ABSTRACT

of the doctoral dissertation entitled: "Populations study of rare and endemic species Almond Ledebour (*Amygdalus ledebouriana* Schltdl.) in conditions of Eastern Kazakhstan" submitted for the degree of Doctor of Philosophy (PhD) on the specialty "6D061300 - Geobotany " ORAZOV AIDYN

Relevance of work. According to the concept of the development of specially protected natural areas of the Republic of Kazakhstan until 2030, the most effective measure to preserve endemic and rare shrub plant species is the creation of a network of specially protected natural areas. According to the new Ecological Code of the Republic of Kazakhstan adopted on January 2, 2021, special attention is paid to the protection of the gene pool of endemic plants.

However, comprehensive studies with population characteristics such as age composition and genetic diversity of endemic and rare shrub species have not been conducted. Rosaceae Juss, one of the rare and endemic plant species of Eastern Kazakhstan, according to the Decree of the Government of the Republic of Kazakhstan No. 1034 of October 31, 2006 on approving the list of rare and endangered species of plants and animals in the Red Book of Kazakhstan. belongs to the family *A. ledebouriana*.

The relevance of the research work is determined by the fact that the comprehensive study of the populations of this plant species, its structural features and the determination of the level of genetic diversity using modern molecular genetic methods and the analysis of the results of conservation by biotechnological methods have not been carried out in the Republic of Kazakhstan.

The object of the study - Rare and endemic Ledebour almond - *Amygdalus ledebouriana* Schlecht.

Research subject - the endangered *Amygdalus ledebouriana* Schlecht. geobotanical, morphological condition, molecular genetic description and biotechnological preservation of populations.

The purpose of the work: Research and biotechnological preservation of rare and endemic *A. ledebouriana* populations in East Kazakhstan based on the current structural condition, morphological differences, genetic diversity parameters.

Research objectives:

1. Determination of rare and endemic features of *A. ledebouriana* populations in East Kazakhstan, plant status, distribution area and relative floral composition of the community in which the plant participates;
2. Study of structural features of *A. ledebouriana* populations in East Kazakhstan and comparison of variability of morphological height of bushes between populations;
3. Based on the nucleotide sequence of ITS and *mat*K DNA markers, analysis of Prunus related species and determination of the position of *A. ledebouriana* in the phylogenetic genealogy;
4. Comparison of plant species populations of *Chamaeamygdalus* section based on SSR DNA markers and assessment of genetic features;
5. *A. ledebouriana* creation of a protocol for obtaining permanent aseptic callus cells by biotechnological (ex situ) method in the in vitro environment of the plant at the tissue level;
6. Propose conservation measures for the rare and endemic species *A. ledebouriana* in the specially protected areas of East Kazakhstan.

The theoretical and methodological base of the research:

A. ledebouriana the so-called plant was first identified and described by the German botanist and mycologist Dierich Franz Leonhard von Schlechtendal. The first information about this species was published in 1854: Schlecht. Abh. Nature. Ges. Halle 2. The species is described in Volume 10 of the Flora of the USSR, Volume 4 of the Flora of Kazakhstan and the first part of the pictorial identifier of the plants of Kazakhstan. *A. ledebouriana* is included in the Red Book of

Kazakhstan as a rare and endangered plant species. In all the sources mentioned above, *A. ledebouriana* is identified as a separate plant species with endemic characteristics.

The second species (*A. nana*) was first described in 1753 by Carl Linnaeus. The first notices of this species are in 1941 volume 10 of Flora of the USSR with a detailed description of A. nana. This species was described in volume 4 in 1961 for the book "Flora of Kazakhstan". The main difference between the two species is the growing environment and the shape of the seed. The seeds of *A. ledebouriana* are elongated horizontally.

However, in the editions of the flora of the Union of Soviet Socialist Republics (USSR) in 1941 and Kazakhstan in 1961 (S.K. Cherepanov, S.A. Abdulina), the almond family is defined as a separate independent family, and the famous "Plantarium" of the Russian Federation (www.plantarium.ru) the atlas of plant species and the online identification system confirm it. The main distinguishing feature of the almond family is the presence of a dry and bony fruit.

The most complete information about species diversity of tarbagatai plants is E.F. Stepanova's work "Flora and plants of the Tarbagatai ridge". The researches of many foreign scientists, Ma Yang and Xi Hua, made it possible to carry out research on the genetic diversity of almonds using AFLP and SSR molecular markers. The study of the life cycles of the A. ledebouriana plant was determined according to the Uranov methodology. The study of ecological and biological features of the species was carried out according to the methodological recommendations made by Golubev and Molchanov. The distribution of the floral composition of *A. ledebouriana* plant by ecological groups and species distribution area is given according to the classification of Cumin. Plant names are listed according to World Wide Web plans (POWO 2021).

M.G. Nikolaeva, I.V. Lianguzov, L. M. Identification of quantitative and qualitative seeds included in the experiment according to the methodological developments of Pozdov. Identification of species in phytocenoses is carried out on the basis of basic data of "Flora of Kazakhstan" (1956-1966, 1999-2002), "Flora of Siberia" (1982-1993), "Determinant of Central Asian Plants" (1968-1994). Determining the geographical distribution of the habitats of A. ledebouriana plant on the territory of EK and creating a map scheme was carried out in the Arc GIS system. Processing of statistical data by G.F. Lakin and N. L. Udolskaya, Zaitsev G. N. methods, as well as using Statistica 6.1 and Microsoft Office Excel 2007 application package.

Herbarium and Identification of Plants: Flora of Kazakhstan (1956), Illustrated Directory of Plants of Kazakhstan (1961), Flora of the USSR (1941). Academician N.V. based on floristic zoning of species. Zoning proposed by Pavlov was used according to Flora of Kazakhstan (1956). Latin names of plants are given according to WCSP (Royal Botanic Gardens, Kew) (2021) electronic resource Plant list and Inaturalist (2013). Cenopopulation types, classification by age composition were determined according to the methods of A.A. Uranov, T.A. Rabotnov, Yu.A. Zlobin, L.A. Zhivotovsky. The Latin names of the plants were adapted to the World Wide Web plans (POWO, 2021). Processing of statistical data by G.F. Lakin (1990) and N. L. It was conducted according to the methods of Udolskaya (1976), G.N. Zaitsev (1976). Statistica 6.1 and Microsoft Office excel 2007 application program package, it was conducted using the R-studio program. Status of endangered and rare species - Camelina R.V. and Sokolova G.G. by classification. (2016). Work with GIS data was carried out in ArcGis 10.6.1 (Esri, Redlands, California, USA) and QGIS 3.10 (QGIS Development Team, 2021). Methodological organization of floristic squares by A.P. It was made in accordance with the works of Seregin (2012).

A method of studying the genetic structure using ITS and *mat*K DNA markers. A method of studying the genetic diversity of populations using the SSR DNA marker. Isolation of genomic DNA from goldenrod leaves was performed by the CTAB protocol (Doyle, 1991). Population variability GenAlex software version 6.5 (Peakall et al., 2012), Past software 4.03, R-studio software (Version 1.3.1093), ClustVis web tool (Metsalu & Vilo, 2015), PopGene version 1.32 (studied by Yeh et al.). al., 1997), STATISTICA 10.0 programs. Clustering was performed using unweighted paired-group averaging (UPGMA) (2014).

All experiments used 0.7% MS agar gel medium with 3-6% sucrose according to Murashige (1977). *In vitro* biology of cultured cells of higher plants and biotechnology Butenko R.G. (1999) methods made according to the morphological structure of the callus was described according to the method of Timofeev and Rumyantseva (2012).

Personal contribution of the author to obtaining a scientific result. The formulation of the problem, the formulation of the goal of the dissertation research, the solution of problems, the processing of materials, the analysis and synthesis of the results were carried out by the author independently. Preparation for publication of scientific papers reflecting the main results of the dissertation was carried out independently and with the participation of co-authors.

Scientific novelty of the dissertation work. Based on a comprehensive study of the characteristics of rare and endemic *A. ledebouriana* populations, new information was obtained, which allows to propose measures for the conservation of the studied *A. ledebouriana* populations.

Populations of *A. ledebouriana* located in the Altai and Tarbagatai ranges have a relatively high level of genetic diversity. The seeds collected from the populations were transferred to the "Seed bank of the natural flora of Kazakhstan" of the Institute of Botany and Phytointroduction with the aim of preserving the gene pool of the *A. ledebouriana* plant species for a long time, and a certificate of transfer was obtained.

A. ledebouriana nucleotide sequence information obtained from biological materials based on ITS and *mat*K DNA markers was uploaded to the NCBI international database under the number MN335241.1, MN453776.1 and MN453777.1, the obtained results make a great contribution to the study of molecular taxonomy of the *Chamaeamygdalus* section.

A. ledebouriana the procedure for obtaining stable aseptic callus cells in vitro from embryonic materials and the chemical composition of the optimal nutrient medium, the concentration of exogenous phytohormones will be used in the future for cryopreservation and microclonal propagation of tissues of rare and endemic plant species. For the first time, the optimal medium composition and regulation with the concentration of IBA 1 mg/l, GA and 6-BAP 0.5 mg/l was determined for obtaining stable aseptic callus cells in vitro from the rare and endemic plant *A. ledebouriana*. A patent was obtained for a model useful for the chemical composition of the nutrient medium.

Basic rules recommended for protection:

- Due to its isolated geographical location and anthropogenic influence in the mountainous regions of Eastern Kazakhstan, the endemic species *A. ledebouriana*, included in the Red Book, is replaced by *A. nana*, which in turn creates a threat of extinction of the *A. ledebouriana* plant.

- *A. ledebouriana* is distinguished by two large distribution areas in the mountainous systems of Altai and Tarbagatai in East Kazakhstan: Naryn Ridge population of the Altai Mountains consisting of two cenopopulations with 250 species of coenoflora; the population of the Kalba ridge of the Altai Mountains, consisting of two cenopopulations with 153 species of coenoflora; population of Tarbagatai Range of Tarbagatai Mountains, consisting of three cenopopulations with 376 species of coenoflora; A population of *A. nana* representative of *Chamaeamygdalus* section, morphologically similar to A. *ledebouriana*, was also identified in the small hilly area of the Ulbi ridge of the Altai mountains, which has a coenoflora of 150 species.

- The statistical difference in plant height between three mountain populations of *A. ledebouriana* was determined to be related to the location above sea level. A statistically significant difference between populations was confirmed for plant height (P-value = 2.3e-15). A t-test showed that all three montane *A. ledebouriana* populations had significantly higher plant height compared to field populations of *A. nana* ($P<0.0001$). Due to the geographical location, a significant difference in plant height was found between representatives of two morphologically similar mountain *A. ledebouriana* and steppe *A. nana* species of *Chamaeamygdalus* section.

- On the basis of ITS and *mat*K DNA-markers, the phylogenetic position of the plant species *A. ledebouriana* of the *Prunus* family and that *A. nana* is genetically close to the plant species *A. ledebouriana* were determined.

- On the basis of SSR DNA-markers, genetic structural differences between the populations of *A. ledebouriana* and *A. nana* representatives of the *Chamaeamygdalus* section were determined based on their geographical location, the total genetic variability of the *A. ledebouriana* plant species was found to be 73% within populations and 27% between populations.

– The optimal nutrient concentration for callus formation with the addition of exogenous phytohormones is IBA 1 mg/l, GA and 6-BAP 0.5 mg/l. In vitro, the relative frequency of formation of stable aseptic callus cells in immature embryo explants without germline increases to 7.79 ± 0.46%.

Conclusion:

1. The flora composition of the plant communities in which the populations of the rare and endemic *A. ledebouriana* plant in East Kazakhstan are present were determined and three new populations of the *A. ledebouriana* plant were identified. Populations are distinguished by two large areas of distribution in the mountain systems of Altai and Tarbagatai: Naryn ridge population of Altai Mountains (3-KA), which consists of two cenopopulations with 250 species of coenoflora; the population of the Kalba range of the Altai Mountains, which consists of two cenopopulations with a coenoflora of 153 species (2-KO); The population of the Tarbagatai Range of the Tarbagatai Mountains, consisting of three cenopopulations with 376 species of coenoflora, was identified.

2. Tarbagatai, Kalba and Naryn ridge populations are dominated by young generative and mature generative plants, which indicates renewal of these populations. Ulbi ridge population of *A. nana* contains representatives of old generative and subsenile plants, this Ulbi ridge is a sign of aging population, i.e., weakness of its renewal. *A. ledebouriana* and *A. nana* studied populations are normal, old individuals are not dominant in all populations. The greatest plant height was recorded for population 3-KA (2.09±0.06 m), which was the highest mountain population above sea level. T-test ($P<0.0001$) confirmed a statistically significant difference between populations in terms of plant height (P-value = 2.3e-15). The rate of this trait in plants depended on the location above sea level and the lowest height for *A. ledebouriana* samples was above 1.7 m and the highest height for *A. nana* plant samples was below 1.5 m.

We evaluated samples of two species using 19 polymorphic SSR loci based on comparison of populations of plant species of section *Chamaeamygdalus* and evaluation of genetic features based on 3 SSR DNA markers. *A. ledebouriana* (1-UR, 2-KO, 3-KA) from 60 samples of UPGMA phylogenetic tree was constructed that clearly separated the 20 *A. nana* (4-UK) samples. This result was also supported by the PCoA plot, where PC2 (41.2%) separated 4-UK samples from 1-UR and 2-KO, and PC1 (49.1%) separated 3-KA from the other three populations. Clustering shows that there is a low level of admixture between populations, which supports the 'isolation by distance' model. Evaluation of the genetic index of heterozygosity (Ney index) shows that the highest genetic diversity is *A. nana* population (0.606), and the lowest indicator was recorded in 3-KA population (0.449). Perhaps high altitude is a strong enough environmental factor to negatively affect the genetic variation of *A. ledebouriana*. Nevertheless, the separation of 3-KA from 1-UR and 2-KO supported a large level of genetic variation within the species. Population 4-UK formed a distinct cluster, and only one sample from that population (4-UK_07) clustered closely with samples from population 3-KA. Population structure estimation using the STRUCTURE package showed that the populations of *A. ledebouriana* and *A. nana* began to separate at steps K=3 and K=4, further indicating that *A. ledebouriana* and *A. nana* are two distinct species. Evaluation of samples in four clusters at K=4 indicated low levels of admixture, supporting a model of isolation by distance with limited gene flow between populations. Thus, the analysis of plant height and the use of SSR markers *A. nana* and *A. ledebouriana* and was successfully used to study the genetic diversity and population structure of the endemic species *A. ledebouriana*.

4. According to the topology of the phylogenetic genealogy dendrogram by ITS and *mat*K DNA marker, it is divided into large cluster III. Cluster I combine three small subclusters. Our phylogenetic analyzes were largely consistent with evolutionary studies of section

Chamaeamygdalus. Based on the results of molecular and morphological research, it was determined that *A. ledebouriana* is genetically close to *A. nana*.

5. In the course of creating a protocol for obtaining permanent aseptic callus cells of *A. ledebouriana* plant in the in vitro environment at the tissue level by biotechnological (ex situ) method, a high rate of callus formation was observed in the culture medium supplemented with phytohormones such as Kinetin, 6-BAP, GA, IBA. Decreasing the kinetin concentration to 0.04 mg/L resulted in a lower intensity but increased formation frequency of 72.00 ± 5.66 % and increased mass. An increase in the concentration of kinetin led to a decrease in the efficiency of cell division. The frequency of callus formation varied between 22.50 ± 3.54 % and 31.25 ± 2.95 %. A mean concentration of 0.5 mg/L of phytoregulators such as GA and 6-BAP gave a high frequency of 65.38 ± 5.44 % to 72.00 ± 5.66 % in both types of explants. Increasing the concentration of IBA to 1 mg/L increased the frequency from 46.00 ± 2.83 % to 72.00 ± 5.66 %. A stable callus was formed on day 15 with little tissue necrosis in these media.

Thus, the frequency of callus formation under in vitro conditions was 38.98 ± 6.01% for the first type of explants (whole embryo) and 46.78 ± 6.47% for the second type of explants (embryo without germinal root). This is 7.79 ± 0.46% more than for the second type of explants. The removal of the germinal root from the embryo inhibits the organogenesis of the root and the first shoot, which allows the accumulation of nutrients for the growth of the callus mass.

Approbation of work. The results and conclusions of the research are presented in 11 works, including 5 articles in journals recommended by the Supervisory Committee of the Republic of Kazakhstan in the field of education and science, 3 articles in the materials of international scientific and practical conferences:

In journals included in the Scopus and Web of Science databases:

1. Orazov A. et al. Callus induction with 6-BAP and IBA as a way to preserve *Prunus ledebouriana* (Rosaceae), and endemic plant of Altai and Tarbagatai, East Kazakhstan // Biodiversitas Journal of Biological Diversity. - 2022. - Vol. 23. - no. 6. (Q-3, percentile-44) DOI: 10.13057/biodiv/d230645

2. Orazov A. et al. Flora accompanying *Prunus ledebouriana* (Schltdl.) YY Yao in the Tarbagatai State National Park in Kazakhstan //International Journal of Biology and Chemistry. - 2021. - Vol. 14. – no. 1. - S. 21-34.https://doi.org/10.26577/ijbch.2021.v14.i1.02