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Morphometric traits and molecular characterization by using ISSR and RAPD markers in *Jatropha curcus* L. accessions grown in Andaman and Nicobar Islands, India

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ABSTRACT

An extensive germplasm exploration survey was undertaken and identified 25 high yielding accessions of Jatropa curcus from different locations of latitudinal and longitudinal spread between 920 and 940 E longitude and 60 to 140 N latitude of the A & N Islands. The genetic diversity among 25 accessions were analysed by using 15 polymerase chain reaction (PCR) markers (07 random amplified polymorphic DNAs (RAPDs) and 08 inter simple sequence repeats (ISSRs). The amplification of genomic DNA of the 25 genotypes by using RAPD analysis has yielded 61 fragments per primer. Number of amplified fragment ranged from two (OPA13) to seven (OPT18). Percentage of polymorphism ranged from 28 .6 % (OPA 13) to maximum of 77.7 %(OPA02). The resolution power ranged from a minimum of 1.20 (OPA13) to maximum of 3.65(OPT 18).Eight ISSR primers generated reproducible amplification of 46 bands across 25 genotypes, of which 26 were polymorphic. The number of amplified fragment ranging from zero (0) to six (12, 13), with an average of 3.25 polymorphic fragment per primer. Percentage of polymorphism ranged from 0 % to a maximum of 100 % (12, 13, and 14). The UPGMA method was used to construct dendrogram and grouped the 25 accessions in six main clusters. Clustering of genotype within groups was not similar between RAPD and ISSR derived dendrogram. The result of the present study shows that J.curcus germplasm within A &N Islands possess diversity up to 55 %.

1. INTRODUCTION

Jatropha is a perennial deciduous shrub, drought resistant and photo insensitive belonging to the family Euphobiaceae, which is probably originated in Central America and is widely distributed in the tropic and sub-tropic regions [1].Jatropha species are essentially cross pollinated, which result in high degree of variation [2] It can grow well under any unfavorable agro-climate condition for its low moisture demand, low fertility requirement and tolerance to high temperature [3].

The seed of Jatropha plant contain viscous, non-edible oil, which besides being source of bio-diesel can also be used for manufacturing other useful products such as candle, high quality soap, cosmetics used as healing several skin disorders.Jatropha is a valuable multipurpose crop used to alleviate soil degradation, desertification and deforestation which can also be used for bio energy to replace/substitute the use of petrol, diesel, raw material for soap and environmental protection [4].DNA marker provides an opportunity to characterize genotypes and to measure genetic relationships more precisely than other markers [5].

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The PCR techniques has offering new markers systems for diagnose of genetic diversity in large scale studies [6]. Over the last 15 years, polymerase chain re action led to the development of two simple and a quick techniques called RAPD and ISSR[7] RAPD is inexpensive and rapid method not requiring any information regarding the genome of the plants and has widely used to ascertain the genetic diversity in several plants [8].The RAPD and ISSR analysis requires only a small amount of genomic DNA and can produce high levels of polymorphism and may facilitate more effective diversity analysis in plants [9]; [10].The objective of this study is to analyse genetic diversity among 25 genotypes collected from different locations of Andaman & Nicobar islands (Table 1.) that could be useful in identification of interspecific hybrids, genetic improvement of the species and genetic resources management.

2. MATERIALS AND METHODS

2.1.Plant material and DNA isolation

Twenty five genotypes were collected from different locations of Andaman and Nicobar Islands (Table 1).Total genomic DNA was isolated from the newly sproused leaves according to

[11]. Leaves were ground using mortar and pestle to fine powder. It was then transferred to pre-warmed extraction buffer and incubated at 65°C for 1 h. An equal amount of chloroform: isoamyl alcohol (1:1) was added, mixed well by gentle inversion and centrifuged. The supernatant was transferred to a fresh tube and DNA was precipitated by adding ¾ volume of isopropanol. After centrifugation, the pellet was washed in 70% ethanol, dried and dissolved in 1X TE buffer. RNA was removed by RNase treatment. DNA was quantified by comparing with uncut λ DNA on the agarose gel, diluted to 12.5 ng $^{-1}$ and used in PCR.

2.1.1. *RAPD analysis*

The conditions for RAPD were: 25 ng of template DNA, 1x PCR-colored Mix (***Eurofins*** Genomics ***India*** Pvt Ltd. Bangalore), 20 ng of primer (***Eurofins*** Genomics**,** Bangalore), in a total volume of 20 µl. Amplification was carried out for denaturation at 94^{o}C for 2 min, primer annealing at 37^{o}C for 1 min, extension at 72^{o}C for 2min, and final extension at 72^{o}C for5 min. The amplified products were separated in 2 % agarose gel contain ethidium bromide in 2X TAE buffer and photographed under UV light.

2.1.2. *ISSR analysis*

The conditions for RAPD were: 25 ng of template DNA, 1x PCR-colored Mix (*Eurofins* Genomics *India* Pvt Ltd. Bangalore), 20 ng of primer (*Eurofins* Genomics, Bangalore), in a total volume of 20 µl. Amplification was carried out for denaturation at 94^{o}C for 2 min, primer annealing at 50^{o}C for 1 min, extension at 72^{o}C for 2min, and final extension at 72^{o}C for 5 min. The amplified products were separated in 2 % agarose gel contain ethidium bromide in 2X TAE buffer and photographed under UV light. Experiment with each primer was done three times and those primers gave reproducible fingerprints were considered for data analysis in Applied Bio systems 2720 thermal cycler.

2.1.3. *Statistical analysis*

The banding patterns obtained from RAPD and ISSR were scored as present (1) or absent (0), each of which was treated as an independent character regardless of its intensity. The binary data so generated were used to estimate levels of polymorphism by dividing the polymorphic bands by the total number of scored bands. The polymorphism information content (PIC) was calculated by the formula: PIC = 2 Pi (1-Pi) [12]. Where, Pi is the frequency of occurrence of polymorphic bands in different primers. Pair-wise similarity matrices were generated by Jaccard's coefficient of similarity [13].by using the SIMQUAL format of NTSYS-pc [14]. The similarity matrix was subjected to cluster analysis by unweighted pair group method for arithmetic mean (UPGMA) and a dendrogram was generated using the programme.

3. RESULTS AND DISCUSSION

3.1. Morphometric parameters

The morphometric parameters are given in the Table 2. The highest plant height was 6.0 and 5 recorded in the accession collected from Ferrargunj followed by the accession Krishnagar and Perka accession as compared to the accession Sipighat lowest. The economic fruits such as 100 pod weight was 325.6 , 293.3 and 100 seed weight 266.6,250 (g) were recorded highest ferragunj and Magultan accession and as compared to the accession Barm nagari lowest This variation in pod yield and seed yield might be due to genetic makeup of the genotypes as well as the prevailing microclimates.

A set of 7 RAPD and 8 ISSR primers were used for screening of 25 genotypes of *Jatropha curcus* for phylogenetic analysis (Table 5.)

3.2. RAPD band pattern

Twenty five genotypes were collected from different locations of Andaman and Niocbar Islands were amplified using the 7 RAPD oligonucleotides indicated in Table 3 and 4. Amplification of Genomic DNA yielded 589 fragments. There were 34 polymorphic bands, out of 61 amplified bands and with an average number of polymorphic bands per primer was 4.85

3.2.1. *Dendrogram analysis for Jatropha curcas as obtained with RAPD markers*

The clustering pattern of 25 genotypes based on UPGMA analysis with Jaccard's similarity coefficient ranging from 0.43 to 0.91(Fig.3). The dendrogram obtained three main cluster are I, II and III respectively. The cluster I and II having the genotypes from the South Andaman and the cluster III has the genotype from the South Andaman, Middle Andaman and Nicobar. The cluster I has four sub-clusters (Ia, Ib, Ic and Id).Sub-cluster Ia has two genotypes from Beachdera and portmort. The Sub-cluster Ib contain two genotypes Calicut and Chidiyatapu .The sub-cluster Ic contain three genotypes Jirgatang, Namunagar & Ferrargunj and sub-cluster Id contain three genotypes Shaitankar, Sipighat and Chouldhari.

The cluster II has again three sub-clusters IIa, IIb and IIc. The IIa consist of three genotypes Mangultan, Templmyo and Wandoor. The IIb again contain three genotypes Tirur, Barmnagar and Shoalbay. The IIC consist of three genotypes Vijaynagar, Govindnagar and Krishnagar. The Cluster III having the one genotype three genotypes from South Andaman and one from Middle and Two from Nicobar are classified into three sub-cluster (IIIa, IIIb and IIIc), IIIa and IIIc contain only one genotype Shamnagar & Mayabandar and IIIb consists of three genotypes Radhanagar, Arong and Perka. The clustering pattern of RAPD, South Andaman genotypes are 56 % similarity between the species and 49 % similarity from the Middle and car Nicobar , middle Andaman genotypes are 73 % similarity with Nicobar genotypes similarly Nicobar genotypes arte 91 % similarity within the species.

3.3.2. *ISSR band pattern*

Eight ISSR oligonucleotides were used for amplification of twenty five genotypes yielded 614 fragments and average number of polymorphic bands per primer was 3.25.

3.3.2.*1. Dendrogram analysis for Jatropha curcas as obtained with ISSR markers*

The clustering pattern of 25 genotypes based on UPGMA analysis with Jaccard's similarity coefficient ranging from 0.67 to 0.96 (Fig.4).The dendrogram obtained three main clusters are I, II and III respectively. The cluster I consisting of genotypes from the South Andaman, cluster II having the genotypes from South Andaman, middle Andaman and Nicobar. The cluster I has three sub cluster Ia, Ib and Ic. The sub cluster Ia consists of four genotypes (Beachdera, Calicut, Sipighat and Chouldhari).The sub cluster Ib contain 6 genotypes (Chidiyatapu, Shaitankari, Jirkatang Kalaphatar,Namunagar and Shoolbay).The sub cluster Ic contain four genotypes (Krishnagar, Mayabandar, Perka and Arong).The cluster II consists of three sub cluster IIa, IIb and IIc. The sub cluster IIa contains three genotypes. IIb consists of five genotypes (Portmort, Tirur, Templemyo, Wandoor and Govindnagar). The sub cluster IIc contain one genotype Shamnagar. The clusters III contain only one genotype Magultan. Based on the dendrogram, South Andaman genotypes are 80 % similarity within the species .Middle Andaman and Nicobar genotypes are 78 % similarity with each other. Middle Andaman and Nicobar genotypes have 93 % similarity.

3.4. Cluster Analysis of RAPD and ISSR

Cluster analysis of combined data of RAPD and IISR obtained three major clusters of 25 genotypes(Fig. 5). The cluster I and Cluster II having the genotypes from the South Andaman and cluster III containing the genotype from the South Andaman, Middle Andaman and Nicobar location. The combined clustering analysis of the RAPD and IISR are similar to the RAPD cluster analysis. In the cluster III South Andaman genotypes mainly Radhanagar are 75 % similarity to Nicobar genotypes and Middle Andaman genotypes. 88% similarity between the genotypes of Nicobar and Middle Andaman. Moreover one genotype (Radhanager) from South Andaman has more than 70 % similarity with Nicobar and Middle Andaman Genotypes.

3.4.1. *Comparative analysis of RAPD and ISSR markers*

RAPD markers were more efficient than the ISSR assay on the basis of percentage of polymorphism, the RAPD showed 56.66% where ISSR showed 56.52 %.

There is no more difference in the polymorphism of both the markers. The genotype from South Andaman showed 74 % and 70% similarity with Nicobar genotype and Middle Andaman genotype, respectively. According to IISR markers used in the study, the Middle Andaman and Nicobar accessions showed 91 % similarity between the genotype and 80 % similarity with South Andaman. The combined analysis of RAPD and ISSR shows that Nicobar and middle Andaman genotype have 91% similarity and 75 % with South Andaman. In all the three dendrogram, the Nicobar and Middle Andaman genotypes are closely related with each other and the polymorphism is more than 80 percent. Similarly, more than 75 % polymorphism to Radhanagar and Krishnagar genotypes from the South Andaman.

The present study shows that amplification of 25 genotypes using RAPD analysis yield 60 fragment of which 34 are polymorphism with an average of 4.89 polymorphic fragments per primer and the Jaccard's Similarity coefficient ranged from 0.43 to 0.91. Similar results were reported by [15].with the use of ISSR and RAPD markers for genetic diversity analysis among different *Jatropha curcus* genotypes in India [16]also reported that both the RAPD and ISSR exhibit more than 60 similarity with castor and 34 % polymorphism across the clones of *Jatropha curcas*. [17] reported that use of 100 RAPD primers revealed 61.8% polymorphism with 7.62 polymorphic bands per primer indicating more genetic diversity in the accessions from diverse regions and use of ISSR primers failed to reveal higher polymorphism in the world collection (35.5%) when compared with Indian accessions (34.1%) of Jatropha curcus where as [18].observed that RAPD and IISR exhibited > 50 % polymorphism in Jatropha curcas). Similarl , [19].found that the *Pogamnia pinnata* showed 94.34 % polymorphism at the species levels either between or within the populations. [20].reported the polymorphism detected with 400 RAPD and 100 ISSR primers was 42.0% and 33.5% with a average polymorphic per primer is 4.82 and 11.7 respectively, the intra-population variation as determined by RAPD primers was 36.0% in *Jatropha curcus L.* Hence, the *Jatropha curcus* genotypes from Andaman & Nicobar are unique which could be useful for breeding programmes to develop the new superior genotypes/ cultivar as well as those accession needs attention for conservation and subsequent utilization.

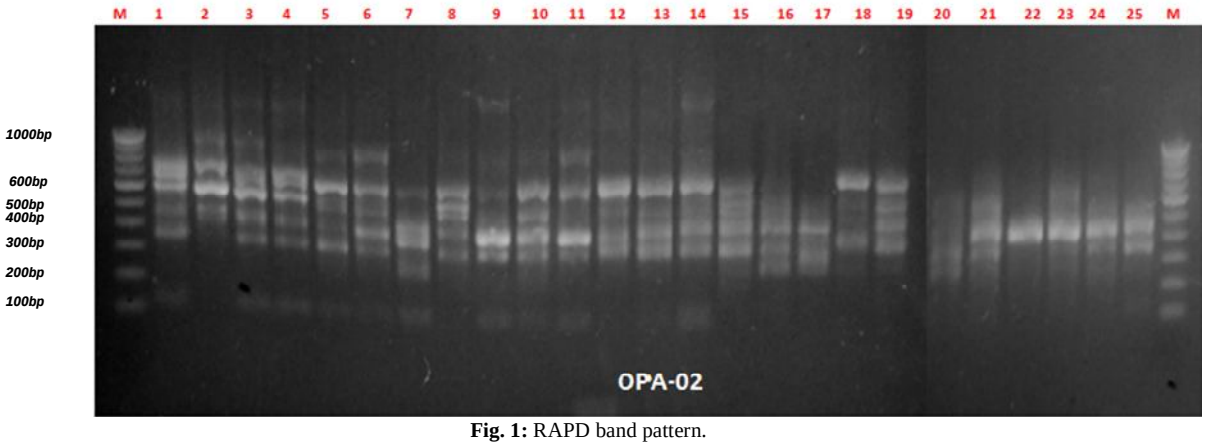

Fig. 1: RAPD band pattern.

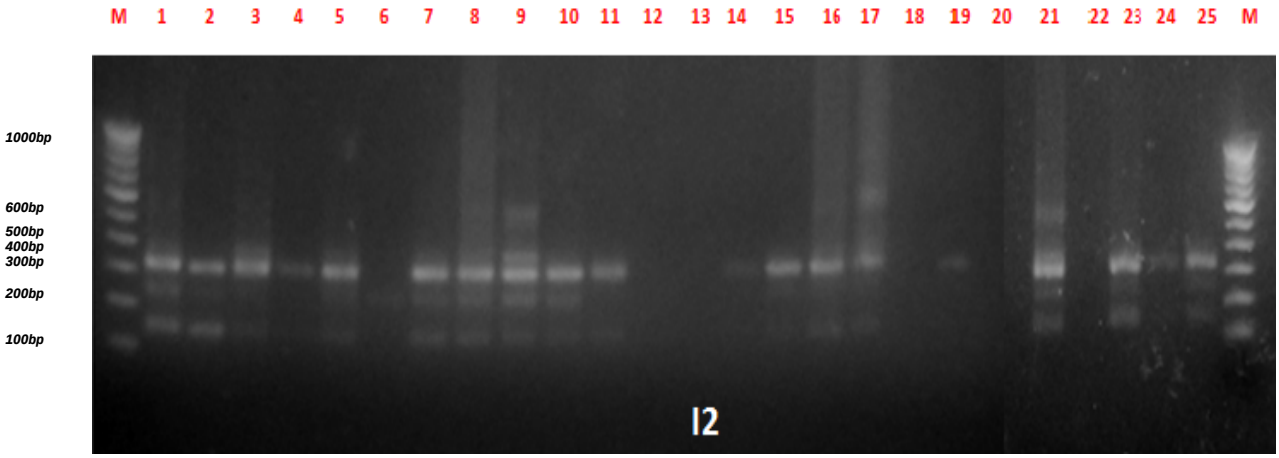

Fig. 2 ISSR band pattern.

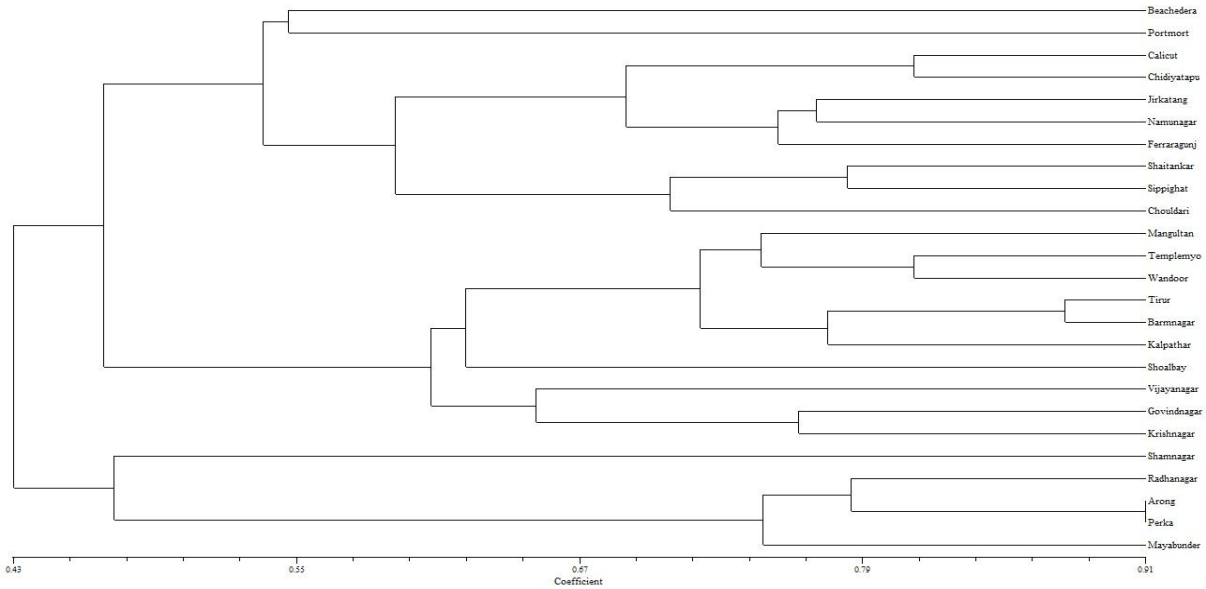

Fig. 3: Dendrogram of RAPD.

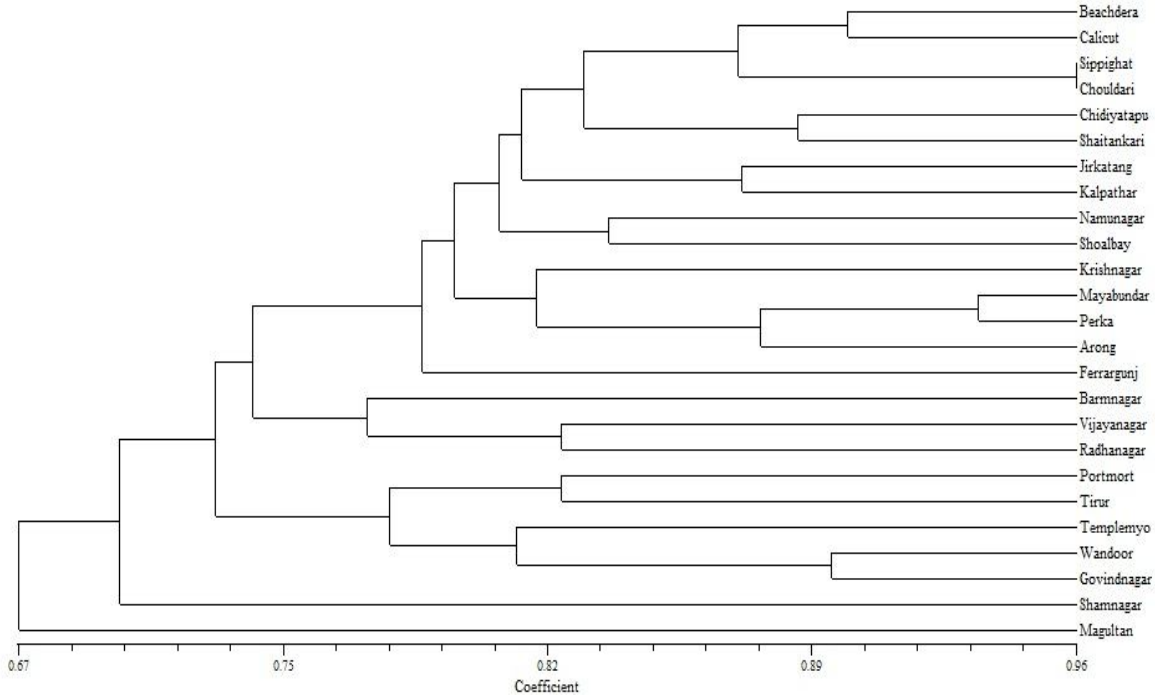

Fig. 4: Dendrogram of ISSR.

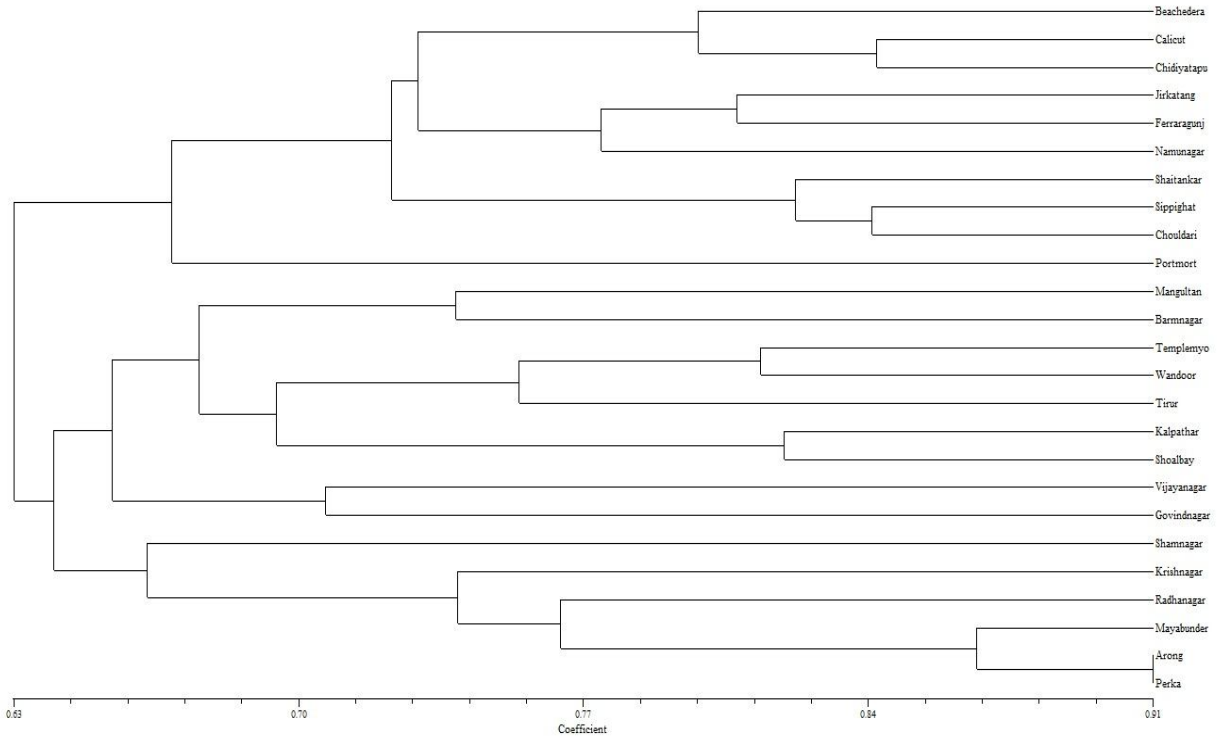

Fig. 5: Dendrogram of RAPD + ISSR.

Table. 1: Geographical locations of various *Jatropha curcus* accessions in Andaman & Nicobar Islands.

No	Collection site	Places	Altitude (MSL)	Latitude	Longitude
1	Beachdera	South Andaman	34m	N11°55'35.9''	E092°39'01.8''
2	Calicut	South Andaman	63 m	N11°35'	E092°42''
3	Chidiyatapu	South Andaman	11m	N11°33'43.6''	E092°43'16.09
4	Jirkatang	South Andaman	5m	N11°50'12.2''	E092°39'15.2''
5	Ferrargunj	South Andaman	71m	N11° 43'22.6''	E092°39'15.8''
6	Namunagar	South Andaman	33m	N11° 40'20.3''	E092°40'54.1''
7	Shaitankar	South Andaman	63m	N11° 43'14.8''	E092°40'05.0''
8	Sippighat	South Andaman	15m	N11° 36'00.6''	E092°41'01.7''
9	Chouldari	South Andaman	6m	N11°38'04.5''	E092°40'04.9''
10	Magultan	South Andaman	13m	N11°33'39'	E092°38'45.9''
11	Portmort,	South Andaman	12 m	N 11^0 39'17.2''	E092^{0}39'35.9''
12	Templemyo	South Andaman	18 m	N 11^0 41' 30.5''	E 092^0 36' 36.1''
13	Wandoor	South Andaman	10m	N11°35'33.3''	E092°36'41.2'
14	Tirur	South Andaman	8m	N11°43'05.1''	E092°36'.47.2''
15	Barmnagari	South Andaman	9m	N11° 41'37.5''	E092°40'49.1''
16	Kalpathar	South Andaman	16m	N11°37'33.7"	E092°40'44.01"
17	Shoalbay	South Andaman	18m	N11°42'53"	E092°43'00''
18	Vijaynagar	South Andaman	12m	N11°59'52.7"	E092°00'30.0"
19	Govindnagar	South Andaman	09m	N11°59'11.7"	E092°00'48.9"
20	Shamnagar	South Andaman	23m	N12°01'00.3"	E092°59'09.5"
21	Krishnagar	South Andaman	17m	N11°00'28.8"	E092°58'33.2"
22	Radhanagar	South Andaman	33m	N11°59'04.2"	E092°37'13.4"
23	Mayabundar	Middle Andaman	67 m	N12'55"	E092^{0}54''
24	Arong	Nicobar	11 m	N 09^{0}10'29.4''	E 092^{0}48'08.2''
25	Perka	Nicobar	12 m	N 09^{0}10'38.2''	E 092^{0}48'54.2''

Table. 2: Morphometric characteristics of *Jatropha curcas* accessions in A & N Islands.

Sl. No	Location	Plant height (m)	Basal girth (cm)	No. of branches	No. of fruits /bunch	No. of flowers/ bunch	100 pods Wt.(g)	Seed Wt./100 pods	No of seed/pod	Fruit set (%)
1	Beachdera	2.6	22.7	6	7	32	263.3	189.2	3	21.08
2	Calicut	3.8	21	7	6	27	194.6	132.4	3	22.22
3	Chidiyatapu	5.4	26.3	7	7	15	256.4	133.8	3	46.66
4	Jirkatang	2.9	28.3	7	6	21	256.4	133.8	3	28.57
5	Ferrargunj	6.0	28.9	8	6	18	325.6	266.6	3	33.33
6	Namunagar	4.1	21	7	7	22	177.3	123.2	3	31.81
7	Shaitankari	3.1	18.3	5	5	26	250.8	190.4	3	19.23
8	Sippighat	1.0	5	5	8	42	193.5	71.2	3	19.04
9	Chouldari	3	15	3	3	9	276.5	230.4	3	33.33
10	Magultan	3.5	16	5	3	9	296.3	250.6	3	33.33

11	Portmort	4.2	13	5	6	8	240.9	198.6	3	75
12	Templemyo	3.9	18	8	5	17	260.9	200.8	3	29.41
13	Wandoor	3.8	21	7	5	16	243.3	186.2	3	31.25
14	Tirur	3.5	27	9	7	31	249.6	183.8	3	22.58
15	Barm nagari	1.5	11	7	8	33	189.4	120.9	3	24.24
16	Kalpathar	3	15	8	7	19	198.2	136.1	3	36.82
17	Shoalbay	3.7	18	7	6	23	192.9	139.4	3	26.08
18	Vijaynagar	3.3	18	6	8	21	286.1	163.4	3	30.08
19	Govindnagar	3.1	15	6	8	22	263.2	159.6	3	36.36
20	Shamnagar	3.6	17.4	8	7	23	294	169.6	3	30.43
21	Krishnagar	5	18.6	7	8	19	256.8	178.3	3	42.10
22	Radhanagar	4.4	15	5	6	-	226.8	116.6	3	-
23	Mayabundar	4.2	16.3	8	9	-	266.4	189.0	3	-
24	Arong	4	24	6	3	25	NR	NR	3	12
25	Perka	5	29	6	3	25	NR	NR	3	12
	Average	3.70	19.15	6.52	6.16	21.90	226.36	154.55	3	

Table. 3: Combined details of fifteen primers (07 RAPD + 08 ISSR) and amplified bands in *J. curcus* accessions.

	No. of total bands	No. of polymorphic bands	No. of Monomorphic bands	% of polymorphic bands	Total No. of Bands amplified	Average No. of Bands	Resolution Power
				ISSR			
I1	5	1	4	20	81	16.2	1.152
I2	6	6	0	100	61	10.16	2.016
I3	6	6	0	100	37	6.166	1.754
I4	5	5	0	100	79	15.8	1.645
I5	7	2	5	28.57	68	9.714	1.216
I6	7	4	3	57.14	142	20.28	1.549
I7	6	2	4	33.33	46	7.66	2.112
I10	4	0	4	0	100	25	0
				RAPD			
OPA01	8	3	5	37.5	37	4.625	2.176
OPA02	9	7	2	77.7	114	12.66	3.126
OPA04	9	5	4	55.5	76	8.44	3.245
OPA08	9	6	3	66.6	76	8.44	2.829
OPA11	8	4	4	50	60	7.5	2.839
OPA13	7	2	5	28.57	95	13.57	1.203
OPT 18	11	7	3	63.63	131	11.9	3.648

Table. 4: A comparative list showing different markers details (RAPD, ISSR and RAPD + ISSR) obtained from 25 *J. curcus* genotypes.

Primers	RAPD	ISSR	RAPD+ISSR
Number of primers used	7	8	15
Total number of polymorphic bands	34	26	60
Total number of monomorphic bands	26	20	46
Total number of bands	60	46	106
Total number of bands amplified	589	614	1203
Percentage polymorphism (%)	56.66	56.52	56.60
Average number of bands/primer	9.81	13.3	11.3
Average number of polymorphic bands/ primer	4.86	3.25	4
Resolving power	19.06	11.44	30.50

Table. 5: Details of all 15primers (seven RAPD and eight ISSR).

S.No.	Sequence Name	Primer Seq.5'to3'	G+C Content
		ISSR	
1.	I1	GAG AGA GAG AGA GAG AGAC	52.63
2.	I2	GAG AGA GAG AGA GAG AGAT	47.37
3.	I3	GAG AGA GAG AGA GAG AGAA	47.37
4.	I4	AGA GAG AGA GAG AGA GC	52.94
5.	I5	GAG AGA GAG AGA GAG AC	52.94
6.	I6	GAG AGA GAG AGA GAG AA	47.06
7.	I7	CTC TCT CTC TCT CTC TG	52.94
8.	I10	GAG AGA GAG AGA GAG AA	47.06
		RAPD	
9.	OPA01	CAG GCC CTT C	70
10.	OPA02	TGC CGA GCT G	70
11.	OPA04	AAT CGG GCT G	60
12.	OPA08	GTG ACG TAG G	60
13.	OPA11	CAA TCG CCG T	60
14.	OPA13	CAG CAC GCA C	70
15.	OPT 18	CAT GCC AGA C	60

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