

## GENETIC VARIABILITY AND DIVERGENCE ANALYSES IN *Jatropha curcas* BASED ON FLORAL AND YIELD TRAITS

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Maurya R., S. Verma, A. Gupta, B.Singh, and H. Kumar Yadav (2013): *Genetic variability and divergence analyses in Jatropha curcas based on floral and yield traits* -. Genetika, Vol 45, No. 3, 655-666.

Genetic variability of 80 accessions of *Jatropha curcas* showed that oil content varied between 20.8-36.1% ( $X=26.2\pm 0.38$ ). Thirty seven accessions showed seed weight/plant above average mean value (180.2g) and 26 accessions showed oil content above average mean (26.2%). The hierarchical clustering grouped all the accessions into 4 clusters. Clustering showed that majority of accessions i.e. 56 (70%) were genetically close to each other and grouped in two clusters. The maximum intra cluster distance was recorded in cluster IV (30.15). The inter cluster distance varied from 47.59 (between cluster I and cluster II) to 211.27 (between cluster III and cluster I). The cluster III showed maximum genetic distance with cluster I, followed by cluster IV and cluster II suggesting comparatively wider genetic diversity among them. The Principal Component Analysis (PCA) showed that first four principal components (PCs) accounted for more than 93% of the total variation. The first principal components accounted for 42.5% of the total variation mainly due to seed length, seed width, seed weight/plant and number of seeds/plant which had maximum and positive weight on this component. Oil content had negative weight on PC1. Thus, PC1 related to the accessions with thick seeds, moderate to high seed yielder with low oil content.

*Key words:* Divergence, Genetic variability, Genetic advance, Heritability, *Jatropha curcas*

### INTRODUCTION

*Jatropha curcus*, also known as physic nut or purging nut, is a perennial shrub belonging to family Euphorbiaceae. It is a diploid ( $2n=22$ ) with relatively smaller genome size of

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416 Mb (CARVALHO *et al.*, 2008) and contains about 175 species worldwide. It is native to tropical America but widely distributed in other tropical and subtropical areas of the world, especially in Africa, India, and Southeast Asia (OPENSHAW 2000, SUJATHA and PRABHAKARAN, 1997). The Portuguese settlers are believed to have introduced *Jatropha* to India during the sixteenth century (GINWAL *et al.*, 2005). Traditionally, the plant is used as fence, to control erosion and reclaim land, and as animal feed and manufacturing of lubricants, soaps, candles, purgative agents, astringents, and coloring dyes (OPENSHAW, 2000). Recently, it gains popularity worldwide as an alternative and renewable source of biodiesel production. The plant can rapidly grow in a wide range of agro-climatic conditions and is not grazed by animals (SUBRAMANIAN *et al.*, 2005). Its hardy nature and high oil content make it a promising oil crop for biodiesel (HENNING, 1998). However, *J. curcas* is still an undomesticated plant and its response to yield and oil content found to be erratic with different agro-climatic zones. Therefore, there is a serious need to take initiative for its genetic improvement for adaptability, agronomically desirable traits, yield and oil content. The assessment of the level and pattern of genetic relationship among germplasm accessions is an important component of genetic improvement program. The informations obtained could be utilized for i) analysis of genetic variability ii) identification of diverse parental combinations to create genetic variability for further selection (BARNETT and KIDWELL, 1998) and iii) introgression of desirable genes from diverse germplasm into the available genetic base (THOMSON *et al.*, 1998). Several types of data sets (morphological, biochemical, molecular markers) and tools have been used for studying genetic variability and relationship among accessions. Currently DNA based molecular markers are being widely used for genetic analysis. In past several genetic diversity studies have also been reported in *J. curcas* using different types of molecular markers like RAPD (GANESH *et al.*, 2008), KUAMR *et al.*, 2009; SUBRAMANYAM *et al.*, 2009), AFLP (QUINTERO *et al.*, 2011; SUN *et al.*, 2008), ISSR (SENTHIL *et al.*, 2009; TANYA *et al.*, 2011) and SSR (WEN *et al.*, 2010). However, the morphological characterization is the first and important step in the description and classification of germplasm. Few preliminary studies based on quantitative genetic variations were reported in *J. curcas*. GINWAL *et al.* (2005) reported some preliminary quantitative genetic variations in seed morphology, germination and seedling growth among ten accessions collected mainly from Madhya Pradesh, India. KAUSHIK *et al.* (2007) evaluated 24 accessions for seed oil content variations and divergence. Genetic association, variability and diversity in seed traits, growth, reproductive and yield traits were reported by RAO *et al.* (2008) among 32 wild accessions of *J. curcas* collected from Andhra Pradesh, India. Most of the morphometric trait based genetic studies carried out earlier were restricted to smaller number of accessions collected from limited areas. However, to explore and exploit the available genetic resources, an extensive survey, collection and evaluation required to find out potential genetic material for genetic improvement of *J. curcas*. Genetic studies based on the multivariate analysis is a powerful tool for determining the degree of divergence between populations, the relative contribution of different components to the total divergence and the nature of forces operating at different levels. Thus, the present investigation was undertaken with 80 accessions of *J. curcas* collected from different states of the India to evaluate genetic variability, assess genetic divergence and identify diverse accessions/groups to facilitate the future breeding strategies and genetic improvement of *J. curcas*.

## MATERIALS AND METHODS

A total of eighty accessions of *J. curcas* representing different eco-geographical and agro-climatic zone of India (Fig.1) were selected from germplasm bank maintained at Banthra Research Center (BRC) of National Botanical Research Institute, Lucknow India. Fifteen cuttings of each accession were raised in polybags filled with soil, cowdung manure and sand in equal proportion during March 2008. The six rooted cuttings of each accession were then transplanted in experimental plot in Randomized Block Design (RBD) with 3 replications and two plants/replication during July 2008. The experimental plot is situated between 26° 40'N latitude and 80° 45' E longitude and at an altitude of 129 m above sea level. The distances between rows and plants were kept 2 meter. The field was irrigated as and when required. Pruning of plants was practiced 2 feet above the ground in the first week of March 2009 and 2010. Data on different morphometric traits were recorded during November 2010- January 2011. Following traits were considered for data recording: *Female flower/plant*: Number of female flower counted during flowering period (November – January), *male flower/plant*: Number of male flower counted per plant, *male/female ratio*: ratio between female and male flower per plant, *Number of fruits/plant*: total number of fruits counted per plant at harvesting time, *Number of seeds/plant*: Total number of seeds counted per plant, *Fruit weight/plant*: total fruit weight measured in gram per plant, *Seed weight/plant*: total seed weight measured in gram per plant, *Seed length and width*: twenty seeds per plant randomly selected and length and width measured in middle with vernier caliper (mm), *Oil content*: twenty five to thirty seeds randomly selected per plant and used to measure oil content in percent through Nuclear Magnetic Resonance (NMR) spectrometer.

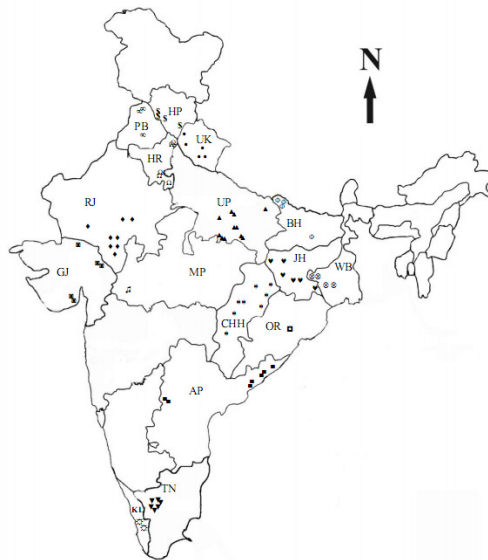


Figure1. Map of India showing collection site of *Jatropha curcas* from different states.

The mean values for each trait were used for statistical analysis and subjected to analysis of variance and covariance using WINDOSTAT software ([www.windostat.org](http://www.windostat.org)). Variance components were estimated from mean square of ANOVA (SINGH and CHAUDHARY, 1985). Heritability in broad sense ( $h_B$ ) was estimated on genotypic mean basis as described by Allard (1999). Expected genetic advance (%) of mean was estimated according to JOHNSON *et al.* (1955). Phenotypic correlation (rp) was calculated using analysis of variance and covariance values as suggested by JOHNSON *et al.* (1955). For divergence studies the variability among population was tested by Wilk's lambda criterion for pooled effect of all the characters. Hierarchical clustering was carried to find out the pattern of similarity/dissimilarity among accessions using ward's minimum variance method (WARD, 1963). The relationships among the clusters were assessed by estimating the intercluster distances using Mahalanobis distance ( $D^2$ ) statistics (RAO, 1952).

## RESULTS AND DISCUSSION

The range and mean value of different traits along with various statistical parameters is presented in Table 1. The number of female flowers/plant varied from 72.6 to 118.0 with an average of  $94.6 \pm 1.39$  and the number of male flowers/plant varied from 16.27.2 to 2960.0 with an average of  $2240.8 \pm 42.92$ . Male/female flower ratio was found variable between 17.2 - 32.1 with an average of  $24.0 \pm 0.37$ . The number of fruits/plant and number of seeds/plant varied between 63.4 - 112.9 and 169.6-297.0 with an average of  $80.5 \pm 1.31$  and  $218.3 \pm 3.89$  respectively. The range of seed weight/plant (g), seed length (mm) and seed width (mm) were 102.7 - 273.8, 13.3 - 18.5 and 7.8 - 11.8 with an average of  $180.2 \pm 4.20$ ,  $16.4 \pm 0.13$  and  $10.7 \pm 0.09$  respectively. The oil content varied between 20.8 - 36.1% with an arithmetic mean of  $26.2 \pm 0.38$ . Out of 80 accessions, 37 accessions have seed weight/plant above average value (i.e.180.2g) and of which only 4 accessions have seed weight/plant above 250g with maximum in accession NBJC1078 (273.08g). Likewise, 26 accessions have oil content above average value of 26.2% and only three accessions namely NBJC1055, NBJC1051 and NBJC1048 having oil content above 35%. So, majority of the accessions in the present investigation were found to be low oil yielder. However, FOIDL *et al.* (1996) and BERCHMANS and HIRATA, (2008) reported oil content upto 40%. In order to assess the heritable portion of total variability, the phenotypic variance ( $\delta^2_p$ ) was partitioned into genotypic ( $\delta^2_g$ ) and error variance ( $\delta^2_e$ ). The values of error variance were found to be higher than those of genotypic variance ( $\delta^2_g$ ) for number of female flowers/plant, male/female ratio, number of fruits/plant, number of seeds/plant and seed weight/plant suggesting much influence of environmental factors on these traits. The phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) varied from 7.51 to 25.48 and 7.05 to 17.87% respectively. Maximum PCV and GCV were noticed for seed weight/plant followed by fruit weight/plant, number of male flower/plant, number of seeds/plant. The PCV was found to higher than that of GCV for all the traits with remarkable differences in their values. The traits seed length, seed width and oil content has very small differences in PCV and GCV values. Similarly, KAUSHIK *et al.* (2007) and RAO *et al.* (2008) also noticed higher PCV over GCV with small differences for seed length, seed width and oil content. The genetic improvement in the traits with small differences in PCV and GCV values can easily be achieved by selection of promising plant types and also through crossing the desirable accessions among themselves followed by selection in segregating generations.

Table 1. Range, mean, estimates of variance components, broad sense heritability and genetic advance in *Jatropha curcas*

	Min	Max	Mean $\pm$ SD	F value	$\sigma^2_g$	$\sigma^2_p$	$\sigma^2_e$	GCV	PCV	Hb	GA	GA%
Female flower /plant	72.6	118.0	94.6 $\pm$ 1.39	2.82**	99.55	263.10	163.5	10.54	17.13	38.0	12.64	13.35
Male flower /plant	1627.2	2960.0	2240.8 $\pm$ 42.92	4.06**	109448.18	216787.51	107339.3	14.76	20.78	50.0	484.24	21.61
male/female ratio	17.2	32.1	24 $\pm$ 0.37	1.76**	4.79	23.69	18.89	9.12	20.27	20.0	2.03	8.45
No. of fruits /plant	63.4	112.9	80.5 $\pm$ 1.31	2.86**	88.95	232.08	143.13	11.72	18.93	38.0	12.03	14.95
No. of seeds /plant	169.6	297.0	218.3 $\pm$ 3.89	3.43**	850.42	1898.14	1047.72	13.35	19.94	45.0	40.21	18.40
Fruit weight /plant	301.1	575.2	397 $\pm$ 8.18	4.5**	4128.07	7665.63	3537.56	16.15	22.01	54.0	97.13	24.42
Seed weight /plant (g)	102.7	273.8	180.2 $\pm$ 4.20	3.90**	1040.43	2116.22	1075.79	17.87	25.48	49.0	46.59	25.81
Seed length (mm)	13.3	18.5	16.4 $\pm$ 0.13	23.32**	1.34	1.52	0.18	7.05	7.51	88.0	2.24	13.64
seed width (mm)	7.8	11.8	10.7 $\pm$ 0.09	20.69**	0.61	0.70	0.09	7.28	7.82	87.0	1.50	13.97
Oil content (%)	20.8	36.1	26.2 $\pm$ 0.38	27.10**	10.84	12.09	1.25	12.60	13.30	90.0	6.42	24.58

The estimate of genetic variability alone is considered as not much helpful in determining the heritable portion of variation unless until coupled with the estimate of heritability. The estimate of heritability along with variability can provide more insight towards the amount of genetic advance to be expected from the selection process. Thus, the knowledge of heritability of a character become important as it indicates the possibility and extent to which improvement is possible through selection. Broad sense heritability varied from 20% to 90% and maximum was observed for oil content (90%) followed by seed length (88%) and seed width (87%). The lowest heritability (20%) was noticed for male/female ratio and other traits have moderate heritability ranging from 38% to 54%. The high heritability noticed for oil content, seed length and seed width indicates that these characters are under genotypic control. However, heritability estimates may differ widely in the same crop and same trait (RASMUSON, 2002) because heritability always refers to a defined population and a specific experimental set up (HOLLAND *et al.*, 2002). Considering high heritability alone is not enough in making efficient selection in advance generation unless accompanied by substantial amount of genetic advance (GA), which provides the information about the degree of gain in a character obtained under a particular selection pressure (JOHNSON *et al.*, 1955). Expected genetic advance, as a function of selection intensity, phenotypic variance and heritability, has an added advantage over heritability as a guiding factor to breeders in a selection program. The genetic advance as percent of mean varied from 8.45 for male/female ratio to 25.81 for seed weight/plant. The higher genetic advance for seed weight/plant, oil content, and number of fruits/plant was might be due to presence of variation in these traits. High heritability coupled with high GA and GCV for oil content suggests that this trait was primarily controlled by additive gene action and any simple selection model would be advantageous to obtain the desired genetic gain. Low heritability with high GA for seed weight/plant, fruit weight/plant and number of seeds/plant and high heritability with low GA for seed length and seed width indicates that these traits might be largely governed by non-additive gene actions and hence much improvement cannot be achieved through

selection. Similar to the present findings, high heritability and low GA for seed length and seed width was also reported by KAUSHIK *et al.* (2007) and RAO *et al.* (2008).

Table 2. Distribution of 80 accessions of *Jatropha curcas* in 4 clusters based on their 10 quantitative traits

Cluster	Number of accessions	Accessions name
Cluster I	27	NBJC1001, NBJC1034, NBJC1044, NBJC1005, NBJC1039, NBJC1054, NBJC1004, NBJC1045, NBJC1060, NBJC1121, NBJC1007, NBJC1031, NBJC1137, NBJC1107, NBJC1127, NBJC1023, NBJC1097, NBJC1008, NBJC1033, NBJC1129, NBJC1064, NBJC1022, NBJC1058, NBJC1124, NBJC1078, NBJC1122, NBJC1130.
Cluster II	29	NBJC1006, NBJC1093, NBJC1083, NBJC1057, NBJC1133, NBJC1082, NBJC1087, NBJC1135, NBJC1003, NBJC1035, NBJC1112, NBJC1036, NBJC1050, NBJC1020, NBJC1021, NBJC1085, NBJC1080, NBJC1131, NBJC1063, NBJC1067, NBJC1065, NBJC1073, NBJC1052, NBJC1071, NBJC1084, NBJC1101, NBJC1138, NBJC1094, NBJC1072.
Cluster III	6	NBJC1009, NBJC1014, NBJC1013, NBJC1019, NBJC1017, NBJC1025.
Cluster IV	18	NBJC1048, NBJC1051, NBJC1055, NBJC1049, NBJC1081, NBJC1089, NBJC1092, NBJC1079, NBJC1141, NBJC1053, NBJC1123, NBJC1069, NBJC1088, NBJC1075, NBJC1076, NBJC1070, NBJC1090, NBJC1077.

Further, the potential accessions could be identified by analyzing genetic diversity in the genetic resources collected/available, which will further facilitate various genetic improvement programs. The simultaneous testing of significance based on Wilk's lambda criterion for pooled effect of all the characters showed significant differences among the population ( $\chi^2 = 790$  df = 3079.06\*\*). A hierarchical cluster analysis (Wards minimum variance) grouped all the 80 accessions into 4 clusters (Table 2, Fig. 2). The number of accessions per clusters varied from 6 (cluster III) to 29 (cluster II). The cluster II was largest with 29 accessions collected from 11 states India i.e. Andhra Pradesh (6 accessions), Rajasthan (5), Jharkhand (4), Uttar Pradesh (2), Chhattisgarh (2), Bihar (2), Punjab (2), Gujarat (1), Haryana (1), Kerala (1) and Tamil Nadu (1). The cluster I is second largest comprising 27 accessions including 5 accessions collected from Uttar Pradesh, 4 from Gujarat, 3 from West Bengal, 2 each from Rajasthan, Tamil Nadu, Himachal Pradesh, Bihar, Chhattisgarh, Haryana, and 1 each from Punjab, Madhya Pradesh, Uttaranchal, Jharkhand and Kerala. The cluster III was the smallest with 6 accessions collected from Uttaranchal (4), Jharkhand (1) and Rajasthan (1). The cluster IV had 18 accessions collected from Chhattisgarh (4), Uttar Pradesh (4), Tamil Nadu (3), Himachal Pradesh (2), Haryana (2), Orissa (1), Andhra Pradesh (1), and West Bengal (1). The clustering of accessions based on multivariate analysis showed that majority of accessions i.e. 56 accessions (70%) were genetically close to each other and grouped only into two clusters. The distribution of accessions from same origin/geo-graphical region into different clusters or vice versa indicated that the geographical origin is not related to genetic divergence.

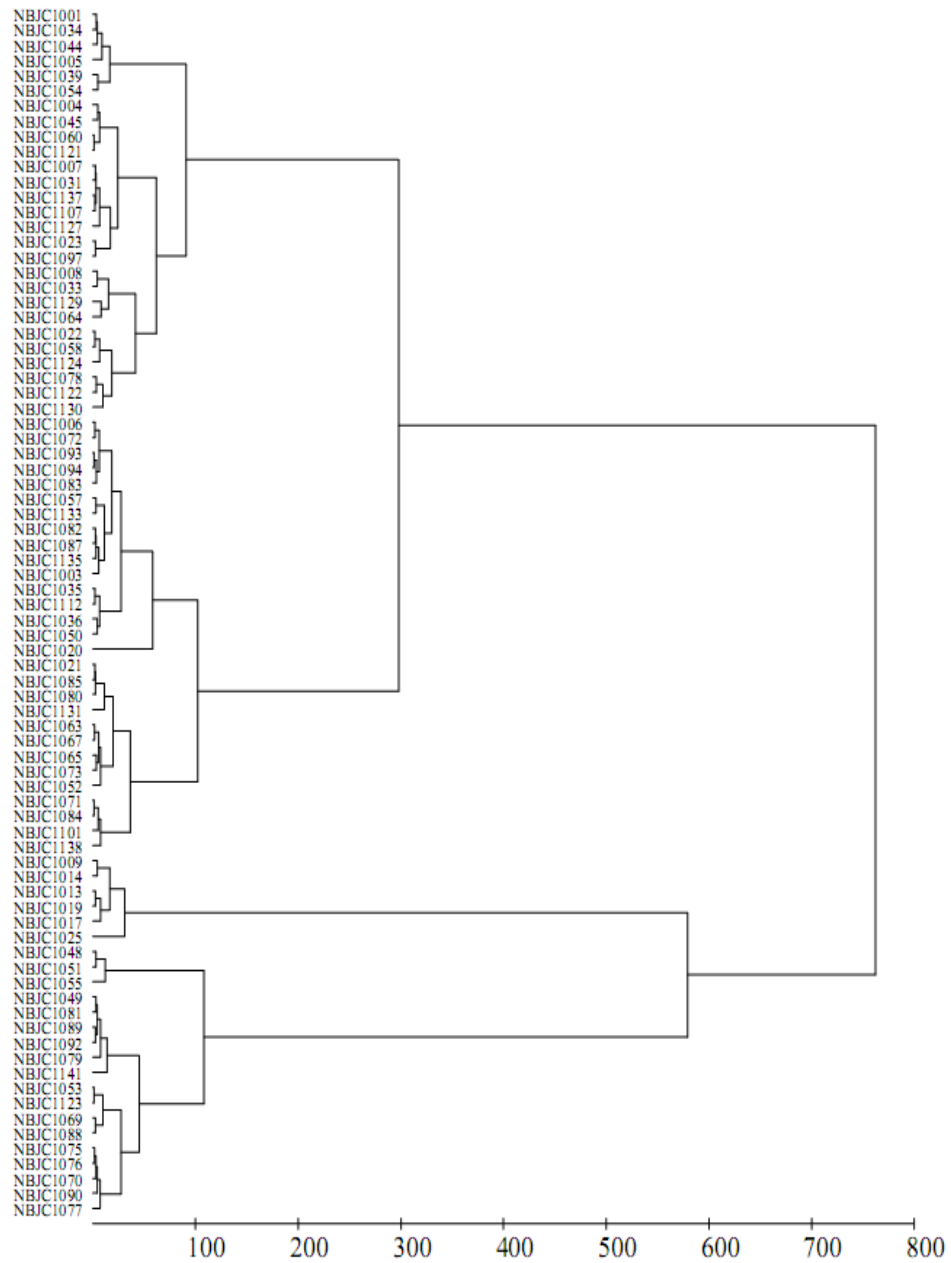


Figure 2. Dendrogram of 80 *Jatropha curcas* accessions derived from the Wards minimum variance cluster analysis using Mahalanobis distances

Similar findings were also reported earlier by RAO *et al.* (2008) and SUDHEER, *et al.* (2010) based on morphological traits and molecular marker studies respectively. The maximum intra cluster distance was noticed in cluster IV (30.15) followed by cluster I (28.61) and cluster II (25.89). The inter cluster distance varied from 47.59 (between cluster I and cluster II) to 211.27 (between cluster III and cluster I). Based on cluster distance, the cluster III showed maximum genetic distance with cluster I, followed by cluster IV and cluster II suggesting comparatively wider genetic diversity among them. The accessions from these clusters could be utilized in hybridization program to get desirable transgressive segregants in their offspring, as there is a higher probability that unrelated genotypes would contribute unique desirable alleles at different loci. Considering the cluster means, the cluster I showed highest mean value for all the traits except oil content. On contrary, the cluster III had lowest mean value for number of male flower/plant, male/female ratio, fruit weight/plant, seed weight/plant, seed length and seed width. The cluster I and cluster III seems to be unique with having highest and lowest cluster mean value for most of the traits respectively and also had highest inter cluster distance among them. The crossing among the accessions of these two clusters may yield hybrids with desirable traits.

Table 3. Intra- (diagonal bold) and inter-cluster Mahalanobis distances for 80 accessions in *Jatropha curcas*

	Cluster I	Cluster II	Cluster III	Cluster IV
Cluster I	<b>28.61</b>	47.59	211.27	91.62
Cluster II		<b>25.89</b>	129.47	66.51
Cluster III			<b>24.77</b>	153.24
Cluster IV				<b>30.15</b>

Table 4a. Cluster means and standard errors of the means of different traits in *Jatropha curcas* L.

	Female flower/plant	Male flower/plant	male/female ratio	No. of fruits/plant	Fruit weight/plant (g)
Cluster I	103.12±2.18	2471.53±68.74	24.30±0.70	89.31±2.37	432.88±14.52
Cluster II	91.71±1.82	2200.53±67.26	24.06±0.65	77.77±1.75	390.95±14.18
Cluster III	91.44±4.54	2018.00±1114.06	22.29±1.07	75.50±3.55	347.50±11.85
Cluster IV	85.91±2.26	2033.94±66.70	24.04±0.68	73.20±1.42	372.94±12.19



Table 4b. Cluster means and standard errors of the means of different traits in *Jatropha curcas* L.

	No. of seeds /plant	Seed weight/plant (g)	Seed length (mm)	Seed width (mm)	Oil content (%)
Cluster I	245.39±7.10	198.61±7.75	17.58±0.10	11.12±0.08	24.97±0.36
Cluster II	209.29±4.90	179.32±6.24	16.17±0.14	10.88±0.06	24.34±0.27
Cluster III	205.28±9.10	151.44±6.98	13.92±0.24	8.29±0.02	25.56±1.02
Cluster IV	196.41±4.11	163.63±7.37	15.98±0.16	10.72±0.07	31.04±0.69

Table 5. Loadings of the first four principal components of genetic divergence in 80 accessions of *Jatropha curcas*

Characters	PC <sub>1</sub>	PC <sub>2</sub>	PC <sub>3</sub>	PC <sub>4</sub>
Female flower/plant	0.40	0.42	0.56	0.09
Male flower/plant	0.37	0.32	0.25	0.21
male/female ratio	0.01	-0.05	-0.00	0.07
No. of Fruits/plant	0.37	0.21	0.48	0.19
No. of seeds /plant	0.51	0.55	0.89	0.55
Fruit weight/plant (g)	0.20	0.14	0.29	0.06
Seed weight/plant (g)	0.61	0.52	0.88	0.59
Seed length (mm)	3.07	-0.18	0.26	-0.87
seed width (mm)	1.95	-0.74	-1.10	0.95
Oil content (%)	-0.32	-3.05	0.72	0.07
<b>Components</b>				
Root	14.49	10.86	4.09	2.44
% Variance explained	42.54	31.87	12.02	7.18
Cum. variance Explained	42.54	74.41	86.43	93.62

In order to assess the patterns of variation, principal component analysis (PCA) was done by considering all the ten variables simultaneously. The first four principal components (PCs) accounted for more than 93% of the total variation (Table 5). PCA is a multivariate technique that allows to find the major patterns within a multivariate data set. Associations between traits emphasized by this method may correspond to genetic linkage between loci controlling traits or a pleiotropic effect. The first principal components accounted for 42.5% of the total variation due to seed length, seed width, seed weight/plant and number of seeds/plant which had maximum and positive weight on this component. Oil content had negative weight on PC1. Thus, PC1 related to the accessions with thick seeds, moderate to high seed yielder with low oil content. The PC2 concentrated 32% of total variation and was positively associated with seed

weight/plant, number of seeds/plant and female flower/plant. The oil content had highest negative weight on PC2 also followed by seed length, seed width and male female ratio. The third PC accounted for 12% variation was mainly due to seed weight/plant, number of seeds/plant, number of fruits/plants and seed width had negative weight. The seed weight/plant invariably had almost equal and positive weight on all four components.

### CONCLUSION

In conclusion, the phenotypic evaluation and characterization of wide range of *J. curcas* accessions showed that the most of the traits have low variability as revealed by various statistical parameters. Though, some sort of variations was noticed and cluster analysis indicated that the accessions from the clusters I and II have some potential towards the development of high oil yielding accessions of *J. curcas*. A planned hybridization programme based on inter crossing of promising accessions of different cluster may facilitate to accumulate favorable genes in hybrids.

### ACKNOWLEDGEMENT

The authors thank the Director, NBRI for providing the necessary facilities during the investigation

Received August 03<sup>th</sup>, 2013

Accepted October 05<sup>th</sup>, 2013

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**ANALIZA GENETIČKE VARIJABILNOSTI I DIVERGENTNOSTI *Jatropha curcas*  
NA OSNOVU OSOBINA CVETA I PRINOSA**

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Izvod

Utvrđena je genetička varijabilnost sadržaja ulja u 80 genotipova *Jatropha curcas* u rasponu u rasponu od 20.8-36.1% ( $X=26.2\pm 0.38$ ) a prosečan sadržaj je 26.2%. Hijerarhijskom analizom grupisanja ispitivani genotipovi su se grupisali u četiri grupe (klastera). Utvrđena je različita distanca unutar i između klastera. Analiza osnovnih komponenata variranja (PCA) je pokazala da prve četiri komponente (PCs) učestvuju sa više od 93 % ukupne varijabilnosti.

Primljeno 03. VIII.2013.

Odobreno 05. X. 2013.