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THE CONSERVATION OF SPECIES *BELLEVALIA SARMATICA* (GEORGI) WORONOV BY THE VITROCULTURE METHOD

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Abstract: *In vitro* bulbification is a method by which bulb formation is stimulated and speeded up in the species that are propagated by bulbs and which need, in the conditions of a classicel culture, a longer period for the formation of flower-bearing bulbs. In this article, the results of research is presented, regarding the conservation, multiplication and maintenance of this vulnerable species included in the Red Book of the Republic of Moldova. This study was carried out in order to re-introduce this species into its native habitats. Optimal media were elaborated for the initiation, multiplication, rhyzogenesis and the maintenance of this species in *in vitro* conditions.

Keywords: conservation, *in vitro*, multiplication, auxins, cytokinins

Introduction

One of the present problems of contemporary botany is the elaboration of strategies for the conservation of the diversity of plant species that are in danger of extinction.

The most successful strategy for long-term protection of biodiversity is the protection and conservation of the phytocenoses and the populations of the spontaneous flora – in situ maintenance. But many rare species have reached a certain limit and in situ conservation alone does not solve the problems of protection against the more and more frequent anthropogenic factors. In such circumstances the most successful method for the prevention of the extinction of the species is the maintenance and multiplication of the respective taxon in artificial conditions that are similar to the natural conditions.

The introduction of the rare and endangered species into culture ensures conservation, facilitates the study of their biology and development, their ecology, methods for propagation and, at the same time, ensures the obtention, conservation and cultivation of planting material and seeds. These methods are of elementary importance for the repatriation (reintroduction) of these species into their natural ecological niches. The conservation of intact plant populations and phytocenoses and genetic biodiversity of the species in botanical gardens offers a multitude of means for the increase of the number of taxa.

The main and stringent problem related to the conservation of some species is the elaboration of the strategy that should stop the diminishing of the populations of the endangered taxons. More and more scientists have the opinion that the biotechnological methods are extremely efficient for the improvement of the situation, as compared to the traditional methods of regeneration. Many rare species from the spontaneous flora of the

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Republic of Moldova are continuously diminishing because of human activities that lead to the destruction of plant populations and the expansion of invasive species. *Bellevalia sarmatica* (Georgi) Woronov is a bulbous decorative species from the steppe included into the Red Book of the R. of Moldova, protected by the State [NEGRU, 2002]. Until recently, the steppe areas occupied 2/3 of the entire territory of Moldova. Presently, the natural steppe populations are maintained as some small territories as areas protected by the State [TELEUȚĂ, 2002].

The use of *in vitro* cultures is in itself a conservation method, representing a viable and efficient alternative for the protection and maintenance of the genetic fund of rare and endangered species. In bulbous species the increased concentrations of sucrose lead to the more rapid growth of the explants, such reducing the time necessary for the obtention of bulbs and the period until the flowering phase [TAKUO & KOJI, 2004].

The aim of the present study was the testing of the reaction in *in vitro* culture of the explants prelevated from a mature *Bellevalia sarmatica* plant in order to initiate tissue cultures for the medium-term conservation of this species and the induction of the regeneration process for the obtention of viable plants.

Material and methods

The material used for *in vitro* culture consisted of flower-bearing bulb explants taken from mature *Bellevalia sarmatica* plants that were collected in the Budjac Reservation in June 2010.

One of the main conditions for successful microcloning is the sterilization of the explant [CAVALLINI & al. 1987]. The explants prelevated from the donor material (bulb, bulb fragments) were washed under running tap water for 15 minutes. The pre-sterilization was carried aut in a solution of $KMnO_4$ (0.05%) + Tween-20 for 10 minutes. Sterilization was done with diacid (0.01%) solution for 6-7 minutes, followed by 3 rinses with sterile distilled water. After the disinfection of plant material, the explants were inoculated on the culture media, in large or small flasks depending on the size of the prelevated fragments, so as the newly formed bulbs should have enough space for development.

For the study of the reactivity of this species in *in vitro* culture, several variants of culture media were tested, all of them with the macro- and microelements according to the MS [MURASHIGE & SCOOG, 1962] formula, presented in Tab. 1.

The introduction into *in vitro* culture was done in nutritive media with various concentrations of plant hormones. For the initiation of *in vitro* cultures, media with low concentrations of auxins and cytokinins were tested (variants B-1, B-2, B-3). For rapid bulb development and root formation two variants of media were tested (B-6, B-9). As carbon source, sucrose was used. All the culture media were gelled with 6 g/l agar. The pH of the media was adjusted to 5.8 before autoclavation.

Results and discussions

The first evident observations were done after 4 weeks, when it was found that a large number of explants generated small protuberances, similar to 0.5 mm bulbils.

The testing of the three variants of media for culture initiation (Tab. 1) evidentiated the fact that the optimal medium for this phase was the one that contained MS (1962) macro- and microelements as basal medium and 1.0 mg/l–6-benzylamilopurine

(BAP) and 0.25 mg/l - α-naphtylacetic acid (NAA) and 30 g/l sucrose (B-1). After 6 weeks of culture, 10-12 bulbs/explant were obtained, with a diameter of about 3.0 mm (Fig. 1, a). On the B-2 medium, with a lower BAP concentration (0.5 mg/l) feweer bulbs fewer bulbs were formed (7-9 bulbs/explant). Regeneration also took place on the medium with Kinetin (B-3), but in a lower percentage (4-6 bulbils/explant). The first de novo regenerated bulbils on this medium were noticed after 8 weeks in culture. This fact demonstrates that the presence of BAP leads to a better growth of isolated tissues and better organogenesis as compared to the variants with Kinetin [VOINOV & al. 2009]

The bulbs obtained were transferred onto culture media with higher concentrations of plant hormones and sucrose. On the media with increased concentration of sucrose a more rapid growth of bulbils was observed and there the bulbs had the largest diameters, of 14-17 mm. After two weeks of culture on B-6, B-9 media the bulbs formed roots and leaves. Between the two variants of media, B-6 and B-9 there were no significant differences regarding growth and organogenesis, so that for this stage the B-6 medium with 60 g/l sucrose can be recommended (Fig. 1, b).

The bulbs that were regenerated *in vitro* were acclimated *ex vitro* and successfully transferred to *ex vitro* conditions. Acclimatization was carried aut in conditions of controlled climate regarding humidity and temperature, in peat and perlite mixture, for 14 days. The survival rate was of 100% and plant growth and development continued.

The results obtained in this species are promising, so the method of vitrocultures will be extended for other important and endangered taxons from the spontaneous flora, which is constantly diminishing and necessitates protection.

Conclusions

On the basis of our research we can conclude that:

For the sterilization of plant material (bulbs) for the initiation of *in vitro* culture of species *Bellevalia sarmatica* (Georgi) Woronow it is recommended to use disinfection with $KMnO_4$ (0.05%) + Tween-20 for 10 minutes and diacid (0.01%) solution for 6-7 minutes.

On the basal MS medium supplemented with BAP and NAA at the concentrations of 1.0 mg/l and respectively 0.25 mg/l the best results were obtained regarding the efficiency of microcloning and morphogenic potential.

The optimal medium for rhyzogenesis and rapid growth was MS basal medium supplemented with BAP (1.0 mg/l), NAA (1.0 mg/l) and sucrose at the concentration of 60 g/l.

For the acclimation of bulbs the optimal conditions were found and the time for flower-bearing bulb formation was reduced with 1-2 years.

The protocol for micropropagation and medium-term maintenance in *in vitro* culture can be successfully used for the conservation of the endangered species *Bellevalia sarmatica* (Georgi) Woronow. Applying this method ensures the possibility of obtaining homogenous planting material in large amounts in a short period of time and in limited space.

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Tab. 1. The composition of the nutritive media for the microclonal propagation of species *Bellevalia sarmatica*

	Variants	Basal medium	Supplementary additives
Media for culture initiation	B-1	MS-100%	1.0 mg/l BAP 0.25mg/l NAA 30 g/l sucrose
	B-2	MS-100%	0.5 mg/l BAP 0.25 mg/l NAA 30 g/l sucrose
	B-3	MS-100%	0.5 mg/l kinetin 0.25 mg/l IAA 30 g/l sucrose
Media for rapid development	B-6	MS-100%	1.0 mg/l BAP 1.0 mg/l NAA 60 g/l sucrose
	B-9	MS-100%	1.0 mg/l BAP 1.0 mg/l NAA 90 g/l sucrose

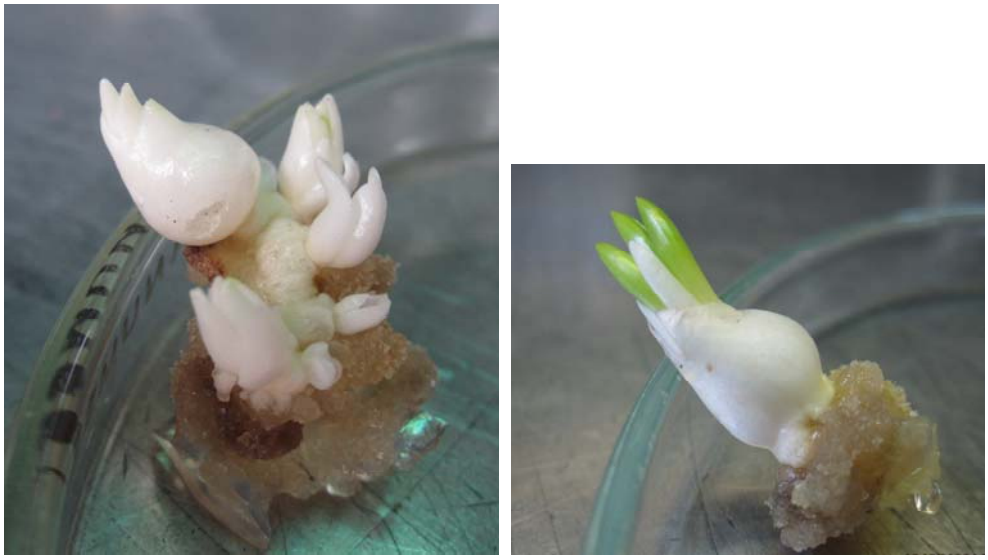

Fig. 1. The *in vitro* culture of species *Bellevalia sarmatica* on B-1 medium (a) and B-6 medium (b)