Caspian Journal of Environmental Sciences
Online ISSN: 1735-3866
Print ISSN: 1735-3033

Molecular genetic analysis of gene pools of rare and endangered plant species *Adonis* L. of Northern Kazakhstan

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ABSTRACT

Adonis L. is one of the largest and most famous genera of the Runanculaceae family. *Adonis* genus includes two parts of annual and perennial species. The latter contains more effective substances than annual species, but among annual species, plants. *Adonis* L. contains more effective substances, which mainly grow in sandy areas with high heat in the central parts of Kazakhstan. The extract of *Adonis* L. plant species has a high total phenolic content and, therefore, requires research on this plant's antioxidant and radical removal properties. Describing plants by molecular markers is a good strategy for improving and protecting plant genetic resources. The aim of this study is molecular genetic analysis to investigate the total phenolic content of the *Adonis* L. hydroalcoholic extract. The phenolic content of this plant extract was determined by the Folin Ciocaltio reagent method (FC) and Gallic acid as a standard. The results showed that the hydroalcoholic extract has a total phenolic content of 26.607 ± 2.35 mg of Gallic acid per gram of extract. The increase in the antioxidant activity of essential oils is significantly related to the increase in the phenolic content of essential oils. The results showed that the presented method is suitable and useful for estimating genetic diversity in *Adonis* L. populations.

Keyword: *Adonis* L., Molecular genetic, Gene pools, Phenolic.
Article type: Research Article.

INTRODUCTION

The genus *Adonis* is one of the most important genera of the Alale genus, which, according to various sources, has 30 to 40 species with annual and perennial vegetative forms and is widely spread in Southwest Asia and Europe, North Africa, and the Mediterranean region. Kazakh annual species of this genus are: (i) Annual species (*A. annua* L), (ii) Perennial species (*A. dentata* Boiss), (iii) Small-seeded species (*A. microcarpa* Dc.), (iv) Summer species (*A. aestivalis* L.), (v) hollow species (*A. scrobiculata*), (vi) spherical species (*A. globasa* C) and (vii) fiery species (*A. flammea* Jacq; Boronnikova & Kalendar 2010; Kropf *et al.* 2020; Karahan *et al.* 2022). Some of the species in this genus, such as *A. microcarpa*, *A. flammea*, and *A annua*, are weeds in fields and gardens and can be found in most areas (edges of streams, roads, gardens and barren fields). These species have little poison in their organs, which is poisonous to animals. Pollinological studies were conducted by Rosche *et al.* (2018) in this genus. Based on the thickness of the pollen grain wall at the pollen pole and other parts and the density of thorns on the surface of the pollen of this genus, the existence of two subspecies was confirmed (Kropf

Caspian Journal of Environmental Sciences, Vol. 22 No. 4 pp. 913-918 Received: April 22, 2024 Revised: July 04, 2024 Accepted: Sep. 13, 2024
DOI: 10.22124/CJES.2024.8139

Publisher: University of Guilan

2012). Morphological traits are discussed. To solve this problem, it can be essential to investigate the separation of species boundaries in this genus with other characteristics, such as pollen micromorphological traits. Therefore, one of the essential goals of this study is pollinological investigations in the species of this genus. Comparative genomics is known as one of the developing research fields that provide important evolutionary insights and useful applied information in the field of understanding genomic similarities and differences between species (Suliyanthini 2023); on this basis, by comparing the genome between two or more species, it is possible to identify the evolutionary plan, conserved domains, gene structure, phylogenetic relationships, orthologous genes and other unique genomic features such as genome size, chromosome number, number of protein-coding genes, microsatellites etc. payment (Rao 2015). Various molecular markers, such as microsatellite markers, are used for genetic diversity studies, population structure, genetic mapping, phylogenetic studies, and identification and selection of plant species, and can provide appropriate population genetic information to executive department managers (Kovács *et al.* 2023). The knowledge of gene homology can facilitate the transfer of genes or genomic regions related to desired traits through genetic engineering, gene editing, or extensive crossing between species. Comparative genomics depends on sequenced genomes and powerful, web-based bioinformatics tools that are freely available and easy to use for experimental biologists. The increasing availability of genomic resources, such as genome sequencing and high-throughput molecular markers, allows comparative genomic studies among various species and provides new insights into the evolution of genes or gene families through whole-genome analysis. Comparative genomics studies can extract a lot of knowledge about the genome function of forest trees. So far, plant species with different economic importance have been well studied in this field, but trees have yet to be studied (Dorogina *et al.* 2023). Many researchers (Kupriyanov 2024; Kupriyanov 2024) reported that comparative genomics is used in plant species breeding, explaining the relationship between the change in the characteristics of this gene and the amount of adaptation of different species of the *Eucalyptus* genus to different environments is still a challenge. Compared to other oak species, the genome of *Quercus mongolica* had many copies of the CER1 gene, so it was suggested that these duplicate copies could help adapt this oak species to drought stress. The research results in Hassan *et al.* (2023) and Zaimenko *et al.* (2024) show that most of the compounds in *A. aestivalis* L are related to phenolic compounds (Kupriyanov 2024). A study (Pacher *et al.* 2024) investigated chemical compounds of essential oil, fatty acids, and extract of *Adonis oogenesis* L plant and its antioxidant and antibacterial properties.The main components of the essential oil of the aerial part of this plant were palmitic acid (32.97%), methyl linolenate (11.91%) and phytol (10.33%). Natural antioxidants are mostly found in plants that contain phenolic compounds. Phenolic content and plant compounds depend on genetic and environmental factors. Plants have many antioxidant compounds, and it is difficult to identify each one. Therefore, the antioxidant capacity of the extracts is evaluated with different assays. The presence of phenolic and tetraterpenoid compounds has been proven in the *Adonis* genus.

MATERIALS AND METHODS

Plant characteristics

Adonis is a herbaceous, perennial plant belonging to the Runanculaceae family. This plant grows widely in central and southern parts of Europe, western Asia, and eastern Siberia. *Adonis* grows mainly on the south-facing slopes of dunes. This plant has a rhizome with a thickness of 2 to 3 cm. The outer layer of the roots is black, and the inner part is white. In autumn, vegetative buds are formed on the surface of the rhizome. Numerous stems come out from the growth of these buds in the spring. The stem is straight, cylindrical, and grooved in the longitudinal area. The height of the stem is different and between 35 and 45 cm. The stem has few branches. The leaves are alternate, toothed, and relatively narrow and root-like. The flowers are more or less large and 4 to 8 cm in diameter. Each plant has 5 to 20 branches, and flowers appear at the end of the main and secondary branches. Each flower has 5 green sepals and 15 to 30 petals. The color of the petals is golden yellow. Flowering begins in spring and from mid-April. The fruit is a capsule and club-shaped. There are brown seeds (light or dark) inside the fruit. Each plant produces 30 to 50 seeds.

Ecological needs

This plant grows in the southern and sunny slopes where the soil is rich in calcium compounds

Plant propagation

The seeds of this plant have weak vegetative power. In many cases, the seeds dry out after germination. Thus, this plant is propagated by seeds and vegetatively.

Propagation by seeds: The seeds are suitable for planting when they are fresh and moist; their germination power is between 30% and 40%. Dried seeds have fragile vegetative power. The seeds are sown directly on the mainland in the spring, and between June and July. The proper distance for planting rows is 50 cm. The seed depth at planting should be 1.5 to 2 cm. Plants grow very slowly in the first years of growth and reach their final growth after 3 to 4 years.

Vegetative propagation: because the seeds of this plant have very weak vegetative propagation, its vegetative propagation is much more suitable. After choosing suitable plants in wild habitats, they are taken out of the ground along with the surrounding soil (it is better to do this after rain or irrigation when the ground is wet) and in the desired fields in rows at a distance of 60 cm. The distance between the two plants is 30 cm each meter in length. The best time for vegetative propagation is autumn (October) or spring (April). A total of 12 samples were collected from the gene bank of the Kazakhstan Pasture Research Institute, which were from different places. Genomic DNA extraction was done from the leaves of each of the *Adonis* stands with some changes (Kropf *et al.* 2020) based on the extraction instructions by Khanuja method (Zaimenko *et al.* 2024). The amount of extracted genomic DNA was calculated by a biophotometer measuring absorbance at 260 nm, and the purity of DNA was also checked. DNA storage was brought to 10 nanograms per microliter for the PCR reaction. DNA purity was also done by loading the samples on 0.8% agarose gel, and its concentration was evaluated by comparing it with a DNA marker, λ (Karahan *et al.* 2022). The method of Kropf (2012) was used to check the total phenolic content, and Gallic acid was used as a standard (Rao 2015). First, 330 microliters of the samples with a concentration of 0.005 g mL^{-1} were poured into the test tube. Then 1.5 mL of Folin-Ciocalteus reagent and 1 mL of 8% sodium carbonate were added to the above solution and stirred well. Next, it was placed at the laboratory temperature for 180 seconds, and then its absorbance at 750 nm was read by an ultraviolet spectrometer. A similar method was performed for all standard gallic acid solutions, and the standard curve for gallic acid was drawn. Using the Gallic acid standard curve drawn, the total amount of phenolic compounds in the extracts can be obtained by the following formula (Kovács *et al.* 2023):

The total amount of phenolic compounds in the extract = (the amount of optical absorption of the essential oil - 0.033)/0.001

Investigating the antioxidant power of the plant extract using the Ferric Reducing Antioxidant Power (FRAP) method

The method of Kovács *et al.* (2023) was used to investigate the antioxidant power of ferric: One mL of different extract concentrations (0.006 g mL^{-1}) was prepared and added to 3 mL of potassium phosphate buffer with a pH of 5.6. An aliquot of 3 mL of 1% potassium-free cyanide was added to the solution of the previous step, which was kept at 45 ℃ for 30 minutes. Then 3 mL of 10% trichloroacetic acid was added to the previous solution. The resulting solution was divided into equal volumes of 3 mL, and 3 mL of distilled water was added to each solution. Afterward, 0.6 mL of iron chloride ($FeCl_3$-$6H_2O$) was added to each of these solutions, and then the tubes were left at room temperature for 30 minutes. The absorbance of each of these tubes was read at a wavelength of 700 nm with a spectrophotometer. Gallic acid was used as a standard for this test. The iron recovery power was stated based on the gallic acid equivalent (mg g^{-1}) of the used sample. The extracts' iron recovery power was obtained using the gallic acid standard curve drawn.

RESULTS

Identification and classification of plants are done based on the sequence of their protected genes. One of their most crucial is ribosomal gene regions, which are used to check the proximity and classification of plants. In these protected sequences, there is no possibility of mutation or horizontal transfer in the genes of the components of large molecular devices such as the ribosome, because these components have complex interactions with each other that have evolved together over millions of years, and a small change in their sequence can cause structural large and changes with its inefficiency. These gene regions are widely used in the classification and identification of plants. These regions are amplified using thermocycler and polymerase chain reaction (PCR), and after checking their quantity and quality, they are sequenced. The sequence obtained from an isolate is compared with the sequences of strains from all species in various online databases, including NCBI, which is an accurate and suitable way for the molecular identification of the *Adonis* plant. However, in many different species of the same genus, due to the high similarity of these gene regions, the use of a multi-gene separation approach (MLSA) and

the comparison of several genes are also widely used. The genes used, and the number of genes needed to identify or classify the *Adonis* plant differ depending on the species type. In this research, the species in different regions of Kazakhstan have been evaluated.

Total flavonoid content of the plant

The extraction efficiency for 85% methanolic extract was equal to 5.95% and for ethyl acetate extract was equal to 5.21% by weight. The total flavonoid content of plant extracts in annual and perennial species was obtained in terms of milligrams of catechin per gram of sample and as shown in Table 1:

Table 1. Determination of total flavonoid content of Adonis extract (mg g^{-1}) of sample.

	Type of extract	sample (mg g^{-1})
One-year species	Methanol 85%	97.88
	Ethyl acetate	91.03
Perennial species	Methanol 85%	98.44
	Ethyl acetate	92.78

Microsatellites, with their high polymorphism and equal inheritance, are a key focus of our research. The primers presented in Table 2 are not just theoretical but can be practically used to perform the ISSR test, demonstrating the real-world relevance of our work.

Table 2. Primer sequence ISSR.

Sequence	Temp	Primer
5-GTGGTGGTGGTGGTG-2	65	GTG5
5-GACGACGACGACGAC-2	61	GAC5
5-CAGCAGCAGCAGCAG-2	59	CAG5
5-GTCGTCGTCGTCGTCGTCGT-2	55	PCMS
5-CACCACCACCACCAC-2	53	ISSR10
5-ACTGACTGACTGACTG-2	52	ISSR2

The table shows the three microsatellites with the highest frequency in the studied species. Based on this, the ten microsatellite motifs with the highest frequency on the genome of *Adonis* species have occupied 69.92% of the total identified microsatellite motifs. So, the most abundant motif in Adonis species was the single nucleotide motif (T; 13.17%) and AT motif (15.3 %), respectively. Among the ten motifs with the highest frequency, GA (6.93%) and AAG (0.83%) motifs were observed in the *Adonis* genome (Table 3).

Table 3. Microsatellites with the highest frequency in the studied species.

Number	One-year species		Perennial species	
	Microsatellite motif	Percent	Microsatellite motif	Percent
1.	T	18.2	AT	16.5
2.	A	17.9	TA	16.1
3.	CA	8.5	A	9.6
4.	AG	6.8	T	8.4
5.	CT	6.3	AG	8.1
6.	AT	6.2	CT	4.5
7.	TA	5.1	CA	4.2
8.	TC	3.9	AAC	3.4
9.	AAC	1.1	TC	2.1
10.	AAG	0.8	TTA	1.9

Fig. 1 vividly illustrates the presence of microsatellites of varying sizes in the genomes of the two species under study. The distribution pattern of these microsatellites, particularly in sequences of two and three nucleotides, is remarkably distinct between the two plant species. However, when it comes to sequences of six and four nucleotides, the similarity is striking. Regarding the repetition unit in the one-year genome, 44.34% were single nucleotides, 36.35% were two nucleotides, 1.5% were three nucleotides, and 0.9% were four nucleotides. Regarding the repeat unit in perennial species, 6.5% were single nucleotides, 7.5% were two nucleotides, 3.18% were three nucleotides, and 2.14% were four nucleotides.

Antioxidant effects of plant extracts

The antioxidant capacity of the plant extract was evaluated by the FRAP antioxidant power-reduction FRAP method, and the results of the antioxidant activity in the FRAP test were reported as mg g^{-1} of the extract (Table 4). Gallic acid was used as an antioxidant standard, and its absorption graph was drawn (Fig. 2).

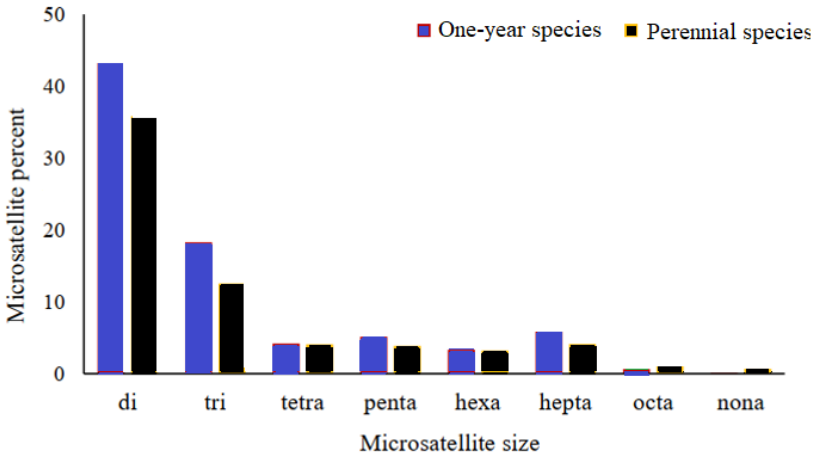

Fig. 1. microsatellites with different sizes in the genome of the two studied species.

Table 4. FRAP ferric reducing power of the extracts in terms of Gallic acid equivalent.

	Type of extract	Sample weight (mg g^{-1})
One-year species	**Methanol 85%**	**3.94**
	Ethyl acetate	3.34
Perennial species	Methanol 85%	4.01
	Ethyl acetate	3.99

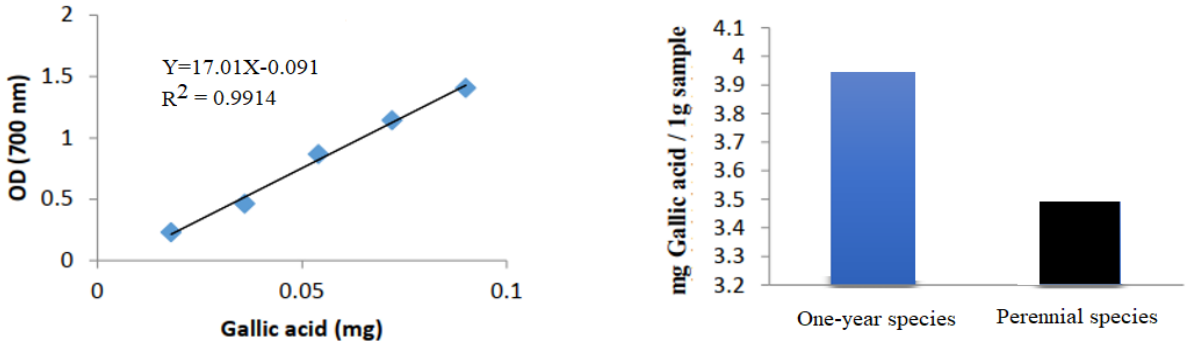

Fig. 2. Determining the FRAP ferric reducing power of the extracts in terms of Gallic acid equivalents (mg per gram of sample.

The results of our research are significant. We found that the extract of *Adonis aestivalis* L. exhibits antioxidant effects. Furthermore, our examination of the plant's total phenolic and flavonoid content revealed a higher concentration of phenolic compounds. This suggests that future projects should focus on identifying, extracting, and comparing the most important phenolic compounds in plant extracts. Additionally, investigating the antioxidant properties of different plant extracts, extracted using various solvents and methods, and evaluating their antioxidant activity in laboratory and clinical models, could yield valuable insights.

CONCLUSION

With the development of sequencing technologies, the possibility of more detailed analyses of genomic features such as genome size, microsatellite sequences, pseudogenes, etc., has been provided, and based on this, it can give a deep insight into genomic features as well as their diversity patterns among different types of plants. Genome size varies among plant species, and the evolutionary and practical significance of this variation is a significant issue. In this research, the different features of the genome and microsatellite sequences of two annual and perennial plant species of *Adonis* were compared with each other. Therefore, it was observed that the genome size of the annual species is larger than the genome size of the perennial species. However, this increase in genome size does not necessarily mean that the species has become more advanced during the evolutionary process. Comparative genomics can help identify breeding targets and design new breeding strategies by providing diverse alleles related to important traits such as cell wall quality and wood formation. Also, comparative genomics can

help preserve genetically distinct and endangered species. Protected genes in different species are a valuable genetic resource that can be used in selection with the help of markers, genetic engineering, and gene editing. By understanding the genomic structure of different species, researchers can identify unique genetic traits and prioritize conservation efforts for genetically distinct or endangered species.

ACKNOWLEDGEMENTS

Research work was carried out based on the project No AP15473108 of the Committee of the Ministry of Science and Higher Education of the Republic of Kazakhstan.

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Bibliographic information of this paper for citing:

Zhumagul, M, Myrzagaliyeva, A, Mukhtubayeva, S, Baibossynova, S, Zhanbyrbayeva, A, Akhmetov, K, Rustemova, A, Bekturova, A 2024, Molecular genetic analysis of gene pools of rare and endangered plant species *Adonis* L. of Northern Kazakhstan, Caspian Journal of Environmental Sciences, 22: 913-918.