International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2019; 7(1): 208-212

Received: 26-11-2018
Accepted: 28-12-2018

R Bhargava
ICAR-Central Institute for Arid Horticulture, Beechwal, Bikaner, Rajasthan, India

Karun Gurjar
ICAR-Central Institute for Arid Horticulture, Beechwal, Bikaner, Rajasthan, India

AK Singh
ICAR-Central Horticultural Experiment Station (CIAH), Vejalpur, Panchmahals, Godhra, Gujarat, India

Sanjay Singh
ICAR-Central Horticultural Experiment Station (CIAH), Vejalpur, Panchmahals, Godhra, Gujarat, India

Correspondence
Karun Gurjar
ICAR-Central Institute for Arid Horticulture, Beechwal, Bikaner, Rajasthan, India

Genetic diversity of wood apple (*Feronia limonia* L.) revealed by random amplified polymorphic DNA

R Bhargava, Karun Gurjar, AK Singh and Sanjay Singh

Abstract
Wood Apple (*Feronia limonia*) belongs to the family Rutaceae. It is also known as monkey fruit, curd fruit, kotha, Kavat and Kathbel. The different parts of plant (leaf, stem, bark, fruit, and seed) have been used for curing various diseases. The fruit is used for curative properties, which make the tree one of the useful medicinal plants of India. It is subtropical and grows in abundance throughout India's drier regions. Due to lack of awareness regarding its significance with respect to their nutritional and therapeutical values there is no systematic cultivation of wood apple in India is not available. A wide genetic diversity in wood apple in terms of fruit shape, size, colour and qualitative characters are found in wild, roadside, in farmhouse garden, and it is still under unexploited condition. Considering the wide gene diversity in wood apple, ten accessions have been collected from Central Horticultural Experiment Station (CIAH), Vejalpur, Panchmahals (Godhra), Gujarat to assess the genetic diversity in wood apple using Random Amplified Polymorphic DNA (RAPD) technique. Twenty five arbitrary random primers were used to determine RAPD polymorphism. A total of 192 amplified bands were scored from the 22 RAPD primers, out of those 139 polymorphic bands (PPB) was found. High rate of polymorphism was observed reasonably for RPID-5, RPID-16, RPID-23, RPID-24 and RPID-25 primers. The size of PCR products ranged from 250 bp to 4500 bp. The number of amplified DNA bands varied between 2 (primer RPID-13) and 16 (primer RPID-24 and RPID-25). The highest genetic similarity value coefficient was observed between genotypes CHESW12 and CHESW4 was 0.915 where as the minimum genetic similarity coefficient was observed between genotypes genotype CHESW9 and CHESW10. The dendrogram based on hierarchical cluster analysis using UPGMA algorithm was prepared. The cultivars were grouped into two main clusters. RAPD profiles developed using primers RPID-3, RPID-4, RPID-6, RPID-9, RPID-10, RPID-11, RPID-16, RPID-18, RPID-19, RPID-20, RPID-21, RPID-23, RPID-25 showed the typical/specific band combinations which can be useful for identification of genotype. In this experiment, RAPD proved to be a rapid, reliable and practicable method for revealing of polymorphism in the wood apple genotypes.

Keywords: *Feronia limonia*, RAPD, PCR, UPGMA, similarity coefficient

Introduction
Wood Apple (*Feronia limonia*) belongs to the family Rutaceae is a well known Traditional herb used since time immemorial. In India, it is known in different vernacular names Hindi (Kaitha, Kath bel), Sanskrit (kapitha), Assamese (bal, bael), Bengali (Koth bael), Gujarati (Kothu), Khmer (Kvet), Kannada (Belada hannu), Malayalam (vilam kai), Marathi (Kava TH), Oriya (Kaitha or Kainth), Telugu (Vellaga pandu), Tamil (Vilam Pazham) and English (Wood apple, Elephant apple, Monkey fruit and Curd fruit) and is a well known Traditional herb used since time immemorial (Singh *et al*., 2016) [17]. The native habitat of wood apple is South India and Sri Lanka (Sharma *et al*., 2014) [15]. It is one of the very hardy trees, mainly found in forest and dry plain area of Indian subcontinents. Different plant parts (leaf, stem, bark, fruit, and seed) have been used for curing various diseases (Joshi *et al*. 2011 and Sharma *et al*. 2012). The fruit exhibits excellent nutritional and medicinal properties. Traditionally the fruit has been used for relief against the diarrhea, dysentery, tumors, asthma, woundsetc (Ilango and Chitra, 2009) [16]. An extensive survey of diversity rich area of Gujarat was made by Singh *et al*., (2016) [17], making an allowance for the wide genetic diversity in wood apple in terms of fruit physical characters (fruit shape, size, colour, number o seeds etc.) and different chemical attributes (TSS, acidity, vitamin C, fruits mineral content, *viz*. P, K and Ca), showed a wide range of variability.

Plant genetic diversity is an important because it can strongly influence the long-term viability of plant populations, and their ability to acclimatize under to altering climatic and environmental conditions. The studies undertaken in hot spot region of wood apple demonstrates a rich genetic diversity with respect to morphological and fruit characters. Accordingly a rich germplasm has been collected from CHES, Vejalpur, Godhra. Since very limited work has been done to ascertain interrelationship among these germplasm, an attempt was made in the present study to unfold thge phylogenetic kinship among elite wood apple genotypes at the molecular level. The data these obtained constitute the text of the present communication.

Material Methods

Plant Material: The present study was carried out at the laboratory of ICAR-Central Institute for Arid Horticulture Beechwal, Bikaner, Rajasthan. The genetic diversity was studied among in 11 genotypes of wood apple. Genotypes were randomly collected from the Indore, Ratlam (Madhya Pradesh) and Vadodara (Gujarat) with an average distance was 50 km between two genotypes within district (Figure 1) These genotypes were maintained at the field of Central Horticultural Experiment Station (ICAR-CIAH), Vejalpur, Panchmahals (Godhra), Gujarat, India. Total genomic DNA was isolated from fresh young leaf material using the Qiagen kit (Qiagen India) method with little modification by adding Sodium dodecyl sulfate (SDS) and Polyvinylpyrrolidone (PVP).

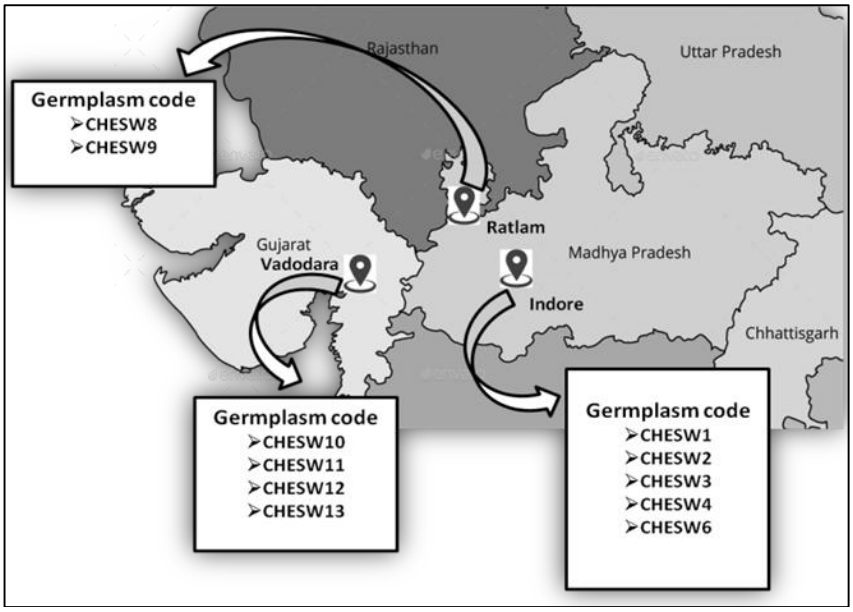

Fig 1: The location of the wood apple genotypes sample used in the study.

DNA amplification

A total of 25 primers (Genei, Bangalore, India) were used in this study (Table 1). The optimum reaction mix for a 25-µl PCR reaction comprised approximately 200 ng of DNA template; dATP, dCTP, dGTP and dTTP, each at 200 µM final concentration; 0.50 ~µM primer; 1.25 x Taq polymerase buffer [12.5 mM TRIs-HC1 (pH 8.0), 62.5 mM KC1, 0.125% triton X-t00], 800 µM $MgC1_2$; 0.5 units of recombinant Taq DNA polymerase (Thermo Fisher Scientific India Pvt. Ltd.). Amplifications were performed in a Genemate series PCR system (960A Analytica Biotech Corp., UK). For RAPD-PCR, the reaction profile consisted of an initial denaturation step of 5 min at 95°C, followed by a 1 min denaturation step at 95°C, annealing for 1 min at 37°C and extension for 2 min at 72°C. A total of 35 cycles were performed. A 7 min extra extension step was used after the last cycle in order to allow completion of incomplete reactions and were separated according to size on 1.4% agarose gels, stained with ethidium bromide and the separated PCR products were then visualized under UV light and the gels were photographed using Gene Genius Imaging System from Syngene, USA.

Data analysis

Amplified fragments were scored for each individual as present (1) or not present (0) of homologous bands. Only clear and reproducible bands were considered for scoring. The binary data matrix was prepared using the present and absence of band. To examine the genetic relationship among genotypes, NTSYS-pc version 2.02c (Rohlf, 2002) [14] was used. Among the various similarity matrices, primarily Jaccard and Dice similarity coefficients were chosen since they do not attribute the coincidence of band absence. The similarity matrices then were used to construct dendrograms using unweighted pair group method with arithmetic average (UPGMA). and sequential agglomerative hierarical nested (SAHN) cluster analysis.

Result and discussion

RAPD PCR Products

Genetic diversity studies in wood apple genotypes have been carried out using RAPD markers. DNA extraction with manual method (Doyle and Doyle, 1990) of wood apple proved difficult due to the presence of secondary metabolites, polysaccharides and phenolic compounds. DNA was successfully isolated with little modifications in Qiagen kit method. The modified method included PVP and SDS and the modifications proved to be fruitful. A total of 192 bands were presented from the 22 selected primers, corresponding to an average of 7.8 bands per primer. The selected primers generated distinctive products sizes in the range of 250-4500 bp. Out of these bands, 139 were polymorphic (72.39%). The banding pattern of RAPD produced by the primers are shown in (Figure 2), The number of polymorphic loci detected by RAPD markers was high. The maximum number of bands (16) were produced by the two primers RPID-24 and RPID-25 whereas, the minimum number of bands (2) were produced by RPID-02, (Table 1). The average percentage of

polymorphic bands (PPB) obtained for these molecular markers was 68.89. Three RAPD primers *viz*, RPID-12, RPID-18, RPID-20 were showing the highest PPB (100%) whereas, the RPID-03 was showing the lowest PPB (37.5%). Our study offers an optimization of RAPD primer screening for assessment of genetic relationship in wood apple genotypes through RAPD analysis, which was not done earlier. The polymorphic information contents (PIC) value of the primers ranged from .087 (RPID-02) to .500 (RPID-25). The gels were also screened for primers reveling RAPD unique fragments to particular genotype (Table 3). For 7 of the 12 genotypes, it was possible to find at least one such primers. Out of 25 RAPD primer set, a total 13 primers (RPID-3, RPID-4, RPID-6, RPID-9, RPID-10, RPID-11, RPID-16, RPID-18, RPID-19, RPID-20, RPID-21, RPID-22 and RPID-25) revealed specific band for the genotypes. The highest numbers of unique bands (four bands) were obtained by the primer RPID-25, whereas The maximum numbers of unique bands (six bands) were observed in the genotype CHESW-09.

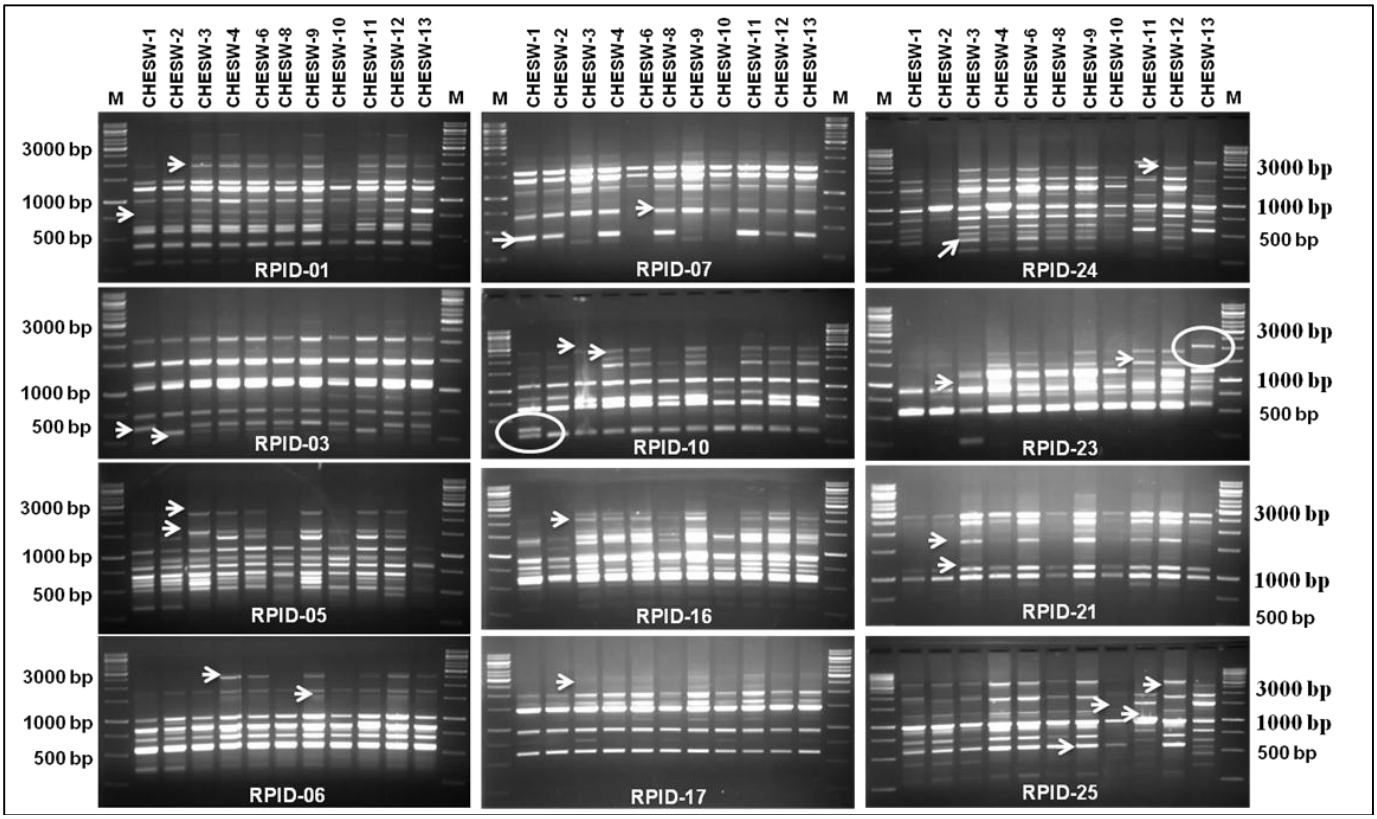

Fig 2: Genetic polymorphism among wood apple genotypes as revealed by RAPD analysis. M: 1 kbp DNA ladder.

Genetic structure and genetic relationship analysis

Since RAPD markers are dominant, so we implicit that each band represented the phenotype at a single biallellic locus (Williams *et al*., 1990). Twenty two primers that gave reproducible polymorphic bands were selected by repeating the two times of the experiment (Table 1). The dendrogram generated (Figure 3) by UPGMA cluster analysis of the data produced by RAPD, could be clearly divided the 11 wood apple genotypes into two clusters, i.e. cluster I had all the 10 genotypes of wood apple (CHESW1, CHESW2, CHESW3, CHESW4, CHESW6, CHESW8, CHESW9, CHESW11, CHESW12 and CHESW13). Cluster II had only the rest genotype CHES-10. However, the cluster I divided further in two major Groups *viz*., group A (CHESW1, CHESW2) and group B (CHESW3, CHESW4, CHESW6, CHESW8, CHESW9, CHESW11, CHESW12 andCHESW13). The group B further divided into the two sub group *viz* group B(I) and group B (II) which contains the 6 (CHESW3, CHESW4, CHESW6, CHESW8, CHESW9 and CHESW12) and 2 (CHESW11 and CHESW13) genotypes respectively. Based on RAPD data, all possible pairwise Jaccard's similarity coefficient values for 11 wood apple genotypes were calculated (Table 2). Which indicated the range of genetic similarity indexed values ranged minimum 0.518 (between CHESW4 and CHESW12) to a maximum value of 0.915 (between CHESW4 and CHESW12), whereas the mean similarity indexed value was observed 0.688.

Massive loss of valuable plant species in the past centuries and its adverse impact on environmental and socioeconomic values has triggered the conservation of plant resources. For this, it is essential to have accurate identification and characterization of plant materials which can subsequently use for the breeding applications and to ensure their sustainable use. In the past few years, developed molecular tools seems to be more effective, easy, less laborious than traditional morphological markers because it allows direct access to the genetic material (Williams *et al*., 1990; Paterson *et al*., 1991) [21, 12] as well as they makes possible to understand the relationships between plants (Barik *et al*. 2005; Ibrahim A. Arif. *et al*. 2010) [3, 2]. Application of different DNA markers are extensively used since DNA markers reflect directly individual differences at the level of DNA molecules (cover both coding and noncoding regions of the genome) (Dandelj *et al*. 2004) [5] and are not affected by environment, developmental stage, certain tissue and organ, and have high-genomic frequency, high polymorphism and mostly a random genomic distribution (Moghaieb *et al*., 2013) [11]. Various DNA markers were used for genetic diversity assessment, such as RFLP (Botstein *et al*., 1980) [4], RAPD (Williams *et al*., 1990) [5], AFLP (Vos *et al*., 1995) [19], SSR

(Akkaya *et al*., 1992), SNP (Jordan and Humphries 1994) [19] etc. RAPD technique is a simple and powerful DNA marker tool, though the technique has also some limitations (Ram G S *et al*. (2007) [19]. It has been widely used in many plant species for varieties analysis, population studies and genetic linkage mapping (Williams *et al*., 1990; Yu *et al*., 1993; Rout *et al*., 2003) [21, 3]. RAPD analysis has been found to be useful in genetic diversity estimation in different genus of *Rutaceae* family such as *Boronia* (Yan *et al*., 2002) [22], *Citrus* (Federici *et al*., 1998; Uchoi *et al*. 2017) [1, 2]. Our results are also confirmatory with the finding of Singh *et al*. (2016) [13] who reported significant (high) genetic variations using the physical and chemical characteristics in wood apple genotypes.

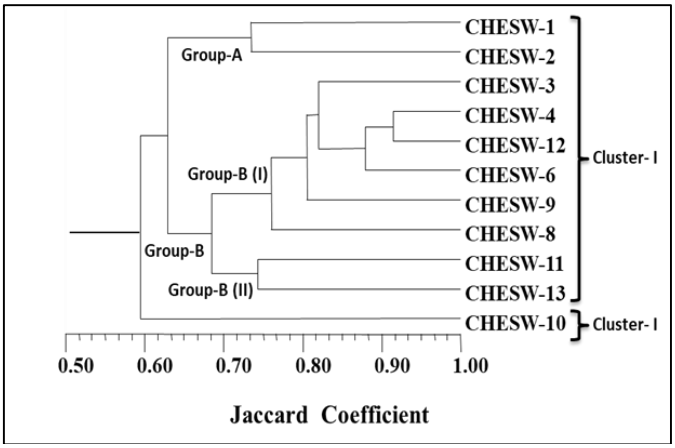

Fig 3: Clustering of 11 wood apple genotypes based on RAPD profiling.

Table 1: Total number of scorable bands, polymorphism % and band size of RAPD markers obtained by 22 RAPD primers.

S. No.	Name of Primer	Size of Bands	No. of Total Band	No. of Polymorphic bands	No. of Monomorphic bands	Polymorphic Pecentage	PIC
1	RPID-1	360-4500	13	8	5	61.54	0.270
2	RPID-2	400-700	2	1	1	50.00	0.087
3	RPID-3	400-2000	8	3	5	37.50	0.425
4	RPID-4	600-1500	5	4	1	80.00	0.492
5	RPID-5	300-2500	13	10	3	76.92	0.426
6	RPID-6	400-2500	10	4	6	40.00	0.397
7	RPID-7	400-1800	8	4	4	50.00	0.252
8	RPID-9	500-1400	8	5	3	62.50	0.407
9	RPID-10	300-2500	9	6	3	66.67	0.451
10	RPID-11	300-1800	8	5	3	62.50	0.425
11	RPID-12	500-3500	8	8	0	100.00	0.312
12	RPID-13	600-1100	2	1	1	50.00	0.298
13	RPID-15	600-2500	8	5	3	62.50	0.351
14	RPID-16	550-2500	11	9	2	81.82	0.364
15	RPID-17	600-2000	7	3	4	42.86	0.372
16	RPID-18	400-2400	8	8	0	100.00	0.494
17	RPID-19	550-2500	7	5	2	71.43	0.419
18	RPID-20	550-1900	8	8	0	100.00	0.449
19	RPID-21	1000-2900	6	3	3	50.00	0.382
20	RPID-23	250-2100	11	9	2	81.82	0.475
21	RPID-24	400-3100	16	15	1	93.75	0.485
22	RPID-25	300-3000	16	15	1	93.75	0.500
Total			192	139	53	Average 68.89	Average 0.388

Table 2: Genetic distances among 11 wood apple genotypes.

Genotypes	CHESW1	CHESW2	CHESW3	CHESW4	CHESW6	CHESW8	CHESW9	CHESW-10	CHESW11	CHESW12	CHESW13
CHESW1	1.000										
CHESW2	0.735	1.000									
CHESW3	0.737	0.617	1.000								
CHESW4	0.709	0.583	0.819	1.000							
CHESW6	0.747	0.618	0.837	0.876	1.000						
CHESW8	0.732	0.593	0.767	0.784	0.777	1.000					
CHESW9	0.631	0.540	0.776	0.825	0.819	0.707	1.000				
CHES-10	0.633	0.534	0.604	0.591	0.616	0.700	0.518	1.000			
CHESW11	0.611	0.577	0.693	0.772	0.755	0.644	0.692	0.584	1.000		
CHESW12	0.687	0.585	0.805	0.915	0.884	0.770	0.800	0.613	0.791	1.000	
CHESW13	0.574	0.526	0.638	0.685	0.648	0.617	0.574	0.554	0.744	0.706	1.000

Table 3: Wood apple genotypes and their specific RAPD markers.

S. No.	Genotypes	Primers revealing specific RAPDs (no. of base pairs of a band)	Total marker
1	CHESW-1	RPID-3 (600); RPID-4 (600); RPID-10- (400); RPID-11 (500).	4
2	CHESW-2	RPID-6(900).	1
3	CHESW-9	RPID-18 (700); RPID-18 (800); RPID-18 1500; RPID-20 (550); RPID-20 (1000); RPID-21 (2000).	6
4	CHESW-10	RPID-9 (500); RPID-19 (1100); RPID-19 (2400).	3
5	CHESW-11	RPID-25 (630).	1
6	CHESW-12	RPID-4 (1500).	1
7	CHESW-13	RPID-16 (1900); RPID-23 (2100); RPID-25 (350); RPID-25 (2100); RPID-25 (2500).	5
Total			21

Conclusion

Owing to nutritional and medicinal importance, wood apple is not exploited at genetic level yet. The study of molecular markers is not known in wood apple so far. therefore, RAPD markers have been identified and profiled first time to determine the genetic diversity in wood apple genotypes. The RAPD analysis resulted into 139 polymorphic bands assay the genotypes which revealed two clusters and each clusters devided into sub-group according to their location. However, two genotypes vis. CHES 12 and CHES 6 are clustered in between. This study can be utilized to develop the specific markers for coop improvement programme in wood apple.

References

1. Akkaya MS, Bhagwat AA, Cregan PB. Length polymorphisms of simple sequence repeat DNA in soybean. Genetics. 1992; 132:1131–1139.
2. Arif IA, Bakir MA, Khan HA, Al Farhan AH, Al Homaidan, AA. A Brief Review of Molecular Techniques to Assess Plant Diversity. International Journal of Molecular Sciences. 2010; 11:2079-2096.
3. Barik S, Senapati SK, Aparajita S, Mohapatra A, Rout GR. Identification and Genetic Variation among *Hibiscus* Species (Malvaceae) Using RAPD Markers. Zeitschrift fur Naturforschung C. 2006 61(1-2):123-8.
4. Botstein D, White RL, Skolnick M, Davis RW. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am J Hum Genet. 1980; 32:314-331.
5. Dandelj D, Jakse J, Javornik B. Assesment of genetic variability of olive variaties by microsatellite and AFPL markers. Euphitica. 2004; 136:93-102.
6. Doyle JJ, Doyle JL. Isolation of plant DNA from fresh tissue. Focus. 1990; 12:13-15.
7. Federici CT, Fang DQ, Scora RW, Roose ML. "Phylogenetic Relationships With in the Genus Citrus (Rutaceae) and Related Genera as Revealed by RFLP and RAPD Analysis. TAG Theoretical and Applied Genetics. 1998; l96:812–822.
8. Ilango K, Chitra V. Wound healing and anti-oxidant activities of the fruit pulp of *Limonia acidissima* Linn. (Rutaceae) in rats. Tropical Journal of Pharmaceutical Research. 2010; 9:223-30.
9. Jordan SA, Humphries P. Single nucleotide polymorphism in exon 2 of the BCP gene on 7q31–q35. Hum Mol Genet. 1994; 3:1915.
10. Joshi RK, Badakar VM, Kholkute SD, m Khatib N. Chemical composition and antimicrobial activity of the essential oil of the leaves of *Feronia elephantum* (Rutaceae) from northwest Karnataka. Natural Product Communications. 2011; 6(1):141-3.
11. Moghaieb REA, Mohammed EHK, Youssie SS. Genetic diversity among some canola cultivars as revealed by RAPD, SSR and AFLP analyses. Biotech, 2014, 403-410.
12. Paterson AH, Lander ES, Hewitt JD, Peterson S, Lincolin SF, Tanksley SD. Resolution of quantitative traits into Mendelian factors by using a complete RFLP linkage map. Nature. 1988; 335:721-726.
13. Ram GS, Parthiban KT, Senthil KR, Thiruvengadam V, Paramathma M. Genetic diversity among Jatropha species as reveled by RAPD markers. Genet. Resour. Crop Evol. 2008; 55(6):80-809.
14. Rohlf FJ. NTSYS-pc 2.0e. Exeter Software, Setauket, New York, USA, 2002.
15. Sharma PH, Patela H, Sharma S. Study of Physico-Chemical Changes During Wood Apple (*Feronia limonia*), Journal of Food Research and Technology. 2014; 2(4):148-152.
16. Sharma P, Bodhankar SL, Thakurdesai PA. Protective effect of aqueous extract of *Feronia elephantum* Correa leaves on thioacetamide induced liver necrosis in diabetic rats. Asian Pacific Journal of Tropical Biomedicine. 2012; 2(9):691-695.
17. Singh AK, Singh S, Yadav V, Sharma BD. Genetic variability in wood apple (*Feronia limonia*) from Gujarat. 2016; 86(11):1504-1508.
18. Uchoi A, Malik SK, Choudhary R, Kumar S, Pal D. 2017. IJBT 16(3):346-356.
19. Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M *et al*. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res. 1995; 23:4407–4414
20. Welsh J, McClelland M. Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res. 1990; 18:7213-7218.
21. Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. 1990; 18:6531-6535.
22. Yan G, Shan F, Plummer AJ. Genomic relationships within Boronia (Rutaceae) as revealedby karyotype analysis and RAPD molecular markers. Plant Syst. Evol. 2002; 233:147-161.