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Genetic diversity studies of apricot of trans-himalaya based on morphological and SARP markers

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ABSTRACT

Forty-seven apricot genotypes were used to assess genetic diversity (GD) based on morphological and SRAP (Sequence-related amplified polymorphism). Six qualitative and 16 quantitative characters were studied among the genotypes. Twenty combinations of SRAP markers were used, and 115 polymorphic bands out of 134 bands were observed. The overall GD estimated, as percentage polymorphic loci (85.06%), Nei's genetic diversity (0.27±0.19), and Shannon's information index (0.40±0.25) were high. Analysis of molecular variance (AMOVA) revealed higher GD (92%) within the groups of apricot. The unweighted pair group method (UPGMA) demonstrated that the apricot genotypes had a similarity range from 0.96 to 0.48, with a mean value of 0.72 similarity coefficient. Furthermore, UPGMA clustering and Bayesian-based STRUCTURE analysis revealed an intermixing in the clustering of apricot genotypes. There is no clear grouping between apricots according to their kernel taste and stone colour, which reveals that these genotypes have a similar genetic background. Knowledge gained from the present study has a practical utility in managing germplasm conservation and breeding programmes.

Keywords: *Prunus armeniaca*, genetic diversity, molecular markers.

INTRODUCTION

Knowledge of genetic diversity (GD) is key for efficient preservation, management, and utilization of plant genetic resources (Barac *et al.*, 3). Knowledge about genetic relationships and diversity among breeding materials could be useful in crop improvement strategies (Mahammadi and Prassanna, 10). Ample GD in plants can provide a broad background for genetic research and crop breeding programmes. Preservation of crop genetic resources is based on the continuous introduction of new genetic material from traditional and wild varieties for the breeding of highly productive and resistant varieties (Mondin *et al.*, 11).

Cultivar characterization and discrimination are required for breeding and commercialization of apricot cultivars (Yilmaz *et al.*, 18). Knowledge of GD and relationship among the germplasm resource will be useful for protecting and utilizing local apricot varieties. Several investigations have been carried out to determine diversity in apricots with pomological, phenological, and morphological characters (Hormaza, 8). These traditional approaches to diversity study are slow and subject to environmental influences. Apricot can be adapted to particular microclimates and shows significantly different morphological changes when proceeding from one microclimate to others (Uzun *et al.*, 17). Therefore, for reliable identification and discrimination of genotype and cultivars, appropriate markers are required. Accordingly, new methods based on molecular studies must be included in breeding programmes and to study the genetic relationships among cultivars. Various types of molecular markers such as AFLP, ISSR, RAPD, SRAP, RFLP, and SSR have been employed for the analysis of plant GD and characterization. Among them, SRAP (Sequence-related amplified polymorphism) marker has been a commonly used method for diversity study and population genetics analysis (Li *et al.*, 9).

The SRAPs is an efficient and simple marker system and has several advantages over other marker system (Ai *et al.*, 2). SRAP targets the coding sequences in the genome and results in a moderate number of co-dominant markers (Ahmad *et al.*, 9). The information received from SRAP markers is more conformable to detect variations and evolutionary history of the morphotypes than that of the AFLP marker (Ferrio *et al.*, 7). SRAP markers are more effective, quicker, and less expensive over SSR markers (Ahmad *et al.*, 1).

The current work aimed to characterize the morphological and GD of apricot genotypes and to investigate the relationship among the genotypes to estimate the extent of GD in apricot genotypes

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between and within classified groups according to kernel taste and stone colour.

MATERIALS AND METHODS

In total, 47 apricot genotypes were studied (Table 1) from experimental orchard (34° 08.21′ N; 77° 34.32′ E, elevation 3345 m using GARMIN GPS 72, USA) at Defence Institute of High Altitude Research in trans-Himalayan Ladakh, India. A model-based clustering method was used to determine the optimal number of genetic and morphometric clustering in the genotypes. To best of our knowledge use of SRAP markers along with morphological characters for assessment of structure and GD in apricots has not been reported yet.

Leaf sample of 47 genotypes was collected from an experimental orchard in Ladakh and kept in -80°C freezer until DNA extraction. Apricot genotypes were grouped into three (Naryal *et al.*, 12). Fourteen genotypes fall under Group-A: brown stone and bitter kernel; 23 genotypes under Group-B: brown stone and sweet kernel, and ten genotypes under Group-C: white stone and sweet kernel (Fig. 1). Genomic DNA was isolated using a CTAB method (Saghai-Maroof *et al.*, 16). The DNA sample was diluted to the final concentration of 30 ng. μl^{-1} before PCR amplification.

Dimensional properties of fruit stone and kernel of apricot were measured with a digimatic caliper (CD-6″CS, Mitutoyo, Japan) to an accuracy of 0.01 mm. Blush area of fruit was drawn on a tracing paper and was further used to determine the blush area using graph paper (Naryal *et al.*, 13). TSS were measured with a refractometer (ATAGO, Tokyo), and values were corrected at 20°C. The total acid (TA) percentage was determined by titration using 0.1 N NaOH and values expressed as per cent mallic acid. Moisture content was determined by the oven drying method until the weight was constant and then expressed as a percentage of fresh weight.

SRAP markers developed previously were adopted in this study (Ai *et al.,* 2). Twenty primer combinations using seven forward (Me 1-7) and nine

Table 1. Summary of the apricot genotypes analysed.

Sl. No.	Genotype	Kernel Taste	Stone Colour	Sl. No.	Genotype	Kernel Taste	Stone Colour
1.	101	Bitter	Brown	25.	162	Sweet	Brown
2.	102	Sweet	Brown	26.	167	Sweet	Brown
3.	103	Sweet	Brown	27.	168	Sweet	Brown
4.	104	Sweet	Brown	28.	169	Bitter	Brown
5.	105	Sweet	White	29.	170	Sweet	Brown
6.	106	Bitter	Brown	30.	171	Bitter	Brown
7.	107	Sweet	White	31.	173	Sweet	Brown
8.	108	Sweet	White	32.	174	Sweet	White
9.	109	Bitter	Brown	33.	175	Bitter	Brown
10.	110	Bitter	Brown	34.	176	Sweet	Brown
11.	113	Sweet	Brown	35.	178	Sweet	Brown
12.	114	Sweet	Brown	36.	179	Sweet	Brown
13.	115	Sweet	Brown	37.	180	Sweet	White
14.	116	Sweet	Brown	38.	181	Sweet	Brown
15.	117	Sweet	White	39.	184	Sweet	White
16.	118	Sweet	Brown	40.	185	Sweet	White
17.	119	Sweet	Brown	41.	186	Sweet	White
18.	121	Sweet	White	42.	187	Sweet	White
19.	123	Sweet	Brown	43.	189	Sweet	Brown
20.	126	Bitter	Brown	44.	190	Sweet	Brown
21.	133	Sweet	White	45.	194	Sweet	White
22.	134	Bitter	Brown	46.	195	Sweet	Brown
23.	135	Sweet	Brown	47.	196	Sweet	White
24.	158	Bitter	Brown				

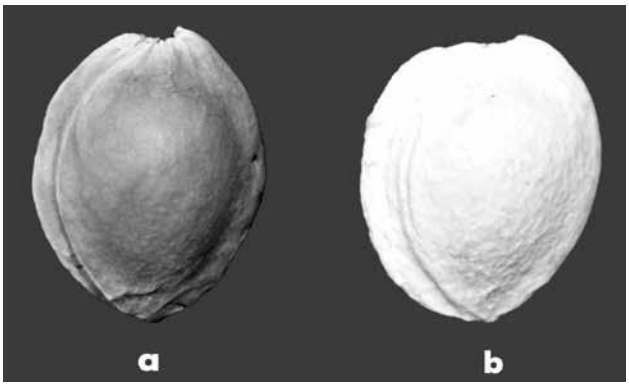

Fig. 1. a) Normal stone of apricot; b) Raktsey Karpo

reverse (Em1-9) (Table 2) were tested and selected based on proper amplification and reproducibility for diversity studies. The PCR amplification was performed with a total volume of 20 µl PCR reactions consisted of 0.9 µM of primers, 0.2 mM of dNTPs, 2.5 mM of $MgCl_2$, 1.5 units of *Taq* DNA polymerase, genomic DNA at 30 ng and nuclease-free water up to 20 µl reaction volume. Amplification was carried out in a 96 well thermocycler (BioRadT100™) programmed with the initial step at 95°C for 3 min. followed by 5 cycles of three steps: 1 min. of denaturing at 94°C, annealing at 35°C for 1 min. and extension at 72°C for 1 min. In the subsequent 35 cycles, the annealing temperature was increased to 50°C, and the extension step consists of one cycle for 5 min. at 72°C. Amplified products were electrophoresed on 1.5% agarose gel, and the molecular size of amplicons was determined using a 50 bp-10 kb DNA ladders. After electrophoresis, the gels were documented in a gel documentation system (BioRad, Gel Doc™XR+).

Six qualitative and 16 quantitative characters were used in the multivariate statistical analysis of morphological data (Table 3). A multivariate approach was used to classify the plant population based on quantitative morphological characters using Gower general similarity coefficient in the PAST software (Paleontological statistics, Version 3.22).

The band with the same mobility was considered as an identical band, whereas polymorphism was scored by the presence (1) or absence (0) of the band. POPGENE version 1.32 was used to calculate the different GD parameters: number of alleles (Na), the effective number of alleles (Ne), Nei's genetic diversity (H), Shannon's information index (I), number of polymorphic loci (NPL), percentage polymorphic loci (PPL). To describe genetic variability within and among the groups, the non-parametric analysis of molecular variance (AMOVA) was performed using squared Euclidean distances among all genotypes to partition the variation into two hierarchical levels; individual and groups (Excoffier *et al.*, 6) using GenAlEx v. 6.3 software (Peakall and Smouse, 14). Inter population genetic distance and genetic identity were calculated by Nei's method using GenAlEx software. STRUCTURE version 2.3 (Pritchard *et al.*, 15) was used to predict the number of clusters (K) and the probability of individual assigned to each cluster. The parameters sets assumed were admixture allele model with correlated allele frequencies and with no prior group's information. The number of clusters set from K = 1 to 10 with five simulations. For each simulation, we have fixed burn-in period of 1,00,000 steps followed by 2,50,000 Monte Carlo Markov chain replicates. Results obtained from STRUCTURE were interpreted by online available tool STRUCTURE HARVESTER (Earl, 14), which implements Evanno's method (Evanno *et al.*, 15) for calculation of a correct number of clusters (K).

RESULTS AND DISCUSSION

The selected 20 SRAP primer combinations pairs result in reproducible amplification, and polymorphic

Table 2. SRAP primers used for diversity studies on+ apricots of trans-Himalayan Ladakh.

Forward primer	Reverse primer
Me1: TGAGTCCAAACCGGATA	Em1: GACTGCGTACGAATTAAT
Me2: TGAGTCCAAACCGGAGC	Em2: GACTGCGTACGAATTTGC
Me3: TGAGTCCAAACCGGAAT	Em3: GACTGCGTACGAATTGAC
Me4: TGAGTCCAAACCGGACC	Em4: GACTGCGTACGAATTTGA
Me5: TGAGTCCAAACCGGAAG	Em5: GACTGCGTACGAATTAAC
Me6: TGAGTCCAAACCGGTAA	Em6: GACTGCGTACGAATTGCA
Me7: TGAGTCCAAACCGGTCC	Em7: GACTGCGTACGAATTGAG
	Em8: GACTGCGTACGAATTGCC
	Em9: GACTGCGTACGAATTTCA

Table 3. Descriptive statistics related to morphological variables among the 47 genotypes (minimum, maximum and mean values measured for each variable, SD: standard deviation, CV: coefficient of variation.

Sl no	Variable	Mean ± SD	Min	Max	CV (%)
1.	Fruit shape lateral	6±3	1	8	43.4
2.	Fruit shape ventricle	4±1	2	5	32.5
3.	Fruit shape of apex	3±1	1	4	32.7
4.	Fruit skin colour	4±1	3	6	25.0
5.	Flesh colour	4±1	3	6	20.9
6.	Stone shape	2±1	1	5	45.2
7.	Fresh fruit wt. (g)	20.2±8.5	5.0	41.9	41.8
8.	Fresh stone wt. (g)	2.0±0.6	0.7	4.2	31.0
9.	Moisture in fruit (%)	71.4±7.6	48.7	84.7	10.7
10.	TSS (°Brix)	23.7±5.5	14.5	36.8	23.3
11.	Fruit length (mm)	32.5±4.9	21.2	43.3	15.0
12.	Fruit width (mm)	32.6±5.0	21.5	44.0	15.3
13.	Fruit thickness (mm)	30.0±4.8	19.6	40.4	16.1
14.	Stone length (mm)	21.7±2.5	14.7	26.4	11.6
15.	Stone width (mm)	17.9±2.3	13.1	25.0	12.8
16.	Stone thickness (mm)	11.4±1.6	8.1	15.9	13.8
17.	Fruit blush area (mm)	223.6±247.7	0.0	831.5	110.8
18.	Kernel wt. (g)	0.5±0.2	0.1	1.0	35.3
19.	Kernel length (mm)	15.1±1.7	10.7	18.3	11.3
20.	Kernel width (mm)	10.8±1.3	7.6	14.4	11.6
21.	Kernel thickness (mm)	6.2±1.3	3.1	9.9	20.6
22.	Seed coat thickness (mm)	1.5±0.2	1.0	2.4	16.0

bands were used to analyze polymorphism in 47 genotypes. A total of 134 bands were scored, out of which 115 (85.8%) bands were polymorphic (Table 4). An average of 6.7 bands per primer set was obtained, and an average 5.75 polymorphic band was obtained per primer set. The number of bands observed was higher than previous reports on apricot by Uzun *et al*. (17) (5.4 bands per primer set and 73% polymorphism rate, 4.9 bands per primer set and 64.3% polymorphism). However, Ai *et al*. (2) obtain 19.1 bands per primer set and 88.11% polymorphism rate, which was higher than that of our study.

At the genotypic level, the GD were higher (NPL=114, PPL%=85.07, Na=1.85±0.36, Ne=1.46±0.37, H=0.27±0.19, I=0.40±0.25) (Table 5). The GD parameters at the level of grouped genotypes were highest in Group-B (NPL=103, PPL%=76.87, Na=1.77±0.42, Ne=1.46±0.38, H=0.26±0.20, I=0.39±0.28). Group-C showed the lowest GD (NPL=82, PPL%=61.19, Na=1.61±0.49, Ne=1.37±0.39, H=0.21±0.21, I=0.32±0.29). AMOVA also showed that the major part of the total variance is partitioned within the population (92%), and only 8% of the variance was partitioned among populations (Table 6 ; Fig. 2). Thus, AMOVA analysis of the SRAP marker suggests a better panmictic and not genetic divergent. Nei's genetic distance and genetic identity of apricot groups are presented in Table 7.

Nei's genetic identity is above diagonal and genetic distances is below diagonal. The highest value of genetic identity (0.9631) and the lowest genetic distance (0.0376) was obtained between Group-A and Group-B, followed by Group-B and Group-C (genetic identity = 0.9344, genetic distance = 0.0679) and the lowest value of genetic identity (0.9343) and highest genetic distance (0.0680) was observed between Group-A and Group-C (Table 7).

The dendrogram generated from the UPGMA cluster analysis based on Gower similarity index from morphological traits classified the 47 genotypes included in this study into two main groups (Fig. 3). The first cluster includes only one genotype with brown stone with the sweet kernel. The second cluster

includes most of the genotypes from Group-A, -B, -C. The second cluster was further grouped into three sub-clusters. The first and third sub-cluster shows the intermixing of genotypes from Group-A and -B, whereas Group-C is clustered into a separate sub-group. Variation was observed for three groups based on phenotypic expression. From the present study, it was also concluded that three groups represents

Table 4. Polymorphism revealed by twenty SRAP primer combinations.

Primer combination	Total bands	Polymorphic bands	Percentage of polymorphic band	Primer combination	Total bands	Polymorphic bands	Percentage of polymorphic band
Me1/Em1	6	5	83.3	Me4/Em6	7	5	71.4
Me1/Em2	9	8	88.9	Me5/Em2	8	7	87.5
Me1/Em4	9	9	100.0	Me5/Em6	6	3	50.0
Me1/Em5	4	3	75.0	Me5/Em8	8	8	100.0
Me2/Em2	7	7	100.0	Me5/Em9	8	7	87.5
Me2/Em6	8	7	87.5	Me6/Em2	4	3	75.0
Me2/Em7	5	3	60.0	Me6/Em5	8	7	87.5
Me3/Em2	6	5	83.3	Me6/Em6	6	5	83.3
Me3/Em4	5	5	100.0	Me7/Em3	8	7	87.5
Me4/Em3	6	5	83.3	Total	134	115	85.8
Me4/Em4	6	6	83.3	Average	6.7	5.75	85.8

Table 5. Summary of genetic variation statistics for all loci of SRAP among the apricot genotypes of trans-Himalayan Ladakh.

Group	Sample size	Na (Mean ± SD)	Ne (Mean ± SD)	H (Mean ± SD)	I (Mean ± SD)	NPL	PPL
Group-A	14	1.63±0.48	1.40±0.39	0.23±0.21	0.34±0.29	85	63.43
Group-B	23	1.77±0.42	1.46±0.38	0.26±0.20	0.39±0.28	103	76.87
Group-C	10	1.61±0.49	1.37±0.39	0.21±0.21	0.32±0.29	82	61.19
Overall genetic variability	47	1.85±0.36	1.46±0.37	0.27±0.19	0.40±0.25	114	85.07

Na = number of alleles; Ne = effective number of alleles; H = Nei's genetic diversity; I = Shannon's information index; NPL= number of polymorphic loci, PPL= Percentage of polymorphic loci. Group-A: brown stone and bitter kernel; Group-B: brown stone and sweet kernel; Group-C: white stone and sweet kernel

Table 6. Total genetic variance analysis (AMOVA) of apricot brought from SRAP results.

Source	Df	SS	MS	Est. Var.	%
Among pops	2	70.352	35.176	1.323	8%
Within pops	44	690.627	15.696	15.696	92%
Total	46	760.979		17.019	100%

Table 7. Inter-population genetic distances and genetic identity calculated by Nei's method in apricot groups.

Group*	A	B	C
A	***	0.9631	0.9343
B	0.0376	***	0.9344
C	0.0680	0.0679	***

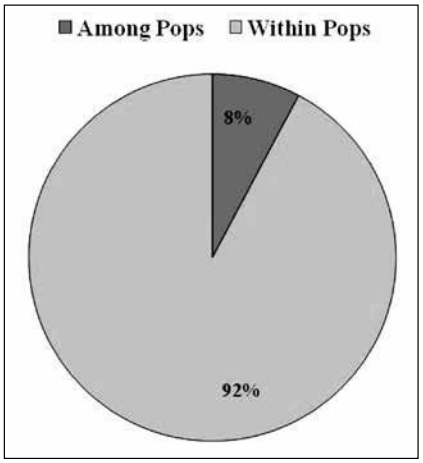

Fig. 2. Total genetic variance analysis (AMOVA) of apricot based on SRAP results.

different characters and can be further used for categorization of morphotypes.

To determine the genetic similarity among the genotypes, a dendrogram for the genotypes was obtained (Fig. 4) with the Jaccard's similarity coefficient and UPGMA cluster analysis using the PAST (Paleontological Statistics, Version3.22). The similarity index values range from 0.49 to 0.96. All these results reflect high genetic variability in apricots grown in the Ladakh region. Apricot genotypes were grouped into non-specific groups and did not follow our earlier assumption of three groups based on kernel taste and stone colour.

The genetic structure investigated 47 genotypes by applying Bayesian model-based clustering algorithm approach in STRUCTURE software. Delta K values were plotted against the K numbers. The modal value of the distribution of K number identified two clusters; DK, when graphed against K, showed a maximum peak at K = 2, dropping down to near zero at K = 3 (Fig. 5). Structure analysis using SRAP markers revealed the independent distribution of genotypes with respect to their groups. The study also indicates that apricot genotypes from the trans-Himalayan region are highly genetically diverse. Structure analysis did not support our grouping of

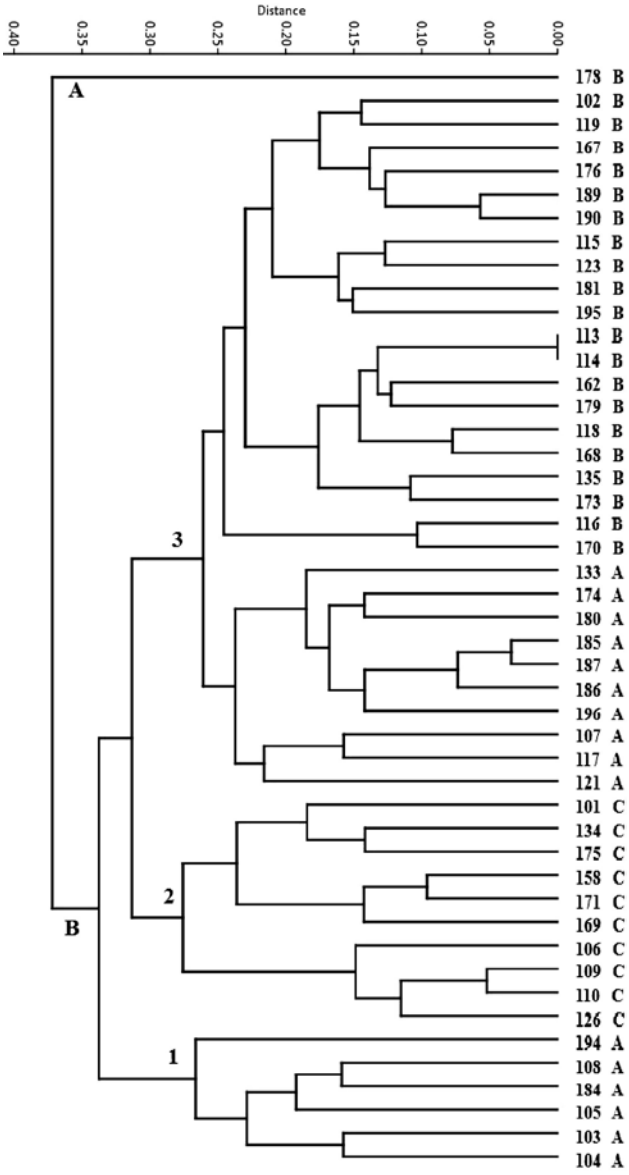

Fig. 3. UPGMA cluster analysis based on Gower's similarity index from morphometric parameters.

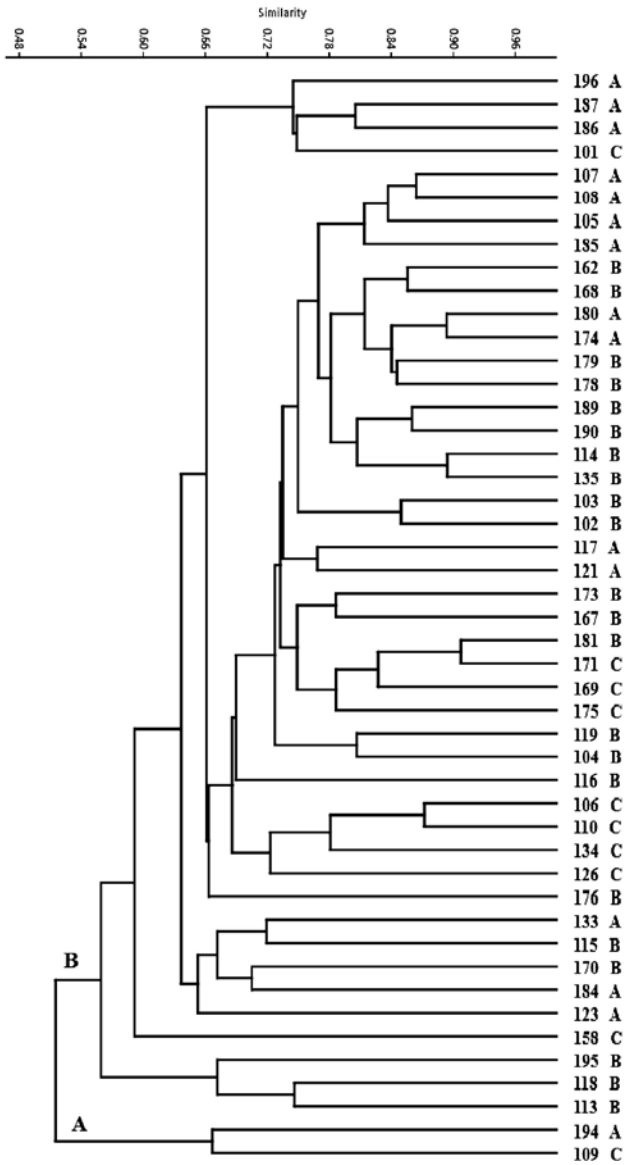

Fig. 4. SRAP based UPGMA cluster analysis by Jaccard's similarity coefficient.

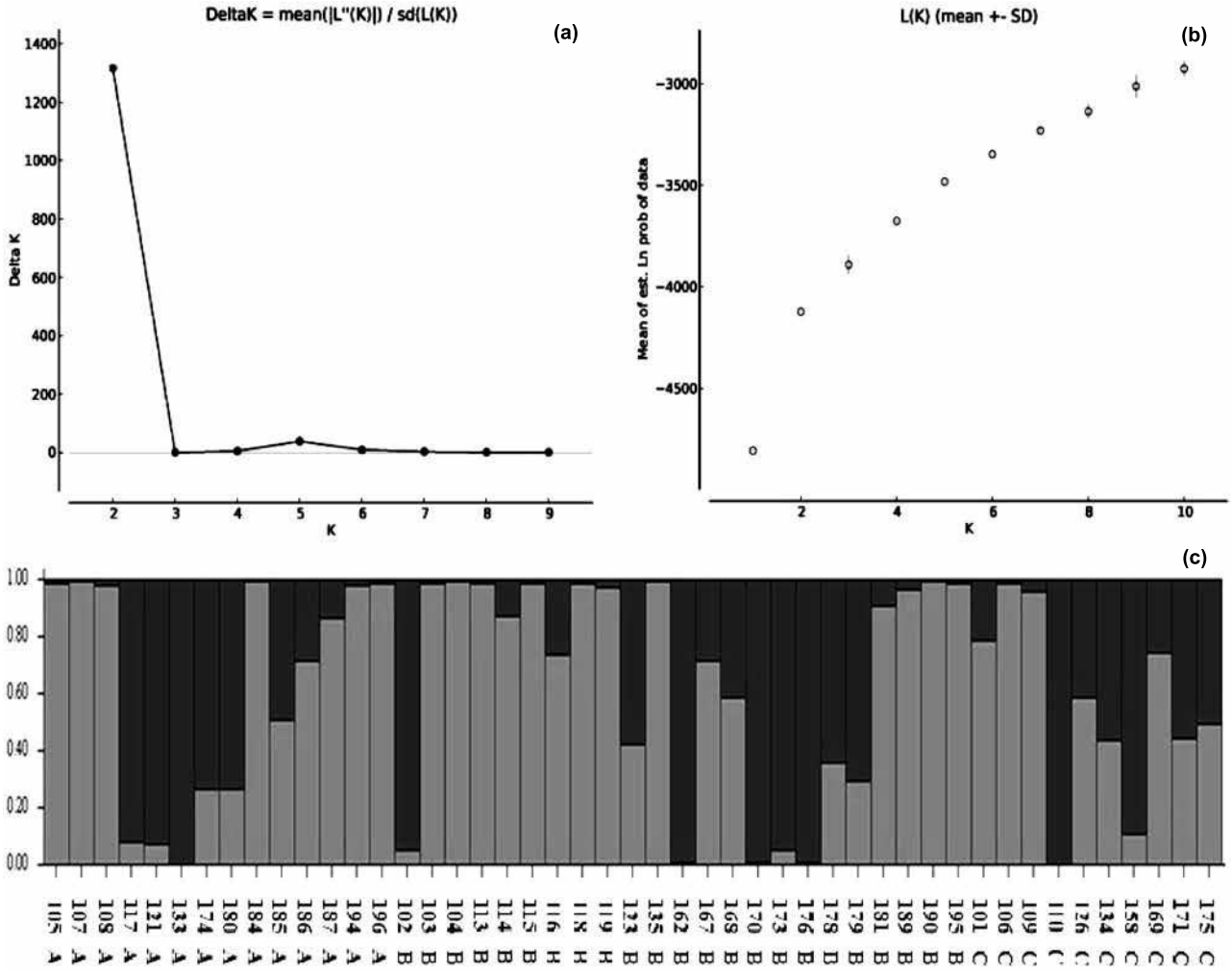

Fig. 5. STRUCTURE analysis (a: the relationship between K and delta K; b: the relationship between K and Ln P (D); c: Membership probability of assigning individuals of the all populations to different clusters when K = 10

apricots into three based on kernel taste and stone colour (Group-A, -B, -C).

SRAP markers efficiently distinguish apricot genotypes with a high level of polymorphism. UPGMA cluster analysis reflects that apricots of the Ladakh region are morphologically divergent. High GD within the group and less among the three groups was observed. The study, therefore, revealed that germplasm conservation should not be done purely based on kernel taste and stone colour.\

AUTHORS' CONTRIBUTION

Conceptualization of research (Tsering Stobdan); Designing of the experiments (Avilekh Naryal, Tsering Stobdan); Contribution of experimental materials (Avilekh Naryal, Ashwani Kumar Bhardwaj, Rohit Kumar); Execution of field/lab experiments and data collection (Avilekh Naryal, Ashwani Kumar Bhardwaj, Rohit Kumar); Analysis of data and interpretation (Avilekh Naryal, Ashish R. Warghat, K.B. Bhushan); Preparation of the manuscript (Avilekh Naryal, K.B. Bhushan, Tsering Stobdan).

DECLARATION

The authors declare no conflict of interest.

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