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Features of Reproduction Rare and Endangered Species of *Lagochilus Proskorjakovii* Ikramov in Culture in Vitro

Abdullaeva D.A[1], Safarov K.S[2]

Tashkent Botanical Garden at the Institute of the Gene Pool of Plants and Animals, Uzbek Academy of Sciences, Uzbekistan

Abstract: ***The features of micro propagation herb Lagohilus proskorjakovii Ikramov - rare endemic of Nuratau mountain. The conditions for the introduction of rare and endangered plant species L. proskorjakovii sterile in vitro culture: the method of sterilization, type of primary explants, saline environments and hormonal preparations. We investigated a number of culture media for culturing to determine the most optimal to maintain the culture. It is shown that only when the sterilization of seeds L. proskorjakovii in solutions of AgNO3 0.1% (15 min.) And 70% ethanol (1 min.) Remained viable in culture conditions. The optimal concentration of auxin in combination with cytokines in the multiplication phase. The conditions for rooting induction in culture in vitro.***

Keywords: *L. proskorjakovii*, culture in vitro, micropropagation, root formation

1. Introduction

In recent years, the conservation of biodiversity of flora and fauna is considered as one of the global environmental problems. Conservation of plant biodiversity offers a variety of ways and means: entering the plants in the Red Book, the organization of specially protected natural areas and the creation of collections in botanical gardens. Along with traditional methods of *ex situ* conservation of plants it is becoming increasingly important to use for the purpose of biotechnological breeding methods.

Study potential *in vitro* cultured plant tissues and cells is important not only to address the conservation of rare and endangered species, but also to expand the resource base and the development of rapid multiplication technology of ornamental and medicinal plants [1].

Lagohilus proskorjakovii Ikramov. the family of *Labiatae*. It is a rare endemic of Nuratau mountain. Distributed in the Jizzakh and Samarkand region (Nuratau Ridge). It grows on dry rocky and gravelly slopes of the middle belt of mountains. Very rare, sporadic, isolated specimens. It is protected in the territory of the Nurata state reserve. Included in the Red Book of the Republic of Uzbekistan [6]. This unique plant belongs to the group of herbs that have important medicinal properties and is used to treat many diseases. Preparations its significantly stimulates the process of blood coagulation, have a sedative, cardio tropic, vasodilator, hypotensive and antispasmodic action [3]. Plants kind *Lagohilus* contains the active ingredient - lagohilin.

Lagohilus reproduces only by seed. Seeds *Lagohilus* species are characterized by low germination and germination is delayed for a long period [3]. Due to the high value of natural resources and reduce the need arises naturally in its plantation establishment in culture.

Recently, for breeding and conservation of plant species used method of micropropagation, which allows you to get the planting material in large quantities and in a short time than using traditional breeding methods.

In this regard, we are carrying out research to develop methods of micropropagation of some rare medicinal plants and ornamental flora of Uzbekistan.

The aim of this work was the development of clonal micropropagation technology *L.proskorjakovii* Ikr.

2. Materials and Methods of Research

In the experiments, the classic techniques for working with cultures of isolated tissues and organs of plants [4]. As used explants *L. proskorjakovii* seeds collected from the southern slope of the mountain Nuratau. Seeds stratified (below 00C) for 40 days.

Sterilization is carried out in various embodiments and with various concentrations of exposure time:
1. 0.5% sodium hypochlorite NaOCl (40 min.) + 96% ethanol (2 min.);
2. H_2SO_4 (sulfuric acid conc.) (30 sec.);
3. 0.5% sodium thimerosal (thimerosal) (20 min);
4. 0,1% $AgNO_3$ solution (15 min.) + 70% ethanol (1 min.).
The results showed that the seed sterilization in *L. proskorjakovii* 0,1% $AgNO_3$ solution (15 min.) and 70% ethanol (1 min.) Is the best method [2]. In this sterilization seeds maintained high viability.

For a successful transfer to sterile plants in vitro culture requires proper selection medium. It should include all the necessary plant macro-and micronutrients, as well as vitamins, plant hormones and carbohydrates. Some nutrient media containing casein hydrolyzed, amino acids. In addition, the composition of culture media include EDTA (ethylenediaminetetraacetic acid), which improves the availability of iron to the cells [5]. Plant tissues were cultured on nutrient media, Murashige Skoog (MS).

For higher multiplication factors, culture media enriched with drugs cytokines and auxin nature [7]. In our

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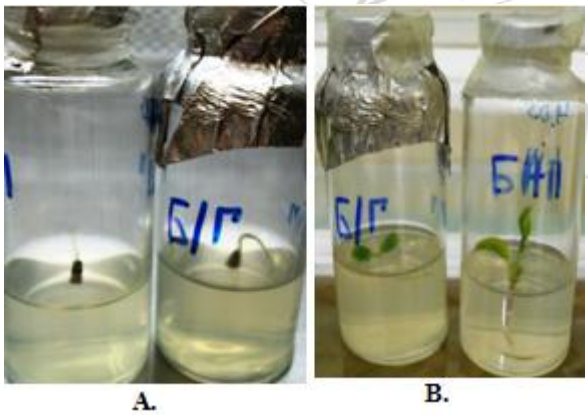
experiments the culture medium for multiplication phase modifications on different concentrations of auxins were used: β-indoleacetic acid (IAA) - 0.1-2 mg / l in combination with 0.1-2.0 mg / l 6-benzylaminopurine (6- BAP), gibberellic acid - 0.1-0.5 mg / l kinetin - 0.25-1 mg / l. At all stages of clonal micropropagation as a source of carbon supply typically use 3% sucrose. However, this concentration is not optimal for all introduced into in vitro culture of plant [7]. In step introduction in vitro culture and micropropagation used in step 4% sucrose, and a low concentration (3% sucrose solution) was used in the step of rooting. As a control, we used MS medium without growth regulators.

To accelerate root formation we studied the effect of three auxin (IAA, NAA - a-naphthylacetic acid, IMC - indole-3-butyric acid) from 0.5 to 5.0 mg / l at intervals of 0.5 mg / l on the ability fundamentally educations *L.proskorjakovii* in tissue culture. Should be chosen not only the composition of the medium, and cultivation conditions for the successful cultivation of isolated cells and tissues [8]. In our experiments, explants were cultured under the following conditions: temperature - 24 ° C, photoperiod of 16/8 h light / dark factors statuesque room for growing plants.

3. Results and Discussion

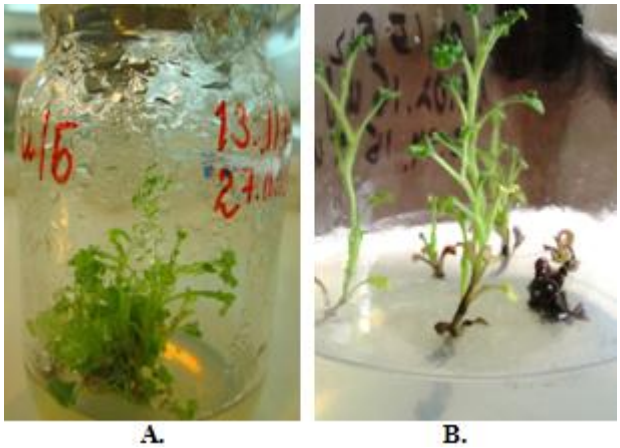
Activation of growth processes in the seeds that germinated on agar without hormone MS medium and hormonal environment with BAP 0.1 mg / l was observed in a week (Fig. 1).

Regenerant grown on a nutrient medium with hormone BAP 0.1 mg / l from the primary explants, cut and transferred to a culture medium containing MS + 0.1 mg / L 6-BAP + 1.0 mg / l IAA (Figure 2). To prevent drying of the culture medium every week explants are transplanted to fresh media.



A. B.

Figure 1: Germination of seed culture in vitro (A) rooted seedling seed (B) *L.proskorjakovii* without hormone medium on Murashige Skoog (MS) medium with BAP and 0.1 mg/l.



A. B.

Figure 2: Micropropagation L. proskorjakovii (A) and regenerated, separated by micropropagation (B)

It is shown that the addition of MS + 0.1 mg / l BAP + 0.8 mg / l IAA; MS + 0.1 mg / l BAP + 1 mg / l IAA; MS + 0.2 mg / l BAP + 1.0 mg / L IAA; MS + 0.5 mg / l BAP + 0.5 mg / L IAA and MS + 0.1 mg / L IAA + 1 mg / l BAP led to the development of shoots and explants second passage gave rise to new shoots.

After several weeks of cultivation in the media described were obtained necessary for developing the next phase of the number of explants. Part plants were transplanted to a medium with BAP 1.0 mg / l BAP and 2.0 mg / L, supplemented with adenine sulfate in a concentration of 50 mg / l. This caused an increase in the pace of recovery and increased multiplication factor *L.proskorjakovii*. In the presence of culture medium and 2.0 BAP 1.0 mg / l, characterized regenerants good development. This affects both the BAP multiplication factor, and the length of the shoots. Some of the plants were transplanted on the medium with IAA 1 mg / l kinetin + 0.25 mg / l IAA and 2 mg / l kinetin + 0.5 mg / L, which was increased regeneration of shoots and increase the size of the explants, but not taking hold.

Obtaining plants with well-formed root system is an important stage of clonal micropropagation. It is known that auxins - rhizogenesis process stimulants in plants in vitro [4]. We have studied the effect of three auxin (IAA, NAA, IBA) from 0.5 to 5.0 mg / l at intervals of 0.5 mg/l root of the educational ability *L.proskorjakovii* in tissue culture. For microplants formed in the culture medium decreased the amount of salts and carbohydrates, excluded cytokinin (6-BAP), which prevents root formation processes. It is known that the efficiency with auxin rooting shoots greatly reduced under the influence of high doses of cytokinins [7].

In all tested nutrient media *L. proskorjakovii* plants have well-developed leaves and sometimes roots. Rooting microshoots having three or more leaves, was observed in two weeks. Where in the concentration of IAA in the 2.0 and 2.5 mg / l promoted more rapid formation of roots *L. proskorjakovii*. When using NAA in a concentration of 2.0 mg / l rooting rates were similar with the embodiment where the IAA concentration was 2.0 mg / l. None of tested phytohormones did not contribute to the formation of roots directly from meristematic tops, t. E. In the beginning there was an increase tension (not less than 4 cm), and then the

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formation of the root. It should be noted that the concentration of IAA 1.0 mg / l, root induction caused and promoted growth. In step rhizogenesis noted CQI no positive effect at low concentrations of 0.5 and 1.0 mg / l. battening.

Thus, the most important moments of the introduction of rare and endangered plants in in-vitro culture are: the choice of the object of research; selection sterility and the sterilization of the algorithm depending on the study of the material; choice of culture medium containing the full composition of the nutrients necessary for life of the object of study; selection of optimal culture conditions.

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