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Genetic relationship assessment of superior accessions of *Garcinia gummi-gutta* L. collected from central Travancore region using RAPD markers

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ABSTRACT : An experiment was conducted at Regional Agricultural Research Station, Kumarakom during 2014-15 with an objective to understand the genetic relationship of some superior *Garcinia* accessions having different geographic origin and maintained at germplasm collections of Regional Agricultural Research Station, Kumarakom, Kerala, India. Germplasm identification and characterization is an important link between conservation and utilization of plant genetic resources. In this study, random amplified polymorphic DNA (RAPD) markers were used to find out the genetic relationship of 30 garcinia accessions. This included two *Garcinia* varieties Amrutham and Haritham which were released during 2015 from this station. The primer OPM16 gave maximum number of polymorphic bands and OPAB16 produced least. Out of the total 397 alleles scored, 68.76 per cent were found to be polymorphic. The polymorphic information content (PIC) ranged between 0.14 (OPC 7) and 0.49 (OPAB 16) and marker index (MI) ranged from 0.01 (OPC 7) to 0.15 (OPM 16 and OPAB 16) among the primers used. Jaccard's similarity co-efficient between genotypes ranged from 0.462 to 0.991. An UPGMA dendrogram was constructed using NTSYS pc 2.02e software and showed two major clusters. The variety Amrutham did not form any cluster and stood alone in the group whereas Haritham clustered in the second group. This is the first report for the molecular based genetic diversity studies for these accessions.

KEY WORDS : *Garcinia gummi-gutta,* Genetic diversity, RAPD markers, Malabar tamarind

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Garcinia gummi-gutta L., is originated from Indonesia and the Western ghats region is considered as a secondary centre of origin. In South India it is known as 'Malabar Tamarind'. The fruits of this tree are rich source of hydroxy citric acid, an important biologically active plant metabolite used as anti-obesity and anti- cholesterol drug. The genus *Garcinia* belongs to the family Clusiaceae (Guttiferae). This family consists around 200 species, among which 36 occur in India. Garcinia is one among the few genera in angiosperms that shows a very high degree of diversity (Osman and Rahman, 2006). *G. gummi-gutta* (L.)

commonly known as Malabar tamarind is grown widely in homesteads of Kerala. The famous regions for the cultivation and export of Malabar tamarind are Kottayam and Ernakulam districts of Kerala (Abraham *et al*., 2006). It's products such as fruit rind, resin, gum and seed oil play pivotal role in the economic development of developing countries such as Africa, India and Malasia (Tharachand *et al.,* 2013). Extensive variability in branching pattern, crown of the tree, characters of fruit and seed are shown widely by the plant (Abraham *et al.,* 2006). The knowledge on the genetic diversity of *Garcinia gummi-gutta* is very meager.

Since large variation is observed among different genotypes of *Garcinia*, the analysis of genetic diversity is essential. Several types of molecular markers have been used to determine genetic divergence within and among plant species. Molecular markers offer greater advantage over the morphological and biochemical markers in species identification and phylogenetic studies (Suh *et al.,* 2011). Among the molecular markers, the most simple, fast and easy to perform assay is the use of RAPD markers to determine the existing genetic diversity and variation among and within the population (Williams *et al.,* 1990). Assessing the genetic variation between the accessions based on RAPD technique is accepted because of its high potential for detecting the polymorphism (Tingey and del Tufo, 1993). A large number of reports have appeared in the literature using RAPD patterns for differentiating varieties, species, etc. of crop plants. RAPD has employed good insight in detecting the polymorphisms in many crop species like *Garcinia gummi-gutta* (Utpala *et al*., 2013); (Tharachand *et al.,* 2015); *Garcinia* sp. (Rao *et al.,* 2003); Mangosteen (Mansyah *et al.,* 2013)*;* Kokum (Sahasrabudhe and Deodhar, 2010) and Mango (Majumdar *et al.,* 2011) etc. The present study was undertaken at RARS, Kumarakom, Kottayam with an aim to understand the relationship of some superior *Garcinia* accessions collected from Kottayam, Pathanamthitta and Alapuzha districts of Kerala which are being maintained at RARS Farm as part of germplasm. The newly released *Garcinia* varieties of Kerala Agricultural University, Amrutham and Haritham have also been included in this sampling as they are part of RARS germplasm. The genetic information of the sampled germplasm was studied using UPGMA clustering and principle co-ordinate analysis.

RESEARCH PROCEDURE

Plant material and sample collection :

This study was conducted at RARS, Kumarakom, under Kerala Agricultural University, with thirty superior accessions of *G. gummi-gutta* which were selected from the germplasm maintained at this station. These accessions were collected from different localities of Kottayam, Pathanamthitta and Alappuzha districts of Kerala (Table A).

Table A: Selected garcinia accessions and their DNA yield

Accessions	Collection location	Concentration of DNA (ng/ml)(260nm)	Accessions	Collection location	Concentration of DNA (ng/ml) (260nm)
3/141	Edakadathy	53.12	4/159	Edathwa	57.1
3/111	Alumthuruthy	62.78	5/28	Aymanam	52.6
3/89	Kavumbhagam	59.2	3/97	Kavumbhagam	59.6
4/193	Thakazhy	65.9	4/152	Perumbalam	61.2
4/151	Perumbalam	61.2	5/58	Thalunkal	63.5
5/53	Vellikkalam	65.5	5/29	Aymanam	59.3
4/160	Thalavady	66.6	5/54	Vellikkulam	60.8
33/90	Thiruvalla	66.1	5/68	Edamaruk	61.9
15/90(Amrutham)	Olassa	62.3	5/69	Plassanal	62.5
16a/90	Kumarakom	58.3	5/78	Monippally	63.1
22/90	Kalaketty	59.5	5/60	Cholathadam	61.0
13/90	Olassa	60.1	5/65	Nadakkal	60.8
64/90 (Haritham)	Vaikom	62.1	5/14	Kilirur	59.1
4a/90	Edathwa	62.0	4/169	Periseri	58.7
2/90	Olassa	63.2	4/158	Thalavady	59.6

DNA extraction :

Young pale green leaves of *Garcinia* were collected in sterile plastic covers and brought to the laboratory in ice buckets just before the extraction. Samples were randomly collected from each tree. The total genomic DNA was isolated using a modified CTAB method (Sahasrabudhe and Deodhar, 2010). Three grams of leaf sample was ground with liquid nitrogen in pre-chilled mortar and pestle. Then 9 ml of CTAB buffer was added and homogenized thoroughly. 50 mg of PVP was added to this and incubated in water bath at 60°C for 1h with intermittent shaking. Then equal volume of chloroform: isoamyl alcohol (24:1) was added, gently mixed by inversion. Mixture was centrifuged at 10000 rpm for 25 min. The supernatant was transferred to a fresh centrifuge tube and this procedure was repeated again to form a clear supernatant. Then half the volume of 5M NaCl and 2 volume of ice cold isopropanol was added to the supernatant. The DNA was collected in micro centrifuge tube and 1.5 ml of 80 per cent ethanol was added. Spined the tubes at 5000 rpm for 5 min at 4°C. The resulting supernatant was discarded and the pellet was air dried. The pellet was finally dissolved in 0.1X TE buffer and stored at-20°C. The isolated and purified DNA was checked for quality and quantity prior to PCR amplification. The quality of the DNA was checked using 0.8 per cent agarose gel electrophoresis. The concentration of DNA was estimated using Qubit 2.0 fluorometer.

PCR amplification :

PCR amplification was carried out to produce RAPD profiles with standard 10 base primers supplied by Operon using thermal cycler (Agilent Sure Cycler 8800). Each tube contained 25µL of reaction mixture which included 40 ng DNA template, 2.5 µL 1X assay buffer, 1µL of dNTPs mixture, 2 µL of primer and 0.4U of Taq DNA polymerase containing 20mM Tris HCl, 100mM KCl, 0.1M EDTA, 1M DTT, 0.5 per cent Tween-20 and 50 per cent glycerol. The PCR programme was designed for 40 cycles with an initial denaturation for 4 min at 94°C followed by denaturation 94°C for 1 min, primer annealing at 50°C for 1 min, extension for 2 min at 72°C and final extension at 72°C for 7 min. The amplified product was visualized in 1.5 per cent Agarose gel with 100bp ladder and was photographed using the gel documentation system (BIO RAD Gel Doc EZ Imager) and all the reactions were repeated to confirm the results.

Data analysis :

The amplification products for each DNA sample with primers were considered as polymorphic when they were present or absent in at least one of the evaluated genotypes. The RAPD bands were scored visually on the basis of their presence (1) or absence (0), separately for each genotypes of *Garcinia* and each primer. Those bands which were clear unambiguous and reproducible only were scored. The sizes of fragments were estimated by using 100-bp ladder marker (Merk India Pvt. Ltd.), which was run along with the amplified products. The polymorphic information content (PIC) of all the primers used were calculated using the formula 2fi (1-fi), where fi is the frequency of amplified allele present (band present) and (1-fi) is the frequency of null allele (band absent) (Anderson *et al.,* 1993). The marker index (MI) was calculated using the formula MI = PIC X E ($E=n\beta$ and $\beta=np/np+nnp$; where np is the number of polymorphic bands and nnp is the number of non-polymorphic bands). (Powel *et al.*, 1996). Marker index calculates the overall utility of a marker system. The binary data of all the scored primers was used to create the binary data matrix. The genetic relationship among the accessions was analyzed by using the software programme Numerical Taxonomy and Multivariate Analysis System for PC (NTSYS-pc version 2.02e [Exeter software, E. Setauket, NY, USA]) (Rohlf, 1998). The similarity for qualitative data (SIMQUAL) programme was used to calculate the Jaccard's similarity co-efficient (Jaccard, 1908). The phylogenetic tree (dendrogram) was constructed based on unweighted pair group method using arithmetic averages (UPGMA) (Sneath and Sokal, 1973) and sequential agglomerative hierarchical non-overlapping (SAHN) clustering. Principle co-ordinate analysis (PCoA) was performed using EIGEN procedure in NTSYS.

Research Analysis and Reasoning

In this study DNA from 30 *Garcinia* accessions were amplified using 30 random decamer primers. Out of this only 15 primers produced clear and reproducible bands (Table 1). These primers generated 599 RAPD bands with size ranging from 100-1300 bp. Out of this 599 bands, 397 (66.28) were polymorphic. The number of amplicons for each primer varied between 24 (OPC 7) and 60 (OPA 9 and OPN -05), with an average of 39.93 amplicon per primer. Utpala *et al.* (2013) reported a total number of 134 bands with 80 polymorphic bands

using 12 RAPD primers for 65 *Garcinia* samples and the percentage of polymorphic loci in RAPD profiling was 60.4 per cent which was lower than our findings. In a study conducted by Tharachand *et al*. (2015) the amplicons for each RAPD primers varied between 12 (OPA 12) and 35 (OPA3) with an average of 18.5 amplicons per primer. In the present study the PIC value ranged between 0.14 and 0.49 among the 15 RAPD primers. The primer OPAB 16 showed the highest (0.49) and the primer OPC7 showed the lowest (0.14) PIC value. The MI among the primers ranged between 0.01 and 0.15. The highest MI values were shown by the

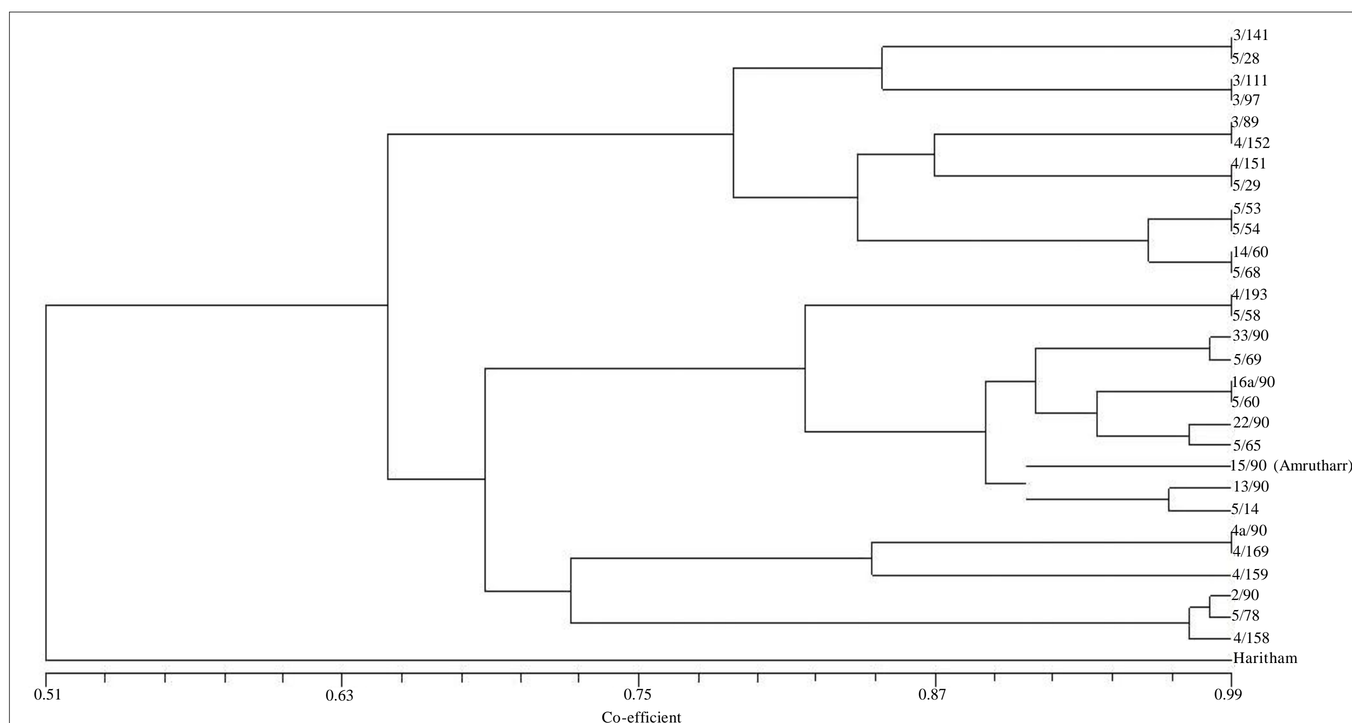

Fig. 1 : UPGMA dendrogram showing genetic relationships between 30 *Garcinia* accessions

Table 1: Scorable DNA bands of *Garcinia* accessions by RAPD primers

Primer	Sequence	Total number of bands	Number of polymorphic bands	Percentage of polymorphic bands	PIC	MI
OPA1	AATCGGGCTG	37	30	81.08	0.21	0.06
OPA2	GGGTAAACGC	35	23	65.71	0.35	0.07
OPC7	GTCCCGACGA	24	18	75.00	0.14	0.01
OPD19	CTGGGGACTT	26	19	73.08	0.29	0.05
OPM16	GAGCCCTCCA	41	35	85.37	0.47	0.15
OPA4	ACTGAACGCC	45	28	62.22	0.37	0.07
OPA9	AGGGCCGTCT	60	32	53.33	0.28	0.04
OPN-05	ACTGAACGCC	60	30	50.00	0.37	0.07
OPG-03	GAGCCCTCCA	30	25	83.33	0.24	0.03
OPG10	AGGGCCGTCT	36	21	58.33	0.28	0.04
OPAB16	CCCGGATGGT	20	17	85.00	0.49	0.15
OPBB-18	CAACCGGTCT	42	26	61.90	0.39	0.08
OPAB-1	CCGTCGGTAG	50	29	58.00	0.27	0.04
OPP-05	CCCCGGTAAC	53	31	58.49	0.28	0.04
OPW-15	ACACCGGAAC	40	33	82.50	0.47	0.13
Total		599	397	66.28		
Average		39.93	26.467	68.73	0.33	0.07

Table 2 : Jaccard's co-efficient of similarity between 30 *Garcinia* accessions based on RAPD markers

3/141	3/111	3/89	4/193	5/53	4/160	33/90	15/90	16a/90	22/90	13/90	64/90	4a/90	2/90	4/159	5/28	3/97	4/152	5/58	5/29	5/54	5/68	5/69	5/78	5/60	5/65	5/14	4/169	4/158		
3/141	1.000																													
3/111	0.857	1.000																												
3/89	0. 866	0.756	1.000																											
4/193	0.639	0.748	0.672	1.000																										
4/151	0.840	0.798	0.874	0.697	1.000																									
5/53	0.748	0.807	0.798	0.790	0.874	1.000																								
4/160	0.748	0.807	0.832	0.756	0.874	0.966	1.000																							
33/90	0.563	0.689	0.613	0.824	0.605	0.714	0.680	1.000																						
15/90	0.563	0.672	0.597	0.824	0.605	0.714	0.680	0.882	1.000																					
16a/90	0.563	0.689	0.613	0.807	0.605	0.714	0.680	0.933	0.915	1.000																				
22/90	0.563	0.689	0.613	0.807	0.605	0.714	0.680	0.916	0.899	0.949	1.000																			
13/90	0.622	0.731	0.655	0.866	0.647	0.756	0.723	0.907	0.924	0.907	0.924	1.000																		
64/90	0.487	0.479	0.470	0.496	0.462	0.487	0.471	0.471	0.504	0.521	0.537	0.495	1.000																	
4a/90	0.563	0.622	0.546	0.773	0.588	0.681	0.647	0.697	0.714	0.748	0.714	0.689	0.622	1.000																
2/90	0.639	0.630	0.605	0.731	0.630	0.639	0.605	0.655	0.689	0.689	0.655	0.680	0.478	0.705	1.000															
4/159	0.579	0.621	0.546	0.739	0.588	0.647	0.613	0.647	0.680	0.697	0.664	0.672	0.655	0.848	0.756	1.000														
5/28	0.991	0.848	0.857	0.630	0.831	0.739	0.739	0.554	0.554	0.554	0.554	0.613	0.495	0.554	0.630	0.571	1.000													
3/97	0.848	0.991	0.747	0.739	0.789	0.798	0.798	0.680	0.663	0.680	0.680	0.722	0.470	0.613	0.621	0.613	0.840	1.000												
4/152	0.857	0.747	0.991	0.663	0.865	0.789	0.823	0.605	0.588	0.605	0.605	0.647	0.478	0.554	0.613	0.554	0.848	0.739	1.000											
5/58	0.630	0.739	0.663	0.991	0.689	0.781	0.747	0.815	0.831	0.798	0.798	0.857	0.504	0.704	0.722	0.731	0.621	0.731	0.655	1.000										
5/29	0.831	0.789	0.865	0.689	0.991	0.865	0.865	0.596	0.596	0.596	0.596	0.638	0.470	0.596	0.638	0.596	0.823	0.781	0.873	0.680	1.000									
5/54	0.739	0.798	0.789	0.781	0.865	0.991	0.957	0.705	0.722	0.705	0.705	0.747	0.495	0.672	0.630	0.638	0.731	0.789	0.781	0.789	0.857	1.000								
5/68	0.739	0.798	0.823	0.747	0.866	0.957	0.991	0.672	0.672	0.672	0.672	0.714	0.478	0.655	0.613	0.621	0.731	0.789	0.831	0.739	0.873	0.949	1.000							
5/69	0.563	0.689	0.613	0.806	0.605	0.714	0.680	0.983	0.865	0.915	0.899	0.890	0.470	0.680	0.638	0.630	0.554	0.680	0.605	0.798	0.596	0.705	0.672	1.000						
5/78	0.638	0.630	0.605	0.731	0.630	0.638	0.605	0.638	0.672	0.672	0.638	0.663	0.495	0.705	0.983	0.756	0.630	0.621	0.613	0.722	0.638	0.630	0.613	0.638	1.000					
5/60	0.571	0.697	0.621	0.798	0.613	0.722	0.689	0.924	0.907	0.991	0.941	0.899	0.512	0.739	0.680	0.689	0.563	0.689	0.613	0.789	0.605	0.714	0.680	0.924	0.663	1.000				
5/65	0.571	0.697	0.621	0.798	0.613	0.722	0.689	0.890	0.873	0.924	0.974	0.899	0.529	0.705	0.630	0.655	0.563	0.705	0.613	0.789	0.605	0.714	0.680	0.890	0.613	0.932	1.000			
5/14	0.605	0.714	0.638	0.848	0.630	0.739	0.705	0.873	0.890	0.873	0.890	0.966	0.529	0.689	0.663	0.672	0.596	0.715	0.647	0.840	0.638	0.731	0.714	0.873	0.663	0.865	0.882	1.000		
4/169	0.571	0.630	0.554	0.764	0.596	0.689	0.655	0.689	0.705	0.739	0.705	0.680	0.613	0.991	0.697	0.840	0.563	0.621	0.563	0.756	0.605	0.680	0.663	0.689	0.697	0.747	0.714	0.680	1.000	
4/158	0.647	0.638	0.613	0.722	0.638	0.647	0.613	0.630	0.663	0.663	0.630	0.655	0.487	0.697	0.974	0.764	0.638	0.630	0.621	0.714	0.647	0.638	0.621	0.630	0.974	0.672	0.621	0.638	0.705	1.000

primers OPM 16 and OPAB 16.

The dendrogram (Fig.1) revealed the diversity among the genotypes clearly where the construction was based on Jaccard's similarity co-efficient. A co-efficient matrix was drawn where it ranged from 0.46 to 0.99 Tharachand *et al.* (2015) reported that the Jaccard's similarity co-efficient ranged between 0.07 and 0.64 among 12 *Garcinia cambogia* accessions. In an another study the similarity co-efficient ranged from 0.60 to 0.96 with RAPD for 65 samples of twelve *Garcinia* species (Parthasarathy *et al.*, 2013) which is in close conformity with our findings. In present study the accession 5/54 and 4/160 were closely related (99.1%) and the accessions 64/90 and 5/53 were distinctly related (46.2%). According to Rao and Hodgkin (2002) outcrossing is one of the factors to maintain high level of genetic diversity.

In *G. indica* low levels of diversity was reported by Thatte *et al.* (2012) using morphological and molecular markers (RAPD and ISSR). Ramage *et al.* (2004) reported extensive variation between *G. mangostana* accession using randomly amplified DNA finger printing (RAF) techniques. A dendrogram based on UPGMA analysis grouped the 30 accessions into 2 groups at similarity index of 0.51 (Fig.1) with the variety Haritham failing to form a cluster. The variety Haritham and accession 3/141 are placed at two extremes of the dendrogram. The main group included all the other 29 accession which fell into two clusters (cluster I and cluster II) at similarity index value of 0.65. The first cluster contains 12 accessions and was again formed two sub clusters at similarity index of 0.78 with the accession 3/141, 5/28, 3/111 and 3/97 in first sub cluster (I A) and 3/89, 4/152, 4/151, 5/29, 5/53, 5/54, 14/60 and 5/68 in second sub cluster (I B) (Table 2).

The second cluster also fell into two sub clusters at similarity index of 0.68 with 11 accession in one sub cluster (II A) and remaining 6 accession in second sub cluster (II B). The variety Amrutham which was collected from Ollassa in Ettumanoor Block of Kottayam district was placed in sub cluster IIA and showed close similarity with 13/90 (Ollassa) and 5/14 (Kiliroor) which were also collected from same block perhaps showing their ancestral relationship. The clustering pattern did not show any correlation with geographical origin. Earlier Tharachand *et al.* (2015) also reported that the twelve *G. cambogia* collections from Kerala clustered into the same clade despite being collected from different geographical locations. The dendrogram clearly shows a higher genetic variation among the selected accessions. The results of this study are also supported by earlier reports indicating extensive genetic variation among different *Garcinia* accessions maintained at NBPGR Regional Centre, Thrissr (Tharachand *et al.*, 2015). The PCoA analysis (Fig.2) showed 4 clear clusters. The variety Haritham stood alone without forming any cluster.

Conclusion :

This study was conducted to assess the genetic diversity of 30 superior accessions maintained at RARS Farm using RAPD markers. The results revealed a high

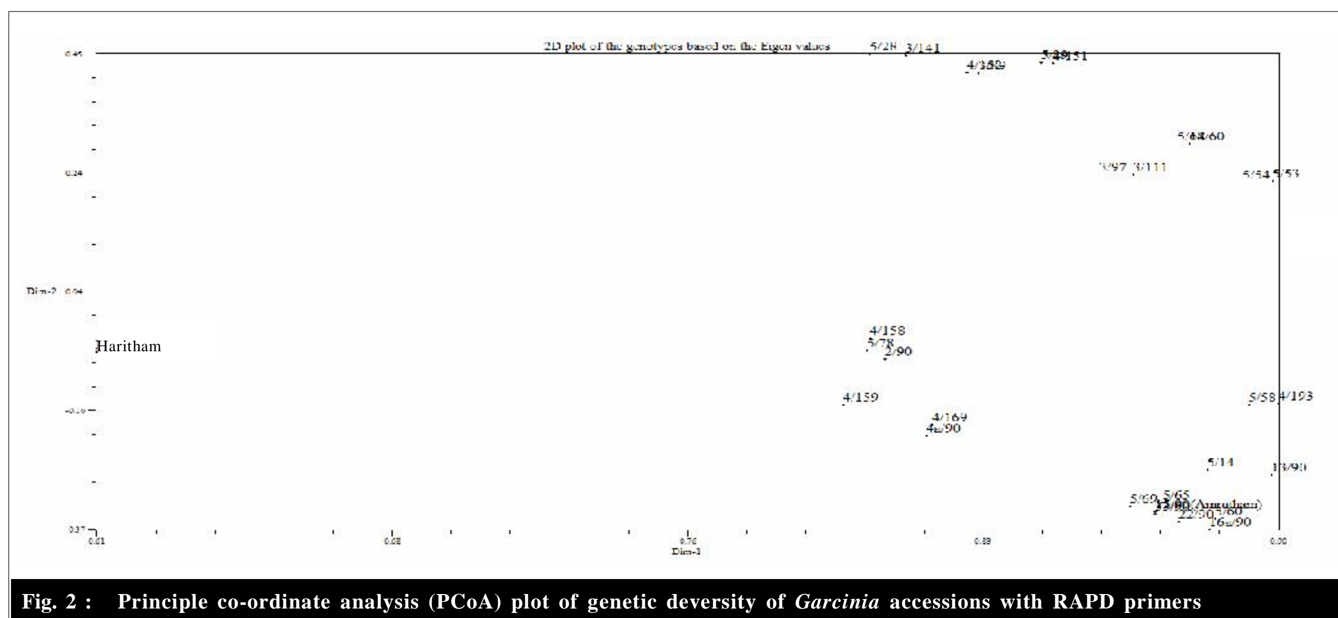

Fig. 2 : Principle co-ordinate analysis (PCoA) plot of genetic deversity of *Garcinia* accessions with RAPD primers

level of genetic diversity among the accessions which was evident from morphological characters. This high variability may be due to the gene flow within and between populations caused by the cross pollinating nature of the crop. The result of this study can be used for the efficient conservation, management and utilization of *Garcinia* germplasm maintained at RARS Farm. Further studies using other molecular markers and more acessions could provide better understanding of genetic diversity of *Garcinia gummi-gutta*.

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