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Research Article

Repetitive element palindromic PCR (rep-PCR) as a genetic tool to study interspecific diversity in Euphorbiaceae family

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ABSTRACT

Background: The classification of diversity in germplasm collections is important for plant breeding. The repetitive element palindromic-polymerase chain reaction (rep-PCR) technique was used to investigate inter-specific diversity within 17 species from the Euphorbiaceae family using REP and BOX primers.
Results: The agglomerative cluster analysis was used to evaluate the scoring data. BOX and REP gave amplification with polymorphism of 98.84% and 100% respectively. REP marker demarcated between the subgenus peltatae. Both markers confirmed *Jatropha tanjorensis* as a natural hybrid between *Jatropha gossypifolia* and *Jatropha curcas*. Five random sequences from the rep-PCR gels were chosen, cloned and sequenced. The blast results demonstrated that the amplified products were from the mitochondrial genomes.
Conclusion: The rep-PCR molecular tool can be used to characterize diversity in plants as they are suitable for distinguishing eukaryotic genomes effectively.

1. Introduction

Establishing correct description and characterization of accessions is important for identification of individuals at the species or sub-species level or distinguishing different varieties or inbred lines for phylogenetic or breeding purposes [1].

The genus *Jatropha* is morphologically diverse with up to 175 species [2] and is an economically important plant in more than 50 countries [3] and, *Jatropha curcas* in particular, has an enormous potential as a biodiesel plant [4]. The full exploitation of the plant has been slow due to a number of factors including unreliable and low seed set, low oil yields, the presence of toxins and antinutritional factors [5]. Knowledge of genetic diversity, relationship and variation in species is a pre-requisite in any breeding programme [6]. Genetic diversity in the *Jatropha* germplasm has been investigated previously based on agromorphological traits [2,6,7], biochemical traits [6], molecular markers including isozymes [6], RAPDs [8,9,10,11], ISSRs [8], AFLPs [11,12,13], single primer amplification reaction (SPAR) [14], SSR [13] and nrDNA ITS [10].

Organellar genomes — chloroplast DNA (cpDNA) and mitochondrial DNA (mtDNA) — have been increasingly used in recent years as markers to assess maternal/or paternal gene flow because of their uniparental mode of inheritance [15]. Molecular markers such as RFLP, and RAPD, PCR-RFLP, which assess genome-wide variability, have been employed to detect variations across mitochondrial genomes or to identify specific cytoplasms [16]. Short repeats are common in the mitochondrial genomes of both lower and higher plants [1] and about 10% of most sequenced plant mitochondrial genomes are constituted by repeats. An alternate strategy to assess the variability in mitochondrial genomes is to exploit the presence of repeated regions within the genome and mutations involving structural rearrangements [17]. Mitochondrial genomes are considered to be of prokaryotic origin and variable number of tandem repeats (VNTRs) have been found in plant mt-DNA [18].

Repetitive sequence based polymerase chain reaction (rep-PCR) technique has been devised for characterization of bacteria and is widely employed to distinguish species, strains, serotypes, among others. The technique was devised [19] and uses three specific primers, designated BOX, ERIC and REP which have been designed to match the conserved sequences distributed in diverse bacterial genomes. These primers amplify genomic regions located between repetitive sequences and have proven extremely useful in the study of microbial diversity. With regard to plants, rep-PCR has been used to determine the different cytoplasmic male sterility (CMS) lines of *Brassica juncea* [20] and for identifying mitochondrial genome diversity in safflower (*Carthamus tinctorius* L.) and its wild relatives [21]. However, mitochondrial diversity studies have not been reported among Euphorbiaceae species.

In this study, the applicability of rep-PCR was demonstrated as an effective tool in the diversity study among the spurge family.

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2. Materials and methods

2.1. Plant materials

Seventeen *Jatropha* species were selected from Mauritius and India (Table 1). For the Indian species, certified seeds were acquired from Odisha following set quarantine procudures in Mauritius. The seeds were germinated and their leaves used in this research work.

2.2. Isolation and quantification of genomic DNA

Genomic DNA was extracted from the leaves crushed to a fine powder by the standard CTAB method. The leaf tissues were ground with a mortar and pestle to a fine powder using liquid nitrogen. Five grams of the leaves powder were homogenized in 20 mL of extraction buffer (2% (w/v) CTAB, 20 mM EDTA, 2% (v/v) PVP, 1.4 M NaCl, 100 mM Tris–HCl (pH 8.0) and 1% (v/v) β-mercaptoethanol and incubated at 65°C for 45 min). The supernatant was treated with RNase A (10 mg/μL), incubated at 37°C for 30 mins and twice extracted with chloroform:isoamylalcohol (24:1 v/v). The DNA was precipitated with isopropanol and washed twice with 70% (v/v) ethanol. The pelleted DNA was air dried and resuspended in 500 μL of sterile millipore water and stored overnight at -20°C. The purity of the extracted DNA was determined by taking the ratio of absorbance at 260 nm and 280 nm.

2.3. Protocols

2.3.1. Rep-PCR

Amplification was carried out in a 20 μL reaction mixture consisting 50 ng/μL genomic DNA, 10× PCR reaction buffer containing 15 mM $MgCl_2$, 10 pM primer, 2.5 mM of each dNTP and 3 U/μL of Taq DNA polymerase (Genei, Bangalore, India). Amplification was performed in a thermal cycler (Eppendorf, Germany) PCR machine. The PCR conditions were 94°C for 3 min, followed by 45 cycles of DNA amplification 20 s at 92°C, 1 min at 52°C for BOXA1R primer and (1 min at 38°C for REP primers) and 8 min at 68°C and 15 min incubation at 68°C.

(1) BoxAIR: CTACGGCAAGGCGACGCTGACG,
(2) REP1R:IIIICGICGICATCIGGC/REP1I: ICGICTTATCIGGCCTAC

The PCR amplifications were done on 2% (w/v) agarose gel for 7–8 h at constant voltage (2 V/cm).

Table 1
Codes of Euphorbias species.

Species	Country	Code
C. variegatum (L.) A. Juss.	Mauritius	Cva
J. integerrima Jack. (pink)	India	JiP
J. gossypifolia L.	India	Jg
J. integerrima Jack (red)	Mauritius	JiRM
J. integerrima Jack (red)	India	JiR
J. mahafalensis JH Perrier	India	Jma
J. manihot L.	Mauritius	Jmh
J. multifida L.	Mauritius	JmM
J. multifida L.	India	Jm
J. podagrica Hook	Mauritius	JpM
J. podagrica Hook	India	Jp
J. tanjorensis Ellis & Saroja	India	Jt
Jatropha curcas L.	Mauritius	JcM
Jatropha curcas L.	India	Jc
R. communis L. (white)	Mauritius	RcWM
R. communis L.(red)	Mauritius	RcRM
R. communis L.	India	Rc

2.4. Cloning

To confirm that the amplicons obtained from rep-PCR were of mitochondrial genomes, six representative amplicons in the range of 575 bp to 1 kb in length for both BOX and REP primers, were extracted using a gel extraction kit (Qiagen, Hilden, Germany) and cloned into the PCR cloning vector, pMiniT Vector (NEB PCR Cloning Kit, new England biolads, UK). All the clones were sequenced at Inqaba (Pretoria, SA) from the Forward primer (5′ ACCTGCCAACCAAA GCGAGAAC 3′) and Reverse primers (5′ TCAGGGTTATTGTCTCATGA GCG 3′) available in the vector and subsequently the cloned sequences were blasted (NCBI, Gene Bank).

2.5. Data scoring and statistical analysis

For scoring and analysis of data from the two molecular markers (BOX and REP primers), the total number of monomorphic and polymorphic bands which were clear, unambiguous and reproducible were scored for the tested primers. Data scoring was carried out using a binary number system for '1' as the presence and '0' as the absence of fragment (band) for both primers. The percentage of polymorphism (PP) for the markers between the species were calculated from the formula PP = total number of polymorphic bands/total number of bands multiplied by 100. To compare the efficiency of each marker polymorphic information content (PIC) as a marker discrimination power, was computed using the formula $PIC = 1 - \Sigma (p_i)^2$, where pi is the frequency of *i*th allele at a given locus [22,23]. The Resolving power (R_p) ($=\Sigma$ IB, where IB, informativeness band, takes the value of $1–2 \times (0.5 - p)$, p being the proportion of the 17 species analyzed containing the band) of the markers, was also computed. Measure of similarity among 17 Euphorbiaceae species was established as matrices of genetic similarity compiled using the SIMQUAL function Jaccard's coefficients. Dendrograms representing the genetic relationship among all *Jatropha* species were generated from the similarity matrices by applying unweighted pair-group arithmetic mean method (UPGMA) (cluster analysis) with the SAHN function system. To measure the goodness of fit for 17 species, specific cluster in the UPGMA algorithm, the relationships between the original similarity coefficients and cophenetic values were evaluated, and the Mantel's test was performed and the Cophenetic correlation coefficients (r) were calculated. Principal component analysis (PCA) was also performed with modules DCENTER (transformation), CORR and EIGEN (ordination) using the Euclidean distances derived from the standardized values of the similarity coefficients for only polymorphic bands. Three dimensional (2-d) plots of the 17 euphorbias (OTU — Operational Taxonominal Unit) were output from by the PCA analysis using the Jaccard's eigen values with MOD3d (graphics) module. Diversity analyses were done in numerical taxonomy and multivariate analysis system (NTSYSpc version 2.21q) (numerical taxonomy system, Applied Biostatistics, NY) [24]. GenAlEx (version 6.5) and PopGen 32. softwares were used to estimate the genetic similarity, diversity indexes and Analysis of Molecular Variance between the different species. Dendrograms generated in this paper were compiled after the data were permutated (n = 10,000) to obtain significance using FreeTree 0.9.1.50. Dendrograms were visualized on MEGA Version 6 (2013) after bootstrapping using UPGMA. To ensure reproducibility the experiment was carried out five times.

3. Results

3.1. BOX and REP Primer polymorphism patterns

The two sets of primer, REP and BOX, gave successful amplification with a total of 183 and 260 bands respectively with band sizes lying between 60 bp–2060 bp and 180 bp–2970 bp respectively. 183 bands from REP and 257 bands were polymorphic band with polymorphism of 100% and 98.84% respectively (Table 2). BOX primer yielded the

Table 2
Markers profiles for the 17 Euphorbiaceae genotypes.

Marker	Box	Rep	Box + Rep
Total number of polymorphic bands	257	183	440
Total number of monomorphic bands	3	0	3
Total number of bands	260	183	443
% Polymorphism	98.84	100	99.32
R_p	4.352	2.142	3.014
PIC	0.357	0.372	0.363

highest number of polymorphic alleles (29) for *Codiaeum variegatum* (yellow croton plant) followed with both the local and Indian species of *Jatropha intergerrima* (red) with 22 bands and the lowest number (1) for *Jatropha manihot* where Rep primer *J. curcas* (India), and *Jatropha mahafalensis* had the maximum (22) and minimum numbers (1) of polymorphic alleles. The mean numbers of allele per species were 15.29 ± 7.87 (BOX) and 10.76 ± 5.77 (REP) maximum, 29 and minimum 1 allele numbers of amplified bands in BOX and REP. PIC value of 0.357 was obtained for BOX primer, 0.372 for REP Primer and combined BOX + REP 0.363 with 4.352, 2.142 and 3.014 for the R*p* values respectively (Table 2).

The observed number of alleles, effective number of alleles, Nei's genetic diversity, Shannon's information index for the species with values ranging from 1.9–2.0, 1.49–1.58, 0.31–0.35 and 0.521–0.528 for BOX, REP and BOX + REP. The mean coefficient of the gene differentiation (Gst) value (71%) indicated that 39% the genetic diversity resided within the genotype (Euphorbiaceae species) (Table 3).

Analysis of the molecular variance showed that the source of variation between the species varied between 65% and 69% and within from 31% to 34% (Table 4). The highest in between the species variation was 68.96%.

3.2. Genetic similarity analysis

Cluster (or phylo) analysis and PCA charts are valuable presentation tool for the determination of the relationships that co-exist between and within species. Both the PCA plots and UPGMA dendrograms generated were comparable and useful to study the inter-diversity for 17 Euphorbiaceae using both REP and BOX primer.

For both the PCA plot and dendogram, three main clusters were generated (Fig. 1). Cophenetic values and the similarity matrices revealed a very good degree of correlation fit ($r = 0.9452$) of the dendrogram. For inter-species genetic relationship among 17 species, the Jaccard's genetic similarity coefficients derived from the BOX data ranged from 0.00 (*Jatropha manihot* with most of the species except *Ricinus communis* from India) to 0.952 (*J. curcas* Mauritius and *J. curcas* India) (Table 5).

Dendrogram (Fig. 2) constructed based on REP Jaccard's similarity matrix demonstrated 4 clusters comparable to the PCA plots. REP showed the lowest genetic similarity coefficient between *J. mahafalensis* with all the *Ricinus* sp., *C. variegatum* (Cassava) and *J.* manihot (yellow croton) (0.0) while the highest value was obtained for *J. integerrima* (Mauritius and India) (0.741) and *J. curcas* Mauritius and *J. curcas* India (Table 6). This result showed that the inter-species genetic similarity of the genus *Jatropha* was fairly similar for two different rep-PCR based

Table 4
Analysis of molecular variance for BOX, REP and BOX + REP between and within the different Euphorbiaceae species.

Source of variance	Markers	Variance component	% of Total variance
Between species	Box	7.799	65.57
	REP	5.579	68.96
	BOX + REP	13.378	66.94
Within species	Box	4094	34.43
	REP	2.512	31.04
	BOX + REP	6.606	33.06

Level of significance based on 1000 permutation iteration steps ($p < 0.001$).

molecular markers. The Mantel test indicated a significant correlation results of REP ($r = 0.8890$).

The scoring data of both primers were pooled and analyzed for the same set of species and a very good Cophenetic correlation dendogram fit was obtained for BOX + REP data ($r = 0.8958$).

Seven to ten clusters were present in the PCA plot/dendrogram of BOX + REP dat (Fig. 3). The level of similarity between *J. mahafalensis* and *C. variegatum* (yellow croton) (0.0), *J. integerrima* (red) (Mauritius and India) (0.862) and the Mauritian and Indian species of *J.* curcas (0.783) ad similar magnitude to that of the REP dendrogram (Table 7).

The 2D plot was generated in order to understand the relationship between the species and it gave three distinct clusters for BOX primer with *Jatropha tanjorensis*, *Jatropha gossypifolia*, *J. podagrica*, *J. integerrima* (red), *J. mahafalensis*, *J. curcas*, *J. manihot* and *Jatropha multifida* forming the major cluster (Fig. 1).

Moreover the PCA plots revealed (a) that the first three most informative PC components explained 96%, 66%, and 73% of the total variation.

3.3. Cloned sequence

The blast search showed homology to both mitochondria and chloroplast genome sequences (Table 8). This may not be an abnormality as mitochondrial genomes are known to acquire sequences from chloroplast through horizontal gene transfer [1].

4. Discussion

Molecular markers have largely been used in the characterization of species, confirmation of hybrids in inter-specific species, determination of crossability success, establishment of phylogenetic relationships, marker assisted selection and construction of framework linkage map [8]. Molecular markers are also a very reliable source of information as they are neutral to environmental influence and reveal differences at the whole genome level. Knowledge of genetic relationship among different species is primordial for studies of phylogenic relationships and evolutionary trend crossability relationships. Understanding the phylogeny of species relies on molecular markers that are able to show effective inferences about the genetic affinity between species. Determination of genetic relationship among species is useful in the management of genetic resources and successful interspecific hybridization [16].

To analyze the genetic relationship of *Jatropha* species, Pamidiamarri et al. [6,10] used AFLP and RAPD and Basha and Sujatha [8] tested ISSR,

Table 3
Mean diversity indexes for the 17 Euphorbiaceae species using BOX, REP and BOX + REP.

Markers	Na	Ne	h	I	G_{st}	Nm
BOX	1.988 (0.021)	1.589 (0.273)	0.352 (0.117)	0.528 (0.138)	0.703	0.211
REP	2.000 (0.00)	1.571 (0.264)	0.345 (0.111)	0.522 (0.128)	0.737	0.178
BOX + REP	1.993 (0.113)	1.491 (0.324)	0.318 (0.102)	0.521 (0.129)	0.721	0.193

Na: observed number of alleles; Ne: effective number of alleles; h: Nei's genetic diversity; I: Shannon's information index; G_{st}: coefficient of gene differentiation; Nm: estimate of gene flow; () ± standard deviation.

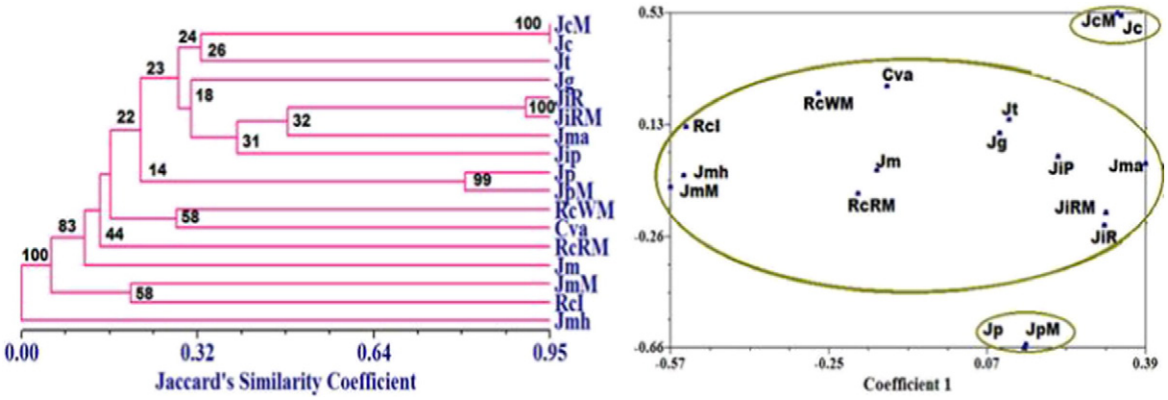

Fig. 1. Genetic relationships among the 17 species based on Jaccard's similarity coefficients (UPGMA) for BOX marker from Euphorbiaceae family. For both the PCA plot and dendogram, three main clusters were generated. Cophenetic values and the similarity matrices revealed a very good degree of correlation fit (r = 0.9452) of the dendrogram. For inter-species genetic relationship among 17 species, the Jaccard's genetic similarity coefficients derived from the BOX data ranged from 0.00 (*J. manihot* with most of the species except *R. communis* from India) to 0.952 (*J. curcas* Mauritius and *J. curcas* India) (Table 5).

Table 5
Jaccard's similarity indexes of 17 Euphorbiaceae species for BOX primer.

	JcM	Jt	Jg	Jm	Jp	JiR	Jip	Jma	Jc	JmM	JpM	JiRM	RcI	RcRM	RcWM	Jmh
JcM																
Jt	0.333															
Jg	0.294	0.241														
Jm	0.125	0.188	0.148													
Jp	0.167	0.176	0.185	0.071												
JiR	0.250	0.185	0.294	0.174	0.273											
Jip	0.300	0.240	0.229	0.083	0.174	0.345										
Jma	0.444	0.348	0.433	0.130	0.350	0.500	0.462									
Jc	0.952	0.320	0.286	0.120	0.160	0.242	0.290	0.429								
JmM	0.043	0.000	0.037	0.000	0.091	0.043	0.045	0.045	0.042							
JpM	0.154	0.158	0.172	0.133	0.800	0.304	0.208	0.318	0.148	0.077						
JiRM	0.273	0.214	0.278	0.160	0.250	0.909	0.367	0.464	0.265	0.040	0.280					
RcI	0.077	0.053	0.103	0.000	0.000	0.120	0.038	0.080	0.074	0.200	0.000	0.111				
RcRM	0.129	0.125	0.147	0.048	0.150	0.207	0.172	0.172	0.125	0.056	0.136	0.194	0.045			
RcWM	0.171	0.138	0.216	0.120	0.000	0.206	0.176	0.176	0.167	0.087	0.033	0.229	0.160	0.091		
Jmh	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.067	0.000	
Cva	0.256	0.108	0.205	0.029	0.088	0.195	0.143	0.200	0.282	0.000	0.083	0.214	0.156	0.100	0.282	0.000

RAPD and chloroplast unilocus primers (ccmps). Prabakaran and Sujatha [6,8] confirmed *J. tanjorensis* as a natural hybrid between *J. curcas* and *J. gossypifolia* based on morphological, cytological and biochemical. In the present investigation, the hybrid nature of *J. tanjorensis* between *J. curcas* and *J. gossypifolia* was further supported by REP and BOX markers. The Jaccard's genetic similarity coefficient generated for *J. curcas*/*J. tanjorensis* was slightly higher than of *J. gossypifolia*/*J. tanjorensis*. The genetic similarity observed was in accordance with Ganesh et al. [9] and Pamidiamarri et al. [10,12].

The clustering patterns indicated that *J. integerrima* pink was situated closer to *J. curcas* than *J. integerrima* red, thus indicating clearly its polymorphae section. *J. multifida* and *J. podagrica* although morphologically different, can be easily identified as the two species are classified under same section, peltatae (Dehgan [2]). The similarity

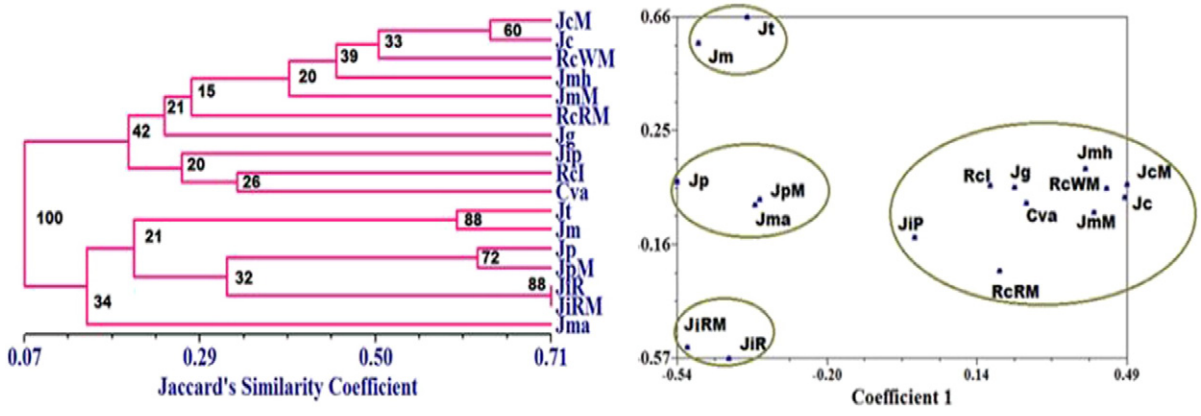

Fig. 2. Genetic relationships among the 17 species based on Jaccard's similarity coefficients (UPGMA) for REP marker from Euphorbiaceae family.

Table 6
Jaccard's similarity Index of 17 Euphorbiaceae species (REP primer).

	JcM	Jt	Jg	Jm	Jp	JiR	Jip	Jma	Jc	JmM	JpM	JiRM	RcI	RcRM	RcWM	Jmh
JcM																
Jt	0.080															
Jg	0.217	0.133														
Jm	0.038	0.600	0.000													
Jp	0.087	0.273	0.000	0.400												
JiR	0.083	0.071	0.067	0.154	0.300											
Jip	0.200	0.056	0.111	0.056	0.214	0.200										
Jma	0.012	0.125	0.020	0.125	0.167	0.143	0.091									
Jc	0.640	0.071	0.192	0.034	0.037	0.115	0.222	0.034								
JmM	0.375	0.048	0.353	0.048	0.053	0.105	0.190	0.020	0.385							
JpM	0.182	0.250	0.067	0.250	0.625	0.273	0.200	0.143	0.115	0.167						
JiRM	0.043	0.083	0.077	0.182	0.375	0.714	0.143	0.200	0.038	0.056	0.333					
RcI	0.200	0.000	0.167	0.000	0.100	0.000	0.231	0.000	0.174	0.188	0.200	0.000				
RcRM	0.240	0.000	0.167	0.000	0.059	0.188	0.095	0.000	0.308	0.368	0.188	0.133	0.063			
RcWM	0.565	0.087	0.300	0.042	0.045	0.091	0.120	0.000	0.444	0.476	0.143	0.048	0.158	0.261		
Jmh	0.500	0.087	0.238	0.087	0.095	0.043	0.217	0.000	0.500	0.348	0.200	0.048	0.222	0.208	0.360	
Cva	0.360	0.045	0.091	0.045	0.167	0.100	0.300	0.000	0.321	0.381	0.222	0.053	0.333	0.174	0.231	0.333

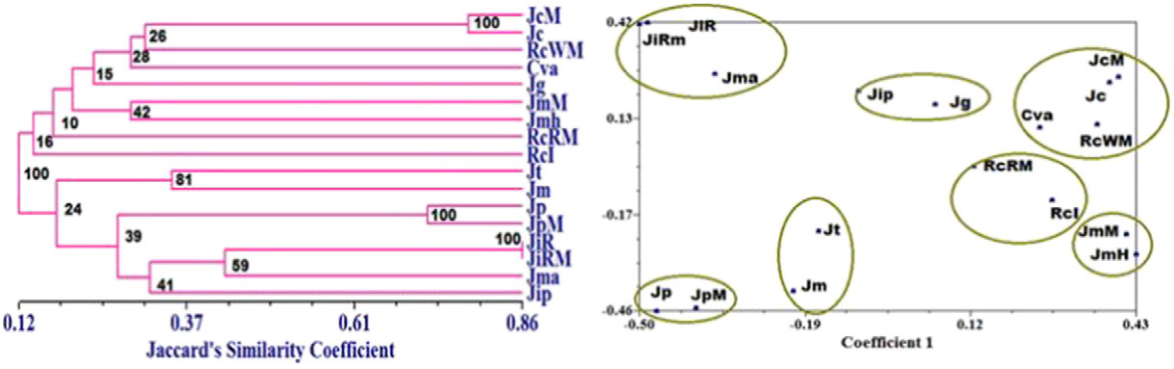

Fig. 3. Genetic relationships among the 17 species based on Jaccard's similarity coefficients (UPGMA) for BOX + REP marker from Euphorbiaceae family.

of *J. multifida* and *J. podagrica* which have been reported by Ganesh et al. [9] and Bahadur et al. [4] was distinguished by the use of a single primer, REP. Although, the clustering pattern depicted in the BOX and REP dendrograms are different, the common information about diversity among *Jatropha* species can still be accounted for. The observations are in line with both Ganesh et al. [9], Pamidimarri et al. [10] and Basha and Sujatha [8]. Variability in diversity of the selected *Jatropha* species was effectively demonstrated by both markers, BOX and REP. Several markers were successful in differentiating between the *Jatropha* species under study and comparing with other species [22]. Information obtained in terms of diversity of the several species has been well documented over the years with different type and quantity of markers which have been used to confirm certain information.

Analysis of data and methods used play and important role when results are reported and conclusions are drawn. Ganesh et al. [9] and Pamidimarri et al. [10] used Jaccard similarity coefficient for the construction of the dendrogram whereas Basha and Sujatha [8] used similarity coefficient. Different pattern of clustering were observed, Ganesh et al. [9] and Pamidimarri et al. [10] shared the same clustering pattern regardless of the number and types of primer used.

Table 7
Jaccard's similarity index of 17 Euphorbiaceae species (BOX + REP).

	JcM	Jt	Jg	Jm	Jp	JiR	Jip	Jma	Jc	JmM	JpM	JiRM	RcI	RcRM	RcWM	Jmh
JcM																
Jt	0.204															
Jg	0.263	0.205														
Jm	0.080	0.346	0.091													
Jp	0.128	0.214	0.119	0.208												
JiR	0.179	0.146	0.224	0.167	0.281											
Jip	0.255	0.163	0.189	0.071	0.189	0.295										
Jma	0.255	0.290	0.325	0.129	0.308	0.424	0.351									
Jc	0.783	0.189	0.246	0.074	0.096	0.186	0.259	0.235								
JmM	0.213	0.027	0.159	0.031	0.067	0.071	0.116	0.027	0.220							
JpM	0.167	0.194	0.136	0.185	0.722	0.294	0.205	0.276	0.132	0.129						
JiRM	0.179	0.175	0.224	0.167	0.281	0.862	0.295	0.424	0.167	0.047	0.294					
RcI	0.130	0.031	0.122	0.000	0.038	0.081	0.103	0.065	0.120	0.192	0.071	0.081				
RcRM	0.179	0.068	0.154	0.024	0.108	0.200	0.140	0.119	0.207	0.216	0.158	0.174	0.053			
RcWM	0.328	0.115	0.246	0.082	0.020	0.161	0.153	0.115	0.286	0.273	0.078	0.161	0.159	0.161		
Jmh	0.267	0.056	0.109	0.065	0.067	0.023	0.116	0.000	0.271	0.286	0.129	0.023	0.148	0.154	0.191	
Cva	0.297	0.085	0.167	0.035	0.115	0.164	0.194	0.143	0.299	0.148	0.130	0.164	0.213	0.127	0.262	0.148

Table 8
Summary of BLAST search of sequenced clones.

Sample no.	Amplicon	Clone size sequence	Homology	Location	Plant
1	BOX	650	(LOC105635464): similar cytochrome P450 71B36	Mitochondria	*Jatropha curcas* L.
2	BOX	890	(LOC105634665), similar to protein-tyrosine-phosphatase MKP1	Mitochondria	*Jatropha curcas* L.
3	BOX	515	(LOC105631770), similar to long chain acyl-CoA synthetase 2	Chloroplast/plastids Mitochondria	*Jatropha curcas* L.
4	BOX	750	utp-glucose-1-phosphate uridylyltransferase, putative	Mitochondria	*R. communis* L.
5	REP	1 kb	secretory peroxidase PX3	mitochondrial electron transport chain	*C. variegatum* (L.) A. Juss.

The results are calculated with various coefficients; many different similarity coefficients are suggested for use in their determination. The consequence of selecting different coefficients affects the clusters obtained by Sesli and Yegenoglu [5]. In this study, the influence of the choice among two different methods of calculating similarity coefficients with and without the negative occurrences, Jaccard and single matching genetic similarity based on the UPGMA, for BOX and REP for the 17 accessions investigated. The Jaccard, Sorensen-Dice, Anderberg, and Ochiai coefficients are recommended because they are easy to explain and they do not consider negative co-occurrences as observed and the pooled scoring data of the primer sets was also effective in generating species inter-specific information.

5. Conclusions

The 17 accessions studied could be unambiguously identified with two sets of rep-PCR primers, BOX and REP. The primers were effective in clustering the *Jatropha* species according to their subgenus and *J. tanjorensis* as a hybrid. The rep-PCR technique proved to be less time consuming, rapid and effective in distinguishing among inter-specific species. It was also demonstrated that the molecular diversity corroborates with the extensive morphological diversity among the species which could be beneficial for agronomically improving *J. curcas*. It is likely that rep-PCR can be used for inter-specific studies but further experiments and resources are needed to complete this study and to confirm the effective application of this method in plants.

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Conflict of interest

The authors declare that they have no conflict of interest.

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References

[1] Alverson AJ, Zhuo S, Rice DW, Sloan DB, Palmer JD. The mitochondrial genome of the legume *Vigna radiata* and the analysis of recombination across short mitochondrial repeats. PLoS One 2011;6: e16404. http://dx.doi.org/10.1371/journal.pone.0016404.
[2] Dehgan B. Phylogenetic significance of interspecific hybridization in *Jatropha* (Euphorbiaceae). Syst Bot 1984;9:467–78. http://dx.doi.org/10.2307/2418796.
[3] Basha SD, Francis G, Makkar HPS, Becker K, Sujatha M. A comparative study of biochemical traits and molecular markers for assessment of genetic relationships between *Jatropha curcas* L. germplasm from different countries. Plant Sci 2009; 176:812–23. http://dx.doi.org/10.1016/j.plantsci.2009.03.008.
[4] Bahadur B, Sujatha M, Carels N. Jatropha, Challenges for a new energy crop. Vol 2: Genetic improvement and biotechnology. New York: Springer; 2013. http://dx.doi.org/10.1007/978-1-4614-4915-7.
[5] Sesli M, Yegenoglu ED. Comparison of similarity coefficients used for cluster analysis based on RAPD markers in wild olives. Genet Mol Res 2010;9:2248–53. http://dx.doi.org/10.4238/vol9-4gmr966.
[6] Prabakaran AJ, Sujatha M. *Jatropha tanjorensis* Ellis & Saroja, a natural interspecific hybrid occurring in Tamil Nadu, India. Genet Resour Crop Evol 1999;46:213–8. http://dx.doi.org/10.1023/A:1008635821757.
[7] Francis G, Edinger R, Becker K. A concept for simultaneous wasteland reclamation, fuel production, and socio-economic development in degraded areas in India: Need, potential and perspectives of *Jatropha* plantations. Nat Res Forum 2005;29: 12–24. http://dx.doi.org/10.1111/j.1477-8947.2005.00109.x.
[8] Basha SD, Sujatha M. Genetic analysis of *Jatropha* species and interspecific hybrids of *Jatropha curcas* using nuclear and organelle specific markers. Euphytica 2009;168: 197–214. http://dx.doi.org/10.1007/s10681-009-9900-0.
[9] Ganesh RS, Parthiban KT, Kumar RS, Thiruvengadam V, Paramathma M. Genetic diversity among *Jatropha* species as reveled by RAPD markers. Genet Resour Crop Evol 2008;55:803–9. http://dx.doi.org/10.1007/s10722-007-9285-7.
[10] Pamidiamarri DVNS, Pandya N, Reddy MP, Radhakrishnan T. Comparative study of interspecific genetic divergence and phylogenic analysis of genus *Jatropha* by RAPD and AFLP. Mol Biol Rep 2009;36:901–7. http://dx.doi.org/10.1007/s11033-008-9261-0.
[11] Reddy MP, Chikara J, Patolia JS, Ghosh A. Genetic improvement of *J. curcas* adaptability and oil yield. Fact seminar on *Jatropha curcas* L. Agronomy and Genetics; 2007. p. 2007.
[12] Sudheer Pamidiamarri, Singh S, Mastan SG, Patel J, Reddy MP. Molecular characterization and identification of markers for toxic and non-toxic varieties of *Jatropha curcas* L. using RAPD, AFLP and SSR markers. Mol Biol Rep 2009;36: 1357–64. http://dx.doi.org/10.1007/s11033-008-9320-6.
[13] Sun QB, Li LF, Li Y, Wu GF, Ge XJ. SSR and AFLP markers reveal low genetic diversity in the biofuel plant *Jatropha curcas* in China. Crop Sci 2008;48:1865–71. http://dx.doi.org/10.2135/cropsci2008.02.0074.
[14] Renade SA, Srivastava AP, Rana TS, Srivastava J, Tuli R. Easy assessment of diversity in *Jatropha curcas* L. plants using two single-primer amplification reaction (SPAR) methods. Biomass Bioenergy 2008;32:533–40. http://dx.doi.org/10.1016/j.biombioe.2007.11.006.
[15] Grivet D, Petit RJ. Chloroplast DNA phylogeography of the hornbeam in Europe: Evidence for a bottleneck at the outset of postglacial colonization. Conserv Genet 2003; 4:47–56. http://dx.doi.org/10.1023/A:1021804009832.
[16] Liu SL, Zhuang Y, Zhan P, Adams KL. Comparative analysis of structural diversity and sequence evolution in plant mitochondrial genes transferred to the nucleus. Mol Biol Evol 2009;26:875–91. http://dx.doi.org/10.1093/molbev/msp011.
[17] Atlan A, Couvet D. A model simulating the dynamics of plant mitochondrial genomes. Genetics 1993;135:213–22.
[18] Nishizawa S, Kubo T, Mikami T. Variable number of tandem repeat loci in the mitochondrial genomes of beets. Curr Genet 2000;37:34–8. http://dx.doi.org/10.1007/s002940050005.
[19] Lupiski JR, Weinstock GM. Short, interspersed repetitive DNA sequences in prokaryotic genomes. J Bacteriol 1992;174:4525–9.
[20] Ashutosh, Dwivedi KK, Kumar VD, Prakash S, Bhat SR. rep-PCR helps to distinguish different alloplasmic cytoplasmic male sterile lines of *Brassica juncea*. Plant Sci 2005;168:1083–7. http://dx.doi.org/10.1016/j.plantsci.2004.12.005.
[21] Viswanathaswamy DK, Nizampatnam NR. Rep-PCR identifies both inter- and intra-specific mitochondrial genome differences in *Carthamus*. Plant Mol Biol Rep 2013;31:1150–6. http://dx.doi.org/10.1007/s11105-013-0580-5.
[22] Kesari V, Sathyanarayana VM, Parida A, Rangan L. Molecular marker-based characterization in candidate plus trees of *Pongamia pinnata*, a potential biodiesel legume. AoB Plants 2010;10. http://dx.doi.org/10.1093/aobpla/plq017.
[23] Powell W, Morgante M, Andre C, Hanafey M, Vogel J, Tingey S, et al. The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. Mol Breed 1996;2:225–38. http://dx.doi.org/10.1007/BF00564200.
[24] Rohlf J. NTSYS-pc: Numerical Taxonomy and Multivariate Analysis System, Version 2.2. Setauket, New York: Exeter Software; 2012.