Genetic diversity analysis of *Jatropha* by Random Amplified Polymorphic DNA (RAPD)

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ABSTRACT

Genetic diversity analysis of *Jatropha* and intraspecific level of *J. curcas* were studied using RAPD technique. Six out of one hundred and seven primers produced polymorphic DNA patterns. A total of 144 bands were scored ranging at 0.25-3.0 kb. Genetic similarity was estimated by the Jaccard coefficient from NTSYSpc 2.20e version and ranged between 0.46-1.000. A dendrogram was constructed using Unweighted Pair Group Method with Arithmetic Mean (UPGMA) which divided *Jatropha* into two major clusters. The first group comprised all the 18 *J. curcas* accessions. *J. curcas* (Myanmar), *J. curcas* (Lumpang) and *J. curcas* (Chiang Mai) were grouped together with high similarity index (1.000), while *J. curcas* (Mukdahan) and *J. curcas* (India) were also clustered together. The second clade included 5 samples of *Jatropha* as *J. gossypifolia*, *J. podagrica*, *J. multifida*, *J. integerrima* (1) and *J. integerrima* (2), with the latter two clustered together and showing genetic similarity according to their morphological character.

Keywords; *Jatropha, RAPD, Genetic diversity*

INTRODUCTION

Increasing petroleum cost and fuel consumption rates have sparked a global energy crisis. In Thailand, diesel oil requirements expand at 4.5% per annum (Dhakshanamoorthy et al., 2011) with subsequent effects on economic, industrial, transportation and agricultural sectors. To rectify this situation, the Thai Government has promoted the generation of alternative energy sources as crop species (Dhakshanamoorthy et al., 2015). *Jatropha curcas*, also called purging nut or physic nut, is a perennial shrub belonging to the genus *Jatropha* and family Euphorbiaceae with around 200 species distributed throughout tropical and subtropical regions. *J. curcas* is native to Mexico and the Central Americas and the plant is cultivated in South America, Asia and Africa as a drought-resistant, salt tolerant, fast-growing crop that can produce seeds for up to 50 years. Average oil content in the seeds ranges from 30 to 50% by weight and contains approximately 80% unsaturated fatty acids (Chhetri, 2008). Diverse geographical origins showed variation in oil content (27.8 to 39.0%) and seed weight (44 to 79 g) in Indian accessions (Rao et al., 2008). Large-scale cultivation of *J. curcas* to produce biodiesel is hampered by poor availability of seeds through limited fruits, non-homogenous fruit ripening, low numbers of female flowers, reduced branching and

inadequate pollination (Ladawan Na Ayudhaya and Garivait, 2011). Previous studies on *Jatropha* focused on biotechnological techniques such as tissue culture, genetic engineering and molecular markers to reduce breeding program duration (Argollo Marques et al., 2013). Therefore, an understanding of genetic population diversity is required to address these problems.

Molecular markers are a powerful technique for investigating genetic diversity in plants as they are independent of environmental influences. Many types of molecular markers have been used to improve genetic understanding including Amplified Fragment Length Polymorphism (AFLP), Inter Simple Sequence Repeat (ISSR) and Expressed Sequence Tag (EST)-SSR. Random Amplified Polymorphic DNA (RAPD) markers are still used for inter- and intra-specific analyses because of their simplicity, low cost, identification speed and lower infrastructure requirements (Kesar and Rangan, 2011). Consequently, RAPD is a highly effective tool for investigating genetic diversity and supporting polymorphism detection in the whole genome of many plant species. *Jatropha* species have been studied using this technique to distinguish individual germplasms from different regions including India, Mexico and China (Basha and Sujatha, 2007; Pamidimarri et al., 2009; Pamidimarri et al., 2010; Chen et al., 2011). To create greater genetic diversity, mutation breeding has also been investigated regarding agronomic traits, using RAPD to determine genetic similarity and selecting mutants in *J. curcas* (Dhakshanamoorthy et al., 2011; Zainudin et al., 2014; Dhakshanamoorthy et al., 2015). The RAPD technique evaluates the grouping pattern of clustering and assesses genetic relationships among *Jatropha* species. Accurate identification of species and varieties is essential for effective selection, genetic resource management and development of further breeding programs associated with biodiesel production.

METHODOLOGY

Plant materials

Fifteen accessions of *J. curcas* (Ja1-Ja15) from different geographical areas were collected and maintained by the Agricultural Research and Development in Nakhonratchasima. Five accessions (Ja16-Ja18), *J. podagrica* (Ja20) and *J. integerrima* (Ja21) were collected from Phitsanulok Province, with two as *J. gossypifolia* (Ja19) and *J. multifida* (Ja23) from Phichit Province. *J. integerrima* (Ja22) was collected from Chiang Mai and *Ricinus communis* (Ja24) was used as an out group (Table 1).

DNA extraction

Genomic DNA was extracted from young leaves following the modified CTAB method **(**Doyle and Doyle, 1987). About 0.5 g of young leaves were ground to a fine powder in liquid nitrogen. Three milliliters of preheated (65°C) 2x CTAB isolation buffer (2% CTAB, 1.4 M NaCl, 20 mM EDTA, 100 mM Tris-HCl, pH 8.0 and 10 mM 2-mercaptoethanol) were added to the sample. The homogenate was incubated at 65°C for 1 h, inverting the tube every 15 min.

Extraction was performed using an equal volume of chloroform:isoamyl alcohol (24:1) and centrifuged at 6,000 g for 10 minute at room temperature. The aqueous phase was collected into a new 1.5 ml microcentrifuge tube. Then, 0.1 volume of 3 M sodium acetate and 2 volumes of absolute ethanol were added to precipitate the DNA and the mixture was kept at -20°C for 30 minutes before being centrifuged at 6,000 rpm for 5 minutes. The liquid phase was discarded and the white DNA pellet was washed with 500 µl of 70% ethanol. The nucleic acid pellet was air-dried and resuspended with deionized water. RNase A was added to the DNA sample at a final concentration of 10 ng/ml and incubated at 37°C for 1 h. DNA quantity and quality were detected on 0.8% agarose gel and by a UV spectrophotometer.

Screening of RAPD primers

One hundred primers were screened for RAPD analysis. PCR was performed for a total volume of 25 µl containing 20 ng of template DNA, 1x PCR buffer, 3.5 mM $MgCl_2$, 200 µM dNTP, 200 nM primers, 1.0 unit of *Taq* polymerase and sterile water. DNA amplifications were performed in a thermocycler with an initial denaturing step of 1 min at 94°C, followed by 45 cycles of 1 min at 94°C, 1 min at 36°C, 2 min at 72°C and a final extension of 5 min at 72°C. PCR products were separated using 1.5% agarose gel electrophoresis with 1x TBE buffer, and DNA bands were visualized by ethidium bromide staining.

Analysis of genetic diversity

Profiles of RAPD primers were scored as presence (1) or absence (0) for each primer. These scores were used to calculate their genetic similarity according to Nei and Li (1979). Dendrograms were constructed using a UPGMA (unweighted pair group method with arithmetic average) in NTSYSpc 2.2 (Rohlf, 2000).

Table 1. The 23 *Jatropha* accessions and collection locations.

Accession number	Species	Location	Collection site
Ja1	*Jatropha curcas*	Myanmar	a
Ja2	*Jatropha curcas*	Lampang	a
Ja3	*Jatropha curcas*	Nakhon Sawan	a
Ja4	*Jatropha curcas*	Chiang Mai	a
Ja5	*Jatropha curcas*	Khon Kaen	a
Ja6	*Jatropha curcas*	Senegal	a
Ja7	*Jatropha curcas*	Loei	a
Ja8	*Jatropha curcas*	Lamphun	a
Ja9	*Jatropha curcas*	Chiang Mai	a
Ja10	*Jatropha curcas*	Mukdahan	a
Ja11	*Jatropha curcas*	India	a
Ja12	*Jatropha curcas*	Chiang Mai	a
Ja13	*Jatropha curcas*	Lamphun	a
Ja14	*Jatropha curcas*	Laos	a
Ja15	*Jatropha curcas*	Chiang Mai	a
Ja16	*Jatropha curcas*	Phitsanulok	a
Ja17	*Jatropha curcas*	Mexico 1 (non-toxic)	b
Ja18	*Jatropha curcas*	Mexico 2 (non-toxic)	b
Ja19	*Jatropha gossypifolia*	Phichit	Phichit
Ja20	*Jatropha podagrica.*	Phitsanulok	b
Ja21	*Jatropha integerrima* (1)	Phitsanulok	b
Ja22	*Jatropha integerrima* (2)	Chiang Mai	Chiang Mai
Ja23	*Jatropha multifida*	Phichit	Phichit
Ja24	*Ricinus communis*	Phitsanulok	Phitsanulok

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RESULTS AND DISCUSSION

One hundred and seven primers were screened for genomic DNA amplification of twenty-three accessions of *Jatropha*. Only six primers presented polymorphic DNA patterns (Table 2). Each polymorphic primer showed from four (2075) to twelve (2393) bands. Total number of polymorphic bands was 46 with an average of 7.67 bands per primer. Size of the amplified fragments ranged from 250 to 3000 bp and level of polymorphism differed between accessions. Average percentage of polymorphism was 32.27% and revealed low intraspecific variation of *J. curcas* accession from Asia (Montes, 2014). According to Basha et al. (2009) studied the genetic relationships among *J. curcas* from different countries using ISSR markers that revealed a low percentage of polymorphism. RAPD analysis showed minimum polymorphism and high genetic similarity in *J. curcas* (Basha and Sujatha, 2007; Ganesh et al., 2008).

Diversity in the genus *Jatropha* was illustrated by the UPGMA method using NTSYSpc version 2.20e. Similarity index varied from 0.486-1.00 (Table 3) suggesting that *Jatropha* in Thailand (*J. curcas*, *J. gossypifolia*, *J. podagrica*, *J. integerrima* and *J. multifida*) represents a genetically diverse sample with intraspecifics of *J. curcas* ranging from 0.778-1.00. Ganesh et al. (2008) studied genetic diversity in 12 accessions of *Jatropha* using the RAPD technique with results also showing low genetic diversity. UPGMA cluster analysis based on Jaccard's similarity coefficient generated a dendrogram which illustrated that all accessions of *J. curcas* were distinguishable from other species in the genus. Similarity index revealed that *J. curcas* was closely related to *J. gossypifolia*, while Dhillon et al. (2009) and Sudheer Pamidiamarri et al. (2009) determined *J. curcas* as closely related to *J. integerrima*. Our dendrogram classified 23 accessions into two distinct clusters (Fig. 1). Group A consisted of all 18 *J. curcas* samples and formed a monophyletic group. *J. curcas* (Myanmar), *J. curcas* (Lampang) and *J. curcas* (Chiang Mai) were placed in the same subgroup in accordance with geographical origin. *J. curcas* (Mukdahan) was grouped to *J. curcas* (India) with high similarity index (1.000) but different geographical origin. *J. curcas* (Mexico 1 and Mexico 2) were the most divergent accessions. Group B consisted of 5 samples; *J. gossypifolia*, *J. podagrica*, *J. multifida*, *J. integerrima* 1 and *J. integerrima* 2 that were divided into two sub-clades. *J. integerrima* 1 and *J. integerrima* 2 formed the first sub-clade while the second comprised *J. gossypifolia*, *J. podagrica* and *J. multifida*. In agreement with morphology of pollen, four species of group B show distinct triangular pattern on their exine wall.

Table 2. RAPD polymorphic primers with numbers of monomorphic and polymorphic bands.

Primer	Total number of bands	Monomorphic bands	Polymorphic bands	% Polymor-Phism
2075	13	9	4	30.77
2391	17	10	7	41.17
2393	27	15	12	44.44
2777	32	24	8	25.00
OPA4	25	17	8	32.00
OPA18	31	24	7	20.23
Total	145	99	46	
Average	24.16	16.5	7.67	32.27

CONCLUSIONS

Results revealed genetic diversity within *Jatropha*, based on RAPD markers. A total of 107 RAPD primers were used for initial screening, only 6 primers were found to give polymorphic patterns. RAPD data were subjected to UPGMA with analysis performed using NTSYSpc version 2.20e. Our dendrogram separated 23 accessions into two major groups. Group A consisted of all accessions of *J. curcas* as a monophyletic group, while group B comprised *J. gossypifolia*, *J. podagrica*, *J. multifida*, *J. integerrima* 1 and *J. integerrima* 2. *J. curcas* accessions from Thailand and other countries revealed very low genetic variation among samples; however, RAPD markers showed potential for use in conventional breeding programs to develop high-yield cultivars.

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Table 3. Similarity indix of 23 *Jatropha* accessions.

Samples	Ja1	Ja2	Ja3	Ja4	Ja5	Ja6	Ja7	Ja8	Ja9	Ja10	Ja11	Ja12	Ja13	Ja14	Ja15	Ja16	Ja17	Ja18	Ja19	Ja20	Ja21	Ja22	Ja23	Ja24
Ja1	1.000																							
Ja2	1.000	1.000																						
Ja3	0.993	0.993	1.00																					
Ja4	1.00	1.00	0.993	1.00																				
Ja5	0.986	0.986	0.979	0.986	1.00																			
Ja6	0.993	0.993	0.986	0.993	0.979	1.00																		
Ja7	0.993	0.993	0.986	0.993	0.979	0.986	1.00																	
Ja8	0.931	0.931	0.924	0.931	0.944	0.938	0.938	1.00																
Ja9	0.958	0.958	0.965	0.958	0.958	0.951	0.965	0.917	1.00															
Ja10	0.972	0.972	0.979	0.972	0.972	0.965	0.965	0.917	0.986	1.00														
Ja11	0.951	0.951	0.958	0.951	0.951	0.944	0.958	0.924	0.979	0.979	1.00													
Ja12	0.972	0.972	0.979	0.972	0.972	0.965	0.965	0.917	0.986	1.00	0.979	1.00												
Ja13	0.944	0.944	0.951	0.944	0.944	0.938	0.938	0.903	0.958	0.972	0.965	0.972	1.00											
Ja14	0.958	0.958	0.965	0.958	0.958	0.951	0.951	0.903	0.972	0.986	0.965	0.986	0.986	1.00										
Ja15	0.979	0.979	0.972	0.979	0.979	0.972	0.972	0.924	0.979	0.993	0.972	0.993	0.965	0.979	1.00									
Ja16	0.861	0.861	0.854	0.861	0.861	0.854	0.854	0.833	0.861	0.875	0.868	0.875	0.875	0.899	0.882	1.00								
Ja17	0.875	0.875	0.868	0.875	0.875	0.868	0.868	0.833	0.875	0.889	0.882	0.889	0.889	0.889	0.896	0.944	1.00							
Ja18	0.792	0.792	0.785	0.792	0.806	0.799	0.785	0.819	0.792	0.806	0.799	0.806	0.819	0.806	0.813	0.778	0.778	1.00						
Ja19	0.563	0.563	0.556	0.563	0.576	0.569	0.556	0.618	0.549	0.563	0.569	0.563	0.576	0.563	0.569	0.590	0.590	0.660	1.00					
Ja20	0.507	0.507	0.514	0.507	0.521	0.500	0.500	0.535	0.521	0.535	0.542	0.535	0.549	0.549	0.528	0.583	0.549	0.549	0.625	1.00				
Ja21	0.549	0.549	0.542	0.549	0.549	0.556	0.542	0.549	0.535	0.549	0.542	0.549	0.563	0.563	0.556	0.569	0.590	0.563	0.583	0.625	1.00			
Ja22	0.493	0.493	0.486	0.493	0.493	0.500	0.486	0.521	0.493	0.493	0.500	0.493	0.521	0.507	0.500	0.514	0.535	0.590	0.542	0.556	0.792	1.00		
Ja23	0.507	0.507	0.500	0.507	0.521	0.500	0.500	0.521	0.521	0.521	0.514	0.521	0.535	0.535	0.528	0.590	0.604	0.590	0.611	0.667	0.569	0.583	1.00	
Ja24	0.521	0.521	0.514	0.521	0.521	0.514	0.514	0.521	0.507	0.521	0.528	0.521	0.535	0.521	0.528	0.542	0.549	0.563	0.444	0.556	0.542	0.500	0.486	1.00

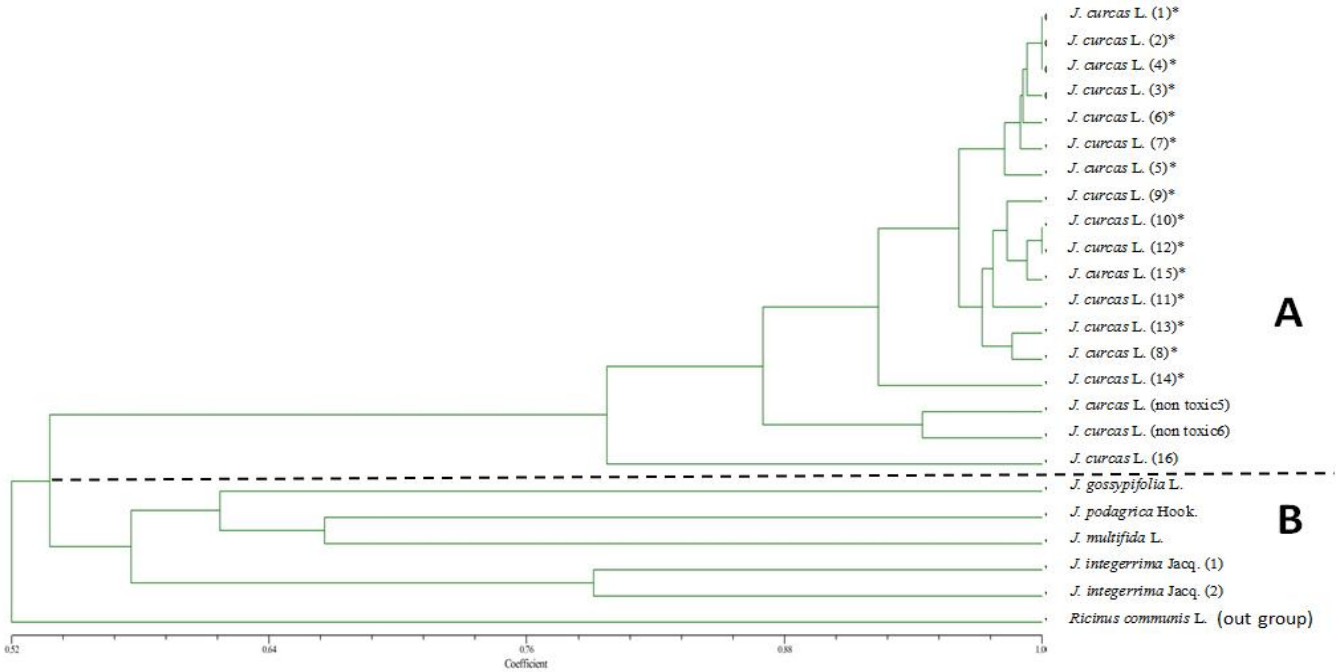

Figure 1. Dendrogram showing the relationships among the 23 *Jatropha* accessions generated from similarity indices based on UPGMA.

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