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PHYSICOCHEMICAL PROPERTIES OF JATROPHA SEED OIL: DISCLOSES POTENTIAL SOURCE OF BIODIESEL PRODUCTION

A.K.M. AMINUL ISLAM[1*], S. CHAKRABARTY[1,2], IBRAHIM JAFRI[3], MOHAMED M. ZAYED[4], ALAA BAAZEEM[5], AMANY S. EL-KHOULY[6], MOHAMED SAKRAN[7], AND AYMAN EL SABAGH[8*]

[1]Department of Genetics and Plant Breeding Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur 1706, Bangladesh
[2]College of Forest Resources and Environmental Science, Michigan Technological University 1400 Townsend Drive, Houghton, MI 49931, USA
[3]Department of Biotechnology, College of Science, Taif University, P.O. Box 11099, Taif 21944, Saudi Arabia
[4]Department of Chemistry, Rabigh College of Sciences and Arts, King Abdulaziz University, Jeddah, Saudi Arabia
[5]Department of Biology, College of Science, Taif University, P.O. Box 11099, Taif 21944, Saudi Arabia
[6]Department of Chemistry, College of Science, King Faisal University, Al-Ahsa, P.O. Box 380, Hofuf, Saudi Arabia
[7]Department of Biochemistry, Faculty of Sciences, University of Tabuk, Tabuk 71491, Saudi Arabia
[8]Department of Field Crops, Faculty of Agriculture, Siirt University, Siirt-56100, Turkey
**Corresponding author's e-mails: aminulgpb@bsmrau.edu.bd, aymanelsabagh@gmail.com*

Abstract

Jatropha is an alternative source of fossil fuel grown in the tropics and subtropics. This research focuses on measuring of physical, chemical and (lubricating) characteristics of Jatropha seed oil extracted from 45 genotypes to explore its potential as biodiesel feedstock. Significant genetical variation was observed from the ANOVA for the genotypes. High coefficient of variation for genotype and phenotype were found for all seed oil properties with moderate differences. The traits reported the highest values of broad sense heritability and genetic advance were oil moisture content (85.77%), free fatty acids (52.99%) and peroxide value (148.84%). Significant correlation coefficient was found at 10 physiochemical characteristics Jatropha seed oil samples. The oil content of seed (%) revealed significant positive correlation coefficient with oil density (0.40**), oil moisture content (0.21*), saponification value (0.26**). Cluster analysis based on seed oil properties; 45 Jatropha genotypes were clustered in six groups. The maximum number of the genotypes (11) were grouped into cluster V. The cluster V integrated the highest number of genotypes while the second and third top-performing genotypes were found in cluster IV (9) and II (8), respectively.

Key words: *Jatropha curcas*, Variability, Correlation, Cluster analysis, Physicochemical properties, Biodiesel.

Introduction

To ensure future energy security, the scientists are looking for the alternate source of fossil fuel due to the increasing demand on fuel, depletion of its reserve and global warming. Vegetable oils have widely been used to produce biofuel as a substitute of petroleum fuel. Biofuel production from edible vegetable oils limits the supply and use of oil as edible food. The genus Jatropha belongs to Euphorbiaceae family, a non-edible source of vegetable oil which can be used as alternative of edible oils as a source of biofuel. Jatropha species is originated in South America (Ramawat, 2010), and has been moved other tropical and sub-tropical countries (Mabberley, 2008). Due to wider geographical adaptability and low cost of production Jatropha seed oil has given a significant attention as viable source of renewable energy (Oliveira *et al.,* 2013). Jatropha seed oil was also used for the preparation of medicine, textile dye, soaps, and cosmetics (Moniruzzaman *et al.,* 2017) and the biomass of Jatropha is used to prepare briquettes, absorbent, resin, bioactive compounds and compost (Primandari *et al.,* 2018). Jatropha has wider genetic variation in plant architecture, fruiting behavior, fruit yield, seed yield and oil content in the kernel (Xu *et al.,* 2012; Guan *et al.,* 2013). This variation in oil content reached 30-65% of Jatropha seed (Basha *et al.,* 2009; Nzikou *et al.,* 2009). The acreage planted with Jatropha worldwide is nearly million ha, with>80% located in Asia, where India is the largest cultivator of Jatropha globally, followed by China, Myanmar, 12% in Africa and 2% in Latin America (Edrisi *et al.,* 2015). Chakrabarty *et al.,* (2019) identified the diversity of 17 morphological traits of *Jatropha curcas* germplasm in Bangladesh, the analysis of genetic diversity may provide inclusive evidence on genetic base which is important for the germplasm utilization, management and conservation (Qibao *et al.,* 2008, Achten *et al.,* 2010). the horizons of Jatropha potential for biodiesel production of Jatropha genotypes in Bangladesh was studied by Khatun *et al.,* (2019). However, variety selection and commercial cultivation for biodiesel industry in Bangladesh is still lacking. For initiating efficient breeding programs towards the genetic improvement of Jatropha that entails wider adaptability to different ecosystems, prerequisite knowledge on genetics and germplasm diversity is a dire need. The present study is mainly focused on the evaluation of physiochemical properties of *Jatropha curcas* to understand the plant potential as a source of feedstock for biodiesel production.

Material and Methods

Experimental site: The experiment was conducted in the Field Laboratory of the Department of Genetics and Plant Breeding, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh. The site is located at the central part of Madhupur Tract (AEZ-28) and was positioned in the sub-tropical climate zone (24.05^0N latitude and 90.25^0E longitude) at an elevation

of 804 m above the sea level. There was significant rainfall in this site (average 70-75 mm) during the month of May to September and scanty of rainfall from September with gradual decrease of temperature up to March (25-130 mm). The site is characterized with silt loam soil texture with pH of 6.7 (Brammer, 1978).

Materials of the experiment: The experiment incorporated 45 Jatropha genotypes previously collected from different parts of Bangladesh, India, Nepal, Malaysia, Thailand, Indonesia and South Africa. The age of the Jatropha plants were 8-10 years from which the seeds were collected to extract seed oil.

Extraction of *J. curcas* oil: *J. curcus* seed oil was extracted through Soxhlet Apparatus using n-hexane as solvent (Sayyar *et al.,* 2009). About 300 ml of n-hexane was poured in a round bottom flask. Ten grams of grinded powder of *J. curcus* seed kernel was packed in a filter paper and placed into a thimble. This thimble with kernel powder was inserted into the extractor center and fixed with round bottom flask and the condenser and placed on heating mantle heated (50-60^{0}C) to boil solvent (Akpan *et al.,* 2006). The total process of extraction continued for 6-8 hrs. The extract with solvent in the round bottom flask was transferred to the rotary evaporator to recover the extracted seed oil at 40^{0}C from the excess solvent. The solvent was recovered by filtering from the oil for further use.

Percentage of oil extracted: The oil content was estimated as a percentage of the oil extracted from the purified seed kernel powder. The seed oil was then stored at 2^{o}C in a refrigerator for subsequent analysis of multiple physical, chemical and fuel properties.

Physical characteristics of seed oil

Oil density (gcm^{-3}): The density of extracted seed oil was determined by Anton-Par DMA 4500 density meter (Graz, Germany) at 20^{o}C.

Chemical characteristics of the seed oil: Free fatty acids (as % of oleic acid), acid value (KOHg^{-1}), iodine value (I_2 100 g^{-1}), saponification value (mgKOH^{-1}) and peroxide value (mMolkg^{-1}) were estimated as per the Standard Tentative Methods of Analysis (Anon., 1991).

Fuel characteristics of the seed oil

Higher heating value (MJkg^{-1}): The higher heating value (HHV) refers to the quantity of heat release after a unit amount of oil or fuel is combusted. Higher heating value of Jatropha oil sample was calculated according to the formula developed Demibras (1998; 2003) using the iodine and saponification value.

$$HHV = 49.43 - (0.041 \times SV) - (0.015 \times IV)$$

Cetane number: Cetane number of the seed oil sample was estimated using the formula given by Bose (2009).

$$CN = 46.3 + \frac{5458}{SV} - 0.225 \times IV$$

Data analysis: The data analysis of all investigated traits of the seed oil of Jatropha samples was performed using Computer based STAR software package (Statistical Tools for Agriculture Research). Genetic components of variability, heritability and genetic gain of the traits were estimated according to the formula suggested by Allard (1960), and Singh & Chaudhary (1985). Principal Component Analysis (PCA), Correlation Coefficients and Boxplot analysis was done using Statistical Package 'R version 3.5.1'. The cluster analysis was performed using PAST Package (Paleontological Statistics) version 3.14.

Results

ANOVA and analysis of variability parameters: Significant variations were observed between the genotypes for all studied characters (Table 1). Oil content of Jatropha seed kernel was 53.75±2.12% and oil moisture content was 0.91±0.07% (Table 1). The average Oil content extracted using Soxhlet method was 30-40% higher than the values reported by Lonazo (2007) and 65-80% lower than the values reported by Reinhard (2007). The average density of the oil sample was 0.88 g per cm^3 (Table 1), this result is in agreement within with the findings of Akbar *et al.,* (2009). The differences may be due to genotype x environment interactions. Average acid value was found 1.73 mg NaOH/g oil and FFA value of the samples was 0.85 which was greatly relevant with the findings of Akbar *et al.,* (2009) and Tint & Mya (2009). This value is an indicator of quality of the fatty acids in the seed oil. The average iodine value (100.84±0.94 mg/g oil) of Jatropha seed oil found in the study was significantly pertinent with the findings of Knothe (2002) and the value reported was 105.47 mg.g^{-1} oil. The average value for saponification number is227.98 ± 0.98 mg KOH.g^{-1} oil. The saponification value observed in this study was higher than the value (182.45 mg KOH.g^{-1}) found by Akbar *et al.,* (2009). Saponification value is the indicator of quality of oil and triglyceride present in the oil sample which is fundamental property for detergents production. The result of peroxide value of oil sample was 9.67±0.63 nMo.kg^{-1} and this result was lower than the findings reported by Akbar *et al.,* (2009). Average value obtained for HHV was 38.57±0.04 MJ.kg^{-1} and it was close to the value found by Becker & Makkar (2009). It was also consistent with the findings reported by Nevase *et al.,* (2008). The value reported in this study aligned with the reports of Nevase *et al.,* (2008). Average cetane number (48.01±0.22) observed for the oil sample was slighter than the cetane number (57.29) found by Bose (2009).

Genotypic Coefficient Variation (GCV) and Phenotypic Coefficient Variation (PCV) for 10 physiochemical and fuel properties are presented in Table 2. The highest value of PCV and GCV were recorded to peroxide Value (mMolkg^{-1}) trait with 73.128% and 72.688%, respectively. followed by Oil Moisture Content (%) with 43.48% and 42.54%, respectively. While low values of PCV and GCV were from oil density (gcm^{-3}) 2.62% and 2.38%, respectively high heating value (MJkg^{-1}) (3.27% and 3.26%, respectively) and oil percentage (%) (9.45% and 8.12%, respectively).

Table 1. ANOVA and mean performance for physicochemical properties of *Jatropha curcas* genotypes.

Oil properties	Replications (df=2)	Genotypes (df=44)	Error (df=88)	CV	Mean ± SE	HSD
Oil Percentage (%)	8.23^{NS}	63.88**	6.75	4.83	53.75 ± 2.12	8.67
Oil density (gcm^{-3})	0.001**	0.001**	0.00	1.10	0.88 ± 0.01	0.03
Oil moisture content (%)	0.01^{NS}	0.45**	0.01	8.96	0.91 ± 0.07	0.27
Free fatty acids (as % oleic acid)	0.003**	0.15**	0.00	0.95	0.85 ± 0.07	0.03
Acid value ($KOHg^{-1}$)	0.01^{NS}	0.50**	0.03	10.00	1.73 ± 0.14	0.58
Iodine value (I_2 100 g^{-1})	8.56**	1021.85**	1.33	1.14	100.84 ± 0.94	3.84
Saponification value ($mgKOH^{-1}$)	28.48**	2703.74**	1.43	0.52	227.98 ± 0.98	3.99
Peroxide value ($mMolkg^{-1}$)	0.15^{NS}	148.96**	0.60	8.01	9.67 ± 0.63	2.59
Cetane number	0.86**	87.76**	0.07	0.57	48.01 ± 0.22	0.91
High heating value (MJ/kg)	0.06**	4.74**	0.00	0.13	38.57 ± 0.04	0.17

** Significant at p<0.01; ns = Non-significant; df = Degrees of freedom; CV: Coefficient of variation (%), HSD: Tukeys's honest significant difference

Table 2. Determination of genetic parameters for physicochemical properties seed oil samples of *Jatropha curcas.*

Oil properties	GCV	PCV	h^2_b	GA	GA (% of mean)
Oil percentage (%)	8.12	9.45	73.83	7.72	14.37
Oil density (gcm^{-3})	2.38	2.62	82.77	0.04	4.47
Oil moisture content (%)	42.54	43.48	95.75	0.78	85.77
Free fatty acids (as % oleic acid)	25.74	25.76	99.87	0.45	52.99
Acid value ($KOHg^{-1}$)	22.71	24.81	83.78	0.74	42.82
Iodine value (I_2 100 g^{-1})	18.29	18.33	99.61	37.92	37.61
Saponification value ($mgKOH^{-1}$)	13.17	13.18	99.84	61.78	27.10
Peroxide value ($mMolkg^{-1}$)	72.69	73.13	98.80	14.40	148.84
Cetane number	11.26	11.28	99.75	11.12	23.17
High heating value ($MJkg^{-1}$)	3.26	3.27	99.84	2.59	6.71

PCV = Phenotypic coefficient of variation; GCV = Genotypic coefficient of variation; h^2b = Broad sense heritability (%); GA = Genetic advance

Table 3. Maximum, minimum and average value of physical, chemical and fuel characteristics of 45 genotypes of *jatropha curcas* seed oils.

	SOP	OD	OMC	FFA	AV	IV	SV	PV	CN	CV/HHV
Max	61.88	0.95	2.00	1.34	2.47	129.82	271.24	31.00	61.83	41.47
Min	19.22	0.79	0.40	0.48	0.79	70.10	160.33	1.00	37.55	36.40
Average	53.75	0.88	0.91	0.85	1.73	100.84	227.98	9.67	48.01	38.57

SOP= Seed oil percentage (%), OD= Oil density (gcm^{-3}), FFA= Free fatty acid (as % oleic acid), OMC= Oil moisture content (%), AV= Acid value ($KOHg^{-1}$), IV= Iodine value (I_2 100 g^{-1}) and SV= Saponification value ($mgKOH^{-1}$), PV= Peroxide value ($mMolkg^{-1}$), HHV= High heating value ($MJkg^{-1}$) and CN= Cetane number

Heritability is the indicator of heritable portion of variation. In the present study, very high (>95%) heritability in broad sense was observed for oil moisture content (95.75%), free fatty acids (99.87%), saponification value (99.84%), iodine value (99.61%), peroxide value (98.80%), cetane number (99.75%), high heating value (99.84%). High heritability (>80%) was recorded for the physiochemical character's oil density (82.77%) and acid value (83.78%) except seed oil percentage (73.83%) that exhibited moderate heritability. Similar findings of low heritability estimates for seed moisture content were also reported by Ngugi *et al.,* (2013). Genetic Advance estimates varied from 61.78% for saponification value ($mgKOH^{-1}$) to 0.04% for oil density ($g.cm^{-3}$). Oil moisture content, free fatty acids, and peroxide value demonstrated high values of heritability and genetic advance (>50%).

Performance of *J. curcas* seed oil for physicochemical properties: Performance of the *J. curcas* genotypes for the all physical, chemical and fuel properties in this study are described in Figure 1 using boxplots. The median value of each parameter is indicated by the line within the boxplot. The horizontal lines (right whiskers), colored boxes (interquartile range boxes) and left whiskers indicated the data range of 25% (lower), 50% (middle) and 25% (higher) genotypes, respectively.

Table 3 indicates variation of genotypic performance in terms of mean for oil density (0.79-0.95 $g.cm^{-3}$ with an average of 0.88 $g.cm^{-3}$), free fatty acid (0.48-1.34 as % oleic acid), oil moisture content (0.4-2.00%), acid value (0.79-2.47 $KOHg^{-1}$), seed oil percentage (19.22-61.88% with an average of 53.75%), peroxide value (1.00-31.00 $mMolkg^{-1}$), iodine value (70.10-129.82 I_2 100 g^{-1}), saponification value (160.33-271.24 $mgKOH^{-1}$), high heating value (36.40-41.47 $MJkg^{-1}$) and cetane number (37.55-61.83). The results found for seed oil content fell within the limits (20-60%) reported by Pramanik (2003) depending on the source of seed samples. The results of El Kinawy (2010) demonstrated 45% oil on kernel basis from Jatropha seed which is attributed to the variation in the seeds sources, growing environment of the plant, stage of ripening, harvesting time and storage condition of the seeds

(Nzikou *et al.*, 2009; Kimbonguila *et al.*, 2010). FFA is one of the most vital characteristics of oil which signifies the worth of seed oil quality. The high value of FFA indicated that the oil is unfeasible for transesterification (Tiwari *et al.*, 2007; Canaki, 2007). The genotype with low free fatty acid content requires lower quantity of base to neutralize free fatty acid available in the oil sample having high FFA (Demibras, 2003; Tiwari *et al.*, 2007; Canaki, 2007). Seed oil samples with high free fatty acid (>1%) require extra process like acid transesterification or pretreated transesterification or two-step process to insure proper use as feedstocks for biodiesel production (Canaki & Grephen, 2001; Dorado *et al.*, 2002). But the base-catalyzed transesterification is faster than the acid-catalyzed transesterification (Crabbe *et al.*, 2001).

Iodine value represents the content of the unsaturated fatty acids in the seed oil sample. High content of unsaturated fatty acid is indicated by high iodine value of the oil sample (Knothe, 2005). The presence of high amount of unsaturated fatty acid such as oleic and linoleic acids is responsible for high iodine value of seed oil. Akbar *et al.*, (2009) reported 78.5% unsaturated fatty acids in the *J. curcas* oil samples from different origin. The prospects of *J. curcas* seed oils in the production of alkyl resin, shoe polish, varnishes etc. depends on its iodine value (Akintayo, 2004). Similar results of *J. curcas* seed oil from Malaysian origin were also reported by Islam *et al.*, (2011; 2012; 2022). High saponification value indicates that oils are normal triglycerides which can be used as raw materials of soap and shampoo production. Oils with low saponification value is not suitable for soap industry and requires more alkaline to neutralize available free fatty acids released by the oil samples.

Boxplot for oil density was normally distributed for this trait with two outliers (Fig. 1). The genotypes G12 and G29 were in the normal range that exhibited higher performance compared to remaining genotypes for this parameter (Fig. 1). The boxplot for free fatty acids and oil moisture content were skewed right on the other hand boxplot for acids value skewed left which revealed uneven dispersion of the genotypes for this physiochemical property of oils of 45 *J. curcas* genotypes (Fig. 1). None of the genotypes displayed higher performance in these parameters.

The box plot for seed oil percentage is said to be symmetrical as the median was equidistant from the maximum and minimum values of this parameter which means values for this parameter is normally distributed among 45 *Jatropha curcas* genotypes. In Figure 1, distance from the median to minimum was less than the distance from median to maximum for peroxide value, cetane number and high heating value which the box plot is positively skewed and the genotypes were not normally distributed for these parameters. On the other hand, the box plot for iodine value and saponification value was negatively skewed or skewed left which indicated asymmetry in the distribution of the genotypes for these parameters.

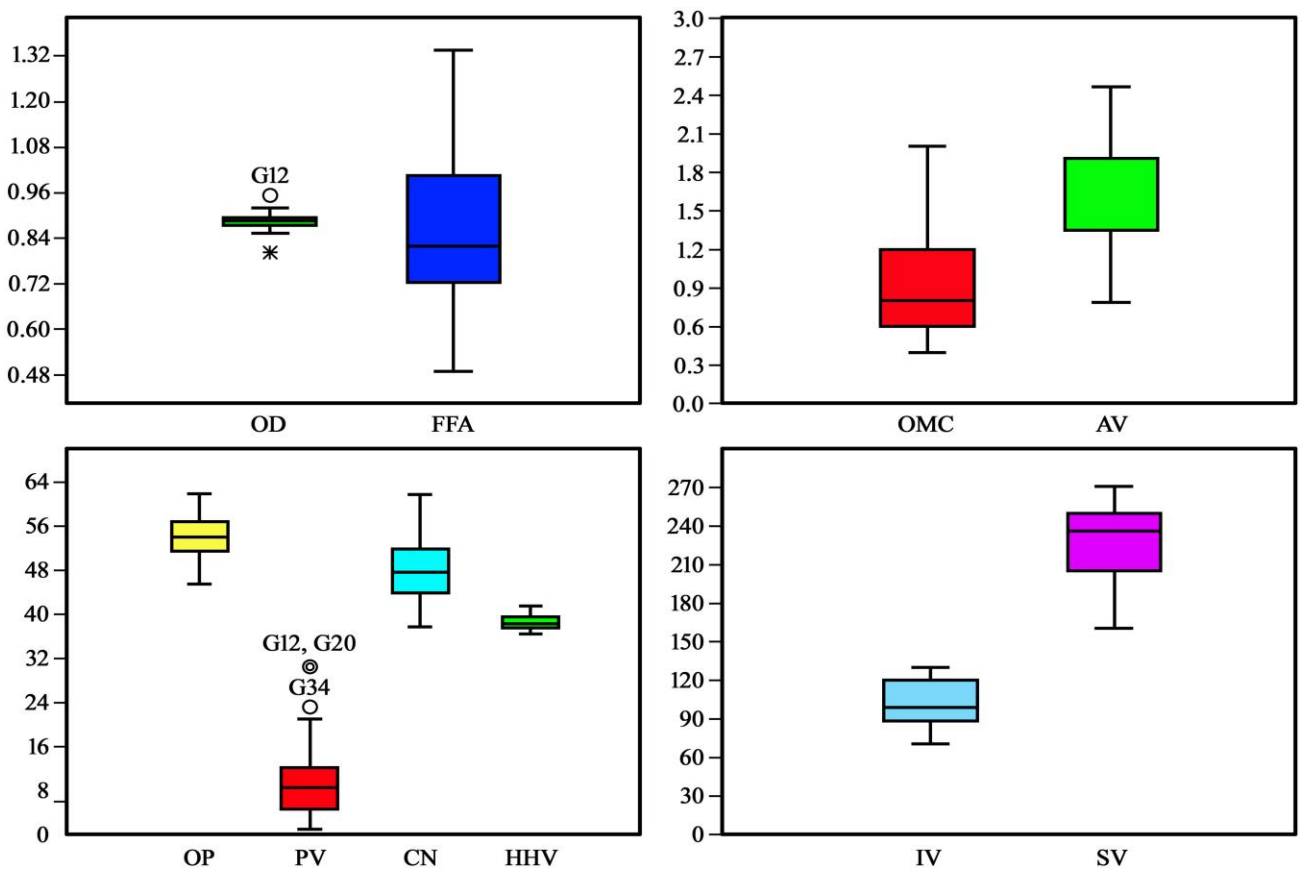

Fig. 1. Performance of the genotypes for the studied physicochemical properties.
OP: Oil percentage (%), OD: Oil density (gcm^{-3}), OMC: Moisture content of Oil (%), FFA: Free fatty acid, AV: Acid value ($KOHg^{-1}$), SV: Saponification value ($mgKOH^{-1}$), IV: Iodine value (I_2 100 g^{-1}), PV: Peroxide value ($mMolkg^{-1}$), HHV: High heating value ($MJkg^{-1}$) and CN: Cetane number.

Table 4. Genotypic correlation coefficient among the physicochemical properties of *Jatropha curcas* genotypes.

	OD	MC	FFA	AV	IV	SV	PV	CN	HHV	OP
OD	1.00	0.20*	0.08^{NS}	-0.03^{NS}	0.05^{NS}	-0.11^{NS}	0.28**	0.05^{NS}	0.10^{NS}	0.40**
OMC		1.00	-0.26^{**}	-0.40**	0.08^{NS}	0.38**	-0.09^{NS}	-0.30**	-0.39**	0.21*
FFA			1.00	0.81**	-0.18*	-0.15^{NS}	0.14^{NS}	0.23**	0.19*	0.10^{NS}
AV				1.00	-0.24**	-0.03^{NS}	0.24**	0.19*	0.08^{NS}	0.03^{NS}
IV					1.00	-0.02^{NS}	0.12^{NS}	-0.75**	-0.20*	-0.18*
SV						1.00	0.00^{NS}	-0.65**	-0.98**	0.26**
PV							1.00	-0.11^{NS}	-0.03^{NS}	0.09^{NS}
CN								1.00	0.80**	-0.03^{NS}
HHV									1.00	-0.21*

* and ** Indicates level of significance ($p<0.05$, $p<0.01$); ns= Non-significant

OP: Oil percentage (%), OD: Oil density (gcm^{-3}), OMC: Moisture content of oil (%), FFA: Free fatty acids, AV: Acid value ($KOHg^{-1}$), IV: Iodine value (I_2 100 g^{-1}), SV: Saponification value ($mgKOH^{-1}$), PV: Peroxide value ($mMolkg^{-1}$), HHV: High heating value ($MJkg^{-1}$) and CN: Cetane number

Table 5. Number of *Jatropha curcas* genotypes grouped into six different clusters.

Clusters	Number of genotypes	Genotypes
I	4	G10, G18, G30, G39
II	8	G1, G7, G8, G11, G12, G21, G36, G45
III	6	G2, G3, G14, G17, G43, G44
IV	9	G5, G6, G13, G15, G16, G19, G20, G22, G24
V	11	G4, G23, G25, G26, G27, G28, G29, G31, G35, G38, G41
VI	7	G9, G32, G33, G34, G37, G40, G42

Analysis of correlation coefficients among 10 physiochemical properties: Significant correlation coefficients were observed among 10 physiochemical properties such as oil content (%), moisture content of oil sample (%), free fatty acid, acid value ($KOHg^{-1}$), iodine value (I_2 100 g^{-1}), saponification value ($mgKOH^{-1}$), high heating value ($MJkg^{-1}$), cetane number except oil density ($g.cm^{-3}$) and peroxide value ($mMolkg^{-1}$) (Table 4). Seed oil content (%) had significant positive correlation coefficient with oil density (0.40**), oil moisture content (0.21*), saponification value (0.26**) and significant negative correlation with iodine value (-0.18*) and high heating value (-0.21*). Oil density had significant positive correlation coefficient with moisture content (0.20*) peroxide value (0.28**). Free fatty acid content had significant positive correlation with acid value (0.81**), cetane number (0.23**), and high heating value (0.19*) and negative significant correlation with iodine value (-0.18*). The acid value had significant positive correlation with peroxide value (0.24**) and cetane number (0.19*). Significant negative correlation coefficient was observed for cetane number with iodine number (-0.75**) and saponification number (-0.65**). Cetane number had significant positive correlation coefficient with high heating number (0.80**).

Principal component analysis: Principal components analysis was done to identify major components of variation and their relative contribution and correlation pattern among the 10 physiochemical traits. Normalized data were used in principal component analysis in controlling existing variation. First four principal components were responsible for maximum variation for 10 physiochemical characters where principal component 1 (PC 1) contributed most of the variation. From Figure 2a, it was observed that PC 1, PC 2, PC 3 and PC 4 were sufficient to explain maximum variations. The principal component analysis was further determined the existence of high level of variation and the Jatropha genotypes and revealed the overall diversity. Shabanimofrad *et al.,* (2013) also reported that first four principal components contributed maximum toward the total variations. There were some sorts of correlation coefficient negative and positive were also found between principal components and the traits (Fig. 2b). Negative correlation was observed between principal component 1 (PC 1) and free fatty acid, acid value, iodine value, cetane number, high heating value whereas positive correlation was observed with seed oil content, oil moisture content, iodine value, saponification value and peroxide value. PC2 was positively correlated with oil moisture content, iodine value, cetane number and high heating value. First principal component scores were plotted against the given genotypes to visualize variation (Fig. 3). Therefore, the extent of similarity or differences among the genotypes in the context of studied characteristics were possible to identify clearly. The oil content, moisture content of oil, saponification value, iodine value and peroxide value were in the same direction as PC1, hence they are positively correlated with PC1. On the other hand, oil density, free fatty acid, acid value, high heating value, cetane number were in opposite direction with PC1, which implied a negative association (Fig. 2b). Hence smaller value PC1 represents for free fatty acid, acid value, oil density, high heating value and cetane number.

Cluster analysis: Cluster analysis was performed using the data on physiochemical properties of 45 Jatropha genotypes which grouped the genotypes into six clusters (Table 5). Cluster V contained the highest number of genotypes (11), cluster IV has the second highest (9 genotypes) and cluster II has the third highest (8 genotypes) whereas cluster I contained the least number (4) of genotypes (Table 5).

Table 6. Cluster distance inter-cluster (Off-diagonal) and intra cluster (diagonal).

Cluster	I	II	III	IV	V	VI
I	**1.324**					
II	9.513	**0.24**				
III	14.197	5.372	**0.561**			
IV	15.384	7.52	2.699	**1.974**		
V	13.409	7.382	4.678	3.156	**0.485**	
VI	11.77	4.346	2.923	3.65	3.099	**2.584**

The results of inter cluster distance indicate to the amount of diversity between the genotypes of two different clusters for seed oil properties while the amount of intra-cluster distance indicates the genetic diversity of the genotypes of a single cluster.

Table 6 represents the intra and inter cluster distances of six clusters. According to the cluster distance, it ranged from 0.240 (cluster II) to 2.584 (cluster VI). The highest inter cluster distance was observed between the cluster I and cluster IV (15.384) trailed by cluster I and III (14.197) and cluster I and V (13.409). The lowest cluster distance (2.699) was found between cluster III and cluster IV followed by III and VI (2.923).

The importance of cluster analysis in genetic diversity analysis of the genotypes has previously been described by Mahalanobis (1936). Genotypes with similar seed oil properties were located in the same cluster and the genotypes with different seed oil properties were clustered into different cluster. Genetically distant genotypes feature high diversity and are candidates for parental selection in breeding Jatropha for enhanced seed oil properties (Rao *et al.*, 2008; Christo *et al.*, 2014; Santos *et al.*, 2016; Chakrabarty *et al.*, 2019).

The cluster mean values of 10 physiochemical properties are presented in Table 7. The higher mean value for the seed oil content was found in cluster IV. The highest mean value was found for the cetane number and high heating value in cluster I. On the other hand, the highest mean value for free fatty acid, acid value and peroxide value were found in the cluster III. Cluster IV had the highest mean value for iodine value and saponification value.

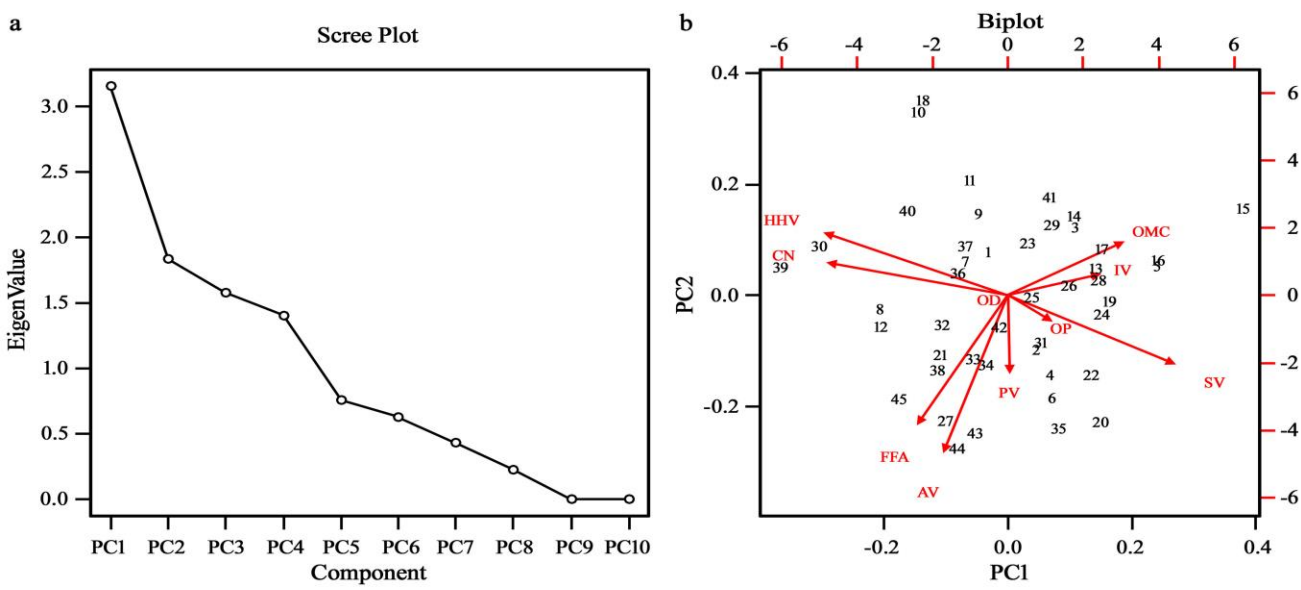

Fig. 2. Principal component analysis showing: **a**, eigenvalues in the scree plot; **b**, biplot of PC 1 and PC 2.

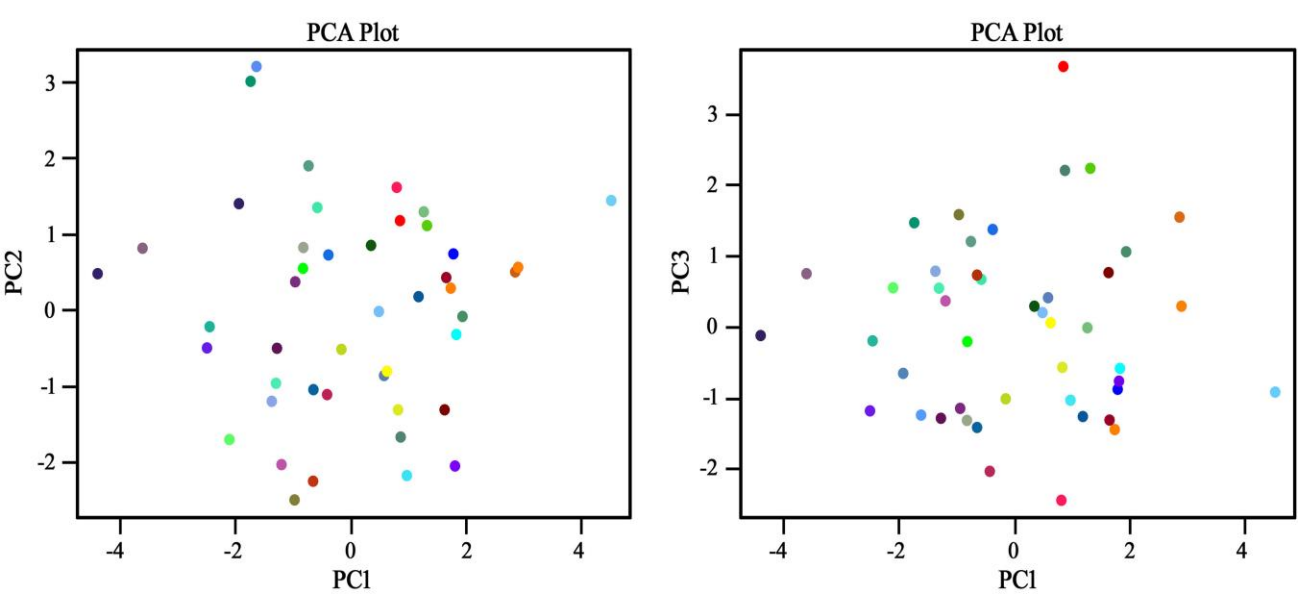

Fig. 3. Principle components scores plotted corresponding to given genotype to visualize genetic variation.

Table 7. Cluster means of 10 physiochemical properties of 45 Jatropha genotypes.

Oil Properties	I	II	III	IV	V	VI
Oil percentage (%)	51.64	53.14	52.21	54.78	54.39	56.59
Oil density ($g.cm^{-3}$)	0.88	0.89	0.88	0.89	0.88	0.89
Oil moisture content	0.8	0.68	0.9	1.09	1.02	0.89
Free fatty acids	0.82	0.95	0.89	0.77	0.85	0.86
Acid value	1.49	1.84	1.82	1.66	1.76	1.67
Iodine value	95.4	111.1	113.85	119.84	85.2	81.6
Saponification value	169.56	194.28	231.74	257.06	253.39	225.16
Peroxide value	4.5	12.59	12.13	9.89	7.24	11.11
Cetane number	57.08	49.44	44.25	40.59	48.7	52.23
High heating value	41.05	39.8	38.22	37.09	37.76	38.97

Conclusion

The study revealed significant genetic diversity study among 45 *Jatropha curcas* genotypes for 10 physiochemical properties of seed oil. Though the oil content (%), moisture content of the oil (%), free fatty acid content, acid value ($KOHg^{-1}$), iodine value (I_2 100 g^{-1}), saponification value ($mgKOH^{-1}$), cetane number, high heating value ($MJkg^{-1}$) except oil density (gcm^{-3}) and peroxide value ($mMolkg^{-1}$) were significantly correlated. Coefficient of variation (CV), broad sense heritability, genetic advanced in percent of mean, and genetic diversity in terms of PCA all collectively indicate that the characters under study could be considered as topmost selection standards for the improvement of *J. curcas*. Genetic clustering of Jatropha genotypes based on 10 physiochemical properties revealed that genotypes of cluster I has the maximum distance from the genotypes of cluster IV. So, our study introduces potential parents of Jatropha genotypes parents from two clusters to be utilized to improve physiochemical properties by selective breeding.

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