RESEARCH ARTICLE

Genetic diversity and structure of *Capparis spinosa* L. in Iran as revealed by ISSR markers

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Abstract *Capparis spinosa* L. (caper bush) is an economically and ecologically important perennial shrub that grows across different regions of Iran. In this study, the genetic diversity and population structure of Iranian genepool of *C. spinosa* is evaluated using Inter Simple Sequence Repeat (ISSR) markers. Using 10 ISSR primers, 387 DNA fragments (bands) were amplified from the genomic DNA of 92 individuals belonging to twenty-one populations of *C. spinosa*, of which 378 (97.7%) were polymorphic. High level of genetic diversity (percentage of polymorphic loci = 98.2%, $h = 0.1382$, $I = 0.243$), high genetic differentiation ($G_{st} = 0.5234$) and low gene flow ($N_m = 0.4553$) among populations were observed. Capers bush populations were divided into 4 groups in the dendrogram, PCoA plot and Bayesian clustering results, mostly corresponded to their geographic regions. The results showed that there are value in sampling Iranian caper bush populations to look for valuable alleles for use in plant breeding programs.

Keywords Capers bush · Genetic diversity · Iran · ISSR

Introduction

Capparis L. (250 species) is the largest genus of the family Capparaceae, distributed in pantropical region (Hall 2008). De Candolle (1824) proposed the first sectional classification for the genus and included all the old world species in

sect. *Eucapparis*. This approach was adopted by Bentham and Hooker (1862) and three new sections were added to the previous sections. Hutchinson (1967) suggested that *Capparis* species must be divided into more than one genus. However, his view was not accepted at that time, due to the lack of knowledge and insufficient collections (Cornejo and Iltis 2008a). Inocencio et al. (2006) divided *Capparis* species into twelve sections. In recent years, the study of flora Mesoamerica and Ecuador revealed that *Capparis* known in that area should be broken into several genera (Cornejo and Iltis 2008a, b, c). *Capparis* species are valuable as a resource for medicine, food, improving soil fertility, stabilizing dunes, fuel, timber and livestock feed (Fici 2014; Mahla et al. 2013).

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. spinosa* 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.

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Table 1 Population names, geographical coordinates, altitude and number of individuals analysed of each population of *C. spinosa*

Geographic region	Population	Latitude (E)	Longitude (N)	Altitude (m)	Sample size
South	1-Zirido	51°26'42.25"	30°14'22.38"	829	4
	2-Jahrom	53°33'41.18"	28°30'37.20"	1045	3
	3- Shahr-e Babak	54°12'3.78"	30°17'15.11"	1560	5
	4-Anar	55°11'3.57"	30°56'40.69"	1420	4
	5-Kerman	57°6'5.19"	30°14'24.31"	1768	4
	6-Parsian	53°31'26.46"	27°4'8.07"	198	5
	7-Bushehr	51°13'15.63"	29°6'15.04"	75	4
	8- Masjed Soleyman	49° 15'55.59"	31° 38'46.92"	180	3
	9-Behbahan	50°15'29.07"	30°35'12.73"	327	4
	10-Yasuj	51°15'40.55"	30°57'18.58	1500	5
Center	11-Isfahan	51° 55'44.12"	32° 33'7.13"	1529	4
	12- Ardakan	53° 57'18.83"	32° 16'52.78"	1061	4
West	13- Harsin	46° 55'44.10"	034° 24'43.29"	1310	6
	14- Urmia	45° 18'32.37"	37° 18'0.99"	1267	5
	15- Maku	44° 34'36.68"	39° 16'23.50"	1133	4
	16- Ahar	46° 59'11.28"	38° 26'34.20"	1369	4
North	17- Darband	51° 25'31.03"	35° 50'0.75"	1900	4
East	18- Azadshahr	55° 11'17.79"	37° 5'7.12"	140	5
	19- Ferdows	58°8'28.30"	33°59'50.85"	1285	5
	20- Shadmehr	59° 2'1.49"	35° 9'45.86"	1194	5
	21- Malek abad	59°35'30.85"	35°59'29.37"	1288	5

Caparis spinosa is a taxonomically complicated species that occur in Europe, Africa, Madagascar, Asia, Australia and Oceania (Jacobs 1965; Fici 2014). This species was first described by Linnaeus (1753) and taxonomic treatment of the species and its allies has been controversial for a long time. Some authors combined some closely related species of *Capparis* into a single species, *C. spinosa* s. l., with a number of infraspecific taxa, whereas others divided them into different species including *C. spinosa* s. s., (Boissier 1867; Zohary 1960; Jacobs 1965; Highton and Akeroyd 1991; Inocencio et al. 2006; Fici 2014, 2015).

There is no strong evidence 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). This species is commercially important and cultivated in Morocco, Italy, and Spain (Barbera and Di Lorenzo 1984). *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). Unripe fruits and flower buds are also pickled in vinegar for home consumption.

Genetic diversity of *C. spinosa* in some regions have been studied (Inocencio et al. 2005; Saifi et al. 2011; Bhoyar et al. 2012; Özbek and Kara 2013; Al- Safadi et al.

2014), but information regarding the genetic diversity of this species in Iran is lacking.

Among the wide diversity of molecular markers, Inter Simple Sequence Repeat (ISSR) markers have been frequently used to detect genetic similarities or differences in plants (Singh et al. 2014; Vashishtha et al. 2013; Gristina et al. 2014; Safaei et al. 2016). In this study, we make use of ISSR markers to infer the genetic diversity within the Iranian genepool of *C. spinosa* s. s. and to elucidate the patterns of diversity related to geography.

Materials and methods

Plant materials

A set of 92 individuals of *C. spinosa* collected from twenty-one localities of different regions of Iran during 2013–2014 were analysed. (Table 1). In each location, we collected 3–6 individuals with at least 2 m interval. Based on our knowledge of ecology and geographical conditions of the localities, populations were divided into 5 geographic groups (south 10, center 2, west 4, north 1 and east 4 populations).

Table 2 Details of ISSR primers used in this study

Primer	Primer sequence (5' → 3')	Total number of bands	Number of polymorphic bands	Percentage of polymorphic bands	Annealing temperature (°C)
ISSR 4	CTCTCTCTCTCTCTGG	38	38	100	60
ISSR 5	ACACACACACACACCG	44	44	100	60
ISSR 6	ACACACACACACACCT	38	37	97.37	56
ISSR 8	CGTCACACACACACACA	60	60	100	61
ISSR 807	AGAGAGAGAGAGAGAGT	48	48	100	55
ISSR 808	AGAGAGAGAGAGAGAGC	28	26	92.86	57
ISSR 810	GAGAGAGAGAGAGAGAT	37	37	100	46
ISSR 811	GAGAGAGAGAGAGAGAC	40	37	92.5	52
ISSR 817	CACACACACACACAAA	18	16	88.89	53
ISSR 880	GGAGAGGAGAGGAGA	36	35	97.22	53

DNA extraction

Genomic DNA was extracted from the youngest dried leaves (1 g) using the CTAB procedure described by Doyle and Doyle 1987 and Cullings 1992 with minor modifications. The quality and quantity of the extracted DNA were measured by a spectrophotometer (Eppendorf AG, Hamburg, Germany) and 0.8% (w/v) agarose gel electrophoresis. DNA samples were diluted to 50 ng/μl and stored at – 20 °C.

ISSR-PCR

A set of 22 ISSR primers (from ISSR primer set published by University of British Columbia, www.scribd.com) were used for PCR. Of these primers, only 10 amplified clear and distinguishable DNA fragments. These primers were selected to amplify DNA fragments from the genomic DNA (Table 2). PCR reactions were carried out in a volume of 15 μl, containing 5.75 μl sterile double-distilled water, 7.5 μl of the 2 × Taq DNA polymerase master mix Red (Amplicon, Cat. No. 180301, 150 mM Tris–HCl pH 8.5, 40 mM (NH₄)₂SO₄, 3.0 mM MgCl₂, 0.4 mM dNTPs, 0.05 units/ml Amplicon Taq DNA polymerase, inert red dye and a stabilizer), 5 pmol of each primer, and 1 μl of template DNA (50 ng/μl). The PCR was carried out at 94 °C for 4 min for initial denaturation, 45 cycles of 1 min denaturation at 94 °C, 1 min for annealing at 46–61 °C depending on the primer (see Table 2), and extension for 2 min at 72 °C. This was followed by a final extension of 10 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 100 bp DNA ladder and gels were visualized under UV light.

Data analysis

A matrix of raw data was constructed based on the presence (1) or absence (0) of each band for all individuals. The similarity matrix was generated using Jaccard similarity coefficient (Jaccard 1908). This matrix was subjected to a cluster analysis based on Neighbor Joining (NJ) method using DARwin5 software version 6.0.14 (Perrier and Jacquemoud-Collet 2006). Genetic diversity parameters such as the percentage of polymorphic loci (PPL), effective number of alleles (Ne), Nei's genetic diversity (h), Shannon's information index (I), gene flow (Nm) and genetic differentiation coefficient (Gst) was measured using POP-GENE ver. 1.32 (Yeh et al. 1999).

Analysis of molecular variance (AMOVA) and principal coordinate analysis (PCA) were performed using GenALEX software ver. 6.4.1 (Peakall and Smouse 2006). The Mantel test was also performed using GenALEX software ver. 6.4.1 to calculate the relationship between genetic distance and geographic distance of populations.

Bayesian clustering analyses using STRUCTURE ver. 2.3.4 (Pritchard et al. 2000) was performed to infer the number of genetic clusters (K) and the probability of individual assigned to each cluster based on delta K (Δk) value (Evanno et al. 2005).

Table 3 Genetic diversity indices for ISSR markers within the populations and geographic regions of *C. spinosa*

Geographic region	population	N	PPL	Ne	I	h	Nm	Gst
South	Zirido	95	24.55	1.14	0.129	0.085	–	–
	Jahrom	53	13.70	1.08	0.075	0.05	–	–
	Shahr-e Babak	102	26.36	1.14	0.13	0.086	–	–
	Anar	102	26.36	1.14	0.135	0.088	–	–
	Kerman	82	21.19	1.11	0.107	0.07	–	–
	Parsian	84	21.75	1.12	0.109	0.07	–	–
	Bushehr	62	16.02	1.08	0.079	0.051	–	–
	Masjed Soleyman	36	9.30	1.05	0.05	0.033	–	–
	Behbahan	82	21.19	1.12	0.112	0.07	–	–
	Yasuj	70	18.09	1.09	0.09	0.05	–	–
							0.5342	–
West	Harsin	103	26.61	1.11	0.114	0.07	–	–
	Ahar	77	19.90	1.10	0.099	0.05	–	–
	Urmia	82	21.19	1.11	0.103	0.06	–	–
	Maku	78	20.16	1.12	0.109	0.07	–	–
							0.8006	–
North	Darband	59	15.25	1.09	0.083	0.055	–	–
East	Azadshahr	71	18.35	1.09	0.089	0.058	–	–
	Ferdows	69	17.83	1.08	0.083	0.053	–	–
	Shadmehr	81	20.93	1.10	0.102	0.066	–	–
	Malekabad	79	20.41	1.11	0.103	0.067	–	–
							0.9895	–
Center	Isfahan	81	20.93	1.10	0.104	0.06	–	–
	Ardakan	97	25.06	1.13	0.126	0.082	–	–
							1.2166	–
Average		78	20.24(4.37)	1.10(0.23)	0.101(0.02)	0.064(0.014)	–	–
Species level				1.194	0.2430	0.1382	0.4553	0.5234

N number of polymorphic loci; *PPL* percentage of polymorphic loci; *Ne* effective number of alleles; *I* Shannon's information index; *h* Nei's gene diversity

The lowest and highest amounts are highlighted in bold

Results

PCRs using 10 ISSR primers amplified 387 clear bands from the genomic DNA of 92 individuals from the twenty-one populations, of these bands, 378 (97.7%) were polymorphic (Table 2). The DNA fragment sizes ranged from 150 to 2000 bp. Number of amplified fragments varied from 18 (ISSR 817) to 60 (ISSR 8) (Table 2), averaging 38.7 per primer. Details of genetic diversity parameters are shown in Table 3. The percentage of polymorphic loci (PPL) was 20.24, ranged from 9.30 (Masjed Soleyman) to 26.61 (Harsin) at the population level. Anar population had the highest level of variability, with $h = 0.088$ and $I = 0.135$, while the Masjed Soleyman population showed the lowest one, with $h = 0.033$ and $I = 0.05$. Populations of the central region showed the highest ($Nm = 1.2166$)

and those of the southern region showed the lowest ($Nm = 0.5342$) level of gene flow (Table 3).

In the dendrogram generated based on the NJ method, individuals were divided into 4 main clusters (Ia, Ib, II, III; Fig. 1) which corresponded to the PCoA grouping (Fig. 2). Cluster I which included two sub-clusters (a, b), with a bootstrap value of 68%, contained the populations that occur from the south to the center of Iran. Cluster II with a bootstrap value of 54%, grouped populations mainly that occur in the west and the Northwest of the country. Isfahan population from the center was clustered with the western and northwestern populations. Cluster III, with a bootstrap value of 72%, grouped individuals collected from the north and the east of Iran. The Mantel test showed moderate correlation between geographic and ISSR-based genetic distances ($r = 0.215$; $P = 0.001$) across all the

Fig. 1 A neighbor joining tree of 92 *Capparis spinosa* individuals generated based on ISSR data (bootstrap values are shown on the cluster nodes)

populations. The PCoA results (Fig. 2) also divided individuals into 4 groups same as grouping in the dendrogram. Group I contained populations of the south and the center of Iran; subgroup a contained individuals mainly originated from the center toward the south and subgroup b included individuals mainly originated from the south and the southwest. Group II included individuals of around Isfahan, west and northwestern regions. Northern and eastern individuals are included in group III (Fig. 2).

AMOVA attributed 61% of the total genetic diversity to the within and 39% to the between populations variation. The STRUCTURE analysis based on the ΔK method showed that the best number of genetic clusters (K) was 4, suggesting that all individuals fell into four clusters (Fig. 3). Most populations were grouped according to their geographical regions and more individuals collected from same locality were clearly included in one genetic cluster,

but individuals of some population such as Darband (from around Tehran), Kerman and Isfahan were assigned to more than one cluster. The red cluster included individuals collected from the south and center of Iran. The yellow cluster included individuals collected from the northern and eastern regions. The blue cluster included individuals originated from the south and southwest of Iran and green cluster contained individuals originated from the west, northwest and center.

Discussion

Many factors such as evolutionary events, breeding system, environmental factors, past bottlenecks and human activities shape the extent and pattern of genetic diversity in a plant species (Rao and Hodgkin 2002). *C. spinosa* has a

Fig. 2 2D PCoA plot of the *C. spinosa* individuals showing relationships based on ISSR data. Group I contained populations of the south and center of Iran; subgroup a contained individuals originated mainly from the center toward south and subgroup b

included individuals mainly originated from the south and southwest. Group II included individuals of around Isfahan, west and north-western regions. Northern and eastern individuals are included in group III

wide geographic distribution range in Iran with a vast range of climatic and ecological conditions; occurring on different substrata (clay, marl and lime), in different elevations from sea level to over 1900 m, from hot deserts to wetlands. This situation strongly strengthen the hypothesis that *C. spinosa* may harbor high level of genetic diversity in Iran.

In this study, most of the measured parameters showed high level of genetic diversity within Iranian germplasm of *C. spinosa*. This level of genetic diversity was comparable with the results of Bhoyar et al. (2012), who used ISSR and RAPD markers to study genetic variability of *C. spinosa* in Trans-Himalayas. Using 8 ISSR primers, Bhoyar et al.

(2012), scored 85 DNA bands from 90 genotypes of *C. spinosa*, whereas in our study using 10 ISSR primers, 385 bands were generated in PCR of 92 genotypes. The primer (GA)₈T in the Bhoyar et al. (2012) study did not amplify any DNA fragments whereas in our study, this primer amplified 37 DNA bands from genomic DNA of all individuals. In Bhoyar et al. (2012) primer (AG)₈T amplified 16 DNA bands whereas in this study, it generated 48 bands. This abundance in ISSR bands in our study and other differences could be resulted from the genotypes studied. Our samples were collected from a wide geographical range with a vast range of ecological conditions whereas in Bhoyar et al. (2012), genotype were collected from a

Fig. 3 Distribution map of the studied populations of *C. spinosa* (color of each population corresponded to genetic cluster in the bar plot). The bar plot show genetic relationship among populations of *C. spinosa* generated using STRUCTURE software

smaller region with lower ecological variabilities (high altitudes and cold and arid environments). Also, it may be resulted from difference in genetic structure of this species in Iran and Trans-Himalayas, from the primers used or annealing temperatures. Despite of these differences, other measures of genetic diversity were comparable between the two studies. The measured parameters of genetic diversity were higher than what was measured in other *Capparis* species including *C. spinosa* and its closely related taxa (Özbek and Kara 2013 using RAPD data; Saifi et al. 2011 using ISSR markers; Inocencio et al. 2005 using AFLP data).

C. spinosa is an andromonoecious species, bearing both male and perfect flowers on the same plant (Zhang and Tan 2008). In the natural populations, we frequently saw both male and perfect flowers on same plant or different plants

from same population. These floral type accompanied with some other factors such as growing in variable and stressful environments increased the cross-pollination rate in caper bush up to more than 90% (Zhang and Tan 2009). In such situation, high level of gene flow and consequently low level of genetic differentiation among populations of a particular species is expected, but unexpectedly, the results of this study showed low level of gene flow ($N_m = 0.4553$) and considerable differentiation ($G_{st} = 0.5234$) among populations of *C. spinosa*. Cross-pollination is mediated by means of pollinators. In many of localities in which the caper bush populations occur, pollinators are infrequent and mostly move in short distances. Based on our field observation and Saifi et al. (2011), flowers of caper bush are visited by several insects, most of which have short travel distance. Therefore, the pollination

could be predominantly local rather than long-distance. Other main source of gene flow is seed dispersal. Anemochory, myrmecochory, and endozoochory by rodents are the main methods of seed dispersal in *C. spinosa* (Wang et al. 2016). Regarding the size of the seeds and the fruit structure, these are not good means for seed dispersal over long distances. In addition, large mountain ranges and vast deserts in Iran might limit dispersal by anemochory and zoochory. Difference in flowering time can be another factor that limit the gene flow among populations from different regions. While the southern and central populations of caper bush in Iran bloom in the mid spring, the northern, northwestern and western populations flower in the early summer. Therefore, despite being an outbreed species, the tools that facilitate the gene flow among populations are inadequate which resulted in more or less genetically differentiated populations. Saifi et al. (2011) in an study of *Capparis* ssp. in the North Morocco using 5 ISSR primers showed considerable genetic differentiation among populations and also groupings related to the geographic origin of populations. They suggested that this population differentiation could be resulted from low gene flow. As the studied populations of *C. spinosa* were mainly located on roadsides and field borders, human activities may affect the pattern of genetic diversity by means of seed dispersal. Probably these factors caused the genetical admixes observed in Kerman, Isfahan and Darband populations. Wang et al. (2016) indicated that high genetic diversity in caper bush populations from the north China was clearly correlated with the complex topographic conditions and altitude. The effect of ecological factors on the level of genetic diversity has also been reported in some plant species (Segarra-moragues et al. 2016). All of the mentioned factors may have roles in creating geographical pattern of diversity in Iranian genepool of caper bush, evident from the STRUCTURE analysis, dendrogram and PCOA plot (Figs. 1, 2 and 3). The STRUCTURE results indicated that there are 4 different gene pools of *C. spinosa* in Iran, mainly corresponding to the geographic region. These gene pools may be originated from accumulation of different adaptation based mutations or each genepool may have been introduced to Iran from different genepools in neighboring countries. Because we have no information regarding the genetic structure of caper bush in the neighboring regions around Iran, we were unable to test these hypotheses. A more comprehensive study with more sampling covering regions from Asia to Africa can provide a clearer image of global genetic structure of this species and consequently the origin of the Iranian genepools of caper bush would be elucidated.

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