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Morphological Characterization of Some Accession of *Jatropha curcas* L. for Biofuel Production

B. Y. Abubakar, D. B. Dangora, A. K. Adamu, D. N. Iortsuun, B.D. Abdul-hadi, M.A. Saidu, S.A. Saminu and S.U. Abubakar

Department of Biological Sciences, Ahmadu Bello University, Zaria-Nigeria

* Corresponding author: E-mail: byabubakar@abu.edu.ng, GSM: +234-806-157-0509

ABSTRACT

The search for alternative energies in the present day realities of crude petroleum resources price fluctuations become pertinent to ensure sustainable expenditure in other government socioeconomic obligations. Plant resources with their basic role in our ecosystem have of recent been used to provide a mitigation avenue in meeting the challenges of our renewable energy needs through biofuel production. Experiences in terms of *Jatropha curcus* L. cultivation elsewhere needs to be considered before adoption in new promising areas. Performance in reproductive yields of plants can be judged through enhanced vegetative responses and growth. Based on these, an investigation on the morphological variation among sixteen *J. curcas* accessions from northern Nigeria was carried out and is hereby reported. The generated data on seed, fruit, germination and seedling attributes were analyzed using the NTSYS pc software, and the 16 plant accessions were clustered into five groups with specification on area of collection. For instance, all the 3 accessions from north eastern Nigeria were grouped in cluster V while cluster IV is composed of only TZZM18 collected from the extreme north western Nigeria. The highest values in seedling height, leaf length and leaf width of (15.851, 9.315 and 7.747)cm were shown by KDKN04, KGKN08 and RNJG13 respectively. Incidentally, these accessions were all from Kano State confirming closeness in terms of geographical location which ensures easy pollen exchange at their reproductive life phase. This study concludes that morphological variability exist in the *J. curcus* from northern Nigeria that would provide the chance for seed and germplasm improvement for better yield in biofuel production.

Key words: *Jatropha curcas*, Accessions, Morphology, Clusters, Variability

INTRODUCTION

The present day challenges in environmental change and its consequences to human and ecosystem have become an eye opener with regards to alternative energy provisions. Dependency on fossil fuels with its attending environmental disadvantages is viewed by not only the industrial economies but, by the producers of these petro-dollar resources. Human civilization is directly linked with energy consumption. The famous "1973 OPEC oil shock" brought into focus the limited supply of fossil fuels (Fig. 1). Also, consequences of environmental change triggered the need for energy conservation and renewable energy (Kishore, 2012). It has now been realized that one of the way out is to take a deeper look at other alternative renewable energy sources from vegetables (biofuels), solar wind geothermal etc. These sources are regarded more environmentally friendly as their discharges are not known to present many consequences to environmental imbalance. Equally, in the cycle of their production, plants for biofuel bring mitigation against climate change through carbon fixation, ensure generation of organic manure and bring about healthy environment.

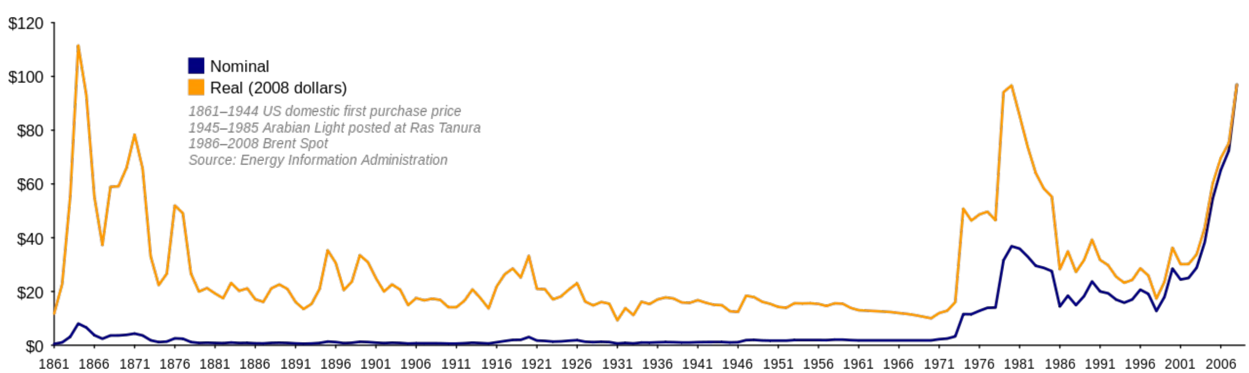

Fig.1. Graph of oil prices showing sharp increase in **1973-** $3-$12/barrel (http://en.wikipedia.org/wiki/1973_oil_crisis (September 3, 2014)

From experiences elsewhere, *J. curcas* as biofuel feedstock is not without challenges. For instance in Kenya, Mogaka *et al* (2014) reviewed Jatropha cultivation and production (2005-2010) in four districts and concluded that Jatropha plantations are uneconomical and risky due to competition for land and labour with food crops. They concluded that its production be abandoned until improved seed material is available, locally adapted agronomic knowledge about its cultivation is available and production becomes economically competitive. In China, Weyerhaeuser *et al* (2007) reviewed Jatropha biofuel production in southwest China and observed the need to increase in acreages does not guarantee oil and seed yield improvement, but, there is the need to significantly scale up research capacity in germplasm improvement and supply chain economics.

J. curcas is a member of the Euphorbiaceae family and one of such alternative vegetable being an oil bearing shrub/tree which grows in arid and semi-arid climates. The plant is traditionally used for medicinal purposes especially in treating toothache among the Hausas (from northern Nigeria), it is also useful for the prevention and control of soil erosion as living fence and presently as source of oil which can be converted to bio-diesel. FAO (2010) confirmed that apart from its use as liquid fuel, the oil has been used to produce soap and biocides (insecticide, fungicide, nemacide and molluscide). During oil production *J. curcas* is transesterified to methyl esters (bio-diesel) and glycerol. The latter is an important by-product, which can be burned for heat or used as a feedstock in cosmetic industry. In northern Nigeria, *J. curcas* is not the only representative of the genus Jatropha; other species include *J. gossypifolia*, *J. podagrica* and *J. atacorensis* (Hutchinson *et al*, 1966).

Despite all of the above mentioned and many other uses of these plants that thrive in most part of the Nigerian northern states, knowledge on the plant genome and its agronomy is poor. It may thus, be difficult to direct systematic breeding of the plant for improved yield. There is also lack of bench mark descriptors and information on genetic variability, effect of environment and genotype x environment (G x E) interaction (Divakara *et al*. 2009). It was equally noted that *J. curcas* growth and management, be it on private, public or community lands, have been poorly documented, with little field experience being share among researchers and farmers

(Ghosh and Singh, 2011). However, some authors (Mohammed-Dabo *et al* 2012) have recently reported that biodiesel sourced from Nigerian *J. curcas* was shown to have properties within the ASTM standard and contained the four major fatty acids (palmic, oleic, linoleic and strearic acids). The aim of this work is therefore, to carry out germplasm characterization of accessions collected from some states of the northern Nigeria with the hope of data documentation for directing future crop improvement.

MATERIALS AND METHODS

Sources of Materials

J. curcas fruit samples were collected from 16 localities across six states of northern Nigeria as shown in the table below:

S/no.	Accession no.	Locality	GPS
1.	GSKN01	Girls' College, Kano State	08^{0}48.680'E 11^0 38.687'N
2.	ZKKN03	Zakarawa, Kano State	08^0 48.369'E 11^0 38.615'N
3.	KDKN04	Kwanar Dam, Kano State	08^0 51.015'E 11^0 38.587'N
4.	FLKN005	Filin Idi, Kano State	08^0 47.770'E 11^0 38.656'N
5.	SGKN07	Sabon Gari,Kano State	08^0 47.770'E 11^0 47.760'N
6.	KGKN08	Kwarin Goje, Kano State	08^0 47.715'E 11^0 39.204'N
7.	AGKN09	Audugawa, KanoState	08^0 48.369'E 11^{0}38.730`N
8.	THKN11	Tsohuwar hanya, Kano State	08^0 48.921`E 11$^0$38.400`N
9.	RNJG13	Roni, Jigawa State	08^{0}15.350'E 12^{0}39.924'N
10.	SKJG15	Sankau, Jigawa State	08^{0}14.618'E 12^{0}40.802'N
11.	RYJG16	Rimiyel, Jigawa State	08^{0}25.043'E 12^{0}42.002'N
12.	TBJG17	Tsibit, Jigawa State	08^{0}12.897'E 12^{0}40.904'N
13.	TZZM18	Tazame, Zamfara State	06^{0}51.075`E 12$^0$06.556`N
14.	PYB21	Potiskum, Yobe State	11̊ 10. 955'E ̊11 41.327'N

15.	NMAD22	Numan, Adamawa State	12° 3.824' E	°9 26.951'N
16.	BAU23	Bauchi, Bauchi State	9° 48.005' N	1°0 18.718' N

Matured fruit samples were collected randomly in polythene bags from 10 parent plants in a population and labeled with accession numbers to maintain their identity.

Determination of morphometric features of the samples

The morphometric features of the *J. curcas* fruits and seeds collected were determined. The parameters of Fruit length (mm), Fruit width (mm), Seed length (mm), Seed width (mm),100 seed weight (g), Germination %, Seedling height (cm), Leaf length (cm) and Leaf width (cm) were measured using meter rule, Vannier caliper as the case may be. Whereas, the weight of 100 seeds from each location was measured using weighing balance (Sartorius ISO 9001) as described by Ghosh and Singh (2011).

Seed Germination Test

Sixty seeds from each location were randomly selected for the germination test at the Botany Lab; Department of Biological Sciences, Ahmadu Bello University, Zaria-Nigeria. Three plastic petri dishes were used for each location, in each petridish 20 seeds from each location were placed, the experiment were replicated 3 times. A double layer of wet absorbent paper (tissues and filter paper) and the petri dishes were labeled. The moisture levels of the filter papers were maintained by adding water as required. Seed was regarded germinated when its radicle has emerged from seed and computed after 11 days. The average of the three replicates over 11 days time period was obtained as and the germination percentage and was calculated using the formula below;

Field Transplanting and data collection

The *J. curcas* seeds germinated at the 9th to 11th day of planting, readings of *J. curcas* seedlings parameters were taken for a period of 3 weeks after transplanting at the Botanical Garden, Ahmadu Bello University, Zaria-Nigeria. The seedlings were transplanted in an opened field. Intervals of 50 meters were given between each seedling within a location and intervals of 25 meters were laid to serve as demarcation between locations to another. Measurement of stem height was taken from the soil surface to the maximum point were the leaf grows. The measurement of leaf length and width starts after the emergence of the leaves 3 weeks after sowing and the result tabulated.

Data analysis

The data collected was transformed using the resample module of the software, STANDARDISED and analysed using the SIMINT module of the NTSYSpc vs 2.2e software package (Rohlf, 2009). This analysis generated a similarity matrix of the individual character variation of each of the accessions or operational taxonomic unit (OTU) with respect to one another. The matrix values from the characters were then clustered at a cophenetic correlation of the distance matrix and a tree constructed based on the Unweigheted Pair Group Method with Arithmetic mean (UPGMA).

RESULTS AND DISCUSSION

The highest seed length was shown by NMAD22 (4.40mm) while, the lowest is 1.38mm (KGKN08) as shown in **Table 2**. Also the highest seed width of 3.50mm was shown by PYB21 and the lowest is 0.82mm (TZZM18) percentage germination ranges from 5 to 75 suggesting a wide range of variation. Highest values in seedling height, leaf length and leaf width of 15.851cm, 9.315cm and 7.747cm were shown by KDKN04, KGKN08 and RNJG13 respectively. Incidentally, these accessions were all from Kano State. This confirms closeness in geographical location ensuring easy pollen exchange at their reproductive life phase. Increase seed weight may account for more oil accumulation to enable for more biofuel yield. The accession with the highest 100 seed weight of 70.4g and 69.0g were shown by AGKN09 and KDKN04 respectively. Whereas, the lowest value in terms of seed weight was shown by TBJG (24.5g) **Table 3**. Medina *et al* (2011) opined that seed weight was among the traits that were strongly influenced by the environment. However, they noted that morphological characterization in *J. curcus* was useful in establishing phylogenetic relationship at genus level.

Table 2. Fruit and seed characters of different accession of *J. curcus*

S/no.	Accession no.	FL (mm)	FW (mm)	SL (mm)	SW (mm)	100SW (g)
1.	GSKN01	2.765±0.026	2.039±0.024	1.86±0.019	1.036±0.007	44.2
2.	ZKKN03	2.704±0.028	1.986±0.014	1.91±0.100	1.025±0.005	42.4
3.	KDKN04	2.660±0.027	2.014±0.022	2.00±1.820	1.012±0.009	69.0
4.	FLKN005	2.302±0.043	1.861±0.031	1.54±0.036	0.845±0.020	29.0
5.	SGKN07	2.371±0.047	1.925±0.031	1.71±0.037	0.952±0.024	31.4
6.	KGKN08	2.058±0.019	1.809±0.154	1.39±0.011	0.845±0.006	40.4
7.	AGKN09	na	na	1.896±0.007	0.843±0.004	70.4
8.	THKN11	na	na	1.886±0.008	0.848±0.004	63.4
9.	RNJG13	2.54±0.026	2.029±0.024	1.750±0.017	1.134±0.008	45.8

10.	SKJG15	2.350±0.024	1.923±0.016	1.641±0.016	1.063±0.007	45.5
11.	RYJG16	2.384±0.032	1.924±0.016	1.641±0.016	1.083±0.006	55.3
12.	TBJG17	2.059±0.024	1.860±0.020	1.485±0.018	1.040±0.008	24.5
13.	TZZM18	na	na	1.753±0.063	0.820±0.004	60.4
14.	PYB21	4.49± 0.180	3.69± 0.180	4.12± 0.190	3.32± 0.180	52.7
15.	NMAD22	4.70± 0.180	3.81± 0.190	4.21± 0.190	3.21± 0.180	51.6
16.	BAU23	4.34± 0.180	3.58± 0.160	4.11± 0.180	2.98± 0.180	56.8

KEY: FL= Fruit Length, FW= Fruit width, SL= Seed length, SW= Seed width, 100SW= 100 Seed weight

Table 3. Germination percentage and seedling characters of the different accession of *J. curcus*

S/no.	Accession no.	Germination %	**SH** (cm)	**LL** (cm)	**LW** (cm)
1.	GSKN01	63	15.427 ± 0.245	8.478 ± 0.212	6.519 ± 0.152
2.	ZKKN03	61	15.460 ± 0.275	8.185 ± 0.238	6.364 ± 0.171
3.	KDKN04	70	15.851 ± 0.273	8.248 ± 0.236	6.356 ± 0.169
4.	FLKN005	53	13.72 ± 0.439	7.450 ± 0.380	6.030 ± 0.273
5.	SGKN07	47	15.289 ± 0.439	8.508 ± 0.380	6.704 ± 0.273
6.	KGKN08	29	12.742 ± 0.374	9.315 ± 0.324	6.712 ± 0.234
7.	AGKN09	75	9.1080.167	9.1080.167	6.4600.145
8.	THKN11	65	15.2900.388	8.8680.189	6.3910.118
9.	RNJG13	22	15.462 ±0.193	9.515 ± 0.177	7.747 ± 0.148
10.	SKJG15	15	13.600 ± 0.576	6.577 ± 0.460	4.817 ± 0.397
11.	RYJG16	63	14.560 ± 0.353	7.058 ± 0.323	5.505 ± 0.248
12.	TBJG17	10	12.868 ± 0.325	6.700 ± 0.242	4.817 ± 0.200
13.	TZZM18	35	na	na	na
14.	PYB21	28	na	na	na

15.	NMAD22	05	na	na	na
16.	BAU23	37	na	na	na

KEY: SH= Seedling height, LL= Leaf length, LW= Leaf width

A total of five clusters were revealed as in (**Figure 1**), with clusters I, II and III comprising of four OTUs all of which from same geographical location. This is similar to the findings of Chen *et al* (2011) which demonstrated that *J. curcus* clones studied from the same place have close affinity and cluster together. However, this is in contrast to the findings of Camellia *et al* (2012), whereby *J. curcus* accessions from Malaysia were grouped using molecular markers of ISSR with no relationship to areas of collection. Also morphological distinctiveness was discerned via RAPD markers in Indian *J. curcus* species (Ram *et al*; 2008). Similarly in the present study, cluster V is comprised of all the OTUs from northeast Nigeria. The only out group if contained in cluster IV is TZZM18 and is geographically isolated from all others. In all of these clustering, parentage relationship was shown due to the pollination biology of the species. Closeness is enhanced by the accessions' geographical location. It is observed that variation do exist even from accession that are geographically close. This may give room for improvement of the stock through hybridization. Since a good deal of morphological variation was encountered in the studied accessions, it is pertinent that genetic markers like RAPD and ISSR be employed in future analyses to relate these variations with genetic affinity. However, a national approach becomes imperative since variation may come up if multilocation trials are conducted.

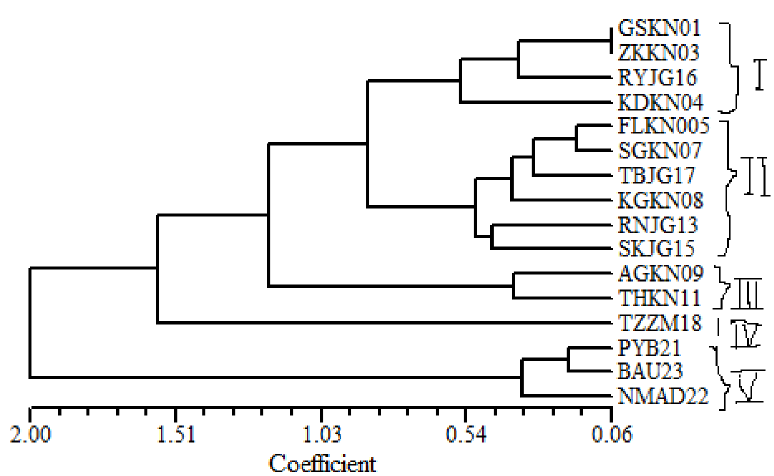

Figure 1. Dendrogram of cluster analysis of *Jatropha curca*s samples from northern Nigeria on Jaccard dissimilarity index using SAHN module

Finally, since a good measure of morphological variability exists among the investigated accessions, a chance for seed and germplasm improvement is thus evident to arrive at the best cultivar with higher productivity. However, we are of the opinion that a national research group needs to be formed for systematic coordination of Jatropha data management. There is also the need for government support in developing Jatropha as one of the sources of renewable energy in Nigeria.

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