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GENETIC DIVERSITY AND INTER-RELATIONSHIP AMONG *Stellaria* L. (CARYOPHYLLACEAE) SPECIES BY ISSR MARKERS

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Stellaria L. (Caryophyllaceae, Alsinoideae) comprises *ca*. 150–200 species across the world. *Stellaria* species are common herbs, preferred humid mountainously slopes, but some grew in desert. Main center of diversification for *Stellaria* is Eurasia, with a center of distribution in the mountains of E. central Asia. Therefore, due to the importance of these plant species, we performed a molecular data analysis for this genus. For this study, we used 65 randomly collected plants from six species in six provinces. Amplification of genomic DNA using 5 primers produced 70 bands, of which 62 were polymorphic (84.00%). The obtained high average PIC and MI values revealed high capacity of ISSR primers to detect polymorphic loci among *Stellaria* species. The genetic similarities of six collections were estimated from 0.69 to 0.85. According to Inter-Simple sequence repeats (ISSR) markers analysis, *S. pallida* and *S. graminea* had the lowest similarity and the species of *S. media* and *S. pallida* had the highest similarity. The aims of present study are: 1) can ISSR markers identify *Stellaria* species, 2) what is the genetic structure of these taxa in Iran, and 3) to investigate the species inter-relationship? The present study revealed that ISSR markers can identify the species.

Keywords: Iran, Species Identification; Structure, *Stellaria* species, ISSR markers

INTRODUCTION

Identifying the accurate boundaries of a species is critical to have a better perspective of any biological studies. Therefore, species delimitation is a subject of extensive part of studies in the framework of biology (COLLARD and MACKILL, 2009; WU *et al.*, 2013). However, defining the criterion which could address the boundaries of species is matter of debate (ESFANDANI-BOZCHALOYI *et al*., 2018a, 2018b). Wild relatives of crops contain genes with the great potential for use in breeding programs and constitute a part of their gene pool (PANDEY *et al*., 2008). In

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addition, the study of intra-specific levels of genetic variation and investigation of genetic structure of wild populations is crucial for development of effective conservation strategies.

Stellaria L. (Caryophyllaceae, Alsinoideae) comprises *ca*. 150–200 species across the world (BITTRICH, 1993). This genus has nine species grouped in two sections. *S. blatteri* Mattf., *S. scaturiginella* Rech.f. and *S. sarcophylla* Rech.f. have an uncertain section (RECHINGER, 1988). According to *Flora Iranica*, *Stellaria* sections in Iran include: *Stellaria* with two annual species [*S. media* (L.) Vill. and *S. pallida* (Dumort.) Pire] and four perennial species which grow in the mountain areas, including *S. holostea* L., *S. persica* Boiss., *S. graminea* L., and *S. nemorum* L. Section *Pseudalsine* Boiss. consists of only one annual species (*S. alsinoides* Boiss. & Buhse) growing in the mountains of Iran. *Stellaria* species are common herbs, preferred humid mountainously slopes, but some grew in desert. Main center of diversification for *Stellaria* is Eurasia, with a center of distribution in the mountains of E. central Asia. Some species are also cosmopolitan (BITTRICH, 1993). There are limited chromosome records for *Stellaria* in the world. Basic Chromosome numbers of x=10, 11, 12 and 13 have been reported for the genus. The genus is characterized by the presence of five sepals and petals which are usually bifid; however, in some species the petals are markedly reduced or absent (FIOR *et al.*, 2006; HARBAUGH *et al*., 2010). Previous study on species delimitation and species relationship performed in this genus. VERKLEIJ *et al.* (1980) obtain some more information about the genetic differences among and between the two species and within S. *media* between the two local sub-populations, by means of the electrophoretically detectable variation in isoenzymes. They showed for five enzymes there was a difference in isoenzyme pattern between the species *Stellaria media* and *Stellaria pallida*. Of these five enzymes two showed an interpopulational variation in *Stellaria media.* The fact that there was almost no variability of the isoenzyme pattern in *Stellaria pallida* could be explained by the permanent autogamous (cleistogamous) state of the species. Effects of polyploidy on the isoenzyme pattern and its activity could not be demonstrated. Literature revealed that studies are mainly dealing with taxonomy, seed and pollen morphology, stem and leaf anatomy (KESHAVARZI and ESFANDANI-BOZCHALOYI, 2014a, 2014b; ESFANDANI-BOZCHALOYI and KESHAVARZI, 2014) of *Stellaria* species but there are no attempt to study genetic diversity, ecological adaptation and intra- and inter-specific differentiation along with morphometric studies on *Stellaria* of Iran. Therefore, we performed morphological and molecular study of 65 collected specimens of 2 section in *Stellaria.*

Among advanced genetic markers, Random Amplified Polymorphic DNA (RAPD) and Inter Simple Sequence Repeats (ISSR) markers have been widely used for diversity analyses (PHARMAWATI *et al*., 2004). RAPD technique is quick, easy and requires no prior sequence information. The technique detects nucleotide sequence polymorphism using a single primer of arbitrary nucleotide sequence (MORENO *et al*., 1998). ISSR marker involves PCR amplification of DNA by a single 16-18 bp. long primer composed of a repeated sequence anchored at the 3' or 5' end of 2-4 arbitrary nucleotides. The technique is rapid, simple, inexpensive and more reproducible than RAPD (ESFANDANI-BOZCHALOYI *et al*., 2017a, 2017b, COLLARD and MACKILL, 2009; WU *et al.*, 2013). The present investigation has been carried out to evaluate the genetic diversity and relationships among different *Stellaria* species using new gene-targeted molecular markers, i.e ISSR markers. This is the first study on the use of ISSR markers in *Stellaria* genus. Therefore, we performed molecular study of 65 collected specimens of 6 *Stellaria* species. We

try to answer the following questions: 1) Is there infra and interspecific genetic diversity among studied species? 2) Is genetic distance among these species correlated with their geographical distance? 3) What is the genetic structure of populations and taxa? 4) Is there any gene exchange between *Stellaria* species in Iran?

MATERIALS AND METHODS

Plant materials

A total of 65 individuals were sampled representing 6 geographical populations belonging to 6 *Stellaria* species in Lorestan, Guilan, Mazandaran, Esfahan, Golestan and Kohgiluyeh and Boyer-Ahmad Provinces of Iran during July-Agust 2016-2019 (Table 1). For ISSR analysis we used 65 plant accessions (Five to twelve samples from each populations) belonging to 6 different populations with different eco-geographic characteristics were sampled and stored in -20 till further use. More information about geographical distribution of accessions are in Table 1.

Table 1. Voucher details of *Stellaria* species in this study from Iran

No	Sp.	Locality	Latitude	Longitude	Altitude (m)
Sp1	*S. persica* Boiss.	Kohgiluyeh and Boyer-Ahmad	38 ° 52'37'	47 ° 23' 92'	1144
Sp2	*S. graminea* L.	Mazandaran, Haraz road, Emam Zad-e-Hashem	32°50′03″	51°24′28″	1990
Sp3	*S. alsinoides* Boiss & Buhse	Guilan, Sangar, Road sid	29◦20′07″	51° 52′08″	1610
Sp4	*S. pallida* (Dumort.) pire	Esfahan:, Ghameshlou, Sanjab	38 ° 52'373	47 ° 23' 92'	1144
Sp5	*S. holostea* L.	Lorestan, Oshtorankuh, above Tihun village	33° 57′12″	47° 57′32″	2500
Sp6	*S. media* (L.) VILL.	Golestan, gorgan	34 ° 52'373	48 ° 23' 92'	2200

Morphological studies

Five to twelve samples from each species were used for Morphometry. In total 18 morphological (11 qualitative, 7 quantitative) characters were studied. Data obtained were standardized (Mean= 0, variance = 1) and used to estimate Euclidean distance for clustering and ordination analyses (PODANI, 2000). Morphological characters studied are: corolla shape, bract shape, calyx shape, calyx length, calyx width, calyx apex, calyx margins, bract length, corolla length, corolla width, corolla apex, leaf length and leaf width, leaf apex, leaf margins, leaf shape, leaf gland and bract margins.

DNA Extraction and ISSR Assay

Fresh leaves were used randomly from one to twelve plants in each of the studied populations. These were dried by silica gel powder. CTAB activated charcoal protocol was used to extract genomic DNA (ESFANDANI-BOZCHALOYI *et al.*, 2019). The quality of extracted DNA was examined by running on 0.8% agarose gel. For the ISSR analysis, 22 primers from UBC (University of British Columbia) series were tested for DNA amplification. Five primers were chosen for ISSR analysis of genetic variability, based on band reproducibly (Table 2). PCR reactions were carried in a 25µl volume containing 10 mM Tris-HCl buffer at pH 8; 50 mM KCl; 1.5 mM $MgCl_2$; 0.2 mM of each dNTP (Bioron, Germany); 0.2 µM of a single primer; 20 ng genomic DNA and 3 U of *Taq* DNA polymerase (Bioron, Germany). The amplifications' reactions were performed in Techne thermocycler (Germany) with the following program: 5 min initial denaturation step 94°C, followed by 40 cycles of 1 min at 94°C; 1 min at 52-57°C and 2 min at 72°C. The reaction was completed by final extension step of 7-10 min at 72°C. The amplification products were observed by running on 1% agarose gel, followed by the ethidium bromide staining. The fragment size was estimated by using a 100 bp molecular size ladder (Fermentas, Germany).

Data Analyses

Morphological Studies

Morphological characters were first standardized (Mean = 0, Variance = 1) and used to establish Euclidean distance among pairs of taxa (PODANI, 2000). For grouping of the plant specimens, The UPGMA (Unweighted paired group using average) ordination methods were used (PODANI, 2000). ANOVA (Analysis of variance) were performed to show morphological difference among the populations while, PCA (Principal components analysis) biplot was used to identify the most variable morphological characters among the studied populations (PODANI, 2000). PAST version 2.17 (HAMMER *et al.*, 2012) was used for multivariate statistical analyses of morphological data.

Molecular Analyses

ISSR bands obtained were coded as binary characters (presence = 1, absence = 0) and used for genetic diversity analysis. Discriminatory ability of the used primers was evaluated by means of two parameters, polymorphism information content (PIC) and marker index (MI) to characterize the capacity of each primer to detect polymorphic loci among the genotypes (POWELL *et al.*, 1996). MI is calculated for each primer as MI = PIC × EMR, where EMR is the product of the number of polymorphic loci per primer (*n*) and the fraction of polymorphic fragments (β) (HEIKRUJAM *et al.*, 2015). The number of polymorphic bands (NPB) and the effective multiplex ratio (EMR) were calculated for each primer. Parameter like Nei's gene diversity (H), Shannon information index (I), number of effective alleles, and percentage of polymorphism (P% =number of polymorphic loci/number of total loci) were determined (WEISING *et al.*, 2005, FREELAND *et al.*, 2011). Shannon's index was calculated by the formula: H' = -Σpiln pi. Rp is defined per primer as: Rp = ∑ Ib, were "Ib" is the band informativeness, that takes the values of 1-(2x [0.5-p]), being "p" the proportion of each genotype containing the band. The percentage of polymorphic loci, the mean loci by accession and by population, UHe,

H' and PCA were calculated by GenAlEx 6.4 software (PEAKALL and SMOUSE, 2006). Nei's genetic distance among populations was used for Neighbor Joining (NJ) clustering and Neighbor-Net networking (FREELAND *et al*., 2011; HUSON and BRYANT, 2006). Mantel test checked the correlation between geographical and genetic distances of the studied populations (PODANI, 2000). These analyses were done by PAST ver. 2.17 (HAMMER *et al.*, 2012), DARwin ver. 5 (2012) software. AMOVA (Analysis of molecular variance) test (with 1000 permutations) as implemented in GenAlex 6.4 (PEAKALL and SMOUSE, 2006) were used to show genetic difference of the populations. Gene flow was determined by (i) Calculating Nm an estimate of gene flow from Gst by PopGene ver. 1.32 (1997) as: Nm = 0.5(1 - Gst)/Gst. This approach considers the equal amount of gene flow among all populations.

RESULTS

Species identification and inter-relationship

Morphometry

ANOVA showed significant differences (P <0.01) in quantitative morphological characters among the species studied. In order to determine the most variable characters among the taxa studied, PCA analysis has been performed. It revealed that the first three factors comprised over 67% of the total variation. In the first PCA axis with 50% of total variation, such characters as corolla shape, calyx shape, calyx length, bract length and leaf shape have shown the highest correlation (>0.7), leaf apex, corolla length, leaf length, leaf width were characters influencing PCA axis 2 and 3 respectively. Different clustering and ordination methods produced similar results therefore, PCA plot of morphological characters are presented here (Figs 1). In general, plant samples of each species were grouped together and formed separate groups. These results show that both quantitative and qualitative morphological characters separated the studied species into distinct groups. In the studied specimens we did not encounter intermediate forms.

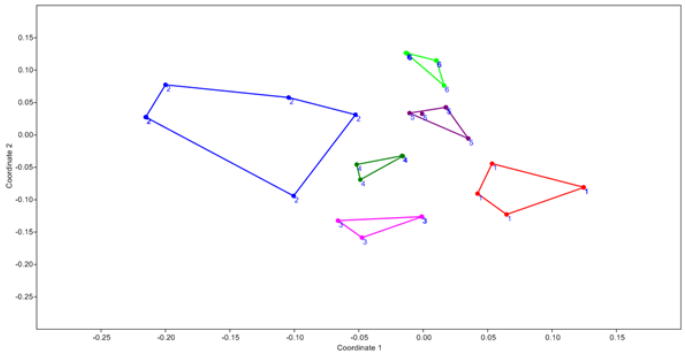

Figure 1. PCA plots of morphological characters revealing species delimitation in the *Stellaria* species.

Species Identification and Genetic Diversity

Five ISSR primers were screened to study genetic relationships among *Stellaria* species; all the primers produced reproducible polymorphic bands in all 6 *Stellaria* species. An

image of the ISSR amplification generated by ISSR-2, ISSR-3 primer is shown in Figure 2. A total of 62 amplified polymorphic bands were generated across 6 *Stellaria* species. The size of the amplified fragments ranged from 100 to 3000 bp. The highest and lowest number of polymorphic bands was 14 for ISSR-5 and 10 for ISSR-4, on an average of 12 polymorphic bands per primer. The PIC of the 5 ISSR primers ranged from 0.44 (ISSR-1) to 0.57 (ISSR-4) with an average of 0.51 per primer. MI of the primers ranged from 3.15 (ISSR-1) to 6.44 (ISSR-3) with an average of 4.9 per primer. EMR of the ISSR primers ranged from 2.25 (ISSR-1) to 5.56 (ISSR-5) with an average of 4.3 per primer (Table 2). The primers with the high EMR values were considered to be more informative in distinguishing the genotypes.

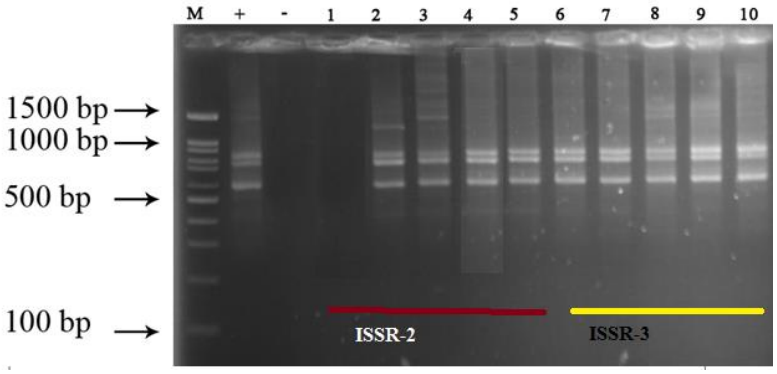

Figure 2. Electrophoresis gel of studied ecotypes from DNA fragments produced by ISSR-2, ISSR-3.

Table 2. ISSR primers used for this study and the extent of polymorphism

Primer name	Primer sequence (5'-3')	TNB	NPB	PPB	PIC	PI	EMR	MI
ISSR-1	DBDACACACACACACACA	13	13	100.00%	0.44	5.66	2.25	3.15
ISSR-2	GGATGGATGGATGGAT	18	12	66.99%	0.55	6.91	5.46	4.85
ISSR-3	GACAGACAGACAGACA	15	12	88.00%	0.53	4.34	4.55	6.44
ISSR-4	AGAGAGAGAGAGAGAGYT	10	10	100.00%	0.57	5.88	5.56	4.85
ISSR-5	ACACACACACACACACC	14	14	100.00%	0.49	5.23	3.23	4.40
Mean		13	12	84.00%	0.51	5.5	4.3	4.9
Total		70	62					

TNB - the number of total bands, NPB: the number of polymorphic bands, PPB (%): the percentage of polymorphic bands, PI: polymorphism index, EMR, effective multiplex ratio; MI, marker index; PIC, polymorphism information content

The genetic parameters were calculated for all the 6 *Stellaria* species amplified with ISSR primers (Table 3). Unbiased expected heterozygosity (*H*) ranged from 0.21 (*S. alsinoides*) to 0.37 (*S. pallida*), with a mean of 0.29. A similar pattern was observed for Shannon's

information index (*I)*, with the highest value of 0.49 observed in *S. pallida* and the lowest value of 0.24 observed in *S. alsinoides* with a mean of 0.31. The observed number of alleles (*N*a) ranged from 0.122 in *S. holostea* to 0.577 in *S. alsinoides.* The effective number of alleles (*N*e) ranged from 1.033 (*S. media*) to 1.347 (*S. persica*).

Table 3. Genetic diversity parameters in the studied Stellaria species. Abbreviations:

SP	N	Na	Ne	I	He	UHe	%P
S. persica Boiss.	5.000	0.358	1.347	0.484	0.27	0.29	44.50%
S. graminea L.	6.000	0.299	1.234	0.281	0.28	0.25	40.38%
S. alsinoides Boiss & Buhse	3.000	0.577	1.062	0.24	0.224	0.213	34.73%
S. pallida (Dumort.) pire	8.000	0.499	1.067	0.49	0.381	0.37	59.26%
S. holostea L.	9.000	0.122	1.034	0.272	0.23	0.23	53.15%
S. media (L.) VILL.	6.000	0.545	1.033	0.25	0.20	0.30	52.53%

(N = number of samples, I= Shannon's information index, He = gene diversity, UHe = unbiased gene diversity, P%= percentage of polymorphism, populations).

AMOVA test showed significant genetic difference (P = 0.001) among studied species. It revealed that 53% of total variation was among species and 48% was within species (Table 4) Moreover, genetic differentiation of these species was demonstrated by significant Nei's GST (0.73, P = 0.001) and D_est values (0.311, P = 0.001). These results revealed a higher distribution of genetic diversity among *Stellaria* species compared to within species.

Table 4. Analysis of molecular variance (AMOVA) of the studied species

Source	df	SS	MS	Est. Var.	%	*ΦPT*
Among Pops	18	1456.364	44.229	23.124	52%	
Within Pops	57	234.443	3.805	11.844	48%	53%
Total	73	1655.807		34.060	100%	

df: degree of freedom; SS: sum of squared observations; MS: mean of squared observations; EV: estimated variance; *Φ*PT: proportion of the total genetic variance among individuals within an accession, (P < 0.001).

Different clustering and ordination methods produced similar results therefore, NJ clustering are presented here (Figure 3). In general, plant samples of each species belong to a distinct section, were grouped together and formed separate cluster. This result show that molecular characters studied can delimit *Stellaria* species in two different major clusters or groups. In the studied specimens we did not encounter intermediate forms. In general, two major clusters were formed in NJ tree (Figure. 3), Populations of *S. alsinoides* were placed in the first major cluster and were placed with great distance from the other species. The second major cluster included two sub-clusters. Plants of *S. holostea , S. persica , S. graminea* comprised the first sub-cluster, while plants of *S. media* and *S. pallida* formed the second sub-cluster.

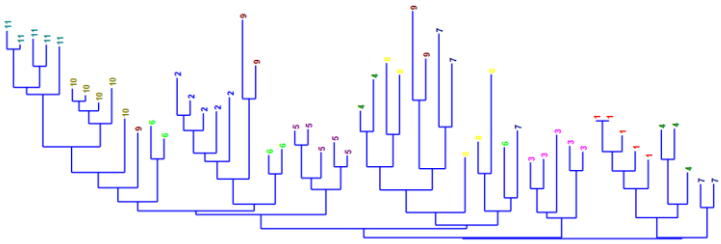

Figure 3. NJ tree of ISSR data revealing species delimitation in the *Stellaria* species

In general, relationships obtained from ISSR data agrees well with species relationship obtained from morphological. This is in agreement with AMOVA and genetic diversity parameters presented before. The species are genetically well differentiated from each other. These results indicate that ISSR molecular markers can be used *Stellaria* species taxonomy. The Nm analysis by Popgene software also produced mean Nm= 0.265, that is considered very low value of gene flow among the studied species.

Mantel test with 5000 permutations showed a significant correlation (r = 0.28, p=0.0002) between genetic distance and geographical distance, so isolation by distance (IBD) occurred among the *Stellaria* species studied.

Nei's genetic identity and the genetic distance determined among the studied species (Table not included). The results showed that the highest degree of genetic similarity (0.85) occurred between *S. media* and *S. pallida*. The lowest degree of genetic similarity occurred between *S. pallida* and *S. graminea* (0.69). The low Nm value (0.265) indicates limited gene flow or ancestrally shared alleles between the species studied and indicating high genetic differentiation among and within *Stellaria* species.

DISCUSSION

Genetic diversity is an important role in biology of long-term evolution of a taxon or a population. The basis of existence, growth, and evolution is taxon. Thus, the study of genetic diversity of taxon is fundamental to recognize the taxonomy, origin, and evolution of taxon. Moreover, such research will provide a theoretical basis for the germplasm resource conservation, development, utilization, and breeding (LUBBERS *et al.*, 1991; JIA *et al*, 2021; BI *et al*, 2021; CHENG *et al*, 2021).

The present research revealed interesting data about its genetic variability, genetic stratification and morphological divergence in north and west part of Iran. Degree of genetic variability within a species is highly correlated with its reproductive mode, the higher degree of

open pollination/ cross breeding brings about higher level of genetic variability in the studied taxon (MEUSEL *et al.,* 1965; MA *et al*, 2021; PENG *et al*, 2021). PIC and MI characteristics of a primer help in determining its effectiveness in genetic diversity analysis. SIVAPRAKASH *et al.* (2004) suggested that the ability of a marker technique to resolve genetic variability may be more directly related to the degree of polymorphism. Generally, PIC value between zero to 0.25 suggest a very low genetic diversity among genotypes, between 0.25 to 0.50 shows a mid-level of genetic diversity and value ≥0.50 suggests a high level of genetic diversity (TAMS *et al.,* 2005). In this research, the ISSR primers' PIC values ranged from 0.44 to 0.57, with a mean value of 0.51, which indicated a high-level ability of ISSR primers in determining genetic diversity among the species of *Stellaria.* All of 5 primer pairs showed a good polymorphism in taxon of *Stellaria.* A total 70 alleles were recognized for the studied species. Total number of bands per primers ranged from 10 to 14 polymorphic bands and the mean of the allele number in loci was 12. *Stellaria media* and *Stellaria pallida* are mainly self-fertile and between these species there exists a crossing barrier (PETERSON, 1936), perhaps mainly due to the diploidy of *S. pallida* (2n = 22) and the hypotetraploidy of *S. media* (2n=40-44) (SCHOLTE, 1978). According to CHINNAPPA and MORTON (1984) the genetic variation and phenotypic plasticity contributing to the population differentiation within the *S. longipes* complex was investigated using isozyme, RFLP, and RAPD analyses, and comparative morphological studies.

In most studies, population size is limited to several vegetative accession (MEUSEL *et al.,* 1965; UOTILA, 1996). This population could be showed genetic drift, whose effect are observed in the high level of F_{IS} and low level of genetic diversity. The isolation of the population and absence the gene flow led to fragmentation of the *Stellaria* populations. Between genetic diversity parameters and population size were showing positive correlations that confirmed various studies (LEIMU *et al.,* 2006). There are two reasons for the positive correlation between genetic diversity and population size (LEIMU *et al.,* 2006). 1- A positive correlation could imply the presence of an extinction vortex, where the drop-in population size lowers genetic diversity, which leads to inbreeding depression. The second reason is the fact that plant fitness differentiates populations based on variations in habitat quality (VERGEER *et al.,* 2003).

According to BOOY *et al.* (2000) the low levels of genetic diversity could reduce plant fitness and restrict a population's ability to respond to changing environmental conditions through selection and adaptation. Genetic diversity (48%) was obtained within populations, whereas 52% of genetic variation obtained between the evaluated populations. One of the key factors determining the distribution of genetic variation is the breeding system in plant species (DUMINIL, 2007). BOOY *et al.* (2000) revealed that one migrant per generation cannot be existed to guarantee long-term survival of small populations and that the number of migrants is demonstrate through life history characters and population genetic (VERGEER *et al.,* 2003).

There are two hypotheses for the absence of differences between isolated populations. The first hypothesis explained that genetic diversity within and between populations demonstrates gene flow processes, which led to the fragmentation of larger populations (DOSTÁLEK *et al.,* 2010). The second hypothesis presented that geographically proximate populations are more efficiently connected through gene flow than populations separated by greater distance. In conclusion, the results of this study showed that to evaluate the genetic diversity of the *Stellaria* genus, the primers derived from ISSR were more effective than the

other molecular markers. Also, *Stellaria* species were clearly separated from each other in the dendrogram and PCA, indicating the higher efficiency of ISSR technique in *Stellaria* species identification.

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GENETIČKI DIVERZITET I MEĐUSOBNI ODNOSI MEĐU VRSTAMA *Stellaria* L. (CARIOPHILLACEAE) PRIMENOM ISSR MARKERA

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Izvod

Stellaria L. (Cariophillaceae, Alsinoideae) obuhvata ca. 150-200 vrsta širom sveta. Vrste *Stellaria* su uobičajene biljke, preferiraju vlažne planinske padine, ali neke su rasle u pustinji. Glavni centar diverzifikacije roda *Stellaria* je Evroazija, sa centrom distribucije u planinama istočne centralne Azije. Zbog toga smo, zbog značaja ovih biljnih vrsta, izvršili molekularnu analizu podataka za ovaj rod. Za ovu studiju koristili smo 65 nasumično sakupljenih biljaka iz šest vrsta u šest provincija. Amplifikacija genomske DNK korišćenjem 5 prajmera dala je 70 traka, od kojih su 62 bile polimorfne (84,00%). Dobijene visoke prosečne vrednosti PIC i MI otkrile su visok kapacitet ISSR prajmera da detektuju polimorfne lokuse među vrstama *Stellaria*. Genetske sličnosti šest kolekcija procenjene su od 0,69 do 0,85. Prema analizi ISSR markera, *S. pallida* i *S. graminea* su imale najmanju sličnost, a vrste *S. media* i *S. palli*¬da su imale najveću sličnost. Ciljevi ove studije su: 1) da li ISSR markeri mogu da identifikuju vrste *Stellaria*, 2) kakva je genetska struktura ovih taksona u Iranu, i 3) da se istraži međuodnos vrsta? Ova studija je otkrila da ISSR markeri mogu identifikovati vrstu.

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