

# Collection of rare and endangered plant species in the meristem bank of the RAS Main Botanical Garden

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**Abstract.** Results of many research years on *in vitro* formation and conservation of genetic plants' bank of rare and endangered species in the Main Botanical Garden of the Russian Academy of Sciences. Peculiarities of plants' cultivation and conservation *in vitro* related to different families were revealed. The main methodological aspects at the stages of obtaining axenic culture, micropropagation and long-term deposition are reflected. The main criterion in choosing the optimal explant type for both taxa introduction into the culture *in vitro* and for long-term reproduction and conservation is the plants' life form. The nutrient media formulations and cultivation factors for slow explants' growth of the studied crops and conservation of their viability were optimized. It was revealed that when selecting optimal conditions of cultivation and conservation it is necessary to consider the biological features of the taxon.

## 1 Introduction

The biodiversity of organisms in the world has recently been declining at an unprecedented rate. In the period from 1996 to 2019, The IUCN red list has been replenished by a total of 15,774 plant species, with 36% of the total being endangered species [1]. The main causes of the decline in plant biodiversity include agricultural intensification, recreational activities, tourism and urban planning, collection of wild plants for medicinal, decorative, and other purposes, aggressive invasive species spread, modification of natural systems and environmental pollution. These factors cannot help but cause concerns and force to use more effective conservation measures for rare plants.

Although the most reliable way to preserve the species' gene pool is to protect habitat and monitor wild growing populations (*in situ*), *ex situ* methods can be used as an addition to *in situ* methods [2, 3].

Biotechnology techniques are now crucial for the management of plant genetic resources, especially for the conservation of rare and endangered species. *In vitro* cultivation can provide significant benefits as it ensures rapid reproduction of endangered

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plant species that have limited reproductive capacity and exist in habitats on the verge of destruction. It is important to note that *in vitro* methods allow to obtain mass revitalized planting material with minimal source material in a short time. *In vitro* methods have been shown to be very effective in preserving many plant species [4-5].

In addition to micropropagation, tissue culture provides opportunities for long-term storage of the plant gene pool in banks *in vitro*. Such banks are created in several botanical gardens of Russia [6].

The main research goal was the formation of a genetic bank *in vitro* of various species of rare and endangered plants and to identify optimal conditions for their cultivation.

## 2 Materials and Methods

The study used seeds and vegetative parts of plants collected in natural habitats and derived from exchange funds.

*In vitro* plants' cultivation was carried out according to the developed methods in the laboratory biotechnology [6, 7]. In the sterilization of seeds and plants' vegetative parts, "Chistotsvet" systemic fungicide was used in a 2-4% concentration (active substance - diphenconazole), 70% ethyl alcohol (C<sub>2</sub>H<sub>6</sub>O). The main sterilizers - 7% solution of calcium hypochlorite (Ca (ClO)<sub>2</sub>), 5% sodium hypochlorite solution (NaClO).

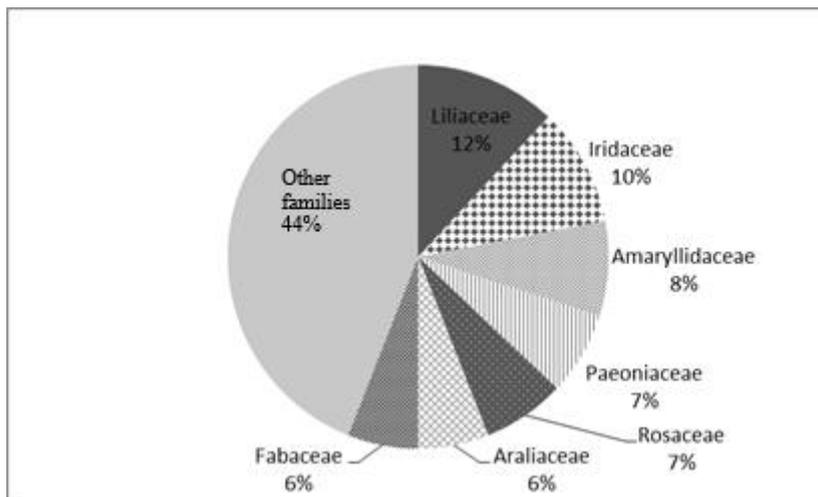
At the stage of micropropagation, nutrient media was used according to the inscription of Murashige and Skoog (MS, 1962), Quoirin and Lepoivre (QL, 1977). The following growth regulators were added to the nutrient media composition: 0.2-12.0 mg/L benzylaminopurin (BAP), 1.0-5.0 mg/L 2-isopenteniladenine (2-ip) or a combination of these preparations with 0.05 mg/L indolyl-3-acetic (IAA), 0.1 mg/L 1-naphthylacetic (NAA), and 0.1-1.0 mg/L gibberelic acid (GA). PVP (polyvinylpyrrolidone) and activated carbon at a concentration of 50,0-100,0 mg/L were used as sorbents, as antioxidants - 250-100,0 mg/L of ascorbic and citric acids, as well as their combination. The passage duration was from 14 to 60 days.

When depositing plants of different life forms, different explants were used. For woody and herbaceous taxa - equalized fragments of microshoots containing 1-2 metamers, for bulbous - bulbs with 1-2 leaves. Depositing was carried out in climate chamber conditions at reduced illumination from 0 to 1500 lx and temperature from 5 to 7 °C, as well as 16-hour photoperiod. MS and ½MS containing benzylaminopurine (0.1-0.3 mg/L) and sucrose (20,0-60.0 g/L) were used as nutrient media.

## 3 Results and Discussion

SBG RAS genetic bank *in vitro* was formed since 1986 and is one of the most representative in Russia. Currently, the plant collection under active growth conditions contains more than 1325 taxa: 155 species, 1,385 varieties and selected forms belonging to 183 genera and 62 families.

The collection of rare and endangered plant species contains 82 taxa. The following families are most represented: Liliaceae (*Cardiocrinum*, *Fritillaria*, *Lilium* and *Tulipa*), Iridaceae (*Belamcanda*, *Gladiolus*, *Iris*), Amaryllidaceae (*Galanthus* and *Leucojum*), Paeoniaceae (*Paeonia*), Rosaceae (*Amygdalus*, *Armeniaca*, *Cotoneaster*, *Potentilla*, *Prinsepia*, *Sanguisorba*), Araliaceae (*Aralia*, *Kalopanax*, *Oplopanax*) Fabaceae (*Calophaca*, *Genista*, *Hedysarum*) (Fig. 1).



**Fig. 1.** Quantitative composition of rare and endangered plant species' collection *in vitro* GBS RAS

The main stage of clonal micropropagation is the production of axenic culture *in vitro*. Depending on the age and origin of plant tissues, various sterilization schemes were used. At the stage of *in vitro* introduction into the culture, the most optimal was the sequential sterilization with “Chistotsvet” (4%), ethanol (70%) for 30 seconds and a solution of calcium hypochlorite (7%) - 5-7 min. Seeds were treated with solution for 7-20 minutes, fragments of vegetative parts of plants - for 3-7 minutes. Increased concentration and exposure led to a decrease in the contamination level, but the percentage of viable explants also decreased.

At the initiation stage and during further cultivation *in vitro* of some species of rare plants, there is a problem of morphogenetic activity decrease due to phenolic exudation. Phenolic exudation varies depending on the species, genotype and physiological state of the plant, nutrient medium composition, conditions, and duration of cultivation. Increased synthesis of medicinal plants' secondary metabolites leads to accumulation and subsequent oxidation of phenolic compounds in tissues and nutrient media, and, as a result, a decreased viability or death of regenerants [8]. Antioxidants (citric and ascorbic acids, cysteine, etc.), sorbents (PVP, charcoal) and polyphenol oxidase inhibitors (sulfur dioxide, sodium chloride, etc.) help reduce the negative effects of polyphenols [9]. Among the studied plants there were species of rare plants with increased synthesis of phenolic compounds during cultivation *in vitro* (*Kalopanax septemlobus* (Thunb.) Koidz., *Armeniaca mandshurica* (Maxim.) Skvorts., *Epimedium colchicum* (Boiss.) Trautv., *Rhodiola rosea* L., *Dioscorea caucasica* Lipsky). It was revealed that in order to reduce phenolic exudation, it is advisable to add antioxidants to the nutrient medium in the amount of 100.0 mg/L of ascorbic acid or a combination of 50.0 mg/L of citric and 50.0 mg/L of ascorbic mg/L acids, as well as polyphenoloxidase inhibitors - 50.0 mg/L PVP.

Morphogenesis and *in vitro* regeneration depend on a complex of factors and there are no universal biotechnological methods for reproduction and conservation of a particular taxon. Reproduction methods chosen for *in vitro* cultivation minimized the risk of somaclonal variability [10, 11].

Before the *in vitro* system can be used to store the gene pool, effective ways of regeneration and clonal micropropagation must be optimized.

During the studies, MS medium was used for most of the rare and endangered plant species studied as nutritional cultivation medium, which contributed to active formation of adventive buds, shoots, and bulbs.

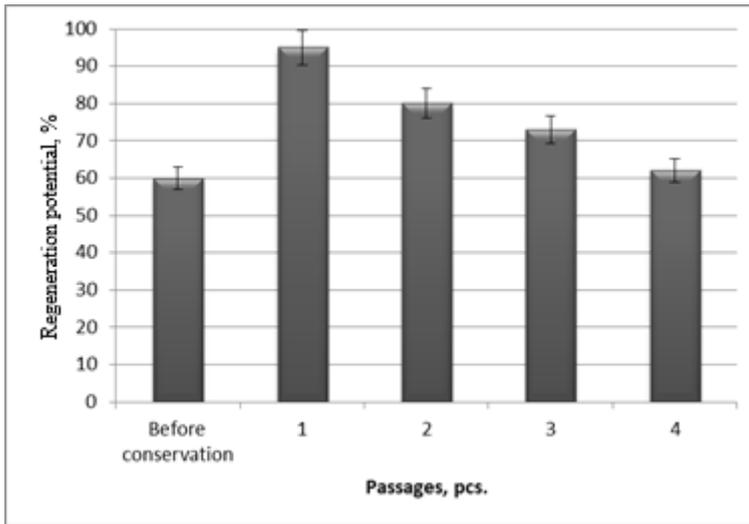
For model plants' objects of different life forms, optimal type and concentration of growth regulators, as well as the passage duration (Table 1) were established.

**Table 1.** The subcultivation period duration of rare and endangered plant species and the nutrient media composition at the stage of micropropagation.

Object	Duration of subcultivation, days	Concentration and type of growth regulator, mg/L
<i>Aristolochia manshuriensis</i> Kom.	25-30	0,8 BAP; 0,01 IAA
<i>Galanthus angustifolius</i> G. Koss <i>Leucojum aestivum</i> L.	30-45	10,0 BAP; 0,1 NAA
<i>Aralia cordata</i> Thunb. <i>Kalopanax septemlobus</i> (Thunb.) Koidz. <i>Oplopanax elatus</i> (Nakai) Nakai	25-30	1,0 BAP; 0,05 IAA
<i>Belamcanda chinensis</i> (L.) DC. <i>Gladiolus palustris</i> Gaudin <i>Iris pumila</i> L.	40-50	10,0 BAP; 0,1 IAA
<i>Cardiocrinum cordatum</i> (Thunb.) Makino <i>Fritillaria meleagris</i> L. <i>Lilium callosum</i> Siebold et Zucc. <i>Tulipa lipskyi</i> Grossh.	45-60	5,0 BAP; 0,1 NAA
<i>Paeonia obovata</i> Maxim.	40-60	1,0 BAP
<i>Amygdalus pedunculata</i> Pall. <i>Armeniaca mandshurica</i> (Maxim.) Skvorts. <i>Cotoneaster lucidus</i> . Schlecht. <i>Potentilla vulgarica</i> Juz. <i>Prinsepia sinensis</i> (Oliv.) Bean <i>Sanguisorba magnifica</i> I. Schischk. et Kom.	20-35	0,5 BAP; 0,01 IAA

The hydration frequency of the explants during prolonged storage was negligible and was 2 -5% depending on the taxonomic belonging of the plants.

For sustainable conservation of crops *in vitro*, it is necessary to maintain their viability with weak dynamics of growth processes. Reduction of growth rate is achieved by reducing temperature and lighting intensity and reducing the mineral base of the nutrient medium, adding osmotically active substances and retardants [12, 13]. During the studies it was established that with the increase in sucrose concentration in the nutrient medium composition there is a tendency to decrease the growth processes dynamics and viability increase. The obtained results made it possible to establish that the optimal conservation condition for most species is cultivation on a nutrient medium containing ½ MS and 40.0 g/L sucrose supplemented by 0.3 mg/L BAP. After 1.5 years of conservation, the model objects were assessed by regeneration potential (Fig. 2).



**Fig. 2.** Change of regeneration potential of *Aristolochia manshuriensis* Kom. after long storage.

After transferring regenerants to standard cultivation conditions, an increase in regeneration potential up to 95% was observed, as well as an increase in the formation of adventive buds and microshoots by 2-3 times. However, with further subcultivations, the regeneration activity decreased, and the dynamics of growth processes went down (by 10 - 15%).

For rare plants of different taxonomic groups, optimal explants' types and their size were determined based on growth and organogenetic indices. Table 2 shows optimal explants' indicators for long-term cultivation of rare plants' species of different life forms.

**Table 2.** Optimum explant type and its size (mm) for plants' storage *in vitro*.

Species	Apical meristems	Microshoots' segments	Bulbs and their segments
Woody			
<i>Rhododendron schlippenbachii</i> Maxim.	0,2-0,3	8,0-12,0	
<i>Aristolochia manshuriensis</i> Kom.	0,3-0,7	7,0-9,0	
<i>Kalopanax septemlobus</i> (Thunb.) Koidz.	3,0-5,0	12,0-15,0	
Herbaceous			
<i>Sanguisorba magna</i> I. Schischk. et Kom.	0,2-0,4	0,5-5,0	
<i>Paeonia obovata</i> Maxim.	2,5-4,0	2,0-3,5	
Bulbous			
<i>Gladiolus palustris</i> Gaudin	0,3-0,5		3,0-5,0
<i>Galanthus angustifolius</i> G. Koss	0,2-0,4		2,0-4,5

The main criterion in choosing the optimal explant type for both taxa introduction into the culture *in vitro* and for long-term reproduction and conservation is the plants' life form. Based on a morphometric indicators' complex, optimal sizes and types of explants for long-term collection storage in *in vitro* conditions were determined.

## 4 Conclusions

In perennial studies on clonal micropropagation in the GBS RAS, an *in vitro* collection of rare and endangered plants was preserved, including 82 species, which is 17.3% of total angiospermous plants number included in the Red Book of the Russian Federation.

It was found that the viability conservation and minimization of explants growth during *in vitro* storage for 1.5 years was facilitated by the combination of reduced temperature and illumination, as well as the addition of osmotics to the nutrient medium composition. Types and sizes of explants for plants of different life forms were substantiated for effective viability conservation in the deposition process at reduced temperature in *in vitro* conditions. Based on the regeneration potential assessment after long-term cultures' storage (at reduced temperature for 1.5 years), a stimulating effect was revealed at the first stages of cultivation, which was expressed in increasing the regeneration potential and dynamics of growth processes.

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