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SPECIES DELIMITATION IN *CAPPARIS* (CAPPARACEAE): MORPHOLOGICAL AND MOLECULAR

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Capparis spinosa L. (Capparidaceae) is the largest genus of the family Capparaceae, distributed in pantropical region. *C. spinosa* is known as a medicinal plant species. In Iran, different parts of caper bush plants are used as diuretics, tonics and in treatment of malaria and joint disease. Till present time, there has been no detailed information available on molecular phylogeny and genetic structure of these species in the country. Therefore, the present study was conducted with the aim to investigate species delimitation by both morphological and molecular data and to reveal genetic diversity and population structure in these five of *Capparis* species. For this study, 108 randomly collected plants from 20 geographical populations in of *Capparis* species were used. We encountered extensive within species genetic and morphological diversity. ISSR molecular markers could delimit the studied species. STRUCTURE analysis revealed the occurrence gene flow between these species. The Mantel test showed correlation between genetic distance and geographical distance of the populations studied. Phylogenetic tree was constructed based on ITS data set which separated out-groups from the studied species. Genetic affinity of the studied species have been discussed.

Keyword: Capparis spinosa, Genetic admixture; ISSR, ITS, Population Structure

INTRODUCTION

Capparis L. is a genus belonging to the Capparaceae (Capparidaceae). It is distributed in the subtropical and tropical regions of the world (RAJA *et al*., 2013) and grows widely in the

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Mediterranean and Western Asia. It is believed to include more than 250 species (FICI, 1993). Caper is the English common name of this genus and it is also known by different names, e.g., Kabbar (Arab), câprier (French), and Alcaparro (Spain) (ZOHARY, 1960; HEYWOOD, 1964; JACOBS, 1965; INOCENCIO *et al*., 2006; SAADAOUI *et al*., 2007). In the Middle East, ZOHARY (1960) regarded *Capparis* as a native flora distributed in Africa and south-western Asia, whereas Jacobs (1965) suggested that the Malaysian and Australian *C. spinosa* were introduced by humans Species belonging to the genus *Capparis* have plesiomorphic features (FICI, 2001). Some available literature treated the botanical description of *Capparis spinosa* and reported the polymorphic aspects of this species and the high degree of heterogeneity in its morphological characters (ZOHARY, 1960; LEGUA *et al*., 2013).

Capparis spinosa a perennial creeping subshrub that is widely distributed in the Mediterranean and in arid West and Central Asia (FICI, 2001), is a xerophytic Tertiary relic (WU *et al*., 2010). In particular, wild *C. spinosa* is mainly distributed on the southern and northern flanks of the Tianshan Mountains and in adjacent desert areas in arid Northwestern China. This plant material has been utilized as a source of traditional medicine and is considered to be an excellent material for wind screens and sandy soil stabilization (PANICO *et al*., 2005; ZHANG and HAI, 2002). Extensive research concerning this species has focused on its ecological reproductive, physiological stress and pharmacological aspects (PANICO *et al*., 2005; RHIZOPOULOU *et al*., 2006; ZHANG and TAN, 2009). Previous reports involving the intraspecific variation of *C. spinosa* have been based on morphological parameters (FICI, 2001) and several molecular markers, i.e., RAPD and ISSR markers (BHOYAR *et al*., 2012; OZBEK and KARA, 2013), which provide little information on the genetic structure of *C. spinosa*. Its representatives, showing plesiomorphic features for the whole genus *Capparis* Linnaeus (1753: 503), were treated in the past or as separate species (BAILLON, 1885; JAFRI, 1956; ST. JOHN, 1965) or at intraspecific rank within the single *C. spinose* (JACOBS, 1965; HEWSON, 1982; WHEELER, 1992; FICI, 2003).

The taxonomy of the genus in Iran was the subject of a long controversy. BOISSIER (1843) introduced 2 new species, *C. parviflora* and *C. mucronifolia*, for the flora orientalis area. Later in 1867, he combined them in the *C. spinosa* as varieties (BOISSIER, 1867). ZOHARY (1960) reported five species with some varieties in Iran. In Flora Iranica, HEDGE and LAMOND (1970) cited two species, *C. cartilaginea* Decne. and *C. spinosa* with three variety, var. *spinosa*, var. *parviflora* and var. *mucronifolia.* These varieties were later recognized as separate species by SAGHAFI KHADEM (2000) in Flora of Iran. INOCENCIO *et al*. (2006) recognized four species for this group in Iran, *C. cartilaginea, C. sicula* Veill. (three subspecies), *C. mucronifolia* and *C. parviflora* (two subspecies). FICI (2014; 2015) combined the above 4 species into *C. spinose* with 2 subspecies and 3 varieties. These different classifications reflect the taxonomic complexities present in this group. Here we followed the classification of the genus presented in the Flora of Iran (SAGHAFI KHADEM, 2000). *Capparis* species are mostly distributed in the south of Iran, but *C. spinosa* is widely distributed in all regions of Iran. *C. spinosa* is known as a medicinal plant species. In Iran, different parts of caper bush plants are used as diuretics, tonics and in treatment of malaria and joint disease (TLILI *et al*., 2011). Genetic diversity of *C. spinosa* in some regions has been studied (INOCENCIO *et al*., 2005; SAIFI *et al*., 2011; BHOYAR *et al*., 2012; ÖZBEK and KARA, 2013).

Genetic diversity among the individuals or populations can be assessed using morphological and molecular markers (WANG *et al*., 2005; KUMAR *et al*., 2006; JAWDAT *et al*., 2010). Molecular tools provide valuable data on genetic diversity through their ability to detect the variation at the DNA level. Identification is very important in biodiversity studies in many different ways. For evaluation of species diversity, it is essential that individuals can be classified accurately. Several types of molecular markers, including random amplified polymorphic DNA (RAPD), simple sequence repeat (SSR) and inter–simple sequence repeat (ISSR) and amplified fragment length polymorphism (AFLP) have been successfully used for germplasm identification and genetic diversity studies (ESFANDANI-BOZCHALOYI *et al.*, 2018a; 2018b; 2018c; 2018d). We used both ISSRs as well as ITS to carry out species delimitation. Similalry, we used ISSRs to investigate population genetic diversity and structure. For this purpose, we collected plants of from *Capparis decidua*; *C. parviflora*; *C. cartilaginea*; *C. spinose* and *C. mucronifolia* the areas they grow and the areas of overlap and delimit these five species.

MATERIALS AND METHODS

Plant materials

A total of 108 individuals were sampled representing 20 natural populations of *Capparis* of different regions of Iran during 2012–2018 (Table 1). For morphometric and ISSR analysis we used 108 plant accessions (four to eleven samples from each populations) belonging to 20 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 and Fig. 1 and Fig 2. Different references were used for the correct identification of species *Capparis* (Flora of Iran (SAGHAFI KHADEM, 2000)). Vouchers were deposited at the herbarium of Islamic Azad University, Science and Research Branch, Tehran, Iran (IAUH).

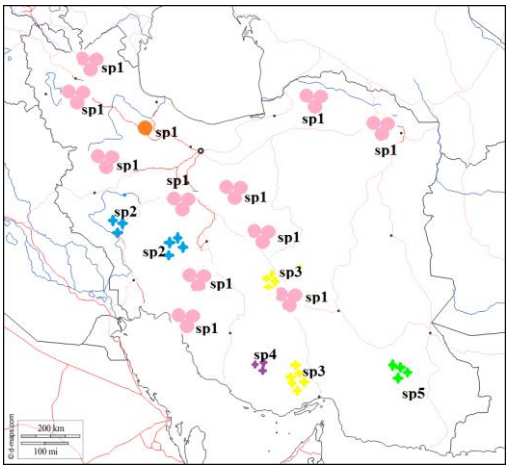

Figure. 1. Distribution map of the populations studied. Sp1: *Capparis spinosa*; sp2*: Cappais parviflora*; sp3: *Capparis mucronifolia;* sp4: *Capparis cartilaginea;* sp5: *Capparis decidua*.

Table 1. Location and herbarium accession numbers of the studied populations of Capparis collected by Najafian in Iran

Pop No.	species	Locality	No. of collected accessions	Voucher No.	Elevation (m)	Longitude	Latitude
1	*Capparis spinosa* L.	Zanjan	5	IAUH-15292	1533	54° 12'56.54"	31° 45'36.23"
2	*Capparis spinosa* L.	Azerbaijan(W), Khoy	5	IAUH-15293	986	54° 21'69.5"	31° 86'07.41"
3	*Capparis spinosa* L.	Azerbaijan(W), Ashoghlou	5	IAUH - 15295	373	55° 33'2.96"	29° 34'38.73"
4	*Capparis spinosa* L.	Khorassan(N), Chenaran	5	IAUH - 15291	1156	53° 19'11.57"	29° 52'32.55"
5	*Capparis spinosa* L.	Golestan, Gorgan	5	IAUH - 15288	83	54° 57'5.11"	36° 57'5.92"
6	*Capparis spinosa* L.	Azerbaijan (E), Siahroud	5	IAUH - 15294	709	55° 43'11.34"	36° 875569
7	*Capparis spinosa* L.	Kerman, Sirjan	5	IAUH - 15286	1715	56° 59'11.86"	37° 34'31.83"
8	*Capparis spinosa* L.	Fars, Arsajan	5	IAUH-15287	1605	59°12'39.47"	36° 19'47.26"
9	*Capparis spinosa* L.	Kermanshah, Kermanshah	5	IAUH-15297	1311	48°9'18.27"	36° 19'36.86"
10	*Capparis spinosa* L.	Yazd, Khezrabad	5	IAUH-15298	1445	45°10'19.35"	38° 52'18.10"
11	*Capparis spinosa* L.	Boushehr, Boushehr	5	IAUH-15296	12	45° 48'9.16"	38° 54'31.33"
12	*Cappais parviflora* Boiss.	Fars, Zarqan	5	IAUH-15300	1700	46° 42'9.77"	38° 59'8.81"
13	*Capparis parviflora* Boiss.	Fars, Firuzabad	5	IAUH-15301	1789	51° 56'48.84"	27° 58'13.40"
14	*Capparis parviflora* Boiss.	Fars, Nurabad	5	IAUH-15302	1655	47° 33'53.77"	34° 26'59.68"
15	*Capparis parviflora* Boiss.	Chaharmahal and Bakhtiari, Doplan	5	IAUH-15303	1200	51° 39'01.99"	35° 20'47.31"
16	*Capparis mucronifolia* Boiss.	Kerman, Jiroft	5	IAUH-15304	1766	57° 22'47.5"	27° 22'58.96"
17	*Capparis mucronifolia* Boiss.	Hormozgan, Bandar Abbas Genow	5	IAUH-15305	1300	57° 46'44.88"	28° 47'42.81"
18	*Capparis mucronifolia* Boiss.	Hormozgan, Minab	5	IAUH-15306	1350	55° 52'56.39"	27° 55'54.45"
19	*Capparis cartilaginea* Decne.	Hormozgan, Tang-E-Zagh	10	IAUH-15307	1100	54° 34'59.5"	25° 22'25.65"
20	*Capparis decidua* (Forssk.) Edgew.	Sistan and Baluchestan, Chabahar	8	IAUH-15308	1656	60° 96'67.71"	27° 60'54.27"

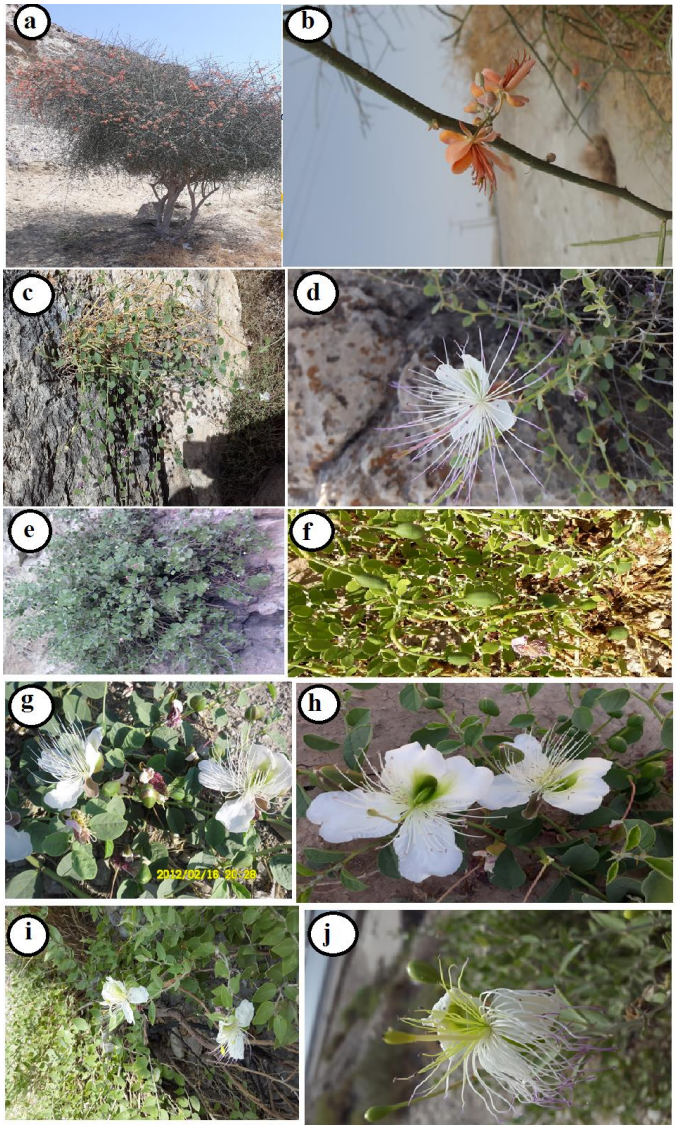

Figure 2. a,b: *Capparis decidua*; c, d: *Cappais parviflora*; e, f: *Capparis cartilaginea*; g, h: *Capparis spinosa;* ij: *Capparis mucronifolia ;* a, c, e, g, i) Habit; b, d, f, h,j) Flower.

Morphological studies

We studied 21 qualitative and quantitative morphological characters containing number of branching, leaf length (cm), leaf width (cm), leaf thickness (mm), petiole length (cm),

flowering pedicel length (cm), sepal length (cm), sepal width (cm), petal lamina length (cm), fruit length (cm), fruit width (cm), fruiting pedicel length (cm), gynophores length (cm), number of seed, stipule length (cm), stipule base width (cm), stipule base length (cm), bud length (cm), plant habit, stipule shape, and leaf abaxial indument. Also, we studied other morphological features such as leaf shape, fruit shape, stipule color, and stipule decurrent that showed variation in individuals. Four to twelve samples from each population were randomly studied for morphological analyses.

DNA extraction and ISSR assay

Fresh leaves were used randomly from 5-12 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 0.7% agarose gel. 10 ISSR primers; (AGG) 5GT, (CA) 7GT, (AGC) 5GG, UBC 832, (CA) 7GT, (GA) 9G, UBC 820, UBC 810, (CA) 9T and (GG) 7CA commercialized by UBC (the University of British Columbia) were used. PCR reactions were carried in a 25µl volume containing 11 mMTris-HCl buffer at pH 8; 50 mMKCl; 2 mM $MgCl_2$; 0.3 mM of each dNTP (Bioron, Germany); 0.4 µM of a single primer; 10 ng genomic DNA and 5 U of *Taq*DNA polymerase (Bioron, Germany). The amplifications, reactions were performed in Techne thermocycler (Germany) with the following program: 4min initial denaturation step 95°C, followed by 40 cycles of 1min at 94°C; 1 min at 55-57°C and 2 min at 72°C. The reaction was completed by final extension step of 5-10 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) and MDS (Multidimensional scaling) were used (PODANI, 2000). 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. Parameter like Nei's gene diversity (H), Shannon information index (I), number of effective alleles, and percentage of polymorphism were determined (WEISING *et al.*, 2005; FREELAND *et al.*, 2011). Nei's genetic distance among populations was used for Neighbor Joining (NJ) clustering, while TCS network (CLEMENT *et al.*, 2002) was used for networking by Popart program (http://popart.otago.ac.nz.). Mantel test checked the correlation between geographical and genetic distance 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), and was used to show genetic difference of the populations. The genetic structure of populations was studied by Bayesian based model STRUCTURE analysis (PRITCHARD *et al*., 2000). For STRUCTURE analysis, data were scored as dominant markers (FALUSH *et al*., 2007). We used the admixture ancestry model under the correlated allele frequency model. A Markov chain Monte Carlo simulation was run 20 times for each value of K after a burn-in period of 10^5. The Evanno test was performed on STRUCTURE result to determine proper number of *K* by using delta *K* value (EVANNO *et al*., 2005). 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.

ITS data of the species studied were obtained from NCBI (The National Center for Biotechnology Information) and used for further analyses. Accession numbers for species retrieved from Gene Bank are presented in the Appendix.

ITS sequences were first aligned and cured by MUSCLE, and then used for constructing phylogenetic trees and TCS networking. MEGA 7 (2017) was used for drawing phylogenetic trees and Popart program (2017) was used for networking. Four species namely *Boscia mossambicensis*, *Boscia angustifolia*, *Crateva formosensis* and *Crateva formosensis* were used as out-group taxa.

RESULTS

Species delimitation and inter-relationship

Morphometry

Different clustering and ordination methods produced similar results therefore, only PCoA plot of morphological characters are presented here (Fig. 3). In general, plant samples of each species were grouped together and formed a separate group. This result show that morphological characters studied can delimit *Cappais* species. In the studied specimens we did not encounter intermediate forms.

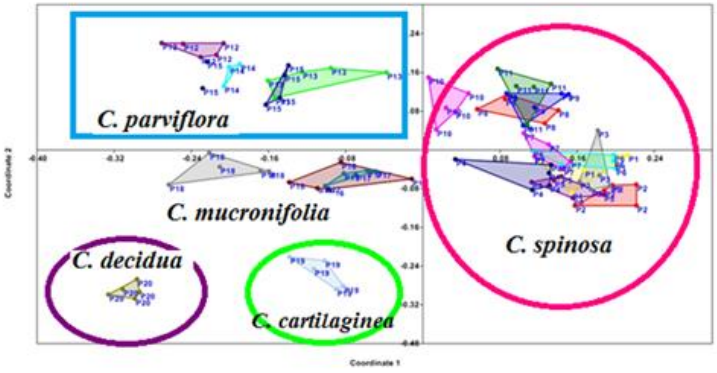

Figure 3. PCoA plot based on both quantitative and qualitative morphological characters delimiting the studied species in *Cappais*.

PCA analysis of morphological traits revealed that the first three PCA components comprised about 67.5% of total variation. Morphological characters like, leaf shape, fruit shape, stipule shape, flower bud, apex, stipule color, and leaf abaxial indument positively correlated with these components and are the most variable morphological characters among the studied species and therefore, they may be used in taxonomy of these taxa.

Molecular analysis

WARD clustering and TCS networking (Figs. 4 and 5), of the studied populations did entirely delimit the studied species and revealed that plants in these species are not inter-mixed. In the studied specimens we did not encounter intermediate forms. In general, two major clusters were formed in WARD tree (Fig. 4), Populations of *Capparis spinosa* 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 *Cappais parviflora* comprised the first sub-cluster, while plants of *Capparis decidua*, *Capparis cartilaginea* and *Capparis mucronifolia* formed the second sub-cluster. This is in agreement with PCoA plot presented before.

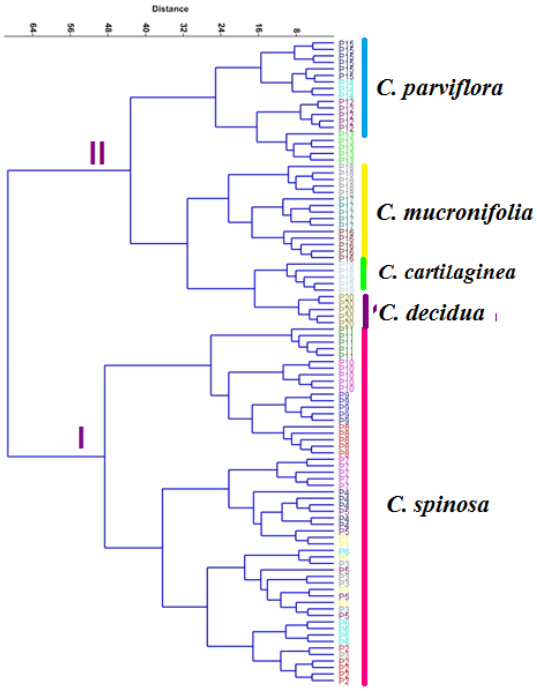

Figure. 4. WARD dendrogram of the studied populations based on ISSR markers.

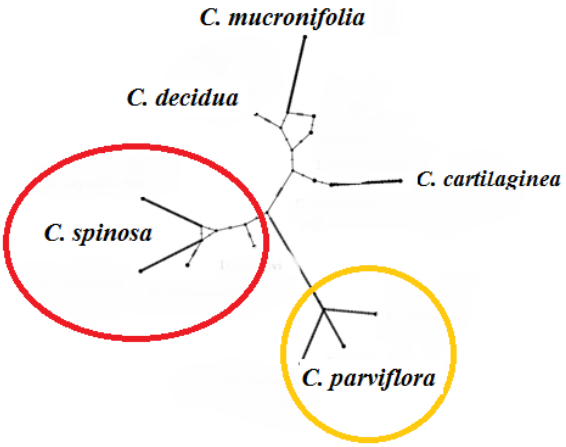

Figure. 5. TCS netwerk of the studied populations based on ISSR markers.

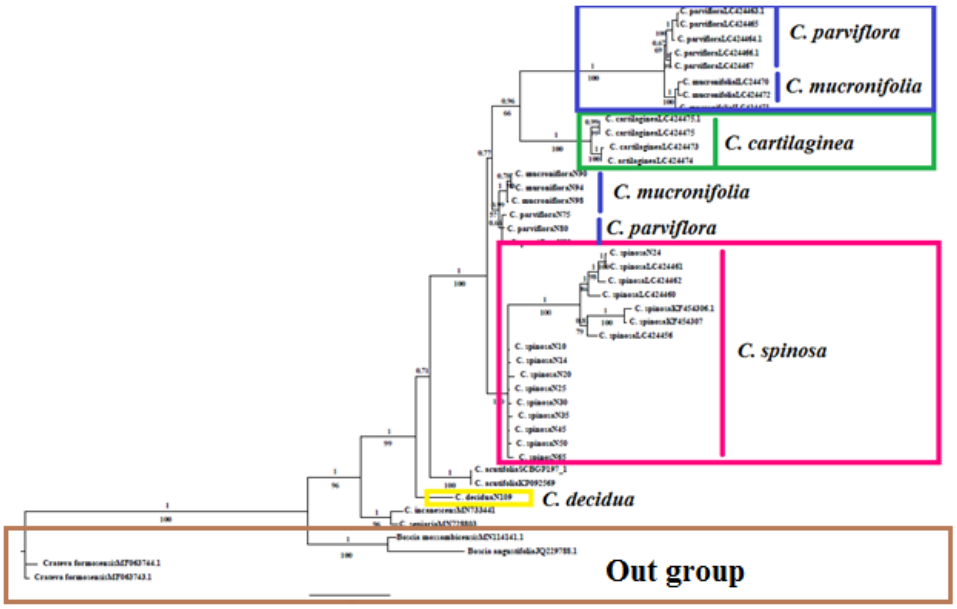

Figure 6. Phylogenetic tree of the species studied based on ITS sequences.

TCS network (Fig. 5) supported WARD clustering relationship, and revealed the presence of 5 main groups. The first group was formed by plants of *Cappais parviflora*, the second haplotype group was composed of Populations of *Capparis spinosa*; while the other studied species formed the third and other major haplotype group.

The phylogenetic tree separated four out-group species namely *Boscia mossambicensis, Boscia angustifolia, Crateva formosensis* and *Crateva formosensis* in a two clade, while the in-group species were distributed with together in one separate clades.

UPGMA trees of data set of ITS supported separation of the five species as their accessions formed separate clusters with high bootstrap value (>0.98) (Fig. 6). In general, UPGMA trees of ITS trees is in agreement with morphology data. It showed affinity between of *Cappais parviflora* and *Capparis mucronifolia.* It also separated *Capparis spinosa* from the other species.

Population genetic analysis (ISSR)

Genetic diversity parameters determined in three studied species (Table 2) revealed that *Capparis spinosa* had the highest level of genetic polymorphism (85.44%), while the lowest level of the same occurred in *Capparis decidua* (15.96%). In general, these species have moderate to high degree of genetic variability which may be due to pollination mode of reproduction in these plants.

Table 2. Genetic diversity parameters based on ISSR data in the studied Capparis species.

Species	*N*	*Na*	*Ne*	*I*	*He*	*UHe*	*%P*
Capparis spinosa	55	1.835	1.248	0.348	0.357	0.227	85.44%
Cappais parviflora	20	1.302	1.136	0.322	0.343	0.151	47.57%
C*apparis* mucronifolia	15	1.240	1.134	0.299	0.232	0.148	55.96%
Capparis cartilaginea	5	1.302	1.136	0.222	0.183	0.151	37.57%
Capparis decidua	5	1.240	1.134	0.277	0.166	0.148	15.96%

*(*N = number of samples, Na = Number of different alleles, Ne = number of effective alleles, I= Shannon's information index, He = gene diversity, UHe = unbiased gene diversity, P%= percentage of polymorphism, populations).

The AMOVA test did produce significant molecular differences (P = 0.02) among the studied species. The low Nm value (2.34) indicates limited gene flow or ancestrally shared alleles between the species studied and supports genetic stratification as indicated by K-Means and STRUCTURE analyses. AMOVA revealed that 12% of total genetic variability occurred among the studied populations while 88% occurred within these species.

Nei's genetic identity and the genetic distance determined among the studied species are presented in Table 3. The results showed that the highest degree of genetic similarity (0.96) occurred between *Cappais parviflora* and *Capparis cartilaginea* and then between *Capparis mucronifolia* and *Capparis cartilaginea* (0.94). As we did not encounter any intermediate plants particularly in the areas of overlap, we consider these species to have low degree of ancestral shared alleles.

Table. 3. Nei's genetic identity (above diagonal) and genetic distance (below diagonal) among the study Population

Population ID	Capparis spinosa	Cappais parviflora	Capparis mucronifolia	Capparis cartilaginea	Capparis decidua
Capparis spinosa	*****	0.8621	0.8850	0.7721	0.8840
C*appais* parviflora	0.0171	*****	0.9231	0.9629	0.8499
C*apparis* mucronifolia	0.0141	0.0049	*****	0.9425	0.8750
Capparis cartilaginea	0.0161	0.0066	0.0095	*****	0.7860
Capparis decidua	0.0188	0.0055	0.0111	0.0086	*****

Mantel test did produce significant correlation (r = 0.01, p = 0.23) between geographical distance and genetic distance of these species and therefore, isolation by distance (IBD) exists between them.

We performed STRUCTURE analysis followed by the Evanno test to identify the optimal number of genetic groups. We used the admixture model to illustrate interspecific gene flow and/or ancestrally shared alleles in the species studied.

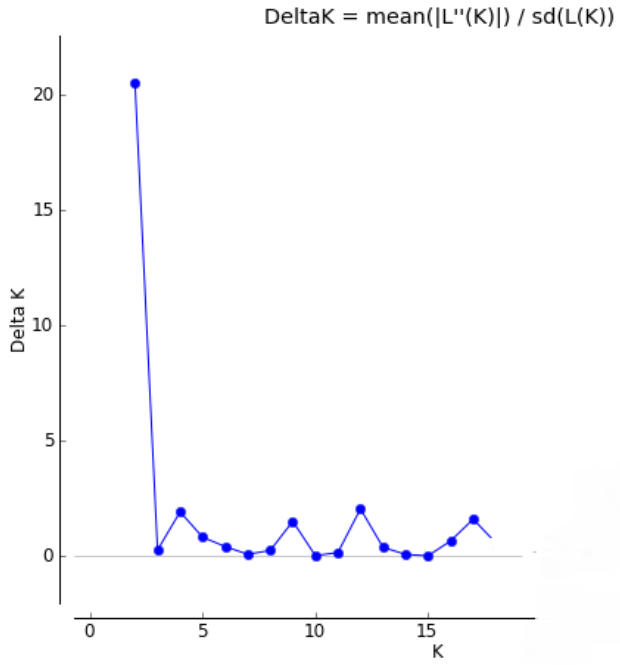

Figure 7. Delta k plot of Evanno's test based on STRUCTURE analysis.

Table 4 Evanno table output

	Mean	N	Std. Deviation	L'K	L"K	L"/STD	
1	-20916.3	25	0.7483				
2	-19540.5	20	5.6786	560.27	-293.455	-51.6774	K=2
3	-20100.8	20	6.29	815.522	-255.252	-40.5806	
4	-19273.7	20	10.6868	266.815	-284.875	-26.6567	
5	-19291.8	20	360.956	-18.06	-473.61	-1.3121	
6	-19783.4	20	1052.895	-491.67	491.67	0.46697	

STRUCTURE analysis followed by Evanno test also produced delta K = 2 (Table 4, Fig 7). The STRUCTURE plot and population assignment revealed some degree of genetic admixture among the studied *Capparis* populations (Fig 8). Gene flow is also important in conservation contexts, particularly for the species with local populations. Fortunately, *Capparis* populations showed good within-population genetic variability and limited amount of among population gene flow. Gene flow among local populations could mitigate losses of genetic variation caused by genetic drift in local populations and thus save them from extinction.

Mantel test revealed isolation by distance in the studied *Capparis* populations. The plant species that form geographical populations, as geographical isolation increases, a reduction in both seed dispersal and pollen flow will result in decreased gene flow between distantly located populations (FREELAND *et al.*, 2011). That is why Evanno test and K-Means clustering identified two different gene pools for *Capparis* in the country.

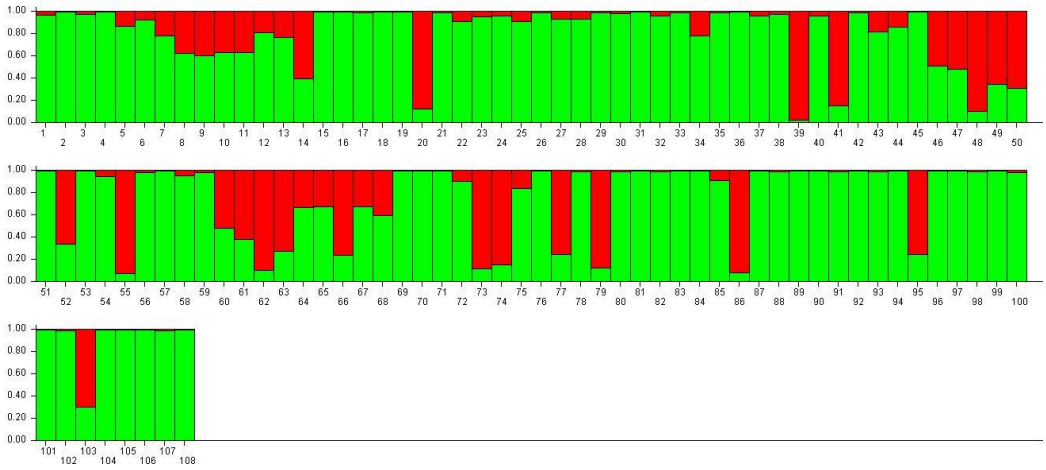

Figure 8. STRUCTURE plot of *Capparis* species based on ISSR data.

DISCUSSION

Species delimitation and taxonomic consideration

The species delimitation in complex groups and in those that the species have different degree of morphological overlap is a tedious and difficult task. In these situations, it is suggested to use different and combined approaches like morphological, molecular, cytological, etc. to determine the species boundaries (CARSTENS *et al*., 2013). In the last few decades the use of molecular markers as tools for species and subspecies delimitation has drastically increased (ESFANDANI-BOZCHALOYI *et al.*, 2017a; 2017b; 2017c; 2017d). The basic premise for the use of molecular markers for species delimitation is that the "species tree" should be inferred from a "gene tree". The present study revealed these species may be delimited by morphological characters as well as of ITS sequences. The species relationship obtained also is in agreement with morphological analysis and supports taxonomic treatment of Flora of Iran (SAGHAFI KHADEM *et al*., 2000).

Morphological analyses of the studied *Capparis* species showed that they are well differentiated from each other both in quantitative measures (ANOVA test result) and qualitative characters (PCA plot result). PCA analysis suggests that characters like, leaf shape, fruit shape, stipule shape, flower bud, apex, stipule color, and leaf abaxial indumenta, may be used in species delimitation.

Inter-specific morphological and genetic variability

Population genetic study provides valuable information about genetic structure of plants, the stratification versus gene flow among the species populations, genetic divergence of the populations, etc. (ESFANDANI-BOZCHALOYI *et al.*, 2017a). This information has different applications, and from pure understanding of biology of the species to conservation of endangered species, choosing of proper parents for hybridization and breeding and phylogeography and mechanism of invasion (FREELAND *et al.*, 2011). AMOVA and STRUCTURE analysis revealed that the species of this genus are not genetically differentiated. The Nm value obtained based on ISSR data, revealed very high amount of gene flow among the studied species that was also supported by STRUCTURE analysis as *Capparis* species mostly had not distinct genetic structure. To conclude, the present study revealed the use of ISSR molecular markers along with morphological characters in *Capparis* species delimitation. We did not observe any intermediate forms in our extensive plant collection and the studied species are strongly differentiated during the speciation process and invasion in new habitats. Genetic drift, strong inbreeding, and local adaptation are effective evolutionary forces operating in *Capparis* species and population divergence and adaptation.

Capparis spinosa shows considerable morphological variation due to various factors such as phenotypic plasticity, ecogeographical differentiation, topographical modifications, and hybridization processes promoting the presence of intermediate phenotypes. This high variability suggests chaotic complex structure within wild forms of *C. spinosa*. Based on Amplified Fragment Length Polymorphism (AFLP) a low genetic distance was revealed among *Capparis* sp. (i.e., C. *spinosa, C. orientalis, C. sicula, C. aegyptia*, and *C. ovata*) from Spain, Morocco and Syria (Inocencio et al., 2005). About 50% of polymorphic frequency was revealed between *C. orientalis, C. spinosa* and *C. sicula* and a low consistency of *C. spinosa*, with 2% unique bands

was marked. In Egypt, the taxonomic identity among and within species of the genus *Capparis* using Random Amplified Polymorphism DNA (RAPD) was conducted by MOUBASHER *et al*. (2011). Eight polymorphic RAPD markers were generated. A considerable genetic variation was identified and revealed the presence of three varieties of *C. spinosa*: var. *spinosa,* var. *canescens,* var. *deserti* and one *inermis* type.

The genetic assessment of Moroccan capers by Inter Simple Sequence Repeat (ISSR) revealed 98.89% distinct profiles based on the geographic origin and indicated remarkable phenotypic plasticity linked to the ecological area and environment (SAIFI *et al*., 2011). This might be explained by a low level of gene flow due to the fragmentation of habitats of these populations that leads to accumulate significant genetic differences (INOCENCIO *et al*., 2005). The genetic study of Azerbi and Iranian Capers using RAPD markers indicated no correlation between genetic variation and geographical distances among populations (NOSRATI *et al*., 2012). BHOYAR *et al.* (2012) analyzed the genetic variability of *C. spinosa* populations growing in the trans-Himalayan region in India for adaptation to high altitude, by using both RAPDs and ISSRs markers. In Turkey, OZBEK and KARA (2013) differentiated five varieties: *C. spinosa* var. *spinosa,* var. *aegyptia* and var. *canescens*, and *Capparis ovate* Desf. var. *palaestina*, and var. *herbacea*. Ten RAPD primers produced 98 loci, 73 of which were polymorphic with 87.42% total genetic variation. Hypothesis of the effect of population size on genetic diversity was confirmed as well as the relation between eco-geographical factors and genetic diversity affecting the number of effective alleles. SILVESTRE *et al*. (2014) investigated capers growing in Sicily and the surrounding islets of Lampedusa, Pantelleria and Salina using ISSR markers. A recent study conducted in Syria correlated the morphological traits to the genetic differentiation and to the geographical distribution of *Capparis* species, using Inter Retro-transposon Amplified Polymorphism (IRAP), ISSR and combined data of IRAP+ISSR. The percentages of polymorphism recorded were 71, 82, and 75%, respectively for the three techniques. A clear separation was revealed among *C. spinosa , C. aegyptia* Lam, and *C. sicula* Duh. Nevertheless, two samples could not be identified and were found at an intermediate position between *C. sicula* and *C. spinosa* indicating a possible hybrid origin between these two species (AL-SAFADI *et al*., 2014).

Plant species delimitation is of central importance in phylogenetic systematics, evolution, biogeography and biodiversity. It is significant to infer patterns and mechanisms of speciation and hybridisation, the evolutionary process by which new biological species arise and gene flow between closely related phylogenetic species can occur (DUMINIL and DI MICHELE, 2009; SCHLUTER, 2001). Isolation by distance, local adaptation and gene flow are different mechanisms responsible for species differentiation and genetic diversity (FREELAND *et al*., 2011; FRICHOT *et al*., 2013).

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Appendix

Species	Herbarium code
Capparis spinosa L.	IAUH-15292
Capparis spinosa L.	IAUH-15293
Capparis spinosa L.	IAUH -15295
Capparis spinosa L.	IAUH -15291
Capparis spinosa L.	IAUH -15288
Capparis spinosa L.	IAUH -15294
Capparis spinosa L.	IAUH -15286
Capparis spinosa L.	IAUH- 15287
Capparis spinosa L.	IAUH- 15297
Capparis spinosa L.	IAUH- 15298
Capparis spinosa L.	IAUH- 15296
Cappais parviflora Boiss.	IAUH- 15300
Capparis parviflora Boiss.	IAUH- 15301
Capparis parviflora Boiss.	IAUH- 15302
Capparis parviflora Boiss.	IAUH- 15303
Capparis mucronifolia Boiss.	IAUH- 15304
Capparis mucronifolia Boiss.	IAUH- 15305
Capparis mucronifolia Boiss.	IAUH- 15306
Capparis cartilaginea Decne.	IAUH- 15307
Capparis decidua (Forssk.) Edgew.	IAUH- 15308

Species	Accession No.
Capparis spinosa	C424456.1
Capparis spinosa	C424460.1
Capparis spinosa	C424461.1
Capparis spinosa	C424462.1
Capparis parviflora	C424463.1
Capparis parviflora	C424464.1
Capparis parviflora	C424465.1
Capparis parviflora	C424466.1
Capparis parviflora	C424467.1
Capparis mucronifolia	C424470.1
Capparis mucronifolia	C424471.1
Capparis mucronifolia	C424472.1
Capparis cartilaginea	424473.1
Capparis cartilaginea	424474.1
Capparis cartilaginea	424475.1
Capparis spinosa	KF454306.1
Capparis spinosa	KF454307.1
Capparis acutifolia	Kp092569
Capparis acutifolia	KP093584()
Capparis incanescens	MN733441

MORFOLOŠKO I MOLEKULARNO RAZDVAJANJE UNATAR VRSTE *CAPPARIS* (CAPPARACEAE)

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Izvod

Capparis spinosa L. (Capparidaceae) je najveći rod porodice *Capparaceae*, rasprostranjen u pantropskom regionu. *C. spinosa* je poznata kao lekovita biljna vrsta. U Iranu se različiti delovi biljke kapra koriste kao diuretici, tonici i u lečenju malarije i bolesti zglobova. Do sada u zemlji nisu postojale detaljne informacije o molekularnoj filogeniji i genetskoj strukturi ovih vrsta. Stoga je ovo istraživanje sprovedeno sa ciljem da se istraži razdvajanje unutar vrste prema morfološkim i molekularnim podacima i da se otkrije genetska raznolikost i struktura populacije u ovih pet vrsta *Capparis.* Za ovu studiju je korišćeno 108 nasumično prikupljenih biljaka iz 20 geografskih populacija vrsta *Capparis*. Naišli smo na opsežne genetske i morfološke diverzitete vrsta. ISSR molekularni markeri mogli bi da se koriste za razdvajanje proučavane vrste. STRUKTURNA analiza otkrila je pojavu protoka gena između ovih vrsta. Mantelov test pokazao je korelaciju između genetske udaljenosti i geografske udaljenosti proučavanih populacija.

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