Pertanika J. Trop. Agric. Sci. 38 (1): 127 - 142 (2015)

PERTANIKA JOURNALS

TROPICAL AGRICULTURAL SCIENCE

Journal homepage: http://www.pertanika.upm.edu.my/

Genetic Divergence and Evaluation of Yield Potential of *Jatropha curcas* Accessions Collected from Peninsular Malaysia

Arolu, I. W.[1], Rafii, M. Y.[1,2]*, Hanafi, M. M.[1], Mahmud, T. M. M.[1,2] and Askani, S.[1,3]

[1]*Institute of Tropical Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*
[2]*Department of Crop Science, Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*
[3]*Department of Agronomy and Plant Breeding, Islamic Azad University of Shahr-e-Rey Branch, Tehran, Iran*

ABSTRACT

Widening of the narrow genetic base of *Jatropha curcas* through germplasm collection, diversity study and evaluation is needed to bring about much needed improvement in its seed yield and oil content. This study was carried out to profile the divergence patterns of 45 *Jatropha curcas* from three populations (Kelantan, Selangor and Terengganu) and to evaluate their yield over a period of three years. Eleven (11) morphological traits together with ISSR markers were used in this study. The percentage of polymorphism for the ISSR markers among the three populations was very high, ranging from 90.38-100%. Shannon information index (I) and expected heterozygosity (He) were found to be highest in the Kelantan population, at 0.58 and 0.40 respectively. Genetic differentiation (Analysis of molecular variance) expressed as fixation index (0.46) revealed that variations within the population accounted for about 100% of the total variation. Interestingly, the cluster analysis based on molecular and morphological traits, as presented in the dendrogram, grouped the 45 accessions into seven and five clusters respectively. For morphological traits, variability in terms of coefficient of variation (CV) was very high, as much as 53.19 and 51 % in total number of seeds and oil yield/ha. Small differences were seen between phenotypic and genotypic coefficient of variation (≤ 10%) for the yield trait. Broad sense heritability for virtually all the yield and yield components was very high (≥ 67.8). Accessions B-01-03, D-01-06, T-01-06, B-06-02 have been identified and recommended for further evaluation under field conditions before they are chosen for future breeding programmes for seed yield and oil improvement.

ARTICLE INFO

Article history:
Received: 5 August 2014
Accepted: 27 August 2014

E-mail addresses:
wasiuarolu@yahoo.com (Arolu, I. W.),
mrafii@upm.edu.my (Rafii, M. Y.),
mmhanafi@upm.edu.my (Hanafi, M. M.),
mtmm@upm.edu.my (Mahmud, T. M. M.),
ashkani.sadegh@upm.edu.my (Askani, S.)
* Corresponding author

ISSN: 1511-3701

Keywords: AMOVA, broad sense heritability, genetic divergence, germplasm collection, ISSR marker, *Jatropha curcas*

INTRODUCTION

With the increasing rise in global warming, efforts have been intensified to bring down emissions of harmful gases into the environment to an acceptable level (Banković-Ilić *et al*., 2012). Various approaches have been devised to implement this. Such approaches include the use of renewable energy sources (green fuel from seed oil), reduction in burning of fossil fuels, tree planting and other environmentally sustainable practices (Kristensen *et al*., 2011). In an attempt to identify environment-friendly and economically-feasible alternative energy feed stocks, *Jatropha curcas* has been chosen, alongside other oil producing crops. The choice of *Jatropha curcas* was due to its high non-edible seed oil production, fast-growing nature and the low-input requirements for its cultivation (Henning, 2004; Chauhan *et al*., 2012).

Jatropha curcas is a medium-to-tall oil producing tree crop from the euphorbiaceae family. It is able to tolerate drought and grows very well on marginal soil. It also thrives very well with little or no agronomical input. It is widely used as an agroforestry plant for live fences and land reclamation in developing countries. The leaves are used as mulch by farmers while its latex is of great dermatological and ethno-botanical importance. The seed oil content varies, ranging from 30-45 % by weight depending on the genotypes or varieties. The non-toxic *Jatropha curcas* seeds are incorporated into animal feed as a protein source due to its high crude protein content of about 50-60% (Tanya *et al*., 2011).

Despite the great potential of *Jatropha curcas*, the in-depth agronomical requirements necessary for successful and profitable commercial cultivation are yet to be standardised. Similarly, the genetic diversity structures and patterns required for breeding and production of high yielding cultivars are yet to be identified. Morphological alongside ISSR markers have been widely employed in genetic diversity studies for different oil producing tree crops such as coconut, olive, canola plants etc. This is a simple and low-cost method of studying the genetic structure, divergence and variance components in a breeding population (Sunil *et al*., 2011). It gives breeders the opportunity to identify individual plants with high potential in a breeding population.

This research therefore aims to achieve the following objectives: (i) to evaluate the yield performance and genetic divergence existing in the 45 accessions of *Jatropha curcas* collected from three states in Peninsular Malaysia; (ii) to estimate the variance components and broad sense heritability; and iii) to calculate the principal component and carry out cluster analyses with the purpose of identifying high yielding *Jatropha curcas* accessions from the germplasm.

MATERIALS AND METHODS

The seeds and cuttings of 45 accessions of *Jatropha curcas* were collected from three states in Peninsular Malaysia viz. Kelantan, Terengganu and Selangor, and planted in Universiti Putra Malaysia's experimental plot for field evaluation. The experimental plot is in a tropical environment with high humidity and sunshine and receiving about 2500 mm of rainfall annually. The site contains a well-drained sandy-to-loamy soil with moderate pH suitable for crop growth. The cuttings were planted using 2 × 3 metre spacing in two blocks with six cuttings planted in each block to represent each accession, resulting in 270 plants per block.

DNA Extraction, ISSR Profiling and Analysis.

Samples of fresh young leaves were obtained from each of the trees representing the different accessions. The leaf samples were finely crushed with ceramic mortar and pestle, in the presence of liquid nitrogen. In order to obtain high quality DNA, CTAB extraction protocol of Doyle and Doyle (1990) was employed with some modification in the quantity of the CTAB buffer. The DNA pellets of the samples were completely dissolved in a TE buffer and quantified using the Nanodrop spectrophotometer (Thermoscientific) before being diluted with sterile distilled water to a concentration of 50 ng/µL for PCR analysis and kept in a refrigerator at -20 °C.

Twenty-five ISSR primers, as listed by Gupta *et al*. (2010), were selected and used for profiling this germplasm. The PCR protocol was performed using a Qiagen PCR master mix kit and a total reaction mixture of 25 µl was adopted, while the temperature settings followed standard protocols as described by Murty *et al*. (2013). Following electrophoresis, the gel picture was captured using a Bio-Rad Image Lab. Binary scoring, 1 or 0 representing "presence or absence" of a specific clear and polymorphic band.

Analysis of genetic diversity parameters such as Shannon information index, Nei's genetic diversity and expected heterozygosity were done using GenAIEX 6.5 (Peakall & Smouse, 2006, 2012). Principal component analysis was performed using NTSYS-PC (Rohlf, 1997). Genetic differentiation through analysis of molecular variance (AMOVA) was performed using GenAIEX 6.5.

Data Collection and Statistical Analysis

Data collection on yield and yield components commenced from year one after planting and was carried out for three years, and the mean values were used for analysis. Eleven quantitative traits were measured, which include plant height (cm), number of primary branches, number of secondary branches, stem diameter (mm), seed yield per plant (g), seed length (mm), seed breadth (mm), number of seeds per plant, seed yield per hectare (kg), oil content (%) and oil yield per hectare (kg). The data collection was carried out following the method of Shabanimofrad *et al*. (2013).

Analysis of variance (ANOVA) was carried out using SAS 9.3 to investigate any

significant differences among the accessions based on the traits. Similarly, a SAS code "proc varcomp method=type1" was used for estimating the variance components. Using the results, other heritability components such as genetic advance, broad sense heritability, phenotypic and genotypic coefficients of variation were calculated following the method of Allard (1960) and Kang (1998). Cluster analysis was done using the NTSYS pc software for the construction of the dendrogram and genetic similarity distances among the accessions.

RESULTS

Genetic Diversity Analysis.

The 45 accessions of *Jatropha curcas* collected were profiled using ISSR markers (Table 1 and Fig.1). The accessions were partitioned into three populations (Table 2). Due to their diverse nature, different allelic variations were observed in the three populations, ranging from 1.82 (Terengganu population) to 2.0 (Kelantan population), with a mean value of 1.93. Polymorphism percentages were very high in the Kelantan, Terenganu and Selangor populations, at 98.08, 90.38 and 100% respectively. The mean percentage of polymorphism for the three populations was found to be 96.3 %. The Shannon information index (I) and observed heterozygosity (Ho) were observed to be the least in the Terengganu population (0.53 and 0.36), while the Kelantan population exhibited the highest at 0.58 and 0.40, respectively.

AMOVA is the genetic differentiation which revealed the allelic variations within and between the Jatropha populations. In this study, about 100% of the variations observed in the germplasm occurred as a result of variations within the populations (Table 3) with fixation index (F-Score) of 0.463.

The cluster analysis was done to construct a dendrogram based on UPGMA analysis using dice similarity index with coefficients ranging from 0.20 to 0.98 (Fig.2). This was done to highlight the overall genetic relationship among the

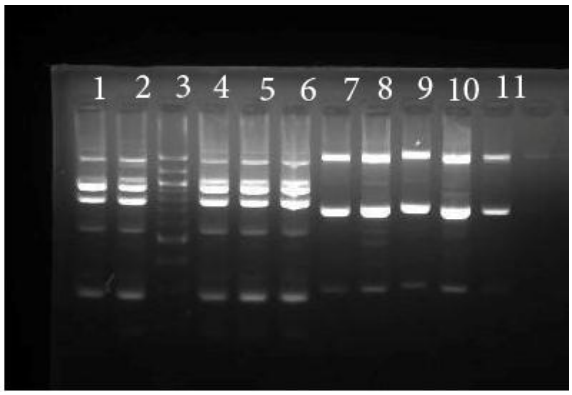

Fig.1: Gel picture showing the polymorphic bands across the eleven accessions of *Jatropha curcas*

TABLE 1
List of 45 accessions from three states with their codes, and locations

Number	Accessions	Population	Origin	Area	Latitude	Longitude
1	B-01-01	Selangor	Seri Serdang	Serdang	3°0'1.24"	101° 43' 1.1994"
2	B-01-02	Selangor	Seri Serdang	Serdang	3°0'38.88"	101° 42' 35.9994"
3	B-01-03	Selangor	Seri Serdang	Serdang	3°0'38.16"	101° 42' 21.6"
4	B-01-04	Selangor	Taman Serdang Raya	Serdang	3°0'38.52"	101° 42' 25.1994"
5	B-01-05	Selangor	UPM-Cemetery	Serdang	2°59' 52.44"	101° 43' 4.8"
6	B-01-06	Selangor	UPM- Kolej 17	Serdang	2°58'45.48"	101° 42' 39.5994"
7	B-01-07	Selangor	UPM- Kolej 17	Serdang	2°58'45.479"	101° 42' 39.5994"
8	B-02-01	Selangor	Ladang Raja Musa	Kuala Selangor	2°24'29.519"	101° 16' 55.1994"
9	B-02-02	Selangor	Bukit Belimbing	Kuala Selangor	2°24' 29.88"	101° 16' 51.6"
10	B-02-03	Selangor	Sri Angala Aman	Kuala Selangor	2°23'48.479"	101° 16' 30"
11	B-02-04	Selangor	Kota Hulu Moram	Kuala Selangor	3°23'33.72"	101° 17' 27.5994"
12	B-02-05	Selangor	Taman Sri Blimbing	Kuala Selangor	3°23'23.639"	101° 16' 19.2"
13	B-02-06	Selangor	Lorong Intan A	Kuala Selangor	3°25'10.92"	101° 13' 15.6"
14	B-03-01	Selangor	Sungai Choh, Rawang	Hulu Selangor	3°20' 45.6"	101° 35' 24"
15	B-03-02	Selangor	Batu 16, Kampong Melayu	Hulu Selangor	3°18'15.839"	101° 35' 45.6"
16	B-04-01	Selangor	Kampong Sungai Buloh	Kuala Selangor	3°14' 44.16"	101° 28' 22.7994"
17	B-04-02	Selangor	Jalan Rahidin	Kuala Selangor	3°11' 6.3994"	101° 32' 56.4"
18	B-05-01	Selangor	Bangi Lama	Hulu Langat	2°54' 5.04"	101° 46' 40.8"
19	B-05-02	Selangor	Bangi Lama	Hulu Langat	2°54' 2.8794"	101° 46' 37.2"
20	B-05-05	Selangor	Pekan Beromang	Hulu Langat	2°52'35.759"	101° 52' 22.8"
21	B-05-06	Selangor	Kampong Sungai Jai	Hulu Langat	2°52' 15.96"	101° 52' 55.2"
22	B-05-11	Selangor	Near Hulu Langat river	Hulu Langat	3°9'52.9194"	101° 50' 59.9994"
23	B-06-01	Selangor	Batu Laut, Banting	Kuala langat	2° 40' 23.52"	101° 31' 19.2"
24	B-06-02	Selangor	Banting	Kuala langat	2°40'22.439"	101° 31' 19.2"
25	B-06-03	Selangor	Taman Changang	Kuala langat	2°49'45.479"	101° 37' 8.3994"

TABLE 1 (*continued*)

Number	Accessions	Population	Origin	Area	Latitude	Longitude
26	D-01-01	Kelantan	PLT. Pasir Puteh	Pasir Puteh	5°49'38.639"	102° 22' 15.5994"
27	D-01-02	Kelantan	PLT. Pasir Puteh	Pasir Puteh	5°49'38.999"	102° 22' 15.5994"
28	D-01-03	Kelantan	PLT. Pasir Puteh	Pasir Puteh	5°49'38.639"	102° 22' 15.5994"
29	D-01-04	Kelantan	PLT. Pasir Puteh	Pasir Puteh	5°49'38.639"	102° 22' 15.5994"
30	D-01-05	Kelantan	PLT. Pasir Puteh	Pasir Puteh	5°49'38.28"	102° 22' 15.5994"
31	D-01-06	Kelantan	PLT. Pasir Puteh	Pasir Puteh	5°49'37.92"	102° 22' 15.5994"
32	D-01-07	Kelantan	Kampong Gong Tinggi	Pasir Puteh	5°48'12.96"	102° 28' 11.9994"
33	D-01-08	Kelantan	Kampong Tebing Tinggi	Pasir Puteh	5°49'33.599"	102° 26' 16.8"
34	D-01-09	Kelantan	Kampong Tok Bali	Pasir Puteh	5°54'28.8"	102° 27' 50.3994"
35	D-02-01	Kelantan	Jabatan Pertanian, Kota Bharu	Kota Bharu	6°6'6.8394"	102° 16' 1.1994"
36	D-02-02	Kelantan	Jabatan Pertanian, Kota Bharu	Kota Bharu	6°6' 6.8394"	102° 16' 1.1994"
37	D-03-01	Kelantan	Jambu Tawar	Machang	5°42'48.599"	102° 12' 39.5994"
38	T-01-01	Terengganu	Merang	Setiu	5°30'24.48"	102° 56' 16.8"
39	T-01-03	Terengganu	Merang	Setiu	5°30'24.48"	102° 56' 9.6"
40	T-01-04	Terengganu	Merang	Setiu	5°30'24.48"	102° 56' 6"
41	T-01-05	Terengganu	Merang	Setiu	5°30'25.199"	102° 56' 9.6"
42	T-01-06	Terengganu	Penarik	Setiu	5°28'14.519"	102° 48' 57.6"
43	T-01-08	Terengganu	Merang	Setiu	5°32'13.199"	102° 57' 39.5994"
44	T-01-09	Terengganu	Batu Rakit	Setiu	5°26'53.16"	103° 2' 59.9994"
45	T-01-10	Terengganu	Batu Rakit	Setiu	5°26'35.879"	103° 3' 21.5994"

TABLE 2
Genetic divergence as revealed by ISSR profiling

Pop	N	Na	Ne	I	He	uHe	P (%)
Pop 1	24	2.000	1.687	0.564	0.385	0.394	100.00
Pop 2	13	1.981	1.699	0.572	0.393	0.408	98.08
Pop 3	8	1.827	1.637	0.521	0.357	0.381	90.38
Total mean		1.936	1.674	0.552	0.378	0.394	96.15
SE		0.027	0.024	0.014	0.011	0.012	2.94

Na = No. of Different Alleles; Ne = No. of Effective Alleles; I = Shannon's Information index. He =Expected Heterozygosity P= Percentage of Polymorphic Loci; Pop 1= Selangor, Pop 2= Kelantan and Pop 3= Terengganu.

TABLE 3
Analysis of Molecular variance of three *Jatropha curcas* populations

Source	d.f	SS	MS	Est. Var.	Variation (%)
Among Populations	2	20.611	10.305	0.003	0%
Within Populations	42	430.856	10.258	10.258	100%
Total	44	451.467		10.262	100%

accessions. The dendrogram classified the 45 accessions into seven distinct groups at the mean coefficient of 0.55. Cluster I had the highest number of accessions (35), followed by cluster VI with three members. Cluster III and V had two members each, while cluster II and VII had one member each respectively. Cluster I members were mainly accessions from Kelantan and Selangor, with only seven accessions from the Terengganu population were found to be in Cluster I.

Genetic Diversity and Analysis of Variance Components

From the analysis of variance (Table 4), it was observed that all the yield traits starting with seed yield per plant, seed yield per hectare, total number of seeds, percentage oil content and total oil yield per plant were highly significant. Additionally, Table 5 also shows the mean, coefficient of variation, range and standard deviation values. The highest coefficient of variation was in total number of seeds (53.19%), followed by oil yield per hectare (51%). Total number of seeds ranged from 31.4 to 1183.1, while minimum seed weight per plant was found to be 36.1 g and the maximum was 850.5 g.

Low differences of less than 10% were found between phenotypic and genotypic coefficient of variations (PCV and GCV), except for stem collar diameter, number of secondary and primary branches with differences of 22.04, 34.36 and 15.42 % respectively. Highest PVC was found in total number of seeds (49.39 %), followed by oil yield per hectare (47.94%) while seed length and seed width were found to have the lowest PVC. As for GVC, the highest was for total number of seeds (42.27 %) while the number of secondary branches

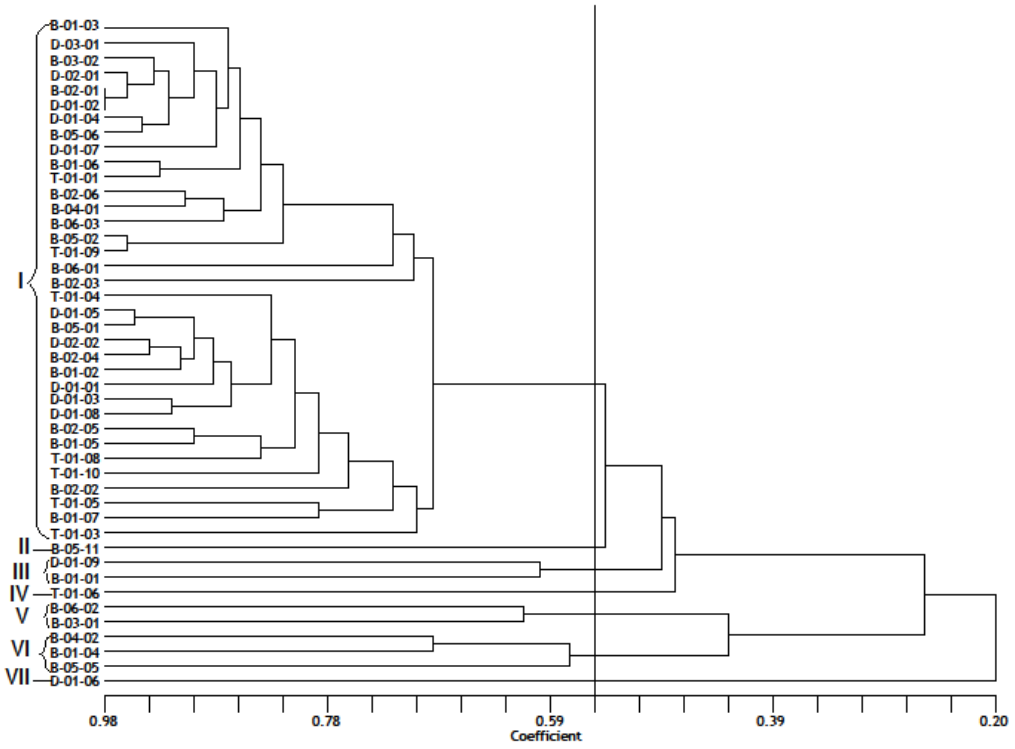

Fig.2: Cluster analysis from ISSR profiling of 45 *Jatropha curcas* accessions based UPGMA and dice similarity index

(0.00), seed length (0.71 %) and seed width (1.61 %) were seen to be the lowest. Broad sense heritability was generally high, with total number of seeds, seed weight per plant, total seed yield per hectare and percentage oil content at 73.25, 70.18, 70.18 and 67.81 % respectively. However, stem collar diameter, number of secondary branches and seed width showed the least amount of broad sense heritability.

From principal components analyses, as shown in Table 6, it was revealed that the first four principal components (PC1-PC4) accounted for 84.3% of the total variations observed in these accessions. PC1 contributed 39%, while PCs 2, 3 and 4 contributed 25.2, 10.9 and 9.3 % respectively. In PC1, the highest positive contribution was made by stem collar diameter followed by number of primary branches, while total number of seeds, seed yield per hectare, oil yield and seed yield per plant contributed negatively. Likewise in Pc 2, all the traits contributed positively except for seed width, seed length and percentage of oil content with 0.011, 0.098 and 0.211 as their positive contributions.

Genetic Distance and Dendrogram Based on Morphological Traits

The genetic distance among the 45 accessions are presented in Table 7. As indicated in the

TABLE 4
Mean squares of all the agro-morphological traits for 45 accessions of *J. curcas*

Source of variation	d.f	collar diameter	no. of Secondary branches	no. of primary branches	Seed width	Seed length	Seed yield/plant	plant height	Oil content	total no. of seed	seed yield/ha	Oil yield/ha
Blocks (b)	1	55373.44**	0.03^{ns}	1.74^{ns}	0.02^{ns}	1.10*	287404.21**	15020.28**	8.68**	569496.86**	782470.08**	69052.78**
Genotypes (g)	44	2165.84^{ns}	12.77^{ns}	1.13^{ns}	0.46^{ns}	0.302^{ns}	33610.95**	561.14^{ns}	8.40**	64823.51**	91505.89**	9038.18**
Error	44	3275.09	13.34	0.73	0.46	0.27	5889.04	536.47	1.61	10009.53	16033.24	1811.47

Note: * = significant at 0.05 level; **= significant at 0.01 level, ns= not significant

TABLE 5
Heritability and variance components of quantitative traits.

Variable	Mean	Std. Error	CV	Mini	Max	PCV	GCV	h_B
Stem collar diameter (mm)	150.15	6.07	38.33	49.60	343.73	34.74	12.69	0.00
No. Secondary branches	10.49	0.38	34.17	3.00	20.00	34.36	0.00	0.00
No. Primary branches	3.35	0.10	28.95	1.50	6.50	28.81	13.39	21.60
Seed width (mm)	11.44	0.07	5.91	5.85	12.40	5.94	1.61	0.46
Seed length (mm)	18.73	0.06	2.89	17.50	19.90	2.85	0.71	6.21
Seed weight (g)	295.90	15.90	50.98	36.11	851.50	47.49	39.79	70.18
Plant height (cm)	152.12	2.81	17.53	101.50	228.00	15.40	2.31	2.25
Oil content (%)	31.41	0.24	7.15	27.20	37.10	7.12	5.87	67.81
Total no. of seeds	391.64	21.96	53.19	31.39	1183.09	49.39	42.27	73.25
Seed yield/ha (kg)	488.24	26.24	50.98	59.58	1404.97	47.49	39.79	70.18
Oil yield/ha (kg)	153.63	8.26	51.00	20.61	431.33	47.94	39.13	66.61

Note: CV = Coefficient of variation (%); h_B = Broad sense heritability (%), PCV = Phenotypic coefficient variation (%); GCV = Genotypic coefficient variation (%).

TABLE 6
Principal component analysis and percentage variation contributed by each of the component

Eigenvalue	PC1(%)	PC2(%)	PC3(%)	PC4(%)
Proportion	0.39	0.252	0.109	0.093
Cumulative	0.39	0.641	0.751	0.843
Stem collar diameter (mm)	0.27	-0.364	-0.008	-0.146
No. Secondary branches	0.161	-0.502	-0.027	0.13
No. Primary branches	0.225	-0.48	-0.028	0.072
Seed width (mm)	0.01	0.011	-0.573	-0.659
Seed length (mm)	0.027	0.098	-0.747	0.208
Seed weight (g)	-0.454	-0.201	-0.016	-0.039
Plant height (cm)	0.102	-0.424	-0.215	0.287
Oil content (%)	-0.12	0.211	-0.247	0.621
Total no. of seeds	-0.449	-0.205	0.021	-0.077
Seed yield/ha (kg)	-0.454	-0.201	-0.016	-0.039
Oil yield/ha (kg)	-0.46	-0.17	-0.055	0.045

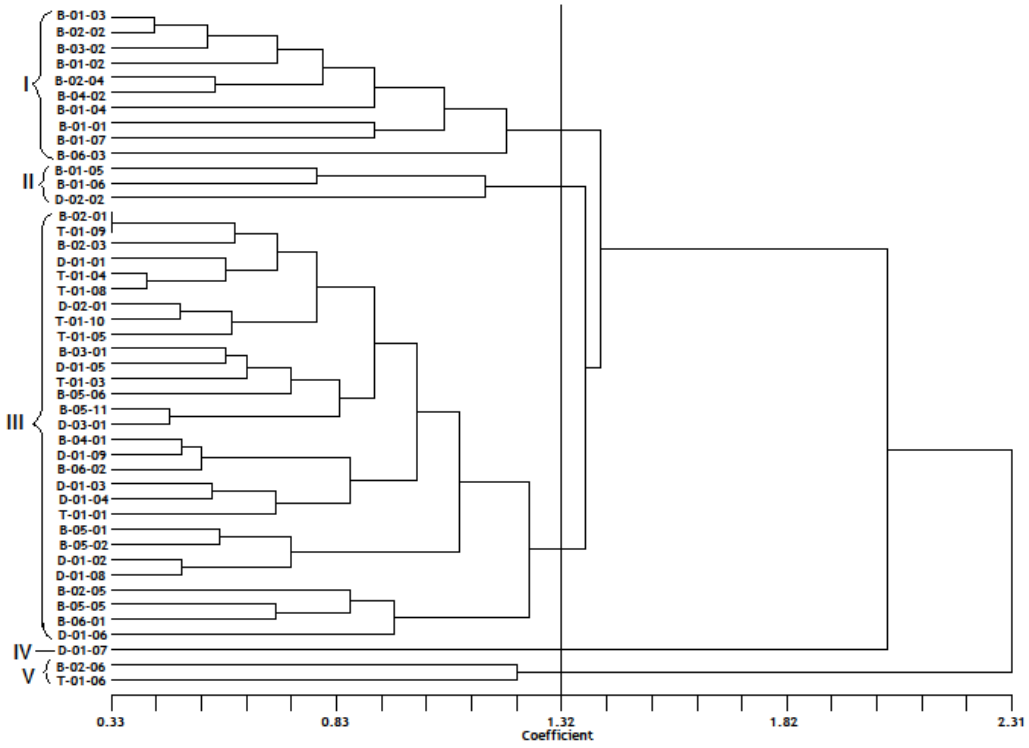

Fig.3: Cluster analysis of 45 *Jatropha curcas* accessions based on 11 quantitative traits

TABLE 7
Dissimilarity matrix showing the Genetic Distance among the Accessions based on Quantitative traits

Genotypes	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1	0.00																													
2	1.11	0.00																												
3	0.79	0.91	0.00																											
4	0.97	1.10	0.72	0.00																										
5	0.87	1.59	0.99	0.91	0.00																									
6	1.21	1.96	1.50	1.40	0.78	0.00																								
7	0.99	0.90	0.94	1.06	1.22	1.52	0.00																							
8	1.16	1.79	1.15	1.16	0.87	1.11	1.1	0.00																						
9	0.42	1.08	0.65	0.90	0.89	1.18	0.87	0.93	0.00																					
10	1.16	1.83	1.18	1.10	0.81	1.05	1.38	0.55	0.92	0.00																				
11	1.09	1.34	0.92	1.13	1.35	1.60	1.18	1.07	0.73	0.97	0.00																			
12	1.38	1.83	1.23	1.26	1.40	1.68	1.74	1.26	1.14	0.94	0.72	0.00																		
13	2.61	3.30	2.64	2.64	1.99	1.69	2.65	1.88	2.53	2.06	2.76	2.74	0.00																	
14	1.29	1.79	1.01	1.39	1.28	1.61	1.48	0.91	1.03	0.97	0.81	0.91	2.32	0.00																
15	0.57	1.27	0.66	0.93	0.64	1.04	0.85	0.77	0.52	0.95	1.06	1.36	2.17	0.99	0.00															
16	0.98	1.92	1.27	1.37	0.86	0.98	1.57	0.95	0.95	0.83	1.18	1.09	2.05	1.00	0.88	0.00														
17	0.75	1.17	0.68	0.73	0.88	1.15	0.96	0.87	0.43	0.75	0.56	0.88	2.47	0.97	0.70	0.96	0.00													
18	1.48	2.20	1.42	1.56	1.12	1.18	1.79	0.99	1.35	1.06	1.42	1.31	1.61	0.91	1.10	0.78	1.26	0.00												
19	1.84	2.62	1.81	1.94	1.34	1.27	2.14	1.22	1.71	1.23	1.81	1.66	1.25	1.26	1.45	1.08	1.64	0.57	0.00											
20	1.15	1.90	1.35	1.22	1.13	1.33	1.78	1.34	1.16	1.09	1.22	0.88	2.52	1.27	1.24	0.73	1.02	1.15	1.55	0.00										
21	0.96	1.58	0.80	1.13	0.85	1.13	1.30	0.93	0.87	1.06	1.18	1.32	1.99	0.84	0.56	0.88	0.91	0.76	1.14	1.21	0.00									
22	1.52	2.12	1.34	1.40	1.24	1.38	1.83	1.04	1.31	0.93	1.16	0.86	2.02	0.78	1.24	0.89	1.10	0.56	0.93	0.96	0.93	0.00								
23	0.83	1.55	0.93	1.13	0.95	1.33	1.51	1.24	0.80	0.99	0.97	0.84	2.58	1.00	0.93	0.68	0.78	1.18	1.54	0.70	0.97	1.06	0.00							
24	1.25	2.18	1.48	1.50	0.84	0.88	1.69	0.78	1.18	0.72	1.43	1.34	1.65	1.13	1.00	0.53	1.17	0.74	0.81	1.09	0.99	0.96	1.09	0.00						
25	1.34	1.57	0.97	1.37	1.51	1.89	1.28	1.04	1.05	1.23	0.87	1.27	2.64	0.69	1.09	1.48	1.08	1.46	1.77	1.71	1.11	1.33	1.39	1.55	0.00					
26	1.42	2.09	1.31	1.41	1.08	1.24	1.61	0.73	1.25	0.93	1.43	1.49	1.64	1.00	1.01	1.15	1.24	0.86	0.95	1.53	0.80	0.99	1.44	0.89	1.14	0.00				
27	2.13	2.77	1.95	2.17	1.73	1.76	2.27	1.34	1.92	1.44	1.83	1.74	1.52	1.17	1.69	1.46	1.83	0.88	0.65	1.89	1.36	1.06	1.82	1.23	1.61	1.05	0.00			
28	1.64	2.29	1.57	1.82	1.49	1.61	1.87	1.07	1.38	0.96	1.12	1.02	2.18	0.73	1.39	0.99	1.27	0.99	1.11	1.38	1.29	0.88	1.21	1.02	1.27	1.24	1.02	0.00		
29	1.41	2.17	1.43	1.57	1.22	1.48	1.73	0.87	1.25	0.82	1.14	0.98	2.11	0.71	1.17	0.72	1.17	0.82	1.06	1.07	1.13	0.77	1.02	0.75	1.26	1.12	1.14	0.56	0.00	
30	1.16	1.89	1.10	1.36	0.98	1.21	1.41	0.60	0.98	0.81	1.10	1.21	1.84	0.58	0.75	0.77	1.00	0.62	0.90	1.26	0.59	0.79	1.08	0.71	0.94	0.57	1.00	0.86	0.70	0.00
31	1.43	2.00	1.46	1.75	1.52	1.64	1.87	1.54	1.31	1.37	1.15	1.01	2.62	1.08	1.39	0.91	1.21	1.18	1.53	1.03	1.31	1.09	0.84	1.34	1.65	1.76	1.70	1.01	1.04	1.28
32	2.16	2.66	2.07	2.10	1.82	1.93	2.55	1.97	1.99	1.60	2.11	1.92	2.65	2.06	2.09	2.00	1.92	2.13	1.97	2.18	2.01	1.98	1.90	1.84	2.20	1.81	2.15	1.95	2.09	1.97
33	1.94	2.70	1.89	2.11	1.67	1.69	2.24	1.30	1.77	1.39	1.73	1.61	1.67	1.07	1.58	1.22	1.74	0.74	0.66	1.65	1.25	0.95	1.64	1.05	1.55	0.99	0.50	0.95	0.96	0.86
34	1.20	2.02	1.37	1.51	0.95	0.99	1.60	0.90	1.03	0.68	1.15	1.10	1.97	1.00	1.02	0.50	0.98	0.90	1.03	1.06	1.05	0.96	0.87	0.57	1.47	1.16	1.32	0.74	0.72	0.81
35	1.33	2.05	1.24	1.18	0.99	1.34	1.69	0.79	1.20	0.73	1.28	1.08	2.01	0.93	1.07	0.96	1.10	0.87	1.10	1.07	0.93	0.74	1.13	0.81	1.21	0.67	1.28	1.16	0.85	0.73
36	1.72	2.35	1.78	1.55	1.14	1.15	1.89	1.35	1.74	1.47	2.17	2.19	1.51	1.95	1.41	1.63	1.73	1.50	1.49	1.87	1.38	1.72	1.93	1.32	2.06	1.12	1.89	2.15	1.89	1.48
37	1.35	2.05	1.36	1.35	1.18	1.29	1.78	1.12	1.24	1.00	1.17	0.88	2.15	0.96	1.19	0.70	1.05	0.67	1.13	0.60	0.99	0.46	0.93	0.92	1.49	1.20	1.39	1.07	0.81	0.92
38	1.71	2.49	1.72	1.78	1.46	1.67	2.19	1.31	1.57	1.03	1.41	0.92	2.29	1.07	1.57	0.92	1.43	1.02	1.15	1.01	1.43	0.79	1.10	0.97	1.64	1.38	1.29	0.78	0.61	1.12
39	1.40	2.10	1.24	1.40	1.13	1.48	1.77	0.96	1.29	0.99	1.31	1.12	1.99	0.69	1.07	0.87	1.22	0.58	0.92	1.10	0.78	0.59	1.06	0.86	1.19	0.81	1.03	0.99	0.68	0.58
40	1.35	2.00	1.24	1.25	0.86	1.04	1.48	0.51	1.15	0.61	1.23	1.21	1.62	0.87	0.92	0.87	1.00	0.61	0.80	1.22	0.80	0.69	1.19	0.64	1.23	0.59	1.01	0.97	0.79	0.53
41	1.27	1.76	1.00	0.99	1.12	1.53	1.44	0.78	1.08	0.87	1.06	1.07	2.26	0.80	0.99	1.19	0.97	1.08	1.41	1.25	0.90	0.89	1.21	1.15	0.84	0.74	1.45	1.28	1.04	0.77
42	2.77	3.15	2.62	2.66	2.23	2.05	2.59	2.18	2.68	2.45	3.57	3.13	1.22	2.60	2.29	2.60	2.66	2.13	1.93	3.02	2.12	2.47	2.95	2.27	2.67	1.84	2.06	2.74	2.70	2.17
43	1.43	2.15	1.44	1.39	0.89	0.94	1.55	0.56	1.28	0.76	1.50	1.53	1.40	1.19	1.01	1.02	1.21	0.83	0.87	1.43	0.97	1.03	1.44	0.64	1.43	0.53	1.15	1.25	1.05	0.71
44	1.08	1.63	0.99	1.03	0.95	1.29	1.09	0.33	0.83	0.62	0.95	1.19	2.13	0.85	0.75	1.08	0.81	1.16	1.42	1.37	0.93	1.11	1.21	0.98	0.80	0.74	1.48	1.17	0.98	0.67
45	1.19	1.98	1.33	1.16	1.04	1.28	1.61	0.81	1.11	0.81	1.30	1.21	2.13	1.16	1.05	0.97	1.09	1.10	1.37	1.04	1.06	1.02	1.21	0.86	1.31	0.84	1.60	1.38	1.03	0.89

TABLE 7 (*continued*)

Genotypes	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45
31	0.00														
32	2.34	0.00													
33	1.53	2.20	0.00												
34	0.96	1.75	1.21	0.00											
35	1.55	1.79	1.13	1.08	0.00										
36	2.41	2.10	1.87	1.73	1.31	0.00									
37	0.97	2.19	1.17	0.96	0.86	1.75	0.00								
38	1.11	1.91	1.09	0.92	0.96	2.06	0.84	0.00							
39	1.28	2.03	0.85	1.04	0.54	1.57	0.74	0.86	0.00						
40	1.42	1.86	1.02	0.82	0.62	1.24	0.87	1.09	0.69	0.00					
41	1.63	1.98	1.34	1.30	0.50	1.49	1.03	1.28	0.73	0.79	0.00				
42	3.14	`2.85	2.28	2.54	2.37	1.56	2.66	2.96	2.39	2.01	2.41	0.00			
43	1.73	1.89	1.13	1.00	0.75	0.96	1.14	1.34	0.94	0.41	0.95	1.80	0.00		
44	1.62	1.92	1.42	1.06	0.76	1.44	1.21	1.38	0.98	0.70	0.59	2.32	0.78	0.00	
45	1.68	1.98	1.37	1.17	0.49	1.28	0.96	1.20	0.89	0.85	0.67	2.49	0.84	0.76	0.00

table, the highest dissimilarity (3.57) was found between accession 42 (T-01-06) and four other accessions (11 (B-02-04), 38 (T-01-01), 23 (B-06-01) and 32 (D-01-07)) at 3.57, 2.96, 2.95 and 2.85, respectively. However, the least genetic distance was 0.33, observed between accessions 44 (T-01-09) and 8 (B-02-01), followed by 0.41 between accessions 43 (T-01-08) and 40 (T-01-04), and 0.42 between accessions 9 (b-02-02) and 1 (b-01-03).

Additionally, a dendrogram constructed using 11 quantitative traits grouped the 45 accessions into five clusters at mean coefficient of 1.32 (Fig.3). Cluster III had the largest number of members (29) followed by Cluster I (10), while Clusters II, V and IV had 4, 2 and 1 accessions respectively. Cluster I and II mainly comprised accessions from the Selangor population, except for D-02-02, which is from the Kelantan population.

DISCUSSION

Genetic diversity study of germplasm resources is considered to be an essential activity undertaken before the commencement of any plant breeding programme. Presence of genetic diversity in a genetic resource helps in breeding and crop improvement programmes by helping to ensure the presence of trait variability for selection of individuals containing desirable traits such as high yield and resistance to various biotic and abiotic stresses in the environment. Germplasm exploration and evaluation of the *Jatropha curcas* are essential to widen the narrow genetic base of this crop. The full economic potential of this crop cannot be realised until high yielding materials are identified and obtained through long-term evaluation and selection.

In this study, molecular markers were combined with morphological markers to obtain finger-printing and genetic relationship information among the accessions. The presence of high amounts of

polymorphism (ranging from 90.38-100%) in the three populations studied suggests that the genetic dissimilarity among the accessions is very high. This makes them promising materials for hybridisation. This observation is in agreement with Tanya *et al*. (2011), who made similar observation while studying the genetic variation among 30 Jatropha accessions in Thailand using ISSR markers. It was reported that polymorphism among the individual populations was very high.

Additionally, genetic differentiation through analysis of molecular variance is necessary to reveal the allelic pattern for an in-depth understanding of the population structure. As seen in this study, virtually all the variations (100%) observed in the populations were due to variations within the three populations. This implies that higher differences were present within the accession from the same population. Variation among the population will be more than variation within the population if difference species are involved in the study. High genetic fixation within populations of Jatropha and its related species has been reported in several studies (Kumar *et al*., 2011a; Barboza *et al*., 2012; Biabani *et al*., 2013). Santos *et al*. (2010) reported that genetic variation among 50 Jatropha plants studied was 72.47 % when profiling the Jatropha germplasm with Amplified fragment length polymorphic markers. This occurs because the genotypic composition or genetic make-up of individual plants differ and this will result in higher variations within a population rather than among the populations.

The clustering patterns as presented in the dendrogram, of both morphological and ISSR, showed that the majority of the plants were clustered in the first group. This implies that the accessions are likely to have a similar genetic background. This observation is in agreement with Sudheer *et al*. (2010), who observed that more than 50% of the Jatropha accessions were clustered into one major group. The presence of a large number of accessions of Jatropha from the same population shows that ISSR is capable in finger-printing and identifying plant populations from diverse or similar genetic backgrounds. This observation on ISSR markers' discriminating ability is also seen in the findings reported in many studies (Tanya *et al*., 2011; Singh *et al*., 2012; Xu *et al*., 2012).

The presence of substantial variability as depicted by high coefficients of variation and range indicates that these accessions are genetically diverse in terms of their yield and other trait potentials. This finding complements the results from the molecular finger-printing using ISSR. This variability could also be a reflection of the wild nature of the accessions. These materials were collected from the wild where the plants were growing in their natural populations with a natural or random mating system. This finding is in line with the observations and conclusions on other wild populations of Jatropha studied in countries like China, Brazil, India and Thailand (Grativol *et al*., 2011; Kumar *et al*., 2011; Shen *et al*., 2012). Wani *et al*. (2012) in his study also observed substantial morphological variations in the Jatropha plant's vegetative traits such

as plant height, number of branches and yield traits, such as seed yield per plant, percentage oil content and 100 seeds' weight. The large genetic distance (> 3.5) displayed by some of the accessions suggest that these populations of Jatropha can be successfully introgressed into breeding programmes to enrich and widen their genetic base.

Furthermore, low differences observed between phenotypic and genotypic coefficients of variation for morphological traits suggest that the influence of environmental factors on the expressions of these traits is low. High broad-sense heritability (> 60%) in most of the yield traits further affirms this claim. Broad sense heritability is the proportion of variation which can be inherited by the offspring (Acquaah, 2007). The magnitude of this parameter affects the response to selection in breeding and crop improvement programmes. Higher broad sense heritability in economically important traits is of significant importance to the breeders, as it helps to increase the pace at which progress is made through selection (Bhargava *et al*., 2007).

Based on all the information obtained from this study, it can be concluded that morphological markers complemented with ISSR markers are suitable for profiling and depicting the genetic diversity of the Jatropha population. The results also show that these populations contain sufficiently divergent materials suitable for introgression into existing breeding programmes. Based on all the results obtained, accessions B-01-03, D-01-06, T-01-06 and B-06-02 are identified and recommended for further evaluation under field conditions before selection or chosen for use in future breeding programmes for seed yield and oil improvement.

REFERENCES

Acquaah, G. (2007). *Principles of Plant Genetics and Breeding* (p. 569). Blackwell Publishing Ltd.

Allard, R. W. (1960). *Principles of Plant Breeding*. New York: John Wiley and Sons, New York.

Banković-Ilić, I. B., Stamenković, O. S., & Veljković, V. B. (2012). Biodiesel production from non-edible plant oils. *Renewable and Sustainable Energy Reviews*, *16*(6), 3621–3647.

Barboza, N., Albertazzi, F. J., Sibaja-Cordero, J. a., Mora-Umaña, F., Astorga, C., & Ramírez, P. (2012). Analysis of genetic diversity of *Cucurbita moschata* (D.) germplasm accessions from Mesoamerica revealed by PCR SSCP and chloroplast sequence data. *Scientia Horticulturae*, *134*, 60–71.

Bhargava, A., Shukla, S., & Ohri, D. (2007). Genetic variability and interrelationship among various morphological and quality traits in quinoa (*Chenopodium quinoa* Willd.). *Field Crops Research*, *101*(1), 104–116.

Biabani, A., Rafii, M. Y., Saleh, G. B., & Latif, A. (2013). Inter- and intra-population genetic variations in *Jatropha curcas* populations revealed by inter-simple sequence repeat molecular markers. *Maydica, 58*, 111– 118.

Chauhan, B. S., Kumar, N., & Cho, H. M. (2012). A study on the performance and emission of a diesel engine fueled with Jatropha biodiesel oil and its blends. *Energy*, *37*(1), 616–622.

Doyle, J. J. & Doyle, J. L. 1990. A rapid total DNA preparation procedure for fresh plant tissue. *Focus* 12:13-15.

Grativol, C., Da Fonseca Lira-Medeiros, C., Hemerly, A. S., & Ferreira, P. C. G. (2011). High efficiency and reliability of inter-simple sequence repeats (ISSR) markers for evaluation of genetic diversity in Brazilian cultivated Jatropha curcas L. accessions. *Molecular Biology Reports*, *38*(7), 4245–56.

Gupta, S., Srivastava M., Mishra, G., Naik P, Chauhan, R., Tiwari, S., Kumar, M., & Singh, R. (2010) Analogy of ISSR and RAPD markers for comparative analysis of genetic diversity among different Jatropha curcas genotypes. *African Journal of Biotechnology*, *7*(23), 4230-4243

Henning, R. (2004). *Jatropha curcas L. in Africa*. http://www.jatropha de/documents/jci manual. pdf8/3/2004 accessed on 11 July, 2012.

Kang, M. S. (1998). Genotype-by-environment interaction for crop development. *Advances in Agronomy*, *62*, 199– 252.

Kristensen, T., Mogensen, L., Knudsen, M. T., & Hermansen, J. E. (2011). Effect of production system and farming strategy on greenhouse gas emissions from commercial dairy farms in a life cycle approach. *Livestock Science*, *140*(1-3), 136–148.

Kumar, S., Kumaria, S., Sharma, S. K., Rao, S. R., & Tandon, P. (2011a). Genetic diversity assessment of *Jatropha curcas* L. germplasm from Northeast India. *Biomass and Bioenergy*, *35*, 3063–3070.

Kumar, S., Kumaria, S., Sharma, S. K., Rao, S. R., & Tandon, P. (2011b). Genetic diversity assessment of *Jatropha curcas* L. germplasm from Northeast India. *Biomass and Bioenergy*, *35*, 3063–3070.

Murty, S. G., Patel, F., Punwar, B. S., Patel, M., Singh, A. S., & Fougat, R. S. (2013). Comparison of RAPD, ISSR, and DAMD markers for genetic diversity assessment between accessions of *Jatropha curcas* L. and its related species. *Journal of Agricultural Biotechnology and Sustainable Developmentcultural Sceince and Technology*, *15*, 1007–1022.

Peakall, R., & Smouse, P. E. (2006). Genalex 6: Genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes*, *6*(1), 288–295.

Peakall, R., & Smouse, P. E. (2012). GenAlEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research--an update. *Bioinformatics (Oxford, England)*, *28*(19), 2537–9.

Rohlf F. J. (1997). NTSYSpc: *Numerical taxonomy and multivariate analysis system, version 2.02*. Setauket (New York): Exeter Publishing.

Santos, C. A. F., Drumond, M. A., Rodrigues, M. A., & Evangelista, M. R. V. (2010). Genetic similarity of *Jatropha curcas* accessions based on AFLP markers. *Crop Breeding and Applied Biotechnology (Online)*, *10*(4), 364–369.

Shabanimofrad, M., Rafii, M. Y., Megat Wahab, P. E., Biabani, A. R., & Latif, M. A. (2013). Phenotypic, genotypic and genetic divergence found in 48 newly collected Malaysian accessions of *Jatropha curcas* L. *Industrial Crops and Products*, *42*, 543–551.

Shen, J., Pinyopusarerk, K., Bush, D., & Chen, X. (2012). AFLP-based molecular characterization of 63 populations of *Jatropha curcas* L. grown in provenance trials in China and Vietnam. *Biomass and Bioenergy*, *37*, 265–274.

Singh, S., Panda, M. K., & Nayak, S. (2012). Evaluation of genetic diversity in turmeric (*Curcuma longa* L.) using RAPD and ISSR markers. *Industrial Crops and Products*, *37*(1), 284–291.

Sudheer, P. D. V. N., Rahman, H., Mastan, S. G., & Reddy, M. P. (2010). Isolation of novel microsatellites using FIASCO by dual probe enrichment from *Jatropha curcas* L. and study on genetic equilibrium and diversity of Indian population revealed by isolated microsatellites. *Molecular Biology Reports*, *37*(8), 3785–93.

Sunil, N., Sujatha, M., Kumar, V., Vanaja, M., Basha, S. D., & Varaprasad, K. S. (2011). Correlating the phenotypic and molecular diversity in *Jatropha curcas* L. *Biomass and Bioenergy*, *35*(3), 1085–1096.

Tanya, P., Dachapak, S., Tar, M. M., & Srinives, P. (2011a). New microsatellite markers classifying nontoxic and toxic *Jatropha curcas*. *Journal of Genetics, 90*(3), 76–78.

Tanya, P., Taeprayoon, P., Hadkam, Y., & Srinives, P. (2011b). Genetic diversity among Jatropha and Jatropha-related species based on ISSR markers. *Plant Molecular Biology Reporter*, *29*(1), 252–264.

Wani, T. A., Kitchlu, S., & Ram, G. (2012). Genetic variability studies for morphological and qualitative attributes among *Jatropha curcas* L. accessions grown under subtropical conditions of North India. *South African Journal of Botany*, *79*(0), 102–105.

Xu, G. H., Su, W. Y., Shu, Y. J., Cong, W. W., Wu, L., & Guo, C. H. (2012). RAPD and ISSR-assisted identification and development of three new SCAR markers specific for the *Thinopyrum elongatum* E (Poaceae) genome. *Genetics and Molecular Research: GMR*, *11*(2), 1741–51.