African Journal of Biotechnology Vol. 11(13), pp. 3048-3054, 14 February, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.1120
ISSN 1684–5315 © 2012 Academic Journals

Full Length Research Paper

Genetic relationships and diversity of *Jatropha curcas* accessions in Malaysia

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Accepted 21 November, 2011

This study has been undertaken to assess the extent of genetic diversity in a representative set of 16 accessions of *Jatropha curcas*. Inter-simple sequence repeat (ISSR) analysis was used to establish the genetic relationship among the accessions. From the eight ISSR primers used, the number of amplicons per primers varied from 2 (I7) to 14(UBC834) and the amplicon size from 151 bp to 2779 bp. Out of a total of 63 bands, 25 (40%) were polymorphic with an average of 4.16 polymorphic bands per primer. Jaccard's coefficient of similarity varied from 0.72 to 1, indicating low level of genetic variation among the studied genotypes. UPGMA cluster analysis indicated five main clusters with the highest number of accession grouped under cluster II. Although, the grouping was not related to location sources, there was a close genetic relationship among the *Jatropha* accessions represented. This indicated that the accessions were derived from the same source when they were introduced to Malaysia.

Key words: ISSR, genetic variation, primers, cluster analysis, biodiesel.

INTRODUCTION

Jatropha curcas L. (Euphorbiaceae), also known as 'Jarak' is an introduced plant in Malaysia which is grown for various purposes such as for its medicinal value and the oil seeds (Achten et al., 2010; Divakara et al., 2010). The oil extracted from the seed is the most valuable end product and an important source for biofuel. In recent years, due to the concerns on fossil fuel depletion, this plant has attracted many attentions of the energy producers as it has the potential to partially replace fossil fuel as biodiesel. *Jatropha* does not compete with food production and it can easily be grown under marginal soil which requires little industrial input (Sunder, 2006). There are more than 200 species of *Jatropha* which are widely distributed in the tropics and cultivated worldwide(Sujatha and Prabakaran, 1997). Only a few species can be found cultivated in Malaysia such as *Jatropha curcas*, *Jatropha gossypifolia*, *Jatropha podagrica*, *Jatropha multifida* and *Jatropha panduraefolia*.

J. curcas native to Mexico, Central and South America, and Africa but has spread to other continents of the world including Asia. However, the true centre of origin is still unclear. From the Caribbean, it was probably distributed by the Portuguese seafarers (Singh et al., 2010; Patil, 2006; Heller, 1996). Previous study reported that the highest genetic differences of *J. curcas* were found in Central America and the genetic profile in other parts of the world is very similar (Henning, 2009). *J. curcas* was believed to be introduced in Malaysia since it was under the Portuguese rule in 1511 from the Caribbean island. Jatropha also has been used as an energy source for aircraft at the time the Japanese conquered this country in year 1940 until 1945 (Bernama, 2007). It is vaguely reported about the forms of propagules when *J. curcas* was firstly introduced in Malaysia. This can be determined by using molecular marker in order to explain Jatropha was cultivated using seeds or cuttings. Natural selection also acts on random mutations in the population. Most tree species produce many more offspring and they will survive and reproduce. Over the course of many generations, those individuals who happen to possess the most favourable traits will dominate the composition of the population. As a result from natural selection,

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species has better adaptation to its environment and more successful at reproduction (Brooker, 2009). When the environment changes, there are three main effects that may happen to the population which is habitat tracking, genetic change or extinction. Of these three effects, only genetic change brings about adaptation. Genetic change occurs in a population when natural selection acts on the genetic variability of the population. Plants change their structures, behaviours and physiology in order to adapt the changing environment. If they do not adapt, they cannot survive through the changes. Various environmental factors may cause the genetic material contained in the deoxyribonucleic acid (DNA) to change, which may alter the function and structure of the DNA. Some of the environmental factors which may cause mutation are radiation, chemicals, abrupt changes in temperature, physical force, or other factors. The large majority of genetic variation within populations of a given species has its origin in mutations that occur in single genes (Hartl and Clark, 1989). Most of these single nucleotide polymorphisms are neutral but some are functional and influence phenotypic differences through alleles. For any single locus, spontaneous change from on allelic state to another that is detectably different occurs in only about 1 in 100,000 to 1 in 1000,000 of the gametes produced in a generation (White et al., 2007). Some alleles encode proteins that provide the individual with a selective advantage. Over time, natural selection may change the allele frequencies of genes and thereby lead to the fixation of beneficial alleles and elimination of detrimental alleles (Brooker, 2009). Hence, screening of some accessions is required in order to detect genetic variation. Three distinct varieties are reported which are the Cape Verde variety that has spread all over the world, the Nicaraguan variety with few but larger fruits and a non-toxic Mexican variety devoid of phorbol esters. India released the SDAUJ1 (Chatrapathi) variety from the selection of local germplasm (Basha and Sujatha, 2007). Variation in fatty acid profiles, oil content, energy content, photoperiod insensitivity, fruit size, flowering and fruiting pattern has been reported in different *Jatropha* species (Basha and Sujatha, 2009). In general, the amounts of oil content are link with the fruit size. Plant classified as *Jatropha multifida* have larger fruits and possess higher oil content (50%) as compared to *Jatropha curcas* (23 to 38%) (Basha and Sujatha, 2009). Previous studies have reported that the oil content was similar among *Jatropha curcas* in different countries. The kernel oil content for India was 58.12% (Ginwal et al., 2005), Nigeria 47.25 to 66.4% (Adebowale and Adedire, 2006; Akintayo, 2004), Nicaragua 52.9 to 57.4% (Foidl et al., 1996), Malaysia 63.16% (Emil et al., 2009) and China 51.3-61.2% (Ye et al., 2009). Jatropha can be a multipurpose oilseed crop and therefore require genetic improvement in order to alter its status to be a cultivable crop with higher yields and oil content (Ram et al., 2007). For the year 2008, the total acreage of *J. curcas* cultivated in Malaysia was 1,712 ha and the cultivation scale may increase to 57,601ha by year 2015 (Gexsi, 2008). The biggest constraint to cultivate Jatropha in Malaysia is the small number of fruits produced per inflorescence and the different ripening time of fruits on the same inflorescence. An intensive conservation program has to be carried out and the germplasm must be characterized to enhance utilization for varietal development. Assessment of genetic diversity using molecular marker is one of the major key for efficient management and conservation of plant genetic resources in gene banks.

Morphological characteristics to distinguish among accessions are minimal. Wider variation may exist among species but low variations are expected among varieties, forms and accessions. To determine this, assessment at molecular level could explain the genetic variation more accurately. There has been little analysis done on the genetic variations of *J. curcas* in Malaysia. Most of the work has been done on Jatropha originating from India (Sun et al., 2008). I In *J. curcas*, molecular markers such as RAPD, SSR, ISSR and AFLP have been employed to characterize the genetic variation and relatedness (Singh et. 2010; Gupta et al., 2008; Sun et al., 2008). From the comparative analysis reported by Gupta et al. (2008) between RAPD and ISSR markers on *J. curcas,* RAPD markers were more efficient than the ISSR with regards to polymorphism detection as they detected 84.26% as compared to 76.54% for ISSR markers. However resolving power (Rp), average bands per primer, Nei's genetic diversity (h), total genotype diversity among population (Ht), within population diversity (Hs), Shannon's Information Index(I) and gene flow (Nm) estimates were more for ISSR as compared to RAPD markers but they were not signicantly different from each other for all the parameters. Inter simple sequence repeats (ISSR) are region lying between the microsatellite repeats, have high capacity to reveal polymorphism as compared to other arbitrary primers such as RAPD (Gupta et al., 2008). ISSR markers target multiple microsatellite loci distributed across the genome while RAPD markers scan the entire genome (Basha and Sujatha, 2007). Problem of the reliability and repeatability of RAPD markers are well recognized. In this investi-gation, ISSR markers were chosen to detect the genetic relationships and diversity among the *J. curcas* accessions. The objective of this study was to detect the genetic relationships and diversity of *J. curcas* accessions in Malaysia.

MATERIALS AND METHODS

A total of 16 *J. curcas* accessions (Table 1) were used in this study. *Ricinus communis* (Euphorbiaceae) was treated as the out-group. Most of the accessions were collected from all over Malaysia with

Table 1. Geographical location of *J. curcas* accession.

S/N	Location	Accession		Latitude and longitude	Sample ID
		State	Country		
1	Kg. Bukit Tunggal, Segamat	Johor	Malaysia	(2°32'14.87"N, 102°42'39.54"E)	A
2	Taman Ehsan, Kepong	Selangor	Malaysia	(3°13'16.53"N, 101°37'59.63"E)	B
3	U.P.M,Serdang	Selangor	Malaysia	(3°00'30.68"N, 101°42'06.27"E)	C
4	Sungai Besi	Selangor	Malaysia	(3°03'77.51"N, 101°42'50.00"E)	D
5	Kg. Kejai, Kuala Nerang	Kedah	Malaysia	(6°12'48.26"N,100°34'38.81"E)	E
6	Chenor	Pahang	Malaysia	(3°28'27.58"N, 102°35'33.36"E)	F
7	Pusat Penyelidikan Tun Razak (PPTR)	Pahang	Malaysia	(3°58'25.38"N, 102° 24'16.59"E)	G
8	Felda Kota Gelanggi, Jerantut	Pahang	Malaysia	(3°52'59.32"N, 102°28'46.69"E)	H
9	Felda Tekam	-	India	(3°58'24.09"N, 102° 24'16.75"E)	I
10	Felda Tekam	Sabah	Malaysia	(3°58'25.62"N, 102° 24'16.33"E)	J
11	Pasir Puteh	Kelantan	Malaysia	(5°49'65.43"N, 102°22'23.81"E)	K
12	Merang	Terengganu	Malaysia	(5°30'41.34"N, 102°56'14.82"E)	L
13	National Tobacco Board	-	India	(5°49'64.83"N, 102°22'23.74"E)	M
14	Apin-apin	Sabah	Malaysia	(5°28'13.39"N, 116°16'12.05"E)	N
15	-	-	Brunei	(4°32'04.55"N, 114°43'39.61"E)	O
16	-	Chiang Mai	Thailand	(18°47'56.58"N, 98°39'36.21"E)	P

one accession from Brunei and Thailand. The accessions were obtained by sampling throughout the states to represent geographic regions. There were two accessions from India planted in Kelantan and Pahang which was introduced by the National Kenaf and the Tobacco Board and the Federal Land Development Authority (FELDA). These accessions were obtained from the germplasm.

Deoxyribonucleic acid (DNA) extraction and polymerase chain reaction (PCR) amplification

DNA was extracted from young leaves of each accessions using GeneAll® DNA Purification Kit (GeneAll® Biotechnology, Korea). DNA obtained was quantified using Nanodrop 200c spectro-photometer (Thermo Scientific). The ISSR primers (Table 2) used for DNA amplification in this study were repeated by kumar et al. (2009) and Basha and Sujatha, (2007). DNA amplifications were performed in a 25 µl reaction volume containing about 6 µl of template DNA, 12.5 µl of 2x Type-it Microsatellite PCR Master Mix (Qiagen, 2008), 2.5 µl of 10 x primer mix, 2 µM and 4 µl of RNase-free water. Amplification was performed in a TProfesional Standard Thermocycler (Biometra). The PCR assay was performed in 35 cycles: 1 cycle of 5 min at 95°C, followed by 34 cycles of 30 s at 95°C, 1.5 min at 60°C, 30 s at 72°C and finishing with an extension step of 10 min at 68°C. The amplification products were separated in 1.4% agarose gels. The gel was stained with 0.3 µl ethidium bromide and allele size was estimated by comparing with 100 bp DNA ladder from Research Biolabs and GeneRuler™ 1kb DNA ladder from Fermentas. DNA fragments were photographed using Gel documentation system (ChemiImager™ 5500) and stored as digital pictures.

Data analysis

The allele profiles obtained from PCR amplification were analyzed using GeneTools software to obtain the similarity of coefficient index and to generate a dendrogram. ISSR bands were used to assign loci for each primer and scored for presence (1) or absence (0). NTSYS-pc version 2.1 was used to analyze the binary data.

Table 2. Primers and their sequences used in the ISSR analysis.

S/N	Oligo name	Primer sequence (5′~3′)	Tm value	GC (%)
1	I1	(GA)9C	48.5	52.6
2	I2	(GA)9T	48.0	47.3
3	I3	(GA)9A	49.3	47.3
4	I7	(CT)8G	44.9	52.9
5	UBC812	(GA)8A	54.8	47.1
6	UBC834	(AG)8YT	56.5	47.2
7	UBC847	(CA)8RC	58.8	52.8
8	UBC 880	GGA GAG GAG AGG AGA	56.2	60.0

Table 3. Fragment size and percentage of polymorphisms of 8 primers on 16 accessions of *J. curcas*.

ISSR Primer	Fragment size range (bp)	Total band	Number of polymorphic band	Percentage (%)
I1	305-2779	7	1	14
I2	151-1629	8	4	50
I3	191-1517	6	0	0
I7	2027-2563	2	0	0
UBC812	179-1491	6	3	50
UBC834	212-2281	14	8	57
UBC847	444-2190	8	5	63
UBC880	461-2350	12	4	33

Similarity matrix from detection of polymorphic fragments was analyzed based on Jaccard's similarity coefficient using the SIMQUAL format of NTSYS-pc (Rohlf, 2002). Dendrograms were constructed using the un-weighted pair-group method with an arithmetic average (UPGMA) method (Sneath and Sokal, 1973).

RESULTS

DNA concentration and quantity

DNA quantity was determined by measuring the UV absorbance spectrum. The A_{260}/A_{280} ratio was determined. Total DNA isolated from all *J. curcas* accessions had A_{260}/A_{280} ratio of below 1.80, General extraction kit method resulted in average pure DNA with A_{260}/A_{280} ratios (1.61-1.86), indicating that the DNA had few of protein and polysaccharides contamination. The concentrations of the isolated DNA ranged from 16.9 to 78.6 indicating that small quantity of genomics DNA was isolated.

Polymorphisms detected by ISSR

The genetic variation of the 16 accessions of Jatropha and *Ricinus* sp. as an out-group, were analyzed based on Inter simple sequence repeat (ISSR) DNA markers. From eight ISSR primers used, number of amplicons per primers varied from 2(primer I7) to 14(UBC834) and the amplicon sizes varied from 151 bp to 2779 bp (Table 3). From the total of 63 bands, 25 (40%) were polymorphic with an average of 4.16 polymorphic bands per primer (Table 3). All primers gave amplification products of which 6 primers, UBC812, UBC834, UBC847, UBC880, I1 and I2 generated a polymorphic banding profile (Table 3). Figure 1 shows the polymorphic banding profile generated using six primers UBC812, UBC834, UBC847, UBC880, I1 and I2 across all the 17 samples.

Genetic relationships and diversity

The matrix of similarity coefficients was used as a basis to cluster the samples in the form of a dendrogram. The Jaccard's coefficient of similarity values ranged from 0.72 to 1.00. The similarity was found to be the lowest (0.72) between accessions from Kelantan and Selangor. The dendrogram constructed based on ISSR marker data resolved five clusters at a threshold of 90% (Figure 2). Generally, the grouping was not related to location sources. Cluster I consisted of accession from Johor (A). Cluster II included 8 accessions, of which 3 accessions were from state of Pahang (G, H and F), 1 accession

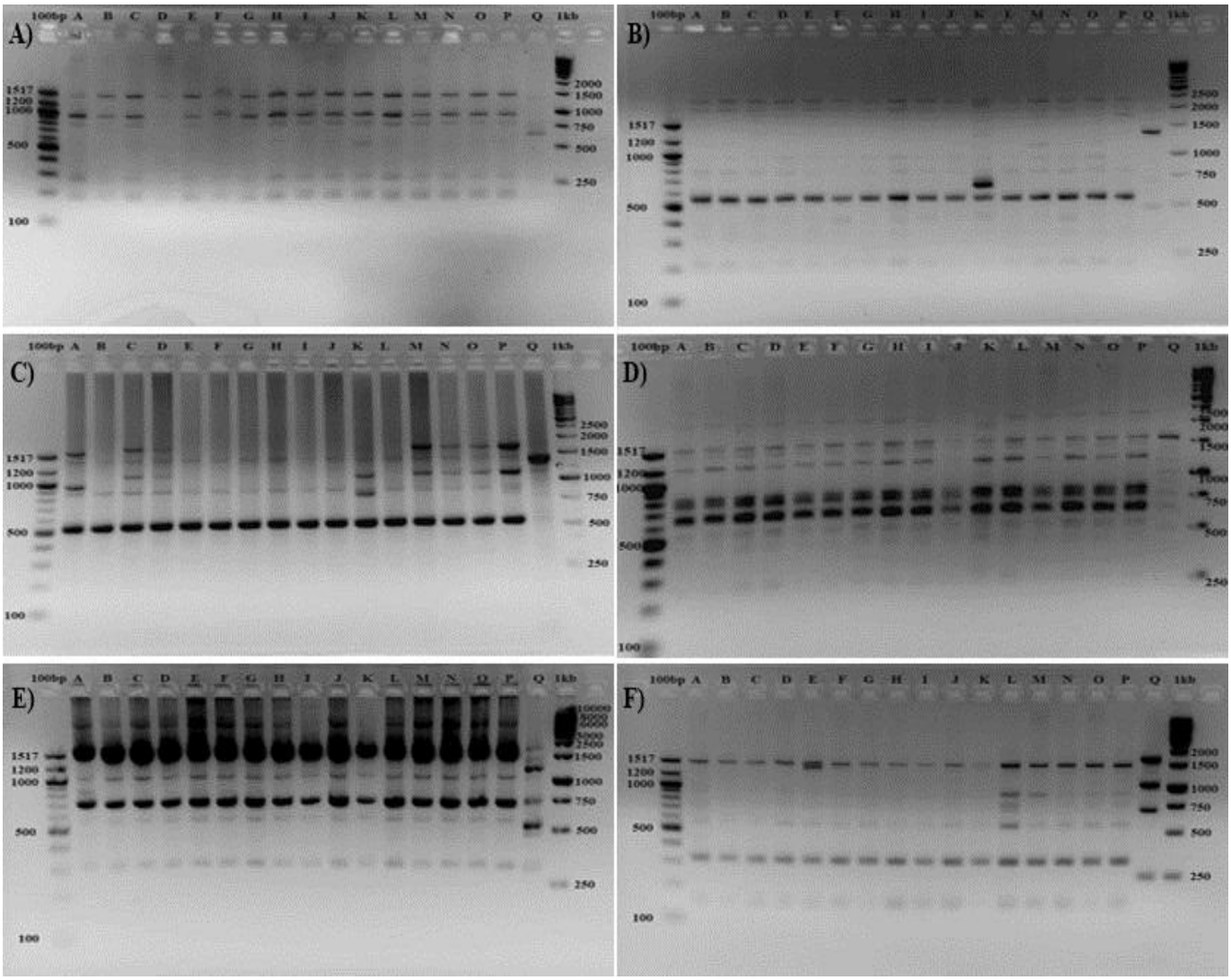

Figure 1. Polymorphic banding patterns of *J. curcas* generated using six ISSR primers (A) UBC812, (B) UBC834, (C) UBC847, (D) UBC880, (E) I1 and (F) I2 across all the 17 samples A to Q. Abbreviations: A, Johor; B, Selangor; C, Selangor; D, Selangor; E, Kedah; F-H, Pahang; I, India; J, Sabah; K, Kelantan; L, Terengganu; M, India; N, Sabah; O, Brunei; P, Thailand; Q, Out-group.

each from state of Selangor (B), Kedah (E), Terengganu (L), Sabah (J) and 1 accession from India (I). Cluster III comprised of 5 accessions which were from Selangor (C), India (M), Sabah (N), Brunei (O) and Thailand (P). Accession from Sabah (N) and Brunei (O) were genetically identical with the markers used. Cluster IV and V included accession from Selangor (D) and Kelantan (K).

DISCUSSION

Molecular marker analysis is a powerful tool for grouping genotypes based on genetic distance data. During the last three decades, classical strategies for the evaluation of genetic variability, such as comparative anatomy, morphology, and physiology have increasingly been complemented by molecular techniques (Weising et al., 2005). Wide ranges of molecular techniques are available to assess genetic variability among populations and individuals. ISSR marker is an efficient marker system in *J. curcas* because of their capacity to reveal several informative bands in a single amplification (Gupta et al., 2008). Survey of different accessions in the country is required to search for superior plants (Shirish et al., 2008). *Jatropha* can be improved through assessment of variation in wild source and selection of superior genotypes. Application of mutagen, inter-specific hybridization and genetic transformation may enable the selection of plant possessing desirable traits such as

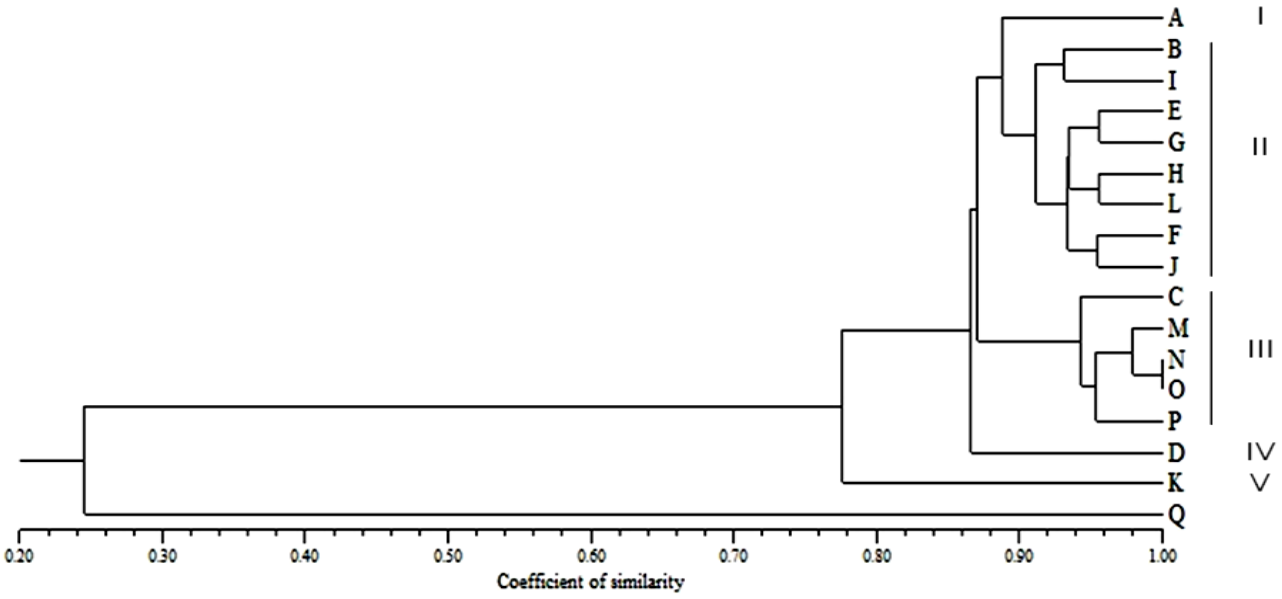

Figure 2. Dendrogram revealed by UPGMA cluster analysis on the 16 accessions of *J. curcas* based on Jaccard's similarity generated from ISSR analysis using 8 primers. Abbreviations: A, Johor; B, Selangor; C, Selangor; D, Selangor; E, Kedah; F-H, Pahang; I, India; J, Sabah; K, Kelantan; L, Terengganu; M, India; N, Sabah; O, Brunei; P, Thailand; Q, Out-group.

higher seed yield and oil content, earlier maturity, reduced plant height, resistance to pests and diseases, drought resistance or tolerance, higher ratio of female to male flowers and improved fuel properties (Divakara et al., 2010; Sujatha et al., 2008).

The study reveals the presence of a low amount of genetic diversity among the 16 *Jatropha* accessions. The Jaccard similarity matrix ranged from 0.72 to 1.00, indicating that the Malaysian *J. curcas* came from a narrow genetic base. The low degree of variation represented showed that most of these accessions were derived from the same source when they were introduced to Malaysia. The source was most likely to be Indian as two of the accessions (I and M) were from India. Recent studies based on molecular markers surprisingly uncovered low levels of genetic diversity in Jatropha landraces from China and only modest levels of diversity in India indicating that the gene pool applied at a large scale may rest on a fairly fragile genetic foundation (Achten et al., 2010). Preliminary studies using simple sequence repeat (SSR) markers within populations of *Jatropha* in its natural distribution in Mexico revealed a very low genetic variation (Achten et al., 2010; Sun et al., 2008). Pamidimarri et al. (2009), applied SSR, amplified fragment length polymorphism (AFLP) and random amplification of polymorphic DNA (RAPD) markers to discriminate between two Mexican accessions of Jatropha (one toxic and one non-toxic). Although, they could discriminate between the accessions, they found no variation between individuals within each accession. Low variation observed in molecular marker among populations of *Jatropha* might confirm to be an indication of a population structure with a high level of genetic homogeneity. The low genetic basis and vegetative propagation may also limit the genetic variation in country where it has been introduced. The narrow genetic base of *J. curcas* in India has been attributed to the small number of introduced plants and their vegetative propagation. Although, Jatropha can produce many seeds and is easy to transport and store, it was usually propagated vegetatively (Sun et al., 2008). Although, mutation is the ultimate source of genetic variation in populations and is an essential process in evolution, it is only weakly influences allele frequencies from one generation to the next. This lack of significant short term influence is because spontaneous mutation rates are very low.

Previous studies in a range of plant species for a variety of single-gene traits showed that rates of detectable mutations are typically only 10^{-5} to 10^{-6} mutations per gene per generation (White et al., 2007).

In conclusion, the Jaccard similarity matrix ranged from 0.72 to 1.00, indicating low genetic variation among the *Jatropha* accessions. This maybe mainly attributed to the fact that *J. curcas* is an introduced plant species in Malaysia and comes from the same source or due to the low number of markers used.

ACKNOWLEDGEMENTS

The authors are grateful to those who have directly and indirectly contributed to this study especially to National

Kenaf and Tobacco Board and Federal Land Development Authority (FELDA). This study was funded by MOHE under the Fundamental Research Grant Scheme (FRGS).

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