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Research article

ISSR-based genetic diversity assessment of five populations of *Juniperus polycarpos* K. Koch in southern habitats of Iran

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Abstract

Juniperus polycarpos K. Koch is a durable conifer species with different growth habit that grow in ecological landscapes. In this study, genetic diversity was investigated for five populations of juniper that grew in Fasa, Sepidan, Khabr, Rabor, and Genow habitat in the south of Iran. For DNA extraction, leaf samples of 10 diverse accessions of each habitat were collected (totally 50 accessions). Populations genetic diversity was evaluated based on 12 inter simple sequence repeat (ISSR) markers. Assessment of ISSR markers predicted 75 loci with 67 (89.33%) polymorphic loci, polymorphic information content (PIC) 0.45, resolving power (Rp) 3.78, and effective multiplex ratio (EMR) and marker index (MI) of primers were 5.50 and 2.47, respectively. Populations Nei's genetic diversity showed higher intra-population genetic diversity (0.25) than inter-population genetic diversity (0.13). Results indicated the highest different loci (Na) for Sepidan population (56) and the highest effective alleles (Ne) were obtained in two populations of Sepidan (1.49) and Genow (1.49). Furthermore, the highest gene diversity (H) observed in the population of Sepidan (0.27) and Genow (0.27). The population of Sepidan has the highest Shannon's information index (I) (0.41) and percentage of polymorphic loci (P) (74.67). Investigated populations showed high total genetic diversity (Ht) (0.38), intra-population genetic diversity (Hs) (0.25), and inter-population genetic diversity (Dst) (0.13), and moderate genetic differentiation among populations (Gst = 0.34). High gene flow (Nm) was obtained between evaluated populations (0.94). Moreover, AMOVA analysis indicated 70% within and 30% among populations genetic variation.
Keywords: Accession, Conifer, Juniper, Molecular markers, Variation.

Introduction

Juniperus polycarpos is an important evergreen genus of the Cupressaceae and this genus is the second most diverse coniferous plants. Depend to taxonomic standpoint, the number of juniper species is in dispute, and so far different numbers have been reported, from 52 (Farjon, 2001), 54 (Ciesla, 2002), and about 68–80 species with 27-28 varieties (Adams, 2008, 2014). There are more variation between juniper species in their color of bark and seed cones, bark thickness, needle-like or scale-like leaves, strobili and strobilus (Adams, 2004, 2008; Farjon, 2005; Mao *et al*., 2010). Geographical distribution, population biodiversity and reproductivity of native juniper forests are influenced by human pressure and activities such as excessive grazing, wood collection for building and fuelwood, wildfire, and expansion of agricultural and urban zones for durable time (Bhattarai *et al*., 2007). Furthermore, the propagation of junipers

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is dependent on sexual reproduction and their propagation takes place via seeds dispersing by wind and gravity or water, normally (Farjon, 2005). Long periods of water shortage as a natural disaster has caused disruption in the reproductive cycle of juniper (Shahabfar and Eitzinger, 2013). Old populations of *J. excelsa* subsp. polycarpos (Persian juniper) that grow in the south of Iran (Miller and Cope, 1996), have been confirmed recently as *J. seravschanica* and *J. polycarpos,* and the Persian juniper (*J. polycarpos*) has widespread distribution from Kazakhstan, Turkmenistan, Azerbaijan to Iran (Adams *et al*., 2014; Hojjati *et al*., 2018).

Genetic diversity is an important factor that allows adaptation of plant species against various environmental situations such as instability in the environment and soil conditions. Moreover, everything of species genetic variability is a useful tool used to recognize the difference within and among the populations genetically and help to maintain the variation to improve population monitoring and conservation planning (Govindaraj *et al*., 2015). The changes of climate, especially drought extension and global warming affects natural habitat alterations and consequently threats natural biodiversity and inhabitants (Williams *et al*., 2007; Füssel, 2010). ISSR markers as a useful approach were applied to evaluate associations among physico-chemical and functional indices and diversity in various plant species of angiosperms and gymnosperms such as *Crocus sativus* L. (Mir *et al*., 2021), *Ziziphus jujuba* Mill. cultivars (Reche *et al*., 2019), *Dendrobium moschatum* SW. (Tikendra *et al*., 2019), *Oryza sativa* L. (Al-Turki and Basahi, 2015), *Pinus squamata* L. (Zhang *et al*., 2005), *Amentotaxus argotaenia* (Hance) Pilger complex (Ge *et al*., 2005), *Pseudotaxus chienii* W.C. Cheng (Su *et al*., 2009). Assessment of species genetic diversity is a beneficial tool that is necessary to recognize the elite genotypes, selection, and conservation of endemic plants in the natural landscapes.

Materials and Methods

Fresh leaf tissues were collected from 50 samples of *Juniperus* spp. from 5 natural populations in Sepidan and Fasa in Fars and Khabr and Rabor in Kerman, and Genow in Hormozgan provinces in the south and southeast of Iran. Ten samples were randomly collected from each of these natural populations, considering 500-1000 m distance between them. Samples were transferred to the laboratory of Tissue Culture and Biotechnology, Department of Horticultural Science, Shiraz University in liquid nitrogen and stored at -80°C inside a freezer (ARCTIKO, Denmark) until used. Modified cetyltrimethyl ammonium bromide (CTAB) method was used for DNA extraction (Lodhi *et al.,* 1994).

The PCR was set up by 24 µl reactions contained 1x PCR buffer, including: VWR Red Tag DNA Polymerase Master Mix, 1.5 mm $MgCl_2$, 0.5 µM of each primer (Table 1), DNA template, and PCR-grade H_2O. PCR cycling was performed in a BIO-RAD MyCycler™ Thermal Cycler (Applied Biosystems, USA) programmed as follows: 94°C/4 min initial denaturation, 35 cycles of 94°C/30 s, primer annealing temperature (Table 1) 52-55. 9°C/45 s and 72°C/10 min and final cycle of 4°C/∞.

Agarose gels were prepared using TBE buffer, the microwave oven was used to prepare 1.5% agarose gel. Agarose gel was stained adding 12 µl SMOBIO nucleic acid gel stain '10,000x' (NS1000 fluorovue™) for 250 ml of warm agarose solution. Prepared gels were located in the electrophoresis gel box (25×15). Enough running buffer (TBE buffer) poured in the electrophoresis buffer tank to cover the surface of the gel completely. DNA samples (15µl of PCR products) slowly and carefully loaded into the gel. DNA size marker (Thermo Scientific,

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Gene Ruler) was loaded to predict the fragment size. The gel ran for 120 min till the dye was migrated to an appropriate distance.

Table 1. List of twelve ISSR primers were used to predict the genetic diversity of *Juniperus* spp.

Primer	Sequence 5´–3´	%GC	Annealing temperature (°C)
UBC807	AGAGAGAGAGAGAGAGT	47.06	52.0
UBC808	AGAGAGAGAGAGAGAGC	52.94	54.4
UBC810	GAGAGAGAGAGAGAGAT	47.06	52.0
UBC811	GAGAGAGAGAGAGAGAC	52.94	54.4
UBC812	GAGAGAGAGAGAGAGAA	47.06	52.0
UBC813	CTCTCTCTCTCTCTCTT	47.06	52.0
UBC815	CTCTCTCTCTCTCTCTG	52.94	54.4
UBC818	CACACACACACACACAG	52.94	54.4
UBC823	TCTCTCTCTCTCTCTCC	52.94	54.4
UBC824	TCTCTCTCTCTCTCTCG	52.94	54.4
UBC825	ACACACACACACACACT	47.06	52.0
UBC835	AGAGAGAGAGAGAGAGY*C	50.0	55.9

*Y=C/T

After accomplishment of electrophoresis, gels were imaged using Gel Logic 212 Pro (Carestream). DNA bands were showing up as fluorescent bands (Fig. 1). DNA fragments that amplified by a specific primer were considered as a trait and the ISSR fragments were scored according to their presence (1) or absence (0).

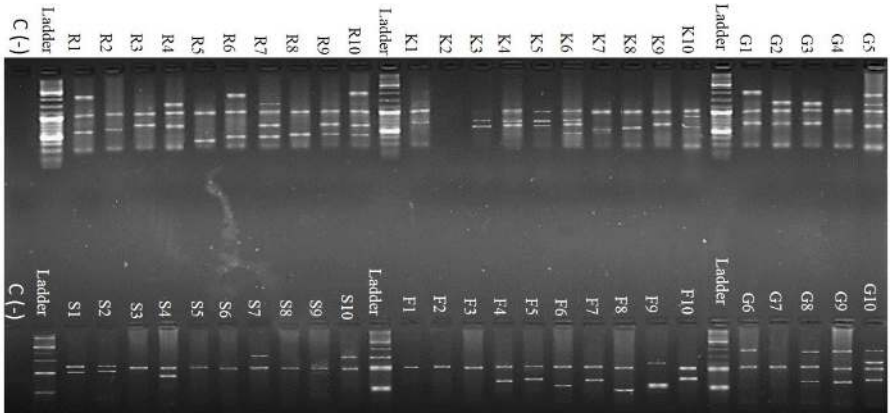

Figure 1. PCR amplification of DNA from different accessions of *Juniperus* spp. Negative control contains no template DNA (C); 100-1000 bp of DNA size marker (Ladder); Rabor accessions (R); Khabr accessions (K); Sepidan accessions (S); Fasa accessions (F); Genow accession (G).

Measured indices for each ISSR marker included: Total loci (TL), Polymorphic loci (PL), Fraction of Polymorphism (FP), Polymorphic Information Content (PIC), ISSR Primer Index (ISPI), Resolving power (Rp), Mean Rp, Multiplex Ratio (MR), Effective Multiplex Ratio (EMR), and Marker Index (MI) (Anderson *et al*., 1993; Powell *et al*., 1996; Ghislain *et al*., 1999; Prevost and Wilkinson, 1999; Varshney *et al*., 2007; Rajwade *et al*., 2010; Sharma *et al*., 2016).

Measured indices for each population included: Number of different loci (Na), Percentage of different loci (percentage of Na), Number of effective alleles (Ne), Nei's gene diversity (H), Shannon's information index (I), Percentage of polymorphic loci (PPL), Total genetic diversity

(Ht), Intra-population genetic diversity (Hs), Inter-population genetic diversity (DST), Estimated genetic differentiation among populations (Gst), and Estimated gene flow (Nm) were assessed with POPGENE and GenAlEx using Nei's diversity statistics (1987). Analysis of Molecular Variance (AMOVA) was also used to obtain the variation component within and among populations. A genetic distance of evaluating populations assessed with POPGENE and NTSYS using Euclidian distance, and genetic similarity of different accessions was predicted with Jaccard's similarity (J) coefficient using a SIMQUAL program of NTSYSpc v. 2.20 (Rohlf, 2009). The J similarity coefficients of different accessions were used to construct UPGMA dendrograms using SAHN program of NTSYSpc v. 2.20 (Rohlf, 2009).

Results

Twelve ISSR marker amplifications produced 75 total loci (TL) with size of 100–1000 bp for 50 accessions of *J. polycarpos* that 67 (89.33%) loci were shown polymorphic (PL), there were differences in detected total and polymorphic loci by different amplified ISSR primers (Table 2). Furthermore, the results of this study showed a logical ability of ISSR markers in the prediction of polymorphic loci of different populations of *J. polycarpos*. Investigation of other indices of ISSR markers showed the fraction of polymorphism (FP) of evaluated primers considered in range of the lowest 0.60 to the highest 1, mean indicated FP was 0.88 (Table 2). Polymorphic information content (PIC) of primers assayed and UBC812 and UBC835 had the highest PIC (0.50) while UBC825 had the lowest PIC (0.38), mean PIC for all primers was 0.45.

Results of ISSR primer index (ISPI) are shown in Table 2, UBC807 and UBC810 showed the highest and lowest ISPI, respectively. Mean suggested ISPI in this study is 2.38 (Table 2). The high resolving power (RP) was exhibited by UBC807 (5.72) and the lowest was belonged to UBC810 (2.16), and total mean RP was 3.78. Moreover, mean RP evaluated for each loci of primers and results showed that UBC808 had the highest mean RP (0.76) for each loci and the lowest mean of each loci was belonged to UBC810 (0.43) (Table 2). Therefore, the multiplex ratio (MR), effective multiplex ratio (EMR), and marker index (MI) were measured and amount of each is presented in Table 2.

Table 2. The indices of ISSR primers were used to assess the genetic diversity of *Juniperus* spp.

ISSR primers	Sequence 5´–3´	TL	PL	FP	PIC	ISPI	RP	Mean RP
UBC807	$(AG)_8T$	8	8	1	0.49	3.57	5.72	0.71
UBC808	$(AG)_8C$	6	6	1	0.48	2.74	4.56	0.76
UBC810	$(GA)_8T$	5	3	0.60	0.48	1.51	2.16	0.43
UBC811	$(GA)_8C$	6	5	0.83	0.43	2.24	3.80	0.63
UBC812	$(GA)_8A$	5	5	1	0.50	2.21	3.68	0.73
UBC813	$(CT)_8T$	5	4	0.8	0.44	1.88	3.16	0.63
UBC815	$(CT)_8G$	7	6	0.85	0.49	2.69	4.04	0.57
UBC818	$(CA)_8G$	7	5	0.71	0.42	2.59	4.32	0.61
UBC823	$(TC)_8C$	8	7	0.87	0.42	2.28	3.66	0.45
UBC824	$(TC)_8G$	7	7	1	0.47	2.78	4.36	0.62
UBC825	$(AC)_8T$	5	5	1	0.38	1.73	2.48	0.49
UBC835	$(AG)_8Y^*C$	6	6	1	0.50	2.39	3.44	0.57
Range/Sum/Mean		75	67	0.88	0.45	2.38	3.78	0.60

MR = TB/TP = 6.25
EMR = MR × Mean FP = 5.50
MI = EMR × Mean PIC = 2.47

TL: Total Loci, PL: Polymorphic Loci, FP: Fraction of Polymorphism, PIC: Polymorphic Information Content, ISPI: ISSR Primer Index, RP: Resolving Power, Mean RP; MR: Multiplex Ratio, TP: Total Primer, EMR: Effective Multiplex Ratio, MI: Marker Index.

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Nei's analysis of genetic diversity were estimated for five *J. polycarpos* populations of Fasa, Sepidan, Khabr, Rabor, and Genow using an ISSR marker approach (Table 3). Results showed the highest polymorphic loci (Na) in Sepidan population (56), and two populations of Sepidan and Genow had the highest effective alleles (Ne). While, the lowest Na and Ne were belonged to Khabr population (Na = 45; Ne = 1.35). Furthermore, the highest gene diversity (H) was observed in populations of Sepidan (0.27) and Genow (0.27) followed by populations of Fasa, Rabor and Khabr, respectively (Table 3). Evaluation of Shannon's information index (I) and percentage of polymorphic loci (PPL) showed the highest I (0.41) and PPL (74.67) in Sepidan population followed by populations of Genow, Fasa, Rabor, and Khabr (Table 3).
Results of Nei's analysis of genetic diversity in subdivided populations showed a high total genetic diversity (Ht) (0.38), intra-population genetic diversity (Hs) (0.25), and inter-population genetic diversity (DST) (0.13). Moreover, these results estimated the moderate genetic differentiation among populations (Gst) (0.34), and evaluated population showed a high estimated gene flow (Nm) (0.94) (Table 3).

Table 3. Genetic variation assessment of *J. polycarpos* populations.

Population	No. of accessions	Na	Ne	H	I	PPL (%)
Fasa	10	52	1.44	0.25	0.38	69.33
Sepidan	10	56	1.49	0.27	0.41	74.67
Khabr	10	45	1.35	0.21	0.31	60.00
Rabor	10	48	1.39	0.22	0.34	64.00
Genow	10	53	1.49	0.27	0.40	70.67
Total	50	Ht = 0.38	Hs = 0.25	DST = 0.13	Gst = 0.34	Nm = 0.94

Na: No. of polymorphic loci, Ne: No. of effective alleles, H: Nei's gene diversity, I: Shannon's information index, PPL: Percentage of polymorphic loci, Ht: Total genetic diversity, Hs: Intra-population genetic diversity, DST: Inter-population genetic diversity, Gst: Estimated genetic differentiation among populations, Nm: Estimated gene flow.

Molecular variance analysis (AMOVA) was performed for 50 accessions of 5 populations and results showed the significant variance of accessions and population variation with PhiPT ($P > 0.001$) (Table 4). Five evaluated populations had significant differences within and among population variations in regard to ISSR markers. Estimated variance from within population variation was 11.60, and variance of among population variation was 4.98 (Table 4). Furthermore, 70% of total predicted variations were belonged to within population variations and 30% were belonged to among population variations (Table 4).

Table 4. Results of Analysis of Molecular Variance (AMOVA).

Source	Def	SS	MS	Est. Var.	%	PhiPT*	
						Value	P (Rand >=data)
Among Pops	4	245.80	61.45	4.98	30		
Within Pops	45	522.20	11.60	11.60	70		
Total	49	768.00		16.58	100	0.30	0.001

*Probability, P (Rand ≥ data), for PhiPT is based on the standard permutation across the full data set.

Nei's genetic distance (D) between five populations was assessed using ISSR markers (Table 5). Results showed a difference in D between 5 assessed populations with a range of 0.10 to 0.33. The lowest D was belonged to the populations of Khabr and Rabor, and the highest D was belonged to populations of Sepidan and Khabr. Furthermore, isolated populations of

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Genow had a roughly similar D (0.24 to 0.26) with other investigated populations from Sepidan, Fasa, Khabr, and Rabor (Table 5). Moreover, Nei's genetic identity (I) estimated the variation of similar proportion of genes in five investigated populations, and its maximum and minimum sum was between populations of Khabr and Rabor (0.89), and Sepidan and Khabr (0.71), respectively (Table 6).

Table 5. Nei's Genetic Distance (D).

Example of binary data	Fasa	Sepidan	Khabr	Rabor	Genow
False	0.000				
Sepidan	0.20	0.000			
Khabr	0.30	0.33	0.000		
Rabor	0.26	0.26	0.10	0.000	
Genow	0.26	0.24	0.25	0.26	0.000

Table 6. Nei's Genetic Identity (I).

Example of binary data	Fasa	Sepidan	Khabr	Rabor	Genow
False	1.000				
Sepidan	0.81	1.000			
Khabr	0.74	0.71	1.000		
Rabor	0.76	0.76	0.89	1.000	
Genow	0.76	0.78	0.77	0.76	1.000

Comparison of population's variation was performed via genetic distance matrices obtained from ISSR marker systems; and similarity dendrogram was prepared by POPGENE software according to D with the UPGMA method based broad clusters. Major differentiation was genetically exhibited between the five evaluated populations of junipers (Figure 1). According to UPGMA of D, all populations were clustered in three major classes: group (1) the populations of Khabr and Rabor which originally their habitat is located in Kerman province, group (2) as the populations of Fasa and Sepidan habitats which their origin is in Fars province, and group (3) as the population of Genow habitat of Hormozgan province (Figure 1).

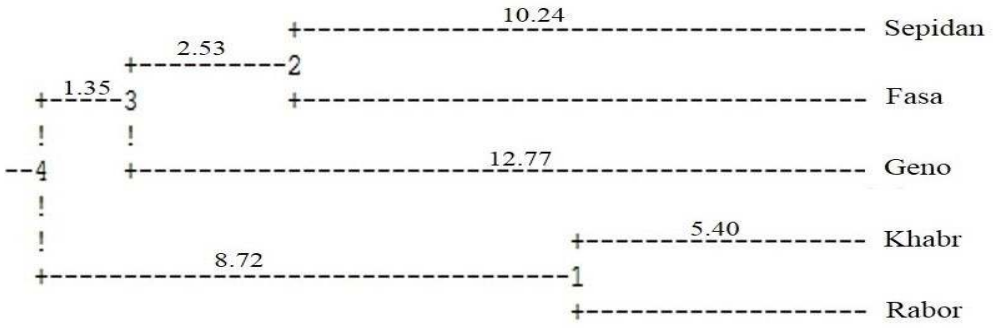

Figure 2. Population variance based on Nei's genetic distance (D): Method = UPGMA modified from the NEIGHBOR procedure of PHYLIP Version 3.5.

The principal component analysis (PCA) was calculated using NTSYSpc to evaluate variations of 50 accessions. PC-I had 23.31 variance (Eigen value) as 46.63% of total variation followed

by PC-II variance 2.41 as 4.5% of total variation, PC-III variance 2.38 as 4.77% of total variation, and PC-IV variance 1.85 as 3.71% of the total variation. Cumulatively, first four components showed 59.94% of the total variation in the data. PC-I had a significantly positive correlation ($P < 0.001$) with other 50 accessions. Detected variation of all accessions of five populations is shown in Plot of PCA analysis (Figure 2).

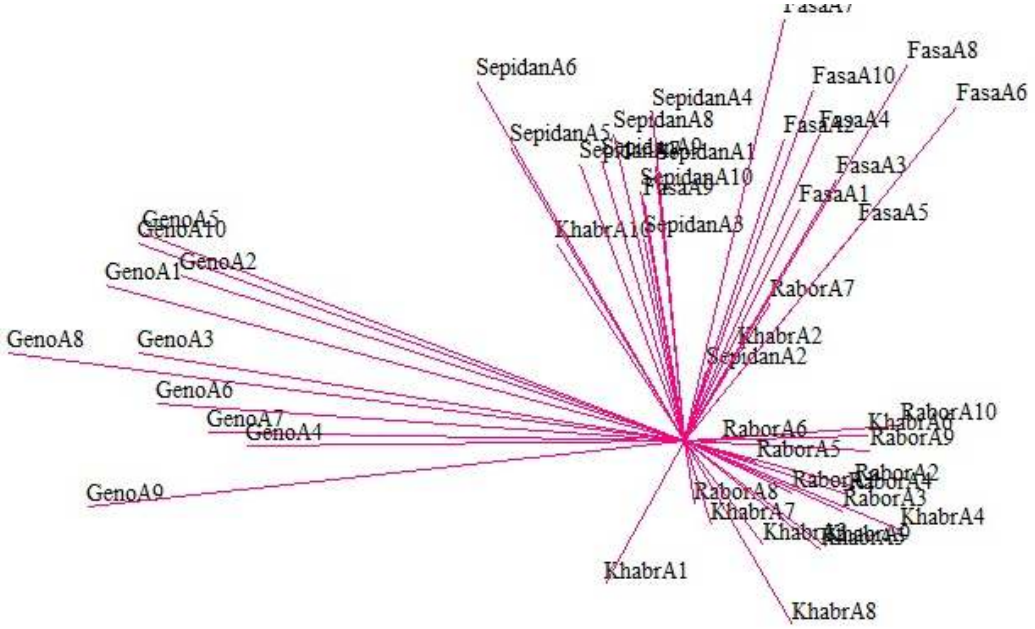

Figure 3. Accessions variation detected by Eigen vector using PCA analysis.

To compare the predicted similarity of accessions by ISSR markers, similarity matrices were performed by the SimQual approach using NTSYSpc, and clustering was performed by SAHN using UPGMA method. Statistical results showed a significant difference between assessed accessions ($r = 0.77$, $t = 18.53$, $p = 1.000$). Results of individual accessions analysis of Jaccard's (J) showed significant variation among accessions (Figure 4-4). According to the reference line of J cluster, all 50 accessions genetically grouped into 18 different groups. An individual accession of Sepidan placed in group 1, accessions of Rabor population placed in six groups (2-7) including: three groups of 2, 3, and 4 consisted individual accessions, four accessions consisted group 5, two accessions placed in group 6, and an accession consisted group 7 (Figure 3). Accessions of Khabr populations divided into six groups (8-12, 17) including: an individual accession separately for group 8 and 9, two accessions in group 10, three accessions in group 11, an accession in group 12, and two accessions in group 17 (Figure 3). All accessions of Genow population placed in two groups (13, 14) including: an accession belonged to group 13, and 9 accessions consisted group 14 (Figure 3). Accessions of Sepidan population placed in three groups (1, 15,16) including: an accession for group 1, four accessions in group 15, and five accessions in group 16. Finally, all accessions of Fasa population were placed in a group (18) (Figure 3).

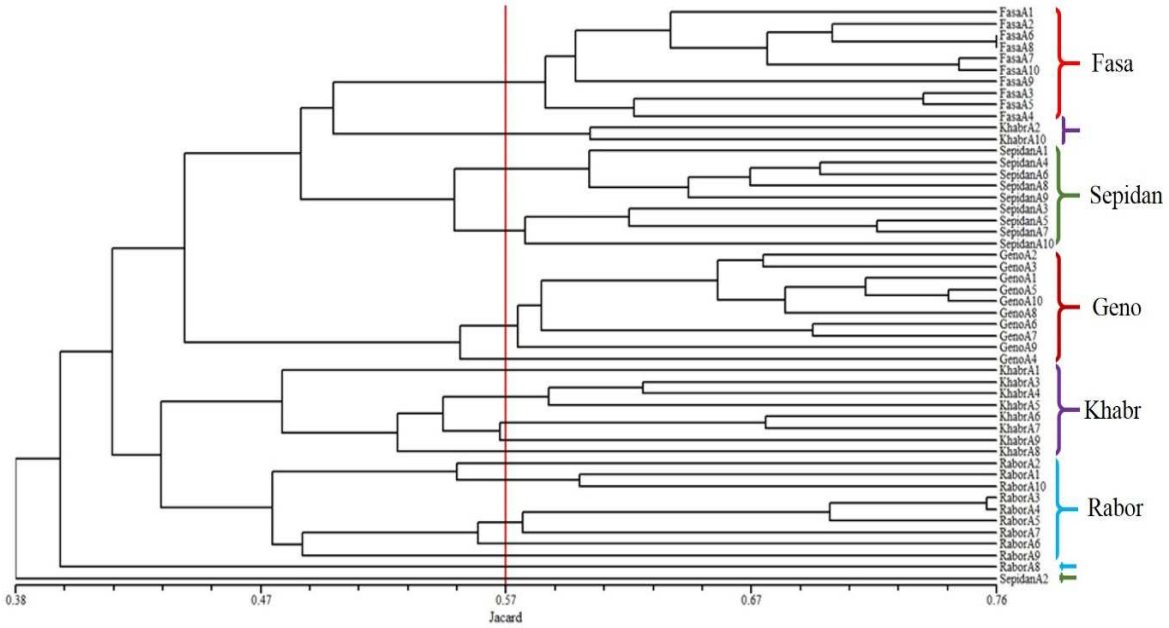

Figure 4. Dendrogram (UPGMA) showing clusters of 50 *Juniperus* accessions based on Jaccard (J) similarity index.

Discussion

In the current study, investigation of ISSR markers has shown the high capability and logical ability of these markers to predict total and polymorphic loci of different populations of *J. polycarpos* that is in accordance with the overall percentage of polymorphics predicted by ISSR markers in the assessment of genetic diversity of *Crocus sativus* L. (Mir *et al*., 2021), *Salvadora persica* L. (Asadi Monfared *et al*., 2018), and *Cymbopogon* sp. (Baruah *et al*., 2017). Furthermore, evaluation of ISSR markers showed that the mean FP of primers and PIC is 0.88, and 0.45, respectively. According to Botstein *et al*. (1980), highly informative markers had the PIC value equal to 0.5, and moderate informative markers had the PIC value from 0.25 to 0.5, and slight informative markers had the PIC value less than 0.25. The results of this study showed that the PIC values for all the ISSRs was more than 0.25 and the average PIC value was 0.45, hence all the ISSRs were considered high or moderate informative markers. The mean of ISPI in the current study is 2.38 and ISSRs had logical RP for each locus. Furthermore, the MR, EMR, and MI in this study had a logical amount and our findings had a reasonable range alike in previous studies using ISSR markers (Sharma *et al*., 2016; Baruah *et al*., 2017; Asadi Monfared *et al*., 2018).

In current study, percentage of total polymorphic ISSR loci (89.33) was higher than the 88.28% loci reported for different *Lycium* varieties by Liu *et al*. (2020), and 87.94% loci reported by Yan *et al*. (2019) for *Mallotus oblongifolius* (Miq.) Muell-Arg, but lower than the 92.59% polymorphic alleles reported by Kumla *et al*. (2012) for *Mystus nemurus* (Cuv. & Val.). Results of the current study showed significant genetic diversity among different populations of *J. polycarpos* that are growing in the habitat of Fasa, Sepidan, Khabr, Rabor, and Genow. These results are in accordance with the results of ISSR markers used to detect genetic diversity and population structure of the endemic Azorean juniper (*Juniperus brevifolia* Seub.) (Bettencourt *et al*., 2015), as well as with the results of the ISSR application to assess the genetic diversity

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of *Hibiscus sabdariffa* L. (Sharma *et al*., 2016). Investigation of NA, Ne, H, I, and PPL at the population level have shown significant diversity of populations and these results are in accordance with the results of ISSR application for population structure analysis of *Mallotus oblongifolius* (Yan *et al*., 2019) and threatened rosewood in the Peruvian Amazon (Guizado *et al*., 2020).

The current study showed higher Ht of specimens (0.38) than Hs (0.25). These results are similar to the findings of Feng *et al*. (2020) for *Juniperus rigida* Sieb. in regard to total genetic diversity (Ht = 0.338) and intra-populations genetic diversity (Hs = 0.308). Moreover, our results indicated the higher Hs than Dst for investigated populations of *J. polycarpos.* In a similar trend, Reim *et al*. (2016) showed a very high genetic diversity of *J. communis* L. populations in Saxony, Germany. Likewise, Bettencourt *et al*. (2015) have shown high genetic diversity within *J. brevifolia* Seub. populations (93%) and Rumeu *et al*. (2014) have shown high genetic diversity within population of Canarian *J. cedrus* Webb & Berthel. Furthermore, this study estimates the moderate Gst (0.34), and low Nm (0.94) among populations. In this regard, previous studies indicated moderate differentiation and low gene flow between populations of some *Juniperus* species (Qin *et al*., 2021; Feng *et al*., 2020; Bettencourt *et al*., 2015).

In addition, the gene flow of investigated populations of *J. polycarpos* in current study was strongly lower than the mean gene flow for heterozygous plants (Nm = 5.380) due to Hamrick (1987) and Liu *et al*. (2014). Therefore, low gene flow among five studied populations in this study could be linked to their pollen grain productivity and their geographical distance. Pollination of *J. polycarpos* has influenced by wind aspect and speed the same as all other conifers (Kling and Ackerly, 2021; Owens *et al*., 1998). Conifer species have larger pollen grain than most angiosperms but they have light pollens that can dispersed in long distances (Owens *et al*., 1998), consequently, the pollen can dispersed from 300–1300 km in the Pinaceae (Potter and Rowley, 1960). However, drought extinction in Southern parts of Iran at recent decades has caused the production of weak cones with irregular maturation and poor pollen grain in *J. polycarpos* (Rahimian Boogar and Salehi, 2020) that can effects this species' pollination and gene flow. Moreover, seed dispersing can influenced the gene flow of plant species (Kling and Ackerly, 2021). Conifer seeds normally disperse via wind and gravity or water (Farjon, 2005), animals and birds (Johnsen, 1962). According to geographic distance between investigated populations in this study, indicating the seed dispersal as an effective factor affecting gene flow among different habitat is impossible.

In addition, the pattern of H of assessed populations of *J. polycarpos* are depended to their origin or natural habitat. Populations of *J. polycarpos* had the H in the range of 0.21 to 0.27, that is similar with populations of *J. communis* from Russia with 0.14 to 0.25 He (Khantemirova and Semerikov, 2010), while, our results are unlike with findings for populations of *J. communis* that grown in Germany with 0.80 to 0.86 He (Reim *et al*., 2016). Giraldo *et al*. (2018) reported positive association among geographical distance and genetic distance of populations. In the current study, populations of Sepidan and Khabr with the highest geographical distance have the highest genetic distance, and populations of Khabr and Rabor with lower geographical distance have the lowest genetic distance. Furthermore, AMOVA analysis for investigated accessions of five populations have shown that within population variation (70%) is higher than among population variation (30%), these findings are in

agreement with the results of Bettencourt *et al*. (2015) on *J. brevifolia*. And investigation of common juniper varieties suggested high within population genetic variation (96%) and low proportion of the variation among the populations (4%) (Khantemirova and Semerikov, 2010).

Conclusions

The population of *J. polycarpos* which grows in the natural habitat of Sepidan has the highest genetic variation compared with populations of Fasa, Khabr, Rabor, and Genow habitats. And the population of Khabr has the minimum genetic diversity. Gene flow affects within-population genetic diversity, pollen grain dispersal, and outcrossing abilities that can influence the gene flow. On the other hand, pollen production and pollination of *J. polycarpos* impressed by different factors such as climatic conditions. The cooler climate and more precipitation of the habitat can be an effective factor causing high genetic diversity of the Sepidan population. Gene flow, differentiation, and Nei's Genetic Distance were affected by geographical distance of populations. Populations of Khabr and Rabor have the lowest geographical distance and they have the highest gene flow and genetic similarity, while the population of Sepidan has the highest geographical distance with Khabr population and they have low gene flow and maximum genetic diversity. Moreover, natural habitats of Khabr, Rabor, Fasa, and Genow are located in warmer regions of Iran with lower precipitation that can be influenced by drought extension in recent decades, and it may negatively affect the populations diversity. Accordingly, breeders and managers of nature conservation should pay more attention to endemic species in the natural habitats. Elite genotypes should be recognized and selected with the aim of breeding, propagating, and cultivation.

References

Adams, R.P. (2004). *Junipers of the World: the genus Juniperus.* Vancouver, BC - Trafford.

Adams, R.P. (2008). *Junipers of the World: the genus Juniperus* (2nd Ed), Vancouver, BC - Trafford.

Adams, R.P. (2014). *Junipers of the world: The genus Juniperus* (3rd Ed). Victoria, BC – Trafford.

Adams, R.P., Al-Farsi, A., Schwarzback, A. (2014). Confirmation of the southernmost population of *Juniperus seravschanica* in Oman by DNA sequencing of nrDNA and four cpDNA regions. *Phytologia*, *96* (3), 218-224.

Al-Turki. T.A., Basahi, M.A. (2015). Assessment of ISSR based molecular genetic diversity of Hassawi rice in Saudi Arabia. *Saudi Journal of Biological Sciences, 22*, 591–599.

Anderson, J.A., Churchill, G.A., Autrique, J.E., Tanksley, S.D., Sorrells, M.E. (1993). Optimizing parental selection for genetic linkage maps. *Genome, 36*(1), 181–186.

Asadi Monfared, M., Samsampour, D., Sharifi-Sirchi, G.R., Sadeghi, F. (2018). Assessment of genetic diversity in *Salvadora persica* L. based on inter simple sequence repeat (ISSR) genetic marker. *Journal of Genetic Engineering and Biotechnology, 16*, 661–667.

Baruah, J., Gogoi, B., Das, K., Ahmed, N.M., Sarmah, D.K., Lal, M., Bhau, B.S. (2017). Genetic diversity study amongst Cymbopogon species from NE-India using RAPD and ISSR markers. *Industrial Crops and Products, 95*, 235-243.

Bettencourt, S.X., Mendonça, D., Lopes, M.S., Rocha, S., Monjardino, P., Monteiro, L., Machado, A.deC. (2015). Genetic diversity and population structure of the endemic Azorean juniper, *Juniperus brevifolia* (Seub.) Antoine, inferred from SSRs and ISSR markers. *Biochemical Systematics and Ecology, 59*, 314 – 324.

Bhattarai, S., Chaudhary, R.P., Taylor, R.S.L. (2007). Plants used as fence and fuelwood in manang district, Central Nepal. *The Scientific World Journal, 5*(5), 107-111.

Botstein, D., White, R.L., Skolnick, M., Davis, R.W. (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *American Journal of Human Genetics, 32*(3), 314-331.

Ciesla, W.M. (2002). Juniper forests - a special challenge for sustainable forestry. *Forests, Trees and Livelihoods, 12*(3), 195-197.

Farjon, A. (2005). *A monograph of Cupressaceae and Sciadopitys*. Royal Botanic Gardens, London - Kew. 648 p.

Farjon, A. (2001). *World Checklist and Bibliography of Conifers* (2nd Ed). Royal Botanic Gardens, London - Kew

Feng, X., Liu, J., Zhang, Y., Wu, W., Pan, Y., Wang, D., Li, D. (2020). Podophyllotoxin profiles combined with SRAP molecular markers in *Juniperus rigida*: A promising alternative source of podophyllotoxin. *Industrial Crops and Products, 153,* 112547.

Füssel, H.M. (2010). Review and quantitative analysis of indices of climate change exposure, adaptive capacity, sensitivity, and impacts. Potsdam Institute for Climate Impact Research (PIK), World Development Report, Germany. 1-35.

Ge, X.J., Zhou, X.L., Li, Z.C., Hsu, T.W., Schaal, B.A., Chiang, T.Y. (2005). Low genetic diversity and significant population structuring in the relict *Amentotaxus argotaenia* complex (Taxaceae) based on ISSR fingerprinting. *Journal of Plant Research, 118*, 415–422.

Ghislain, M., Zhang, D., Fajardo, D., Huamann, Z., Hijmans, R. (1999). Marker-assisted sampling of the cultivated Andean potato *Solanum phurejacollection* using RAPD markers. *Genetic Resources and Crop Evolution, 46*, 547–555.

Giraldo, R., Caballero, W., Camacho-Tamayo, J. (2018). Mantel test for spatial functional data. *AStA Advances in Statistical Analysis, 102*, 21–39.

Govindaraj, M., Vetriventhan, M., Srinivasan, M. (2015). Importance of Genetic Diversity Assessment in Crop Plants and Its Recent Advances: An Overview of Its Analytical Perspectives. *Genetics Research International,* ID 431487.

Guizado, S.J.V., Nadeem, M.A., Ali, F., Barut, M., Habyarimana, E., Gómez, T.P., Santillan, J.A.V., Canales, E.T., Gómez, J.C.C., Chung, G., Baloch, F.S. (2020). Genetic diversity and population structure of endangered rosewood from the Peruvian Amazon using ISSR markers. *Acta Amazonica, 50*(3), 204-212.

Hamrick, J.L. (1987). *Gene Flow and Distribution of Genetic Variation in Plant populations / Urbanska K. Differentiation Patterns in Higher Plants*. New York - Academic Press.

Hojjati, F., Kazempour-Osaloo, S., Adams, R.P., Assadi, M. (2018). Molecular phylogeny of *Juniperus* in Iran with special reference to the *J. excelsa* complex, focusing on *J. seravschanica. Phytotaxa, 375* (2), 135–157.

Johnsen, T.N. (1962). One-seed Juniper invasion of northern Arizona grasslands. *Ecological Monographs, 32*, 187–207.

Khantemirova, E.V. Semerikov, V.L. (2010). Genetic variation of some varieties of common juniper *Juniperus communis* L. inferred from analysis of allozyme loci. *Russian Journal of Genetics, 46* (5), 546–554.

Kling, M.M., Ackerly, D.D. (2021). Global wind patterns shape genetic differentiation, asymmetric gene flow, and genetic diversity in trees. *PNAS, 118* (17). e2017317118.

Kumla, S., Doolgindachbaporn, S., Sudmoon, R., Sattayasai, N. (2012). Genetic variation, population structure and identification of yellow catfish, Mystus nemurus (C&V) in Thailand using RAPD, ISSR and SCAR marker. *Molecular Biology Reports, 39*, 5201–5210.

Liu, H., Hu, S., Dong, L., Zhang, H., Zeng, Y., Yu, W., Dai, W., (2014). SRAP analysis of genetic diversity of four female populations in *Torreya grandis*. *Nonwood Forest Research, 3*, 30–35. (In Chinese)

Liu, X., Du, J., Khan, M.D.A., Cheng, J., Wei, C., Mei, Z., Chen, H., He, T., Fu, J. (2020). Analysis of genetic diversity and similarities between different *Lycium* varieties based on ISSR analysis and RAMP-PCR markers. *World Academy of Sciences Journal, 2*, 83-90.

Lodhi, M.A., Ye, G.N., Weeden, N.F., Reisch, B.I. (1994). A Simple and Efficient Method for DNA Extraction from Grapevine Cultivars and Vitis Species. *Plant Molecular Biology Reporter, 12* (1), 6-13.

Mao, K., Hao, G., Liu, J., Adams, R.P., Milne, R.I. (2010). Diversification and biogeography of *Juniperus* (Cupressaceae): variable diversification rates and multiple intercontinental dispersals. *New Phytologist, 188* (1), 254-272.

Miller, A., Cope, T. (1996). *Flora of the Arabian Peninsula and Socotra*, vol. 1. Edinburgh - Edinburgh University Press,

Mir, M.A., Mansoor, S., Sugapriya, M., Alyemeni, M.N., Wijaya, L., Ahmad, P. (2021). Deciphering genetic diversity analysis of saffron (*Crocus sativus* L.) using RAPD and ISSR markers. *Saudi Journal of Biological Sciences, 28*, 1308–1317.

Nei, M. (1987). *Molecular Evolutionary Genetics*. New York - Columbia University Press.

Owens, J.N., Takaso, T., Runions, C.J. (1998). Pollination in conifers. *Trends in Plant Science, 3*(12), 479-485.

Potter, L.D., Rowley, J. (1960). Pollen rain and vegetation, San Augustin Plains, New Mexico. *Botanical Gazette, 112*, 1–25.

Powell, W., Machray, G.C., Provan, J. (1996). Polymorphism revealed by simple sequence repeats. *Trends in Plant Science, 1*, 215–222.

Prevost, A., Wilkinson, M.J. (1999). A new system of comparing PCR primers appliedto ISSR fingerprinting of potato cultivars. *Theoretical and Applied Genetics, 98*, 107–112.

Qin, A., Ding, Y., Jian, Z., Ma, F., Worth, J.R.P., Pei, S., Xu, G., Guo, Q., Shi, Z. (2021). Low genetic diversity and population differentiation in *Thuja sutchuenensis* Franch., an extremely endangered rediscovered conifer species in southwestern China. *Global Ecology and Conservation, 25*, e01430.

Rajwade, A.V., Arora, R.S., Kadoo, N.Y., Harsulkar, A.M., Ghorpade, P.B., Gupta, V.S. (2010). Relatedness of Indian flax Genotypes (*Linum usitatissimum* L.): An inter-simple sequence repeat (ISSR) primer assay. *Molecular Biotechnology, 45*, 161–170.

Reche, J., García-Martínez, S., Carbonell, P., Almansa, M.S., Hernández, F., Legua, P., Amorós, A. (2019). Relationships between physico-chemical and functional parameters and genetic analysis with ISSR markers in Spanish jujubes (*Ziziphus jujuba* Mill.) cultivars. *Scientia Horticulturae, 253*, 390-398.

Reim, S., Lochschmidt, F., Proft, A., Tröber, U., Wolf, H. (2016). Genetic structure and diversity in *Juniperus communis* populations in Saxony, Germany. *Biodiversity; Research and Conservation, 42*, 9-18.

Rohlf, F.J. (2009). NTSYS-pc: Numerical Taxonomy and Multivariate Analysis System, Version 2.2. Exeter Software, New York - Applied Biostatistics Inc.

Rumeu, B., Vargas, P., Molina, R., Nogales, M., Caujapé-Castells, J., (2014). Phylogeography and genetic structure of the threatened Canarian Junipeurs cedrus (Cupressaceae). *Botanical Journal of the Linnean Society, 175* (3), 376e394.

Shahabfar, A., Eitzinger, J. (2013). Spatio-Temporal Analysis of Droughts in Semi-Arid Regions by Using Meteorological Drought Indices. *Atmosphere, 4*, 94-112.

Sharma, H.K., Sarkar, M., Choudhary, S.B., Kumar, A.A., Maruthi, R.T., Mitra, J., Karmakar, P.G. (2016). Diversity analysis based on agro-morphological traits and microsatellite based markers in global germplasm collections of roselle (*Hibiscus sabdariffa* L.). *Industrial Crops and Products, 89*, 303–315.

Su, Y, Wang, T., Ouyang, P. (2009). High genetic differentiation and variation as revealed by ISSR marker in *Pseudotaxus chienii* (Taxaceae), an old rare conifer endemic to China. *Biochemical Systematics and Ecology, 37*, 579–588.

Tikendra, L., Amom, T., Nongdam, P. (2019). Molecular genetic homogeneity assessment of micropropagated *Dendrobium moschatum* Sw. - A rare medicinal orchid, using RAPD and ISSR markers. *Plant Gene, 19*, 100196.

Varshney, R.K., Chabane, K., Hendre, P.S., Aggarwal, R.K., Graner, A. (2007). Comparative assessment of EST-SSR EST-SNP and AFLP markers for evaluation of genetic diversity and conservation of genetic resources using wild, cultivated and elite barleys. *Plant Science, 173*, 638–649.

Williams, J.W., Jackson, S.T., Kutzbach, J.E. (2007). Projected distributions of novel and disappearing climates by 2100 AD. *PNAS, 104* (14), 5738-5742.

Yan, W., Li, J., Zheng, D., Friedman, C., Wang, H. (2019). Analysis of genetic population structure and diversity in *Mallotus oblongifolius* using ISSR and SRAP markers. *PeerJ, 7*, e7173.

Zhang, Z.Y., Chen, Y.Y., Li, D.Z. (2005). Detection of low genetic variation in a critically endangered Chinese pine, *Pinus squamata*, using RAPD and ISSR markers. *Biochemical Genetics, 43*, 239–249.

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