Electronic Journal of Plant Breeding, 1(3): 268-278 (June 2010)

Research Article

Molecular genetic diversity analysis in seed sources of Jatropha (*Jatropha curcas* L) using ISSR markers

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Abstract :
The extent of genetic diversity among 17 seed sources of *Jatropha curcas* was studied using 13 ISSR primers along with its combinations. The number of bands produced by 13 individual primers ranged from 1 to 10. The percentage polymorphism or polymorphic percentage ranges from 100 to 33.3 and the PIC value varied from 0.89 to 0.65. Among 13 ISSR primers, two primers *viz*., 895 and 899 produced 100% polymorphism.These genotypes were also tested with 78 combination primers for its amplification. Out of seventy eight combinations, eight combinations were found to exhibit 100% polymorphism and its PIC value ranged from 0.86 to 0.38. The genetic similarity index was used to construct a dendrogram which illustrated that the 17 genotypes fall into five clusters at 0.72 Jaccard's similarity coefficient and the same data was used to construct the radial tree using Darwin software. Both these software identified the two genotypes CJC 1 and CJC 19 as diverse. These diverse accessions can be utilized in breeding programme to obtain more variability.

Key words:
Jatropha curcas, molecular genetic diversity, cluster analysis, ISSR Primers

Introduction

The genus *Jatropha* L. belongs to the family Euphorbiaceae. It is a native species of Central America and has been spread to other tropical and subtropical countries.It is mainly grown in Asia and Africa. In India, it was first introduced by Portuguese navigators in the sixteenth century. It occurs in almost all parts of India including Andaman Island. It is locally known as Ratanjyot, Bhagrenda (Hindi), Kattamanakku (Tamil) and Physic nut or Purging nut (English). It is a drought tolerant crop which grows quickly and survives in all types of soil. It can also be grown on waste and marginal land. It is a morphologically diverse genus which comprises of 176 species of rhizomatous sub shrubs and herbs (Paramathma *et al.,*2004).Jatropha seeds contain 46-58% of oil on kernel weight and 30-40% on seed weight (Subramanian *et al*. 2005). The oil is also a rich source of hydrocarbon (27.0-48.5% of seed oil) and aroused the interest of the researchers on the biofuel crop for its genetic improvement. Considering its vast semi - wild distribution and cross pollination nature, it is expected to have considerable genetic variation.

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The genetic diversity studies will aid the breeder to assess the variability of the trait of importance and to choose the parents for hybridizatio n program. Since variability is a prerequisite for selection programme, it is necessary to detect and document the amount of variation existing within and between the populations. Assessment of genetic diversity with molecular markers can distinguish the individual accessions's rapidly using small amount of DNA virtually with no environmental influence. Among the DNA markers, non anchored Inter Simple Sequence Repeats (ISSR) markers are arbitrary multiloci marker produced by PCR amplification with microsatellite primer. They are advantageous because no prior genomic information is required for their use. (Bornert and Branchard, 2001). The threat of genetic erosion led to a significant interest in the assessment of genetic diversity in germplasm collections (Manifesto *et al*. 2001). PCR Amplification is a relatively novel technique and has proved to be a powerful, rapid, simple, reproducible and inexpensive way to assess genetic diversity or

identify closely related cultivars in many species. ISSR is a dominant marker, though occasionally exhibit codominance. In this method, Simple Sequence Repeats (SSR) is used as a primer to amplify mainly the inter -SSR regions which are the most potent region for producing cultivar specific marker. This marker reveals a much larger number of fragments per primer than RAPD analysis (Anju Bajpai *et al*., 2008). The analysis of genetic diversity in the Jatropha cultivars is scanty using molecular markers. Only few reports were available on using ISSR marker (Basha and Sujatha, 2007; Shweta Gupta *et al*., 2008). Hence the present investigation has been initiated to study the molecular genetic diversity on seventeen seed sources of *Jatropha curcas* (L) using ISSR marker.

Materials and Methods

Plant materials DNA isolation

Plant material used for this study consisted of seed sources of 17 accessions of *Jatropha curcas* (L) collections. The material had been collected from different parts of Tamil Nadu (India) and one from Zimbabwe (Table 1) and maintained in the Nursery, Centre of excellence in Biofuels, Tamil Nadu Agricultural University, Coimbatore.

DNA isolation

Young fresh leaves of 5-10 days old were collected and the genomic DNA isolation was carried out using cetyl-trimethyl ammonium bromide (CTAB) buffer with some modifications. The Genomic DNA was quantified using the spectrophotometer by measuring the sample OD at 260 nm against blank. The ratio A260/A280 is used to estimate the purity of nucleic acid and the DNA was diluted to make a working solution of 20 ng/µl for PCR analysis. The quality of the DNA was also checked using 0.8% agarose gel electrophoresis.

PCR amplification

The PCR reaction was performed out in total volume of 10 µl using 13 ISSR primers from university of British Columbia (UBC), along with its 78 combinations used for this study. (Table 2) The reagents that required for performing PCR reaction were 1X buffer + 1.5mM magnesium chloride, 2 mM of dNTPs, 0.3 IU of Taq polymerase, 1 mM of ISSR Primer, 20 ng of template DNA and 4.3 µl of sterile double distilled. Following PCR profile was followed: after a denaturation step for 1.50 min at 94.0 °C, the amplification reactions were carried out for 35 cycles. Each cycle comprised of 40 Sec 94.0 °C, 45 Sec 44.0°C, and 1.50 min 72.0°C. The cycle again starts with another denaturation 45 sec at 94.0°C and annealing 45 sec at 44°C. The final elongation step was extended to 5.0 min at 72°C and kept for final hold at 4°C at infinite. Amplification products were separated on 1.5% agarose gels in 1 X TBE buffer at 90 volts for 2.30 hours using horizontal gel electrophoresis system. Gels were stained with ethidium bromide. The 100 bp DNA ladder run along the sides of the amplified product to determine their approximate size. The amplified fragments were visualized under ultraviolet light and photographed with gel documentation system.

Statistical Analysis of Banding Pattern

Polymorphic bands were scored visually for their presence or absence with each primer. The scores were obtained in the form of matrix with '1' and '0', which indicate the presence and absence of bands in each genotype respectively. Polymorphism information content (PIC) values were calculated for ISSR markers, in order to characterize the capacity of each primer to reveal or detect polymorphic loci among the genotypes. PIC value was calculated using the formula PIC = $1-\sum pi^2$, where pi is the frequency of the ith allele (Smith *et al*., 1997). The binary data scoring was used to construct a dendrogram. The genetic associations between accessions were evaluated by calculating the Jaccard's similarity coefficient for pair wise comparisons based on the proportions of shared bands produced by the primers. Similarity matrix was generated using the SIMQUAL programme of NTSYS-pc software, version 2.02 (Rohlf, 2000). The similarity coefficients were used for cluster analysis and dendrogram was constructed by the unweighted Pair-Group method (UPGMA) (Sneath and Sokal, 1973). The dissimilarity index of Darwin's (Ver 5.0) software (Pierrier *et al*., 2003) was used to compute the radial tree in the present study. The categorical data for which the presence of bands were coded as 1 and 0 for absence was used to construct the tree.

Results and Discussion

The advantages available with microsatellite markers are certain to find increased usage in efforts to quantify genetic diversity and to characterize accessions in plant germplasm collections. The usefulness of these markers for germplasm characterization has been demonstrated in Sweet potato, Soybean, Rapeseed, Rice, Phaseolus (Gupta *et al*., 1996) and Jatropha (Basha and Sujatha, 2007; Shweta Gupta *et al*., 2008; Qi -Bao *et al*., 2008). Reports on the analysis of genetic diversity in the Jatropha cultivars are scanty using molecular markers. Only few reports were available on using ISSR marker (Senthil kumar *et al*., 2009). In order to analyze the extent of genetic diversity among the seed sources of seventeen accessions of *Jatropha curcas*, 13 ISSR primers and their 78 combinations primers were used in the study. Totally 91 primers were studied. The details of the primers, percentage

polymorphism, PIC value were presented in the (Table 2). The numbers of bands (produced by individual primers) ranges from 1 to 10. The polymorphism percentage ranges from 33.33 (Primer 895) to 100 (Primer 895 and 899). The PIC value of these primers ranged from 0.65 (Primer 899) to 0.89 (Primer 825). With regard to the primer combinations, eight combinations were found to exhibit 100% polymorphism and PIC value ranges from 0.86 to 0.38. The ISSR profiles of the primer (840) and the combination primer (900 Vs 810) were shown with polymorphism (Plate 1a ans 1 b).

Jaccard's similarity coefficient of UPGMA cluster analysis was used to construct a dendrogram which illustrated the overall genetic relationship among the 17 accessions of *Jatropha curcas*.L. The dendrogram resulting from the NTSYS program (Rohlf, 2000) was depicted in the Fig. 1. Based on the dendrogram, the 17 seed sources of *Jatropha curcas* were grouped into five clusters at 0.72 coefficients. Among the five clusters, cluster IV was the largest one comprising of 11 genotypes which is further subdivided into three sub clusters. The first sub cluster includes five genotypes *viz*., CJC 28, TNMC-7, CJC 31, CJC 30, and CJC 25, the second sub cluster includes two genotypes CJC 23, CJC 24 and the third sub cluster includes four genotypes such as CJC 21, CJC 22, CJC 2, and CJC 20. The cluster II includes the genotypes CJC 17, CJC 18 and III includes CJC 3 and CJC 4. Cluster II and III were second largest having two genotypes each followed by cluster I (CJC 19) and cluster V (CJC 1) with one genotype.

The same molecular data was used to construct radial tree based on Darwin software. Darwin is mainly focused on diversity structure description, based on distance methods. It was depicted in the Fig. 2 with four clusters. Cluster I, II and IV comprised with five genotypes each and cluster III had two genotypes. Among the genotypes CJC 1 (cluster I) and CJC 19 (cluster II) showed largest distance from central axis. Based on radial tree method the CJC 1 and CJC 19 genotypes considered as more diverse from other accessions. These genotypes may be inter crossed to generate more variability. Moreover to realize greater variation, genotypes should be selected among the clusters and not within the cluster. Clustering pattern of both software differs to some extent only and broadly comparable. In general, the clustering pattern shown by Darwin software helps to assess the genotypes clustering more clearly than NTYSys software. Both softwares helped in identifying most diverse genotypes namely CJC 1 and CJC 19. Further, the genetic diversity analysis also depict the 21 informative ISSR primers (Table 2) with PIC value of more than 0.8 which may be useful to select those primers to study the Jatropha molecular diversity system.

The efficiency of ISSR marker revealed considerable level of DNA polymorphism among the Jatropha genotypes. The molecular genetic diversity study using this marker will pave the scope for selection of diverse genotypes and polymorphic markers for the future crop improvement programme.

References

Anju, B., Srivastava, N., Rajan, S., Chandra, R., 2008. Genetic diversity and discrimination of mango accessions using RAPD and ISSR markers. Indian J. Hort. 65(4), 377- 382.

Basha, S.D. and Sujatha, M., 2007. Inter and intra population variability of *Jatropha curcas* characterized by RAPD and ISSR markers and development of population specific SCAR markers. Euphytica. 156(3), 375-386.

Bornet B.and Branchard M. 2001**.** Nonanchored inter simple sequence repeat (ISSR) markers: reproducible and specific tools for genome fingerprinting**.** Plant Mol. Biol. Rep., 19: 209–215.

Gupta, P.K., Balyan, H.S., Sharma, P.C., Ramesh, B., 1996. Microsatellites in plants:A new class of molecular markers. Curr Sci. 70(1), 45-54.

Manifesto, M.M., Schlatter, A.R., Hopp, H.E., Suarez, E.Y., Dubcovsky, J., 2001. Quantitative evaluation of genetic diversity in wheat germplasm using molecular markers. Crop Sci. 41, 682-690.

Paramathma, M., Parthiban, K.T., Neelakantan, K.S., 2004. *Jatropha curcas*, Bharat press, Mettupalayam, Tamil Nadu, pp 1-45

Perrier, X., Flori, A., Bonnet, F., 2003. Data analysis methods. Genetic diversity of cultivated tropical plants. Eds. In Hamon, P, Seguin, M, and. Glaszmann, J.C., Enfield, Science publisher, Montpellier, pp. 43-76.

Qi-Bao Sun, Lin-Feng Li, Yong Li, Guo-Jiang Wu, Xue-Jun Ge, 2008. SSR and AFLP Markers reveal low genetic diversity in the biofuel plant *Jatropha Curcas* in China. Crop Sci, 48, 1865-1871.

Rohlf, J., 2000. NTSYSpc: Numerical taxonomy and multivariate analysis system. Version 2.1. Users Guide, Exeter Software, Setauket, 38, New York.

Senthil Kumar, K.T, Parthiban, and M. Govinda Rao M. 2009. Molecular characterization of Jatropha genetic resources through Inter Simple Sequence Repeats (ISSR) for comparative analysis of genetic diversity. Mol. Biol. Rep**.,** 36: 1951-1956.

Shweta Gupta, Mani Srivastava, Mishra, G.P., Naik, P.K., Chauhan, R.S., Tiwari, S.K.,Kumar, M., Singh, R., 2008. Analogy of ISSR and RAPD markers for comparative analysis of genetic diversity. African J. Biotechnol 7(23), 4230-4243

Smith, J.S., Chin, C.L., Shu, H., Smith, O.S., Wall, S., Senior, M.L.,Mitchell, S.E., Kresovich, S., Zeigle, J., 1997. An evaluation of the utility of SSR loci as molecular markers in maize (*Zea mays* L.): comparisons with data from RFLPs and pedigree. Theor. Appl. Genet. 95, 163-173.

Sneath, P.H.A., Sokal, R.R., 1973. Numerical Taxonomy: The Principles and Practice of Numerical Classification**.** W.F. Freeman and Co, San Fransico, 573.

Subramanian, K.A., Singal, S.K., Saxena, M., Singhal, S., 2005. Utilization of liquid biofuels in automotive diesel engines: an Indian perspective. Biomass Bioenergy 29, 65-72.

Table 1. List of accessions of Jatropha seed source collections

Sl. No	Code. No	Location	State	Country
1	CJC 1	Vellore	Tamil Nadu	India
2	CJC 2	Coimbatore	Tamil Nadu	India
3	CJC 3	Coimbatore	Tamil Nadu	India
4	CJC 4	Ambasamudram	Tamil Nadu	India
5	CJC 17	Thondamuthur	Tamil Nadu	India
6	CJC 18	Velliankadu	Tamil Nadu	India
7	CJC 19	Anaikatty	Tamil Nadu	India
8	CJC 20	Satyamangalam	Tamil Nadu	India
9	CJC 21	Tholampalayam	Tamil Nadu	India
10	CJC 22	Mettupalayam	Tamil Nadu	India
11	CJC 23	Sulur	Tamil Nadu	India
12	CJC 24	Sirumugai	Tamil Nadu	India
13	CJC 25	Theni	Tamil Nadu	India
14	CJC 28	Victoria	Victoria	Zimbabwe
15	CJC 30	Kallapuram	Tamil Nadu	India
16	CJC 31	Vazhukuparai	Tamil Nadu	India
17	TNMC 7	Mettupalayam	Tamil Nadu	India

Table 2. Percentage polymorphism and Polymorphic Information Content (PIC) value for the ISSR primer

Sl. No	Primers	Total No. of bands	No. of polymorphic bands	Polymorphism %	PIC value
1	810	10	9	90.0	0.89
2	812	7	5	71.4	0.85
3	825	9	8	88.9	0.89
4	834	8	6	33.3	0.83
5	840	7	4	57.1	0.73
6	843	2	0	-	-
7	847	7	6	85.7	0.86
8	867	3	2	66.7	0.65
9	879	3	0	-	-
10	880	1	0	-	-
11	895	7	7	100.0	0.80
12	899	3	3	100.0	0.66
13	900	5	4	80.0	0.80
	Combinations				
1	810 vs840	7	3	42.9	0.86
2	812vs879	3	2	66.7	0.64
3	812vs899*	-	-	-	-
4	812vs900	1	0	-	-
5	812vs810	8	4	50.0	0.83
6	812vs840	7	5	71.4	0.84
7	812vs825	7	5	71.4	0.83
8	834vs895	6	3	50.0	0.80
9	834vs843	4	3	75.0	0.74
10	834vs867	5	2	40.0	0.78
11	834vs847	6	4	66.7	0.79
12	834vs880	1	0	-	-
13	834vs812	7	6	85.7	0.85
14	834vs900	3	3	100.0	0.46
15	834vs879	1	0	-	-
16	834vs899*	-	-	-	-
17	834vs810*	-	-	-	-
18	834vs840	1	0	-	-
19	834vs 825	1	0	-	-
20	843vs880*	-	-	-	-

Table 2. Contd.,

Sl. No	Primers	Total No. of bands	No. of polymorphic bands	Polymorphism %	PIC value
21	843vs867	2	2	100.0	0.38
22	843vs847*				
23	843vs812	2	0	-	-
24	843vs900	6	3	50.0	0.82
25	843vs879	6	3	50.0	0.42
26	843vs810*	-	-	-	-
27	843vs840	1	0	-	-
28	843vs899	1	0	-	-
29	843vs82*	-	-	-	-
30	840vs825	6	4	66.7	0.83
31	847vs900	5	5	100.0	0.74
32	847v840	6	4	66.7	0.83
33	847vs812	8	6	75.0	0.83
34	847vs899	2	0	-	-
35	847vs899*	-	-	-	-
36	847vs810	2	0	-	-
37	847vs825*	-	-	-	-
38	867vs847*	-	-	-	-
39	867vs812	1	0	-	-
40	867vs879	8	6	75.0	0.83
41	867vs899*	-	-	-	-
42	867vs900	4	3	75.0	0.73
43	867vs810*	-	-	-	-
44	867vs840	2	0	-	-
45	867vs825	6	3	50.0	0.83
46	867vs812	1	0	-	-
47	879vs899	3	3	100.0	0.66
48	879v810	4	3	75.0	0.74
49	879vs840*	-	-	-	-
50	879vs900	3	3	100.0	0.67
51	879vs825*	-	-	-	-
52	880vs867	7	5	71.4	0.81
53	880vs847	2	1	50.0	0.50
54	880vs812	5	5	100.0	0.79
55	880vs900	6	1	16.7	0.83
56	880vs899	1	0	-	-
57	880vs879	2	0	-	-

Table 2. Contd.,

Sl. No	Primers	Total No. of bands	No. of polymorphic bands	Polymorphism %	PIC value
58	880vs810	9	3	33.3	0.89
59	880vs840	2	0	-	-
60	880vs825	5	4	80.0	0.78
61	895vs880	4	1	25.0	0.75
62	895vs867	3	3	100.0	0.63
63	895vs843	1	0	-	-
64	895vs847	3	2	66.7	0.42
65	895vs900	1	0	-	-
66	895vs812*	-	-	-	-
67	895vs879	2	0	-	-
68	895vs899*	-	-	-	-
69	895vs840	4	3	75.0	0.74
70	895vs810	1	0	-	-
71	895vs825	5	3	60.0	0.80
72	899vs900	8	7	87.5	0.86
73	899vs810	2	0	-	-
74	899vs840	3	3	100.0	0.59
75	899vs825	6	5	83.3	0.72
76	900vs810	7	5	71.4	0.86
77	900vs840	4	2	50.0	0.69
78	900vs 825	1	0	-	-

*….. Not amplified

Figure 1. Dendrogram of 17 seed sources of Jatropha curcas L. based on Jacard's similarity coefficient

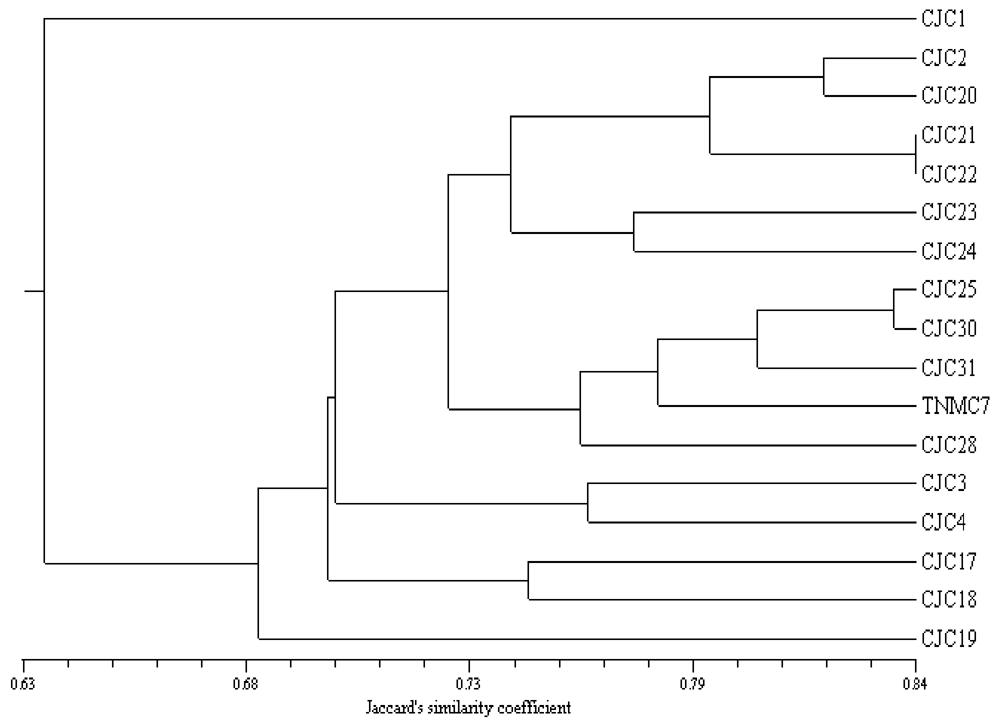

Figure 2. Radial tree view of 17 seed sources of Jatropha curcas L

TNMC7
CJC28
CJC25
CJC30
CJC31
CJC22
CJC21
CJC23
CJC18
CJC24
CJC2
CJC17
CJC20
CJC3
CJC1
CJC4
CJC19
0
0.1

Plate 1a. ISSR Profiles of the primer UBC 840 for 17 seed sources of *Jatropha curcas* L.

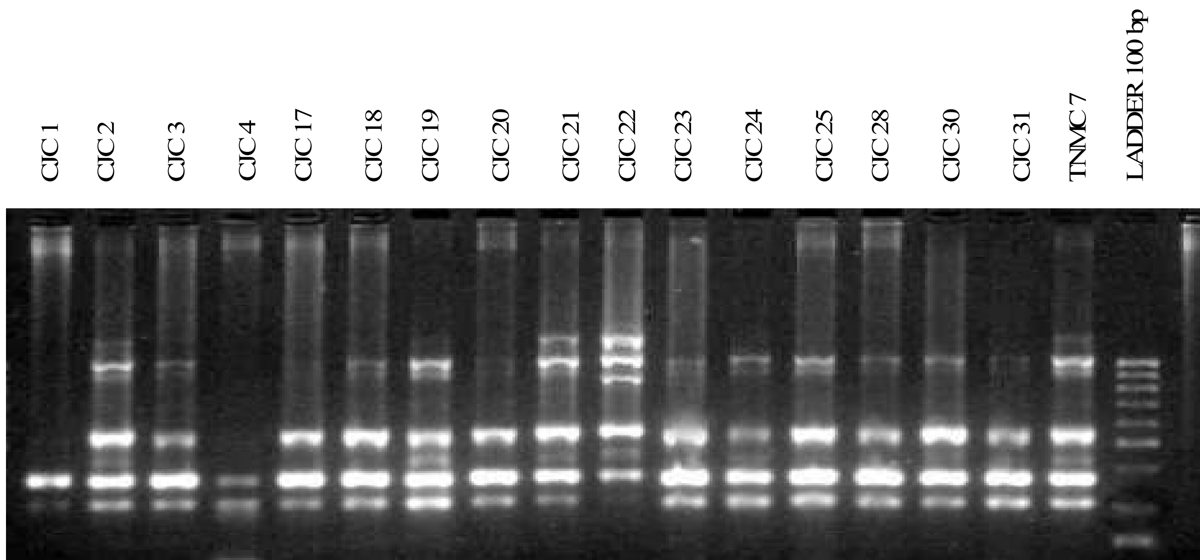

Plate. 1b. ISSR Profiles of the combination primer 900 Vs 810 for 17 seed sources of *Jatropha curcas* L.