J Bangladesh Agril Univ 17(4): 437–445, 2019 https://doi.org/10.3329/jbau.v17i4.44603

ISSN 1810-3030 (Print) 2408-8684 (Online)

Journal of Bangladesh Agricultural University

Journal home page: http://baures.bau.edu.bd/jbau, www.banglajol.info/index.php/JBAU

Genetic diversity analysis of some local biodiesel plant (*Jatropha curcas* L.) in Bangladesh

Fahmida Khatun[1]✉, Md. Mahfuzur Rahman[1], Md. Ahasun Habib[1], Md. Shahidul Haque[1], Harun-or Rashid[2], Sumitra Saha[1], Arrafy Rahman[3]

[1]Department of Biotechnology, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh
[2]Biotechnology Division, Bangladesh Institute of Nuclear Agriculture, Mymensingh 2202, Bangladesh
[3]Department of Microbiology, International Institute of Applied Science and Technology, Rangpur 5400, Bangladesh

ARTICLE INFO

Article history:
Received: 18 June 2019
Accepted: 27 October 2019
Published: 31 December 2019

Keywords:
Jatropha curcas, Genetic diversity,Biodiesel plant, RAPD

Correspondence:
FahmidaKhatun
✉: fahmida.meem@bau.edu.bd

ABSTRACT

The present study was conducted to analyze genetic diversity among biodiesel producing plant *Jatropha curcas* genotypes using six RAPD primers. The *J. curcas* samples were collected from ten agro climatic regions in Bangladesh. The six primers produced 31 DNA bands. All the DNA bands (31) showed 100% polymorphism. Overall gene frequency of 10 genotypes of *J. curcas* was ranged from 0.10 to 0.90. The average Nei's gene diversity and Shannon's Information Index for all loci were estimated 0.2994 and 0.4650, respectively. The gene diversity value was ranged from 0.18 to 0.50 and Shannon's Information Index was ranged from 0.3251 to 0.6931. Inter-germplasm similarity indices (Si) ranged from 5.56 to 66.67% with an average of 33.906%. The similarity coefficient range varied from 0.00 to 0.971. The UPGMA dendrogram constructed from Nei's (1972) genetic distance group indicated segregation of the ten *J. curcas* germplasms into three main clusters. Cluster I, II and III possessed 8, 1 and 1 germplasms, respectively. This study revealed that at least three different *J. curcas* genotypes are available in Bangladesh. The RAPD technique is, however, found to be useful in studying genetic variation among *J. curcas* genotypes of different regions in Bangladesh.

Introduction

Jatropha curcas L. (Biodiesel plant) is a perennial and monoecious plant belonging to Euphorbiaceae family; native to America but distributed widely in the tropical and subtropical areas (Koushik *et al.*, 2007). In Bengali, *J. curcas* is known as Verenda, Arenda, Chanda, Jamalgota etc. This plant can grow well under adverse soil and climatic conditions. *J. curcas* has magnetized a great deal of attention due to its potential as an incipient energy plant. Its seeds contain 30-45% oil in which a high percentage of polyunsaturated linoleic and monounsaturated oleic acid were found (Gubitz *et al.*, 1999).

It is a minuscule tree which attaining a height of 5m and unisexual flowers and infrequently hermaphrodite flowers. Mature seeds are black, 2cm long and 1cm thick. Seed kernel contains about 33 – 60% oil. After extracting from mechanical expeller, oil is filtered; processed and 20% of this oil can be utilized as biodiesel for running diesel Engineer. It is a renewable and safe energy source and a viable substitute to fuel wood, coal, furnace oil, kerosene, LPG, and diesel (Martin and Mayeux, 1985).

J. curcas is a second generation, drought tolerant biofuel crop. It can supersede fossil fuels in developed and in developing countries like Bangladesh while bringing superfluous income to poor farmers. Moreover, the country has a sizably voluminous area of uncultivable land which should be taken into consideration for propagating this consequential bioenergy crop to engender biofuel. It can be cultivated easily on wastelands and marginal land. As an oil bearing biomass feedstock, it can ascertain an alternative source of energy and reduce our dependency on fossil fuel. This plant can grow anywhere and can live for about 50 years (Henning, 2010). As a mature plant or tree, it can absorb around 18 lbs of carbon dioxide (CO_2) per year, the plant plays an important role in climate change issues. So, *Jatropha* can sequester around 20 tons of CO_2 annually in one hectare of land (Muok, 2008). *J. curcas* is as a sustainable source of second generation biodiesel feedstock species and different propagation technologies are used to increase their overall supply. In addition, the plant can grow in stressful environment including

Cite this article

Khatun, F., Rahman, M.M., Habib, M.A., Haque, M.S., Rashid, H.O., Saha, S., Rahman, A. 2019. Genetic diversity analysis of some local biodiesel plant (*Jatropha curcas* L.) in Bangladesh. *Journal of Bangladesh Agricultural University*, 17(4): 437–445. https://doi.org/10.3329/jbau.v17i4.44603

drought and its cultivated land pattern is suitable in Bangladesh (Nahar, 2011).

Since *J. curcas* plays an important role for the manufacture of biodiesel, researchers perceived a demand to develop noble cultivars. The breeding procedures of *Jatropha* are difficult, compared to breeding procedures for other oil producing species viz. peanut, cotton, castor, sunflower and soybean (Freitas *et al.*, 2011). The success of a breeding procedure relies on the knowledge of estimates of genetic parameters within the main traits and of the genetic variability available. The existence of genetic variability is consequential in a breeding procedure so that breeders can select different genotypes to engender hybrids and analogous genotypes to engender lines. Now-a days, the DNA markers become the marker of choice for the study of genetic diversity has become routine, to revolutionized the plant biotechnology. RAPD, for Random Amplification of Polymorphic DNA, is a type of PCR reaction, where the segments of DNA that are amplified are random. The RAPD analysis is a commonly used molecular marker in genetic diversity studies. No knowledge of the DNA sequence for the targeted gene is required, as the primers will bind somewhere in the sequence, but it is not certain exactly where. This makes the method popular for comparing the DNA of biological systems that have not had the attention of the scientific community, or in a system in which relatively few DNA sequences are compared (Rafalski *et al.*, 1994). Information about of genetic variability among *J. curcas* is not commonly available in our country. Therefore, the present study was designed to estimate the genetic variability among *J. curcas* found in Bangladesh.

Materials and Methods

Sample collection

Leaves of *J. curcas* samples were collected from 10 different regions of Bangladesh and listed in the Table 1.

Table 1. Collection of *J. curcas* samples from different regions of Bangladesh

Sl. No.	Sample no.	Agro climatic zones	Collection localities	Latitude (N)	Longitude (E)
1	Sample 1	South region (sample no.1-4)	Shyamnagar, Satkhira	21.9502^0	89.1706^0
2	Sample 2		JUST, Jessore	23^0 14' 0"	89^0 7' 31"
3	Sample 3		Rupsha, Khulna	22.8221^0	89.6346^0
4	Sample 4		PSTU, Patuakhali	22.4652^0	90.3825^0
5	Sample 5	North region (sample no.5-7)	BRU, Rangpur	25^0 43' 4"	89^0 15' 33"
6	Sample 6		Sadar, Mymensingh	24.7851^0	90.356^0
7	Sample 7		Nakla, Sherpur	24.9753^0	90.2057^0
8	Sample 8	Middle region	Shariatpur	23.2423^0	90.4348^0
9	Sample 9	North-east region	SAU, Sylhet	24.9092^0	91.902^0
10	Sample 10	West region	RU, Rajshahi	24.3684^0	88.6376^0

JUST=Jessore University of Science and Technology, PSTU= Patuakhali Science and Technology University, BRU= Begum Rokeya University, SAU=Sylhet Agricultural University, RU= Rajshahi University

Extraction of genomic DNA

Genomic DNA from each individual sample was extracted from young leaf tissue by using ATP™ Genomic DNA Mini Kit (Plant) following the described protocol. Young and fresh leaves from each genotype were sliced with scissors and then distilled water and 100% ethanol were added to wash them before drying on fresh tissue paper for removal of microbial spore and any other foreign DNA. Protocol mentioned in ATP™ Genomic DNA Mini Kit (Plant) (ATP biotech Inc., Taiwan) was followed accordingly. After DNA extraction, presence of genomic DNA was confirmed by agarose gel electrophoresis and DNA quantification was done by spectrophotometer.

Primer selection

Eight primers were primarily used to produce polymorphic DNA bands, of which six primers (Table 2) i.e. OPP13, S1155, OPB02, S1239, S1027, and OPA02 gave reproducible, clear, bright and distinct polymorphic DNA bands (Tabassum *et al.*, 2013; Kolade *et al.*, 2016). These primers were finally selected for determination of genetic diversity across the 10 *J. curcas* genotypes collected from different regions of Bangladesh.

Preparation of PCR reaction mixture for final amplification

During the experiment the Thermo scientific PCR master mix (2x) were gently vortexed and then thawed and centrifuged. Then PCR tube was placed on ice. Working solution of DNA samples were mixed gently after thawing out. Dilute primers were ready for use and prepared working solution with nuclease free water and mixed gently. PCR reactions were carried out on each DNA sample in a 25 µl reaction mix containing 12.5 µl of 2x PCR master mixes, 2 µl of 10 pmol/µl primer, 2 µl of *J. curcas* genomic DNA and an appropriate amount of nuclease free water. All the pre-mixes were vortexed shortly, and then they were aliquot into the tubes. The PCR tubes were set on the wells of (BioRad-PTC200)

thermocycler plate. Then the PCR reactions were programmed for 35 cycles with denaturation at 95°C for 1 min, annealing at 35°C for 1 min, extension at 72°C for 2 min and final elongation at 72°C for 10 min. The amplified PCR products were run using 1.5% agarose gel electrophoresis and photographed for documentation.

Genetic data analysis

According to bands position on gel, all bands of RAPD markers were scored visually on the basis of their presence (1) or absence (0), separately for each individual and each primer. The size of each band was calculated using molecular weight ladder. The scores obtained using the primer in the RAPD analysis was then pooled to create a single data matrix. This was used to estimate population differentiation, genetic distance (D^2) (Nei, 1972), polymorphic loci, Nei's (Nei, 1978) gene diversity, gene frequency (Elo *et al.*, 1997) and to construct a UPGMA dendrogram among populations using a computer program, POP GENE (Yeh *et al.*, 1999).

Similarity index values between the RAPD profiles of any two individuals on the same gel were calculated from RAPD markers by using following formula:

Similarity index (SI) = $2N_{xy}/N_x + N_y$

Where, N_{xy} is the number of RAPD bands shared by individuals x and y respectively, and N_x and N_y are the number of bands in individual x and y, respectively (Chapco *et al.*, 1992; Wilde *et al.*, 1992; Lynch, 1990).

Results

The results that were obtained from the experiment using six RAPD markers on 10 *J. curcas* are mentioned below. Some of the data have been presented and expressed in tables and figures for ease of understanding.

Primer selection and RAPD pattern

Eight primers were initially screened. Within 8 primers, six primers (OPP13, S1155, OPAB02, S1239, S1027 and OPA02) produced distinct and clear bands. These six primers were selected for their better quality band and efficiency in detecting polymorphism. Then these six primers were used to screen all of the 10 *J. curcas* accessions. The RAPD profiles of *J. curcas* accessions using OPP13, S1155, OPAB02, S1239, S1027 and OPA02 primers are shown in Figure 1.

Score of polymorphic bands observed

All six primers produced a total of 31DNA loci with size ranging from 100bp to 1000bp; DNA fragments were found to be polymorphic. Parameters of six primers studied are presented in the Table 2. Primer OPP13 produced the highest number of polymorphic bands (9). Thus it showed a high level of polymorphism. S1155 primers produced 7 polymorphic bands. Similarly OPAB02, S1239, S1027 and OPA02 primers produced 5, 4, 3 and 3 bands, respectively. The banding patterns of *J. curcas* from different regions using primers OPP13, S1155, OPAB02, S1239, S1027 and OPA02 primers are shown in Figure 1(a), 1(b), 1(c), 1(d), 1(e) and 1(f), respectively. Among ten *J. curcas* germplasm, some of them showed inter monomorphic bands.

Frequencies of polymorphic loci

Overall gene frequency often *J. curcas* is presented in Table 3. The highest value (0.9000) of gene frequency was obtained among OPAB02and S1027. The lowest value (0.1000) of gene obtained in case of locus $OPAB02_1$, $OPAB02_3$, $OPAB02_5$, $OPP13_1$, $OPP13_2$, $OPP13_4$, $OPP13_6$, $OPP13_8$, $S1027_1$, $S1155_2$, $S1155_3$, $S1155_5$, $S1155_6$, $S1155_7$, and $S1239_2$. Among 31 loci, 29 had a less value than 0.9000 which suggest that there have a moderate degree of genetic variation among *Jatropha* genomes.

Gene diversity for the RAPD primer

Genetic diversity values of 10 *Jatropha* genomes for six primers are given in Table 4. The average Nei's gene diversity and Shannon's Information Index for all loci were estimated 0.2994 and 0.4650, respectively. The highest level of gene diversity value (0.5000) was found in three different loci ($OPA02_1$, $OPA02_3$ and $OPP13_5$) and Shannon's Information Index (0.6931) was found in three different loci ($OPA02_1$, $OPA02_3$ and $OPP13_5$). The lowest level of Nei's gene diversity (0.1800) and Shannon's Information Index (0.3251) was found in 17 loci ($OPAB02_1$, $OPAB02_2$, $OPAB02_3$, $OPAB02_5$, $OPP13_1$, $OPP13_2$, $OPP13_4$, $OPP13_6$, $OPP13_8$, $S1027_1$, $S1027_2$, $S1155_2$, $S1155_3$, $S1155_5$, $S1155_6$, $S1155_7$, and $S1239_2$.). Hardy-Weinberg expectation of average heterozygosity in sub-population (Ht) was 0.2994 (Table 5). The result suggests that significant levels of genetic variations exist among this *Jatropha* genome.

Inter-germplasm similarity indices

Accessions which show higher similarity indices are likely to have less heterogeneity. In this study inter accessions similarity (Si) ranged from 5.56 to 66.67% with an overall average Si value of 33.906% (Table 6). The Si value for primer OPA02 ranged from 33.33 to 66.67% with an average of 46.666. The Si value for primer OPAB02 ranged from 20 to 60% with an average of 36.222. The Si value for primer OPP13 ranged from 5.56 to 44.44% with an average of 25.307. The Si value for primer S1027 ranged from 33.33 to 66.67% with an average of 39.997. The Si value for primer S1155 ranged from 7.14 to 50% with an average of 25.237. The Si value for primer S1239 ranged from 12.50 to 50% with

an average of 30. The highest number of *Jatropha* accession pair showed 66.67% similarity by primers OPA02 and S1027 and for this, average inters accessions similarity by this primer was 46.666 and 39.997 respectively, while other primers showed average Si value of (OPAB02, OPP13, S1155, and S1239) 36.222, 25.307, 25.237 and 30 respectively. S_1 vs. S_3; S_1 vs. S_5; S_1 vs. S_6;S_4 vs. S_6; S_4 vs. $S_7$and S_6 vs. S_7, showed 66.67% similarity for OPA02 primer and S_3 Vs. S_7 showed 66.67% similarity for primer S1027.S_1 vs. S_6;and S_1 vs. S_8 showed 5.56% similarity for primer OPP13. The germplasms pair S_1vs. S_9 showed the lowest average Si value (26.13%) considering all six primers.

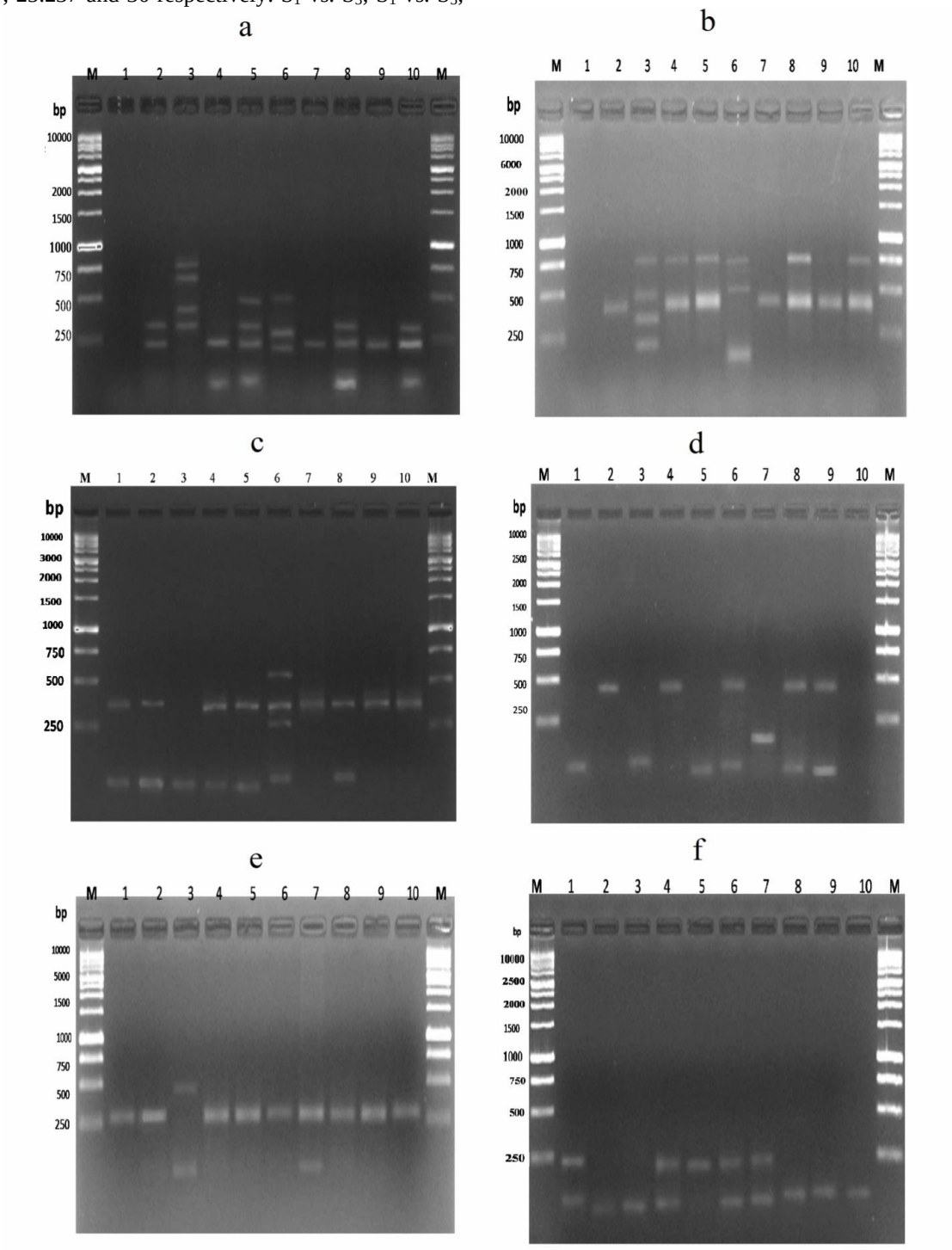

Fig. 1 RAPD profile of 10 *J.curcas* genotypes using RAPD primer (a) OPP13, (b) S1155, (c) OPAB02, (d) S1239, (e) S1027 and (f) OPA02. Bands of lane 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 indicates DNA bands of Sample 1, Sample 2, Sample 3, Sample 4, Sample 5, Sample 6, Sample 7, Sample 8, Sample 9 and Sample 10, respectively. M: Molecular Weight Marker (1Kb DNA ladder).

Table 2. RAPD primers with corresponding bands score and their size range together with polymorphic bands observed in 10 *J. curcas* genomes

Primer code	Sequences (5´-3´)	GC content %	Size range (bp)	Total number of bands scored	Number of polymorphic bands	Proportion of polymorphic loci (%)
OPA02	GAA GGC TCC C	70%	100-250	3	3	100
OPAB02	GGA AAC CCC T	70%	100-550	5	5	100
OPP13	GGA GTG CCT C	70%	100-750	9	9	100
S1027	ACG AGC ATG G	60%	100-500	3	3	100
S1155	GAA GGC TCC C	70%	200-750	7	7	100
S1239	TGA CAG CCC C	60%	100-500	4	4	100
Average			-	5.17	5.17	100
Total				31	31	-

Table 3. Overall gene frequencies of polymorphic RAPD markers in *J. curcas* genomes

Sl. no.	RAPD Markers	Gene frequency	Sl. no.	RAPD Markers	Gene frequency	Sl. no.	RAPD Markers	Gene frequency
1	OPA02-1	0.5	12	OPP13-4	0.1	23	S1155-3	0.1
2	OPA02-2	0.4	13	OPP13-5	0.5	24	S1155-4	0.7
3	OPA02-3	0.5	14	OPP13-6	0.1	25	S1155-5	0.1
4	OPAB02-1	0.1	15	OPP13-7	0.7	26	S1155-6	0.1
5	OPAB02-2	0.9	16	OPP13-8	0.1	27	S1155-7	0.1
6	OPAB02-3	0.1	17	OPP13-9	0.4	28	S1239-1	0.4
7	OPAB02-4	0.6	18	S1017-1	0.1	29	S1239-2	0.1
8	OPAB02-5	0.1	19	S1027-2	0.9	30	S1239-3	0.3
9	OPP13-1	0.1	20	S1027-3	0.2	31	S1239-4	0.3
10	OPP13-2	0.1	21	S1155-1	0.6			
11	OPP13-3	0.2	22	S1155-2	0.1			

Table 4. Summary of genetic diversity and Shannon information index statistics for all loci (Nei, 1978)

Loci	Sample size	No. of alleles observed	Effective no. of alleles	Gene diversity (h)	Shannon information index (i)
OPA02-1	10	2.0000	2.0000	0.5000	0.6931
OPA02-2	10	2.0000	1.9231	0.4800	0.6730
OPA02-3	10	2.0000	2.0000	0.5000	0.6931
OPAB02-1	10	2.0000	1.2195	0.1800	0.3251
OPAB02-2	10	2.0000	1.2195	0.1800	0.3251
OPAB02-3	10	2.0000	1.2195	0.1800	0.3251
OPAB02-4	10	2.0000	1.9231	0.4800	0.6730
OPAB02-5	10	2.0000	1.2195	0.1800	0.3251
OPP13-1	10	2.0000	1.2195	0.1800	0.3251
OPP13-2	10	2.0000	1.2195	0.1800	0.3251
OPP13-3	10	2.0000	1.4706	0.3200	0.5004
OPP13-4	10	2.0000	1.2195	0.1800	0.3251
OPP13-5	10	2.0000	2.0000	0.5000	0.6931
OPP13-6	10	2.0000	1.2195	0.1800	0.3251
OPP13-7	10	2.0000	1.7241	0.4200	0.6109
OPP13-8	10	2.0000	1.2195	0.1800	0.3251
OPP13-9	10	2.0000	1.9231	0.4800	0.6730
S1017-1	10	2.0000	1.2195	0.1800	0.3251
S1027-2	10	2.0000	1.2195	0.1800	0.3251
S1027-3	10	2.0000	1.4706	0.3200	0.5004
S1155-1	10	2.0000	1.9231	0.4800	0.6730
S1155-2	10	2.0000	1.2195	0.1800	0.3251
S1155-3	10	2.0000	1.2195	0.1800	0.3251
S1155-4	10	2.0000	1.7241	0.4200	0.6109
S1155-5	10	2.0000	1.2195	0.1800	0.3251
S1155-6	10	2.0000	1.2195	0.1800	0.3251
S1155-7	10	2.0000	1.2195	0.1800	0.3251
S1239-1	10	2.0000	1.9231	0.4800	0.6730
S1239-2	10	2.0000	1.2195	0.1800	0.3251
S1239-3	10	2.0000	1.7241	0.4200	0.6109
S1239-4	10	2.0000	1.7241	0.4200	0.6109
Mean	10	2.0000	1.4898	0.2994	0.4650
St. Dev		0.0000	0.3257	0.1397	0.1626

Table 5. Nei's analysis of gene diversity in subdivided populations

Loci	Sample Size	Ht	Hs	Gst	Nm*
OPA02-1	10	0.5000	0.0000	1.0000	0.0000
OPA02-2	10	0.4800	0.0000	1.0000	0.0000
OPA02-3	10	0.5000	0.0000	1.0000	0.0000
OPB02-1	10	0.1800	0.0000	1.0000	0.0000
OPB02-2	10	0.1800	0.0000	1.0000	0.0000
OPB02-3	10	0.1800	0.0000	1.0000	0.0000
OPB02-4	10	0.4800	0.0000	1.0000	0.0000
OPB02-5	10	0.1800	0.0000	1.0000	0.0000
OPP13-1	10	0.1800	0.0000	1.0000	0.0000
OPP13-2	10	0.1800	0.0000	1.0000	0.0000
OPP13-3	10	0.3200	0.0000	1.0000	0.0000
OPP13-4	10	0.1800	0.0000	1.0000	0.0000
OPP13-5	10	0.5000	0.0000	1.0000	0.0000
OPP13-6	10	0.1800	0.0000	1.0000	0.0000
OPP13-7	10	0.4200	0.0000	1.0000	0.0000
OPP13-8	10	0.1800	0.0000	1.0000	0.0000
OPP13-9	10	0.4800	0.0000	1.0000	0.0000
S1017-1	10	0.1800	0.0000	1.0000	0.0000
S1027-2	10	0.1800	0.0000	1.0000	0.0000
S1027-3	10	0.3200	0.0000	1.0000	0.0000
S1155-1	10	0.4800	0.0000	1.0000	0.0000
S1155-2	10	0.1800	0.0000	1.0000	0.0000
S1155-3	10	0.1800	0.0000	1.0000	0.0000
S1155-4	10	0.4200	0.0000	1.0000	0.0000
S1155-5	10	0.1800	0.0000	1.0000	0.0000
S1155-6	10	0.1800	0.0000	1.0000	0.0000
S1155-7	10	0.1800	0.0000	1.0000	0.0000
S1239-1	10	0.4800	0.0000	1.0000	0.0000
S1239-2	10	0.1800	0.0000	1.0000	0.0000
S1239-3	10	0.4200	0.0000	1.0000	0.0000
S1239-4	10	0.4200	0.0000	1.0000	0.0000
Mean	10	0.2994	0.0000	1.0000	0.0000
St. Dev		0.0195	0.0000		

* Ht = Hardy-Weinberg average heterozygosity expectedin subpopulation, * Nm = estimate of gene flow from Gst or Gcs. E.g., Nm = 0.5(1 - Gst)/Gst; The number of polymorphic loci is: 31. The percentage of polymorphic loci is: 100

Table 6. Pair-wise inter-genotype similarity indices (Si) among ten *J. curcas* genotypes

Germplasm combinations	OPA02	OPAB02	OPP13	S1027	S1155	S1239	Average
S_1Vs S_2	50.00	40.00	11.11	33.33	7.14	25.00	27.76
S_1Vs S_3	66.67	40.00	11.11	33.33	14.29	25.00	31.73
S_1Vs S_4	50.00	40.00	22.22	33.33	14.30	25.00	30.81
S_1Vs S_5	66.67	60.00	16.67	33.33	21.43	37.50	39.27
S_1Vs S_6	66.67	30.00	5.56	50.00	7.14	25.00	30.73
S_1Vs S_7	50.00	40.00	16.67	33.33	14.29	37.50	31.97
S_1Vs S_8	50.00	30.00	5.56	33.33	7.14	37.50	27.26
S_1Vs S_9	50.00	30.00	16.67	33.33	14.30	12.50	26.13
S_1Vs S_{10}	50.00	40.00	11.11	33.33	7.14	25.00	27.76
S_2Vs S_3	33.33	30.00	33.33	50.00	35.71	25.00	34.56
S_2Vs S_4	50.00	40.00	22.22	33.33	21.43	25.00	32.00
S_2Vs S_5	33.33	40.00	33.33	33.33	21.40	25.00	31.07
S_2Vs S_6	50.00	60.00	27.78	33.33	28.57	37.50	39.53
S_2Vs S_7	50.00	30.00	16.67	50.00	14.29	25.00	30.99
S_2Vs S_8	33.33	40.00	27.78	33.33	21.43	37.50	32.23
S_2Vs S_9	33.33	30.00	16.67	33.33	14.29	37.50	27.52
S_2Vs S_{10}	33.33	30.00	27.78	50.00	21.40	12.50	29.17
S_3Vs S_4	50.00	30.00	33.33	50.00	42.86	25.00	38.53
S_3Vs S_5	33.33	30.00	44.44	50.00	42.86	25.00	37.61
S_3Vs S_6	50.00	50.00	38.89	50.00	50.00	37.50	46.07
S_3Vs S_7	50.00	20.00	27.78	66.67	35.71	25.00	37.53
S_3Vs S_8	33.33	30.00	38.89	50.00	42.86	37.50	38.76
S_3Vs S_9	33.33	20.00	27.78	50.00	35.71	37.50	34.05

Table 6 continues

Germplasm combinations	OPA02	OPAB02	OPP13	S1027	S1155	S1239	Average
S_3Vs S_{10}	33.33	20.00	38.89	50.00	42.86	12.50	32.93
S_4Vs S_5	50.00	40.00	33.33	33.33	28.57	25.00	35.04
S_4Vs S_6	66.67	60.00	27.78	33.33	35.71	37.50	43.50
S_4Vs S_7	66.67	30.00	16.67	50.00	21.43	25.00	34.96
S_4Vs S_8	50.00	40.00	27.78	33.30	28.57	37.50	36.19
S_4Vs S_9	50.00	30.00	16.67	33.33	21.43	37.50	31.49
S_4Vs S_{10}	50.00	30.00	27.78	33.33	28.57	12.50	30.36
S_5Vs S_6	50.00	60.00	38.89	33.33	35.71	37.50	42.57
S_5Vs S_7	50.00	30.00	27.78	50.00	21.43	25.00	34.04
S_5Vs S_8	33.33	40.00	38.89	33.33	28.57	37.50	35.27
S_5Vs S_9	33.33	30.00	27.78	33.33	21.43	37.50	30.56
S_5Vs S_{10}	33.33	30.00	38.89	33.33	28.57	12.50	29.44
S_6Vs S_7	66.67	50.00	22.22	50.00	28.57	37.50	42.49
S_6Vs S_8	50.00	60.00	33.33	33.33	35.71	50.00	43.73
S_6Vs S_9	50.00	50.00	22.22	33.33	28.57	50.00	39.02
S_6Vs S_{10}	50.00	50.00	33.33	33.33	35.71	25.00	37.90
S_7Vs S_8	50.00	30.00	22.20	50.00	21.43	37.50	35.19
S_7Vs S_9	50.00	20.00	11.10	50.00	14.29	37.50	30.48
S_7Vs S_{10}	50.00	20.00	22.20	50.00	21.43	12.50	29.36
S_8Vs S_9	33.33	30.00	22.22	33.33	21.43	50.00	31.72
S_8Vs S_{10}	33.33	30.00	33.33	33.33	28.57	25.00	30.59
S_9Vs S_{10}	33.33	20.00	22.22	33.33	21.43	25.00	25.89
Average	46.666	36.222	25.3077	39.997	25.237	30	33.90578

Genetic distance

Pair-wise Nei's genetic distances ranged from 0.0667 to 0.9714 (Table 7). The value of highest genetic distance (0.9714) was found between *Jatropha* genotypes sample number 3 vs. sample number 6 and sample number 9; while the lowest (0.0667) was found between Sample numbers 8 vs. sample number 10. Comparatively higher levels of genetic distance (0.9361) were observed in sample 3 vs. sample 5 and sample 3 vs. sample 9. Genetic distance 0.7949 found in sample 3 vs. sample 1 and sample 3 vs. sample 8.

UPGMA dendrogram

A dendrogram based on Nei's genetic distance Unweighted Pair Group Method of Arithmetic Means (UPGMA) indicated segregation of the 10 *J. curcas* germplasms into three main clusters (Figure 2). Cluster I was subdivided into three sub-clusters IA, IB, and IC. Sub-cluster IA comprised of sample 1 and sample 9; Sub-cluster IB comprised of sample 2, sample 7 and sample 4; and Sub-cluster IC comprised of sample 5, sample 8 and sample 10 of *J. curcas*. The cluster II comprised of only *J. curcas* sample 6 which was collected from Sadar, Mymensingh and cluster III has one sample i.e.; sample 3 which was collected from Rupsha, Khulna. The similarity coefficient range varied from 0.00 to 0.971. The highest value of similarity coefficient range is 0.971 and the lowest value of similarity coefficient is 0.00.

Table 7. Summary of Nei's genetic identity (above diagonal) and genetic distance (below diagonal) values among 10 *Jatropha* Genotypes

Pop ID	1	2	3	4	5	6	7	8	9	10
1	****	0.7419	0.4516	0.7419	0.6452	0.6774	0.7097	0.7419	0.8387	0.7419
2	0.2985	****	0.5161	0.8065	0.7742	0.5484	0.8387	0.8065	0.8387	0.8065
3	0.7949	0.6614	****	0.5161	0.3548	0.3226	0.4839	0.4516	0.3548	0.4516
4	0.2985	0.2151	0.6614	****	0.7742	0.6129	0.8387	0.8065	0.7097	0.8065
5	0.4383	0.2559	0.9361	0.2559	****	0.5161	0.7419	0.7742	0.7419	0.8387
6	0.3895	0.6008	0.9714	0.4895	0.6614	****	0.5161	0.4839	0.5161	0.4839
7	0.3429	0.1759	0.7259	0.1759	0.2985	0.6614	****	0.6452	0.8065	0.7097
8	0.2985	0.2151	0.7949	0.2151	0.2559	0.7259	0.4383	****	0.7742	0.9055
9	0.1759	0.1759	0.9361	0.3429	0.2985	0.6614	0.2151	0.2559	****	0.8387
10	0.2985	0.2151	0.7949	0.2151	0.1759	0.7259	0.3429	0.0667	0.1759	****

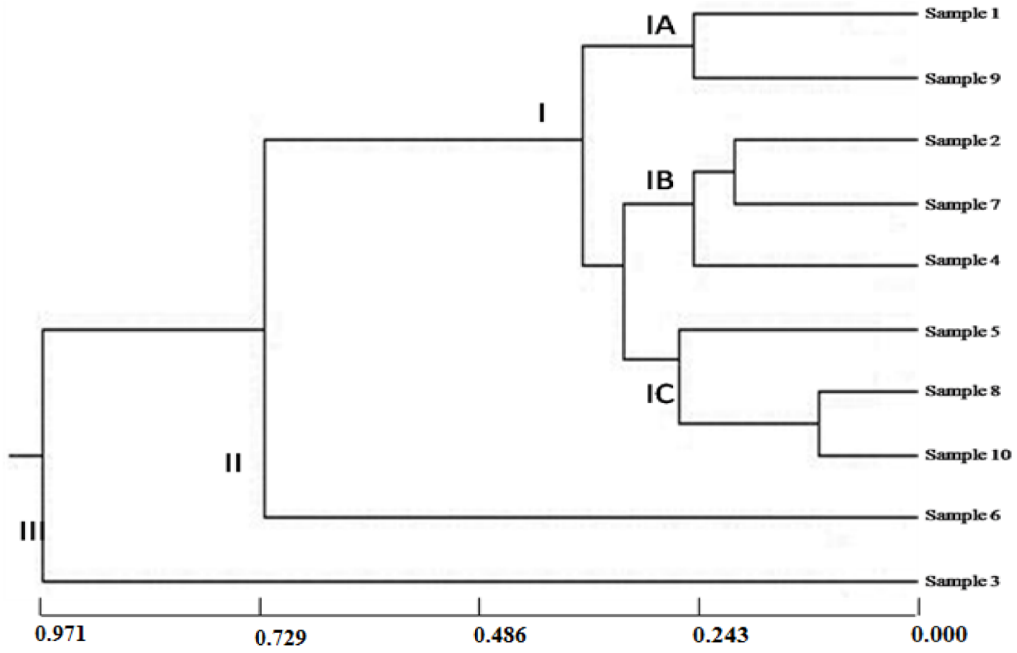

Fig. 2 Unweighted pair group method of arithmetic mean (UPGMA) dendrogram based on Nei's (1972) genetic distance, summarizing data on differentiation among 10 *J. curcas* genomes according to RAPD analysis

Discussion

In this study, genetic variation among ten (10) *J. curcas* germplasms collected from ten agro climatic regions was revealed using six RAPD primers. The primers generated 31 stable and reproducible DNA bands among 10 *Jatropha* genotypes. The band size varied from 100bp to 1000 bp, with average number of bands per primer was 5.17. A total of 31 bands were scored of which 100% (31 bands) were polymorphic across the 10 *Jatropha* genotypes. The number of polymorphic bands observed on an average 5.17, so the level of polymorphism observed in present study was fairly high (100%) indicating that a wide and diverse genetic distance existed among the genotypes of *J. curcas* found in Bangladesh.

Among the six primers, two primers produced several bands such as OPP13 primer produced total 9 bands and S1155 primer produced 7 bands. While the other primers OPAB02 produced 5 bands, S1239, OPA02 and S1027 produced 4, 3 and 3 bands, respectively. These findings revealed that all the six primers produced bands against the 10 *J. curcas* genomes which were collected from 10 agro climatic regions showed 100% polymorphism. Similar conclusion was made by Gupta *et al.* (2008) while accessing genetic variation in 14 accessions of *J. curcas* from several agro climatic zones of India using RAPD primers. They observed polymorphism (80.2%) in *Jatropha* species with 26 RAPD primers. 10 primers were used in five accessions of *J. curcas* from four agro climatic zones of Maharashtra to detect 125 DNA bands of which 96 (74.62%) were polymorphic (Gopale and Zunjarrao, 2013).

The genetic distance values observed in this study ranged from 0.0667 to 0.9714. These findings are in line with the results of Pamidiamarri *et al*. (2008) where the highest genetic polymorphism was observed in *J. curcas* through RAPD analysis. However, in another study moderate level of genetic variability was found in *J. curcas* germplasms in India (Basha *et al*., 2007). The findings from the present study revealed that *J. curcas* germplasms from different regions of Bangladesh have a wide range of genetic variation.

Pair-wise inter-genotype similarity indices (Si) is a measure of relatedness among genome pool that also demonstrated 10 *Jatropha* genotypes using the six primers. The overall average inter-genotype similarity index was 33.906%. The average highest inter-genotype similarity indices were found in six pair of genotypes for OPA02 primer and one pair of genotypes for S1027 and the value was 66.67% which showed less heterozygosity in other words they are homogenous. The germplasm pair S_1vs. S_9 showed the lowest average Si value (26.13%) considering all six primers. Pair wise similarity induces coefficient ranges varied from 0.00 to 0.971. Dhillon *et al.* (2006) reported that such a wide range in similarity coefficient values indicated that *J. curcas* germplasms represent a genetically diverse population and this might be attributed to a high level of cross pollination in this species. The diversity revealed by RAPD is in agreement with the conclusion that our breeding plant species retain considerable variability. The maintenance of a high genetic variance within population was favored by genetic systems of the species like gene flow, out breeding, mutation, high genetic load, etc.

The UPGMA dendrogram (Figure 2) showed that 10 genotypes were clustered into three groups. Cluster I was subdivided into three sub-clusters IA, IB, and IC. The cluster II comprised of only *Jatropha* sample 6 and cluster III comprised of sample 3. The cluster II and cluster III were collected from Sadar, Mymensingh and Rupsha, Khulna respectively. The cluster II and III showed more genetic diversity. The cluster I comprised other eight genotype samples, which were subdivided into three sub-clusters. The genotypes of same sub-cluster might be of same genotypic or parental source were revealed comparatively less genetic diversity. *J. curcas* is an economically important and promising plant for biodiesel production in Bangladesh. This importance necessitates its molecular characterization. The genetic diversity that has been revealed in the present study could be informative in the improvement, management and conservation point of view. The genotype pairs which showed high level of genetic distance could be used in the future research programme. Moreover, diversified gene pool of the studied *J. curcas* genotypes should be conserved effectively. So this study can be used as a relevant information source for further genetic diversity analysis.

Acknowledgement

This research work was financially supported by Ministry of Science and Technology, Government of the People's Republic of Bangladesh.

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