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Diversity of physic nut (*Jatropha curcas*) in Malaysia: application of DIVA-geographic information system and cluster analysis

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Abstract

A sum of 59 accessions of physic nut, *Jatropha curas* were collected from different locations of Selangor, Kelantan and Terengganu states of Malaysia to assess genetic diversity using multivariate analysis and DIVA-geographic information system (GIS). Six quantitative characters, seed length, seed width, fruit length, fruit width, 100 seed weight and oil content were recorded. Based on 6 quantitative characters, 59 accessions were grouped into three clusters at a coefficient level of 3.7. Highly positive correlations were found between fruit length and fruit width, fruit length and seed length, fruit width and seed length, fruit length and seed width, fruit width and seed width and seed length and seed width. DIVA-GIS showed the highest diversity index for 100 seed weight in the *J. curcas* accessions which were collected from the central parts of Selangor state. On the other hand, the highest diversity index for oil content was observed in the accessions of northern parts and costal region of Terengganu state, followed by the central parts of Selangor state in Malaysia.

Keywords: Correlations; Genetic diversity; *Jatropha curcas*; multivariate analysis; quantitative traits.
Abbreviations: DIVA-GIS-A geographic information system for the analysis of biodiversity; GPS-Global positioning system.

Introduction

Physic nut, *Jatropha curcas* is a potential source of vegetable oil as a replacement for petroleum and in particular, the production of biodiesel (King *et al.*, 2009). Physic nut is native to South America (Ramawat, 2010) and it was introduced in Asia by the Portuguese (Sunil et al., 2008). Collection of germplasm is done to obtain material for bio-systematic research or for genetic diversity studies, for conservation, and for immediate use in breeding programs (Von Bothmer et al., 1995). The utilization of these tree germplasm accessions warrants extensive study in the form of multi-location trials, which is both time and resource consuming. Molecular markers, such as amplified length polymorphism and microsatellites, were employed previously by a number of investigators to understand the genetic diversity of different plants (Theocharis et al., 2010; Singh et al., 2010). Geographic information system (GIS) mapping is a powerful but simple way to visually validate location of species (Flemons et al., 2007), preliminary diversity analysis and identify gaps in collection (Pradesh et al., 2010). DIVA-GIS is a statistical sotfware and designed to assist the plant genetic resources and biodiversity communities to map the range of distribution of species in which they are interested (Hijmans et al., 2002). An example in which DIVA-GIS was used extensively is that of Hijmans and Spooner (2001), who described the geographic distribution of wild potato species in North, Central and South America. It has been successfully used with different crops such as *Phaseolus* bean (Jones et al., 1997), wild potatoes (Hijmans et al., 2000) and *Piper* (Parthasarathy et al., 2006). As a tropical country, Malaysia could provide suitable conditions for this exotic species to grow. Despite the economic importance of *J. curcas* and plantation size of 750,000 acres of this plant in Malaysia (Shuit et al., 2010), very little experimentation on the provenance trials and genetic resources of *J. curcas* have been done in this country while knowledge of genetic variability is completely necessary for introducing the breeding programs. Here we have attempted to use cluster analysis and DIVA-GIS software to assess the genetic diversity of wild *J. curcas* germplasm using phenotypic traits.

Materials and methods

Collection of plant accessions

A total of 59 accessions of *J. curcas* were collected from Selangor (35), Kelantan (13) and Terengganu (11) states of Peninsular Malaysia following a random sampling procedure during 2008-2009. The values of latitude, longitude and altitude of collection sites were recorded using the Global Positioning System (Garmin GPS-12) (Table 1and Fig. 1).

Data collection and statistical analysis

Data were recorded on 6 quantitative characters, seed length, seed width, fruit length, fruit width, 100 seed weight and oil content. A total of 20 fruits were harvested randomly from each accession for recording fruit length and width. The oil

Table 1. *Jatropha curcas* accessions collected from different part of Selangor, Terengganu and Kelantan states of Malaysia.

Accession	Latitude	Longitude	Source Location	Disrict	State
B-01-01	3.0059	101.7166	Seri Serdang	Serdang	Selangor
B-01-02	3.0108	101.7102	Seri Serdang	Serdang	Selangor
B-01-03	3.0106	101.7059	Seri Serdang	Serdang	Selangor
B-01-04	3.0107	101.7065	Taman Serdang raya	Serdang	Selangor
B-01-05	2.9979	101.7176	Persiaran universiti1	Serdang	Selangor
B-01-06	2.9793	101.7114	UPM-Near Kolej-17	Serdang	Selangor
B-01-07	2.9793	101.7114	UPM-Near Kolej-17	Serdang	Selangor
B-01-08	2.9795	101.7115	UPM-Near Kolej-17	Serdang	Selangor
B-02-01	3.4082	101.2820	Jalan Raja Musa	Kuala Selangor	Selangor
B-02-02	3.4083	101.2806	Jalan Raja Musa,Bukit Belimbing	Kuala Selangor	Selangor
B-02-03	3.3968	101.2752	Kampung Bukit Belimbing	Kuala Selangor	Selangor
B-02-04	3.3927	101.2913	Jalan Raja Musa,Bukit Belimbing	Kuala Selangor	Selangor
B-02-05	3.3899	101.2724	Kampung Bukit Belimbing	Kuala Selangor	Selangor
B-02-06	3.4197	101.2212	Kampung Parit Serong	Kuala Selangor	Selangor
B-03-01	3.3460	101.5895	Kampung Hilir Indah	Hulu Selangor	Selangor
B-03-02	3.3044	101.5959	Jalan Sentosa	Hulu Selangor	Selangor
B-04-01	3.2456	101.4726	Jalan Kuala Selangor	Kuala Selangor	Selangor
B-04-02	3.1990	101.5493	Jalan Rahidin	Kuala Selangor	Selangor
B-04-03	3.1952	101.5472	Kampung Paya Jaras Dalam	Kuala Selangor	Selangor
B-04-04	3.1950	101.5381	Kampung Paya Jaras Hilir	Kuala Selangor	Selangor
B-05-01	2.9014	101.7776	Pekan Bangi	Hulu Langat	Selangor
B-05-02	2.9008	101.7772	Pekan Bangi	Hulu Langat	Selangor
B-05-03	2.8912	101.8270	Kampung Sungai Kembong Ulu Bangi	Hulu Langat	Selangor
B-05-04	2.8730	101.8436	Kampung Kuala Pajam	Hulu Langat	Selangor
B-05-05	2.8766	101.8727	Pekan Beromang	Hulu Langat	Selangor
B-05-06	2.8711	101.8823	Kampung Sungai Jai	Hulu Langat	Selangor
B-05-07	2.9603	101.8484	Kampung Sungai Macang	Hulu Langat	Selangor
B-05-08	3.1644	101.8847	Kampung Sungai Pagoh	Hulu Langat	Selangor
B-05-09	3.1733	101.8704	Kampung Tanjong Paoh	Hulu Langat	Selangor
B-05-10	3.1771	101.8563	Kampung Kuala Perdik	Hulu Langat	Selangor
B-05-11	3.1647	101.8504	Pekan Batu Lapan Belas	Hulu Langat	Selangor
B-05-12	3.1487	101.8371	Batu 16 Dusun.Tua	Hulu Langat	Selangor
B-06-01	2.6732	101.5223	Kampung Jangin	Kuala langat	Selangor
B-06-02	2.6729	101.5222	Batu Laut	Kuala langat	Selangor
B-06-03	2.8293	101.6187	Kampung Bukit Changgang	Kuala langat	Selangor
T-01-01	5.5068	102.9381	Kampung Merang	Setiu	Terengganu
T-01-02	5.5068	102.9385	Kampung Merang	Setiu	Terengganu
T-01-03	5.5068	102.9359	Kampung Merang	Setiu	Terengganu
T-01-04	5.5068	102.9353	Kampung Merang	Setiu	Terengganu
T-01-05	5.5070	102.9359	Kampung Merang	Setiu	Terengganu
T-01-06	5.4707	102.8156	Kampung Rahmat	Setiu	Terengganu
T-01-07	5.4371	102.8156	Penarik	Setiu	Terengganu
T-01-08	5.5370	102.9609	Kampung Merang	Setiu	Terengganu
T-01-09	5.4481	103.0502	Kampung Batu Rakit	Kuala Terengganu	Terengganu
T-01-10	5.4433	103.0560	Kampung Tanjong	Kuala Terengganu	Terengganu
T-01-11	5.3922	102.8631	Kampung.Sungai Bari	Kuala Terengganu	Terengganu
D-01-01	5.8274	102.3707	Kampung Cherang Tuli	Pasir Puteh	Kelantan
D-01-02	5.8275	102.3708	Kampung Wakaf Berangan	Pasir Puteh	Kelantan
D-01-03	5.8274	102.3708	Kampung Wakaf Berangan	Pasir Puteh	Kelantan
D-01-04	5.8274	102.3709	Kampung Wakaf Berangan	Pasir Puteh	Kelantan
D-01-05	5.8273	102.3711	Kampung Wakaf Berangan	Pasir Puteh	Kelantan
D-01-06	5.8272	102.3712	Kampung Wakaf Berangan	Pasir Puteh	Kelantan
D-01-07	5.8036	102.4700	Kampung Gong Tinggi	Pasir Puteh	Kelantan
D-01-08	5.8260	102.4384	KampungTebing Tinggi	Pasir Puteh	Kelantan
D-01-09	5.9080	102.4635	KampungTok Badi	Pasir Puteh	Kelantan
D-01-10	5.8989	102.4750	KampungTok Badi	Pasir Puteh	Kelantan
D-02-01	6.1019	102.2667	Jabatan Pertanian Kota Bharu	Kota Bharu	Kelantan
D-02-02	6.1019	102.2666	Jabatan Pertanian Kota Bharu	Kota Bharu	Kelantan
D-03-01	5.7135	102.2115	Kampung Pangkal Payong	Machang	Kelantan

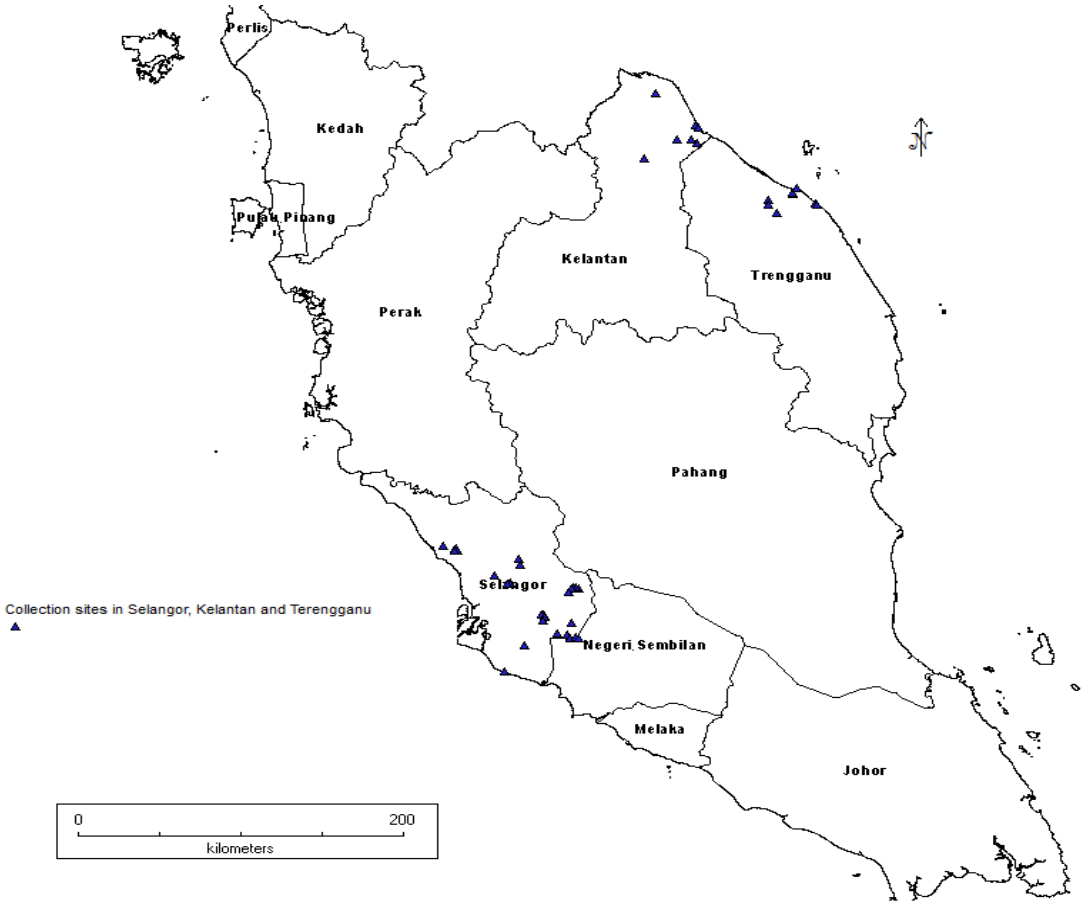

Fig 1. DIVA-GIS mapping of collection sites of *Jatropha curcas* from Selangor, Kelantan and Terengganu states, Malaysia.

content of the germplasm accessions was analyzed using the Soxhlet method (Kaushik et al., 2007). To evaluate the relationship among the different characters, correlation coefficients were determined using SPSS 15 software.

Cluster and principal component analysis

The morphological data were subjected to principal component analysis (PCA) using the NTSYS-Pc versions 2.1 (Rohlf, 2002) program. The eigenvectors and eigenvalues were determined in PCA. Eigenvectors are the weights in a linear transformation when computing principal component scores while eigenvalues indicate the amount of variance explained by each principal component. Cluster analysis was done and a UPGMA dendrogram was constructed using Jaccard's similarity coefficient.

DIVA-GIS for diversity analysis

DIVA-GIS software allows analysis of gene bank and herbarium databases to elucidate genetic, ecological and geographic patterns in the distribution of crops and wild species (Hijmans et al., 2001). Here, DIVA-GIS software version 7.2.1 (www.DIVAGIS.org) was used for the analysis of diversity in quantitative traits coordinated with geographical coordinates.

Results

All the accessions of *J. curcas* exhibited variability in all the 6 quantitative traits that were studied. Mean data of quantitative traits and its descriptive statistical analysis are provided in Tables 2 and 3. The largest fruit and seed length were recorded in B-04-03 (Selangor state) with an average length of 27.09 and 20.87 mm respectively, while the smallest fruit and seed length were recorded in B-06-02 (Selangor state), with a mean length of 19.69 and 14.96 mm respectively. B-05-01 from Selangor state possessed maximum fruit width (23.66 mm) while B-06-02 (Selangor state) recorded the least with a mean width of 17.34 mm. Weight of 100 seed was minimum in B-02-06 (40.42 g) and maximum was in B-04-02 (88.79 g) accessions which were recorded from Selangor state. The highest coefficient of variation was found in oil content followed by 100 seed weight, fruit length and fruit width. Estimated correlation coefficient (Table 4) revealed highly significant (p=0.01) positive correlations between fruit length and fruit width, fruit length and seed length, fruit width and seed length, fruit length and seed width, fruit width and seed width and seed

Table 2. Descriptive statistical analysis of six quantitative characters of *Jatropha curcas.*

Traits	Mean	Median	Min.	Max.	Sdv.	CV
Fruit width (mm)	21.85	22.01	17.34	23.66	1.17	5.34
Fruit length (mm)	24.58	24.65	19.69	27.09	1.42	5.77
Seed width (mm)	11.41	11.42	9.54	12.39	0.46	4.04
Seed length (mm)	18.47	18.60	14.96	20.87	0.98	5.33
100 seed weight (g)	71.06	72.44	40.42	88.79	10.93	15.38
Oil content (%)	34.15	34.21	17.37	42.75	5.43	15.89

Sdv- Standard deviation; CV-Coefficient of variation

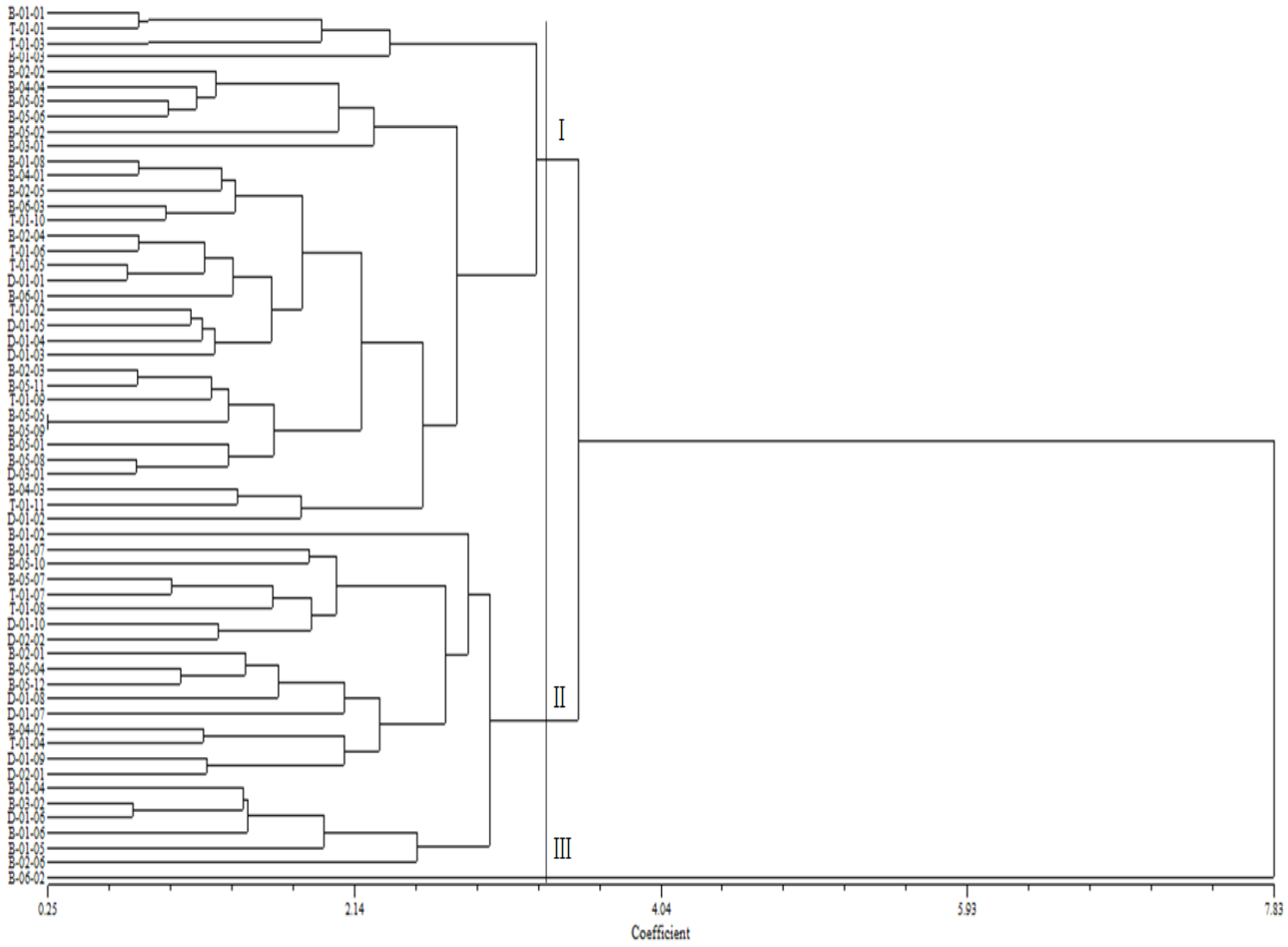

Fig 2. Dendrogram constructed by UPGMA method based on 6 quantitative traits of 59 *Jatropha curcas* accessions.

length and seed width. According to Table 5, PCA was conducted for the six phenotypic traits in order to summarize the obtained data. The first 2 principal components explained 72.1% variation among the accessions. In cluster analysis, 59 *J. curcas* accessions were grouped into 3 clusters at a co-efficient level of 3.7 (Fig 2). The first cluster consisted of 35 accessions which were vigorous in fruit length, fruit width, seed length, seed width and 100 seed weight. The second cluster consisted of 23 accessions which were vigorous in oil content and the third cluster consisted of only one accession collected from Selangor state (B-06-02), which was lower fruit length, fruit width, seed length and seed width compared to other accessions. Grid maps were generated for the diversity index for 2 important traits, 100 seed weight and oil content using DIVA-GIS software. The highest diversity index for 100 seed weight was observed in the accessions collected from central parts of Selangor state (Fig. 3). The highest diversity index for oil content was observed in the *J. curcas* accessions collected from the northern parts and costal region of Terengganu state followed by central parts of Selangor state (Fig. 4). The highest coefficient of variation for 100 seed weight was recorded in three parts of Selangor followed by Kelantan and Trengganu states (Fig. 5).

Discussion

According to six quantitative characters, 59 *J. curcas* accessions were grouped into 3 clusters. The genotypes belonging to the distant clusters could be used in hybridization programs for obtaining a wide spectrum of variation among the segregates. Similar reports were also made by Bansal et al. (1999), Mokate et al. (1998) and Kumari and Rangasamy (1997). The genotypes belonging to clusters I and III having greater cluster distance might be

Table 3. Mean data of six quantitative characters of 59 *Jatropha curcas* accessions.

Accessions	Fruit length (mm)	Fruit width (mm)	Seed length (mm)	Seed width (mm)	100 Seed Weight (g)	Oil content (%)
B-01-01	25.96	22.66	19.30	11.66	72.20	24.76
B-01-02	23.58	18.93	18.70	11.18	82.65	31.20
B-01-03	24.15	21.78	18.24	11.40	55.47	17.37
B-01-04	25.06	22.31	18.29	11.06	57.89	34.21
B-01-05	23.22	22.26	17.13	10.58	49.81	32.14
B-01-06	24.06	21.58	17.65	11.09	64.67	29.72
B-01-07	22.38	20.28	17.16	10.98	62.35	31.60
B-01-08	25.29	22.34	19.17	11.42	64.63	32.34
B-02-01	22.80	21.40	18.16	11.34	70.07	35.92
B-02-02	26.29	23.08	19.66	11.80	51.79	35.67
B-02-03	26.37	22.27	19.05	11.49	67.54	39.13
B-02-04	24.40	22.10	18.60	12.08	76.54	30.77
B-02-05	25.39	22.90	18.71	11.30	73.68	31.21
B-02-06	23.23	20.90	17.60	11.38	40.42	28.10
B-03-01	24.30	22.60	19.22	11.68	49.83	29.80
B-03-02	23.18	21.73	17.76	11.01	56.98	36.33
B-04-01	24.37	22.01	19.26	11.25	63.72	32.50
B-04-02	22.81	21.39	18.06	11.65	88.79	29.83
B-04-03	27.09	23.08	20.87	11.85	80.34	35.74
B-04-04	25.70	22.55	19.79	11.55	60.70	37.19
B-05-01	26.57	23.66	19.24	11.91	73.08	39.72
B-05-02	26.78	23.59	19.77	12.39	54.24	37.76
B-05-03	25.46	22.98	18.80	11.67	53.31	38.43
B-05-04	23.88	20.45	18.29	11.19	70.62	42.75
B-05-05	25.66	22.18	18.85	11.35	80.08	41.52
B-05-06	25.70	23.07	19.36	11.48	58.09	41.22
B-05-07	22.59	20.60	17.31	10.99	81.50	33.39
B-05-08	25.40	22.60	18.90	11.69	72.44	39.34
B-05-09	25.54	22.15	19.02	11.35	81.20	40.88
B-05-10	23.10	21.30	17.34	10.41	71.71	33.65
B-05-11	26.23	22.90	19.05	11.27	71.12	39.40
B-05-12	24.20	20.90	18.22	11.45	68.42	38.62
B-06-1	25.09	22.38	18.22	11.82	85.05	34.47
B-06-2	19.69	17.34	14.96	9.54	68.70	30.82
B-06-3	24.29	22.37	18.69	11.53	68.73	36.71
T-01-01	25.44	22.39	19.14	11.38	69.37	24.76
T-01-02	25.01	21.67	18.76	11.68	73.64	31.20
T-01-03	24.65	21.44	19.63	11.67	67.87	17.37
T-01-04	23.32	21.46	18.39	11.44	82.25	34.21
T-01-05	25.30	22.65	19.35	11.79	80.28	32.14
T-01-06	24.20	22.78	18.52	11.90	79.97	29.72
T-01-07	23.57	21.28	17.22	10.89	83.87	31.60
T-01-08	22.02	20.28	16.11	11.09	78.73	32.34
T-01-09	26.49	22.59	18.46	11.13	76.23	35.92
T-01-10	24.73	21.82	18.61	11.40	76.55	35.67
T-01-11	26.85	23.43	19.91	11.68	87.45	39.13
D-01-01	25.17	22.84	19.31	12.08	78.00	30.77
D-01-02	26.77	23.55	19.28	11.61	82.77	31.21
D-01-03	25.22	21.91	18.62	11.69	85.05	28.10
D-01-04	25.85	21.85	18.80	11.36	81.73	32.50
D-01-05	24.80	21.22	18.44	11.26	77.33	29.83
D-01-06	23.82	21.24	18.11	10.97	59.88	35.74
D-01-07	23.52	19.84	18.60	11.72	60.54	37.19
D-01-08	24.54	21.82	17.83	10.96	76.31	39.72
D-01-09	24.14	21.71	17.88	12.05	80.65	37.76
D-01-10	23.43	20.32	16.61	10.73	81.33	38.43
D-02-01	23.28	21.62	17.87	11.82	78.40	42.75
D-02-02	23.16	20.10	17.36	10.98	74.29	41.52
D-03-01	25.23	22.91	18.58	11.95	71.67	41.22

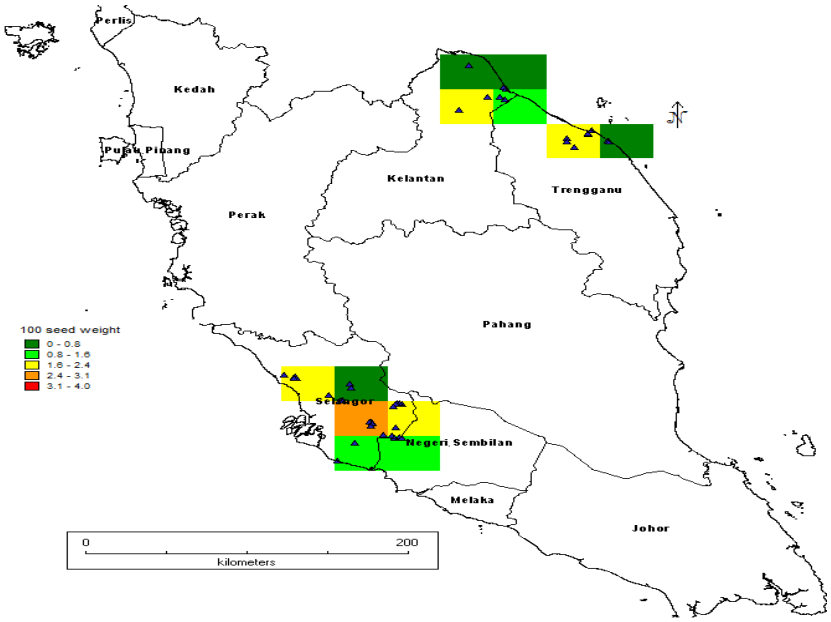

Fig 3. Grid map showing diversity index for 100 seed weight in *Jatropha curcas* germplasm collected from Selangor, Kelantan and Terengganu states, Malaysia.

Table 4. Correlation coefficients among six quantitative characters of *Jatropha curcas.*

	Fruit length	Fruit width	Seed length	Seed width	100 Seed weight	Oil content
Fruit length	1.000					
Fruit width	0.837**	1.000				
Seed Length	0.855**	0.738**	1.000			
Seed width	0.615**	0.645**	0.727**	1.000		
100 Seed weight	0.059	-0.060	-0.019	0.085	1.000	
Oil content	0.160	0.110	0.026	0.042	0.098	1.000

**Correlation is significant at p= 0.01 level

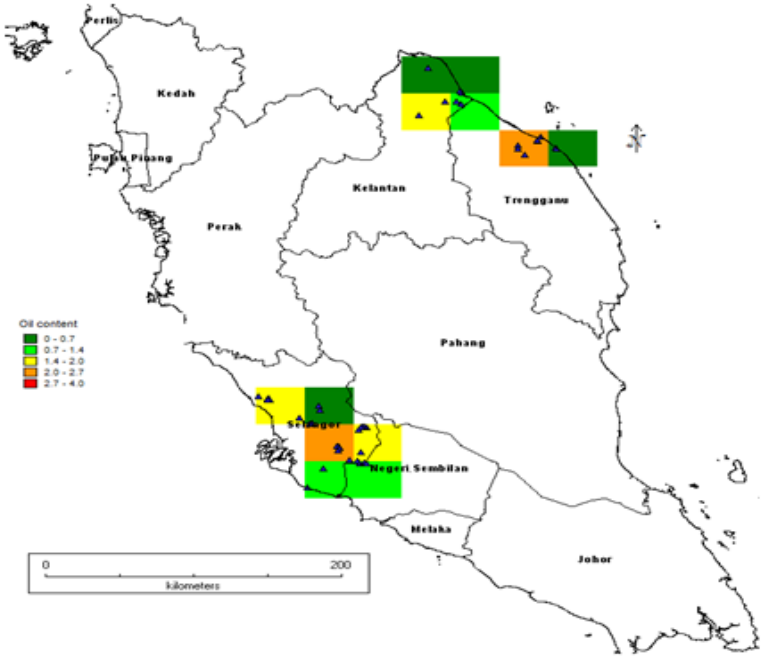

Fig 4. Grid map showing diversity index for oil content in *Jatropha curcas* germplasm collected from Selangor, Kelantan and Terengganu states, Malaysia.

Table 5. Eigenvectors and values of the first two principal components for six quantitative characters of 59 *Jatropha curcas* accessions.

Variables	Eigen vectors	
	PC1	PC2
Cumulative (Eigen values)	0.538	0.721
Fruit length	0.931	0.048
Fruit width	0.900	-0.073
Seed Length	0.925	-0.106
Seed width	0.823	-0.002
100 Seed weight	0.030	0.766
Oil content	0.139	0.705

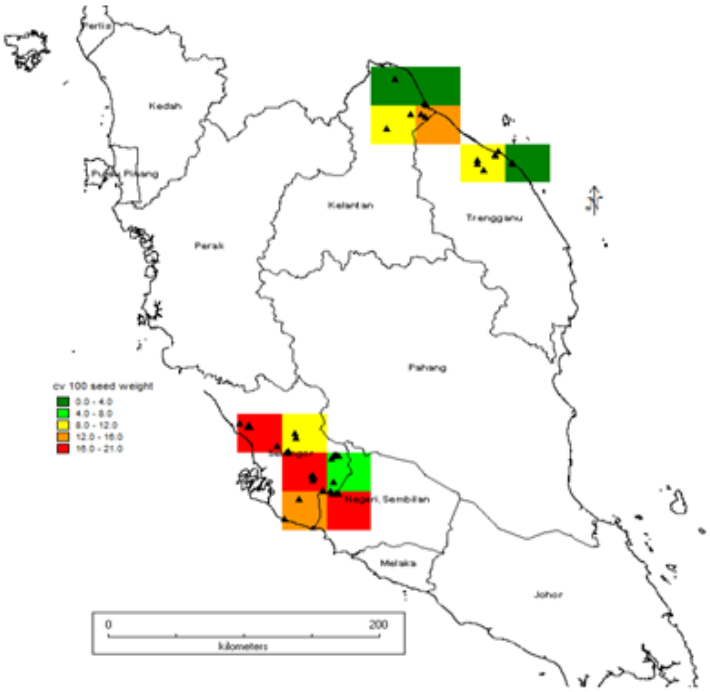

Fig 5. Coefficient of variation for 100 seed weight in *Jatropha curcas* accessions using DIVA-GIS.

recommended for inclusion in a hybridization program as they are expected to produce good segregants. Research on phenotypic diversity and suggested use of germplasm of *J. curcas* in hybridization programs in Malaysia are scanty. The findings reporting a limited number of germplasms of *J. Curcas* and their suggested use in hybridization was also reported by Divakara et al. (2010). The highest diversity index for oil content was observed in the *J. curcas* accessions collected from the northern parts and costal region of Terengganu state. Similar studies using DIVA-GIS have also been reported by several authors in *Jatropha* and other crops (Parthasarathy et al., 2006; Sunil et al., 2009). Sunil et al. (2009) generated grid maps for the distribution and diversity of *J. curcas* in the southeast coastal zone of India based on phenotypic traits to find the potential area for germplasms with high oil content. In piper, 15 morphological characters of 16 wild species from southern India were plotted for the hierarchical clusters and compared using DIVA-GIS to identify the areas or used to map species richness and diversity (Parthasarathy et al., 2006). The highest coefficient of variation for 100 seed weight was recorded in 3 parts of Selangor state, indicating that diverse accessions are available in this state. Application of GIS mapping has been successfully used in the recent past in assessing the genetic diversity and in identifying areas of high diversity of different crops or areas, such as *Phaseolus* bean, wild potatoes, forest vegetation, agro-biodiversity, medicinal plants and *Piper* (Pradesh et al., 2010). Analysis of our phenotypic diversity of *J. curcas* in germplasm could be facilitated further reliable classification of accessions and its identification with future utility for specific breeding purposes.

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