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Genetic divergence studies in indigenous *Malus baccata* biotypes by using the random amplified decamer primers

Vikrant and Manju Modgil

Abstract
Study of genetic diversity is an important aspect to be covered for proper utilization of the germplasm for breeding purpose and crop improvement programme. Keeping in view, genetic divergence studies in indigenous crab apple biotypes (*Malus baccata* var. Himaliaca) maintained at two field gene banks of Himachal Pradesh state of India was carried out by using the RAPD molecular markers. A total of 119 decamer primers were initially screened to check these biotypes during the genotypic screening out of which 94 showed clear and scorable bands. In samples collected from IARI Regional Station, Shimla, these primers revealed 67.47% of polymorphism and PIC value ranged between 0.497 to 0.867, whereas average number of alleles per primer was 4.84. Jaccard's similarity coefficient ranged from 0.44 to 0.65 which showed the divergence among the biotypes. Comparatively low percentage polymorphism (53.37%) was observed in seven biotypes maintained at NBPGR, Regional Station, Shimla, while almost similar PIC value range 0.47-0.87 was obtained as in case of former. Similarity coefficient values ranged from 0.62-0.74 which was less than that of IARI, RS. Here, *M. baccata* came as an outliner and found to be distinct among all the biotypes. Combined analysis of fourteen samples of crab apple biotypes maintained at two gene banks resulted in 75.5% polymorphism and highest PIC value of 0.867. UPGMA clustering figured all the biotypes in one major cluster with some subclusters except *Malus baccata* Kinnaur which is the most divergent among all and came out as an outliner. It shows similarity of more than 40 per cent with rest of all the biotypes. Thus, in the present studies, considerable variation was found among the indigenous wild crab apple biotypes which is in line with previously reported morphological studies. Further, RAPD primers were found to be useful in evaluating the genetic diversity in wild apple biotypes.

Keywords: Apple, genetic diversity, *Malus baccata*, RAPD markers

Introduction
Asia Minor, the Caucasus, Central Asia, Himalayan India, Pakistan and Western China were known to be the origin of cultivars of *Malus* (Watkins, 1995; Zhang *et al*. 1993) [37 40].in which at least 25 native species of *Malus* occur and it was suggested by Rehder (1940)[27]. that *Malus sylvestris* Mill, *Malus prunifolia* Borkh *and Malus baccata* Borkh are involved to some degree in the development of large fruited cultivated apple. It is the most important temperate fruit crop of North Indian Himalayan region with regard to production and economic value. India is the fifth-largest apple producer in the world and approximately 60 and 40 per cent of apple production accounts from the Himalayan region of Jammu and Kashmir and Himachal Pradesh respectively. A large number of wild and indigenous species are growing extensively in the entire Himalayan region. To protect the valuable genetic resources, which contains interesting diversity for important horticultural traits, from further degradation and to secure them for further development of cultivars, seed and wood collections have been made in the wild apple forests (Hokanson*et al*. 1998)[10]. Out of five wild *Malus* species in the world, *Malus sikkimensis* and *Malus baccata* were reported from Himalayas (Hooker, 1879: Anon, 1962)[11, 1]. There are two ideotypes of *Malus sikkimensis* and six distinct biotypes of *Malus baccata*Borkh var. *himalaica* growing wild in different agroclimatic regions of India. Over the years, these species have developed resistance against various biotic and abiotic stresses. During a survey in late 1960s, different species and types of various genera including *Malus* were collected from wild forests, maintained and conserved as *M. baccata* biotypes at the research farms of IARI and NBPGR Regional Research Stations, Shimla (Randhawa, 1987)

[26]. with a view to investigate breeding rootstocks having wider ecological adaptability and resistance to various diseases than the rootstocks that are now in use for apples. During the process of selection longtime back, little attention was paid to important traits because these were not of immediate concern at that time (Kishore and Randhawa, 1993)[14]. Thus, valuable germplasm were neglected which are now facing extinction. The nomenclature of *M. baccata* biotypes is being maintained by suffixing the name of native place from where these were originally collected.

During different studies conducted on these biotypes at IARI Regional Research Station Shimla, large variability was exhibited in various horticultural traits like adaptability to agroclimatic conditions, vigour, winter hardiness, drought tolerance, chilling requirement, resistance to disease and pests (Kishore *et al*. 1990; Sharma*et al*. 2011)[15, 29]. These were also evaluated as rootstocks for different commercial cultivars. Morphological and biochemical (Kumar *et al*., 2018; Kishore and Randhawa, (1993) [18, 14]. characterization of indigenious wild apple biotypes have been carried out which showed variability in different characters. However these characters are highly influenced by changing environment conditions. In such cases, molecular analysis will necessarily need to be done to make decision regarding trueness to type, level of diversity and the utility of these wild biotypes. Availability of diverse *Malus* germplasm helps in preserving genetic traits and is a primary requirement for pursuing successful apple breeding programs, as it increases genetic diversity and allows for development of new apple cultivars with desirable traits. Despite the presence of high level of genetic diversity in cultivated apple, most of the apple orchards are dominated by only a few cultivars and many breeding programs utilize only a few well known cultivars in crosses for commercial apple production (Noiton and Shelbourne, 1992)[20]. While, there are over 6000 important cultivars and land races documented across the world, commercial apple production relies on a few major cultivars, such as 'Delicious', 'Golden Delicious', 'Granny Smith', 'Fuji' and 'Gala', which account for 61% of the total fruit production (O'Rourke, 2003)[21]. The genetic pool of the traits to be incorporated into the domesticated apple would certainly be expanded if breeders include wild *Malus* species in development of cultivars and rootstocks suited to diverse environmental conditions. Almost all the apple cultivars released since 1970 have derived their resistanceto apple scab from *M.* × *floribunda* '821' Siebold ex Van Houte (Vf), (Hough *et al*. 1953) [12], while other scab resistant genes have been incorporated from *M. baccata* 'Hansen's 2' (V_b), (Dayton and Williams, 1968; William and Kuc, 1969) [7, 39], *M. baccata*'Jacckii Dg27T1' (V_{bj}) (Dayton and Williams, 1968)[7]. *M. micromalus* Makino '245-38' (V_m), (Dayton and Williams, 1970) [8]. Similarly, other traits like cold hardiness has been derived from *M. baccata* and closely related *M. prunifolia*, as well as *M. sieversii* (Stepanov, 1974; Strang and Studhnoff, 1975) [33, 34]. It has been reported in various studies that the population of *Malus pumila* Mill., indigenious to northwest Spain showed a wide range of ripening periods and fruit quality and was an unexploited resource for breeding programmes which has now been studied (Ramos-Carberet al. 2007)[25]. Molecular characterization and genotyping in apple cultivars (Ramos-Carber*et al*. 2007) [25]. Have been performed using different markers such as isozymes (Pancaldi *et al*. 1995) [22]. RAPDs (Mitre *et al*. 2009) [19]. ISSRs (Smolik *et al*. 2004)[32].And SSRs (Kithara *et al*. 2005; Cavanna *et al*. 2008)[17, 5]. RAPD markers are simple, cost effective and easy to use because no prior DNA sequence information is required as needed with other PCR based markers. RAPD is still in use for estimation of genetic variability of different plant species in moderate laboratories (Danilovic *et al*. 2015; Pinar *et al*. 2015) [6, 23].And it has been shown to be a tool for the differentiation and identification of various apple cultivars (Kaya *et al*. 2015; Mitre *et al*. 2009) [13, 19]. Keeping in view, the present investigation was undertaken to assess the level of genetic divergence in indigenous crab apple biotypes using RAPD primers.

Materials and Methods

Plant material

A total of fourteen samples collected from two gene banks were analysed in this study (Table 1). Newly expanded leaves from tree of crab apple biotypes were collected fromIARI and NBPGR Regional Research stations, Shimla (HP) India, and stored at -80 ^{0}C until use. Total genomic DNA from the collected leaves of each biotype was isolated from approximately 200 mg of frozen leaves by using CTAB method by VirscekMarn *et al.* (1999)[36]. With some modifications. RNaseA 100 µg/ml was used to remove RNA contaminants in all the samples for 30 minutes at 37^{0}C. DNA quality was assessed by running the DNA samples on 0.8% agarose gel and its concentration using Picometer (Picodrop Ltd., Cambridge U.K.). To make all the DNA samples concentration uniform, they were diluted to a concentration of 50ng/µl before use.

DNA amplification and RAPD analysis

For DNA amplification, total 15 µl reaction volume were made for each marker analysis containing Taq DNA polymerase buffer (1X) with 1.5 mM $MgCl_2$, Taq DNA polymerase (3U/reaction), primers (10 pmol/reaction), deoxynucleotide triphosphate (dNTPs) (25 mM) of GeNei^{Tm}, Bangalore, India and template DNA (50 ng/reaction). A total of 119 RAPD primers were used for amplification of fourteen samples of crab apple (*Malus baccata*) biotypes at their respective annealing temperatures using a PCR amplification machine (Eppendorf Thermal cycler) by setting the amplification program to initial cycle of 5 min at 94°C followed by 35 cycles of 1 min at 94°C, annealing temperature depending upon Tm value of primer for 1 min, elongation step of 2 min at 72°C, and a final extension step of 8 min at 72°C. Recovered amplified products from the PCR were verified on agarose gel (GeNei, Bangalore, India) electrophoresis at concentrations of 1.2% to 2% in 1X TAE buffer stained with ethidium bromide (10 mg/ml). The products were visualized by UV light on Biovis Gel Documentation system and images were captured. The size of the amplified products were determined by comparing these with DNA ruler of 100 bp standard molecular weight markers (GeNei, Bangalore, India). The data on band position on agarose gel was recorded by binary analysis assigning '0' for the absence of band and '1' for presence of band. The similarity matrix generated using Jaccard coefficient was used for unweighted pair-group method based on arithmetic average (UPGMA) using software package NTSYS-PC ver.2.02i (Rohlf, 1998)[28]. And the output data was graphically represented as dendrogram.

Results and Discussion

The level of polymorphism was analysed among seven crab apple biotypes collected from two sites with 119 RAPD primers. Out of total 119 RAPD primers initially screened for

amplification of fourteen samples, ninety four produced clear and scorable amplified products (Fig. 1, a-h). In the past, RAPD markers were extensively used for the characterization of wild apple accessions, species and cultivars (Kaya *et al*. 2015; Antic *et al*. 2016) [13, 2].

RAPD and cluster analysis within site IARI, RS, Shimla

A total of 455 scorable bands were amplified out of which 307 were found to be polymorphic showing 67.4% polymorphism. 100 percent of polymorphism was generated by twenty nine primers, whereas eight primers resulted in more than 80% polymorphism. Polymorphic information content (PIC) values or genetic diversity for seven samples of biotypes collected from IARI, RS Shimla varied from 0.497 to 0.864 (Table 2). Out of total 94 primers amplified, twenty two primers showed PIC value more then 0.8, which means that these primers were more informative among all (Table. 5). Highest PIC value of 0.864 was found with primer OPA-07, whereas primer TIBMBB-05 showed lowest PIC value of 0.497.The highest number of alleles per locus were eight with two primers i.e. S92 and OPA-07, while the lowest number was two with primers OPAG-20 and TIBMBB-05, with an average number of alleles per primer 4.84. The amplified products ranged between 100 to 2000bp.

According to the data obtained from similarity matrix analysis, similarity cofficient values ranged from 0.44 to 0.65 (Table 2) between the biotypes collected. Highest similarity of 0.65 was found between the *M. baccata* Pangi and *M. baccata* Shillong followed by *M. baccata* Rohru and *M. baccata* Pangi (0.64); *M. baccata* Rohru and *M. baccata* Shillong (0.63). Whereas the coefficient of genetic similarity between *M. baccata* Khrot and *M. baccata* Kashmir; *M. baccata* Kinnaur and *M. baccata* Kashmir were in the range of 0.44-0.47, which means these are the most likely diverse genotypes. UPGMA clustering analysis is used for comparing the genetic relatedness among seven biotypes (Fig. 2) of this site. The dissimilarity in dendrogram grouped all the biotypes into one cluster, in which *M. baccata* Khrot, *M. baccata* Kashmir, *M. baccata* Kinnaur (Dhack), *M. baccata* Pangi, *M. baccata* Shillong, *M. baccata* Rohru were present and *M. baccata* Kinnaur found to be distinct from all the biotypes and came out as an outliner. Rahman *et al*. (1997)[35].While studying the genetic relationship among the various Annona genotypes indicated that the cultivated and wild species were grouped separately.

RAPD and cluster analysis within NBPGR, RS, Shimla

During the genotypic analysis of the sample collected from NBPGR, RRS, Shimla, 429 amplified alleles were obtained out of which 229 were found to be polymorphic showing 53.37% of polymorphism (Table 5). The polymorphic percentage generated by the fifteen primers was 100%, while five primers generated more than 80% polymorphism. High level of polymorphism (89.29%) has been demonstrated in 35 native genotypes of apple using RAPD markers (Kaya *et al*. 2015) [13]. Ur- Rahman *et al*. (1997) [35]. Reported 60% polymorphic bands out of total 215 products in *M. hupehensis* and 40% out of 271 amplified bands in *M. toringoides* with 20 RAPD primers. Polymorphic information content values ranged from 0.42-0.87 among seven biotypes which showed wide genetic diversity. Twenty three primers showed PIC values higher than 0.8 which has high discriminating power. The highest number of alleles produced was eight with the primer OPA-16, whereas, lowest number of alleles was two with eight primers S91, OPC-02, OPC-01, OPAG-20, OPAC-10, OPAT-03, TIBMBB-05 and TIBMBD-16 with an average number of 4.56 alleles per primer. The length of fragments obtained by PCR with 94 primers in seven samples ranged from 100 to 2000bp. It has been observed that the similarity cofficient values ranged from 0.62 - 0.74 (Table 3). The highest similarity value (0.74) was found between *M. baccata* Khrot and *M. baccata* Shillong followed by 0.70 observed between *M. baccata* J&K and *M. baccata* Kashmir. Similar result was found between *M. baccata* Chamba and *M. baccata* J&K (0.70). While comparing genetic similarity coefficient values among biotypes, high diversity was found between *M. baccata* and *M. baccata* Kashmir. The dendrogram shows that all the biotypes within the site of NBPGR fall in one cluster with two subclusters, depending upon the position of each biotype in the phenogram (Fig. 3) where *M. baccata* was found to be distinct among all and came out as an outliner and shows similarity of more than 65 per cent with rest of all the biotypes but is divergent. The correct location and identification of this biotype has not been known. *M. baccata* Khrot and *M. baccata* Shillong shows highest similarity in the phenogram that is more than 74 per cent. Whereas *M. baccata* Assam, *M. baccata*Chamba, *M. baccata* J&K and *M. baccata* Kashmir fall in same sub cluster.

Combined Analysis

Amplification results of fourteen samples of biotypes collected from two sites at Shimla shows that a total of 466 loci were amplified out of which 352 were found to be polymorphic resulting in 75.5% polymorphism. The number of polymorphic alleles per primer ranged from 2 to 8. The highest PIC value was obtained from the primer OPA-07 with 0.867 (Table 5) which was found to be more informative whereas primer TIBMBB-05 resulted in lowest PIC value of 0.497. Primer pairs C4, S92, C5, OPA-20, OPE-19, OPB-05, OPC-05, OPC-20, OPD-13, OPC-09, OPB-04, OPE-01, OPC-18, OPAC-02, OPAG-06, OPG-03, OPG-11, UBC-43, UBC-667, OPT-14, TIBMBD-17 and OPV-14 showed more than 0.8 PIC value and thus can also be considered as informative during the present studies. Average number of alleles per primer and maximum number of alleles detected among the present study of wild apple biotypes were low in comparison to previous studies (Sikorskaite *et al*. 2012) [31]. which demonstrated the average number of alleles 7.55 and range 5-12. Hokanson *et al*. (2001)[9]. reported higher number of alleles during their studies, and comparatively decreased genetic diversity. It has been observed that the coefficient of genetic similarity (Table 4) ranged from 0.49 to 0.74. Similar biotypes i.e. *M. baccata* Shillong collected from two Regional Research Stations shows 0.63 similarity values among themselves. Likewise, the similar biotypes of *M. baccata* Khrot and *M. baccata* Kashmir shows low similarity values of 0.54 and 0.55 respectively. Combined UPGMA clustering analysis (Fig. 4) comparing to all possible pairs between the fourteen samples of *M. baccata* biotypes figured the *M. baccata* Kinnaur as most divergent among all and shows similarity of more than 40 per cent. This may be due to the reason that it was collected from high altitude (2900mts) and has different environmental conditions as compared to other locations. Rest of all the biotypes grouped into one cluster where *M. baccata* Shillong and *M. baccata* Khrot collected from NBPGR, RS showed highest similarity of 74 per cent. *M. baccata* Rohru and *M. baccata* Shillong of NBPGR, RS grouped in the similar subcluster with more than 60 per cent similarity. On other hand, *M. baccata* Kashmir, *M. baccata* Khrot and *M. baccata* Kinnaur (Dhack) of IARI, RRS figured

outside in the cluster from rest of the biotypes in the same cluster which showed their divergence. It has been further observed that *M. baccata* of NBPGR, RS lied close to *M. baccata* Pangiof IARI, RRS with 61% similarity in same sub cluster and can be assumed that *M. baccata* might have bought from Chamba Distt. *M. baccata* Assam, *M. baccata* Chamba, *M. baccata* J&K and *M. baccata* Kashmir of NBPGR, RS aligned in same subcluster with more than 65 per cent similarity which shows the similar result as found in individual cluster analysis of NBPGR samples. Here, the biotypes of Kashmir maintained in both the genebanks donot group in same subcluster, the reason may be that there were wide altitudinal variations (1400-2800mts.) in Jammu & Kashmir state and also may be some temporal variations to collection. Variation may also have arisen as a result of natural mutations because apple is known for its ability to produce sports or strains at random (Brown 1975) [4]. This is also clear from low similarity coefficient values. Our result supports the findings of Hokanson *et al*. (2001) [9]. That depending upon the age cohort from which the respective accessions arose, and the size and genetic make-up of the surrounding *Malus* populations at that time, the genetic constitution of the accessions could be considerably different. It has already been reported that around the world, wild population of *Malus* are being reduced in size and eliminated due to human activities (Hokanson *et al*. 1997; Way *et al*. 1991) [38]. In the present study, there was 0.68-0.70 similarity coefficient between *M. baccata* Kashmir, *M. baccata* J&K and *M. baccata* Shillong in NBPGR, RS samples which are in agreement with the previous report that these two biotypes have some common traits like tolerance to root rot under low disease pressure as compared to other (Sharma and Pramanick 2012)[30]. However, in other site these two biotypes resulted in lower similarity coefficient (0.54) which may be due to the reason that the accessions may be collected in different years from different place of location in the respective state. Low divergence between *M. baccata* Kashmir and *M. baccata* Shillong supports the previous study that former is vigorous and latter imparts semi vigorous growth to grafted Commercial scion cultivars (Sharma and Pramanick 2012) [30].

M. baccata Shillong, biotypes showed 4-5 unique and polymorphic bands with more than four primers, while *M. baccata* Khrot and *M. baccata* Kashmir also revealed two unique bands. *M. baccata* Shillong also has the lowest similarity with rest of the wild crab apple biotypes which is in agreement with earlier report that *M. baccata* Shillong has phenolics, high level of resistance against apple scab, powdery mildew and woolly aphids (Kishore and Randhawa, 1993) [14].

By selecting diverse parents, progress can be made in apple breeding efforts towards developing new cultivars with economically valuable traits, including those with enhanced fruit quality, disease and pest resistance. The present studies are supported by Hokanson *et al*. (2001) [9] who reported more dispersion among the accessions of *M. baccata*, *M. micromalus* and *M. siieversii* while analyzing the SSR variations in a collection of 142 *Malus* species and hybrids. Similar trend has been reported in wild apple and pear genetic resources in the Starcevica Forest Park (Antic *et al*. 2016) [2].The present investigation showed sufficient genetic diversity and confirm the previous report that crab apple biotypes collected from different locations of Indian Himalayan states are suspected to be variants of *M. baccata*. It has also been suggested that the existing genetic diversity in wild apple biotypes warrants them distinct species or varietal status (Kishore *et al*. 2005) [16]. Recently, the wild apple biotypes were well evaluated for their graft compatibility, chilling requirement, disease and pest resistance and showed considerable variability, which could make them suitable rootstocks or parent genotype in hybridization (Kumar *et al*. 2018)[18]. The present study supplements it by demonstrating considerable variability which shows richness in wild crab apple biotypes maintained at both the gene banks.

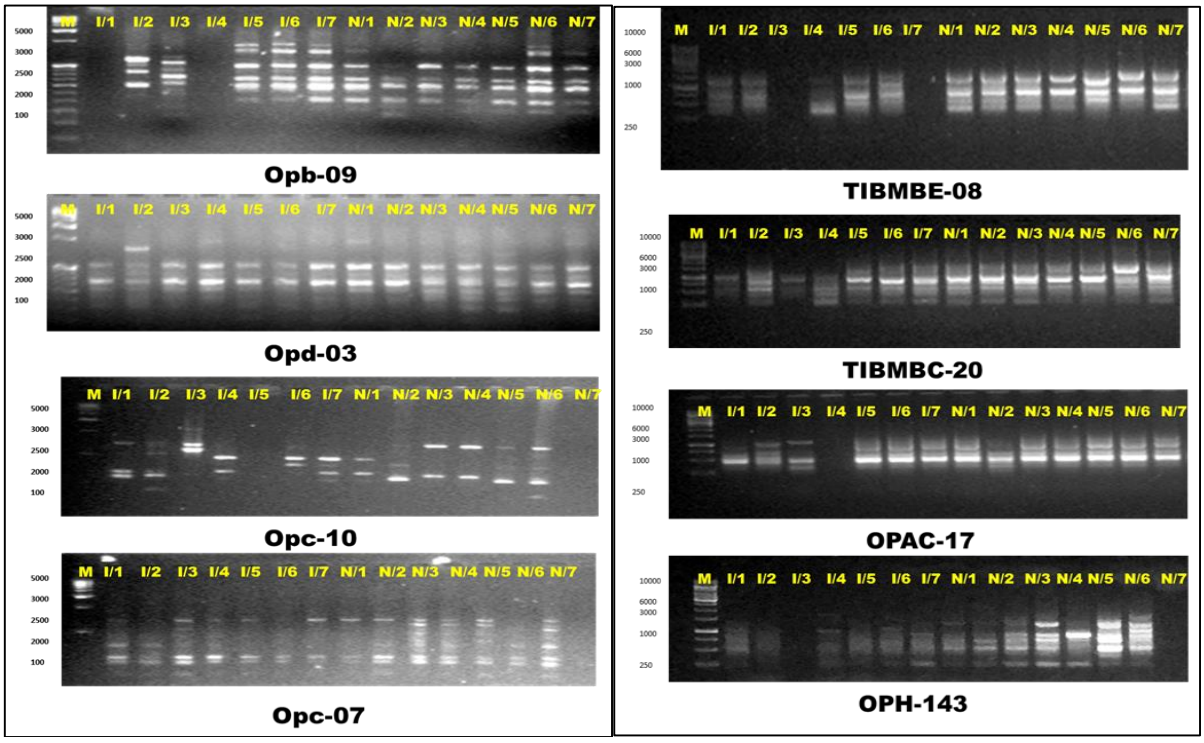

Fig1: (a-h) RAPD profiles of indigenous crab apple biotypes collected from two gene banks, with primers Opb-09, Opd-03, Opc-10, Opc-07, TIBMBE-08, TIBMBC-20, OPAC-17 and OPH- 143

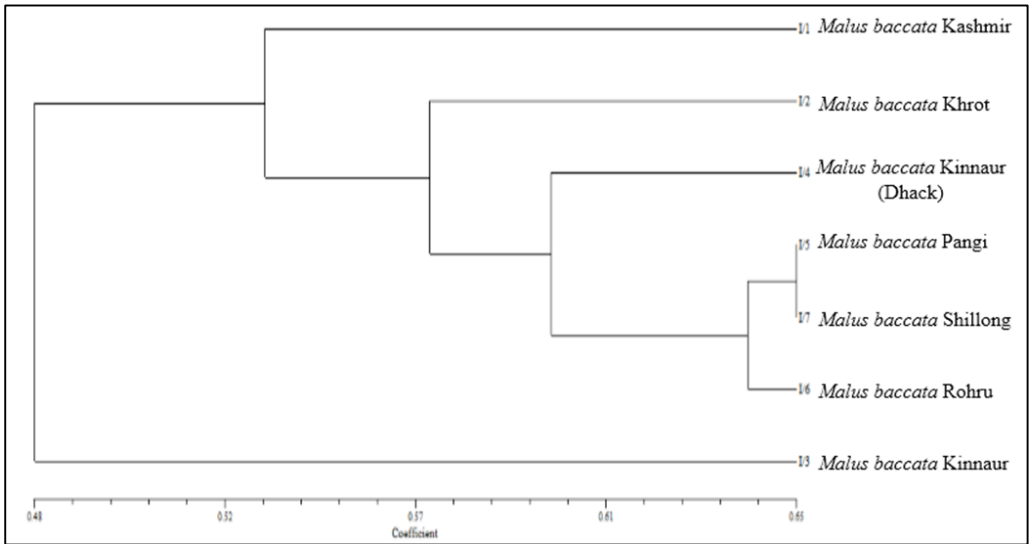

Fig2: Dendrogram showing clustering of biotypes maintained at IARI Regional Research Staiton, Shimla

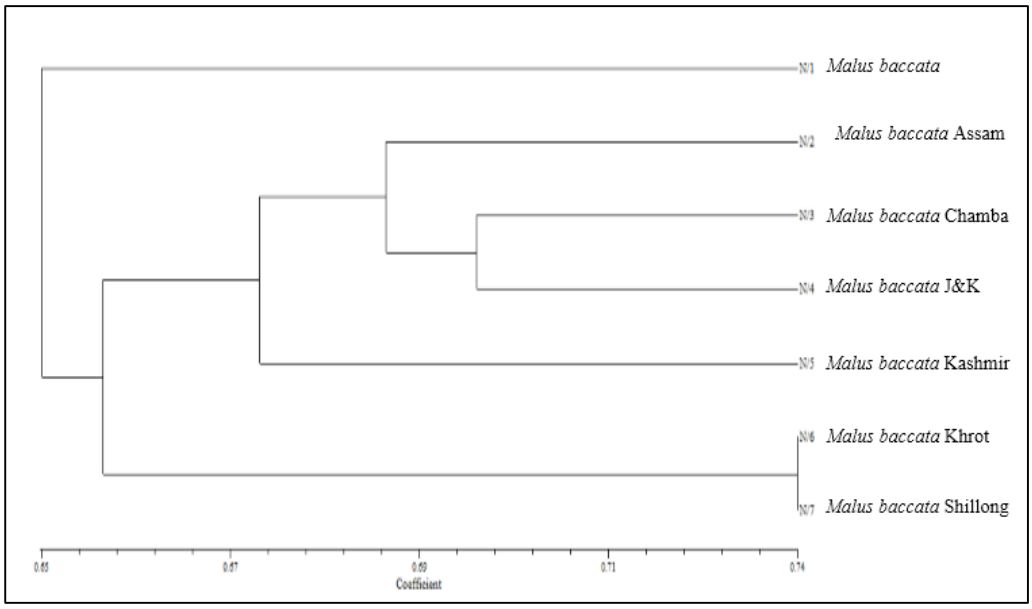

Fig3: Dendrogram showing clustering of biotypes maintained at NBPGR Research Staiton, Phagli and Shimla

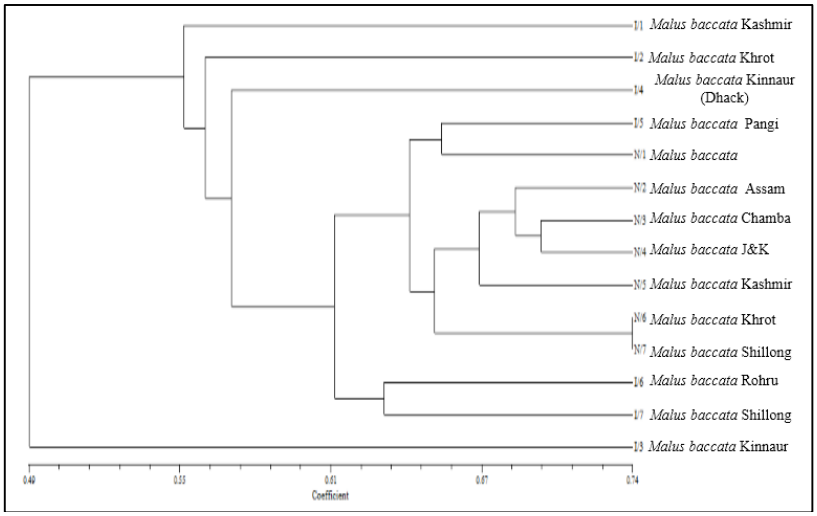

Fig4: Dendrogram showing the variations among different biotypes of crab apple i.e. *Malus baccata* collected from both Regional Research Stations

Table 1: Study of indigenous crab apple biotypes, field gene banks and origin

Sr. No.	Name of the biotype (sample)	Code No.	Field gene banks	Origin

1.	*Malus baccata* Kashmir (A)	I/1	ICAR- Indian Agriculture Research Station (IARI-RS), Dhanda, Shimla	Kashmir Valley, J&K
2.	*Malus baccata*Khrot	I/2	-do-	Khrot, Distt. Shimla H.P.
3.	*Malus baccata*Kinnaur	I/3	-do-	Kinnaur, Distt. Kinnaur H.P.
4.	*Malus baccata*Kinnaur(Dhak)	I/4	-do-	Dhak, Distt. Kinnaur H.P.
5.	*Malus baccata*Pangi	I/5	-do-	Pangi, Distt. Chamba H.P.
6.	*Malus baccata*Rohru	I/6	-do-	Rohru, Distt. Shimla H.P.
7.	*Malus baccata*Shillong	I/7	-do-	Shillong, Meghalaya
8.	*Malus baccata*	N/1	ICAR- National Bureau of Plant genetic Resources Regional Station (NBPGR-RS),Phagli, Shimla	-
9.	*Malus baccata*Assam	N/2	-do-	Assam, (India)
10.	*Malus baccata*Chamba	N/3	-do-	Chamba, Distt. Chamba H.P.
11.	*Malus baccata*J&K	N/4	-do-	Srinagar, J&K
12.	*Malus baccata*Kashmir(1)	N/5	-do-	Kashmir Valley, J&K
13.	*Malus baccata*Khrot	N/6	-do-	Khrot, Distt. Shimla H.P.
14.	*Malus baccata*Shillong	N/7	-do-	Shillong, Meghalaya

Table 2: Similarity matrix of the biotypes maintained at IARI Regional Station, Shimla

Rw/Cl	I/1	I/2	I/3	I/4	I/5	I/6	I/7
I/1	1.00						
I/2	0.51	1.00					
I/3	0.47	0.44	1.00				
I/4	0.51	0.53	0.51	1.00			
I/5	0.57	0.58	0.48	0.62	1.00		
I/6	0.52	0.59	0.49	0.61	0.64	1.00	
I/7	0.54	0.57	0.47	0.55	0.65	0.63	1.00

Code given to different biotypes: I/1-*M. b.* Kashmir; I/2-*M. b.* Khrot ; I/3-*M. b.* Kinnaur; I/4- *M. b.* Kinnaur (Dhack); I/5-*M. b.* Pangi; I/6-*M. b.* Rohru ; I/7-*M. b.* Shillong

Table 3: Similarity matrix of the biotypes maintained at NBPGR Research Station, Phagli, Shimla

Rw/Cl	N/1	N/2	N/3	N/4	N/5	N/6	N/7
N/1	1.00						
N/2	0.67	1.00					
N/3	0.67	0.68	1.00				
N/4	0.66	0.69	0.70	1.00			
N/5	0.62	0.65	0.68	0.70	1.00		
N/6	0.65	0.64	0.64	0.68	0.68	1.00	
N/7	0.62	0.65	0.64	0.65	0.65	0.74	1.00

Code given to different biotypes: N/1-*M. b.* ; N/2-*M. b.* Assam; N/3- *M. b.* Chamba; N/4-*M. b.* J&K ; N/5-*M. b.* Kashmir (1); N/6-*M. b.* Khrot ; N/7-*M. b.* Shillong

Table 4: Combined similarity matrix of the biotypes maintained at both Regional Research Stations, Shimla

Rw/Cl	I/1	I/2	I/3	I/4	I/5	I/6	I/7	N/1	N/2	N/3	N/4	N/5	N/6	N/7
I/1	1.00													
I/2	0.51	1.00												
I/3	0.47	0.44	1.00											
I/4	0.51	0.53	0.51	1.00										
I/5	0.57	0.58	0.48	0.62	1.00									
I/6	0.52	0.59	0.49	0.61	0.65	1.00								
I/7	0.54	0.57	0.47	0.55	0.65	0.63	1.00							
N/1	0.60	0.56	0.48	0.57	0.66	0.62	0.63	1.00						
N/2	0.55	0.59	0.46	0.55	0.66	0.63	0.66	0.68	1.00					
N/3	0.58	0.52	0.50	0.56	0.64	0.57	0.60	0.67	0.68	1.00				
N/4	0.54	0.57	0.53	0.60	0.66	0.62	0.60	0.66	0.69	0.70	1.00			
N/5	0.55	0.57	0.45	0.52	0.62	0.54	0.60	0.62	0.65	0.68	0.70	1.00		
N/6	0.57	0.54	0.54	0.56	0.63	0.62	0.56	0.65	0.64	0.64	0.68	0.68	1.00	
N/7	0.56	0.54	0.51	0.57	0.64	0.63	0.63	0.62	0.65	0.64	0.65	0.65	0.74	1.00

Code given to different biotypes: I/1-*M. b.* Kashmir; I/2-*M. b.* Khrot ; I/3-*M. b.* Kinnaur ; I/4- *M. b.* Kinnaur (Dhack); I/5-*M. b.* Pangi; I/6-*M. b.* Rohru ; I/7-*M. b.* Shillong; N/1-*M. b.* ; N/2-*M. b.* Assam; N/3- *M. b.* Chamba ; N/4-*M. b.* J&K ; N/5-*M. b.* Kashmir (1); N/6-*M. b.* Khrot ; N/7-*M. b.* Shillong

Table 5: Total number of allelic forms and PIC values obtained through RAPD primers in *Malus baccata* biotypes

S. No.	Primers Name	IARI, RRS, Shimla			NBPGR, RRS, Shimla			Combined Analysis			Amplicons size (bp)
		T.A.	P.A.	PIC	T.A.	P.A.	PIC	T.A.	P.A.	PIC	
1	C4	6	6	0.815	6	4	0.828	6	6	0.826	100 - >1000
2	C5	6	6	0.824	7	5	0.834	8	7	0.843	100 - >1000
3	OLIGO-01	5	2	0.765	5	1	0.778	5	2	0.775	100 - 2000

4	OLIGO-04	5	2	0.770	4	3	0.722	5	5	0.758	100 - 2000
5	OLIGO-05	4	2	0.749	4	1	0.749	4	2	0.749	100 - 1000
6	OLIGO-07	4	4	0.722	4	3	0.730	4	3	0.732	100 -1000
7	OLIGO-08	4	1	0.748	4	2	0.730	4	2	0.744	100 - 2000
8	OLIGO-09	3	3	0.571	3	3	0.611	3	3	0.604	100 - 2000
9	OLIGO-11	5	4	0.793	5	0	0.800	5	4	0.799	200 - 1000
10	OLIGO-13	5	0	0.750	4	1	0.729	4	1	0.744	100 - 2000
11	OLIGO-14	5	4	0.748	5	1	0.793	5	4	0.784	100 - 2000
12	OLIGO-16	4	3	0.627	4	1	0.729	4	3	0.713	100 - 2000
13	OPA-01	5	3	0.798	5	4	0.799	5	4	0.799	100 - 1000
14	OPA-02	5	2	0.734	5	1	0.785	5	5	0.773	250 - 1000
15	OPA-03	5	5	0.793	4	4	0.727	5	5	0.788	100 - 1000
16	OPA-04	7	4	0.844	7	7	0.851	7	7	0.850	100 - 2000
17	OPA-06	4	1	0.749	4	0	0.750	4	1	0.750	250 - 1000
18	OPA-07	8	4	0.864	8	2	0.870	7	3	0.867	100 - 1000
19	OPA-09	5	4	0.790	5	0	0.800	5	4	0.798	250 - 2000
20	OPA-11	4	2	0.731	4	2	0.658	4	2	0.709	100 - 1000
21	OPA-14	5	1	0.781	5	1	0.793	5	1	0.788	100 - 1000
22	OPA-16	4	3	0.606	3	1	0.610	4	4	0.610	100 - 2000
23	OPA-17	5	3	0.785	5	5	0.798	5	4	0.794	100 - 1000
24	OPA-19	3	1	0.664	4	2	0.722	4	3	0.710	100 - 1000
25	OPA-20	7	1	0.856	7	6	0.852	7	6	0.856	100 - 800
26	OPAC- 07	4	4	0.741	4	3	0.724	4	4	0.736	100 -1000
27	OPAC-02	6	1	0.828	6	1	0.828	6	1	0.828	100 -1000
28	OPAC-10	4	4	0.734	2	1	0.486	4	4	0.683	100 -1000
29	OPAC-11	5	5	0.796	5	5	0.734	5	5	0.791	100 -1000
30	OPAC-17	4	3	0.745	4	2	0.749	4	3	0.749	250 - 1000
31	OPAG-16	6	2	0.823	6	1	0.824	6	2	0.824	100 -1000
32	OPAG-20	2	2	0.500	2	0	0.500	2	2	0.500	100 -1000
33	OPAT-03	3	2	0.660	2	0	0.500	3	2	0.619	200 - 1000
34	OPB-03	3	1	0.664	3	0	0.667	3	1	0.666	100 - 1000
35	OPB-04	5	3	0.765	6	3	0.830	6	4	0.812	100 - 2000
36	OPB-05	7	3	0.844	7	3	0.848	7	3	0.847	100 - 800
37	OPB-06	3	1	0.560	3	1	0.659	3	1	0.630	2000 - 2500
38	OPB-09	6	6	0.826	6	3	0.766	6	6	0.812	100 - 2000
39	OPB-10	5	3	0.749	5	4	0.695	5	4	0.736	100 - 800
40	OPB-11	4	4	0.747	4	1	0.727	4	4	0.746	100 - 2000
41	OPB-13	5	3	0.792	5	2	0.763	5	3	0.789	100 - 2000
42	OPB-15	3	1	0.664	4	2	0.722	3	3	0.587	100 - 1000

*T.A. –Total alleles; P.A. – Polymorphic alleles

**PIC – Polymorphic Information Content

Conclusion

In conclusion, considerably high degree of genetic diversity was found among indigenous wild apple biotypes as revealed by RAPD molecular markers which demonstrates the usefulness of germplasm collection at gene banks and could play important role in selection of desirable biotypes for apple breeding as well as of suitable rootstock. Investigation of genetic variation indicates that RAPD markers proved useful and suitable technique to assess the polymorphic loci and to estimate the genetic distances between wild *Malus baccata* biotypes.

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