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# APPLICATION OF *IN VITRO* MICROCLONAL PROPAGATION FOR RARE AND ENDANGERED PLANT SPECIES

**Annotation.** This article examines the specifics of microclonal propagation of rare and endangered plant species found in the Ulytau region through the application of *in vitro* technologies.

Microclonal propagation is a progressive biotechnological method aimed at the conservation and reproduction of rare and specially protected plant species. *In vitro* technology enables the large-scale production of genetically uniform plants under laboratory conditions, which is particularly relevant for species with limited natural populations.

The analysis of literature sources allowed for the systematization of accumulated scientific experience related to microclonal propagation of rare plant species in Kazakhstan. As a result, the most effective propagation methods were identified. Furthermore, the analysis revealed that certain species remain insufficiently studied. The development of species-specific cultivation protocols and the improvement of ex vitro acclimatization methods are proposed as key directions for future research.

**Keywords:** literature sources; plants; rare species; biotechnological methods; microclonal propagation; in vitro; Ulytau.

## *Introduction*

Kazakhstan possesses remarkable botanical diversity, including many endemic and rare species, most of which remain insufficiently studied. The conservation of rare and endangered plant species is crucial for maintaining biodiversity and ecosystem resilience. A significant number of such species, including those found in the Ulytau region, are listed in the Red Book of Kazakhstan.

Among the most effective approaches to the preservation and propagation of these species is the application of biotechnological methods, particularly the establishment of in vitro cultures

Involvement of biotechnology methods based on cultivation of isolated plant organs, tissues and cells to solve problems of biodiversity conservation has advantages over traditionally used approaches. They enable the production of disease-free material, mass propagation of valuable genotypes, year-round cultivation, and the creation of genetic repositories. Notably, such techniques allow for the recovery of vegetative progeny from species that are difficult to reproduce under natural conditions and facilitate the preservation of genotypes without removing plants from the juvenile phase (Vysotsky, 1986) [1].

The use of cell culture techniques was pioneered by J. Morel in the late 1950s for orchid micropropagation, and later adapted for large-scale propagation of ornamental, fruit, and forest species. However, limitations such as genotype dependence and adaptation difficulties still hinder widespread application, especially for woody plants.

Since the 1970s, in vitro culture methods have been increasingly applied for the mass propagation of trees and shrubs. However, compared to herbaceous plants, the success rate in woody species has been relatively limited. Despite this, considerable progress has been achieved, and various in vitro technologies now serve as viable alternatives to traditional propagation methods for numerous economically important and ornamental species, including spruce, larch, eucalyptus, apple, plum, cherry, lilac, birch, and maple

Nevertheless, the widespread use of biotechnological approaches remains constrained by the strong dependence of morphogenetic potential and regeneration capacity on genotype and cultivation conditions. Furthermore, the absence of reliable acclimatization techniques for regenerants to *ex vitro* environments often leads to high mortality rates and limits the efficiency of such approaches in both biodiversity conservation and high-quality plant production.

It should be emphasized that research in the field of plant tissue culture for gene pool conservation has its specific challenges. Often, researchers face limitations in choosing target species and a shortage of initial plant material.

Moreover, growing anthropogenic pressure on natural ecosystems, combined with the progressive loss of rare and vulnerable species of plants, fungi, and algae, heightens the risk of biodiversity degradation. Steppe, semi-desert, and desert landscapes of Kazakhstan, including the Ulytau region, are not exceptions to this trend [3].

Herbaceous and woody plants differ significantly in the level of totipotency (ability of cells to differentiate into any cell type) of cells and regeneration potential. This necessitates a differentiated approach to the application and improvement of clonal micropropagation technologies [2].

Despite notable progress in plant biotechnology, the reproduction of many economically important or ornamental plant species remains challenging. Traditional methods often fail to meet the propagation demands for certain genotypes. However, advances in the cultivation of isolated cells, tissues, and organs have led to the development of clonal micropropagation - a novel form of vegetative propagation. In many cases, it represents not only a practical alternative, but the only viable option for mass propagation of such species [3].

*In vitro* propagation is commonly referred to as “micropropagation” because, unlike traditional “macro methods” that utilize large segments of plants, this technique works with much smaller tissue samples. Typically, plant material used in *in vitro* culture ranges in size from 0.2-0.5 cm to 2.0-5.0 cm. The small scale allows for precise control of growth conditions and enables the regeneration of numerous genetically uniform plantlets.

The process of in vitro micropropagation generally consists of the following stages:

1. **Selection and sterilization of explants,** along with the selection and optimization of the nutrient medium that ensures optimal growth and development;
2. **Shoot multiplication**, achieved through the proliferation of shoots on a suitable propagation medium;
3. **Rooting of microshoots** under sterile conditions;
4. **Transfer of regenerant plants to *ex vitro* conditions** for acclimatization and further growth.

The success of clonal micropropagation depends on multiple factors, including the genotype of the donor plant, the epigenetic and physiological status of the explant, the composition of the mineral and organic components of the nutrient medium – especially the type and concentration of phytohormones – and the cultivation conditions. Accumulated research indicates that a universal cloning protocol is unlikely to be effective. Instead, empirical optimization is necessary for each species to ensure high propagation efficiency [2].

Table - 1 – *Methods of plant micropropagation*

| Purpose of application | Propagation object | Explant used | Propagation method |
|---|---|---|---|
| **Clonal micropropagation** | 1. Valuable genotypes (especially when the amount of starting material is limited); 2. Hybrid forms that segregate under generative reproduction; 3. Species that are difficult to propagate by traditional methods | Shoots (buds, apical meristems, stem nodes); leaves; seeds (embryos and seedling segments) | **Activation of apical and axillary meristems.** Common but time-consuming method. |
| | | | **Direct organogenesis or embryogenesis from differentiated cells** of vegetative organs. Mainly used for herbaceous species. |
| | | | **Callus induction with subsequent organogenesis/embryogenesis.** High risk of somaclonal variation. |
| | | | **Embryogenic suspension cultures followed by regeneration from somatic embryos.** Technically demanding and suitable for only a limited number of species. |
| Free from viruses | Species that | Apical | Activation of apical |

| | | | |
|---|---|---|---|
| and pathogens | reproduce well traditionally but are virus-infected or pathogen-susceptible | meristems from buds or shoots | meristems. The very small size of explants complicates establishment in sterile culture. |

Compiled by the authors according to: [2, c. 34]

To date, numerous formulations of nutrient media have been developed for plant tissue culture, but the most widely used remains the Murashige and Skoog (MS) medium (1962). This medium contains an optimally balanced composition of macro- and micronutrients essential for plant growth and morphogenesis. Variations among other media primarily relate to the ammonium-to-nitrate nitrogen ratio and specific hormonal requirements of different species. Among the notable media modifications are those by Linsmaier and Skoog (1965), WPM (Woody Plant Medium) by Lloyd and McCown (1980), QL medium by Quoirin and Lepoivre (1977), DVM-1 by Gresshoff and Doy (1972), as well as formulations by Nitsch (1974), Heller (1953), and Schenk and Hildebrandt (1972) [4; 7].

**Table - 2 – Recommendations for modifying the composition of MS medium at various stages of woody plant micropropagation**

| **Micropropagation stage** | **Mineral salts** | **Phytohormones (mg/L)** | **Organic additives** |
|---|---|---|---|
| **Initiation** | 0.5-1.5× MS | Cytokinins (CK) at low concentrations (e.g., 0.05–0.5 BAP), or CK + Auxin (e.g., 0.5 BAP + 0.1 IAA) | Sucrose: 1–5% Agar: 0.7–0.8% |
| Propagation | **1) maintained at the level of the initiation medium;<br>2) in case of darkening and/or vitrification of explant tissues, the concentration of salts is reduced by 1.5–2 times.** | CK concentration is increased several times until abnormal shoots appear, or A (1/2–1/10 of the amount of CK) is additionally added at the same time as CK* is increased. | Sucrose and agar concentrations are usually kept at the level of the initiation medium. |
| **Rooting** | 1) maintain at the level of the breeding medium;<br>2) concentrati on | 1) reduce or eliminate CK*;<br>2) auxins are administered in small concentrations; | Sucrose concentration is reduced; agar concentration is increased to 1.1%. |

| | | | |
|---|---|---|---|
| | salt concentration is reduced, 2-3 times | 3) completely eliminate hormones from the environment | |

Compiled by the authors according to: [2, c. 35]

*Note:* MS = standard Murashige & Skoog medium; CK = cytokinin; A = auxin; BAP = 6-Benzylaminopurine; IAA = Indole-3-acetic acid.

The development of microclonal propagation techniques serves as a foundation for establishing *in vitro* genetic banks of rare and endangered plant species. It also represents one of the most promising strategies for the long-term conservation of biodiversity [8].

To better understand the current research landscape, we conducted a literature review focusing on the use of biotechnological methods applied to the study of rare and vulnerable species found in the Ulytau region.

*Results*

Numerous plant species registered in Kazakhstan are classified as rare or endangered [9]. Based on the Red Book of Kazakhstan (2014), more than 20 such species have been identified within the Ulytau region. These include, among others:

*Tulipa biebersteiniana* Schult. & Schult.fil., *Tulipa borszczowii* Regel, *Tulipa schrenkii* Regel, *Tulipa biflora* Pall., *Tulipa patens* C.Agardh, *Scirpus kasachstanicus* Dobroch, *Tanacetum ulutavicum* Tzvel., *Alnus glutinosa* (L.) Gaertn., *Anabasis turgaica* Iljin & Krasch., *Berberis karkaralensis* Kornilov & Potapov, *Adonis wolgensis* Stev., *Pulsatilla flavescens* (Zucc.) Regel, *Pulsatilla patens* (L.) Mill., *Drosera rotundifolia* L., *Oxytropis subverticillaris* C.A.Mey., *Stipa pennata* L., *Ornithogalum fischerianum* Krasch, *Astragalus sumneviczii* Pavlov, *Hedysarum bectauatavicum* Bajtenov, *Betula kirghisorum* Sawicz, *Nymphoides peltata* (S.G.Gmel.) Kuntze, *Craniospermum echioides* (Schrenk) Bunge, *Crambe tataria* Sebeók, and *Berberis karkaralensis* Kornil. & Potapov.

Analysis of literature sources has shown that *in vitro* methods for the conservation of rare and endangered plant species are actively developed and applied in our country. For example, the Institute of Plant Biology and Biotechnology is working on creating *in vitro* collections of rare species [10] listed in the Red Book of Kazakhstan [9]. This includes the development of clonal micropropagation protocols to obtain sufficient numbers of plants to restore natural populations and preserve the gene pool.

Studies of species of the genus *Tulipa*:

In Kazakhstan, 18 species of the genus *Tulipa* are under state protection, with five of them found within the Ulytau region.

M. Maślanka et al. [11] studied the inital stages of *in vitro* propagation of *Tulipa tarda*. While *in vitro* cultures of tulips are commonly initiated from scale bulbs (Nishiuchi, 1980; Gude & Dijkema, 1997), floral stems (Rice et al., 1983; Hulscher et al., 1992; Podwyszyńska & Marasek, 2003), or ovaries (Ptak & Bach, 2007), this particular study successfully initiated cultures from seeds.

The findings revealed that seed-derived bulbs and additional bulb structures served as suitable explants for the propagation of *T. tarda*. Sucrose and BAP (6-

benzylaminopurine) promoted bulb formation, whereas ABA (abscisic acid) and cold treatment inhibited this process. Although further optimization of cultivation conditions is still required to enhance bulb development and propagation efficiency, the presented protocol demonstrates potential for the successful in vitro propagation of *T. tarda*.

Additionally, Podwyszyńska et al. [12] investigated *in vitro* cultivation of *Tulipa gesneriana* L. Shoot regeneration from stem explants was achieved using a medium containing 5 μM zeatin and 5 μM α-naphthaleneacetic acid. However, approximately 90% of regenerated structures lacked meristematic tissue and were unsuitable for further propagation. Meristem formation was moderately enhanced with silver thiosulfate and significantly improved with paclobutrazol or methyl jasmonate. Pre-culturing shoots in liquid media further increased multiplication rates.

Studies of species of the genus *Scirpus*:

The presence of *Scirpus kasachstanicus* has been recorded in the Ile-Balkhash Nature Reserve, underscoring the importance of its conservation [13]. Developing and applying in vitro culture techniques for this species would provide an effective means to preserve its genetic diversity and support the restoration of natural populations.

At the Zhezkazgan Botanical Garden, research has been conducted to assess the introduction potential of *Tanacetum ulutavicum*. This included a preliminary evaluation of seed productivity and seed quality indicators [14–15]. However, no specific data on the use of in vitro culture methods for this species have been found in available sources.

S. Garton et al. [16] investigated the micropropagation and regeneration features of *Alnus glutinosa* under *in vitro* conditions. Their research focused on the morphogenetic potential of different plant organs and tissues within the *Alnus* genus, aiming to identify optimal parameters for successful propagation and regeneration. The findings contributed valuable insights for establishing species-specific protocols for woody plants.

In her study, N.V. Romadanova [17] optimized *in vitro* cultivation conditions for various species of the *Berberis* genus. As a result, an in vitro collection of barberry was established, consisting of 59 accessions, including: 1 form of *B. amurensis*, 12 - *B. iliensis*, 27 - *B. integerrima*, 1 - *B. koreana*, 1 - *B. nummularia*, 2 - *B. oblonga*, 1 - *B. sibirica*, 12 - *B. sphaerocarpa*, 1 - *B. thunbergii*, 1 - *B. vulgaris*. We should also note the absence of data on the application of *in vitro* culture methods for the rare species *Berberis karkaralensis*. However, no published data were found regarding the application of in vitro techniques to the rare species *Berberis karkaralensis*, highlighting a gap in current research.

E.G. Martyushova et al. [18] examined the *in vitro* introduction of rare and endangered species of the Ural region, including *Pulsatilla flavescens*. The study focused on the sterilization of explants and the optimization of culture conditions for growth and development under *in vitro* conditions.

In a related study, K. Danova et al. [19] applied *in vitro* cultivation techniques for *Pulsatilla patens*, with a specific focus on root induction. A suitable rooting medium was developed for this difficult-to-root species, demonstrating its potential for improved conservation and propagation.

K. Kukulczanka et al. [20] demonstrated that *Drosera* seeds germinate readily under *in vitro* conditions, with seedlings showing vigorous branching and rapid root development. Among the tested media, the RM medium yielded the best results. Continuous growth of *Drosera* plants *in vitro* allows for the production of hundreds of plantlets annually through subculturing from axillary buds or leaf segments. These in vitro-grown plants can also be stored at 1-8°C for several months, extending up to 18 months if necessary.

P. Jadczak et al. [21] further investigated micropropagation of *Drosera rotundifolia*. Adventitious shoots derived from established *in vitro* cultures were transferred to full-strength MS medium as well as to nutrient-reduced variants. The results showed that optimal growth and development strongly depended on the composition of the medium.

M.A. Nikiforova et al. [22] explored strategies to overcome seed dormancy in rare *Oxytropis* species, including *O. subverticillaris*. The study revealed that the seeds exhibit physical dormancy, requiring specific pre-germination treatments to initiate successful sprouting.

In vitro cultures have also been explored to increase the production of pharmacologically active compounds such as saponins in species of the genus *Astragalus* [24]. These biotechnological approaches provide a controlled environment for metabolite analysis and the potential for large-scale production of secondary compounds of interest.

A.A. Erst et al. [25] successfully established regenerated plants, callus cultures (from both shoot and root origins), and hairy root cultures of *Hedysarum theinum*. The optimal medium for large-scale propagation was MS medium supplemented with 5 µM BAP, 200 mg/L glutathione, and 200 mg/L casein hydrolysate. For the rooting stage, ½-strength MS with 7 µM NAA proved effective. The study highlighted that nutrient medium composition, explant type, and explant orientation significantly influence callus formation. The optimal conditions included an inverted explant position and the use of either B5 medium with 10 µM 2,4-D or B5 medium with 10 µM NAA. The growth index, in terms of dry biomass, was 4.8 for stem explants and 2.7 for root explants. Biochemical analysis of secondary metabolites confirmed that cell, tissue, and organ cultures of *H. theinum* can serve as a valuable source of medicinal raw materials. Furthermore, the findings point to promising prospects for future studies aimed at developing high-yielding *H. theinum* lines with enhanced biosynthesis of biologically active substances (BAS) in vitro.

In a study by the authors N. Pushkarova et al. [27] examined the *in vitro* cultivation methods of Crambe tatagris and their effect on the biochemical composition of the plant. The results showed that plants grown in vitro have a high content of α-linolenic acid and the absence of erucic acid, which makes them promising for use in the food and chemical industries.

When establishing *in vitro* collections of rare and endangered species, strict identification protocols must be followed. Passport data should include GPS coordinates, photographs of the natural habitat, and information about environmental conditions [28]. Ideally, samples should be collected from multiple natural populations to ensure genetic representativeness and support protocol development for *in vitro* conservation [29, 30].

The passport data of specimens are necessary for the formation of a unified database of *in vitro* culture collections, which will allow not only to use the obtained clones to replenish living collections and reintroduction, but also to include them in international exchange programs.

Many rare species exhibit lower genetic diversity than widespread taxa, making them more vulnerable to environmental changes [31, 32]. Understanding both intra- and interpopulation genetic variation is essential for designing effective conservation strategies [33, 34]. The application of modern molecular genetic techniques allows researchers to assess the genetic structure of plant populations, aiding in the formulation of national biodiversity preservation programs [35].

*Conclusion*

The application of in vitro biotechnological techniques for conserving rare and endangered plant species listed in the Red Book of Kazakhstan has proven to be a highly effective approach for both species preservation and population restoration. Particular attention is given to species endemic to the Ulytau region, contributing to the protection of the region's unique flora and biodiversity on a national scale.

The studies reviewed in this paper draw upon the experience of both national and international researchers and highlight the potential of plant biotechnology as a tool for conservation. When selecting *in vitro* conservation strategies, the biological characteristics of each taxon must be considered to ensure success. It is also crucial to collect a sufficient number of samples from across the species' range to capture genetic variability.

Scientific information on the application of *in vitro* techniques to rare and endangered plant species found in the Ulytau region remains limited. Therefore, we conducted a literature review focusing on species closely related to, or belonging to the same genera as, our target taxa. The methodologies and results reported in these studies may provide a valuable basis for the development of species-specific *in vitro* protocols applicable to the plants of the Ulytau region.

General recommendations are available for all stages of micropropagation – including *in vitro* initiation, shoot multiplication, rooting, and storage. However, due to species-specific responses, individualized protocols must be developed for each species listed in the Red Book of Kazakhstan and particularly those found in the Ulytau region.

Biotechnological methods make it possible to produce large quantities of genetically uniform planting material in a short time from a minimal amount of initial tissue. The literature analysis presented here revealed both well-established methodologies and underexplored areas, indicating the need for further research. The resulting plant material can be used for *ex situ* conservation, live collections, and reinforcement of depleted natural populations. These findings can inform national programs focused on preserving Kazakhstan's floral gene pool and support reintroduction initiatives targeting endangered taxa.

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# СИРЕК ЖӘНЕ ЖОЙЫЛЫП КЕТУ ҚАУПІ БАР ӨСІМДІК ТҮРЛЕРІ ҮШІН *IN VITRO* ЖАҒДАЙЫНДА МИКРОКЛОНАЛДЫ КӨБЕЙТУ ӘДІСІН ҚОЛДАНУ

**Аңдатпа.** Мақалада Ұлытау облысында таралған сирек және жойылып кету қаупі бар өсімдік түрлерін *in vitro* технологияларын қолдану арқылы микроклоналды көбейту ерекшеліктері қарастырылады.

Микроклоналды көбейту — сирек және ерекше қорғалатын өсімдіктерді сақтау мен көбейтуге бағытталған прогрессивті биотехнологиялық әдіс. *In vitro* технологиясы генетикалық жағынан біртекті өсімдіктердің зертханалық жағдайда көп санын алуға мүмкіндік береді, бұл әсіресе табиғи популяциясы аз түрлер үшін аса өзекті.

Әдеби дереккөздерге жасалған талдау Қазақстандағы сирек кездесетін өсімдіктерді микроклоналды көбейтуге қатысты жинақталған ғылыми тәжірибелерді жүйелеуге мүмкіндік берді. Жүйелеу нәтижесінде тиімді көбейту әдістері айқындалды. Сонымен қатар, талдау барысында жекелеген түрлерге қатысты зерттеулердің жеткілікті жүргізілмегендігі белгілі болды. Түрге бейімделген өсіру хаттамаларын әзірлеу мен өсімдіктерді *ex vitro* жағдайларына бейімдеу әдістерін жетілдіру - болашақ зерттеулердің негізгі бағыттары ретінде ұсынылады.

**Кілт сөздер:** әдеби деректер; сирек өсімдіктер; биотехнологиялық әдістер; микроклоналды көбейту әдісі; in vitro; Ұлытау.

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## ПРИМЕНЕНИЕ МЕТОДА МИКРОКЛОНАЛЬНОЕ РАЗМНОЖЕНИЕ В УСЛОВИЯХ *IN VITRO* ДЛЯ РЕДКИХ И ИСЧЕЗАЮЩИХ ВИДОВ РАСТЕНИЙ

**Аннотация.** В статье рассматриваются особенность метода микроклонального размножения редких и исчезающих видов растений, встречающихся в Улытауской области с применением технологий *in vitro*.

Микроклональное размножение представляет собой прогрессивный биотехнологический метод, направленный на сохранение и воспроизводство редких и особо охраняемых видов растений. Технология *in vitro* позволяет получать большое количество генетически однородных растений в лабораторных условиях, что особенно актуально для видов с ограниченными природными популяциями.

Анализ литературных источников позволил систематизировать накопленный научный опыт по микроклональному размножению редких растений в Казахстане. В результате систематизации были определены наиболее эффективные методы размножения. Кроме того, анализ выявил недостаточную изученность отдельных видов. Разработка видоспецифичных протоколов культивирования и совершенствование методов акклиматизации растений к условиям *ex vitro* рассматриваются как приоритетные направления будущих исследований.

**Ключевые слова:** литературные источники; растения; редкие виды; биотехнологические методы; микроклональное размножение; in vitro; Улытау.