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Original Paper

Impact of habitat fragmentation on genetic structure of *Capparis spinosa* populations revealed by ISSR markers

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Houshang Nosrati*, Hanife Akbarian

Department of Plant, Cell and Molecular Biology, Faculty of Natural Sciences, University of Tabriz, Tabriz, East Azerbaijan Province, Iran

*Corresponding author, E-mail: hnosrati@tabrizu.ac.ir

Abstract

The results from empirical studies on the impact of habitat fragmentation on genetic variation of populations are controversial, ranging from negative to positive effects. This contradiction has been attributed to either species characteristics (e.g. plant life history) or environmental components (e.g. the scale of habitat fragmentation). To examine the role of DNA marker types on these effects, we carried out a comparative study of seven populations of *Capparis spinosa* L. (caper, Capparaceae) using ISSR markers and compared these results with those obtained previously using RAPD markers. Although both ISSR and RAPD markers showed high consistency and indicated that genetic variation in populations was not correlated with either geographical distance ($P \geq 0.47$, $P \geq 0.83$; for ISSR and RAPD, respectively) or altitude ($P \geq 0.535$, $P \geq 0.419$; for ISSR and RAPD respectively), the levels of variation were related to population size. ISSR analysis showed that genetic variation did not significantly differ between small and large populations ($P \geq 0.4149$), while RAPD analysis indicated significant effect of population size on this variation ($P \leq 0.002$). This may suggest that, in addition to species and environmental components, types of DNA markers used for assessing population genetic variations affect results on the impact of fragmentation on genetic variation.

Key words: *Capparis spinosa*, habitat fragmentation, ISSR markers, population genetic variation, RAPD markers.
Abbreviations: ISSR, inter-simple sequence repeats; RAPD, random amplified polymorphic DNA.

Introduction

Human anthropogenic activities, especially transformation and degradation of habitats through land-use change cause habitat fragmentation, as a consequence decreasing global biodiversity (Newbold et al. 2015; Wilson et al. 2016; Schlaepfer et al. 2018). Habitat fragmentation imposes negative effects on the persistence of populations and species by increasing geographical isolation between populations and decreasing effective population size, which give rise to increased inbreeding depression and genetic drift within populations (Leimu et al. 2006; Honnay, Jacquemyn 2007; Aguilar et al. 2008; Leimu et al. 2010; Leonardi et al. 2012; Yuan et al. 2012), and decreasing gene flow frequency through decreasing pollen-seed dispersal among populations (Hermansen et al. 2017; Browne, Karubian 2018; Schlaepfer et al. 2018). All these effects can result in decreased fitness in fragmented populations and consequently increase risk of extinction (Schlaepfer et al. 2018; Yu et al. 2020).

In recent decades there has been increased interest in assessing the genetic consequences of habitat fragmentation in plant populations. The published results have been discussed in several extensive reviews (e.g. Fahring 2003; Ouborg et al. 2006; Honnay, Jacquemyn 2007; Aguilar et al. 2008; Newbold et al. 2015; Fahrig, 2017; Fletcher et al. 2018; Schlaepfer et al. 2018; Fahrig et al. 2019; Raffard et al. 2019). The resulting outcomes are controversial. For example, in an extensive review of landscape-scale empirical studies, Fahrig (2017) concluded that habitat fragmentation more often had a positive rather than negative effect on biodiversity. However, Fletcher et al. (2018) disagreed with the conclusion proposed by Fahrig (2017) by arguing that both empirical and theoretical evidence indicate generally negative ecological effects of habitat fragmentation on biodiversity.

Capparis spinosa L. (caper, Capparaceae) is a long-lived perennial creeping xerophytic subshrub, which is widely distributed in the Mediterranean basin and in arid West and Central Asia (Fici 2014). It is a consistent floristic element of Mediterranean ecosystems (Rhizopoulou, Psaras 2003). Caper is andromonoecious and mostly an outcrossing species (Zhang, Tan 2008; Zhang, Tan 2009; Wang et al. 2016). The plant has several important classes of chemicals, including alkaloids, polyphenols, flavonoids, indole, glycosides, and aliphatic glucosinolates in the fruit of the plant (Mollica et al. 2019), and hence commercial use in traditional medicine due to its diuretic, antihypertensive,

poultice and tonic properties (El-Shahaby et al. 2019; Mollica et al. 2019; Wojdyło et al. 2019).

The taxonomic status of *C. spinosa* in Iran is a subject of debate. According to the Flora Iranica, *C. spinosa* consists of three varieties (Hedge, Lamond 1970); combined morphometric and ISSR analyses indicated that *C. spinosa* is comprised of several species (Ahmadi et al. 2020). However, recent morphological investigation on quantitative and qualitative characters showed that all accessions are the same taxon (Najafian et al. 2021). According to Flora Iranica (Hedge, Lamond 1970), the geographical distribution range of the plant was very wide and covered almost all regions of the country as a wild species. However, as the fruit of wild plants have been widely collected during last few decades by locals across the country for different medicinal and culinary purposes, the distribution range of the species has been fragmented. This fragmentation was noted in long-term field observation and monitoring of the geographical distribution range of the species in the study region (Nosrati, unpublished results).

We aimed to investigate whether the discrepancy on the impact of habitat fragmentation on population genetic structure, such as one between Fahrig (2017) and Fletcher et al. (2018), originates from methodology differences. Previously we used RAPD markers to show that population size had large impact on the genetic diversity of *C. spinosa* (Nasrati et al. 2012). In the present study, compared results obtained using ISSR and RAPD markers in assessing the effect of population geography, altitude and size on the levels of genetic variations in seven eco-geographically different populations of *C. spinosa* in East-Azerbaijan Province, Iran.

Materials and methods

Study sites and sampling

Seven eco-geographical populations of *C. spinosa* of different sizes were investigated in East-Azerbaijan Province, Iran (Fig. 1): Qaraqaya (39.20372 N, 47.32234 E), Eskanlo (39.20974 N, 47.10774 E), Tatar (39.05297 N, 46.79797 E), Kalaleh (38.94203 N, 46.76446 E), Saivan (38.32929 N, 45.88401 E), Khoja (38.16321 N, 46.56837 E), Tabriz (38.09065 N, 46.34648 E). The geographical distance between populations ranged from 12 to 152 km. The smallest populations had approximately 100 individual plants and were restricted to small areas, while the largest populations were at least 10 times larger and distributed in very wide areas. Ten individual plants were randomly sampled from each population and therefore, a total of 70 individuals from seven sites were included in the study.

DNA extraction and PCR amplification profile

Genomic DNA was extracted from either 100 mg leaves or several seeds of sampled plants. The plant material was first washed with distilled water to remove any contamination, then ground with liquid nitrogen in a mortar. The plant material was quickly transferred into a microtube containing 2 mL extraction buffer (consisting of 16 mL of 0.5 M Na_2EDTA, 80 mL of 1 M Tris-HCL, 32.72 g NaCl, at pH 8) and stored at 65 °C for 20 min and inverted every 5 min. Polyvinylpolypyrrolidone was added at 1% (w/v) concentration when extracting DNA from leaves to remove secondary metabolites such as phenols. The solution was centrifuged at 10 000 rpm for 5 min. The upper phase of the solution was transferred into another microtube containing the same amount of chloroform isoamyl alcohol (at 24:1 ratio), and then the microtube was inverted 20 times. The solution was centrifuged at 10 000 rpm for 5 min, the upper phase was transferred into the same amount of 96% ethanol at –20 °C and then inverted. The solution was centrifuged at 5 000 rpm for 5 min in order to precipitate the DNA and to remove contamination. The solute was discharged and the microtube containing precipitated DNA was put upside down for one hour in an incubator to allow the ethanol to evaporate. Finally, the purified DNA was resuspended and stored in either Tris-EDTA buffer or sterile distilled water to protect the DNA from degradation, for subsequent use of DNA for running PCR. The concentration of the DNA samples was measured by spectrophotometry, and subsequently adjusted at 10 ng mL^{-1}. Ten ISSRs primers were examined in PCR of the DNA samples of the plants, of which five primers (5'-(AC)8T-3'; 5'-(AC)8G-3'; 5'-(AC)8CG-3'; 5'-(AG)10C-3'; 5'-(AG)8GC-3') producing the most polymorphic loci were selected for further use.

The PCR solution contained 7 µL master mix (consisting of PCR buffer, $MgCl_2$, dNTP, Taq-DNA polymerase), 1 µL templet DNA (20 ng μL^{-1}), 1 µL primer (1 pm μL^{-1}), and 4 µL distilled/deionized water. The PCR amplification profile consisted of the following steps: initial denaturation at 94 °C for 4 min, followed by 35 cycles of sequential steps of denaturation at 94 °C for 30 s, primer annealing at 52 °C for 45 s, DNA elongation at 72 °C for 45 s, with final extension step at 72 °C for 4 min.

Fig. 1. Map of the study regions showing populations sites of *Capparis spinosa* in East Azerbaijan Province, Iran. 1, Qaraqaya; 2, Eskanlo; 3, Tatar; 4, Kalaleh; 5, Saivan; 6, Tabriz; 7, Khoja; Az, Azerbaijan. Map taken from Nosrati et al. (2012).

Table 1. Population size, altitude, and components of genetic diversity based on RAPD and ISSR analysis among different populations of *Capparis spinosa* distributed in East-Azerbaijan Province, Iran. Data on RAPD analysis are from Nosrati et al. (2012)

Size	Population Name	Altitude (m)	Components of genetic diversity					
			RAPD			ISSR		
			Polymorphic loci (%)	Nei's diversity	Shannon's diversity	Polymorphic loci (%)	Nei's diversity	Shannon's diversity
Small	Eskanlo	300	48.84	0.1667	0.2519	27.66	0.086	0.133
	Tabriz	1450	52.33	0.1788	0.2713	53.19	0.169	0.261
	Saivan	1600	61.63	0.1809	0.2837	43.62	0.143	0.218
Large	Tatar	400	68.64	0.2386	0.3598	42.55	0.145	0.220
	Qaraqaya	450	68.60	0.2340	0.3536	32.98	0.100	0.155
	Kalaleh	650	81.40	0.2630	0.4017	54.26	0.197	0.292
	Khoja	1500	67.44	0.2158	0.3316	37.23	0.118	0.182

The PCR amplification products were separated on 1% (w/v) agarose gel. Photographs of ethidium bromide-stained agarose gels of ISSR loci were scored as 1 as present and 0 for absent of a band. Consequently, the obtained dataset was entered in a binary matrix for cluster analysis using NTSYS-pc (Numerical Taxonomy and Multivariate Analysis System, ver. 2.02). The ISSR amplification was repeated three times to insure the reproducibility of the banding patterns.

Genetic variation

The polymorphism levels of ISSRs loci and genetic variation within populations were measured using Nei's diversity (Nei 1973) and Shannon information (Shannon 1948) indices. The total genetic variation was partitioned into two components i.e. within- and among-populations on the basis of analysis of molecular variance (AMOVA) using Arlequin ver. 3.11.

Relationship of population genetic variations with size, geography and altitude

The characteristics of populations, including size, altitude and geographical distance, are presented in Table 1 and Table 2. Three populations were considered as small while the other four were regarded as large populations, based on number of individuals. The level of genetic variations between small and large populations was compared using the *t*-test for two independent means. Lack of significant difference in genetic variation between small and large populations was interpreted no effect of fragmentation on genetic variation of populations. The relationship of population genetic variation with altitude and geographical distance was investigated using the Pearson correlation test. In addition, genetic similarity among populations was tested using UPGMA cluster analysis of a matrix of Nei's distance through SHAN (sequential, hierarchical, the NTSYS-pc) with 100 bootstrap replications. The results obtained from ISSR markers were directly compared with the results previously reported from the same populations using RAPD markers (Nosrati et al. 2012).

Results

Genetic variations

A total of 80 clear and reproducible bands produced by using five ISSR primers on 70 randomly sampled individual plants from seven different populations of *C. spinosa*. The levels of polymorphic ISSR loci ranged from 27.66% in Eskanlo to 54.26% in Kalaleh populations. Nei's genetic diversity for ISSR markers ranged from 0.086 to 0.1970 among populations and for RAPD markers – 0.1667 to 0.2630 (Table 1).

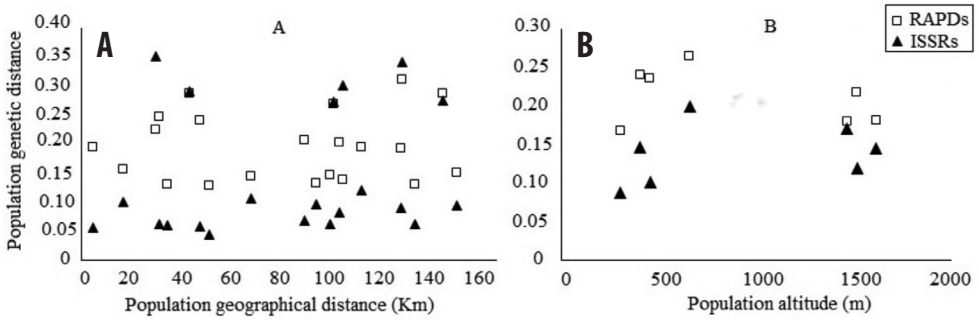

Fig. 2. Population genetic distances were not correlated with either population geographical distances (A; ISSR: $n = 21$, $P > 0.47$; RAPD: $n = 21$, $P > 0.83$) or with population altitude (B; ISSR: $n = 7$, $P > 0.535$; RAPD: $n = 7$, $P > 0.419$) in *Capparis spinosa*. Data on RAPD analysis are from Nosrati et al. (2012).

Table 2. Matrix of Nei's genetic distances based on ISSR and RAPD (in parentheses) markers and geographical distances (in brackets as km) in population pairs of *Capparis spinosa*. Data on RAPD analysis are from Nosrati et al. (2012)

Population	Kalaleh	Eskanlo	Qaraqaya	Saivan	Tatar	Khoja
Eskanlo	0.060 (0.1283) [34.7]					
Qaraqaya	0.044 (0.1265) [52]	0.099 (0.1544) [17]				
Saivan	0.061 (0.1453) [100.2]	0.061 (0.1291) [135]	0.094 (0.1478) [152]			
Tatar	0.055 (0.1914) [12]	0.062 (0.2434) [31.5]	0.057 (0.2366) [48]	0.081 (0.2004) [104.5]		
Khoja	0.068 (0.2031) [90.6]	0.119 (0.1919) [90.6]	0.090 (0.1897) [129.6]	0.105 (0.1423) [68.7]	0.095 (0.1316) [95]	
Tabriz	0.269 (0.2644) [102]	0.335 (0.3061) [130]	0.271 (0.2825) [146.7]	0.287 (0.2820) [43.6]	0.297 (0.1373) [106]	0.346 (0.2208) [30]

Relationship of population genetic diversity with size, geography and altitude

Comparison the levels of genetic variations between small and large populations using ISSR and RAPD markers produced different results. ISSR analysis showed that genetic variation was not significantly different between small and large populations ($P \geq 0.4149$). However, RAPD analysis indicated significant impact of population size on this variation ($P \leq 0.002$).

There was no significant correlation between population genetic and geographical distances based on both ISSR and RAPD markers (Fig. 2). Similarly, population genetic variation was not correlated with altitude of the population both in ISSR and RAPD analyses (Fig. 2). The UPGMA dendrogram based on Nei's matrices of both ISSR and RAPD markers (Table 2) clustered the geographically distant populations together in the same cluster (Fig. 3).

Partitioning the total ISSR variation by an AMOVA test showed that 62% of the variation was within populations, while 38% of total variability was assigned to among-population components; for RAPD – 67 and 33%, respectively (Table 3).

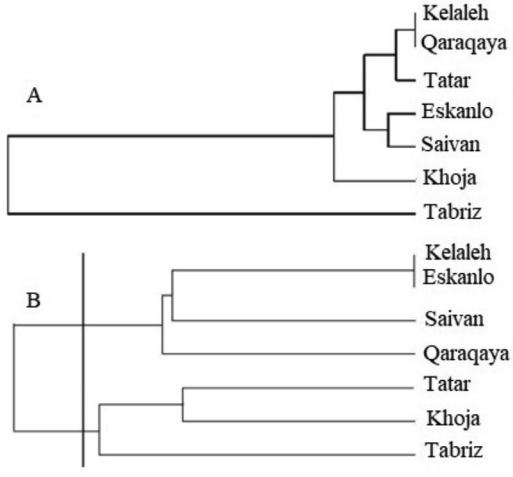

Fig. 3. UPGMA dendrograms showing genetic relationship among populations of *Capparis spinosa* based on ISSR (A) and RAPD (B) markers. Data on RAPD analysis are from Nosrati et al. (2012).

Discussion

The results obtained from analysing of components of population genetic structure in seven populations of *C. spinosa* using ISSR and RAPD markers showed a contradictory relationship between population size and genetic variations. While ISSR variation did not significantly differ between small and large populations, RAPD diversity significantly differed depending on population size. However, the results of ISSR and RAPD analysis were consistent in all other cases. Both markers indicated that population genetic variation was not correlated with population geographical distance and altitude. Moreover, the lowest and highest genetic variation was observed in the same populations by both ISSR and RAPD, and the shortest and greatest genetic distances were detected between the same population pairs by both markers. The contradictory relationship between habitat fragmentation and genetic variation among populations of *C. spinosa* between ISSR and RAPD analyses in our study most likely can be explained by use of different types of DNA markers for assessing the population genetic variation, which was the only difference between two analyses of the same species and populations. We therefore concluded that, in addition to frequently reported factors such as species biological characteristics and populations size and fragmentation scale, the type of DNA markers used for assessing levels of population genetic variations may play important role on assessment of the effect of habitat fragmentation on population genetic variation, and may cause contradictory effect of habitat fragmentation on population genetic structure.

Conflicting results on the relationship between habitat fragmentation and population genetic variations have been frequently reported from various experimental studies

Table 3. Partitioning of total genetic variations to the components of among- and within-populations in seven populations of *Capparis spinosa* using AMOVA test. Data on RAPD analysis are from Nosrati et al. (2012)

Genetic marker	Source of variation	Degree of freedom	Variance component	Sum of squares	Variance (%)	*P* value
ISSR	Among populations	6	4.39	306.34	38	< 0.01
	Within-populations	63	7.14	449.6	62	< 0.01
RAPD	Among populations	6	4.93	356.29	32.8	< 0.01
	Within-populations	63	10.09	635.50	67.2	< 0.01

(e.g. reviews by Fahrig 2017; Fletcher et al. 2018). The contradictory effects of habitat fragmentation on genetic diversity were attributed to various factors including species characteristics e.g. phylogeny, habitat requirements and biology (Heinken, Weber 2013; Narumi, Ohara 2018), population size (Vandepitte et al. 2007; Heinken, Weber 2013; Matesanz et al. 2017; Narumi, Ohara 2018), plant life-history traits such as annual vs perennial (Tewksbury et al. 2002; González et al. 2020), woody vs herbaceous (Chung et al. 2020), pollination mode and breeding system (Aguilar et al. 2008; Jacquemyn et al. 2012), seed dispersal modes (Hamrick 2004; Ozawa et al. 2013; Browne, Karubian 2018; Chybicki, Oleksa 2018) and the scale of habitat fragmentation (Duminil et al. 2013; Yuan et al. 2015; Yu et al. 2020), and now we can add type of DNA markers to the list.

The higher within-population genetic variations in Capparis spinosa (62 and 67%, based on ISSR and RAPD markers, respectively) indicates that the higher level of outcrossing could be attributed to its andromonoecy breeding system of, which promoting outcrossing, while limiting selfing. The cross-pollination rate in this species was even reported to be more than 90% (Zhang, Tan 2008; Zhang, Tan 2009). Although our studies based on ISSR and RAPD, alongside others (Bhoyar et al. 2012; Gristina et al. 2014) using the same markers, clearly indicate that *C. spinosa* is predominantly an outcrossing species, a study on 31 natural populations of *C. spinosa* based on analysing three cpDNA fragments reported that 83.4% of the variation was allocated to inter-populations and only 16.6% to intra-populations components (Wang et al. 2016). The authors attributed the very high level of among-population genetic diversity to the geographical isolation between populations because of complex mountains and deserts, resulting in limitation of gene exchange between populations.

Our studies alongside the others suggest that in analysing population genetic structure, all aspects including species life story, fragmentation scale, and type of DNA markers used for measuring population genetic diversity should be considered in interpreting the results.

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