

Evaluation of Molecular and Morphological Diversity of *Capparis Spinosa*

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Abstract

The objective of this study was the evaluation of molecular and morphological diversity among 80 caper (*Capparis spinosa* L.) genotypes from the 12 regions of the central Zagros Mountains located in the west of Iran. The results showed a high level of morphological genetic variation among the caper samples. According to the morphological cluster analysis, 80 genotypes were clustered into five main groups. The 15 factors justified 78.7 % of the total variation based on factor analysis. ISSR primers produced a total of 108 polymorphic bands (85.04%) from 127 bands and the PIC for primers ranged from 0.01 to 0.52. SCoT primers produced a total of 165 polymorphic bands (86.84%) from 190 bands and the PIC for primers ranged from 0.06 to 0.55. Ordination and cluster analysis by ISSR markers showed that the genetic relationships among all accessions could be separated into three major groups and by SCoT markers separated into six groups. The results did not show a perfect match between the molecular diversity groupings and geographical regions, because many natural factors and human activities shape the amount and pattern of genetic diversity in a plant population. SCoT markers were more informative than ISSR markers for the assessment of genetic diversity of caper germplasm. The combined (ISSR+SCoT) markers haven't shown more information of genetic diversity than single analysis of ISSR and SCoT. The results indicated the existence of dispersion and different levels of morphological variation and molecular genetic diversity in the genotypes collected from west of Iran.

Introduction

Recently, medicinal plants have been known for possess multiple health-promoting effects, and the high efficacy and low adverse effects for the treatment of different human diseases (Nabavi et al. 2016). Also, it is well known that synthetic drugs can cause a wide range of serious adverse events (Bertrand et al. 2014). Therefore, in recent years, medicinal plants have received much attention (Schulz 2006).

C. spinosa belonging to the family Capparidaceae is a xerophytic plant. *C. spinosa* had been a part of the Mediterranean diet for over 5,000 years (Muharrem et al. 2009). Its commercial name is *C. spinosa* and its brand is caper. This plant is capable of growing in a broad range of climatic conditions, varying from dry deserts to cooler altitudes of mountains, this plant has developed special mechanisms in order to survive in the semiarid lands conditions and consequently its introduction may help to prevent the disruption of the equilibrium of those fragile ecosystems and the soil degradation. (Lansky et al. 2014; Pugnaire 1989; Manikandaselvi et al. 2016). *C. spinosa* is used as a traditional medicine for lowering blood sugar and blood fat, diuresis, as well as a rheumatism and arthritis treatment. Studies have shown that many of its chemical constituents have antimicrobial, anti-oxidative, anti-inflammatory, immunomodulatory and antiviral properties (Tlili et al. 2010; Patel et al. 2014; Zhang et al. 2012). Caper population structure has been low studied. Different taxonomists have recognized 250 morphologically different species in the genus caper.

All different parts of the caper like its young shoots, flower buds, fruits and seeds are used for human diet. Previous studies on the chemical composition of *C. spinosa* have reported the presence of a large number of beneficial compounds such as vitamins, minerals, alkaloids, and lipids (Matsuyama et al. 2009; Tlili et al. 2010; Patel et al. 2014). Capers (flower buds), caper berries (fruits), leaves, roots, and seeds of this plant are used medicinally (Anonymous 1999). Fruit and the root of the plant were used in gout and also as diuretics, astringents, and tonic. It is known that its glucosinolate compositions possess anti-carcinogenic properties, anti-lipid peroxidation and antioxidant effects (Tlili et al. 2010; Patel et al. 2014; Zhang et al. 2012). The whole plant is anti-microbial and used in rheumatism (Aliyazicioglu et al. 2013). Flavonoids and polyphenols of *C. species* (Mansour et al. 2016; Musallam et al. 2012), are known to possess many beneficial biological activities (Schmidt et al. 2016).

C. spinosa displays huge agro-based potentialities and a highly demand for exploitation due to a diversified international market. Today, it seems necessary to focus on the possibility of selection and improvement of this specie, especially in the east Mediterranean countries (Sozzi and Vicente 2006; Tlili et al. 2011; Nabavi et al. 2016).

Aromatic and medicinal plants have less arable land compared to other crops. However, are contains a large number of plant species used, with the greatest variation in morphological traits and characteristics (Salamat et al. 2014). Patterns of morphological variation observed within and between plants populations indicate that morphology may vary in apparently random directions. Exactly unclear for the geographic origin of *C. spinosa*, however, it seems to be originated from somewhere in China, India or Central Asia (Liu et al. 2015). Zohary (1960) reported five species with some varieties in Iran. Zokian (2015) reported the high diversity of morphological and anatomical characteristics of *C. spinosa* that grown wildy in Iraq. Musallam et al. (2012) collected twenty four populations of *C. spinosa* that covered different geographical regions of Jordan, and Reported that the phenotypic diversity of *C. spinosa* in Jordan was found to be high.

Increasingly, molecular marker technologies are playing an important role in assessing genetic diversity, identifying genetic relationships, and aiding germplasm fingerprinting in plant collections. Over the last few decades a variety of different genetic analytical techniques have emerged in the field of molecular genetics along with several PCR-based genetic markers that have now been established and are used to provide information on genetic variations in plant species. Recently, studies were reported about a high genetic diversity in *C. spinosa* using DNA markers such as RAPD (O'zbek and Kara 2013; Bhoyar et al. 2012), ISSR (Liu et al. 2015; Rhimi et al., 2019; Ahmadi and Saeidi, 2018; Tamboli et al. 2018), AFLP (Yousfi et al. 2016; Inocenco et al., 2003) and IRAP (Al-safadi et al. 2014). Inter Simple Sequence Repeat (ISSR) markers (Zietkiewicz et al. 1994) and Start Codon Targeted (SCoT) markers (Bertrand et al. 2009) are dominant marker, which can be used for assessment of genetic diversity within crop germplasm, genetic analysis, bulked segregant analysis, and quantitative trait loci mapping. Gristina et al. (2014) studied nineteen wild populations collected from different regions in Italy and belonging to two different subspecies, *C. spinosa* subsp. *spinosa* and subsp. *rupestris*, were evaluated by the ISSR marker. Ahmadi and Saeidi (2018) Studied 21 populations of caper in Iran using ISSR markers, and the populations of plants divided into 4 clusters.

SCoT a novel method for generating plant DNA markers were designed based on the ATG start sequences and the regions between the start codons are amplified during the polymerase chain reaction and show the differences. The SCoT primers are typically 18–24 nucleotides and their G and C content is 50–72% (Bertrand et al. 2009). In a study, 16 *Foeniculum vulgare* populations in Iran were evaluated using SCoT markers, and the results showed that the studied populations were divided into 4 clusters (Nikkerdar *et al.* 2017).

In the present study, genetic diversity of 80 *C. spinosa* samples collected from 12 regions in west of Iran were evaluated, and characterized by using morphological characteristics, ISSR, and SCOT markers.

Material Method

Plant materials

The plant materials, including 80 caper genotypes collected from 12 locations of the central Zagros Mountains located in the west of Iran (Kermanshah, Charmelah, Ilam, Sarpolzahab, Qasreshirin, Goresefid, Gilanegharb, Ivan, Somar, Naftshahr, Khosravi, and Kerend) from May 22, 2016 to June 29, 2017 (Fig. 1.). Geographic coordinates and elevation of each genotype habitat were determined by GPS (Table 1). The 80 genotypes from 12 populations were evaluated for 43 morphological characteristics in the place (Table 2).

DNA extraction

Fresh young leaf samples were taken from each caper genotype to assess the molecular variation and genetic relationships of 80 genotypes. The samples were stored at -80°C temperature until DNA was extracted. Genomic DNA was extracted using a modified CTAB protocol by Doyle and Doyle (1987). Quantitative amount of DNA was determined with spectrophotometer at the wavelength of 260 nm and quality of DNA was determined with agarose gel electrophoresis.

Table 1
General features of the 12 sampling locations of *C. Spinosa* L. in west of Iran

Sample code	Population	Population size	Sampling location	Sample number/ population	Geographical location		
					Latitude	Longitude	Altitude (m)
1	Pop 1	5	Kermanshah	1	47°12.620	34°31.633	1319
2				2	47°17.164	34°57.101	1406
3				3	47°17.132	34°57.026	1356
4				4	47°17.220	34°57.035	1361
5				5	47°17.252	34°57.115	1372
6	Pop 2	4	Charmelah	1	46°17.387	33°56.586	1332
7				2	46°17.382	33°56.593	1338
8				3	46°17.366	33°56.606	1338
9				4	46°16.732	33°56.489	1304
10	Pop 3	10	Ilam	1	46°23.731	33°36.492	1306
11				2	46°23.718	33°36.034	1333
12				3	46°23.736	33°36.038	1338
13				4	46°23.711	33°36.031	1329
14				5	46°23.707	33°36.030	1329
15				6	46°23.693	33°36.024	1330
16				7	46°23.702	33°36.026	1331
17				8	46°23.718	33°35.989	1332
18	Pop 4	12	Sarpolzahab	9	46°18.408	33°39.798	1131
19				10	46°18.413	33°39.814	1127
20				1	45°52.042	34°27.846	555
21				2	45°52.018	34°27.876	553
22				3	45°52.087	34°27.811	555
23				4	45°52.142	34°27.787	573
24				5	45°52.157	34°27.789	567
25				6	45°52.157	34°27.789	570
26				7	45°52.153	34°27.786	573
27				8	45°52.154	34°27.785	574
28				9	45°52.142	34°27.771	571
29				10	45°52.124	34°27.752	560
30	Pop 5	6	Qasereshirin	11	45°52.128	34°27.755	561
31				12	45°52.131	34°27.756	561
32				1	45°36.057	34°31.671	388
33				2	45°36.042	34°31.670	376
34				3	45°36.033	34°31.666	376
35				4	45°36.033	34°31.670	376
36				5	45°36.052	34°31.691	372

Sample code	Population	Population size	Sampling location	Sample number/ population	Geographical location		
					Latitude	Longitude	Altitude (m)
37	Pop 6	6	Goresefid	6	45°36.057	34°31.688	372
38				1	45°48.271	34°15.933	632
39				2	45°48.212	34°15.936	633
40				3	45°48.215	34°15.930	633
41				4	45°48.238	34°15.926	628
42				5	45°48.211	34°15.928	628
43	Pop 7	4	Gilanegharb	6	45°48.218	34°15.941	628
44				1	45°58.551	34°72.560	867
45				2	45°58.556	34°72.580	867
46				3	45°61.964	34°51.570	1053
47	Pop 8	5	Ivan	4	45°61.954	34°51.460	1049
48				1	45°38.634	33°52.552	300
49				2	45°37.171	33°57.095	1399
50				3	45°37.630	33°57.111	1385
51				4	45°37.540	33°57.115	1388
52	Pop 9	14	Somar	5	45°37.278	33°57.132	1388
53				1	45°69.810	33°57.422	1121
54				2	45°57.216	33°57.364	712
55				3	45°57.069	33°57.429	713
56				4	45°56.913	33°57.415	695
57				5	45°50.485	33°56.310	507
58				6	45°50.483	33°56.304	515
59				7	45°50.492	33°56.304	514
60				8	45°50.477	33°56.301	514
61				9	45°38.631	33°52.547	301
62	Pop 10	4	Naftshahr	10	45°38.629	33°52.551	299
63				11	45°64.631	33°82.552	299
64				12	45°64.240	33°87.732	295
65				13	45°64.242	33°87.730	295
66				14	45°64.500	33°87.725	295
67				1	45°70.722	34°03.107	622
68	Pop 11	4	Khosravi	2	45°70.725	34°03.103	622
69				3	45°70.730	34°03.102	622
70				4	45°70.732	34°03.102	622
71				1	45°73.328	34°39.925	305
72				2	45°73.328	34°39.925	310
73				3	45°73.330	34°39.930	307

Sample code	Population	Population size	Sampling location	Sample number/ population	Geographical location		
					Latitude	Longitude	Altitude (m)
74	Pop 12	6	Kerend	4	45°73.335	34°39.931	375
75				1	46°29.426	34°23.841	1215
76				2	46°29.521	34°23.877	1215
77				3	46°29.501	34°23.877	1215
78				4	46°29.580	34°23.821	1215
79				5	46°29.495	34°23.945	1215
80				6	46°29.687	34°23.877	1215

Table 2
Mean, Maximum, Minimum, Variance, Standard Deviation and Coefficient of the Variation values for each characteristic
evaluated among the 12 populations of *C. spinosa* L.

evaluated among the 12 populations of <i>C. spinosa</i> L.										
No.	Characteristic			abbreviation	Min	Max	Mean	SD ^a	Variance	CV % ^b
1	Herb	Growth Power	GP	3	9	5.90	1.67	2.79	28.31	
2		Branchesis	B	3	9	5.50	1.72	2.95	31.23	
3		Growth Habit	GH	1	4	3.33	0.80	0.64	24.14	
4		Growth 1 Year Branch	GYB	1	3	2.04	0.83	0.69	40.65	
5		Middle Node Length	MNL	3	7	3.93	1.05	1.09	26.65	
6		Lenticels Number	LN	1	4	2.74	0.85	0.72	30.97	
7		Stem Color	HSC	1	10	2.34	2.58	6.65	110.31	
8		Type stem	HTS	1	2	1.05	0.22	0.05	20.76	
9		Fruit Length	FLH	3	9	5.98	1.58	2.50	26.46	
10	Fruit	The Maximum Diameter	FTD	3	7	4.73	1.33	1.77	28.19	
11		Length to Diameter Ratio	FLD	3	7	4.15	1.09	1.18	26.15	
12		Tail Length	FTL	3	7	6.05	1.09	1.20	18.09	
13		Pedicel	P	3	7	5.85	1.22	1.48	20.78	
14		Thick Tail	FTT	3	7	4.38	1.08	1.16	24.61	
15		Edge Profiles	FEP	1	2	1.23	0.42	0.17	34.09	
16		Symmetry	FS	1	2	1.21	0.41	0.17	33.74	
17		Number of Lines on The Fruit	NF	3	7	3.93	1.22	1.49	31.15	
18		Curved Tail	FCT	1	9	5.65	1.94	3.78	34.40	
19		Percentage Tail curvature	PTC	1	7	3.35	2.23	4.98	66.60	
20		Position of Maximum Diameter	FPM	1	3	1.54	0.87	0.75	56.27	
21		Aqueous Meat	FAM	1	10	5.10	2.32	5.39	45.52	
22		Fruit Tail Color	FTC	1	2	1.15	0.36	0.13	31.05	
23		Bump Lines	FBL	1	2	1.33	0.47	0.22	35.35	
24		Size	SS	1	3	1.66	0.65	0.42	39.15	
25		Seed	Shape	SSH	1	2	1.86	0.34	0.12	18.49
26	Color		SC	1	2	1.53	0.50	0.25	32.75	
27	Leaf	Flower Size	FLS	1	2	1.05	0.22	0.05	20.76	
28		Branch Quil Density	SBOD	1	7	4.73	1.75	3.07	37.11	
29		Leaf Quil Density	LQD	1	7	4.58	1.81	3.27	39.52	
30		Leaf Blade Length (Cm)	LBL	3	9	5.23	1.55	2.40	29.65	
31		Leaf Blade Width (Cm)	LBW	3	9	5.40	1.43	2.04	26.45	
32		Ratio Leaf Blade Length/Width	RLB	3	9	5.43	1.21	1.47	22.34	
33		Petiole Length	PL	3	9	4.38	1.63	2.66	37.27	
34		Shape of Base	LSB	1	5	2.33	1.16	1.34	49.87	
35		Shape of Apex	LSA	1	5	2.63	1.25	1.56	47.57	
36		Keen Beak Length	LKB	1	7	4.15	1.89	3.58	45.58	
37		Petiole Stipule	LPS	1	2	1.60	0.49	0.24	30.62	
^a Standard deviation										
^b CV, coefficient of variation										

No.	Characteristic	abbreviation	Min	Max	Mean	SD ^a	Variance	CV % ^b
38	Flower Bud Length	LFB	1	2	1.63	0.48	0.23	29.79
39	Flag Color	LFC	1	4	1.71	0.74	0.55	43.50
40	Flower Shape Tip Buds	STB	1	2	1.31	0.46	0.21	35.32
41	Flag Length	FL	3	9	6.58	1.54	2.37	23.41
42	Petal Size	PS	3	9	6.68	1.43	2.04	21.42
43	Sepal Length	SL	1	3	2.28	0.79	0.62	34.73
^a Standard deviation								
^b CV, coefficient of variation								

ISSR-PCR

A set of 34 ISSR primers were used for PCR. Of these primers, only 10 primers were selected based on amplification of clear and distinguishable DNA fragments (Table 3). PCR reactions were carried out in a volume of 25 µl, containing 14.75 µl sterile double-distilled water, 2.5 µl of the PCR buffer (Amplicon, Cat. No. 180301), 150 mM Tris–HCl pH 8.5, 40 mM (NH₄)₂SO₄, 1.6 mM MgCl₂, 0.5 mM dNTPs, 0.2 units/ml Amplicon Taq DNA polymerase (Sigma- Aldrich, USA), 2.5 pmol of primer, and 3 µl of template DNA (50 ng/ µl). The PCR was carried out at 94°C for 3 min for initial denaturation, 45 cycles of 1 min denaturation at 94°C, 1 min for annealing at 50–57°C depending on the primer (Table 3), and extension for 1 min 30 second at 72°C. This was followed by a final extension of 6 min at 72°C. Generated products were separated on 2% agarose gel electrophoresis in 1×TBE buffer and stained with ethidium bromide (10 mg/ml). Fragment size was estimated by using a 1 kb DNA ladder and gels were visualized under UV light.

SCoT-PCR

Ten primers were used for SCoT amplification (Table 3). Amplification reaction was performed in volumes of 26 µl containing 15.75 µl sterile double-distilled water, 2.5 µl of the PCR buffer (Amplicon, Cat. No. 180301, 150 mM Tris–HCl pH 8.5, 40 mM (NH₄)₂SO₄), 1.5 mM MgCl₂, 0.5 mM dNTPs, 0.25 units/ml Amplicon Taq DNA polymerase (Sigma- Aldrich, USA), 2.5 pmol of each primer, and 3 µl of template DNA (50 ng/ µl). The PCR was carried out at 94°C for 3 min for initial denaturation, 35 cycles of 1 min denaturation at 94°C, 1 min for annealing at 50°C depending on the primer (Table 3), and extension for 1 min 30 second at 72°C. This was followed by a final extension of 6 min at 72°C. Generated products were separated on 2% agarose gel electrophoresis in 1×TBE buffer and stained with ethidium bromide (10 mg/ml). Fragment size was estimated by using a 1 kb DNA ladder and gels were visualized under UV light.

Table 3
Characteristics of the ISSR and SCoT primers used in this study

ISSR Primers	Annealing temperature	SCoT Primers	Annealing temperature
UBC 873	54	SCoT 1	50
UBC 880	54	SCoT 12	52
USB 835	56	SCoT 13	50
UBC 864	56	SCoT 18	52
USB 884	55	SCoT 22	52
UBC 856	54	SCoT 29	50
UBC 825	54	SCoT 30	50
UBC 841	50	SCoT 31	52
UBC 807	54	SCoT 33	50
UBC 808	57	SCoT 36	54

Statistical analysis

Frequency and percentage distribution of morphological traits were specified to qualitative descriptors. The Principal components analysis (PCA) based on the covariance matrix of the coefficients and factor analysis were performed by the SPSS version 22.0 software (SPSS Inc. 2004). Cluster analyses were conducted to specify the dissimilarity indices measure to be used in clustering with the Neighbour joining (NJ) method by the software DARwin5 (version: 5.0.145). PCR-amplified ISSR and SCoT fragments detected on gels were scored as absent (0) or present (1). The dissimilarity matrix was generated using Jaccard indices (Jaccard 1908). The DARwin program was used for cluster analysis based on a dissimilarity matrix. The Mantel test was performed using XLSTAT software. The matrix was analyzed by the Neighbour joining method (NJ) and relationships between the cultivars were

illustrated as a dendrogram. Genetic diversity parameters such as the number of polymorphic loci (NPL), the percentage of polymorphic loci (PPL), effective number of alleles (Ne) (Kimura and Crow, 1964), Nei's genetic diversity (h), Shannon's information index (I) (Lewontin 1972), gene flow (Nm) and genetic differentiation coefficient (Gst) were calculated using POPGENE ver. 1.32 (Yeh *et al.* 1999). Analysis of molecular variance (AMOVA) and principal coordinate analysis (PCoA) were performed using GenALEX software ver. 6.5 (Peakall and Smouse 2006).

Results

Assessment of genetic variability and relationships among the caper genotypes using morphological traits

Twelve *C. spinosa* populations were characterized according to general morphological characteristics. The genotypes collected from "Gilanegharb" had medium to large size shrubs with green stem, medium flowers with white petals and flag, ovate shape dark green leaves and glabrous, medium, non-symmetrical and oblong fruits with the largest diameter in the middle, green fruit tail (Fig. 2A). The genotypes belong to "Goresefid" had medium shrubs with violet stem, medium to small, non-symmetrical and oblong fruits with the largest diameter in the middle, green fruit tail, moderate or relatively large, glabrous dark green leaves, low inter node length, medium flowers with white flags (Fig. 2B). "Khosravi" population had shrubs with medium growth power, wooden trunk, small and round leaves with abundant trichome, low internode length, small flowers with white petals and purple flags, small and round fruits with a bright green color, and the largest diameter of the fruit in the middle (Fig. 2C). The genotypes collected from the "Naftshahr", "Kerend" and "Somar" had large shrubs with yellow stem, large flowers with white flags, bold green, large and crusty leaves, ovate shape leaves and glabrous, sunk apex leaves, short middle node length, and medium fruits with the highest fruit diameter at the end of the fruit (Fig. 2D). "Qasreshirin" population had medium shrubs, hairless purple to green colored stem, round and small dark green leaves, Medium internode length, medium fruits and flowers. The color of their fruit is dark green and their fruit tail color is green. They have ovate shape fruit with the largest diameter in the bottom, and red and white or cream colored ripe fruit. The genotypes collected from the "Sarpulzahab", "Charmeleh" and "Kermanshah" had large shrubs with large leaves, internode length, and petals. They had purple to green colored stem, white petals, pink and white colored flag, round hairy bright green leaves, bright ovate fruits, and green fruit tail (Fig. 2E, 2F).

The collected *C. Spinosa* fruits showed persistent variation (Fig. 3). Of the studied characteristics HSC, FTC, FPM and LSB showed higher coefficients of variation (CV), indicating a high level of variation, and the characteristics of HTS, FTL, P, SSH, LBRL, FL, and PS showed the least coefficients of variation (CV), representing the lowest level of changes (Table 2).

The relationship between 80 *C. Spinosa* genotypes was shown in the dendrogram of a hierarchical cluster analysis using Euclidean dissimilarity with the Neighbour joining (NJ) method as amalgamation rules (Fig. 4). According to the results of cluster analysis based on morphological characteristics, 80 genotypes were clustered into five main groups. The first group consisted of genotypes from "Ivan" population (48–51), and the some genotypes "Sarpulzahab" (numbers 24–25), "Qasreshirin" (numbers 33, 34, 35), and one genotype from "Gilanegharb" (number 46). These genotypes were similar in GB, B, GH, HTS, FLD, FTL, FTC, SSH, SBOD, LQD, LBW, LPS, FL, and PS characteristics.

The genotypes from "Khosravi" population, and the most genotypes "Somar" (numbers 53, 62–70), and one genotype from "Gilanegharb" (number 44) and "Sarpulzahab" (number 26) were belonged to the second group. These genotypes were similar in MNL, FLD, FTT, FTC, SSH, RLB, PL, FL, and PS. The third main group contains the genotypes of "Somar" (numbers 55–57, 59, 60), "Ilam" (numbers 10–13, 15, 16, 18 and, 19), and one genotype from "Charmeleh" (number 8), and "Ivan" (number 52), and "Qasreshirin" (number 37), and two genotypes of "Sarpulzahab" (number 27 and 31). These genotypes were similar in GH, HTS, FLD, FTL, FBL, SS, SSH, FLS, LBW, LFC, FL, and PS characteristics. The fourth main group contains the two genotypes of "Charmeleh" (numbers 6 and 7), "Goresefid" (number 39 and 40), and one genotype from "Sarpulzahab" (number 23), and "Gilanegharb" (number 45) population. These genotypes were similar in HSC, HTS, FLH, FTD, FLD, FEP, FCT, PTC, FPM, FTC, SS, SSH, FLS, LBL, PL, and LFC characteristics.

The fifth main group consisted of genotypes from "Kermanshah" population and "Kerend" population, and the most of genotypes of "Sarpulzahab" (numbers 20–22, 28–30), "Goresefid" (number 38, 41–43), and some of genotype from "Somar" (number 54, 58, 61), "Ilam" (number 14, 17), "Qasreshirin" (number 32, 36), and one genotype from "Charmeleh" (number 9), and "Gilanegharb" (number 47) population. These genotypes were similar in GH, MNL, HSC, HTC, FLD, FTL, P, FTT, NF, FTC, SSH, FLS, SBOD, LPS, LFB, STB, and PS characteristics.

Principal component analysis was performed due to reduction of data for transparency relation between two or more of the characteristics. The first three components justified 27.90 % of the total changes (Table 4). The first and second components explained 13.15 and 8.73 % of the changes, respectively. The factor analysis was performed based on principal component analysis and using varimax rotation with eigenvalues greater than one and reduced the 43 variables into 15 factors. Fifteen factors justified about 78.7 % of the total variation found among genotypes (Table 5). The loading factors greater than 0.5, regardless of the respective sign were considered as significant coefficients. The first factor accounted for 6.70 % of the variation in total. This factor included type stem, fruit length, the maximum diameter, tail length, pedicel, aqueous meat, flower size, flag color, flag length and petal size. Therefore, the second factor explained 3.40 % of the total variation. Total growth power and branchesis were the main traits in second factor. The third factor explained 3.10 % of the total variation.

Table 4

The results of principal component analysis (PCA) performed according to Pearson's correlation (one-tailed) matrix with Eigen values, percentage of variance explained and cumulative percentage of variance

PC	Eigenvalue	variance%	Cumulative variance (%)	PC	Eigenvalue	Variance %	Cumulative variance (%)
1	13.151	18.43	18.43	21	0.490	0.69	68.33
2	8.732	12.24	30.67	22	0.434	0.61	68.74
3	6.022	8.44	27.90	23	0.408	0.57	69.10
4	5.201	7.29	33.11	24	0.362	0.51	69.40
5	4.553	6.38	37.66	25	0.303	0.42	69.66
6	4.460	6.25	42.12	26	0.264	0.37	69.89
7	3.975	5.57	46.09	27	0.222	0.31	70.10
8	3.750	5.26	49.84	28	0.215	0.30	70.29
9	3.061	4.29	52.90	29	0.188	0.26	70.46
10	2.728	3.82	55.63	30	0.172	0.24	70.60
11	2.148	3.01	57.78	31	0.139	0.20	70.73
12	1.573	2.20	59.35	32	0.131	0.18	70.84
13	1.513	2.12	60.87	33	0.107	0.15	70.94
14	1.366	1.91	62.23	34	0.101	0.14	71.03
15	1.151	1.61	63.38	35	0.087	0.12	71.11
16	1.113	1.56	64.50	36	0.077	0.11	71.18
17	0.862	1.21	65.36	37	0.071	0.10	71.23
18	0.779	1.09	66.14	38	0.054	0.08	71.28
19	0.668	0.94	66.81	39	0.049	0.07	71.32
20	0.598	0.84	67.40	40	0.038	0.05	71.34

Table 5
The results of factor analysis performed with 43 characteristics in the 80 caper genotypes

Characteristics		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Growth Power	GP	.03	.60	.04	-.53	.13	.09	-.03	.07	-.10	.13	.15	.15	.17	.20	-.24
Branchesis	B	.06	.62	-.16	-.39	.13	.06	-.11	.00	-.01	.29	-.02	.17	.29	.01	-.23
Growth Habit	GH	.32	.28	-.15	.35	.13	.05	-.17	-.22	.09	-.36	.30	.07	-.01	.02	.16
Growth 1 Year Branch	GYB	-.08	-.73	.15	.17	.10	.08	-.12	.11	.14	-.09	-.07	-.05	.20	-.03	-.21
Middle Node Length	MNL	.38	-.10	-.14	.40	-.12	.06	.18	-.21	-.05	.38	-.01	-.04	.14	-.20	-.01
Lenticels Number	LN	-.34	.17	-.14	.05	.00	.13	.43	-.55	.13	.19	.01	-.21	.04	-.02	.02
Stem Color	HSC	-.06	-.61	.17	-.27	-.28	.15	.12	-.07	.03	.05	-.29	.19	-.10	.18	-.05
Type stem	HTS	-.87	.18	.05	.19	-.15	.08	-.08	-.04	.14	.14	-.08	.04	-.09	-.05	.00
Fruit Length	FLH	.61	-.03	.05	.43	-.36	-.16	-.18	.06	.12	.13	.00	-.03	.07	.16	.04
The Maximum Diameter	FTD	.50	.12	.45	.31	-.10	.02	-.32	.14	.07	-.05	.10	-.07	.09	-.03	-.29
Length to Diameter Ratio	FLD	.31	-.20	-.54	.13	-.31	-.35	.11	.17	.08	.15	-.19	-.10	-.01	.07	.01
Tail Length	FTL	.55	-.09	-.02	.31	.23	-.06	-.27	-.34	-.14	.17	-.32	.00	.05	.16	-.11
Pedice	P	.54	.34	-.10	.22	-.08	.17	-.27	-.27	-.08	.02	-.21	-.09	-.10	.20	-.03
Thick Tail	FTT	.36	.16	.48	-.09	-.09	.23	-.24	.20	.15	.32	-.10	-.37	.03	.11	.11
Edge Profiles	FEP	.01	-.37	.02	-.27	.20	-.43	.24	-.12	.15	.35	.03	-.11	.11	.01	-.12
Symmetry	FS	.05	-.38	-.06	.00	.10	-.46	.16	-.09	.01	.40	.24	.04	-.14	.27	-.09
Number of Lines on The Fruit	NF	.20	.18	.12	-.19	.19	.15	-.37	.03	.26	.40	.16	-.06	-.27	-.02	.43
Curved Tail	FCT	.47	-.28	-.18	-.42	.22	.00	.05	.10	-.04	.16	.14	-.01	-.07	-.45	.04
Percentage Tail curvature	PTC	-.40	-.20	.02	-.14	.00	.16	.05	-.14	-.02	-.05	.25	-.18	.56	.22	.25
Position of Maximum Diameter	FPM	.05	.22	.46	.10	-.12	-.33	.36	.09	-.16	.11	.08	.13	-.34	.07	.20
Aqueous Meat	FAM	-.54	-.18	-.06	-.14	.01	.17	-.28	.35	.26	.19	.23	-.17	.00	.14	.08
Fruit Tail Color	FTC	.14	-.39	.18	-.11	-.15	.36	.01	-.27	.38	.16	.08	.05	.01	.10	.03
Bump Lines	FBL	-.27	.23	.43	.14	.33	-.05	.01	-.24	.27	.11	.08	.19	-.04	.07	-.32
Size	SS	-.37	.33	.15	.53	-.12	-.20	.06	.02	-.16	.23	.14	.14	.07	-.12	.07
Shape	SSH	-.12	.04	.22	-.23	.54	.06	-.03	.04	-.12	.05	-.37	-.05	-.05	-.31	.08
Color	SC	-.01	.10	-.37	.00	.65	-.20	.15	.08	.13	-.08	-.15	.07	-.07	.38	.03
Flower Size	FLS	-.87	.18	.05	.19	-.15	.08	-.08	-.04	.14	.14	-.08	.04	-.09	-.05	.00
Branch Quil Density	SBOD	-.43	-.31	-.05	.30	.27	.21	-.19	.23	-.47	.16	.06	.20	.05	.10	.09
Leaf Quil Density	LQD	-.46	-.40	.02	.29	.26	.17	-.17	.17	-.41	.18	.05	.25	.05	.06	.09
Leaf Blade Length (Cm)	LBL	.44	.18	-.35	.25	.11	.42	.17	.28	.06	.27	.02	.17	.00	.05	-.08
Leaf Blade Width (Cm)	LBW	.45	-.12	-.23	.11	.08	.60	.28	.13	.01	.15	-.09	.20	-.15	.02	-.05
Ratio Leaf Blade Length/Width	RLB	.16	.22	-.42	.14	-.09	-.33	.00	.37	.26	.20	.13	.09	.13	-.20	.02

Characteristics		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Petiole Length	PL	.26	.09	.10	-.17	-.38	.33	.51	.23	.01	-.16	.19	.20	.01	.22	.07
Shape of Base	LSB	.12	-.20	-.25	.08	.20	.09	-.09	.05	.23	-.21	.39	-.07	-.35	-.05	-.34
Shape of Apex	LSA	.05	-.08	-.06	.13	-.03	.31	.07	-.31	.47	.01	-.05	.36	.16	-.26	.09
Keen Beak Length	LKB	.40	-.08	.07	-.11	.27	-.17	-.17	.00	.47	-.16	-.15	.28	-.04	.14	.28
Petiole Stipule	LPS	.04	.22	.28	.31	.36	-.20	.38	.17	.23	-.13	-.14	.01	.26	.02	.22
Flower Bud Length	LFB	.20	.13	-.25	-.44	-.45	-.14	-.23	.08	-.18	.02	-.23	.24	.06	.00	.06
Flag Color	LFC	-.67	.16	.38	-.07	-.19	.00	.07	.00	.12	.09	-.17	.08	-.21	.04	-.12
Shape Tip Buds	STB	.04	.22	-.08	.16	.18	.38	.42	.32	-.05	-.01	-.18	-.45	-.07	.06	-.05
Flag Length	FL	.57	-.12	.68	-.04	.04	-.03	.12	.15	-.09	.01	.12	.05	.05	-.04	-.06
Petal Size	PS	.64	-.08	.60	-.02	.07	-.04	.15	.06	-.14	.02	.05	.08	.09	-.09	.00
Sepal Length	SL	.37	.05	-.06	-.15	.08	.11	.03	-.57	-.46	.07	.22	-.05	-.12	.04	.14
Eigenvalues		6.7	3.4	3.1	2.7	2.4	2.3	2.0	2.0	1.9	1.6	1.3	1.2	1.1	1.1	1.0
% of variance		15.7	8.0	7.2	6.3	5.5	5.3	4.7	4.6	4.5	3.7	3.0	2.8	2.6	2.5	2.4
Cumulative variance		15.7	23.7	30.9	37.3	42.8	48.1	52.7	57.3	61.8	65.5	68.4	71.2	73.8	76.2	78.7

Assessment of genetic variability and relationships among the caper genotypes using molecular markers

Molecular analysis based on ISSR markers

Interpretation of obtained bands from gel electrophoresis showed a total of 127 ISSR bands amplified from 10 used ISSR primers. The mean number of band per assay was 12.7. The size of ISSR fragments generated by the different primers in this study ranged from 240 to 2500 bp, and the number of bands produced by the different primers ranged from 6 (UBC 864) to 18 (UBC 807). A total of 108 bands of 127 bands (85.04%) were polymorphic. The ISSR primers and their produced fragments in 80 caper genotypes are characterized in Table 6. The maximum number of polymorphic bands was amplified with the UBC 856, UBC 825, UBC 807 and UBC 808 primers, identifying 100% polymorphism and the minimum number of polymorphic bands were amplified with the ISSR864 primer, identifying 50% polymorphism.

Table 6
ISSR primers and the bands characteristics and interpretation in the 80 genotypes from 12 caper populations

Row	Primer name	NA	NP	PP	MI	RP	PS	PIC
1	UBC 873	11	8	72.7	1.22	5.82	14.38	0.21
2	UBC 880	10	6	60	0.72	3.60	15.55	0.20
3	USB 835	12	10	83.3	2.11	8.33	13.10	0.25
4	UBC 864	6	3	50	0.03	1.50	11.88	0.02
5	USB 884	11	8	72.7	1.12	5.82	13.35	0.19
6	UBC 856	17	17	100	5.08	17	16.98	0.30
7	UBC 825	15	15	100	4.89	15	13.98	0.33
8	UBC 841	10	6	60	0.56	3.60	16.33	0.17
9	UBC 807	18	18	100	4.55	18	22.33	0.25
10	UBC 808	17	17	100	3.80	17	20.23	0.22
Average		12.7	10.8	85.039	2.41	9.57	15.81	0.22
NA = the Number of Amplified Fragments, NP = the Number of Polymorphic Fragments, PP = Percentage of Polymorphism, MI = Marker Index, RP = the Ratio of Polymorphism, PS = Power Separation, PIC = Polymorphic Information Content								

Genetic diversity analysis based on 10 ISSR markers was carried out through POPGEN software on 12 caper populations of the central Zagros Mountains. Population characteristics and their diversity indices (NPL, PPL, Ne, h, I, NM, Gst) were assayed base on ISSR markers and summarized in

Table 7. Percentages of polymorphic loci (PPL) were ranged from 5.51 in “Kerend” population to 57.48 in “Somar” population among the studied populations. “Somar” population showed the highest level of variability, with NPL = 73, PPL = 57.48, Ne = 1.324, h = 0.193 and I = 0.291, while in “Kerend” population was observed the lowest one, with NPL = 7, PPL = 5.51, Ne = 1.041, h = 0.023 and I = 0.034. The mean values of genetic differentiation (Gst), and gene flow (Nm) between populations were 0.491, and 0.518, respectively.

Table 7
Genetic diversity analysis of 12 caper populations assessed with ISSR markers

Population	Geographic region	Sample size	NPL	PPL	Ne	h	I
Pop1	Kermanshah	5	38	29.92	1.213	0.117	0.171
Pop2	Charmeleh	4	41	32.28	1.205	0.119	0.177
Pop3	Ilam	10	68	53.54	1.303	0.178	0.269
Pop4	Sarpolzahab	12	69	54.33	1.320	0.184	0.276
Pop5	Qasreshirin	6	57	44.88	1.275	0.166	0.240
Pop6	Gorsefid	6	43	33.86	1.228	0.130	0.191
Pop7	Gilanegharb	4	46	36.22	1.251	0.141	0.208
Pop8	Ivan	5	12	9.45	1.053	0.032	0.048
Pop9	Somar	14	73	57.48	1.324	0.193	0.291
Pop10	Naftshahr	4	36	28.35	1.212	0.117	0.169
Pop11	Khosravi	4	44	34.65	1.246	0.138	0.201
Pop12	Kerend	6	7	5.51	1.041	0.023	0.034
Average	-	-	45	35.04	1.222	0.128	0.189
NPL = the Number of Polymorphic Loci, PPL = the Percentage of Polymorphic Loci, Ne = Effective Number of Alleles, h = Nei's Genetic Diversity, I = Shannon's Information Index.							

The 12 populations were subjected to analysis of molecular variance (AMOVA) to estimate the percentage of variation among populations and within population. The AMOVA demonstrated a highly significant ($P < 0.01$) genetic differentiation within the sampled caper populations by ISSR markers, 67 % of the total genetic variance was attributed to between the populations, and 33% were explained by individual differences within populations (Table 8).

Table 8
Summary of nested AMOVA based on ISSR, SCoT and ISSR + SCoT markers, among the 12 caper populations

Source of variation	Among populations			Within population		
Marker	ISSR	SCoT	ISSR + SCoT	ISSR	SCoT	ISSR + SCoT
df	11			68		
Variance component	5.164	5.576	10.74	10.323	16.162	26.486
Percentage	33	26	29	67	74	71
P value*	0.001	0.001	0.001	0.001	0.001	0.001
* Levels of significance are based on 1,000 iteration steps						

The Jaccard dissimilarity indices ranged from 0.01 of 0.52. The hierarchical cluster analysis based on ISSR markers using the neighbour joining method generated a dendrogram with three main clusters (Fig. 5), which corresponded to the PCoA grouping (Fig. 6). The first group consists of two subgroups. The first subgroup includes the genotypes of populations “Naftshahr” (numbers 67–70), “Khosravi” (numbers 71–74), “Kerend” (numbers 75–80), and most parts of “Somar” (numbers 53 and 56–66), and the second subgroup includes the genotypes of population “Sarpolzahab” (numbers 20 and 25–27).

The second group of clusters analysis, similar with the first group, consists of two subgroups. The first subgroup includes the genotypes of populations “Gilanegharb” (numbers 44–47), “Ivan” (numbers 48–52), and most parts of “Sarpolzahab” (numbers 23–24 and 30–31), and “Gorsefid” (numbers 38–40), “Qasreshirin” (numbers 32, 34–35, and 37), “Ilam” (numbers 17 and 19), “Somar” (numbers 54–55), and one genotype from “Kermanshah” (number 2). The second subgroup includes the genotypes of populations “Kermanshah” (numbers 1 and 3–5), “Charmeleh” (numbers 6–9), “Ilam” (numbers 10–16), “Gorsefid” (numbers 41–43), “Sarpolzahab” (numbers 21–22 and 28–29), and one genotypes from “Qasreshirin” (number 33). The third group includes one genotype of “Ilam” (number 18) and one genotype of “Qasreshirin” (number 36). Two-dimensional PCoA plot of the *C. spinosa* also divided individuals into three groups same as grouping in the dendrogram (Fig. 6).

The Mantel test showed moderate correlation between morphological traits and ISSR-based genetic similarity ($r = 0.289$; $P = 0.002$) across all the genotypes (Table 9).

Table 9
Mantel test based on Euclidean coefficients for morphological traits, and Jaccard coefficients for ISSR and SCoT markers

		ISSR	SCoT	ISSR + SCoT
Morphological traits	Correlation	0.289	0.465	0.399
	P (uncorr; onetailed)	0.0002	0.0002	0.0002
Permutation N 5000				

Molecular analysis based on SCoT markers

SCoT primers generated 190 bands which 165 bands (86.84%) were polymorphic. The mean number of band per assay was 19. The size of SCoT fragments generated by the different primers, ranged from 150 to 3000 bp and the number of bands produced by the different primers ranged from 14 (SCoT29) to 24 (SCoT1). Table 10 shows the obvious differences in the total bands amplified by various SCoT primers. The maximum number of polymorphic bands was observed in the SCoT13 primer with 22, identifying 95.65 percentage of polymorphism and the minimum number of polymorphic bands was observed in the SCoT29 primer with 10, identifying 71.43 percentage of polymorphism.

Table 10
SCoT primers characteristics in 80 genotypes from 12 caper populations

Row	Primer name	NA	NP	PP	MI	RP	PS	PIC
1	SCoT1	24	21	87.50	3.60	18.38	28.20	0.20
2	SCoT12	22	16	72.73	2.08	11.64	28.80	0.18
3	SCoT13	23	22	95.65	4.58	21.04	27.70	0.22
4	SCoT18	19	16	84.21	2.90	13.47	23.70	0.22
5	SCoT22	18	15	83.33	2.66	12.50	21.60	0.21
6	SCoT29	14	10	71.43	1.13	7.14	20.40	0.16
7	SCoT30	18	17	94.44	3.82	16.06	23.20	0.24
8	SCoT31	15	13	86.67	2.65	11.27	18.20	0.24
9	SCoT33	15	14	93.33	3.08	13.07	21.00	0.30
10	SCoT36	22	21	95.45	5.19	20.05	24.80	0.26
Average	-	19	16.5	86.48	3.17	14.46	23.76	0.22
NA = the Number of Amplified Fragments, NP = the Number of Polymorphic Fragments, PP = Percentage of Polymorphism, MI = Marker Index, RP = the Ratio of Polymorphism, PS = Power Separation, PIC = Polymorphic Information Content								

Analysis of molecular variance (AMOVA) showed a highly significant ($P < 0.01$) genetic differentiation within the sampled caper populations by SCoT markers, 74 % of the total genetic variance was attributed to between the populations, and 26 % were explained by individual differences within populations (Table 8).

The mean of the percentage of polymorphic loci (PPL) was 35.22, ranged from 11.58 ("Ivan" population) to 58.42 ("Sarpolzahab" population) at the population level. "Ivan" population had the lowest level of variability, with NPL=22, PPL=11.58, $N_e=1.085$, $h = 0.048$ and $I = 0.070$, while the "Sarpolzahab" population showed the highest one, with NPL=111, PPL=58.42, $N_e=1.344$, $h = 0.200$ and $I = 0.300$ (Table 11). The mean values of genetic differentiation (G_{st}), and gene flow (N_m) between populations were calculated as 0.449 and 0.613, respectively.

The dissimilarity coefficients ranged from 0.06 of 0.55. The dendrogram based on neighbour joining method grouped the 80 individuals into six major clusters (Fig. 7). The first group contains the populations from "Kerned" (numbers 75-80), "Naftshahr" (numbers 67 and 69), and some parts of "Ghasreshirin" (numbers 32-33 and 35) population and one genotype from "Sarpolzahab" (number 31). The genotypes from "Ivan" (numbers 48-52), "Ilam" (numbers 10-14, 16), and some parts of "Somar" (numbers 53-56), and one genotype from "Sarpolzahab" (number 22) were belonged to the second group. The third group includes "Kermanshah" population (numbers 1-5) and some parts of "Somar" (numbers 62-64), and one genotype from "Ilam" (numbers 17) populations. The fourth group includes "Charmelah" (numbers 6-9) population, and some parts of "Naftshahr" (numbers 70, 68), "Somar" (numbers 57-61, and 65-66) populations and one genotype from "Ghasreshirin" (numbers 34) population. The fifth group includes the "Khosravi" population (numbers 65-68), and most parts of "Sarpolzahab" (numbers 20-21, and 23-30), and some parts of "Ilam" (numbers 15, and 18-19). The sixth group includes "Gorsefid" (numbers 38-43), "Gilanegharb" (numbers 44-47) and two genotype from "Ghasreshirin" (numbers 36-37) populations. Two-dimensional PCoA plot divided 80 caper individuals into two groups (Fig. 8). The Mantel test showed the significant correlation between morphological characteristics and SCoT-based genetic distances ($r = 0.462$; $P = 0.002$) across all the genotypes (Table 9).

Table 11
Genetic diversity analysis of 12 caper populations assessed with SCoT markers

Population	Geographic region	Sample size	NPL	PPL	Ne	h	I
Pop1	Kermanshah	5	38	20.00	1.143	0.080	0.117
Pop2	Charmeleh	4	55	28.95	1.198	0.112	0.164
Pop3	Ilam	10	96	50.53	1.320	0.182	0.270
Pop4	Sarpolzahab	12	111	58.42	1.344	0.200	0.300
Pop5	Qasreshirin	6	79	41.58	1.269	0.154	0.228
Pop6	Goresefid	6	59	31.05	1.193	0.111	0.165
Pop7	Gilanegharb	4	59	31.05	1.207	0.119	0.176
Pop8	Ivan	5	22	11.58	1.085	0.048	0.070
Pop9	Somar	14	106	55.79	1.558	0.197	0.293
Pop10	Naftshahr	4	63	33.16	1.256	0.130	0.202
Pop11	Khosravi	4	89	46.84	1.290	0.169	0.252
Pop12	kerend	6	26	13.68	1.099	0.056	0.081
Average			66.92	35.22	1.247	0.130	0.193
NPL = the Number of Polymorphic Loci, PPL = the Percentage of Polymorphic Loci, Ne = Effective Number of Alleles, h = Nei's Genetic Diversity, I = Shannon's Information Index.							

Molecular analysis of combined (ISSR + SCoT) markers

Genetic diversity parameters as NPL, PPL, Ne, h and I were calculated for the populations using the combined ISSR and SCoT markers. The obtained results based on the combined ISSR + SCoT data indicated the most variability in "Sarpolzahab" population and the least in "Ivan" and "Kerend" populations (Table 12). The mean values of genetic differentiation (G_{ST}), and gene flow (N_m) between populations were calculated as 0.470, and 0.563, respectively.

The dissimilarity coefficients ranged from 0.04 of 0.51. The dendrogram, constructed from combined ISSR + SCoT markers indicated that the caper cultivars grown in the western region of Iran could be clearly divided into three groups (Fig. 9). The first group in this study contains the populations of "Naftshahr" (numbers 67–70), "Khosravi" (numbers 71–74), "Kerend" (numbers 75–80), and the most of "Somar" population (numbers 53, 56–66), and one genotype from "Qasreshirin" population (numbers 36). The genotypes from "Goresefid" (numbers 38–43), "Gilanegharb" (numbers 44–47), "Ivan" (numbers 48–52) populations, and two genotypes from "Somar" population (numbers 54 and 55), and one genotype from "Qasreshirin" (numbers 37) population were belonged to the second group. The third group includes the genotypes from "Kermanshah" (number 1–5), "Charmelah" (numbers 6–9), "Ilam" (numbers 10–19), and "Sarpolzahab" (numbers 20–31) populations. Two-dimensional PCoA plot divided the 80 caper individuals into three groups (Fig. 10) same as grouping in the dendrogram. Both ISSR and SCoT clusters showed partial similarity with dividing by combined ISSR + SCoT data.

The Mantel test demonstrated the significant correlation between morphological traits and ISSR + SCoT genetic distances ($r = 0.289$; $P = 0.001$) across all the genotypes (Table 9).

Table 12
Genetic diversity analysis of 12 caper populations assessed with combined ISSR + SCoT markers

Population	Geographic region	Sample size	NPL	PPL	Ne	h	I
Pop1	Kermanshah	5	75	23.66	1.170	0.094	0.137
Pop2	Charmeleh	4	96	30.28	1.201	0.115	0.169
Pop3	Ilam	10	164	51.74	1.313	0.181	0.270
Pop4	Sarpolzahab	12	180	56.78	1.334	0.194	0.290
Pop5	Qasreshirin	6	136	42.90	1.281	0.156	0.233
Pop6	Goresefid	6	102	32.18	1.207	0.118	0.175
Pop7	Gilanegharb	4	105	33.12	1.224	0.128	0.189
Pop8	Ivan	5	33	10.41	1.069	0.040	0.059
Pop9	Somar	14	179	56.47	1.335	0.196	0.292
Pop10	Naftshahr	4	99	31.23	1.238	0.131	0.189
Pop11	Khosravi	4	133	41.96	1.272	0.156	0.232
Pop12	kerend	6	33	10.41	1.076	0.043	0.062
Average			111.3	35.09	1.226	0.129	0.191
NPL = the Number of Polymorphic Loci, PPL = the Percentage of Polymorphic Loci, Ne = Effective Number of Alleles, h = Nei's Genetic Diversity, I = Shannon's Information Index.							

Discussion

For evaluation of morphological diversity in the 80 caper genotypes were recorded 43 morphological traits. Factor analysis was used to decrease data and showing the role of each characteristic in the genetic diversity. This analysis reduced the 43 variables into 15 factors. Fifteen factors justified about 78.7 % of the total variation found among genotypes. The results have shown genetic relationships between the samples collected from the west of Iran. A large variation was observed in the current set of caper based on morphological characteristics. There was no complete agreement between the results of cluster analysis and geographical areas (Fig. 4). The individuals collected from same locality were clearly included in one genetic cluster such as Kermanshah, Kerend, Naftshahr and Khosravi, but individuals of some populations such as Sarpolzahab, Somar, Gilanegharb and Charmelah were assigned to more than one cluster, in agreement with the result of Ahmadi and Saeidi study (2018). Having a hard seed shell makes the digestive tract of some animals and birds unable to digest the seeds of this plant, and seeds of these plants spread by the feces of animals and spread in different geographical locations. Also, being aware of the medicinal properties of this plant for thousands of years can play a role in the movement of seeds of different species and ecotypes of this plant. Overall locating some of the genotypes collected from the west of Iran habitats in separate groups and as well as the grouping of ecosystems in this region may be due to germplasm displacement and high plant diversity. The most of the traits examined had CV values greater than 30%, indicating high variation among the studied caper genotypes based on majority of the characteristics evaluated. Many natural factors and human activities shape the extent and pattern of genetic diversity in a plant species (Rao and Hodgkin 2002). In many plants, the effect of low temperature on the reduction of characteristics has been proven (Omidbaigi 2000). While with increasing altitude the most morphological characteristics could be increased significantly (Fakhri et al. 2008). In this study, the altitude difference of almost 1000 meters between the investigated locations could be one of the environmental causes of high variation among the genotypes studied.

In current study, most of the measured parameters showed high level of genetic diversity in Iranian germplasm of *C. spinosa*. This level of genetic diversity in Iran was previously reported by Ahmadi and Saeidi (2018), who used ISSR markers to study genetic variability of *C. spinosa*. The Both of ISSR and SCoT markers revealed the similar levels of polymorphism. Efficiency of ISSR and SCoT markers for the detection of polymorphism and genetic relationships in caper genotypes is in agreement with the results of previous studies (Bohyar *et al.* 2012; Nosrati, et al. 2012; O'zbek and Kara 2013; Moubasher et al. 2011; Gristina et al. 2014; Kumar et al. 2013, and Ahmadi and Saeidi 2018). Genetic diversity parameters such as the number of polymorphic loci (NPL), the percentage of polymorphic loci (PPL), effective number of alleles (Ne), Nei's genetic diversity (h), Shannon's information index (I) were used for measure the information of ISSR and SCoT markers, all these parameters were found Equal for SCoT and ISSR markers (Tables 6 and 11), but, the number of bands amplified by the SCoT method was higher than the ISSR method. The obtained results highlight the distinctive nature of these markers, so, it could be concluded that the SCoT and ISSR markers had the same potency for study of the genetic diversity of caper, such as the study of Bohyar *et al.* (2012), who used RAPD and ISSR markers to study genetic variability of *C. spinosa* in Trans-Himalayas. Using 8 ISSR primers scored 85 DNA bands from 90 genotypes of *C. spinosa* in China (Liu *et al.* 2016), by 10 ISSR primers scored 313 DNA bands from 94 genotypes of *C. spinosa* in Iran (Ahmadi and Saeidi 2018), s, whereas in our study using 10 ISSR primers, 127 bands were generated in PCR of 80 genotypes. This difference in ISSR bands in our study and other could be resulted from diverse in the genotypes studied. Also, it may be resulted from difference in the primers used or annealing temperatures. The level of genetic diversity is related to the marker used and to the caper population and its size (Inocencio et al. 2005).

The combined (ISSR + SCoT) markers compared to alone ISSR and SCoT markers were found same efficient with regards to polymorphism detection, and haven't shown more information of genetic diversity than single analysis of ISSRs and SCoTs. Molecular marker diversity studies did not show a perfect match between the molecular diversity groupings and geographical regions, but the combined (ISSR + SCoT) markers shown a perfect match between the molecular diversity groupings and geographical regions, Except Somar and Qasreshirin individuals were assigned to more than one cluster. Mantel test demonstrated moderate correlation between the genetic relationships estimated using ISSR and SCoT data and morphological data. In both dendrograms for ISSR and SCoT markers, samples belonging to each population were almost clustered into one group. The samples clustered with morphological traits were classified in different groups from grouping based on molecular data and even from geographical groups. Grouping based on morphological traits could be influenced by environmental factors (such as sea level elevation, amount of light exposure, air humidity, soil moisture content, soil texture, etc).

This study showed considerable gene flow for ISSRs ($N_m = 0.518$) and SCoTs ($N_m = 0.613$), and low level of differentiation of ISSRs ($G_{st} = 0.491$) and SCoTs ($G_{st} = 0.449$) among populations of *C. spinosa*. But, the results of Ahmadi and Saeidi (2018) study showed low level of gene flow ($N_m = 0.455$) and considerable differentiation ($G_{st} = 0.523$) among populations of *C. spinosa*. *Caper* is an andromonoecious species, bearing both male and perfect flowers on the same plant (Zhang and Tan 2008), which causes high level of gene flow and consequently low level of genetic differentiation among populations.

The existence of dispersion and different levels of morphological variation and molecular genetic diversity in the studied genotypes indicates that *C. spinosa* germplasm in west of Iran is applicable and useful for breeding programs. *Caper* is long lived and it is perennials, possible that actual rate of out-crossing and gene flow are enough to maintain observed level of genetic variation. Hence, individuals belonging to populations with sufficient genetic distance could be introduced as potentially appropriate parents in different caper breeding programs. Of course, further populations are needed to introduce the best populations in the natural habitats of this species and studying other species of this genus.

Declarations

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Data availability statement

All data underlying the results are available as part of the article and no additional source data are required

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We have used from general grant of the Vice Chancellor for Research of University of Hormozgan,

Ethical statement

This material is the authors' own original work, which has not been previously published elsewhere.

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Figures

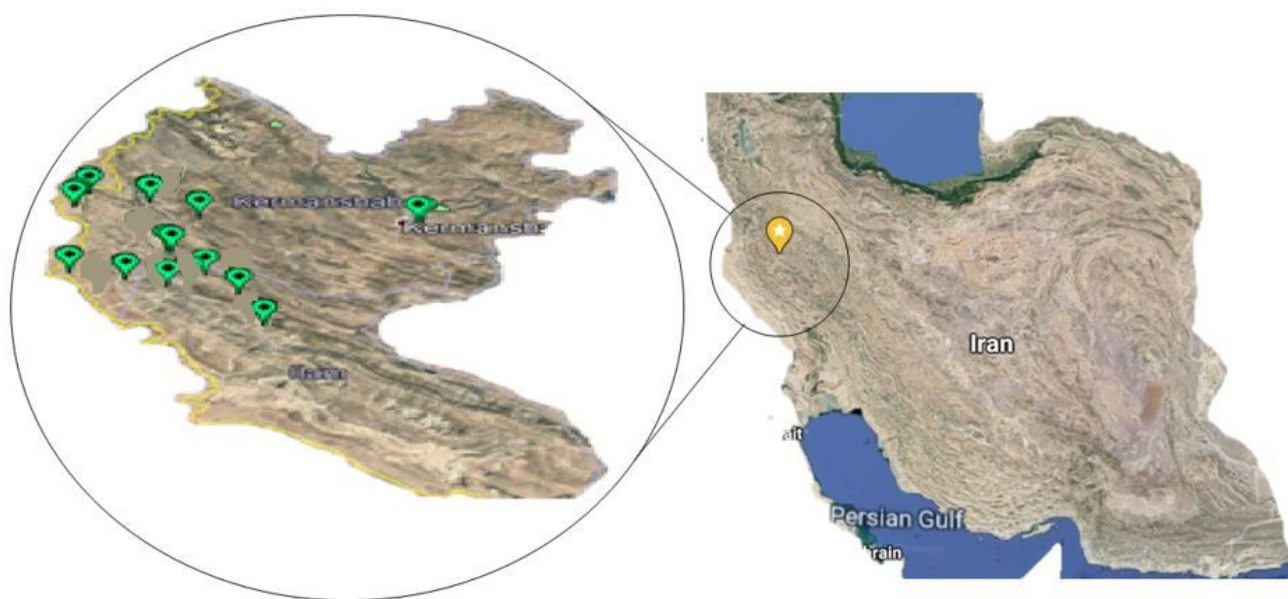


Figure 1

Geographic zones of the 12 studied populations of *C. spinosa* Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.



Figure 2

A representative picture of various caparis populations. A: Gilanegharb, B: Goresefid, C: Khosravi, D: Somar, E: Charneleh, F: Sarpolzahab. The ability of caper bushes to grow on rock



Figure 3

Morphological diversity on fruit characteristics in some studied caper genotypes

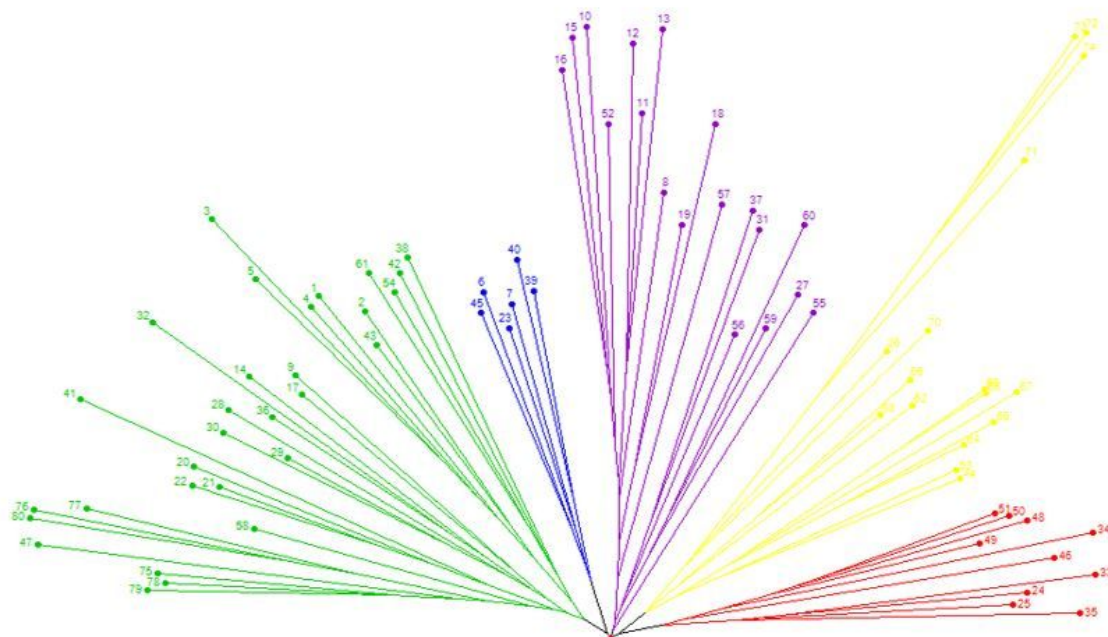


Figure 4

Dendrogram of a hierarchical cluster analysis using Euclidean with the Neighbour joining method as showing relationships between the 12 caper populations based on 43 morphological characteristics

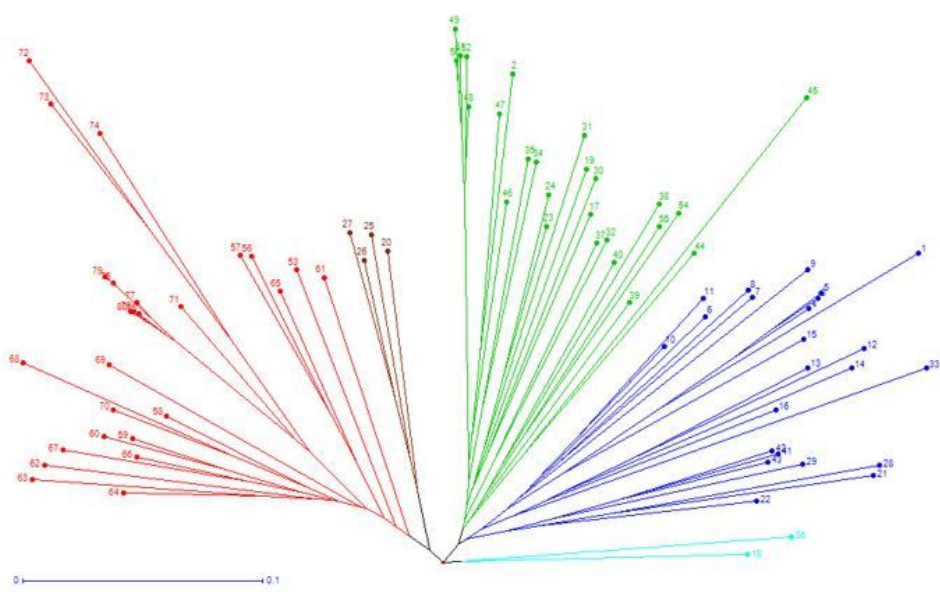


Figure 5

A Dendrogram generated using NJ clustering showing relationships between 80 genotypes from 12 caper populations based on ISSR data

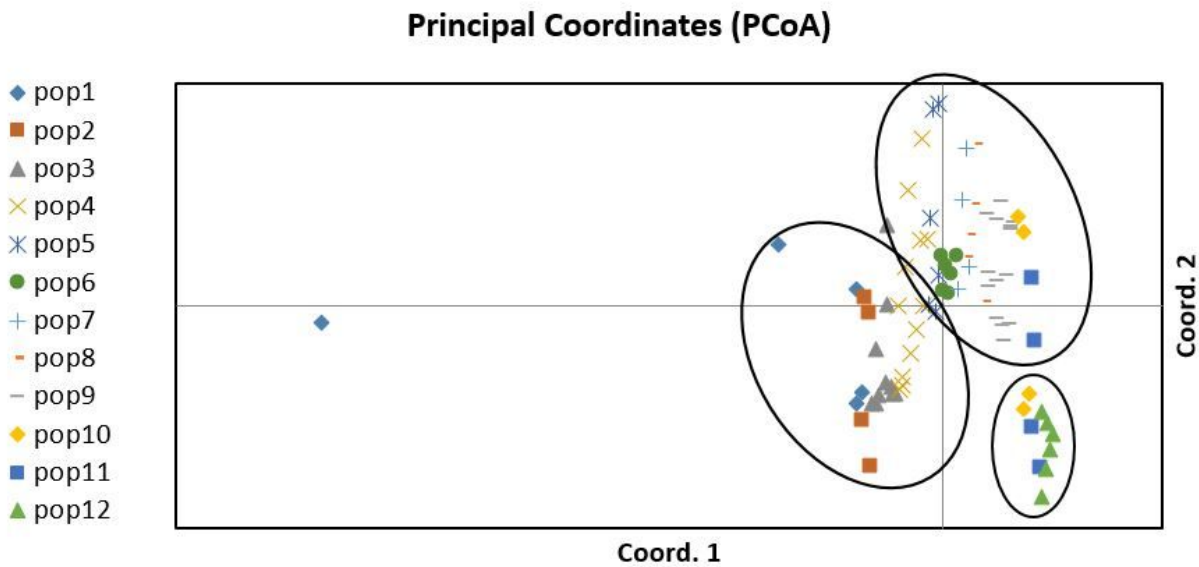


Figure 6

Two dimensional PCoA plot of the 80 genotypes from 12 caper populations based on ISSR data

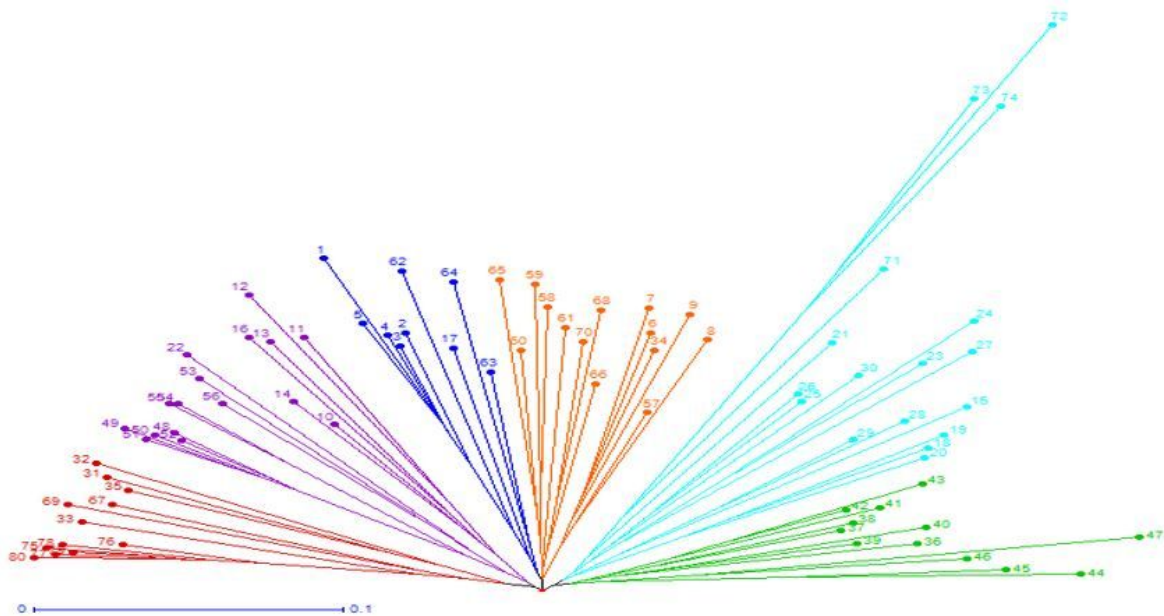


Figure 7

A Dendrogram generated using NJ clustering showing relationships between 80 genotypes from 12 caper populations based on SCoT data

Principal Coordinates (PCoA)

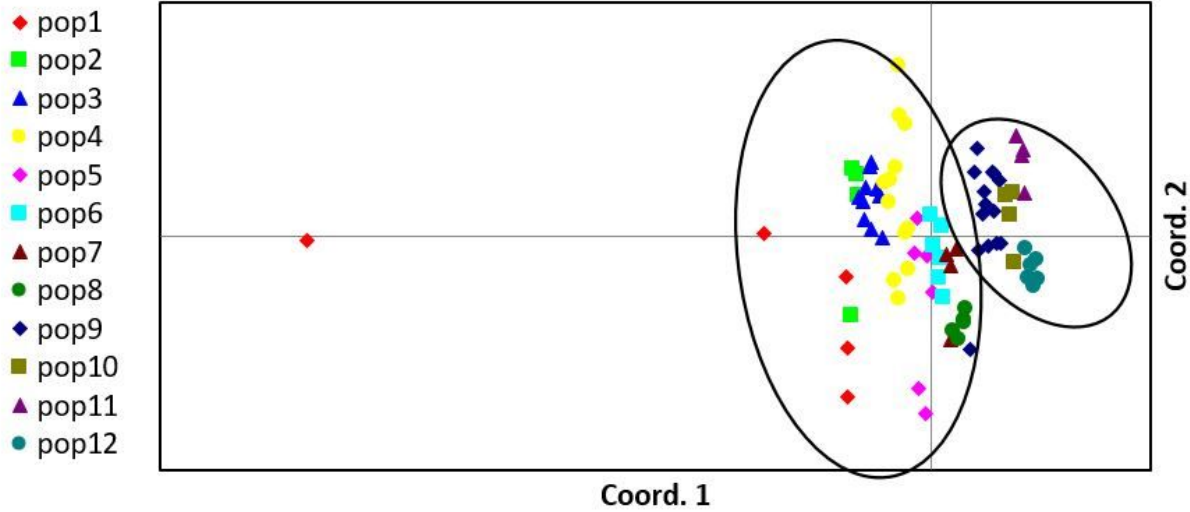


Figure 8

Two dimensional PCoA plot of the 80 genotypes from 12 caper populations based on SCoT data

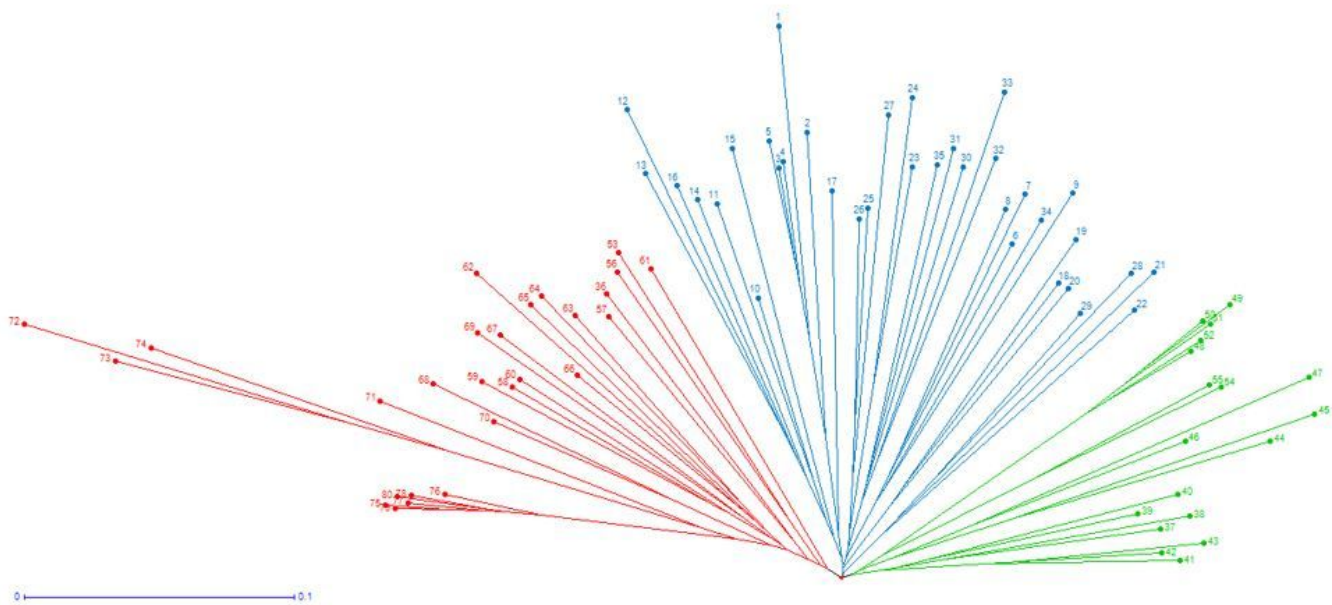


Figure 9

A Dendrogram generated using NJ clustering showing relationships between 80 genotypes from 12 caper populations based on ISSR+ SCoT data

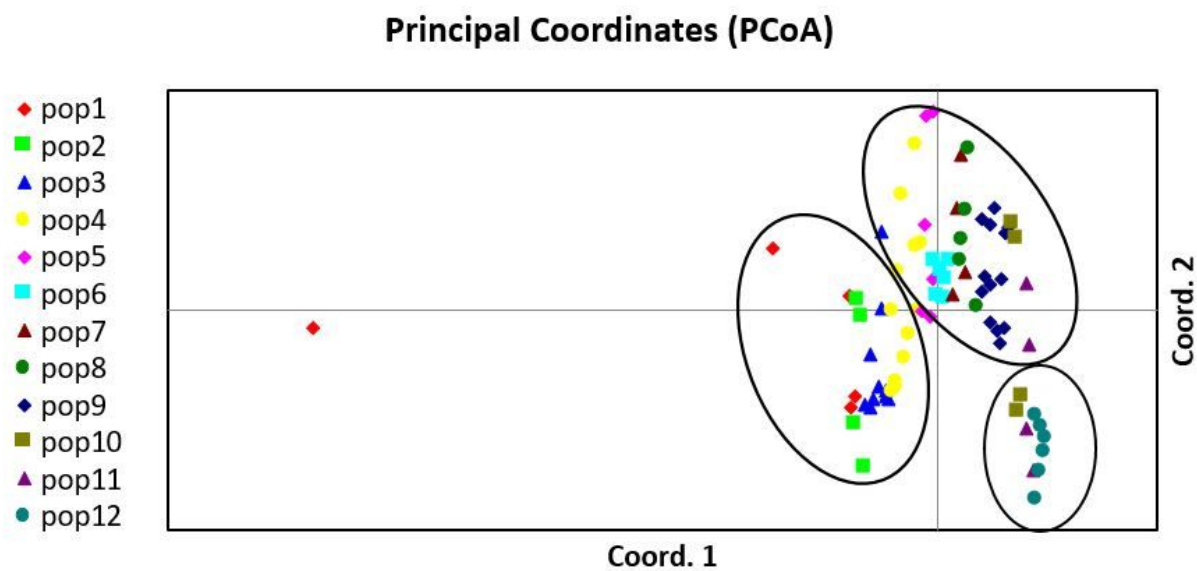


Figure 10

Two dimensional PCoA plot of the 80 genotypes from 12 caper populations based on SCoT data