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Genetic Diversity of *Hippophae rhamnoides* in Technogenic Areas of Northern Kazakhstan

Genetic diversity is a key indicator of a species' evolutionary development, adaptability to environmental changes, and long-term survival. Globally, the sustainable use and conservation of genetic resources — including *in situ* protection and population-level genetic studies — is a major scientific priority. Sea buckthorn (*Hippophae rhamnoides* L., Elaeagnaceae) has been traditionally used for centuries but has only recently been recognized for its significant economic value. Its berries are rich in biologically active compounds, making the species increasingly important in medicine, agriculture, food production, and cosmetics across Europe and Asia. This study evaluates the genetic diversity of *Hippophae rhamnoides* in technogenic zones of Northern Kazakhstan using ISSR markers. Seed samples were collected from two plant populations in the Prigorodny Forest District, Kostanay Region, for molecular analysis. ISSR analysis revealed high within-population polymorphism (87%) and lower between-population polymorphism (13%) in Hippophae *rhamnoides*. UPGMA clustering indicated genetic differentiation both between and within populations, suggesting distinct genetic origins and potential cross-pollination. The observed high within-population polymorphism and clustering pattern highlight genetic differentiation of Hippophae *rhamnoides* forms according to their origin. This study is preliminary, and further research should include populations from other technogenically affected regions, as well as comparative analyses with populations from protected areas.

Keywords: ISSR markers, molecular analysis, polymorphism, population, *Hippophae rhamnoides*

Introduction

Genetic diversity or variability of living organisms is an important indicator of the evolutionary development of any species, its ability to adapt to environmental conditions, and its preservation as a species by forming a phylogenetic tree.

Monitoring this indicator is especially important for species that are rare or endangered, have small populations, as well as for species that are domesticated or grown in special botanical gardens. Currently, in all countries of the world, the sustainable use of natural genetic resources, including the study of the genetic diversity of various populations in their range and the conservation of endangered species in situ, is one of the most important issues [1].

Although people have used sea buckthorn (*Hippophae* L., Elaeagnaceae) for thousands of years, its significant economic importance has only recently been appreciated. *Hippophae rhamnoides* (sea buckthorn) is a member of the berry family and its fruits contain a high level of active biological compounds [2–5]. It is currently used in many areas of medicine, food, agriculture, and cosmetology in Europe and Asia [6].

In addition, sea buckthorn is currently the subject of food security and sustainable use of genetic resources programs [7-8]. Therefore, it is necessary to take measures to preserve and rationally use agrobiologically important sea buckthorn species in their natural habitat.

Despite the widespread use of sea buckthorns in our country, research on the distribution of its species and their genetic diversity is much less than research on the phytochemical composition of sea buckthorns. In Kazakhstan, detailed information is available only for a single species of sea buckthorn and its subspecies [9-10]. Therefore, investigating populations of this highly valuable and climate-resilient member of the Elaeagnaceae family using ISSR (Inter Simple Sequence Repeat) markers will allow for the assessment of its

genetic diversity and distribution in Kazakhstan, reconstruction of its phylogenetic relationships, and the establishment of a DNA bank.

The aim of our study is to evaluate the genetic diversity of *Hippophae rhamnoides*, a poorly studied sea buckthorn species distributed across technogenic zones in Kazakhstan, using ISSR markers.

Experimental

Plant seed samples were collected during a field botanical study conducted in the spring–autumn period of 2023-2024 in the Prigorodny Forest District, Kostanay Forestry of the Kostanay region, located in Northern Kazakhstan.

The research was carried out in the Laboratory of Molecular Genetics and Plant Biotechnology at the Institute of Botany and Phytointroduction under the Committee of Forestry and Wildlife, Ministry of Ecology and Natural Resources of the Republic of Kazakhstan.

A total of six seed and fruit samples were collected, representing two plant populations. The first population included three forms: Hr 80, Hr 81, and Hr 82; the second population comprised three forms as well: Hr 78, Hr 32, and Hr 34. The map shows sample collection points (Fig. 1).

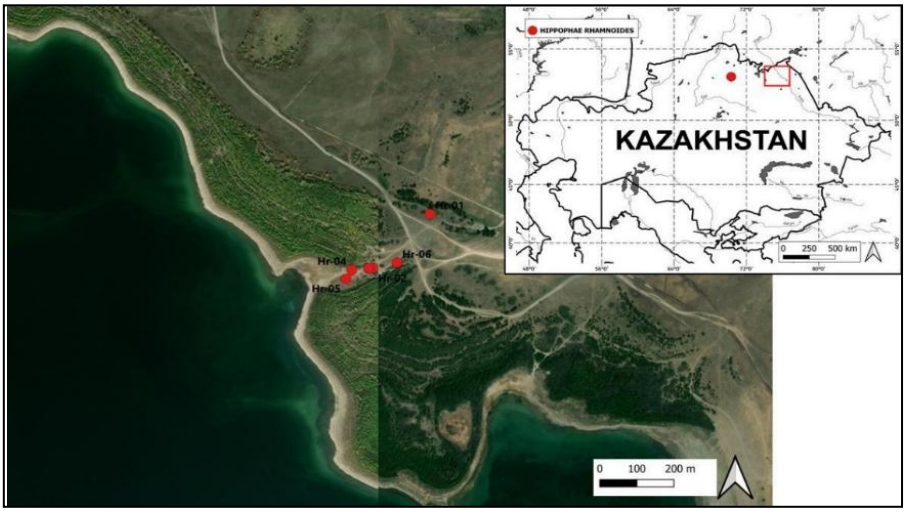

Figure 1. Area for collecting of *Hippophae rhamnoides* forms

Obtaining seedlings as biological material from Hippophae rhamnoides seed samples

To obtain seedlings from *Hippophae rhamnoides* seeds, a special coconut substrate was used. Initially, the seed samples were rinsed with distilled water to remove various contaminants or residual substances. After rinsing, the seeds were placed in a refrigerator at +5 °C for 30–40 minutes. While the seeds were undergoing cold stratification, 100 g of dense, solid coconut substrate was softened using tap water and left to swell. The prepared substrate was then divided into six plastic containers. The chilled seed samples were placed on the surface of the substrate, avoiding deep placement, as light exposure is essential for sea buckthorn seed germination. The containers were covered and stored in a dark place at a temperature of 20–25 °C for one week, until germination began. Seed germination was observed after one week, with the appearance of the first leaves. The plastic containers were placed in a special room with a lamp, without a cover. The substrate was monitored every 4-5 days and watered with 5–10 mL of tap water as needed. Within two weeks, the first seedlings were obtained for use as biological material.

DNA extraction from seedling samples

For DNA extraction, seedling samples were carefully removed from the coconut substrate using sterilized tweezers and rinsed thoroughly with distilled water. DNA was extracted using the DiamondDNA^{TM} Plant DNA Extraction Kit (Altaibiotech, USA), specifically designed for isolating genomic DNA from plant tissues.

To determine the exact concentration of DNA molecules extracted from the plant, a MAXLIFE fluorimeter from Diamond DNA^{TM} was used. DNA concentrations were measured between 0.5 and 2000 ng/μl (Tab. 1).

Table 1

Concentration of DNA molecules extracted from *Hippophae rhamnoides* seeds, measured using the MAXLIFE fluorimeter

Form	Population	DNA concentration (0,5-2000 ng/µl)
Hr80	1	56.0
Hr81	1	56.0
Hr82	1	53.3
Hr78	2	40.0
Hr32	2	66.6
Hr34	2	40.0

The presence or absence of DNA fragments was verified using 1.5 % agarose gel electrophoresis in a horizontal electrophoresis chamber at 45 V and 0.45 A for 20 minutes (Fig. 2). For visualization of DNA on the gel, 2.5 µL of the DNA sample was mixed with 1.5 µL of a specialized 6x DNA Loading Buffer (Glycerine, Xylencyanol) and 7 µL of ddH_2O. As a control, *Thermo Scientific 6x DNA Loading Dye* was used to ensure proper migration and comparison.

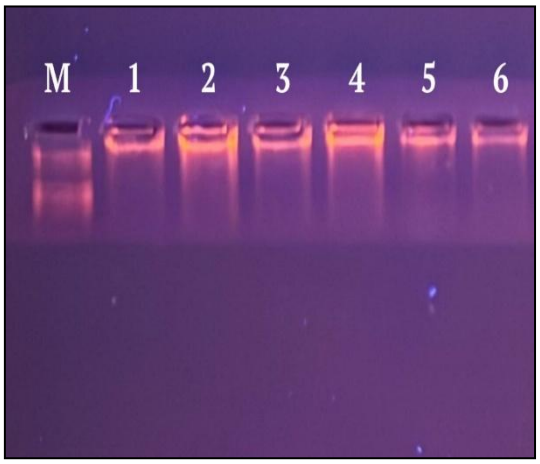

Figure 2. Electrophoresis of DNA isolated from sea buckthorn seedlings

Polymerase Chain Reaction

PCR was performed on a VeritiPro thermocycler (Thermo Fisher Scientific, USA). For 20 µl of the ISSR-PCR solution, a mixture was prepared containing: 1 µl of DNA, 10 µl of 2x HS Taq Red Mix, 1.3 µl of 10 pM primer, 7.7 µl of ddH2O. ISSR-PCR mode: initial denaturation — 94 °C, 01:30 min., denaturation — 94 °C, 00:40 min., primer annealing — 45 °C, 00:45 min., elongation — 72 °C, 01:30 min x 36 cycles, final elongation step — 72 °C for 6 min. The names and nucleotide sequences of the ISSR primers used in the study, along with information on their annealing temperatures, are shown in Table 2.

Table 2

ISSR primer sequences used in the study

№	Primer Name	Primer Sequence (5′ → 3′)	Tm, ^{0}C
1	UBC-810	5'- GAG AGA GAG AGA GAG AT -3'	45
2	UBC-812	5'- GAG AGA GAG AGA GAG AA -3'	45
3	ISSR-3A37	5'- CAC ACA CAC ACA CAT GA -3'	45
4	HB-12	5'-CAC CAC CAC GC -3'	45
5	17899B	5'-CAC ACA CAC ACA GG-3'	45
6	X10	5'-AGC ACG ACG ACG ACG ACG C -3'	45
7	814	5'-CTC TCT CTC TCT CTC TTG-3'	45
8	MAO	5'-TCA GAC GAT GCG TCA TCT CCT CCT CCT CRC -3'	45

Horizontal agarose gel electrophoresis

To confirm the successful synthesis of specific marker loci in the PCR products, electrophoretic analysis was performed. A 1.5 % agarose gel was prepared using 3 g of agarose, 200 mL of 0.5x TBE buffer, and 35 μL of ethidium bromide solution. The PCR products (10 μL) were loaded onto the gel, and electrophoresis was carried out in 0.5x TBE buffer for 2 hours and 15 minutes. The resulting gel was visualized using a UV transilluminator to detect DNA bands.

Results and Discussion

Analysis of genetic diversity of Hippophae rhamnoides using ISSR markers

To select the most polymorphic ISSR-PCR primers, a preliminary screening of eight ISSR primers, selected according to previously published studies [11-12], was performed on six samples of Hippophae rhamnoides. The selected primers proved to be effective in our research [13]. One of the advantages of ISSR markers is that their application does not require prior knowledge of the genome's nucleotide sequence [14-15].

Among the tested markers, four primers—ISSR-3A37 (Fig. 3), HB-12, UBC-810, and UBC-812 were identified as the most informative and were selected for further analysis. A total of 197 ISSR DNA fragments were identified from the DNA fragments studied, of which 39 fragments were polymorphic for the ISSR-3A37 marker, 38 fragments were polymorphic for the HB-12 marker, 44 fragments were polymorphic for the UBC-810 marker, and 76 fragments were polymorphic for the UBC-812 marker. The most polymorphic marker was the UBC-812 marker.

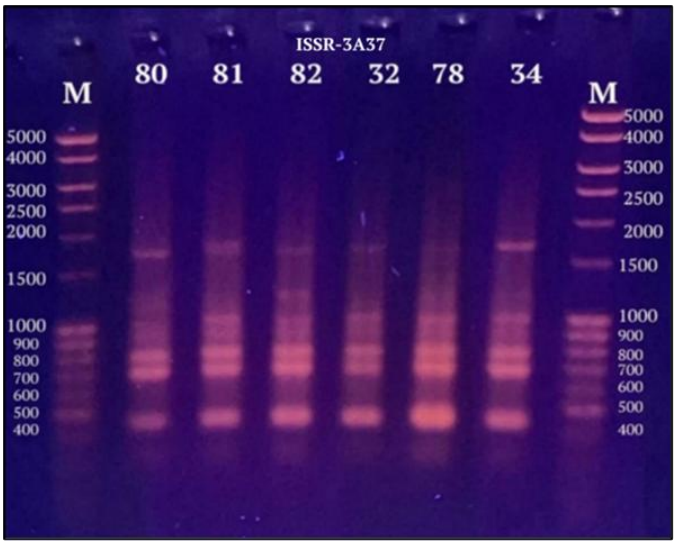

Figure 3. Electrophoregram of the ISSR-PCR product for the ISSR-3A37 marker

Based on the frequencies of DNA fragments, key indicators of genetic diversity for two *Hippophae rhamnoides* populations collected from compartments 62 and 66 of the Prigorodny Forest District (Kostanay Region) were calculated using GenAlEx 6.5 software.

For ISSR markers, the mean number of alleles (Na) typically varies between 0 and 1, though in some cases it may exceed 1. In this study, the Na value for the first population was 0.957 ± 0.08, while for the second population, it was 1.304 ± 0.06. The overall mean Na across both populations was 1.130 ± 0.058.

The effective number of alleles (Ne) reflects not only the number of alleles per locus but also their evenness in distribution. According to the results, Ne was 1.122 ± 0.04 in the first population and 1.243 ± 0.05 in the second population, demonstrating consistency between the effective and average allele numbers.

Shannon information index (I) [16] is one of the indices that assess the diversity of species in an ecosystem. It calculates whether the research population is always full and whether there are many or few species. The Shannon information index (I) for the first population is 0.097 ± 0.034. The Shannon information index (I) for the second population is 0.194 ± 0.044. The Shannon information index (I) for the two populations in total was 0.145 ± 0.028.

In addition, the unbiased diversity (uh) index based on alleles was considered. This index allows us to assess genetic diversity based on allele frequencies and the size of the analyzed samples. The values of this index range from 0 to 1, where 0 indicates low diversity and suggests that all analyzed samples belong to the same species. A value of 1 indicates the presence of multiple species. For the first population, the unbiased diversity index (uh) was 0.101 ± 0.036. For the second population, the unbiased diversity index (uh) was 0.203 ± 0.046. The overall unbiased diversity index (uh) across the two populations of sea buckthorn was 0.152 ± 0.029. These results suggest that the plant samples from both populations are likely to belong to the same species, *Hippophae rhamnoides*. If the uh index is close to 1, it can be assumed that there are subspecies of the species *Hippophae rhamnoides*.

The Shannon diversity index and the unbiased diversity index (uh) are similar to each other. This is because both consider the diversity of species in the study area. The main difference between these indices is that the unbiased diversity (uh) based on alleles is used to analyze genetic diversity, while the Shannon information index is used to assess species diversity in an ecosystem.

The sea buckthorn plant samples exhibited 87 % within-population variation, while the between-population variation accounted for 13 % (Fig. 4).

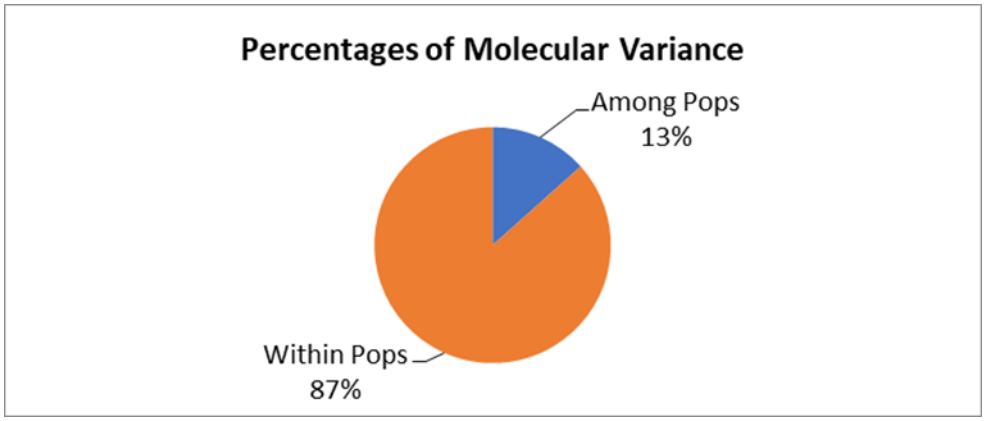

Figure 4. Within-population and between-population variation in *Hippophae rhamnoides*

As a result of the UPGMA cluster analysis, the studied forms of the sea buckthorn plant were divided into two separate groups: the first population and the second population.

The forms in the first group were further divided into three clusters. The first cluster included the Hr81 and Hr82 forms. These forms belong to the same population, which suggests a close genetic origin. The second cluster consisted solely of the Hr32 form. Although Hr32 belongs to the second population, its clustering with the first group may be explained by cross-pollination. The third cluster contained only the Hr80 form, which was genetically distinct from Hr81 and Hr82, likely due to differences in origin. The second group comprised the Hr78 and Hr34 forms, both belonging to the second population. This group was divided into two separate clusters, indicating that despite originating from the same population, these forms exhibit signs of genetic differentiation (Fig. 5).

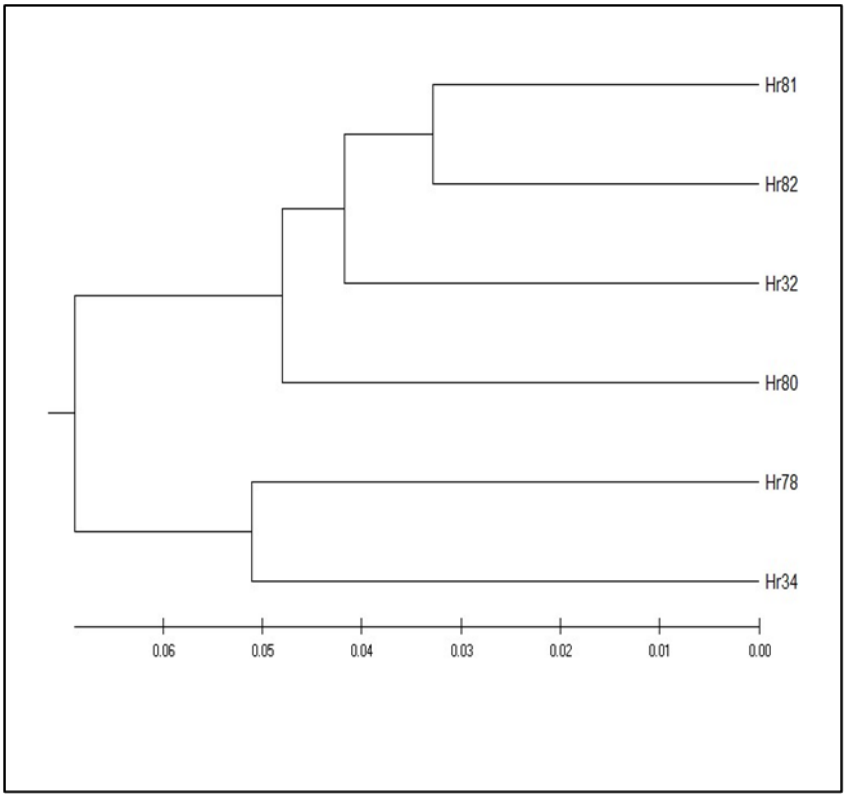

Figure 5. UPGMA dendrogram of the plant forms of the sea buckthorn using the program MEGA 7

Conclusion

Based on the obtained ISSR DNA fragments, the level of within-population polymorphism was 87 %, while the between-population polymorphism was 13 %.

The UPGMA cluster analysis revealed that the studied forms of *Hippophae rhamnoides* were divided into two separate groups corresponding to the first and second populations. The forms in the first group were further separated into three clusters. The first cluster included the Hr81 and Hr82 forms. The second cluster consisted solely of the Hr32 form, which belongs to the second population. Its placement in the first group is explained by the possibility of cross-pollination. The third cluster included only the Hr80 form.

The second cluster group consisted of the Hr78 and Hr34 forms of the second population, which formed separate individual clusters.

The high level of within-population polymorphism (87 %) and the clustering pattern indicate that the *Hippophae rhamnoides* forms exhibit genetic differentiation associated with their origin. However, this study represents only a preliminary stage in the genetic assessment of sea buckthorn populations. To obtain a more comprehensive understanding of the species' genetic diversity and adaptive potential, it is necessary to expand the analysis to include populations from other technogenically-transformed regions, as well as to compare them with populations growing in protected areas.

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Author contribution

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript: **Shadmanova L.Sh. —** conceptualization, data curation, investigation, methodology, editing; **Kulboldin T.S. and Token A.I. —** data curation, formal analysis, supervision, writing draft; **Kanapin Ch.B., Mukan G.S., Akhatov K.Zh., Yeszhanova A.S. and Razhanov M.R. —** conducting the fieldwork and sample collection.

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К.Ж. Ахатов, Г.С. Мұқан, А.С. Есжанова, М.Р. Ражанов

Солтүстік Қазақстанның техногендік экожүйелеріндегі *Hippophae rhamnoides* популяцияларының генетикалық әртүрлілігі

Генетикалық әртүрлілік түрдің эволюциялық дамуының, қоршаған орта өзгерістеріне бейімделуінің және ұзақ мерзімді сақталуының негізгі көрсеткіші. Әлемдік деңгейде генетикалық ресурстарды орнықты пайдалану мен сақтау, соның ішінде *in situ* қорғау және популяциялық-генетикалық зерттеулер басты ғылыми басымдықтардың бірі саналады. Итшомырт шырғанақ (*Hippophae rhamnoides* L., Elaeagnaceae) ғасырлар бойы дәстүрлі түрде қолданылып келген, алайда оның жоғары экономикалық құндылығы тек соңғы уақытта мойындалды. Оның жемістері биологиялық белсенді қосылыстарға бай, сондықтан бұл түр Еуропа мен Азияда медицинада, ауыл шаруашылығында, тамақ өнеркәсібінде және косметологияда барған сайын маңызға ие болуда. Осы зерттеуде Солтүстік Қазақстанның техногендік аймақтарында өсетін итшомырт шырғанақтың (*Hippophae rhamnoides*) генетикалық әртүрлілігі ISSR-маркерлерді қолдану арқылы бағаланды. Молекулалық талдау үшін Қостанай облысындағы Пригородный орманшылығынан екі популяциядан тұқым үлгілері жиналды. ISSR-талдау *Hippophae rhamnoides* үшін жоғары деңгейдегі ішкіпопуляциялық полиморфизмді (87 %)

және төмен деңгейдегі аралық популяциялық полиморфизмді (13 %) көрсетті. UPGMA кластерлеуі популяциялар арасында және олардың ішінде генетикалық дифференциацияны анықтады, бұл әртүрлі генетикалық шығу тегі мен ықтимал айқас тозаңдануды көрсетеді. Жоғары ішкіпопуляциялық полиморфизм және кластерлеу сипаты *Hippophae rhamnoides* формаларының шығу тегіне байланысты генетикалық дифференциациясын айқындайды. Бұл зерттеу алдын ала сипатқа ие, әрі қарайғы жұмыстар басқа техногендік аймақтардағы популяцияларды және қорғалатын аймақтарда өсетін популяциялармен салыстырмалы талдауды қамтуы тиіс.

Кілт сөздер: ISSR-маркерлер, молекулалық талдау, полиморфизм, популяция, *Hippophae rhamnoides*

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Генетическое разнообразие популяций *Hippophae rhamnoides* в техногенных экосистемах Северного Казахстана

Генетическое разнообразие является ключевым показателем эволюционного развития вида, его адаптивности к изменениям окружающей среды и долгосрочного выживания. Во всем мире устойчивое использование и сохранение генетических ресурсов, включая *in situ* охрану и популяционно-генетические исследования, является важнейшим научным приоритетом. Облепиха крушиновидная (*Hippophae rhamnoides* L., Elaeagnaceae) традиционно используется на протяжении веков, но лишь недавно была признана видом с высоким экономическим потенциалом. Ее ягоды богаты биологически активными соединениями, что делает вид все более востребованным в медицине, сельском хозяйстве, пищевой промышленности и косметологии в Европе и Азии. В данном исследовании оценено генетическое разнообразие облепихи (*Hippophae rhamnoides*), произрастающей в техногенных зонах Северного Казахстана, с использованием ISSR-маркеров. Для молекулярного анализа были собраны семена из двух природных популяций Пригородного лесничества Костанайской области. ISSR-анализ показал высокий уровень внутрипопуляционного полиморфизма (87 %) и низкий уровень межпопуляционного полиморфизма (13 %) у *Hippophae rhamnoides*. Кластеризация методом UPGMA выявила генетическую дифференциацию между и внутри популяций, что указывает на различное генетическое происхождение и возможное перекрестное опыление. Высокий уровень внутрипопуляционного полиморфизма и характер кластеризации указывают на генетическую дифференциацию форм *Hippophae rhamnoides* в зависимости от их происхождения. Данное исследование носит предварительный характер и является лишь первым шагом в генетической оценке популяций облепихи. Для более полного понимания генетического разнообразия и адаптивного потенциала вида необходимо расширить анализ, включив в него популяции из других техногенно трансформированных регионов, а также провести сравнение с популяциями, произрастающими в охраняемых зонах.

Ключевые слова: ISSR-маркеры, молекулярный анализ, полиморфизм, популяция, *Hippophae rhamnoides*

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