Journal of Tropical Biology and Conservation 13: 57–70, 2016 ISSN 1823-3902

Research Article

SRAP Markers Based Genetic Analysis of *Silene* Species

Tahereh Aghaee Bargish, Fatemeh Rahmani*

Department of Biology and Institute of Biotechnology, Faculty of Sciences, Urmia University, Urmia, Iran.
*Corresponding author: F.Rahmani@urmia.ac.ir

Abstract
Plant genetic resources are an essential part of the world's natural resources, and knowledge of genetic diversity and its distribution is essential for plant conservation. In the present work, the genetic variation was studied among thirteen species of *Silene* genus. Samples were collected from different locations of West Azarbijan and Khorasan provinces in Iran. This investigation, for the first time, has studied sequence-related amplified polymorphism (SRAP) marker to assess the genetic diversity and genetic similarity among 13 species of *Silene* in Iran. These species belonge to eight sections including *Sclerocalycinae, Melandriforms, Lychnidiforms, Inflatae, Lasiocalycinae, Spergolifoliae, Auriculatae* and *Conoimorpha*. Fifteen SRAP primer combinations generated 62 fragments, of which 46 (71.90 %) were polymorphic. Percentage of polymorphism ranged from 50 % to a maximum of 100 % and Jaccard's similarity coefficient ranged from 0.48 to 0.91. Minimum Evolution analysis revealed four main clusters. The study indicates that SRAP markers with targeting ORFs, high reproducibility and optimal marker distribution could be good candidates for assessing genetic variation in *Silene*.

Keywords: Genetic variation, *Silene*, polymorphism, SRAP

Introduction
Silene belongs to silenoideae subfamily, caryophyllaceae family, and is composed of 700 species around the world (Jurgens et al., 2002; Jurgens, 2004). This genus is distributed in Turkey, Russia, Italy, Iraq, Iran, east of Mediterranean, Europe, Japan, Spain and England (Boissier, 1884; Tutin, 1964; Zohary, 1966; Groshkova, 1970; Davis, 1965-1985; Anzalone, 1982; Melzheimer, 1988). In Iran, *Silene* includes 110 species of which 35 are endemic (Melzheimer, 1988).

Plant genetic diversity represents the heritable variation within and between species which is the basis for selection and plant improvement. Hence, genetic

Received 04 January 2016
Reviewed 18 May 2016
Accepted 01 August 2016
Published 15 October 2016

diversity conservation is essential for the future of humans. Plant genetic diversity changes in time and space with extent and distribution depending on breeding system, ecological and geographical factors, past bottlenecks, and human factors. A better knowledge of genetic diversity will help us to understand the taxonomy, origin and evolution of plant species. Morphological characteristics often do not result in a clear identification of species due to effects of different environmental conditions (Ebrahimi et al., 2011). However, DNA markers offer many advantages over morphological characters for the determination of genetic diversity and the identification of species, such as not being influenced by the environment, and detection directly at the DNA level (Joy et al., 2007; Pradeepkumar et al., 2003).There are a variety of DNA marker techniques, such as random amplified polymorphic sequence (RAPD) (Juchum et al., 2007; Ro et al., 2007), amplified restriction fragment polymorphism (AFLP) (Percifield, 2007;Yuan et al., 2007) and microsatellite markers (SSR) (Legesse et al., 2007; Tommasini et al., 2003). The sequence-related amplified polymorphism (SRAP) technique is simple, reliable with moderate throughput ratio and facile sequencing of selected bands. The marker is highly reproducible DNA marker useful for both mapping and gene tagging in plants (Li & Quiros 2001).

SRAP has been shown to be more informative than other PCR-based techniques in detecting genetic diversity (Budak et al., 2004) and has been successfully used to study the genetic diversity of, and relationships among, several species (Ferriol et al., 2003; Budak et al., 2004; Riaz et al., 2004; Esposito et al., 2007; Fu et al., 2008; Feng et al., 2009; Uzun et al., 2009; Castonguay et al., 2010; Talebi et al., 2011b; Abedian et al., 2012). In SRAP, the first ten bases of the forward primers consist of a core sequence with no specific constitution which is followed by CCGG and then by three selective bases at the 3′ end. The reverse primers consist of the same core sequence as the forward primers with the following AATT and then three selective nucleotides to the 3′ end of the primer. The CCGG motif falls usually into exons and exonic sequences are conserved among individuals. In contrast, AATT sequence is frequently found in promoters and introns (Li & Quiros 2001).

Although the genetic diversity of *Silene* genus has been studied by use of molecular markers in the world (Rettig et al., 1992; Richards et al., 2003; Tero et al., 2003; Welch et al., 2006; Minder et al., 2007; Jolivet & Bernasconi, 2007), there is not much systematic study on the genetic relationship among the species of *Silene* based on molecular markers in Iran (Sheidai et al., 2008; Sheidai et al., 2010; Aghaee Bargish & Rahmani, 2015). Specifically, no

attempts have been made to use SRAP markers, which target ORFs as functional regions of the genome resulting in a moderate number of co-dominant markers. SRAP works like a random amplified polymorphic DNA (RAPD) marker, but targets specific regions of the genome (Dalong et al., 2010). The SRAP markers have been shown to be sustainable and less complex compared to other molecular marker systems. The genetic diversity potency of RAPD, ISSR, SSR and SRAP has been reported in the following order: SRAP> SSR> ISSR> RAPD (Budak et al., 2004). Thus, the objective of this study was to assess the genetic relationship between thirteen species of *Silene* genus in Iran using SRAP marker.

Materials and methods

Plant materials. Species of *Silene* genus were collected from their natural growing regions of West Azerbijan and Khorasan area (Figure 1).The details on sample collection are given in Table 1. Fresh young leaves of 5 to 6 plants were collected from the field-grown plants and stored at -80 °C prior to DNA extraction. Plant leaves of the same species were mixed prior to DNA extraction.

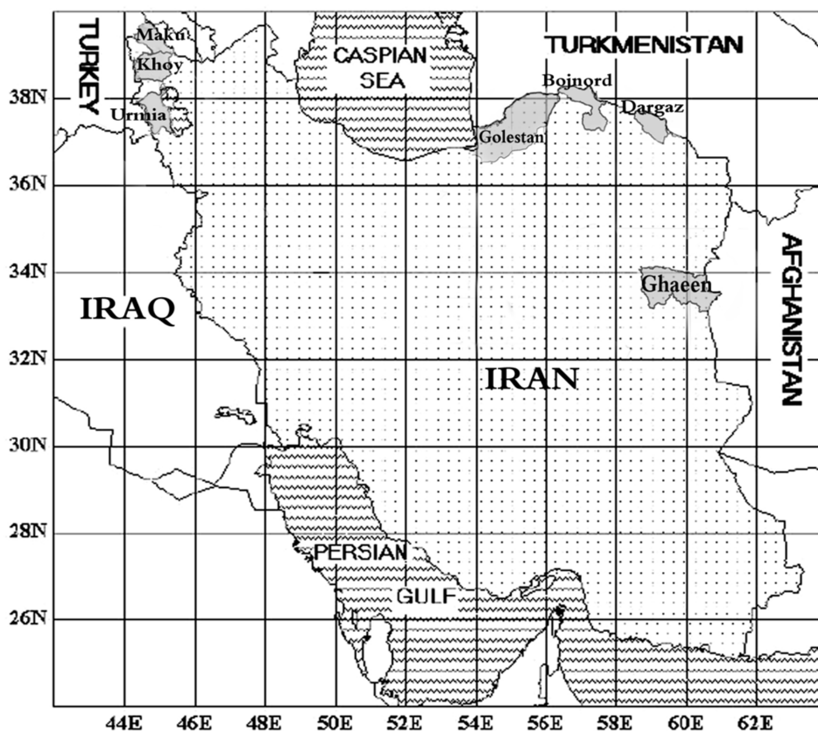

Figure 1. Geographical distribution of selected *Silene* spp. used in this study. The collection area has been highlighted in grey colour.

Table 1. Location of *Silene* species used in the present study.

Bil.	Species	Location
1	*S. bupleuroides*	Urmia, Salmas road, Ghoshchi 1800 m.
2	*S. vulgaris*	Maku Between GarehKhaj and Dibak, 1850 m.
3	*S. aucheriana*	Maku Between GarehKhaj and Torkan 1850 m.
4	*S. dichotoma*	Urmia, Jade Sero, Nazlu, 1273 m.
5	*S. latifolia*	Urmia, Jade Emamzade, 1273 m.
6	*S. chlorifolia*	Urmia, Ghoshchi, koheSomakh, 1800 m.
7	*S. araratica*	Maku Between GarehKhaj and Dibak, 1850 m.
8	*S. conoidea*	Khoy, Psak Village,1700 m.
9	*S. spergulifolia*	Urmia,45 km Oshnaviyeh, 1300 m.
10	*S. parjumanensis*	Western Khorasan, W-Ghaeen, Tajan mountains, 2000 m.
11	*S. noctiflora*	North Khorasan, W-S Bojnord, Rien, 1700 m.
12	*S. indeprensa*	Khorasan, Daregaz, International Park Tounduoreh, 2400 m.
13	*S. coronaria*	Goulestan, E-N International Park Goulestan, 1200 m.

DNA extraction. The genomic DNA was extracted following the modified CTAB method (Liu et al., 2003). The concentration of each DNA sample was determined spectrophotometrically at 260 nm (Biophotometer 6131; Eppendorf, Hamburg, Germany). The quality of genomic DNA was determined by electrophoresis on 1.0 % (w/v) agarose gel and then diluted to 10 ng /µl for PCR reactions.

SRAP-PCR amplification. In total, 5 forward and 9 reverse primers (Guo et al., 2013) were purchased from Cinnagen, Tehran, Iran. Nineteen random SRAP primer combinations were selected for SRAP analysis (Table 2). SRAP amplifications were performed in 25 µl reaction volumes containing 12.5 µl Master mix (Taq DNA polymerase, 10x PCR buffer, dNTPs and $MgCl_2$ and 0.5 µl primer (100 µM) and 9 µl ddH2O and 3 µl of genomic DNA template. Amplification conditions were as follows: initial denaturation at 95 °C for 3 minutes, 5 cycles of 95 °C for 1 minute, 32 °C for 1.5 minute and 72 °C for 1.5 minute; 35 cycles of 95 °C for 1 minute, 55 °C for 1.5 minute and 72 °C for 1.5 minute; followed by a final 10 minutes extension at 72 °C. Amplification products were visualized by 5 hours running on 3 % agarose gel with constant voltage of 50 V, following ethidium bromide staining (10 µg/ ml). The size of DNA fragments were estimated by comparison with the 50 bp Gene Ruler DNA size marker (Fermentas).

Table 2. Primer sequences used for SRAP analysis in this study.

SRAP primers			
Forward primers		**Reverse primers**	
ME1	5'-TGAGTCCAAACCGGATA-3'	EM1	5'-GACTGCGTACGAATTCAAT-3'
ME2	5'-TGAGTCCAAACCGGAGC-3'	EM2	5'-GACTGCGTACGAATTCTGC-3'
ME3	5'-TGAGTCCAAACCGGAAT-3'	EM3	5'-GACTGCGTACGAATTCGAC-3'
ME4	5'-TGAGTCCAAACCGGACC-3	EM4	5'-GACTGCGTACGAATTCTGA-3'
ME5	5'-TGAGTCCAAACCGGTGC-3'	EM6	5'-GACTGCGTACGAATTCGCA-3'
-	-	EM17	5'-GACTGCGTACGAATTCGAG-3'
-	-	EM18	5'-GACTGCGTACGAATTCGCC-3'
-	-	EM19	5'-GACTGCGTACGAATTCTCA-3'
-	-	EM20	5'-GACTGCGTACGAATTCTCC-3'

Table 3. Genetic diversity of *Silene* species revealed by SRAP.

Primers	Total number of bands	Number of polymorphic bands	Number of monomorphic bands	Percentage of polymorphic bands (PPB)	PIC value
ME1-EM2	3	2	1	66. 6	0.44
ME1-EM17	3	3	0	100	0.71
ME2-EM20	4	4	0	100	0.58
ME3-EM19	6	4	2	66.6	0.59
ME4-EM17	2	2	0	100	0.56
ME4-EM18	3	3	0	100	0.37
ME5-EM6	3	3	0	100	0.43
ME1-EM3	6	5	1	83.3	0.79
ME1-EM4	6	5	1	83.3	0.81
ME3-EM4	3	2	1	66.6	0.67
ME4-EM2	4	2	2	50	0.50
ME5-EM1	3	2	1	66.6	0.64
ME5-EM2	3	2	1	66.6	0.64
ME1-EM6	2	1	1	50	0.50
ME5-EM3	2	1	1	50	0.48
ME4-EM1	3	2	1	66.6	0.34
ME3-EM3	2	1	1	50	0.50
ME2-EM6	2	1	1	50	0.36
ME5-EM4	2	1	1	50	0.49
Total	62	46	16	-	-
Mean	3.2	2.4	-	71.90	0.54

Data analysis. The amplified DNA fragments were recorded as either (1) or (0), representing the presence or absence of the band, respectively. Data analysis was conducted using polymorphic bands. Faint or unclear bands were not considered. Amplified fragments were scored to create binary data matrices. Data analyses were performed using the numerical taxonomy multivariate analysis system software package (NTSYS-pc); version 2.02 (Rohlf, 2002) and MEGA4 (Tamura et al., 2007). Polymorphism information content (PIC) values were estimated according to the formula: $PIC = 1 - \Sigma (P_{ij})^2$, where P_{ij} is the frequency of the ith pattern revealed by the jth primer summed across all patterns revealed by the primers (Botstein et al., 1980). Dendrogram was constructed later based on Minimum Evolution method. The representativeness of the dendrogram was evaluated by estimating cophenetic correlation coefficient for the dendrogram and comparing it with the similarities matrix using Mantel matrix correspondence test (Mantel, 1967). For statistical support, bootstrap analysis was performed using MEGA4 with 1000 replicates to obtain the confidence of the tree (Tamura et al., 2007).

Results

SRAP analysis. Nineteen SRAP primer combinations generated a total of 62 bands of which 46 (71.90 %) were polymorphic (Table 4). The number of scorable markers produced per primer ranged from 2 (ME4-EM17, ME1-EM6, ME5-EM3, ME3-EM3, ME2-EM6& ME5-EM4) to 6 (ME1-EM4, ME1-EM3 & ME3-EM19), with an average of 3.2 fragments per primer. The size of the amplified products ranged from 100 bp to 600 bp. Figure 2 represents the SRAP profile of 13 *Silene* species using ME1-EM4 primer.

The PIC values ranged from 0.34 (ME4-EM1) to 0.81 (ME1-EM4) with a mean of 0.54. Jaccard's similarity coefficients based on 62 SRAP markers ranged from 0.48 to 0.90. *S. vulgaris* and *S. indeprensa* showed the lowest genetic similarity (0.48), and *S. conoidea* and *S. araratica* revealed the highest similarity (0.91).

Table 4. Jaccard's similarity coefficient between thirteen *Silene* species based on SRAP molecular marker.

	S. bupleuroides	*S. vulgaris*	*S. aucheriana*	*S. dichotoma*	*S. latifolia*	*S. chlorifolia*	*S. araratica*	*S. conoidea*	*S. spergulifolia*	*S. parjumanensis*	*S. noctiflora*	*S. indeprensa*	*S. coronaria*
S. *bupleuroides*	1.00												
S. vulgaris	0.63	1.00											
S. aucheriana	0.63	0.55	1.00										
S. *dichotoma*	0.72	0.66	0.74	1.00									
S. latifolia	0.66	0.57	0.62	0.74	1.00								
S. chlorifolia	0.78	0.66	0.74	0.82	0.75	1.00							
S. araratica	0.75	0.64	0.67	0.78	0.72	0.84	1.00						
S. conoidea	0.67	0.62	0.62	0.76	0.71	0.76	0.90	1.00					
S. spergulifolia	0.64	0.59	0.78	0.66	0.55	0.72	0.65	0.64	1.00				
S. parjumanensis	0.66	0.57	0.69	0.75	0.65	0.76	0.73	0.66	0.63	1.00			
S. noctiflora	0.67	0.62	0.62	0.82	0.80	0.72	0.74	0.76	0.64	0.66	1.00		
S. indeprensa	0.55	0.48	0.65	0.57	0.54	0.65	0.63	0.62	0.63	0.53	0.54	1.00	
S. coronaria	0.78	0.72	0.70	0.82	0.70	0.76	0.78	0.72	0.72	0.76	0.76	0.54	1.00

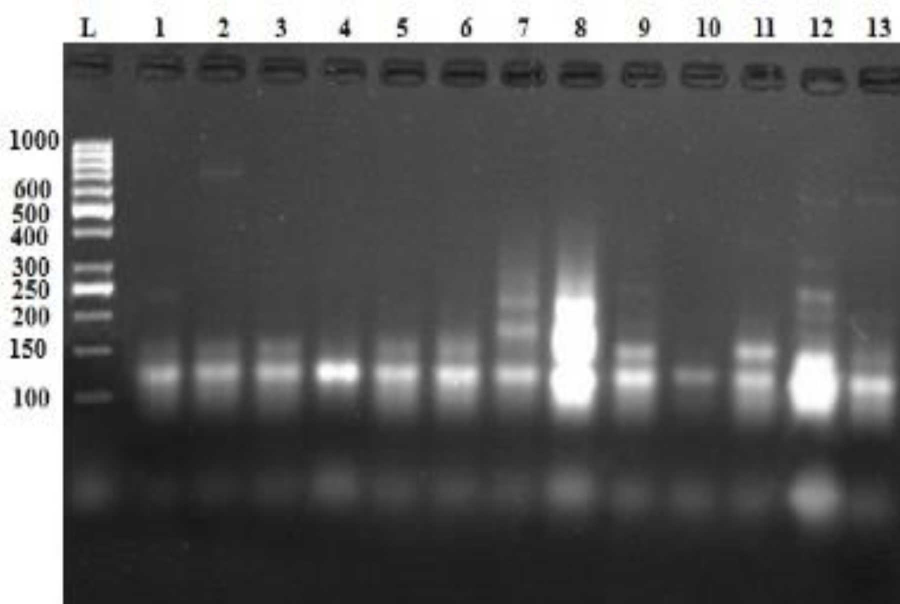

Figure 2. Amplification profile of 13 species of *Silene* based on ME1-EM4 primers combination. The numbers from left to right are representative of 1) *S.bupleuroides;* 2) *S.vulgaris;* 3) *S.aucheriana;* 4) *S.dichotoma*; 5) *S.latifolia;* 6) *S.chlorifolia;* 7) *S.araratica;* 8) *S. conoidea;* 9) *S.spergulifolia;* 10) *S.parjumanensis;* 11) *S.noctiflora;* 12) *S.indeprensa;* 13) *S.coronaria.* L represents 50bp DNA ladder.

The dendrogram, constructed based on Minimum Evolution method using MEGA4 software distinguished four main groups among 13 species of *Silene* (Figure 3). Cluster I comprised of 8 species that were delineated into two sub-clusters. Sub-cluster I included *S.latifolia, S. noctiflora, S.dichothoma, S.araratica* and *S.conoidea*. Sub-cluster II consisted of *S. parjumanensis, S.vulgaris and S.coronaria*. Clusters II and III contained *S.bupleuroides* and *S.chlorifolia*, respectively. Cluster IV comprised of *S.indeprensa, S.aucheriana* and *S.spergulifolia*.

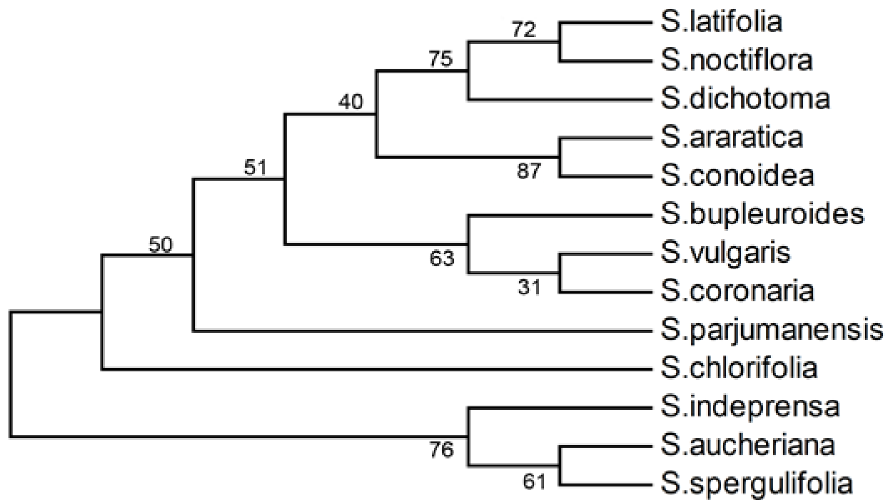

Figure 3. Dendrogram obtained by Minimum Evolution cluster analysis based on SRAP

Discussion

There are many examples of successful application of molecular markers in genetic diversity analysis. Study of genetic relationship among plant taxa at species or/and genera level is very important because it provides information about the direction and sequential scale for plant evolution (Savolainen & Chase, 2003). SRAP marker with the advantages of RAPD markers and AFLP markers is a relatively new type of molecular marker and is more suitable for application in practice because of its features such as simple, low-cost test, security and rich in polymorphism (Li & Quiros, 2001; Li & Zhang, 2005). Rich polymorphism of these features is the most important characteristics. SRAP has also been reported as the most powerful DNA marker due to polymorphism

detection in coding sequences which are usually conserved among closely related species and varieties with low mutation rate (Mishra et al., 2011).

In this study, 19 SRAP primer combinations generated 62 fragments, of which 46 (71.90 %) were polymorphic. This value appeared to be lower than other SRAP based studies, detecting higher polymorphism, e.g. 95.76 % for *Dianthus* accessions (Xiao et al., 2008) and 93 % for coffee species (Mishra et al., 2011). However, ISSR molecular analysis detected higher genetic variability (98.85 %) among these *Silene* species (Aghaee Bargish & Rahmani, 2015). The reason behind this difference could be explained by association of ISSR markers to both coding and non-coding genome sections (Roldan-Ruiz et al., 2001), while SRAP marker targets the coding sequence (Liaol et al., 2012). Detection of relatively high polymorphism indicates efficiency of SRAP markers for analyzing polymorphism in *Silene*. In the SRAP study, the mean number of alleles obtained for each primer was 2.4. The obtained value appeared to be much lower than 14.3 and 18.6 reported for *Dianthus* accessions (Xiao et al., 2008) and *Eremochloa ophiuroides* (Milla-Lewis et al., 2012), respectively, which could be related to difference in species and populations. Our genetic variability analysis detected similarity coefficient ranged from 0.48 to 0.90 (Table 4) reflecting sufficient amount of diversity among *Silene* species in Iran. It has been known that genetic variation is influenced by a number of evolutionary factors such as mating system, seed dispersal, geographic range, natural selection and gene flow (Hamrick & Godt, 1989). Jolivet & Bernasconi (2007) also studied genetic and morphological diversity in six populations of *S. latifolia* and reported significant molecular and genetic differentiation.

Polymorphic information content (PIC) as a diagnostic capacity of the marker is associated not only to polymorphic alleles, but also to polymorphic allele frequencies. Based on this definition, values > 0.5 are classified as highly informative diversity loci (Botstein et al., 1980). In our study, the average PIC value for SRAP marker primer combinations obtained 0.54 revealing the high efficiency of applied primers in the isolation of *Silene* samples which could be proposed for similar studies. The ME4-EM1 (0.34) and ME1-EM4 (0.81) primer combinations produced the lowest and highest PIC value, respectively.

For accurate detection of genetic diversity between species, cluster analysis was performed by help of the Minimum Evolution method which revealed four main clusters (Figure 3). In general, SRAP clustering was in accordance with morphological classification. Based on morphological classification, *S. latifolia* and *S.noctiflora* belong to *Sect. Melandriformes* (Boissier, 1867) and *S.*

coronaria belong to *Sect. Lychnidiformes* (Melzh, 1988). Clustering based on SRAP grouped the *S.latifolia* and *S.noctiflora* in one cluster (Sub-cluster I) and *S. coronaria* was placed in cluster II. Morphologically, they are the closest relatives and *Sect. Melandriformes* differs from *Sect. Lychnidiformes* based on calyx veins and indumentum (Edalatiyan et al., 2010). The SRAP marker placed *S.bupleuroides* and *S.coronaria* in one group (Cluster I, sub-cluster II). Although, the ISSR and SRAP markers target different regions of the genome, clustering based on ISSR also placed these two species in one group (Aghaee Bargish & Rahmani, 2015). The *S. araratica* and *S. conoidea* appeared to have the maximum similarity (0.90). These two species belong to close geographical area according to distribution map of *Silene* in Iran (Table 1).

According to our SRAP molecular marker investigation, S. *indeprensa, S. aucheriana, and S. spergulifolia* were grouped in cluster IV. *S. spergulifolia* belongs to *Sect. Spergulifoliae* and *S. aucheriana* and *S. indeprensa* belong to *Sect. Auriculatae* (Boissier, 1867). These two sections are morphologically close with difference in flower type and inflorescence (Edalatiyan et al., 2010). These species were also grouped in one cluster according to our ISSR clustering (Aghaee Bargish & Rahmani, 2015).
The statistical support for clustering exhibited bootstrap values greater than 31 % (Figure 3). The branch point that grouped *S. araratica* and *S. conoidea* had the highest value (87 %). These two species are of close geographical origin. Up to now, only a few studies have looked at genetic diversity in *Silene* (Rettig et al., 1992; Richards et al., 2003; Tero et al., 2003; Welch et al., 2006; Minder et al., 2007; Jolivet & Bernasconi, 2007). The present study demonstrated that genetic fingerprinting based on SRAP is informative for estimating the extent of genetic diversity, as well as to determine the pattern of genetic relationships. Our results revealed sufficient level of genetic distance (0.10 to 0.52) among 13 different *Silene* species in Iran. However, application of more molecular markers is proposed in generating future information (Mishra et al., 2011). In this study, the existence of a relatively high polymorphism level was sufficient enough to establish fingerprints with relatively few primer sets. Additional phylogenetic studies using appropriate nuclear, chloroplast and mitochondrial gene sequences could also help to evaluate the systematic position of *Silene* species.

Acknowledgment

This research was financially supported by the Ministry of Sciences and carried out in the Biotechnology Research Center of Urmia University.

References

Abedian M, Talebi M, Golmohammdi HR, Sayed Tabatabaei BE. 2012. Genetic diversity and population structure of mahaleb cherry (*Prunus mahaleb* L.) and sweet cherry (*Prunus avium* L.) using SRAP markers. *Biochemical Systematics and Ecology* **40:**112–117

Aghaee Bargish T, Rahmani F. 2015. Assessment of Genetic Diversity of *Silene* (Caryophyllaceae) Species Using ISSR Molecular Marker. Jordan Journal of Agricultural Sciences **11(4)**:1037-1047

Anzalone B, Becherer A, Ehrendorfer F, Merxmuller H, Metlesics H, Montelucci G, Rasetti F, Ichstein T, Segelberg I. 1982. Flora De Italia (*Silene*). *Edagricole* **1:**238-263

Boissier E. 1867."Flora Orientalis, Caryophyllaceae", Vol. 1, Geneva et Basieer Aputh. Georg, Bibliopolam: pp. 567–656

Boissier E. 1884. Flora Orientalis **5:**537-678

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

Budak H, Shearman RC, Parmaksiz I, Dweikat I. 2004. Comparative analysis of seeded and vegetative biotype buffalo grasses based on phylogenetic relationship using ISSRs, SSRs, RAPDs, and SRAPs. *Theoretical and Applied Genetics* **109(2):**280–288

Castonguay Y, Cloutier J, Bertrand A, Michaud R, Laberge S. 2010. SRAP polymorphisms associated with superior freezing tolerance in alfalfa (Medicago sativa spp. sativa). *Theoretical and Applied Genetics* **120(8):**1611–1619

Chowdhuri PK. 1957. Studies in the genus *Silene*. *Notes from the Royal Botanic Garden, Edinburgh* **22:**221–278

Davis, Peter Hadland. 1965. *Flora of Turkey and the east Aegean islands*. Vol. 10. Edinburgh University Press.

Ebrahimi A, Fatahi R, Zamani Z. 2011. Analysis of genetic diversity among some Persian walnut genotypes (*Juglans regia* L.) using morphological traits and SSRs markers. *Scientia horticulturae* **130(1):**146-151

Edalatiyan MN, Ghahermaninejad F, Attar F, Joharchi MR. 2010. A taxonomic study on the genus *Silene* (Caryophyllaceae) in Iran, *Rostaniha* **11(2):**133-149

Esposito MA, Martin EA, Cravero VP, Cointry E. 2007. Characterization of pea accessions by SRAP's markers. *Scientia horticulturae* **113(4):**329–335

Feng F, Chen M, Zhang D, Sui X, Han S. 2009. Application of SRAP in the genetic diversity of Pinus koraiensis of different provenances. *African Journal of Biotechnology* **8(6):**1000–1008

Ferriol M, Pico B, Nuez F. 2003. Genetic diversity of a germplasm collection of Cucurbita pepo using SRAP and AFLP markers. *Theoretical and Appllied Genetics* **107(2):**271–282

Fu X, Ning G, Gao L, Bao M. 2008. Genetic diversity of Dianthus accessions as assessed using twomolecularmarker systems (SRAPs and ISSRs) and morphological traits. *Scientia horticulturae* **117(3):**263–270

Gorshkova SG, Illin MM, Knorring OE, Kuzeneva OI, Murav OA, Tolmachev AI, Shishkin BK, Shtenberg EI, Vasil chenko IT. 1970. Flora of the U.S.S.R Vol. VI, *Centrospermae, Izdatel Stvo Akademii Nauk SSSR Moskva Leningard*: 447-528

Guo LL, Liu XJ, Liu XC, Yang ZM, Kong DY, He YJ, Feng ZY. 2013. Advance in Barley Sciences. In: Zhang G, Li C, Liu X. (Eds.) *The construction of molecular genetic map of barley using SRAP markers*. Springer Dordrecht Heidelberg New York London. Pp. 433-457

Hamrick JL, Godt MJW. 1989. Allozyme diversity in plant species. In: Brown AHD, Clegg MT, Kahler AL, Weir BS. (Eds.) *Plant Population Genetics, Breeding, and Germplasm Resources*. Sinauer Associates, Sunderland. Pp. 43-63

Jolivet C, Bernasconi G. 2007. Molecular and quantitative genetic differentiation in European populations of *Silene latifolia* (Caryophyllaceae). *Genetics* **177(2):**1239-1247

Joy N, Abraham Z, Soniya EV. 2007. A preliminary assessment of genetic relationships among agronomically important cultivars of black pepper. *BMC Genetics* **8(1)**:42

Jürgens A, Witt T, Gottsberger G. 2002. Flower scent composition in night flowering *Silene* species (Caryophyllaceae). *Biochemical Systematics and Ecology* **30(5):**383-397.

Jürgens AT. 2004. Flower scent composition in diurnal *Silene* species (Caryophyllaceae): Phylogenetic constraints or adaptation to flower visitors. *Biochemical Systematics and Ecology* **32(10):** 841-859

Juchum FS, Leal JB, Santos LM, Almeida MP, Ahnert D, Corrêa RX. 2007. Evaluation of genetic diversity in a natural rosewood population using RAPD markers. *Genetic and Molecular Biology* **6:**543-553

Legesse BW, Myburg AA, Pixley KV, Botha AM. 2007. Genetic diversity of African maize inbred lines revealed by SSR markers. *Hereditas* **144:**10-17

Liao L, Guo Q, Wang ZL, Zhu Z. 2012. Genetic diversity analysis of *Prunella vulgaris* in China using ISSR and SRAP markers. *Biochemical Systematics and Ecology* **45:**209-217

Li G, Quiros CF. 2001. Sequence-related amplified polymorphism (SRAP), a new marker system based on a simple PCR reaction: its application to mapping and gene tagging in Brassica. *Theoretical and Applied Genetics* **103(2-3):**455–461

Li Y, Zhang C. 2005. Studies on genetic diversity with a molecular marker SRAP of watermelon hybrids. *Acta Horticulturae Sinica* **32(4):**643-647

Liu L, Guo W, Zhu X, Zhang T. 2003. Inheritance and fine mapping of fertility restoration for cytoplasmic male sterility in *Gossypium hirsutum* L. *Theoretical and Applied Genetics* **106(3):**46-469

Mantel NA. 1967. The detection of disease clustering and a generalized regression approach. *Cancer Research* **27(2 part 1):**209–220

Melzheimer V. 1988. Flora Iranica (*Silene*-Caryophyllaceae).Rechinger KH. (Ed), **163:**341-508

Milla-Lewis SR, Kimball JA, Carolina ZN, Harris-Shultz KR, Schwartz BM, Hanna WW. 2012. Use of sequence-related amplified polymorphism (SRAP) markers for comparing levels of genetic diversity in centipedegrass (*Eremochloa ophiuroides* (Munro) Hack.) germplasm. *Genetic resources and crop evolution* **59(7):**1517–1526

Minder AM, Rothenbuehler C, Widmer A. 2007. Genetic structure of hybrid zones between *Silene latifolia* and *Silene dioica* (Caryophyllaceae): evidence for introgressive hybridization. *Molecular Ecology* **16(12):**2504-2516

Mishra KM, Nishani S. 2011. Molecular identification and genetic relationships among coffee species (*Coffea* L.) inferred from ISSR and SRAP marker analyses. *Archives of Biological Sciences* **63(3)**:667-679

Percifield RJ, Hawkins JS, McCoy J, Widrlechner MP, Wendel JF. 2007. Genetic diversity in *Hypericum* and AFLP Markers for species-specific identification of *H. perforatum* L. *Planta Medica* **73:**1614-1621

Pradeepkumar T, Karihaloo JL, Archak S, Baldev A. 2003. Analysis of genetic diversity in *Piper nigrum* L. using RAPD markers. *Genetic Resources and Crop Evolution* **50(5)**:469-475

Rettig JH, Wilson HD, Manhart JR. 1992. Phylogeny of the Caryophyllales: Gene sequence data. *Taxon* **41:**201-209

Riaz A, Potter D, Stephen M. 2004. Genotyping of peach and nectarine cultivars with SSR and SRAP molecular markers. *Journal of the American Society for Horticultural Science* **129(2):**204–211

Richards CM, Emery SN, McCauley DE. 2003. Genetic and demographic dynamics of small populations of *Silene latifolia*. *Heredity* **90(2):**181-186

Rohrbach P. 1869. Monographie der Gattung *Silene*. Leipzig.

Ro HS, Kim SS, Ryu JS, Jeon C, Lee TS, Lee H. 2007 Comparative studies on the diversity of the edible mushroom *Pleurotus eryngii*: ITS sequence analysis, RAPD fingerprinting, and physiological characteristics. *Mycological research* **111(6):**710-715

Roldan-Ruiz FA, Gilliland TJ, Dubreuil P, Dillmann C, Lallemand J. 2001. A comparative study of molecular and morphological methods of describing relationships between perennial ryegrass (*Lolium perenne* L.) varieties. *Theoretical and Applied Genetics* **103(8):**1138-1150

Rohlf FJ. 2002. NTSYSpc: Numerical taxonomy system, ver. 2.1.*Setauket, NY: Exeter Publishing Ltd.*

Savolainen V, Chase MW. 2003. A decade of progress in plant molecular phylogenetics. *Trends in Genetics* **19(12)**:717-724

Sheidai M, Gholipour A, Noormohammadi Z. 2010. Species relationship in the genus Silene L. Section Auriculatae (Caryophyllaceae) based on morphology and RAPD analyses. *Acta Biologica Szegediensis* **54(1)**:25-31

Sheidai M, Nikoo M, Gholipour A. 2008. Cytogenetic variability and new chromosome number reports in *Silene* L., species (Sect. Lasiostemones, Caryophyllaceae). *Acta Biologica Szegediensis.* **52(2):**313-319

Talebi M, Hajiahmadi Z, Rahimmalek M. 2011b. Genetic diversity and population structure of four Iranian alfalfa populations revealed by sequence-related amplified polymorphism (SRAP) markers. *Journal of Crop Science and Biotechnology* **14(3):**173–178

Tamura K, Dudley J, Nei M, Kumar S. 2007. MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Molecular biology and evolution* **24(8)**:1596-1599

Tero N, Aspi J, Siikamaki P, Jakalaniemi A, Tuomi J. 2003. Genetic structure and gene flow in a metapopulation of an endangered plant species, *Silene tatarica. Molecular Ecology* **12(8):**2073-2085

Tolmachev B, Shishkin K, Shtenberg EI, Vasil chenko IT. 1970. Centrospermae, Izdatel Stvo Akademii Nauk SSSR Moskva Leningard. Flora of the U.S.S.R Vol. VI:447-528

Tommasini L, Batley J, Arnold GM, Cooke RJ, Donini P, Lee D, Law JR, Lowe C, Tutin TG. 1964. Flora Europaea (*Silene*-Caryophyllaceae) Heywood VH, Burges NA, Valentined H, Walters SM, Webb DA. (Eds). **1:**158-181

Uzun A, Yesiloglu T, Aka-Kacar Y, Tuzcu O, Gulsen O. 2009. Genetic diversity and relationships within Citrus and related genera based on sequence related amplified polymorphism markers (SRAPs). *Scientia Horticulturae* **121(3)**:306-312

Xiao Peng F, GuoGui N, LiPing G, ManZhu B. 2008. Genetic diversity of *Dianthus* accessions as assessed using two molecular markers system (SRAPs and ISSRs) and morphological traits. *Scientia Horticulturae* **117(3):**263-270

Welch ME, Darnell MZ, McCauley DE. 2006. Variable Populations Within Variable Populations: Quantifying Mitochondrial Heteroplasmy in Natural Populations of the Gynodioecious Plant *Silene vulgaris. Genetics* 174**(2):**829–837

Zohary M. 1966. Flora of Palaestina (Caryophyllaceae). The Academy of Sciences and Humanities. *Jerusalem* **1:**81-100

Yuan Z, Yin Y, Qu J, Zhu L, Li Y. 2007. Population genetic diversity in Chinese pomegranate (*Punica granatum* L.) cultivars revealed by fluorescent-AFLP markers. *Journal of Genetics and Genomics* **34(12):**1061-1071