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Genetic diversity of Ambri apple variants of Jammu region in India

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ABSTRACT

The distinct germplasm of any crop species constitutes a vital genetic resource for extracting genes or alleles necessary to meet future nutritional and disease-resistance needs. In the present study, a total of 29 SSR markers were used to interpret genetic diversity among fifty Ambri apple variants of the Jammu region, wherein polymorphic information content (PIC), polymorphic percentage, and number of alleles per locus were calculated. Seventeen polymorphic SSR loci amplified 54 alleles, and the number of alleles ranged from 3 to 10, with an average of 5.35 alleles per locus. The mean PIC value for all loci was 0.64. The highest polymorphic percentage (62.50) was observed in primer CH04g10 and the lowest (14.20) in primer CH03d12. Jaccard's similarity coefficient among Ambri apple variants ranged from 0.14 to 0.74, which indicated a broad genetic base. The cluster dendrogram partitioned the cultivars into two main clusters. Of the 50 collected variants, a wide range of diversity was observed in respect of fruit weight (158.21 to 292.23 g), fruit length (4.35 to 6.37 cm), fruit width (4.62 to 7.62 cm), TSS (13 to 15.91°Brix), titratable acidity (0.30 to 1.7 percent), and ascorbic acid (2.10 to 4.80 mg/100g) content. The highest coefficient of variation was observed in titratable acidity (44.31 percent). Of the selected population, two variants, SKJAD-29 and SKJAD-30, proved promising for commercial cultivation.

Keywords: *Malus* × *domestica* Borkh., cluster analysis, SSR markers, variability

INTRODUCTION

The cultivated apple has believed to be originated from South West Asia. In India apple is cultivated on 307 thousand hectares area with the annual production of 2371 thousand metric tonnes (Anon,5). *Ambri* apple occupies a minuscule area and the acreage, this variety is dwindling fast due to genetic erosion, but its plantation still exist on higher altitudes. The plantation is old, and has continued to yield without any application of scientific package of practices. Around 33 apple varieties are grown in India, but due to poor shelf life, the accessibility of Indian apple (fresh) in markets is only restricted between July to November. *Ambri* an indigenous eminent dessert variety of apple and carry on with its supremacy by virtue of its sweet flesh, excellent aroma, crisp texture and prolonged storability (up to six months in ordinary storage under typical temperate areas).In Jammu and Kashmir, profile of fruit industry has been dominated by Red Delicious apple along with other delicious group varieties which occupies 60 per cent of the total area under apple. Presently *Ambri* apple existing in the form of different types is facing greatest loss of gene pool due to natural mortality or replacement by exotic precocious spur bearing cultivars. The introductions (exotic) have eliminated many locally adapted apple varieties in general and Ambri in particular. Special emphasis on saving genetically important indigenous *Ambri* types from extinction is therefore to be given utmost priority. Doda and Kishtwar being a temperate hot spot for apple diversity in Jammu region, therefore the survey was conducted to explore the diversity of the *Ambri* apple genotypes.

The traditional methods for characterization and assessment of genetic variability in perennial fruit crop species are time consuming and affected by the environment. The long juvenile period of apple makes the task of genotype characterization for different traits more difficult. To overcome these limitations, molecular markers have been used to differentiate, characterize, and identify apple accessions. Among the DNA-based markers, microsatellites or SSRs (simple sequence repeats) allow a high level of resolution in genetic studies due to their high polymorphism, co-dominant inheritance, reproducibility, and easy detection by PCR (Gupta *et al*.,12). These markers also have proven useful in the repository setting to examine potential redundancies and propagation errors within collections (Dangl *et al*.,8).The main objective of this study was to examine the degree of genetic diversity of *Ambri* apple population collected from Doda and Kishtwar districts of Jammu province to understand the genetic relatedness, and select the most promising accessions

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for its conservation and popularization for future apple improvement programme.

MATERIALS AND METHODS

The survey was done during the year 2017 - 2018 of districts Doda and Kishtwar of Jammu region, including the areas like Bhaderwah, Thathri, Gandoh, Bhagwah, Kishtwar, Dool, Nagseni, Mughalmadain and Chatroo ranging from 1272m to 1798m amsl (Table 1). Initially, a total of 150 naturally growing seedling trees were surveyed and 50 were selected on the basis of preliminary field observations. The fruits of *Ambri* apple variants with divergent characters were selected at commercial maturity of their growing sites. Total genomic DNA was extracted from young, fresh, disease and insect free leaves samples using the cetyl trimethylammonium bromide (CTAB) protocol of (Doyle and Doyle, 9). Quality and quantity of genomic DNA was estimated through agarose gel electrophoresis and nanodrop (Thermo scientific USA). A set of 29 SSR marker were used in the amplification of genomic DNA as listed in Table 2 along with primer sequences and annealing temperature. PCR was carried out an initial denaturation step of 5 min, followed by a loop of 35 cycles each consisting of denaturation (at 94ºC for 1 min), annealing (at 53 to 60ºC for 1 min and 40 sec), elongation (at 72ºC for 40 sec) and the final extension was performed (at 72ºC for 5 min). Then samples were held at 4ºC until the SSR fragments were separated by electrophoresis using 3 per cent agarose gel in 0.5X TBE buffer and visualized with ethidium bromide (1.0 μgml^{-1}) under UV light. The DNA bands were scored on the basis of relative mobility in gel, the allele difference was determined according to their fragment size (bp) corresponding to the 100 bp standard marker (Sigma Aldrich, U.S.A). The molecular data obtained were used to study in computer software program DARwin 5 (Perrier and Jacquemoud-Collet,17) for dendrogram construction. In order to check the informartiveness and discriminatory power of SSR markers used in this study, certain parameters like polymorphism information content, polymorphism percentage and alleles per locus were calculated. Fruit weight was recorded with the help of electronic weighing balance as average of 10 random mature fruits. TSS was calculated with the help of Erma hand refractometer (0-32^{o}C) according to standard to standard procedure as given in (A.O.A.C. 1) and titratable acidity (TA) was determined by neutralization to pH 7.0 with 0.1N NaOH. Ascorbic acid was estimated by titration method using 2,6-dichloroindophenol as suggested by (A.O.A.C. 2). The data were analysed using software Windowstat 9.3 version.

RESULTS AND DISCUSSION

A total of 54 alleles were amplified by 17 polymorphic SSR loci and the number of allelesranged from 3 to 10 with an average of 5.35 alleles per locus

Table 1. Location of fifty Ambri apple genotypes in Jammu region.

Genotypes	Place	Elevation (m amsl)	Coordinates
SKJAB 01, SKJAB 02, SKJAB 03, SKJAB 04, SKJAB 05	Bhaderwah	1753	N= 32°58.270 E =075°42.983
SKJAT 06, SKJAT 07, SKJAT 08, SKJAT 09	Thatri	1594	N= 33°11.079 E=075°28.575
SKJAG 10, SKJAG 11, SKJAG 12, SKJAG 13, SKJAG 14, SKJAG 15, SKJAG 16, SKJAG 17,	Gundoh	1669	N= 33°08.872 E=075°46.559
SKJAG 18, SKJABh 19, SKJABh 20	Bhagwah	1580	N= 33°08.851 E=075°46.580
SKJAK 21, SKJAK 22, SKJAK 23, SKJAK 24, SKJAK 25, SKJAK 26, SKJAK 27	Kishtwar	1739	N= 28°08.751 E=075°46.580
SKJAD 28, SKJAD 29, SKJAD 30, SKJAD 31, SKJAD 32, SKJAD 33, SKJAD 34, SKJAD 35, SKJAD 36	Dool	1652	N= 28°08.751 E=075°46.580
SKJAN 37, SKJAN 38, SKJAN 39, SKJAN 40, SKJAN 41, SKJAN 42	Nagseni	1561	N= 28°08.751 E=075°46.580
SKJAM 43, SKJAM 44, SKJAM 45, SKJAM 46, SKJAM 47	Mugalmaidan	1272	N= 28°08.751 E=075 46.580
SKJAC 48, SKJAC 49, SKJAC 50	Chatroo	1798	N= 28°08.753 E=075 46.580

Table 2. List of selected SSR primers along with their primer sequence.

S. No.	Primers	Forward primer (5'-3')	Reverse primer (5'-3')	Annealing Temperature (°C)
1	CH03g07	AAT AAG CAT TCA AAG CAA TCC G	TTT TTC CAA ATC GAG TTT CGT T	55
2	CH04e03	TTG AAG ATG TTT GGC TGT GC	TGC ATG TCT GTC TCC TCC AT	56
3	CH04g10	CAA AGA TGT GGT GTG AAG AGG A	GGA GGC AAA AAG AGT GAA CCT	55
4	CH05c02	TTA AAC TGT CAC CAA ATC CAC A	GCG AAG CTT TAG AGA GAC ATC C	55
5	CH05d11	CAC AAC CTG ATA TCC GGG AC	GAG AAG GTC GTA CAT TCC TCA A	55
6	CH05e03	CGA ATA TTT TCA CTC TGA CTG GG	CAA GTT GTT GTA CTG CTC CGA C	55
7	CH02h11a	CTGTTTGAACCGCTTCCTTC	CGTGGCATGCTTATCATTTG	50
8	CH03d12	ATTGCTCCATGCATAAAGGG	GCCCAGAAGCAATAAGTAAACC	50
9	CH03e03	AAAACCCACAAATAGCGCC	GCACATTCTGCCTTATCTTGG	53
10	CH03g12z	CAAGGATGCGCATGTATTTG	GCGCTGAAAAAGGTCAGTTT	50
11	CH04a12	ATCCATGGTCCCATAAACCA	CAGCCTGCAACTGCACTTAT	50
12	CH05d04	TCCG GGTATGCTTCGATT	ACTTGTGAGCCGTGAGAGGT	50
13	CH05d11	GAGAAGGTCGTACATTCCTCAA	CACAACCTGATATCCGGGAC	51
14	CH05e03	CAAGTTGTTGTACTGCTCCGAC	AAGTGCACCCACACCCTTAC	51
15	Hi01d06y	GGAGAGTTCCTGGGTTCCAC	GTTTAAGTTCGCCAACATCGTCTC	53
16	Hi02d04	TGCTGAGTTGGCTAGAAGAGC	GTTTGTTGCTGTTGGATTATGCC	51
17	Hi03e03	ACGGGTGAGACTCCTTGTTG	GTGCAGAGTCTTTGACAAGGC	53
18	CH03c02	TCACTATTTACGGGATCAAGCA	TGTCTCAAGAACACAGCTATCACC	51
19	Hi02b10	GTTTCTTGGAGGCAGTAGTGCAG	TGCTGAGTTGGCTAGAAGAGC	60
20	Hi02d04	GTTTAAGTTCGCCAACATCGTCTC	TGATGCATTAGGGCTTGTACTT	60
21	CH05c07	GGGATGCATTGCTAAATAGGAT	TTGTGGACGTTCTGTGTTGG	59
22	CH03a02	TTGTGGACGTTCTGTGTTGG	CAAGTTCAACAGCTCAAGATGA	60.6
23	CH02b12	GGCAGGCTTTACGATTATGC	CCCACTAAAAGTTCACAGGC	59.7
24	Hi03e03	ACGGGTGAGACTCCTTGTTG	GTTTAACAGCGGGAGATCAAGAAC	60
25	CN444636	CACCACTTGAGTAATCGTAAGAGC	GTTTAACAGCGGGAGATCAAGAAC	60
26	CH05g07	CCCAAGCAATATAGTGAATCTCAA	TTCATCTCCTGCTGCAAATAAC	60
27	CH02c02	CTTCAAGTTCAGCATCAAGACAA	TAGGGCACACTTGCTGGTC	60
28	Hi03a03	ACACTTCCGGATTTCTGCTC	GTTTGTTGCTGTTGGATTATGCC	60
29	CN444542	ATAAGCCAGGCCACCAAATC	GTTTGCAGTGGATTGATGTTCC	60

(Table 3).The PIC value in the present study ranged from 0.5 to 0.8 with an average of 0.64. Maximum (0.80) PIC value was observed in primer CH05d11 and Hi03a03 which showed this primer have power for analyzing the genetic variability in *Ambri* apple selections. In similar study was conducted by (Galli *et al*.11) recorded 55 polymorphic alleles were detected at the 6 SSR loci (average 9.2 alleles per locus) and the polymorphism information content (PIC) averaged 0.72. Farrokhi *et al.,* 10) also reported in Iranian apple that PIC varied from 0.18 to 0.76. The mean PIC value for all loci was 0.49. The PP (Polymorphic Percentage) value in the present study ranged from 14.20 to 50.00 with an average of 0.64 (Table 3). The dendrogram (Fig. 1) classified all genotypes into two main clusters, cluster I and cluster II with further sub clusters. The first group consisted of twenty six genotypes and second group consists of twenty three genotypes. One genotype SKJAG-12 does not the part of any cluster. Maximum number of genotypes were accommodated into cluster I (SKJAB-01, SKJAB-02, SKJAB-05, SKJAT-06, SKJAG-11, SKJAG-10, SKJAT-09, SKJAT-08, SKJAT-07, SKJAB-04, SKJAB-03, SKJABh-19, SKJABh-18, SKJAG-17, SKJAG-15, SKJAG-16, SKJAG-14, SKJAG-13, SKJAK-26, SKJAK-27, SKJAK-25,

Table 3. Genetic characterization of 17 microsatellite loci for fifty *Ambri* apple genotypes native to Jammu province.

S. No.	Primer	PIC (Polymorphism Information Content) value	PP (Polymorphic Percentage)	Allele per locus
1	CH04e03	0.50	50.00	6
2	CH05c02	0.50	20.30	5
3	CH05d11	0.80	40.00	10
4	CH05e03	0.70	20.00	5
5	CH02h11a	0.60	33.33	3
6	CH03d12	0.70	14.20	7
7	CH03e03	0.70	33.31	9
8	Hi02d04	0.76	33.33	3
9	Hi03e03	0.71	33.30	3
10	Hi02b10	0.60	40.00	5
11	Hi02d04	0.70	25.00	5
12	CH05c07	0.62	33.38	3
13	CH03a02	0.52	33.35	3
14	CH05g07	0.50	40.00	5
15	CH02c02	0.61	42.85	7
16	Hi03a03	0.80	25.00	8
17	CN444542	0.61	25.00	4
	Range	0.5-0.8	14.20-50.00	3-10

SKJAK-24, SKJAK-23, SKJAK-22, SKJAK-21 and SKJABh-20), followed by 23 genotypes in cluster II (SKJAC-50, SKJAC-49, SKJAM-48, SKJAM-47, SKJAM-46, SKJAM-45, SKJAM-44, SKJAM-43, SKJAN-42, SKJAN-41, SKJAN-40, SKJAN-39, SKJAN-38, SKJAN-37, SKJAD-35, SKJAD-34, SKJAD-33, SKJAD-36, SKJAD-32, SKJAD-31, SKJAD-30, SKJAD-29 and SKJAD-28). Cluster I was further divided into two sub clusters containing 8 and 19 variants. Cluster II was further divided into two sub cluster with 9 and 15 variants. Dendrograms are efficient means of summarizing microsatellite data can reveal relationships including identical genotypes.. On the basis of the cluster analysis, *Ambri* apple genotypes used in the present study showed a wide genetic base owing to the cross pollination behaviour of apple. The similarity matrix suggesting a rich genetic variation, hence can be used as prospective parents in further breeding programme to get segregates. Similar results were obtained by (Farrokhi *et al.*, 10) who revealed that 45 alleles were generated at sixteen SSR loci with polymorphism information content (PIC) content ranged between 0.18 to 0.76 in apple. Mean polymorphism information content (0.49) was recorded for all loci. Jaccard's similarity coefficient varied from 0.19 to 0.79 in apple cultivars. Polymorphism percentage among fifty *Ambri* apple genotypes revealed the highest polymorphic percentage (62.50 per cent) in primer

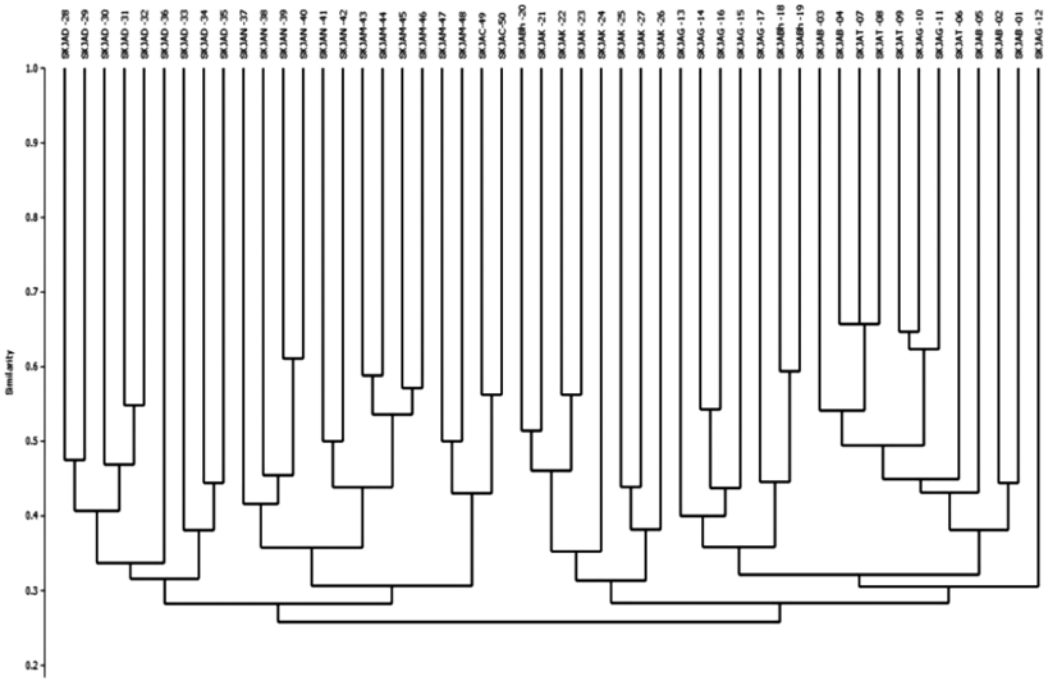

Fig. 1. Dendrogram showing clustering pattern of fifty Ambri apple genotypes using UPGMA analysis based on SSR genotyping.

CH04g10 while lowest polymorphic percentage (14.20 per cent) was observed in primer CH03d12. Similar results were obtained by (Cokran *et al*., 7) in apple where thirty RAPD primers amplified 207 bands, of which 91 were polymorphic (40.1 per cent), 10 SSR primers produced 33 bands and 26 of them were polymorphic (78.78 per cent) and 5 AFLP combinations amplified 183 bands of which 88 were polymorphic (48.08 per cent).

During the present study, huge variations were observed in respect of fruit length (4.35 cm to 6.37 cm) and fruit width (4.62 cm to 7.62 cm) and fruit weight (158.21 g to 292.23 g) in the tested population (Table 4). These results are in agreement with the results of (Sharma *et al.,18*) who found wide range of diversity in *Ambri* apple in respect of fruit weight (86.90 to 334.97 g), fruit length (59.13 to 84.00 mm), fruit width (68.21 to 94.31 mm). It is a well known fact that the genetics, environment and cultural practices all interact to determine fruit weight.. The maximum TSS (15.91 °Brix) was recorded in genotype SKJAD-30 whereas, minimum TSS (12.57 °Brix) was recorded in SKJABh-18. Similar observations were recorded by (Sharma *et al*.,18) who revealed that among the thirty four collected variants of Ambri apple, a wide range of TSS (10.90 to 20.00 ºBrix) was observed. (Bostan,6) reported that SS (Soluble Solids) values from 10.50 to 15.00 per cent in apple. (Mratinic, 16) also reported in apple that soluble solid content (SS) varied from 12.55 to19.24 per cent and total solids (TS) were between 8.65 and 12.18 per cent. TSS is influenced by environmental factors such as temperature, light (duration and intensity), rainfall/supply of water and locations (Ahmed, 3)

Titratable acidity among the different *Ambri* apple genotypes showed wide variability. SKJAG-17 variant exhibited the highest titratable acidity (1.72 per cent) while minimum titratable acidity (0.30 per cent) was observed in genotype SKJAD-29 followed by the genotypes SKJAK -22 (0.31 per cent), SKJAD -32 (0.32 per cent) and SKJAD -31 (0.33 per cent). (Mratinic, 16)) also reported that titratable acidity (TA) content was observed between 0.10 and 0.82 per cent in apple. (Hassan *et al.,* 13) who studied morphological characterization of apple accessions observed that acidity varied from 0.06 to 0.79 per cent in Kashmir region.

Ascorbic acid content ranged from 2.10 mg/100g (SKJAG -17) to 4.80mg/100g (SKJAN-39). Similar results were obtained by (Ahmed *et al*.,4)) in pear who found significant differences among the genotypes in respect of vitamin C. Vitamin C content in fruits can be regulated by various factors such as genotypic variation, pre-harvest climatic factors, maturity and harvesting methods. Alteration in day temperature at different localities might be responsible for variability for vitamin C contents. Fruits of high altitude with low temperatures showed better in terms of ascorbic acid than low lying and warm localities.

An insight of data revealed that highest TSS/Acid ratio (49.38) was observed in genotype SKJAK-22, while it was lowest (8.52) in SKJAD-29. These results are close conformity with the results of (Misger *et al.,*15) who reported that Coscia cultivar of pear exhibited the highest TSS/acid ratio (48.80) and lowest (28.260)in Red Anjou.

Two genotypes SKJAD-29 and SKJAD-30 showed superiority over selected genotypes with respect to leaf, fruit and quality parameters (Plate 1). The existing biochemical diversity could be explored as pre indices for identification of biotic and abiotic stress tolerance ability of the studied *Ambri* apple genotypes. All the observations made in this study will provide valuable evidence for decision making in choosing of markers for future work, characterization of germplasm, breeding and apple germplasm management.

AUTHORS' CONTRIBUTION

Conceptualization of research (KD, KK, PB), Designing of the experiments(KK, PB), Contribution of experimenta materials (KD, KK, PB), Execution of field/lab experiments and data collection(KD),

Table 4. Physico-chemical fruit traits variation in *Ambri* apple genotypes.

Character	Range	Mean	Standard deviation	Coefficient of variation (%)
Fruit weight (g)	158.21-292.23	228.93	26.78	11.74
Fruit length (cm)	4.35-6.37	5.60	0.46	8.21
Fruit width (cm)	4.62-7.62	6.22	0.81	13.02
Total Soluble Solids (°Brix)	13.00-15.91	15.01	0.86	5.72
Titratable acidity (%)	0.30-1.7	0.88	0.39	44.31
Ascorbic acid (mg/100g)	2.10-4.80	2.77	0.71	25.92
TSS/Acid ratio	8.52-49.38	22.02	10.52	47.77

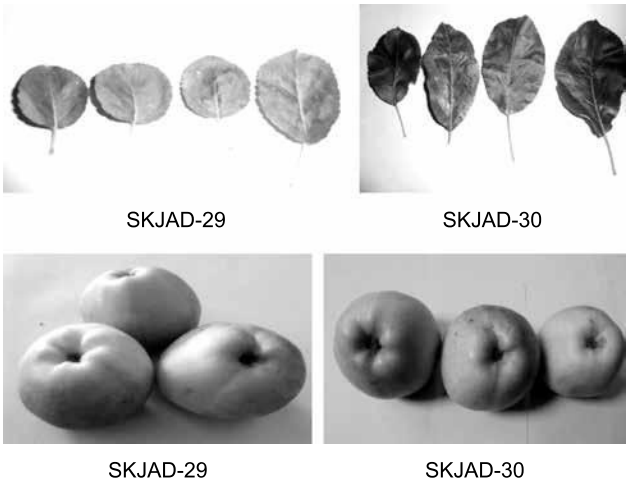

Plate 1. Morphological variations in leaf and fruit characteristics between promising *Ambri* variants.

Analysis of data and interpretation (KK, MS, BC, BKS), Preparation of the manuscript (KD, KK, PB)

DECLARATION

The authors declare no conflict of interest.

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