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Inter- and intra-population genetic variations in *Jatropha curcas* populations revealed by inter-simple sequence repeat molecular markers

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Abstract

Inter simple sequence reaction (ISSR) was employed to assess diversity in six Jatropha populations that were collected in Malaysia, Indonesia, the Philippines and India. A total of 144 accessions (24 accessions per population) were selected and analysed with 10 ISSR primer combinations to generate a total of 143 polymorphic fragments. The number of bands generated per primer varied from 4 to 27. The percentages of polymorphic bands for the Indonesia1, Indonesia2, Malaysia1, Malaysia2, Philippines and India Jatropha populations were 54.6, 59.4, 46.2, 53.2, 60.8 and 56.4%, respectively, with an average of 55.1%. The Nei's total genetic diversity (H_T), the intra-populations genetic diversity (H_S) and the coefficient of genetic differentiation (G_{ST}) were 0.1136, 0.0989 and 0.1295, respectively. The coefficient of genetic differentiation (G_{ST}) was 0.1295, which indicated a frequency of approximately 13% in genetic variations observed in the inter-Jatropha populations, while an 87% variation corresponded to the intra-Jatropha populations. Analysis of molecular variance (AMOVA) analysis indicated highly significant ($p \leq 0.001$) genetic differences among the six Jatropha populations. An UPGMA dendrogram was constructed, and the Jatropha populations were grouped into four major clusters at a coefficient level of 0.28. The genetic similarities between the populations ranged from 0.31 to 0.25. The principal component analysis showed a relatively similar grouping of the populations. To obtain high heterotic responses, hybridisation should be made between the two distant populations of Malaysia 1 and Indonesia1.

Keywords: Jatropha curcas, diversity, ISSR, molecular marker

Introduction

The potential of *Jatropha curcas* L as a new energy source is evident, given that the interest in biofuel production is at an all-time high. The manufacturing of biofuel potentially could position developing nations to become net exporters of fuel, which could greatly promote their goals of economic independence (Openshaw, 2000; Tatikonda et al, 2009). Jatropha is a tropical shrub plant that belongs to the Euphorbiaceae family and exists as more than 200 species that are widely distributed throughout Central America, Africa and Asia (Basha and Sujatha, 2007; Basha et al, 2009; Ambrosi et al, 2010; Singh et al, 2010). Of these species, the most significant are *J. curcas*, *J. gossypifolia*, *J. glandulifera*, *J. multifida*, and *J. podagrica*. Of this latter group, *Jatropha curcas* is one of the most significant biodiesel yielding crops (Jubera et al, 2009). *J. curcas* adapts well to different types of marginal lands, such as wastelands, and the ability of some Jatropha species to tolerate high levels of drought and pests has generated interest in the cultivation of these species (Kaushik et al, 2003; Cai et al, 2010; Kumar et al, 2011). Some researchers have described morphological diversity among the genotypes of *J. curcas* (Heller, 1996; Kaushik et al, 2007; Rao et al, 2008; Gohil and Pandya, 2008; Sunil et al, 2009; Shabanimofrad et al, 2011).

The analysis of molecular markers might help to improve Jatropha plants through the use of sustainable marker-assisted breeding (Ganesh Ram et al, 2008; Kumar and Sharma, 2008; Wang et al, 2011). DNA molecular markers provide credible information about polymorphisms, as the genetic composition is unique for each species and is not influenced by physiological conditions, age or environmental causes. The development and use of molecular markers for the discovery and exploitation of DNA polymorphisms is an important achievement in the field of molecular genetics and permits the acceleration of breeding programs. DNA-based markers can be used to assist breeding programs through Marker Assisted Selection, in which prospective varieties are selected at the seedling stage (Divakara et al, 2010; Ashkani et al, 2012). Previously, researchers used ISSR markers to understand the genetic diversity among and within *J. curcas* populations and to group plants according to genetic similarity. Tanya et al (2011) reported that the average Nei's total genetic diversity of Jatropha populations (H_T) was 0.355 and that the intra-population genetic diversity (H_S) was 0.068, while the inter-population genetic differentiation coefficient (G_{ST}) was 0.807 as a result of the low level of genetic varia-

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tion among the populations, as determined with ISSR markers. Grativol et al (2010) studied genetic diversity in different accessions of *J. curcas* from Brazil and used ISSR markers to demonstrate a high level of genetic diversity among these accessions. Additionally, Khurana-Kaul et al (2012) reported low levels of variation in J. curcas from India, as determined with ISSR markers, that was attributed to the small number of introductions and vegetative propagation.

Sudheer Pamidimarri et al (2009) reported values of 84.91 and 83.51% genetic similarity among the toxic and non-toxic genotypes of *J. curcas* by RAPD and AFLP, respectively, as well as the specific RAPD and AFLP markers for both strains. Wen et al (2010) reported the genetic variation among 45 *J. curcas* accessions, as determined with ISSR markers, and classified 45 Jatropha strains into six groups for which the genotypes correlated with the geographic origin. Liu et al (2011) and Sato et al (2011) developed ISSR markers for Jatropha and indicated a low level of genetic variation within *J. curcas*.

Subramanyam et al (2010) used RAPD markers to report a 75% polymorphism rate amongst 40 accessions that were collected from different geographical regions of India. The genetic diversity among the Jatropha species and Jatropha accessions was analysed with ISSR markers that indicated a high level of genetic variation among the genotypes (Senthil Kumar et al, 2009). Basha and Sujatha (2007) indicated high levels of genetic variation within Jatropha populations and low levels of inter-population genetic variation. To estimate the genetic diversity in seven *J. curcas* accessions, PCR-based random amplified polymorphic DNA (RAPD) markers were used by Jubera et al (2009). The genetic distance (%), based on Jaccard's similarity coefficient, ranged from 81.8-100%, which revealed a narrow range of genetic variability among the accessions, according to the RAPD markers.

In summary, this research was conducted to evaluate the level of polymorphism generated from the ISSR technique to determine the applicability of this technique to *J. curcas* population genetics studies. The ISSR technique was employed to investigate the presence of genetic variations among and within *J. curcas* populations that were collected from different countries. Ultimately, appropriate Jatropha populations or accessions could be selected and used as parental lines in future breeding programs.

Materials and Methods

Plant material

A total of 144 accessions were collected from six populations in four countries (Malaysia, the Philippines, India and Indonesia). Twenty-four accessions per population were randomly selected for the present study. Collection sites, population codes and sample sizes are shown in Table 1. All accessions were planted at the University Agriculture Park, Universiti Putra Malaysia in 2009. The experimental site is located at 3.0059N, 101.71655E and at an altitude of 88 masl.

DNA extraction

Total genomic DNA was extracted from the young and healthy leaves of each of the 144 accessions according to the CTAB method from Doyle and Doyle, with minor modifications (1990). DNA was extracted by combining 0.2 g of ground leaf tissue and 850 µl of extraction buffer (100 mM Tris-HCl, pH 8.0; 20 mM EDTA, pH 8.0; 1.4 mM NaCl; 2% (w/v) CTAB; 2% (w/v) PVP and 2-mercaptoethanol) in a 2 ml microcentrifuge tube. The leaf sample and extraction buffer were thoroughly mixed and incubated at 65°C for 60 min with agitation by hand at 15 min intervals. The samples were centrifuged at 13,000 rpm for five min to precipitate the polysaccharides, and then supernatants were transferred to a 1.5 ml micro centrifuge tube. To each incubated sample, 650 µl of chloroform:isoamyl alcohol (24:1) was added, and the samples were mixed well by gentle shaking and centrifuged at 13,000 rpm for five min. The resultant supernatant was carefully transferred to a new microcentrifuge tube.

Isolated DNA was precipitated from the aqueous phase by adding an equal volume of 4°C isopropanol at least twice; after each addition, the tubes were shaken gently, incubated at -25°C for 30 min and centrifuged at 13,000 rpm for 10 min. The precipitated DNA was washed twice with 500 µl of ice-cold 70% ethanol. The DNA pellets were air-dried and resuspended in 50 µl of TE buffer (10 mM Tris-HCl, pH 8.0; 1 mM EDTA, pH 8.0) and treated with one µl of RNase.

DNA quantification

The purity and concentration of the isolated genomic DNA were quantified with a Nanodrop spectrophotometer. The quality of the isolated genomic DNA was determined by electrophoretic separation on an 0.8% agarose gel in 1x TBE buffer.

ISSR primers

Initially, 35 primers were screened for amplifica-

Table 1 - Collection sites, population codes and numbers of samples per population for diversity analysis.

Countries of collection	Population codes	Number of accessions
Indonesia (2 populations)	Indonesia1 (In1)	24
	Indonesia2 (In2)	24
Malaysia (2 populations)	Malaysia1 (My1)	24
	Malaysia2 (My2)	24
Philippines (1 population)	Philippines1(Ph1)	24
India (1 population)	India1(Id1)	24

Table 2 - ISSR primers and sequences and the numbers and size ranges of bands observed among the Jatropha populations.

Primer	Sequence (5'-3')	NAB	NPB	Size range (bp)
ISSR1	AGAGAGAGAGAGAGAGT	24	24	150-1500
ISSR4	TGTGTGTGTGTGTGTGA	28	28	250-1600
ISSR5	AGAGAGAGAGAGAGAGYT	13	13	200-1300
ISSR16	GAGAGAGAGAGAGAGAGAT	22	22	150-1750
ISSR18	AGAGAGAGAGAGAGAGC	4	4	300-500
ISSR22	ACACACACACACACACT	23	23	250-1700
ISSR25	GAGAGAGAGAGAGAG AT	13	12	100-1000
ISSR29	GAGAGAGAGAGAGAGAC	6	6	300-850
ISSR30	DBDACACACACACACAC	5	5	450-800
ISSR33	HVHGTGTGTGTGTGTGT	5	5	700-1100
Total		144	143	

Note: Single-letter abbreviations for mixed base positions: R= (A, G), Y= (C, T), H= (A, C, T) (i.e., not G), V= (A, C, G) (i.e., not T). NAB, number of amplified bands; NPB, number of polymorphism bands.

tion efficiency with four high quality DNA samples. According to the amplification efficiencies and reproducibility rates, ten ISSR primers (Table 2) were chosen as appropriate for the evaluation of the 144 accessions from the six Jatropha curcas populations.

PCR amplification

PCR amplifications were performed in a total reaction volume of 25 µl. The PCR reactions contained 12.5 µl of 2x Type-it Multiplex PCR Master Mix, 3 µl of 5x Q-Solution (QIAGEN), 4 µl of DNA template, 2.5 µl of the ISSR primer and 3 µl of RNase-free water.

The amplification reactions were performed with an initial denaturation temperature of 95°C for 5 min, followed by 35 cycles of 95°C for 30 s, 60°C for 90 s and 72°C for 30 s, and a final single-cycle elongation step of 68°C for 10 min.

Agarose gel electrophoresis

The amplified PCR products were mixed with 2 µl of Loading Dye BLUE and were separated on a 2% agarose gel with 1× TBE buffer at 70 volts for approximately three hours. The gels were visualised under a UV light after ethidium bromide staining. An imaging system was used to save images of the gels as JPEG files.

Data scoring

The digital image files were analysed with UVI-Doc software, and the fragment sizes were estimated based on the DNA ladder mix. For all samples, only clear and non-ambiguous amplicons were scored. The alleles were designated based on the fragment sizes; for each sample, the amplified DNA fragments from the ISSR-PCR technique were scored as 1 for the presence of fragment and 0 for the absence of fragment. The agarose gel electrophoresis data were used to calculate the number of bands produced by each primer. The data were also used to calculate the percentages of polymorphic loci (P).

The resulting presence/absence data matrix was analysed with POPGENE software, version 1.3.1 (Yeh et al, 1997) to estimate the level of genetic diversity. A band presence was assumed to represent a dominant genotype, while a band absence was assumed to represent a homozygous recessive genotype. The following genetic diversity parameters, including the percentage of polymorphic bands (PPB), Shannon's information index (I) and Nei's gene diversity (H; Nei 1973), were evaluated at both the inter- and intra-population levels.

The percentage of polymorphic bands (PPB), the effective allele number (n_e), gene diversity (h), and Shannon's information index were calculated to estimate the genetic variation. The gene differentiation between populations was analysed by the coefficient of gene differentiation (G_{ST}) and the gene flow (N_m), which was estimated as $N_m = (1/4)(1- G_{ST})/G_{ST}$ (Nei, 1987) with POPGENE, version 1.3.1.

By applying the above formula, the gene flow between populations, Nm, was estimated, where N is the effective population size and m is the fraction of individuals in a population that are immigrants (Moyano et al, 2003). Using this formula, it is assumed that if $N_m < 1$, then the local populations tend to differentiate; if $N_m \geq 1$, then there will be little differentiation among the populations, and migration is more significant than genetic drift (Moyano et al, 2003). In other words, the movement of one individual per generation among the populations impedes significant differentiation between those populations, regardless of the population size. All computations were performed with POPGENE, version 1.31 (Yeh et al, 1999).

The total genetic diversity (H_T), the within-population genetic diversity (H_S), the among-populations genetic diversity (D_{ST}), and the coefficient of genetic differentiation (G_{ST}) are dependent on the expressions $H_T = H_S + D_{ST}$ and $G_{ST} = D_{ST}/H_T$. An evaluation of the overall population differentiation is achieved from the GST value. The values of G_{ST} range from 0 to 1, with $G_{ST} = 1$ revealing that the populations are fixed for different alleles. G_{ST} is equivalent to Wright's between-populations differentiation coefficient (F_{ST}; Nei, 1973).

Nei's (1973) G_{ST} statistics are explained as a ratio of the inter-subpopulation gene diversity. G_{ST} is an expansion of Wright's F_{ST} to incorporate multiple alleles, as it is a weighted mean of F_{GT} over all extant alleles (Takahata and Nei, 1984). As with F_{ST}, G_{ST} values range from 0 - 1, with a value of 0 revealing no population differentiation and a value of 1 revealing the maximum differentiation at which populations become fixed for particular alleles (Wright 1951). Both Nei's gene diversity and G_{ST} can be employed in any population without regard to the number of alleles at a locus or to the patterns of evolutionary forces (Nei, 1973).

Additionally, a nonparametric AMOVA from the GenAlEx program, version 6.2, was applied to describe the genetic structure and variability among populations. The number of permutations for significant AMOVA testing was set at 1,000 replicates. The effects of spatial separation on genetic structure were

evaluated by the Mantel test on matrices of the genetic (Nei, 1978) and geographic distances between populations. The binary data (matrix) prepared were applied for the computations of Jaccard's coefficient of genetic similarity between all possible pairs of accessions. Estimated similarity coefficient values were used to construct a dendrogram (cluster diagram) according to the method of Unweighted Pair Group with Arithmetic Averages (UPGMA), and Principal Coordinate Analysis (PCA) analysis was executed with the software package NTSYS-pc, version 2.02 (Rohlf, 2002).

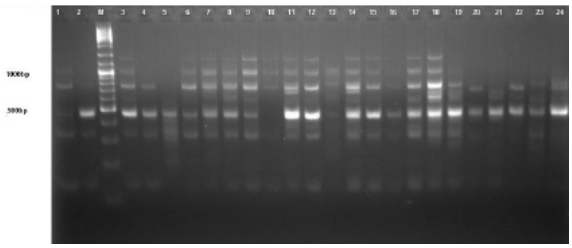

Figure 1 - Amplified products of the Indonesia1 population (In1) from primer ISSR1. The agarose gel demonstrates the DNA banding pattern for ISSR1 polymorphisms. M= molecular markers (DNA Ladder mix); 1-24 numbers of accession in the Indonesia1 population.

Results and Discussion

Primer screening and reproducibility

The primer sequences indicated that the following repeats that contained reiterated dinucleotides were more frequent in Jatropha: $(GA)_n$, $(AG)_n$, $(AC)_n$, $(CA)_n$, $(TG)_n$, and $(GT)_n$. The bands that contained different repeat nucleotides were most frequently produced with the poly (TG) primers (ISSR4) and with the poly (AG) primers (ISSR1) (Table 2). In the present investigation, the primers that were based on the poly (TG) and poly (AG) motif produced more polymorphisms on average than the primers based on any other motifs. The primer sequences that did not produce amplicons in the present investigation contained the dinucleotide repeat sequences $(AT)_n$, $(TC)_n$, $(TA)_n$, and $(CT)_n$. Similarly, Gupta et al. (2008) reported that the ISSR primers for poly $(GA)_n$ and poly $(AG)_n$ motifs produced more polymorphisms than primers for any other motif, while primers for the $(AT)_n$, $(GT)_n$ and other motifs did not produce any amplicons. A total of 144 samples were assayed for the ability to produce DNA amplicons with 10 ISSR primers. The amplified products of a representative Jatropha curcas population (In1) with primer ISSR1 are shown in Figure 1.

ISSR variation

The ten selected primers generated 143 polymorphic bands, and the sizes of the amplified products ranged from 100 to 1750 base pairs. The number of bands generated per primer varied from 4 to 27. The lowest number of bands was generated by primer ISSR18, while the the highest band was generated by primer ISSR4. Primer ISSR4 produced the highest number of polymorphic bands in all Jatropha populations, followed by ISSR1, ISSR22 and ISSR16 (Table 2). The highest number of polymorphic bands was observed in the Philippines group, followed by Indonesia2, India and Indonesia2. The individual primers produced 1 to 19 bands per population (Table 3).

Genetic diversity within Jatropha populations

The percentages of polymorphic bands for the Indonesia1, Indonesia2, Malaysia1, Malaysia2, Philippines and India Jatropha populations were 54.6, 59.4, 46.2, 53.2, 60.8 and 56.4%, respectively, with an overall average of 55.1% (Table 4). Among the Jatropha populations, the average number of alleles per locus (n_a) varied from 1.4615 (Malaysia1) to 1.6084 (Philippines), with the mean number of 1.5513. The effective number of alleles per locus (n_e) ranged from 1.1218 (Malaysia1) to 1.1706 (Malaysia2), with the mean number of 1.1453. Assuming Hardy-Weinberg equilibrium, the mean Nei's gene diversity (h) was 0.0989, with a range from 0.0816 (Malaysia1) to 0.111 (Malaysia2). The mean Shannon's Information index (I) was 0.1671, with a range from 0.1376 (Malaysia1) to 0.1779 (India).

To study the distributions of genetic variation in the Jatropha populations, Nei's (1973) gene diversity statistics were applied. Nei's (1973) total genetic diversity (H_T), the intra-populations genetic diversity (H_S), the coefficient of genetic differentiation (G_{ST}) and gene flow (N_m) were calculated to be 0.1136, 0.0989, 0.1295 and 3.3612, respectively (Table 4). The coefficient of genetic differentiation (G_{ST}) was 0.1295, thus indicating that a genetic variation rate of nearly 13% was observed in inter-Jatropha population analyses and that a genetic variation rate of 87% corresponded to intra-Jatropha population analyses. Similar re-

Table 3 - Number of polymorphic bands per *J. curcas* population from ten ISSR primers.

		Number of amplified bands per primer										
Population	N	ISSR1	ISSR4	ISSR5	ISSR16	ISSR18	ISSR22	ISSR25	ISSR29	ISSR30	ISSR33	NPB*
Indonesia1	24	18.0	18.0	5.0	8.0	1.0	13.0	8.0	2.0	3.0	3.0	79.0
Indonesia2	24	18.0	19.0	7.0	6.0	2.0	16.0	6.0	5.0	3.0	4.0	86.0
Malaysia1	24	16.0	15.0	7.0	4.0	2.0	13.0	2.0	2.0	4.0	5.0	70.0
Malaysia2	24	12.0	11.0	8.0	14.0	2.0	15.0	4.0	5.0	3.0	2.0	76.0
Philippines	24	16.0	18.0	9.0	13.0	3.0	18.0	4.0	3.0	3.0	2.0	89.0
India	24	19.0	14.0	6.0	9.0	2.0	17.0	8.0	2.0	3.0	4.0	84.0
Mean	---	16.0	16.2	7.2	9.0	2.0	15.3	4.8	3.4	3.2	3.3	80.7
Total	144	99.0	95.0	42.0	54.0	12.0	92.0	32.0	19.0	19.0	20.0	484.0

N: number of accession; NPB: number of polymorphism bands; *NPB for all 10 primers

Table 4 - Estimated genetic diversity of the Jatropha populations.

Population	Polymorphic bands (%)	Observed number of alleles (n_a)	Effective number of alleles (n_e)	Nei's (1973) gene diversity (h)	Shannon's information index (I)
Indonesia1	54.6%	1.5455±0.4997	1.1501±0.2473	0.1000±0.1447	0.1666±0.2138
Indonesia2	59.4%	1.5944±0.4927	1.1264±0.1887	0.0929±0.1181	0.1640±0.1830
Malaysia1	46.2%	1.4615±0.5003	1.1218±0.2317	0.0816±0.1334	0.1376±0.1994
Malaysia2	53.2%	1.5315±0.5008	1.1706±0.2707	0.1110±0.1538	0.1810±0.2265
Philippines	60.8%	1.6084±0.4898	1.1478±0.2282	0.1026±0.1350	0.1756±0.2015
India	56.4%	1.5664±0.4973	1.1552±0.2468	0.1053±0.1397	0.1779±0.2079
Mean	55.1%	1.5513±0.4968	1.1453±0.2356	0.0989±0.1375	0.1671±0.2054

Total gene diversity (H_T)	Average gene diversity within populations (H_S)	Coefficient of gene differentiation (G_{ST})	Estimate of gene flow (N_m)
0.1136±0.0146	0.0989±0.0105	0.1295	3.3612

sults were reported previously by Gupta et al (2008), Cai et al (2010) and Pecina-Quintero et al (2011). These results could be attributed to high levels of cross-pollination and interactions of materials from different genetic sources (Ikbal and Dhillon, 2010). The high diversity within the population, revealed in this study by molecular markers, is in agreement with the general belief that outbred plant species, such as those in the present study, always exhibit considerable diversity. Similar results were obtained by Subramanyam et al (2009). Additionally, it is generally believed that the availability and maintenance of higher genetic diversity within populations are favoured by genetic systems such as gene flow, mating systems and mutations. Therefore, the outbred nature of *J. curcas* might have promoted higher diversity within the observed populations (Ikbal and Dhillon, 2010; Phong et al, 2011; Khurana-Kaul et al, 2012).

The partitioning of genetic diversity into within-population and between-population analyses, based on Shannon's diversity index, also revealed more variation within populations (0.81) than between populations (0.19) (Supplementary Table 1).

Genetic structure is an important feature that reflects gene flow, mating systems in a population and the extent of population diversity (Song et al, 2006). Nybom and Bartish (2000) computed mean Gst values of 0.59, 0.19, and 0.23 for self-mating, mixed mating and outcrossing plants, respectively. Based on a general understanding of the association between genetic structure and breeding systems, the current study revealed that *J. curcas* is a mixed mating species because the value of G_{ST} =0.13 is near that for mixed mating species (0.19). Similar results were also obtained by Rafii et al from an analysis of *J. curcas* (2012).

AMOVA analysis

The ISSR profiles of the Jatropha populations were analysed by an analysis of molecular variance (AMOVA) to estimate the inter-population genetic variances. The inter- and intra-genetic variances in the Jatropha populations were 17% and 83%, respectively (Supplementary Table 2). Additionally, AMOVA analysis demonstrated highly significant ($p \leq 0.001$) genetic differences among the six Jatropha populations. The AMOVA results also demonstrated highly significant genetic differences among the samples within populations. Of the total genetic variation among the 144 *J. curcas* samples from six *J. curcas* populations from four countries, 83% was due to genetic differences within populations. These data indicate high genetic dissimilarities among the J. *curcas* plants sampled from a single population. Cai et al (2010) previously used AMOVA to determine inter-group genetic variances. The results demonstrated that Chinese Jatropha populations exhibited high genetic variance within geographical groups and low genetic variance among geographical groups. On the basis of this result, Cai et al (2010) assumed that each group was representative of its source population. Therefore, the differentiation between the overall population and the geographical groups truly occurred and resulted in a relatively high genetic diversity within the Chinese Jatropha populations Gupta et al (2008) reported that AMOVA analysis showed a molecular variance of 31% among populations and a variance of 69% within populations, which indicated greater within-population variance. Pecina-Quintero et al (2011) and Basha and Sujatha (2007) reported that the percentage of intra-population genetic variation was higher than that of inter-population variation. Our results are also in agreement with previous reports. Such low genetic variation among the populations of *J. curcas* could be due to the few introductions that spread across the countries, primarily through vegetative propagation, which permits the avoidance of gene exchange among the individuals in a population. Programmes launched in Brazil, Nicaragua and India to introduce *s* for varied purposes exhibited limited success due to wide gaps between the potential and actual yields. This species has not yet been improved for productivity, and most of the projects relied on naturally occurring unadapted populations that resulted from a few initial introductions, as reported by Basha and Sujatha (2007).

Cluster analysis

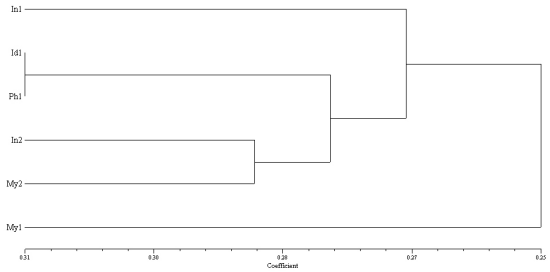

Figure 2 - Dendrogram of the *J. curcas* populations according to Jaccard's similarity matrix and based on the current ISSR data. In1= Indonesia1, In2= Indonesia2, My1= Malaysia1, My2= Malaysia 2, Ph1= Philippines1 and Id1= India1.

Based on Jaccard's similarity coefficient, the genetic variation among the Jatropha populations ranged from 0.234 to 0.313 (Supplementary Table 3). The highest genetic similarity coefficient (0.313) was computed between the groups Id1 and Ph1, and the lowest coefficient (0.234) was observed between the groups In1 and My1. The lowest genetic similarities (i.e., the highest genetic distance) were recorded between the groups In1 (Indonesia) and My1 (Malaysia). The details of Jaccard's similarity coefficient are shown in the Supplementary Table 4. Genotypes with higher genetic distances could be used as parents in hybridisations to obtain maximum heterosis. Several authors previously described similar results (Latif et al, 2011a,b; Abdullah et al, 2011).

A cluster analysis with Jaccard's similarity coefficient produced a UPGMA dendrogram (Figure 2), which clarified the overall genetic relationship between the Jatropha populations. Based on the genetic similarities, the six *J. curcas* populations were grouped into four major clusters. Cluster I consisted of one population collected from Indonesia (In1), cluster II consisted of two populations collected from the Philippines (Ph1) and India (Id1), cluster III consisted of two populations collected from Indonesia (In2) and Malaysia (My2), and cluster IV consisted of one population collected from Malaysia (My1). The UPGMA dendrogram, based on genetic similarities, indicated that samples from different geographic regions could be classified together. Sun et al (2008) reported that a high level of genetic similarity existed between two samples from Malaysia and samples from China. Grativol et al (2010) reported that the same profile of principal clusters was produced in both the UPGMA (un-weighted pair group method using arithmetic averages) phenogram and the multidimensional scaling analysis, with some variations in the subclusters. The accessions from geographically proximal locations were classified more closely in both cases.

Principal component analysis (PCA)

The principal component analysis (Supplementary Figure 1) revealed a similar grouping of the populations to that obtained from the cluster analysis. Three principal components (PCs) accounted for 63% of the total variation in the six populations of J. curcas; of these, the first three PCs exhibited variations of 39.24, 12.88 and 12.83% (Supplementary Table 5). Globally, several authors have used PCA to determine distinct variations in *J. curcas* populations (Ambrosi et al, 2010; Ikbal and Dhillon, 2010; Yi et al, 2010; Rafii et al, 2012). The analysis of eigenvectors supplied information on the features of the populations that were responsible for the distinct intra-population differences. The first principal component (PC1) data set was found to account for 39.24% of the total variation. All six populations contributed positively to the PC1. This principal component was strongly responsible for the differences between the populations.

Based on the dendrogram (Figure 2) Id1, Ph1 and My2 were grouped into clusters II or III. However, the three-dimensional PCA revealed that these three populations were closely related in one direction (Supplementary Figure 1). The distribution of the populations in the three-dimensional graph, based on the first three principal components, was similar to that obtained from the cluster analysis, in which Id1, Ph1 and My2 were clearly segregated from the other populations.

Bibiani et al (2012) reported morphological variations in six populations from Malaysia, Indonesia, the Philippines and India. The phenotypic variations, based on morphological traits of the six populations of *J. curcas*, were quite different from the genotypic variations that are based on our present ISSR data analysis of the same populations. Several authors reported that phenotypic traits are controlled significantly by non-genetic or environmental factors (Bonduriansky and Day, 2009; Wong et al, 2005; de Souza et al, 1998). The relationship between the genotype and the phenotype is not simple. Occasionally, dominant alleles are silenced by other genes that can minimise the appearance of the phenotype (Miko, 2008). The phenotype of a developing or developed organism is thought to be the result of interactions between the inherited genotype (the genetic makeup of the individual), transmitted epigenetic factors (changes in genome function that do not alter the nucleotide sequence within the DNA), and non-hereditary environmental variations.

Conclusions

The ISSR technique allowed us to estimate the genetic relationships of the *J. curcas* populations from Malaysia, Indonesia, India and the Philippines to detect high levels of polymorphism within populations and low levels of polysmorphism among populations. To improve and broaden the genetic base of *J. curcas*, populations with the lowest genetic similarities could be selected as parents. Therefore, pairs of distant populations, such as Malaysia 1 and Indonesia1, should be hybridised. The analysis of molecular variance showed that 83% of the total genetic variations were due to differences within populations, while 17% were due to genetic variation among pop-

ulations. The low intra-population genetic variation of J. curcas from Indonesia, the Philippines, India and Malaysia could be due to the lesser introductions of plant materials that were domesticated across the countries, primarily through vegetative propagation.

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References

Abdullah N, Rafii Yusop M, Ithnin M, Saleh G, Latif MA, 2011. Genetic variability of oil palm parental genotypes and performance of its' progenies as revealed by molecular markers and quantitative traits. C R Biol 334: 290-299

Ambrosi DG, Galla G, Purelli M, Barbi T, Fabbri A, Lucretti S, 2010. DNA Markers and FCSS Analyses Shed Light on the Genetic Diversity and Reproductive Strategy of *Jatropha curcas* L Diversity 2: 810-836

Ashkani S, Rafii MY, Rusli I, Sariah M, Abdullah SNA, Rahim HA, Latif MA, 2012. SSRs for marker-assisted selection for blast resistance in rice (*Oryza sativa* L) Plant Mol Biol Rep 30: 79-86

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 Science 176: 812-823

Basha S, Sujatha M, 2007. Inter and intra-population variability of *Jatropha curcas* (L) characterized by RAPD and ISSR markers and development of population-specific SCAR markers. Euphytica 156: 375-386

Biabani A, Rafii MY, Saleh GB, Shabanimofrad M, Latif MA. 2012. Phenotypic and genetic variation of *Jatropha curcas* L populations from different countries. Maydica 57: 164-174

Bonduriansky R, Day T, 2009. Non-genetic inheritance and its evolutionary implications. Ann Rev Ecol Evol Systematics 40:103-25

Cai Y, Sun D, Wu G, Peng J, 2010. ISSR-based genetic diversity of *Jatropha curcas* germplasm in China. Biomass and Bioener 34: 1739-1750

de Souza VAB, Byrne DH, Taylor JF, 1998. Heritability, Genetic and Phenotypic Correlations, and Predicted Selection Response of Quantitative Traits in Peach: II. An Analysis of Several Fruit Traits. J Am Soc Hortic Sci 123: 604-611

Divakara B, Upadhyaya H, Wani S, Gowda C, 2010. Biology and genetic improvement of *Jatropha curcas* L: A review. Applied Energy 87: 732-742

Ganesh Ram S, Parthiban K, Senthil Kumar R, Thiruvengadam V, Paramathma M, 2008. Genetic diversity among Jatropha species as revealed by RAPD markers. Genet Resour Crop Ev 55: 803-809

Gohil R, Pandya J, 2008. Genetic diversity assessment in physic nut (*Jatropha curcas* L). Int J Plant Prod 2: 321-326

Grativol C, da Fonseca Lira-Medeiros C, Hemerly AS, Ferreira PCG, 2011. High efficiency and reliability of inter-simple sequence repeats (ISSR) markers for evaluation of genetic diversity in Brazilian cultivated *Jatropha curcas* L accessions. Mol Biol Rep 38: 4245-4256

Gupta S, Srivastava M, Mishra G, Naik P, Chauhan R, Tiwari S, 2008. Analogy of ISSR and RAPD markers for comparative analysis of genetic diversity among different *Jatropha curcas* genotypes. Afr J Biotechnol 7: 4230-4243

Heller J, 1996. Physic nut *Jatropha curcas* L. Promoting the conservation and use of underutilized and neglected Crops (IPGRI), no 1. International Plant Genetic Resources Inst, Rome

Ikbal K, Dhillon R 2010. Evaluation of genetic diversity in Jatropha curcas L. using RAPD markers. Indian J Biotechnol 9: 50-57

Jubera MA, Janagoudar B, Biradar D, Ravikumar R, Koti R, Patil S, 2009. Genetic diversity analysis of elite *Jatropha curcas* (l.) genotypes using randomly amplified polymorphic DNA markers. Karnataka J Agric Sci 22: 293-295

Kaushik N, Kaushik J, Kumar S, 2003. Response of Jatropha seedlings to seed size and growing medium. J Non-Timber Forest Prod 10: 40-42

Kaushik N, Kumar K, Kumar S, Roy S, 2007. Genetic variability and divergence studies in seed traits and oil content of Jatropha (*Jatropha curcas* L) accessions. Biomass and Bioener 31: 497-502

Khurana-Kaul V, Kachhwaha S, Kothari S 2012. Characterization of genetic diversity in *Jatropha curcas* L germplasm using RAPD and ISSR markers. Indian J Biotech 11: 54-61

Kumar A, Sharma S, 2008. An evaluation of multipurpose oil seed crop for industrial uses (*Jatropha curcas* L): A review. Ind Crop Prod 28: 1-10

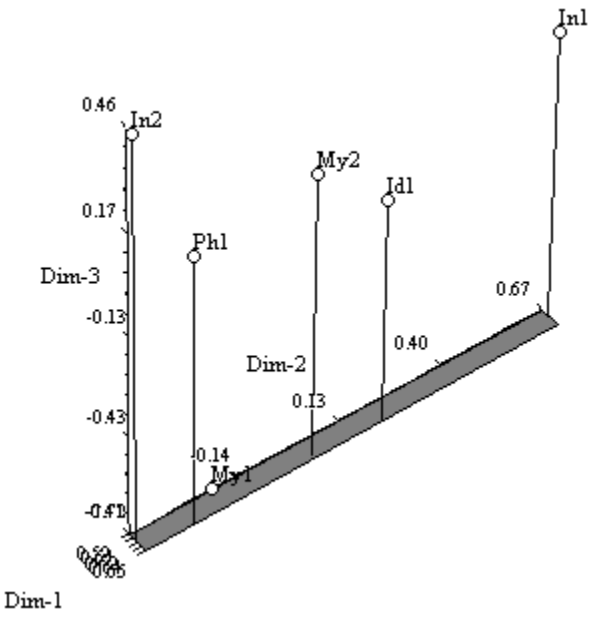
Kumar S, Kumaria S, Sharma SK, Rao SR, Tandon P, 2011. Genetic diversity assessment of *Jatropha curcas* L germplasm from Northeast India. Biomass and Bioener 35: 3063-3070

Latif MA, Rafii Yusop M, Motiur Rahman M, Bashar Talukdar M, 2011a. Microsatellite and minisatellite markers based DNA fingerprinting and genetic diversity of blast and ufra resistant genotypes. C R Biol 334:282-289

Latif MA, Rahman MM, Kabir MS, Ali MA, Islam MT, Rafii MY, 2011b. Genetic diversity analyzed by quantitative traits among rice (*Oryza sativa* L) genotypes resistant to blast. Afr J Microbiol Res 5: 4383-4391

Liu P, Wang C, Li L, Sun F, Yue G, 2011. Mapping QTLs for oil traits and eQTLs for oleosin genes in

Jatropha. BMC Plant Biol 11: 132
Miko I, 2008. Phenotype variability: penetrance and expressivity. Nat Educ 1(1)
Moyano C, Alfonso C, Gallego J, Raposo R, Melgarejo P, 2003. Comparison of RAPD and AFLP marker analysis as a means to study the genetic structure of *Botrytis cinerea* populations. Eur J Plant Pathol 109: 515-522
Nei M, 1973. Analysis of gene diversity in subdivided populations. Proc Natl Acad Sci USA 70: 3321
Nei M, 1987. Molecular evolutionary genetics: Columbia Univ Press
Openshaw K 2000. A review of Jatropha curcas: an oil plant of unfulfilled promise. Biomass and Bioener 19: 1-15
Pecina-Quintero V, Anaya-Lopez JL, Colmenero AZ, Garcia NM, Nunez Colin CA, Solis Bonilla JL, 2011. Molecular characterisation of *Jatropha curcas* L genetic resources from Chiapas, México through AFLP markers. Biomass and Bioener 35: 1897-1905
Phong D, Hien V, Thanh T, Tang D, 2011. Comparison of RAPD and ISSR markers for assessment of genetic diversity among endangered rare *Dalbergia oliveri* (Fabaceae) genotypes in Vietnam. Genet Mol Res 10: 2382-2393
Rafii MY, Shabanimofrad M, Puteri Edaroyati MW, Latif MA 2012. Analysis of the genetic diversity of physic nut, *Jatropha curcas* L accessions using RAPD markers. Mol Biol Rep 39: 6505-6511
Rao G, Korwar G, Shanker A, Ramakrishna Y 2008. Genetic associations, variability and diversity in seed characters, growth, reproductive phenology and yield in *Jatropha curcas* (L) accessions. Trees-Struct and Funct 22: 697-709
Rohlf F, 2002. NTSYS-pc: Numerical Taxonomy System, version 2.1 Exeter Publishing Ltd, Setauket, New York, USA
Sato S, Hirakawa H, Isobe S, Fukai E, Watanabe A, Kato M 2011. Sequence analysis of the genome of an oil-bearing tree, *Jatropha curcas* L. DNA Res 18: 65
Senthil Kumar R, Parthiban K, Govinda Rao M 2009. Molecular characterization of Jatropha genetic resources through inter-simple sequence repeat (ISSR) markers. Mol Biol Rep 36: 1951-1956
Shabanimofrad M, Yusop, MR, Saad MS, Megat Wahab PE, Biabanikhanehkahdani A, Latif MA, 2011. Diversity of physic nut (*Jatropha curcas*) in Malaysia: Application of DIVA-geographic information system and cluster analysis. Aust J Crop Sci 5: 361-368
Singh P, Singh S, Mishra SP, Bhatia SK, 2010. Molecular Characterization of Genetic Diversity in *Jatropha curcas* L. Genes, Genomes and Genomics, Ikenobe, Japan, 4: 1-8
Subramanyam K, Muralidhararao D, Devanna N, 2010. Genetic diversity assessment of wild and cultivated varieties of *Jatropha curcas* (L) in India by RAPD analysis. Afr J Biotechnol 8: 1900-1910
Sudheer Pamidiamarri D, Pandya N, Reddy M, Radhakrishnan T, 2009. Comparative study of interspecific genetic divergence and phylogenic analysis of genus Jatropha by RAPD and AFLP. Mol Biol Rep 36: 901-907
Sun QB, Li LF, Li Y, Wu GJ, Ge XJ, 2008. SSR and AFLP markers reveal low genetic diversity in the biofuel plant Jatropha curcas in China. Crop Sci 48: 1865-1871
Sunil N, Sivaraj N, Anitha K, Abraham B, Kumar V, Sudhir E, (2009). Analysis of diversity and distribution of *Jatropha curcas* L germplasm using Geographic Information System (DIVA-GIS). Genet Resour Crop Ev 56: 115-119
Tanya P, Taeprayoon P, Hadkam Y, Srinives P, 2011. Genetic diversity among Jatropha and Jatropha-related species based on ISSR markers. Plant Mol Biol Rep 29: 252-264
Tatikonda L, Wani S, Kannan S, Beerelli N, Sreedevi T, Hoisington D, 2009. AFLP-based molecular characterization of an elite germplasm collection of *Jatropha curcas* L, a biofuel plant. Plant Sci 176: 505-513
Wang CM, Liu P, Yi C, Gu K, Sun F, Li L, 2011. A first generation microsatellite-and SNP-based linkage map of jatropha. PloS one 6(8): e23632
Wen M, Wang H, Xia Z, Zou M, Lu C, Wang W, 2010. Developmenrt of EST-SSR and genomic-SSR markers to assess genetic diversity in *Jatropha curcas* L. BMC Res Notes 3: 42
Wong AHC, Gottesman II, Petronis A, 2005. Phenotypic differences in genetically identical organisms: the epigenetic perspective, Human Mol Genet 14(1) R11–R18
Yeh F, Yang R, Boyle T, 1999. POPGENE Microsoft Windows-Based Freeware for Population Genetic Analysis Release 1.31. Alberta, Canada University of Alberta



Note: In1= Indonesia1, In2= Indonesia2, My1= Malaysia1, My2= Malaysia 2, Ph1= Philippines1 and Id1= India1

Supplementary Figure 1. Three-dimensional plot of the principal components from the results of the ISSR marker data among the populations of *Jatropha curcas.*

Supplementary Table 1. Partitioning of the genetic variation within and between populations according to Shannon's information index

Parameters	Means
Mean genetic variation for the populations (Hpop)	0.17
Mean genetic variation for the entire data (Hsp)	0.21
Proportion of genetic variation within populations (Hpop/Hsp)	0.81
Proportion of genetic variation between populations ((Hsp/Hpop)Hsp)	0.19

Supplementary Table 2. Molecular variations between and within the *J. curcas* populations

Source of variation	df	Sum square	Mean square	Variance component	Percentage	P- Value
Inter-populations	5	262.96	52.59	1.76	17%	<0.001
Intra-populations	138	1249.36	9.05	8.68	83%	<0.001
Total	143	1512.32		10.43		

Df- degree of freedom

Supplementary Table 3. Jaccard's similarity coefficients matrix for the six *J. curcas* populations

Population	Indonesia1	Malaysia1	India1	Philippines1	Indonesia2
Malaysia1	0.234				
India1	0.296	0.267			
Philippines1	0.251	0.265	0.313		
Indonesia2	0.244	0.235	0.263	0.294	
Malaysia 2	0.285	0.267	0.273	0.281	0.287

Supplementary Table 5. Component loading of the first three principal components for the six Jatropha populations, according to the ISSR markers

Population	First three principal components		
	PC 1	PC 2	PC 3
Variation (%)	39.24	12.88	12.8
Indonesia1	6.11	6.66	1.15
Malaysia1	5.93	-1.99	-7.22
India1	6.49	2.02	-6.63
Philippines1	6.47	-2.85	7.05
Indonesia2	6.16	-4.09	4.63
Malaysia 2	6.41	2.59	1.10

Note: PC 1, PC 2 and PC 3: The three axes represent the first three principal components of the Jatropha populations