CHAPTER 6

MORPHOLOGICAL AND SRAP MARKERS BASED GENETIC DIVERSITY STUDIES OF APRICOTS OF TRANS-HIMALAYA

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 revealed that these genotypes have a similar genetic background. Knowledge gained from the present study has practical utility in the management of germplasm conservation and in breeding programs.

6.1 Introduction

Knowledge of genetic diversity (GD) is key for efficient preservation, management, and utilization of plant genetic resources [105]. Knowledge about genetic relationship and diversity among breeding materials could be useful in crop improvement strategies [106]. Ample GD in plants can provide a wide background for genetic research and crop breeding programmes [107]. Preservation of crop genetic resources is based on the continuous introduction of new genetic material from traditional and wild varieties for breeding of highly productive and resistant verities [108].

Cultivar characterization and discrimination are required for breeding and commercialization of apricot cultivars [109]. Knowledge of GD and relationship among the germplasm resource will be useful for protecting and utilizing local apricot varieties [110]. Several investigations have been carried out to determine diversity in apricots with pomological, phenological and morphological characters [66], [111]. 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 to one microclimate to others [112]. Therefore, for reliable identification and discrimination of genotype and cultivars, independent markers from the environmental factor are required. Accordingly, new methods based on the molecular studies must be included in breeding programs 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 analysis of plant GD and characterization. Among them, SRAP (Sequence-related amplified polymorphism) marker has been commonly used method for diversity study and population genetics analysis [113].

The SRAPs is an efficient and simple marker system and has several advantages over other markers system [113], [114]. SRAP targets coding sequences in the genome and results in a moderate number of co dominant markers [115]. The information given by SRAP markers are more conformable to morphological variability and evolutionary history of the morphotypes than of AFLP marker [116]. SRAP markers are more effective, quicker and less expensive over SSR marker [115].

The aim of the current work was to characterize the morphological and GD of apricot genotypes and to investigate the genetic and morphometric relationship among the genotypes to estimate the extent of GD in apricot genotypes between and within classified groups

according to kernel taste and stone colour. Furthermore, 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 marker along with morphological characters for assessment of structure and GD in apricots has not been reported.

6.2 Materials and methods

6.2.1 Plant material and DNA extraction

Leaf sample of 47 genotypes was collected from an experimental orchard in Ladakh and kept in -80^{0}C freezer until DNA extraction. Apricot genotypes were grouped into three. Fourteen genotypes fall under Group-A: brown stone and bitter kernel; 23 genotypes under Group-B: brown stone and sweet kernel and 10 genotypes under Group-C: white stone and sweet kernel. Genomic DNA was isolated using a CTAB method [117]. The DNA sample was diluted to the final concentration of 30 ng.µl^{-1} before PCR amplification. Further, the genotypic data was also used for a coalition with earlier determined morphological data of these 47 individuals as mentioned in Chapter 2.

6.2.2 SRAP analysis and PCR amplification

SRAP markers developed previously were adopted in this study [114]. Twenty primer combinations using seven forward (Me 1-7) and nine reverse (Em1-9) (Table 6.1) were tested and selected based on proper amplification and reproducibility for diversity studies. The PCR amplification was performed in PCR tubes with total volume of 20 µl SRAP PCR reaction consisted 0.9µM of primers, 0.2mM 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 (BioRad T100^{TM}) programmed with the initial step at 95^{0}C for 3 min followed by 5 cycles of three steps: 1 min of denaturing at 94^{0}C, annealing at 35^{0}C for 1 min and extension at 72^{0}C for 1 min. In the subsequent 35 cycles, the annealing temperature was increased to 50^{0}C and extension step consist of one cycle for 5 min at 72^{0}C. Amplified products were electrophoresed on 1.5 % agarose gel and 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^{TM} XR+).

Table 6.1: SRAP primers used for diversity studies of 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

6.2.3 Morphological and genetic data analysis

Six qualitative and 16 quantitative characters were used in the multivariate statistical analysis of morphological data (Table 6.2). A multivariate approach was used to classify the plant population based on quantitative morphological characters using Gower general similarity coefficient [118] in the PAST software (Paleontological statistics, Version 3.22).

The band with 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 [119] was used to calculate the different GD parameters: number of alleles (Na), effective number of alleles (Ne), Nei's genetic diversity (H), Shannon's information index (I), number of polymorphic loci (NPL), percentage polymorphic loci (PPL). In order 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 [120] using GenAlEx v. 6.3 software [121]. Interpopulation genetic distance and genetic identity were calculated by Nei's method using GenAlEx software. STRUCTURE version 2.3 [122], [123] was used to predict 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 K and for each simulation we have fixed burn-in period of 100000 steps followed by 250000 Monte Carlo Markov chain replicates. Results obtained

Table 6.2: 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

Variables	Mean ±SD	Min	Max	CV (%)
Fruit shape Lateral	6±3	1	8	43.4
Fruit shape Ventricle	4±1	2	5	32.5
Fruit shape of apex	3±1	1	4	32.7
Fruit skin colour	4±1	3	6	25.0
Flesh colour	4±1	3	6	20.9
Stone shape	2±1	1	5	45.2
Fresh fruit Wt. (gm)	20.2±8.5	5.0	41.9	41.8
Fresh stone Wt. (gm)	2.0±0.6	0.7	4.2	31.0
Moisture fruit %	71.4±7.6	48.7	84.7	10.7
TSS	23.7±5.5	14.5	36.8	23.3
Fruit Length (mm)	32.5±4.9	21.2	43.3	15.0
Fruit Width (mm)	32.6±5.0	21.5	44.0	15.3
Fruit Thickness (mm)	30.0±4.8	19.6	40.4	16.1
Stone Length (mm)	21.7±2.5	14.7	26.4	11.6
Stone Width (mm)	17.9±2.3	13.1	25.0	12.8
Stone Thickness (mm)	11.4±1.6	8.1	15.9	13.8
Blush area (mm)	223.6±247.7	0.0	831.5	110.8
Kernel Wt.(gm)	0.5±0.2	0.1	1.0	35.3
Kernel Length (mm)	15.1±1.7	10.7	18.3	11.3
Kernel Width (mm)	10.8±1.3	7.6	14.4	11.6
Kernel Thickness (mm)	6.2±1.3	3.1	9.9	20.6
Seed coat thickness (mm)	1.5±0.2	1.0	2.4	16.0

from STRUCTURE were interpreted by online available tool STRUCTURE HARVESTER [124] which implements Evanno's method [125] for calculation of a correct number of clusters (K).

6.3 Result and discussion

6.3.1 SRAP amplification

The selected 20 SRAP primer combinations pairs results in reproducible amplification and polymorphic bands were used to analysis polymorphism in 47 genotypes. A total of 134 bands were scored, out of which 115 (85.8%) bands were polymorphic (Table 6.3). 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. [112] (5.4 bands per primer set and 73% polymorphism rate), Pinar et al [126] (4.9 bands per primer set and 64.3% polymorphism rate). However, Ai et al. [114] obtain 19.1 bands per primer set and 88.11% polymorphism rate, which was greater than that of our study.

6.3.2 Genetic diversity

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 6.4). 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 total variance is partitioned resides within the population (92%) and only 8% of the variance was partitioned among populations (Table 6.5 and Figure 6.1). Thus, AMOVA analysis of SRAP marker suggests a better panmictic and not genetic divergent. The Nei's genetic distance and genetic identity of apricot groups are presented in Table 6.6. The highest value of genetic identity (0.9631) and the lowest genetic distance (0.0376) were 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) were observed between Group-A and Group-C.

Table 6.3: Polymorphism revealed by twenty SRAP primer combinations

Primer combination	Total band	Polymorphic band	Percentage of polymorphic band	Primer combination	Total band	Polymorphic band	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 6.4: 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 6.5: 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%

Figure 6.1: Total genetic variance analysis (AMOVA) of apricot based on SRAP results

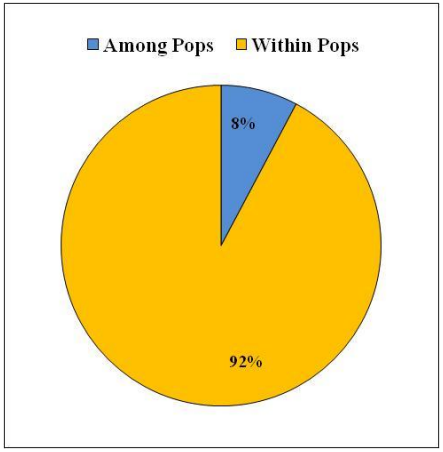


Table 6.6: Inter-population genetic distances and genetic identity calculated by Nei's method in apricot groups

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

Nei's genetic identity is above diagonal and genetic distances is below diagonal.

6.3.3 Morphometric and genetic relationship

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 (Figure 6.2). 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 subcluster. First and third subcluster shows intermixing of genotypes from Group-A and B, whereas Group-C is clustered into separate sub group.

To determine the genetic similarity among the genotypes, a dendrogram for the genotypes were obtained (Figure 6.3) with Jaccard similarity coefficient and UPGMA cluster analysis using 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 Ladakh region. Apricot genotypes were grouped into nonspecific groups and did not follow our earlier assumption of three groups based on kernel taste and stone colour.

Figure 6.2: UPGMA cluster analysis based on Gower similarty index from morphometric parameters

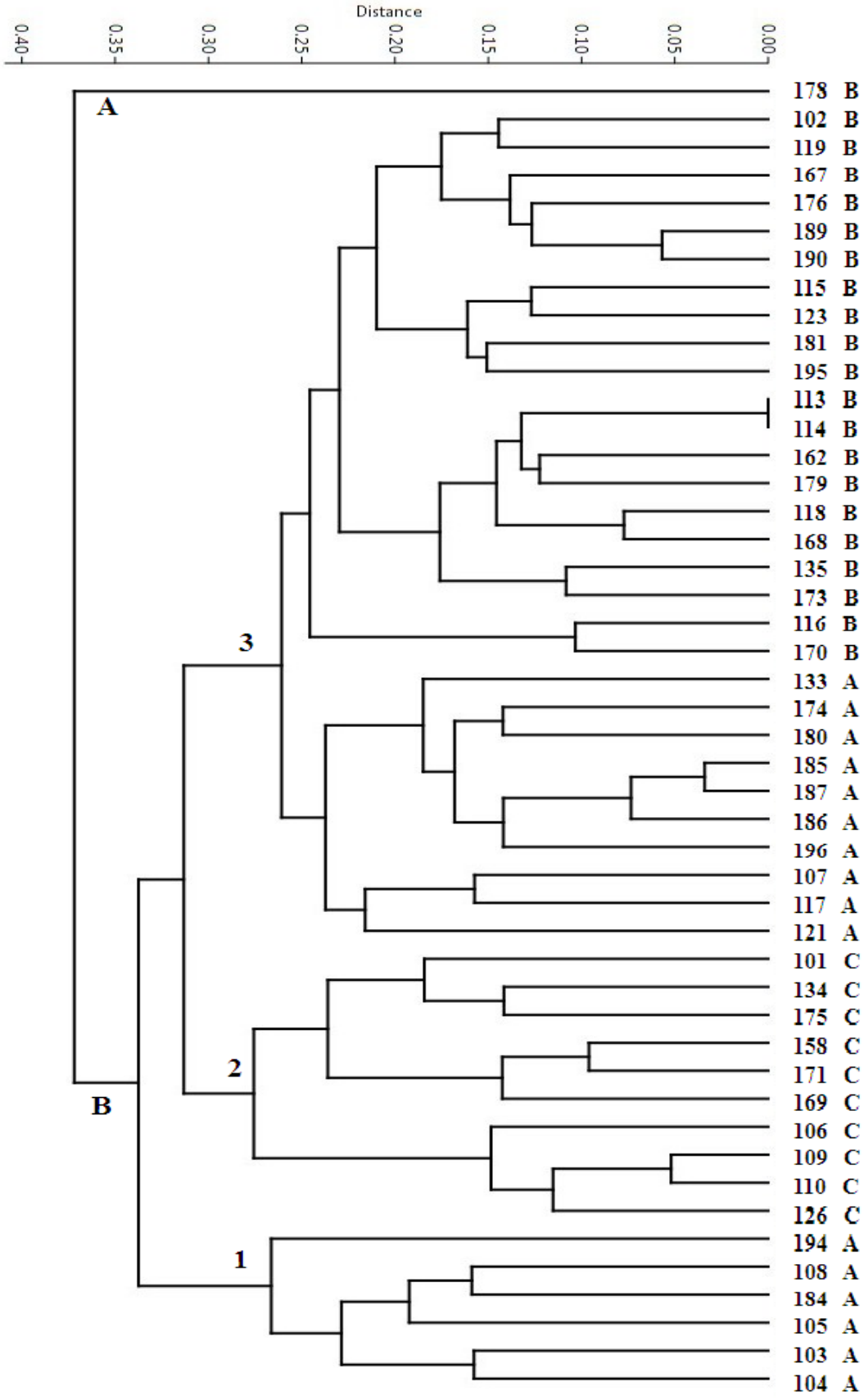
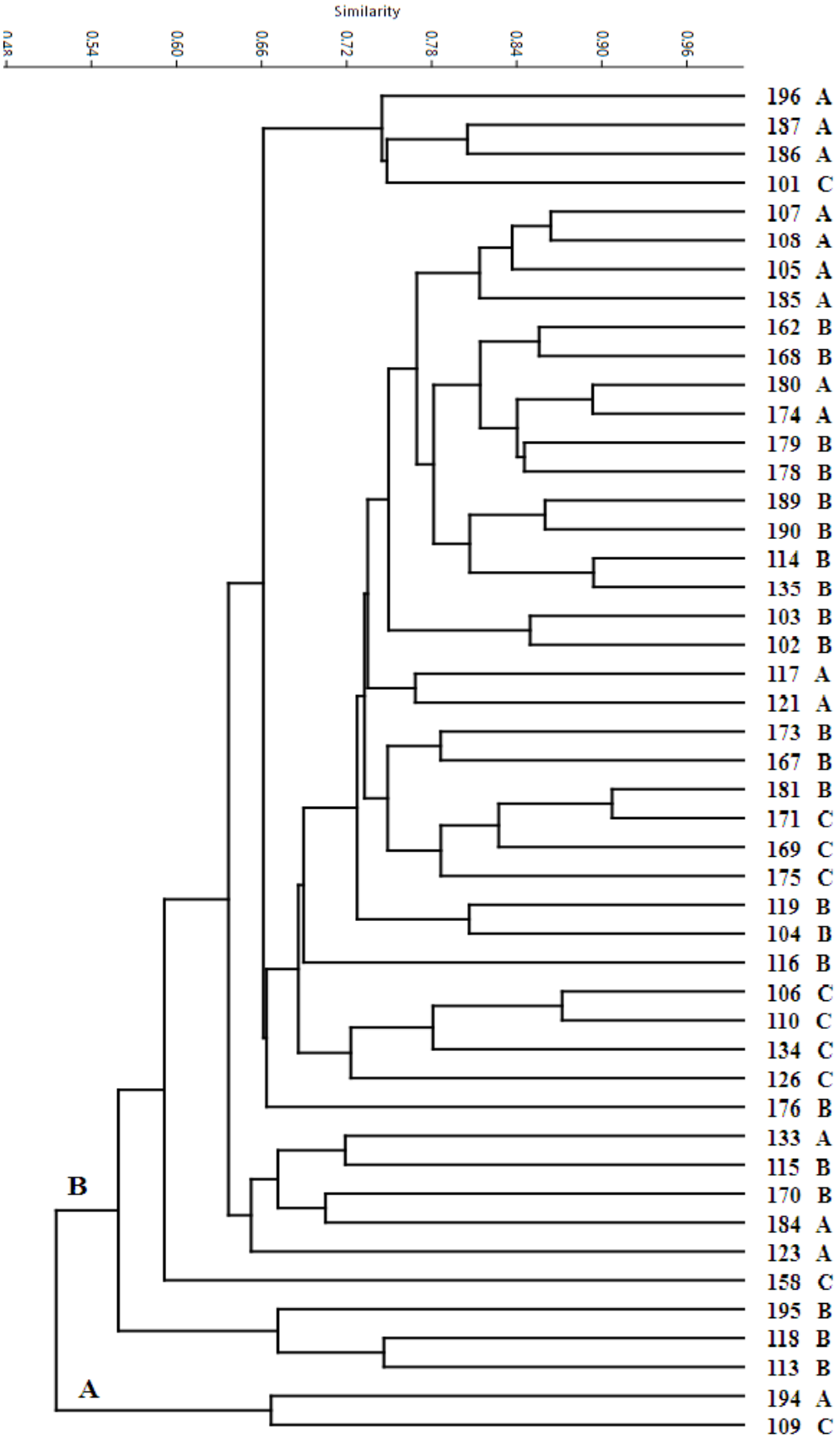


Figure 6.3 : SRAP based UPGMA cluster analysis by Jaccard similarity coefficient



6.3.4 Genetic structure

The genetic structure investigated on 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 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 (Figure 6.4). Structure analysis using SRAP markers revealed independent distribution of genotypes with respect to their groups. The study also indicates that apricot genotypes from trans-Himalayan region are highly genetically diverse. Structure analysis did not support our grouping of apricots into three based on kernel taste and stone colour (Group-A, -B, -C).

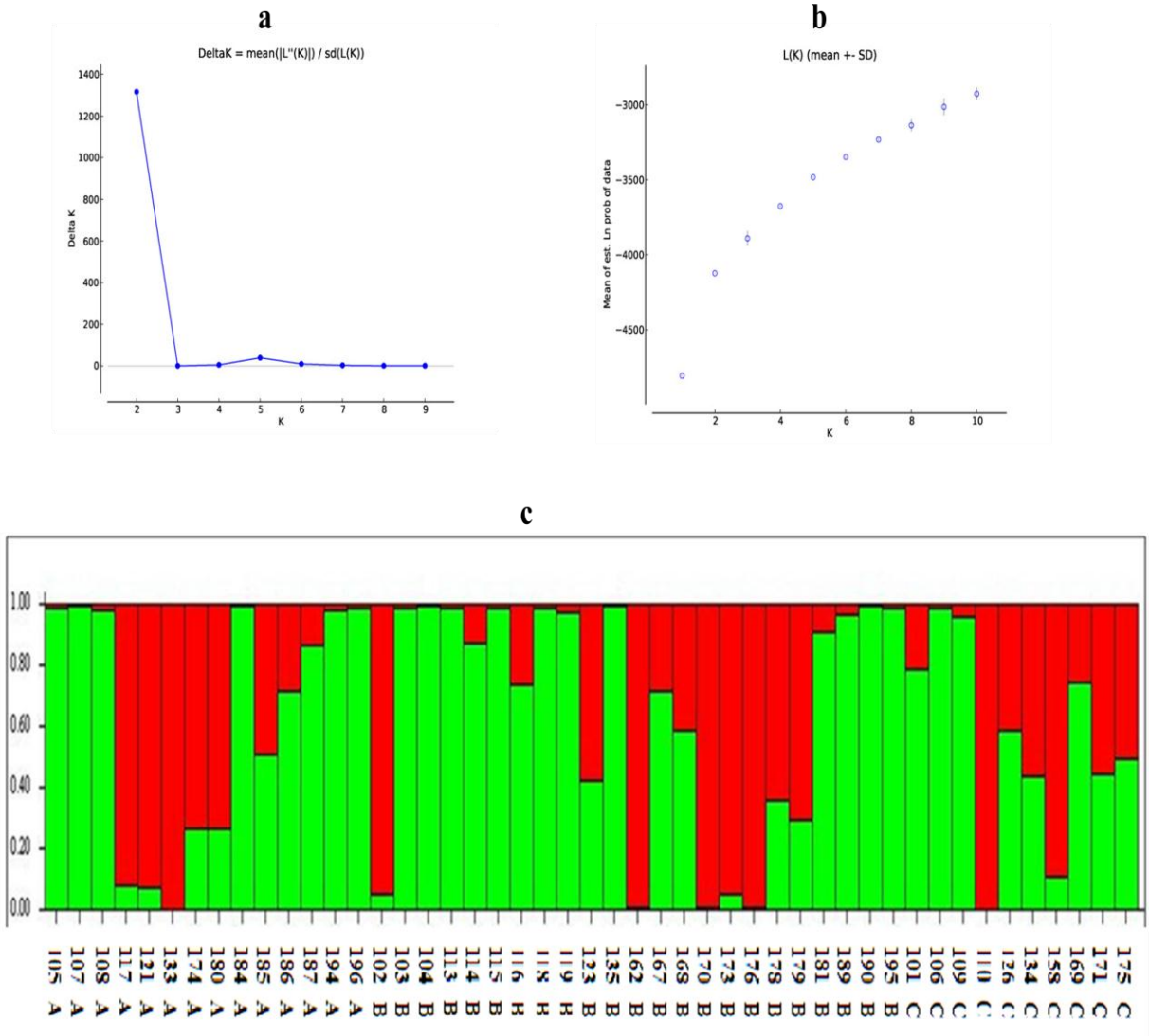


Figure 6.4: STRUCTURE analysis (a): the relationship between K and delta K; (b): the relationship between K and Ln P (D); (c): Membership probability of assigning individuals of the all populations to different clusters when K=10

6.4 Conclusion

SRAP markers efficiently distinguish apricot genotypes with a high level of polymorphism. UPGMA cluster analysis reflects that apricots of 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.