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Genetic Diversity and Structure of Iranian Teucrium (*Teucrium polium* L.) Populations Assessed by ISSR Markers

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ABSTRACT

Understanding of genetic diversity is essential in breeding programs and plant genetic resources management. In this study, the genetic diversity of 77 individuals of Teucrium from different regions of Iran was investigated using 18 ISSR markers. A total number of 198 bands were detected by ISSR primers, of which 184 (92.9%) bands with an average of 10.2 bands per primer were polymorphic. The Percentage of Polymorphic bands (PPL) ranged from 80 (UBC834) to 100% (UBC811, 812, 818, 820, 825, 826, and UBC855). The average Polymorphic Information Content (PIC), Shannon's Information index (I), and Number of effective alleles (Ne) were 0.39, 0.526, and 1.6, respectively. The Analysis of Molecular Variance (AMOVA) revealed the higher level of genetic variation within populations (77%) compared to among populations (23%). Cluster analysis separated the individuals into three major groups using WPGMA based on Nei's genetic distance coefficients. In addition, a model-based Bayesian approach subdivided the individuals into three major subgroups. The results of this study revealed that estimation of population genetics parameters using ISSR markers can be applied for assessing the differences between Teucrium populations and management of the genetic resources.

Keywords: AMOVA, Model-based cluster, Shannon's information index, Teucrium polium L.

INTRODUCTION

Medicinal plants are considered as a rich source of chemical substances with potential therapeutic effects which can be used in drug development and synthesis (Rasool Hassan, 2012). *Teucrium polium* L. (Lamiaceae family) is a perennial and outcrossing species. The plant grows as a wild plant principally in different geographical locations around the world but mainly in the Mediterranean region (Boulila, *et al*., 2010; Khanahmadi and Rezazadeh, 2010). *T. polium*, (named in Persian "Kalpooreh") is abundantly found in Iran and has long been widely used in traditional medicine for treatment of many health disorders (Gorgini Shabankare, *et al*., 2015; Parsaee and Shafiee, 2006).

From a series of researches, it has been found that aerial parts, roots, and seeds of *Teucrium polium* contain many active phytochemical compounds. Also, it has been found that the extract and individual components isolated from different parts of *T. polium* plant possess a broad spectrum of pharmacological and biological effects such as antioxidant, anticancer, antiinflammatory, antinociceptive, antispasmodic, hypoglycemic, hypotensive, antiulcer, antibacterial, and antifungal (Bahramikia and Yazdanparast, 2012;

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Jaradat, 2015). Moreover, *T. polium* has recently been introduced as a new hyperaccumulator plant and suggested for phytoextraction of Ni in contaminated soils (Yaman, 2014).

Genetic diversity patterns can provide insights into evolutionary and demographic history of a taxon. Exploitation, evaluation, and strategies for *in situ* and *ex situ* conservation of genetic diversity present in natural populations are imperative to guarantee sustainable development (Smolik *et al*., 2011). Also, availability of sufficient knowledge on genetic diversity and distributional pattern of genetic resources are necessary for plant breeding programs (Jain *et al*., 2002). Molecular markers play an important role in achieving these goals. Molecular markers provide information ranging from diversity at the nucleotide level to gene and allele frequencies, extent and distribution of genetic diversity, and population structure. Such information can be useful for management and the development of effective conservation strategies (Sarwat *et al*., 2012), and efficient breeding and improvement of breeding programs (Lörz and Wenzel, 2007).

Inter-simple sequence repeat (ISSR) technique is one of the most widely used Polymerase Chain Reaction (PCR)-based DNA marker systems (Reddy *et al*., 2002). ISSR technique is based on the amplification of DNA segments between two microsatellite repeated regions (Zietkiewicz *et al*., 1994). This technique has a better reproducibility than Random Amplified Polymorphic DNA (RAPD), is easy to detect, and can be produced at lower costs than Amplified Fragment Length Polymorphism (AFLP). It is simpler to use than the Simple Sequence Repeat (SSR) technique and less restrictive than Restriction Fragment Length Polymorphism (RFLP), and may offer considerable variation among species (Archak *et al*., 2003). ISSR combines the advantages of AFLP and SSR markers with the convenience of RAPD (Zietkiewicz *et al*., 1994). Therefore, ISSR is an ideal technique for fingerprinting and a useful alternative to single-locus or hybridization-based methods (Aghaei *et al*., 2012; Dashchi *et al*., 2012; Modareskia *et al*., 2012). ISSR has been widely used to study genetic variation in *Salvia miltiorrhiza* (Song *et al*., 2010), *Passiflora alata* and *P. edulis* (Santos *et al*., 2011), *Ocimum basilicum* (Aghaei *et al*., 2012), *Foeniculum vulgare* (Bahmani *et al*., 2012), *Carum copticum* (Modareskia *et al*., 2012), *Chamomilla recutita* (Okon *et al*., 2013), *Ricinus communis* (Goodarzi *et al*., 2015), and *Papaver bracteatum* and *P. somniferum* (Mohseni *et al*., 2015). It has also been used in characterization of genetic structure in *Podophyllum hexandrum* (Alam *et al*., 2008), quality control of the medicinal plant *Armillaria mellea* (Zheng *et al*., 2009), authentication of medicinal plant *Swertia chirayita* (Tamhankar *et al*., 2009) and to study genetic diversity in two wild populations of *Emmenopterys henryi* (Li and Jin, 2008) and *Glycyrrhiza uralensis* (Yao *et al*., 2008). A few studies have been performed on the genetic diversity of *Teucrium polium* using molecular markers. RAPD markers were used to assess the genetic diversity within and among seven Tunisian diploid and polyploid populations of *T. polium* (Boulila *et al*., 2010), six Iranian populations of *T. polium* (Pesaraklu *et al*., 2013), and selected *T. polium* populations in Jordan (Al-Rawashdeh, 2015). Also, Djabou *et al*. (2012) investigated genetic diversity of two *T. polium* subspecies from western Algeria and Corsica using chemical, chloroplast and ribosomal nuclear markers.

The main objectives of the present study were: (1) To evaluate the potential of ISSR technique to detect the level of diversity present in populations of *T. polium* from

different geographical regions of Iran, (2) To assess the genetic diversity and population structure of this medicinal plant species, and (3) To provide basic information for conservation, breeding, and genetic improvement programs.

MATERIALS AND METHODS

Plant Materials and DNA Isolation

In this study, 77 individuals belonging to eight *Teucrium polium* populations were surveyed (Table 1). The seeds of four Populations (P1-P4) were collected from Urmia region and the seeds of the other four Populations (P5-P8) were provided from the Research Institute of Forest and Rangelands, Tehran, Iran. DNA was extracted from young leaves based on the procedure described by Doyle and Doyle (1990). The quantity and quality of extracted genomic DNA samples were determined by spectrophotometer and 1% agarose gel electrophoresis, respectively.

ISSR Analysis

Thirty ISSR primers were screened using a few DNA samples, of which 18 with high polymorphic and discernible banding patterns were selected for further analysis (Table 2).

Polymerase Chain Reactions (PCRs) were carried out in a Bio-Rad thermocycler (Bio-Rad Laboratories Inc., Hercules, CA, USA). The 20 µL PCR reactions contained 40 ng of genomic DNA, 1X PCR buffer (10 mMTris-HCL, 50 mM KCL, pH= 8.3), 1.5 mM $MgCl_2$, 0.2 µM dNTP, 0.5U Taq polymerase and 10 pmol primer. The thermal profile for PCR was as follows: 94°C initial denaturation for 4 minutes, then 37-35 cycles of 94°C for 40 seconds, 50 to 55°C for 40 seconds, and 72°C for 2 minutes, then, 72°C for 5 minutes. ISSR bands were separated by electrophoresis in 1.8% agarose gels (Trade Mark) and visualized with ethidium bromide (1.0 µg mL^{-1}). The electrophoretic patterns of the PCR products were photographed under UV light using a Gel-Doc image analysis system (Gel Logic 212 PRO).

Data Analysis

The amplification products were scored for the presence (1) and absence (0) of bands for 77 individuals and a binary matrix was constructed for future analysis. Number of polymorphic bands, Number of private bands (Np), percentage of polymorphic bands, Nei's genetic diversity (h), the Total genetic diversity from all genotypes (H_T), and mean of within population diversity (H_S) were calculated using GenAlEx 6.5 (Peakall and Smouse, 2012). The coefficient of gene differentiation (G_{ST}) was calculated as described by Nei (1973).

Table 1. *Teucrium polium* populations from different geographical regions of Iran.

Code	Region collected	Accession number	Number of individuals
P1	Urmia-Shahrchai	U11	10
P2	Urmia-Garehbag	U21	10
P3	Urmia-Garehbag	U22	10
P4	Urmia-Garehbag	U23	10
P5	Yazd-Bafgh	24191	10
P6	Yazd-Mehrriz	24202	10
P7	Bushehr	24511	10
P8	Isfahan-Khorbyabanak	27213	7

Table 2. Diversity and differentiation obtained by primers of ISSR marker applied to eight populations of *Teucrium polium*.

Primer	Sequence (5'-3')	Bands				Diversity					differentiation	
		Size (bp)	Total	Polymorphic	PPL	Ne [a]	I [b]	PIC [c]	H_T [d]	Hs [e]	Gst [f]	Nm [g]
UBC808	$(AG)_8C$	500-1500	10	8	80.00	1.710	0.556	0.43	0.385	0.287	0.255	1.462
UBC811	$(GA)_8C$	500-2000	12	12	100	1.605	0.532	0.39	0.352	0.285	0.192	2.107
UBC812	$(GA)_8A$	500-2000	13	13	100	1.572	0.515	0.41	0.338	0.258	0.237	1.613
UBC816	$(CA)_8T$	300-2000	14	13	92.86	1.671	0.567	0.39	0.385	0.286	0.258	1.440
UBC818	$(CA)_8G$	500-2000	8	8	100	1.642	0.539	0.35	0.362	0.231	0.363	0.877
UBC820	$(GT)_8C$	400-2000	13	13	100	1.661	0.568	0.44	0.384	0.266	0.308	1.124
UBC822	$(TC)_8A$	500-2500	9	8	88.89	1.514	0.483	0.45	0.320	0.265	0.172	2.406
UBC825	$(AC)_8T$	500-1500	9	9	100	1.565	0.506	0.34	0.339	0.207	0.388	0.789
UBC826	$(AC)_8C$	500-2000	13	13	100	1.647	0.553	0.40	0.368	0.231	0.372	0.845
UBC834	$(AG)_8YT$	300-1500	10	8	80.00	1.604	0.502	0.41	0.339	0.207	0.388	0.789
UBC836	$(AG)_7YA$	75-2000	14	12	85.71	1.471	0.465	0.29	0.298	0.247	0.172	2.405
UBC840	$(GA)_8YT$	300-1000	9	8	88.89	1.585	0.508	0.38	0.343	0.214	0.375	0.834
UBC848	$(CA)_8RG$	500-2000	9	8	88.89	1.633	0.530	0.34	0.359	0.250	0.304	1.146
UBC855	$(AC)_8YT$	400-3000	12	12	100	1.589	0.536	0.39	0.352	0.248	0.297	1.183
UBC857	$(AC)_8YG$	500-2000	7	6	85.71	1.400	0.408	0.35	0.264	0.233	0.118	3.731
UBC864	$(ATG)_6$	500-3000	11	10	90.91	1.705	0.585	0.41	0.401	0.298	0.257	1.448
UBC880	$(GGAGA)_3$	300-1500	14	13	92.86	1.689	0.566	0.40	0.384	0.259	0.328	1.026
A12	$(GA)_6CC$	300-2000	11	10	90.91	1.512	0.476	0.39	0.311	0.245	0.213	1.848
Total	-	-	198	184	-	-	-	-	-	-	-	-
Means	-	-	11	10.22	92.93	1.604	0.526	0.39	0.352	0.252	0.283	1.268

[a] Effective Number of alleles; [b] Shannon's Information index; [c] Polymorphism Information Content; [d] Total diversity; [e] Within population diversity; [f]Coefficient of Gene differentiation, [g] Gene flow. R= Purine (A/G), Y= Pyrimidine (C/T).

$G_{ST} = (H_T - H_S)/H_T$ (1)

The Polymorphism Information Content (PIC) was calculated using the formula: $PIC = 2 f_i (1-f_i)$ (2)

Where, f_i is the frequency of the i^{th} amplified band present (Roldan-Ruiz *et al*., 1993). Moreover, observed Number of alleles (Na), effective Number of alleles (N_e), mean of expected Heterozygosity (He), Shannon's information index, Number of total bands (N) for each population and Nei's unbiased genetic distance were obtained by the PopGene V1.31 (Yeh *et al*., 1999). PopGene software was also used to calculate the pairwise Nei's genetic distances among populations. Analysis of Molecular Variance (AMOVA) was performed using GenAlEx V6.5 (Peakall and Smouse, 2012) to partition the total molecular variance into within and among populations variation. Genetic distance between each pair of genotypes was calculated (Nei, 1972), and a dendrogram based on the Nei's genetic distance matrix was constructed by application of the Weighted Pair Group Method with Arithmetic average (WPGMA) cluster analysis using DARwin V6 (Perrier and Jacquemoud-Collet, 2006). DARwin was also used to perform a Principal Coordinate Analysis (PCoA) based on the Nei's distance matrix.

Population structure analysis was carried out through a Bayesian-based model using Structure 2.3.4 software (Pritchard *et al*., 2000) using a burn-in period of 10,000 and 10,000 Markov Chain Monte Carlo iterations (MCMC) runs and a model allowing for admixture and correlated allele frequencies. At least five runs of structure were done by setting the number of subgroups (K) from 1 to 10, and an average Likelihood value *L(K)* was calculated for each *K* across all runs. The model choice criterion to detect the most probable value of *K* was *ΔK*, an *ad hoc* quantity related to the second-order change of the log probability of data with respect to the number of clusters inferred by Structure (Evanno *et al*., 2005; Zhao *et al*., 2009).

RESULTS AND DISCUSSION

In the present study, out of 30 ISSR primers, 18 selected primers amplified 198 clear and discernible bands, of which 184 (92.9%) were polymorphic, showing the high discriminative and resolving power of the used ISSRs on the studied germplasm. Gel electrophoresis pattern obtained by using primer UBC822 is illustrated in Figure 1. The number of total bands per primer ranged from seven (UBC857) to 14 (UBC816, UBC836 and UBC880), with an average of 11.0. The number of polymorphic bands per primer varied from 6 (UBC857) to 13 (UBC812, UBC816, UBC820, UBC826 and UBC880), with an average of 10.22. The band sizes of amplified products were found between 200 to 3,000 bp. The highest size difference (300 to 3,000 bp) between the amplified products was obtained with the primers UBC855, UBC857 and UBC836, while the lowest one (400 to 1,000 bp) was obtained with primer UBC818 (Table. 2).

To characterize the capacity of each primer to detect polymorphism and to evaluate the discriminating ability of each primer in the studied germplasm, various diversity indices such as the highest percentage of polymorphic bands, *Ne*, *I* and *PIC* were calculated .The highest percentage of polymorphic bands was produced by UBC811, UBC812, UBC818, UBC820, UBC825, UBC826, UBC855 primers (100%), while the primers UBC808 and UBC834 produced the lowest percentage of polymorphic bands (80%). The value of effective mean Number of alleles (Ne) per primer ranged from 1.71±0.345 (UBC808) to 1.40±0.266 (UBC857) with an average 1.604. *PIC* values across all primers averaged 0.39. UBC808 and UBC836 showed the highest (0.43) and the lowest (0.29) *PIC* values, respectively. The mean

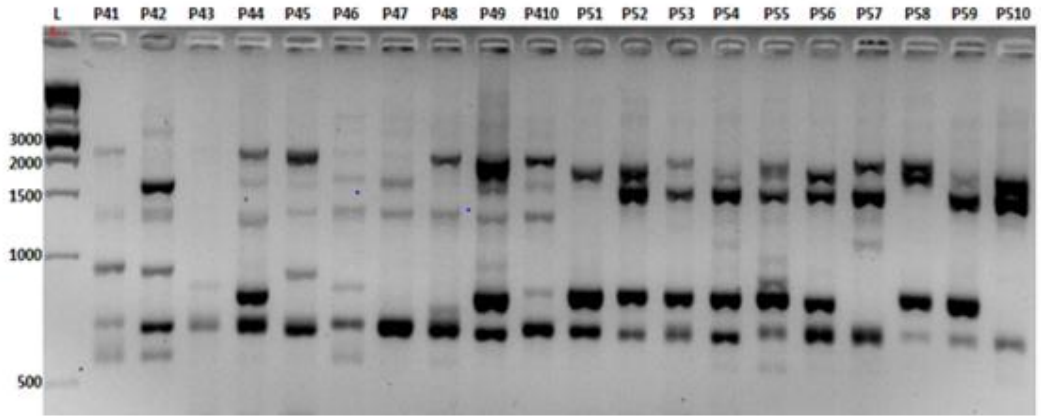

Figure 1. Polymorphism detected by ISSR primer UBC822 in *Teucrium polium* genotypes. Lanes from left to right, (P41 to P410): Individuals from population Urmia-Garehbag (U23), (P51 to P510): Individuals from population Yazd-Bafgh (24191), and (L): 1 Kb DNA ladder in base pair.

Nei's gene diversity (h) ranged from 0.4±0.102 (UBC864) to 0.261±0.151 (UBC857), with an average of 0.352±0.017. Shannon's information index (I) varied from 0.585±0.102 (UBC865) to 0.408±0.216 (UBC857), with an average of 0.526±0.166 (Table 2).

The coefficient of Genetic differentiation (Gst) ranged from 0.388 (UBC825 and UBC834) to 0.118 (UBC857) with an average of 0.283. The level of gene flow (Nm), which was computed based on Gst, was estimated and varied from 0.789 (UBC834) to 3.731 (UBC857), with an average of 1.268 (Table 2).

Genetic diversity statistics for the populations are summarized in Table 3. At the population level, a relatively high genetic diversity was detected. The highest values of Percentage of Polymorphic bands (PPL), expected Heterozygosity (He), and Shannon's Information index (I) were observed in the P2 population. In contrast, the P8 population showed the lowest values for all indices. Population P2 with the high level of genetic diversity can be considered as a good genetic resource for parental selection in *Teucrium polium* breeding programs. Also, a private ISSR band was observed in the P2 population. Generally, the private and unique bands could be transformed as distinct fingerprint into STS (Sequence-Tagged Site) and SCAR (Sequence Characterized Amplified Regions) markers in order to develop the species specific marker for the best management and accurate identification of the plant genetic materials

The Nei's genetic distance between populations ranged from 0.076 (between P6 and P7 populations) to 0.234 (between P5 and P6 populations), with an average of 0.166 (Table 4). The genetic distance among populations is a valuable parameter to conserve and use them in breeding programs. It was proved that crosses between unrelated parents belong to genetically diverse populations may show more heterosis (Reif *et al*., 2007). Hence, individuals belonging to populations with sufficient genetic distance could be introduced as potentially appropriate parents in different *Teucrium polium* breeding programs to explore more heterosis as well as to produce mapping populations.

Table 3. Genetic diversity indices on *Teucrium polium* populations obtained by 18 ISSR primers.

Index[a]	P1	P2	P3	P4	P5	P6	P7	P8	Average (SE[h])
Na [a]	1.556	1.631	1.515	1.57	1.470	1.611	1.551	1.328	1.530
(SE)	(0.050)	(0.049)	(0.056)	(0.052)	(0.055)	(0.049)	(0.053)	(0.060)	(0.019)
N_e [b]	1.372	1.465	1.416	1.442	1.379	1.442	1.410	1.347	1.409
(SE)	(0.026)	(0.027)	(0.026)	(0.027)	(0.027)	(0.027)	(0.026)	(0.027)	(0.009)
He [c]	0.222	0.268	0.245	0.254	0.221	0.257	0.243	0.203	0.239
(SE)	(0.014)	(0.014)	(0.014)	(0.014)	(0.014)	(0.014)	(0.014)	(0.014)	(0.005)
I [d]	0.337	0.399	0.367	0.378	0.331	0.383	0.366	0.303	0.358
(SE)	(0.019)	(0.019)	(0.019)	(0.020)	(0.020)	(0.019)	(0.019)	(0.020)	(0.007)
N_P [e]	0	1	0	0	0	0	0	0	0
N [f]	174	175	162	170	163	174	167	150	166.88
P_P [g]	67.68	74.75	69.70	71.72	64.65	73.23	70.71	57.07	68.69

[a] Observed Number of alleles; [b] Effective Number of alleles; [c] Expected Heterozygosity; [d] Shannon's Information index; [e] Number of private bands; [f] Number of polymorphic bands; [g] Percentage of Polymorphic bands, [h] Standard Error.

Table 4. Genetic distance among 8 populations of *Teucrium polium* based on ISSR data.

Populations	P1	P2	P3	P4	P5	P6	P7
P2	0.187						
P3	0.137	0.149					
P4	0.181	0.077	0.158				
P5	0.171	0.162	0.140	0.129			
P6	0.204	0.204	0.179	0.181	0.234		
P7	0.199	0.187	0.165	0.164	0.221	0.076	
P8	0.123	0.204	0.126	0.197	0.167	0.166	0.171

Distribution of total genetic variation by AMOVA for ISSR dataset attributed 77% of the genetic diversity to differences between individuals within populations (intra-population) in comparison to a low proportion (23%) of genetic diversity allocated to inter-population differentiation, indicating that overall genetic differentiation among populations was relatively low, (P= 0.001, PhiPT= 0.230) (Table 5).

Allogamous and cross-pollinated nature of species are essentially explained by more within population level of genetic variation (Rossetto *et al*., 1995), whereas self-pollinated plants or plants with vegetative reproduction are often presented by more among population level of genetic variation (Tachida *et al*., 1996). Similar results have been reported in other cross-pollinated medicinal plant species such as *Ricinus*

Table 5. Analysis of Molecular Variance (AMOVA) for within and among diversity of *Teucrium polium* populations.

Source	df [a]	MS [b]	Estimated variance	%	*PhiPT* Value	*PhiPT* Prob
Among Pops	7	128.539	9.923	23%	0.230	0.001
Within Pops	69	33.175	33.175	77%		
Total	76		43.098	100%		

[a] Degrees of freedom; [b] Mean of Squares.

communis (Goodarzi *et al*., 2015) and also *Teucrium polium* (Boulila *et al*., 2010). Genetic variation is due to numerous factors including mating system, gene flow, genetic drift, seed and pollen scattering, human activities, long-term evolutionary history, natural selection, and breeding systems (Hamrick *et al*., 1996). Considering the importance of medicinal plants in our country, we believe that human activities (seed transformation) and cross pollination are probably the main reasons of high genetic diversity in Iranian *T. polium* populations.

The WPGMA dendrogram based on Nei's genetic distance clustered the 77 assessed individuals into three main groups (Figure 2).

Populations coming from Yazd-Mehrriz (P6) and Bushehr were classified in group I and Urmia-Garehbag (P2) and Urmia-Garehbag (P4) populations were clustered in group II. Group III divided to four subgroups (P5, P3, P8 and P1) containing Yazd-Bafgh, Urmia-Garehbag, Isfahan-Khorbyabanak and Uremia-Shahrchai

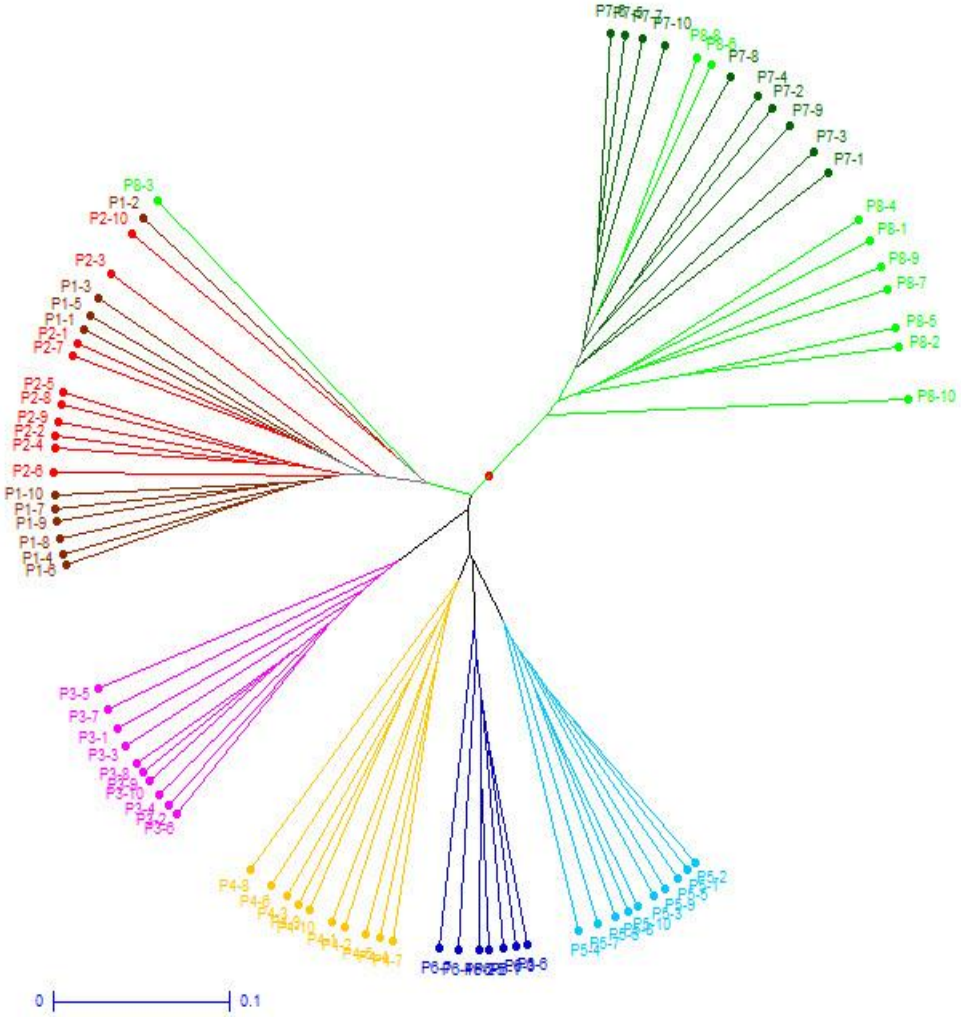

Figure 2. Dendrogram of 77 *Teucrium polium* individuals generated by WPGMA method based on Nei's genetic distance.

populations. The distribution of 77 individuals belonging to eight different populations across respective groups did not reflect the geographic origins perfectly. The high degree of genetic variation revealed in dendrogram may be due to the out-crossing nature of the studied plant material. Knowledge about the genetic relationships of genotypes provides useful and helpful information for germplasm management and planning intraspecific crosses in *Teucrium polium* breeding programs. Also, the PCoA using Dice genetic similarity values of Teucrium populations mostly supported the WPGMA cluster analysis (Figure 3) and again revealed that there was not a perfect association between genetic relationships of *T. polium* populations and their geographic locations.

In addition to genetic diversity analysis, effective analysis of the population structure and accurate classification of individuals to appropriate subpopulations were carried out by using Bayesian method in the Structure software (Pritchard *et al*., 2000).

This clustering method is based on the allocation of individual to *K* clusters in such a way that Hardy–Weinberg equilibrium and linkage equilibrium are valid within clusters, whereas these kinds of equilibrium are absent between clusters (Pritchard *et al*., 2000).

The results showed a significant population structure in the studied *Teucrium polium* germplasm. Since the distribution of *L(K)* did not show a distinct mode for the true number of *K*, to overcome difficulties in determining the real value of *K*, another

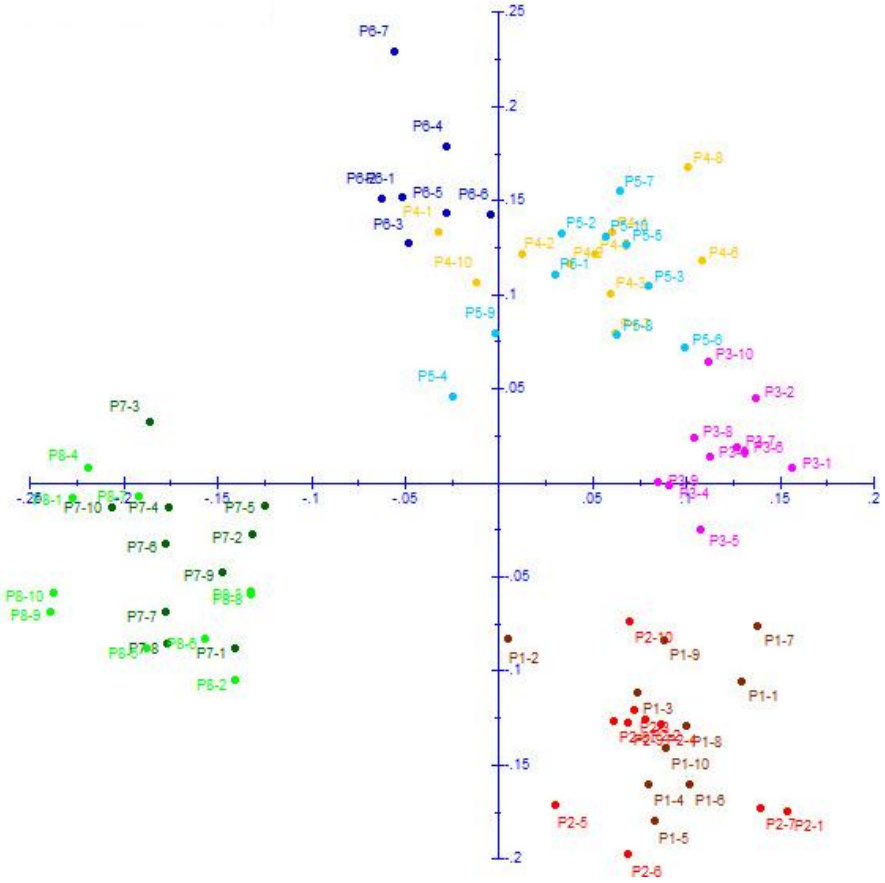

Figure 3. Two dimensional plot of the genetic relationship among Iranian *Teucrium polium* genotypes as revealed by Principal Coordinate Analysis (PCoA).

measurement (ΔK) was used (Evanno *et al*., 2005). In our study, the collection of 77 assayed *T. polium* genotypes was partitioned into three subgroups (K= 3) (Figure 4), approximately similar to the results obtained from cluster analysis based on WPGMA algorithm, with the consideration of the highest value of *ΔK* (Figure 5).

In conclusion, knowledge on the level of genetic variation among accessions, especially on medicine plants, is a prerequisite for their germplasm conservation. Our study revealed the usefulness and utility of ISSR marker system as a molecular diagnosis tool to characterize the genetic diversity, and determin the pattern of genetic structure within and among different *Teucrium polium* populations.

Although, the obtained data from this study might be useful in the future for designing *Teucrium polium* breeding programs and defining strategies for its germplasm conservation, but, a successful management program of *T. polium* cannot be based only on ISSRs. Thus, a combination of molecular, quantitative, physiological, and chemical analyses can be a powerful tool for efficient conservation strategies.

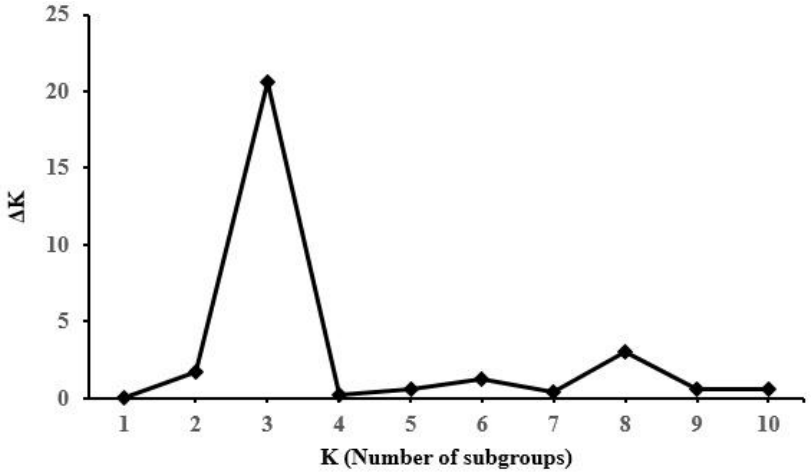

Figure 4. Bilateral charts to determine the optimal number of *K* identified by Structure program.

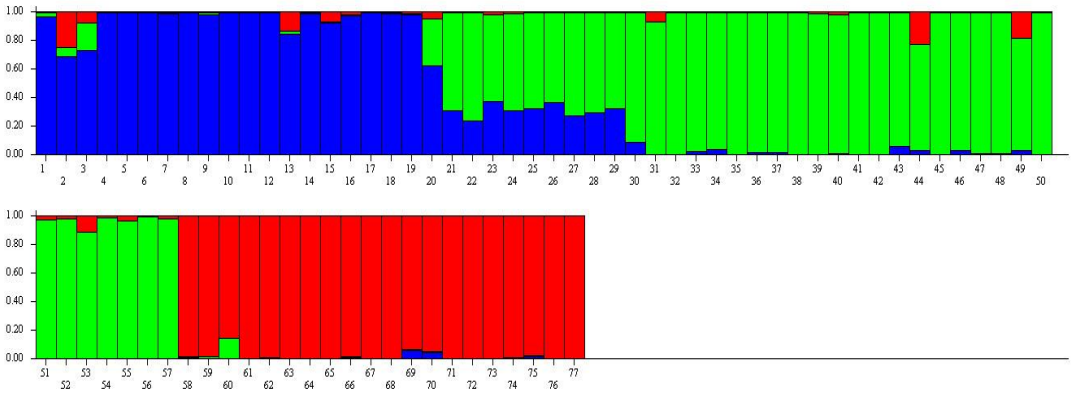

Figure 5. Genetic relatedness of 77 individuals of *Teucrium polium* using 18 ISSR primers as analyzed by the Structure program. The different color of the bars indicates the three different subpopulations identified.

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تنوع ژنتیکی و ساختار جمعیت کلپوره ایرانی (*Teucrium polium* L.) بر اساس نشانگرهای ISSR

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چکیده

آگاهی از تنوع ژنتیکی در برنامه های اصلاحی و مدیریت منابع ژنتیکی گیاهی ضروری است. در این مطالعه، تنوع ژنتیکی ۷۷ فرد کلپوره از نواحی مختلف ایران با استفاده از ۱۸ نشانگر ISSR مورد بررسی قرار گرفت. در مجموع ۱۹۸ نوار با استفاده از آغازگرهای ISSR تکثیر شد که ۱۸۴ (۹۲/۹٪) نوار با میانگین ۱۰/۲ نوار برای هر آغازگر چند شکل بودند. درصد مکان‌های چند شکل (PPL) برای آغازگرها از ۸۰ (UBC834) تا ۱۰۰ در صد (UBC811، 812، 818، 820، 825، 826 و UBC855) متغیر بود. میانگین محتوای اطلاعات چندشکلی (*PIC*)، شاخص شانون اطلاعات (*I*)، و تعداد آلل موثر (*NE*) به ترتیب برابر ۰/۳۹ ۰/۵۲۶ و ۱/۶ بود. تجزیه واریانس مولکولی (AMOVA) سطح بالاتری از تنوع ژنتیکی درون جمعیت‌ها (۷۷ در صد) را در مقایسه با بین جمعیت‌ها (۲۳ در صد) نشان داد. تجزیه کلاستر با استفاده از روش WPGMA بر اساس ضرایب فاصله ژنتیکی نی، افراد را در سه گروه اصلی قرار داد. علاوه بر این یک رویکرد بیزین مبتنی بر مدل، افراد را به سه زیر گروه اصلی تقسیم کرد. نتایج این مطالعه نشان داد که برآورد پارامترهای ژنتیک جمعیت با استفاده از نشانگرهای ISSR می‌تواند برای ارزیابی تفاوت بین جمعیت‌های کلپوره جهت مدیریت منابع ژنتیکی مورد استفاده قرار گیرد.