Acta Botanica Hungarica 65(3–4), pp. 385–398, 2023
DOI: 10.1556/034.65.2023.3-4.9

GENETIC DIVERSITY AND POPULATION STRUCTURE OF *HALOXYLON APHYLLUM* IN IRAN BY ISSR MARKERS

A. Pourmeidani[1], E. Amini[2], F. Nasrollahi[3]* and Y. Mohammadi[4]

[1]*Research Division of Natural Resources, Agricultural and Natural Resources Research and Education Centre, AREEO, Qom, Iran*
[2]*Department of Biology, Faculty of Sciences, Gonbad Kavous University, Gonbad, Iran*
[3]*Department of Biology, Faculty of Sciences, University of Qom, Qom, Iran; *E-mail: nbotanist@yahoo.com (corresponding author)*
[4]*Research Institute of Forests and Rangelands, Agricultural Research, Education and Extension Organization (AREEO), Tehran, I. R. Iran*

(Received 2 February 2023; Accepted 22 August 2023)

Haloxylon aphyllum is a significant species adapted to salinity conditions and plays an important role in stabilising soil, providing forage, and serving as a source of firewood for residents. In this study, the genetic diversity of four populations of *H. aphyllum* in Iran was examined using four primers to assess genetic diversity, which produced a total of 41 bands. The AMOVA test showed that the studied populations differed in their genetic content. Specifically, 46% of genetic variability occurred within populations, while 54% arose between populations, indicating a high degree of genetic variation among *H. aphyllum* populations. The Mantel test presented a significant correlation between genetic distance and geographic distance. Additionally, the STRUCTURE analysis presented comprehensive information on the genetic structure of the studied *H. aphyllum* populations. The presence of genetic diversity and heterozygosity among *H. aphyllum* populations suggests local adaptation among populations, which may be due to the heterogeneity of environmental factors such as soil moisture and nutrients that create genetic heterogeneity.

Key words: AMOVA, genetic, Iran, populations

INTRODUCTION

Deserts in the Irano-Turanian region are abundant with *Haloxylon* Bunge ex Fenzl plant. A tree or large shrub is their life form (Iljin 1936, Netchaeva *et al.* 1973). *Haloxylon* species has a special photosynthetic apparatus. The leaves are reduced, and the anatomical structure shows C4 photosynthesis. Desert areas are known as fragile ecosystems (Jafari *et al.* 2003). One of the biological methods to reduce desertification is using the biological stabilisation of *Haloxylon* sp. (Xu *et al.* 2014). *Haloxylon aphyllum* (Minkw.) Iljin and *H. persicum* Bunge (having the common name of black and white saxauls) previously belonged to the family Chenopodiaceae but were reclassified to the family Amaranthaceae in the APG IV (Angiosperm Phylogeny Group) classification system (2016). These succulent plants drop salt-rich stems and leaves to protect themselves and adapt to salinity conditions. *Haloxylon aphyllum* is an im-

portant species that adapts to salinity conditions and plays an important role in soil stabilisation (Gintzburger *et al.* 2003, Nikitin 1966).

The morphological diversity of *Haloxylon* communities has shown remarkable variations due to wind erosion and land degradation. Genetic diversity and population structure information are needed for this community to aid conservation and restoration efforts. As populations evolve, they achieve a defined optimum of genetic diversity, which causes disruptions in the genetic balance and disrupts ecosystem conditions (Altukhov 2003). Identifying ecological elements in such microevolutionary processes is crucial to gain deep insight into them (Mitton *et al.* 1998, Nevo *et al.* 1994, Prentice *et al.* 2000). In the presence of undulate biological and abiotic factors, genetic diversity plays an important role in species adaptation, so this factor is so vital to the survival of the species (Vrijenhoek 1994).

Over the past few decades, various genetic techniques have emerged in molecular genetics, including several PCR-based genetic markers that provide valuable information on genetic variations in plant species. Among these markers, the ISSR (inter simple sequence repeat) molecular markers are stable, highly reproducible, and easy to work with, making them useful in various biological investigations (Minaeifar *et al.* 2015, Sheidai *et al.* 2012, 2013, 2014, Tahmasebi and Nasrollahi 2021). Despite their utility, little research has been conducted on assessing *Haloxylon* genetic diversity. Shuyskaya *et al.* (2012) studied the genetic differentiation of *Haloxylon aphyllum* in the Kyzylkum land and found that populations were noticeably deficient in heterozygotes, despite a medium degree of genetic diversity.

In the current study, we used an ISSR marker that has not been previously applied to *H. aphyllum*. The main objectives of our study were (1) to evaluate the genetic relationships within Iranian *Haloxylon* using molecular data and (2) to identify the population genetic structure and gene flow in four local populations of *H. aphyllum*. To the best of our knowledge, this is the first study of Iranian *Haloxylon aphyllum*, and the information obtained from this study can be utilised for the conservation, breeding, and sustainable management of this plant species.

MATERIAL AND METHODS

Plant material

Genetic and morphological data considered in this study are based on 61 samples from four populations in *Haloxylon aphyllum* (Fig. 1). The plant samples were collected from four geographical populations of Iran, and their habitat is shown in Figure 2. GPS specified the geographical coordinates and elevation of each genotype habitat (Table 1).

Table 1
Investigated *Haloxylon* populations.

Pop.	Code	Locality	No. of samples	Longitude	Latitude	Altitude (m)
1	Y	Yazd	18	E 54.3569°	N 31.8974°	1,216
2	S	Semnan	19	E 53.3798°	N 35.5788°	1,130
3	C	Sistan and Baluchestan	12	E 60.5821°	N 27.5300°	1,224
4	K	Kerman	12	E 57.0834°	N 30.2839°	1,775

DNA extraction – The genomic DNA was extracted by CTAB-activated charcoal protocol (Amini *et al.* 2018, Cullings 1992, Doyle and Dickson 1987, Doyle and Doyle 1987, Nasrollahi *et al.* 2019). The quality and quantity of extracted were tested by running on 0.8% agarose.

ISSR analysis – In the current study, a total of eight ISSR primers were screened (Al Salameen *et al.* 2018). Among eight used ISSR primers, four primers revealed the amplified DNA fragments. The PCR reaction was done with 61

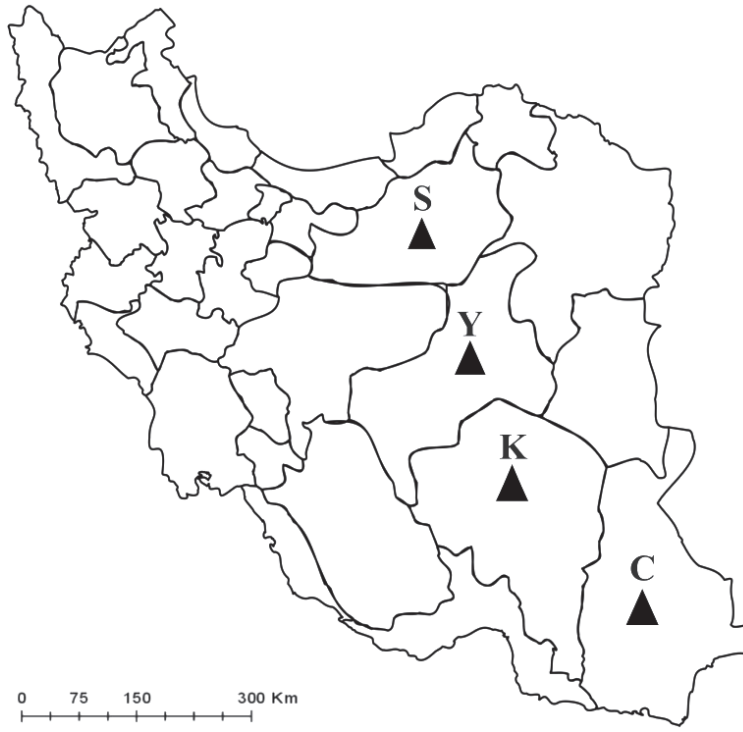

Fig. 1. Distribution map of the studied *Haloxylon aphyllum* populations in Iran. Populations are marked with codes S, Y, K, and C, according to Table 1

Table 2
The used ISSR primers

Code	Sequences
ISSR13	$(ACG)_6$ GA
ISSR826	CCCGGATCC $(CA)_8$
ISSR810	$(GA)_8$ T
ISSR7	$(AC)_8$ AG

Table 3
PCR program for ISSR primers

Step	Temperature	Time	Cycling
Initial denaturation	94 °C	5 min	–
Denaturation	94 °C	60 s	–
Annealing	52–55 °C	60 s	40
Extension	72 °C	90 s	–
Final extension	72 °C	6 min	1

samples and four ISSR primers (Table 2) in a 25 μL reaction volume, including 2.5 mM $MgCl_2$ (Cinna Gen Co, Iran), 0.2 μM of primer (Cinna Gen Co, Iran), 10 mM Tris-HCl, pH 8.3, 1 mM dNTP mix (Cinna Gen Co, Iran), 1 U of Taq DNA polymerase-500 (Cinna Gen Co, Iran), and 15 40 ng of template DNA. ISSR-PCR was performed in the thermocycler (Biorad, USA) with the program in Table 3. The amplified product was checked in 1% agarose gel electrophoresis.

Molecular analysis

The obtained ISSR bands were treated as binary characters, with the presence of a band coded as 1 and absence as 0. Genetic diversity parameters, such as Nei's gene diversity (H), Shannon information index (I), number of effective alleles, and percentage of polymorphism, were determined for each population using the methods described by Freeland *et al.* (2011). Nei's genetic distance was used to perform clustering algorithms, including Neighbour-Joining (NJ), WARD, and Unweighted Paired Group Average (UPGMA). Principal Coordinate Analysis (PCoA) and Multidimensional Scaling (MDS) methods were also used for grouping the populations after 100 times permutation (Freeland *et al.* 2011).

Fig. 2. The natural habitat of *Haloxylon aphyllum*

Table 4
Items of ISSR bands in *Haloxylon aphyllum* populations (populations 1–4 matching Figure 1)

	Pop1	Pop2	Pop3	Pop4
No. bands	26	23	21	15
No. bands freq. (≥ 5%)	20	29	23	19
No. private bands	3	2	0	0
No. common bands (≤ 25%)	0	2	1	0

To calculate the correlation between the genetic and geographical distances of the studied populations, Mantel's test was performed using PAST ver. 2.17 (Hammer *et al.* 2012) and DARwin ver. 5 (Perrier and Jacquemoud-Collet 2006) programs. To determine the genetic differentiation of the species, AMOVA analysis (Analysis of Molecular Variance) was performed using GenAlex 6.4 (Peakall and Smouse 2006). Gene flow was estimated using the Nm value, which is an estimate of gene flow from GST using Pop Gene ver.1.32 (Nm = 0.5 (1–GST)/GST) and by the least square method, as performed in T-REX (Boc *et al.* 2012).

The genetic structure of the populations was analysed using a model-based clustering approach implemented by the STRUCTURE software ver. 2.3 (Pritchard *et al.* 2000). The optimal value of K in the population studied was determined using the STRUCTURE Harvester website (Earl and von Holdt 2012).

RESULTS

ISSR assay and genetic diversity

The results of the DNA extraction process showed the acceptable quality of the obtained molecules for use in the genetic diversity study. The gel electrophoresis results showed that the resulting nucleic acid molecules do not have any smears and can be used in the polymerase chain reaction. ISSR primers produced 41 reproducible bands/loci (Table 4). The discriminating power of ISSR loci shows that approximately all ISSR loci have great discriminating authority. Therefore, ISSR markers in plant population differentiation of *Haloxylon aphyllum* studied are efficient.

The DCA plot (Detrended Correspondence Analysis) revealed that the loci are located in different positions, so the ISSR molecular markers are distantly related (Fig. 3). This analysis shows that these markers are related to different genome regions, making them effective molecular markers for differentiating the studied populations. Genetic diversity parameters determined in *H. aphyllum* are reported in Table 5. The percentage of genetic polymorphism taken ranged from 7.43% in population 2 to 52.62% in population 4. Ne, genetic distance and genetic identity determined among *H. aphyllum*

Table 5
Genetic diversity details in *Haloxylon aphyllum* populations (populations 1–4 are according to Figure 1)

Pop	N	Na	Ne	I	He	uHe	%P
Pop1	18.000	0.603	1.177	0.158	0.106	0.127	28.02
Pop2	19.000	0.233	1.063	0.054	0.037	0.049	7.43
Pop3	12.000	0.726	1.147	0.154	0.096	0.105	36.68
Pop4	12.000	1.082	1.224	0.224	0.142	0.148	52.62

N = number of studied plants, Na = number of different alleles, Ne = number of effective alleles, I = Shannon's information index, He = expected heterozygosity, uHe = unbiased gene diversity, and P% = percentage of polymorphic loci

populations showed that genetic similarity among populations ranged from 1.063 to 1.244. Populations 2 and 3 show the most genetic similarity (Table 6).

Genetic differentiation

AMOVA proved that studied populations differ markedly in their genetic content (P = 0.001). This analysis identified that 46% of genetic variation occurred within populations, and 54% arose between populations. These results displayed a great degree of genetic variability among *Haloxylon aphyllum* populations. As well as paired-sample AMOVA indicated remarkable differences between the mentioned populations (Table 7).

STRUCTURE analysis (Fig. 4) represented the genetic structure of the populations above. The studied populations included some specific genetic

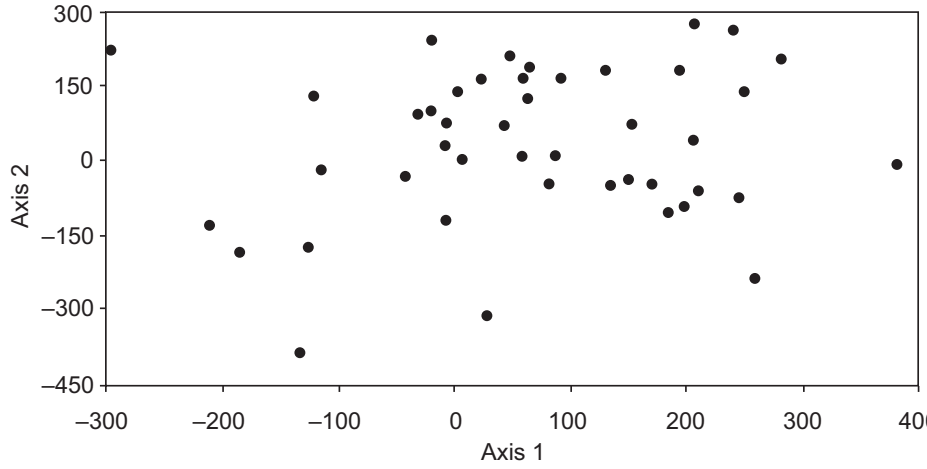

Fig. 3. DCA plot of ISSR loci in *Haloxylon aphyllum*

Table 6
Nei's genetic identity (above diagonal) and genetic distance (below diagonal) (populations numbers matching to Figure 1)

Pop ID	1	2	3	4
1	***	0.7121	0.7482	0.7129
2	0.6876	***	0.8771	0.8233
3	0.6621	0.7681	***	0.8595
4	0.3311	0.2267	0.1551	***

content and allele combinations (different colour segments). This analysis showed a low level of gene flow and common ancestral alleles (same colour segments). These populations contained some specific genetic content and allele combinations (differently coloured segments). This analysis indicated a slight degree of gene flow and ancestral common shared alleles in *H. aphyllum* populations (similarly coloured segments). STRUCTURE analysis displayed comprehensive information on the genetic structure of the studied *H. aphyllum* populations (Fig. 4). The populations S and Y show similarly coloured segments, so they are genetically similar due to a great level of ancestral alleles. Populations K and C differed in colour segments and contained distinct allele combinations.

Genetic grouping and population affinity

UPGMA clustering of *Haloxylon aphyllum* trees by ISSR data nearly placed trees of each population in distinct clusters (Fig. 6). In a few exceptions, individuals with common alleles were mixed together. This result shows that ISSR

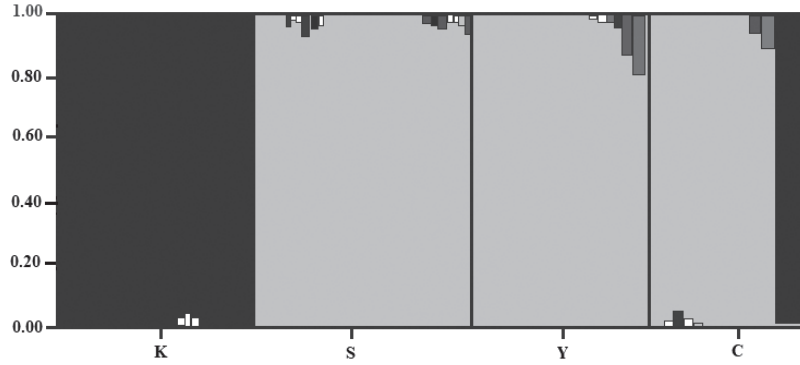

Fig. 4. STRUCTURE plot of the studied *Haloxylon aphyllum* based on ISSR molecular data (populations 1–4 match Table 1)

Table 7
Pair-wise AMOVA between *Haloxylon aphyllum* populations (PhiPT Values below diagonal. Probability values based on 99 permutations are shown above diagonal) (populations 1–4 are presented in Figure 1)

AMOVA	Pop 1	Pop 2	Pop 3	Pop 4
Pop1	0.000	0.010	0.020	0.010
Pop2	0.434	0.000	0.010	0.010
Pop3	0.586	0.230	0.000	0.010
Pop4	0.843	0.572	0.324	0.000

molecular markers are a powerful molecular tool to differentiate *H. aphyllum* populations.

The PcoA plot determined the genetic affinity of *H. aphyllum* populations after 1,000 times permutation (Fig. 5). So the populations are placed in two major groups. The first group includes the populations of Yazd and Semnan. The populations of Sistan and Baluchestan, and Kerman form the second major group.

After 10,000 permutations, the Mantel test revealed a considerable correlation (P =< 0.01) between geographical and genetic distance in the populations. Thus, as the populations deviate from each other, they become more divergent in genetic characteristics.

Neighbour-Net network revealed between and within-population genetic variability in *H. aphyllum* (Fig. 7). The length of edges and sidebars indicate genetic differences of the studied *H. aphyllum* trees.

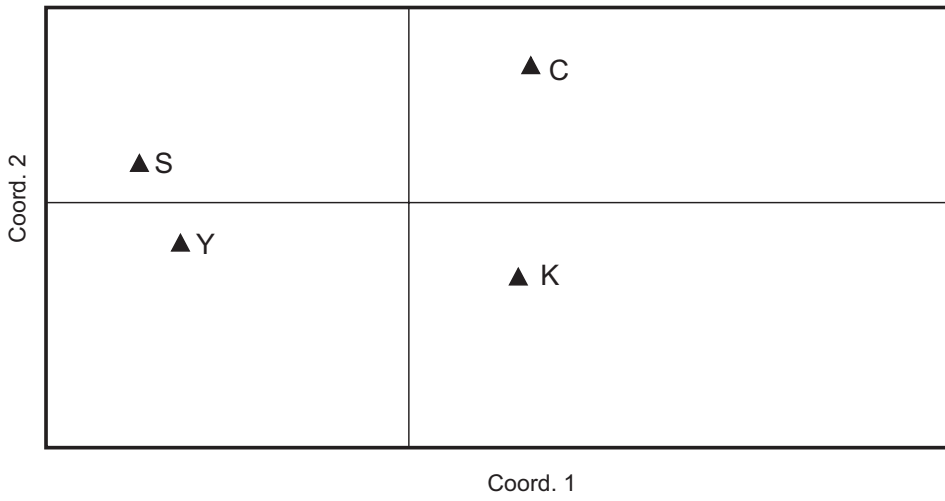

Fig. 5. PCoA plot after 1,000 times permutation

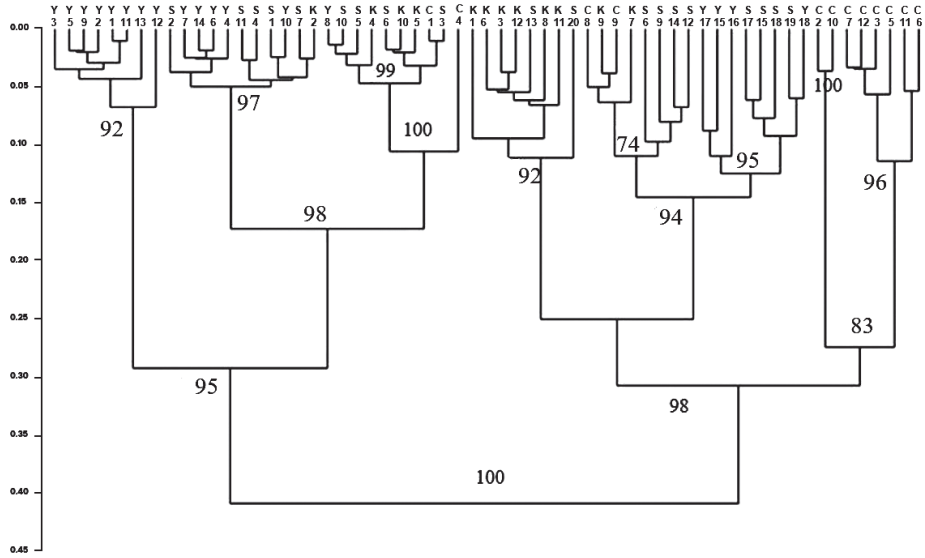

Fig. 6. UPGMA clustering of the studied populations based on ISSR data

DISCUSSION

In terms of genetic and breeding studies, *Haloxylon aphyllum* is of great importance. This tree species grows in different geographical regions of Iran, so genetic studies need to be carried out on its populations. Using popula-

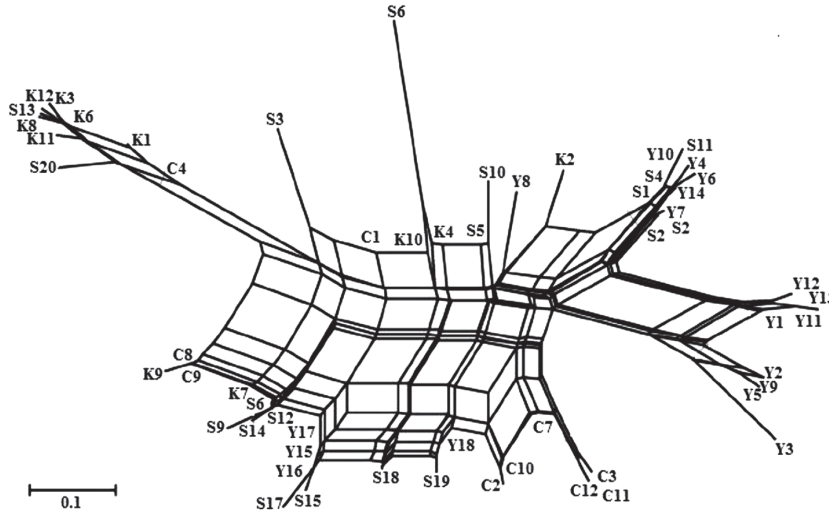

Fig. 7. Neighbour-Net of the studied populations based on ISSR data. (Populations matching Table 1)

tion genetics, we can better understand the genetic structure, classification vs. gene flow, and genetic divergence of populations, which can be used in future conservation and remediation programs (Freeland *et al.* 2011).

In the present study, ISSR molecular markers and diverse bioinformatics approaches were used to demonstrate that *H. aphyllum* is a genetically diverse species within a population and among populations, allowing the plant to adapt to changes in its environmental conditions. Thus, diversity is crucial to a species' survival (Çalişkan 2012, Sheidai *et al.* 2013, 2014).

Because *H. aphyllum* is a species that forms several geographic populations, this is especially important. In a plant population, genetic diversity is defined as the existence of different alleles of a gene. These alleles are often present with different frequencies in different communities and, as a result, genetic diversity in a plant species can be observed both within a population and between several populations of that species. Evaluating the genetic diversity and kinship of different plant populations is one of the first steps that must be taken in correctly implementing breeding programs. Sufficient information on the genetic diversity of plant populations is also essential for a successful breeding program, and molecular markers are one of the best available tools to achieve these goals. In the observation section, variation among *H. aphyllum* populations was observed. Overall, the results confirmed that the molecular data used have a differential value. Genetic diversity is essential in the continuation of plant species because it is used to create obligatory adaptations to deal with environmental changes. Populations with high genetic diversity have a higher chance of survival compared to populations with lower genetic diversity (Sheidai *et al.* 2012, 2013, 2014).

Our study revealed a high level of inter-population genetic diversity (54%) in *H. aphyllum*, which is important for understanding the microevolutionary processes of the species. The AMOVA test showed a significant genetic difference among the studied populations ($P = 0.001$), indicating that the populations are genetically distinct. Given that *H. aphyllum* is wind-pollinated and has a wide distribution and range of traits in a large part of the ancient world, it is expected to have a high inter-population genetic flow to maintain its intra-species continuity and species identity.

When the level of gene flow decreases, genetic drift can create a high level of genetic homogeneity in the population, which can lead to adaptation to the habitat (Hou and Lou 2011). Many plant species grow in specific habitats and have developed adaptive strategies appropriate to their habitat (Schneller and Liebst 2007). Our results suggest that the dispersion of *H. aphyllum* populations may be limited by distance, and gene flow is primarily formed between neighbouring populations. As a result, closer populations tend to be more genetically similar to each other (Medrano and Herrera 2008, Slatkin 1993).

Currently, limited scientific information is available on the genetic diversity of different populations of *H. aphyllum* using molecular markers. Similarly, the genetics and breeding of species such as *H. aphyllum* are not well-studied. In agreement with our results, Shuyskaya *et al.* (2012) investigated the genetic differentiation of black saxaul and the effect of soil salinity in the Kyzylkum desert of Uzbekistan, during which they found that *H. aphyllum* populations in this region have low heterozygosity and a moderate level of genetic diversity. Moreover, soils with medium salinity levels appear to be optimal for the growth of black saxaul, as subpopulations growing on such soils are the main source of ecological flexibility for the entire population.

The presence of genetic diversity and heterozygosity among *H. aphyllum* populations suggests local adaptation among populations due to the heterogeneity of environmental factors, such as soil moisture and nutrients, which can result in genetic heterogeneity. Pollination barriers can also hinder gene flow and contribute to genetic differentiation among subpopulations. However, due to the small distance between *H. aphyllum* subpopulations, the lack of obstacles for pollination, and the dispersal of winged seeds by wind, genetic differentiation is unlikely due to geographic isolation.

CONCLUSIONS

The present study is a molecular study that examined the ISSR data of the studied *Haloxylon aphyllum* populations. Our results showed a high inter-population genetic variability in *H. aphyllum*, which is significant for assessing the species' genetic diversity. The UPGMA dendrogram based on the molecular data provided valuable information on the relationships among the studied populations.

Multilocus molecular markers, including ISSR, SSR, AFLP, RAPD, etc., do not act as adaptive genes in population genetic studies and are considered neutral molecular markers. However, they are useful in assessing the genetic diversity and relationships among populations. Morphological data will also be needed to understand the relationships among *H. aphyllum* populations better.

*

Acknowledgement – The authors would like to thank Qom Agricultural and Natural Resources Research Centre.

REFERENCES

Al Salameen, F., Habibi, N., Kumar, V., Al Amad, S., Dashti, J., Talebi, L. and Al Doaij, B. (2018): Genetic diversity and population structure of Haloxylon californium moq. in Kuwait by ISSR markers. – *PLoS ONE* **13**(11): e0207369. https://doi.org/10.1371/journal.pone.0207369

Altukhov, Yu. P. (2003): *Geneticheskie protsessy v populyatsiyakh*. (Genetic processes in populations). – Aka demkniga, Moscow.

Amini, E., Kazempour-Osaloo, Sh., Maassoumi, A. A. and Zare-Maivan, H. (2018): Phylogeny, biogeography, and divergence times of Astragalus section Incani DC. (Fabaceae) inferred from nrDNA ITS and plastid rpl32-trnL$_{(UAG)}$ sequences. – *Nordic J. Bot.* **37**(2): 1–20. https://doi.org/10.1111/njb.02059

APG IV (2016) An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV. – *Bot. J. Linn. Soc.* **181**: 1–20. https://doi.org/10.1111/boj.12385

Boc, A., Diallo, A. B. and Makarenkov, V. (2012): T-REX: a web server for inferring, validating and visualizing phylogenetic trees and networks. – *Nucleic Acid Res.* **40**: W573–W579. https://doi.org/10.1093/nar/gks485

Çalişkan, M. (2012): *Genetic diversity in plants*. – Intechopen, London.

Cullings, K. W. (1992): Design and testing of a plant-specific PCR primer for ecological and evolutionary studies. – *Mol. Ecol.* **1**(3): 233–240. https://doi.org/10.1111/j.1365-294x.1992.tb00182.x

Doyle, J. J. and Dickson, E. E. (1987): Preservation of plant samples for DNA restriction endonuclease analysis. – *Taxon* **36**(4): 715–722. https://doi.org/10.2307/1221122

Doyle, J. J. and Doyle, J. L. (1987): A rapid DNA isolation procedure for small quantities of fresh leaf tissue. – *Phytochem. Bull.* **19**: 11–15.

Earl, D. A. and Von Holdt, B. M. (2012): STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. – *Conserv. Gen. Res.* **4**: 359–361. https://doi.org/10.1007/s12686-011-9548-7

Freeland, J. R., Kirk, H. and Peterson, S. D. (2011): *Molecular ecology*. 2nd ed. – Wiley-Blackwell, UK, 449 pp.

Gintzburger, G., Toderich, K. N., Mardonov, B. K. and Makhmudov, M. M. (2003): *Rangelands of the arid and semi arid zones in Uzbekistan*. – CIRAD, Montpellier.

Hammer, Q., Harper, D. A. T. and Ryan, P. D. (2012): PAST: Paleontological Statistics software package for education and data analysis. – *Pal. Electron.* **4**: 9.

Hou, Y. and Lou, A. (2011): Population genetic diversity and structure of a naturally isolated plant species, Rhodiola dumulosa (Crassulaceae). – *PLoS One* **6**(9): e24497. https://doi.org/10.1371/journal.pone.0024497

Iljin, M. M. (1936): *Chenopodiaceae Less.* – In: Komarov, V. L. and Shishkin, B. K. (eds): Flora of the USSR. Vol. VI. Centrospermae. Israel Program for Scientific Translation, Jerusalem. (English translation by N. Landau, 1970), pp. 4–272.

Jafari, M., Niknahad, H. and Erfanzadeh, R. (2003): Effect of Haloxylon plantation on some soil characteristics and vegetation cover (Case study: Hossein Abade-E-Mishmast region of Qom province). – *J. Desert* (Biaban) **8**(1): 152–162.

Medrano, M. and Herrera, C. M. (2008): Geographical structuring of genetic diversity across the whole distribution range of Narcissus longispathus, a habitat-specialist, Mediterranean narrow endemic. – *Ann. Bot.* **102**: 183–194. https://doi.org/10.1093/aob/mcn086

Minaeifar, A., Sheidai, M. and Attar, F. (2015): Genetic and morphological diversity in Cousinia cylindracea (Asteraceae) populations: identification of gene pools. – *Biodiversitas* **16**: 288–294. https://doi.org/10.13057/biodiv/d160225

Mitton, J. B., Grant, M. C. and Yoshino, A. M. (1998): Variation in allozymes and stomatal size in pinyon (Pinus edulis, Pinaceae), associated with soil moisture. – *Amer. J. Bot.* **85**: 1262–1265. https://doi.org/10.2307/2446636

Netchaeva, N. T., Vasilevskaya, V. K. and Antonova, K. G. (1973): *Life forms of plants of Karakum Desert*. – Nauka, Moscow, 241 pp. [In Russian]

Nevo, E., Krugman, T. and Beiles, A. (1994): Edaphic natural selection of allozyme polymorphisms in Aegilops peregrine at a Galilee Microsite in Israel. – *Heredity* **72**: 109–112. https://doi.org/10.1038/hdy.1994.16

Nasrollahi, F., Kazempour-Osaloo, Sh., Saadati, N., Mozaffarian, V. and Zare-Maivan, H. (2019): Molecular phylogeny and divergence times of Onosma (Boraginaceae s.s.) based on nrDNA ITS and plastid rpl32-trnL$_{(UAG)}$ and trnH–psbA sequences. – *Nordic J. Bot.* **37**(1): 1–12. https://doi.org/10.1111/njb.02060

Nikitin, S. A. (1966): *Drevesnaya i kustarnikovaya rastitel'nost' pustyn' SSSR*. (Tree and shrub vegetation in deserts of the Soviet Union). – Nauka, Moscow.

Prentice, H. C., Lonn, M., Lager, H., Rosen, E. and van der Maarel, E. (2000): Changes in allozyme frequencies in Festuca ovina populations after a 9-year nutrient/water experiment. – *J. Ecol.* **88**: 331–347. https://doi.org/10.1046/j.1365-2745.2000.00454.x

Peakall, R. and Smouse, P. E. (2006): GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. – *Mol. Ecol. Notes* **6**: 288–295. https://doi.org/10.1111/j.1471-8286.2005.01155.x

Perrier, X. and Jacquemoud-Collet, J. P. (2006): *DARwin Software*. – http://darwin.cirad.fr/darwin.

Podani, J. (2000): *Introduction to the exploration of multivariate data*. – Backhuys, Leiden, Netherlands, 455 pp.

Pritchard, J. K., Stephens, M. and Donnelly, P. (2000): Inference of population structure using multilocus genotype data. – *Genetics* **155**: 945–959. https://doi.org/10.1093/genetics/155.2.945

Shuyskaya, E. V., Gismatullina, L. G., Toderich, K. H., Voronin, P. Y. and Soldatova, N. V. (2012): Genetic differentiation of black saxaul, Haloxylon aphyllum (Chenopodiaceae), along a soil salinity gradient in the Kyzylkum Desert. – *Russ. J. Ecol.* **43**(4): 302–306. https://doi.org/10.1134/s1067413612040157

Sheidai, M., Seif, E., Nouroozi, M. and Noormohammadi, Z. (2012): Cytogenetic and molecular diversity of Cirsium arvense (Asteraceae) populations in Iran. – *J. Jap. Bot.* **87**: 193–205.

Sheidai, M., Zanganeh, S., Haji-Ramezanali, R., Nouroozi, M., Noormohammadi, Z. and Ghsemzadeh-Baraki, S. (2013): Genetic diversity and population structure in four Cirsium (Asteraceae) species. – *Biologia* **68**: 384–397. https://doi.org/10.2478/s11756-013-0162-x

Sheidai, M., Ziaee, S., Farahani, F., Talebi, S. M., Noormohammadi, Z. and Hasheminejad-Ahangarani Farahani, Y. (2014): Infra-specific genetic and morphological diversity in Linum album (Linaceae). – *Biologia* **69**: 32–39. https://doi.org/10.2478/s11756-013-0281-4

Schneller, J. and Liebst, B. (2007): Patterns of variation of a common fern (Athyrium filix-femina; Woodsiaceae). population structure along and between altitudinal gradients. – *Amer. J. Bot.* **94**(6): 965–971. https://doi.org/10.3732/ajb.94.6.965

Slatkin, M. (1993): Isolation by distance in equilibrium and non-equilibrium populations. – *Evolution* **47**: 264–279. https://doi.org/10.2307/2410134

Tahmasebi, A. and Nasrollahi, F. (2021): Morphologic and genetic study of Halocnemum strobilaceum in rangeland ecosystems of Golestan province (North of Iran). – *Rostaniha* **22**(1): 134–146.

Vrijenhoek, R. C. (1994): Unisexual fish: model systems for studying. – *Annu. Rev. Ecol. Evol. Syst.* **25**: 71–96. https://doi.org/10.1146/annurev.es.25.110194.000443

Weising, K., Nybom, H., Wolff, K. and Kahl, G. (2005): *DNA fingerprinting in plants: principles, methods, and applications*. 2nd ed. – CRC Press, Boca Rayton, Fl, USA, 472 pp.

Xu, G. Q., Yu, D. D., Xie, J. B., Tang, L. S. and Li, Y. (2014): What makes Haloxylon persicum grow on sand dunes while H. ammodendron grows on interdune lowlands: a proof from reciprocal transplant experiments. – *J. Arid Land* **6**(5): 581–591. https://doi.org/10.1007/s40333-014-0029-1