Natl. Acad. Sci. Lett.
DOI 10.1007/s40009-012-0086-y

RESEARCH ARTICLE

Genetic Variability Studies Among Natural Populations of *Capparis spinosa* from Cold Arid Desert of Trans-Himalayas Using DNA Markers

Manish S. Bhoyar · Gyan P. Mishra · Pradeep K. Naik · Ashutosh A. Murkute · R. B. Srivastava

Received: 27 June 2012 / Revised: 21 August 2012 / Accepted: 17 October 2012

Abstract The phylogenetic relationships of 90 wild grown *Capparis spinosa* genotypes which are collected from nine sampling sites from three valleys viz. Suru (3,309 m above mean sea level [amsl]), Nubra (2,926 m amsl) and Indus (3,505 m amsl) of trans-Himalayan region were analyzed using 40 PCR markers (20 random amplified polymorphic DNAs [RAPDs] and 20 inter simple sequence repeats [ISSRs]). RAPD analysis yielded 223 fragments, of which 220 were polymorphic while, ISSR produced 85 bands, of which all are found polymorphic, with an average of 11.0 and 10.62 polymorphic fragments per primer respectively. ISSR markers were found more efficient in relation to polymorphism detection. Clustering of individuals within groups was not similar when RAPD and ISSR derived dendrogram were compared, whereas the pattern of clustering of the individuals remained more or less the same in RAPD and combined data of RAPD + ISSR. analysis of molecular variance analysis showed that total variation within the population was maximum, followed by among population and least for among valley in all the three cases. Pertaining to the management of caper, the high genetic differentiation of population indicated the requisite of conserving the utmost possible number of populations from different valleys of trans-Himalayas.

Keywords Caper · Genetic diversity · RAPD · ISSR · AMOVA

Introduction

Fragmented distributions of plant populations are caused not only by human activity but also by natural factors, such as long term, large-scale climate oscillations, topographical changes, the isolation of suitable habitats, or other ecological changes. Habitat fragmentation is a significant threat to the maintenance of biodiversity which is expected to reduce genetic diversity and increases inter population genetic divergence [1]. However, habitat fragmentation does not always lead to reduced genetic variation [2] but it may increase the genetic diversity of a fragmented population than continuously distributed population [3]. This is because the effects of habitat fragmentation on genetic diversity and population structure can be affected by other factors, such as population size, gene flow and the time scale of fragmentation. Studies of the genetic diversity of naturally fragmented populations may not only reveal the ecological consequences of population fragmentation over long periods of time but also provide a frame of reference for predicting the consequences of habitat fragmentation by human activities [2].

Capparis spinosa L. (Capparidaceae) also called 'Caper' and locally known as '*Kabra*' is one of the oldest known medicinal plant in 'Amchi system' (local medicinal

---

Electronic supplementary material The online version of this article (doi:10.1007/s40009-012-0086-y) contains supplementary material, which is available to authorized users.

M. S. Bhoyar · G. P. Mishra (✉) · A. A. Murkute · R. B. Srivastava
Defence Institute of High Altitude Research, DRDO, C/o 56 APO, Leh 901 205, Kashmir, India
e-mail: gyan.gene@gmail.com

P. K. Naik
Jaypee University of Information Technology, Waknaghat, Solan 173 215, Himachal Pradesh, India

Present Address:
G. P. Mishra
Directorate of Groundnut Research, Junagadh 362001, Gujarat, India

system) which is occasionally used by local people of Ladakh as a leafy vegetable and forage. In India, it is found in inner valleys of trans-Himalaya between 3,020 and 3,890 m above mean sea level (amsl) which includes Indus, Nubra and Suru valleys of Ladakh region. This plant has multiple uses in cuisine as salad, pickle and condiments. Bio-chemical studies have reported the presence of alkaloids, lipids, flavonoids and glucosinolates, cancer preventing agents and biopesticides in *C. spinosa* [4, 5].

In Ladakh, *C. spinosa* habitat has been extremely threatened, mainly by anthropogenic action, and its distribution has been reduced to a very restricted area. Earlier, caper leaves were used only in small quantities by local people for vegetable purpose but, recently commerce and demand have increased. Heavy extraction from the wild along with heavy grazing at high altitude pasture in the trans-Himalayas has now threatened its survival. Since ages, the caper which is growing wild in Ladakh has developed considerable variability. The genotypes in this region are area specific, suitably adapted for survival since it grows at high altitudes (3,000–4,000 m amsl) along with temperature and nutrient stress that they are subjected under the cold arid environment [5].

PCR based marker system like random amplified polymorphic DNAs (RAPDs) and inter simple sequence repeats (ISSRs) have been used extensively both for DNA fingerprinting and population genetic studies [6, 7]. Limited reports are available on the molecular characterization of caper [8, 9]. The reduced number of *Capparis* individuals in trans-Himalayas makes the species highly susceptible to extinction, and conservation measures should be implemented immediately. Information on levels of genetic diversity is essential for successful management and preservation of populations of threatened species like *C. spinosa*.

In this study, we used RAPD and ISSR analysis to assess the genetic diversity, with the aim to determine population differentiation and structure of isolated populations of *C. spinosa* from three valleys across its distribution in the trans-Himalayan range of India in a scenario of local adaptation at high altitudes along with providing insight to facilitate conservation management of these populations.

Materials and Methods

Plant Materials

Ninety wild grown individual plants from nine different locations were collected from three valleys (Indus, Nubra and Suru) with an altitude ranging from 3135 m (Nubra),

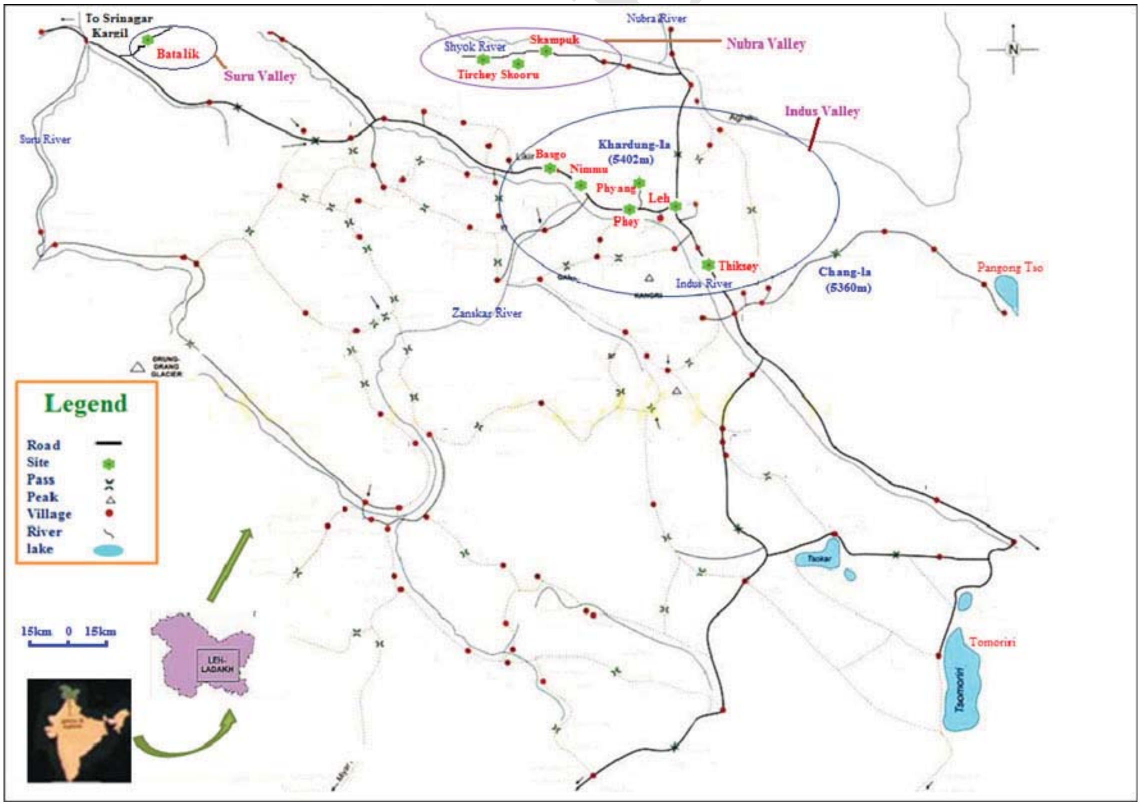


Fig. 1 Collection sites of 90 *Capparis* individuals from three valleys (Indus, Suru and Nubra) and nine villages located in Ladakh (Jammu and Kashmir, India)

Table 1 Nine populations of *C. spinosa* collected from three valleys of trans-Himalayan region

Valley	Site (villages)	Latitude (°E)	Longitude (°N)	Altitude (m)	Samples
Suru	Batalik	76.2465	34.5541	3,309	1–8
Indus	Nimmu	77.3434	34.1926	3,319	9–12
	Basgo	77.2917	34.2139	3,241	13–16
	Phyang	77.4669	34.1615	3,347	17–31
	Phey	77.4815	34.1399	3,185	32–46
	Thiksey	77.6648	34.0554	3,435	47–62
Nubra	Skampuk	77.4158	34.6383	3,197	63–68
	Skuru	77.3685	34.6485	3,135	69–78
	Tirchey	77.3513	34.6579	3,159	79–90

3309 m (Suru) to 3435 m (Indus) from cold arid desert of trans-Himalayas (Fig. 1; Table 1) during 2009–2010. The interval between samples was 100–500 m, the pair wise distance between populations was 5–35 km, whereas, the pair wise distance between valley divisions was 50–250 km.

DNA Extraction, PCR Amplification and Gel Electrophoresis

Total genomic DNA was extracted from frozen leaves (5 g) by the CTAB method [10] with minor modifications. Twenty random decamer primers from IDT Tech, USA (Table 2) were used for RAPD amplification. Amplification reaction were performed in volumes of 25 µl containing 10 mM Tris–HCl (pH 9.0), 1.5 mM $MgCl_2$, 50 mM KCl, 200 µM of each dNTPs, 0.4 µM primer, 20 ng template DNA and 0.5 unit of *Taq* polymerase (‘Sigma-Aldrich, USA’). The first cycle consisted of denaturation for 5 min at 94 °C, primer annealing at 37 °C for 1 min and extension at 72 °C for 2 min. In the next 40 cycles denaturation period is 1 min at 92 °C, while annealing and extension parameters remained same as in the first cycle while final extension step at 72 °C for 7 min.

In case of ISSR, primers were obtained from ‘Applied Biosciences, India’ (Table 3) and PCR amplification was performed in reaction cocktail and amplification cycle similar to RAPD except specific annealing temperature (±5 °C of T_m). The amplification for each primer was

Table 2 List of RAPD primers used with its amplification details

Primer	Nucleotide sequence (5′–3′)	T_m (°C)	Total number of loci	Percentage of polymorphic loci	Total number of fragments amplified	Resolving power
S-21	CAGGCCCTT C	36.4	07	100.00	426	9.46
S-22	TGCCGAGCT G	40.7	16	100.00	802	17.82
S-23	AGTCAGCCA C	34.3	13	92.30	952	21.15
S-24	AATCAG CCA C	30.1	14	100.00	992	22.04
S-25	AGGGGTCTT G	32.6	12	100.00	599	13.31
S-26	GGTCCCTGA C	35.2	11	90.90	546	12.13
S-27	GAAACGGGT G	33.2	14	92.85	703	15.62
S-28	GTGACGTAG G	31.1	09	100.00	339	7.53
S-29	GGGTAACGC C	37.4	10	100.00	486	10.8
S-30	GTGATCGCA G	33.1	11	100.00	580	12.8
S-31	CAATCGCCG T	36.7	06	100.00	323	7.17
S-32	TCGGCGATA G	34.0	05	100.00	219	4.86
S-33	CAGCAGCCA C	37.7	13	100.00	818	18.17
S-34	TCTGTGCTG G	34.3	11	100.00	634	14.08
S-35	TTCCGAACC C	34.2	10	100.00	502	11.15
S-36	AGCCAGCGA A	38.3	12	100.00	646	14.35
S-37	GACCGCTTG T	35.7	14	100.00	357	7.93
S-38	AGGTGACCG T	36.2	12	100.00	659	14.64
S-39	CAAACGTCG T	34.2	11	100.00	484	10.75
S-40	GTTGCGATC C	33.5	12	100.00	675	15.00
Total		–	223	98.65	11,742	–

Table 3 List of ISSR primers used with its amplification details

Primers	Nucleotide sequence	G+C (%)	T_m (°C)	Total number of loci	NPL	Percentage of polymorphic loci	Total number of bands amplified	Resolving power
ISSR 1	$(AG)_8T$	47.0	47.0	16	16	100	797	17.71
ISSR 2	$(AC)_8T$	47.0	51.4	08	08	100	205	4.55
ISSR 3	$(TG)_8A$	47.0	51.3	05	05	100	56	1.24
ISSR 4	$(AG)_8YT$	47.2	49.2	10	10	100	407	9.04
ISSR 5	$(GA)_8YT$	47.2	47.4	13	13	100	509	11.31
ISSR 6	$(GT)_8YC$	52.7	52.7	16	16	100	735	16.33
ISSR 7	$(ACC)_6$	66.6	60.6	12	12	100	807	17.93
ISSR 8	$(GGC)_6$	100	77.3	05	05	100	279	6.20
Total				85	85	100	3,795	

Y = C, T; R = A, G

ISSRs 9–20 did not amplify with the individuals used in the present investigation. Individual primers sequences were given in the parentheses. ISSR 9, [$(AT)_8T$]; ISSR 10, [$(TA)_8RT$]; ISSR 11, [$(AT)_8YA$]; ISSR 12, [$(CT)_8T$]; ISSR 13, [$(TC)_8A$]; ISSR 14, [$(GT)_8A$]; ISSR 15, [$(TGC)_6$]; ISSR 16, [$(TGCA)_4$]; ISSR 17, [$(CTAG)_8$]; ISSR 18, [$(GA)_8T$]; ISSR 19, [$(CT)_8RA$] and ISSR 20, [$(CCG)_8$]

performed twice independently with same procedure in order to ensure the fidelity of RAPD and ISSR markers. Amplification products were electrophoresed on 1.5 % agarose gel (Life Science Technologies, USA) then run at constant voltage (50 V) in 1× TBE for approximately 2 h, visualized by staining with ethidium bromide (0.5 µg ml^{-1}) and documented on a gel documentation system (Alpha Innotech, Alphaimager, USA).

Data Collection and Analysis

The banding patterns obtained from RAPD and ISSR were scored as present (1) or absent (0), which was treated as an independent character. The data was subjected to cluster analysis by Neighbor Joining (NJ) and dendrograms were generated using DARwin5 software package Version 5.0.158 [11]. POPGENE was used to calculate within species diversity (H_s), total genetic diversity (H_t) and Nei's unbiased genetic distance among different individuals. Data for Nei's genetic diversity (H), Shannon's information index (I), number of polymorphic loci (NPL) and percentage polymorphic loci (PPL) across all the nine populations were also analyzed [12].

The RAPD and ISSR data were subjected to a hierarchical analysis of molecular variance (AMOVA) [13], using three hierarchical levels; individual, population and their regions. GenAlEx was used to calculate the principal coordinates analysis (PCA) that plots the relationship between distance matrix elements based on their first two principal coordinates [14]. According to Prevost and Wilkinson [15] the resolving power (R_p) of a primer is: $R_p = \Sigma IB$ where IB (band informativeness) takes the value of: $1 - [2 \times (0.5 - P)]$, P being the proportion of the 90 individuals containing the band.

In order to determine the utility of each of the marker systems, diversity index (DI), effective multiplex ratio (EMR) and marker index (MI) were calculated according to Powell et al. [16]. DI for genetic markers was calculated from the sum of the squares of allele frequencies: $DI_n = 1 - \Sigma pi^2$ (where 'pi' is the allele frequency of the ith allele). EMR (E) is the product of the fraction of polymorphic loci and the NPL for an individual assay. EMR (E) = n_p (n_p/n). MI is defined as the product of the average DI for polymorphic bands in any assay and the EMR for that assay, MI = $DI_{avp} \times E$.

Additionally we performed Bayesian clustering analyses using STRUCTURE v2.2 [17] to infer the number of cluster (K) and the probability of individual assigned to each cluster. We executed analyses with no a priori information on collection sites or patch structure. Nine independent simulations were run, with a 100,000 burn-in period length, testing from one to nine clusters (K I–IX) for each. We calculated the average of each K likelihood values through all runs as well as ΔK statistics to verify the correct number of segregate groupings.

Results and Discussion

RAPD Marker: Genetic Variability and Dendrogram Analysis

All the chosen primers yielded 11,742 fragments which range from 5 (S-32) to 16 (S-22). Out of 223 amplified bands, 200 were found polymorphic, with average numbers of polymorphic bands per primer as 11.0 (Table 2). A dendrogram based on NJ analysis grouped the 90 individuals into five major clusters (I–V) (Fig. 2a) which corresponds with NJ tree at population level (Fig. 2b) and

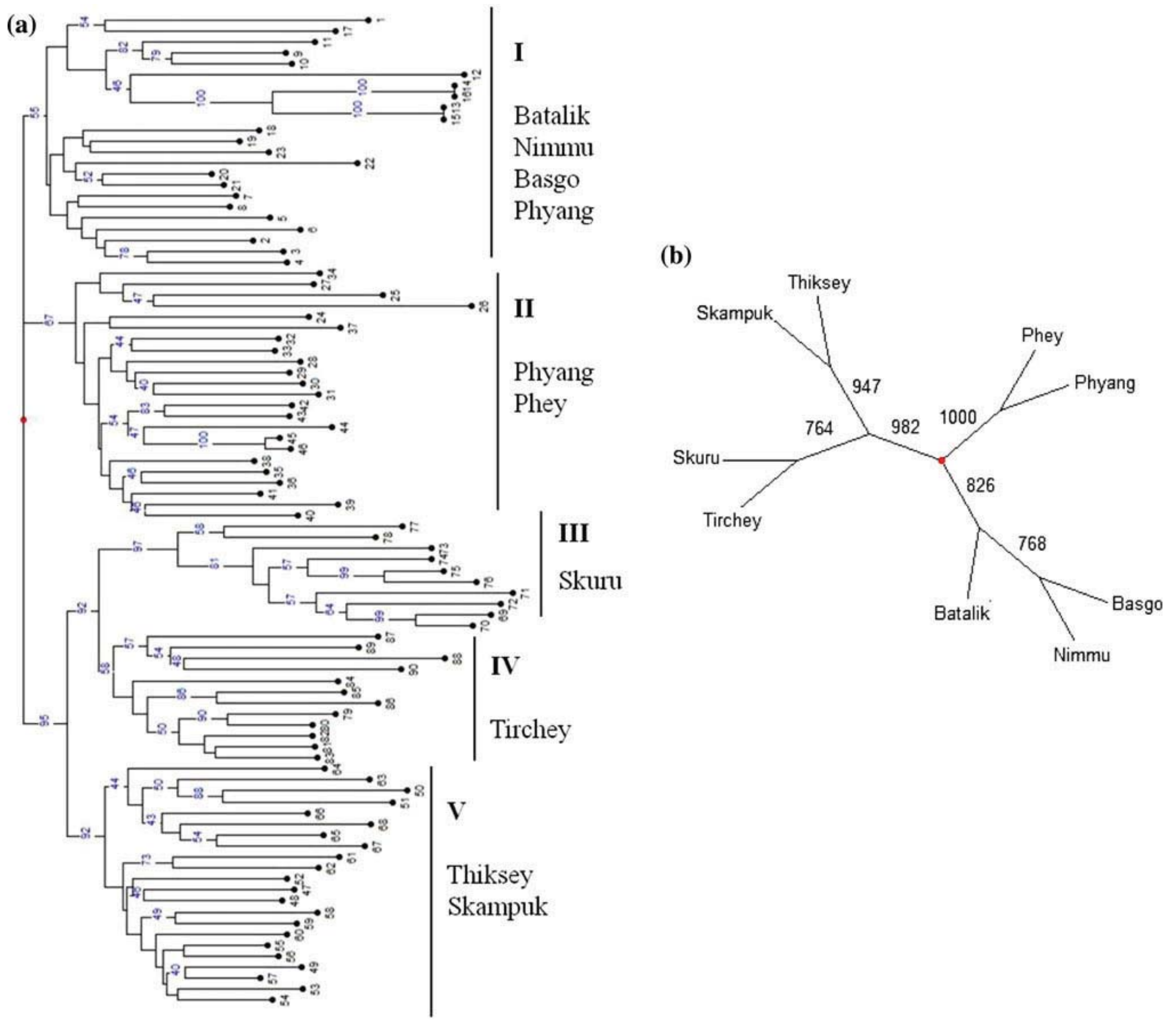


Fig. 2 RAPD profiling. **a** Dendrogram generated using NJ clustering showing relationships between 90 *Capparis* genotypes, where *numbers* indicate their bootstrap values, **b** NJ *tree* at population level

STRUCTURE derived unbiased clustering of genotypes at individual level [Fig. S1(a)]. Cluster I contains individuals from Batalik (Suru), Nimmu and Basgo (Indus) villages, cluster II represents individuals from Phey and Phyang (Indus). However, clusters III and IV contains individuals from Skuru and Tirchey (Nubra), cluster V have the individuals from Thiksey (Indus) and Skampuk (Nubra).

ISSR Marker: Genetic Variability and Dendrogram Analysis

Out of 20 ISSR primers used, only eight amplified which produced average 85 bands, of which all were found polymorphic. Number of amplified fragments varied from 5 (ISSRs 3 and 8) to 16 (ISSRs 1 and 6) and both average numbers of bands and polymorphic bands per primer is 10.62 (Table 3).

The sequences of these 20 primers seem to indicate that microsatellites more frequent in caper contain the repeated di-nucleotides (AG)n, (AC)n, (TG)n, (GA)n, (GT)n, and tri-nucleotides (ACC)n, and (GGC)n. The number of bands produced with different repeat nucleotide were more with the (AG)nT, (GT)nYC and (ACC)n primers (ISSRs 1, 6 and 7). In the present investigation, the primers that were based on the (AG)n, and (GT)n motif produced more polymorphism (16 bands per primer) than the primers based on any other motifs. However (AT)n and some other primers gave no amplification products (Table 3), despite the fact that (AT)n di-nucleotide repeats are thought to be the most abundant motifs in plant species [6, 18]. Possible explanation could be

Journal : Large_Springer-India40009 Dispatch : 20-10-2012 Pages : 11
Article No. : 86 ☐ LE ☐ TYPESET
MS Code : SCLE-D-12-00131 ☑ CP ☑ DISK

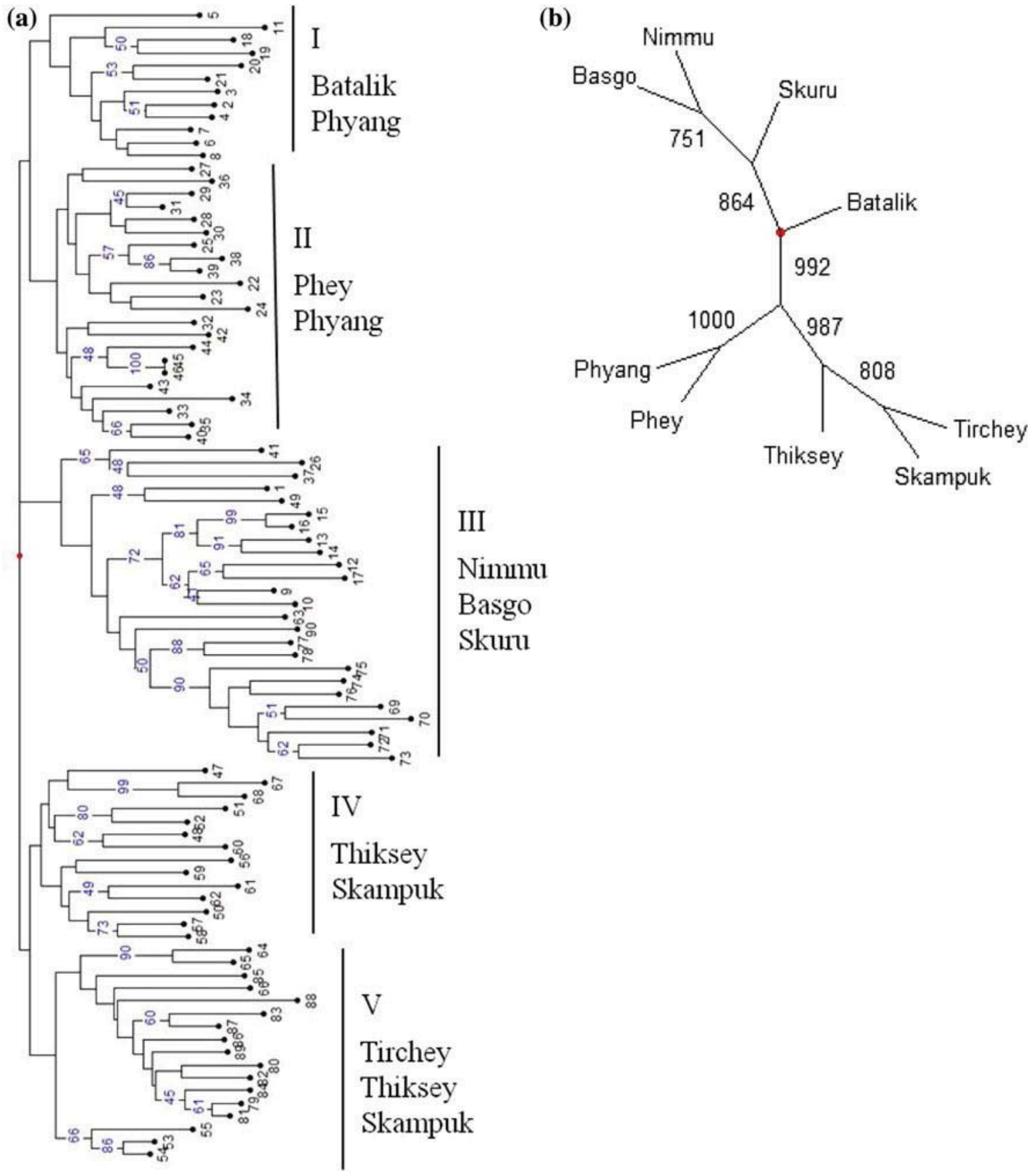


Fig. 3 ISSR profiling. **a** Dendrogram generated using NJ clustering showing relationships between 90 *Capparis* genotypes, where *numbers* indicate their bootstrap values, **b** NJ *tree* at population level

that ISSR primers having (AT) or (TA) motifs due to sequence complementarity may form dimers during PCR amplification or it may not be annealing with template DNA due to low T_m. Reason behind non-amplification of other repeats may be either its absence or absence of motifs complementary to the primers in the genome of Caper genotypes used in the present investigation.

The complete data was based on a total of 3,795 bands and a dendrogram based on NJ analysis grouped the 90 individuals into five major clusters (I–V) (Fig. 3a) which corresponds with NJ tree at population level (Fig. 3b) and unbiased clustering of genotypes at individual level [Fig. S1(b)]. Cluster I contains individuals from Batalik (Suru) and Phyang (Indus) villages, cluster II represents individuals from Phey and Phyang (Indus). However, cluster III is a mixed cluster having majority of individuals from Nimmu, Basgo (Indus) and Skuru (Nubra), clusters IV and V have the individuals from Thiksey (Indus), Skampuk (Nubra) and Thiksey (Indus), Tirchey, Skampuk (Nubra) respectively.

Table 4 Summary of genetic variation statistics for all loci of RAPD, ISSR and RAPD + ISSR among the *Capparis* populations with respect to their distributions among three valleys

Marker type	Sample size	H	I	H_t	PPL
RAPD					
Suru	8	0.239 ± 0.196	0.358 ± 0.277	0.239 ± 0.038	65.92
Indus	54	0.35 ± 0.142	0.52 ± 0.18	0.350 ± 0.02	96.86
Nubra	28	0.354 ± 0.157	0.52 ± 0.205	0.354 ± 0.025	93.27
Mean	–	0.315	0.466	0.315	–
ISSR					
Suru	8	0.421 ± 0.11	0.603 ± 0.143	0.421 ± 0.012	96.3
Indus	54	0.487 ± 0.017	0.680 ± 0.017	0.487 ± 0.001	100
Nubra	28	0.460 ± 0.044	0.651 ± 0.047	0.460 ± 0.002	100
Mean	–	0.456	0.645	0.456	–
RAPD + ISSR					
Suru	8	0.401 ± 0.119	0.581 ± 0.15	0.401 ± 0.014	96.71
Indus	54	0.478 ± 0.024	0.671 ± 0.024	0.478 ± 0.001	100
Nubra	28	0.483 ± 0.022	0.676 ± 0.023	0.483 ± 0.001	100
Mean	–	0.454	0.643	0.454	–

H Nei's gene diversity, *I* Shannon's information index, H_t heterogeneity, *PPL* percentage polymorphic loci

Thus, our RAPD and ISSR data suggest that although an isolation-by-distance pattern may be detected across the whole range of *C. spinosa*, but the relationships between geographical and genetic distances have different patterns at different spatial scales. Similarly distinct patterns at different spatial scales were also found for some other plant species [19].

RAPD, ISSR and RAPD + ISSR: Genetic Variability Details for Valley Divisions

When H, I, H_t, NPL and PPL were studied for valley divisions (i.e. Suru, Indus and Nubra valleys) using RAPD then, all these respective values were found higher for Nubra and least for Suru valley, while ISSR indicated more variability in Indus and least in Suru valley individuals. However, RAPD + ISSR combined data indicated more variability in Nubra and least in Suru (Table 4) which is same as observed by Kumar et al. [18] for apricot.

RAPD + ISSR Combined Data for Cluster Analysis

Dendrogram obtained from the NJ analysis showed that all the individuals were clustered into six major clusters (I–VI) (Fig. 4a) which corresponds with NJ tree at population level (Fig. 4b) and STRUCTURE derived clustering of genotypes at individual level [Fig. S1(c)]. The clusters I and II contains individuals from Tirchey (Nubra) and Thiksey (Indus) respectively. However clusters III and IV represents individuals from Skuru (Nubra) and Skampuk (Nubra), Thiksey (Indus) villages respectively. Phyang, Basgo, Nimmu (Indus) and Batalik (Suru) samples were clustered together in cluster V. However, cluster VI is having individuals from Phyang and Phey (Indus). Both RAPD and ISSR clusters showed partial similarity with combined data of RAPD + ISSR. Almost similar result was observed by Kumar et al. [18] in case of apricot. Besides this PCA analysis were also comparable to the cluster analysis (Fig. 5). This is further confirmed through unbiased structuring of genotypes using STRUCTURE analysis at population level (Fig. S2) where collection sites were represented with different color bars along with their probabilities. In RAPD, ISSR and RAPD + ISSR the first three most informative PC components explained 31.87, 37.59, 30.99 % of the total variations respectively.

Comparative Analysis of RAPD, ISSR and RAPD + ISSR Markers

In the present study, ISSR markers were found more efficient with regards to polymorphism detection, as they detected 100.0 % as compared to 98.68 % for RAPD markers. This is in agreement with the result of plant species like *Vigna* [20]. A lower number of clusters are detected in all the three cases i.e. RAPD, ISSR and RAPD + ISSR than the number of populations sampled as also reported by Montes et al. [21]. Given that *C. spinosa* are long lived perennials and fragmentation has been more intense during the past 20–30 years, the expected effects of fragmentation on genetic diversity may take longer to express itself than in a shorter lived species. It is also possible that actual rate of out-crossing and gene flow are sufficient to maintain observed level of genetic variation within fragmented populations. Our results suggests that the strong genetic structure of this species makes it

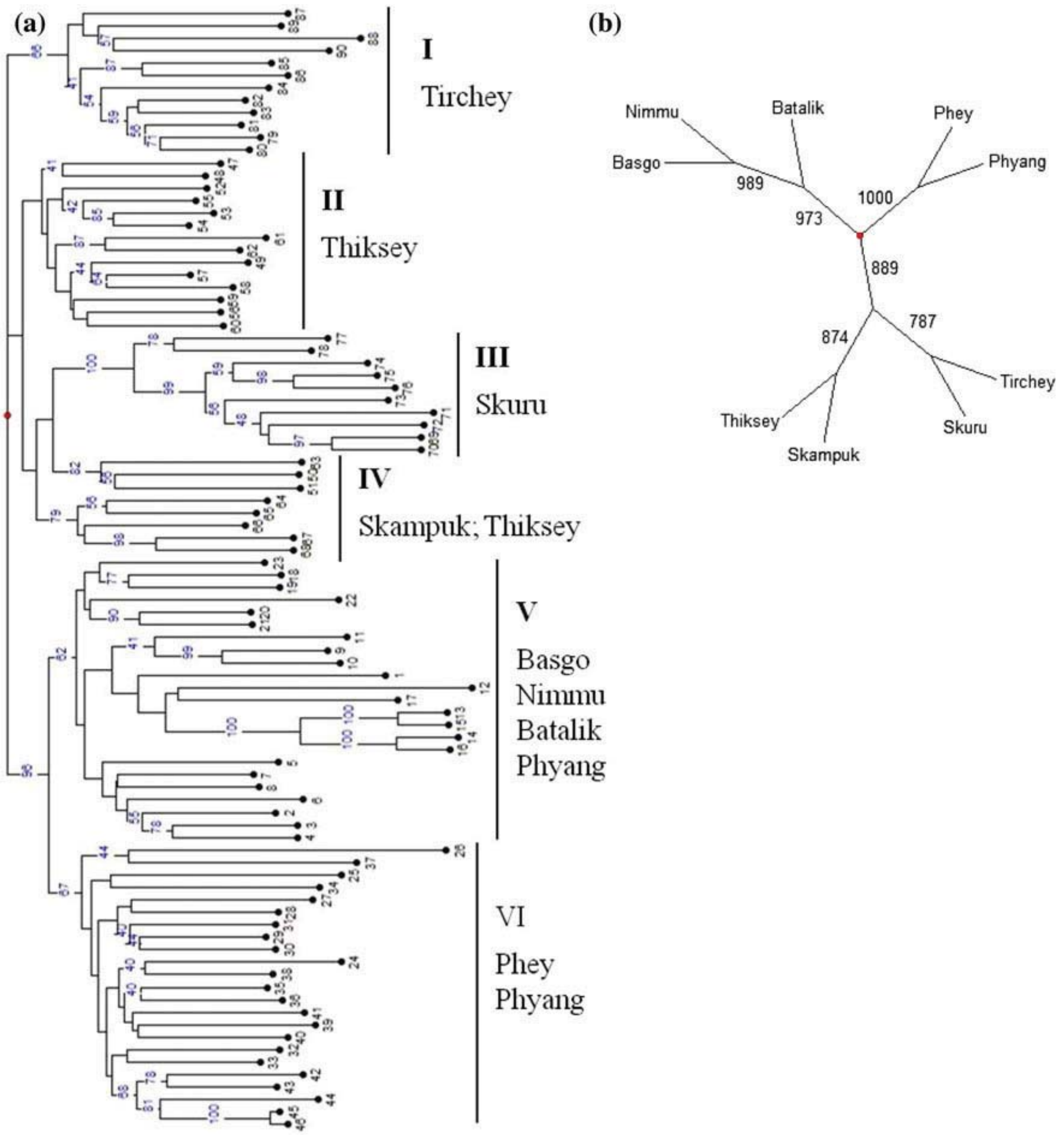


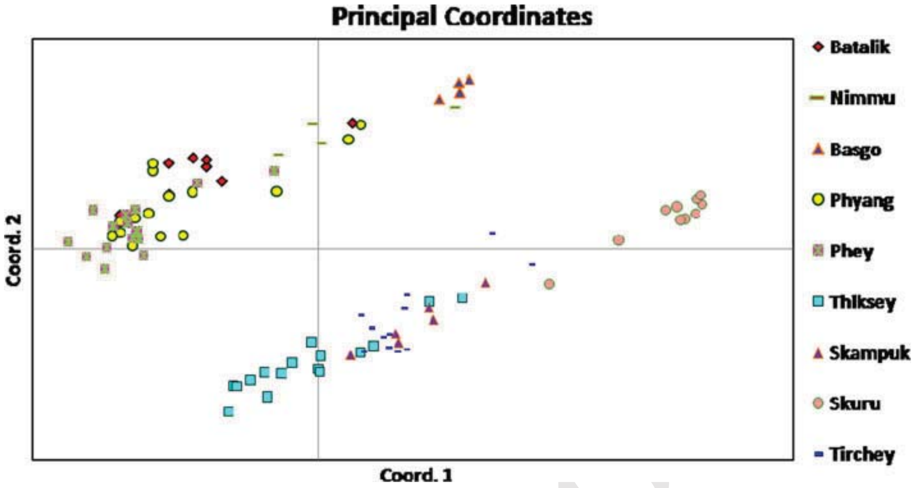
Fig. 4 RAPD + ISSR profiling. **a** Dendrogram generated using NJ clustering showing relationships between 90 *Capparis* genotypes, where *numbers* indicate their bootstrap values, **b** NJ *tree* at population level

potentially susceptible to variations in the mating system (inbreeding) and the effects of drift induced by reductions in population size and isolation.

The differences found among the dendrograms generated by RAPDs and ISSRs could be partially explained by the different number of PCR products analyzed (11,742 for RAPDs and 3,795 for ISSRs) reinforcing again the importance of the number of loci and their coverage of the overall genome [18]. Dendrograms in the present study did not indicated clear pattern of clustering for within valley samples but, almost clear pattern was observed in all the three cases for between valley samples of Indus and Nubra valley. However, Suru valley genotypes showed similarity to Indus valley individuals and few genotypes from Thiksey (Indus) were found more close to Nubra genotypes. The genetic closeness among the Indus and Suru valleys cultivars can be explained by the high degree of commonness in their individuals which is same as observed in blackgram [22] and apricot [18].

To further test this population structure, a model-based clustering method was implemented using program STRUCTURE. Without prior information about the populations and under an admixed model, STRUCTURE showed three as the most likely number of populations for RAPD, ISSR and RAPD + ISSR data i.e., ΔK reached its maximum at $K = 3$ (Fig. 6a, b), suggesting that all populations fell into one of the

Fig. 5 Two-dimensional *plot* of principle component analysis of 90 *C. spinosa* individuals using RAPD + ISSR analysis. The *shapes* plotted represent individual genotypes



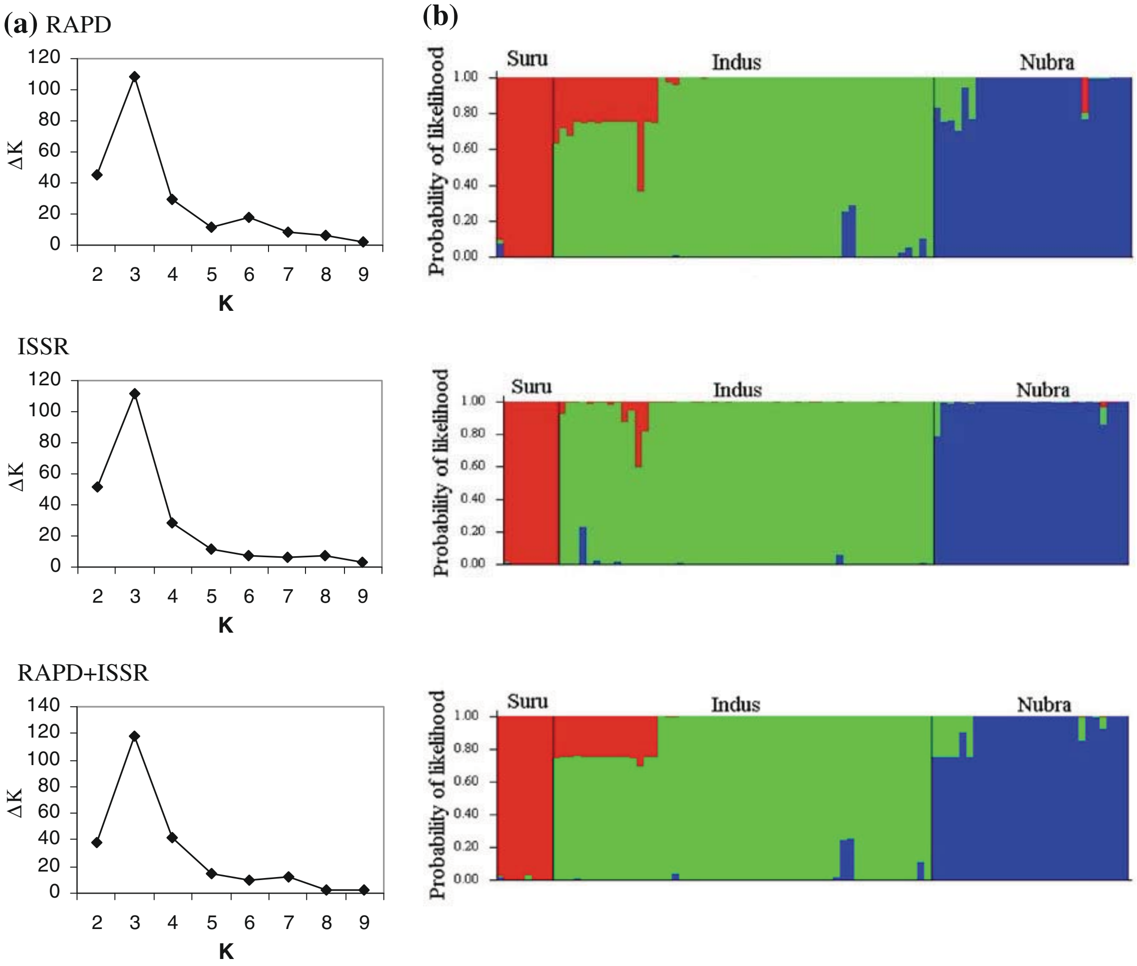


Fig. 6 Analyses of STRUCTURE results for the determination of unique clusters with in the distribution range of *C. spinosa.* **a** Average of ΔK for each K of independent runs, showing three as the likely number of populations for RAPD, ISSR and RAPD + ISSR data, **b** unbiased grouping when $K = 3$

three clusters. These three genetically distinct clusters primarily correspond to the geographic distribution of these populations (Table 1). The red cluster covered the genotypes from Suru valley, green cluster represent populations from Indus valley and the remaining populations were grouped in the blue cluster which is primarily consisting of genotypes from Nubra valley (Fig. 6a, b). The congruence between the geographical distribution of populations and their genetic relationships is generally

Table 5 Summary of nested AMOVA based on RAPD, ISSR individually and in combination, among the populations of *Capparis*. Levels of significance are based on 1,000 iteration steps

Source of variation	Among valley			Among population/valley			Individual/within population		
df	2			6			81		
Markers	RAPD	ISSR	RAPD + ISSR	RAPD	ISSR	RAPD + ISSR	RAPD	ISSR	RAPD + ISSR
Variance component	2.450	1.211	3.661	15.387	5.459	20.845	27.227	10.275	37.503
Percentage	5.44	7.14	5.90	34.14	32.21	33.61	60.41	60.63	60.48
P value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

interpreted as sign of a longstanding pattern of restricted gene flow [23].

Diversity index (DI), Effective Multiplex Ratio (EMR) and Marker Index (MI)

DI, EMR and MI are used to measure and compare the informativeness of one marker with other viz. RAPD and ISSR [24, 25]. In the present investigation all these parameters were found high for ISSR than RAPD (Table S1) which highlights the distinctive nature of these markers. The higher number of fragments per primer combination in RAPD and ISSR provides higher EMR however Baghizadeh et al. [25] found that although SSR markers had the lowest values of the EMR and MI, but they had the highest level of polymorphism in pistachio cultivars. However, MI as a measure of overall marker utility is applicable to any experimental situation where *H* and *E* may be calculated and hence MI may be used to predict the relative utilities of the various marker types for unknown germplasm [16]. Along with MI value, Prevost and Wilkinson [15] used resolving power (R_p) to compare the informativeness of AFLP. Lack of correlation between EMR, MI and R_p in our study, or lack of consistency in the correlation in other studies [24], makes it clear that probably a single parameter is not a good indicator to assess the informativeness of any primer.

G_{st} and AMOVA

The G_{st} value from RAPD, ISSR and RAPD + ISSR were 0.119, 0.033 and 0.032 respectively indicating that 88.1 %, 96.7 % and 96.8 % of the genetic diversity resided within the population (Table S1). Molecular variance from RAPD, ISSR and RAPD + ISSR among valley (5.44 %, 7.14 % and 5.9 %), among population (34.14 %, 32.21 % and 33.61 %) and within the population (60.41 %, 60.63 % and 60.48 %) indicated that there are more variations within the population (Table 5). Besides this it again reconfirm the equal efficacy of both the markers for genetic diversity studies in *C. spinosa*. This is helpful in making strategy for germplasm collection and evaluation. Thus, the expectation that genetic variability would decline due to population fragmentation was not supported in this case as observed by Hou and Lou [19]. This result might be observed because *C. spinosa* still occurs in medium or large population sizes (50 to over 100 individuals) in some localities. Another explanation could be that this high genetic diversity is a reflection of high historic genetic variability, which is quite common in long lived perennial plant species [26].

In addition to demographic history, a great number of factors relative to life history and species biology viz. pollen and seed dispersal, successional stages, geographic distribution range, and mating systems can shape the levels and distribution of genetic variability among and within populations [27]. In trans-Himalayas, at more than 3,000 m amsl, there are an array of factors which leads to deviation in partitioning of total genetic variation of a plant species, such as short vegetation period (about 120 days), wide temperature range (−40 to +35 °C) and high UV ‘B’ radiations. Under such unique environmental conditions, no regular pattern of seed dispersal was observed in perennial plants like *C. spinosa*. Besides this germination rates are very low under natural conditions (2–4 %), although under experimental conditions the rates are up to 62 % [28]. In this context, the genetic diversity within population is mostly depended on the first cloning plants.

Conservation Measures

Considering that all *C. spinosa* plants occur wild in trans-Himalayas with no conservation measures, we propose the establishment of new populations in private areas like farmers field and areas under governmental protection. As the plants of *C. spinosa* produce many fruits containing several seeds each, a strategy such as ex situ preservation of seeds in seed bank is recommended. For that, DIHAR has taken initiative and conserved the seeds of different *C. spinosa* populations under its permafrost based national germplasm conservation facility at Chang-La, Ladakh (5,360 m amsl). Considering the low diversity of Suru valley populations, we propose that saplings to be introduced in this valley population should preferably come from the same population in order to maintain the population distinctiveness. Another important measure is to carry out surveys to uncover more individuals and populations in other localities. Additionally, in some regions like Indus valley villages where possibility of habitat destruction or exploitation of plants for local consumption are high, it is necessary to establish sustainable management plans through local government agencies and Non-Government Organizations. Considering the critical situation of *C. spinosa*, probably the safest way to preserve the species is through all of the methods mentioned above.

In conclusion, the total genetic diversity of *C. spinosa* is high, and both RAPD and ISSR markers were equally useful for studying the genetic relationships of *Capparis* individuals from the trans-Himalayan region of Ladakh. The geographical distribution of populations and their genetic relationships were quite consistent and most likely due to the natural geographic fragmentation of this species.

Acknowledgments The authors are thankful to DRDO HQ for its support and funding the project.

References

1. Aguilar R, Quesada M, Ashworth L, Herreriasdiego Y, Lobo J (2008) Genetic consequences of habitat fragmentation in plant populations: susceptible signals in plant traits and methodological approaches. Mol Ecol 17:5177–5188
2. Chen KX, Wang R, Chen XY (2008) Genetic structure of *Alpinia japonica* populations in naturally fragmented habitats. Acta Ecol Sin 28:2480–2485
3. Young AG, Merriam HG, Warwick SI (1993) The effects of forest fragmentation on genetic variation in *Acer saccharum* Marsh. (Sugar maple) population. Heredity 71:277–289
4. Mishra GP, Singh R, Bhoyar MS, Singh SB (2009) *Capparis spinosa*: unconventional potential food source in cold arid deserts of Ladakh. Curr Sci 96:1563–1564
5. Bhoyar MS, Mishra GP, Naik PK, Srivastava RB (2011) Estimation of antioxidant activity and total phenolics among natural populations of *Capparis spinosa* leaves collected from cold arid desert of trans-Himalayas. Aust J Crop Sci 5:912–919
6. Gupta S, Srivastava M, Mishra GP, Naik PK, Chauhan RS, Tiwari SK, Kumar M, Singh R (2008) Analogy of ISSR and RAPD markers for comparative analysis of genetic diversity among different *Jatropha curcas* genotypes. Afr J Biotechnol 23:4230–4243
7. Alam A, Naik PK, Mishra GP (2009) Congruence of RAPD and ISSR markers for evaluation of genomic relationship among 28 populations of *Podophyllum hexandrum* from Himachal Pradesh. Turk J Bot 33:1–12
8. Khouildi S, Pagnotta MA, Tanzarella OA, Porceddu E, Ghorbel A (1999) Assessment of the genetic variation in natural populations of *Capparis spinosa* L. using RAPD analysis. CWANA Newsl 8
9. Inocencio C, Cowan RS, Alcaraz F, Rivera D, Fay MF (2005) AFLP fingerprinting in *Capparis* subgenus *Capparis* related to the commercial sources of capers. Genet Res Crop Evol 52:137–144
10. Saghai-Maroof MA, Soliman KM, Jorgensen RA, Allard RW (1984) Ribosomal spacer length in barley: Mendelian inheritance, chromosomal location and population dynamics. Proc Natl Acad Sci USA 81:8104–8118
11. Perrier X, Jacquemoud-Collet JP (2006) DARwin software. http://darwin.cirad.fr/darwin
12. Zhao WG, Zhang JQ, Wangi YH, Chen TT, Yin Y, Huang YP, Pan Y, Yang Y (2006) Analysis of genetic diversity in wild populations of mulberry from western part of Northeast China determined by ISSR markers. J Genet Mol Biol 7:196–203
13. Excoffier L, Smouse PE, Quattro JM (1992) Analyses of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131:479–491
14. Peakall R, Smouse PE (2001) GenAlEx V5: Genetic Analysis in Excel. Population genetic software for teaching and research. Australian National University, Canberra. http://www.anu.edu.au/BoZo/GenAlEx/
15. Prevost A, Wilkinson MJ (1999) A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars. Theor Appl Genet 98:107–112
16. Powell W, Morgante M, Andre C, Hanafey M, Vogel J, Tingey S, Rafalski A (1996) The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. Mol Breed 2:225–238
17. Falush D, Stephens M, Pritchard JK (2007) Inference of population structure using multilocus genotype data: dominant markers and null alleles. Mol Ecol Notes 7:574–578
18. Kumar M, Mishra GP, Singh R, Kumar J, Naik PK, Singh SB (2009) Correspondence of ISSR and RAPD markers for comparative analysis of genetic diversity among different apricot genotypes from cold arid deserts of trans-Himalayas. Physiol Mol Biol Plants 15:225–236
19. Hou Y, Lou A (2011) Population genetic diversity and structure of a naturally isolated plant species, *Rhodiola dumulosa* (Crassulaceae). PLoS ONE 6(9):e24497. doi:10.1371/journal.pone.0024497
20. Ajibade SR, Weeden NF, Chite SM (2000) Inter simple sequence repeat analysis of genetic relationships in the genus *Vigna*. Euphytica 111:47–55
21. Montes PS, Fornoni J, Farfan NJ (2011) Conservation genetics of the endemic Mexican *Heliconia unpanapensis* in the Los Tuxtlas tropical rain forest. Biotropica 43:114–121
22. Gaffor A, Sharif A, Ahmad Z, Zahid MA, Rabbani MA (2001) Genetic diversity in blackgram (*Vigna mungo* L. Hepper). Field Crop Res 69:183–190
23. Schaal BA, Hayworth DA, Olsen KM, Rauscher JT, Smith WA (1998) Phylogeographic studies in plants: problems and prospects. Mol Ecol 7:465–474
24. Laurentin H, Karlovsky P (2007) AFLP fingerprinting of sesame (*Sesamum indicum* L.) cultivars: identification, genetic relationship and comparison of AFLP informativeness parameters. Genet Resour Crop Evol 54:1437–1446
25. Baghizadeh A, Noroozi S, Javaran MJ (2010) Study on genetic diversity of some Iranian Pistachio (*Pistacia vera* L.) cultivars using random amplified polymorphic DNA (RAPD), inter sequence repeat (ISSR) and simple sequence repeat (SSR) markers: a comparative study. Afr J Biotechnol 9:7632–7640
26. Geert A, Rossum F, Triest L (2008) Genetic diversity in adult and seedling populations of *Primula vulgaris* in a fragmented agricultural landscape. Conserv Genet 9:845–853
27. Nybom H (2004) Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants. Mol Ecol 13:1143–1155
28. Bhoyar MS, Mishra GP, Singh R, Singh SB (2010) Effects of various dormancy breaking treatments on the germination of wild caper (*Capparis spinosa* L.) seeds from the cold arid desert of trans-Himalayas. Indian J Agric Sci 80:620–624

Journal : Large_Springer-India40009
Dispatch : 20-10-2012
Pages : 11
Article No. : 86
☐ LE
☐ TYPESET
MS Code : SCLE-D-12-00131
☑ CP
☑ DISK