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Innovative technologies for cloned plants adaptation

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ABSTRACT

Chrysanthemum plants are popular with amateur gardeners and breeders. It is widely used in landscaping cities, parks, and also for bouquets. Plants are notable for longer flowering and a bright color of flowers. Extracts of chrysanthemum plants contain various biologically active substances with bactericidal properties and are used in pharmacology and dentistry. The objective is to develop an effective technology for rapid *in vitro* reproduction and adaptation of chrysanthemum microclones. The objects of the study were chrysanthemum plants of three varieties: Bacardi, Korean Dawn, and Snow White. Semifloscules and flower buds were used as primary explants. The explants were cultured on a Murashige and Skoog (MS) nutrient medium containing mineral salts, as well as various growth regulators: 1) 1 mg L^{-1} BAP + 0.5 mg L^{-1} IAA; 2) 15% Aminoven solution 3 mL L^{-1} ; 3) Stimul 1 mL L^{-1} ; and 4) a hormone-free medium. Microshoots were adapted using an aeroponic system. Unrooted microshoots were used for adaptation. The GrowPlant X-Stream 120 propagator was used as equipment for adaptation. The frequency of adventious budding depended on the hormonal composition of the nutrient medium. Aminoven in the nutrient medium led to the induction of the formation of adventitious buds on average in 85% of cases. The same indicator averaged 72% on the Stimul-containing medium. In a BAP-containing medium, the considered indicator was the same with the control variant (hormone-free medium). The Snow White explants had the highest regenerative activity, Bacardi was second, and Korean Dawn was third. Studies have shown that the efficiency of adaptation of unrooted microshoots under aeroponic conditions is significantly influenced by the composition of the nutrient medium during micropropagation. Thus, preliminary cultivation of microshoots *in vitro* on Stimul and Aminoven-containing media led to the rapid formation of the root system and active growth of the aerial part. In these variants, the shoot growth index (I) was 0.65-0.71, and specific rate (μ) was 0.89-1.1 mm day^{-1}.

Keywords: Chrysanthemum, Clonal micropropagation, Semifloscules, Adaptation, Aeroponics.
Article type: Research Article.

INTRODUCTION

Globalization and the information revolution have exacerbated the major problems in the world economy. This circumstance must be considered in the Russian Federation, as the modern development of the country's economy is associated with import substitution. This issue became particularly urgent back in 2015, when the United States and the countries of the European Union and other Western partners extended economic sanctions against Russia. Therefore, it is necessary to revise the production management systems and receive their competitive high quality products. This direction is of particular relevance for floriculture, as the basis of the Russian flower market is still import at all levels: seeds, bulbs, planting material, and cut flowers. Holland accounts for over 60% of the total volume of imported flowers to Russia, as well as Ecuador, Kenya, and Zambia. However, imported planting material is often of poor quality, due to an infectious background in plants in the form of pathogens, microorganisms or entomophages (Chalinee & Kamnoon 2011; Das *et al.* 2014). Therefore, as the Russian

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economy is transiting to import substitution, it is necessary to stop the supply of imported plant material and switch to mass propagation of plants of domestic selection. Chrysanthemum plants are highly popular among flowering plants; many of their species and varieties are valuable ornamental plants, widely used in landscaping cities, parks, as well as for bouquets (Deein *et al.* 2013; Labade *et al.* 2016; Kalashnikova 2017). Such popularity of chrysanthemums is due to their long bloom period and a bright color of flowers. In addition, plants and their extracts have bactericidal properties and are used in pharmacology, dentistry, food industry, due to the content of various biologically active substances (Murashige & Skoog 1962; Malaeva 2015). Currently, there are more than 10 thousand varieties of chrysanthemum, which vary in different morphological, biological and other characteristics. It has been reported that it is necessary to have high-quality seed or planting material, which requires certain growing conditions, to obtain well-flowering plants. The use of traditional reproduction methods does not always lead to the genetic stability of plants. Therefore, it is necessary to conduct research on the conservation of plants of various taxonomic groups and search for alternative methods of their reproduction (Barakat *et al.* 2010; Zahrae Redouan *et al.* 2020; Abolhasani *et al.* 2021). The use of biotechnology methods, in particular, clonal micropropagation, not only preserves and reproduces valuable specimens but also creates an *in vitro* collection aimed at preserving plant biodiversity (Chae 2014). Based on the above, the objective is to develop a rapid propagation technique and obtain a high-quality planting material for chrysanthemums *in vitro*.

MATERIAL AND METHODS

The objects of the study were chrysanthemum plants of three varieties: Bacardi, Korean Dawn, and Snow White (Fig. 1).

a b c

Fig. 1. Studied varieties of chrysanthemum plants: a - Bacardi, b - Korean Dawn, c - Snow White.

The primary explants were semifloscules and flower buds isolated from cut plants. All explants were divided into 4 groups by: 1) the location of semifloscules in the bud (group I - isolated from the peripheral part of the bud, group IV - from the center, II and III groups - from the middle of the bud) (Figs. 1-2) the stage of a flower bud development (group I - full-blown buds, group IV - unblown buds, groups II and III - half-blown buds) (Fig. 2).

Fig. 1. Semifloscules isolated from chrysanthemum flowers.

Group I Group II Group III Group IV

Fig. 2. Groups of buds isolated from chrysanthemum plants.

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Semifloscules and flower buds were sterilized with 0.1% mercuric chloride solution for 4.5 minutes (Borodulina *et al.* 2019), after which they were washed in three portions of sterile distilled water. The explants were cultivated on a Murashige and Skoog (MS) nutrient medium containing mineral salts (Murashige & Skoog 1962), as well as various growth regulators: 1) 1 mg L^{-1} BAP + 0.5 mg L^{-1} IAA; 2) 15% Aminoven solution 3 mL L^{-1} ; 3) Stimul 1 mL L^{-1} ; 4) a hormone-free medium. pH of the medium in all variants was 5.5-5.8. The formed adventious buds were transplanted onto a hormone-free MS medium for further growth and formation of microshoots. During micropropagation, all microshoots were put in a culture room, at a temperature of +21-23 ºC , a 16-h photoperiod, under illumination with white fluorescent lamps (OSRAM AG, Germany) with 3-3.5 thousand lux. Microshoots were adapted using an aeroponic system. For this, unrooted microshoots were used. GrowPlant X-Stream 120 (Netherlands), an aeroponic cloner for 120 plants, was used as equipment for adaptation. The machine operated with Rastvorin granular mineral fertilizer (Russia), which included potassium (18-28%), nitrogen (8-18%), phosphorus (5-18%), manganese 0.1%, boron 0.01%, copper 0.01%, zinc 0.01%, molybdenum 0.001%. In addition, 3 General Hydroponics liquid complex mineral fertilizers (Flora Series) were used: FloraGrow, FloraBloom, FloraMicro [General Hydroponics Europe (GHE), France]. The results of biometric indicators (height of the aboveground part, cm) were considered over time after planting microshoots in aeroponics. The growth index (I) and the specific growth rate (μ) were calculated using the formulas:

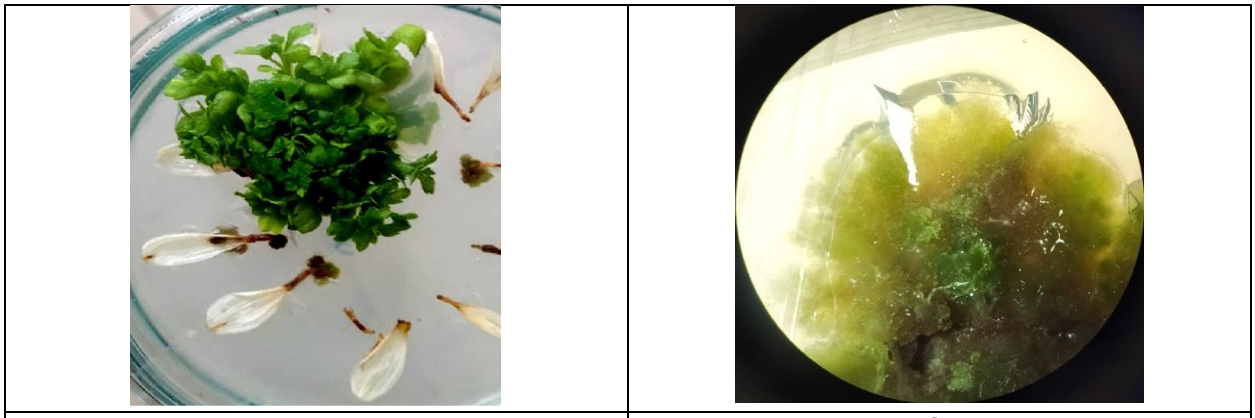
$$I = \frac{X_{max} - X_0}{X_0},\ \mu = \frac{\ln X_2 - \ln X_1}{t_2 - t_1},$$

where X_{max} and X_0 are maximum and initial values of the height of microshoots, cm, X_2 and X_1 are the height of microshoots (cm) at the time t_2 and t_1, days, respectively. Experimental data were processed on the basis of the methods of mathematical statistics (Adedji 2020; Dospekhov 2011). Analysis of variance and regression was carried out in MS Excel.

RESULTS

Culturing of semifloscules in vitro

The main regulatory factor of all morphogenetic processes is the hormonal composition of the nutrient medium. The correct selection of hormones, their concentrations and combinations leads to the maximum effect. However, this process also depends on the studied genotype (Barakat *et al.* 2010; Chae 2014; Kalashnikova 2017; Milekhin 2015). Our experiments found that, regardless of the studied variety, the formation of morphogenic structures was observed as early as 10 days from the beginning of culturing. As a rule, adventious buds were formed in the basal part of semifloscules. This process took place either directly on the primary explant or in the callus tissue (Fig. 3). The rate of adventious buds depended on the hormonal composition of the nutrient medium (Fig. 4).



a b

Fig. 3. Formation of adventious buds (a) or callus tissue (b) at the base of the semifloscules of chrysanthemum, Snow White variety.

Aminoven in the nutrient medium led to the induction of the formation of adventitious buds on average in 85% of cases. The same indicator averaged 72% on the Stimul-containing medium. In a BAP-containing medium, the considered indicator was the same with the control variant (hormone-free medium). The stimulating effect of

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Aminoven can be explained by 17 nonessential and essential L-amino acids (alanine, arginine, tyrosine, proline, glycine, etc.) contained in the solution, the effect of which on the somatic cells morphogenetic potential was proved by R.G. Butenko (Barakat *et al.* 2010)

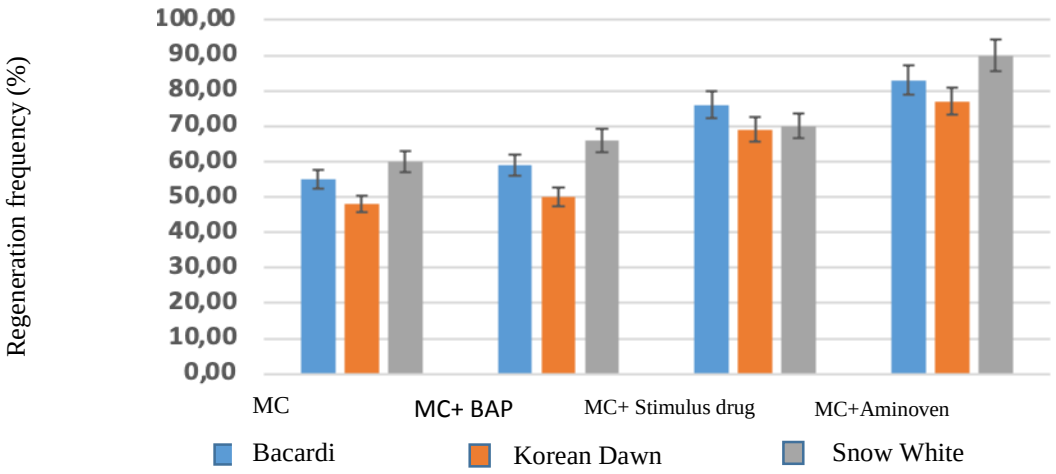

Fig. 4. The effect of nutrient medium composition on the rate of formation of adventious buds on chrysanthemum semifloscules.

Semifloscules of the Snow White variety showed to have the highest regenerative activity, Bacardi plants were second, and Korean Dawn ones were third. Probably, such a ranking of varieties by morphogenetic activity is associated with the color of semifloscules. Brightly colored flowers are known to synthesize more phenolic compounds, which have a certain inhibitory effect on the subsequent activity of somatic cells (Chae 2014) The rate of regeneration was experimentally proved to depend not only on the varietal characteristics of the explants but also on its size and age. Adventious buds developed more intensively in semifloscules of group I. The ones closer to the flower center (groups III and IV) were uncapable of morphogenesis. Therefore, this group is not advisable to use as a primary explant when introducing them into *in vitro* culture because they quickly died due to their small size.

Culturing of flower buds in vitro

Initially, flower buds of groups I-IV were cultured on MS nutrient medium containing BAP 1 mg L^{-1} in combination with IAA 0.5 mg L^{-1} . The formation of adventious buds was observed already on the 12^{th} day from the beginning of culturing. As a rule, *de novo* buds formed in the basal part of flower buds, which later developed into microshoots (Fig. 5).

Morphogenesis was found to depend on the studied variety and the type of primary explant. Thus, explants isolated from Snow White chrysanthemum plants had the highest morphogenetic activity. This variety was characterized by the formation of 7-8 adventious buds in the basal part of flower buds. For other varieties, the considered indicator did not exceed 2-3 pieces. Buds of groups II and III were the most active. In these variants, isolated explants stayed highly viable, with 87% regeneration rate.

The formed adventitious buds on semifloscules and flower buds were subsequently separated from the primary explant and independently cultured on a hormone-free MS nutrient medium. Under these conditions, the formation of microshoots was observed, which were transferred to *ex vitro* conditions for adaptation. GrowPlant X-Stream 120 was used for this purpose. Chrysanthemum microshoots were taken out of test tubes, fixed with special holders, which were then transferred to the propagator (Fig. 6). The composition of the culture medium during micropropagation was found to have a significant effect on the unrooted microshoots adaptation efficiency in aeroponic conditions. Thus, preliminary culturing of microshoots *in vitro* on the Stimul and Aminoven-containing media led to the rapid formation of the root system and active growth of the aerial part. The use of the studied substances had a positive effect on the growth index of shoots (I) and their specific growth rate (μ; Table 1). The variant with preliminary cultured microshoots on a hormone-free medium had the worst indicator.

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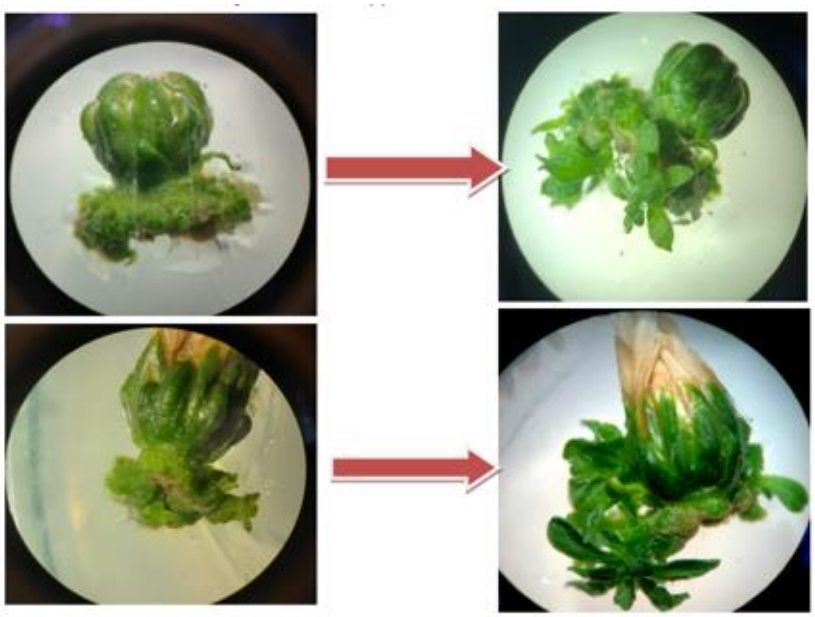
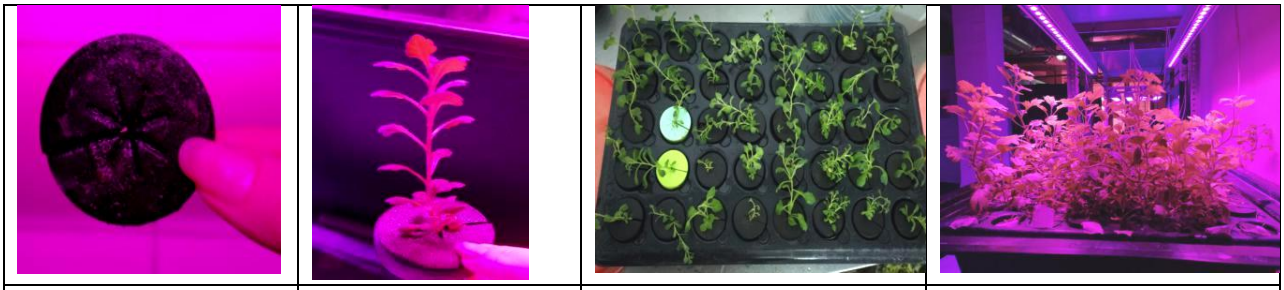


Fig. 5. Formation of adventious buds, Snow White variety.



a b c d

Fig. 6. Adaptation of microshoots of chrysanthemums in the aeroponic system: a: Holder, b: Fixed microshoots in the holder, c: Plants at the beginning of adaptation, d: Plants at the end of adaptation.

Table 1. Characteristics of the growth of microshoots in the aeroponic system.

Culture media *in vitro*	**Growth index (I)**	**Specific growth rate (μ), mm day^{-1}**
Hormone-free MS medium	0.29	0.39
MS + BAP + IAA	0.58	0.77
MS + Stimul	0.65	0.89
MS + Aminoven	0.71	1.1

The positive effect of Aminoven and Stimul on the growth and rooting rate of chrysanthemum microcuttings can be explained by the presence of free L-α-amino acids (12-17) and microelements in these substances. It should be noted that no formation of roots was observed in the case of adaptation of unrooted microshoots in the soil substrate. Under these conditions, microcuttings died already on the 4th day of adaptation. Thus, it has been experimentally established that at the last stage of clonal micropropagation, it is advisable to use an aeroponic system to create favorable conditions for both rooting and growth of microcuttings.

CONCLUSION

As a result of the research carried out, a new method of rapid *in vitro* propagation and obtaining high-quality planting stock of chrysanthemum plants was developed and proposed. The technology involves the use in small quantities of readily available raw material (semifloscules and flower buds) for cloning. The presence of 15% Aminoven (3 mL L^{-1}) in the culture medium causes a significant increase in the morphogenetic potential of cultured explants. The proposed method is easy to implement in comparison with the previously proposed techniques by Russian and foreign authors. The use of the developed method will increase by 18 times the yield

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of genetically stable, high-quality chrysanthemum planting stock, which makes it possible to produce plants in amounts susufficient for industrial floriculture of valuable domestic varieties and hybrids.

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