IRANIAN JOURNAL OF BOTANY 26 (2), 2020
DOI: 10.22092/ijb.2020.342824.1281

GENETIC DIVERSITY AMONG POPULATIONS OF THE TAMARIX SPECIES USING CDDP MOLECULAR MARKER

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Received 2020. 05. 03; accepted for publication 2020. 08. 02

Ghavampour, M. A, Mirjalili, S. A., Jafari, M., Azarnivand, H. & Javadi, S. A. 2020. 12. 30. Genetic diversity among populations of the *Tamarix* species using cddp molecular marker. *-Iran. J. Bot. 26 (2): 123-133*. Tehran.

Tamarix L. is a problematic genus in taxonomy of Tamaricaceae due to the uncertainty of species number, distribution, and ecological conditions in Iran. In this study, we investigated the genetic diversity and taxonomic relationships of 34 individuals from 8 populations including 3 species of *Tamarix* in Isfahan province, Iran. Ten primers using CDDP molecular marker were used to survey the genetic diversity of the genus. One hundred and twenty-five bands were created from ten primers, of which 102 (80.16%) were polymorphic. Cluster analysis classified individuals into three distinct groups. High gene flow among *Tamarix* species using PCoA analysis showed high variability among the three *Tamarix* species, so that samples from different species were grouped together. Molecular analysis of variance showed that intra-population genetic diversity (90%) was greater than inter-population genetic diversity (10%). The highest mean Nei′s genetic diversity (H) and Shannon diversity index (I) was observed in the Habib Abad population. Data analysis showed that morphological traits and DNA sequencing data in the genus *Tamarix* were not fully correlated, which could be justified by the large number of hybrids between species and the lack of genetic differentiation between the studied species.

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Key words: *Tamaricaceae*; genetic diversity; CDDP marker; hybridization; Iran

تنوع ژنتیکی در میان جوامع گونه‌های گز با استفاده از نشانگر مولکولی CDDP

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سرده Tamarix به دلیل عدم قطعیت تعداد گونه‌ها، پراکنش و اهمیت اکولوژیکی در ایران، یکی از مهم‌ترین موضوعات در رده‌بندی تیره گز است. در این مطالعه، تنوع ژنتیکی و روابط خانوادگی ۳۴ فرد از ۸ جمعیت از ۳ گونه Tamarix در استان اصفهان مورد بررسی قرار گرفت. ده آغازگر از

نشانگر مولکولی CDDP برای بررسی تنوع ژنتیکی این سرده استفاده شد. ۱۲۵ باند از ده آغازگر ایجاد شد، که از این تعداد ۱۰۲ (۸۰/۱۶ درصد) چند شکلی بودند. آنالیز خوشه‌ای جمعیت‌ها را به سه گروه مجزا دسته‌بندی کرد .جریان بالای ژنی در میان گونه‌های Tamarix با استفاده از تجزیه و تحلیل PCoA تنوع زیادی در بین سه گونه Tamarix نشان داد، به طوری که نمونه‌های گونه‌های مختلف با هم گروه‌بندی شدند. تجزیه واریانس مولکولی نشان داد که تنوع ژنتیکی بین جمعیت (۹۰٪) بیشتر از تنوع ژنتیکی درون جمعیت (۱۰٪) است. بالاترین میانگین تنوع ژنتیکی نای (H) و شاخص تنوع شانون (I) در جمعیت حبیب‌آباد مشاهده شد .تجزیه و تحلیل داده‌ها نشان داد که صفات ریخت‌شناختی و داده‌های توالی DNA در سرده Tamarix کاملاً با هم ارتباط ندارند، که می‌تواند با وجود تعداد زیاد دورگه بین گونه‌ها و عدم تمایز ژنتیکی بین گونه‌های مورد مطالعه توجیه شود.

INTRODUCTION

Tamarix L. (Tamaricaceae) is the largest genera of this family. The monograph of Baum accepted 54 species, a number that has increased to 62 by newly described species (Baum, 1978). The natural geographic dispersion of the genus reported from the Middle East, south Europe, north Africa to Pakistan, India, and China. The main speciation centers of this genus are Pakistan, Afghanistan, Iran, Turkmenistan, southern Uzbekistan, eastern China, and eastern Mediterranean (Al-Mefarrej & al., 2014). *Tamarix* is one of the five largest genera of the Tamaricaceae that successfully grow in temperate regions (Gaskin and Schaal, 2003). *Tamarix* plants are effective in immobilizing soil in desert areas due to their extensive root system (Gaskin and Schaal, 2003). There are differences in species numbers in different authors accounts. In Flora Iranica, 35 species (Reschinger, 1970), in Iranian Trees and Shrubs, 39 species (Sabeti, 1976), in Flora of Iran, 22 species this genus are mentioned (Assadi & al., 1989). The genus has many problems in taxonomy and classification (DeLoach & al., 2004). In addition, there are problems in identifying *Tamarix* species due to mistakes in available botanical descriptions and keys (Arianmanesh & al., 2016). The presence of hybrids is a strong reason for taxonomic bewilderment in the genus *Tamarix*, so that, the appropriate method for studying hybrids among species of *Tamarix* is the use of molecular markers (Gaskin and Schaal, 2003).

Investigating the genetic structure of wild *Tamarix* species populations as well as the genetic diversity of species within a population is essential for planning conservation of plant species genetic resources as well as selective crosses.

Measuring the level of genetic variation within and between populations is the first important step in evaluating the genetic dynamics of a species. In recent years, marker techniques based on conserved regions of the gene, such as the CDDP marker (Conserved DNA Derived Polymorphism), have received more attention. This marker targets genes that are directly involved in the plant's response to biotic and abiotic stresses and readily provide functional markers that are dependent on the plant phenotype (Poczai & al., 2011). This method is similar to the ISSR because it uses single primers as forward and backward primers (Collard and Mackill, 2009). DNA conserved regions have often conserved in different plant species. This method is performed on an agarose gel and is a relatively inexpensive method (Wang & al., 2014). CDDP markers are longer primers with a higher binding temperature of 50 to 70 degrees as compared with RAPD markers, which improves replication capability. It also focuses on genomic regions and provides complete information on the genome compared to the randomized RAPD marker (Hajibarat & al., 2015). Plants examined with CDDP markers include *Solanum dulcamara* (Poczai & al., 2011), *Chrysanthemum indicum* (Wang & al., 2014), *Triticum spp.* (Guo & al., 2016), *Rose spp.* (Jiang and Zang, 2018) and *Anthurium andraeanum* (Saidi & al., 2018). The molecular markers including AFLP (Gaskin and Kazmer, 2009), SSR microsatellite (Terzoli & al., 2014), ISSR (Ijbari & al., 2014), ITS spacer (Arianmanesh & al., 2016; Sun & al., 2016) and chloroplast markers (Gaskin and Shafroth, 2005) have used to investigate the genetic diversity and structure of species in the genus *Tamarix* in different areas. Arianmanesh *& al.* (2016) using internal transcribed spacers (ITS) showed that Iranian *Tamarix* species are a monophyletic group and the use of morphological features along with molecular data would be effective in determining the evolution of *Tamarix* species. Gaskin and Kazmer (2009) reported a correlation between *Tamarix spp.* with latitude using the AFLP marker, as well as a high percentage of new hybrids that may have implications for biological control measures. Terzoli *& al.* (2014) using microsatellites on the genus *Tamarix* showed the lack of genetic diversity between *T. gallica* and

T. canariensis. Salehi Shanjani & al. (2010) studied genetic structure of Iranian populations of beech, *Fagus orientalis* (Fagaceae) and detected a close genetic relationship between adult trees from seed generation of each population, which revealed by un-weighted pair group method based on arithmetic average (UPGMA) and supported by an analysis of molecular variance (AMOVA). Because of the complexities of the species, the high diversity among populations, and even the diversity of individuals within a single species in the genus *Tamarix*, further investigation using molecular markers seem necessary.

MATERIALS AND METHODS

Plant materials

34 specimens from eight populations belonging to three *Tamarix* species (*T. androssowii* Litw., *T. ramosissima* Ledeb. and *T. kotschyi* Bge.) were collected from different geographical locations including Najafabad (2 populations), Habibabad (2 populations), Segzi (2 populations), Mobarkeh (one population), and West Gavkhooni swamp (one population) in Isfahan province (Iran) in spring 2018. They were identified by botanists from Isfahan Agricultural Research Center, division of Natural Resources and herbarium experts of the Isfahan University of Technology using authentic Persian, English, and Latin references, including flora of Iran (No. 1) (Assadi 1989) and Flora Iranica (Reschinger 1970), then analyzed to assess genetic diversity (table 1). Species identified included *T. androssowii* Litw., *T. ramosissima* Ledeb. and *T. kotschyi* Bge. The voucher specimens are preserved in the herbarium of Isfahan Research & Education Center for Agriculture and Natural Resources (SFAHAN).

DNA extraction

Genomic DNA extractions were performed using the modified Bi & al. (1996) method. The quality and quantity of DNA were determined using a Spectrophotometer (SHIMADZU, UV-2550) at a wavelength of 260 nm and 0.8% agarose gel. The extracted DNA samples were placed at -20 °C until use in the PCR reaction. In this study, 10 CDDP primers (Metabion International Company) were used to evaluate genetic variation intra-population and inter-population (table 2). Polymerase Chain Reaction (PCR) with a final volume of 10 µL including 5 µL of Master Mix (Amplicon Company), 3.2 µL of deionized water, 0.8 µL of primer and 1 µL of genomic DNA at a concentration of 25-50 ng using the device Thermocycler (Bio-Rad) the following was done: initial denaturation step at 94°C for 1 min, followed by 35 cycles each of 1 min at 50 °C, 2 min at 72 °C and 5 min at 72 °C for the final extension. PCR reaction products were identified using electrophoresis on 1.5% agarose gel with the Gel Documentation system (Bio-Rad, Hind II).

Molecular data analysis

Clear reproducible DNA markers were scored as binary data from gel photos (absent=0 or present=1). NTSYS V2.02 (Rohlf, 1996), GenAlex 6.4 (Peakall and Smouse, 2006), and PowerMarker V3.25 (Liu and Muse, 2005) Softwares were used for data analysis. The average polymorphic information content (PIC) was calculated using PowerMarker software based on the $PIC = 1-\sum fi^2$, where f_i is equal to the frequency of the ith allele (Botstein & al., 1980). The marker index (MI) was also calculated according to MI= PIC.N.β. for all primers, where PIC is the polymorphic information content, N: the number of polymorphic bands, and β: the percentage of polymorphism for each primer. Mantel test was performed for Jaccard, Dice, and SM (Simple Matching) similarity indices based on the UPGMA method using NTSYS software (V2.02) to draw a tree graph (dendrogram). The results showed that the SM coefficient has the highest correlation (r=0.9674). Principal Coordinate Analysis (PCoA was performed) as a complementary cluster analysis method to extract more information from the data by GenALEX6.3 software. Molecular variance analysis (AMOVA) was also performed to study genetic diversity and polymorphism of intra-population and inter-population using GenAlEX software (V6.3). The intra-population genetic diversity analysis of the genus *Tamarix* was performed using GenALEX6.3 software. In this evaluation, the number of observed alleles (Na), number of effective alleles (Ne), Nei's genetic diversity index (H), and Shannon index (I) were calculated for each population.

Table 1: Geographical characteristics of *Tamarix spp.* Collected in Isfahan Province. The specimens are documented and kept in the herbarium of Isfahan Research & Education Center for Agriculture and Natural Resources (SFAHAN).

Species	ID	Geographical coordinated	Location	Herbarium code	Species	ID	Geographical coordinates	Location	Herbarium codes
T. ramosissima	H1	32°49'41"N 51°49'12"E	Habibabad	17858	*T. ramosissima*	N1	32°32'11"N 51°23'8"E	Najafabad	17860
T. androssowii	H2	32°49'39"N 51°51'33"E	Habibabad	17863	*T. ramosissima*	N2	32°39'9"N 51°18'28"E	Najafabad	17860
T. ramosissima	H3	32°49'39"N 51°50'18"E	Habibabad	17858	*T. ramosissima*	N3	32°38'13"N 51°24'41"E	Najafabad	17860
T. androssowii	H4	32°49'33"N 51°54'31"E	Habibabad	17863	*T. ramosissima*	N4	33° 4'56"N 50°40'13"E	Najafabad	17860
T. ramosissima	H5	32°49'40"N 51°49'37"E	Habibabad	17858	*T. ramosissima*	N5	32°38'6"N 51°20'5"E	Najafabad	17860
T. androssowii	H6	32°49'2"N 51°56'18"E	Habibabad	17863	*T. ramosissima*	N6	32°38'13"N 51°24'41"E	Najafabad	17860
T. androssowii	H7	32°54'29"N 51°56'14"E	Habibabad	17863	*T. ramosissima*	N7	32°38'52"N 51°19'15"E	Najafabad	17860
T. androssowii	H8	32°49'35"N 51°53'13"E	Habibabad	17863	*T. androssowii*	N8	33° 6'52"N 50°42'28"E	Najafabad	17864
T. androssowii	H9	32°49'36"N 51°52'33"E	Habibabad	17863	*T. ramosissima*	Mob1	32°22'4.11"N 51°28'4.57"E	Mobarakeh	17861
T. androssowii	H10	32°49'38"N 51°51'34"E	Habibabad	17863	*T. ramosissima*	Mob2	32°22'4.13"N 51°28'4.59"E	Mobarakeh	17861
T. androssowii	H11	32°49'18"N 51°56'31"E	Habibabad	17863	*T. ramosissima*	Mob3	32°22'4.18"N 51°28'4.59"E	Mobarakeh	17861
T. ramosissima	H12	32°49'42"N 51°49'2"E	Habibabad	17858	*T. androsowii*	SG1	32°42'45"N 52° 1'28"E	Segzi	17857
T. androssowii	H13	32°49'57"N 51°47'34"E	Habibabad	17863	*T. androssowii*	SG2	32°42'45"N 52° 1'29"E	Segzi	17857
T. androssowii	H14	32°49'38"N 51°51'33"E	Habibabad	17863	*T. androssowii*	SG3	32°42'44"N 52° 1'29"E	Segzi	17857
T. androssowii	H15	32°49'18"N 51°56'31"E	Habibabad	17863	*T. kotschyi*	SG4	32°40'39"N 51°58'34"E	Segzi	17859
T. androssowii	H16	32°49'30"N 51°56'41"E	Habibabad	17863	*T. kotschyi*	SG5	32°40'40"N 51°58'35"E	Segzi	17859
T. androssowii	BA1	32°7'36"N 52° 39'30"E	West of Gavkhooni swamp (BA)	17862					
T. androssowii	BA2	32°7'36"N 52° 39'30"E	West of Gavkhooni swamp (BA)	17862					

Table 2. Primers characters used to study the genetic diversity of populations of *Tamarix* spp. (Collard and Mackill, 2009).

Gene	Gene function	Primer name	Primer sequence(5´–3´)	٪GC	Tm°c
WRKY	Transcription factor for evolutionary and physiological functions	WRKY-R2	GCC GTC GTA SGT SGT	66.7	52
		WRKY-R3	GCA SGT GTG CTC GCC	73.3	54
		WRKY-F1	TGG CGS AAG TAC GGC CAG	66.7	61
ABP1	Coding toxin-binding protein	ABP1-1	ACS CCS ATC CAC CGC	73.3	54
ERF	Transcription factor involved in disease resistance pathway	ERF2	GCS GAG ATC CGS GAC CC	76.5	62
MYB	Unknown (involved in secondary metabolism, abiotic and biotic stresses, cellular morphogenesis)	MYB2	GGC AAG GGC TGC CGG	80.0	57
KNOX	Homeobox genes that function as transcription factors with a unique Homeodomain	KNOX-2	CAC TGG TGG GAG CTS CAC	66.7	61
MADS	Involved in controlling floral organ initiation and development	MADS-1	ATG GGC CGS GGC AAG GTG C	73.7	66
		MADS-2	ATG GGC CGS GGC AAG GTG G	73.7	66
		MADS-4	CTS TGC GAC CGS GAG GTG	72.2	63

RESULTS AND DISCUSSION

The CDDP marker was used to investigate the genetic diversity of 34 samples of three species of the genus *Tamarix*. A total of 125 bands were produced using 10 primers, of which 102 were polymorphism (%80.168) (Fig. 1). The KNOX-2 and WRKY-F1 primers each produced 16 bands, identifying more gene locations than the other primers. The WRKY-R3 primer with 8 bands produced the lowest number of bands. The lowest and highest polymorphism were related to WRKY-R3, MADS-4 primers with 5 bands (62.50% and 45.45%), and ABP1-1 with 15 bands (100%), respectively. On average, each primer produced 7.85% of the polymorphic band. The average polymorphic information content (PIC) was 0.308, so that the highest value was observed for MADS-2 primer (0.455) and the lowest value 0.0975 for KNOX-2 primer. The marker index was also calculated for each primer, so that, its mean was 3.865 and the highest and lowest values related to ABP1-1 (6.664) and KNOX-2 (1.56) primers, respectively (Table 3). The polymorphism of each of the studied populations showed that the Habibabad population (H) with 79.20%, Najafabad (N) with 34.40%, and Segzi (SG) with 12% had polymorphism.

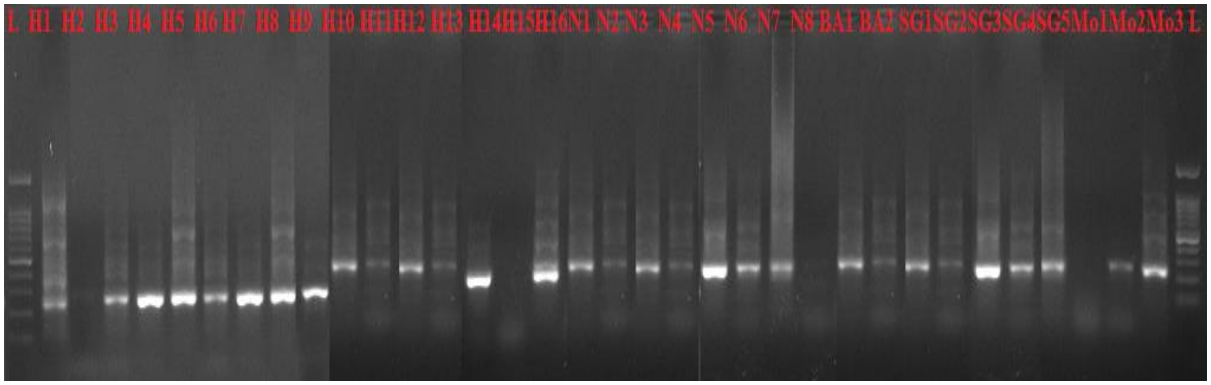

Fig. 1. Bands amplified by the MYB2 primer, CDDP marke for three species of *T. androssowii*, *T. kotschyi* and *T. ramosissima*

Table 3. Duplication results of the 10 CDDP primers in populations *Tamarix spp*. TNB: Total Number of Bands, NPB: Number of Polymorphic Bands, PPB: Percentage of Polymorphic Bands, PIC: Polymorphic Information Content, MI: Marker Index.

Primer	TNB	NPB	PPB%	PIC	MI
WRKY-R2	9	8	88.88	0.2308	2.0772
WRKY-R3	8	5	62.50	0.3243	2.5944
MADS-4	11	5	45.45	0.3500	3.85
ABP1-1	15	15	100	0.4443	6.6645
WRKY-F1	16	14	87.50	0.3617	5.7872
ERF2	15	13	86.66	0.2294	3.441
MYB2	13	8	61.53	0.4296	5.5848
KNOX-2	16	14	87.50	0.0975	1.56
MADS-1	10	9	90	0.1634	1.634
MADS-2	12	11	91.66	0.4555	5.466
Total	125	102	-	-	-
Mean	12.5	10.2	80.168	0.3086	3.8659

In the resulting dendrogram individuals were grouped into 3 clusters according to 70% similarity (Fig. 2). The first group included all populations of Najafabad, Segzi, Gavkhooni swamp, Habibabad, and Mobarakeh, comprising *T. androssowii*, *T. ramosissima* and *T. kotschyi.* Group 2 contained H10, H16, H4, H5, N1, and N2 specimens of *T. androssowii* and *T.ramosissima* species and finally, group 3 included H14 and H7 specimens of *T. androssowii*.

The results of the variance analysis showed (Table 4) that the share of intra-populations diversity (90%) was greater than the inter-populations diversity (10%). Principal coordinate analysis (PCoA) was used to examine more closely the relationship intra-populations and inter-populations (Fig. 3). The first and second axes (components) explained 31.48% and 24.98% of the total variance, respectively (Table 5). According to the results, the first, second, and third axis justified 69.98% of the total diversity.

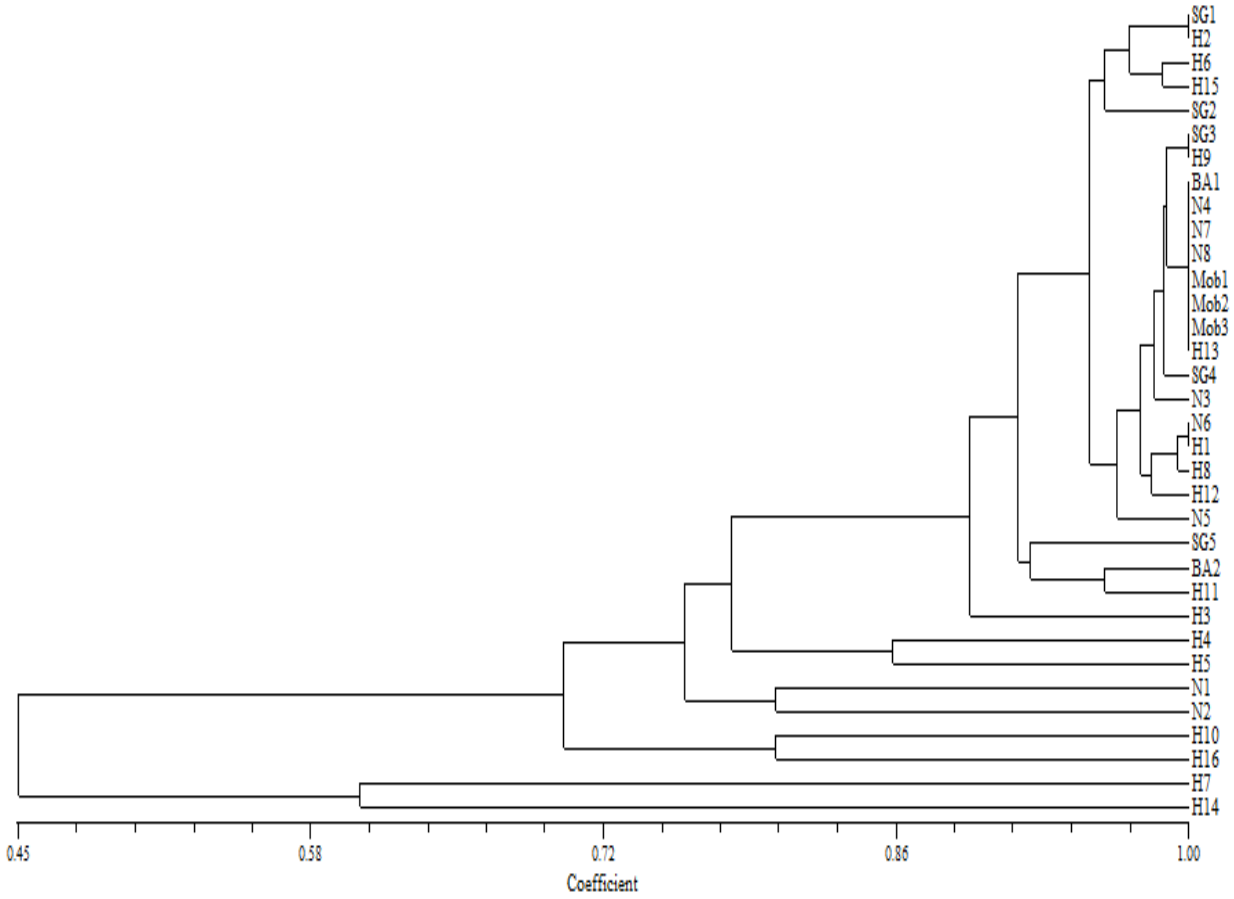

Fig. 2. Dendrogram from NTSYS software and similarity coefficient (SM) and UPGMA method in populations of *Tamarix spp.*

Table 4. Molecular Variance Analysis (AMOVA) data from CDDP molecular marker for populations of *Tamarix.*

Source of variation	df	Variance ratio(%)	Variance components	MS	SS
Inter-populations	4	10	2.434	35.652	142.608
Intra-populations	29	90	21.373	21.373	619.804
Total	33	100	23.806	-	762.412

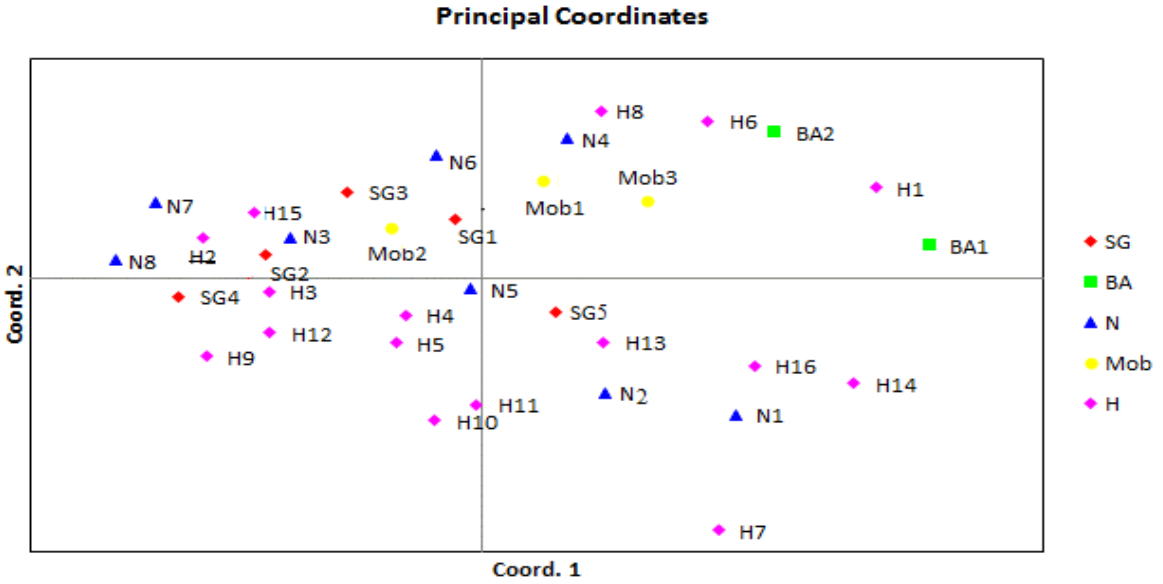

Fig. 3. PCoA diagram based on Principal Coordinate Analysis of GenAlex software in 34 individuals from 5 populations of *Tamarix*.

Table 5. Percentage of variation explained by the three axes for CDDP primers in PCoA.

Axis / Parameters	1	2	3
Percentage of variation	31.8	24.98	13.53
Cumulative percentage of variation	31.48	56.45	69.98

Genetic diversity indices were also calculated in the studied populations. The highest number of alleles (Na) was observed in Habibabad (H) population (1.616) and lowest in the Mobarakeh population (0.080). The highest effective alleles (Ne), i.e. alleles with equal frequency and well distributed, were observed in the Habibabad population (H) with 1. 219 and the least in the populations of Gavkhooni swamp (BA) and Mobarakeh (Mob) with 1. The amount of diversity in the population of Habibabad (H) was more than other populations on the Shannon index (I= 0.226) and Nei index (H= 0.157). This population had higher diversity and dispersion rate than other populations (Table 6).

Table 6. Genetic diversity indices in studied populations of *Tamarix* spp.

Population	N	Na	Ne	I	H
SG	5	0.304	1.056	0.056	0.036
BA	2	0.112	1.00	0.00	0.00
N	8	0.728	1.106	0.122	0.073
Mob	3	0.080	1.00	0.00	0.00
H	16	1.616	1.219	0.266	0.157
Mean	6.8	0.568	1.076	0.089	0.053

In the Pairwise Population Matrix of Nei Genetic Distance using GenAlex software, the most similarity was found between the populations of Gavkhooni swamp (BA) and Mobarkeh (Mob) with a distance of 0.920. Najaf Abad (N) and Segzi (SG) populations with a distance of 0.991 showed the highest difference among the studied populations.

In surveying the variation inter-population and intra-population of the genus *Tamarix* using the CDDP marker, significant polymorphic results (80.16%) were obtained in band patterns. The marker index (MI) showed a good estimate of the efficiency of primers that

is attributed to the number of polymorphic bands and the high genome coverage of the marker (Milbourne & al., 1997). The high marker index indicates the production of more polymorphic bands and the provision of more information from the genome (Spooner, 2005). In this study, the highest marker index (6.66) was observed in ABP1-1 primer, indicating high efficiency of this primer compared to other primers. Polymorphic information content (PIC) is one of the important indicators for comparing the differentiation power of primers. High values of this index imply multiple polymorphisms in one location that are important in differentiating individuals (Santhosh & al., 2009). The MADS-2 primer with 0.455 and the highest PIC had a higher level of differentiation and more ability to detect polymorphism than the other primers studied. The results of the present study and high polymorphism rate (80.16%) indicate that the CDDP marker is suitable for the taxonomic and phylogenetic study of the genus *Tamarix.*

The results of molecular variance (AMOVA) clearly showed the intra-population genetic variations in the genus *Tamarix.* The results showed that of the total variance observed, 10% was related to inter-population variation and 90% to intra-population variation. Arianmanesh & al. (2016) reported that inter-population variation was 15% and intra-population variation was 85%, indicating gene exchange between individuals. Ijbari & al. (2014) with the study of *Tamarix* species using the ISSR marker observed 15% inter-population genetic variations and 85% intra-population variations. The high genetic diversity can be used for local adaptation as well as preventing the homozygosity and genetic extinction of the studied *Tamarix* species (Ijbari & al., 2014).

In screening genetic variation through molecular data, it is advisable for primers to have a monotonous distribution at the genome level to cover the whole genome as much as possible. Therefore, the correlation between the results would be low and a larger number of axes would be needed to justify their diversity, if primers were selected from different parts of the genome (Hedrick, 2011). The principal coordinate analysis showed that the primers used partially cover the whole genome because, the first, second, and third axes justified 69.98% of the total variation, indicating the proper distribution of primers in the genome.

The results of the present study showed relatively favorable genetic variation among populations. The Nei index (H) equal to 0.157% and the Shanon index (I) equal to 0.266% were calculated. The study of diversity in the populations studied with the CDDP marker indicates that the greatest genetic similarity was observed between the populations of Gavkhooni swamp (BA) and Mobarakeh (Mob). The genetic similarity between these populations originates from the proximity of the genesis, the origin of the same distribution, and the genetic affinities of these populations. The highest genetic distance was observed between the Segzi (SG) and Najafabad (N) populations.

In general, based on the results of the CDDP method, the molecular similarity of the three species in a group may be due to hybridization. The results obtained from the grouping of *Tamarix* species are consistent with the results of previous studies based on ITS and microsatellite markers. The range of these species is similar overlap and concurrency (Gaskin and Schaal, 2003). Gaskin and Shafroth (2005) in the study of *Tamarix* species observed a close correlation between *T. karkalensis* and *T. kotschyi* using chloroplast markers. Ijbari & al. (2014) by analyzing *Tamarix* species stated that the species are somewhat different from each other due to high genetic exchange, grouping of species. The high degree of gene flow between neighboring trees even prevents the genetic differentiation of the species. The high occurrence of hybridization among *Tamarix* species may be the main cause of some classification confusions in this genus (Villar & al., 2015). According to Brotherson and Winkel (1986), the pollination in this genus occurs with the wind and according to Stevens (1985) through the insects. In both cases, pollination results in high genetic diversity and it may be the reason for the formation of interspecies hybrids and the success factors in the widespread distribution in this genus.

The results of this study showed high gene flow among individuals of species and different species of genus *Tamarix* were placed next to each other in the dendrogram. High hybridization among *Tamarix* species indicated a wide range of hybrid genotypes. Data analysis showed that the morphological traits and DNA sequence data were not permanently correlated in the genus *Tamarix*, given the high number of hybrids between the species and the lack of genetic differentiation between the studied species. Thus, further studies using other molecular markers as well as the combination of morphological and molecular results for more accurate identification of hybrid species in Iran is also important. It should also be considered to investigate the genetic diversity of each *Tamarix* species separately using molecular markers and morphological traits.

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