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RESEARCH ARTICLE

Genetic diversity, population structure and genetic relationships in apricot (*Prunus armeniaca* L.) germplasm of Jammu and Kashmir, India using ISSR markers

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Abstract The collection and characterization of apricot germplasm is an important step for its utilization, conservation, and breeding programs. In the present study, the genetic diversity and population structure of 106 accessions of apricot including 82 indigenous and 24 exotic accessions collected from various geographical locations of Jammu and Kashmir, India were evaluated using 14 inter simple sequence repeat markers. The PCR amplification produced 365 loci of which 356 (97.53%) were polymorphic. The average number of alleles, effective number of alleles, expected heterozygosity, Shannon's information index indicated high level of genetic diversity in the collected apricot accessions, and the polymorphism information content (0.82) revealed that these markers were highly polymorphic. The results of UPGMA dendrogram and model-based STRUCTURE analysis clearly divided the 106 apricot accessions into two main groups; one group included the 78 accessions of Kashmir province and the other included 28 accessions of Jammu province indicating the distinction of two genetic pools of apricot in the region. Nonetheless, PCoA also revealed a similar grouping of accessions except few exotic accessions that formed a small separate group. Moreover, analysis of molecular variance showed high genetic variation within the population (67%) and low among the population (33%). To our knowledge, this study represents the first comprehensive report on the ISSR based genetic diversity, relationship, and the structure of apricot accessions of Jammu and Kashmir. Further, the results of the present study will assist in efficient utilization, conservation, and MAS based breeding programs of apricot.

Keywords Apricot · Genetic diversity · Population structure · ISSR · AMOVA · Cluster analysis

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Introduction

Apricot (*Prunus armeniaca* L.) of the family Rosaceae is one of the most important plants of the genus

Prunus, commonly cultivated for its high nutritional value (Shangguan et al. 2012). It is one of the four economically important fruit crops of the genus *Prunus*, such as *P. persica* L., *P. domestica* L., and *P. avium* L. (Burgos et al. 2007). It is an interfertile diploid species with 8 chromosome pairs ($2n = 16$) and has an intermediate genome between that of peach and cherry i.e. (5.9×10^8 bp/$2n$) (Arumuganathan and Earle 1991). Apricot is a temperate zone fruit that can be grown under climatically variable conditions in many regions of the world such as Central Asia, Japan, Siberia, and China (Asma 2007). In North–Western Himalayan regions, wild apricot is found growing in the southern part of China, cold deserts of Tibet and in northern India it is found growing in temperate regions of Jammu and Kashmir, Himachal Pradesh and Uttarakhand at 2000–2500 m above average sea level (Sharma 2000). Moreover, in northern India, Jammu & Kashmir is the leading producer of apricots contributing a total of 20456 MT in the year 2018–2019 (http://hortikashmir.gov.in/).

Jammu and Kashmir particularly Kashmir valley holds a rich diversity of both indigenous as well as exotic cultivars. Indigenous accessions originated in the region, are locally grown, seedling origin trees (wild), or locally grafted (cultivated) accession, while the exotic accessions are those which have been introduced from other countries mostly of European origin (Bhat et al. 2013). The main purpose of apricot cultivation is fruit production for fresh consumption that offers high profit to the growers, while a part of the produce is destined for the processing industry. However, lack of cultivation practices, poor shelf life, lack of awareness in perceiving apricot quality, and poor market opportunities has led to erosion of genetic diversity of landraces in apricot. This depletion of apricot germplasm in the region may lead to the loss of many elite landraces which represent a natural repository of certain novel genotypes in terms of color, flavor, and taste of fruit, disease resistance, and high yield. Information on genetic diversity and population structure is therefore desired for this crop to assist the conservation, determine genetic relationships between accessions and characterization of germplasm for breeding purposes.

A variety of methods and techniques such as morphological, molecular, biochemical have been developed to expedite work on population genetic diversity, among which molecular tools play a vital role in genetic resource management and use (Chen et al. 2020). Such molecular tools increase our possibilities to study genetic diversity and to advance genome-wide association studies of complex characters and select suitable donors for breeding purposes (Aliyev et al. 2007; Hajiyev et al. 2015). Furthermore, these molecular markers have been in use for more than 30 years to estimate genetic diversity values due to their ability to produce more accurate information (Reddy et al. 2002; Madhumati 2014).

ISSR is one of the DNA marker systems involving the amplification of a DNA segment between two (SSR sequences) identical microsatellites in the opposite direction (Gebrehiwet et al. 2019). They provide a detailed genome coverage, high performance, time efficiency, and cost-efficiency (Ganopoulos et al. 2011; Yilmaz et al. 2012). Moreover, ISSR markers are highly reproducible and are one of the most widely used markers, revealing a variety of information bands in a single amplification (Gelotar et al. 2019). Several studies have been undertaken to study the genetic diversity of apricot germplasm using different molecular markers in the other parts of the world such as RAPD, AFLP, SSR, ISSR, and SRAP (Ercisli et al. 2009; Lamia et al. 2010; Yilmaz et al. 2012; Ai et al. 2011). However, in Jammu and Kashmir including Ladakh province, morphological tools have been mostly used to study the variability of apricot germplasm (Bhat et al. 2013; Malik et al. 2010; Girish et al. 2012; Abdul et al. 2016; Angmo et al. 2017; Kumar et al. 2015; Wani et al. 2017) and to the best of our knowledge, very limited work is available using molecular markers such as RAPD (Kumar et al. 2009a; Mir et al. 2012) and except for the study of Kumar et al. (2009b) from Ladakh region, no report is available on the evaluation of genetic diversity of apricot germplasm of Jammu and Kashmir using ISSR markers. Furthermore, these studies were often restricted by the limited number of accessions and specific locations. Therefore, genetic diversity data on the major apricot germplasm of the Jammu and Kashmir region are by and large lacking. Hence, the present study was conducted on 106 accessions of apricot collected from different areas of Jammu and Kashmir using 14 ISSR markers. This is the first report on genetic diversity analysis of apricot germplasm using ISSR markers that include indigenous (wild and cultivated) accessions as well as exotic accessions grown throughout the Jammu and Kashmir region.

The generated information could be used as a baseline in the future to support conservation, marker-assisted breeding, crop improvement, and comparative genomic studies.

Materials and methods

Collection site and sample collection

In the present study, 106 apricot accessions were collected from 22 geographical areas of Jammu and Kashmir, India (Table 1, Fig. 1). The young leaf samples were collected and stored at − 80 °C after snap freezing in liquid nitrogen until subjected to DNA extraction.

DNA extraction

Genomic DNA extraction from the young and healthy leaves of collected apricot accessions was carried out using the DNeasy Plant Mini Kit (Qiagen, Hilden GmbH, Germany), according to the manufacturer's instructions. In order to check the quality of extracted genomic DNA, 0.8% w/v agarose gel with ethidium bromide staining and 1X TAE as running buffer were used and the quantity was calculated by spectrophotometer at 260 nm.

PCR amplification

A total of 14 ISSR markers chosen from the literature (Yılmaz et al. 2009) obtained from IDT (Integrated DNA Technologies, USA) were used to access the genetic diversity of 106 apricot accessions. Details of 14 ISSR markers with their sequence and annealing temperature (°C) are presented in Table 2. For each amplification, the reaction mixture was prepared in a final volume of 20 µl which consists of 2 µl 10× reaction buffer, 1.5 µl of 25 mM $MgCl_2$, 0.3 µl 25 mM of dNTP mix (dATP, dGTP, dCTP, and dTTP, 3 µl of primer (10 µM), 0.3 µl *Taq polymerase* (5U/µl) (Thermo Scientific, USA), 11.9 µl of sterilized distilled water and 1.0 µl of template DNA (50 ng). The amplification was carried out in Applied Biosystems thermocycler, USA using the following PCR conditions: preheating and initial denaturation at 94 °C for 5 min followed by 35 cycles of denaturation at 94 °C for 1 min; primer annealing at specific annealing temperature for each primer (Tm) 45 s; extension at 72 °C for 2 min and a final extension at 72 °C 10 min and 4 °C hold. After the completion of the PCR, 2.5 µl of 6× loading dye (Thermo Scientific, USA) was added to the amplified products and separated in 1.8% (w/v) agarose gel with ethidium bromide staining using 1× TAE (Tris-acetate EDTA) as running buffer. Finally, the size of the fragments was measured against the 100 bp DNA ladder (Thermo Fisher Scientific, USA).

Data scoring and analysis

The well separated and intense bands were scored in binary code with 1 and 0, indicating the presence and absence of bands respectively. The informativeness of the ISSR marker system in defining, differentiating, and evaluating the apricot diversity was evaluated by the following parameters for each assay unit; polymorphic information content (PIC), resolving power (RP), and marker index (MI). PIC was calculated according to Smith et al. (1997); $PIC = 1 - \Sigma Pi2$, RP was calculated according to Prevost and Wilkinson (1999); $RP = \Sigma Ib$, similarly marker index (MI) according to Powell et al. (1996); $MI = PIC \times \beta \times \alpha$. Furthermore, band frequency, observed number of alleles (Na), effective number of alleles (Ne), Shannon's information index (*I*), expected heterozygosity (He), and unbiased heterozygosity (uHe) were estimated by using GenAlEx version 6.5 (Peakall and Smouse 2012). Based on Jaccard's genetic similarity coefficient (Rohlf 2000), a dendrogram of 106 accessions of apricot was constructed using the NTSYS-pc Version 2.1 program with the UPGMA (unweighted pair-group method with arithmetic means) approach. Besides, PAST software was used to calculate the principal coordinate analysis (PCoA) (Hammer et al. 2001). A model-based Bayesian clustering analysis program STRUCTURE (Pritchard et al. 2000) determined the genetic structure and the number of clusters in the data set of apricot accessions. The number of possible populations (*K*) was estimated between 1 and 10 and the analysis was repeated twice. For each run, the burn-in and Markov Chain Monte Carlo (MCMC) were set to 50,000 each, and iterations were set to 5. The run with the highest likelihood was used to

Table 1 List of 106 apricot accessions used in the present study collected from the different geographical locations of Jammu and Kashmir, India

S. no	Collection site	GPS coordinates	Accessions
Accessions collected from Kashmir Province			
1	Srinagar: Rangreth, CITH	33° 59′ 4.37″ 74° 48′ 0.38″	SNC01, SNC02, SNC03, SNC04, SNC05, SNC06, SNC07, SNC08, SNC09, SNC10, SNC11, SNC12, SNC13, SNC14, SNC15, SNC16, SNC17,SNC18, SNC19
2	Srinagar: Hazratbal, KUBG	34° 7′ 51.2 74° 50′ 1.41″	SNW20, SNC21, SNC22
3	Srinagar: Zakura, Govt. Hort. Nursery	34 9 21.68″ 74° 50′ 25.1″	SNC23, SNC24
4	Srinagar: Zakura.	34° 9′ 21.68″ 74° 50′ 25.1″	SNC25
5	Ganderbal: Rangil.	34° 12′ 55.53″ 74° 48′ 32.85″	GBC01, GBC02
6	Ganderbal: Raipora, Govt. Hort. Nursery	34° 15′ 05.85″ 74° 44′ 33.91″	GBC03, GBC04, GBC05
7	Ganderbal: Watlar.	34° 15′ 47.94″ 74° 46′ 45.96″	GBC06, GBC07
8	Ganderbal: Badampora.	34° 13′ 30.67″ 74° 41′ 29.46″	GBC08, GBC09
9	Ganderbal: Manasbal.	34° 15′ 23.32″ 74° 41′ 12.65″	GBW10, GBW11, GBW12, GBW13, GBW14, GBW15, GBW16, GBW17, GBW18, GBW19.
10	Bandipora: Guroora.	34° 22′ 9.89″ 74° 40′ 14.17″	BPC01
11	Bandipora: Nesbal.	34° 14′ 40.18″ 74° 40′ 15.18″	BPW02, BPW03, BPW04, BPW05, BPW06
12	Bandipora: Chewa.	34° 16′ 22.86″ 74° 41′ 9.97″	BPW07, BPW08, BPW09, BPW10, BPW11, BPW12, BPW13, BPW14, BPW15, BPW16, BPW17, BPW18, BPW19, BPW20, BPW21
13	Baramulla: Pattan, Govt. Hort. Nursery	34° 9′ 40.68″ 74° 33′ 22.67″	BMC01, BMC02
14	Kupwara: Wahipora.	34° 4′ 12.78″ 74° 27′ 20.97″	KWC01
15	Budgam: Charar e Sharief.	33° 50′ 51.1″ 74° 45′ 20.59″	BGC01, BGC02, BGC03, BGC04, BGC05, BGW06
16	Shopian: Zainpora, Govt. Hort. Nursery	33° 47′01.33″ 74° 49′ 54.41″	SPC01, SPC02, SPC03, SPC04
Accessions collected from Jammu Province			
17	Rajouri: Rajdhani.	33° 30′ 39.89″ 74° 20′ 55.04″	RJW01, RJW02, RJW03, RJC04, RJC05
18	Rajouri: Thanamandi.	33° 32′ 11.29″ 74° 22′ 12.009″	RJC06, RJW07, RJC08, RJC09, RJW10
19	Rajouri: Shahdara Sharief.	33° 33′ 2.6″ 74° 20′ 40.23″	RJW11, RJW12, RJW13, RJW14, RJC15
20	Rajouri: Main Town.	33° 22′ 37.69″ 74° 18′ 48.85″	RJW16, RJW17, RJW18, RJW19, RJC20

Table 1 continued

S. no	Collection site	GPS coordinates	Accessions
21	Rajouri: Darhal.	33° 29′ 4.45″ 74° 24′ 46.14″	RJW21, RJW22, RJW23, RJC2, RJC25
22	Poonch: Bafliaz.	33° 34′ 33.09″ 74° 23′ 27.72″	PNC01, PNC02, PNW03

designate individual accessions into groups and true K was estimated according to the method defined by Evanno et al. (2005). Within a group, accessions with inferred ancestry based on probability values $P \geq 70\%$ were assigned to a different group, and those with $< 70\%$ were treated as ''admixture'', i.e., these accessions tend to have mixed ancestry from the parents belonging to various geographical origins or gene pools. The results were further processed and the accessions were assigned to different clusters using CLUMPP software v1.1.2 (Jakobsson and Rosenberg 2007). The output of CLUMPP was directly used as input for STRUCTURE software (Pritchard et al. 2000) to obtain results in graphical format. Further, the analysis of molecular variation (AMOVA) among and within populations was performed by GenAlEx version 6.5 (Peakall and Smouse 2012).

Results

ISSR polymorphism

In the present study, the ISSR markers showed a high polymorphism and reproducible fragments on the 106 accessions of apricot. A representative image illustrating the banding pattern of 106 apricot accessions using ISSR markers BC840 is shown in Fig. 2. A total of 365 alleles were amplified ranging from 3 for BC825 to 36 for BC827 with an average of 26.07 alleles per locus among which 356 alleles were polymorphic (97.53%) and 9 alleles were monomorphic. Furthermore, among 14 markers, most of the markers showed 100% polymorphism except BC825, BC835, BC873, BC880, and BC888. The allelic size of these 14 ISSR markers ranged from 150 bp in BC835, BC841, BC843, and BC888 to 3500 bp in BC807. The PIC value recorded revealed the lowest PIC value 0.67 in marker BC825 and the highest PIC value 0.92 in marker BC835 and BC843. Other parameters like RP, MI, and EMR were also studied for all 14 ISSR markers. RP ranged from 2.01 in BC825 to 23.24 in BC827, MI was recorded 1.35 in BC825 to 31.09 in BC827 and EMR ranged from 2 in BC825 to 36 in BC827 (Table 2).

Genetic diversity analysis

Band frequency, observed number of alleles (Na), effective number of alleles (Ne), Shannon's information index (I), expected heterozygosity (He), and unbiased heterozygosity (uHe) were used to evaluate the genetic diversity of collected apricot accessions (Table 3). The highest band frequency was observed for BC880 (0.958) followed by BC888 (0.844) and BC868 (0.806). The average value of observed number of alleles was 2.960, ranging from 2.500 (BC873) to 3.448 (BC812) while the effective number of alleles ranged from 2.252 (BC825) to 2.830 (BC841) with an average value of 2.646. In addition highest, Shannon's information index (*I*) was recorded for marker BC827 (0.793) followed by BC812 (0.762) and BC888 (0.749). The highest expected heterozygosity (He = 0.515) and unbiased heterozygosity (uHe = 0.521) were recorded for BC827 followed by BC888 respectively. BC825 showed the least expected heterozygosity (He = 0.189) as well as unbiased heterozygosity (uHe = 0.192) while 0.403 and 0.408 were the average values of expected heterozygosity and unbiased heterozygosity recorded for all the 14 ISSR markers (Table 3).

Genetic relationship and population structure

ISSR data was used to calculate pairwise distances between all possible pairs of 106 accessions to identify

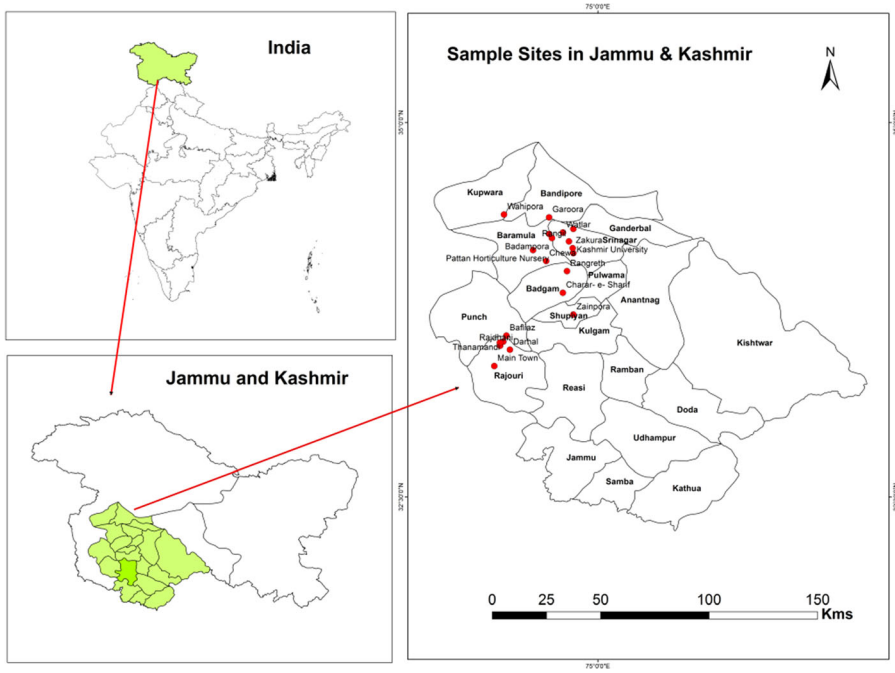

Fig. 1 Map of Jammu and Kashmir showing different collection sites of apricot accessions used in the present study

the genetic relationship among the apricot accessions. The similarity coefficients among the collected accessions ranged from 0.16 (RJC20 and BPW06) to 0.90 (SNC01and SNC03). The UPGMA dendrogram obtained divided apricot accessions into two main clusters, namely cluster I and cluster II. Most of the accessions grouped in individual clusters belonged to a particular geographical location (Fig. 3). Cluster I includes all accessions collected from different locations of Kashmir province while cluster II comprises accessions collected from different locations of Jammu province. Cluster I which includes most of the accessions (78) was divided into two main subgroups IA and IB at a genetic similarity coefficient level of 0.37. IA formed a smaller cluster consisting of 16 accessions from Srinagar, Baramulla, and Shopian districts of Kashmir province. These all 16 accessions are commonly grown exotic apricot accessions in Kashmir province which are mostly of European origin. However, IB formed the largest group of 62 accessions consisting of both wild and cultivated accessions of apricot, and was further subdivided into two sub-clusters (IB1 and IB2) at a genetic similarity coefficient level of 0.52. Subgroup IB1 was further divided into two clusters (IB1a and IB1b); IB1a includes 16 cultivated accessions of Srinagar, Shopian, and Budgam districts of Kashmir province while IB1b again formed two sub-clusters with one sub-cluster containing cultivated accessions of Srinagar, Ganderbal, Bandipora, and Kupwara, two wild accessions (BGW06 and SNW20) of Budgam and Srinagar and another sub-cluster containing 15 wild accessions of Bandipora and one wild accession from Ganderbal district. Similarly, the main sub-cluster IB2 was further divided into two subgroups in which one group comprises 09 wild accessions from the Ganderbal district and 04 accessions from Bandipora district while its 2nd subgroup contains only one wild accession BPW06 from Bandipora district.

All the 28 accessions collected from different locations of Jammu province formed the main cluster II, which further separated into two subgroups IIA and IIB. Subgroup IIA contained 10 wild and 06 cultivated accessions of Rajouri district of Jammu province while IIB formed two sub-clusters in which one sub-cluster comprised of 11 accessions from Rajouri district and 2nd sub-cluster contains only one accession of Poonch district of Jammu province (Fig. 3).

Principal coordinate analysis (PCoA) was performed by PAST software and graphically showed two distinct clusters except for a few exotic accessions

Table 2 Details of 14 ISSR markers, number of bands and polymorphism content for 106 apricot accessions

S. no	Marker	Sequence of marker (5′–3′)	Annealing temp (°C)	Range of amplicon in bp	NL	ML	PL	% of PL	RP	PIC	MI	EMR
1	BC807	$(AG)_8T$	48	300–3500	24	0	24	100	11.90	0.89	21.56	24
2	BC812	$(GA)_8$ A	50	290–1500	29	0	29	100	16.77	0.88	25.70	29
3	BC818	$(CA)_8G$	50	290–3000	32	0	32	100	16.98	0.88	28.16	32
4	BC825	$(AC)_8T$	50	500–700	3	1	2	66.66	2.01	0.67	1.35	2
5	BC827	$(AC)_8G$	50	200–3000	36	0	36	100	23.24	0.86	31.09	36
6	BC835	$(AG)_8YC$	50	150–1800	34	4	30	88.23	13.50	0.92	24.45	26.47
7	BC840	$(GA)_8YT$	48	220–1500	32	0	32	100	18.00	0.85	27.22	32
8	BC841	$(GA)_8YC$	50	150–1400	28	0	28	100	22.32	0.75	21.05	28
9	BC843	$(CT)_8GA$	48	150–2000	30	0	30	100	11.77	0.92	27.69	30
10	BC847	$(CA)_8RC$	50	180–1600	22	0	22	100	18.56	0.74	16.37	22
11	BC868	$(GAA)_6$	45	180–1400	25	0	25	100	17.03	0.81	20.39	25
12	BC873	$(GACA)_4$	45	450–1800	16	1	15	93.75	7.90	0.86	12.14	14.06
13	BC880	$(GGAGA)_3$	45	270–2000	25	2	23	92.00	20.73	0.72	15.23	21.16
14	BC888	$BDB(CA)_7$	50	150–1600	29	1	28	96.55	22.60	0.77	20.92	27.03
15	Min				3	1	2	66.66	2.01	0.67	1.35	2
16	Max				36	4	36	100	23.24	0.92	31.09	36
17	Average				26.07	0.64	25.42	97.53	15.95	0.82	20.95	24.90

*R = (A, G), Y = (C, T), B = (C, G, T), D = (A, G, T)

(*NL* number of loci, *ML* monomorphic loci, *PL* polymorphic loci, *% of PL* percentage of polymorphic loci, *RP* resolving power, *PIC* polymorphism information content, *MI* Marker index, *EMR* effective multiplex ratio)

that formed a small separate cluster (Fig. 4). The PCoA revealed the first two most informative principal components with a percentage of variability of 18.21% and 15.33% respectively, which together accounted for 33.54% of the total genetic variation. Figure 4 showed similar patterns of clustering with that of the UPGMA dendrogram except for the subcluster IB which formed a separate cluster. As seen in Fig. 4, three groups were obtained and all the three groups clustered according to their geographical location. Group I include accessions collected from Kashmir province which all are exotic accessions while group II consists of 65 wild and cultivated accessions of Kashmir province among which most of the accessions are indigenous except few exotic accessions. Similarly, group III includes 28 accessions collected from different locations of Jammu province.

To find out the number of populations from 106 accessions using 14 ISSR markers, STRUCTURE analysis was carried out. Our results showed a clear peak for ΔK at K = 2 with the next largest peaks at K = 4 and K = 6 (Fig. 5A). For the first level of clustering at K = 2, the apricot accessions were differentiated into two clusters, the first one consisted of accessions from Kashmir province while the second cluster included all accessions of Jammu province (Fig. 5B; Table S1). We used the second level of clustering at K = 4 to define the clusters considered in subsequent analyses. At K = 4, the 78 accessions of Kashmir province got separated into 3 clusters while the accessions from the Jammu region remained as a single cluster. Cluster 1 (16 accessions; in blue) included exotic accessions of those of European origin. Cluster 2 (30 accessions; in yellow) grouped few exotic and indigenous cultivated accessions from Srinagar, Baramulla, Shopian, Budgam and wild accessions from Ganderbal and Bandipora districts. Cluster 3 (32 accessions; in green) grouped cultivated accessions from Ganderbal, Bandipora, Srinagar, Kupwara, and wild accessions from Bandipora districts, and Cluster 4 (28 accessions; in red) grouped all

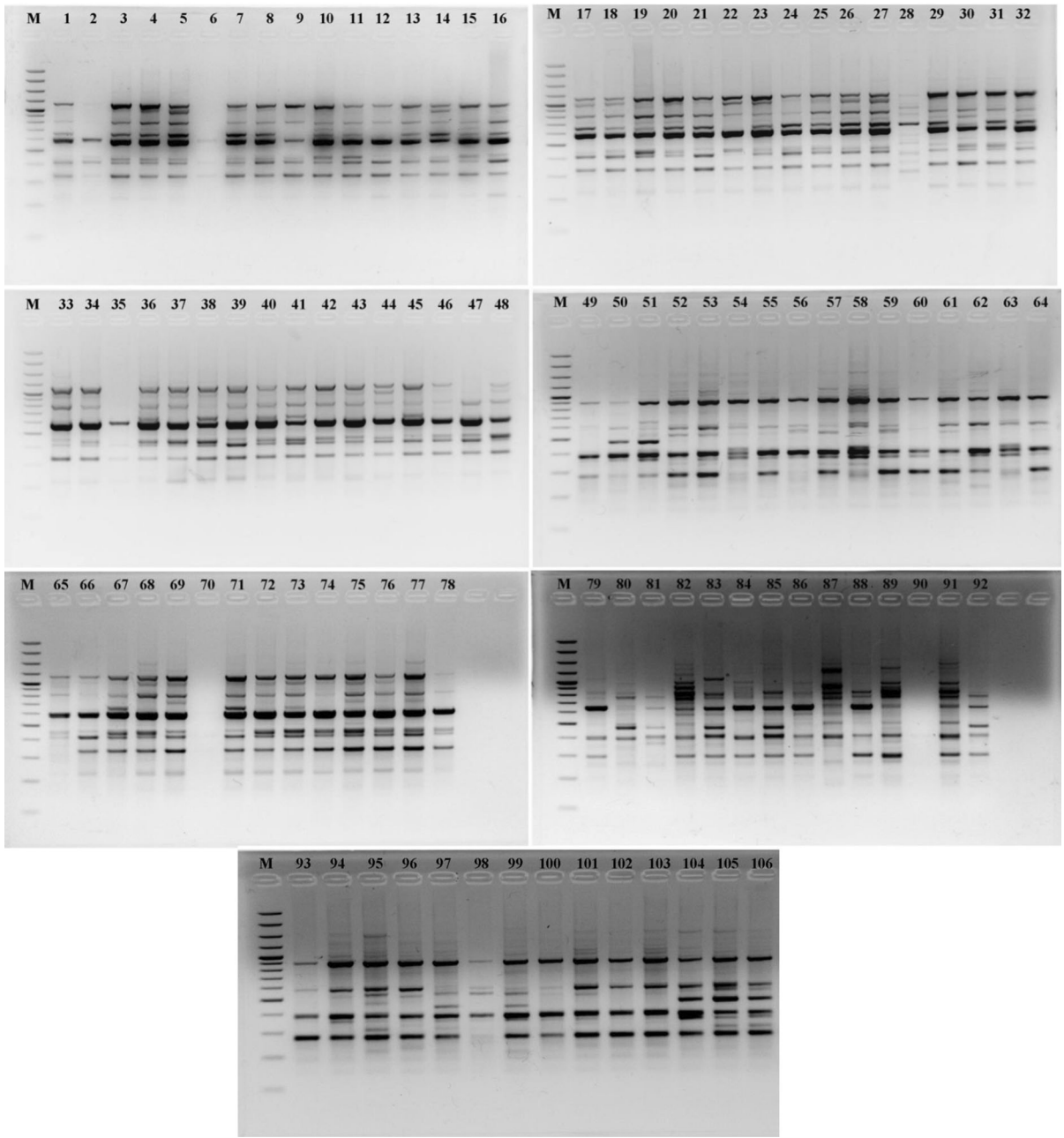

Fig. 2 ISSR profiles generated for 106 apricot accessions using ISSR marker BC840. Lane M shows 100 bp standard DNA ladder and lanes 1-106 represent apricot accessions

the wild and cultivated accessions of Jammu province (Table S2). At the same time, the third-largest ΔK at K = 6 was much larger than the previous ones. When K = 6, there was a further division of 78 apricot accession collected from Kashmir province while no further clustering was recorded in the accessions collected from Jammu province. Therefore, at K = 6, cluster 1 (16 accessions; in pink) grouped exotic accessions collected from different districts of Kashmir province, Cluster 2 (16 accessions; in yellow) grouped few exotic and cultivated accessions of Kashmir province, Cluster 3 (16 accessions; in

Table 3 Genetic diversity parameters as revealed through 14 ISSR markers among 106 apricot accessions

Marker	Band freq.	Na	Ne	I	He	uHe
BC807	0.496	2.833	2.573	0.552	0.350	0.355
BC812	0.586	3.448	2.743	0.762	0.482	0.487
BC818	0.437	2.625	2.565	0.534	0.342	0.346
BC825	0.671	2.667	2.252	0.332	0.189	0.192
BC827	0.616	3.389	2.828	0.793	0.515	0.521
BC835	0.438	2.941	2.569	0.566	0.357	0.362
BC840	0.560	3.063	2.649	0.641	0.409	0.414
BC841	0.753	3.000	2.830	0.737	0.489	0.495
BC843	0.370	2.800	2.447	0.479	0.292	0.296
BC847	0.720	3.045	2.763	0.716	0.466	0.471
BC868	0.806	2.880	2.744	0.689	0.454	0.460
BC873	0.665	2.500	2.567	0.543	0.353	0.358
BC880	0.958	3.280	2.701	0.703	0.447	0.453
BC888	0.844	2.966	2.813	0.749	0.493	0.500
Minimum	0.370	2.500	2.252	0.332	0.189	0.192
Maximum	0.958	3.448	2.830	0.793	0.515	0.521
Average	0.637	2.960	2.646	0.628	0.403	0.408

Na: Observed number of alleles, Ne: Effective Number of alleles, I: Shannon's information index, He: Expected heterozygosity, uHe: Unbiased heterozygosity

turquoise) grouped 14 cultivated accessions from Ganderbal, Bandipora, Srinagar, Kupwara districts and 2 wild accessions from Budgam and Srinagar districts of Kashmir province. Cluster 4 (16 accessions; in green) grouped the wild accessions collected from the Bandipora district of Kashmir province, while cluster 5 (14 accessions; in blue) grouped 9 wild accessions from Ganderbal and 5 wild accessions from Bandipora districts of Kashmir province and the last cluster 6 (28 accessions; in red) grouped all the wild and cultivated accessions collected from Jammu province (Fig. 5B; Table S3).

Model-based Bayesian clustering of 106 accessions revealed a similar pattern as inferred with the UPGMA dendrogram and PCoA, with clear genetic discrimination of two major clusters where major Cluster 1 formed several sub-clusters separating the exotic, cultivated (grafted accessions) and wild (seedling origin) accessions and formed the most diversified cluster. Overall, the observed cluster distribution reflected the clear separation of two main genetic clusters in Jammu and Kashmir, India.

Based on the grouping of accessions in two populations defined by the model-based Bayesian clustering algorithm using the STRUCTURE program, the expected heterozygosity for the two populations ranged from 0.202 for population II to 0.256 for population I with an average value of 0.229, and the *Fst* values ranged from 0.267 for population I to 0.558 for the population II with an average value of 0.412 (Table S4). Besides, the genetic differentiation based on *Fst* value for both populations identified by STRUCTURE analysis was recorded as 0.1382 (Table S5).

Analysis of molecular variance (AMOVA)

The analysis of molecular variance (AMOVA) was done to study the variation among and within the populations after the grouping of accessions in two populations by STRUCTURE analysis (Table 4). The results demonstrated that 67% of the total variation occurred within populations whereas 33% of the total variance was distributed among the populations. These results suggested that there is a substantial gene flow within the populations and the presence of different combinations of alleles as well the allelic exchange rates within the populations are very high than among the populations.

Discussion

Detailed genetic characterization of plants is a crucial step in the implementation of breeding programs, successful conservation, and usage of plant genetic resources (Tripathi et al. 2012; Izzatullayeva et al. 2014). The most effective and efficient technique for this characterization is the molecular marker analysis. Various properties, particularly non-responsiveness towards environmental, pleiotropic, and epistatic effects, make these molecular markers extremely profitable and useful in comparison with traditional phenotypic or morphological markers (Mondini et al. 2009). The ISSR markers are one of the most frequently used molecular markers for the assessment of genetic diversity and the relationship in the number of crop plants (Reddy et al. 2002). In the present

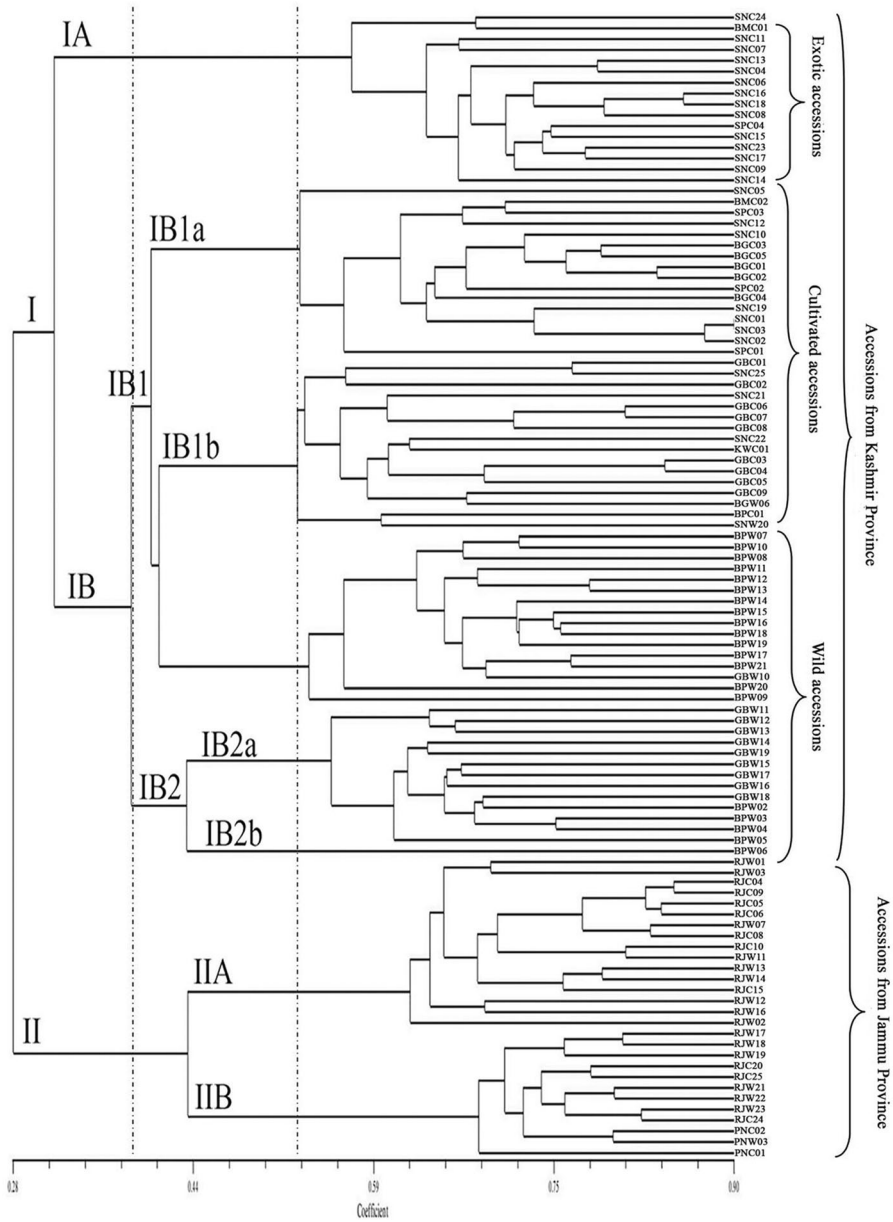

Fig. 3 Dendrogram generated by UPGMA cluster analysis illustrating genetic relationship among 106 apricot accessions using 14 ISSR markers

investigation, as many as 14 ISSR markers were used to study the genetic diversity, population structure, and the relationship between 106 accessions of apricot collected from the different locations of Jammu and Kashmir, India.

ISSR polymorphism

All the 14 ISSR markers used in the present study were selected based on their high polymorphism, ability to amplify a single locus and their high discriminating power that allows differentiation between the accessions (Yilmaz et al. 2009, 2012; Li et al. 2013, 2014a, b; Liu et al. 2015, 2016). These ISSR

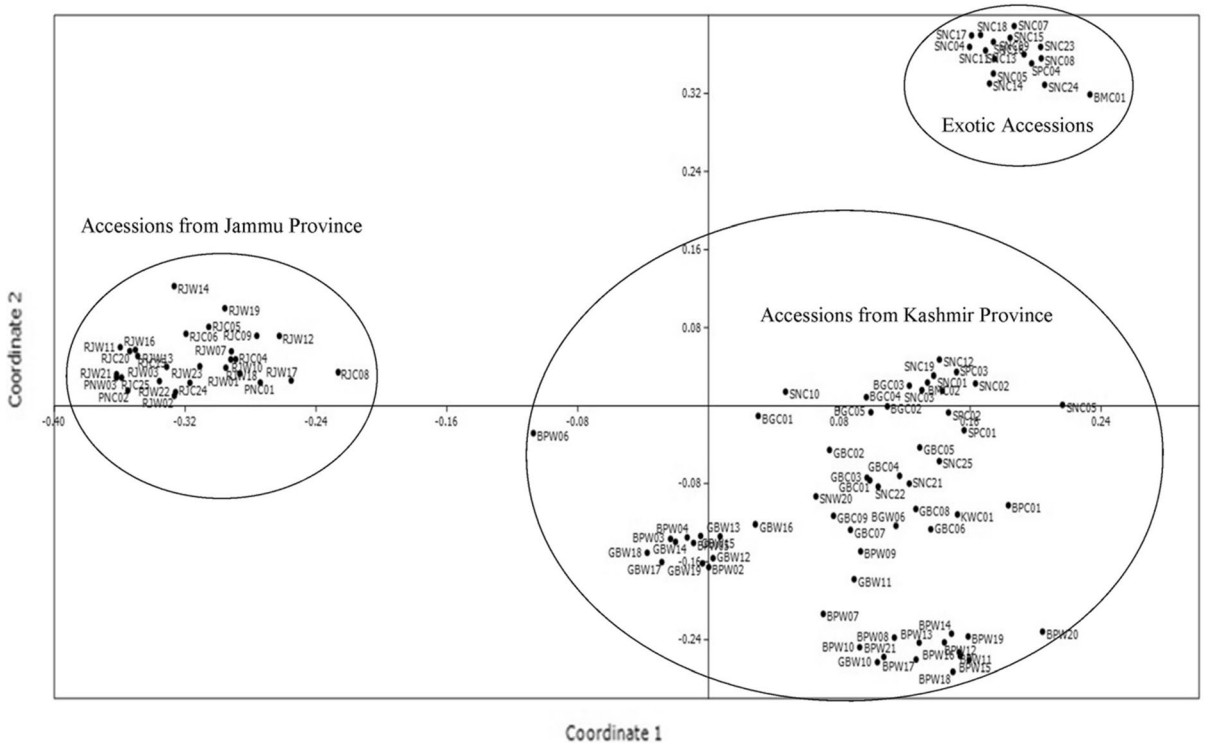

Fig. 4 Principal coordinate analysis (PCoA) of 106 apricot accessions according to the first two Eigen vectors constructed by PAST software

markers showed an overall high level of polymorphism (97.53%) which suggests that there is a considerable level of genetic variation present in the accessions of apricot grown in both Jammu and Kashmir provinces. This percentage of polymorphism is higher than reported by Yilmaz et al. (2009) (88%); Li et al. (2014b) (90.37%) in Turkish apricot germplasm and Siberian apricots in China respectively. The ISSR markers used in the present study generated 356 highly informative polymorphic loci with an average PIC value of 0.82 which is considerably higher than found by (Yilmaz et al. 2009, 2012) (PIC = 0.564) in the Turkish apricot germplasm and in the *Prunus* spp. using 20 ISSR markers. The higher PIC value indicates that the set of ISSR markers used in the present study was highly efficient and suitable for the diversity analysis of apricot accessions from the Jammu and Kashmir region. Similarly, in comparison to other studies, the average MI = 20.95 observed in our study was higher than those reported on apricot by Kumar et al. (2009b) (MI = 3.74) and sweet cherry by Ivanovych et al. (2017) (MI = 2.46). In a similar way, the average values of the other parameters such as R.P (15.95) and EMR (24.90) were also observed to be high as compared to the studies of Yilmaz et al. (2009) (RP = 0.829), Dvin et al. (2020) (R.P = 7.882) and Kumar et al. (2009b) (EMR = 4.81). In the diversity analysis of apricot by ISSR markers, the majority of previous studies did not involve PIC, MI, RP, and EMR calculations, however, very low values were recorded in a few studies. The reason for the high level of polymorphism obtained in the present study is because of a rich genetic diversity of apricot germplasm in both Jammu and Kashmir provinces, the large number of accessions selected (sample size), and the high efficiency of ISSR markers used in the present study. Besides, high allelic diversity in the studied germplasm may be due to the presence of wild apricot accessions that grow locally and provide opportunities for cross-pollination with the cultivated accessions. The presence of allelic richness in any germplasm is a valuable tool for obtaining desirable accessions for commercial cultivation as well as in the breeding programs.

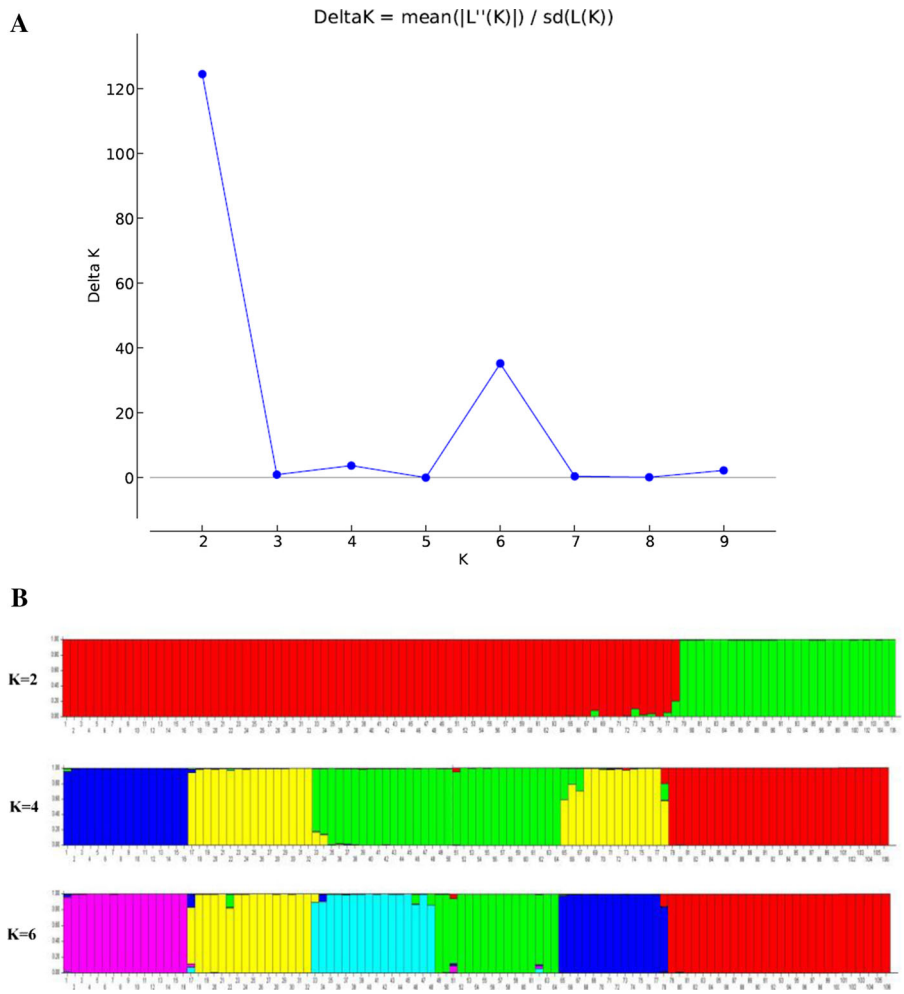

Fig. 5 **A** Graphical representation of mean likelihood L (K) and variance per K values generated by model based-STRUCTURE on dataset containing 106 apricot accessions across 14 ISSR markers. **B** STRUCTURE plot of membership coefficients for all the 106 apricot accessions in the study sample sorted in the same order and classified according to successive preset K values ranging from 2 to 10. Each individual is represented by a thin vertical line, which is partitioned into K colored segments that represent the individual's estimated membership fractions in K clusters

Table 4 Analysis of molecular variance (AMOVA) among the 106 apricot accessions classified as two populations.

Source of variation	*df*	SS	MS	Est. var.	% Var.	*P* value
Among Pops	1	961.105	961.105	22.220	33%	< 0.001
Within Pops	104	4729.055	45.472	45.472	67%	< 0.001
Total	105	5690.160		67.692	100%	

df degrees of freedom, *SS* sum of squares, *MS* mean of squares, *Est. Var.* estimated variance, *% Var. p*ercentage of variation

Genetic diversity analysis

There are different types of measures of genetic diversity such as the observed number of alleles, the effective number of alleles, Shannon's information index, expected heterozygosity, and unbiased heterozygosity. All these parameters were used to

study the genetic diversity of collected apricot accessions of Jammu and Kashmir. The average value of the observed number of alleles (Na) per locus was 2.960 while the average value of the effective number of alleles (Ne) per locus was 2.646, which appears to be higher than the previous studies on apricot germplasm. Effectively, Liu et al. (2015) recorded a mean value of 1.73 (Na) and 1.40 (Ne) using 9 ISSR markers for sweet kernel apricots of China and Li et al. (2013) obtained a mean value of 1.62 (Na) and 1.36 (Ne) for 14 wild apricot populations of Ili Valley area of northwestern China using 15 ISSRs. Similarly, the average values of Shannon's information index ($I = 0.628$) were higher than the average value reported by Li et al. (2014a), who recorded the average value of Shannon's information index (I) as 0.414 for 76 accessions using 15 ISSR markers and Li et al. (2013) who obtained the average value as 0.317 for 14 wild apricots. The average value of expected heterozygosity in the present study was 0.403 indicating the substantial genetic diversity in the apricot accessions of Jammu and Kashmir. By comparison, the expected heterozygosity obtained from *Prunus avium* was lower (He = 0.238) by Ivanovych et al. (2017). Apricot is a long-lived woody tree with a broad geographical range in Jammu and Kashmir. The accessions have most likely acquired major genetic variations in fruit weight and dimensions, fruit color, fruit shape, fruit firmness, etc., due to the adaptation to the environment, selection through domesticating processes, and different pollination mechanisms. All such factors in combination or alone have resulted in a high degree of genetic diversity in the presently studied apricot accessions. Moreover, the high genetic diversity observed in the present study is because the area surveyed falls in the Central Asian region (from the Tianshan mountains to Kashmir) which is one of the primary domestication centers of the commercial apricot and is considered as one of the oldest and richest reservoirs of apricot germplasm (Vavilov 1951; Faust et al. 1998; Hormaza et al. 2007).

Genetic relationship and population structure

The UPGMA method was used for the cluster analysis based on Jaccard's genetic similarity coefficient (Rohlf 2000). Two main clusters distinguished 78 apricot accessions (73.59%) collected from Kashmir province, from the remaining 28 apricot accessions (26.41%) collected from Jammu province, which could be related to the existence of two genetic stocks (gene pools) in Jammu and Kashmir. The major cluster I contained two subgroups structured according to the geographical origin with cultivated exotic accession in subgroup IA and indigenous accessions (wild and cultivated) in subgroup IB. The obtained genetic relationship was in agreement with the results of Hu et al. (2018) based on their diversity analysis of apricot germplasm collected from the Tien-Shan Mountains of China using SSR markers and Bourguiba et al. (2010a) using mapped SSR markers on the genetic diversity of apricot in Tunisia. The further division of subgroup IB into sub-clusters IB1 and IB2 according to the mode of propagation was also revealed in the dendrogram. Evidence of this structure according to the mode of propagation (grafting vs. seedling origin trees) was also obtained by (Bourguiba et al. 2010b, 2012a) when studying Tunisian apricot germplasm and genetic diversity of grafted and seed propagated apricot in the Maghreb region respectively. However, in the sub-cluster IB1, clustering of a few exotic accession and wild accessions with the cultivated accessions can be explained by the fact that these accessions present large adaptability as well as a substantial gene exchange among materials originating from the different regions as described by Crossa-Raynaud (1960), Carraut and Crossa- Raynaud (1974). Similar results were also shown using PCoA analysis which agreed with the separation of apricot accessions of Kashmir province from Jammu province. Although the grouping corresponded largely to the geographical provinces, there was a notable exception with the 16 accessions of the main cluster I which have formed a subgroup IA in UPGMA tended to form a separate cluster which all are exotic accessions. Besides the PCoA analysis also suggested that apricot accessions of Kashmir province were more diverse as they had a wider distribution in the PCoA plot as compared to the apricot accessions collected from Jammu province. The genetic structure of 106 apricot accessions was studied by model-based Bayesian clustering by STRUCTURE analysis and the results indicated the existence of clear two group-structure. The two major cluster structures revealed the presence of two different apricot genetic stocks present in the apricot germplasm of Jammu and Kashmir. A similar result of the two-group structure was reported in the previous study conducted on Tunisian apricot accessions

(Krichen et al. 2008, 2014). Li et al. (2014b), Gürcan et al. (2015) and Bourguiba et al. (2012b) also confirmed this structure and clearly allocated apricot accessions into two groups with the Bayesian method using STRUCTURE software.

From the analysis of UPGMA dendrogram, PCoA, and Bayesian clustering, two genetic clusters were clearly indicated among the apricot accessions of Jammu and Kashmir. Moreover, the estimate of genetic variation reflected in the AMOVA was higher within the populations than among the populations. These estimates indicate that significant genetic diversity exists in apricot accessions of both Jammu and Kashmir provinces that formed two genetic clusters in which isolation by distance may have played a determining role. Besides, there could be a lot of factors responsible for the separation of these two genetic clusters, such as landscape variability, environmental and human-mediated transport of reproductive material which becomes barriers to the migration and gene flow among the two genetic clusters. However, the geographical topology, acting as an extrinsic barrier between the two provinces may play an important role. On the other hand, existing genetic barriers have possibly arisen as a result of changes in the local climate and vegetation that might cause genetic discontinuity among the two populations (Pacheco-Olvera et al. 2012). Several previous studies also reported the existence and influence of geographical barriers on the genetic structure patterns (Lusini et al. 2014; Meng et al. 2016; Bhandawat et al. 2019; Torokeldiev et al. 2019).

Conclusion

This study represents the first major effort to characterize the apricot germplasm collected from various geographical locations of Jammu and Kashmir, India. The results of the high variability parameters obtained by the ISSR markers indicated that the apricot germplasm of Jammu and Kashmir maintained a high level of genetic diversity. Our study showed that the collected 106 apricot accessions were divided into two main genetic clusters by UPGMA, PCoA, and STRUCTURE analysis according to the geographical location/distribution. The distinction of two genetic clusters may be attributed to human selection as well as the ecological constraints. This study provided a precise picture in understanding the genetic diversity and the relationship between these 106 apricot accessions of Jammu and Kashmir which will be important for identification, utilization, and conservation of germplasm as well as in molecular marker-assisted breeding programs.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Human and animals rights This article does not contain any studies with human participants or animals performed by any of the authors.

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