Research Article

Correspondence of ISSR and RAPD markers for comparative analysis of genetic diversity among different apricot genotypes from cold arid deserts of trans-Himalayas

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ABSTRACT

The phylogenetic relationships of 36 locally grown *Prunus armeniaca* genotypes which are collected from nine sampling sites from two valleys *viz*. Nubra (9,600 ft) and Leh (11,500 ft) of trans-Himalayan region were analyzed using 31 PCR markers (20 RAPDs and 11 ISSRs). This is the first report of molecular genetic diversity studies in apricot from this region of the world. RAPD analysis yielded 139 fragments, of which 136 were polymorphic, with an average of 6.8 polymorphic fragments per primer. ISSR analysis produced 58 bands, of which 56 were polymorphic, with an average of 5.09 polymorphic fragments per primer. The primers based on (CT)n produced maximum number of bands (nine) while, (AT)n and many other motifs gave no amplification. RAPD markers were found more efficient with regards to polymorphism detection, as they detected 97.84 % as compared to 96.5 % for ISSR markers. Clustering of genotypes within groups was not similar when RAPD and ISSR derived dendrogram were compared, whereas the pattern of clustering of the genotypes remained more or less the same in RAPD and combined data of RAPD + ISSR. The results of PCA analysis were comparable to the cluster analysis. These analyses, allowed us to identify the groups corresponding to the two apricot collection sites. **[Physiol. Mol. Biol. Plants 2009; 15(3) : 225-236]** *E-mail : gyan.gene@gmail.com*

Key words : *Prunus armeniaca, Apricot, Genetic Diversity, RAPD, ISSR, AMOVA*

INTRODUCTION

Apricot (*Prunus armeniaca* L.) is the most important fruit crop of cold arid regions of India which cover 3,200 ha with a total production of 5,200 MT (Dwivedi and Attrey, 2002). It is intimately associated with the culture and traditions of the Ladakh people. The crop is one of the most important sources of livelihood and economy for this cold desert region. Almost every part of the plant fruit is used by the local inhabitants. Ripe apricot is an excellent desert fruit. It is dehydrated with or without seed since ancient time which is still practiced world over. Wild apricot popularly called as "*Zardalu*" appears to have originated in Indian Himalayas. In fruit crops, three regions have been identified as the origin center for cultivated apricot (1) Chinese Center (China and Tibet) (2) Central Asian countries (from Tien-Shan to Kashmir, India) (3) Near-Eastern Center (Iran, the Caucasus and Turkey) (Vavilov 1951). The Central Asian group is the oldest and richest in biodiversity that includes local apricots from central Asia, Xinjiang, Afghanistan, Baluchistan, Pakistan and Northern India (Kashmir). Most of them are self-incompatible, produce small to medium-sized fruits. It is believed to have been introduced in cold arid Ladakh via Baltistan though some experts opined that it was introduced directly from China via Tibet. Apricot thrives well in dry cold region of Ladakh having marginal, rocky and sandy soils. It also has the capability to tolerate as low as -35 ºC during winter, when it is dormant. This perhaps makes it an ideal fruit crop for the cold arid region (Dwivedi *et al.*, 2007).

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Since ages, the apricot in the region has developed considerable variability. However little attention has been paid by the researchers to evaluate and conserve the existing genotypes of apricot in the cold arid region. The genotypes found in the cold arid regions have been observed to differ drastically from those growing in other temperate regions. These are very area specific cultivars suitably adapted to survival at high altitudes of 2,500- 6,000 m above mean sea level (MSL) and the temperature, nutrient and environmental stress that they are subjected to under the cold arid conditions. Limited reports are available about the cultivars and detailed investigation is required. Classical approaches for identification and analysis of genetic variability in fruit crops are based on morphological, physiological and agronomic traits (UPOV, 1976). However, these traits have limitations as they are few in numbers and influenced by environmental factors.

Over the last 18 years, PCR technology has led to the development of two simple and quick techniques called random amplified polymorphic DNA (RAPD) (Williams *et al.,* 1990) and inter simple sequence repeats (ISSR) (Zietkiewicz *et al.*, 1994) which are independent of environmental conditions and are unaffected by developmental stages of the plant. These markers have been used both for DNA fingerprinting (Gupta *et al*., 2008) and population genetic studies (Alam *et al.*, 2009). Molecular genetic markers would aid the long-term objective of identifying diverse parental lines to generate segregating populations for tagging important traits, such as gene(s) for high content of sugars or vitamins from these apricot genotypes. Messina *et al.* (2004) used SSR markers to screen the polymorphism in Apricot cultivars where they observed number of alleles from two to nine and expected heterozygosity (*H*e) from 0.26 to 0.82. *He* is the estimated fraction of all individuals who would be heterozygous for any randomly chosen locus. The same SSRs also showed an appreciable transferrability across different *Prunus* species (Messina *et al*., 2004).

In the present investigation, RAPD and ISSR marker techniques were used to determine the genetic variability in apricot accessions from two valleys i.e. Leh and Nubra of Ladakh region in India. These valleys were separated from each other through natural mountain barrier. To our knowledge, no report has been published on the genetic diversity, population structure among the local populations of apricot from the trans-Himalayan region with molecular markers like RAPD and ISSR for diversity analysis, with the aim to providing insight to facilitate conservation management of the populations.

MATERIALS AND METHODS

Plant materials

Thirty-six locally grown genotypes collected from 9 different villages (4 genotypes from each village) were obtained from two valleys (Leh and Nubra) with altitude ranging 9,600 m (Nubra) to 11,500 m (Leh) from the cold arid desert of Trans-Himalayas (Figure 1; Table 1). 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. Apricot in Ladakh is having immense morphological variability like fruit shape, fruit color, earliness to flowering, kernel features, etc. While selecting the plants on morphological basis, only those genotypes (from each village) were taken for the investigations, which were morphologically very distinct for above mentioned parameters so as to avoid any chance of duplication.

DNA extraction and PCR amplification

Total genomic DNA was extracted from frozen leaves (5 g) by the CTAB method (Saghai- Maroof *et al.*, 1984) with minor modifications, which included the use of 200 mg per sample polyvinyl pyrollidone. Twenty random decamer primers from IDT Tech, USA (Table 2a) were used for RAPD amplification following the protocol of Williams *et al.* (1990). 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

Table 1. Nine populations of apricot collected from two valleys and different sites at different altitudes covering 36 sampling sites and their altitude.

Name of Valley	Accession No. (Village)	Sampling Site	Altitude (m)
Leh valley	Leh 1-4	Leh	3505
	Stok 5-8	Stok	3414
	Hemis 9-12	Hemis	5500
	Stakna 13-16	Stakna	3371
	Nimu 17-20	Nimu	3900
Nubra valley	Khardung 21-24	Khardung	3960
	Khalsar 25-28	Khalsar	3500
	Hundar 29-32	Hundar	3017
	Thoise 33-36	Thoise	2900

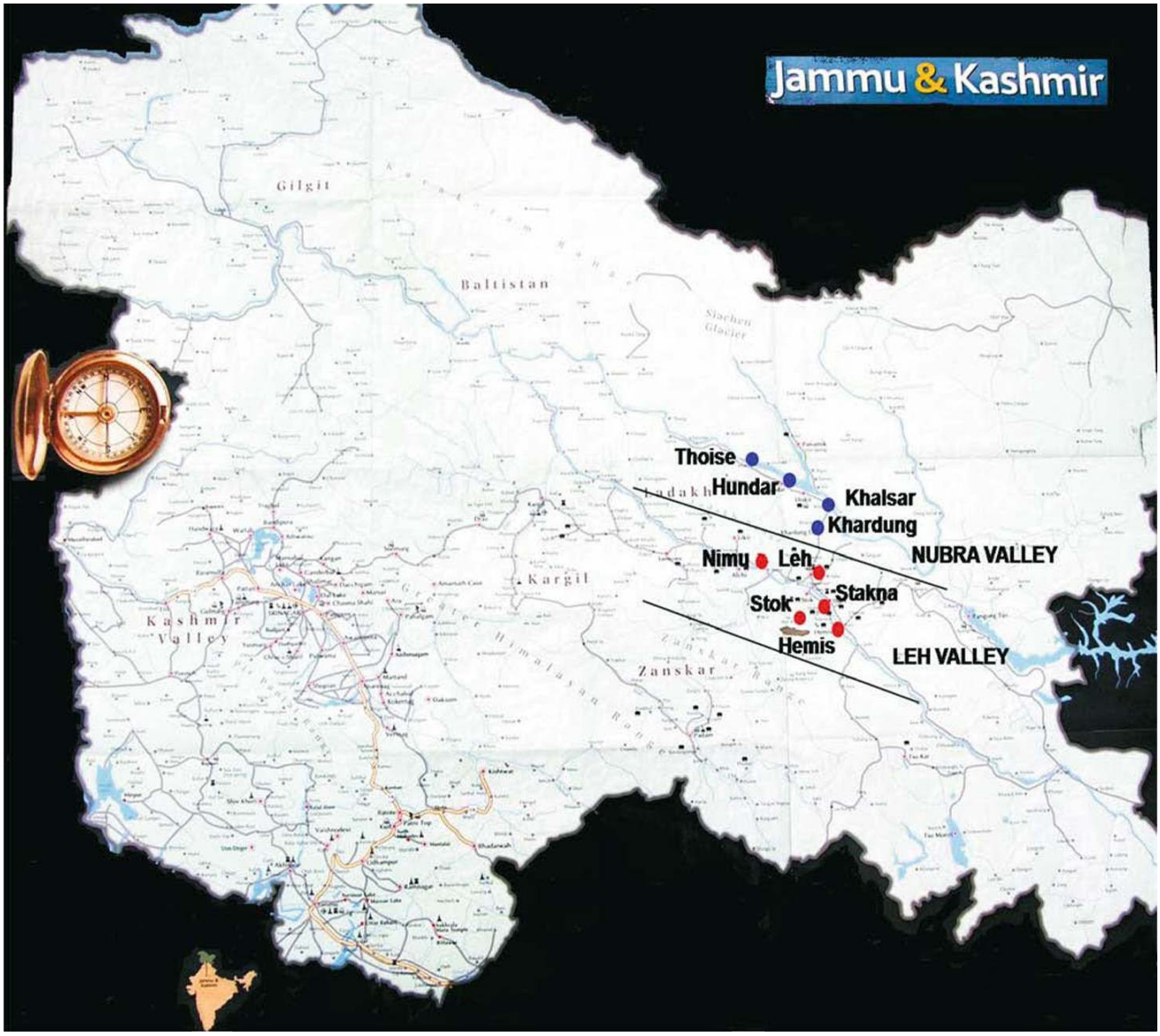


Fig. 1. Collection sites of 36 apricot genotypes from two valleys (Leh and Nubra) and nine villages located in Ladakh (Jammu and Kashmir, India).

unit of *Taq* polymerase ('Sigma-Aldrich, USA'). The first cycle consisted of denaturation of template DNA at 94 °C for 5 min, primer annealing at 37 °C for 1 min, and primer extension at 72 °C for 2 min. In the next 40 cycles the period of denaturation was reduced to 1 min at 92 °C, while the primer annealing and primer extension time remained the same as in the first cycle. The last cycle consisted of only primer extension (72 °C) for 7 min. In case of ISSR, the primers were obtained from 'Applied Biosciences, India' and PCR amplification was performed in reaction cocktail similar to RAPD. Initial denaturation for 5 min at 94 °C was followed by 40 cycles of 1 min at 94 °C, 1 min at specific annealing temperature (± 5 °C of Tm), 2 min at 72 °C and a 10 min final extension step at 72 °C (Table IIb). PCR products were stored at 4 °C before analysis. The amplification for each primer was performed twice independently with same procedure in order to ensure the fidelity of RAPD and ISSR markers.

Agarose gel electrophoresis

Amplification products were electrophoresed on 1.5 % agarose gel (Life Science Technologies, USA) and run

Table IIa. List of primers used for RAPD amplification, GC content, total number of loci, the level of polymorphism and resolving power.

Primer	Primer Sequence (5'~3')	GC (%)	Tm (°C)	Total number of loci	Number of polymorphic loci	Percentage of polymorphic loci	Total number of fragments amplified	Resolving power
S21	CAGGCCCTT C	70	36.4	13	13	100	241	13.39
S22	TGCCGAGCT G	70	40.7	12	12	100	193	10.72
S23	AGTCAGCCA C	60	34.3	6	6	100	149	8.28
S24	AATCAGCCA C	50	30.1	7	7	100	164	9.11
S25	AGGGGTCTT G	60	32.6	4	4	100	118	6.56
S26	GGTCCCTGA C	70	35.2	8	8	100	169	9.39
S27	GAAACGGGT G	60	33.2	7	7	100	170	9.44
S28	GTGACGTAG G	60	31.1	4	4	100	71	3.94
S29	GGGTAACGC C	70	37.4	8	6	75	232	12.89
S30	GTGATCGCA G	60	33.1	5	5	100	151	8.39
S31	CAATCGCCG T	60	36.7	6	6	100	99	5.50
S32	TCGGCGATA G	60	34.0	6	6	100	87	4.83
S33	CAGCACCCA C	70	37.7	5	4	80	130	7.22
S34	TCTGTGCTG G	60	34.3	7	7	100	163	9.06
S35	TTCCGAACC C	60	34.2	8	8	100	188	10.44
S36	AGCCAGCGA A	60	38.3	8	8	100	188	10.44
S37	GACCGCTTG T	60	35.7	4	4	100	54	3.00
S38	AGGTGACCG T	60	36.2	5	5	100	89	4.94
S39	CAAACGTCG G	60	34.2	7	7	100	73	4.06
S40	GTTGCGATC C	60	33.5	9	9	100	275	15.28
	Total	–	–	**139**	**136**	**97.84**	**3004**	–

at constant voltage (50 V) in 1X TBE for approximately 2 h, visualized by staining with ethidium bromide (0.5 μg ml^{-1}) and a total of 2.5 μl loading buffer (6X) was added to each reaction before electrophoresis. After electrophoresis, the gels were documented on a gel documentation system (Alpha Innotech, Alphaimager, USA). Molecular size of amplicons was estimated using a 100 bp and 1 Kb DNA ladders ('Bangalore Genei, India').

Data collection and analysis

The banding patterns obtained from RAPD and ISSR were scored as present (1) or absent (0), each of which was treated as an independent character. Jaccard's similarity coefficient (J) was used to calculate similarity between pairs of accessions. The similarity matrix was subjected to cluster analysis by unweighted pair group method with arithmetic means (UPGMA) and a dendrogram was generated using the program NTSYS-pc (Rohlf, 1992). POPGENE software was used to calculate Nei's unbiased genetic distance among different genotypes with all markers. Data for observed number of alleles (Na), effective number of alleles (Ne), 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 (Zhao *et al.*, 2006). Within species

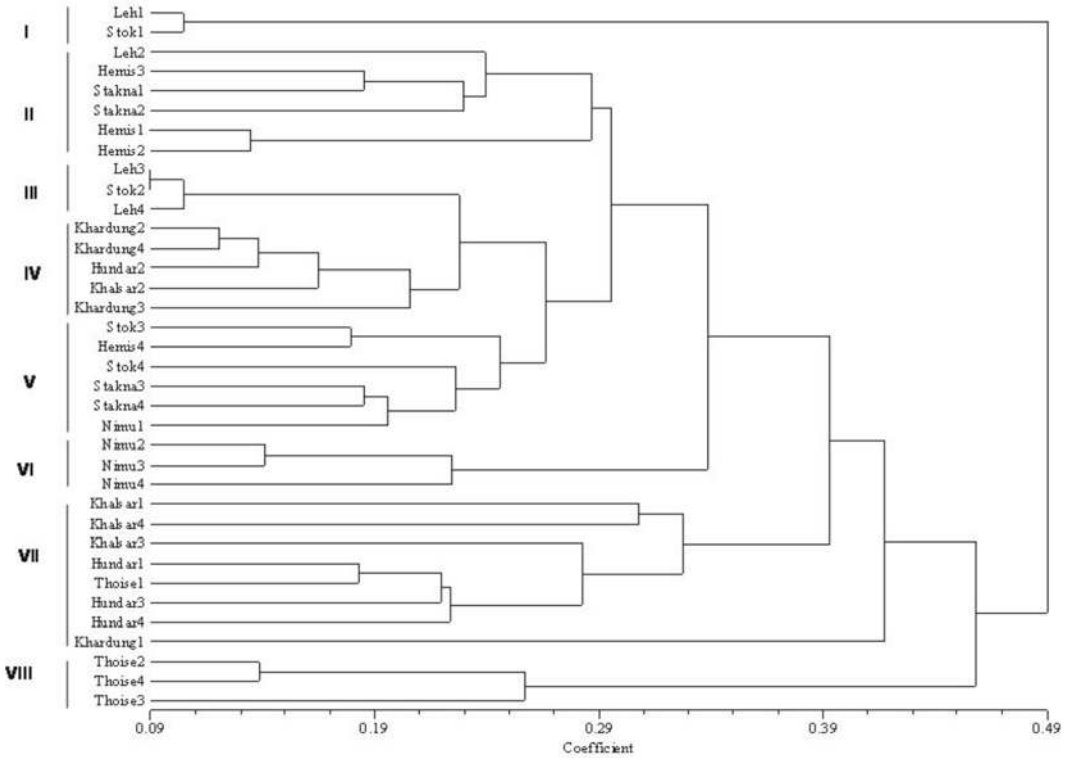
Table IIb. List of primers used for ISSR amplification, sequence, GC content, total number of loci, the level of polymorphism, size range of fragments and resolving power. Where, (Y = C, T; R = A,G).

Primer	Primer Sequence (5'~3')	GC (%)	Tm (°C)	Total number of loci	Number of polymorphic loci	Percentage of polymorphic loci	Total number of fragments amplified	Resolving power
ISSR 1	$(AG)_8$ T	47.0	47	4	4	100	111	6.17
ISSR 2	$(GA)_8$ T	45.4	47	5	5	100	136	7.56
ISSR 3	$(AC)_8$ T	51.4	47	3	3	100	45	2.50
ISSR 4	$(TG)_8$ A	51.3	47	4	4	100	125	6.94
ISSR 5	$(AG)_8$YT	49.2	47.2	6	6	100	96	5.33
ISSR 6	$(GA)_8$YT	47.4	47.2	7	6	85.7	159	8.83
ISSR 7	$(CT)_8$ RA	47.1	47.2	7	7	100	156	8.67
ISSR 8	$(GT)_8$ YC	52.7	52.7	7	7	100	174	9.67
ISSR 9	$(ACC)_6$	60.6	66.6	5	4	80	154	8.56
ISSR 10	$(CCG)_6$	76.8	100	5	5	100	104	5.78
ISSR 11	$(GGC)_6$	77.3	100	5	5	100	107	5.94
ISSR 12	$(AT)_8$ T	23.1	0	0	–	–	–	–
ISSR 13	$(TA)_8$ RT	25.6	2.7	0	–	–	–	–
ISSR 14	$(AT)_8$ YA	26.0	2.7	0	–	–	–	–
ISSR 15	$(CT)_8$ T	45.7	47	0	–	–	–	–
ISSR 16	$(TC)_8$ A	47.0	47	0	–	–	–	–
ISSR 17	$(GT)_8$ A	49.4	47	0	–	–	–	–
ISSR 18	$(TGC)_6$	62.4	66.6	0	–	–	–	–
ISSR 19	$(TGCA)_4$	53.3	50	0	–	–	–	–
ISSR 20	$(CTAG)_4$	43.9	50	0	–	–	–	–
	Total	–	–	**58**	**56**	**96.5**	**1367**	–

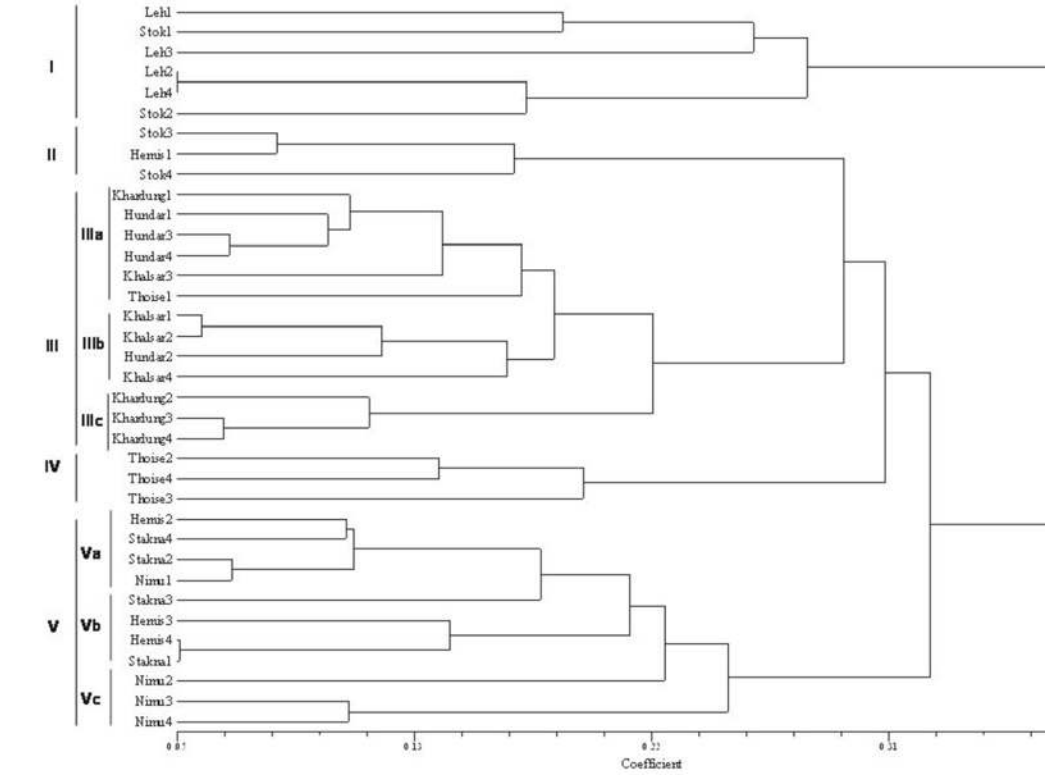
diversity (Hs) and total genetic diversity (Ht) (Nei, 1978) were calculated within the species and within four major groups (as per their collection site) using POPGENE software. The RAPD and ISSR data were subjected to a hierarchical analysis of molecular variance (AMOVA) (Excoffier *et al.*, 1992), using three hierarchical levels; individual, population and their regions. GenAlEx software was used to calculate a principal coordinates analysis (PCA) that plots the relationship between distance matrix elements based on their first two principal coordinates (Peakall and Smouse, 2001). Regression (R^2) between two matrices obtained with two marker types was estimated using Nei's genetic diversity. In this instance, the matrix regression corresponds to two independently derived dendrograms. According to Prevost and Wilkinson (1999) the resolving power (Rp) of a primer is: $Rp = \Sigma\, IB$ where *IB* (band informativeness) takes the value of: 1–[2* (0.5–P)], P being the proportion of the 36 genotypes 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.* (1996). DI for genetic markers was calculated from the sum of the squares of allele frequencies: $DI_n = 1\text{-}\Sigma\, pi^2$ (where 'pi' is the allele frequency of the ith allele). The arithmetic mean heterozygosity, Di_{av}, was calculated for each marker

(2.1) RAPD



(2.2) ISSR



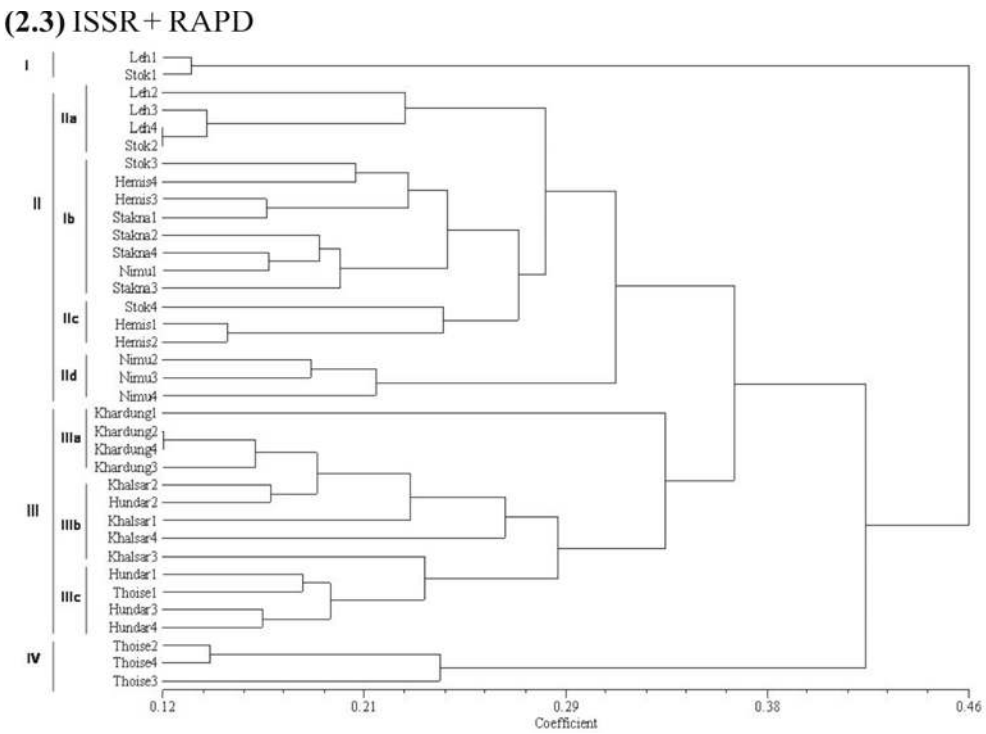


Fig. 2. Dendrograms generated using unweighted pair of group method with arithmetic average analysis, showing relationships between 36 *Apricot* genotypes, using (2.1) RAPD, (2.2) ISSR and (2.3) ISSR + RAPD data.

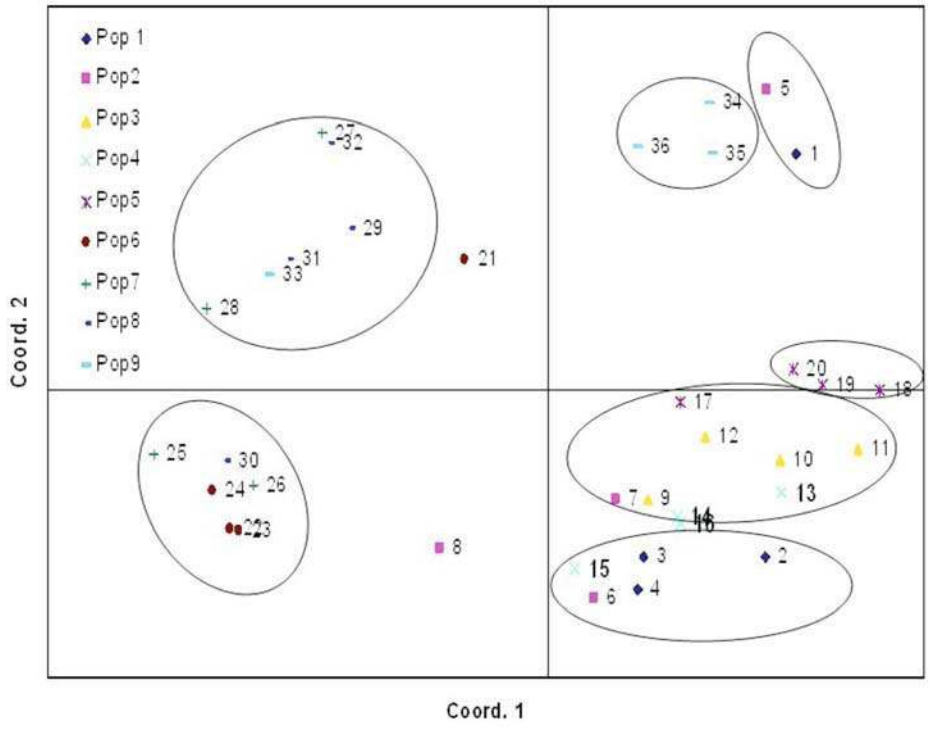


Fig. 3. Two-dimensional plot of principal component analysis of thirty-six local apricot genotypes using RAPD + ISSR analysis. The numbers plotted represents individual cultivars. (Where, Pop 1 = *Leh*, Pop 2 = *Stok*, Pop 3 = *Hemis*, Pop 4 = *Stakna*, Pop 5 = *Nimu*, Pop 6 = *Khardung*, Pop 7 = *Khalsar*, Pop 8 = *Hundar*, Pop 9 = *Thoise*)

Table IIIa. Summary of genetic variation statistics for all loci of RAPD, ISSR and RAPD + ISSR among the apricot populations with respect to their distributions among two valleys.

Markers & Valley Divisions	Sample size	Na	Ne	H	I	Ht	Hs	Gst	NPL	PPL
RAPD										
Leh valley	20	1.9353 (0.2470)	1.5292 (0.3209)	0.3149 (0.1523)	0.4759 (0.1979)	0.3149 (0.0232)	0.00 (0.00)	1.00	130	93.53
Nubra valley	16	1.8993 (0.3020)	1.5406 (0.3322)	0.3172 (0.1603)	0.4752 (0.2134)	0.3172 (0.0257)	0.00 (0.00)	1.00	125	89.93
Mean		**1.917**	**1.535**	**0.316**	**0.475**	**0.316**	–	–	–	–
ISSR										
Leh valley	20	1.9138 (0.2831)	1.5282 (0.3630)	0.3065 (0.1706)	0.4611 (0.2222)	0.3065 (0.0291)	0.00 (0.00)	1.00	53	91.38
Nubra valley	16	1.7414 (0.4417)	1.3970 (0.3715)	0.2355 (0.1895)	0.3587 (0.2629)	0.2355 (0.0359)	0.00 (0.00)	1.00	43	74.14
Mean		**1.828**	**1.463**	**0.271**	**0.409**	**0.271**	–	–	–	–
RAPD+ISSR										
Leh valley	20	1.9289 (0.2576)	1.5289 (0.3329)	0.3124 (0.1575)	0.4716 (0.2049)	0.3124 (0.0248)	0.00 (0.00)	1.00	183	92.89
Nubra valley	16	1.8528 (0.3552)	1.4983 (0.3495)	0.2931 (0.1730)	0.4409 (0.2345)	0.2931 (0.0299)	0.00 (0.00)	1.00	168	85.28
Mean		**1.891**	**1.514**	**0.303**	**0.456**	**0.303**	–	–	–	–

Na = Observed number of alleles; Ne = Effective number of alleles; H = Nei's gene diversity; I = Shannon's Information index; Ht = Total genetic diversity; Hs = Genetic diversity in population; Gst = Genetic diversity between population; NPL = No of Polymorphic Loci; PPL = % Polymorphic Loci

class: $Di_{av} = \Sigma Di_{n/n}$, (where 'n' is the number of markers (loci) analyzed). The DI for polymorphic markers is: $(Di_{av)}p = \Sigma Di_n/n_p$ (where 'np' is the number of polymorphic loci and n is the total number of loci). EMR (E) is the product of the fraction of polymorphic loci and the number of polymorphic loci for an individual assay. EMR (E) = n_p (n_p/n).

MI is defined as the product of the average diversity index for polymorphic bands in any assay and the EMR for that assay, MI = DI_{avp} * E. Wright's inbreeding coefficient (Fst) was also calculated using the programme *AFLP-SURV* (Weir, 1990). Bayesian method allows the direct estimates of Fst from dominant markers without assuming previous knowledge of the degree of within-population inbreeding and that genotypes within populations are in Hardy-Weinberg proportions (Holsinger *et al*., 2002).

RESULTS AND DISCUSSION

Genetic variability details from RAPD markers

All the chosen primers amplified with the number of amplified fragments ranging from four (S 25, 28 and 37) to thirteen (S 21) and which varied in size from 200 – 2,500 bp and yielded 3004 fragments. Out of 139 amplified bands, 136 were found polymorphic, with an average number of bands per primer and average numbers of polymorphic bands per primer as 6.95 and 6.8 respectively (Table IIa). The other primer amplification details are shown in the Table IIa. When Na, Ne, H, I, Ht, Hs, NPL

and PPL were studied for valley divisions (i.e. Leh and Nubra valleys) then all these respective values were found higher for Leh valley genotypes indicating more variability in Leh valley than in Nubra valley genotypes (Table IIIa). The respective values for overall genetic variability for Na, Ne, H, I, Ht, Hs, Fst, DI, EMR and MI across all the 36 genotypes were given in Table IIIb. The Gst value 0.3674 indicated that 63.26 % of the genetic diversity resided within the population. AMOVA helps in partitioning of the overall RAPD variations among groups and among populations within the group. Molecular variance among valley (11 %), among population (12 %) and within the population (77 %) (Table IV) indicating that there are more variations within the population. This is helpful in making strategy for germplasm collection and evaluation.

RAPD derived dendrogram analysis

A dendrogram based on UPGMA analysis grouped the 36 genotypes into eight main clusters (I - VIII) with Jaccard's similarity coefficient ranging from 0.09 to 0.49 (Figure 2.1). Cluster I, II, III, V and VI represents the genotypes from Leh valley while, cluster IV, VII and VIII have all the genotypes from Nubra valley. The results of PCA analysis were comparable to the cluster analysis. The first three most informative PC components explained 34.82 % of the total variation.

Genetic variability details from ISSR markers

The 11 primers on an average produced 58 bands across 36 genotypes, of which 56 bands were polymorphic, accounting for 96.55 %. Number of bands varied from three (ISSR 3) to seven (ISSR 6, 7 and 8), and sizes ranged from 200 – 2,500 bp. Average numbers of bands and polymorphic bands per primer were 5.27 and 5.01 respectively. Other primer amplification details are shown in Table IIa.

The sequences of these 20 primers seem to indicate that microsatellites more frequent in apricot contain the repeated di-nucleotides (AG)n, (GA)n, (TG)n, (CT)n, (AC)n, (GT)n, and tri-nucleotides (ACC)n, (CCG)n, (GGC)n. The number of bands produced with different repeat nucleotide were more with the (GT)n, (GA)n, (CT)n, and (AC)n primers (ISSR 8, 6, 7 and 9). The primers that were based on the (GA)n, (CT)n and (GT)n motif produced more polymorphism on average (7 bands per primer) than the primers based on any other motifs used in the present investigation. The primer sequences which did not amplify in the present investigation contain the di-nucleotides repeats as (AT)n, (GT)nA, (TC)n, (TA)n, (CT)nT; tri-nucleotides repeats (TGC)n

Table IIIb. Overall genetic variability across all the 36 genotypes of apricot based on RAPD, ISSR and RAPD+ISSR analysis.

Marker Type	Na	Ne	H	I	Ht	Hs	Gst	NPL	PPL	Fst	DI	EMR	MI
RAPD	1.9784 (0.1458)	1.5999 (0.2972)	0.3510 (0.1325)	0.5239 (0.1644)	0.3510 (0.0176)	0.2220 (0.0086)	0.3674	136	97.84	0.102	0.815	6.69	5.45
ISSR	1.9483 (0.2234)	1.5683 (0.3281)	0.3310 (0.1552)	0.4948 (0.2000)	0.3310 (0.0241)	0.1803 (0.0115)	0.4552	55	94.83	0.182	0.777	4.81	3.74
RAPD+ISSR	1.9695 (0.1723)	1.5906 (0.3061)	0.3451 (0.1395)	0.5153 (0.1756)	0.3451 (0.0195)	0.2097 (0.0097)	0.3922	191	96.95	0.121	–	–	–

Fst = Wright's inbreeding coefficient; DI = Diversity Index; EMR = Effective Multiplex Ratio; MI = Marker Index

and the repeated tetra-nucleotides sequences (CTAG)n and (TGCA)n (Table IIb). We obtained good amplification products from primers based on (CT)n and (GT)n repeats while (AT)n and some other primers gave no amplification products (Table IIb), despite the fact that (AT)n di-nucleotide repeats are thought to be the most abundant motifs in plant species (Martín and Sánchez-Yélamo, 2000). Similar results were obtained in grapevine (Moreno *et al.,* 1998), rice (Blair *et al.,* 1999), Vigna (Ajibade *et al.*, 2000) and wheat (Nagaoka and Ogihara, 1997). In case of non amplifying ISSR primers we went for different annealing temperatures (± 5 °C of Tm) for various PCR reactions but no amplification was observed. A possible explanation of these results is that ISSR primers based on AT motifs are self-annealing, due to sequence complementarity, and would form dimers during PCR amplification (Blair *et al.*, 1999) or it may be due to its-non annealing with template DNA due to its low Tm. Reason behind non amplification of other repeats may be their absence in the genome or due to the absence of motifs complementary to the primers in the genome.

For valley divisions the respective values of Na, Ne, H, I, Ht, Hs, NPL and PPL were found higher for Leh valley genotypes indicating that there is more variability in Leh valley than in Nubra valley genotypes (Table IIIa). Gst value 0.4552 indicated that 54.48 % of the genetic diversity resided within the population (Table IIIb). The details of overall genetic variability across 36 genotypes were given in Table IIIb. AMOVA for among valley (22 %), among population (16 %) and within the population (62 %) indicated that there are more variations within the population (Table IV).

ISSR derived dendrogram analysis

The complete data was based on a total of 1367 bands and Jaccard's similarity coefficient ranged from 0.05 to 0.39. The genotypes were clustered into five clusters (I – V) where, cluster I, II and V represents all the genotypes from Leh valley while, cluster III, and IV contains all the genotypes from Nubra valley (Figure 2.2). The results of PCA analysis were comparable to the cluster analysis. The first three most informative PC components explained 56.39 % of the total variation.

Genetic variability details from RAPD + ISSR combined data

When Na, Ne, H, I, Ht, Hs, NPL and PPL parameters were analyzed for valley divisions, then the values were found higher for Leh valley genotypes (Table IIIa). Gst value 0.3922 indicated that 60.78 % of the genetic diversity resided within the population (Table IIIb). The details of overall genetic variability of 36 genotypes were given in Table IIIb. AMOVA for among valley (15 %), among population (13 %) and within the population (72 %) indicated that there are more variations within the population (Table IV).

RAPD and ISSR combined data for cluster analysis

The UPGMA dendrogram obtained from the cluster analysis of RAPD and ISSR combined data gave near similar clustering pattern, with Jaccard's similarity coefficient ranging from 0.12 to 0.46 (Figure 2.3). All the genotypes were clustered into four major clusters (I - IV) with 2, 18, 13 and 3 genotypes respectively. Cluster I and II represents all the genotypes from Leh valley

Table IV. Summary of nested analysis of molecular variance (AMOVA) based on RAPD, ISSR individually and in combination, among the populations of apricot. Levels of significance are based on 1000 iteration steps.

Source of variation	Among valley			Among populations/valley			Individual/within populations		
d.f.	1			7			27		
Marker	RAPD	ISSR	RAPD + ISSR	RAPD ISSR	ISSR ISSR	RAPD +	RAPD	ISSR	RAPD +
S.S.D.	87.661	58.528	146.189	234.95	98.75	333.700	555.5	188.25	743.750
Variance component	3.043	2.499	5.542	3.248	1.784	5.031	20.574	6.972	27.546
Percentage	11	22	15	12	16	13	77	62	72
P-value	<0.001	<0.001	< 0.001	<0.001	<0.001	<0.001	<0.001	<0.001	< 0.001

Where, d.f.: degree of freedom; S.S.D.: sum of square deviation; P-value: probability of null distribution.

while, cluster III, and IV contains all the genotypes from Nubra valley. Both RAPD and ISSR clusters showed partial similarity with combined data of RAPD + ISSR. The results of PCA analysis were comparable to the cluster analysis. The first three most informative PC components explained 33.73 % of the total variation. The two-dimensional ordination confirms the cluster analysis results showing that two genotypes i.e. *Stok* 4 and *Khardung* 1 (8 and 21) were separated (Figure 3). Other genetic variation studies were also performed on ISSR and RAPD combined data which are represented in different tables (Tables IIIa and IIIb).

The differences found among the dendrograms generated by RAPDs and ISSRs could be partially explained by the different number of PCR products analyzed (3004 for RAPDs and 1367 for ISSRs) reinforcing again the importance of the number of loci and their coverage of the overall genome, in obtaining reliable estimates of genetic relationships among apricot cultivars as observed by Loarce *et al.* (1996) in barley. Another explanation could be the low reproducibility of RAPDs (Karp *et al.*, 1997).

Dendrograms in the present study did not indicate very clear pattern of clustering for within valley samples but, a clear pattern was observed in all the three cases for between valley samples. Similar results were obtained in Azukibean (Fernandez *et al.*, 2002) and groundnut (Dwivedi *et al.*, 2001). The genetic closeness among the Leh valley and Nubra valley cultivars can be explained by the high degree of commonness in their genotypes which is same as observed in blackgram (Gaffor *et al.*, 2001). In all the dendrograms, *Nimu* (2, 3, 4) and *Thoise* (2, 3, 4) genotypes were found clustered together. The genetic similarity of these genotypes is probably associated with their similarity in the genomic and amplified region.

Comparative analysis of RAPD with ISSR markers

RAPD markers were found more efficient with regards to polymorphism detection, as they detected 97.84 % as compared to 96.5 % for ISSR markers. Also, the number of polymorphic loci, % polymorphic loci, diversity index, effective multiplex ratio and marker index are more for RAPD than for ISSR markers (Table IIIb). This is in contrast to the results as obtained for several other plant species like wheat (Nagaoka and Ogihara, 1997) and Vigna (Ajibade *et al.*, 2000). More polymorphism in case of RAPD than ISSR markers might be due to the fact that out of 20 ISSR primers used in the study only 11 primers amplified 1367 number of fragments (Table IIb). While in case of RAPD, all the 20 primers which were used in the investigation amplified 3004 number of fragments (Table IIa). Same polymorphism pattern was also observed in case of Jatropha (Gupta *et al.*, 2008) and Podophyllum (Alam *et al.*, 2009).

The regression test between the two Nei's genetic diversity indices resulted in low regression between RAPD and ISSR based similarities ($R^2 = 0.1996$), moderate for ISSR and ISSR + RAPD (0.5366), while it is maximum for RAPD and ISSR + RAPD based similarities (0.8766). This shows that RAPD data is more close to ISSR + RAPD combined data. A possible explanation for the difference in resolution of RAPDs and ISSRs is that the two-marker techniques target different portions of the genome.

There was some consensus between the RAPD and ISSR based grouping of the 36 apricot genotypes. Range of similarity index for RAPD markers (0.09 – 0.49), ISSR markers (0.05 – 0.39) and RAPD + ISSR marker data (0.12 – 0.46) indicating more diversity in case of RAPD (Figures 2.1, 2.2, 2.3). Although within the valley genotypic differences were there but the genotypes from different villages did not fall into a distinct clustering pattern. But the genotypes from two valleys (i.e. Leh and Nubra valley) were present in distinct clusters in all the three cases showing that there is more variation between valley genotypes.

With this study, we can conclude that the molecular analyses of both RAPD and ISSR markers were extremely useful for studying the genetic relationships of local apricot genotypes from the trans-Himalayan region of Ladakh. The results indicate the presence of great genetic variability among local genotypes of apricot where between valley variations are more than within the valley variations, which should be exploited for the future conservation and breeding of apricot from this region.

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