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RESEARCH ARTICLE

ISSR and DAMD markers revealed high genetic variability within *Flavoparmelia caperata* in Western Himalaya (India)

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Abstract *Flavoparmelia caperata* (L.) Hale is medicinally very important and possesses antifungal and antibacterial activities. *F. caperata* is the only species found in India. Inter simple sequence repeat (ISSR) and Directed amplification of minisatellite DNA (DAMD) methods were used to analyze the genetic variability within *F. caperata* from the Western Himalayan region of India. Eleven ISSR and 10 DAMD primers produced 139 and 117 polymorphic bands, and detected 91.44 and 82.34 % polymorphisms, respectively. Cumulative band data generated for ISSR and DAMD markers resulted in 86.86 % polymorphism across all the accessions of *F. caperata*. The average Polymorphic information content (PIC) value obtained with ISSR, DAMD, and cumulative band data were 0.28, 0.27, and 0.27, respectively. The clustering of the *F. caperata* accessions in the UPGMA dendrogram showed that these accessions are intermingled with each other in different subclusters irrespective of their geographical affiliations. The pattern of genetic variations within *F. caperata* accessions could be due to free exchange of spores that might have taken place among these accessions in the wild. ISSR and DAMD markers efficiently and reliably resulted in discrete banding patterns and polymorphic profiles. These markers despite targeting different regions of genome, revealed almost similar levels of polymorphism across all the accessions. The wide range of genetic distance and high level of polymorphism detected by ISSR and DAMD reflected a high genetic variability among the different accessions of *F. caperata*.

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Introduction

The genus *Flavoparmelia* belongs to a medium to large foliose lichenized fungi within the family Parmeliaceae. The genus is characterized by a distinctive pale or yellow-green upper cortex when dry (Nash et al. 2002). There are about 38 species under the genus *Flavoparmelia* in the world, which predominantly grow on bark of trees and rocks (Crespo et al. 2010), and *Flavoparmelia caperata* (L.) Hale is the only species found in India. The species is distributed in the states of Andhra Pradesh, Assam, Himachal Pradesh, Jammu and Kashmir, Manipur, Meghalaya, Nagaland, Sikkim, Tamil Nadu, Uttarakhand, and West Bengal in India. *F. caperata* is medicinally very important and reported to possess antifungal and antibacterial activities (Pennington 1963; Cobanoglu et al. 2010). Secondary metabolites like usnic acid, atranorin, protocetraric acid, and caperatic acid have also been reported in *F. caperata*, which shows anti-inflammatory activities (Nash et al. 2002). Orange-brown to yellow dye is obtained from the thallus of *F. caperata*, which is used to dye animal skins, fabrics, crafts, and hair (Uphof 1959).

A large number of methods varying from morphological to molecular have been used to analyze the individuals, populations, and species. Most species in lichens do not show any unambiguous infraspecific morphological attributes (Printzen 2002). Phenotypic variability of the thallus has been used very often to discriminate among lichenized fungi; it can, however, be somewhat variable within a species and can clearly be influenced by the habitat and environment (Grube and Hawksworth 2007). Molecular markers could, therefore, be helpful to explore the mode and effectiveness of dispersal, gene exchange, and intra as well as infraspecific studies in

lichens (Printzen 2002). DNA fingerprinting methods are known to generate markers that are simple, rapid, and unaffected by the environment, and have been successfully employed to determine genetic variability and relationships at species and population level in many lichenized fungi (Murtagh et al. 1999; Honegger et al. 2004; Bayraktar et al. 2008; Yuzbasioglu et al. 2011; Fontaine et al. 2013). In the present study, we used inter simple sequence repeats (ISSR) (Provost and Wilkinson 1999) and directed amplification of minisatellite DNA (DAMD) (Heath et al. 1993) methods to estimate the genetic variability in *F. caperata*. ISSR and DAMD profiles are generated from a single primer PCR reaction. As they are distributed throughout the genome, therefore, the chances of amplifying large numbers of polymorphic bands are reasonably high (Hamada and Kakunaga 1982; Tautz and Renz 1984; Heath et al. 1993; Zietkiewicz et al. 1994; Zhou et al. 1997). The main advantages of the ISSR and DAMD methods are that they are quick, inexpensive, highly polymorphic, and randomly distributed throughout the genomes. As both these markers are PCR-based, therefore, they only require small amounts of template DNA. Furthermore, they do not require any sequence information for primer construction. As such, there is no information available on application of ISSR and DAMD markers in *F. caperata*; therefore, the objective of the present study was to estimate the genetic variability within *F. caperata* in the Western Himalayan region of India using these markers.

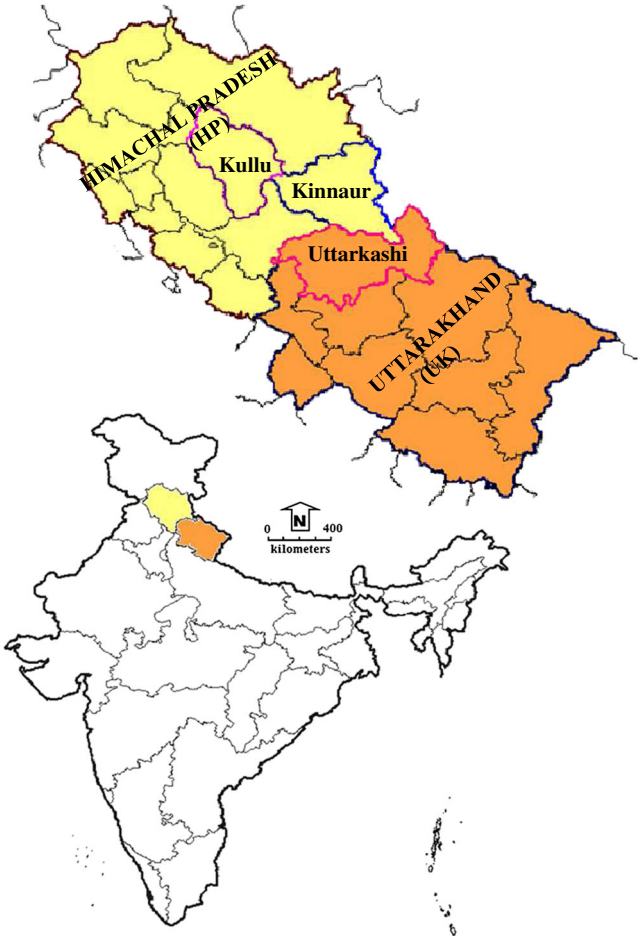

Fig. 1 Map showing the collection sites of *Flavoparmelia caperata* in the Western Himalayan region (*HP* and *UK*) of India

Materials and methods

Plant materials and DNA isolation

Thirty-nine accessions of *F. caperata* considered in the present study were collected in the wild from the Western Himalayan region particularly Himachal Pradesh (HP) and Uttarakhand (UK) states of India. *Everniastrum cirrhatum* (Ec), a closely related taxon, was considered as the out group. Each individual was collected, segregated, and kept in a separate packet in the field itself. The sampling sites and geographic coordinates of the accessions are provided in Fig. 1 and Table 1. Voucher specimens for each accession have also been prepared for all the collected materials and deposited in the herbarium of the CSIR-National Botanical Research Institute (LWG), Lucknow. Total genomic DNA was isolated using the DNeasy Plant Mini Kit (Qiagen, Hilden Germany), according to the instructions of the manufacturer. Isolated genomic DNA was checked for its quality and quantity by gel electrophoresis on 0.8 % agarose gel, staining with ethidium bromide, and comparison was made with a set of known DNA concentration standard, and also by UV spectroscopy using a Nanodrop ND-1000 Spectrophotometer (NanoDrop Technologies Inc. USA).

ISSR analysis

In ISSR analysis, two accessions of *F. caperata* were selected at random to carry out preliminary experiments to determine optimal amplification reaction conditions (annealing temperature and cycling time); reaction components like quantity of template, primers, Deoxynucleotide triphosphates (dNTPs), and Mg^{+2} ions; and primer screening reactions for ISSR. One hundred ISSR primers (University of British Columbia, Canada) were initially screened with two template DNAs. Screening of the primers revealed that 24 primers resulted in inconsistent profiles, 39 primers gave fuzzy profiles, 26 primers did not result in any amplification, and only 11 primers out of the 100 primers screened, resulted in discrete profiles consisting of well-separated fragments (Table 2). PCR amplification of 30 ng DNA was performed in 20-µl reaction containing 1.6 µl (10 mM) dNTP mix (2.5 mM each dNTP), 1.6 µl (25 mM) $MgCl_2$, 2 µl (Buffer F) 10× assay buffer supplied along with the enzyme, 0.33 µl (15 mM) primer,

Table 1 Geographical details of *Flavoparmelia caperata* accessions used in the present study

S. no.	Sample code	Voucher no.	Locality	Latitude (°′N)	Longitude (°′E)	Altitude (m)	Substratum
1	Fc01	12-017421	UK; Uttarkashi (GWLS)	31° 06′ 50.66″	78° 20′ 25.58″	2,592	Rock
2	Fc02	12-018567	UK; Uttarkashi (GWLS)	31° 06′ 55.09″	78° 20′ 38.03″	2,582	Bark
3	Fc03	12-018568	UK; Uttarkashi (GWLS)	31° 06′ 59.35″	78° 20′ 45.02″	2,586	Rock
4	Fc04	12-016606	UK; Uttarkashi (GWLS)	31° 04′ 25.51″	78° 06′ 51.44″	1,536	Bark
5	Fc05	12-018963	UK; Uttarkashi (GWLS)	31° 04′ 46.31″	78° 07′ 20.56″	1,582	Rock
6	Fc06	12-018736	UK; Uttarkashi (GWLS)	31° 04′ 35.49″	78° 08′ 07.46″	1,870	Bark
7	Fc07	12-018964	UK; Uttarkashi (GWLS)	31° 04′ 44.93″	78° 11′ 04.26″	1,915	Bark
8	Fc08	12-018980	UK; Uttarkashi (GWLS)	31° 00′ 54.21″	78° 02′ 11.06″	1,205	Bark
9	Fc09	12-016605	UK; Uttarkashi (GWLS)	31° 04′ 44.97″	78° 11′ 04.91″	1,908	Bark
10	Fc10	12-018863	UK; Uttarkashi (GWLS)	31° 07′ 25.67″	78° 04′ 41.71″	1,560	Bark
11	Fc11	12-018906	UK; Uttarkashi (GWLS)	31° 07′ 26.71″	78° 04′ 41.67″	1,564	Bark
12	Fc12	12-016618	UK; Uttarkashi (GWLS)	31° 04′ 44.91″	78° 11′ 19.39″	1,714	Rock
13	Fc13	12-018790	UK; Uttarkashi (GWLS)	31° 06′ 22.75″	78° 04′ 46.02″	1,512	Bark
14	Fc14	12-018909	UK; Uttarkashi (GWLS)	31° 04′ 36.53″	78° 10′ 50.21″	1,912	Bark
15	Fc15	12-018876	UK; Uttarkashi (GWLS)	30° 55′ 08.82″	78° 03′ 18.17″	2,009	Bark
16	Fc16	12-018775	UK; Uttarkashi (GWLS)	31° 01′ 56.70″	78° 07′ 44.70″	2,340	Bark
17	Fc17	12-018891	UK; Uttarkashi (GWLS)	31° 00′ 55.33″	78° 01′ 55.76″	1,260	Bark
18	Fc18	12-018988	UK; Uttarkashi (GWLS)	31° 00′ 39.66″	78° 01′ 43.56″	1,252	Rock
19	Fc19	12-018809	UK; Uttarkashi (GWLS)	31° 04′ 44.93″	78° 11′ 03.61″	1,915	Bark
20	Fc20	12-018871	UK; Uttarkashi (GWLS)	30° 52′ 43.01″	78° 04′ 27.61″	1,468	Bark
21	Fc21	12-016615	UK; Uttarkashi (GWLS)	31° 04′ 27.64″	78° 06′ 18.88″	1,344	Rock
22	Fc22	12-018800	UK; Uttarkashi (GWLS)	31° 04′ 44.98″	78° 11′ 05.49″	1,906	Bark
23	Fc23	13-019821	UK; Uttarkashi (GWLS)	31° 06′ 41.34″	78° 20′ 16.84″	2,664	Rock
24	Fc24	13-019822	UK; Uttarkashi (GWLS)	31° 06′ 41.56″	78° 19′ 05.99″	2,507	Bark
25	Fc25	13-019483	UK; Uttarkashi (GWLS)	31° 07′ 15.37″	78° 21′ 20.23″	2,723	Bark
26	Fc26	13-019821	UK; Uttarkashi (GWLS)	31°06′ 41.38″	78° 19′ 40.00″	2,476	Rock
27	Fc27	13-019825	UK; Uttarkashi (GWLS)	31° 04′ 51.47″	78° 14′ 53.01″	2,075	Bark
28	Fc28	13-019803	UK; Uttarkashi (GWLS)	31° 06′ 36.63″	78° 14′ 46.43″	2,587	Bark
29	Fc29	13-019486	UK; Uttarkashi (GWLS)	31° 04′ 43.76″	78° 15′ 04.53″	2,104	Bark
30	Fc30	13-023658	HP; Kinaur (Chitkul)	31° 21′ 01.15″	78° 26′ 03.99″	3,420	Bark
31	Fc31	13-019779	HP; Kullu (Kot)	31° 30′ 55.40″	77° 25′ 19.80″	2,311	Bark
32	Fc32	13-019793	HP; Kullu (Jaloritop)	31° 32′ 36.86″	77° 22′ 46.96″	2,951	Bark
33	Fc33	13-023364	HP; Kullu (Kothi)	32° 19′ 19.11″	77° 11′ 38.47″	2,555	Bark
34	Fc34	13-023259	HP; Kullu (Marhi)	32° 20′ 47.11″	77° 13′ 14.07″	3,270	Bark
35	Fc35	13-023266	HP; Kullu (Gulaba)	32° 19′ 57.17″	77° 11′ 48.35″	2,620	Bark
36	Fc36	13-019791	HP; Kullu (Gulaba)	32° 19′ 53.89″	77° 11′ 50.40″	2,653	Bark
37	Fc37	13-019787	HP; Kullu (Kasol)	32° 00′ 39.19″	77° 19′ 05.15″	1,580	Rock
38	Fc38	13-023355	HP; Kullu (Kasol)	32° 00′ 53.29″	77° 19′ 37.06″	1,670	Bark
39	Fc39	13-019765	HP; Kullu (Manikaran)	32° 01′ 31.32″	77° 21′ 17.99″	1,770	Bark
40	Ec	13-019803	UK; Uttarkashi (GWLS)	31° 06′ 36.63″	78° 14′ 46.43″	2,587	Bark

GWLS Govind Wild Life Sanctuary, *HP* Himachal Pradesh, *UK* Uttarakhand, *Ec Everniastrum cirrhatum* out group taxon used in the present study

and 0.33 μl (3 U/μl) *Taq* DNA polymerase (Merck Specialities Pvt. Ltd. India.) using PTC 200 thermo cycler (MJ Research, Inc., USA). After initial denaturation at 94 °C for 4 min, each cycle consisted of 1 min denaturation at 94 ° C, 1 min of annealing at 54 °C, 2 min extension at 72 °C along with 7 min extension at 72 °C at the end of 35 cycles.

DAMD analysis

The DAMD primers were custom synthesized from Sigma Aldrich Chemicals Pvt. Ltd. India, and are listed in Table 2. DNA amplification was carried out according to Zhou et al. (1997). The reaction (20 μl) contained 1.6 μl (10 mM) dNTP

Table 2 ISSR and DAMD primers, total number of bands, polymorphic bands, and polymorphic information content (PIC)

Primer name	Sequence (5′—3′)	Loci amplified	Polymorphic loci	Percentage polymorphism	PIC	Approx band size (bp)
ISSR						
UBC 807	(AG)8T	17	15	88.24	0.20	280–1,200
UBC 808	(AG)8C	12	11	91.67	0.34	300–1,500
UBC 809	(AG)8G	13	12	92.31	0.28	200–2,000
UBC 810	(GA)8T	15	15	100	0.27	290–1,200
UBC 811	(GA)8C	14	13	92.86	0.26	250–2,500
UBC 826	(AC)8C	13	11	84.62	0.23	360–2,000
UBC 835	(AG)8YC	17	17	100	0.30	280–2,500
UBC 836	(AG)8YA	14	14	100	0.36	280–1,200
UBC 840	(GA)8YT	12	10	83.33	0.27	260–1,700
UBC847	(CA)8RC	11	9	81.82	0.40	290–1,600
UBC 861	(ACC)6	14	12	85.71	0.21	300–2,000
Total	11	152	139	91.44	0.28	200–2,500
DAMD						
14C2	GGCAGGATTGAAGC	12	9	75	0.25	200–1,500
M-13	GAGGGTGGCGGTTCT	14	12	85.71	0.19	300–2,500
33.6	AGGGCTGGAGG	9	7	77.78	0.32	200–1,500
HVA	AGGATGGAAAGGAGGC	12	9	75	0.29	200–2,200
6.2H+	AGGAGGAGGGGAAGG	11	8	72.73	0.38	120–2,000
OGRB01	AGGGCTGGAGGAGGGC	23	18	78.26	0.12	200–1,800
FVII ex8	ATGCACACACACAGG	14	14	100	0.34	280–2,000
FVII ex8c	CCTGTGTGTGTGCAT	16	15	93.75	0.28	200–1,800
HBV3	GGTGAAGCACAGGTG	16	15	93.75	0.28	200–2,200
HVR	CCTCCTCCCTCCT	14	10	71.43	0.20	200–1,500
Total	10	141	117	82.34	0.27	120–2,500
ISSR+DAMD	21	293	256	86.86	0.27	120–2,500

Y=(C, T); R=(A, G)

mix (2.5 mM each dNTP), 0.4 µl (25 mM) MgCl2, 2 µl (Buffer A) 10× assay buffer with (15 mM) $MgCl_2$ supplied along with the enzyme, 0.8 µl (20 uM) primer and 0.40 µl (3 U/µl) *Taq* DNA polymerase (Merck Specialities Pvt. Ltd. India), and approximately 40 ng genomic DNA. Optimal DNA amplification was obtained through 40 cycles (92 °C for 1 min, 55 °C for 2 min, and 72 °C for 2 min) in a thermal cycler (PTC 200, MJ Research, Inc., USA).

Agarose gel electrophoresis

The amplified PCR products were resolved on 1.5 % agarose gel using 0.5X TBE buffer (1X TBE buffer: Tris-borate 89 mM; 2 mM EDTA, pH 8.3) at constant voltage of 5 V/cm. After electrophoresis, the gel was stained in ethidium bromide and then visualized and archived using Gel Documentation System (UV Tech, UK). The gel profiles were photographed and stored as digital images in gel documentation system.

Data analysis

The binary data were scored as presence (1) or absence (0) of a band, and only distinct and well-separated bands were considered in the present analysis. The polymorphic information content (PIC) was calculated according to Botstein et al. (1980). Pair wise distances were computed using Jaccard's coefficient in the Free Tree program (ver. 0.9.1.5) (Pavlicek et al. 1999). Jaccard's coefficient of similarity (J_{sim}) and dissimilarity (J_{dist}) was calculated by using the formula $J_{dis}=1-J_{sim}$. Unweighted pair group method with arithmetic mean (UPGMA) tree was generated after allowing a 1,000 replicate bootstrap test using the same program. All the trees were viewed annotated and printed using Tree View (ver.1.6.5) (Page 2001). The Principal coordinate analysis (PCA) and The Mantel Z-statistic were used to test the correlation between two datasets of the molecular markers used in the present study using the NTSYS-pc software version 2.02e (Rohlf 1998).

Results and discussion

ISSR analysis

In the present study, 100 ISSR primers were screened to examine their amplification efficiency and 11 ISSR primers were chosen for further DNA amplification of *F. caperata* samples. The selected ISSR primers amplified a total of 152 DNA bands, of which 139 bands were polymorphic, with 91.44 % polymorphism throughout the *F. caperata* accessions. Primers 807 and 835 revealed the highest number of bands (17), while primer 847 produced the lowest number of bands (11). The primers 810, 835, and 836 gave 100 % polymorphic bands, whereas primer 847 had fewer polymorphic bands (81.82 %). The PIC were calculated for each primer, and primer 847 revealed maximum PIC value (0.40), while corresponding minimum PIC value (0.20) was obtained with primer 807. The mean PIC value obtained for 11 ISSR primers was 0.28 (Table 2). This level of intraspecific polymorphism is significant and suggests that populations of *F. caperata* that grow in the Western Himalayan region have significant genetic variability.

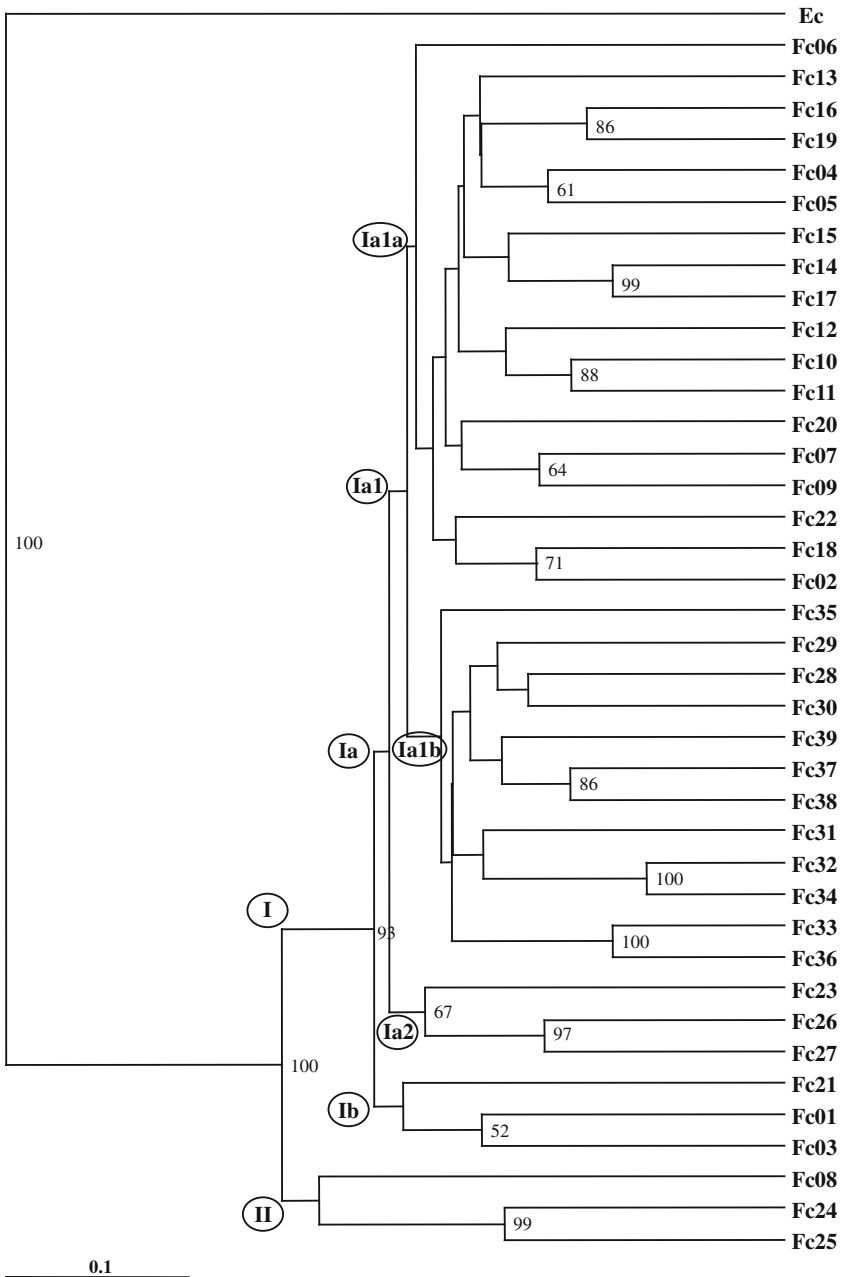

Fig. 2 UPGMA dendrogram generated for the cumulative data of ISSR and DAMD methods. *Numbers at the node* are bootstrap values. *Fc1 to Fc39* denotes the accession numbers of *Flavoparmelia caperata* and *Ec* (*Everniastrum cirrhatum*) is the out group taxon

Table 3 Mantel correlation between the genetic distances obtained from ISSR and DAMD and cumulative data among *Flavoparmelia caperata* accessions used in the present study

Marker pairs	Correlation coefficient (*r*)	*p* value
ISSR vs DAMD	0.6465	0.0769
ISSR vs cumulative[a]	0.9042	0.0769
DAMD vs cumulative[a]	0.9102	0.0769

[a] Combined ISSR and DAMD band data

DAMD analysis

Initially, 20 DAMD primers were screened with two template DNAs, out of which 10 DAMD primers produced clear and reproducible banding patterns. The 10 selected DAMD primers amplified a total 141 DNA bands, of which 117 bands were polymorphic, with 82.34 % polymorphism across the *F. caperata* accessions. The highest number of amplified bands (23) was obtained with primer OGRB-01, whereas the primer 33.6 resulted in the lowest number (9) of amplified bands. The primer FvII ex8 gave 100 % polymorphic bands, whereas primer HVR revealed 71.34 % polymorphism. Primer 6.2H+ produced the highest PIC value (0.38) while primer OGRB-01 resulted in minimum PIC value (0.12). The mean PIC value recorded for 10 DAMD primers was 0.27 (Table 2).

Cumulative analysis

A total of 21 primers (11 ISSR and 10 DAMD) resulted in to 293 bands, of which 256 bands were polymorphic, with 86.86 % polymorphism across the *F. caperata* accessions. The mean PIC value recorded for 21 cumulative primers was 0.27. The approx band size range for 21 cumulative primers was 120–2,500 bp. The cumulative (ISSR+DAMD) genetic distance showed a distance range from 0.15 to 0.66 with an average value of 0.42 among all the accessions of *F. caperata*. The maximum intraspecific average genetic distance was (0.66) between Fc08, Fc26, and Fc31 accessions, while the corresponding least genetic distance was (0.15) between Fc32 and Fc34 accessions, respectively (Data not shown).

The genetic distance data were used to generate the UPGMA dendrogram, and UPGMA dendrogram showed two major clusters, cluster I and cluster II. The out group taxon (*E. cirrhatum*) was separated out clearly from the rest of the *F. caperata* accessions. Cluster I was divided into six subclusters (Ia, Ib, Ia1, Ia2, Ia1a, Ia1b) with low genetic differences between them. Subcluster Ia was further divisible into four subclusters (Ia1, Ia2, Ia1a, and Ia1b), and Ia1a grouped all the accessions from Uttarakhand (UK), whereas subcluster Ia1b clustered together all the accessions from Himachal Pradesh (HP) in two distinct groups except accession Fc35, which did not show any affinity with other accessions from HP. The accessions Fc01, Fc02, and Fc21 from UK grouped together in subcluster Ia2, and did not show any

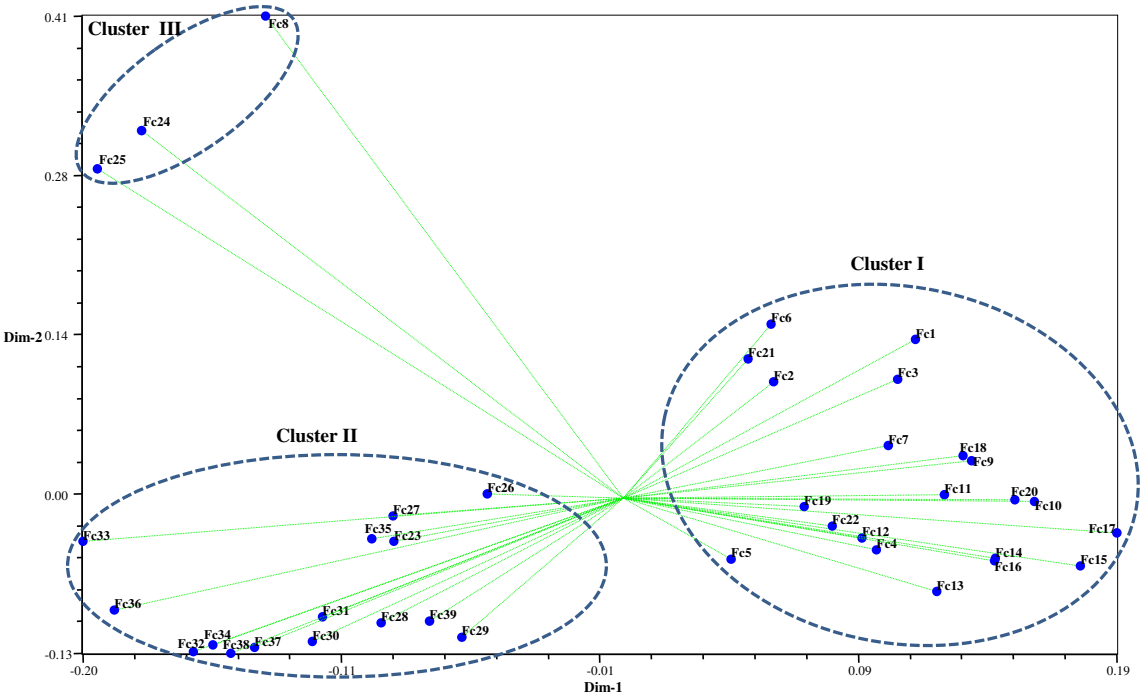

Fig. 3 Principal coordinates analysis representing relationships among *Flavoparmelia caperata* accessions

affinity with other accessions of the UK state. Cluster II consisted of three accessions (Fc08, Fc24, Fc25) from Naitwar, Osla, and Harki Doon (UK), respectively (Fig. 2). The clustering of the *F. caperata* accessions in the UPGMA dendrogram revealed that the groupings are, however, in congruence with the geographical affinities of the accessions but with weak bootstrap support. In the present investigation, accessions did not show any altitudinal specific preferences in UPGMA dendrogram and PCA plot.

Mantel's test (Mantel 1967) was performed for three data sets (ISSR, DAMD, and cumulative), which revealed a significant correlation between cumulative versus DAMD and showed the maximum (0.91) correlation coefficient (*r*), followed by cumulative versus ISSR (0.90), and ISSR versus DAMD (0.65), respectively. These values revealed that DAMD and cumulative data have good correlation and are best fit to each other (Table 3). Two-dimensional plot generated from principal coordinate analysis (PCA) of ISSR and DAMD data showed two major clusters. Cluster I grouped together all the accessions from Uttarakhand (UK), except Fc23 to Fc29, and some of these accessions (Fc23, Fc26, Fc27, Fc28, and Fc29) are intermingled with Cluster II, which is primarily a Himachal Pradesh (HP) cluster. Accession numbers Fc8, Fc24, and Fc25 from UK did not show any relationship with accessions of HP and UK (Fig. 3). Two-dimensional plot generated from principal coordinate analysis (PCA) of ISSR and DAMD data also supported the clustering patterns in UPGMA dendrogram.

ISSR and DAMD data were pooled together and analyzed to generate a cumulative UPGMA dendrogram, (Fig. 2), based on Jaccard's similarity coefficient. ISSR and DAMD methods revealed information for *F. caperata* genomes in the regions rich in microsatellite and minisatellite repetitive DNA sequences, respectively, ensuring wider coverage of the genomes and providing a better reflection of the relationships and affinities of the *F. caperata* accessions used in this study. All the accessions of the *F. caperata* separated into two major clusters and five subclusters. The clustering of the *F. caperata* accessions in the UPGMA dendrogram revealed that these accessions are intermingled with each other in different subclusters irrespective of their geographical affiliations. This pattern of distribution of genetic variation among *F. caperata* accessions might have been due to free exchange of spores that have taken place among these accessions in the wild. ISSR and DAMD markers efficiently and reliably resulted in discrete banding patterns and polymorphic profiles. These markers, despite targeting different regions of genome, revealed almost similar levels of polymorphism across all the accessions. The wide range of genetic distance and high level of polymorphism detected by ISSR and DAMD reflected a high genetic variability among the different accessions of *F. caperata*. Similar level of polymorphism was also revealed with RAPD and ISSR markers in *Lobathalia radiosa* (Yuzbasioglu et al. 2011) and *Diplosphaera chodatii* based on internal transcribed spacer (ITS) of ribosomal DNA (rDNA) and actin gene sequences (Fontaine et al. 2013). The present study establishes that the ISSR and DAMD are the suitable markers to study the genetic variation in *F. caperata.* Genetic variability forms the basis of the evolutionary potential of a species to respond to environmental changes. Loss of genetic variation reduces the ability of a species to adopt in response to environmental changes (Lande 1988; Frankham 1996, 1999). Therefore, maintenance of genetic variation within a species is very important for long term survival (Lande 1988; Shah et al. 2008). The present study significantly revealed high genetic variability within *F. caperata* in the Western Himalayan region of India, and appears to be a maiden attempt in this direction.

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