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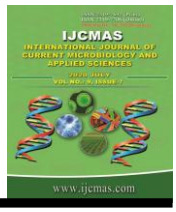

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ISSR Marker Based Genetic Diversity Analysis of 35 Garcinia Accessions (*Garcinia gummi-Gutta* (L) Roxb)

Anu G. Krishnan*, Anu Cyriac, K. Bini, Sible George Varghese

Regional Agricultural Research Station, Kumarakom, Kottayam, Kerala, India

**Corresponding author*

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A B S T R A C T

Genetic diversity was assessed for 35 garcinia (*Garcinia gummi-gutta* (L) Roxb) germplasm maintained at Regional Agricultural Research Station, Kumarakom collected from different geographical locations using ISSR markers. Out of the 34 ISSR primers screened, 18 primers were polymorphic and used for further diversity analysis. The products were detected by electrophoresis and analysed using the program NTSYS-PC. Seventy two loci were polymorphic out of the 99 loci amplified. The average Polymorphic Information Content (PIC) obtained for 18 primers was 0.33 and the average marker index was found to be 1.3. Two major clusters were identified at 64% similarity. The clustering pattern did not follow any geographic distribution pattern. The genetic similarity index ranged from 0.55 to 0.95 indicating a genetic diversity of 45% within the germplasm accessions.

Introduction

Garcinia gummi-gutta (L) Roxb., popularly known as *Camboge* (Gaertn.) Desr. or Malabar tamarind (*Kudampuli*) belongs to the family Clusiaceae. The family consists of nearly 200 species and shows a very high degree of diversity (Osman and Rahman 2006). Among the members G.*gummigutta (L.)* is originated from Indonesia. It is an endemic, fruit yielding tree species widely distributed in the Western Ghats of India. Recently the plant is having very high importance in the commercial market due to the presence of hydroxy citric acid in the fruit rind and leaves of the plant, which is reported to be an anti- obesity compound (Shara *et al.*, 2004; Saito *et al.*, 2005).

Moreover in India, the fruit rind is used as a sour flavoring spice in preparation of many dishes. In this context strengthening of research on genetic diversity of *Garcinia gummy-gutta* has great scientific importance for the conservation of germplasm resources. Characterization of germplasm is important

for the effective utilization and conservation of the genotypes. Molecular markers such as RAPD, ISSR and SSR markers are very efficient tools for genotyping. The most important character of the DNA markers is that they are not affected by environmental factors and the variation (polymorphism) can be detected at DNA level using simple technologies. The micro satellite based molecular marker system, Inter-sample sequence repeat (ISSR) developed by Zietkiewicz *et al.,* (1994), provides ample polymorphism, high reproductibility, great stability, simple operation, low cost, low DNA usage and safety. Parthasarathy *et al.*, (2013) reported that both ISSR markers and RAPD are very effective in Garcinia to study about the diversity. Earlier Tharachand *et al.*, (2015) observed a high level of genetic diversity among the genotypes studied using RAPD markers. He stated that the data is supported by the large variations observed in the morphological features of the sampled accessions. Recently Ravishankar *et al.*, (2017) developed a huge number of SSR markers for Garcinia using the next generation sequencing technologies. They have selected randomly 32 numbers of markers and tested in thirty accessions of *Garcinia gummi-gutta.* The markers were very effective and the number of alleles per locus ranges from 12-27. It reflects the high level of heterozygosity among the genotypes. The present study was aimed to analyze the genetic diversity among 35 accessions of *Camboge* using ISSR markers which may be useful for further utilization of germplasm and genetic breeding.

Materials and Methods

Plant materials and DNA isolation

Thirty five superior genotypes of '*Kudampuli'* which were identified from the field germplasm maintained at RARS, Kumarakom were exploited in this study (Table 1). These were collected from different geographical locations of Kerala, in Kottayam, Alappuzha and Pathanamthitta districts. DNA was isolated in the laboratory of Regional Agricltural Research Station, Kumarakom using the modified Doyle and Doyle CTAB DNA extraction method. The quantity of the DNA isolated was checked using the Qubit fluorometer and the quality was assured based on the 260/280 ratio. It was further checked on a 0.8% w/v agarose gel. The working sample of each DNA was adjusted to 50 ng/microlitre

Amplification of ISSR primers

A total of 34 ISSR primers in UBC series(UBC 900) synthesised by Sigma genosys which were reported in the studies of Parthasarathy *et al.*, 2016 were tested for their amplification in 35 accessions of *garcinia.* The PCR reactions were carried out in a Agilent Thermocycler with 25 µl reaction volume which contains 1X PCR buffer, 1.5 mM $MgCl_2$, 400µMdNTPs, 50ng template DNA, 10 picomol primer and 1u Taq DNA polymerase (TaKaRa). The PCR program consisted of 35 cycles with an initial denaturation of 94°C for 2 min. Each cycle was programmed for a denaturation at 94°C for 40 seconds, annealing temperature of 50°C for 1 minute and the primer elongation at 72°C for 1.5min. The final primer extension was set at 72°C for 10 minutes and hold at 4^0 C till the tubes are taken. Amplified DNA was resolved in a 1.5% Agarose (Sigma) gel along with a 1kb DNA ladder (Thermo Scientific).

Data analysis and scoring

Only clear and reproducible bands were considered for scoring. The data were scored from the gel images using '1' for presence and '0' for absence of band.

The polymorphic information content for each marker was calculated using the formula: PIC = 1- (Weir 1990), where Pi is the frequency of the i^{th} allele in the genotypes studied. For dominant markers, this formula can be simplified to PIC= 2PiQi (Roldan-Ruiz *et al.,* 2000) where Pi is the frequency of presence and Qi is the frequency of absence of a particular band and the maximum value is 0.5. To calculate PIC value for a primer, the PIC values for all the polymorphic bands produced by the primer were averaged (Rana and Bhat 2004; Tehrani *et al.,* 2008). Marker index were calculated by multiplying PIC with number of polymorphic bands(Powell *et al.,* 1996). For the assessment of genetic diversity, jaccards similarity coefficient was used to calculate the similarity between sample sets for generating similarity matrix. The cluster analysis was performed using the unweighted pair group method for arithmetic mean (UPGMA) and the dendrogram was generated using the program NTSYS-PC.

Results and Discussion

ISSR analysis

Out of random 34 ISSR primers tested, 18 primers produced clear and consistent polymorphism among the 35 garcinia accessions. All these ISSR primers were composed of di nucliotide repeat sequences (Table 1). Fig.1 shows the banding pattern of 35 garcinia accessions using ISSR primers UBC 848a and TC10G. A total of 99 DNA loci were generated, out of which 72 bands were polymorphic and 27 were monomorphic. The polymorphic loci percentage obtained in this study was 72.7%. The number of loci amplified ranged from two to thirteen with an average of 5.5 loci per marker (Table 2). The number of polymorphic loci ranged between 1 to 12 and the ratio of polymorphic loci was between 50% to 87.5% with an average of 72.7% which indicates the effectiveness of ISSR markers. Average number of DNA fragments amplified by ISSR primers in this study was slightly lower when compared to the ISSR amplication reported in G. mangostana and garcinia spp by Sobir *et al.,* 2011, which was 6.1. The marker 840a amplified highest number of loci (13) of them 12 (92.3%) were polymorphic. Out of the 99 bands scored four were genotype specific. The polymorphic information content (PIC) value ranged from 0.08 to 0.46. The highest and lowest PIC values were observed for the markers TC10G and 841a respectively. The marker index (MI) for the primers were ranged from 0.24(841a) to 3.06(843a) with an average of 1.3. ISSR markers have also been successfully used for genetic variability studies in garcinia spp (Sobir *et al.,* 2011, Parthasarathy *et al.,* 2013). In a study on mangosteen diversity using ISSR markers Mansyah *et al.,* (2010) obtained 58% polymorphic bands out of 72 bands generated by 11 random ISSR primers in 23 accessions. In the present study 18 ISSR markers generated 72 polymorphic bands with an average of 4 bands per primer. Which is comparable with 3.82 bands on study of *Garcinia mangostana* by Mansyah *et al.,* (2010).

The amplification patterns of different ISSR primers were different in this study and these ISSR primers effectively revealed the genetic diversity among the studied garcinia accessions.

Genetic relationship analysis

Genetic diversity analysis was carried out based on the dominant scoring of the data. The similarity index values were ranged from 0.55 to 0.95 (Table 3) indicating significant level of diversity among the accessions studied. Maximum similarity was observed for acc. no. 13/90 (181) and 15/90 (194 - Amrutham).

Table.1 *Garcinia gummi-gutta* genotypes used for the study

Si.No.	Accession no.	Tree no.	Place of collection
1	3/88	252	Kavumbhagam, Mannankarachira
2	3/91	250	Kavumbhagam, Mannankarachira
3	3/93	247	Manippuzha
4	3/108	254	Alumthuruthy
5	3/116	248	Podiyadi
6	3/137	144	Melukara, Kozhencherry
7	3/130	65	Varayanoor, Pullad
8	3/132	24	Chenganoor
9	4/151	234	Perublem
10	4/154	139	Perublem
11	4/156	209	Perublem
12	4/172	170	Perlassery
13	4/177	233	Mahadevikkad
14	4/160	10	Thalavadi
15	4/187	141	Kelamangalam, Thakazhi
16	5/15	114	Kummanam
17	5/19	116	Kummanam
18	5/31	47	Olassa
19	5/36	9	Udayanapuram
20	5/66	164	Nadakkal
21	64/90	173(Haritham)	Madapally, Vaikom
22	4- A/90	174	Attipeedika,Kumarakom
23	2/90	176	Attipeedika, Kumarakom
24	4/90	178	Attipeedika, Kumarakom
25	6/90	180	Alumchottil
26	13/90	181	Olassa
27	16/90	183	Olassa
28	10/90	185	Olassa
29	9-D/90	187	Olassa
30	4/1/90	189	Edathua
31	45/90	191(Nithya)	Edathua
32	33/90	193	Valanjavattom, Pathanamthitta
33	15/90	194(Amrutham)	Olassa
34	16A/90	196	Olassa
35	22/90	197	Thiruvarppu

Table.2 Characteristics of polymorphic ISSR markers amplified in Garcinia

S.No.	Primer	Total no. of bands amplified	Number of polymorphic bands	Percentage of polymorphism	PIC	MI
1	807	4	2	50	0.36	0.72
2	809	6	3	50	0.19	0.57
3	810	4	2	50	0.42	0.84
4	812	3	3	100	0.38	1.14
5	835	4	2	50	0.28	0.56
6	836a	3	2	66.6	0.29	0.58
7	840a	13	12	92.3	0.25	3.0
8	840b	6	4	66.6	0.30	1.2
9	841a	3	2	66.6	0.08	0.24
10	841b	5	3	60	0.35	1.75
11	842b	7	6	85.7	0.36	2.52
12	843a	9	7	77.7	0.34	3.06
13	844a	7	4	57.1	0.38	1.52
14	848a	8	7	87.5	0.35	2.45
15	850a	8	7	87.5	0.28	1.96
16	857b	2	1	50	0.45	0.45
17	860	2	1	50	0.45	0.45
18	TC10G	5	4	80	0.46	1.84
	Total/Average	**99 (5.5)**	**72 (4)**	**72.7**	**0.33**	**1.3**

Table.3 Genetic similarity coefficient between Garcinia accessions

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17						
1	1.000																					
2	0.671	1.000																				
3	0.681	0.679	1.000																			
4	0.766	0.737	0.688	1.000																		
5	0.656	0.662	0.644	0.714	1.000																	
6	0.650	0.580	0.617	0.650	0.648	1.000																
7	0.763	0.757	0.671	0.847	0.686	0.654	1.000															
8	0.691	0.671	0.700	0.722	0.681	0.663	0.696	1.000														
9	0.740	0.806	0.642	0.696	0.690	0.566	0.671	0.718	1.000													
10	0.701	0.726	0.688	0.711	0.667	0.628	0.662	0.718	0.707	1.000												
11	0.679	0.692	0.686	0.659	0.649	0.553	0.622	0.722	0.718	0.757	1.000											
12	0.650	0.707	0.676	0.750	0.671	0.609	0.711	0.688	0.747	0.726	0.724	1.000										
13	0.722	0.737	0.753	0.744	0.690	0.610	0.705	0.639	0.675	0.711	0.744	0.770	1.000									
14	0.720	0.736	0.707	0.720	0.701	0.689	0.693	0.675	0.693	0.732	0.675	0.689	0.743	1.000								
15	0.788	0.738	0.711	0.702	0.641	0.616	0.688	0.702	0.741	0.803	0.702	0.725	0.744	0.700	1.000							
16	0.707	0.700	0.635	0.687	0.680	0.619	0.691	0.785	0.704	0.709	0.728	0.731	0.707	0.705	0.750	1.000						
17	0.731	0.770	0.654	0.731	0.681	0.638	0.705	0.722	0.773	0.711	0.709	0.701	0.709	0.753	0.732	0.829	1.000					
18	0.725	0.740	0.734	0.663	0.632	0.654	0.688	0.738	0.659	0.684	0.704	0.692	0.725	0.701	0.706	0.821	0.756					
19	0.684	0.767	0.760	0.705	0.725	0.675	0.711	0.727	0.701	0.764	0.684	0.716	0.750	0.800	0.750	0.756	0.760					
20	0.692	0.684	0.724	0.737	0.686	0.662	0.697	0.692	0.646	0.775	0.692	0.649	0.714	0.736	0.695	0.659	0.658					
21	0.642	0.633	0.737	0.684	0.710	0.654	0.688	0.763	0.658	0.684	0.727	0.697	0.663	0.703	0.628	0.734	0.671					
22	0.741	0.734	0.750	0.785	0.761	0.612	0.747	0.808	0.738	0.753	0.741	0.753	0.785	0.740	0.783	0.790	0.728					
23	0.691	0.622	0.700	0.713	0.635	0.666	0.676	0.757	0.688	0.747	0.734	0.760	0.734	0.688	0.714	0.748	0.659					
24	0.750	0.718	0.658	0.689	0.727	0.584	0.699	0.712	0.708	0.776	0.703	0.671	0.680	0.779	0.737	0.757	0.736					
25	0.692	0.707	0.658	0.737	0.671	0.662	0.720	0.705	0.646	0.716	0.714	0.693	0.737	0.667	0.655	0.722	0.724					
26	0.716	0.667	0.704	0.759	0.653	0.709	0.722	0.667	0.651	0.718	0.655	0.722	0.738	0.737	0.678	0.682	0.704					
27	0.747	0.720	0.714	0.747	0.634	0.600	0.730	0.650	0.675	0.764	0.737	0.740	0.773	0.703	0.738	0.700	0.724					
28	0.718	0.757	0.705	0.787	0.686	0.605	0.795	0.667	0.692	0.720	0.614	0.781	0.740	0.740	0.741	0.683	0.750					
29	0.605	0.616	0.667	0.658	0.636	0.595	0.630	0.704	0.600	0.671	0.704	0.639	0.649	0.611	0.600	0.689	0.694					
30	0.778	0.689	0.658	0.730	0.677	0.722	0.693	0.662	0.703	0.662	0.649	0.736	0.730	0.718	0.718	0.737	0.778					

31	0.659	0.692	0.777	0.722	0.681	0.630	0.684	0.756	0.740	0.724	0.700	0.795	0.722	0.675	0.744	0.728	0.776	
32	0.803	0.708	0.703	0.703	0.647	0.653	0.703	0.720	0.736	0.712	0.726	0.676	0.740	0.729	0.727	0.770	0.740	
33	0.707	0.679	0.738	0.728	0.623	0.700	0.734	0.699	0.643	0.709	0.647	0.731	0.728	0.727	0.709	0.714	0.716	
34	0.627	0.591	0.627	0.646	0.574	0.641	0.631	0.612	0.597	0.625	0.703	0.694	0.652	0.641	0.562	0.647	0.636	
35	0.773	0.654	0.714	0.705	0.639	0.633	0.720	0.654	0.638	0.684	0.642	0.707	0.750	0.726	0.728	0.691	0.692	

	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
1																		
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15																		
16																		
17																		
18	1.000																	
19	0.753	1.000																
20	0.675	0.817	1.000															
21	0.709	0.646	0.654	1.000														
22	0.744	0.769	0.713	0.792	1.000													
23	0.716	0.718	0.727	0.787	0.753	1.000												
24	0.707	0.809	0.743	0.645	0.724	0.680	1.000											
25	0.718	0.768	0.753	0.675	0.713	0.705	0.686	1.000										
26	0.720	0.700	0.688	0.679	0.674	0.750	0.707	0.667	1.000									
27	0.753	0.733	0.697	0.684	0.744	0.727	0.732	0.684	0.731	1.000								

28 0.722 0.724 0.646 0.638 0.782 0.646 0.699 0.667 0.734 0.720 1.000
29 0.708 0.700 0.671 0.671 0.712 0.694 0.662 0.639 0.653 0.686 0.644 1.000
30 0.781 0.690 0.653 0.676 0.714 0.707 0.676 0.746 0.747 0.699 0.750 0.639 1.000
31 0.725 0.750 0.650 0.750 0.808 0.713 0.667 0.671 0.716 0.760 0.740 0.704 0.740 1.000
32 0.870 0.746 0.718 0.685 0.757 0.764 0.761 0.676 0.831 0.797 0.736 0.676 0.746 0.716 1.000
33 0.753 0.734 0.679 0.671 0.706 0.741 0.6971 0.659 0.959 0.722 0.747 0.667 0.737 0.750 0.819 1.000
34 0.667 0.631 0.694 0.672 0.672 0.677 0.597 0.651 0.677 0.683 0.656 0.672 0.694 0.636 0.688 0.692 1.000
35 0.731 0.757 0.720 0.625 0.725 0.718 0.708 0.675 0.753 0.675 0.819 0.686 0.746 0.705 0.809 0.766 0.667 1.000

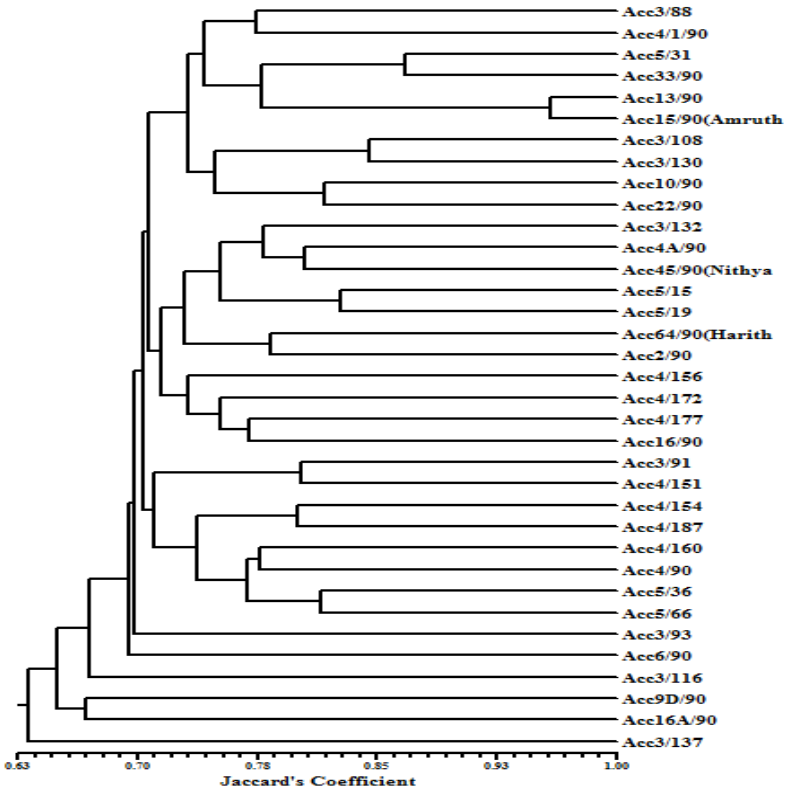

Fig.1 Dendrogram showing genetic relationship between 35 garcinia accessions

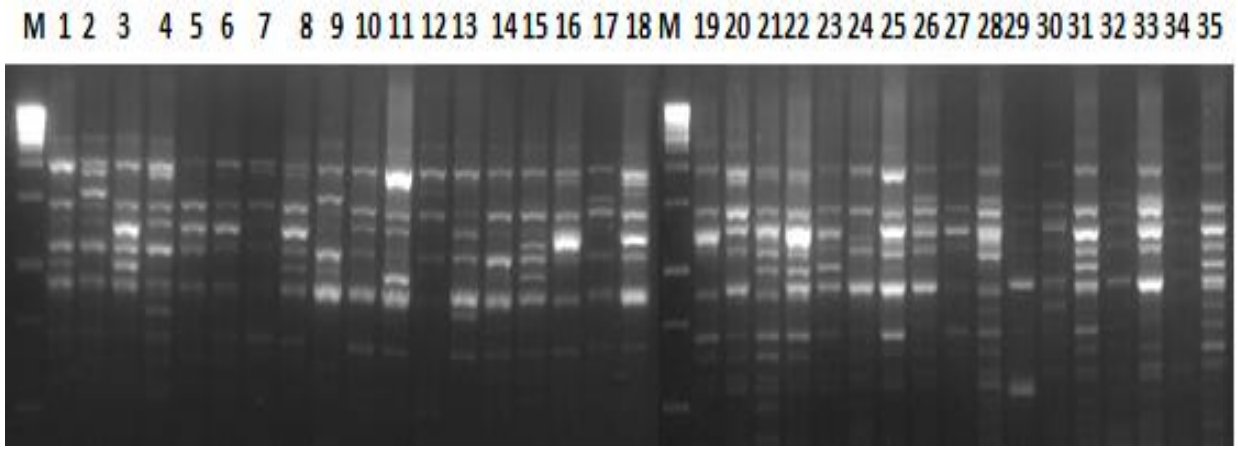

Fig.2 ISSR profiling of selected *Garcinia* genotypes using UBC848a

UBC848a

Lane M: 1 Kb DNA Ladder; Lane 1: Acc. 252; Lane2: Acc. 250; Lane 3:Acc.247; Lane 4 Acc. 254; Lane 5 : Acc.248; Lane 6 : Acc.144; Lane 7 : Acc.65; Lane 8: Acc.24; Lane 9: Acc.234; Lane 10: Acc.139; Lane 11: Acc.209; Lane 12: Acc.170; Lane 13: Acc.233; Lane 14: Acc.10; Lane 15: Acc.141; Lane 16: Acc.114; Lane 17: Acc.116; Lane 18: Acc.47; Lane 19: Acc.9; Lane 20: Acc.164; Lane 21: Acc.173 (Haritham); Lane 22: Acc.174; Lane 23: Acc.176; Lane 24: Acc.178; Lane 25: Acc.180; Lane 26: Acc.181; Lane 27: Acc.183; Lane 28: Acc.185; Lane 29: Acc.187; Lane 30: Acc.189; Lane 31: Acc.191; Lane 32: Acc.193; Lane 33: Acc.194; Lane 34: Acc.196; Lane 35: Acc.197

UPGMA cluster analysis separated the 35 accessions mainly into two major clusters at 64% similarity. Cluster II contained only one genotype, Acc.no. 3/137 and all other genotypes were grouped into cluster I. Cluster I was further divided into six sub clusters at 70% similarity.

The sixth subcluster was again divided to 5 major clusters at 0.72% similarity. Among that acc.no.3/93 (248), 6/90 (180) and 3/ 116 (247) were formed separate individual clads. The released varieties Haritham and Nithya were grouped in to same subcluster whereas the variety Amritam was placed in a different subcluster.

The Haritham and Nithya are grouped as varieties suitable for homestead cultivation while Amrutham is considered as suitable for commercial cultivation. These were the high yielding varieties released from Kerala Agricultural University and they are grouped under one major cluster. The maximum similarity for Haritham was observed with the acc.no. 2/90 (79%). The variety Nithya has maximum similarity with the acc.4A/90 (80%) and Amritam showed 95% similarity with the Acc 13/90.

The genotype 3/137 which was separated alone forming the cluster II was collected from a different geographical location *viz*. Kozhencherry. The clustering pattern did not follow any geographic distribution pattern. Similar results were obtained for mangosteen accessions where clustering pattern did not represent their origin (Sinaga, 2008; Sobir *et al.,* 2011). According to Murthy and Arunachalam (1966) genetic drift and selection in different environments can cause greater diversity among genotypes than geographic distance. Hence selection of parental material based in geographic diversity in breeding programmes may not be effective.

The result of this study indicates that 18 ISSR

primers used were suitable for the genetic diversity analysis of garcinia as they were highly polymorphic. The findings also showed that there exists a great amount of diversity among the 35 garcinia accessions.

References

Mansyah, E., Sobir, S., Santosa, E., and Roedhy, P. 2010. Assessment of inter simple sequence repeat (ISSR) techniques in mangosteen (*Garcinia mangostana* L) grown in different Sumatra region. J. Hortic For. 2(6): 127-134.

Murty, B. R., and Arunachalam, V. 1966. The nature of divergence in relation to breeding systems in some crop plants. *Indian J. Gent. Pl.Breed.* 26 : 188-198.

Osman, M. B., and Milan, A. R . 2006. *Mangosteen: Garcinia mangostana L.* Williams, J.T., Smith, R.W., Haq, N. and Dunsiger, Z. (eds.). In: *Fruits for the future*. Southampton, UK. 170pp.

Parthasarathy, U., Nandakishore, O.P., Babu, N.K., Kumar, S., and Parthasarathy, V.A. (2013). Comparative effectiveness of inter-simple sequence repeat and randomly amplified polymorphic DNA markers to study genetic diversity of Indian Garcinia. *Afr. J. Biotechnol*., 12(46):6443-6451.

Parthasarathy, U., Nandakishore, O.P., Rosana, O.B., Babu, K.N., Kumar, R.S., Parthasarathy, V.A. 2016. *Indian J Exp Biol*. 54(6):400-405.

Powell, W., Morgante, M., Andre, C. 1996. The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. *Mol Breeding* 2: 225–238.

Rana, M. K., and Bhat, K. V. 2004. A Comparison of AFLP and RAPD Markers for Genetic Diversity and Cultivar Identification in Cotton. *J. Pl. Biochem and Biotechnol.* 13(1), 19–24.

Ravishankar, K. V., Vasudeva, R., hemanth, B., sandya, B. S., Sthapit, B. R., Parthasarathy, V. A., and Rao, V. R. 2017. Isolation and characterization of microsatellite markers in *Garcinia gummi*-gutta by next-generation sequencing and cross-species amplification. *J. Genet.* 96(2): 213–218.

Roldán-Ruiz, I., Dendauw, J., and Van Bockstaele, E. (2000).AFLP markers reveal high polymorphic rates in ryegrasses (Lolium spp.). *Mol Breed.* 6:125–134.

Saito, M., Ueno, M., Ogino, S., Kubo, K., Nagata, J. and Takeuchi, M. 2005. High dose of *cambogia*is effective in suppressing fat accumulation in developing male Zucker obese rats, but highly toxic to the testis. *Food Chemi. Toxic.* 43: 411–419.

Shara, M., Ohia, S. E., Schmidt, R. E., Yasmin, T., Zardetto-Smith A., and Kincaid A. 2004. Physico-chemical properties of a novel (-)-hydroxycitric acid extract and its effect on body weight, selected organ weights, hepatic lipid peroxidation and DNA fragmentation, hematology and clinical chemistry, and histopathological changes over a period of 90 days. *Mol. Cell. Biochem.* 260: 171–186.

Sinaga, S. 2008. Morphological and genetic variability analysis of mangosteen (*Garcinia mangostana* L.) and its close related species. Dissertation. Bogor Agricultural University. Bogor.

Sobir,S., Poerwanto,R., Santosa, E., Sinaga,S., and Mansyah, E. 2011. Genetic variability in apomictic. Mangosteen (*Garcinia mangostana*) and its close relatives Garcinia SPP based on ISSR markers *Biodiversitas*. 12(2): 59-63.

Tehrani, M.S., Mardi, M., Saeidi, H.,

Gharehyazi, B., and Assadi, M. 2008. Transferability of genomic and EST-microsatellites from *Festuca arundinacea* Schreb. to *Lolium persicum* Boiss. and Hohen. ex Boiss. *Int J of Bot.* 4:476–480.

Tharachand, C., Immanuel. S.C. and Abraham, Z. (2015). Molecular insights into the genetic diversity of Garcinia cambogia germplasm accessions. Braz. Arch. Biol.Technol., 58(5): 765-772.

Weir, B.S .1990. Genetics data Analysis, Methods for discrete population genetic data. Sunderland, USA, Sinaur Associates.

Zietkiewicz, E., Rafalski, A., and Labuda, D. 1994. Genome Fingerprinting by Simple Sequence Repeat (SSR)-Anchored Polymerase Chain Reaction Amplification. *Genomics.* 20(2), 176–183.

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