

УДК 581.143.6

MOLECULAR GENETIC CHARACTERIZATION AND OPTIMIZATION OF EX VITRO ADAPTATION AND ACCLIMATIZATION OF ENDANGERED *BERBERIS ILIENSIS* M. POP. MICROSHOOTSNurtaza A.^{1,2}, Yessimseitova A.^{1,2}, Dyussebekova D.^{1,2}, Abileva G.¹, Bayanbay S.¹, Narmakhan K.¹, Nurumov D.³ and Kakimzhanova A.^{1,2,*}¹National Center for Biotechnology, 13/5, Korgalzhyn Road, Astana 010000, Kazakhstan;²LLP«Greenlab», Astana 010000, Kazakhstan;³Charyn State National Nature Park, Kydyrbaev street, 4, Chundzha village, Uyghur district, Almaty Region, 041800, Kazakhstan.

*Correspondence: kakimzhanova@biocenter.kz

ABSTRACT

The conservation of rare and endangered plant species requires the development of effective approaches for obtaining viable planting material suitable for ex situ preservation and subsequent reintroduction. *Berberis iliensis* M. Pop. is a rare endemic species with limited natural regeneration, which necessitates the application of biotechnological methods. The aim of this study was to obtain viable planting material of *Berberis iliensis* using in vitro culture followed by adaptation and ex vitro acclimatization. Prior to micropropagation, the source material was characterized molecularly using the nuclear ITS region and the chloroplast rbcL gene. Nine DNA preparations were sequenced at both loci; BLAST analysis against GenBank returned 98.36–99.84 % nucleotide identity for ITS and 98.44–99.83 % for rbcL, assigning the material unambiguously to the genus *Berberis*. Species-level assignment could not be derived from these loci, because no reference sequences of *B. iliensis* are currently deposited in GenBank and both markers are known to lack discriminatory power within *Berberis*; species identity was therefore established morphologically from voucher material, and the molecular data served to exclude misidentification with unrelated taxa and cross-contamination between preparations. Neighbour-joining analysis of the ITS alignment placed all nine samples in a single clade with *B. integririma* (85 % bootstrap support), three of them being identical to the reference sequence KC575602.1. Rooted microshoots obtained by micropropagation according to a previously published protocol were used in the experiments. The effect of substrate composition on plant adaptation was evaluated. The highest growth and development parameters were obtained on a soil:sand substrate, where shoot height reached 6.53 ± 0.14 cm, the number of leaves was 6.30 ± 0.23 , root number was 4.93 ± 0.14 , and root length was 5.11 ± 0.20 cm, with all differences being statistically significant compared to the control. During acclimatization, the influence of mineral nutrition was assessed. The most pronounced positive effect was observed with calcium nitrate treatment, resulting in maximum plant height (16.84 ± 0.42 cm), number of leaves (20.45 ± 0.61), and crown diameter (15.83 ± 0.62 cm). The results demonstrate that the successful transition of in vitro-derived plants to ex vitro conditions depends on substrate properties and mineral nutrition, which affect plant growth and survival. The proposed approach enables the production of viable planting material and can be applied for conservation and restoration of *Berberis iliensis* populations.

Keywords: *Berberis iliensis*; rare and endangered species; in vitro culture; ex vitro acclimatization; planting material; micropropagation; adaptation; substrate composition; mineral nutrition.

INTRODUCTION

Plants of the genus *Berberis* L. (*Berberidaceae*) are widely distributed in temperate and subtropical regions and comprise approximately 500 species, many of which are of high economic and pharmacological value. Members of the genus are used in medicine, the food industry, and ornamental landscaping due to their high content of biologically active compounds, including alkaloids, flavonoids, and phenolic compounds, which determine their antioxidant, anti-inflammatory, and antimicrobial properties [1], [2].

Berberis iliensis M. Pop. holds a special place among the species of this genus—it is an endemic species of Kazakhstan's flora, found in the floodplain ecosystems of the Ili and Charyn rivers. The species is listed in the Red Book of the Republic of Kazakhstan [3] and included in the IUCN Red List [4], reflecting its vulnerable status and the need to develop conservation measures. Furthermore, a significant reduction in tugai ecosystems—exceeding 90%—further exacerbates the decline in the size of this species' wild populations [5].

In recent decades, there has been a significant decline in

the range and population size of this species, caused by human-induced factors, including changes in the hydrological regime, the degradation of tugai ecosystems, and habitat fragmentation [6]. Similar processes are characteristic of rare floodplain ecosystems in the region, where falling water levels and changes in soil and vegetation cover lead to a decline in population health and the transformation of plant communities [7].

Despite its limited range, *B. iliensis* is characterized by a high level of genetic diversity and a complex population structure. ISSR analysis revealed a high level of polymorphism and significant intrapopulation variability, while the presence of hybrid forms with closely related species was established, which complicates the population structure and underscores the need to conserve the species' genetic resources [8], [9]. At the same time, reproductive processes in natural conditions are unstable: seed quality and germination rates vary significantly depending on environmental conditions, which limits the effectiveness of natural population regeneration [6].

The species' rich phytochemical composition further enhances its significance. Dozens of secondary metabolites, in-

cluding phenolic acids, flavonoids, and alkaloids, have been identified in various parts of *B. iliensis*, which account for the plant's high biological activity [10]. It has been established that the leaves and fruits exhibit significant antioxidant activity, with IC_{50} values reaching 0.008 mg/mL, indicating the species' high potential as a source of pharmacologically valuable compounds. At the same time, the leaves represent a renewable source of biologically active substances, making it possible to use them without destroying the plants [2].

The conservation of such valuable, rare, and endemic plant species is one of the key objectives of modern science and conservation efforts, carried out at both the national and international levels using *in situ* and *ex situ* strategies. This issue is of particular importance for species with limited ranges and high levels of human impact, including *Berberis iliensis* [11].

Despite the high genetic diversity and significant pharmacological value of species in the genus *Berberis*, the natural reproduction of these plants is limited by a number of factors [12]. These include low and inconsistent seed germination rates, the presence of embryonic dormancy, irregular fruiting, and a high dependence of reproductive processes on environmental conditions. Additionally, it has been established that the laboratory germination rate of barberry seeds can vary significantly—from high values to extremely low rates in certain species and forms—which is associated with the structural characteristics of the seed coat and limited water permeability [5].

Vegetative propagation is also not a universal solution, as it requires a significant amount of source plant material and may increase the anthropogenic pressure on natural populations [13]. In addition, seed propagation is associated with high genetic variability in the offspring, which makes it difficult to preserve valuable genotypes and to use the plants for breeding and pharmacological purposes [14]. Taken together, these factors create systemic constraints on the reproduction of *Berberis* species and necessitate the search for alternative approaches to their conservation.

Currently, one of the most promising approaches involves the use of biotechnological methods, particularly *in vitro* culture, which enables the production of aseptic plants, the preservation of genetic diversity, and the establishment of collections that serve as a basis for cryopreservation and subsequent reintroduction. Micropropagation protocols have been developed for several species of the genus *Berberis*, ensuring high regenerative capacity, increased propagation rates, and successful acclimatization of plants under controlled conditions. It has been demonstrated that the use of optimized culture media and growth regulators allows for achieving significant propagation rates and obtaining high-quality plants [5]. Furthermore, some studies have noted an increase in the content of bioactive compounds in plants grown *in vitro* compared to wild populations [13].

However, despite the progress made, the processes of *in vitro* culture and subsequent micropropagation remain complex and multifaceted. Existing micropropagation protocols are highly species-specific and cannot be directly applied even to closely related species [14]. Although *in vitro* propagation systems have been developed for certain species within the genus, micropropagation methods for *Berberis iliensis* remain insufficiently optimized and require further refinement to ac-

count for its biological characteristics [15]. Moreover, a crucial step in determining the practical effectiveness of micropropagation is the successful adaptation of plants to *ex vitro* conditions and the production of viable planting material. It is known that plants obtained *in vitro* culture are characterized by altered morphological and physiological traits, including poorly developed cuticles, non-functional stomata, and an underdeveloped root system, which leads to high mortality rates when transferred to natural conditions [16].

It has also been established that such plants exhibit abnormalities at the anatomical, physiological, and biochemical levels, including low photosynthetic activity, increased transpiration, and inadequate water balance regulation, which accounts for their low resistance to abiotic and biotic stresses when transferred to an uncontrolled environment. In this regard, a key step is gradual acclimatization aimed at restoring the morphophysiological structure of plants, forming a functional photosynthetic system, and developing mechanisms of resistance to external factors [17]. It has been established that optimizing acclimatization conditions—including controlling humidity and light levels and transitioning from a heterotrophic to an autotrophic mode of nutrition—significantly improves plant survival and ensures the production of high-quality planting stock suitable for subsequent introduction and restoration of natural populations [16].

Thus, the conservation of *Berberis iliensis* requires a comprehensive approach that includes the use of biotechnological propagation methods and the subsequent production of viable planting stock. An important stage is not only the production of plants *in vitro* but also their successful transition to *ex vitro* conditions, since it is precisely the stages of adaptation and acclimatization that largely determine the effectiveness of the plants' subsequent use for the introduction and restoration of natural populations. In this regard, the optimization of the adaptation processes of rooted microshoots and their acclimatization under protected ground conditions becomes particularly relevant.

The aim of this study is to characterize the source material of *Berberis iliensis* molecularly using the ITS and *rbcL* markers, to evaluate the adaptability of rooted microshoots obtained through micropropagation, and to analyze the effects of various substrates and nutrient conditions on their acclimatization processes under greenhouse conditions.

MATERIALS AND METHODS

The source material consisted of one-year-old shoots of *Berberis iliensis* (Fig. 1), which were collected in the Charyn State National Nature Park (Almaty Region).

Molecular-genetic characterization of Berberis iliensis

The following methods were used for molecular genetic characterization: DNA extraction and purification, polymerase chain reaction, and determination of nucleotide sequences of the nuclear and chloroplast genomes using DNA primers. Three shrubs were studied for molecular genetic characterization. DNA was isolated in triplicate from each shrub, yielding nine DNA preparations (designated BI-1–BI-9), each of which was amplified and sequenced at both the ITS and the *rbcL* locus.

The CTAB method was used to extract plant DNA. Leaf



a – source material



b – one-year-old shoots

Figure. 1. Plant material from *Berberis iliensis* used in the study

tissue was used for DNA isolation [18]. The optical density of the DNA solution was measured at a wavelength of 260 nm using a Nanodrop 1000, which allowed us to determine the concentration and purity of the nucleic acids. The ratio of the D_{260} value to the D_{280} value provided an indication of the nucleic acid's purity. Pure DNA preparations had D_{260}/D_{280} ratios of 1.8 and 2.0, respectively. The isolated mitochondrial DNA from plant samples is stored at -80°C , and working solutions at a concentration of 30 ng/ μL are stored at -20°C . Additionally, leaf material is stored in bags at -20°C .

For molecular genetic identification, the universal primers ITS1 (forward primer 5'AGAAGTCGTAACAAGGTTTC-CGTAGG3') and ITS4 (reverse primer 5'TCCTCCGCT-TATTGATATGC3') with an annealing temperature of 55°C (fragment size 700 bp) were used [19]. To amplify the chloroplast genome, the *rbcL* primers (forward primer 5'ATGT-CACCACAAACAGAGACTAAAGC3') and *rbcL* (reverse primer 5'GTAATCAAGTCCACCACG3'), with a fragment size of 599 bp and an annealing temperature of 55°C , were used [20]. The primers were synthesized in the organic synthesis laboratory at the National Center for Biotechnology.

PCR was performed in a 30 μL reaction mixture containing: 1 $\times$ PCR buffer; 1.5 mM MgCl_2 ; 0.2 mM of each dNTP; 0.5 μM of each forward and reverse ITS or *rbcL* primer; Tag DNA polymerase (0.04 U/ μL); 30 ng DNA. Amplification was performed under the following conditions: initial denaturation at 95°C for 4 min; 95°C for 40 seconds, 55°C for 40 seconds, 72°C for 1 minute; 35 cycles. Final extension for 4 minutes at 72°C on an Eppendorf MasterCycler Pro (Germany).

The amplification products obtained using primers targeting the nuclear and chloroplast genomes were separated on a 1.5% agarose gel in the presence of ethidium bromide using a horizontal electrophoresis chamber in 1 $\times$ TAE buffer. Electrophoresis was performed at 100 volts for 40 minutes. A 700 bp DNA Ladder (Fermentas) was used as a molecular weight marker. Electrophoresis results were photographed under UV light using a GelDoc XR (BioRad, USA).

The PCR product was purified using Sap and ExoI enzymes (Thermo Scientific). These reagents are used for enzymatic purification of the amplified product: the first incubation hydrolyzes excess primer and dephosphorylates the nucleotides; the second, high-temperature incubation inactivates the enzymes, which minimizes loss of the PCR product and allows for subsequent sequencing without additional column

purification. The reaction was performed in 20 μL of reaction mixture containing 1 $\times$ Sap buffer, 10 μL of PCR product, 3 units of ExoI exonuclease, 1 unit of Sap alkaline phosphatase at 37°C for 30 minutes, followed by enzyme inactivation at 75°C for 15 minutes.

DNA sequencing was performed using the ABI PRISM® BigDye™ Terminator v. 3.1 kit, followed by analysis of the reaction products on an Applied Biosystems 3730 DNA Analyzer. Following sequencing, the nucleotide sequences of the analyzed samples were assembled and edited using the SeqMan software [21].

The consensus sequences were compared with the GenBank nucleotide collection (NCBI) using the BLASTn algorithm. For each query the three highest-scoring hits were recorded together with their accession numbers and percentage nucleotide identity. Because the database currently contains no ITS or *rbcL* reference sequences deposited under the name *Berberis iliensis*, the BLAST output was interpreted at the genus level, while species identity was assigned morphologically from the collected voucher material in accordance with the Red Data Book of the Republic of Kazakhstan [3].

For phylogenetic analysis, the nine ITS consensus sequences were aligned with 18 ITS reference sequences of eight *Berberis* species retrieved from GenBank (*B. integririma*, *B. amurensis*, *B. vulgaris*, *B. dielsiana*, *B. feddeana*, *B. canadensis*, *B. subsessiliflora* and *B. aristata*) using the FAMSA algorithm; the alignment was trimmed to a common region of 607 bp. Evolutionary distances were calculated under the Kimura 2-parameter model with pairwise deletion of ambiguous positions, and the tree was inferred by the neighbour-joining method with 1000 bootstrap replicates. The tree was midpoint-rooted, as no ITS sequence suitable as an out-group was available; bootstrap values below 50 % are not shown. No phylogenetic analysis was performed for *rbcL* owing to the absence of informative variation at this locus. It should also be noted that GenBank contains no ITS or *rbcL* sequences for *Berberis sphaerocarpa* Kar. et Kir., the congener known to hybridize with *B. iliensis* in the Ili and Charyn valleys [9]; this species could therefore not be included in the analysis.

Adaptation of Berberis iliensis microshoots

Microplants of *Berberis iliensis* were obtained in accordance with the method described by Karimova et al. [22]. For

adaptation of the clones to soil, the rooted microshoots were first hardened off for 24 hours on a nutrient medium in open culture vessels. This procedure improved plant survival rates under non-sterile soil conditions. The plant roots were rinsed to remove agar residues using a weak solution of potassium permanganate. Four types of substrate were tested for microshoot adaptation: 1 – peat; 2 – soil; 3 – peat:sand (1:1); 4 – soil:sand (1:1). Each substrate variant was tested using 20 microshoots ($n = 20$ per treatment). Plants were grown in a controlled environment chamber under SMD5050 LED grow lights. To assess the effectiveness of the conditions, the following parameters were studied: shoot height, number of leaves, number of roots, and length of the longest root. Phenological observations were conducted on the first day of planting and on the 40th day. During the observation period, watering was performed with water as needed.

Acclimatization of *Berberis iliensis* plantlets

To acclimate the plants to open-field conditions, the seedlings were transferred to a plastic-covered greenhouse. To promote plant growth and development, the effects of various fertilizers were studied. Thus, seven irrigation fertilizer treatments were tested: Treatment 1 – control (water); Treatment 2 – calcium nitrate; Treatment 3 – potassium nitrate; Treatment 4 – magnesium nitrate; Treatment 5 – ammophos; Treatment 6 – Yara Mila Complex; Treatment 7 – ammonium nitrate. Each fertilizer treatment was applied to 30 plantlets ($n = 30$ per treatment).

The fertilizers used in the study provided the plants with the essential macronutrients and micronutrients required for growth and development. Calcium nitrate contained nitrogen and calcium (N 15.5%, NH_4 1.1%, NO_3 14.4%, CaO 26.5%), which promotes active growth of vegetative mass and the formation of cell walls. Potassium nitrate contained nitrogen and potassium (N 13.7%, NO_3 13.7%, K_2O 46.3%) and ensured the regulation of water metabolism and plant resilience. Magnesium nitrate served as a source of nitrogen and magnesium (N 11%, NO_3 11%, Mg 9%, MgO 15%), which are essential for chlorophyll synthesis and photosynthetic activity. Ammophos (monoammonium phosphate) contained nitrogen and phosphorus (N 10–12%, P_2O_5 50–52%) and promoted root system development and energy metabolism. The YaraMila complex fertilizer included a balanced set of macro- and micronutrients (N 12%, P_2O_5 11%, K_2O 18%, MgO 2.7%, SO_3 20%, as well as micronutrients B 0.015%, Mn 0.02%, Zn 0.02%), providing comprehensive plant nutrition. Ammonium nitrate was characterized by a high content of available nitrogen (N 34.4%) in nitrate and ammonium forms, which promotes intensive plant growth. All fertilizers were applied as

aqueous solutions at a concentration of 1 g/L (except ammophos, applied at 0.5 g/L due to its lower solubility). Fertilizer application was carried out by watering at the root zone at a rate of 50 mL per plant every 10 days for 50 days (5 applications total). To assess fertilizer effectiveness, the following parameters were studied: shoot height, number of leaves, and crown diameter. Phenological observations were conducted on the first day after transferring the plants to the greenhouse and on the 50th day. The temperature in the greenhouse was maintained at 22–28°C using a heating and ventilation system. Watering, spraying, and loosening the soil were carried out as needed.

Statistical analysis

The experimental results were analyzed using one-way analysis of variance (ANOVA), and significant differences were identified using Tukey's post hoc test. The data were analyzed using the SPSS 23.0 statistical software package (IBM Inc., New York, USA). Data are presented as means $\pm$ standard error.

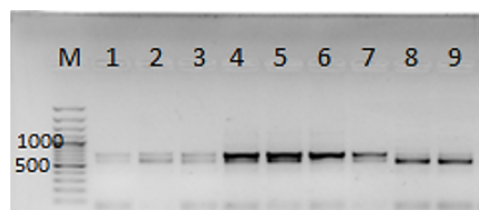
RESULTS

MOLECULAR-GENETIC CHARACTERIZATION OF *BERBERIS ILIENSIS*

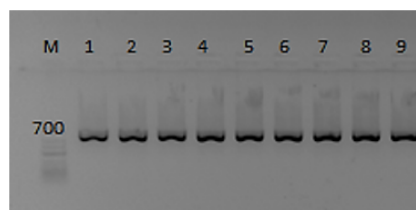
For molecular genetic analysis, DNA was isolated in triplicate from each of the three shrubs studied (nine preparations, BI-1–BI-9). DNA concentration ranged from 140.3 to 356.3 ng/ μL , with most values falling within the range of 178.2–274.1 ng/ μL , indicating a sufficient yield of nucleic acid for subsequent molecular analyses without the need for additional concentration. The OD260/OD280 ratios ranged from 1.8 to 1.9 for all samples, which meets generally accepted criteria for DNA purity and indicates the absence of significant impurities of proteins and secondary metabolites characteristic of the genus *Berberis*, including phenolic compounds and alkaloids capable of inhibiting enzymatic reactions.

DNA amplification using a universal ITS primer yielded fragments approximately 700 bp in length (Fig. 2a). When using the *rbcL* primer, amplicons of approximately 600 bp were formed (Fig. 2b), which fully corresponds to the expected sizes for these marker regions and confirms the correct choice of primers. Electrophoretic analysis of the PCR products revealed the presence of clear and intense bands in the absence of pronounced nonspecific amplicons and primer dimers, indicating high reaction specificity and optimal amplification conditions.

Sequencing yielded nucleotide sequences for all nine DNA preparations at both loci. Following assembly and editing in



a - Electrophoregram of DNA profiles of *Berberis iliensis* using the ITS region



b - Electrophoregram of DNA profiles of *Berberis iliensis* using the *rbcL* region

Figure 2. Electrophoregram of amplicons from *Berberis iliensis*. M – molecular weight marker (Fermentas, 50–700 bp); 1–9 – samples of *Berberis iliensis*

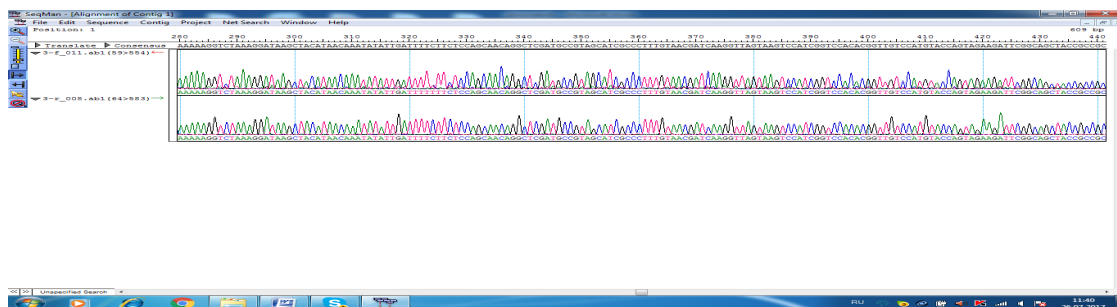


Figure 3. A fragment of the *rbcl* primer nucleotide sequence in the SeqMan program

SeqMan, the ITS consensus sequences were 636–679 bp long and the *rbcl* sequences 563–593 bp, with ambiguous positions accounting for 0.16–0.88 % and 0.17–1.21 % of the sequence length, respectively (Fig. 3). All consensus sequences were subsequently queried against the GenBank nucleotide collection using the BLASTn algorithm (Table 1).

All eighteen sequences returned *Berberis* as the top-scoring genus, and no hits to other genera occurred among the highest-scoring alignments. Nucleotide identity to the closest reference sequence ranged from 98.36 % to 99.84 % (mean 99.13 %) for ITS and from 98.44 % to 99.83 % (mean 99.42 %) for *rbcl*. The generic assignment of the source material is therefore unambiguous.

Species-level assignment, by contrast, could not be derived from the BLAST output, for two independent reasons. First, GenBank contains no ITS or *rbcl* sequences deposited under the name *Berberis iliensis*, so no conspecific reference exists against which the samples could be matched; each query was consequently matched to whichever congener is represented in the database – most frequently *B. feddeana* (MH711425.1, MH711787.1) and *B. dielsiana* (MH711421.1) for ITS, and *B. aristata* (NC_043829.1, MK714340.1) for

rbcl. Second, the identity values themselves demonstrate the absence of species-level resolution at these loci: for ITS the three highest-scoring hits in most samples were three different species sharing an identical percentage identity, while individual *rbcl* sequences reached 99.8 % identity to *B. aristata*, a Himalayan species geographically and morphologically remote from the material studied. Both observations are consistent with published evidence that neither *rbcl* nor ITS discriminates species within *Berberis*, a genus characterized by reticulate evolution and interspecific hybridization [24].

Notably, sample BI-5 matched *Berberis integerrima* Bunge (KC575602.1) with 99.84 % identity – the highest value obtained in the present study. This result is taxonomically consistent rather than contradictory: *B. iliensis* Popov is placed in synonymy under *B. integerrima* Bunge in the current global taxonomic backbone [25], whereas the Kazakhstani floristic tradition and the Red Data Book of the Republic of Kazakhstan [3] maintain it as an independent endemic species. Hybridization between *B. iliensis* and the sympatric *B. sphaerocarpa* in the Ili and Charyn valleys has likewise been documented by ISSR analysis [9], which further limits the applicability of single-locus barcoding within this complex.

Table 1. BLAST identification of the ITS and *rbcl* sequences of the *Berberis iliensis* source material against the GenBank nucleotide collection

Sample	ITS, bp	Closest GenBank match (ITS)	Identity, %	<i>rbcl</i> , bp	Closest GenBank match (<i>rbcl</i>)	Identity, %
BI-1	679	MH711425.1 <i>B. feddeana</i>	98.82	589	NC_043829.1 <i>B. aristata</i>	99.83
BI-2	658	MH711787.1 <i>B. feddeana</i>	99.39	590	NC_043829.1 <i>B. aristata</i>	99.49
BI-3	671	MH711425.1 <i>B. feddeana</i>	98.63	590	NC_043829.1 <i>B. aristata</i>	99.83
BI-4	676	MH711425.1 <i>B. feddeana</i>	99.10	563	NC_043829.1 <i>B. aristata</i>	99.27
BI-5	636	KC575602.1 <i>B. integerrima</i>	99.84	580	NC_043829.1 <i>B. aristata</i>	98.44
BI-6	649	MH711787.1 <i>B. feddeana</i>	99.07	592	NC_043829.1 <i>B. aristata</i>	99.32
BI-7	670	MH711787.1 <i>B. feddeana</i>	99.40	589	NC_043829.1 <i>B. aristata</i>	99.14
BI-8	662	MH711425.1 <i>B. feddeana</i>	99.54	575	NC_043829.1 <i>B. aristata</i>	99.82
BI-9	676	MH711425.1 <i>B. feddeana</i>	98.36	593	NC_043829.1 <i>B. aristata</i>	99.66

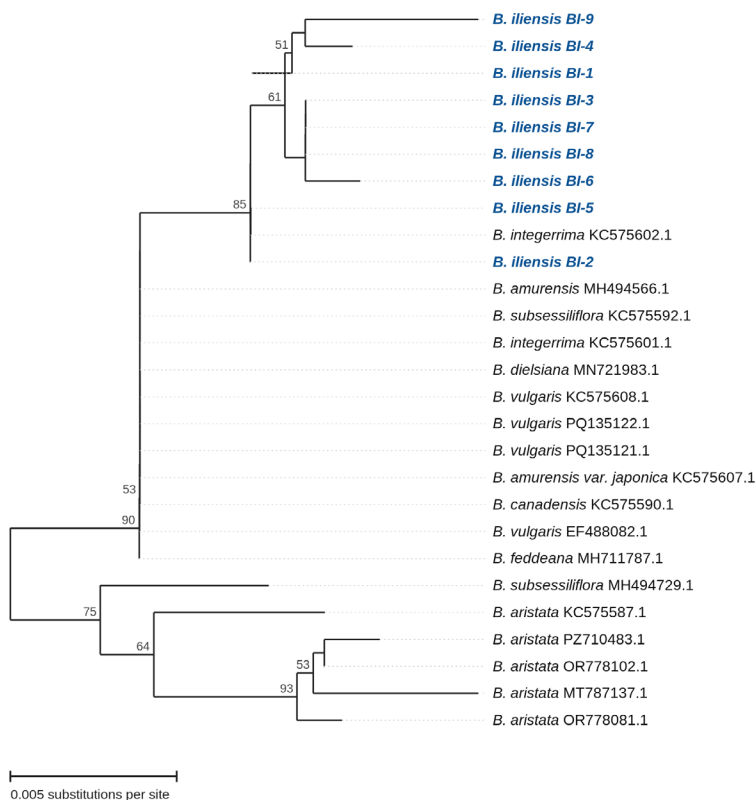


Figure 4. Neighbour-joining tree based on ITS sequences of the *Berberis iliensis* source material (BI-1–BI-9, in bold) and reference sequences of *Berberis* species from GenBank. Kimura 2-parameter distances, 1000 bootstrap replicates; bootstrap values below 50 % are not shown. The tree is midpoint-rooted. Scale bar: 0.005 substitutions per site.

Taken together, the molecular data confirm that the source material belongs to the genus *Berberis*, exclude misidentification with unrelated taxa and cross-contamination between DNA preparations, and provide a verifiable genetic record of the accessions entered into *in vitro* culture.

The closest match is the highest-scoring BLASTn hit. For most ITS queries the second- and third-ranked hits showed identical percentage identity to different *Berberis* species (*B. dielsiana* MH711421.1, MH711231.1); for *rbcL* the second- and third-ranked hits were *B. aristata* MK714340.1, *B. amurensis* var. *latifolia* KM057377.1 and *B. diaphana* MH457122.1 at the same identity value. Full BLAST output is available from the authors on request.

To visualize the relationships of the studied material to congeneric taxa, a neighbour-joining tree was constructed from the ITS alignment (607 bp) comprising the nine *B. iliensis* sequences and 18 reference sequences of eight *Berberis* species (Fig. 4). All nine samples were placed in a single clade supported by 85 % bootstrap, which contained no reference sequence other than *B. integrerrima* KC575602.1. This accession was not resolved as sister to the studied material but was nested among the samples: the Kimura 2-parameter distance between KC575602.1 and samples BI-1, BI-2 and BI-5 was zero, that is, the sequences were identical over the aligned region, whereas distances among the *B. iliensis* samples themselves reached 0.0083. Genetic distances from the studied material to other *Berberis* species ranged from 0.0033 to 0.0285.

The topology therefore does not separate the studied material from *B. integrerrima* at the species level, since intraspe-

cific and interspecific distances overlap completely. At the same time, *B. aristata* – the species that showed 98–100 % identity in the *rbcL* comparison – was resolved as a well-supported clade (93 % bootstrap) clearly separated from the studied samples, confirming that the *rbcL* matches reflect the conservatism of that locus rather than genuine affinity. The second *B. integrerrima* accession (KC575601.1) fell outside the 85 % clade, indicating that this species is not monophyletic in the ITS data either. The phylogenetic analysis thus reproduces the pattern established by the BLAST comparison: the material is placed within *Berberis* and shows closest affinity to *B. integrerrima*, while species-level delimitation is not achievable at the ITS locus.

The use of two marker systems – nuclear (ITS) and chloroplast (*rbcL*) – provides mutually independent lines of evidence, since the two loci are differently inherited and differ in their level of evolutionary conservatism. The ITS region is the more variable of the two and is widely used to distinguish closely related species, whereas *rbcL* is conservative and informative mainly at the genus level and above [19, 20]. In the present study both markers gave concordant results at the genus level, which increases the reliability of the assignment; neither, however, resolved the material to species, in agreement with earlier findings for *Berberis* [24].

No polymorphism was detected among the nine analyzed DNA preparations at either locus. This is expected given the small number of individuals sampled (three shrubs) and the conservative nature of the loci employed, and it does not permit any inference regarding the level of intraspecific genetic

Table 2. Results of the adaptation of *Berberis iliensis* microshoots

Treatment	Day 40				Accretion			
	Shoot height, cm	Number of leaves, pcs	Number of roots, pcs	Root length, cm	Shoot height, cm	Number of leaves, pcs	Number of roots, pcs	Root length, cm
1 – peat	3.20±0.12	2.93±0.13	2.87±0.14	1.54±0.07	1.24	-0.27	0.63	0.92
2 – soil	4.27±0.15*	3.53±0.15	3.00±0.11	1.88±0.06*	2.34	0.93	0.77	1.34
3 – peat:sand (1:1)	4.96±0.17*	4.87±0.14*	4.13±0.13*	2.40±0.08*	2.75	1.87	2.10	1.89
4 – soil:sand (1:1)	6.53±0.14*	6.30±0.23*	4.93±0.14*	5.11±0.20*	4.52	3.30	3.13	4.68

*. The mean difference is statistically significant at the 0.05 level.

variability in the population. Resolving intraspecific structure in *B. iliensis* requires markers of higher variability, such as the RAPD and ISSR systems previously applied to this species [8, 9].

ADAPTATION OF *BERBERIS ILIENSIS* MICROSHOOTS

The results of the adaptation of *Berberis iliensis* microshoots showed a marked dependence of morphometric parameters on substrate composition (Table 2). After 40 days of cultivation, the lowest growth values were recorded when using peat: shoot height was 3.20 ± 0.12 cm, number of leaves was 2.93 ± 0.13 , number of roots was 2.87 ± 0.14 , and root length was 1.54 ± 0.07 cm. At the same time, growth in individual parameters was limited, and a decrease (-0.27) was even observed in the number of leaves, indicating the insufficient effectiveness of this substrate during the adaptation stage.

The use of soil contributed to improved growth parameters: shoot height reached 4.27 ± 0.15 cm, root length— 1.88 ± 0.06 cm, with an increase in growth observed across all parameters. However, the most pronounced positive effect was observed when mixed substrates were used. In the peat:sand variant, shoot height was 4.96 ± 0.17 cm, the number of leaves was 4.87 ± 0.14 , the number of roots was 4.13 ± 0.13 , and root length was 2.40 ± 0.08 cm, accompanied by significant growth in all indicators (height +2.75 cm, leaves +1.87, roots +2.10, root length +1.89 cm).

The maximum values for all parameters studied were obtained when using a soil-sand substrate. Shoot height reached 6.53 ± 0.14 cm, the number of leaves was 6.30 ± 0.23 , the

number of roots was 4.93 ± 0.14 , root length was 5.11 ± 0.20 cm, and growth was the highest among all variants (respectively, +4.52 cm, +3.30 pcs., +3.13 pcs., and +4.68 cm). All differences compared to the control were statistically significant.

The results indicate that adding sand to the substrate significantly improves the adaptation efficiency of *Berberis iliensis* microshoots (Fig. 5). This is likely due to improved aeration and water-air conditions, which are particularly important for root system development. Pure peat, on the other hand, is characterized by excessive water-holding capacity and insufficient air permeability, which can limit plant growth and development. Soil provides a more balanced nutrient supply; however its combination with sand creates optimal conditions for both above-ground growth and active root formation.

Thus, a soil-sand substrate is the most effective for the acclimatization of *Berberis iliensis* microshoots, ensuring maximum growth of both the upper and lower parts of the plant.

ACCLIMATIZATION OF *BERBERIS ILIENSIS* PLANTLETS

The results of acclimatizing *Berberis iliensis* plants under greenhouse conditions showed a significant effect of mineral nutrition on the morphometric indicators of growth and development (Table 3). After 50 days of cultivation, the control plants (watered with water) exhibited the lowest values: height was 10.34 ± 0.09 cm, number of leaves was 12.90 ± 0.46 , and crown diameter was 8.41 ± 0.12 cm. Growth in the control group was also limited (respectively, +4.20 cm, +6.55 leaves, and +4.84 cm), indicating an insufficient sup-



a – rooted microshoots in *in vitro* culture (MS + IBA 1.0 mg/L)



b – prepared microshoot for planting in soil



c – Adapted microshoots on the 40th day

Fig. 5 – Adaptation of *Berberis iliensis* microshoots

Table 3. Results of the acclimatization of *Berberis iliensis* plantlets

Treatment	Day 50			Accretion		
	Shoot height, cm	Number of leaves, pcs	Shoot height, cm	Number of leaves, pcs	Shoot height, cm	Number of leaves, pcs
1 – control (water)	10.34±0.09	12.90±0.46	8.41±0.12	4.20	6.55	4.84
2 – calcium nitrate	16.84±0.42*	20.45±0.61*	15.83±0.62*	10.96	15.10	12.23
3 – potassium nitrate	13.45±0.11*	12.65±0.40*	11.73±0.09*	7.75	6.50	8.12
4 – magnesium nitrate	11.73±0.09*	10.75±0.34*	9.91±0.08*	5.73	3.95	6.11
5 – ammophos	12.84±0.22*	12.75±0.24*	11.56±0.19*	6.69	6.30	7.94
6 – Yara Mila Complex	12.17±0.17*	11.55±0.22*	10.70±0.20*	6.19	4.45	7.13
7 – ammonium nitrate	10.57±0.24*	14.00±0.47*	12.34±0.27*	4.56	6.45	8.67

*. The mean difference is statistically significant at the 0.05 level.

ply of nutrients.

Calcium nitrate had the most pronounced stimulating effect on plant growth. In this treatment, the maximum values of all studied parameters were recorded: plant height reached 16.84 ± 0.42 cm, the number of leaves was 20.45 ± 0.61 , and crown diameter was 15.83 ± 0.62 cm. Growth was also the highest among all variants ($+10.96$ cm in height, $+15.10$ leaves, $+12.23$ cm in crown diameter), with the differences compared to the control being statistically significant. The data obtained indicate the key role of calcium in the formation of aboveground biomass and active cell division, especially during the acclimatization stage.

The application of potassium nitrate also contributed to a significant increase in growth parameters: plant height was 13.45 ± 0.11 cm, and crown diameter was 11.73 ± 0.09 cm, with growth increments of $+7.75$ cm and $+8.12$ cm, respectively. However, the effect on leaf development was less pronounced (12.65 ± 0.40 leaves; increase of 6.50 leaves), which may indicate that potassium primarily affects water balance and tissue turgor rather than the intensity of organogenesis.

The magnesium nitrate treatment showed moderate growth parameters (11.73 ± 0.09 cm in height, 10.75 ± 0.34 leaves,

9.91 ± 0.08 cm in crown diameter), with relatively low growth increments ($+5.73$ cm, $+3.95$ leaves, and $+6.11$ cm, respectively). This may be due to the fact that magnesium, being a central element of the chlorophyll molecule, has a greater influence on photosynthetic activity; however, under conditions of a limited acclimatization period, its effect is less pronounced compared to nitrogen and calcium nutrition.

The application of ammophos yielded intermediate results: plant height was 12.84 ± 0.22 cm, the number of leaves was 12.75 ± 0.24 , and crown diameter was 11.56 ± 0.19 cm, with growth ranging from $+6.30$ to $+7.94$. This indicates a positive effect of phosphorus on plant development; however, its effect was less pronounced compared to nitrate forms of nitrogen.

The Yara Mila complex fertilizer ensured balanced but moderate plant growth (12.17 ± 0.17 cm in height, 11.55 ± 0.22 leaves, 10.70 ± 0.20 cm crown diameter), accompanied by an increase of $+6.19$ – 7.13 . Despite the presence of macro- and micronutrients, this variant was inferior to calcium nitrate, likely due to the lower availability of certain elements during the initial acclimatization period.

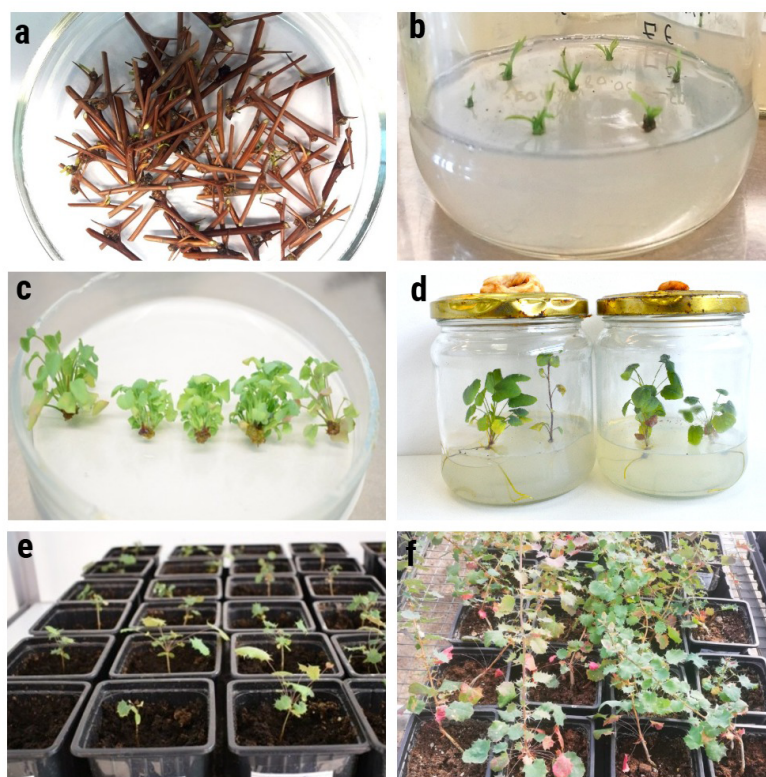
The application of ammonium nitrate resulted in a relatively modest increase in plant height (10.57 ± 0.24 cm; in-



a – adapted shoots before being transferred to the greenhouse for acclimatization

b – acclimatized plants on the 50th day

Fig. 6. Acclimatization of *Berberis iliensis* in a greenhouse



a – Source material (shoot segments with apical and lateral buds); b – Stage of establishment of *in vitro* culture and initial development of explants on nutrient medium; c – Stage of microshoot multiplication; d – *In vitro* rooting of microshoots; e – Stage of plant adaptation to *ex vitro* conditions; f – Acclimatized plants that have formed viable planting material.

Fig. 7. Stages of micropropagation, adaptation and acclimatization of *Berberis iliensis*

crease of +4.56), but was accompanied by a higher number of leaves (14.00 ± 0.47) and a significant increase in crown diameter (12.34 ± 0.27 cm; increase of +8.67). This may indicate a redistribution of growth toward the formation of leaf mass when using the ammonium form of nitrogen.

Overall, the acclimatization results show that the greatest effectiveness is achieved when using calcium nitrate, which ensures maximum plant growth across all parameters studied (Fig. 6). Other forms of fertilizer also have a positive effect, but their impact is more limited or specific in nature. The data obtained confirm the importance of selecting the appropriate form of mineral nutrition during the plant acclimatization stage, as this period is characterized by active formation of aboveground biomass and adaptation to *ex vitro* conditions.

The entire workflow of *Berberis iliensis* conservation biotechnology – from molecular characterization of source material through *in vitro* micropropagation [22], adaptation, and greenhouse acclimatization to the production of viable planting stock, is illustrated in Fig. 7.

DISCUSSION

Molecular characterization of the source material using the ITS and *rbcL* markers assigned the studied plants unambiguously to the genus *Berberis*: all eighteen sequences returned *Berberis* references as the highest-scoring hits, with 98.36–99.84 % (ITS) and 98.44–99.83 % (*rbcL*) nucleotide identity. Species-level identification, however, proved unattainable at these loci, for reasons independent of the quality of the sequence data obtained. On the one hand, GenBank holds

no ITS or *rbcL* entries deposited under the name *B. iliensis*, so no conspecific reference exists against which the samples could be matched, and the algorithm necessarily returns the most similar congener that happens to be represented in the database. On the other hand, the discriminatory power of the standard barcode loci is inherently low in this genus: Roy et al. [24] demonstrated that neither ITS, *rbcL*, *matK* nor *trnH-psbA*, singly or in combination, could separate species of Indian *Berberis*, and attributed this to probable reticulate evolution. The present data reproduce that pattern directly – several samples were equally similar to three different congeners, and one *rbcL* sequence matched the Himalayan *B. aristata* at 99.8 % identity.

The highest identity value recorded in this study, 99.84 % between sample BI-5 and *B. integerrima* (KC575602.1), merits separate comment. Rather than contradicting the identification of the material, it is consistent with it: *B. iliensis* Popov is placed in synonymy under *B. integerrima* Bunge in the current global taxonomic backbone [25], while the Kazakhstani floristic tradition and the Red Data Book of the Republic of Kazakhstan [3] maintain it as a distinct endemic species. The occurrence of hybrids between *B. iliensis* and the sympatric *B. sphaerocarpa* in the Ili and Charyn valleys, established by ISSR analysis [9], adds a further layer of complexity that single-locus barcoding cannot resolve.

The phylogenetic analysis corroborated this interpretation. The nine samples and *B. integerrima* KC575602.1 formed a single clade with 85 % bootstrap support, within which the reference accession was nested rather than sister, and three

of the samples proved identical to it across the 607 bp ITS alignment. Since distances among the studied samples (up to 0.0083) exceeded their distance to the reference (0.0000–0.0066), intraspecific and interspecific variation overlap completely and no species-level boundary can be drawn from these data. The contrast with *B. aristata* is instructive: at the *rbcL* locus this species matched the studied samples at 98–100 % identity, yet in the ITS analysis it formed a well-supported clade (93 %) far removed from them, which demonstrates that the *rbcL* similarity is an artefact of locus conservatism rather than evidence of relationship. A further constraint is that GenBank holds no sequences of *B. sphaerocarpa*, so the congener documented to hybridize with *B. iliensis* [9] could not be included in the comparison.

For the purposes of the present work these limitations do not compromise the conclusions. Species identity of the source material was established morphologically from voucher material collected in the Charyn State National Nature Park, while the role of the molecular data is to document the genetic identity of the accessions entered into in vitro culture, to exclude misidentification with unrelated taxa, and to exclude cross-contamination between DNA preparations – the standard quality-control function of molecular verification in conservation micropropagation programmes. At the same time, the results highlight a practical gap: the absence of reference sequences for *B. iliensis* in public databases. Closing this gap will require verified reference sequences of *B. iliensis* to be deposited in public repositories, which remains a task for future work. Reliable molecular delimitation of *B. iliensis* relative to *B. integerrima* and *B. sphaerocarpa* will, however, require multilocus or genome-scale approaches rather than the standard barcode loci.

The results of the adaptation experiment demonstrated a clear dependence of microshoot survival and growth on substrate composition. The soil:sand mixture (1:1) proved most effective, yielding the highest values for all measured parameters. This can be explained by the improved water-air regime of the mixed substrate: the addition of sand reduces excessive moisture retention characteristic of pure soil, while maintaining sufficient nutrient supply. Plants derived from *in vitro* culture are known to possess poorly developed cuticles and non-functional stomata, which compromises their ability to regulate water exchange after transfer to non-sterile conditions [16]. Under these circumstances, substrate aeration becomes critical for root function and plant survival. The superiority of mixed substrates over single-component media has been documented for other woody species and is consistent with the results obtained here [17]. Pure peat showed the weakest performance, likely due to its high water-holding capacity and low pH, which may be suboptimal for *Berberis* root development.

The acclimatization experiment revealed a significant effect of mineral nutrition on plant growth under greenhouse conditions. Calcium nitrate produced the most pronounced stimulating effect across all measured parameters – shoot height, leaf number, and crown diameter. This is consistent with the known role of calcium in cell wall formation and cell division, as well as the importance of nitrate nitrogen for vegetative growth during the early post-transfer period [23]. The superior response to calcium nitrate compared to other nitro-

gen sources suggests that Ca^{2+} availability may be a limiting factor during acclimatization, when active cell division and tissue expansion are occurring. Potassium nitrate and ammonium phosphate also improved growth relative to the control, reflecting the importance of balanced macronutrient supply. The weakest response was observed for ammonium nitrate, which may be attributed to the potentially inhibitory effect of the ammonium form of nitrogen on root development in young transplants.

Overall, the results confirm that the success of ex vitro establishment of micropropagated *Berberis iliensis* depends on two key factors at successive stages: substrate physical properties at the adaptation stage, and mineral nutrition composition at the acclimatization stage. The approach developed in this study – molecular characterization of source material followed by optimized substrate and fertilizer selection – provides a practical basis for the production of viable planting stock suitable for reintroduction and restoration of natural populations of this endangered endemic species.

CONCLUSIONS

This study investigated three interconnected aspects of *Berberis iliensis* conservation: molecular genetic characterization of the source material, adaptation of rooted microshoots to ex vitro conditions, and acclimatization of plantlets under greenhouse conditions.

The source material was characterized molecularly using two independent marker systems – the nuclear ITS region and the chloroplast *rbcL* gene. Both markers yielded amplicons of the expected size (approximately 700 bp and 600 bp, respectively), and BLAST analysis of the nine sequenced DNA preparations returned 98.36–99.84 % (ITS) and 98.44–99.83 % (*rbcL*) nucleotide identity to *Berberis* reference sequences, confirming the generic assignment of the material and excluding misidentification with unrelated taxa. Species-level assignment could not be obtained from these loci, since no reference sequences of *B. iliensis* are currently deposited in GenBank and the standard barcode markers are known to lack resolution within *Berberis*; species identity was therefore established morphologically from voucher material. Neighbour-joining analysis of the ITS alignment placed all nine samples in a single clade with *B. integerrima* (85 % bootstrap support), three of them being identical to the reference sequence, whereas *B. aristata* – the closest *rbcL* match – formed a well-supported clade separate from the studied material. No polymorphism was detected among the analyzed samples, which reflects the limited number of individuals sampled and the conservative nature of the loci used.

It has been established that the effectiveness of plant adaptation depends significantly on the composition of the substrate. The best results were obtained using a soil-sand mixture, which provides optimal conditions for water regime and aeration. In this variant, the highest indicators of plant growth and development were recorded, including shoot height (6.53 ± 0.14 cm), number of leaves (6.30 ± 0.23), number of roots (4.93 ± 0.14) and root length (5.11 ± 0.20 cm), which significantly exceeded the control.

During the acclimatization stage, it was found that mineral nutrition has a significant effect on the further development of plants. The most pronounced stimulating effect was

observed with the use of calcium nitrate, which yielded the highest growth parameters: plant height— 16.84 ± 0.42 cm, number of leaves— 20.45 ± 0.61 , crown diameter— 15.83 ± 0.62 cm, which indicates the high effectiveness of this element in ensuring physiological stability and active plant growth under greenhouse conditions.

Thus, the proposed set of measures, which includes molecular characterization of the source material and optimized conditions for adaptation and acclimatization, ensures the production of high-quality planting stock of *Berberis iliensis*. The implementation of this approach has significant practical importance and can be used to conserve the gene pool of rare species, introduce them and restore natural populations.

AUTHOR CONTRIBUTIONS

A. Nurtaza – conceptualization, molecular genetic analysis, *in vitro* culture experiments, data curation, writing of the original draft. D. Dyussembekova – *in vitro* culture and micropropagation, adaptation experiments, investigation. A. Yessimseitova – DNA extraction, PCR and sequencing, analysis of nucleotide sequences. G. Abileva – *ex vitro* adaptation experiments, phenological observations, statistical analysis. S. Bayanbay – greenhouse acclimatization experiments, morphometric measurements. K. Narmakhan – preparation of nutrient media and substrates, technical support of the experiments. D. Nurumov – Collection of plant material. A. Kakimzhanova – conceptualization, methodology, project administration, funding acquisition, supervision, review and editing of the manuscript. All authors have read and agreed to the published version of the manuscript.

FUNDING

This work was supported by the scientific and technical program BR28713087-OT-25 “Fundamental and applied aspects of obtaining and targeted synthesis of biologically active substances from medicinal plants *in vitro* culture”.

LITERATURE

1. Romadanova N. V., Karasholakova L. N., Eshbakova K. A. et al. Study of the component composition of seven *Berberis* species // Int. J. Biol. Chem. – 2022. – Vol. 15, No. 2. – P. 96–104. DOI: 10.26577/ijbch.2022.v15.i2.11
2. Romadanova N. V., Karasholakova L. N., Eshbakova K. A., Ozek G., Ozek T., Yur S., Kushnarenko S. V. Phytochemical analysis and antioxidant activity of *Berberis iliensis* M. Pop and *Berberis integerrima* Bunge fruits pulp // Res. Crops. – 2021. – Vol. 22, No. 4. – P. 940–947. DOI: 10.31830/2348-7542.2021.154
3. Байтулин И.О., Ситпаева Г.Т. Красная книга Казахстана. – Астана: ТОО «АртPrintXXI». 2014. – Т. 2. – 53 с.
4. IUCN Red List of Threatened Species [Electronic resource]. – International Union for Conservation of Nature and Natural Resources. 2026. – URL: <https://www.iucnredlist.org/species/63407/a12666327>
5. Romadanova N. V., Mishustina S. A., Karasholakova L. N., Kushnarenko S. V. Optimization of barberry micropropagation // Biotechnol. Theory Pract. – 2017. – No. 2. – P. 47–

55. DOI: 10.11134/btp.2.2017.7

6. Begenov A., Mukhitdinov N., Ametov A., Nazarbekova S., Kuatbayev A., Tynybekov B., Abidkulova K., Ydyrys A. Assessment of the current status of populations of Kazakh rare plants (*Berberis iliensis* M. Pop.) // Int. J. Biol. Chem. – 2014. – Vol. 7, No. 1. – P. 55–60.
7. Childibayeva A., Zhunussova G., Zhushupova G., Seitkali A., Begimbekova I., Ydyrys A., Mukhitdinov N. Structural characteristics of *Rosa iliensis* Chrshan. under conditions of the floodplains of the rivers Ili and Sharyn // J. Ecol. Eng. – 2022. – Vol. 23, No. 1. – P. 296–304. DOI: 10.12911/22998993/143716
8. Akishev Z. D., Smekenov I. T., Usenova A. N., Duisenova A. B., Smailov B. B., Mukhidinov N. M., Bissenbaev A. K. RAPD analysis of genetic diversity in *Berberis iliensis* a species endemic to Kazakhstan // Int. J. Biol. Chem. – 2012. – Vol. 3. – P. 48–53.
9. Pozharskiy A. S., Chekalin S. V. Molecular study of *Berberis iliensis* M. Pop. and *Berberis sphaerocarpa* Kar. et Kir. wild populations in South-East Kazakhstan using ISSR markers // Int. J. Biol. Chem. – 2015. – Vol. 8, No. 1. – P. 15–20. DOI: 10.26577/2218-7979-2015-8-1-15-20
10. Abdykerimova S., Sakipova Z., Nakonieczna S., Koch W., Biernasiuk A., Grabarska A., Malm A., Kozhanova K., Kukula-Koch W. Superior antioxidant capacity of *Berberis iliensis* – HPLC-Q-TOF-MS based phytochemical studies and spectrophotometric determinations // Antioxidants. – 2020. – Vol. 9, No. 6. – P. 504. DOI: 10.3390/antiox9060504. PMID: 32517366
11. Жарасова Д.Н., Толеп Н.А. Особенности введения в культуру *in vitro* барбариса илийского (*Berberis iliensis* M. Pop.) // Bull. Karaganda Univ. “Biology Med. Geogr. Series”. – 2022. – Т. 108, № 4. – С. 29–33. DOI: 10.31489/2022bmg4/29-33
12. Saeidnia S., Manayi A., Gohari A. R., Abdollahi M. Phytochemistry and pharmacology of *Berberis* species // Pharmacogn. Rev. – 2014. – Vol. 8, No. 15. – P. 8–15. DOI: 10.4103/0973-7847.125523. PMID: 24600194
13. Pandey A., Brijwal L., Tamta S. *In vitro* propagation and phytochemical assessment of *Berberis chitria*: an important medicinal shrub of Kumaun Himalaya. India // Asian Pac. J. Trop. Biomed. – 2014. – Vol. 4(1). – P. S218–S224. DOI: 10.12980/APJTB.4.2014C997
14. Molinar F., Ayala-Silva T., Yoo K. S., Stein L. A. Micropropagation of Agarita (*Berberis trifoliata* Moric.) // HortScience. – 1996. – Vol. 31, No. 6. – P. 1030–1032. DOI: 10.1273/HORTSCI.31.6.1030
15. Brijwal L., Pandey A., Tamta S. *In vitro* propagation of the endangered species *Berberis aristata* DC. via leaf-derived callus // In Vitro Cell. Dev. Biol. Plant. – 2015. – Vol. 51, No. 6. – P. 637–647. DOI: 10.1007/s11627-015-9724-5
16. Chandra S., Bandopadhyay R., Kumar V., Chandra R. Acclimatization of tissue cultured plantlets: from laboratory to land // Biotechnol. Lett. – 2010. – Vol. 32, No. 9. – P. 1199–1205. DOI: 10.1007/s10529-010-0290-0. PMID: 20411389
17. Grzelak M., Pacholczak A., Nowakowska K. Challenges and insights in the acclimatization step of microprop-

agated woody plants // Plant Cell Tissue Organ Cult. – 2024. – Vol. 159, No. 3. – P. 72. DOI: 10.1007/s11240-024-02923-1

18. Doyle J. J., Doyle J. L. A rapid total DNA preparation procedure for fresh plant tissue // Focus. – 1990. – Vol. 12. – P. 13–15.

19. White T. J., Bruns T., Lee S., Taylor J. W. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics // PCR Protocols: A Guide to Methods and Applications / eds. Innis M. A. et al. – New York: Academic Press. 1990. – P. 315–322.

20. Costion C., Ford A., Cross H., Crayn D., Harrington M., Lowe A. Plant DNA barcodes can accurately estimate species richness in poorly known floras // PLoS ONE. – 2011. – Vol. 6, No. 11. – P. e26841. DOI: 10.1371/journal.pone.0026841. PMID: 22073205

21. Alex C. F., Shavlik J. W., Blattner F. R. Neural network input representations that produce accurate consensus sequences from DNA fragment assemblies // Bioinformatics. – 1999. – Vol. 15, No. 9. – P. 723–728. DOI: 10.1093/bioinformatics/15.9.723. PMID: 10498776

22. Karimova V. K., Baktybai B. N., Magzumova G. K., Sartbaev Z. T., Imanbaeva A. A., Kakimzhanova A. A. Optimization of *in vitro* cultivation conditions for rare and endangered species *Berberis iliensis* and *Berberis karkaralensis* // Bulletin of the L. N. Gumilyov Eurasian National University. BIOSCIENCE Series. – 2020. – Vol. 132, No. 3. – P. 43–56.

23. Mohammed M., Munir M., Ghazzawy H. S. Design and evaluation of a smart *ex vitro* acclimatization system for tissue culture plantlets // Agronomy. – 2022. – Vol. 13, No. 1. – P. 78. DOI: 10.3390/agronomy13010078

24. Roy S., Tyagi A., Shukla V. et al. Universal plant DNA barcode loci may not work in complex groups: a case study with Indian *Berberis* species // PLoS ONE. – 2010. – Vol. 5, No. 10. – P. e13674. DOI: 10.1371/journal.pone.0013674

25. Plants of the World Online. *Berberis integerrima* Bunge [Electronic resource]. – Royal Botanic Gardens, Kew. 2026. – URL: <https://powo.science.kew.org/taxon/urn:l-sid:ipni.org:names:106778-1> (accessed 28.07.2026)

REFERENCES

1. Romadanova N. V., Karasholakova L. N., Eshbakova K. A. et al. Study of the component composition of seven *Berberis* species. Int. J. Biol. Chem.. 2022. Vol. 15. no. 2. pp. 96–104. DOI: 10.26577/ijbch.2022.v15.i2.11

2. Romadanova N. V., Karasholakova L. N., Eshbakova K. A., Ozek G., Ozek T., Yur S., Kushnarenko S. V. Phytochemical analysis and antioxidant activity of *Berberis iliensis* M. Pop and *Berberis integerrima* Bunge fruits pulp. Res. Crops. 2021. Vol. 22. no. 4. pp. 940–947. DOI: 10.31830/2348-7542.2021.154

3. Bajtulin I. O., Sitpaeva G. T. Krasnaya kniga Kazakhstana [Red Data Book of Kazakhstan]. Astana: Art-PrintXXI. 2014. Vol. 2. 53 p. (In Russian)

4. IUCN Red List of Threatened Species. International Union for Conservation of Nature and Natural Resources. 2026. URL: <https://www.iucnredlist.org/species/63407/a12666327>

5. Romadanova N. V., Mishustina S. A., Karasholakova L. N., Kushnarenko S. V. Optimization of barberry micropropagation. Biotechnol. Theory Pract.. 2017. no. 2. pp. 47–55. DOI: 10.11134/btp.2.2017.7

6. Begenov A., Mukhitdinov N., Ametov A., Nazarbekova S., Kuatbayev A., Tynybekov B., Abidkulova K., Ydyrys A. Assessment of the current status of populations of Kazakh rare plants (*Berberis iliensis* M. Pop.). Int. J. Biol. Chem.. 2014. Vol. 7. no. 1. pp. 55–60.

7. Childibayeva A., Zhunussova G., Zhusupova G., Seitkali A., Begimbekova I., Ydyrys A., Mukhitdinov N. Structural characteristics of *Rosa iliensis* Chrshan. under conditions of the floodplains of the rivers Ili and Sharyn. J. Ecol. Eng.. 2022. Vol. 23. no. 1. pp. 296–304. DOI: 10.12911/22998993/143716

8. Akishev Z. D., Smekenov I. T., Usenova A. N., Duisenova A. B., Smailov B. B., Mukhidinov N. M., Bissenbaev A. K. RAPD analysis of genetic diversity in *Berberis iliensis* a species endemic to Kazakhstan. Int. J. Biol. Chem.. 2012. Vol. 3. pp. 48–53.

9. Pozharskiy A. S., Chekalin S. V. Molecular study of *Berberis iliensis* M. Pop. and *Berberis sphaerocarpa* Kar. et Kir. wild populations in South-East Kazakhstan using ISSR markers. Int. J. Biol. Chem.. 2015. Vol. 8. no. 1. pp. 15–20. DOI: 10.26577/2218-7979-2015-8-1-15-20

10. Abydkerimova S., Sakipova Z., Nakonieczna S., Koch W., Biernasiuk A., Grabarska A., Malm A., Kozhanova K., Kukula-Koch W. Superior antioxidant capacity of *Berberis iliensis* – HPLC-Q-TOF-MS based phytochemical studies and spectrophotometric determinations. Antioxidants. 2020. Vol. 9. no. 6. p. 504. DOI: 10.3390/antiox9060504. PMID: 32517366

11. Zharasova D. N., Tolep N. A. Osobennosti vvedeniya v kul'turu *in vitro* barbarisa ilijskogo (*Berberis iliensis* M. Pop.) [Features of introduction to *in vitro* culture of *Berberis iliensis* M. Pop.]. Bull. Karaganda Univ. “Biology Med. Geogr. Series”. 2022. Vol. 108. no. 4. pp. 29–33. (In Russian) DOI: 10.31489/2022bmg4/29-33

12. Saeidnia S., Manayi A., Gohari A. R., Abdollahi M. Phytochemistry and pharmacology of *Berberis* species. Pharmacogn. Rev.. 2014. Vol. 8. no. 15. pp. 8–15. DOI: 10.4103/0973-7847.125523. PMID: 24600194

13. Pandey A., Brijwal L., Tamta S. *In vitro* propagation and phytochemical assessment of *Berberis chitria*: an important medicinal shrub of Kumaun Himalaya. India. Asian Pac. J. Trop. Biomed.. 2014. Vol. 4. suppl. 1. pp. S218–S224. DOI: 10.12980/APJTB.4.2014C997

14. Molinar F., Ayala-Silva T., Yoo K. S., Stein L. A. Micropropagation of Agarita (*Berberis trifoliata* Moric.). Hort-Science. 1996. Vol. 31. no. 6. pp. 1030–1032. DOI: 10.21273/HORTSCI.31.6.1030

15. Brijwal L., Pandey A., Tamta S. *In vitro* propagation of the endangered species *Berberis aristata* DC. via leaf-derived callus. In Vitro Cell. Dev. Biol. Plant. 2015. Vol. 51. no. 6. pp. 637–647. DOI: 10.1007/s11627-015-9724-5

16. Chandra S., Bandopadhyay R., Kumar V., Chandra R. Acclimatization of tissue cultured plantlets: from laboratory to land. Biotechnol. Lett.. 2010. Vol. 32. no. 9. pp. 1199–

1205. DOI: 10.1007/s10529-010-0290-0. PMID: 20411389

17. Grzelak M., Pacholczak A., Nowakowska K. Challenges and insights in the acclimatization step of micropropagated woody plants. *Plant Cell Tissue Organ Cult.* 2024. Vol. 159. no. 3. p. 72. DOI: 10.1007/s11240-024-02923-1

18. Doyle J. J., Doyle J. L. A rapid total DNA preparation procedure for fresh plant tissue. *Focus.* 1990. Vol. 12. pp. 13–15.

19. White T. J., Bruns T., Lee S., Taylor J. W. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *PCR Protocols: A Guide to Methods and Applications*. eds. Innis M. A. et al. New York: Academic Press. 1990. pp. 315–322.

20. Costion C., Ford A., Cross H., Crayn D., Harrington M., Lowe A. Plant DNA barcodes can accurately estimate species richness in poorly known floras. *PLoS ONE.* 2011. Vol. 6. no. 11. p. e26841. DOI: 10.1371/journal.pone.0026841. PMID: 22073205

21. Allex C. F., Shavlik J. W., Blattner F. R. Neural network input representations that produce accurate consensus sequences from DNA fragment assemblies. *Bioinformatics.*

1999. Vol. 15. no. 9. pp. 723–728. DOI: 10.1093/bioinformatics/15.9.723. PMID: 10498776

22. Karimova V. K., Baktybai B. N., Magzumova G. K., Sartbaev Z. T., Imanbaeva A. A., Kakimzhanova A. A. Optimization of *in vitro* cultivation conditions for rare and endangered species *Berberis iliensis* and *Berberis karkaralensis*. *Bulletin of the L. N. Gumilyov Eurasian National University. BIOSCIENCE Series.* 2020. Vol. 132. no. 3. pp. 43–56.

23. Mohammed M., Munir M., Ghazzawy H. S. Design and evaluation of a smart *ex vitro* acclimatization system for tissue culture plantlets. *Agronomy.* 2022. Vol. 13. no. 1. p. 78. DOI: 10.3390/agronomy13010078

24. Roy S., Tyagi A., Shukla V. et al. Universal plant DNA barcode loci may not work in complex groups: a case study with Indian *Berberis* species. *PLoS ONE.* 2010. Vol. 5. no. 10. p. e13674. DOI: 10.1371/journal.pone.0013674

25. Plants of the World Online. *Berberis integririma* Bunge. Royal Botanic Gardens, Kew. 2026. URL: <https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:106778-1> (accessed 28.07.2026)