties Neznakomka and Baltica White, on the contrary, had maximum reproduction rate (4.4 ± 0.7 and 5.1 ± 0.9 , respectively) in the eighth variant of the culture medium with half the composition of mineral salts. Thus, in our studies, the variety-specificity of *Chrysanthemums* during clonal micropropagation was confirmed, which is also noted in the works of Granda Hamillo (2009). The height of *Chrysanthemum* microshoots in an *in vitro* culture varied depending on the composition of the culture medium (Figure 2).

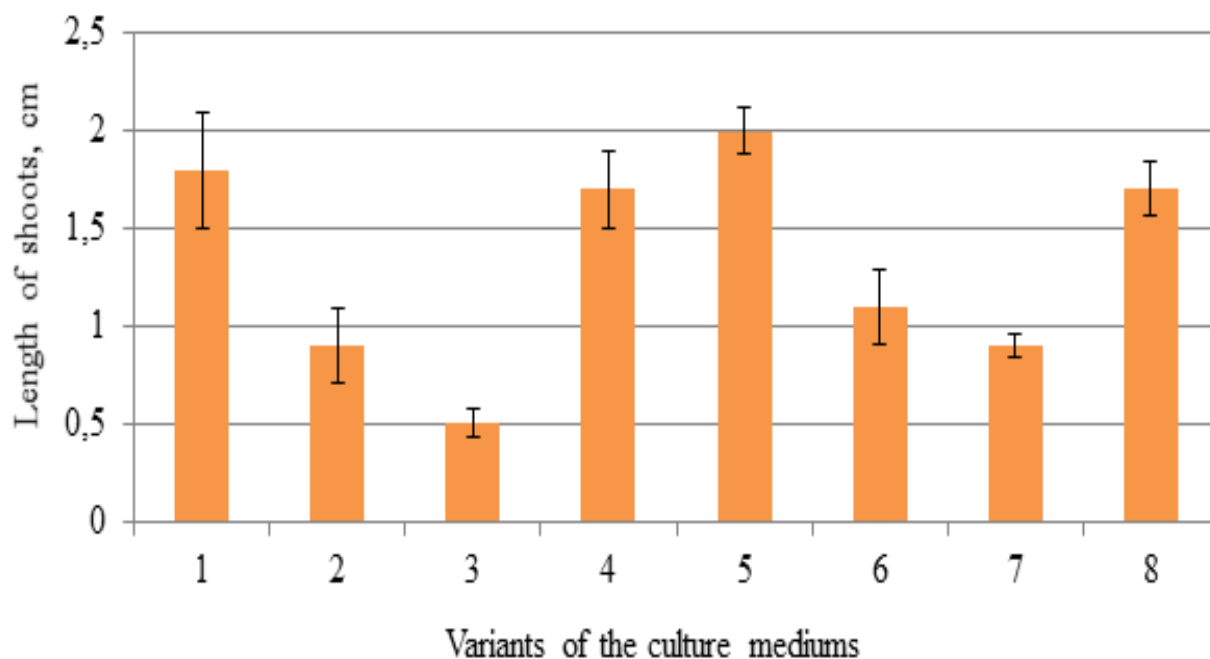


Figure 2. *Chrysanthemum* shoot height *in vitro* culture.

In our experiments, larger microshoots with a height of about 2 cm were formed in the control version and in fourth and eighth variants with the full and half composition of mineral salts. On nutrient media with other compositions, the height of the microprobe did not exceed 1 cm (Figure 3). An important parameter is the foliage of the shoots, which leads to the production of a large number of microshoots and new regenerants. The maximum number of leaves (8.0 ± 1.1 pcs/shoot) was formed in the control variant and in the fourth variant. A decrease in the content of mineral salts in the culture medium did not lead to a significant decrease in the leafiness of microshoots.

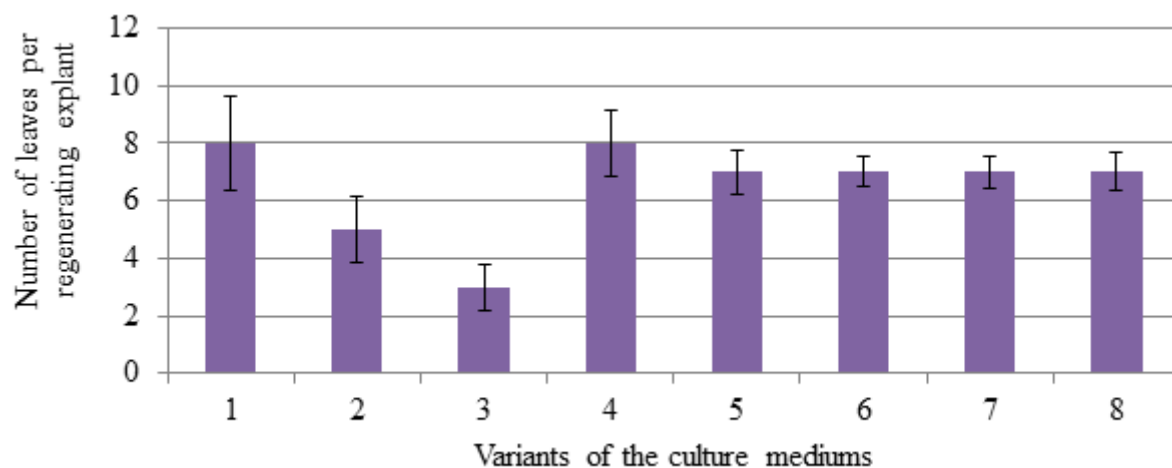


Figure 3. Obligacy of *Chrysanthemum* *in vitro* culture.

Conclusion

The universal culture medium for the clonal micropropagation of Snow White, Stranger, and Baltica White *Chrysanthemum* varieties is 0.5 MS + Kn 1.0 μM + IBA 0.2 μM + agar 4.0 g L⁻¹ + starch 20.0 g L⁻¹ (variety multiplication factor was 5.4 ± 1.3 , 4.4 ± 0.7 , 5.1 ± 0.9 , respectively). The optimal culture medium were identified for each variety: for Snow White – MS + Kn 1.0 μM + IBA 0.2 μM + agar 4.0 g L⁻¹ + starch 20.0 g L⁻¹; for the Stranger and Baltica White – 0.5 MS + Kn 1.0 μM + IBA 0.2 μM + agar 4.0 g L⁻¹ + starch 20.0 g L⁻¹. Higher proliferative activity is characterized by the Neznakomka variety.

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