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RESEARCH ARTICLE

Molecular phylogenetic analysis of key *Jatropha* species inferred from nrDNA ITS and chloroplast (*trn*L-F and *rbc*L) sequences

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Abstract The genus *Jatropha* (Euphorbiaceae) contains species that are of significant economic and ornamental value. However, *Jatropha* breeding material is rather limited due to incomplete information regarding phylogenetic relationships among germplasm resources. Phylogenetic analyses were performed based on the internal transcribed spacer of nuclear ribosomal DNA (nrDNA ITS), two chloroplast regions (*trn*L-F and *rbc*L), and the combined (ITS+*trn*L-F+*rbc*L) dataset among twenty-five specimens representing six key *Jatropha* species. Phylogenetic relationships of *Jatropha* were well resolved between subgenus *Curcas* and subgenus *Jatropha*, and demonstrated the intermediate position of section *Polymorphae* among sections of both subgenera. *Jatropha curcas* and *J. integerrima* demonstrated a close phylogenetic relationship. The molecular data agreed with the morphological classification that recognized *J. multifida* and *J. podagrica* in sec. *Peltatae*. The distinct intraspecific divergence that occurred in *J. curcas* could be attributed to restricted gene flow caused by geographical isolation and different ecological conditions. Phylograms produced with *trn*L-F and *rbc*L sequence data suggested slow rates of sequence divergence among *Jatropha* spp., while the ITS gene tree had good resolution suggesting high genetic variation of ITS among *Jatropha* species.

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Introduction

Jatropha L. (Euphorbiaceae) is morphologically diverse and geographically widespread, with about 175 species (Dehgan and Webster 1979; Dehgan 1984). *Jatropha* species are separated into two subgenera: *Curcas* and *Jatropha*. Subgenus *Curcas* contains sect. *Curcas* and sect. *Platyphyllae*, while subgenus *Jatropha* contains sect. *Jatropha*, sect. *Peltatae* and sect. *Polymorphae*, to accommodate the Old and New World species according to their morphological characters and cytological studies (Dehgan and Webster 1979; Dehgan 1984; Hemming and Radcliffe-Smith 1987; Olowokudejo 1993).

Jatropha species are morphologically diverse and can be trees, shrubs, tuberous perennial herbs, geophytes, and facultative annuals. They are widely distributed in tropical and sub-tropical regions of America, Africa, and Asia (Dehgan 1984). *Jatropha curcas* was postulated to be the most primitive form of *Jatropha* based on morphological and anatomical grounds, and species in other sections are thought to have evolved from *J. curcas* (Physic nut) or another ancestral form, with changes in growth habit and flower structures (Dehgan and Webster 1979; Dehgan and Schutzman 1994). *J. curcas* is native to Central America, and was introduced for cultivation into tropical and sub-tropical regions of South America, Asia, and Africa where it was grown as a hedge crop (Heller 1996). *J. curcas* is a potential high quality biodiesel crop, which has increased interest in this plant in recent years, but narrow genetic base and low productivity limit its usefulness (King et al. 2009; Zhang et al. 2011; Pamidimarri and Reddy 2014; Edrisi et al. 2015).

The genus *Jatropha* has many potential species while their genetic diversity and phylogenetic relationships are still indefinite. Phenotypic variation for fatty acid profiles, photoperiod sensitivity, and flowering and fruiting pattern have been reported in different *Jatropha* species (Banerji et al. 1985; Sujatha 1996). Determination of genetic relationships among *Jatropha* species is critical for the improvement of genetic resources and interspecific hybridization (Sujatha et al. 2013). Molecular markers have been utilized to assess genetic diversity and deduce phylogenetic relationships in *Jatropha* species (Ram et al. 2008; Basha and Sujatha 2009; Kumar et al. 2009; Pamidimarri et al. 2009; Sudheer et al. 2011). However, as a recognized biofuel crop with a huge potential economic value, *Jatropha* breeding material is rather limited due to low or incomplete information about germplasm resources. Therefore, it is necessary to increase the demonstration and understanding of interspecific and intraspecific variability and phylogenetic relationships of *Jatropha*.

Currently, phylogenetic relationships and intraspecific divergence analyses of *Jatropha* using chloroplast and nuclear DNA data have rarely been reported. In this study, the internal transcribed spacer of nuclear ribosomal DNA (nrDNA ITS) and the chloroplast regions *trn*L-F and *rbc*L were used to reconstruct the phylogeny of six key *Jatropha* species. The objectives of this study were to evaluate infrageneric relationships of *Jatropha*, assess and determine the phylogenic relationship of *Jatropha* species, and reveal the genetic divergence among intraspecies of *J. curcas*.

Materials and methods

Plant materials

Twenty-five specimens representing six *Jatropha* species were collected from the Tropical Eco-agriculture Institute, Yunnan Academy of Agricultural Sciences and the South China Botanical Garden, Chinese Academy of Sciences, including twenty accessions of *J. curcas*, and one accession of the following: *J. gossypiifolia*, *J. integerrima*, *J. multifida*, *J. podagrica*, and *J. platyphylla*. *Croton draco* was included as an outgroup species based on previous research (Tokuoka and Tobe 2006). The taxa names and numbers, GenBank accession numbers, and collection information are listed in Table 1.

DNA extraction and sequencing

The fresh leaf tissues were collected and quickly frozen in liquid nitrogen in May 2014. The total genomic DNA was extracted with a Plant Genomic DNA Kit (Tiangen Biotech, Beijing, China). The primer sequences used for PCR amplification and sequencing were nrDNA ITS and chloroplast *trn*L-F and *rbc*L (Table 2). The PCR was conducted in a 50 μL mixture reaction volume containing 5.0 μL 10 × Taq Buffer, 1 μL dNTP Mix (10 mM each), 0.5 μL Taq DNA Polymerase (5 U/μL), 3.0 μL 25 mM $MgCl_2$, 2.0 μL of each primer (10 μM), 2.0 μL of template genomic DNA (2.5 ng/μL), and with an addition of ddH_2O to the final volume (Vazyme Biotech, Nanijing, China). The PCR amplification was performed using the following protocol: an initial pre-denaturing at 95 °C for 4 min, followed by 35 cycles for 1 min denaturing at 94 °C, 1 min annealing at 56 °C, 1 min for primer elongation at 72 °C, and a final extension step at 72 °C for 10 min on BIO-RAD S1000^{TM} Thermal cycler. The *trn*L-F and *rbc*L genes were amplified following the same conditions as for the ITS region, except the annealing temperature was different (Table 2). The amplified products were checked by electrophoresis in 1.5 % agarose gel stained with Gold view and subjected to purification for further sequencing. The sequencing of gene fragments was conducted by BGI China.

Data analysis

The obtained sequences were assembled initially using the SeqMan package (DNASTAR, Inc., Madison, WI, USA) and confirmed through BLAST nucleotide alignment on the NCBI database. Multiple sequences were aligned using the Clustal W program, and refined by manual adjustment with MegAlign (Thompson et al. 2002). The homogeneity of the base composition was detected for Id-test, nucleotide substitutions, transition/transversion ratio, and variability in different taxa and was calculated with MEGA version 6.0 (Tamura et al. 2013). Nucleotide diversity was estimated for haplotype diversity by Hd (Nei and Li 1979), Tajima's π (Tajima 1989), and Watterson's θw (Watterson 1975) and gaps in the alignments were treated as missing data and indels were not coded. Neutrality testing was performed by Tajima 's D and Fu and Li 's D statistics (Fu and Li 1993), using the software DnaSP version 5.10 (Rozas et al. 2003).

Models for Bayesian inference (BI) analyses of nrDNA, cpDNA, and combined datasets were selected using MrModel test (Nylander 2004). Bayesian analysis was performed using MrBayes version 3.1.2 (Ronquist and Huelsenbeck 2003). The GTR+G model, F81+I model, and HKY+I+G model were identified as optimal models for nrDNA ITS, cpDNA, and the combined dataset analyses, respectively. Four MCMC (Markov chain Monte Carlo) chains (one cold and three hot) were run for 1,000,000 generations and two simultaneous analyses were performed and trees were sampled every 100 generations. The first 5000 trees were discarded as burn-in and the

Table 1 The source of genes and resources of *Jatropha* used in this study

Species	Abbr.	ITS No.	*trn*L-F No.	*rbc*L No.	Location
J. curcas	JCC03	KP190942	KP868726	KP898363	Zhenfeng, Guizhou, China
J. curcas	JCC04	KP190943	KP868727	KP898364	Ceheng, Guizhou, China
J. curcas	JCC17	KP190956	KP868728	KP898365	Haikou, Hainan, China
J. curcas	JCC18	KP190957	KP868729	KP898366	Jianfeng, Hainan, China
J. curcas	JCC20	KP190959	KP868730	KP898367	Lazha, Sichuan, China
J. curcas	JCC22	KP190961	KP868731	KP898368	Miyi, Sichuan, China
J. curcas	JCC23	KP191042	KP868732	KP898369	Lazha, Sichuan, China
J. curcas	JCC33	KP190971	KP868733	KP898370	Pingguo, Guangxi, China
J.curcas	JCC38	KP190976	KP868734	KP898371	Nanning, Guangxi, China
J. curcas	JCC44	KP190982	KP868735	KP898372	Honghe, Yunnan, China
J. curcas	JCC51	KP190989	KP868736	KP898373	Jingdong, Yunnan, China
J. curcas	JCC59	KP190997	KP868737	KP898374	Yuanmou, Yunnan, China
J. curcas	JCC60	KP190998	KP868738	KP898375	Yongping, Yunnan, China
J. curcas	JCE15	KP191024	KP868714	KP898351	India
J. curcas	JCE20	KP191029	KP868715	KP898352	Backan, Vietnam
J. curcas	JCE22	KP191031	KP868716	KP898353	Phutho, Vietnam
J. curcas	JCE24	KP191033	KP868722	KP898359	Deaougou, Burkina Faso
J. curcas	JCE25	KP191034	KP868723	KP898360	Bamako, Mali
J. curcas	JCE26	KP191035	KP868724	KP898361	Taunggyi, Burma
J. curcas	JCE27	KP191036	KP868725	KP898362	Mao Ting, Burma
J. gossypiifolia	JGOSS	KP191041	KP868719	KP898356	Guangdong, China
J. gossypiifolia	JGOS1	EU340792*	–	–	
J. gossypiifolia	JGOS2	EU340793*		–	
J. gossypiifolia	JGOS3	KF500510*	–	–	India
J. gossypiifolia	JGOS4	–	–	GU441785*	
J. integerrima	JINTE	KP868739	KP868717	KP898354	Guangdong, China
J. integerrima	JINT1	–	AY794685*	AY794902*	
J. integerrima	JINT2	–	–	AB233879*	
J. integerrima	JINT3	EU340795*	–	–	
J. integerrima	JINT4	EU881729*	–	–	
J. integerrima	JINT5	EU881730*	–	–	
J. integerrima	JINT6	EU881731*	–	–	
J. multifida	JMULT	KP868741	KP868720	KP898357	Guangdong, China
J. multifida	JMUL1	EF599630*	–	–	
J. multifida	JMUL2	EU340789*	–	–	
J. podagrica	JPODA	KP868740	KP868718	KP898355	Yunnan, China
J. podagrica	JPOD1	EU881716*	–	–	
J. podagrica	JPOD2	KF500509*	–	–	India
J. podagrica	JPOD3	EU881714*	–	–	
J. podagrica	JPOD4	EU881715*	–	–	
J. podagrica	JPOD5	–	–	GQ436323*	China
J. platyphylla	JPLAT	KP868742	KP868721	KP898358	Yunnan, China
Croton draco		EF421776*	EF408114*	EF405840*	

– Not determined; * indicate accession data retrieved from GenBank

remaining trees were used to construct a 50 % majority rule consensus trees.

Phylogenetic reconstructions of ITS, *trn*L-F, *rbc*L and combined datasets were performed by maximum parsimony (MP) methods with PAUP version 4.0b10 (Swofford 2002). The MP analyses used heuristic searches with 1000 random additional sequence replicates, ten trees were held at each step, and TBR was used to swap

Table 2 Primers used for PCR and sequencing in this study

Primer	Locus	Sequence (5′–3′)	Tm (°C)	Source
ITS	ITS 4	TCCTCCGCT TAT TGA TAT GC	56	(White et al. 1990)
	ITS 5	TCC GTA GGT GAA CCT GCG G		
trnL-F	c	CGAAATCGGTAGACGCTACG	55	(Mason-Gamer et al. 2002)
	f	ATTTGAACTGGTGACACGAG		
rbcL	1F	ATGTCACCACAAACAGAAAC	53	(Fay et al. 1997)
	724R	TCGCATGTACCTGCAGTAGC		

branches. All characters were equally weighted with gaps as missing data. Maximum parsimony analyses were used to generate the 50 % majority-rule and strict consensus trees. Topological robustness was assessed by bootstrap analysis with 10,000 replicates using simple taxon addition. The incongruent length difference (ILD) test was performed to assess the combinability of the three regions as implemented in PAUP (Farris et al. 1994). The test was conducted with exclusion of invariant characters using heuristic search involving simple addition sequence and TBR branch swapping with 1000 homogeneity replicates.

Phylogenetic network reconstruction method was used to study relationships between ancestral and derived haplotypes. The media-joining (MJ) network analysis has not previously been reported to reveal relationships among *Jatropha* species based on chloroplast gene haplotypes. A maximum parsimony network was constructed following the MJ calculation based on chloroplast sequences using Network version 4.6.1.2 (Bandelt et al. 1999).

Results

Nucleotide sequences variation

We obtained the ITS, *trnL-F,* and *rbcL* sequences from 25 samples of six *Jatropha* species. The length and average G+C content of aligned sequences were as follows: 660 bp and 62.6 % for ITS, 1029 bp and 30.8 % for *trn*L-F region, and 675 bp and 42.4 % for *rbc*L, respectively. Multiple sequences were aligned and sequence characteristics of the three regions (ITS, *trn*L-F, and *rbc*L), and combined matrices are presented in Table 3. Show in Table 4 are: estimates of nucleotide polymorphisms of ITS, *trn*L-F, *rbc*L, and the combined datasets included the number of sites (n), excluded sites with gaps/missing data, the number of polymorphic sites (s), haplotype diversity (Hd), the average pairwise diversity (π), and the diversity of segregating sites (θw). Neutrality tests of Tajima's, Fu and Li's D generated negative values for *Jatropha* species.

ITS phylogenetic analyses

A 50 % majority-rule consensus tree based on ITS sequence data were inferred from the Bayesian analysis with posterior probabilities (PP). The parsimony analysis of ITS sequences data retrieved nine most parsimonious trees with 504 steps, with a consistency index (CI) of 0.8075, and retention index (RI) of 0.8971(not shown). The BI tree was congruent with the MP strict consensus tree, and more statistically well resolved than the MP strict tree. The posterior probabilities and bootstrap values (BS) are shown above branches (Fig. 1). The phylogenetic relationship was well-resolved. Thirty-eight *Jatropha* samples were distinctly divided into two groups based on ITS sequence data: subgenus *Curcas* and subgenus *Jatropha*, and they were composed of five monophyletic clades with well support values. Subgenus *Curcas* contained one clade (Clade I), which consisted of *J. curcas* and *J. platyphylla*. The population of *J. curcas* was mainly clustered into two distinct subclades. Subgenus *Jatropha* comprised four major clades: Clade II, Clade III, Clade IV, and Clade V. Clade II included four accessions of *J. integerrima*, while *J. integerrima* (JINTE) were separately divided into a subclade. Clade III consisted of four accessions of *J. gossypiifolia*. Clade IV consisted of five accessions of *J. podagrica* and one accession of *J. multifida*. Clade V included two accessions of *J. multifida*.

Phylogenetic analyses of plastid regions

The parsimony analysis resulted in one most parsimonious tree with 42 steps, with a consistency index of 0.9286 and retention index of 0.9516 (not shown). The Bayesian tree was identical to the MP strict tree, and more statistically well resolved and supported than the MP strict tree. The posterior probabilities (PP) and bootstrap values (BS) are shown above branches (Fig. 2). The *trn*L-F phylogeny was mainly congruent with the ITS phylogram except for the distribution of *J. platyphylla* and *J. integerrima*. By MP and BI analyses, the 26 *Jatropha* taxa were mainly divided into two groups: subgenus *Curcas* (PP = 93, BS = 85) and subgenus *Jatropha* (PP = 97, BS = 57). Subgenus

Table 3 The statistics from separate and combined analyses of the nuclear and two chloroplast regions

Gene	No. taxa	Aligned length	Conserved characters	Variable characters	Parsim-informative characters	ii	si	sv
ITS	39	660	321	329	245	563	36	22
trnL-F	27	1029	842	103	21	899	5	7
rbcL	30	675	658	17	5	673	1	1
Combined (nr + cp)	25	2426	1973	340	93	2224	19	21

ii Identical pairs, *si* transitionsal pairs, *sv* transversional pairs

Table 4 Estimates of nucleotide diversity and statistics test for the separate and combined datasets

Gene	n	s	π	Hd	θw	Fu and Li 's D	Tajima 's D
ITS	581	291	0.092595	0.896	0.118466	−0.39976 (P > 0.10)	−1.38084 (P > 0.10)
trnL-F	881	91	0.010607	0.792	0.026798	−4.19310 (P < 0.02)	−2.41031 (P < 0.01)
rbcL	675	17	0.002772	0.356	0.006357	−2.69385 (P < 0.05)	−1.92941 (P < 0.05)
Combined	2177	291	0.01645	0.905	0.03503	−3.25349 (P < 0.02)	−2.28406 (P < 0.01)

Curcas were divided into two clades. In Clade I, 10 accessions of *J. curcas* formed a single subclade with good support (PP = 98, BS = 62), while *J. integerrima* (JINTE) nested with the remaining *J. curcas* samples. *Jatropha integerrima* (JINT1) formed the separate lineage of Clade II. Subgenus *Jatropha* consisted of *J. multifida*, *J. platyphylla*, *J. podagrica,* and *J. gossypiifolia*. The MJ network analysis was implemented by describing the genealogical relationship among ten *Jatropha* haplotypes and recovered haplotype groupings generally corresponding to the BI and MP analyses (Fig. 3). The H3 haplotype was composed of nine accessions of *J. curcas* and one accessions of *J. integerrima* (JINTE), eight accessions of *J. curcas* shared the H4 haplotype, while the remaining haplotypes formed a star-like radiation. The MP and BI analyses of *rbc*L resulted in a poorly resolved and weakly supported phylogenetic tree compared to *trn*L-F datasets (not shown).

Combined phylogenetic analyses

The ILD test indicated that the three molecular datasets were congruent (P = 0.01). The combined dataset included 26 taxa and 2426 nucleotide sites, of which 93 characters were parsimony informative. The parsimony analysis based on the combined dataset generated 371 most parsimonious trees with 437 steps with a consistency index of 0.8719 and a retention index of 0.7128 (not shown). A 50 % majority-rule consensus tree was obtained using Bayesian analysis with posterior probabilities based on the combined data matrix of 26 taxa (Fig. 4). The Bayesian tree was identical to the MP strict consensus tree, and more statistically well resolved and supported than the MP strict consensus tree (Fig. 4). Posterior probabilities and bootstrap values are above the branches on the tree.

The combined phylogeny was mainly congruent with the ITS and *trn*L-F gene trees except for some nodes presenting different statistical support, and the distribution of *J. platyphylla* and *J. integerrima*. With the MP and BI analyses, 25 *Jatropha* taxa were divided into two groups: subgenus *Curcas* and subgenus *Jatropha*. Subgenus *Curcas* was well resolved and formed a high supported group (PP = 100, BS = 100) composed of ten accessions of *J. curcas,* which formed an independent lineage. The remaining members of *J. curcas* and *J. integerrima* were resolved as a sister clade to this clade. Subgenus *Jatropha* formed an well resolved group containing *J. multifida*, *J. gossypiifolia*, *J. platyphylla,* and *J. podagrica* with moderate statistic support (PP = 70, BS = 51).

Discussion

The phylogenetic relationship among *Jatropha* species based individually on ITS, *trn*L-F, and *rbc*L and the combined datasets were subjected to BI and MP phylogenetic reconstruction. The MP and BI analyses of *rbc*L gene tree (not shown) was poorly resolved and weakly supported compared to those inferred from ITS and *trn*L-F datasets.

Genetic variation analyses

The present estimates of nucleotide diversity and haplotype diversity revealed a higher diversity of the nrDNA ITS

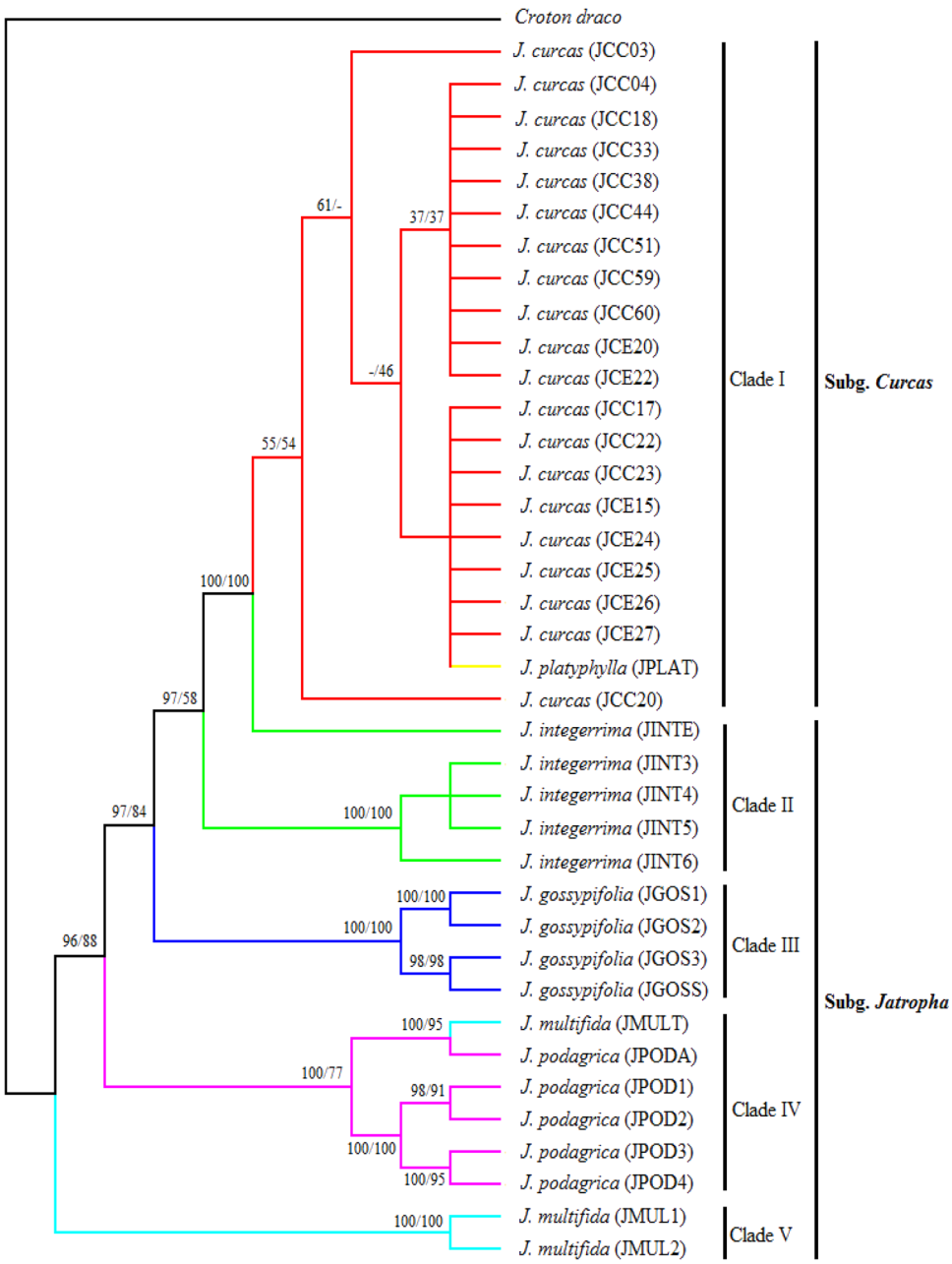

Fig. 1 Fifty percent majority rule BI tree of *Jatropha* species inferred from the ITS sequences. *Numbers* above branches are posterior probability and bootstrap values (PP/BS)

($\pi = 0.092595$, Hd = 0.896) sequences, the cpDNA *trn*L-F ($\pi = 0.010607$, Hd = 0.792) sequences, and the combined dataset ($\pi = 0.01645$, Hd = 0.905), which indicated that ITS, *trn*L-F and the combined regions had a higher evolutionary rate and could reveal genetic diversity and variation in *Jatropha* effectively. The Tajima's D and Fu and Li's D for ITS, *trn*L-F, and *rbc*L of *Jatropha* species were significantly negative estimates, which suggested that the variations deviated from neutrality. Thus, *Jatropha* species might be affected by selective elimination or suffer from a past genetic bottleneck.

Relationships within *Jatropha*

Our investigations with ITS were highly consistent with previous investigations that delimited *Jatropha* into two subgenera (*Curcas* and *Jatropha*). *Jatropha* sec. *Curcas* (*J. curcas*) and sec. *Platyphyllae* (*J. platyphylla*) are in subgenus *Curcas*, while sec. *Polymorphae* (*J. integerrima*), sec. *Jatropha* (*J. gossypiifolia*), and sec. *Peltatae* (*J. podagrica* and *J. multifida*) belong to subgenus *Jatropha* (Dehgan 1984; Hemming and Radcliffe-Smith 1987; Dehgan and Schutzman 1994; Sujatha 1996, 2006).

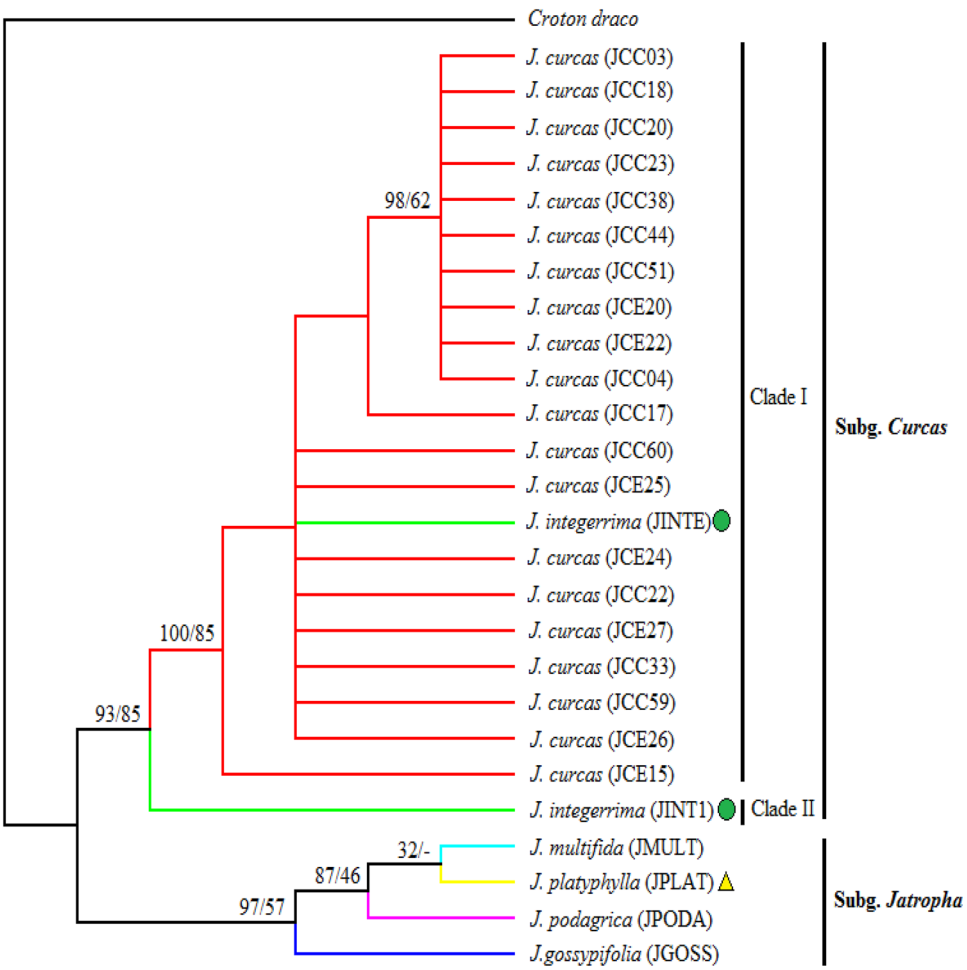

Fig. 2 Fifty percent majority rule BI tree of *Jatropha* species inferred from the *trnL-F* sequences. *Numbers* above branches are posterior probability and bootstrap values (PP/BS)

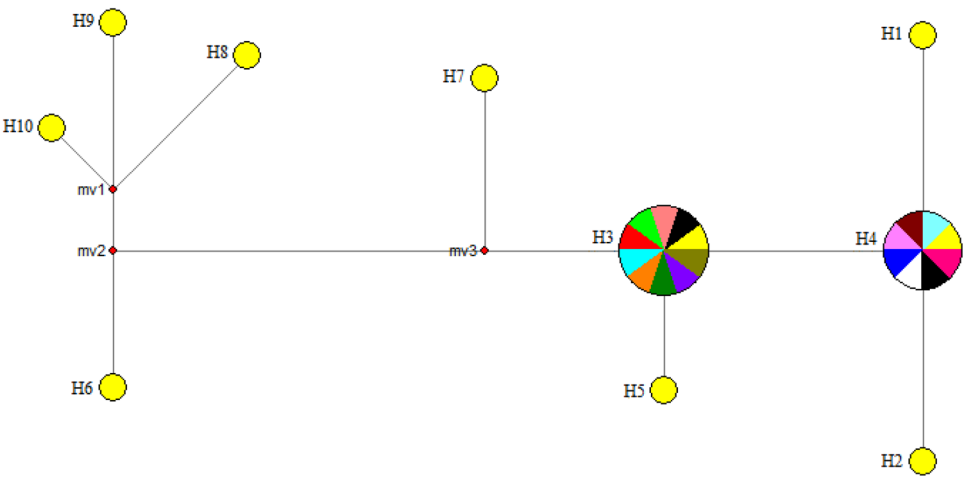

Fig. 3 Maximum parsimony median-joining network of 10 *Jatropha* Haplotypes based on trnL-trnF gene. Mv (median vectors) representing missing intermediates. Node size being proportional to the number of isolates sharing with haplotype

Phylogenetic analyses of *trn*L-F and the combined datasets mainly supported this demonstration, except for the inconsistently clustering clades of *J. platyphylla* (sec. *platyphylla*) and *J. integerrima* (sec. *Polymorphae*). ITS, *trnL-F* and the combined phylogenies showed that *J. integerrima* of sec. *Polymorphae* was clustered with *J. curcas* of sec. *Curcas*, which suggested a close relationship between sec. *Polymorphae* and sec. *Curcas*, and demonstrated the intermediate position of sec. *Polymorphae* among sections of both subgenera (Dehgan and Schutzman 1994; Sujatha 1996).

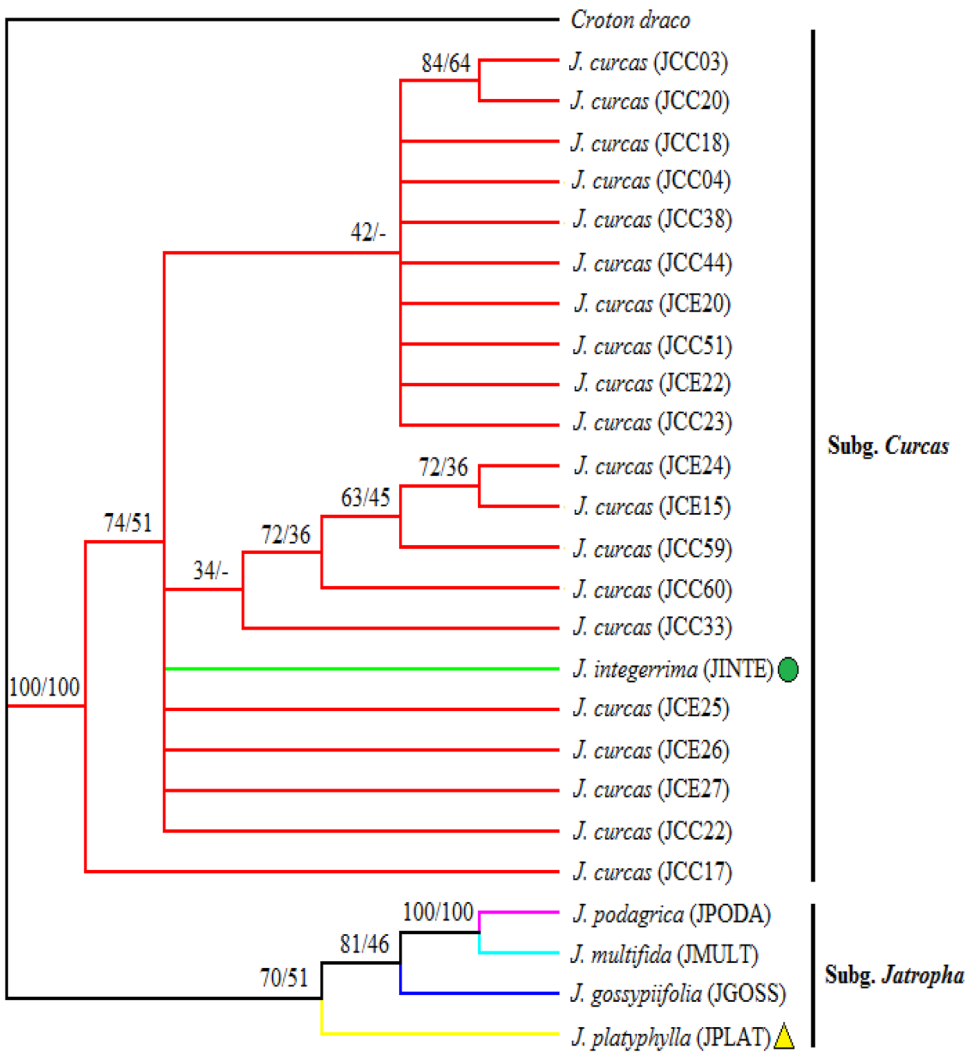

Fig. 4 Fifty percent majority rule consensus tree inferred from Bayesian analysis of the combined ITS+*trnL-F*+*rbcL* dataset. *Numbers* above branches are posterior probability and bootstrap values (PP/BS)

Phylogenetic relationship in *Jatropha* species

The interspecific relationship in some *Jatropha* species has been studied based on morphological characteristics and genome homology studies based on multilocus molecular markers (Reddy et al. 1987; Dehgan and Schutzman 1994; Sujatha and Prabakaran 2003; Sujatha 2006; Parthiban et al. 2009). This phylogenetic analyses showed that *J. integerrima* (especially was JINTE) and *J. curcas* were clustered together with robust bootstrap support, suggesting their close phylogenetic relationship and better compatibility for intersectional hybridization between *J. integerrima* and *J. curcas* (Sujatha and Prabakaran 2003; Basha and Sujatha 2009; Dhillon et al. 2009; Pamidimarri et al. 2009; Sudheer et al. 2011; Tanya et al. 2011). In the present subgenus *Jatropha* group, *J. multifida* clustered with *J. podagrica* and formed a well-supported subclade (PP = 100, BS = 100), which indicated that *J. multifida* was closely related to *J. podagrica.* This supported the morphological taxonomy, which placed *J. multifida* and *J. podagrica* into sec. *Peltatae* (Dehgan 1982; Dehgan and Schutzman 1994; Basha and Sujatha 2009; Pamidimarri et al. 2009; Sudheer et al. 2011).

At present, *J. platyphylla* has been included in few phylogenetic studies. Our data showed that *J. platyphylla* nested in the accessions of *J. curcas* in the ITS gene tree, while in phylogenies of *trn*L-F and the combined datasets *J. platyphylla* grouped with *J. podagrica*, but relationships were not the same between these trees. This inconsistency might be attributed to the different inheritance modes between nuclear and chloroplast genes. In this study, most *Jatropha* species were well resolved and monophyletic in the ITS phylogenetic analysis.

Intraspecific divergence in *J. curcas*

Genetic diversity and phylogeography revealed that the *J. curcas* accessions were geographically differentiated

(Basha et al. 2009; Pamidimarri and Reddy 2014). Our phylogenetic analyses showed that all the individuals of *J. curcas* were divided into several divergent lineages. One distinct lineage contained the main Chinese accessions, while the other lineages were composed of accessions from Burma (Myanmar), China, India, Mali, and Burkina Faso. The intraspecific divergence suggested that the Chinese accessions of *J. curcas* encountered a geographic separation in the process of propagation, speculating that the distinct geographic separation might attribute to the special dry-warm valley climate in the mountainous areas of southwestern China (Ye et al. 2009). The distinct intraspecific divergence that occurred in *J. curcas* could be attributed to restricted gene flow caused by geographic isolation and different ecological conditions.

Comprehensive genetic divergences and phylogenetic analysis using nuclear (ITS) and chloroplast (*trn*L-F and *rbc*L) data are successful in identification and phylogenetic reconstruction of the genus *Jatropha.* Phylogenetic relationships among *Jatropha* species are confirmed consistent with the morphological classification. Phylogenetic analyses suggested that ITS gene in *Jatropha* species was evolutionarily distinct and of higher discriminatory power, which could clarify lineages and phylogenetic relationships in *Jatropha*. This study also demonstrated that the ITS gene was a preferred and efficient marker with the monophyletic associations of taxa for identification at the *Jatropha* species level.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Bandelt HJ, Forster P, Röhl A (1999) Median-joining networks for inferring intraspecific phylogenies. Mol Biol Evol 16:37–48

Banerji R, Chowdhury A, Misra G, Sudarsanan G, Verma S, Srivastava G (1985) Jatropha seed oils for energy. Biomass 8:277–282

Basha S, Sujatha M (2009) Genetic analysis of Jatropha species and interspecific hybrids of *Jatropha curcas* using nuclear and organelle specific markers. Euphytica 168:197–214

Basha S, Francis G, Makkar H, Becker K, Sujatha M (2009) A comparative study of biochemical traits and molecular markers for assessment of genetic relationships between *Jatropha curcas* L. germplasm from different countries. Plant Sci 176:812–823

Dehgan B (1982) Comparative anatomy of the petiole and infrageneric relationships in Jatropha (Euphorbiaceae). Am J Bot 69:1283–1295

Dehgan B (1984) Phylogenetic significance of interspecific hybridization in Jatropha (Euphorbiaceae). Syst Bot 9:467–478

Dehgan B, Schutzman B (1994) Contributions toward a monograph of neotropical Jatropha: phenetic and phylogenetic analyses. Ann Mo Bot Gard 81:349–367

Dehgan B, Webster GL (1979) Morphology and infrageneric relationships of the genus Jatropha (Euphorbiaceae), vol 74. University of California Press, California

Dhillon R, Hooda M, Jattan M, Chawla V, Bhardwaj M, Goyal S (2009) Development and molecular characterization of interspecific hybrids of *Jatropha curcas*× *J. integerrima*. Indian J Biotechnol 8:384–390

Edrisi SA, Dubey RK, Tripathi V, Bakshi M, Srivastava P, Jamil S, Singh H, Singh N, Abhilash P (2015) *Jatropha curcas* L.: a crucified plant waiting for resurgence. Renew Sustain Energy Rev 41:855–862

Farris JS, Källersjö M, Kluge AG, Bult C (1994) Testing significance of incongruence. Cladistics 10:315–319

Fay MF, Swensen SM, Chase MW (1997) Taxonomic affinities of Medusagyne oppositifolia (Medusagynaceae). Kew Bull 52:111–120

Fu YX, Li WH (1993) Statistical tests of neutrality of mutations. Genetics 133:693–709

Heller J (1996) Physic nut. *Jatropha curcas* L. Promoting the conservation and use of underutilized and neglected crops.1. PhD Thesis, Institute of Plant Genetic and Crop Plant Research, Gatersleben, and International Plant Genetic Resource Institute, Rome

Hemming C, Radcliffe-Smith A (1987) A revision of the Somali species of Jatropha (Euphorbiaceae). Kew Bull 42:103–122

King AJ, He W, Cuevas JA, Freudenberger M, Ramiaramanana D, Graham IA (2009) Potential of *Jatropha curcas* as a source of renewable oil and animal feed. J Exp Bot 60:2897–2905

Kumar RS, Parthiban K, Rao MG (2009) Molecular characterization of Jatropha genetic resources through inter-simple sequence repeat (ISSR) markers. Mol Biol Rep 36:1951–1956

Mason-Gamer RJ, Orme NL, Anderson CM (2002) Phylogenetic analysis of North American Elymus and the monogenomic Triticeae (Poaceae) using three chloroplast DNA data sets. Genome 45:991–1002

Nei M, Li WH (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc Natl Acad Sci 76:5269–5273

Nylander J (2004) MrModeltest v2.2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University, Uppsala

Olowokudejo J (1993) Comparative epidermal morphology of West African species of Jatropha L. (Euphorbiaceae). Bot J Linn Soc 111:139–154

Pamidimarri DS, Reddy MP (2014) Phylogeography and molecular diversity analysis of *Jatropha curcas* L. and the dispersal route revealed by RAPD, AFLP and nrDNA-ITS analysis. Mol Biol Rep 41:3225–3234

Pamidimarri DS, Chattopadhyay B, Reddy MP (2009) Genetic divergence and phylogenetic analysis of genus Jatropha based on nuclear ribosomal DNA ITS sequence. Mol Biol Rep 36:1929–1935

Parthiban K, Kumar RS, Thiyagarajan P, Subbulakshmi V, Vennila S, Rao MG (2009) Hybrid progenies in Jatropha-a new development. Curr Sci 96:815–823

Ram SG, Parthiban K, Kumar RS, Thiruvengadam V, Paramathma M (2008) Genetic diversity among Jatropha species as revealed by RAPD markers. Genet Resour Crop Evol 55:803–809

Reddy K, Swamy N, Bahadur B (1987) Cross-incompatibility between Ricinus and Jatropha. Plant Cell Incompat Newslett 19:60–65

Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574

Rozas J, Sánchez-DelBarrio JC, Messeguer X, Rozas R (2003) DnaSP, DNA polymorphism analyses by the coalescent and other methods. Bioinformatics 19:2496–2497

Sudheer PD, Mastan SG, Rahman H, Prakash CR, Singh S, Reddy MP (2011) Cross species amplification ability of novel microsatellites isolated from *Jatropha curcas* and genetic relationship with sister taxa. Mol Biol Rep 38:1383–1388

Sujatha M (1996) Genetic and tissue culture studies in castor (*Ricinus communis* L.) and related genera. Ph.D. thesis, Osmania University, Hyderabad

Sujatha M (2006) Genetic improvement of *Jatropha curcas* L. possibilities and prospects. Indian J Agrofor 8:58–65

Sujatha M, Prabakaran A (2003) New ornamental Jatropha hybrids through interspecific hybridization. Genet Resour Crop Evol 50:75–82

Sujatha M, Bahadur B, Reddy TP (2013) Interspecific hybridization in the genus Jatropha. In: Bahadur B, Sujatha M, Carels N (eds) Jatropha, challenges for a new energy crop, vol 2. Springer, New York, pp 423–443

Swofford DL (2002) PAUP*: phylogenetic analysis using parsimony, Version 4.0b10. Sinauer Associates, Sunderland

Tajima F (1989) Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123:585–595

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 30:2725–2729

Tanya P, Taeprayoon P, Hadkam Y, Srinives P (2011) Genetic diversity among Jatropha and Jatropha-related species based on ISSR markers. Plant Mol Biol Report 29:252–264

Thompson JD, Gibson T, Higgins DG (2002) Multiple sequence alignment using ClustalW and ClustalX. Curr Protoc Bioinformatics, Chapter 2: Unit 2.3

Tokuoka T, Tobe H (2006) Phylogenetic analyses of Malpighiales using plastid and nuclear DNA sequences, with particular reference to the embryology of Euphorbiaceae sens. str. J Plant Res 119:599–616

Watterson G (1975) On the number of segregating sites in genetical models without recombination. Theor Popul Biol 7:256–276

White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR Protoc 18:315–322

Ye M, Li C, Francis G, Makkar HP (2009) Current situation and prospects of Jatropha curcas as a multipurpose tree in China. Agrofor Syst 76:487–497

Zhang Z, Liu B, Tang L, Chen F (2011) Genetic diversity and genetic relationship of *Jatropha curcas* between China and Southeast Asian revealed by amplified fragment length polymorphisms. Afr J Biotechnol 10:2825–2832