



# **Genetic Variability and Character Association for Oil Yield and Its Components in Physic Nut (*Jatropha curcas* L.)**

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## **Authors' contributions**

*This work was carried out in collaboration by both authors. Authors ACO and EFA designed the study and performed the statistical analysis. Author ACO wrote the protocol. Author EFA wrote the first draft of the manuscript. Authors ACO and EFA managed the literature searches, read and approved the final manuscript.*

## **Article Information**

DOI: 10.9734/JEAI/2019/v38i330303

### Editor(s):

(1) Dr. T. Muthukumar, Root & Soil Biology Laboratory, Department of Botany, Bharathiar University, Oimbatore 641 046, Tamil Nadu, India.

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Complete Peer review History: <http://www.sdiarticle3.com/review-history/49522>

**Received 30 March 2019**

**Accepted 14 June 2019**

**Published 02 July 2019**

**Original Research Article**

## **ABSTRACT**

The need to find suitable alternative to fossil fuel necessitated the search for non-food oil crops like physic nut (*Jatropha curcas* L.). Improvement of the crop requires adequate knowledge of extent of genetic variability present. Therefore, a study was carried out to determine genetic variability and character association among forty local and exotic accessions of physic nut. The accessions were evaluated in two years in randomized complete block design with three replications. Results indicated highly significant variation among the accessions for all characters studied except plant height and number of seeds per fruit. High estimates of heritability were observed in number of leaves (84.10%) and seed oil (94.97%) while heritability was low in number of seeds (11.30%) and plant height (13.36%), other characters had moderate heritability. High heritability estimates were accompanied by relatively low genetic advance in all the traits except kernel oil, an indication that the character has high selection value with less environmental influence.

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Correlation analysis showed that tall plants produced bigger seeds because of the positive association between plant height and seed weight. Therefore, seed weight can be used as selection index.

*Keywords: Variation; correlation; Jatropha curcas; heritability; seed oil; seed weight.*

## 1. INTRODUCTION

The global demand for energy is ever increasing [1]. Fossil fuels have been the major energy source in the world over time. However, the environment is currently challenged by the continuous use of fossil fuels as it causes serious environmental problems such as air pollution, global warming, and environmental degradation amongst others [2]. In recent times, attention has been shifted to the use of biofuel which has less environmental problems. However, majority of crops used in biofuel production are food crops [3]. This can greatly impact on food production negatively, arising from competition with food supply and can lead to food shortage especially in the developing countries. Thus, there is need to focus on non-edible oil plants to serve as feedstock for biofuel. *Jatropha curcas* is one of many of these promising non-edible oil plants under consideration.

*J. curcas*, also known as the physic nut, is a shrub which can grow up to 5-7 meters [4,5]. It originates from Mexico or Central America, and was introduced to Africa by the Portuguese sailors in the 16th century [1,6]. The physic nut is one of the leading non-edible oil plants [7] that produces a substantial quantity of oil from its seeds. The oil content has been reported to range between 25 and 50% [8,9,10]. The use of *J. curcas* oils has increased in importance and has, in some cases, replaced the use of fossil fuels. Apart from the prominence gained in oil production, *J. curcas* is important in carbon sequestration [10]. Nahar and Sunny [11] showed that a mature plant absorbs CO<sub>2</sub> at a rate of 18lbs per year, while Becker et al. [12] in their study spanning over a 20-year period revealed that one hectare of *J. curcas* could sequester up to 25 tons of CO<sub>2</sub> from the atmosphere every year.

An important plant like *J. curcas* needs genetic improvement in order to alter its status from wild to a cultivable crop with higher yield and oil content. One of the major constraints in achieving genetic improvement is absence of information about genetic variability. Genetic variability is a pre-requisite for improvement of

any crop because variability precedes for selection. According to Jubera [13] without the presence of sufficient genetic variability of the desired traits that are of economic interest, an attempt to improve this crop will be unsuccessful or a failure.

Currently in Nigeria crop improvement work in this plant is very limited. To fully exploit this multiple utility biofuel crop, research work is required to select the best genotypes for oil content and quality. The genetic diversity in the natural population appears narrow since no remarkable genetic variability has been observed [14]. Therefore, in commencing any crop improvement programme, there is need to determine the amount, cause and nature of variation that is present in the species of interest. In view of the foregoing, the main objective of this study is to evaluate the magnitude of genetic diversity, heritability and correlation between yield and its contributing characters in thirty two accessions of *J. curcas* from six states in Nigeria and one exotic genotype from Brazil.

## 2. MATERIALS AND METHODS

This experiment was conducted at the Laboratory of the Department of Crop, Soil and Pest Management and Teaching and Research Farm of the Federal University of Technology Akure (7°16' N, 5°12' E) located in the rainforest area of Southwestern Nigeria. The location is characterized by a bimodal pattern of rainfall with an annual mean of about 1300 mm with a mean temperature of 27°C and the climate is of the sub-humid type. The vegetation of the experimental plot was mixture of *Panicum maximum* and *Chromolaena odorata* plants.

Mature fruits were collected from thirty-two accessions of *J. curcas* from six states in Nigeria namely, Edo, Osun, Kogi, Ekiti, Kwara and Ondo (Table 1), and fruits from eight trees from the State of Minas Gerais, Brazil. Seeds from the Nigerian states between May and July, 2010. Fruits from Brazil were received in January 2009, seeds were extracted immediately and planted for seed multiplication. Seeds for experimentation were harvested in May 2010.

The experimental plot was manually cleared and seeds were planted directly on flat ground. The experimental design was a randomized complete block design replicated three times. Individual plots were single row plots measuring 1 metre by 10 metres. Alleys of 1 m were created between replications. Seeds were selected randomly from the fruits of each accession and hand planted on the plot allocated to each accession. Spacing was 1 m between hills. Planting was done on 18<sup>th</sup> August, 2010 for the first experiment and 1<sup>st</sup> October, 2011 for the second. Glyphosate was used to control newly germinated weeds 2 weeks before planting. Thereafter manual weeding was carried out at 3 weekly intervals. The following quantitative data were collected on each genotype for agronomic and yield characters from 12 weeks after planting (WAP);

Plant height (cm); measured from base of plant at soil level to the tip of the plant with a meter tape

Stem girth (cm); measured at 50 cm from the base of the plant with the aid of vernier caliper.

Number of branches; the total number of branches per plant was counted.

Number of leaves; the total number of leaves per plant was counted.

Leaf length (cm); measured from base of the leaf to the tip of the leaf. The mean length of matured leaves (20 leaves/plant) was recorded.

Petiole length (cm); measured from point of attachment at the stem to the base of leaf. The mean length of matured leaves (20 leaves/plant) was recorded.

Number of seeds; the total number of seeds harvested per plant throughout the fruiting period (2-3 months) was counted.

Seed weight (g); 100 seeds per plant were weighed using electronic balance.

Percentage oil content; percentage seed, kernel and shell oil was estimated using a Soxhlet extraction method as described by Akbar et al. (2009) [15].

**Table 1. List of states, towns and genotypes collected**

State	Town	Number of genotypes collected	Longitude	Latitude
Ekiti	Ido-Ekiti	2	7°50'43.0"N	5°11'15.6"E
	Ikole	1	7°56'12.1"N	5°34'58.9"E
	Ado Ekiti	1	7°36'44.8"N	5°13'52.0"E
Kwara	Omu Aran	1	8°08'10.7"N	5°06'10.1"E
	Offa	1	8°08'09.3"N	4°42'58.1"E
	Oro	1	8°13'29.9"N	4°53'32.2"E
	Ilorin	1	8°27'31.9"N	4°33'49.8"E
Ondo	Ikare	2	7°31'33.4"N	5°45'20.7"E
	Ifon	1	7°11'49.9"N	5°35'32.8"E
	Owo	2	7°11'55.7"N	5°35'39.4"E
	Akure	2	7°15'28.7"N	5°11'33.9"E
Kogi	Ankpa	2	7°27'08.5"N	7°37'07.1"E
	Okene	1	7°33'44.2"N	6°13'56.9"E
	Anyigba	2	7°29'32.1"N	7°10'17.2"E
	Lokoja	1	7°33'48.5"N	6°14'40.3"E
Osun	Oyan	1	8°02'57.3"N	4°45'50.9"E
	Ile-Ife	2	7°29'04.2"N	4°33'52.4"E
	Osogbo	1	7°47'02.5"N	4°31'58.3"E
	Ilesa	1	7°38'27.8"N	4°44'50.3"E
Edo	Uhíere	1	6°44'55.6"N	5°46'24.5"E
	Ibillo	2	7°26'40.2"N	6°04'09.9"E
	Benin City	1	6°22'36.8"N	5°32'14.6"E
	Auchi	2	7°03'49.0"N	6°16'58.8"E
	Ekpoma	1	6°44'54.5"N	6°04'14.4"E

The data obtained were subjected to analysis of variance using PROC GLM of SAS [16]. Genotypic and phenotypic coefficients of variation were computed according to the procedure of Singh and Chaudhary [17] as follows:

Genotypic coefficient of variation (GCV)

$$= \frac{\sqrt{\sigma_g^2}}{\bar{x}} \times 100.$$

Phenotypic coefficient of variation (PCV) =

$$\frac{\sqrt{\sigma_p^2}}{\bar{x}} \times 100.$$

GCV and PCV values obtained were categorized as low, moderate and high according to Siva-Subramanian and Menon [18]:

0 – 10% : Low  
 10 – 20% : Moderate  
 21% and above : High

Broad sense heritability was estimated as the ratio of genotypic variance to the phenotypic variance and expressed in percentage by Singh and Chaudhary [17] as:

$$\text{Heritability (H)} = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_e^2} \times 100$$

where:

$\sigma_g^2$  = genotypic variance  
 $\sigma_p^2$  = phenotypic variance  
 $\sigma_e^2$  = environmental variance  
 $\sigma^2_p = \sigma^2_g + \sigma^2_e$

Heritability percentage was categorized as low, moderate and high as indicated by Elrod and Stanfield [19] as follows:

0 – 20%: Low  
 20 – 50%: Moderate  
 50% and above: High

Genetic advance (GA) was computed according to the formula given by [20] Johnson et al. (1955):

$$GA = \frac{\sigma_g^2}{\sqrt{\sigma_p^2}} \times K$$

where:

K = 2.06 (selection differential at 5%)  
 $\sigma_g^2$  = Genotypic variance  
 $\sigma_p^2$  = Phenotypic variance  
 $\bar{x}$  = Sample mean of the character

Analysis of covariance was carried out on pairs of variables which exhibited significance ( $P \leq 0.05$ ) in the regression analysis. The generated components of the covariance were used to estimate the phenotypic and genotypic correlation coefficients as suggested by Singh and Chaudhary [17]:

$$\text{Phenotypic Correlation Coefficient (r}_p\text{)} = \frac{COV_p(X_1, X_2)}{\sqrt{\sigma_p^2(X_1) \times \sigma_p^2(X_2)}}$$

$$\text{Genotypic Correlation Coefficient (r}_g\text{)} = \frac{COV_g(X_1, X_2)}{\sqrt{\sigma_g^2(X_1) \times \sigma_g^2(X_2)}}$$

where:

$COV_g(X_1, X_2)$  = Genetic covariance among traits  $X_1$  and  $X_2$ .

$COV_p(X_1, X_2)$  = Phenotypic covariance among traits  $X_1$  and  $X_2$ .

$\sigma_g^2(X_1)$  and  $\sigma_g^2(X_2)$  = Genetic variance for traits  $X_1$  and  $X_2$ , respectively.

$\sigma_p^2(X_1)$  and  $\sigma_p^2(X_2)$  = Phenotypic variance for traits  $X_1$  and  $X_2$ , respectively.

Test of significance of correlation was done by comparing the computed values against tabular 'r' values given by Fisher and Yates [21].

### 3. RESULTS

The mean squares from the analysis of variance for the eleven plant characters that were investigated are shown in Table 2. Mean square for replicate was significant in most characters tested including number of branches, plant height, stem girth, number of seed and seed oil. Highly significant differences for genotype were observed in all the characters except plant height and number of seeds. The means, standard deviation and ranges of eleven characters among the forty genotypes of *J. curcas* are presented in Table 3. Plant height ranged from 110 cm to 279 cm with an overall mean value of 180.29 cm. Wide variation was observed for number of leaves, number of branches and number of seed as they varied from 82 to 632, 3 to 45 and 2 to 513 respectively. Moderate variation was observed in stem girth, percentage kernel oil and percentage seed oil. Low variation was recorded for leaf length and seed weight as they ranged from 12 cm to 18 cm and 62.08 grams to 79.38 grams respectively.

**Table 2. Mean squares from analysis of variance (ANOVA) for eleven agronomic characters of forty *Jatropha curcas* genotypes**

Source	DF	Number of branches per plant	Petiole length (cm)	Number of leaves per plant	Plant height (cm)	Stem girth (cm)	Number of seeds per plant	Leaf length (cm)	Kernel oil (%)	Shell oil (%)	Seed oil (%)	Seed weight (g)
Rep	2	135.36*	2.71ns	957.59ns	3223.80*	36.60**	34193.89**	0.40ns	0.54ns	1.75ns	4.04*	0
Genotype	42	60.79*	11.66**	36128.71**	1463.77ns	12.78**	6034.32ns	1.67**	40.05**	12.57**	69.16**	70.99**
Error	84	33.59	3.43	490.68	1000.9	6.63	4366.73	0.78	0.36	0.70	1.20	0

\*, \*\* - significant at 0.05, 0.01 probability level respectively

**Table 3. Means, standard deviation and range of eleven characters among forty genotypes of *J. curcas***

Characters	Mean $\pm$ SD	Minimum	Maximum
Number of branches	14.21 $\pm$ 6.64	3.00	45.00
Petiole length (cm)	19.02 $\pm$ 2.47	13.90	30.70
Number of leaves	293.22 $\pm$ 110.42	82.00	632.00
Plant height(cm)	180.29 $\pm$ 34.46	110.00	279.00
Stem girth (cm)	23.54 $\pm$ 3.02	16.70	31.70
Number of seeds per plant	86.05 $\pm$ 73.35	2.00	513.00
Leaf length(cm)	14.88 $\pm$ 1.03	12.00	18.00
Kernel oil (%)	61.87 $\pm$ 3.66	51.88	71.88
Shell oil (%)	11.53 $\pm$ 2.15	3.75	15.00
Seed oil (%)	73.40 $\pm$ 4.80	61.25	83.76
Seed weight (g)	14.21 $\pm$ 6.64	62.08	79.38

Estimates of genotypic coefficient of variation, phenotypic coefficient of variation, genotypic variance, phenotypic variance, heritability, genetic advance and expected genetic gain for eleven agronomic characters of forty genotypes of *J. curcas* are presented in Table 4. Number of branches, number of seeds and seed weight showed high genotypic and phenotypic coefficients of variation (21.19%, 45.96%, 27.40%, 81.54% and 21.22%, 39.82%) respectively. Number of leaves, leaf length, kernel oil and seed oil had low genotypic and phenotypic coefficients of variation. Low genotypic coefficient of variation were observed for petiole length, plant height and stem girth. Phenotypic coefficients of variation for these characters were moderate. Phenotypic coefficient of variation was higher than genotypic coefficient of variation in all the characters evaluated. High estimates of heritability were observed in number of leaves and seed oil, while heritability was low in number of seeds (11.30%) and plant height (13.36%). Other characters had a moderate heritability. Genetic advance and expected genetic gain were observed to be low for all the characters studied.

The phenotypic ( $r_p$ ) and genotypic ( $r_g$ ) correlation coefficients among the eleven characters in the forty *J. curcas* genotypes are presented in Table 5. Number of branches showed significant and positive phenotypic and genotypic correlations with stem girth, number of seeds and leaf length, respectively but correlated significantly and positively with plant height only at the genotypic level, and with kernel oil at the phenotypic level only. Petiole length had significant and positive  $r_p$  and  $r_g$  with plant height, stem girth and leaf length respectively but also with shell oil only at the genotypic level. However, it had significant negative correlation at phenotypic level with number of seeds. Number of leaves had

significant and positive  $r_g$  with number of seeds but significant and negative  $r_g$  with seed weight. At genotypic level, plant height showed significant positive correlation with number of seeds and seed weight, but correlated significantly and negatively with leaf length. At the phenotypic level, plant height correlated significantly and positively with stem girth. Stem girth on the other hand, had significant positive  $r_p$  and  $r_g$  with leaf length, and also showed significant positive  $r_g$  with seed weight, but negative with number of seeds. Leaf length showed significant and negative  $r_g$  with kernel oil. Kernel oil had significant and positive genotypic correlations with shell oil, seed oil and seed weight, and significant positive phenotypic correlations with shell oil, seed oil only. Shell oil also showed a significant and positive  $r_p$  and  $r_g$  with seed oil and  $r_g$  with seed weight. Seed oil showed significant positive  $r_g$  with seed weight.

#### 4. DISCUSSION

Crop improvement is achieved when there is significant genetic variability among genotypes for the desired traits. The observed differences among the genotypes evaluated in this study differed in yield characters tend to suggest that breeding work can be initiated through selection or hybridization. The high level of variability among the genotypes suggests that heterosis could be utilized to produce superior hybrids which can further be used in breeding programme to develop superior genotypes [22]. Development of such genotypes requires that overall variability should be partitioned into heritable and non-heritable components with the aid of genetic parameters such as genotypic and phenotypic coefficients of variation and heritability [23]. In this study, phenotypic variance was greater than genotypic variance in all the characters for yield parameters. Similar findings

**Table 4. Estimates of genetic coefficient of variation, phenotypic coefficient of variation, genotypic variance, phenotypic variance, heritability, genetic advance and expected genetic gain for eleven agronomic characters in *Jatropha curcas* genotypes**

<b>Characters</b>	<b>Genotypic coefficient of variation (%)</b>	<b>Phenotypic coefficient of variation (%)</b>	<b>Genotypic variance <math>\sigma_g^2</math></b>	<b>Phenotypic variance <math>\sigma_p^2</math></b>	<b>Environmental Variance <math>\sigma_e^2</math></b>	<b>Heritability (%)</b>	<b>Genetic advance</b>	<b>Expected genetic gain (%)</b>
Number of Branches per plant	21.19	45.96	9.07	42.66	33.59	21.26	2.86	20.12
Petiole length (cm)	8.72	13.08	2.74	6.17	3.43	44.44	2.27	11.97
Number of Leaves per plant	1.35	1.47	15.66	18.62	490.68	84.10	7.48	2.55
Plant height (cm)	6.89	18.85	154.29	1155.18	1000.89	13.36	9.35	5.19
Stem girth (cm)	6.08	12.52	2.05	8.68	6.63	23.62	1.43	6.09
Number of seeds per plant	27.40	81.54	555.86	4922.59	4366.73	11.30	16.32	18.97
Leaf length (cm)	3.66	6.97	0.30	1.08	0.78	27.55	0.59	3.96
Kernel oil (%)	0.54	1.11	0.11	0.47	0.36	23.94	0.34	0.55
Shell oil (%)	12.11	24.22	1.95	7.80	5.85	25.00	1.44	12.47
Seed oil (%)	6.48	6.65	22.65	23.85	1.2	94.97	9.55	13.02
Seed weight (g)	21.22	39.82	22.08	77.71	55.63	28.42	5.16	23.31

**Table 5. Genetic correlation (rg) and phenotypic correlation (rp) for eleven characters of forty *Jatropha curcas* genotypes**

		<b>Petiole length (cm)</b>	<b>Number of leaves per plant</b>	<b>Plant height (cm)</b>	<b>Stem girth (cm)</b>	<b>Number of seeds per plant</b>	<b>Leaf length (cm)</b>	<b>Kernel oil (%)</b>	<b>Shell oil (%)</b>	<b>Seed oil (%)</b>	<b>Seed weight (g)</b>
<b>Number of Branches per plant</b>	$r_g$	0.21	0.04	0.36*	0.72**	0.49**	0.29*	-0.17	-0.02	-0.14	0.14
	$r_p$	0.28	0.07	0.26	0.70**	0.33*	0.31*	-0.34*	-0.05	-0.28	0.14
<b>Petiole length (cm)</b>	$r_g$		-0.06	0.40**	0.53**	-0.76**	0.70**	-0.19	0.29*	-0.03	0.18
	$r_p$		-0.01	0.34*	0.37**	-0.14	0.36*	-0.13	0.23	0.01	0.06
<b>Number of leaves</b>	$r_g$			0.23	-0.12	0.61**	0.03	0.05	-0.21	-0.05	-0.40**
	$r_p$			0.16	-0.03	0.24	0.06	0.05	-0.18	-0.04	-0.2
<b>Plant height (cm)</b>	$r_g$				0.11	0.29*	-0.35*	-0.02	0.04	0.01	0.62**
	$r_p$				0.36*	0.12	0.28	-0.02	0.06	0.01	0.11
<b>Stem girth (cm)</b>	$r_g$					-0.47**	0.66**	-0.21	0.04	-0.14	0.41**
	$r_p$					0.22	0.35*	-0.09	0.03	-0.05	0.1
<b>Number of seeds per plant</b>	$r_g$						-0.27	-0.41**	-0.98**	-0.79*	-0.79**
	$r_p$						0.03	-0.11	-0.32*	-0.23	-0.13
<b>Leaf length (cm)</b>	$r_g$							-0.34*	0.1	-0.21	0.07
	$r_p$							-0.18	0.06	-0.11	0.02
<b>Kernel oil (%)</b>	$r_g$								0.38*	0.92**	0.31*
	$r_p$								0.35*	0.91**	0.15
<b>Shell oil (%)</b>	$r_g$									0.71**	0.57**
	$r_p$									0.71**	0.26
<b>Seed oil (%)</b>	$r_g$										0.48**
	$r_p$										0.23

\*, \*\* - significant at 0.05, 0.01 probability level respectively



were reported in okra [22], in cucumber [24] and in the fluted pumpkin [25]. The high genotypic and phenotypic coefficients of variation observed in number of seeds and seed weight suggests that greater potential might be expected in selection for these characters. The observed high heritability values in number of leaves and seed oil content indicate that the characters were under genetic control and are less influenced by the environment. According to Ojo and Amanze [26], high heritability in a trait strongly suggests that there is potential for large genetic determination for that character. This can be said of number of leaves and seed oil content, which can be exploited for improvement of oil yield in this crop.

Since high heritability does not always indicate a high genetic gain, heritability with genetic advance considered together should be used in predicting the ultimate effect of selecting superior genotypes [27,28]. In this study, high heritability estimates were accompanied by relatively low genetic advance in all the traits except kernel oil, an indication that the character has high selection value with less environmental influence. Therefore, improvement by direct phenotypic selection is possible. Whenever a high heritability value is accompanied by high genetic advance, it may be governed by additive gene action and improvement with respect to these characters could be brought about by phenotypic selection [29,25]. Moderate heritability with low genetic advance was observed for some characters in this study. They include petiole length, stem girth, kernel oil, shell oil and seed weight. This indicates the existence of intermediate additive and non-additive gene action. Consequently improvement of these characters can be achieved through careful selection. The low heritability and low genetic advance recorded for plant height and number of seeds suggest that these characters governed by non-additive gene action [30]. The correlation coefficients among characters were determined at the phenotypic and genotypic levels. In general, most genotypic correlation coefficients were higher in magnitude than phenotypic correlation coefficients. This can be interpreted as a strong inherent genotypic relationship between characters studied, though their phenotypic expression was influenced by environmental influence. Direct selection for improving some of these traits may not be possible since variation among the genotypes is attributed largely to the environment.

Significant and positive correlation between two characters suggests that these characters can be improved simultaneously in a selection programme [28,25]. This is because it shows mutual relationships among characters and selection for one will translate to selection of the other [25]. For the improvement of oil yield in this crop, it was necessary to determine the magnitude and the direction of relationship between the oil yield and its components. Studies on the character associations showed that tall plants might produce bigger seeds because of the positive association between plant height and seed weight. The leaves of tall plants can easily intercept more light than short plants. High light interception will lead to greater accumulation of photosynthate. Accumulation of photosynthate in leaves has been reported to increase seed weight of okra [23]. The reduction in oil yield caused by big branches and broad leaves could be attributed to diversion of photosynthate for seed yield to vegetative growth (branch and leaf). This is in agreement with the findings of Patil et al. [31]. Therefore, pruning should be carried out on this crop to ensure good yield. Seed weight was observed to have significant genotypic relationship with seed oil and shell oil also. An increase in weight may therefore lead to an increase in the latter two traits. As such, seed weight may be used as selection index. Prasanthi et al. [32] reported that the weight of seed per plant and numbers of seeds per plant were important selection indices. This evaluation may be useful in developing reliable selection indices for important agronomic traits in *J. curcas*. It is recommended that future research explore molecular means to further confirm the outcome of this research and establish the relationship between the two methods.

## 5. CONCLUSION

High GCV and PCV observed in number of seeds per plant and seed weight indicates greater potential for the improvement of the mentioned characters in *J. curcas*. Also in this study, high heritability was observed for number of leaves and seed oil yield among other characters investigated. These traits may be improved by direct selection. In addition, seed weight had positive and significant correlated with seed oil, kernel oil and shell oil. Consequently, improvement of seed weight will result in improved oil yield. Therefore these characters can be selected as traits of interest in

the development of an improvement programme for the crop.

### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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