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Original article

Assessment of the Genetic Diversity of Apple (*Malus* × *domestica* Borkh.) Cultivars Grown in the Kashmir Valley using Microsatellite Markers

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ABSTRACT

The diverse germplasm of any crop species represents an important genetic resource for mining genes or alleles necessary to meet future nutritional and disease resistance needs. A total of 29 SSR markers were used to elucidate genetic diversity among nineteen apple cultivars for the first time in the Kashmir valley. Different parameters like polymorphic information content, resolving power and marker index were calculated. A total of 218 polymorphic fragments were obtained. A high level of genetic diversity was observed in these 19 cultivars with 218 polymorphic fragments, between three and 14 alleles per primer pair, averaging 7.51 alleles per SSR. Cultivars differentiated through mutations like Oregon Spur, Reeka Red and Siliver Spur were also used as experimental cultivars in the present study and had identical allelic compositions at all loci. The cluster dendrogram and principal component analysis partitioned the cultivars into two main clusters based on Jaccard's similarity coefficient. These findings will have impact on apple breeding and conservation programs as the present sample of apple cultivars are commercially very important at national and international level. So their characterization at morphological, biochemical, cytological and molecular level will help the apple breeders to use these in apple breeding.

1. Introduction

Apple is an important fruit crop in Kashmir Valley and ranks first in production as well as export among all the fruits in the region (hortikashmir.gov.in). It is one of the four most important fruit crops after citrus, grapes and banana, and one of the commercially most important horticultural crops in the temperate parts of the world (O'Rourke, 2003). Apple varieties are grown throughout the world including Central and West Asia, India, Western provinces of China, Europe and parts of America and Africa (Juniper et al., 1999). In India, apple is mainly grown in Jammu and Kashmir (the leading area), Himachal Pradesh, Uttarkhand, Arunachal Pradesh and Nagaland.

The cultivated apple in Kashmir is comprised of different groups of cultivars such as Delicious, Ambri, and Trel etc. In each type one or few cultivars are only commercially successful e.g. Kashmiri Ambri, American Trel, and Red Delicious etc. The rest of the cultivars in each group are sold in the market under the trade name of well-known cultivars. The monoculture of a few cultivars like Red Delicious, Kullu Delicious, Golden Delicious, American etc. associated with other constraints in the state like Apple Scab, Alternaria, Powdery Mildew and lack of cold chain storage have resulted in loss of diversity and depletion of indigenous apple germplasm and a number of apple cultivars are at the brink of extinction (Bhat et al., 2011). It is therefore important to characterize cultivars of each group so that well known cultivars are clearly distinguished from less known and commercially unsuccessful cultivars. The new cultivars with better characteristics could be identified and promoted to commercial level. The objective of this work was to analyze the genetic diversity of 19 apple cultivars in Kashmir with special reference to Ambri and Delicious cultivars using molecular markers. The information generated will help unambiguously to identify cultivars from each other.

Different types of molecular markers like RAPD, SSR, ISSR, AFLP, RFLP etc. have been used to assess the genetic diversity in crop species. The choice of the technique depends upon the objective of the study, financial constraints, skills and available facilities (Kafkas et al., 2008; Pavlovic et al., 2012). Among the different types of

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molecular markers, microsatellites have proved to be more reliable for DNA fingerprinting due to co-dominant inheritance, high polymorphism, abundance (Fernandez et al., 2009), reproducibility and relative ease of analysis (Schlotterer, 2004). SSR markers have been used to identify and determine genetic diversity and relationships among *Malus* × *domestica* accessions (Gasi et al., 2010; Patzak et al., 2012). Fougat (1984) and Raina (1989) characterized apple germplasm of Kashmir valley on the basis of morphology and cytology whereas Najar (2007) evaluated some apple germplasm of Kashmir Valley by ISSR based molecular markers. In the present study, SSR markers were used for the first time to identify and assess genetic diversity of apple germplasm from the Kashmir Valley.

2. Materials and methods

A total of nineteen apple cultivars (Table 1) were selected for the present study on the basis of high commercial importance in the apple market. They are sold at very high price and are also exported outside of the state to India and consist of the 'Delicious' (indicated by D) and the 'Ambri' (indicated by A) groups. These cultivars were identified in private orchards and at the Govt. horticultural Nurseries of Kashmir. A single tree of each cultivar was selected and labeled with an accession number for collection of leaf samples for DNA extraction.

2.1. DNA extraction and purification

DNA was isolated from young leaf samples using the cetyl trimethyl ammonium bromide (CTAB) protocol of Doyle and Doyle (1990). The extracted DNA was treated with RNase to remove the RNA. The DNA quantity was estimated after separation in 0.7% agarose gel stained with ethidium bromide in the presence of different known concentrations of lambda (λ) DNA. The final concentration of all the DNA samples was adjusted to50 ng μl^{-1} for subsequent PCR.

2.2. SSR analysis

For SSR analysis, PCR reaction mixture was prepared in 200 μl tubes. Final concentrations of the reagents were as follows: 1x PCR buffer, 1.5 mM $MgCl_2$, 200 μM of each dNTP, 0.5 μM of each

Table 1
Apple cultivars, codes, geo-coordinates, accession numbers, collection sites and districts of investigated cultivars.

Cultivar	Code	Latitude	Longitude	Accession No.	Collection Site	District
Red Delicious	D_1	34°02′N	74°53′E	RED DEL ZOU	Zoura	Srinagar
Kullu Delicious	D_2	33° 57′N	74° 30′E	KUL DEL HAR	Hardu suresh	Budgam
Shimla Delicious	D_3	34° 15′N	74° 83′E	SHIDEL ZAK	Zakura	Srinagar
Golden Delicious	D_4	34° 09′N	74° 33′E	GOL DEL ZAN	Zangam Pattan	Baramullah
Cross Delicious	D_5	34°02′N	74°53′E	CRO DEL ZOU	Zoura	Srinagar
Molies Delicious	D_6	34° 18′N	74° 83′E	MOL DEL HOD	Hodura	Gandarbal
Gole Delicious	D_7	34°18′N	74° 86′E	GOL DEL WAD	Wadimohalla	Srinagar
Balgarian Delicious	D_8	34° 18′N	74° 83′E	BALDEL BAK	Bakura	Ganderbal
Oregon Spur	D_9	34° 09′N	74° 33′E	ORE SPU ZAN	Zangam pattan	Baramullah
Reeka Red	D_{10}	33° 72′N	74° 82′E	REE RED DAS	Dashpora Shopian	Shopian
Siliver Spur	D_{11}	34° 09′N	74° 33′E	SIL SPUZAN	Zangam Pattan	Baramullah
Kashmiri Ambri	A_1	34°02′N	74°53′E	KAS AMB ZOU	Zoura	Srinagar
Lal Ambri	A_2	34°02′N	74°53′E	LAL AMB ZOU	Zoura	Srinagar
Ambri Cross	A_3	34°02′N	74°53′E	AMB CRO ZOU	Zoura	Srinagar
Balgarian Ambri	A_4	33° 72′N	74° 82′E	BAL AMB SHO	Shopian	Shopian
Vilayati Ambri	A_5	34° 09′N	74° 33′E	VIL AMB ZAN	Zangam pattan	Baramullah
Delicious Ambri	A_6	33° 72′N	74° 82′E	DEL AMB SHO	Shopian	Shopian
Dudh Ambri	A_7	34°02′N	74°53′E	DUD AMB ZOU	Zoura	Srinagar
High Density Ambri	A_8	33° 72′N	74° 82′E	HIG AMB SHO	Shopian	Shopian

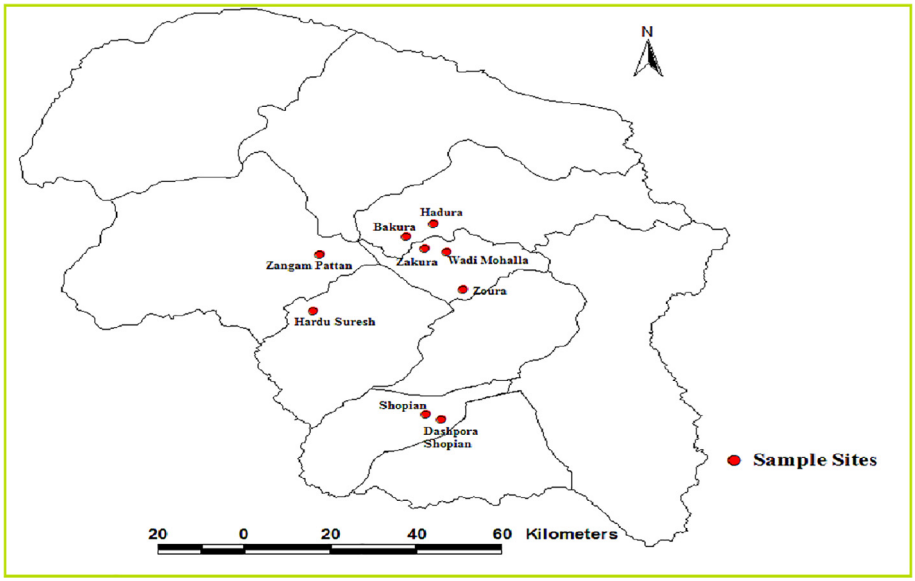

Fig. 1. Map of Kashmir Valley showing collection sites of apple cultivars. Source: SOI Toposheet 1971.

Table 2
SSR primers with various parameters revealing the discriminatory power of each primer.

Primer	Forward sequence (5′–3′)	Allele Range	NA	NPA	PIC	MI	RP
Hi05c06	F ATTGGAACTCTCCGTATTGTGC R ATCAACAGTAGTGGTAGCCGGT	143–183	5	5	0.45	2.25	2.319
Hi05d10	F AATGGGTGGTTTGGGCTTA R GTTTCTTTGGCTATTAGGCCTGC	147–362	13	13	0.31	4.03	5.263
Hi06f09	F AACCAAGGAACCCACATCAG R GTTTCACTTACACACGCACACACG	290–297	3	3	0.48	1.44	1.265
GD147	F TCCCGCCATTTCTCTGC R GTTTAAACCGCTGCTGCTGAAC	158–172	8	8	0.32	2.56	3.269
Hi08h03	F GCAATGGCGTTCTAGGATTC R GGTGGTGAACCCTTAATTGG	150–172	4	4	0.37	1.48	1.897
Hi02a07	F TTGAAGCTAGCATTTGCCTGT R TAGATTGCCCAAAGACTGGG	129–300	8	8	0.19	1.52	1.894
Hi01c06	F TTAGCCCGTATTTGGACCAG R GTTTCACCTACACACACGCATGG	144–163	5	5	0.40	2.00	3.241
Hi06b06	F GGTGGGATTGTGGTTACTGG R GTTTCATCGTCGGCAAGAACTAGAG	171–283	10	10	0.27	2.70	3.473
Hi02d11	F GCAATGTTGTGGGTGACAAG R GTTTGCAGAATCAAAACCAAGCAAG	210–275	6	6	0.33	1.95	3.157
Hi08c05	F TCATATAGCCGACCCCACTTAG R GTTTCACACTCCAAGATTGCATACG	173–265	9	9	0.34	3.06	4.315
Hi08a04	F TTGTCCTTCTGTGGTTGCAG R GTTTGAAGGTAAGGGCATTGTGG	178–266	4	4	0.46	1.84	1.371
Hi08f12	F GGTTTGTAACCCGTCTCTCG R GTTTCGTAGCTCTCTCCCGATACG	129–235	14	14	0.22	3.08	3.894
Hi08e06	F GCAATGGCGTTCTAGGATTC R GTTTGGCTGCTTGGAGATGTG	150–184	5	5	0.36	1.80	3.055
Hi23b12	F TGAGCGCAATGACGTTTTAG R GTTTCAGGCTTTCCCTTCAGTGTC	157–222	6	6	0.21	1.26	2.21
Hi11a01	F ACCGCCAAATGCTTTGTTAC R GTTTCCTCCATTAAACTCCTCAGTG	227–240	4	4	0.45	1.80	2.002
AU223486-SSR	F TGACTCCATGGTTTCAGACG R AGCAATTCCTCCTCCTCCTC	222–228	5	5	0.36	1.80	2.424
Hi23d02	F CCGGCATATCAAAGTCTTCC R GTTTGATGGTCTGAGGCAATGGAG	174–234	4	4	0.42	1.68	2.423
CH03b01	F ACAAGGTAACGTACAACTCTCTC R GTCACAAAACCGCCAGATG	158–234	10	10	0.29	2.90	3.684
U78948-SSR	F GATCGTCCGCCACCTTAAT R AGGGTTTTCATCATGCACATT	231–265	6	6	0.39	2.34	2.53
CH03ho6	F TTGTCCCTTTTTACGTCTTTCC R GTTATTGAGCAAGGCGGAGA	163–191	13	13	0.26	3.38	4.210
CH02e12	F CCAACTTTTTCTGCGGTAGTG R TGGGACCCATATGGTTGAATAC	178–234	9	9	0.25	2.25	2.842
CH04C03	F TGCACACCAAACACAGGACT R TATCAAACATTGGGGCACTG	212–246	4	4	0.42	1.68	2.423
CH04a06	F AGAAAATCTAAGAGCAGCAG R TAAAACTCAAGTCGCCCGTC	123–252	8	8	0.29	2.32	2.947
CH04d11	F ATTAGGCAATACACAGCAC R GCTGCTTTGCTTCTCACTCC	110–163	8	8	0.26	2.08	2.441
CH04d08	F AATTCCACATTCACGCATCT R TTGAAAGACGGAAACGATCA	131–159	8	8	0.32	2.56	3.473
CH04F03	F CTTGCCCTAGCTTCAAATGC R TCGATCCGGTTAGGTTTCTG	177–207	10	10	0.30	3.00	2.894
CH04e12	F CCTGAAATCTGCACAACTACCA R GGTGGTGAAGAAGTAGACAGCC	242–251	6	6	0.34	2.04	2.425
CH04F07	F CAGATCATGAATGATTGAAA R GAAAATCACACCCTCAAACCAT	96–202	10	10	0.22	2.20	2.631
CH04F04	F GTCGGTCACAACTCAGGACC R CGACGTTCGATCTTCCTCTC	166–240	13	13	0.25	3.25	4.105
Average/primer			7.51	7.51	0.32	2.28	2.89

NA: Number of alleles; NPA: Number of polymorphic alleles; PIC: Polymorphic information content; MI: Marker index; RP: Resolving power

Table 3
Allelic composition of nineteen apple cultivars amplified by 19SSR primer pairs.

Primer	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11
Hi05c06	173,178	173,178	173,178	173,183	173,178	173,183	173	173,183	173,178	173,178	173
Hi05d10	260,270,357,362	268,270,338,362	268,270,338,362	229,339	246,268,338,362	339	246,260,357	229,339	268,338	268,270,338,362	268,270
Hi06f09	297	297	297	290,297	291,297	290,297	291	297	297	297	297
GD147	158	158,172	158,172	158,160	162,172	158,170	158,162	158,168	Nil	158	Nil
Hi08h03	172	172	172	171	172	171	172	171	172	172	172
Hi02a07	300	Nil	Nil	129,135	277	129,131,133	277	133	Nil	Nil	Nil
Hi01c06	145,163	145,163	145,163	160	145,163	144,156,160	163	160	145,163	145,163	145,163
Hi06b06	252,275	258,275	258,275	251	252,258	251,270	252	251	258,275	258,275	258,275
Hi02d11	215,275	265,275	265,275	214	215,275	210	215,261	214	265,275	265,275	265,275
Hi08c05	247,257	247,251,257	247,251,257	253,265	247,257	256	247,251	250,256	251,257	247,251,257	247,251,257
Hi08a04	263,266	263	263	263,266	263	263	263,266	263,266	263	263	Nil
Hi08f12	159	159,172	147,159,172	129	162,172	129,231	159,162	145	147,159,172	147,159,172	159,172
Hi08e06	151,172	151,172	151,172	150	151,172	150	151,172	150	151,172	151,172	151,172
Hi23b12	157	159	159	170	Nil	170	186	157,170	159	159	159
Hi11a01	231,234	234	234	231,234,240	234,240	234	231,234	231,240	234	234	234
AV223486-SSR	223,227	227	227	222,225	223,227	222	223	225	227	227	227
Hi23d02	231,234	234	234	174,180	234	174	231,234	174	234	234	234
CH03b01	158,172,180,198	158,198	158,198	179	180,198	179,181	180	177,183	158,198	158,198	158,198
U78948-SSR	231,234	234	234	262,265	234,263	262	231,234	262,265	234	234	234
CH03ho6	174,184	174,190	174,190	163,173,183	170,172,174	173,191	180,182,184	163,183	174,190	174,190	174,190
CH02e12	180,218	180	180	178	180,218	208,216	218	214	180	180	180
CH04C03	215	215	215	212	215	212	215	212,214	215	215	215
CH04a06	124	124	124	123,125	124,142	123,141	128,142	123	124	124	124
CH04d11	155	155	155	154	155	110,154	143,155,160	147	155	155	155
CH04d08	148	131,148	131,148	142,152	131,159	132,152	152	132,154	131,148	131,148	131,148
CH04F03	202	202,204	202,204	201,207	193,202	201,203	177,202	195,201	202,204	202,204	202,204
CH04e12	243	243	243	242,246	243,251	242	243,251	246,250	243	243	243
CH04F07	124	124,159	124	104,112	124	110,112	178,202	96	124	124	159
CH04F04	167,180,218	180,234	180	166,178	180,218	184,208	167,218,231,234	166	180,185	180,185	180,185,234
Primer	A1	A2	A3	A4	A5	A6	A7	A8			
Hi05c06	173,178	173,183	145,178	143,178	173,178,183	173,183	173,183	183			
Hi05d10	240,256,338	229,339	151,160,270,338	147,256,268,338	256,268,338	339	339	339			
Hi06f09	291	297	291,297	297	291,297	290,297	290	297			
GD147	162,166	158,168	166,172	158,168	162,166,172	166,172	162,166	158,168			
Hi08h03	172	150	158	158	172	171	Nil	171			
Hi02a07	277	129,133	Nil	263	298	135	131,135	133			
Hi01c06	163	160	145,163	163	145,163	144,160	160	160			
Hi06b06	252,283	251,276	171,272	171,275	252,275	251,274	251	251			
Hi02d11	215	214	215,265	215,275	215	214,262	214	214			
Hi08c05	247,251,257	250,256	173,251,257	173,282	251	250	256	250,256			
Hi08a04	263,266	263,266	180,266	178	263,266	263,266	263,266	263,266			
Hi08f12	162,166	145	180,176	182,168,176	166,172	145,235	145	145			
Hi08e06	151,155,172	150	184,172	184,172	151,155,172	150,154	150,154	150			
Hi23b12	159	157,170	222	222,172	159	157	170	170			
Hi11a01	234	231,240	227	227	231,234	234	234	231,240			
AV223486-SSR	223,227	225	Nil	228,227	227	225	222,225	225			
Hi23d02	234	174	Nil	231	231,234	174,180	174,180	174			
CH03b01	180	177,183	234,180	234,180	158,180,198	179,197	179	Nil			
U78948-SSR	234	262,265	234	231,240	234	262,265	262,265	Nil			
CH03ho6	172,182	163,183	174,182	164,184	172,174,182	173,181	171,183	163,183			
CH02e12	216	214	180,216,234	208,210,216,218	180,216	178	214	204			
CH04C03	215	212,214	246	246	215	212,214	212,214	212,214			
CH04a06	142	123	251,128,142	252,126	124,142	123,141	141	123			
CH04d11	143,155	147	163	163	143,155	154	154	147			
CH04d08	159	132,152	148,159	133	148,159	148,158	158	132,154			

(*continued on next page*)

Table 3 (*continued*)

Primer	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11
CH04F03	177,193	195,201	177,204	197	177,202,204	177,203	177,191	195,201			
CH04e12	243,251	246,250	243	243	243	242	242,250	246,250			
CH04F07	124	96	124	197,202	159	96,122	96	96			
CH04F04	216,234	166	180,185,216	167,216,231,240	180,234	178	214	166			

D_1-Red Delicious, D_2-Kullu Delicious, D_3- Shimla Delicious, D_4-Golden Delicious, D_5-Cross Delicious, D_6-Molies Delicious, D_7-Gole Delicious, D_8-Balgarian Delicious, D_9-Oregon Spur, D_{10}-Reeka Red, D_{11}- Siliver Spur
A_1-Kashmiri Ambri, A_2-Lal Ambri, A_3-Ambri Cross, A_4- Balgarian Ambri, A_5-Vilayati Ambri, A_6- Delicious Ambri, A_7-Dudh Ambri, A_8-High Density Ambri

primer, 1 unit of taq DNA polymerase 5 U/μl and ultrapure water to reach the final volume of 20 μl. The volume of DNA used as template was 1.5 μl. PCR program was set as follows- initial denaturation: 95 °C for 5 min; denaturation: 95 °C for 30 s; annealing: 55 °C for 30 s; elongation: 72 °C for 60 s; repetition: 35 cycles. The last step was a final extension of 72 °C for 10 min.

The fluorescently-labeled PCR products were mixed with 0.3 μl of Gene Scan-500 ROX size standard (Applied Biosystems) and 12 μl of Hi-Di Formamide (Applied Biosystems) and separated by capillary electrophoresis on an ABI PRISM 3100. The experiment was replicated at least three times to verify the reproducibility of markers. The amplified fragments were scored with GeneScan 3.7 and Genotyper 3.7 software (Applied Biosystems) as 1 for presence and 0 for the absence of allele.

2.3. Data analysis

The following parameters were considered for each assay unit as described by Zargar et al. (2016); Number of polymorphic alleles (NPA); Number of monomorphic alleles (NMA); Fraction of polymorphic loci (β) = NPA/(NPA + NMA); Effective multiplex ratio (EMR) = nβ, where n is the total number of bands and β is the fraction of polymorphic loci;

Polymorphic information content (PIC) = 2fi (1-fi), where fi is the frequency of present bands and 1-fi is the frequency of absence bands;

Marker index (MI) = PIC × EMR; Resolving power (RP) = ∑Ib, where Ib can be calculated by the formula as Ib = 1- [2 × (0.5-p)], where p is the frequency of individual band present.

The scored binary data generated from SSR with present alleles scored as 1 and absent alleles as 0 was used for the construction of dendrogram by Jaccard's similarity coefficient using NTSYS- pc version 2.02e (Rohlf, 1998). The principal component analysis was also performed to differentiate the cultivars. (See Fig. 1)

3. Results

In the present study a highly informative set of 29 SSR primers (Table 2) was used to distinguish 19 apple cultivars from Kashmir valley. A total of 218 alleles were obtained by 29 SSR primers. The allele number for each primer varied from 3 (Hi06f09) to 14 (Hi08f12) with a mean number of 7.51 alleles per primer (Table 2). In general the size of the amplified DNA fragments scored ranged from 96 to 362 bp. The largest number of alleles was generated by Hi08f12 (14 alleles) followed by Hi05d10, CH03h06 and CH04f04 (13 alleles each). Primer pairs Hi06b06, CH03b01, CH04f03 and CH04f07 produced 10 alleles each in all the nineteen apple cultivars. On the other hand, the minimum number of alleles was amplified by Hi06f09 (3 alleles) followed by Hi08h03, Hi08a04, Hi11a01, Hi23d03 and CH04C03, each amplified 4 alleles in all the cultivars. In order to identify the most efficient primers that could distinguish all the cultivars either individually or in combination, three different indices like Polymorphic Information Content (PIC), Markers Index (MI) and Resolving Power (RP) were applied in the present study (Table 2). Allelic composition for each cultivar is presented in Table 3.

3.1. Cultivar relationships based on SSR analysis

The UPGMA separated the apple cultivars into two main clusters (Fig. 2). Cluster I consisted of twelve cultivars while the remaining seven of the cultivars were found in cluster II. Both the clusters were divided into sub clusters. The 'Red Delicious' sub-group consisted of six cultivars: Red Delicious, Kullu Delicious, Shimla Delicious, Reeka Red, Oregon Spur and Siliver Spur. Kullu

Delicious, Shimla Delicious had the same allele composition at all SSRs while 'Reeka Red' was closely related with difference at two of the 29 SSRs. Cross Delicious, Kashmiri Ambri and Vilayati Ambri also grouped together in a separate sub-cluster. In cluster II, two small sub-clusters were again formed. The Golden Delicious sub-cluster consisted of Golden Delicious, Molies Delicious, Delicious Ambri and Dudh Ambri whereas the remaining three cultivars, Balgarian Delicious, Lal Ambri and High Density Ambri formed the second sub-group within cluster II. The Jaccard's similarity coefficient based on SSR data ranged from 0.05 to 0.93. (Fig. 2). The three cultivars: Oregon Spur (D_9), Reeka Red (D_{10}) and Siliver Spur (D_{11}) which are said to be sports of Red Delicious were different from each other and grouped together with Kullu Delicious (D_2) and Shimla Delicious (D_3) in one sub cluster.

The UPGMA cluster analysis revealed that some Ambri and Delicious cultivars form a separate subgroup. There are no possible reasons as the present study is just a preliminary survey in which only 19 cultivars and 29 SSR primers were used. The limited number of primers has generated little information. So the use of maximum number of primers to cover most of the linkage groups can provide more and more information. As such we can not say that the Ambri apple cultivars have developed from Delicious group due to some hybridizations events taking place in the orchards because there is no literature available regarding the origin of most of Ambri as well as Delicious cultivars. It may be possible that some Ambri cultivars would have been developed from Delicious by natural hybridisation events in the orchards.

PCA also supported the groups obtained with cluster analysis. Most of the Delicious cultivars grouped together along with few cultivars from the Ambri group. Five cultivars from the Delicious group namely Kullu Delicious (D_2), Shimla Delicious (D_3), Reeka Red (D_{10}), Oregon Spur (D_9) and Siliver Spur (D_{11}) formed a separate group at one corner in PCA plot, thus indicating close similarity to each other. On the other hand, the second group consisted of seven cultivars which include Kashmiri Ambri (A_1), Gole Delicious (D_7), Ambri Cross (A_3), Balgarian Ambri (A_4), Vilayati Ambri (A_5), Cross Delicious (D_5) and Red Delicious (D_1). The third group also was comprised of seven cultivars which includes Molies Delicious (D_6), Golden Delicious (D_4), Delicious Ambri (A_6), Dudh Ambri (A_7), High Density Ambri (A_8), Lal Ambri (A_2) and Balgarian Delicious (D_8) (Fig. 3).

4. Discussion

Assessment of genetic diversity within a cultivated crop has important consequences in breeding and the conservation of genetic resources. Several molecular markers have been used widely for the analysis of genetic diversity and cultivar identification in large number of species. Molecular markers have succeeded in differentiating cultivars, classifying synonyms, identifying mislabeled cultivars, establishing genetic relationships and giving hints about the process of domestication (Anand, 2000; Wunsch and Hormaza, 2002). SSR markers are the preferred DNA markers for the analysis of genetic relationships and diversity within crop species due to their high polymorphism level, abundance, co-dominant inheritance (Fernandez et al., 2009), reproducibility and relative ease of analysis (Schlotterer, 2004). Hundreds of microsatellite markers have been developed in apple and some have been placed on genetic linkage maps (Liebhard et al., 2002; Silfverberg-Dilworth et al., 2006). Microsatellites have been also used as markers to predict important traits like resistance to apple scab (Vinatzer et al., 2004).

In the present investigation SSR data for 19 apple cultivars revealed a total of 218 polymorphic fragments with 29 primer pairs. The mean number of alleles per primer obtained was 7.51 which is similar to the results reported earlier by different groups (Wichmann et al., 2007; Pereira-Lorenzo et al., 2007). Gasi et al. (2010) selected ten genomic SSRs to assess genetic diversity in 39 cultivars of apple and reported that the average number of alleles per SSR is 10.4. Gao et al. (2007) analyzed 59 apple cultivars using 12 SSRs and detected an average of 14.7 alleles per primer. The higher average number of alleles per SSR primer may be attributed to multi allelic nature of SSR primers. The multi allelic SSRs produce more than two alleles even in diploid cultivars. Multi locus SSRs indicates how many alleles are present in the genome. There is nothing like triploid and tetraploid nature of these cultivars as the present samples were analysed based on cytology which proved all these cultivars diploid with 2n = 34.

Marker indices like PIC, MI, RP etc. are informative parameters to detect the levels of genetic diversity in an organism. In the current study, the primers with highest marker indices values will help in the screening of genetic polymorphism among apple cultivars. The respective values for each informative index have been

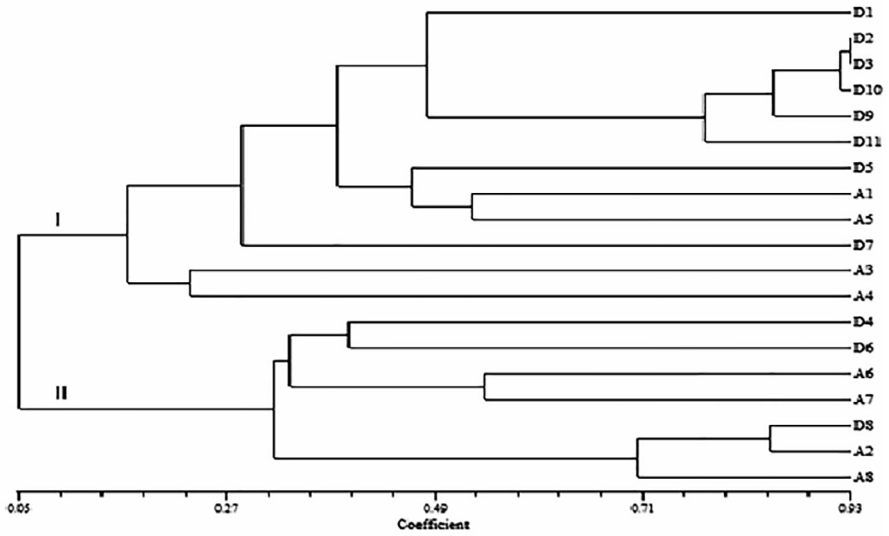

Fig. 2. UPGMA cluster analysis based on Jaccard's similarity coefficient. D_1-Red Delicious, D_2-Kullu Delicious, D_3-Shimla Delicious, D_4-Golden Delicious, D_5-Cross Delicious, D_6-Molies Delicious, D_7-Gole Delicious, D_8-Balgarian Delicious, D_9-Oregon Spur, D_{10}-Reeka Red, D_{11}-Siliver Spur, A_1-Kashmiri Ambri, A_2-Lal Ambri, A_3-Ambri Cross, A_4-Balgarian Ambri, A_5-Vilayati Ambri, A_6-Delicious Ambri, A_7-Dudh Ambri, A_8-High Density Ambri.

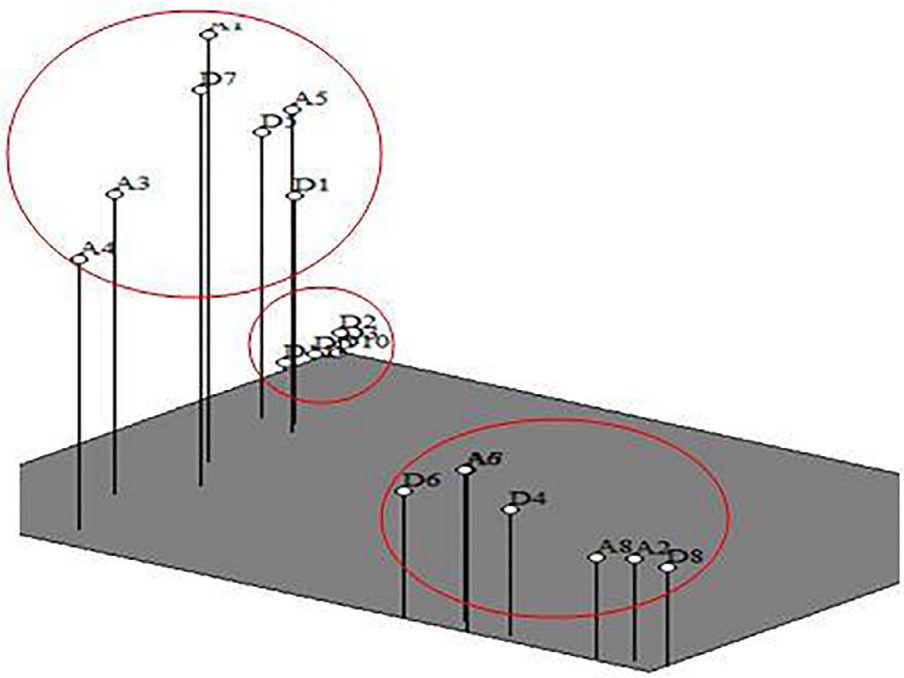

Fig. 3. 3D PCA plot of apple cultivars.

reported in Table 2. It is anticipated that these primers would help apple researchers to pick up and conduct further downstream studies related to genetic amelioration.

Allelic compositions of most of the primer pairs have proved that Kullu Delicious and Shimla Delicious resemble the three sports (Oregon Spur, Reeka Red and Siliver Spur) investigated in the present study. By screening 29 SSR primers for their informativeness, the present study demonstrates that four primers Hi05d10, Hi08c05, CH03h06 and Ch04F04 have highest resolving power i.e. these detect enough base pair variation among nineteen apple cultivars to allow their distinction. Due to close interrelationships and narrow gene pool of the accessions in this study, additional markers/primers will be needed to fully characterize and distinguish a large set of cultivars. This study will enable us to identify a standard set of primers that can be used to distinguish the apple germplasm of our state.

5. Conclusion

The purpose of our study was to assess the genetic diversity of the apple germplasm of Kashmir Valley. SSR analysis based on 29 primer pairs have separated the cultivars of Delicious group and it was also found that Kullu Delicious and Shimla Delicious resemble in allelic composition with the sports like Oregon Spur, Reeka Red and Siliver Spur. 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.

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Compliance with ethical standards

Conflict of interest

All the authors declare that they have no conflict of interest.

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