# CONSERVATION OF THE DIVERSITY OF BOTANICAL GARDEN COLLECTION WITH THE USE OF *IN VITRO* TECHNIQUES

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**Abstract.** The results of in vitro reproduction of species from the collection of the Tashkent Botanical Garden are presented in this article. All information on introducing the species into in vitro culture is scientifically based.

Plant biotechnology makes it possible to preserve rare and endangered plant species. Programs for the creation and maintenance of cell and tissue culture collections are carried out in many botanic gardens. Such programs have been established and are operating effectively in a number of countries. In vitro collections of cells, organs and plants, cryobanks were created, where samples of plant material belonging to different groups are stored in liquid nitrogen. Many specimens of the collection, which are preserved as national treasures, are among rare and endangered plants protected by law. The National Clonal Germplasm Repository USDA in the USA (Corvallis, Oregon) stores 500,000 samples of economically valuable as well as rare and endangered plants covering 10,000 species. In Germany, samples of more than 700 cell culture lines belonging to 80 plant families are stored, and most of these cultures synthesize pharmacologically important secondary metabolites. This article provides information on the development of protocols for in vitro microcloning reproduction of single-sample plants at the Botanical Garden College of the Republic of Uzbekistan.

Research object. A species from the North American exposition was selected as the research object.

Purpose of work. Introduction of selected species from the collection of the Tashkent Botanical Garden to in vitro culture.

Methods and methodology

Different parts of the plant were used as explants for in vitro reproduction of the selected species:

– apical and lateral buds, buds from 1-year branches were used as explants.

– the apical bud is cut, two pairs of lateral buds remain, an oblique incision is made in the place of the opposite lower second pair of lateral buds, and one of the lateral buds is removed.

Optimization of sterilization. Sterilization agents Belizna+Tween 20,70% ethanol and Sodium hypochlorite + tween 20, in different concentrations. The choice of sterilization agent is the number of explants included in the culture. More than 16 sterilization tools were tested. Based on the obtained results, the best tools for sterilization were selected and the following sterilization protocol was developed:

1. Explants are placed in a 20% sterilizing soap solution for 20 minutes. 2. Wash thoroughly with distilled water. 3. The composition is placed in a 0.01% fungicide solution with propikanazol (20*10–4%) – 10 minutes. 4. Wash thoroughly with distilled water – 3–4 times. 5. Placed in 2% "Belizna" solution (0.36% active chlorine) – 10 minutes. 6. Wash thoroughly with distilled water – 3 times. 7. Placed in 70% ethanol – 30 seconds. 8. Wash thoroughly with distilled water – 3 times. Explants are dried in sterile Petri dishes and planted in nutrient medium. In this case, up to 80% sterile explants were obtained that could be planted in nutrient media.

Selection of feeding medium. Different nutrients were tested for the following subjects. Various nutrient media were tested for research objects (Murashige and Skoog, 1962; Chu et al., 1975; Hamburg et al., 1968), WPM (Lloyd and McCown, 1980). The WPM medium (Lloyd and McCown, 1980) was the most optimal among the studied media, in which explant viability reached 90%. Good results were also obtained in Murashige and Skoog nutrient media.

Selection of phytohormones. Different combinations of Auxins and Cytokinins were tested for research objects: auxins (2,4-D, IAA, NAA, IBA) dissolved in 10 ml of 70% ethyl alcohol, cytokinins (BAP, kinetin) in a small amount of alkali or acid solution, and then dissolved in 10 ml of distilled water.

Inducing in vitro culture can also be carried out on a nutrient medium with the phytohormone 6-benzylaminopurine. Incubation was carried out at a standard photoperiod of 16/8 (16 hours day, 8 hours night) and a temperature of 23–24 °C. At all stages of passaging, microcuttings were placed in a container with a nutrient medium in such a way that the lowest point of the explant was located at a depth of 0.5 mm in the nutrient medium, while the lower bud was also located in the nutrient medium.

## Summary

1. From the tested sterilization agents, up to 80% of the explants turned out to be viable, the most optimal are the following: During our study, explants were placed in a 25% sterilizing soap solution for 20–30 minutes, then thoroughlysoaped with distilled water, then placed in a 0.01% fungicide solution with propikanazole (20*10–4%), washed thoroughly with distilled water 2–3 times, immersed in 2% Belizna solution for 15 minutes, thoroughly washed with water – 3 times, placed in 70% ethanol for 30 seconds, and finally thoroughly washed with sterilized water in an autoclave – 3 times.

2. The following nutrient media were selected for the development of mature plants through in vitro micropropagation: Forestiera neo-mexicana Gray. nutrient medium WPM (IBA; TDZ + NAA; BAP; Zea).

## References

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