



## Genetic Potential of Agronomic Traits and Seed Physicochemical Properties of Novel *Jatropha* Lines Developed from Complex Interspecific Hybridization

Narathid Muakrong<sup>1)</sup> and Peerasak Srinives<sup>2,3\*)</sup>

<sup>1)</sup> Faculty of Agriculture, Princess of Naradhiwas University, Narathiwat 96000, Thailand

<sup>2)</sup> Department of Agronomy, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University, Kamphaeng Saen, Nakhon Pathom 73140, Thailand

<sup>3)</sup> The Royal Society of Thailand, Sanam Suea Pa, Dusit, Bangkok 10300, Thailand

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\*) Corresponding author:  
E-mail: agrpss@gmail.com

### ABSTRACT

Fifteen *Jatropha* lines were obtained from complex crossing between progenies derived from interspecific hybridization of *Jatropha curcas* and *J. integerrima*. They were evaluated for variability and association in yield, yield components, oil content, fatty acid composition, phorbol esters content, and growth characters. The major fatty acids in seed were oleic and linoleic acids, with a negative correlation among them. Seed yield showed a positive correlation with number of fruits per inflorescence and seeds per fruit, 100 seed weight and canopy size. A path coefficient analysis showed that number of fruits per inflorescence and canopy width had high positive direct relationship with seed yield per plant. Most characters showed high broad-sense heritability. Clustering by traits classified the breeding lines into five groups with a large distance between groups. The members in each group comprised lines of the same or similar pedigrees. Cluster I was the high seed yield group comprising two accessions (KUJL23 and KUJL18). Clustering based on principle components classified them into four groups. The lines in cluster II and III from cluster analysis were included in one group, while members of the other groups were the same in both clustering methods.

### INTRODUCTION

*Jatropha* or physic nut (*Jatropha curcas* L.) is a small to medium perennial shrub setting oil-bearing seed. The plant is native to Latin America around Mexico and spread worldwide to the tropical, sub-tropical and arid areas mainly with the migration and introduction of the Portuguese seafarers (Heller, 1996). It is a non-edible crop containing toxic substances, especially phorbol esters in seed (Li et al., 2010). The seed is oil rich with high unsaturated fatty acids (40% oleic acid and 39% linoleic acid) (Montes, Technow, Bohlinger, & Becker, 2013). The oil is suitable for producing biodiesel and bio-jet fuel (Montes, Technow, Bohlinger, & Becker, 2013).

*Jatropha* is easily planted in most of soil fertility levels and climate conditions. The plant has an enormous capacity to absorb and utilize nutrients under low-fertility conditions even it grows

on the poorest, mostly P deficient and acid soils. Research of Djajadi & Hidayati (2013) found that rain-fed *Jatropha curcas* had poorest growth among the treatment supporting that irrigation as one of limiting factor in *Jatropha curcas* cultivation for high biomass production.

The current *Jatropha* varieties have low seed yield of about 107-745 g/plant using Malaysian accessions planted at 2 m × 4 m spacing (Shabanimofrad, Rafii, Megat Wahab, Biabani, & Latif, 2013), 19-697 g/plant using Indian accessions under a 3 m × 3 m spacing (Tripathi, Mishra, & Shukla, 2013). While Tar, Tanya, & Srinives (2011) reported that the Myanmar accessions gave seed yield of 57-91 g/plant, Thai accessions 42-55 g/plant, and a Mexican accession 78 g/plant, when all grown under a 2 m × 2 m spacing. Most *Jatropha* accessions are collected from naturally undomesticated plants which

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had never been genetically improved and thus it is difficult for their yield to reach the economic threshold in commercial production (Divakara, Upadhyaya, Wani, & Gowda, 2010). Yet, the collected germplasm has low phenotypic and genetic variation (Rosado et al., 2010; Shen et al., 2010). Yue et al. (2014) could not detect the genetic variation using 29 microsatellites to assess 276 jatropha accessions from five countries in South America, Asia and Africa. The narrow genetic diversity in natural germplasm is the main barrier in current jatropha improvement (King et al., 2009).

The genetic variation can be created by interspecific hybridization (Parthiban et al., 2009). Interspecific hybridization between *J. curcas* and its related species is an effective tool in transferring important traits from the wide relatives to their progenies (One, Tanya, Muakrong, Laosatit, & Srinives, 2014). Genetic improvement through interspecific cross between *J. curcas* and *J. integerrima* has played important roles in improving jatropha yield and yield components (Parthiban et al., 2009), plant architecture (One, Muakrong, Phetcharat, Tanya, & Srinives, 2014), woody biomass (Muakrong, One, Tanya, & Srinives, 2014), and horticultural characters for ornamental purpose (Muakrong, Phetcharat, Tanya, & Srinives, 2014; Sujatha & Prabakaran, 2003). However, jatropha germplasm improvement by *J. curcas* × *J. integerrima* has faced with some bottleneck traits from *J. integerrima* such as small fruits and seeds (One, Tanya, Muakrong, Laosatit, & Srinives, 2014).

In our previous research, interspecific hybridization between *J. curcas* and *J. integerrima* was successful only when *J. curcas* served as female parent, with the suitable crossing duration spans from late morning to early afternoon. Their progenies showed high variation in flower colors, particularly in the F<sub>2</sub> population which could be classified into nine color groups (Muakrong, Tanya, & Srinives, 2014).

Thus the interspecific progenies should be selected and evaluated before using as germplasm in the breeding program. The objective of this research is to evaluate 15 diverse interspecific jatropha breeding lines for genetic variability, heritability and genetic advance from selection in major agronomic traits. The germplasm was developed by the project on Breeding to Accelerate Domestication of Novel Jatropha for Fuel and Feeds, Kasetsart University, Kamphaeng Saen campus, Thailand.

## MATERIALS AND METHODS

### Plant Materials and Experimental Design

Fifteen breeding lines of novel jatropha germplasm were developed from crossing and selecting among the elite progenies derived from interspecific crossing between *Jatropha curcas* (jatropha) and *J. integerrima* (peregrina). Their numbers are assigned after the coding of Kasetsart University Jatropha Breeding Line (KUJL). Their pedigrees are presented in Table 1. For a rapid increase of the breeding lines, the selected accessions were propagated by grafting shoot tips (~ 5 cm long) of each line on two-month-old stocks (1-1.5 cm stem base diameter) of local *J. curcas* accessions 'Chai Nat'. The grafted seedlings were kept in a small plastic tunnel to control the air humidity at about 80% and sun light at 65%. At 75 days after grafting, the seedlings were transplanted into an experimental field following a randomized complete block design (RCBD) with three replications. There were 5 plants in each plot (15 plants per accession). The plants were grown under a high density with the distance between plant was arranged at 1 m and between rows was 1.5 m (ca 6,667 plants per ha).

**Table 1.** Code and pedigrees of 15 interspecific jatropha breeding lines.

No.	Line code*	Pedigree**
1	KUJL5	(Cn/Jid)-4(X)-191
2	KUJL8	(Cn/Jid)-4(X)-319
3	KUJL9	(Cn/Jid)-4(X)-340
4	KUJL10	(Me/(Cn/Jid)-5)-2
5	KUJL11	(Nr/(Cn/Jid)-6)-3
6	KUJL14	(Cn/(Cn/Jid)-1)-29
7	KUJL18	(Ph/(Cn/Jid)-1)-2(X)-2
8	KUJL23	(Ph/(Cn/Jid)-6)-38(X)-2
9	KUJL26	(Cn/(Cn/Jid)-1)-2(X)-10
10	KUJL27	(Nr/(Cn/Jid)-1)-55(X)-2
11	KUJL74	(Me/(Cn/Jid)-2)-15(X)-22
12	KUJL104	[Me/(Cn/(Cn/Jid)-5)-31]-1
13	KUJL109	[Me/(Cn/(Cn/Jid)-9)-78]-30
14	KUJL111	[Nr/(Cn/(Cn/Jid)-2)-6]-5
15	KUJL113	[Me/(Me/(Cn/Jid)-3)-12]-9

Remarks: \* KUJL = Kasetsart University jatropha line. \*\* Cn, Ph and Nr are local varieties of *J. curcas* from Chai Nat, Phrae, and Nakhon Ratchasima provinces, Thailand, and Me is *J. curcas* from Mexico. Jid is dwarf *J. integerrima* from Thailand. The symbol /, //, /// represent the first, second and third crossings. (X) is a selfing symbol. The number in the pedigree indicates the selected plant number

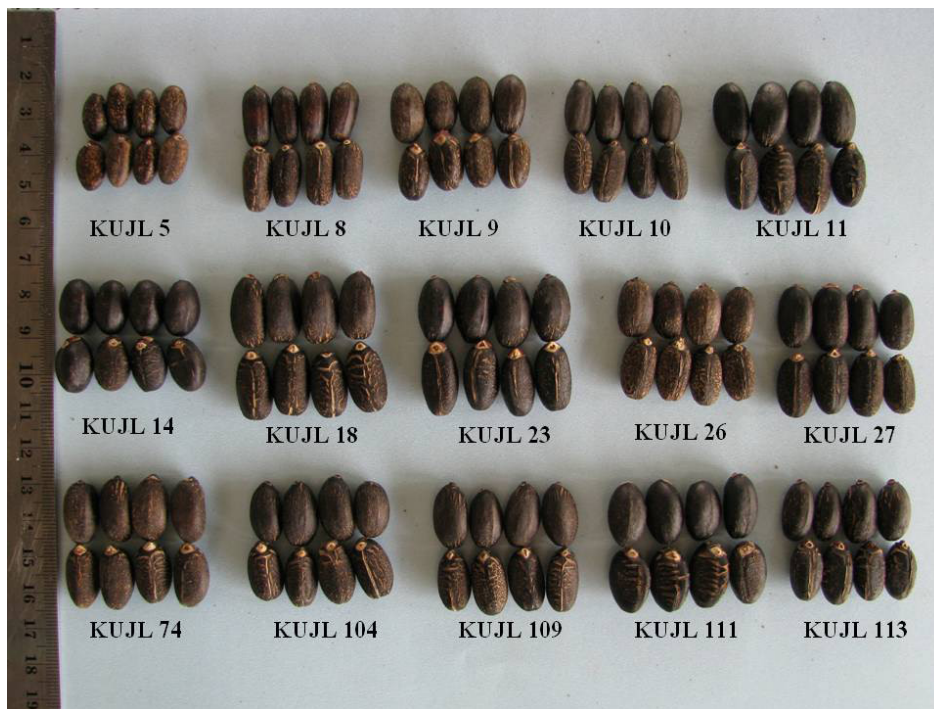
The experimental field belongs to the Agronomy Department, Kasetsart University, Kamphaeng Saen campus. The field located at the latitude 14.01° N and longitude 99.58° E, with the annual average temperature of 29.3°C, relative humidity of 71.4% and rainfall of 1,273 mm. The soil type at the site was sandy clay with the pH ranging between 6.5 - 7.6 and EC of 0.6 mS/cm. Each plant was basally applied with a commercial compost at 100 g per hill prior to transplanting. The plots were weeded every four months. A compound fertilizer of 15-15-15 (N-P2O5-K2O) with the dosage of 20 g/plant was applied at 6 and 12 months after transplanting. The experimental field was irrigated twice, the first-time right after transplanting, the second time at one month later. The experiment was conducted for 18 months starting from May 2016 until November 2017.

**Data Collection**

The leaf samples for each accession from the fiYield and yield components as well as growth characters were recorded on all 15 plants of each accession in three replicates. Seed yield (g per plant) was collected consecutively for full one year, starting from 4-5 months after transplanting (Fig. 1). Oil yield (g per plant) was determined from [(seed yield per plant × seed oil percentage)/100].

Seed shelling percentage was obtained from [(dry seed weight × 100)/dry fruit weight]. Weight of 100 seeds (g) was determined from 100 dry seeds in each plot. Number of seeds per fruit were recorded from a sample of 30 fruits from each plot. Seed length, seed width and seed thickness (mm) were measured from a sample of 30 seeds from each plot. Number of fruits per inflorescence were obtained by counting the number of fruits from 4 random inflorescences of each plant. At 18 months after transplanting, all plants were measured for canopy height and width (cm), and counted for number of primary and secondary branches per plant.

Total oil was extracted using an accelerated solvent extractor (ASE 350®, Dionex, USA); the solvent was evaporated by GeneVac®, EZ-2 series, USA. The content (Wt) was obtained from  $Wt (\%) = [(W2 - W1)/Ws] \times 100$ . Where W1 is weight of the empty bottle (g), W2 is weight of the bottle containing oil after drying (g), and Ws is dry weight of the sample (g). Major fatty acid composition of the oil was analyzed by a GC-FID Agilent 6890 series, following the method of Akbar, Yaakob, Kamarudin, Ismail, & Salimon (2009). While the phorbol esters content was analyzed by a HPLC (KONTRON Instrument) as suggested by Makkar, Becker, Sporer, & Wink (1997).



**Fig. 1** Seed of 15 interspecific jatropha breeding lines

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**Data Analysis**

The genotypic and phenotypic components of variance were estimated from the expected mean squares in the analysis of variance table as originally given by Falconer & Mackay (1996).

$$V_g = (\text{TrMS} - \text{ErMS})/r \dots\dots\dots 1)$$

Where:  $V_g$  : genotypic variance

$$V_p = (\text{TrMS} - \text{ErMS})/r + \text{ErMS} \dots\dots\dots 2)$$

Where :  $V_p$  : phenotypic variance

$$V_e = [(\text{TrMS} - \text{ErMS})/r + \text{ErMS}] - [(\text{T} - \text{ErMS})/r] \dots\dots\dots 3)$$

Where:  $V_e$  : environmental variance);  
 TrMS : mean square among accessions,  
 ErMS : mean square of error  
 r : number of replications

$$\text{PCV} = \frac{\sqrt{V_p}}{\bar{x}} \dots\dots\dots 4)$$

Where : PCV: phenotypic coefficient of variability

$$\text{GCV} = \frac{\sqrt{V_g}}{\bar{x}} \dots\dots\dots 5)$$

Where: GCV : genotypic coefficient of variability

$$\text{ECV} = \frac{\sqrt{V_e}}{\bar{x}} \dots\dots\dots 6)$$

Where: ECV: environmental coefficient of variability;  
 $\bar{x}$  : the grand mean of the trait.

Broad-sense heritability of each trait was estimated following Falconer & Mackay (1996).

$$\text{Broad-sense heritability } (h_b^2) = \frac{V_g}{V_p} \dots\dots\dots 7)$$

Where:  $h_b^2$  : Broad-sense heritability

Genetic advance and genetic advance as percentage of mean were estimated from the following equations:

$$\text{GA} = \left[ \frac{V_g}{V_p} \right] \times (\sqrt{V_p}) \times k \dots\dots\dots 8)$$

Where: GA : genetic advance  
 Where:  $k$  = selection differential at 5 percent  
 selection intensity = 2.06 (Allard, 1960).

$$\text{GA\%} = \frac{\text{GA}}{\bar{x}} \times 100 \dots\dots\dots 9)$$

Where: GA%: genetic advance as percentage of mean;  $\bar{x}$  = grand mean of the trait

The genetic and phenotypic correlations ( $r_g$  and  $r_p$ ) between two traits,  $X_1$  and  $X_2$ , were estimated as:

i) Genotypic correlation ( $r_g$ )

$$r_g = \frac{\text{Cov}_g(X_1 X_2)}{\sqrt{V_g(X_1) \cdot V_g(X_2)}} \dots\dots\dots 10)$$

Where:  $\text{Cov}_g(X_1 X_2)$  = genetic covariance among trait  $X_1$  and  $X_2$ ; and  $V_g(X_1) = V_g(X_2)$  genetic variance for trait  $X_1$  and  $X_2$ , respectively

ii) Phenotypic correlation ( $r_p$ )

$$r_p = \frac{\text{Cov}_p(X_1 X_2)}{\sqrt{V_p(X_1) \cdot V_p(X_2)}} = \dots\dots\dots 11)$$

Where:  $\text{Cov}_p(X_1 X_2)$  = genetic covariance among trait  $X_1$  and  $X_2$ ;  $V_p(X_1) = V_p(X_2)$  = genetic variances for trait  $X_1$  and  $X_2$ , respectively.

Path coefficients were analyzed based on the significance of phenotypic correlation coefficients to further investigate direct and indirect effects of the contributing traits on seed yield using the method outlined by Dewey & Lu (1959). All statistical analyses were performed using the R freeware program (R Development Core Team, 2010). Cluster analysis and principle components analysis were analyzed using the package R-Commander (Rcmdr) version 1.9-6 (Fox, 2005) in the R program version 2.15.2 (R Development Core Team, 2010). The dendrogram was constructed following Ward method (Ward Jr, 1963). The Euclidean distances were determined using standardized values of the 22 agronomic traits.

**RESULTS AND DISCUSSION**

**Yield and Yield Components**

The analyses of variances showed that the jatropha lines were varied in all traits (Table 2). KUJL23 and 18 gave the highest mean seed yield per plant of 673 and 657 g, respectively. While KUJL 9, 14 and 8 were the lowest at 246, 250 and 279 g/plant, respectively. KUJL74 and 104 gave the highest shelling percentage (70.0 and 69.6 %), while KUJL11 was the lowest at 51.2 %. KUJL23 gave the highest 100 seed weight (69.0 g). Although KUJL5 showed the lowest 100 seed weight (27.6 g), it had the highest number of inflorescences per plant (313), while KUJL27 had the highest number of fruits per inflorescence (12.6). KUJL104, 109 and 18 had the highest number of seeds per fruit with the values of 2.8, 2.8 and 2.7, respectively. KUJL111 showed large seed size with 11.2 mm wide and 9.2 mm thick, while KUJL18, 23 and 11 gave the longest seed of 18.0, 17.9 and 17.6 mm, respectively.

**Table 2.** Mean yield and yield components of 15 interspecific jatropha breeding lines.

Accessions	Yield (g/plant)	No. inflorescences per plant	No fruits per inflorescence	100 Seed weight (g)	Seed shelling (%)	No. seeds per fruit	Seed width (mm)	Seed length (mm)	Seed thickness (mm)
KUJL5	397 d	313.0 a	5.6 g	27.6 h	56.1 g	1.7 f	7.8 h	13.2 h	6.3 g
KUJL8	279 g	69.7 e	6.4 f	46.4 g	62.2 de	1.7 f	7.8 h	16.2 e	7.2 f
KUJL9	246 g	49.6 g	6.5 f	46.5 g	63.0 de	1.7 f	9.7 ef	15.5 f	8.5 c
KUJL10	469 c	83.3 d	9.4 d	45.6 g	56.7 g	2.1 e	8.6 g	16.4 de	7.3 ef
KUJL11	392 de	60.9 efg	10.4 b	55.0 e	51.2 h	2.1 e	10.2 bc	17.6 ab	7.6 de
KUJL14	250 g	58.3 efg	5.9 fg	49.4 f	58.1 g	1.7 f	10.2 b	14.8 g	8.5 c
KUJL18	657 a	100.0 c	11.0 b	65.4 b	59.0 fg	2.7 a	10.4 b	18.0 a	8.9 b
KUJL23	673 a	103.3 c	10.3 b	69.0 a	61.5 ef	2.2 de	10.1 bcd	17.9 a	8.6 bc
KUJL26	351 ef	61.0 efg	7.7 e	49.5 f	65.1 cd	2.3 cd	9.7 def	15.2 fg	7.5 de
KUJL27	482 c	55.3 fg	12.6 a	56.0 de	61.9 ef	2.5 b	10.1 bcde	16.6 de	7.4 ef
KUJL74	476 c	59.7 efg	10.6 b	57.6 d	70.0 a	2.3 c	10.5 b	16.9 cd	8.5 c
KUJL104	376 de	56.3 fg	10.2 bc	61.0 c	69.6 a	2.8 a	10.4 b	17.3 bc	8.8 bc
KUJL109	555 b	82.3 d	10.7 b	58.3 d	66.1 bc	2.8 a	9.7 cdef	16.7 de	8.5 c
KUJL111	458 c	125.3 b	9.5 cd	65.5 b	66.1 bc	2.3 cd	11.2 a	17.4 bc	9.2 a
KUJL113	328 f	66.0 ef	9.5 cd	51.2 f	69.0 ab	2.1 e	9.3 f	16.3 e	7.8 d
F-test	**	**	**	**	**	**	**	**	**
CV (%)	5.93	6.93	4.69	2.5	2.73	3.36	2.6	1.85	2.45

Remarks: \* Significant at P ≤ 0.01; Mean values of each trait followed by the same letters are not significantly different at ≤ P 0.05 by DMRT

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**Table 3.** Mean values of growth characters of 15 interspecific jatropha breeding lines.

Breeding lines	No. primary branches	No. secondary branches	Canopy height (cm)	Canopy width (cm)
KUJL5	5.2 c	87.4 a	170 ef	120 bcd
KUJL8	4.2 d	50.9 c	125 g	113 cd
KUJL9	2.9 ef	19.4 f	112 gh	84 e
KUJL10	6.0 b	65.3 b	207 cd	103 de
KUJL11	4.5 c	22.5 ef	240 ab	100 de
KUJL14	3.9 d	30.7 e	119 g	105 de
KUJL18	5.4 c	40.8 d	248 a	150 a
KUJL23	4.4 d	43.6 cd	182 e	137 abc
KUJL26	3.3 e	26.8 ef	97 h	103 de
KUJL27	4.9 c	31.2 e	224 bc	119 bcd
KUJL74	3.4 e	27.7 ef	172 ef	124 bcd
KUJL104	4.6 c	31.0 e	180 e	114 cd
KUJL109	5.4 c	48.4 cd	153 f	124 bcd
KUJL111	7.8 a	49.2 cd	188 de	141 ab
KUJL113	2.6 f	23.3 ef	215 c	110 d
<i>F</i> -test	**	**	**	**
CV (%)	3.96	12.71	6.97	11.29

Remarks: \* Significant at  $P \leq 0.01$ ; Mean values of each trait followed by the same letters are not significantly different at  $\leq P 0.05$  by DMRT.

In this study, KUJL23 and 18 were observed to have higher in yield and 100 seed weight under high population density (planting space of 1 x 1.5 m) which gave a comparable seed yield per plant as the Indian local accession "AFRI50" when planted with the space of 3 x 3 m (697.5 g/plant) (Tripathi, Mishra, & Shukla, 2013). While a Malaysian accession "B-03-02" gave 674.6 g/plant when planted under 2 x 4 m planting space. (Shabanimofrad, Rafii, Megat Wahab, Biabani, & Latif, 2013), and a new jatropha variety "JO S2" derived from an open pollination of accessions from Malaysia and Thailand gave 658.8 g/plant at 2 x 2 m planting space (Yi et al., 2014). KUJL5 in this experiment had the highest number of inflorescences per plant, but some of them dropped during fruit development stage.

#### Growth Characters

Scion and root stock of all accessions were well compatible. Table 3 shows the mean numbers of primary and secondary branches, canopy height and canopy width. The breeding lines were varied in all characters. KUJL111 gave the highest number of primary branches (7.75), while KUJL5 gave the highest number of secondary branches (87.4).

KUJL18 was the tallest (248 cm) whereas KUJL26 was the shortest (97 cm). KUJL18 had the widest canopy (150 cm) and KUJL9 was the narrowest (84 cm). An ideal jatropha varieties should have a smaller canopy which can be grown in a higher population density and yet easy to harvest (Achten et al., 2010). KUJL26 is a promising germplasm for improvement of plant type and harvest index. The previous report by One, Muakrong, Phetcharat, Tanya, & Srinives (2014) confirmed that *J. integerrima* is a good source of dwarf plant type, which is controlled by a recessive gene.

#### Oil Content and Seed Physicochemical Properties

Seed oil content, oil yield per plant, contents of four major fatty acids and phorbol esters (PEs) were significantly different among the lines (Table 4). KUJL113, 74 and 10 had the highest seed oil content at 37.0, 36.9 and 36.7%, respectively, while KUJL14 gave the lowest content at 19.58%. However, the oil content per plant was high in KUJL18 (206.2 g) and KUJL23 (190.5 g), while oleic acid content was high in KUJL26, 10, 18, 14 and 74 at 52.0, 50.7, 50.3, 49.4 and 49.1%, respectively. Linoleic acid content was detected higher in KUJL27, 9, 5, 23 and 11 at 45.6, 43.8, 42.9, 42.6 and 41.6%, respectively.

**Table 4.** Mean values of seed oil content, fatty acid composition and phorbol esters content of 15 interspecific jatropha breeding lines

Breeding lines	Seed oil content (%)	Oil yield per plant (g)	Oleic (%)	Lin-oleic (%)	Palmitic (%)	Stearic (%)	Unsaturated fatty acid (%)	Saturated fatty acid (%)	Phorbol esters (mg/g)
KUJL5	28.1 e	111.7 e	36.5 cd	42.9 ab	10.3 g	10.4 a	79.4 abc	20.6 cde	3.1 a
KUJL8	33.4 b	93.1 f	43.1 b	35.2 c	13.9 bc	7.8 bc	78.3 bc	21.7 cd	1.5 cdef
KUJL9	33.0 b	81.0 f	33.8 d	43.8 ab	13.9 bc	8.5 abc	77.6 cd	22.4 bc	2.1 bc
KUJL10	36.7 a	171.9 c	50.7 a	26.8 ef	12.7 de	9.8 ab	77.5 cd	22.5 bc	1.6 cdef
KUJL11	30.7 cd	120.5 de	36.2 cd	41.6 ab	13.4 cd	8.8 abc	77.8 cd	22.2 bc	1.7 bcdef
KUJL14	19.6 g	49.0 g	49.4 a	29.5 de	13.4 cd	7.7 c	79.0 bc	21.0 cd	1.3 def
KUJL18	31.4 c	206.2 a	50.3 a	25.1 f	14.7 a	9.9 ab	75.4 e	24.6 a	1.6 cdef
KUJL23	28.3 e	190.5 b	33.6 d	42.6 ab	15.0 a	8.8 abc	76.2 de	23.8 ab	2.3 b
KUJL26	31.3 c	109.8 e	52.0 a	27.9 ef	11.8 f	8.3 abc	80.0 ab	20.1 de	1.2 ef
KUJL27	27.8 ef	133.8 d	35.5 cd	45.6 a	11.9 f	7.0 cd	81.1 a	18.9 e	1.9 bcd
KUJL74	36.9 a	175.3 c	49.1 a	29.4 de	12.8 d	8.6 abc	78.5 bc	21.5 cd	1.8 bcde
KUJL104	29.9 d	112.5 e	44.0 b	34.0 c	13.1 d	8.9 abc	78.0 bcd	22.0 bcd	1.1 f
KUJL109	32.9 b	182.8 bc	45.7 b	32.8 cd	14.0 bc	7.6 c	78.4 bc	21.6 cd	1.1 f
KUJL111	26.8 f	122.6 de	38.6 c	41.4 b	14.6 ab	5.4 d	80.0 ab	20.0 de	1.8 bcd
KUJL113	37.0 a	121.4 de	45.3 b	34.0 c	12.1 ef	8.5 abc	79.4 abc	20.6 cde	1.5 cdef
F-test	**	**	**	**	**	**	**	**	**
CV (%)	2.5	5.58	18.55	6.32	2.96	12.99	1.37	4.97	18.56

Remarks: \*\* Significant at  $P \leq 0.01$ ; Mean value of each trait followed by the letters are not significantly different at  $\leq P 0.05$  by DMRT

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Palmitic and stearic acid contents were lower than 15% in all accessions. Higher total unsaturated fatty acid (oleic and linoleic acid) contents were found in KUJL27, 111, 26, 113 and 5 at 81.1, 80.0, 80.0, 79.4, and 79.4%, respectively. All tested accessions had less than 25% total fatty acid content (palmitic and stearic acids). The breeding line with the highest phorbol esters content was KUJL5 (3.06 mg/g), and the lowest was detected at three accessions namely, KUJL109, 104, and 26 with the values of 1.13, 1.14 and 1.19 mg/g, respectively.

This study showed that seed oil content and oil yield per plant were diverse among these breeding lines. High quality vegetable oil for biodiesel production should have high content of mono-unsaturated fatty acids (MUFA), and low saturated and poly-unsaturated fatty acids. In this study, all interspecific lines showed low saturated fatty acids, while KUJL26, 10 and 18 have high MUFA, such as over 50% oleic acid (C18:1).

These accessions are the elite germplasm for improving high oleic acid content. However, the phorbol esters contents in these lines are classified as toxic (PEs > 1mg/g), and thus they cannot be used for improving low PEs. A non-toxic jatropha plant should have the PEs content of lower than 0.01mg/g (Francis, Oliver, & Sujatha, 2013).

#### **Genetic Associations among the Agronomic Traits**

This set of interspecific jatropha breeding lines showed wide ranges of phenotypic values in many traits, viz. seed yield per plant (246-673 g), number of inflorescences per plant (50-313), 100 seed weight (28-69 g), oil yield per plant (49-206 g), number of secondary branches (19-87), plant height (97-248 cm), and plant width (84-150 cm). Our research, ranges are much wider than the reports in *J. curcas* by Rao, Korwar, Shanker, & Ramakrishna (2008), Shabanimofrad, Rafii, Megat Wahab, Biabani, & Latif (2013), and Tripathi, Mishra, & Shukla (2013). Variance components, broad-sense heritability, and genetic advance (response to selection) of the jatropha lines are shown in Table 5, confirming high variability among the accessions.

The number of inflorescences per plant give the highest PCV (73.23%) and GCV (72.90%), both values are very close revealing that this trait is less affected by the environment. The traits with rather high ECV are phorbol esters content (5.67%), stearic acid (5.50%), seed yield (5.17%) and plant width (4.16%), revealing relatively high environmental

effects. Most traits give high broad-sense heritability of over 0.90; except for seed yield (0.72), phorbol ester (0.69), total unsaturated fatty acids (0.61), total saturated fatty acids (0.61), plant width (0.58) and stearic acid content (0.48). The traits with high heritability are number of inflorescences per plant, fruits per inflorescence and seeds per fruit, as well as 100 seed weight, seed shelling percentage, seed width, seed length, seed thickness, seed oil content, oil yield per plant, number of primary and secondary branches, plant height, contents of oleic, linoleic and palmitic acids. Traits with moderate to high heritability are responsive even to phenotypic selection.

Shabanimofrad, Rafii, Megat Wahab, Biabani, & Latif (2013) suggested that the estimates of heritability and genetic advance can be used for predicting response to selection of the superior individuals. Genetic advance as percentage of mean (GA%) was the highest in number of inflorescences per plant (149.50%) and lowest in content of total unsaturated fatty acids (2.76%). The GA% of seed yield, oil yield per plant, and oil content were observed at 51.1, 68.0 and 29.9%, respectively. The high  $h^2_b$  estimates and the low GA (and GA%) indicate that non-additive gene effects and/or genotype  $\times$  environment interaction play significant role in expression of number of fruits per inflorescence, number of seeds per fruit, 100 seed weight, seed width, seed length, seed thickness, and contents of seed oil, oleic, linoleic and palmitic acids. Number of inflorescences and secondary branches, and oil yield per plant showed high  $h^2_b$  and GA. Thus GCV,  $h^2_b$  and GA of these traits can be used as indicators of a potential germplasm source. The number of inflorescences, primary and secondary branches, yield of seed and oil, and plant height showed high to moderate PCV, suggesting that it is not difficult to transfer these traits to their progenies and phenotypic selection to improve the traits is effective.

#### **Correlation and Path Coefficient Analyses**

Phenotypic and genotypic correlation coefficients ( $r_p$  and  $r_g$ ) among 12 agronomic traits are given in Table 6. Seed yield showed high positive  $r_p$  and  $r_g$  with number of fruits per inflorescence, number of seeds per fruit, 100 seed weight, seed length, plant width, number of primary branches and plant height. This suggested that seed yield per plant would increase with the improvement of these characters.



**Table 5.** Genetic variance estimates of 22 agronomic traits of 15 interspecific jatropha breeding lines

Traits	Range	$V_p$	$V_g$	$V_e$	PCV	GCV	ECV	$h^2_B$	GA (%)
Seed yield per plant (g)	246 - 673	21402.55	15445.30	5957.25	34.35	29.18	5.17	0.72	51.06
No. inflorescences per plant	50 - 313	4298.17	4259.67	38.51	73.23	72.90	0.33	0.98	149.50
No. fruits per inflorescence	5 - 12	4.66	4.47	0.18	23.73	23.26	0.47	0.96	46.90
No. seeds per fruit	1.68 - 2.81	0.15	0.14	0.01	17.63	17.30	0.32	0.96	33.88
100 seed weight (g)	28 - 69	108.45	106.66	1.80	19.43	19.27	0.16	0.98	39.37
Seed shelling (%)	51 - 70	31.59	28.70	2.89	9.01	8.59	0.42	0.91	16.87
Seed width (mm)	7 - 11	1.01	0.94	0.06	10.32	9.99	0.33	0.94	19.84
Seed length (mm)	13 - 18	1.75	1.66	0.09	8.06	7.84	0.21	0.95	15.75
Seed thickness (mm)	6 - 9	0.68	0.65	0.04	10.30	10.00	0.30	0.94	20.21
Seed oil content (%)	20 - 37	21.24	20.65	0.60	14.91	14.70	0.21	0.97	29.86
Oil yield per plant (g)	49 - 206	2007.28	1952.96	54.33	33.90	33.44	0.46	0.97	67.95
No. primary branch	3 - 8	1.76	1.73	0.03	29.13	28.86	0.27	0.98	58.97
No. secondary branch	19 - 87	356.11	330.43	25.67	47.33	45.59	1.74	0.93	90.46
Plant height (cm)	97 - 248	2325.39	2175.53	149.86	27.47	26.57	0.90	0.94	52.95
Plant width (cm)	84 - 150	410.58	237.71	172.87	17.39	13.23	4.16	0.58	20.74
Unsaturated fatty acid (%)	75 - 81	2.96	1.81	1.15	2.20	1.72	0.48	0.61	2.76
Saturated fatty acid (%)	19 - 25	2.96	1.81	1.15	7.98	6.24	1.74	0.61	10.05
Oleic acid (%)	34 - 52	46.44	43.27	3.17	15.88	15.33	0.55	0.93	30.48
Linoleic acid (%)	25 - 46	51.36	46.31	5.04	20.18	19.16	1.02	0.90	37.48
Palmitic acid (%)	10 - 15	1.74	1.58	0.15	10.01	9.56	0.45	0.91	18.74
Stearic acid (%)	5 - 10	2.31	1.12	1.19	18.10	12.60	5.50	0.48	18.06
Phorbol ester (mg/g)	1.13 - 3.06	0.32	0.22	0.10	33.20	27.53	5.67	0.69	46.98

Remarks:  $V_p$  = Phenotypic variance,  $V_g$  = Genotypic variance,  $V_e$  = Environmental variance, PCV = Phenotypic coefficient of variation, GCV = Genotypic coefficient of variation, ECV = Environmental coefficient of variation,  $h^2_B$  = Broad-sense heritability, and GA (%) = Genetic advance as percentage of mean

**Table 6.** Phenotypic and genotypic (in parentheses) correlation coefficients among 12 agronomic traits of 15 interspecific jatropha breeding lines.

	2	3	4	5	6	7	8	9	10	11	12	
1	Yield (g/ plant)	0.152 (0.263)	0.690** (0.897**)	0.621** (0.831**)	0.599** (0.813**)	0.315 (0.428)	0.602** (0.850**)	0.296 (0.433)	0.455* (0.694**)	0.249 (0.359)	0.554* (0.839**)	0.786** (0.892**)
2	No. inflores- cences per plant	-0.361 (-0.364)	-0.243 (-0.242)	-0.506* (-0.508)	-0.448* (-0.451)	-0.503* (-0.506)	-0.443* (0.450)	0.375 (0.372)	0.823** (0.826**)	0.070 (0.069)	0.307 (0.351)	
3	No. fruits per inflorescence		0.809** (0.814**)	0.720** (0.718**)	0.557* (0.552)	0.799** (0.812**)	0.359 (0.355)	0.246 (0.246)	-0.277 (0.293)	0.711** (0.735**)	0.457* (0.543)	
4	No. seeds per fruit			0.648** (0.718**)	0.510* (0.552)	0.617* (0.812**)	0.451* (0.592**)	0.303 (0.246)	-0.149 (-0.278)	0.438 (0.735**)	0.518* (0.543)	
5	100 seed weight (g)				0.807** (0.814**)	0.899** (0.907**)	0.817** (0.822**)	0.229 (0.229)	-0.442* (0.457)	0.350 (0.357)	0.569* (0.617**)	
6	Seed width (mm)					0.606** (0.597*)	0.838** (0.839**)	0.156 (0.157)	-0.591* (0.610**)	0.229 (0.243)	0.330 (0.388)	
7	Seed length (mm)						0.614** (0.602**)	0.257 (0.268)	-0.361 (0.364)	0.575* (0.587)	0.472* (0.508)	
8	Seed thick- ness (mm)							0.146 (0.146)	-0.465* (0.480)	0.038 (0.035)	0.387 (0.462)	
9	No. primary branches								0.594** (0.579)	0.365 (0.369)	0.554* (0.658**)	
10	No. second- ary branches									0.054 (0.056)	0.323 (0.379)	
11	Plant height (cm)										0.442* (0.530')	
12	Plant width (cm)											

Remarks: \* Significant at  $P=0.05$ , \*\* Significant at  $P=0.01$

**Table 7.** Phenotypic and genotypic (in parentheses) correlation coefficients between fatty acid compositions and phorbol ester composition in 15 interspecific jatropha breeding lines.

Traits	Linoleic	Palmitic	Stearic	Phorbol ester
Oleic	- 0.977** (- 0.954**)	0.169 (0.221)	- 0.077 (- 0.109)	- 0.649** (- 0.714**)
Linoleic		- 0.060 (- 0.024)	- 0.280 (-0.237)	0.617** (0.665**)
Palmitic			- 0.305 (-0.329)	- 0.268 (- 0.219)
Stearic				0.312 (0.436)

Remarks: \* Significant at  $P=0.05$ , \*\* Significant at  $P=0.01$

**Table 8.** Path coefficient analysis of the direct and indirect effects of seven traits related to seed yield in 15 interspecific jatropha breeding lines.

Traits	Correlation coefficient	Direct effect	Indirect effect							Total
			1	2	3	4	5	6	7	
No. fruits per inflorescence	0.690**	0.687	-	- 0.108	0.197	- 0.266	0.007	- 0.153	0.326	0.003
No. seeds per fruit	0.621**	- 0.134	0.556	-	0.152	- 0.239	0.009	- 0.094	0.370	0.754
Seed length (mm)	0.602**	0.274	0.548	- 0.083	-	- 0.331	0.008	- 0.124	0.337	0.355
100 seed weight (g)	0.599**	- 0.369	0.495	- 0.087	0.222	-	0.007	- 0.075	0.406	0.968
No. primary branches	0.455*	0.030	0.169	- 0.041	0.063	- 0.084	-	- 0.079	0.396	0.424
Plant height (cm)	0.554*	- 0.215	0.488	- 0.059	0.142	- 0.129	0.011	-	0.316	0.769
Plant width (cm)	0.787**	0.714	0.314	- 0.069	0.116	- 0.210	0.017	- 0.095	-	0.073

Remarks: \* Significant at  $P=0.05$ , \*\* Significant at  $P=0.01$

However, there was no agronomic traits found correlated with oil content (data not shown). Number of inflorescences per plant showed negative  $rp$  and  $rg$  with 100 seed weight, seed width, seed length and seed thickness revealing that high number of inflorescences per plant tended to reduce seed size and seed weight. The findings were similar with Nietzsche et al. (2015), that reported the number of secondary branches had negative correlations with 100 seed weight, seed width and seed thickness.

Table 7 showed  $rp$  and  $rg$  between fatty acid compositions and phorbol esters content. Oleic acid content had negative correlations with linoleic acid and PEs contents, while linoleic acid showed a high positive correlation with PEs. Thus both linoleic acid and phorbol esters contents would decreased when selection was practiced for high oleic acid content in this population.

Considering both direct and indirect effects of agronomic traits on seed yield through path

coefficient analysis (Table 8), number of fruits per inflorescence and canopy width had high positive direct relationship with seed yield (0.690 and 0.714), but low direct negative relationship with number of seeds per fruit (- 0.134), 100 seeds weight (- 0.369), and plant height (- 0.215).

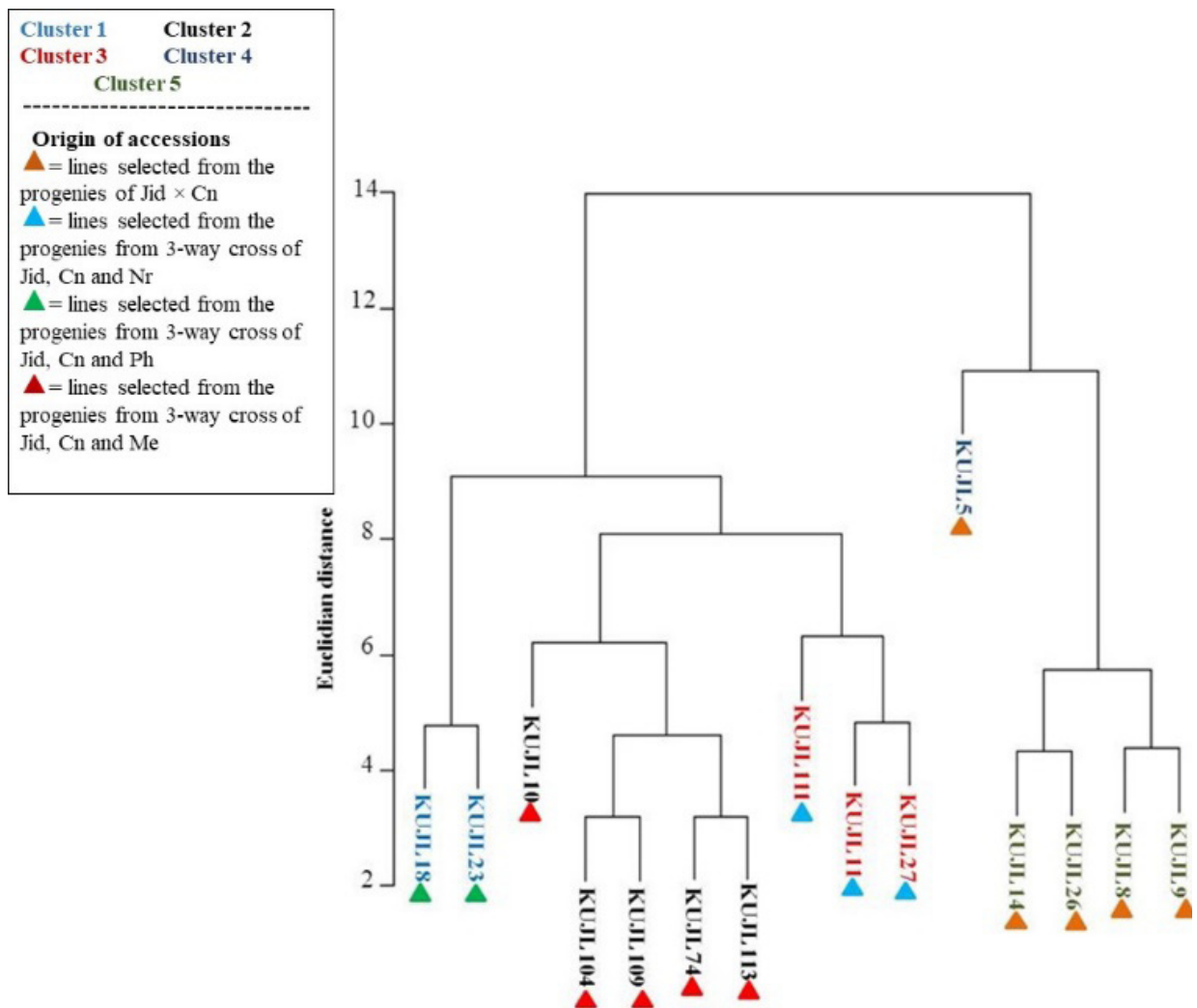
Number of seeds per fruit and seed length gave the highest positive indirect effect through number of fruits per inflorescence (0.556 and 0.548), while 100 seed weight and plant height had high positive indirect effect through number of fruits per inflorescence (0.495 and 0.488) and plant width (0.406 and 0.316). The total indirect effects on seed yield were high in 100 seed weight (0.968), plant height (0.769) and number of seeds per fruit (0.754), respectively. The number of fruits per inflorescence is an effective trait to indirectly select for seed yield per plant, similar to the report in local jatropha germplasm by Das, Misra, Mahapatra, Gantayat, & Pattnaik (2010).

**Clustering and Principal Components Analyses**

Twenty-two agronomic traits of this jatropha germplasm were hierarchically clustered according to Ward’s method using Euclidean distance (Fig. 2). The dendrogram groups the breeding lines into five clusters at the coefficient level of 7. Cluster I has two accessions (KUJL18 and 230, cluster II has five accessions (KUJL10, 74, 104, 109 and 113), cluster III has three accessions (KUJL11, 27 and 111), cluster IV has only one accession (KUJL5), while the last cluster comprises four accessions (KUJL8, 9, 14 and 26). The members of each group can be traced for their pedigree as captioned in Fig. 2. Both accessions in cluster I derived from three parents i.e. Jid, Cn and Ph; five accessions in cluster II derived from Jid, Cn

and Me; accessions in cluster III derived from Jid, Cn and Nr; accessions in cluster IV and V derived from Jid and Cn.

The mean values of agronomic traits in each cluster are presented in Table 9. The members in cluster I had the highest average seed yield per plant (665 g), number of fruits per inflorescence (10.65), number of seeds per fruit (2.43), 100 seed weight (67.21 g), seed length (17.96 mm), seed thickness (8.75 mm), oil yield per plant (198.35 g), canopy height (215.21 cm), canopy width (143.65 cm), saturated fatty acid content (24.22%) and palmitic acid content (14.85%). Cluster II gave the highest seed shelling percentage (66.27), seed oil content (34.68%) and oleic acid content (46.95%), but lowest phorbol esters content (1.43 mg/g).



**Fig. 2.** Cluster analysis constructed using Ward’s method based on Euclidean distance estimated from 22 agronomic traits of 15 interspecific jatropha breeding line

**Table 9.** Number of accessions and mean value of each cluster classified from 22 agronomic traits of 15 interspecific jatropha breeding lines.

Traits	Clusters				
	I (n=2)	II (n=5)	III (n=3)	IV (n=1)	V (n=4)
Seed yield per plant (g)	665.00	440.72	444.14	397.08	281.54
No. inflorescences per plant	101.58	69.36	80.50	312.69	59.68
No. fruits per inflorescence	10.65	10.09	10.83	5.58	6.64
No. seeds per fruit	2.43	2.43	2.29	1.74	1.85
100 seed weight (g)	67.21	54.73	58.81	27.61	47.93
Seed shelling (%)	60.25	66.27	59.71	56.09	62.09
Seed width (mm)	10.26	9.71	10.49	7.76	9.34
Seed length (mm)	17.96	16.74	17.21	13.24	15.41
Seed thickness (mm)	8.75	8.17	8.08	6.29	7.91
Seed oil content (%)	29.86	34.68	28.42	28.15	29.30
Oil yield per plant (g)	198.35	152.79	125.65	111.67	83.23
No. primary branch	4.88	4.38	5.72	5.23	3.57
No. secondary branch	42.21	39.13	34.31	87.38	31.94
Plant height (cm)	215.21	185.58	217.22	170.00	113.23
Plant width (cm)	143.65	115.00	119.93	120.21	101.30
Unsaturated fatty acid (%)	75.78	78.37	79.63	79.39	78.70
Saturated fatty acid (%)	24.22	21.63	20.37	20.62	21.30
Oleic acid (%)	41.94	46.95	36.77	36.50	44.58
Linoleic acid (%)	33.84	31.42	42.86	42.88	34.12
Palmitic acid (%)	14.85	12.95	13.28	10.27	13.24
Stearic acid (%)	9.37	8.69	7.09	10.35	8.07
Phorbol esters (mg/g)	1.94	1.43	1.81	3.06	1.52

This cluster has some genetic background from a low phorbol esters Mexican (Me) accession (Tanya, Dachapak, Tar, & Srinives, 2013). The members of cluster II also showed the least dissimilarity value of approximately 7% between KUJL104 and 109 as well as KUJL74 and 113. Cluster III expressed the highest mean values in number of fruits per inflorescence (10.83), seed width (10.49 mm), number of primary branches (5.72), plant height (217.22 cm), unsaturated fatty acid (80.56%), and lowest saturated fatty acid (20.37%). Cluster IV has only one accession giving the highest mean value in number of inflorescences per plant (312.69), number of secondary branches (87.38), as well as contents of linoleic acid (42.88%), stearic acid (10.35%) and phorbol esters (3.06 mg/g). Cluster V had the breeding lines with dwarf plant type (113.23 cm in height) and narrow

plant canopy (101.30 cm width). The inter-cluster distance in this study also indicated that these 15 breeding lines had high variations in agronomic traits and can serve as a potential gene source in jatropha breeding.

A principal component analysis (PCA) was used to confirm the existence of high genetic variation among the jatropha genotypes based on 22 agronomic traits. The first five components explained 85.60% of the total variation among the accessions (Table 10). Nietsche et al. (2015) evaluated 18 morphological characters of 15 jatropha accessions and showed the first three components explaining 73.5% of the total variation. Vijayanand, Senthil, Vellaikumar, & Paramathma (2009) studied 19 morphological characters of 5 jatropha accessions and found the first three components contributing 84.8%.

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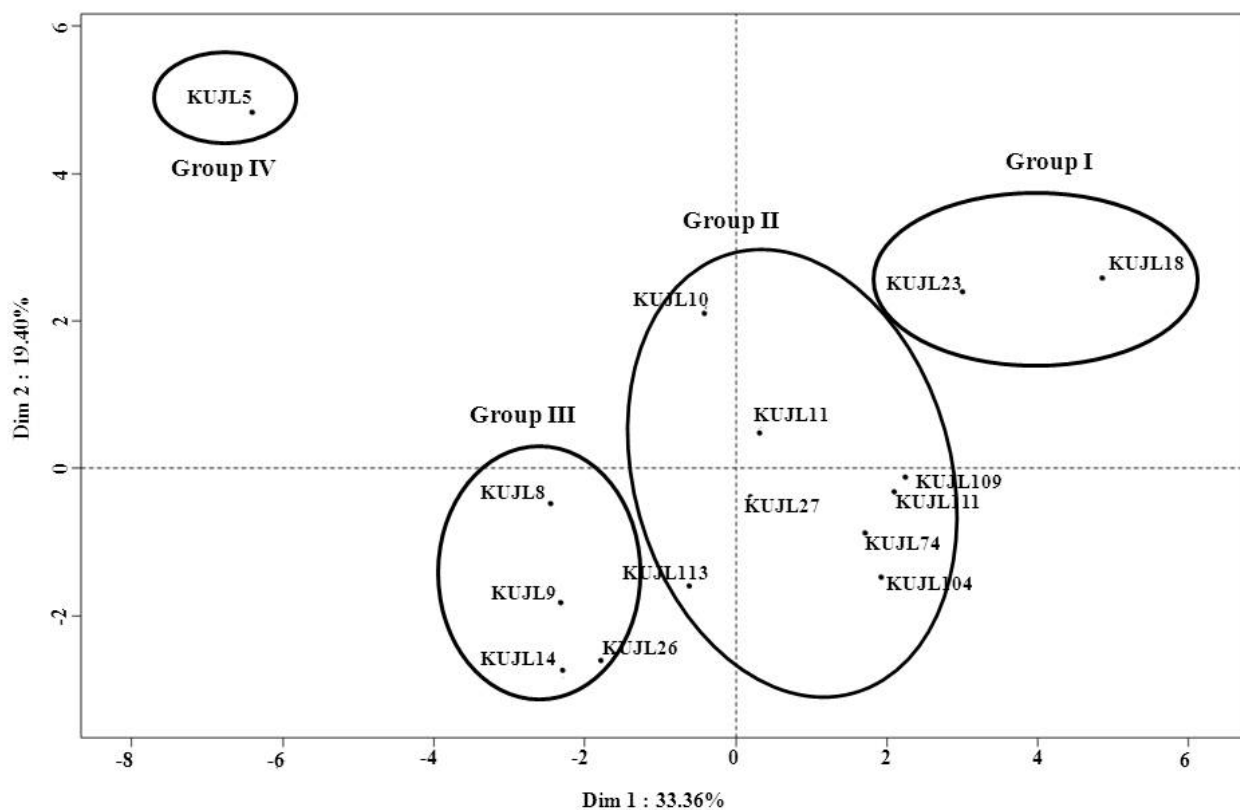
**Table 10.** Eigenvectors and eigenvalues of the first five principal components classified from 22 agronomic traits of 15 interspecific jatropha breeding lines.

Variable	Eigenvectors				
	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
Eigen value	7.34	4.27	3.12	2.32	1.79
Proportion variance (%)	33.36	19.40	14.19	10.54	8.11
Cumulative variance (%)	33.36	52.76	66.95	77.49	85.60
Yield (g/plant)	0.72	0.59	0.06	0.14	-0.01
No. inflorescences/plant	-0.44	0.75	0.22	0.15	-0.24
No fruits/ inflorescence	0.81	0.08	0.15	0.32	0.42
No. seeds/ fruit	0.77	0.00	0.01	0.49	0.04
100 Seed weight (g)	0.94	-0.17	0.20	-0.12	-0.03
Seed shelling (%)	0.29	-0.52	0.02	0.39	0.00
Seed width (mm)	0.71	-0.37	0.36	-0.10	-0.06
Seed length (mm)	0.92	0.01	0.04	-0.13	0.22
Seed thickness (mm)	0.75	-0.35	0.14	-0.27	-0.29
Seed oil content (%)	0.13	0.06	-0.57	0.28	0.50
Oil yield per plant (g)	0.70	0.54	-0.21	0.25	0.12
Phorbol esters (mg/g)	-0.39	0.70	0.34	-0.22	0.19
Palmitic acid (%)	0.69	-0.08	0.04	-0.58	-0.26
Stearic acid (%)	-0.17	0.56	-0.69	-0.09	0.19
Oleic acid (%)	0.15	-0.27	-0.67	0.45	-0.44
Linoleic acid (%)	-0.24	0.17	0.76	-0.31	0.44
Saturated fatty acid (%)	0.46	0.39	-0.54	-0.57	-0.07
Unsaturated fatty acid (%)	-0.46	-0.39	0.54	0.57	0.07
No. primary branches	0.29	0.48	0.45	0.19	-0.43
No. secondary branches	-0.32	0.77	0.05	0.24	-0.39
Plant height (cm)	0.50	0.49	0.10	0.17	0.41
Plant width (cm)	0.62	0.47	0.26	0.21	-0.34

While Shabanimofrad, Rafii, Megat Wahab, Biabani, & Latif (2013) reported that the first four components explained 75.8% of the total variation in 14 morphological characters of 48 Malaysian jatrophas. In this study, factor 1 to factor 5 comprise 33.36%, 19.40%, 14.19%, 10.54% and 8.11% of the total variation (Table 10).

Factor 1 shows high positive variation in seed yield per plant, oil yield per plant, 100 seed weight, seed length, seed width, seed thickness, number of fruits per inflorescence, number of seeds per fruit, plant height and plant width. Variation in

Factor 2 comes mainly from number of primary and secondary branches, number of inflorescences per plant, and contents of total saturated fatty acid and phorbol esters. Factor 3 includes the variation in linoleic content, while Factor 4 is determined by contents of total unsaturated fatty acid and oleic acid, and shelling percentage. Factor 5 includes mainly the variation in seed oil content. Distribution of the jatropha accessions is also given in the PCA dispersion chart (Fig. 3), with the axes of Factor 1 and Factor 2 explaining the variation in major agronomic traits at 33.4% and 19.4%, respectively.



**Fig. 3.** A two-dimensional graph showing relationship among groups of 15 newly interspecific jatropha breeding lines as classified by principal component analysis (PCA).

A two-dimensional graph classifies the breeding lines into 4 groups, where cluster II and III are aggregated into the same group in the PCA with the same distance, while the other groups correspond well with the results from the cluster analysis. Cluster I is a high seed yield group suitable as the recipient parents to cross with the other clusters such as cluster II to improve seed oil content, with cluster IV to increase number of inflorescences per plant, and with cluster V to decrease canopy height. Crossing between groups of different pedigrees can help avoiding inbreeding depression in the progenies. The hybrid plants from crossing among the groups should be heterogeneous because these breeding lines are derived from complex crosses. Mishra (2009) suggested that clonal selection based on individual plant is effective for selection of elite progenies derived from crossing of non-inbred lines.

**CONCLUSION**

Some major agronomic traits showed high or medium level of the predicted values of genetic coefficient of variation, broad-sense heritability, and genetic advance. These agronomic traits can be used to improve and enhance the genetic variation of the existing jatropha germplasm. High positive genetic correlations were observed between seed yield per plant with other traits. Some characters also had high positive indirect effect on seed yield per plant through number of fruits per inflorescence and plant width. Accessions in cluster I are high seed yield group and should be used as the core parents to cross with breeding lines from the other clusters such as cluster II to increase seed oil content, cluster III to increase number of fruits per inflorescence, cluster IV to increase number of inflorescence per plant, and cluster V to decrease plant height. All breeding lines can also be used to improve the existing *J. curcas* accessions as well.

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