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Genetic variability and relationship among different accessions of *Froriepia subpinata* Bail (Gijavash) an endangered medicinal plant from Iran revealed by ISSR and IRAP markers

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Key words: Endemic plant, genetic conservation, threatened species.

Abstract: The genetic variability of *Froriepia subpinata* Ledeb. Bail., an endangered Iranian endemic species, has been estimated with a total of 52 accessions using 20 markers including ISSR and IRAP. The results showed the polymorphic band produced by primers was 82.3%. The best mean values of genetic diversity parameters observed in ISSRs markers, being UBC873, UBC811, and UBC873 the best primers tested. The similarity range among accessions was 34.45% to 93.3%. The cluster analysis classified the accessions into five main groups that in totally, accessions with similarity in region generally were clustered in the same group. Overall, present study could provide elementary information for formulation of conservation strategies and invaluable elementary genetic information for next breeding or designing conservation programs.

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All relevant data are within the paper and its Supporting Information files.

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The authors declare no competing interests.

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1. Introduction

Froriepia subinata Ledeb. Bail. syn= *Buplerum subinatum, Froriepia nuda* is a biennial medicinal and aromatic plant, locally known as Gijavash, that belong to the Apiaceae family. It is a self-pollinated plant with white flowers and small achene fruits. Gijavash leaves are used in diet people and have antimicrobial, antifungal properties and high antioxidant activity (Salmanian and Sadeghi, 2012). This species is the only endemic threatened one of *Froriepia* genus in northern Iran. In this genus, somatic chromosome number ranged between 2n= 14 and 2n=16. Some of its phonological characteristics like irregular and delay germinations and also excessive and improper harvest have exposed it to mortality and annihilation (Mozaffarian, 2015).

Nowadays, due to increased human activities and excessive growth in residential area, plants habitats have been destroyed. So, investigation and study on each threatened plant for identifying best way to protect should be considered. Although pastures protection ways as well as molecular finger-

printing, micropropagation and other biotechnological methods and beneficial techniques (Glover and Abbott, 1995; Sudha *et al.*, 1998) are the most important method for conservation of rare and endangered plants. Therefore, genetic diversity of endangered species is the first step in conservations strategies and selection for domesticating process (Vicente *et al*., 2011).

Moreover, recently an increasing number of studies for plant conservation biology, especially in rare endemic species have demonstrated the value of genetic data (Gaudeul *et al*., 2000; Bellusci *et al*., 2008; González-Pérez *et al*., 2009).

One of the most important features for long-term survival and adaptation to environment conditions of population or species is genetic variation within their taxon (Frankham, 2010).Having information on genetic diversity of a plant species is very necessary for its conservation (Höglund, 2009; Frankham, 2010; Laikre, 2010) as losses of genetic diversity are likely to have consequences for plant fitness (Reed and Frankham, 2003; Dostálek *et al*., 2010). Preserving rare species endangered especially those which have restricted geographic distributions is main concern of scientist because of their habitat destruction and fragmentation. On the other hand, losing allelic richness or genetic diversity in fragmented populations due to their genetic drift and inbreeding depression have increased population differentiation (Buza *et al*., 2000; Tomimatsu and Ohara, 2003).

Thus, an accurate estimate on the level and distribution of genetic diversity of threatened and endangered species seems necessary for designing conservation programs (Smith and Wayne, 1996; Höglund, 2009). In addition, understanding the chance of species survival in the short-term, formulation of conservation strategy for long-term survival need to population genetic information (Cires *et al.*, 2013). Meanwhile, knowledge of population genetic structure can provide important information to understand the evolution of rare and endangered different species. For example, by identifying populations of greatest evolutionary potential as well as populations best suited for source material for *ex situ* preservation or reintroduction (Furches *et al.*, 2009).

According to the fragmentation distribution and the endangered status of this endemic species, there are no population genetic studies and conservation management plans. As an initial step in developing such plan, we have assessed the genetic variability of 52 natural populations of *F. subpinata* using inter simple sequence repeat (ISSR) and inter-retrotransposon amplified polymorphism (IRAP).These populations were naturally grown in their own habitat. To avoid from possibility pollination between different populations, the distance between the populations was considered and tried to elect population which had more difference between each other due to morphological and environmental characteristics.

ISSR and IRAP were chosen because of their advantages over other DNA polymorphism analysis methods, as they do not require prior sequence knowledge, cloning procedures or characterized probes. It is also generally accepted that they have a comparatively high reproducibility (Jones *et al*., 1997). Therefore, both techniques have been successfully used in plant population genetic studies, especially for endangered species (Li and Jin, 2007; Gong *et al.*, 2010; Noroozisharaf *et al*., 2015).

Froriepia subpinata is commonly used in traditional foods in Iran for its bioactive compounds and antioxidant potential. However, the destruction of its natural habitats by human activity has put a strain on its survival. Therefore, the study of genetic diversity among accessions collected from different areas of Iran would be very useful in the biodiversity management and conservation plans organization.

2. Materials and Methods

Plant Material

Fresh leaves of Gijavash (*F. subpinata*) accessions were gathered from 52 different localities of Guilan province, Iran. To accurate estimate the genetic variability, based on local people's knowledge and distribution of the plant, we elected 52 locations throughout several cities (Fig. 1).

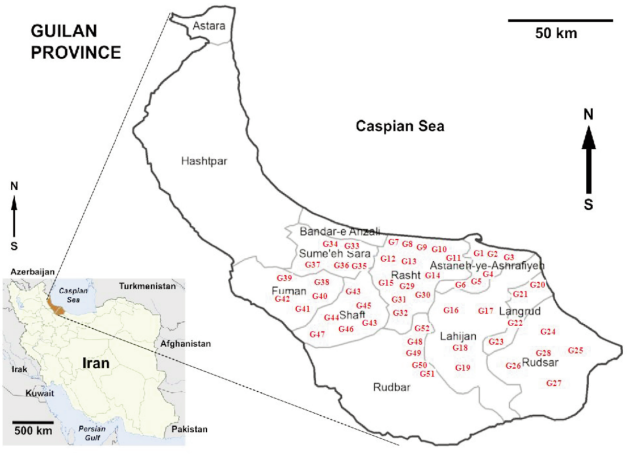

Fig. 1 - Geographical map of 52 *F. subpinata* accessions location collected from Guilan province, Iran.

All the accessions are listed in Table 1 with the location and the altitude of each one. Plants were randomly selected from two or three individuals of each site. To reduce the probability duplicate sampling fresh leaves were taken from each individual separated at least 3 m apart. Samples were immediately frozen in liquid nitrogen and kept at -80°C for genomic DNA extraction. In addition, voucher specimens were collected, dried by pressing in absorbent paper, stored at room temperature, and lodged at the herbarium of the University of Guilan.

DNA extraction

DNA extraction was carried out using the CTAB method described by (Doyle, 1990) with minor modifications as follow: approximately 50-70 mg leaf material was ground in liquid nitrogen, then 600 µL of hot (65°C) extraction buffer 2X (100 mM Tris HCl, pH 8; 20 mM EDTA; 1.4 M NaCl; 2% CTAB; 1% PVP) was added. Subsequently, an equal volume of cold chloroform/Isoamyl alcohol (24:1) was added and mixed by gentile inversion of the tube until a light green single phase emulsion is performed. In the next step, the emulsion centrifuged at 10000 rpm for 10 min. Then, the aqueous phase transferred into a clean tube and addition 100 µL of CTAB solution (10% CTAB, 0.7 M NaCl) and the extraction was repeated. This step may takes several times until no precipitate can be detected at phenol/aqueous layer interface. The aqueous phase is removed and the rest mixed with an equal volume of hot (65°C) CTAB precipitation buffer (50 mM Tris HCl, pH 8.5, 10 mM EDTA, 1% CTAB). The solution mixed gently and incubated every 3-5 min at room temperature for 30 minutes. The resulting CTAB/DNA complex is immediately platted by centrifugation at 12000 rpm for 10 min. The resulting pellet resuspended in 650 µL high salt buffer (10 mM Tris HCl, pH 8; 1 mM EDTA; 1 M NaCl) and the DNA precipitated by addition 1300 µL of cold 100% ethanol. The precipitation gently mixed and incubated every 3-5 min on ice for 30 min. then, it centrifuged at 12000 rpm for 10 min. The pellet was washed three times with 1 ml of cold 70% ethanol, and then dried at room temperature. Finally, pellet was resuspended in 100 µL TE buffer (10 mM Tris HCl, pH 8; 1mM EDTA). Extracted DNA was qualified using 1% (w/v) agarose gel electrophoresis. Afterwards, the DNA concentration and contamination rate was evaluated by NanoDrop spectrophotometers (Thermo Fisher scientific, 5225 Verona Rd, USA). For PCR reaction, only template of DNA was used which had a purity of 2 in a dilution of 15 ng/ml.

Table 1 - Different heat treatments used to enhance seed germination of *Cycas revoluta*

Accession name	Gathered site	Voucher number	Altitude (m)
G1	Poushal	APF53098	-14
G2	Reshvandeh	APF53099	-11
G3	Lashkam	APF54000	-20
G4	Loskehkelayeh	APF54001	-18
G5	Pasgahfarhad	APF54002	-5
G6	Kisom	APF54004	2
G7	Touchipaybast	APF54005	1
G8	Seghaleksar	APF54007	35
G9	Lakan	APF54008	62
G10	Selkisar	APF54011	90
G11	Aziz kiyan	APF54013	174
G12	Hasan kiadeh	APF54014	-22
G13	Kiashahr forest	APF54015	-22
G14	Kiashahr	APF54016	-24
G15	Koshkbijar	APF54017	-25
G16	Ghasabmahaleh	APF54018	-17
G17	Goharsara	APF54019	145
G18	Sheykhan bar	APF54020	124
G19	Toustan	APF54022	-12
G20	Salkuyeh	APF54024	-19
G21	Taleshmahaleh	APF54025	73
G22	Langrud1	APF54026	4
G23	Langrud2	APF54027	8
G24	Chafjir	APF54028	-25
G25	Sahnehsara	APF54030	-15
G26	Karaj posht	APF54031	-21
G27	Chinijan	APF54029	-21
G28	Rudsar	APF54032	-20
G29	Darehposht	APF54033	89
G30	Saravan park	APF54035	141
G31	Tekhsem	APF54036	114
G32	Saravan	APF54037	93
G33	Tahergurab	APF54038	24
G34	Ziabar	APF54039	31
G35	Shanderman	APF54034	49
G36	Sheykhneshin	APF54040	43
G37	Someehsara	APF54038	3
G38	Fuman1	APF54041	35
G39	Sehpiranpayin	APF54042	20
G40	Dobakhshar	APF54044	98
G41	Kohnehgurab	APF54043	14
G42	Fuman2	APF54046	41
G43	Shaft	APF54045	43
G44	Khartum	APF54047	71
G45	Mozhdeheh	APF54048	83
G46	Shah khal	APF54049	48
G47	Dastkhat Chamacha	APF54050	122
G48	Dozdak	APF54052	51
G49	Ezberem	APF54051	62
G50	Bidrun	APF54053	24
G51	Siyah Kal1	APF54054	37
G52	Siyah Kal2	APF54055	42

PCR amplification

PCR reactions were done in 1500 µL reaction volumes containing 750 µL of sterile double distilled water, 150 µL of Taq polymerase reaction buffer (10×), 1 mM $MgCl_2$, 150 µL of dNTPs (10 mM), 100 µL of each primer at 5 mM, 0.5 unit of Taq DNA polymerase, and 200 µL of plant DNA. The planning of thermal cycling was as follows: initial template denaturation at 94°C for 4 min, 35 cycles of denaturation 94°C for 1 min, annealing at 42-50°C (depending on primer used) (Table 2) for 1 min, extension at 72°C for 90s, and final extension at 72°C for 5 min.

The PCR products were loaded on 1.5% (w/v) agarose gel in 1× TAE buffer at voltage of 70 for 90 min. The gel's images were captured using the Biometra gel documentation system (Whatman Biometra, Gottingen, Germany). The produced fragments size in comparing to size marker was distinguished (GeneRuler 1 kb DNA ladder, SM0241, Fermentase, Ontario, Canada).

Data analysis

In all, 20 individual ISSR and IRAP primers with their combinations were used (Table 2). Only reproducible and well clear bands in the replications were considered as potential polymorphic markers. It was assumed that each band represented the phenotype at a single biallelic locus, because the ISSR and IRAP markers are dominant (Williams *et al*., 1990). Amplified fragments were scored for presence (1) or absence (0) of homologous bands. According to PCR banding patterns, a data matrix was created for each reaction. Polymorphism information content (PIC), Effective multiplex ratio (EMR) and Marker index (MI) were calculated (Smith and Wayne, 1996).

Effective number of alleles (Ne), Nei's gene diversity (Nei, 1972) and Shannon's information index (Shannon and Weaver, 1949) were estimated for total accessions using POPGENE software version 1.31 (Yeh, 1999). Similarity matrix based on simple matching coefficient was constructed from the ISSR and IRAP

Table 2 - Polymorphism detected with ISSR and IRAP marker in 52 germplasm accession

Marker type	No.	Primer name	Annealing temperature (˚C)	Sequence (5'-3')	No. of bands	No. of polymorphic bands	% of polymorphic bands
IRAP	1	TOC-1	48.8	TGTTGGGAATAGTCCCACA	9	7	77.77
	2	TOC-2	45.2	TGTTGAATAGTTCCACATT	7	7	100.00
Mean					8	7	88.88
ISSR	3	UBC808	47.4	$(AG)_8$ C	8	6	75.00
	4	UBC811	41	$(GA)_8$ C	10	9	90.00
	5	UBC812	41.2	$(GA)_8$ A	5	4	80.00
	6	UBC813	42.8	$(CT)_8$T	8	7	87.5
	7	UBC816	49.2	$(CA)_8$T	9	8	88.88
	8	UBC817	48.8	$(CA)_8$A	7	5	71.42
	9	UBC824	46.6	$(TC)_8$G	8	8	100.00
	10	UBC825	50	$(AC)_8$T	6	6	100.00
	11	UBC826	50	$(AC)_8$ C	8	6	75.00
	12	UBC873	45.8	$(AG)_8$CTT	7	7	100.00
Mean					7.6	6.6	86.78
ISSR+ISSR	13	UBC808+UBC817	45.2	$(AG)_8$C+ $(CA)_8$A	6	6	100.00
	14	UBC812+UBC813	41	$(GA)_8$A+ $(CT)_8$T	7	5	71.42
	15	UBC811+UBC813	42	$(GA)_8$C+ $(CT)_8$T	6	5	83.33
	16	UBC816+UBC817	46	$(CA)_8$T+ $(CA)_8$A	7	4	57.14
	17	UBC825+UBC826	47	$(AC)_8$T+ $(AC)_8$C	9	5	55.55
Mean					7	5	73.48
ISSR+IRAP	18	UBC817+TOC-1	45.8	$(CA)_8$A+ TGTTGGGAATAGTCC-	5	4	80.00
	19	UBC812+TOC-2	42.8	$(GA)_8$ A+ TGTTGAATAGTTCCA-	7	5	71.42
	20	UBC813+TOC2	44.2	$(CT)_8$T+ TGTTGAATAGTTCCA-	8	7	87.50
Mean					6.66	5.33	79.64
Total Mean					7.3	5.98	82.19
Total					147	121	82.31

data. It was used for the cluster analysis and construction of dendrogram through unweighted pair-group method using arithmetic average (UPGMA), performed by NTSYS-PC software (Rohlf, 2000). In order to evaluate fitness between the dendrogram and similarity matrix, the cophenetic correlation coefficient was calculated. Principal coordinate analysis (PCoA) was accomplished using GenStat (GenStat v12, VSN International Ltd, UK) on a similarity matrix.

3. Results

Twenty individuals ISSR and IRAP and their combinations (ISSR+ISSR; ISSR+IRAP) produced 147 distinguish-able fragments out of which 121 (82.31%) were polymorphic. The polymorphic rang was from 4 in UBC812 to 9 in UBC811 with an average number of 6.05 polymorphic bands per primer. The products number varied from 5 in UBC812 to 10 in UBC811. The mean of polymorphic band percent were 88.88, 86.78, 73.48 and 79.64 for IRAPs, ISSRs, ISSR+ISSR and ISSR+IRAP, respectively. The TOC-2, UBC824, UBC825, UBC873 and UBC808+UBC817 primers had the maximum of polymorphic bands (100%). The minimum of polymorphic bands produced by UBC825+UBC826 primer (55.55%) (Table 2).

The means of PIC value for the amplification products was 0.30 (Table 2). UBC816+UBC817 and UBC873 showed the lowest (0.19) and the highest (0.45) PIC values, respectively. The means of PIC for IRAPs, ISSRs, ISSR+ISSR and ISSR+IRAP were 0.27, 0.32, 0.27 and 0.26, respectively (Table 3).

On the whole, among the 20 used primers, maximum of the EMR, MI, *Ne*, *H* and *I* recorded in UBC811 (8.1), UBC873 (3.16), UBC825 (1.75), UBC825 (0.41) and UBC825 (0.60), respectively .Also it must be considered that the total mean of EMR, MI, *Ne*, *H* and *I* were 5.11, 1.59, 1.49, 028 and 0.43 respectively (Table 3).

Table 3 - Genetic diversity detected with ISSR and IRAP markers in 52 germplasm accessions

Marker type	No.	Primer name	PIC	EMR	MI	*Ne*	*H*	*I*
IRAP	1	TOC-1	0.26	5.44	1.46	1.50	0.29	0.43
	2	TOC-2	0.28	7	1.98	1.57	0.34	0.52
Mean			0.27	6.22	1.72	1.53	0.31	0.47
ISSR	3	UBC808	0.23	4.5	1.06	1.52	0.28	0.41
	4	UBC811	0.34	8.1	2.77	1.56	0.32	0.47
	5	UBC812	0.36	3.2	1.16	1.54	0.31	0.46
	6	UBC813	0.32	6.12	2.00	1.46	0.23	0.38
	7	UBC816	0.35	7.11	2.55	1.60	0.34	0.51
	8	UBC817	0.26	3.57	0.94	1.53	0.29	0.42
	9	UBC824	0.31	8.00	2.54	1.38	0.24	0.40
	10	UBC825	0.35	6.00	2.10	1.75	0.41	0.60
	11	UBC826	0.27	4.5	1.22	1.40	0.24	0.38
	12	UBC873	0.45	7.00	3.16	1.60	0.36	0.55
Mean			0.32	5.81	1.95	1.53	0.30	0.45
ISSR+ISSR	13	UBC808+UBC817	0.35	6.00	2.11	1.73	0.40	0.58
	14	UBC812+UBC813	0.22	3.57	0.78	1.40	0.24	0.36
	15	UBC811+UBC813	0.38	4.16	1.58	1.45	0.28	0.44
	16	UBC816+UBC817	0.19	2.28	0.44	1.25	0.16	0.26
	17	UBC825+UBC826	0.22	2.77	0.61	1.30	0.18	0.28
Mean			0.27	3.75	1.10	1.43	0.25	0.38
ISSR+IRAP	18	UBC817+TOC-1	0.23	3.2	0.76	1.55	0.31	0.45
	19	UBC812+TOC-2	0.26	3.57	0.93	1.29	0.18	0.29
	20	UBC813+TOC2	0.30	6.12	1.84	1.48	0.28	0.43
Mean			0.26	4.29	1.17	1.43	0.25	0.39
Total Mean			0.30	5.11	1.59	1.49	0.28	0.43

PIC= polymorphism information content; EMR= effective multiplex ratio; MI= marker index; *Ne*= effective number of alleles; *H*= Nei's gene diversity; *I*= Shannon's information index.

In addition, there was a significant correlation at P≤0.01 probability level between most of these indices, so that only between EMR and *Ne* as well as EMR and *H* were significant at P≤0.05 level of probability (Table 4).

Table 4 - Correlation between genetic diversity parameters

	PIC	EMR	MI	*Ne*	*H*	*I*
PIC	1					
EMR	0.59 **	1				
MI	0.81 **	0.93 **	1			
Ne	0.58 **	0.49 *	0.56 **	1		
H	0.64 **	0.52 *	0.61 **	0.98 **	1	
I	0.70 **	0.60 **	0.69 **	0.96 **	0.98 **	1

PIC= polymorphism information content; EMR= effective multiplex ratio; MI= marker index; Ne= effective number of alleles; H= Nei's gene diversity; I= Shannon's information index.
** P ≤0.01; * P≤0.05 according to Tukey test.

Principal coordinate analysis (PCoA) was constructed based on simple matching coefficient of similarity. The results showed that the first twelve principal coordinates account for 70.29% of total variation. The first and second extracted component accounted for 26.43% and 9.11% of the variation, respectively (Fig. 2).

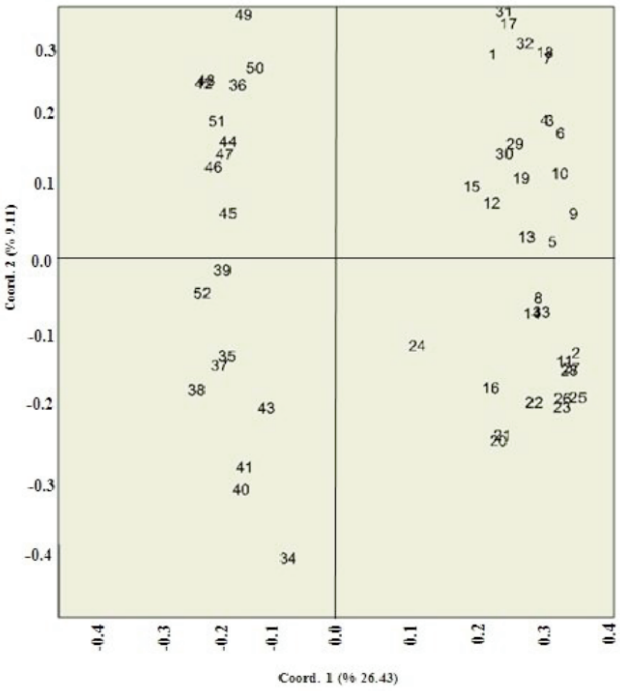

Fig. 2 - Principal coordinate analysis (PCoA). The plot shows the first two principal components (coord. 1 and coord. 2). Accessions codes are identified in Table 1.

To draw cluster analysis for 52 Gijavash accessions, the obtaining data from ISSR and IRAP analysis were used. Figure 3 presents the dendrogram of genetic relationships among the accessions as revealed by the UPGMA method. The 52 accessions of Gijavash classified into 5 main groups. The similarity coefficient range varies from34.45% to 93.27%. The highest similarity was related to G4 and G6 and the lowest similarity observed between G26 and G38 (Fig. 3). Also, high amount of calculated cophenetic correlation coefficient (r=95.2%) showed that UPGMA method was useful in the clustering plant accessions.

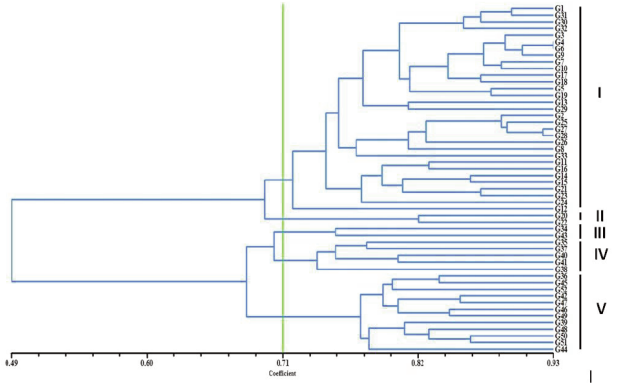

Fig. 3 - UPGMA dendogram of ISSR and IRAP analyses on 52 germplasm accessions of *Froripia subpinata* Ledeb. Bail based on simple matching coefficient.

4. Discussion and Conclusions

Using dominant molecular markers for assessing genetic diversity is usually similar and directly comparable (Nybom, 2004). So that, these dominant markers widely have been used for earning genetic information in large number of endemic and endangered species from different plant families (Jeong *et al.*, 2010; Brütting *et al.*, 2012; Cires *et al.*, 2013; Noroozisharaf *et al.*, 2015) and also for formulation and implementation conservation strategies, along with testing genetic relationships between species (González-Pérez *et al.*, 2009). We applied 20 ISSR and IRAP primers to examine the genetic diversity of 52 accessions from the natural distribution of wild *F. subpinata*. The results showed that a high genetic diversity has been achieved in this species (*H*= 0.28, *I*= 0.43) in comparison with the corresponding genetic coefficients of other endangered species (Hamrick and Godt, 1996; Nybom, 2004; Zheng *et al.*, 2008).

The results of ISSR and IRAP markers demonstrated similar overall trends for genetic diversity. Nevertheless, the genetic diversity indices from IRAP approximately are lower than those from ISSR due to IRAP tending to produce somewhat low estimates of within-population variation (Nybom, 2004).

According to the attributes of *F. subpinata* accessions (i.e. fragmented, endemic) it could expected that there should be low genetic diversity, but in general, it seems that the total genetic diversity based on ISSR and IRAP markers is similar to, or slightly higher, than most of those used by different authors in other plants like *Primula heterochroma*, *Bupleurum rotundifolium*, *Changium smyrnioide, Cycas guizhouensis*. Nei's genetic diversity accounts in other ISSR and IRAP studies ranged from 0.10-0.28 (Qiu *et al.*, 2004; Wu *et al*., 2004; Xiao *et al.*, 2004; Shao *et al*., 2009; Jeong *et al*., 2010; Brütting *et al.*, 2012; Noroozisharaf *et al*., 2015). Base on this result and high polymorphism rate (82.31%), our research has manifested the potential of ISSR and IRAP markers, reproducible and useful methods for classifying different accessions.

Principal coordinate (PCoA) showed that accessions were divided into two groups, (i) the first group of accessions who collected from East of Guilan Province and (ii) the second group belong to West of Guilan Province origination. Many biological factors can influence both the species genetic diversity and its distribution among populations. Among these, the geographic distribution has been considered as one of the most important (Hamrick and Godt, 1990).

In contrast, in another study the geographical range had no significant influence on genetic diversity (Nybom, 2004). Our finding may be related to self-pollination character of this plant, that cause, accessions with less distance from each other had more genetic similarity.

The result of cluster analysis (Fig. 2) also showed that accessions with same region had more similarity to each other, so that maximum of similarity (93.27%) was between G4 and G6, and also the lowest of similarity (34.45%) was between G26 and G38. The G26 and G38 accessions originated from the east (Rudsar city) and west (Fuman city) of Guilan province, respectively (Fig. 1) and it could confirm the relative between genetic similarity and geographic distance in this research.

Overall, present study could provide invaluable elementary genetic information for next breeding plan.

Genetic diversity of different Gijavash accessions was analyzed using ISSR and IRAP molecular markers for the first time.

Results revealed that using of ISSR and IRAP markers had high efficiency for differentiating among the various accessions. Among all used primers, the highest PIC value, EMR and MI was belonging to UBC873, UBC811 and UBC873, respectively. The maximum of *Ne*, *H* and *I* observed in UBC825. With respect to these findings the UBC873, UBC811, UBC873 and specially UBC825 were the most informative primers which could be used to determine the diversity of Gijavash accessions.

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References

BELLUSCI F., PALERMO A.M., PELLEGRINO G., MUSACCHIO A., 2008 - *Genetic diversity and spatial structure in the rare, endemic orophyte* Campanula pseudostenocodon *Lac.(Apennines, Italy), as inferred from nuclear and plastid variation*. - Plant Biosyst., 142: 24-29.

BRÜTTING C., MEYER S., KÜHNE P., HENSEN I., WESCHE K., 2012 - *Spatial genetic structure and low diversity of the rare arable plant* Bupleurum rotundifolium *L. indicate fragmentation in Central Europe*. - Agric., Ecosyst. Environ., 161: 70-77.

BUZA L., YOUNG A., THRALL P., 2000 - *Genetic erosion, inbreeding and reduced fitness in fragmented populations of the endangered tetraploid pea* Swainsona recta. - Biol. Conservation, 93: 177-186.

CIRES E., CUESTA C., PRIETO J.A.F., 2013 - *Genetic diversity and structure in fragmented populations of the endangered species* Ranunculus cabrerensis (*Ranunculaceae*): *implications for conservation*. - Biologia, 68: 30-40.

DOSTÁLEK T., MÜNZBERGOVÁ Z., PLAČKOVÁ I., 2010 - *Genetic diversity and its effect on fitness in an endangered plant species,* Dracocephalum austriacum *L.* - Conservation Genetics, 11: 773-783.

DOYLE J.J., 1990 - *Isolation of plant DNA from fresh tissue*. - Focus, 12: 13-15.

FRANKHAM R., 2010 - *Challenges and opportunities of*

genetic approaches to biological conservation. - Biol. Conservation, 143: 1919-1927.

FURCHES M.S., WALLACE L., HELENURM K., 2009 - *High genetic divergence characterizes populations of the endemic plant* Lithophragma maximum (*Saxifragaceae*) *on San Clemente Island.* - Conservation Genetics, 10: 115-126.

GAUDEUL M., TABERLET P., TILL-BOTTRAUD I., 2000 - *Genetic diversity in an endangered alpine plant,* Eryngium alpinum *L.(Apiaceae), inferred from amplified fragment length polymorphism markers*. - Mol. Ecol., 9: 1625-1637.

GLOVER B.J., ABBOTT R.J., 1995 - *Low genetic diversity in the Scottish endemic* Primula scotica *Hook.* - New Phytologist, 129: 147-153.

GONG W., GU L., ZHANG D., 2010 - *Low genetic diversity and high genetic divergence caused by inbreeding and geographical isolation in the populations of endangered species* Loropetalum subcordatum *(Hamamelidaceae) endemic to China*. - Conservation Genetics, 11: 2281-2288.

GONZÁLEZ-PÉREZ M.A., SOSA P., BATISTA F., 2009 - *Genetic variation and conservation of the endangered endemic* Anagyris latifolia *Brouss. ex Willd. (Leguminosae) from the Canary Islands*. - Plant System. Evol., 279: 59-68.

HAMRICK J.L., GODT M.W., 1990 - *Allozyme diversity in plant species*, pp. 43-63. - BROWN A.H.D., and M.T. CLEGG, A.L. KAHLER, and B.S. WEIR (eds.) *Plant population genetics, breeding, genetic resources*. Sinauer, Sunderland, MA, USA, pp. 449.

HAMRICK J.L., GODT M.W., 1996 - *Effects of life history traits on genetic diversity in plant species.* - Phil. Trans. R. Soc. Lond. B, 351: 1291-1298.

HÖGLUND J., 2009 - *Evolutionary conservation genetics.* - Oxford University Press, Oxford, UK, pp. 200.

JEONG J.H., KIM E.H., GUO W., YOO K.O., JO D.G., KIM Z.S., 2010 - *Genetic diversity and structure of the endangered species* Megaleranthis saniculifolia *in Korea as revealed by allozyme and ISSR markers*. - Plant System. Evol., 289: 67-76.

JONES C., EDWARDS K., CASTAGLIONE S., WINFIELD M., SALA F., VAN DE WIEL C., BREDEMEIJER G., VOSMAN B., MATTHES M., DALY A., 1997 - *Reproducibility testing of RAPD, AFLP and SSR markers in plants by a network of European laboratories*. - Mol. Breeding, 3: 381-390.

LAIKRE L., 2010 - *Genetic diversity is overlooked in international conservation policy implementation.* - Conservation Genetics, 11: 349-354.

LI J., JIN Z., 2007 - *Genetic variation and differentiation in* Torreya jackii *Chun, an endangered plant endemic to China.* - Plant Science, 172: 1048-1053.

MOZAFFARIAN V., 2015 - *Recognition of medicinal and aromatic herbs of Iran, Tehran*. - Moaser Press, Farhange, Iran, pp. 1316.

NEI M., 1972 - *Genetic distance between populations*. - Amer. Naturalist, 106: 283-292.

NOROOZISHARAF A., HATAMZADEH A., LAHIJI H.S., BAKHSHI D., 2015 - *Genetic diversity of endangered primrose (*Primula heterochroma *Stapf.) accessions from Iran revealed by ISSR and IRAP markers*. - Scientia Horticulturae, 190: 173-178.

NYBOM H., 2004 - *Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants*. - Mol. Ecol., 13: 1143-1155.

QIU Y.-X., HONG D.-Y., FU C.-X., CAMERON K.M., 2004 - *Genetic variation in the endangered and endemic species* Changium smyrnioides (*Apiaceae*). - Bioch. System. Ecol., 32: 583-596.

REED D.H., FRANKHAM R., 2003 - *Correlation between fitness and genetic diversity*. - Conservation Biol., 17: 230-237.

ROHLF F., 2000 - *NTSYS-PC, Numerical Taxonomy and Multivariate Analysis System*, V 2.1. Applied Biostatistics Inc.

SALMANIAN S., SADEGHI A., 2012 - *Measurement of total phenol, flavonoid, carotenoid and antioxidant activity of Gijavash*. - Third national symposium of Iran agricultural biotechnology. University of Ferdowsy, Mashahd, Iran.

SHANNON C., WEAVER W., 1949 - *The mathematical theory of communication.* - The University of Illinois Press, Urbana, IL, USA.

SHAO J.-W., CHEN W.-L., PENG Y.-Q., ZHU G.-P., ZHANG X.-P., 2009 - *Genetic diversity within and among populations of the endangered and endemic species* Primula merrilliana *in Chin*a. - Bioch. System. Ecol., 37: 699-706.

SMITH T.B., WAYNE R.K., 1996 - *Molecular genetic approaches in conservation*. - Oxford University Press, Oxford, UK, pp. 498.

SUDHA C., KRISHNAN P., PUSHPANGADAN P., 1998 - In vitro *propagation of* Holostemma annulare *(Roxb.) K. Schum., a rare medicinal plant*. - In Vitro Cell. Devel. Biol. - Plant, 3: 57-63.

TOMIMATSU H., OHARA M., 2003 - *Genetic diversity and local population structure of fragmented populations of* Trillium camschatcense (*Trilliaceae*). - Biol. Conservation, 109: 249-258.

VICENTE M.J., SEGURA F., AGUADO M., MIGLIARO D., FRANCO J.A., MARTÍNEZ-SÁNCHEZ J.J., 2011 - *Genetic diversity of* Astragalus nitidiflorus*, a critically endangered endemic of SE Spain, and implications for its conservation*. - Bioch. System. Ecol., 39: 175-182.

WILLIAMS J.G., KUBELIK A.R., LIVAK K.J., RAFALSKI J.A., TINGEY S.V., 1990 - *DNA polymorphisms amplified by arbitrary primers are useful as genetic markers*. - Nucleic Acids Res., 18: 6531-6535.

WU C.-J., CHENG Z.-Q., HUANG X.-Q., YIN S.-H., CAO K.-M., SUN C.-R., 2004 - *Genetic diversity among and within populations of* Oryza granulata *from Yunnan of China revealed by RAPD and ISSR markers: implications for conservation of the endangered species*. - Plant Sci.,

167: 35-42.

XIAO L.Q., GE X.J., GONG X., HAO G., ZHENG S.X., 2004 - *ISSR variation in the endemic and endangered plant* Cycas guizhouensis *(Cycadaceae)*. - Annals Bot., 94: 133-138.

YEH F.C., 1999 - *POPGENE (version 1.3. 1). Microsoft Window-Bases Freeware for Population Genetic Analysis.* - http://www. ualberta. ca/~ fyeh/.

ZHENG W., WANG L., MENG L., LIU J., 2008 - *Genetic variation in the endangered* Anisodus tanguticus (Solanaceae)*, an alpine perennial endemic to the Qinghai-Tibetan Plateau*. - Genetica*,* 132: 123-129.