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GENETIC DIVERSITY AMONG CANDIDATE PLUS PLANTS (CPPs) IN JATROPHA (*Jatropha curcas*)

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The present study was conducted to evaluate genetic diversity among the progenies of 28 candidate plus plants (CPPs) selected from 487 populations of 20 accessions collected from seven different origins (South Africa, Cape Verde, India, Thailand, Vietnam, Indonesia and Malaysia). The progenies of CPPs were considered as genotypes and after diversity analysis they were grouped into five clusters. Eight genotypes were included in cluster V and were maximum number followed by seven genotypes in cluster III. The minimum number of genotypes was contained in cluster IV. The first eight principal component axes were accounted for 97.78% of the total variation with eigen values of above unity. The distance between cluster I with cluster II was maximum and the minimum distance revealed between cluster III and cluster V. The intra cluster distance within cluster I was maximum and close to cluster II. The lowest mean value for days to first flowering and first fruit maturity was found in Cluster I. The high mean values for most of the desirable characters exhibited by cluster I and II. It has been suggested that the genotypes from most diverge clusters could be utilized directly or used as parents in future breeding program.
Keywords: Physic nut, *Jatropha curcas*, clustering, genetic distance, D^2 analysis, seed yield, oil content, renewable energy.

INTRODUCTION

Physic nut (*Jatropha curcas*) belongs to the family Euphorbiaceae, known as 'jarak' in Malaysia, native to tropical America and domesticated to tropical and subtropical regions of Africa and Asia due to its ability to grow in a number of climatic zones particularly in marginal lands (Baker, 1877). Many plant species are reported to produce oils. Jatropha seed oil has attracted as an alternative source of fossil fuel among oil producing plants (Takeda, 1982; Benerji *et al.,* 1985; Martin and Mayeux, 1985; Openshaw, 2000). Rapeseed, soybean, oil palm, sunflower, wheat and Jatropha are known as a source of bio-diesel in many countries (USA, Germany, France, Bulgaria, Greece, Spain, Denmark, Brazil, Malaysia, Nicaragua and South America) of the world (Jayasingh, 2004; www.biomassfutures.eu). The uses of Jatropha oil range from herbal medicine, pesticide, dye, cosmetics, soap production, bio-absorbent etc. to viable alternatives of fossil fuel (Openshaw, 2000).

The seed and oil yield of currently using Jatropha is relatively low and unprofitable to farmers. Wide gap between potential and actual yield is due to the lack of high yielding plant material. So identification of new plant types with high seed yield and oil content are being prime objectives for further selection from the existing germplasm and multiplication as quality planting material (Islam *et al.,* 2009). The limitations of currently using germplasm in Malaysia are extent of diversity in the germplasm, poor yield, lack of knowledge on the genetic base and vulnerability to a wide range of pests and diseases. Development of high yielding variety is not possible without determining the extent of genetic diversity present in the existing germplasm, degree of influence on environment, heritability and genetic gain of the traits of interest (Dierig *et al.,* 2001).

Jatropha germplasm from Chinese origin showed low levels of genetic diversity (Sun *et al.,* 2008) and landraces from India showed modest levels of diversity (Basha and Sujatha, 2007; Ranade *et al.,* 2008). Based on AFLP markers 680 polymorphic fragments were found in 48 accessions from India (Tatikonda *et al.,* 2009) which classify germplasm accessions into five clusters. Published data on genetic diversity of African gene pool are limited. Jatropha accessions from Egypt and Uganda were found closer cluster to the Asian landraces compared with the Mexican accessions (Basha *et al.,* 2009). Several genetic diversity studies showed lower genomic diversity of gene pools of *Jatropha* from different origin (Yue *et al.,* 2013; Achten *et al.,* 2010). Systematic studies on breeding of high yielding genotypes have not been initiated in Malaysia. Large scope present for the development of high yielding Jatropha variety through genetic diversity study. Therefore, the present study was undertaken to study genetic diversity of Jatropha germplasm available in National University of Malaysia and selection of diverse genotypes for further breeding program.

MATERIALS AND METHODS

Seeds of twenty Jatropha accessions obtained from seven different sources (South Africa, Cape Verde, India, Thailand, Vietnam, Indonesia and Malaysia) were used as experimental materials. The seeds of 20 Jatropha accessions were sown in polybags to grow 487 seedlings as basic population. Seven weeks old healthy seedlings were transplanted in the field with a spacing of 2m × 2m. Experiment was conducted at Teres A, Rumah Tumbuhan, National University of Malaysia (UKM) during 2008-2010. Soil was marginal land previously under Acacia plantation (Islam *et al.*, 2010). The climate in Malaysia is generally tropical warm round the year with temperatures ranging from 21 to 32°C. Monsoon season is on its peak from November to February and receive annual rainfall of 2,500 mm. The relative humidity is high (70 to 90%) throughout the year and receives about six hours of sunshine per day (McGinley and Clough, 2010).

Twenty eight CPPs were selected on the basis of phenotypic traits and multiplied by cuttings from healthy branch. The stem cuttings were sown directly in polybags filled with mixture of sand, soil and farm yard manure (1:1:1). Eight weeks old healthy seedlings produced from stem cuttings were planted in the field at 2m × 2m spacing in completely randomized block design with three replications. Manures and fertilizers were applied in the pit @ FYM (2-3 kg), Urea (20 g), Single Super Phosphate (120 g) and Murate of Potash (16 g) (Punia, 2007). Necessary cultural practices were followed for raising good stand of Jatropha.

Data were recorded on five randomly selected plants in each replication on 21 yield related agronomic traits *viz.*, first flower bud appearance (days), first male flowering (days), first female flowering (days) and first fruit maturity (days), plant height (cm) and primary branches per plant at first flowering (no.), inflorescence per plant (no.), fruits per plant (no.), fruits per inflorescence (no.), seeds per plant (no.), seeds per fruit (no.), seed length (mm), seed width (mm), seed thickness, hundred seed coat weight (g), hundred kernel weight (g), hundred seed weight (g), moisture content (%), seed yield per plant (g), oil content (%), oil yield per plant (g). Jatropha seed oil was extracted from kernel powder by solvent extraction method using hexane (Sayyar *et al.,* 2009). Hexane was evaporated on a water bath until remained and extracted oil was filtered and recovered by removing the solvent. The oil yield was expressed in term of percentage of powdered sample.

Mean data for each trait was subjected to multivariate analysis by using GENSTAT 5.13, a computer based program, copyright 1987 (Mahalonobis, 1936; Rao, 1952; Digby *et al.*, 1989; Jeger *et al.,* 1983). More than one multivariate technique is required to discuss more clearly the results of genetic diversity (Singh and Chaudhary, 1985; Junned *et al.*, 1988; Ariyo, 1987; Patil *et al.,* 1987).

RESULTS

High genetic variability among the genotypes for all characters was noticed from the significant values of analysis

Table 1. Maximum, minimum and mean performance of 21 component characters of 28 CPPs of Jatropha with eigen values and percentage of variation of principal component analysis.

Traits	Min	Max	Mean	PCA axis	Eigen value	%Variation accounted for	Cumulative % of variation
First flower bud appearance (days)	118.00	157.70	135.50	A	9.70	52.54	52.54
First male flowering (days)	129.00	187.70	154.20	B	4.67	25.32	77.86
First female flowering (days)	130.30	189.00	155.10	C	1.53	8.32	86.18
First fruit maturity (days)	179.00	299.00	210.30	D	1.18	6.39	92.57
Plant height at first flowering	66.00	110.00	88.55	E	0.36	1.94	94.51
Primary branches per plant (no.)	0.33	6.00	2.36	F	0.22	1.18	95.69
Inflorescence per plant (no.)	3.00	11.00	6.15	G	0.20	1.07	96.76
Fruits per plant (no.)	35.00	93.50	61.41	H	0.19	1.02	97.78
Fruits per inflorescence (no.)	6.01	14.71	10.18	I	0.12	0.65	98.43
Seeds per plant (no.)	67.20	255.10	67.20	J	0.10	0.56	98.99
Seeds per fruit (no.)	1.73	3.00	2.59	K	0.07	0.38	99.37
Seed length (mm)	15.47	17.92	16.89	L	0.06	0.31	99.68
Seed width (mm)	10.43	12.07	10.97	M	0.03	0.19	99.87
Seed thickness (mm)	8.07	9.25	8.54	N	0.10	0.07	99.94
Hundred seed coat weight (g)	20.55	28.37	23.07	O	0.01	0.05	99.99
Hundred kernel weight (g)	31.66	42.83	36.78	P	0.01	0.01	100.00
Hundred seed weight (g)	44.00	64.00	56.36	Q	0.00	0.00	100.00
Moisture content (%)	50.80	62.17	56.11	R	0.00	0.00	100.00
Seed yield per plant (g)	40.43	147.95	90.09	S	0.00	0.00	100.00
Oil content (%)	32.37	48.32	42.61	T	0.00	0.00	100.00
Oil yield per plant (g)	13.34	71.49	38.94	U	0.00	0.00	100.00

of variance. It was also confirmed when aggregate effect of all traits was tested against Wilk's criterion.

***Principal component analysis (PCA)*:** The first eight principal component axes with eigen values above unity accounted for 97.78% of the total variation whereas the first two principal axes accounted for 77.86% of the total variation among the 21 yield related traits describing 28 CPPs (Table 1).

***Principal coordinate analysis (PCO)*:** The principal coordinate (PCO) analysis for selective combinations showed that the highest inter genotypic distance (2.877) was observed between the genotypes CPP01 and CPP26 followed CPP01 and CPP27 (2.732), CPP03 and CPP27 (2.585), CPP03 and CPP26 (2.399), CPP04 and CPP26 (2.256). The lowest distance was found between CPP16 and CPP21 (0.347), followed by CPP09 and CPP10 (0.432), CPP08 and CPP15 (0.458), CPP11 and CPP17 (0.482) and CPP11 and CPP23 (0.488) (Table 2).

The inter-genotypic distance matrix value of PCO was used to compute intra-cluster distances as suggested by Singh and Chaudhary (1985). No marked variation was observed in intra-cluster distances, which ranged from 0.200 to 0.314 (Table 3). The highest distance was found between CPPs of clusters I and cluster IV followed by cluster III and cluster IV. The CPPs of cluster I had the maximum intra cluster distance and cluster IV had the minimum distance.

***Canonical variate analysis (CVA)*:** Canonical variate analysis was performed to obtain the inter-cluster distances (Mahalanobis's D^2 values). These values of inter-cluster distance (D^2) represent the index of genetic diversity among the clusters and are presented in the Table 3. With regard to inter-cluster distance (Table 3), cluster II, showed maximum genetic distance from cluster I (40.80) followed by cluster II with cluster IV (30.38).

***Cluster analysis*:** Non-hierarchical clustering was done by using co-variance matrix and 28 CPPs under present study were grouped into five clusters. The clustering pattern indicates wide range of diversity among the CPPs for most of the traits. Five different clusters with their corresponding CPPs and their origin included in parentheses are presented in Table 4. It was observed that cluster V contained maximum number (8) of CPPs although its intra-cluster distance was minimum (0.275). The cluster IV (3) contained minimum number of CPPs with the lowest intra-cluster distance (0.200). The cluster I and cluster II contained 4 and 6 CPPs, respectively.

A wide range of variation was observed for all the traits for their cluster means (Table 5). The cluster I produced the

Table 2. Five highest and five lowest distance among the genotypes of Jatropha.

S. No.	Genotypic combination	Distance	S. No.	Genotypic combination	Distance
	Highest inter genotypic distance			**Lowest inter genotypic distance**	
1	CPP01 – CPP26	2.827	1	CPP16 – CPP21	0.347
2	CPP01 – CPP27	2.732	2	CPP09 – CPP10	0.432
3	CPP03 – CPP27	2.585	3	CPP08 – CPP15	0.458
4	CPP03 – CPP26	2.399	4	CPP11 – CPP17	0.482
5	CPP04 – CPP26	2.256	5	CPP11 – CPP23	0.488

Table 3. Inter and intra-group distances for combinations of 28 CPPs in Jatropha.

Clusters	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V
Cluster I	**0.314**				
Cluster II	40.80	**0.303**			
Cluster III	28.23	14.00	**0.277**		
Cluster IV	22.61	30.38	18.73	**0.200**	
Cluster V	18.26	25.90	12.98	13.32	**0.275**

Table 4. Grouping of 28 CPPs of Jatropha using Tocher's method.

Clusters	Number of genotypes	Genotype
Cluster I	4	CPP-01 (Indonesia), CPP-02 (Indonesia), CPP-03 (Malaysia), CPP-04 (Indonesia)
Cluster II	6	CPP-07 (Malaysia), CPP-08 (Malaysia), CPP-13 (India), CPP-18 (India), CPP-22 (Malaysia), CPP-24 (Indonesia)
Cluster III	7	CPP-06 (Malaysia), CPP-09 (Thailand), CPP-10 (Indonesia), CPP-11 (India), CPP-12 (Indonesia), CPP-14 (Malaysia), CPP-15 (Malaysia)
Cluster IV	3	CPP-25 (Cape Verde), CPP-26 (Vietnam), CPP-27 (The Phillipines)
Cluster V	8	CPP-05 (Malaysia), CPP-16 (Indonesia), CPP-17 (Indonesia), CPP-19 (Malaysia), CPP-20 (Malaysia), CPP-21 (Indonesia), CPP-23 (Malaysia), CPP-28 (South Africa)

Table 5. Group wise mean values of twenty one characters of 28 candidate plus trees in Jatropha.

Traits	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V
First flower bud appearance (days)	126.75	145.78	134.14	150.00	127.96
First male flowering (days)	142.67	169.22	152.19	170.45	144.33
First female flowering (days)	143.00	170.61	153.48	171.33	144.92
First fruit maturity (days)	193.25	221.17	211.71	261.33	190.46
Plant height at first flowering (cm)	91.75	93.67	79.57	90.22	90.33
Primary branches per plant at first flowering (no.)	2.33	2.61	3.14	2.33	1.50
Inflorescence per plant (no.)	7.33	5.61	6.62	5.78	5.71
Fruits per plant (no.)	81.32	61.89	63.48	38.28	57.98
Fruits per inflorescence (no.)	11.89	10.33	9.87	6.87	10.73
Seeds per plant (no.)	220.64	171.65	170.70	75.48	146.50
Seeds per fruit (no.)	2.72	2.77	2.69	1.97	2.54
Seed length (mm)	16.90	17.12	16.66	16.84	16.93
Seed width (mm)	10.87	11.18	10.85	11.09	10.92
Seed thickness (mm)	8.56	8.58	8.38	8.74	8.58
100 seed coat weight (g)	22.53	24.31	22.72	22.58	22.91
100 seed kernel weight (g)	36.06	37.55	35.98	35.19	37.86
100 seed weight (g)	56.27	59.67	51.67	60.81	56.35
Moisture content (%)	57.27	53.77	55.97	54.76	57.91
Seed yield per plant (g)	123.84	102.62	88.17	45.91	82.05
Oil content (%)	45.85	43.22	43.45	36.67	42.02
Oil yield per plant (g)	57.09	44.52	38.27	17.09	34.47

Table 6. Latent vectors for 21 characters of Jatropha genotypes.

Traits	Vector I	Vector II	Traits	Vector I	Vector II
First flower bud appearance (days)	3.58	0.26	Seed length (mm)	0.79	2.03
First male flowering (days)	-2.89	-0.63	Seed width (mm)	38.33	-3.09
First female flowering (days)	1.45	0.52	Seed thickness (mm)	-51.20	3.39
First fruit maturity (days)	-0.41	-0.13	100 seed coat weight (g)	-4.21	0.07
Plant height at first flowering (cm)	-0.11	-0.07	100 seed kernel weight (g)	2.55	-0.63
Primary branches per plant (no.)	-0.03	0.20	100 seed weight (g)	1.24	0.11
Inflorescence per plant (no.)	2.47	-0.82	Moisture content (%)	-2.38	-0.28
Fruits per plant (no.)	2.83	0.30	Seed yield per plant (g)	-1.31	-0.05
Fruits per inflorescence (no.)	1.36	-0.15	Oil content (%)	-1.26	0.16
Seeds per plant (no.)	-0.75	-0.07	Oil yield per plant (g)	0.79	0.29
Seeds per fruit (no.)	92.18	6.39			

lowest mean for first flowering (days), first fruit maturity (days) and the highest mean for inflorescence per plant (No.), fruits per plant (no.), fruits per inflorescence (No.), seeds per plant (No.), seeds per fruit (No.), seed yield per plant (g), oil content (%) and oil yield per plant (g). Cluster II composed of six genotypes and produced the highest seeds per fruit (No.), seed size (length, width in mm), hundred seed coat weight (g), hundred seed kernel weight (g) and hundred seed weight (g). The CPPs of cluster III produced the shortest plant height at first flowering and the highest primary branches per plant at first flowering (No.). Cluster IV had the highest mean value for seed thickness and composed of six CPPs. The genotypes of cluster V was important for seed kernel weight as they produced the highest cluster mean for hundred kernel weight (g).

***Contribution of each traits towards divergence of CPPs*:** It was revealed from Vector 1 (Z_1) that the important traits responsible for genetic divergence in the major axis of differentiation were seeds per fruit (92.18), seed width (38.33), fruits per plant (2.83), fruits per inflorescence (2.47) and days taken for flower bud appearance (3.58). In vector II (Z_2), the important traits were seeds per fruit (6.39), seed thickness (3.39), seed length (2.03) and days taken for first female flower (0.52). The values of days taken for first flower bud appearance, days taken for first female flowering, fruits per plant (No.), seeds per fruit (No.), seed length (mm), hundred seed weight (g) and oil yield per plant (g) was positive for both the vectors (Table 6) indicates their important role on the genetic divergence components.

DISCUSSION

***Genetic diversity and non-hierarchical clustering*:** There was no noticeable variation within CPPs grouped in the same cluster. The extent of diversity among the genotypes within the cluster was not always proportion to their number. Cluster I composed of four genotypes revealed the highest intra-cluster distance which indicates that the CPPs in this cluster might have diverged traits those contributes to the formed this cluster. Hence the CPPs fall into cluster I was the most heterogeneous and identified as the best parent for hybridization. The CPPs from cluster I could also be utilized in hybrid breeding due to the highest intra-cluster distances.

Inter-cluster distances (D^2) represent the index of genetic diversity between the genotypes of different clusters. The larger inter-cluster distances compared to intra-cluster distance indicates wider genetic diversity among the CPPs of different clusters. The maximum inter-cluster distance between cluster I and cluster II indicates the wide genetic distance between CPPs belongs to cluster I and cluster II. Hybridization could be done among the genotypes belonging to the distance clusters for obtaining wide range of segregants. Gohil and Pandya (2006) also pointed out that parents should be selected from two clusters with wider inter-cluster distance for hybridization to get maximum variability in *Salicornia brachiata* Roxb (a nontraditional oilseeds). The results obtained in the present study are in consistence with the findings of Basha and Sujatha (2007) in Jatropha and Nafees *et al*. (2015) in pomegranate.

Clustering pattern of the CPPs obtained from the principal component analysis was confirmed by non-hierarchical clustering. The pattern of clustering of CPPs in this study showed that the CPPs from the same origin were grouped into different clusters. For example, the CPPs of Malaysian origin was grouped into four different clusters out of five clusters. The genetic diversity of Jatropha accessions were found to be low and provenances from same regions were grouped into different cluster but provenances from distant regions grouped into same group (Satyawan and Tasma, 2011). The present study did not show any relationship between genetic diversity and geographical origin from the clustering pattern of CPPs. This geographic diversity is due to disparity of adoption, selection pressure, genetic drift and environment (Selvakumar *et al.*, 1989; Shahzad *et al*., 2013; Mehmood *et al*., 2013, 2014; Altaf *et al*., 2014; Khalil Ur Rahman *et al*., 2015). Swain and Diskhit (1997) also reported similar results in sesame.

A wide range of variation was found for all the traits for cluster means. The CPPs of cluster II possessed good contribution for seed related characters. The CPPs of cluster I and II had the most desirable traits which could be directly selected and utilized for the development of high yielding Jatropha variety. It was appeared from the PCA results that contribution of number of seeds per fruit was the highest followed by first flower bud appearance (days), seed length, fruits per plant (No.) in Jatropha genotypes. Significant genetic variation was reported in the literature among accessions, provenances and clones for plant growth, seed yield, seed oil content etc. (Ginwal *et al.*, 2004; Pant *et al.*, 2006; Kaushik *et al.*, 2007; Sunil *et al.*, 2008; Popluechai *et al.*, 2009). Seventy two jatropha accessions collected from 13 different countries including Mexico provides genetic diversity only among Mexican genotypes and other accessions (Basha *et al.*, 2009). Low genetic variation was also found in African and Indian accessions of Jatropha on the other hand Guatemalan and other Latin American accessions showed high genetic variation (Jongschaap *et al.*, 2007; Montes *et al.*, 2008). Surprisingly low levels of genetic diversity was found based on genetic markers revealed in Jatropha landraces from China (Sun *et al.*, 2008) and Indian landraces showed modest levels of genetic diversity (Basha and Sujatha, 2007; Ranade *et al.*, 2008).

***Comparison of different multivariate technique*:** Results obtained from different multivariate analysis were super imposed in Figure 1 and showed more or less similar results and each technique confirmed and supplemented the results of the other technique. The cluster pattern of D^2 analysis through non-hierarchical clustering has been taken care of simultaneous variation in all the characters under study. On the other hand the distribution of CPPs followed more or less similar trend of the PCA I (Z_1) and PCA II (Z_2) of the principal component analysis in different clusters of the D^2 analysis. The D^2 and principal component analysis were found to be alternative methods for getting information on genetic diversity of the genotypes. However, the canonical vector analysis (CVA) gave information on the contribution of each trait towards the divergence of the genotypes.

***Selection of genotypes for hybridization*:** No common criterion was followed during the selection of parents for hybridization. Parents could be selected on the basis of specific objectives of the variety development program. Generally, the parents from distant cluster were selected and believe to be able to produce higher heterosis or a wide range of segregants (Falconer, 1960; Moll *et al.*, 1962; Ramanujam *et al.*, 1974; Ghaderi *et al.*, 1984). Six CPPs (CPP-01, CPP-04, CPP-05, CPP-06, CPP-07 and CPP-18) from the present study were considered to perform batter if used in hybridization program depending on their magnitude of genetic distance, contribution of the different traits and *per se* performance. The CPPs of cluster I could be selected for the first flowering and fruit maturity (days), the highest inflorescences per plant (No.), fruits per plant (No.), fruits per inflorescence (No.), seeds per plant (No.), seeds per fruit (No.), seed yield per plant (g), oil content (%) and oil yield per plant (g). The CPPs of cluster II could be selected for the highest seeds per fruit (no.), seed size (mm), hundred seed coat weight (g), hundred kernel weight (g) and hundred seed weight (g). The CPPs of cluster III produced the highest plant

height at first flowering, primary branches per plant (No.). The CPPs of cluster IV had the highest seed thickness and CPPs of cluster V had the highest 100 seed kernel weight (g). ***Conclusion*:** From the above study, it was found that CPPs of cluster I and II have most desirable yield related traits and could be directly utilized as variety after necessary trial or selected as a parent for hybridization program for further improvement. However, crossing between the CPPs of distant clusters especially from cluster I, II and V may help to produce wide range of segregants in F_2 progeny. Thus the information might be helpful to manage upcoming limitations pertaining to the improvement of Jatropha for bio-diesel industry.

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