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Research Article

Genetic diversity assessment and population genetic studies in Indian Rough Lemon (*Citrus jambhiri* Lush) using RAPD and ISSR markers

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Abstract
An investigation was undertaken to ascertain the extent of genetic diversity and population differentiation among *Citrus jambhiri* accessions. Thirty eight accessions of *C. jambhiri* collected from four different geographical locations (North-east India, Himachal Pradesh, Punjab and Karnataka) were evaluated and characterized using 23 RAPD and 17 ISSR primers. Both RAPD and ISSR markers revealed 79.34% and 80.72% polymorphism, respectively among the accessions. Pair wise comparison of DNA profiles of thirty eight accessions based on RAPD, ISSR and pooled data gave an average similarity value of 0.76, 0.78 and 0.77, respectively. The highest Nei's genetic distance value based on RAPD and ISSR (0.0970, 0.1056) revealed that populations from Himachal Pradesh and Karnataka were most distinct genetically. The genetic differentiation (G_{st}) and Gene flow (N_m) value of RAPD (0.2393, 1.5894) and ISSR (0.2439, 1.5496) markers indicated less genetic differentiation among the collected populations of *C. jambhiri*.

Key words: *Citrus jambhiri*, genetic diversity, population differentiation, RAPD, ISSR

Introduction

Citrus (L.) is one of the most economically important fruit crops of the world, belonging to the subfamily Aurantioideae of the family Rutaceae. It is widely distributed throughout the tropical and subtropical regions of the world and believed to have originated in Southeast Asia, particularly northeast India, Malayan archipelago, China, Japan, and Australia. *Citrus* occupies the second position in terms of area (1064 ha) and third position in terms of production (9.94 mt) of fruit crops in India (NHB database, 2013-2014). North-eastern India is thought to be the area of origin of several *Citrus* species and abundant diversity of various citrus types is available in the north-eastern and north-western states. India has an enormous diversity of Citrus genetic resources, both cultivated and wild. The rich genetic diversity particularly in mandarins, sweet oranges, lime and lemons immensely contributed in improvement of citrus cultivation and industry in India.

Rough lemon (*Citrus jambhiri* Lush.) is one of the indigenous species of probable hybrid origin. It is believed to have originated in India and found throughout the country up to an altitude of 1000 m. In India, rough lemon has largely been used as a rootstock for commercial plantations of citrus; it is one of the major rootstock for mandarin, oranges, Kagzi nimbu, Kinnow etc. Trees on these stocks are vigorous, high yielding, and tristeza- and drought-tolerant. It has good adaptability for light sandy soils and is also fairly tolerant to salts. Apart from its use as a rootstock, it can also be used just like the common lemon, as a garnish on fish or meat or with iced or hot tea, to be squeezed for the flavoured juice. Jamir can be eaten fresh, which can solve this Vitamin-C deficiency partially. It also contains some organic compounds which work against asthma, antidepressant, stress relief, aids digestion, colds, flue, fever, nosebleeds, mouth ulcers, throat infection and boils. Various kinds of food items like Jam, Jelly, pickle and salad can also be prepared from it. The rough lemon has been found to show a very high percentage of nucellar embryony, which would indicate a hybrid origin. Based on the morphological and biochemical studies, Scora (1975) suggested that *C. jambhiri* is a species of hybrid origin between citron and mandarin. Later, molecular markers (RAPD, SSR, cpDNA) also supported this view (Nicolosi *et al*. 2000; Federici *et al*. 1998; Barkley *et al*. 2006, Jena *et al*., 2009). Recently, *C. taitensis* Risso is used as accepted botanical name for *C. jambhiri* (Mabberley, 2008). An array of diverse cultivars is available in this species and there are no published studies on detailed genetic diversity assessment of *C. jambhiri* in India or elsewhere yet. Assessment of genetic diversity would help in understanding the extent and pattern of genetic diversity within and among its populations and would prove to be a potential gene source for Citrus breeding and crop improvement programmes.

Materials and Methods

Plant Material:

A total of thirty eight accessions of rough lemon were collected from four geographical regions of

India. Germplasm for study was collected from North-Eastern India- Sikkim, Arunachal Pradesh, Manipur, Upper Assam and Nagaland (19 accessions), Himachal Pradesh (6 accessions), Punjab(5 accessions) and South India- Karnataka, CHES, Chettali (8 accessions) (Table 1). Standard procedures for survey, sampling, collection and documentation were followed (Guarino *et al.*, 1995). Sample collected from single plant (treated thereafter as individual accession) was given a unique collection number or national identity. The research work was undertaken at cryolaboratory of Tissue Culture and Cryopreservation Unit (TCCU) at National Bureau of Plant Genetic Resources (NBPGR), New Delhi.

DNA Extraction:

Extraction of total genomic DNA was carried out as per the modified protocol of Doyle and Doyle (1990). Quantification of isolated DNA was done spectrophotometrically and its quality was checked by electrophoresis on 1.2% agarose gel.

RAPD Analysis:

The RAPD primers used in the present study were procured from Operon Tech. Inc. Alameda, CA, USA. A total of 50 primers of the Operon series OPA, OPB, OPC, OPD, OPF, OPG, OPO, OPM, OPU were screened with selected templates, out of which 23 primers which gave polymorphic bands were selected for the study. Final optimized amplification reactions were performed in 25 µl reaction volume containing 1X Assay buffer (100 mM Tris HCl (pH 8.3); 500 mM KCl), 2.5 mM $MgCl_2$, 0.2 mM dNTP each, 1 U *Taq* DNA polymerase, 10 pmoles RAPD primer and 20 ng genomic DNA. The amplification was performed in a Bioer Xp thermocycler with reaction conditions programmed as pre-denaturation at 94˚C for 4 min followed by 40 cycles of denaturation at 94˚C for 1 min, annealing at 35˚C for 2 min and extension at 72˚C for 2 min and final extension at 72˚C for 7 min. PCR products containing 3µl loading dye were separated by electrophoresis on 1.5% agarose gel. Gene ruler of 1kb size (Fermentas, International, Inc) was loaded in the first lane of each gel to determine the size of amplified bands. Electrophoresis was carried out at 80 V for 3 h and photographed using UV transilluminator (Mega Biosystematica, U.K).

ISSR Analysis:

For ISSR-PCRs, a total of 50 primers procured from Geno-Biosciences Pvt. Ltd. were screened and out of which, 17 primers which generated clear and unambiguous amplification products were selected for final analysis in selected accessions of *C. jambhiri*. PCR-amplification was carried out in 25 ml reaction volume containing 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 1.0–2.5 mM $MgCl_2$, 0.2 mM dNTP each, 1.0 U Taq DNA polymerase, 0.2 mM primer and 25–30 ng genomic DNA. The amplification was performed in a Bioer Xp thermocycler with reaction conditions programmed as pre-denaturation at 94˚C for 4 min followed by 35 cycles of denaturation at 94˚C for 1 min, annealing at optimized temperature for 1 min and extension at 72˚C for 2 min and final extension at 72˚C for 7 min. PCR products containing 3 µl loading dye were separated by electrophoresis on 1.8% agarose gel. Gene ruler of 1 kb size (Fermentas, International, Inc) was loaded in the first lane of each gel to determine the size of amplified bands. Electrophoresis was carried out at 80 V for 3 h and photographed using UV transilluminator (Mega Biosystematica, U.K).

Data Analysis:

Amplified fragments of RAPD and ISSR were scored for each individual as presence (1) or absence (0) of homologous bands on the basis of size comparison with low range DNA ruler (Genei, Bangalore).A pairwise genetic similarity matrix between accessions was estimated using Jaccard's similarity coefficient (Jaccard, 1908). Dendrograms were constructed using UPGMA of NTSYS-pc version 2.10e (Rohlf, 2000). Mantel test (Mantel, 1967) for goodness of fit for UPGMA cluster analysis to the data was also performed. Polymorphism information content (PIC) of each primer was determined as described by Smith *et. al.* (1997) as PIC = 1 –Σ*fi2*, where *fi* is the frequency of the *i*th allele. Genetic variation among the four populations was analysed on the basis of the banding profile using various parameters such as percentage polymorphism, observed no of alleles (n_a), effective no of alleles (n_e), the total heterozygosity (Ht), heterozygosity within population (H_S), gene diversity (h), coefficient of gene differentiation (G_{st}), estimation of gene flow (N_m) and Shannon's information index (I) using the software POPGENE 1.31 (Yeh *et al*., 1999).

Results and Discussion

RAPD-PCR amplification of the genomic DNA from 38 rough lemon accessions yielded a total of 99 bands, of which 77 were polymorphic. The total number of amplified DNA bands ranged from 2 to 10 with an average of 4.3 bands per primer. The polymorphism percentage ranged from 50 (primer OPD-11 and OPF-01) to 100% (OPB-13, OPB-16, OPC-08, OPD-12, OPD-15, OPF-02, OPH-15, OPM-13, OPU-05 and OPU-20) (Table 2). Average polymorphism across all 38 accessions was 79.34% and also five RAPD primers gave six unique bands in specific *C. jambhiri* accessions. These unique fragments can be used as a marker for identification of these accessions, which will be useful for future conservation, maintenance and breeding programme and can also be used for developing core collection

of *C. jambhiri* germplasm (Table 3). The high level of polymorphism indicated that RAPD markers could be considered as effective tools for estimating genetic diversity in different accessions of Rough lemon. Seventeen primers were selected for the ISSR analysis based on the reproducibility and banding patterns and a total of 83 bands were generated, of which 65 bands were polymorphic. Each primer amplified 3-8 polymorphic amplicons with an average of 4.88 amplicons per primer. The polymorphism percentage ranged from 37.5% (primer UBC-841) to 100% (UBC-807, UBC-812, UBC-827, UBC-855 and UBC-880) and the average polymorphism across all the accessions was 80.72% (Table 4) which is on par with RAPD data. For RAPD, the pair wise Jacquard's similarity values obtained between 38 accessions ranged from 0.55-0.99 with an average similarity of 0.76 and for ISSR it ranged from 0.52 - 0.97 with an average similarity of 0.78. Based on combined RAPD and ISSR data similarity among the accessions of *C. jambhiri* ranged from 0.58- 0.95 with an average of 0.77. Maximum similarity (95%) was observed between accessions MR-09 and MR-10, while minimum was observed between MD-76 and MD-67 with the similarity value of 0.58. This shows that both the primers were on par in grouping the accessions according to their genetic similarity.

RAPD-dendrogram generated based on UPGMA method grouped all the 38 accessions into three major clusters. First cluster comprised of 2 accessions MR-01 and MR-02 and the second cluster comprised of 3 accessions MD-76, MD-2 and MSA-34. The biggest formed cluster was the third cluster which consisted of remaining 33 accessions (Fig1). For ISSR also, dendrogram generated based on UPGMA method grouped all the 38 accessions into three major clusters. First cluster comprised of only two accessions MD-67 and MD-81. Second cluster comprised only one accession MD-37. Rest of the 35 accessions was grouped into the third cluster (Fig 2). Pooled dendrogram generated based on combined RAPD and ISSR data grouped all the thirty eight accessions into four major clusters. First, second and third clusters had only one accession each, MD-81, MR-02 and MD-76 respectively. Fourth cluster was divided into 7 small clusters (Fig 3).

In all, the dendrograms (RAPD, ISSR and Combined), two accessions MD-81(Nimbu Tenga) and MD-76(Gol Nimbu) collected from Upper Assam and Nagaland region and two accessions MR-01 and MR-02 collected from Himachal Pradesh were the most distinct genetically among all other accessions. But this distinctness is not correlated with their morphological characters. The genetic distinctness may be due to their particular area of adaptation and some free genetic exchange between these accessions and their close relatives. Accessions MD-81 and MD-76 were collected from the wild and they had a rare distribution which may also account for their distinctness. Accessions MR-09, MR-10 and MR-11 collected from Karnataka region showed the maximum similarity among themselves suggesting that these accessions are a result of natural selection and chance seedlings derived from *C. jambhiri*, which were also found to have similar morphology and adapted to higher altitude in particular climatic condition. All the clustering patterns in the present study did not accord with geographical sources since having genetically diverse germplasm resources. However, separation of the accessions studied through major clusters could be explained by broad context of molecular as well as ecological divergences and formation of subsequent narrow distant sub-clusters could be explained by narrow range of diversification and comparatively strong relationship in respect of these factors within and between the rough lemon germplasm resources.

Population genetic analysis using RAPD and ISSR markers were very useful and informative in the differentiation and estimation of genetic variability among and within populations. ISSR markers could detect greater heterozygosity (H_t-0.2436), Nei's genetic distance value (G.D-0.0609-0.1056) and genetic differentiation value (G_{st}-0.2439) within *C. jambhiri* populations than those detected by RAPD markers (Ht-0.2081, GD-(0.0449-0.0970), G_{st}-0.2393) (Tables 5 and 7). Nei's (1978) unbiased measures of genetic identity and genetic distance among 4 populations of *C. jambhiri* generated by both RAPD and ISSR markers revealed that populations collected from Himachal Pradesh and Punjab were most similar genetically owing to their similar ecological conditions of adaptation and population collected from Himachal Pradesh and Karnataka were the most distinct genetically (Tables 6 and 8). Gene flow (N_m) with a value of less than one (less than one migrant per generation into a population) and genetic differentiation (G_{st}) with a value more than 0.25 is generally regarded as the threshold quantity beyond which significant population differentiation occurs. The lower G_{st} values (RAPD-0.2393, ISSR-0.2439) and high gene flow values (RAPD-1.5894, ISSR-1.5496) indicated less genetic differentiation among four populations of *C. jambhiri* (Tables 5 and 7). This indicates that sufficient outbreeding is occurring between the individuals which is maintaining the gene flow and genetic diversity among and between the populations. Clustering of some accessions with the accessions of different populations indicate that all accessions originated from a single large population and diverged to different geographical areas.

Thus it is found that, based on RAPD and ISSR marker analysis, a wide variability is present among

the collected accessions of *C. jambhiri* and there is very less genetic differentiation observed between different populations collected from different parts of India. The selection of distinct and superior genotypes from the collected accessions will be helpful to increase production through breeding program which in turn could improve varietal characteristics.

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http://www.nhb.gov.in

Table 1. *Citrus jambhiri* accessions used for the study

S. No.	Accession name	Common name	Region of collection
1	MD-8	Jamir	Sikkim
2	MD-21	Naity Jamir	Sikkim
3	MD-24	Jamir	Sikkim
4	MD-34	Kachai Lemon	Manipur
5	MD-37	Kachai Lemon	Manipur
6	MD-40	Gol Nimbu	Upper Assam & Nagaland
7	MD-41	Hathi Nimbu	Manipur
8	MD-53	Champra	Upper Assam & Nagaland
9	MD-54	Sinduri Lemon	Upper Assam & Nagaland
10	MD-57	Rough Lemon	Upper Assam & Nagaland
11	MD-67	Soh Bitter	Upper Assam & Nagaland
12	MD-69	Adha Jamir	Upper Assam & Nagaland
13	MD-76	Nimbu Tenga	Upper Assam & Nagaland
14	MD-81	Gol Nimbu	Upper Assam & Nagaland
15	MD-92	Hasu	Upper Assam & Nagaland
16	MD-96	Gangen	Upper Assam & Nagaland
17	MD-100	Rough Lemon	Upper Assam & Nagaland
18	MD-2	Rough Lemon	Arunachal Pradesh
19	MD-7	Rough Lemon	Arunachal Pradesh
20	MSA-34	Rough Lemon	Himachal Pradesh
21	MSA-41	Rough Lemon	Himachal Pradesh
22	MR-01	Rough Lemon	Himachal Pradesh
23	MR-02	Rough Lemon	Himachal Pradesh
24	MR-03	Rough Lemon	Himachal Pradesh
25	MR-17	Jamir	Himachal Pradesh
26	MR-12	Jatti Khatti	Punjab
27	MR-13	Jullandir Khatti	Punjab
28	MR-14	Florida Rough	Punjab
29	MR-15	Italian Rough	Punjab
30	MR-16	Jatti Khatti	Punjab
31	MR-04	Jatti Khatti	Karnataka
32	MR-05	Karna Khatta	Karnataka
33	MR-06	Jambhiri kodur sri	Karnataka
34	MR-07	Poona Jambhiri	Karnataka
35	MR-08	Moogu Nimbu	Karnataka
36	MR-09	Jatti Khatti	Karnataka
37	MR-10	Jullandir Khatti	Karnataka
38	MR-11	Jambir Kodur	Karnataka

Table 2. Details of amplified bands generated based on 23 RAPD primers

Sl.no	Primer	Sequence(5'-3')	Total no. of amplified bands	No. of polymorphic bands	Polymorphism (%)	PIC
1	OPA-01	CAGGCCCTTC	4	3	75	0.139
2	OPA-04	AATCGGGCTG	3	0	0	0
3	OPB-04	GGACTGGAGT	6	4	66.66	0.138
4	OPB-13	TTCCCCCGCT	4	4	100	0.153
5	OPB-16	TTTGCCCGGA	4	4	100	0.164
6	OPB-17	AGGGAACGAG	4	3	75	0.062
7	OPC-08	TGGACCGGTG	2	2	100	0.383
8	OPD-03	GTCGCCGTCA	6	5	83.33	0.185
9	OPD-11	AGCGCCATTG	4	2	50	0.025
10.	OPD-12	CACCGTATCC	3	3	100	0.427
11.	OPD-15	CATCCGTGCT	2	2	100	0.392
12.	OPF-01	ACGGATCCTG	4	2	50	0.182
13.	OPF-02	GAGGATCCCT	5	5	100	0.270
14.	OPF-06	GGGAATTCGG	4	3	75	0.267
15.	OPG-08	TCACGTCCAC	4	3	75	0.074
16.	OPH-04	GGAAGTCGCC	5	4	80	0.192
17.	OPH-15	AATGGCGCAG	5	5	100	0.355
18.	OPO-04	AAGTCCGCTC	4	3	75	0.038
19.	OPO-06	CCACGGGAAG	5	3	60	0.205
20	OPM-13	GGTGGTCAAG	3	3	100	0.466
21	OPU-05	TTGGCGGCCT	3	3	100	0.201
22.	OPU-16	CTGCGCTGGA	10	6	60	0.131
23.	OPU-20	ACAGCCCCCA	5	5	100	0.244
Total			99	77		
Mean			4.30	3.34	79.34	0.204

Table 3. List of RAPD primers generated unique bands for specific *C. jambhiri* cultivars

Sl. No.	Primer	Fragment size (bp)	Cultivar
1	OPB-04	450	MD-92
2	OPD-03	900, 400	MD-76, MR-01
3	OPG-08	600	MR-02
4	OPO-04	300	MSA-41
5	OPU-16	150	MD-21

Table 4. Details of amplified bands generated based on 17 ISSR primers

Sl no	Primer code	Sequence 5'-3'	Total no. of bands	PB	PPB	PIC
1.	UBC-807	(AG)8T	4	4	100	0.207
2.	UBC-808	(AG)8C	7	6	85.71	0.214
3.	UBC-809	(AG)8G	5	4	80	0.188
4.	UBC-810	(GA)8T	5	4	80	0.217
5.	UBC-811	(GA)8C	6	5	83.33	0.247
6.	UBC-812	(GA)8A	3	3	100	0.39
7.	UBC-827	(AC)8G	3	3	100	0.099
8.	UBC-834	(AG)8YT	7	6	85.71	0.195
9.	UBC-836	(AG)8YA	5	4	80	0.175
10.	UBC-840	(GA)8YT	6	5	83.33	0.221
11	UBC-841	(GA)8YC	8	3	37.5	0.116
12	UBC-842	(GA)8YG	4	3	75	0.236
13	UBC-855	(AC)8YT	3	3	100	0.083
14	UBC-868	(GAA)6	4	3	75	0.131
15	UBC-880	(GGGTG)3	5	5	100	0.097
16	UBC-889	DBD(AC)7	5	2	40	0.02
17	UBC-890	VHV(GT)7	3	2	66.66	0.162
Total			83	65		
Mean			4.88	3.82	80.72	0.176

Table 5. Genetic structure data for four populations of *C. jambhiri* derived from RAPD markers

Population	Sample size	Mean n_a (St. Dev.)	Mean n_e (St. Dev.)	Mean h (St. Dev.)	Mean I (St. Dev.)	Mean H_t (St. Dev.)	Mean H_s (St. Dev.)	PPB (%)
North East India	19	1.5960(0.4932)	1.3797(0.3993)	0.2150(0.2120)	0.3168(0.2994)	0.2150(0.0450)	0.2150(0.0450)	59.60%
Himachal Pradesh	6	1.5455(0.5005)	1.3540(0.3962)	0.2017(0.2086)	0.2984(0.2961)	0.2017(0.0435)	0.2017(0.0435)	54.55%
Punjab	5	1.2828(0.4527)	1.2095(0.3609)	0.1165(0.1935)	0.1690(0.2768)	0.1165(0.0374)	0.1165(0.0374)	28.28%
Karnataka	8	1.2727(0.4476)	1.1775(0.3368)	0.1001(0.1803)	0.1477(0.2582)	0.1001(0.0325)	0.1001(0.0325)	27.27%
Average (at pop. level)		1.4243	1.2802	0.1583	0.2330	0.1583	0.1583	
Total (at species level)	38	1.7778(0.4179)	1.3707(0.3704)	0.2196(0.1924)	0.3363(0.2662)	0.2081(0.0346)	0.1583(0.0222)	77.78%

G_{st}-0.2393 N_m-1.5894

n_a - Observed number of alleles ; n_e - Effective number of alleles ; h - Gene diversity (Nei, 1973);
I - Shannon's Information index; H_t- Total heterozygosity, estimated as $H_t = 1-\Sigma Pi^2$, where Pi is the frequency of i^{th} allele (Nei, 1978);
H_s- Mean heterozygosity within population; PPB- Percentage of polymorphic bands;
G_{st} - Coefficient of gene differentiation, calculated as $G_{st} = H_t-H_s/H_t$ (Nei, 1978);
N_m - Estimate of gene flow, calculated as $N_m = 0.5(1 - G_{st})/G_{st}$

Table 6. Nei's (1978) unbiased measures of genetic identity (above diagonal) and genetic distance (below diagonal) among 4 populations of *C. jambhiri* generated by RAPD markers

Pop ID	1	2	3	4
1	****	0.9539	0.9420	0.9156
2	0.0472	****	0.9560	0.9076
3	0.0598	0.0449	****	0.9334
4	0.0882	0.0970	0.0690	****

Table 7. Genetic structure data for four populations of *C. jambhiri* derived from ISSR markers.

Population	Sample size	Mean n_a (St. Dev.)	Mean n_e (St. Dev.)	Mean h (St. Dev.)	Mean I (St. Dev.)	Mean Ht (St. Dev.)	Mean Hs (St. Dev.)	PPB (%)
North East India	19	1.7108(0.4561)	1.5133(0.3895)	0.2883(0.2030)	0.4195(0.2866)	0.2883(0.0412)	0.2883(0.0412)	71.08
Himachal Pradesh	6	1.5060(0.5030)	1.4141(0.4475)	0.2206(0.2318)	0.3148(0.3249)	0.2206(0.0537)	0.2206(0.0537)	50.60
Punjab	5	1.2410(0.4303)	1.2044(0.3844)	0.1076(0.1978)	0.1528(0.2781)	0.1076(0.0391)	0.1076(0.0391)	24.10
Karnataka	8	1.2771(0.4503)	1.2200(0.3715)	0.1203(0.2000)	0.1725(0.2846)	0.1203(0.0400)	0.1203(0.0400)	27.71
Average (at pop. level)		1.4337	1.3379	0.1842	0.2649	0.1842	0.1842	
Total (at species level)	38	1.7831(0.4146)	1.4533(0.3453)	0.2706(0.1795)	0.4075(0.2523)	0.2436(0.0300)	0.1842(0.0201)	78.31%

G_{st}-0.2393 N_m-1.5894

n_a - Observed number of alleles ; n_e - Effective number of alleles ; h - Gene diversity (Nei, 1973);
I - Shannon's Information index; H_t- Total heterozygosity, estimated as $H_t = 1-\Sigma Pi^2$, where Pi is the frequency of i^{th} allele (Nei, 1978);
H_s- Mean heterozygosity within population; PPB- Percentage of polymorphic bands;
G_{st} - Coefficient of gene differentiation, calculated as $G_{st} = H_t-H_s/H_t$ (Nei, 1978);
N_m - Estimate of gene flow, calculated as $N_m = 0.5(1 - G_{st})/G_{st}$

Table 8. Nei's (1978) unbiased measures of genetic identity (above diagonal) and genetic distance (below diagonal) among 4 populations of *C. jambhiri* generated by ISSR markers.

Pop ID	1	2	3	4
1	****	0.9179	0.9097	0.9093
2	0.0856	****	0.9409	0.8998
3	0.0947	0.0609	****	0.9362
4	0.0950	0.1056	0.0660	****

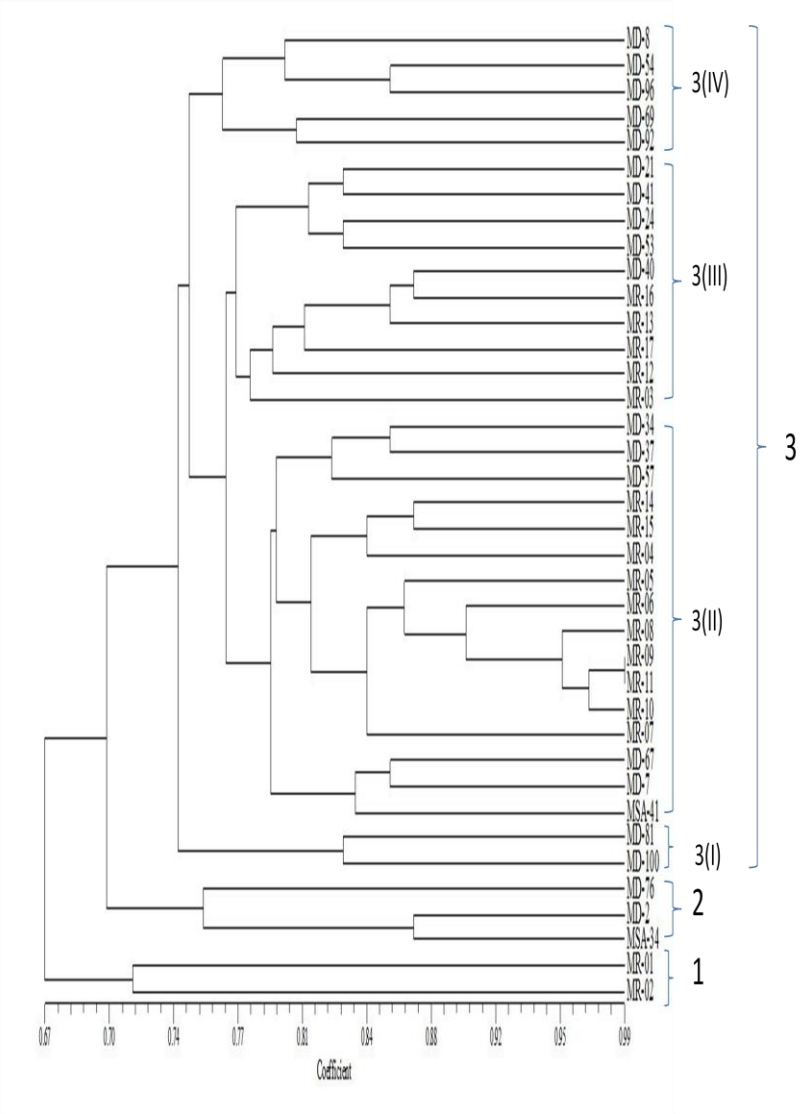

Figure 1. UPGMA dendrogram generated based on RAPD data of 38 accessions of *C. jambhiri*.

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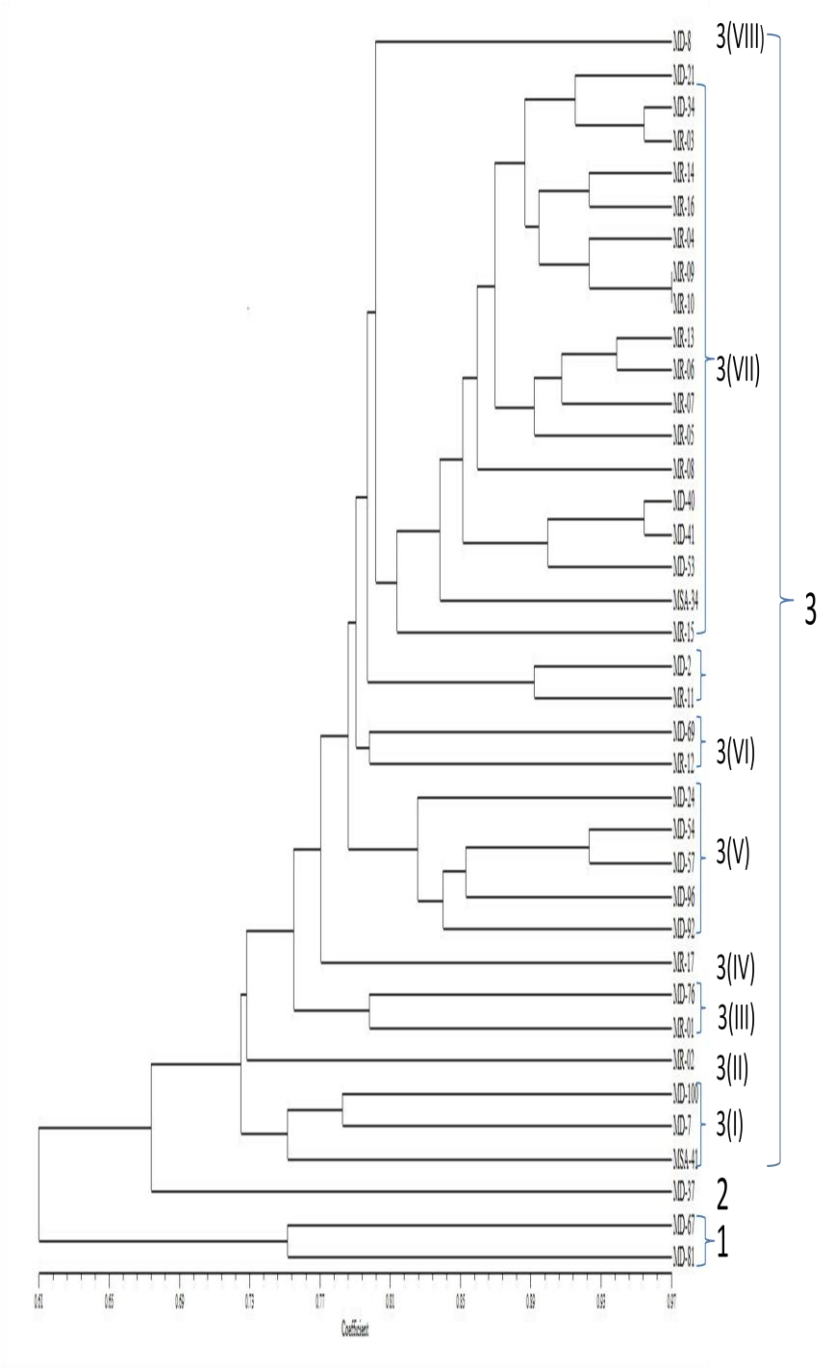

Figure 2. UPGMA dendrogram generated based on ISSR data of 38 accessions of *C. jambhiri*

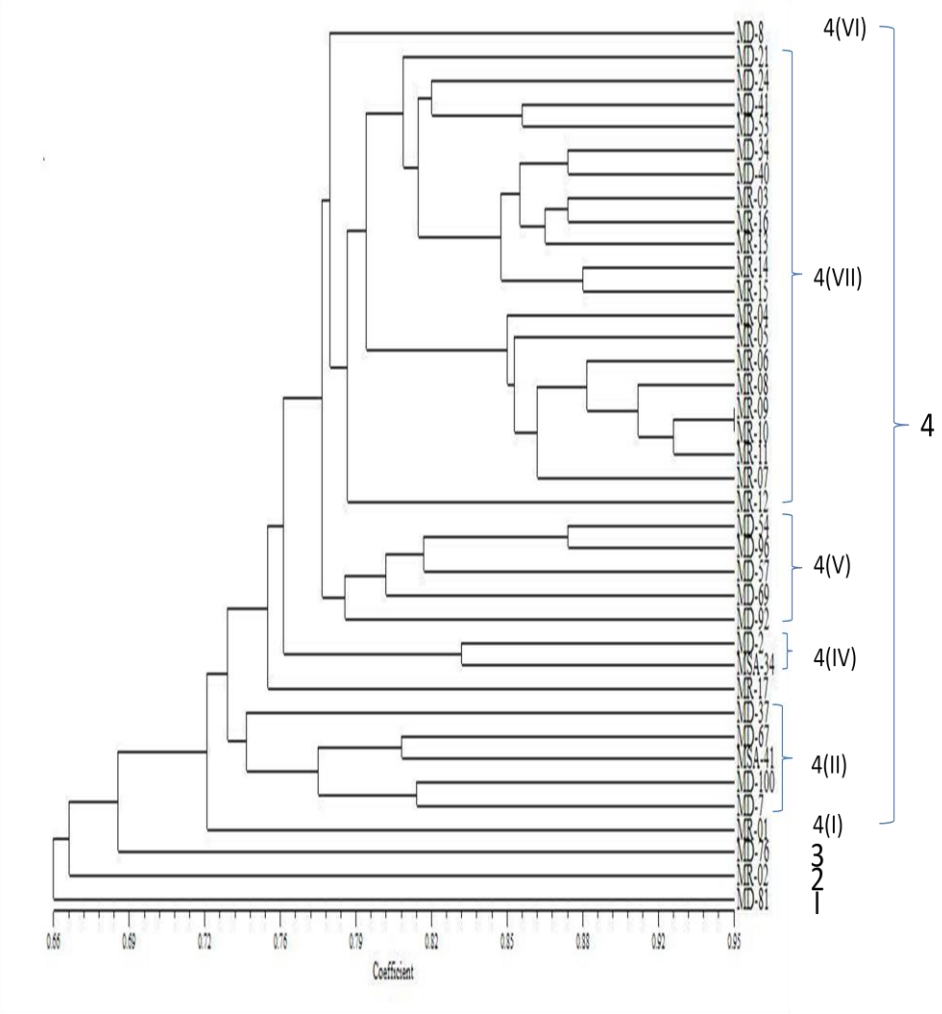

Figure 3. UPGMA dendrogram generated based on pooled data of 38 accessions of *C. jambhiri*