Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

European Journal of Experimental Biology, 2013, 3(6):276-284

ISSN: 2248 –9215
CODEN (USA): EJEBAU

Jatropha curcas in Burkina Faso: Chemical characteristics of seeds and genetic variability of its ecotypes for better adaptability to the needs of populations

Hemayoro Sama[1], Barthélémy Yélémou[2*], Adama Hilou[1], Jeanne Millogo/Rasolodimby[3] and Victor Hien[4]

[1]*Laboratoire de Biochimie et Chimie Appliquées (LABIOCA), Université de Ouagadougou, 01 BP 7021 Ouaga 01, Burkina Faso*
[2]*Institut de l'Environnement et de Recherches Agricoles (INERA), Département Gestion des Ressources Naturelles et Système de Production, INERA-Saria, BP 10 Koudougou, Burkina Faso*
[3]*Laboratoire de Biologie et Ecologie Végétale, Université de Ouagadougou, 01 BP 7021, Ouaga 01, Burkina Faso*
[4]*INERA, Laboratoire Sol Eau Plante, 01 BP 476 Ouaga 01, Burkina Faso*

ABSTRACT

To reduce the country's dependence on fossil fuels, the Government of Burkina Faso has launched a program to promote Jatropha curcas L. Given the enthusiasm of the production of biofuels, Jatropha industry has a problem of availability of seeds for both quantity and quality. For a better understanding of Jatropha curcas to improve the capacity of supply raw materials, the study of morphometric characteristics, oil content and the genetic diversity of different local ecotypes was conducted. In a transect from the southern Sudan region to sub-Sahelian regions of the country, six sources were characterized. The morphometric characteristics of plants and their seeds were evaluated. The oil content was measured by the Soxhlet method. The solids content, the mineral, sugars, nitrogen compounds were determined. The molecular characterization of different sources was also carried out. The thickness of the seeds is related to the gradient while the width and the thickness of the seed and their weight vary depending on its origin. The oil content of sources varies from 48.8% (Torokoro 1) to 56.7% (Kaya and Boni). On the 6 sources analyzed with 5 primers (0PB-01 0PB-03-05 0PB, 0PB-07-10 0PB), each primer has detected a polymorphism whose average varies from 2 to 7 loci. In addition, the source has an effect on the variation of the polymorphism of the primers. En effet, les échantillons de Boni ont présenté le plus fort pourcentage de polymorphisme (54,54%), tandis que ceux de Imkouka a la plus faible (32,81%). L'analyse des sources locales leur donne une variation de la distance génétique de 0,50 à 0,81. Les écotypes étudiés ici sont de bons producteurs de pétrole, mais ils semblent être moins diversifié.

Keywords: *Jatropha curcas* L., biofuel, phytogeographic zones, seeds, genetic diversity

---

INTRODUCTION

Being a sahelian country and not producing oil, Burkina Faso has a strong dependence on outside through the importation of petroleum products. During the period 2006-2009, oil imports was estimated on average per year

over 360 billion CFA francs [1] In addition, the National Electricity Company of Burkina (SONABEL), the main company in charge of distributing electricity, has a coverage rate of only 26% and almost 80% of household's energy consumption is provided the use of wood [2] In addition, the price of oil on the world market knows, large fluctuations, which weakens the economy of poor countries. Nowadays, the trend is for the promotion of biofuels which production deals in fact with respecting the protection of the environment. Thus, in many underdeveloped countries (Burkina Faso, Mali, Senegal, etc.) appears an incentive for the cultivation of *Jatropha curcas* L., species rich in seed oil [3] and commonly used for biofuel production [4,5,6].*Jatropha curcas* is also used in medicinal preparations [7,8] Introduced in Burkina Faso before colonization by French missionaries, *Jatrophacurcas*, constitutes presently a part of the flora. With the involvement of political power in the promotion of *Jatropha curcas*, its industry is experiencing a great development. Many associations, entrepreneurs and private producers are getting involvedin *Jatropha* production, this to diversify crops or to market seeds or for the production of biofuel. The use of Jatropha biofuel should in a long-term replace oil and gas. However, the availability of seeds and or seed quality is a recurring issue raised by the promoters of *Jatropha curcas*. In Burkina Faso, several studies have been conducted on *Jatropha curcas* [9,10,11], however few studies have concerned the chemical composition of the plant as well as the genetic variability of its ecotypes. So our study aimed to identify the best varieties ecotypes of J. curcas in view to allow a better biofuel production.

MATERIALS AND METHODS

Location and site characteristics

Our study involved six sites that cover a climatic gradient with rainfall ranging from 500 - 1200 mm [12]. These sites are located in the Sub Sahelian, North Sudanian and South Sudanian. The age of the plantations goes from 4 to 8 years.

Sampling and morphological characterization

For an overview of the diversity of ecotypes existing in Burkina Faso, the fruits of *Jatropha curcas* L. were collected in localities which rainfall and sols are contrasting (Table 1). In each plantation, ten (10) young plants are chosen haphazardly, each young plant separated by at least 10m are randomly selected in each field. In total six (06) sources distributed as follows were selected for the study:

Table 1: Summary of datas of the locations of the sources collected

Phytogeographical areas	Site	origins	Years of plants
South Sudanian zone	Torokoro	Torokoro 1	8
		Torokoro 2	4
	Boni	Boni	5
North sudanian zone	Imkouka	Imkouka	4
	Ziniaré	Ziniaré	5
Sub Sahelian zone	Kaya	Kaya	5

The morphological characteristics of the harvested plant have been recorded: plant height, root collar diameter, height of the first branch and crown diameter of the plant.

Method of collecting seeds: For each plant, tent fruits were selected for the study. Fruits from one plantation were mixed to form a source. The fruits are dried in the same conditions of temperature and humidity and then shelled and stored at room temperature in the laboratory in glass boxes.

Determination of morpho-metric characteristics of seeds: For each source, three batches of 10 seeds were randomly selected and their dimensions (length, width and thickness) were determined using an electronic calliper and their weight using an electronic balance.

Quantification of the oil content of seeds:

The Soxhlet method [13] was used to quantify the oil content of seeds with petroleum ether as solvent

Determination of dry matter: The dry matter content is determined on the crushed seeds according to French standard (03-603 V).

Determining the mineral content: The mineral content (MM), or ash is determined by weight loss of the dry matter incinerated in a muffle furnace, electrically heated to 550 ° C for three (3) hours (French standard NF V 03 - 922).

Determination of protein content*:* The protein content is measured by the Kjeldahl method.

Determination of carbohydrate content*:* The carbohydrate content is determined by differential calculus using the following formula: total carbohydrate content (%) = 100 - (protein + fat + ash) Molecular characterization

Extraction of total DNA: The total genomic DNA from six sources (Torokoro1 and 2 Boni Imkouka, Kaya and Ziniaré) was extracted from young leaves freshly picked. To do this, the method of the FTA card was used for DNA extraction. It is a method to move quickly from the extraction of DNA PCR. It involves taking young fresh leaves and crush them on the rough part of the FTA card. The sample was dried and 1mm diameter disks that are placed in Eppendorf tubes are punched. By diskette 200 µl of FTA buffer is added. The whole is incubated at room temperature for 5 minutes. This is repeated three times by renewing the buffer. At the end rinsed each diskette with 200 µl of TE buffer (10 mMTris-HCl, 0.1 mM EDTA, pH8.0), and then is dried 5 minutes before transferring directly to each disc in an Eppendorf tube for the PCR reaction.

PCR amplification of genomic DNA: The DNA amplification was performed using the Eppendorf Master Cycler Gradient thermocycler with primer RAPD (0PB-01, OPB-03, OPB-05, OPB-07, OPB-10) in a volume of 20 µl containing 1 µl of each primer and 19 µl of ultra pure water and DNA to each tube. To this, add content of Eppendorf tubes (Taq polymerase, dNTPs, Tris-HCl, KCl, MgCl2). A molecular weight marker of 50 bp (base pairs) of reference and a control without DNA were used. The method was based primarily on the use of the technique of amplification by polymerase chain reaction (PCR). The thermocycler was programmed for initial denaturation of 4 min at 94 ° C, followed by 45 cycles of denaturation of 1 min at 94 ° C, 1 min of annealing at 40° C, 1 min of extension at 72° C and final extension at 72° C, and a hold temperature of 4° C at the end.

Electrophoretic separation and visualization of PCR products RAPD fragments were separated electrophoretically and The PCR products were separated on an agarose gel concentration on 1.8% agarose gels in 0.5 x TBE buffer, stained with ethidium bromide using fluorescent revealing. The migration time was 1:20 minutes and is performed in 85V and 50 mA (milliamps). The revelation of the amplification products was carried out with ultraviolet (UV) light in a dark room and photographs were made using a camera (Canon Power Shot A620 7.1 Mega Pixel).

Estimation of Genetic Diversity by using RAPD Maker RAPD markers usually lead to a binary coding absence (0) / presence (1) to calculate a matrix of presence / absence. Typically is calculated from the matrix of presence / absence, the similarity index of each individual couple. The similarity between two individuals i and j is calculated using the Jaccard index [14]. Once the similarity matrix obtained, we performed either a classification tree type is a principal component analysis.

Data Analysis
Data from the morphological characterization of plants and seeds as well as their chemical composition were entered in Excel and been analyzed of variance (ANOVA) with STATA, SPSS and XL STAT.

RESULTS

Morphological Characterization agro plants Jatropha curcas L.
The morphological characteristics of the plants studied vary according to the sources (Table 2). Analysis of variance showed a significant difference made between the feet of various sites at the height parameters of seedlings and root collar diameter ($P < 0.05$). However the height of the setting of the first branch and the crown diameter do not vary according to the sources. In addition, the gradient factor phytogeographical has no effect on the morphological characteristics of the plants studied.

Table 2: Results of morphological characterization of *Jatropha curcas* plants

Localités	Years of plants	Height of Plants(cm)	Collar diameter	Height of first branch	Crown diameter
Torokoro 1	8	236.80±22.5b	10.90±1.8b	13.26±4.4a	253.3±42.1a
Torokoro 2	4	253.80±32.3b	10.78±1.2b	14.28±4.1a	281.2±34.6a
Boni	5	302.90±51.8a	12.89±1.5a	10.58 ±3.6a	275.3±60,3a
Imkouka	4	218.70±54.1b	12.03±1.6a,b	9.90±4.1a	250.1±55,6a
Kaya	5	233.5±21.7a	10.6±1.3a	10.9±4.3a	239.7± 36.7a
Ziniare	5	243.7±27.3a	10.7±1.9a	10.6±4.7a	247.6±38.4a

For each variable, the different letters represent significantly different values (P <0.05)

Morphological characteristics of seeds

The length of the seeds has a small variations according to its provenances (Table 3). In addition, analysis of variance showed no significant differences between provenances. Variable width, thickness and weight of the seeds showed significant differences between provenances (P< 0.001). The width of seeds from Kaya (Sub Sahelian zone) which show the highest value, varies from 0.7 ± 0.1cm to 1.1 ± 0.1 cm.

The variation of this parameter is not related to climate gradient. The same is true for the variable seed weight. In contrary the thickness of the seed is higher in southern Sudan region in northern Sudanian and Sub Sahelian.

Table 3: Characterization results of morpho metric seeds

Localités	Lenght (cm)	Width (cm)	Thickness(cm)	Weight(g)
Torokoro 1	1.67± 0.1a	0.79± 0.1b	1.02± 0.1a	5.39±1.3b
Torokoro 2	1.72± 0.1a	0.81± 0.2b	1.01±0.1a	6.35±1.1a
Boni	1.72± 0.1a	0.78±0.1b	1.02±0.1a	6.56±.1.5a
Imkouka	1.69±0.1a	0.74±0.1b	1.00±0.1a	6.27±1.2a
Kaya	1.73±0.1a	1.04±0.2a	0.83±0.1b	6.63±1.1a
Ziniare	1.70±0.1a	1.06±0.1a	0.79±0.1c	6.10±1.0a

For each variable, the different letters represent significantly different values (P <0.05)

Chemical composition of seed

The oil content varies from 48.1 to field 1 Torokoro to 56% from the Kaya (Table 4). The higher rate of dry matter, protein, minerals and carbohydrates are held respectively by Torokoro (for the first two parameters), Ziniaré and Imkouka (for the others) while the lowest rates are held by the locality of Kaya.

The composition of the seeds that vary according to origin does not seem to be related to the North-South gradient. Indeed, the analysis of variance gives highly significant values (P <0.01) of the oil content of dry matter content, the rate of carbohydrates, protein and mineral matter according to the sources.

Table 4: Chemical composition of seeds

Localities	Oil content	Dry matter content	Mineral content	Protein content	Carbohydrates content
Torokoro 1	(48.8±0.4)%b	(5.3±0.2)%a	(6.3±0.6)%a	(18.4±0.7)%a	(32.8±0.3)%a
Torokoro 2	(52.2±0.4)%b	(4.5±0.1)%b	(5.3±0.6)%a,	(17.4±0.3)%b	(30.4±0.6)%a
Boni	(55.2±0.4)%a	(4.5±0.1)%b	(5.0±0.5)%b	(13.6±0.3)%d	(31.2±0.5)%a
Imkouka	(50.7±0.1)%b	(4.0±0.1)%c	(4.6±0.4)%c	(15.8±0.3)%c	(33.5±0.3)%a
Kaya	(56.7±0.5)%a	(3.5±0.1)%d	(4.4±0.2)%c	(13.6±0.2)%d	(29.7±0.8)%a
Ziniaré	(51.1±0.3)%b	(4.5±0.1)%b	(6.0±0.3)%a	(16.3±0.1)%b	(31.1±0.8)%a

Genetic Diversity of Jatropha curcas On 6 sources analyzed with 5primers (0PB-01 0PB-03-05 0PB, 0PB-07-10 0PB ¬), each primer has detected a polymorphism whose average varies from 2 to 7 loci (Figure 2).

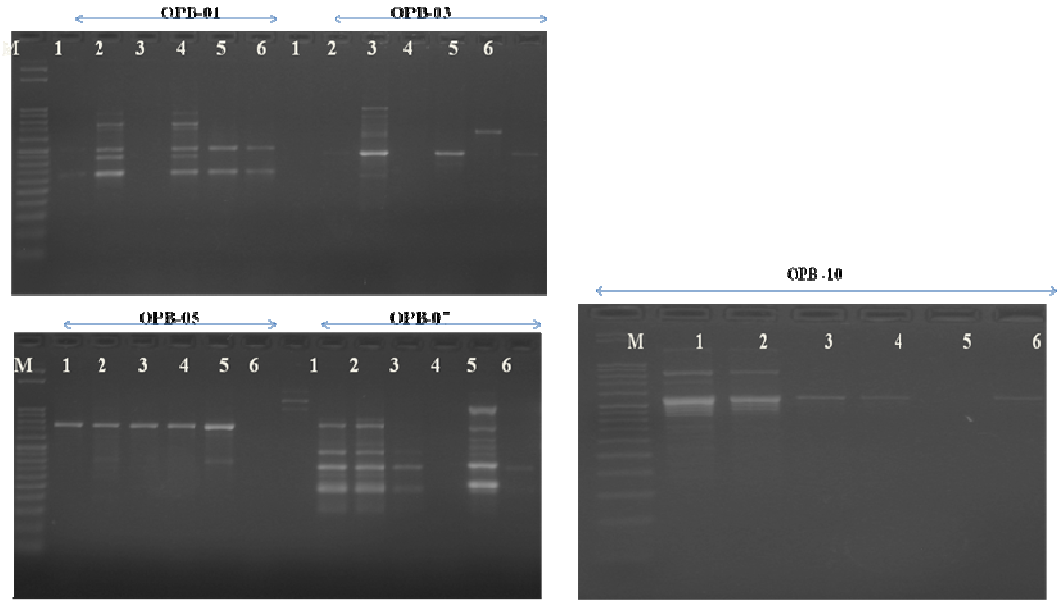


Figure 2: Photo(a, b, c) of RAPD with five primers

To read the tapes, we have considered that the criterion presence / absence (1/0). A total of 22 polymorphic bands were scored with five primers. The number of bands varies from 2 to 0PB-05 to 7 0PB 07. The average bands per primer were 4.4. From the 22 bands, 15 were polymorphic 68% polymorphism. The average number of polymorphic bands per primer is 3 (Table 5).

Table 5: List of markers and percentage of polymorphism obtained Issue

Number of primer	Primers	Séquences ofprimers	number of bands per primer	number of polymorphic bands per primer	Polymorphism (%)
1	0PB-01	GTTTCGCTCC	5	4	80%
2	OPB-03	CATCCCCCTG	4	2	50%
3	OPB-05	TGCGCCCTTC	2	1	50%
4	OPB-07	GGTGACGCAG	6	4	67%
5	OPB-10	CTGCTGGGAC	5	4	80%
total **moyenne**			22 15	4,4 3	68%

In addition, the source has an effect on the variation of polymorphism primers (Table 6). Indeed samples from Boni have presented the highest percentage (54.54%), while those from Imkouka have the lowest percentage of polymorphism (32.81%).

Table 6: Percentage of polymorphism calculated from the number of bands by source (expressed as percentage of polymorphism)

sources	number of bands by source	percentage of polymorphism
Kaya	11	50,00%
Boni	12	54,54
Imkouka	7	32,81%
Ziniaré	9	40,90%
touroukoro 1	10	45,45%
touroukoro2	8	36,36%
moyenne	9,5	43,18%

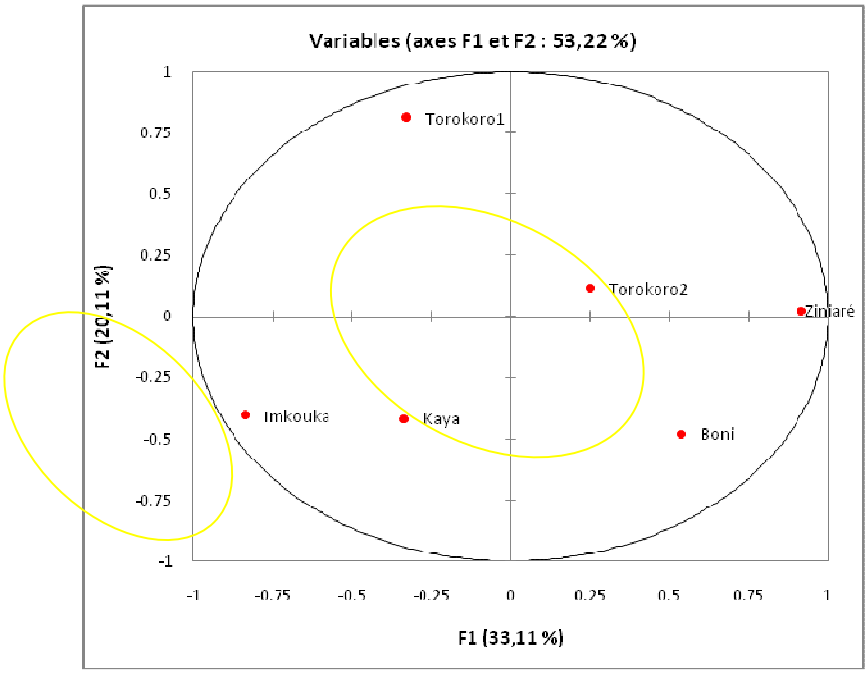
Comparative analysis of sources

Factor Analysis on Table Distance (AFTD)

The results of factor analysis on table distance (Figure 3) shows that the sources can be grouped into three groups.

Group A consists of sources of Kaya and Imkouka. Group B consists of provenance Torokoro2, Boni and Ziniaré. The third group is made only from the Torokoro1 that deviates from others.

Figure 3: Factor Analysis



Similarity index

The similarity matrix calculated from the Jaccard (1908) dissimilarity index has traced the dendrogram of the figure. The distribution of individuals in the dendrogram reflects the degree of relationship between them. Individuals from Boni and Kaya are closest genetically and the Torokoro1 generally shows the greatest genetic distance from the other sources (between 0.682 and 0.813), which confirms the results of the factor analysis. The observation of the dendrogram (Figure 4) allows to distinguish two groups :

Group 1 consists of provenance Torokoro2 and Ziniaré.

Group 2 consists of two subgroups A and B.

The subgroup A includes only individuals from Imkouka.

The subgroup B which includes individuals from Torokoro1, Kaya and Boni.

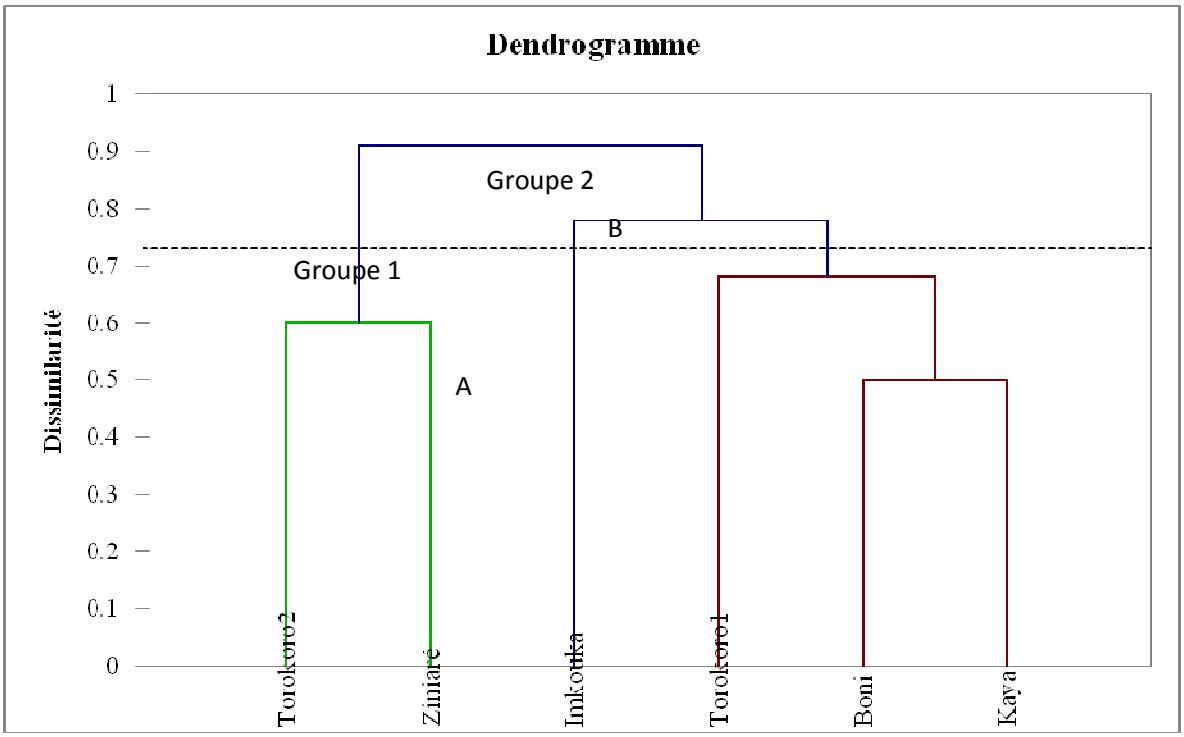
Genetic distances

Genetic distances ranged from 0.50 to 0.81 (Table 7). The lowest value was obtained from sources of Boni and Kaya while the more elevated comes from individuals from Torokoro1and Ziniaré.

Table 7: results of the determination of genetic distances of six local sources

	Torokoro1	Torokoro2	Boni	Imkouka	Kaya	Ziniaré
Torokoro1	0					
Torokoro2	0,692	0				
Boni	0,682	0,667	0			
Imkouka	0,750	0,750	0,778	0		
Kaya	0,667	0,636	0,500	0,556	0	
Ziniaré	0,813	0,600	0,556	0,909	0,786	0

Figure 4: Dendrogram accessions of *Jatropha curcas* L.



DISCUSSION

The morphological characteristics of *Jatropha curcas* aren't related to phytogeographical area but depend on the origin. In addition, the diameter of the crown, the main characteristic influencing fruit production [15] is invariant in all provenances. The analyzes conducted variables have noted significant differences ($P < 0.05$) for most of the morphological, morphometric and chemical composition of the seeds according to the sources. However, only the thickness variation seems to follow the North-South gradient. Other parameters do not follow this gradient. Furthermore, these characters do not vary with the age of the plants. The variability of these characteristics according to sources may be related to the fact that these plants grow in zones contrasting climatic conditions and soil types. These changes related to housing have also been observed in many tree species by other authors [16, 17, 18]. A similar change was also noted in *Azadirachta indica* and *Acacia nilotica* [19, 20]. This diversity of seeds morpho metric characteristics of different ecotypes may not promote a significant potential biofuel and other byproducts. That is why it seems necessary genetic selection to obtain at the same ecotype, the most important morphological characteristics metrics to positively impact the production of biofuel and other byproducts.

The ecotypes identified are oil producers (48.8 % to 56.7 %). Ecotypes Boni (in south Sudan zone) and Ziniaré (north Sudanian zone), two contrasting climatic zones , are producing more oil. These two ecotypes are similar to the high weight of seeds. In addition, the ecotype producing less oil contains the highest in sub-products such as dry matter content, the mineral proteins and carbohydrates. This could be an asset to this in the case of recovery from other byproducts from the seeds of *Jatropha curcas*. The valuation of seed cake after extraction of bio compost, fuel, animal feed, is a practice increasingly common , which gives added value to the Jatropha industry. The values obtained oil contents ranged from 48.8% for Torokoro 1 to 56.7% from Kaya. These values are within the range of values obtained by other authors for other regions of the world. The values of oil content ranging between 46.22 and 58.12% for sources have been obtained in central regions of India [17]. Studying the influence of altitude on the oil content of provenances of Jatropha in India, some researchers, obtained values ranging from 42.34 to 45% [13]. In Senegal studying the oil content of seeds depending on the North-South climatic gradient, the values ranging from 42.64 to 58.6%, have been obtained [18].

The analysis of molecular diversity with Nei parameters showed an average percentage of polymorphic loci of 43.18%. Similar results were observed by other authors in the Jatropha in the world. Thus, a percentage of 42% [21] and a polymorphism of 42.46% [16] have been obtained by some researchers. From these results we can say that from Boni (54.54%) are the most diverse in plant genetics and that of the less diversified Torokoro 2 (36.36%). Factor analysis on table distance allowed to highlight the strong factors that distinguish the fundamental changes in the sources studied. The small variation in genetic distances between 0.50 and 0.81 could be explained by a close genetic relationship between the six sources. The low genetic distances may be due to the low introduction of species and its important vegetative propagation. These findings were also obtained by a lot of authors [18, 22, 23]. Indeed, with irregular rainfall, the production of *Jatropha curcas* nursery is usually done from cuttings, which have fast lifted. This method also has the advantage of economy of seeds acquisition. The reproduction by seeds must be promote in order to permit a better genetic expression of this species [24, 25]. The diversity of analysis was performed from five RAPD primers with the Jaccard index brought together the sources into two groups with oil contents varied within each group. This of kind distribution was also observed by other authors in the world shows that in general these groups are not based on the oil content of seeds. If we consider that the dendrogram in Figure brings people according to their degree of similarity, we can say that the oil content of the seeds is not related to genetic factors of the plant; it would be rather correlated to extrinsic factors.

CONCLUSION

Morpho metric seed characteristics studied, only the thickness of the seed is related to the climatic gradient. Width, thickness and weight vary depending on sources. Different provenances have important oil content. The oil content factor is not related to the climatic gradient. The most productive local sources of oil are those of Boni and Kaya. The ecotype Torokoro though less oil producer, has the highest levels of dry matter, minerals and protein, which could make her interesting operation in the case of the valuation of other products in the oil. The genetic distance showed no genetic variability. However, a study taking into account a larger number of ecotypes would have greater representation of genetic diversity.

Acknowledgements
The authors are grateful to “African Union” and to European Union through the “10 th european fund for Development” and the program of subvention to the research of African Union [HRST/ST/AURG/CALL1/2011], for financial support. We also thank the Laboratory of molecular genetic of INERA- Kamboinsin for technical assistance in biological analysis.

REFERENCES

[1] Direction de la Prospective et de l’Intelligence Economique (DPIE),. Note sectorielle sur l’énergie au Burkina Faso, Ministère des mines et de l’énergie, Ouagadougou, Burkina Faso, **2009**.
[2] J. Blin, M.H. Dabat, G. Faugere, E. Hanff, N. Weisman; Opportunités de développement des biocarburants au Burkina Faso, Ministère de l’Agriculture, de l’Hydraulique et des Ressources Halieutiques, Burkina Faso, **2008**.
[3] E.H.M. Leye, M. Ndiaye, F. Ndiaye, B. Diallo, A.S. SARR, M. Diouf et T. Diop, *Revue des énergies renouvelables*, **2009**, 12, 269-278.
[4] G.I. El Dwani, Sh.A. El Rafael, S.I. Hawash, *Advances in Applied Sciences Research*, **2011**, 2, 221-232.
[5] S. Rejila, N. Vijayakumar, M. Jayakumar, *Asian Journal of Plant Sciences and Research*, **2012**, 2, 123-128.
[6] M. Kannahi, Arulmozhi, *Asian Journal of Plant Sciences and Research*, **2013**, 3, 60-64.
[7] T.H. Nazeema and Girija, *European Journal of Experimental Biology*, **2012**, 2, 421-426.
[8] A.O. Ajayi, A.I. Okoh, *European Journal of Experimental Biology*, **2013**, 3, 16-21.
[9] M. Ouédraogo, Master thesis, Université de Ouagadougou (Ouagadougou, Burkina Faso, **2000)**.
[10] F. Sanou, Engineering thesis, Université Polytechnique de Bobo, (Bobo-Dioulasso, Burkina Faso, **2010**).
[11] P. Bazongo, Master thesis, Université Polytechnique de Bobo, (Bobo- Dioulasso, Burkina Faso, **2011**).
[12] J. Fontes & S. Guinko (19**9**5) ; Carte de la végétation et de l’occupation du sol du Burkina Faso. Ministère de la Coopération Française : projet campus, **1995**.
[13] K.S. Pant, V. Khosla, D. Kumar, S. Gairola, Lyonia, **2006**, 11, 31- 34.
[14] P. Jaccard, *Bull Soc Vaud Nat*,**1908**, 44, 223-270.
[15] B. Yélémou, R. Zougmoré, B.A. Bationo, J. Millogo/Rasolodimby, V. Hien, *Cameroon Journal of Experimental Biology*, **2009**, 5, 10-20.
[16] N. Kaushik, S. Kumar ; *Jatropha curcas* L. Silviculture and uses, Agrobios (India), Jodhpur, **2004**.

[17] H.S. Ghinwal, S.S. Phartyal, P.S. Rawat, R.L. Srivastava, *Silvae Genetica*, **2005**, 54,76-80.
[18] M.M. Kane, Engineering Thesis, Université de Thiès, (Thiès Sénégal, **2010)**.
[19] S.K. Jindal, Satyavir, A. Pancholy, *Journal of Tropical Forest Science*, **1999**, 11, 320–2.
[20] S.K. Kundu, P.M.A. Tigerstredt, *Silvae Genetica* , **1997**, 46,129–37.
[21] S.D. Basha, G. Francis, H.P.S. Makkar, K. Becker, M. Sujatha, *Plant Science*, **2009**, 176, 812-823.
[22] S.R. Ganesh, K.T. Parthiban, R. Senthil Kumar, V. Thiruvengada m and M. Paramathma, *Genet .Res. Crop. Evol*., **2007**, 9285-7.
[23] S. Basha, and M. Sujatha, *Euphytica*, **2007**, 156, 375-386.
[24] A. Ouédraogo, PhD thesis, Université de Ouagadougou, (Ouagadougou, Burkina Faso, **2006**).
[25] B. Yélémou, G. Yaméogo, B.A. Bationo, J. Millogo/Rasolodimby et V. Hien, *Int. J. Biol. Chem. Sci*., **2012**, 6, 2083-**2096.**