

## ANNOTATION

**Of the dissertation work of Beyshov Rustem Saltanovich on the theme «Molecular-genetic studies of populations of rare plant species in Northern Kazakhstan using intermicrosatellite analysis of DNA polymorphism», presented for the degree of Doctor of Philosophy (PhD) in the specialty 6D060700 – «Biology»**

### **General characteristics of the work.**

The dissertation work is dedicated to the molecular-genetic study of populations of rare plant species of Northern Kazakhstan using microsatellite analysis of DNA polymorphism.

### **Relevance of the research topic.**

Preservation of the biological diversity of the plant and animal world and the rational use of its genetic potential is recognized as a global priority.

The reduction of species and genetic diversity poses a real threat to the biosphere, since the sustainability of the reproduction of natural ecosystems is directly related to their genetically determined potential for adaptation to changing environmental conditions.

Floristic research is the necessary basis for the development of recommendations for the protection of the plant world and the compilation of a list of rare plant species. By the middle of this century, the number of endangered plant species is predicted to increase from 7 to 60,000. Reduction of species and genetic diversity poses a real threat to the biosphere, since the sustainability of reproduction of natural ecosystems is directly linked to their genetically determined potential for adaptation to changing environmental conditions. Support of genetic diversity, i.e. genotypic heterozygosity, polymorphism and other genotypic variability due to the need for adaptation in natural populations plays an important role in the integrated assessment of biodiversity.

Currently, in order to preserve the gene pool of rare plants, along with traditional research methods, it is necessary to use modern molecular-genetic methods. Nowadays, DNA banks of valuable, rare plant species have been created, studies on intraspecific variability of protected objects, clarification of controversial issues arising during their systematics and classification, development of methods of molecular-genetic identification and population passportization and study of genetic stability of preserved taxons *in situ* are being conducted.

Moreover, the efficiency of using traditional molecular-genetic markers (structural genes, mini and microsatellite loci) to study gene pools is still insufficient due to the limited number of loci suitable for simultaneous genotyping of individuals. Therefore, this circumstance requires the search for new ways of simultaneous molecular labeling of many genomic parts, which could allow the creation of a «genome portrait» of each individual. As a result, it becomes possible to objectively assess the specificity of gene pools of populations. The development of new methods is crucial for the selection of typical representatives of populations and the development of genetically based programs for their conservation, as well

as for the determination of intraspecific genetic diversity, the assessment of heterozygosity, the reconstruction of phylogenetic relationships between species and intergroup spatial relationships - in solving the main problem of biodiversity conservation.

Many botanists have noticed the floristic features of the territory. This area belongs to the steppe zone, but the proximity to the forest-steppe zone of the West Siberian Plain leaves an imprint on its flora and vegetation. E.I. Rachkovskaya, Z.V. Karamysheva believe that a secluded area in the lowland mountains was created here.

Northern Kazakhstan is unique not only in the diversity of flora and vegetation, but also in the high concentration of its rare plant species.

Identification and study of flora in any territory takes an important place in connection with the changes occurring in the modern environment under the influence of increasing unfavorable factors, primarily human activity.

However, this environment, under the influence of anthropogenic factors, is changing significantly, leading to the extinction of not only local populations, but also entire species.

**The purpose of the dissertation research:** Study of biological features of rare plant species of Northern Kazakhstan, assessment of the status and development of approaches to the conservation of their gene pools using the technology of molecular-genetic identification and passportization based on molecular marking of their genomes.

**Research objectives:**

1. Determination of the volume of field work, expedition routes covering a variety of ecotopes;
2. Study of geographical, ecological and phytocenotic features of habitats, population characteristics and parameters of individuals; selection of biological material;
3. Determination of the age composition of the effective population size; Calculation of the total and effective population size;
4. DNA extraction from dried leaves;
5. Selection, validation and detection of the efficiency of primers for polylocus marking of plants;
6. Study of genetic diversity in populations of rare plant species based on polylocus marking data;
7. Identification of the state of gene pools of the studied populations based on polylocus marking;
8. Identification of molecular markers; population identification;
9. Determination of parameters and scales of the state of gene pools of rare plant species populations based on polylocus marking;
10. Development of recommendations for the conservation of rare plant species of Northern Kazakhstan.

**Object of research.** The objects of the research were 20 natural cenopopulations of four rare plant species of the Northern part of the Republic of Kazakhstan: 5 cenopopulations of *Pulsatilla patens* (L.) Mill., 5 cenopopulations of

*Pulsatilla flavescens* (Zucc.) Juz., 5 cenopopulations of *Adonis vernalis* L. and 5 cenopopulations of *Adonis wolgensis* Stev.

**Methods of research.** Field studies were conducted during flowering and fruiting of plants. Researches were carried out by a detailed route method. DNA isolation from plant samples, determination of DNA polymorphism by ISSR-method using polymerase chain reaction (PCR) were performed during the project.

**Place of research.** Research work and experiments on the thesis were carried out in the laboratory of molecular-genetic studies of the Research Institute of applied biotechnology of Kostanai Regional University named after A. Baitursynov and in the laboratories of «Perm State National Research University», Perm.

**The main points of the defense:**

1. *Adonis wolgensis* Stev., *A. vernalis* L., *Pulsatilla multifida* (Pritz.) Juz., *P. uralensis* (Zam.) Tzvel. it was found that the state of the cenopopulations is satisfactory.

2. The complex morphological and genetic intraspecific structure of the studied populations was determined.

3. *Adonis wolgensis*, *A. vernalis*, *Pulsatilla multifida*, *P. uralensi* it was found that conservation of cenopopulations helps to reduce the anthropogenic load in places of mass growth of these species.

**Novelty of the study:**

The study showed, *Adonis vernalis* L., *Adonis wolgensis* Stev., *Pulsatilla patens* (L.) Mill., *Pulsatilla flavescens* (Zucc.) Juz., that grows in the North Kazakhstan region, the habitat, population characteristics and parameters of individuals were studied. The age composition of populations, the effective criterion of populations was determined, the number of populations was calculated. More than 600 botanical materials were collected from the collection of herbarium leaves. For the first time the genetic diversity and genetic structure of the studied cenopopulations were studied. For the first time carried out genomic marking, assessed the state of the gene pool, developed molecular-genetic formulas, line codes and passports for the studied cenopopulations.

**Practical relevance of the results.** Preservation of biodiversity is one of the most important problems of the modern world. Studying the genetic diversity of rare species (interpopulation, intrapopulation polymorphism, genetic differentiation of populations), along with studying their biology and systematics, geobotanical description of populations and determining the features of their age spectrum and limiting factors, allows a fuller understanding of the nature and features of rare species and, finally, to choose the right conservation mechanism. The conservation of many genetically distinct local populations is the primary goal of preventing the extinction of the species and preserving its evolutionary potential. However, it is not always possible to conserve all existing populations. Often it is necessary to isolate a particular population or select specimens for conservation under artificial conditions and/or genetic banks.

Modern molecular-genetic methods make it possible to determine the level of genetic diversity within and between populations, to determine the population-

genetic structure of a species, so their use is very important when choosing a strategy for the conservation of rare plant species.

**Compliance with the directions of development of science or state programs.** The dissertation work was carried out within the scientific project of the grant funding of the Committee of Science of the Ministry of Education and Science of the Republic of Kazakhstan entitled «AP05132458 Molecular genetic analysis of gene pools of rare plant species populations of Northern Kazakhstan» (registr. № 0118RK00404). Materials of interim reports for 2018, 2019, 2020 and the final report were used in preparation of the thesis.

**Approbation of the results of the dissertation.**

The main statements of the dissertation were considered and discussed at the scientific and technical meetings at the V. Dvurechensky Agricultural Institute of Kostanai Regional University on the expanded meeting of the Department of Biology and Ecology. The main results of the thesis on the topic were published at international scientific and practical conferences: XXX international scientific and practical conference «Innovative approaches in modern science» (Moscow, 2018), international scientific and practical conference «Methodology, theory and practice of modern biology» (Kostanai, 2019), Symbiosis-Russia 2019: XI All-Russian Congress with international young scientists biologists. (Perm, 2019), the international scientific and practical conference «Multifaceted Great Steppe: modernization of social consciousness, education and innovation» (Kostanay, 2019).

The results of the thesis research are included in the interim and final report of the project AP05132458 (2018-2020).

**Publications.** On the topic of the dissertation work 14 scientific articles were published: 4 articles in the journals recommended by the CQAES MES RK, 2 articles in the journals included in the Scopus database, 1 patent for a utility model (Patent № 5485 from 11.03.2020) (Annex B), 6 articles in the collections of international conferences, 2 articles in the electronic journal issued. Published a monograph «Rare plant species of Northern Kazakhstan», as well as recommendations for the conservation of rare plant species of Northern Kazakhstan.

**Scientific internship.** Passed a scientific internship in FSBSI «Federal research center of coal and coal chemistry, Siberian branch of the Russian Academy of Sciences» on the basis of the Kuzbass botanical garden, Kemerovo.

**Personal contribution of the applicant.** The author together with scientific advisors carried out work on the selection of the object, obtaining biological samples, preparation of herbarium herbs, staging experiments, structure and planning the dissertation work. The author personally performed the selection of research methods, generalization and interpretation of 20 cenopopulations of rare plant species of the North Kazakhstan region, using field and laboratory studies, subsequent processing, analysis, including a number of computer programs. The results were published by the co-authors in 16 publications.

**Results of the study:** The following conclusions were made based on the results of the research carried out during the dissertation:

1. As a result of the research, data on the species diversity of populations of 4 rare plant species were obtained and systematized. A total of 20 cenopopulations were studied during flowering of plants.

2. The cenoflora of *Pulsatilla patens* (L.) Mill. s.l. in Northern Kazakhstan, which includes 168 species belonging to 42 families and 141 genera, has been studied. The cenoflora of *Adonis wolgensis* includes 119 species belonging to 28 families and 75 genera. The cenoflora of *Adonis vernalis* includes 77 species belonging to 24 families. More than 6 thousand leaves from 595 plant specimens were collected.

3. The cenopopulation efficiency index (CE) of *Pulsatilla* s.p. is high and is in the narrow range of 0.73-0.88. No differences were found between species and intermediate forms. Population efficiency index *Adonis wolgensis* Stev. is in the range 0.59-0.86, *Adonis vernalis* in the range 0.70-0.84.

4. During the project, high quality DNA was isolated from samples of 300 plants collected in Akmola, Kostanay, and Pavlodar regions of the Republic of Kazakhstan.

5. For molecular genetic analysis using the intermicrosatellite method, ISSR-primers were selected according to the efficiency of detection of DNA polymorphism.

6. Studies have shown that the studied species of the genus *Pulsatilla* Mill. are characterized by a fairly high level of genetic diversity  $P_{95}$  ranges from 0.728 to 0.904;  $H_E$  – from 0,131 to 0,245. The indicators of genetic diversity are higher in the studied cenopopulations of *P. patens* ( $P_{95}$  – 0,965,  $H_E$  – 162,  $I$ –0,373), than in the cenopopulations of *P. flavescens* ( $P_{95}$  – 0,904,  $H_E$  – 0,204,  $I$ –0,307), except for the index of expected heterozygosity. At the same time, the level of interpopulation differentiation is comparable in *P. flavescens* ( $G_{ST}$ =0,256) and *P. patens* ( $G_{ST}$ =0,300).

The studied species of the genus *Adonis* L. are also characterized by a high level of genetic diversity  $P_{95}$  from 0,424 to 0,888;  $H_E$  – from 0,171 to 0,300. Genetic diversity rates are higher in the CP of *A. wolgensis*, than in the CP of *A. vernalis*. At the same time, the level of interpopulation differentiation is significantly higher in *A. vernalis* ( $G_{ST}$  =0,348), as compared to *A. wolgensis* ( $G_{ST}$  =0,131).

7. Based on the analysis of genetic diversity parameters, it was found that the typical characteristics of gene pools have cenopopulations Pf1 and Pf2 *P. flavescens* from the Kostanai region of Northern Kazakhstan, and specific gene pools exist in the cenopopulations Pf3, Pf4 and Pf5 of Akmola region. The state of the gene pools of all five studied *P. flavescens* cenopopulations is satisfactory. Among the *P. patens* cenopopulations, the first and fourth cenopopulations are characterized by typical gene pools (Pp1 and Pp4), and the second, third, and fifth are specific (Pp2, Pp3, Pp5). In the first cenopopulation of *P. patens* in the Pavlodar region there is an impoverishment of the gene pool, the gene pools of the other studied cenopopulations of this species in a satisfactory condition. Among the cenopopulations of *A. wolgensis*, the basic characteristics have cenopopulation Aw3 and Aw5, and specific - Aw1, Aw2 and Aw4. The condition of all five *A. wolgensis* cenopopulations is satisfactory. Among *A. vernalis* cenopopulations, the first and

third cenopopulations have typical gene pools (Av1, Av3), and the second, third, and fifth are specific (Av2, Av4, Av5). In the fourth cenopopulation of *A. vernalis* of Akmola region is depleted gene pool, gene pools of other cenopopulations of this species in a satisfactory condition.

8. The gene pools of the studied 20 cenopopulations were documented in the form of formulas and barcodes reflecting the composition of alleles in individual loci of their genomes. The principle of compiling and recording molecular genetic formulas and barcodes based on the identification of DNA identification markers using the ISSR method of DNA polymorphism analysis, covering most of the plant genomes and suitable for genetic passportization of plants, was used.

9. Based on the analysis of the complex of the main indicators of gene diversity, the state of the gene pools of 18 of the 20 studied cenopopulations is estimated as satisfactory, the reasons for this are the lack of insect pollinators during flowering and fragmentation of the species area. Depletion of the gene pool was noted in the population of prostrate open, located in the Pavlodar region near the village of Bayanaul, and in the population of spring adonis of Akmola region in the forestry Mirnoe.

10. Recommendations for the conservation of 4 rare plant species of northern Kazakhstan were developed: *Adonis vernalis* L., *Adonis wolgensis* Stev., *Pulsatilla patens* (L.) Mill., *Pulsatilla flavescens* (Zucc.) Juz.

**Scope and structure of the dissertation.** The thesis consists of an introduction, two chapters, a conclusion, a list of references. The work contains 235 pages of computer text, 29 figures and 29 tables. The list of references consists of 167 titles.

**Information about the metrological support of the thesis work.** The research work was carried out in the Laboratory of molecular-genetic research of Research Institute of applied biotechnology of Kostanay Regional University named after A. Baitursynov GOST ISO / IEC 17025-2009 «General requirements for the competence of testing and calibration laboratories» accredited by the accreditation system of the Republic of Kazakhstan №KZ.T.11.1949 21 August 2020 and instruments and laboratory equipment used in the research work were verified by the National Centre for Expertise and Certification.