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COMPARATIVE ASSESSMENT OF THE SEED OIL QUALITY AND MICROPROPAGATION OF ELITE JATROPHA CURCAS

Mamadou Bado TRAORE[1,2] , Hamidou SENOU [1, 2*], Lassana TRAORE[1] , Sabake T DIARRA[1]

1 Institut Polytechnique Rural de formation et de Recherche Appliquée (IPR/IFRA), Katibougou Bp: 06, Koulikoro, Mali

2 College of Biological Sciences and Biotechnology, Beijing Forestry University Beijing 100083. P. R. China

* Corresponding author: SENOU Hamidou (email: ha_senou@yahoo.fr)

Abstract: Rural development and climate change can promote the cultivation of Jatropha curcas, a biodiesel feedstock that has been identified as suitable for achieving biofuel. This study was aimed to evaluate the seed oils content of different ecotypes of Jatropha curcas from Mali and China. Seed oils were analyzed for physico-chemical parameters such as iodine value, free fatty acid, saponification and peroxide values. The fatty acid profiles were also determined and identified in quantifiable amount in the seed oils by using the gas chromatography-mass spectrometry (GC-MS). The medium supplemented by kinetin and benzyl amino purine with three levels of variation (0.1; 0.3 and 0.5 mg/L) was used to initiate the axillary buds of elite ecotype. To determine the oil content of the seeds of J. curcas the Soxhlet extraction method was used. We found that the best seed oil concentration was observed in M3 with 64.82±0.78 % which was significantly different to the lowest one observed in M2 (58.49± 1.42). Among these, unsaturated fatty acids were predominant. Furthermore, oleic acid and linoleic acid were dominant with a higher level of methyl oleate (monounsaturated fatty acid). Murashige and Skoog (MS) medium supplemented with BAP (BAP3) was the best for shoot propagation with a higher number of leaves. The highest percentage (45.23±0.85) of the root was achieved on MS/2 supplemented with 0.5 mg of NAA and IBA, which was more favorable to the rooting of shoots with many long roots. The highest among unsaturated acids were therefore observed in C2 with 73.23 which are considered to be best ecotype for the production of biodiesel.

Keywords: Jatropha curcas; Micropropagation; seed oil; Elite Ecotype.

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1 Introduction

Traditional oils shortage with their unstable costs are fueling a great debate and real concern worldwide. Vegetable oils are known as an alternative to these problematic issues and are regarded as the first possibilities for the future engine, as their production technology has already been launched. They are also well known as economically viable. However, the variation in vegetable seed oil composition is attributed to environmental factors which was

reported to influence its biosynthesis. Temperature, precipitation, and light intensity, which are factors commonly investigated in conventional oil seeds are all factors that can usually affect the biosynthesis of vegetable seed oil. The trends of seed saturated fatty acids profile have been reported to increase with increasing temperature [18]. On the other hand, more polyunsaturated acids are synthesized in high quantities at low temperatures.

Jatropha curcas L. has been reported to quickly emerged as a potential biodiesel crop due to the fact that its crude oil can be easily converted into biodiesel, meeting US and European standards (Azam et al. 2005; Tiwari et al. 2007). The use of crude oils of J. curcas has been reported to be safe and with a positive environmental impact (decrease in pollution and greenhouse effect). Moreover, their combustion only releases the amount of carbon dioxide that the plant has consumed during its growth. Due to its importance, various techniques for the breeding of new J. curcas varieties have been reported (King et al. 2009), among which, in vitro propagation has been shown to play an important role for germplasm improvement through the nontransgenic approach. Pure vegetable oils are a possible solution to the problem of many developing countries (Senou et al. 2017). Further, it is important to emphasize that J. curcus has the requisite potential for providing a promising and commercially viable alternative to diesel oil since it has desirable biochemical characteristics compared to diesel. Unfortunately, there are currently no standards in Africa to certify the quality of vegetable oils intended for carburation.

The present study appears to be the first attempt to examine the variation of oil content and composition of J.curcas seed collected from different regions in Mali and China by considering the climatic condition and the wide geographical distribution. Furthermore, this study was designed to assess the seed oils content of different ecotypes by determining the physio-chemical content with fatty acid profiles. The in-vitro generations of the best ecotype were further determined by combining Kinetin and BAP.

2 Materials and Methods

2.1 Study Site and Environmental Conditions

Mature seeds of jatropha curcas were collected from different regions of Mali and China as indicated in the above map under different climatic conditions. Temperature and precipitation were determined according to meteorological service data; the longitude, latitude, and altitude were measured by GPS (eTrex H–Garmin International Inc., Olathe, KS,) and all information's are summarized in table 1

2.2 Table. Geomorphological description of each region

Physiographic and climatic characteristics	Mali			China	
	Katibougou (M1)	Teriyabougou (M2)	Konseguela (M3)	Guizhou (C1)	Sichuan (C2)
Climatic types	Tropical south sudanian	dry tropical climate of the north Sudanian type	Tropical wet sudanian	hot humid sub-tropical	humid sub-tropical monsoonal climate
Latitude	12.9303°	13.206826°	12.40947°	26.64699°	31.456781°
Longitude	-7.52833°	-5.530296°	-5.87665°	106.629997°	102.843018°
Altitude (m)	423	290	357	99	481
Annual average temperatures (^{0}C) (max.)	36,02	36,3	34,6	19,4	21,2
Annual average temperatures (^{0}C) (min.)	21,37	23,3	22,6	11,5	13,8
Annual average temperatures (^{0}C) (mean)	28,70	29,75	28,55	15,4	17,45
Average annual rainfall (mm)	779,4	730	880	1075	855

2.3 Study Area

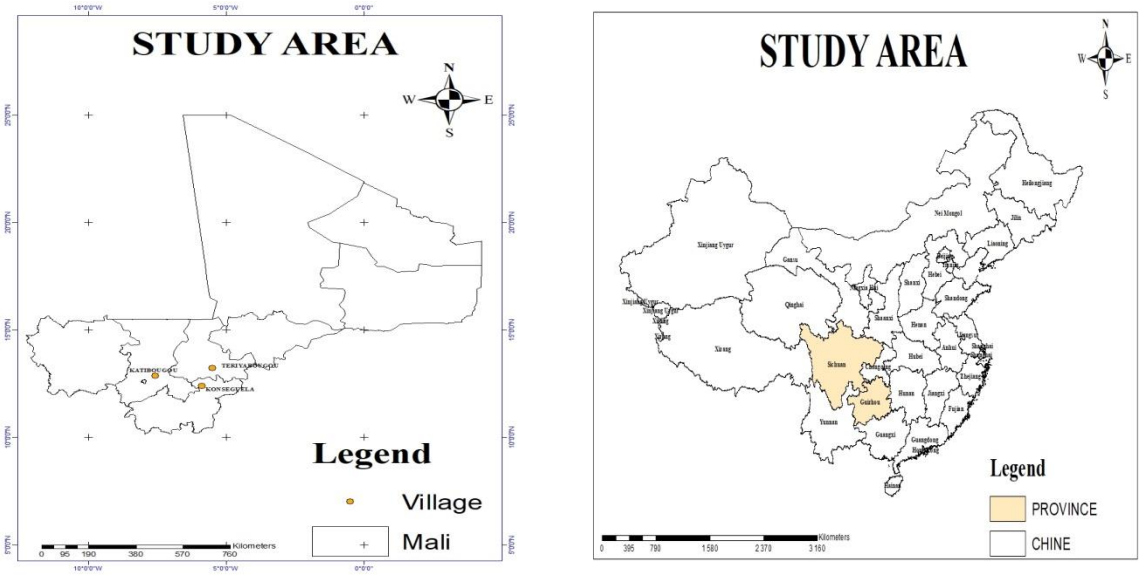


Figure 1. Study Area

2.4 Seed preparation

Five ecotypes of jatropha curcas were collected for their high level of yield in different regions of Mali and China in 2014. Mature seeds were collected and the damaged seeds were removed and discarded. The shells were firstly separated from the almonds and Chopper Grinder (Multi-Function Food Fruit Processor 3 in 1 Electric Blender Mixer) was used for 1 min to ground the selected seed into powder before oil extraction (Senou et al. 2017).

2.5 Oil Extraction

The extraction was performed according to a procedure reported in our previous study (Senou et al. 2017).

The Soxhlet method is a reference method used for extracting vegetable oils from solid dehydrated almonds. It is a gravimetric method, since the sample is weighed at the start and the oil at the end of the extraction. The oil was extracted from the seeds of Jatropha curcas using n-hexane as a solvent. The extraction was carried out on 5 grams of J. curcas powder in a 250 mL round bottom pyrex flask, to which 180 mL of n-hexane is added. The assembly was placed on a glas-Col heating sleeve communicated to a voltage regulator which is extracted at a constant temperature of 80 ° C for a period of 3 hours. The solid / solvent ratio is then carried out to determine the amount of oil as a percentage.

$$\boldsymbol{Percentage\ of\ oil} = \frac{\textbf{Weight of oil obtained in gm}}{\textbf{Weight of seed taken in gm}} \times \mathbf{100}$$

2.6 Fatty acids profiles

Gas chromatography (GC) was used to determine the Fatty acid composition of the seed oil.

The fatty acid composition of the oils is determined by analysis of their methyl esters. Briefly, 0.5 ml of 2N KOH and 1.5 ml of n-hexane in methyl alcohol were used to convert two drops of oil before being injected into the GC system. The analyzes were carried out according to the following temperature programming: The injection temperature is 280 ° C and that of the detector is 300 ° C. The column temperature was programmed from 70 ° C to 200 ° C with a temperature increasing rate of 8 ° C / min. Helium was used as a carrier gas.

The apparatus was fitted with a flame ionization detector (GC-FID 17A, Shimadzu, Kyoto, Japan) under thesame conditions as those described above. The identification and quantification by NIST11.LIB and NIST11S.LIB in mass spectrometry of each fatty acid methyl ester (FAME) was calculated from the integrated area of each peak and expressed as a percentage of the area total of all peaks.

2.7 Micropropagation study

Jatropha curcas axillary buds were disinfected with sodium hypochlorite (0.3%), mercuric chloride (0.1%) and 70% ethanol. The medium used for the proliferation of the buds was Murashige and Skoog medium (MS medium) supplemented with 6-benzylaminopurine (BAP) and kinetin (0.1–0.3 mg / l). The number of leaves and buds and the percentage of the plant initiated were recorded after 6 weeks of culture. Finally, for rooting, half of the MS medium enriched with NAA / IBA due to (0.5 to 1mg / l) was used.

2.8 Statistical analysis

The experiments were performed in replication and the data were graphed using Graph pad Software (Inc., La Jolla, California). Statistical analysis was performed to determine the significant differences between groups by using Analysis of variance (ANOVA) followed by Tukey's multiple comparisons test for means comparison. All data points are expressed as mean ± standard deviation (SD) and p value of ≤0.05 is considered significant.

3 Results

3.1 Oil Content

Means of the seed oil contents are shown in Figure 1. The total oil content of J.curcas varied significantly among the locations with temperature and annual rainfall. After extraction, oil concentration was respectively 61.88 64± 1.27; 58.49± 1.42; 64.81± 0.78; 63.1± 0.48; 62.41 ± 1.79 for M1, M2, M3, C1, C2. The highest oil content was observed in M3 at 28.55ºC and 880mm as temperature and rainfall value respectively with no statistically significant difference with M1, C1 and C2. However, the lowest value was observed in M2 which had the highest temperature 29.75ºC and lowest annual rainfall value 730mm, and was significantly different (one way ANOVA, F (4, 5) = 6.965, *p = 0.0282) compared to M1, M3, C1 and C2.

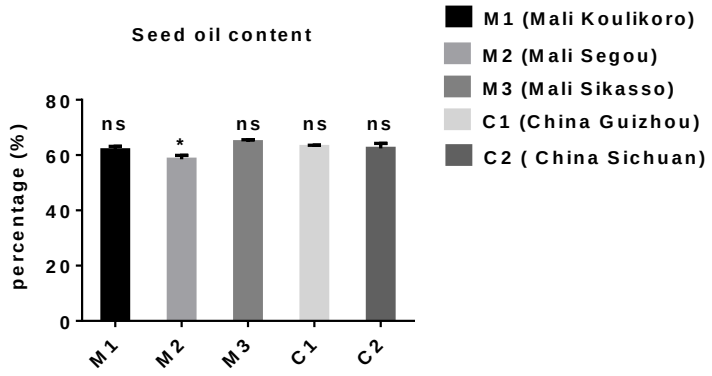


Figure 2. Seed oil content (one way ANOVA, F (4, 5) = 6.965, *p = 0.0282). Each data was replicated and the average was determined. Data are presented as standard deviation of the means. ns indicates absence of significance

3.2 Physicochemical Properties

The physical and chemical properties of the extracted seed oil of jatropha from different regions were determined and the results are shown in figure 2. The iodine values were obtained in order to determine the highest amounts of unsaturated fatty acids levels in the jatropha curcas seeds oil. The values ranged from 102.50±0.71, 101.50±0.71 and 102.00±1.41 (for M1, M2, M3, C1, C2, respectively). No statistically significant difference was observed among different ecotypes (one way ANOVA, F (4, 5) = 0.7727, p = 0.5869). In opposite of iodine value, the highest peroxide value is an indication of the presence of high amounts of polyunsaturated fatty acids. Based on our results, the values of peroxide were respectively 1.57±0.16, 1.81±0.09 and 1.56±0.16 for M1, M2, M3, C1, C2, with no statistical different between the values (one way ANOVA, F (4, 5) = 5.463, p = 0.05).
The saponification values of jatropha curcas seeds oil for the different Ecotype were 193.5, 196.5, 194.0, 193.0, and 190.6 for M1, M2, M3, C1, and C2 respectively.

The highest values of acid were observed in two ecotypes (M2, M3) but not statistically significant compared to M1, C1, C2 (one way ANOVA, F (4, 5) = 3.405, p = 0.1057)

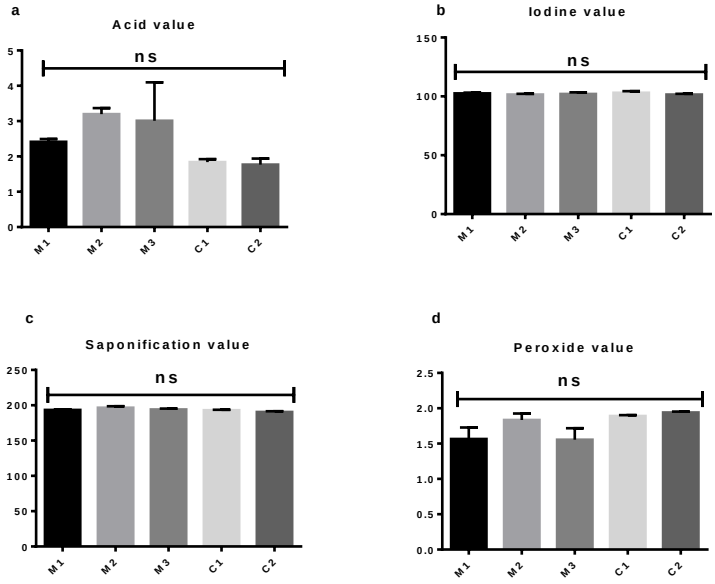


Figure 3. (a) Acid value (one way ANOVA, F (4, 5) = 3.405, p = 0.1057); (b) Iodine value (one way ANOVA, F (4, 5) = 0.7727, p = 0.5869); (c) Saponification value (one way ANOVA, F (4, 5) = 5.573, p = 0.0437); (d) Peroxide value (one way ANOVA, F (4, 5) = 5.463, p = 0.0454). Values are presented as mean ± standard deviation. ns indicates no significance**.**

3.3 Fatty Acids Composition

The fatty acid composition was analyzed, and the results are summarized in Table 2. The fatty acids in J. curcas seed oil are in length between 14 and 25 carbon atoms and contain 0 to 2 double bonds. According to our findings, the content of total unsaturated fatty acids varied from 70.51, 70.63, and 70.68 for M1, M2, and M3 respectively. However, the higher values of 72.29 and 73.23 were observed in C1 and C2 respectively with lower temperature and higher rainfall value (15.4 and 17.45 with 1075 and 855 respectively).

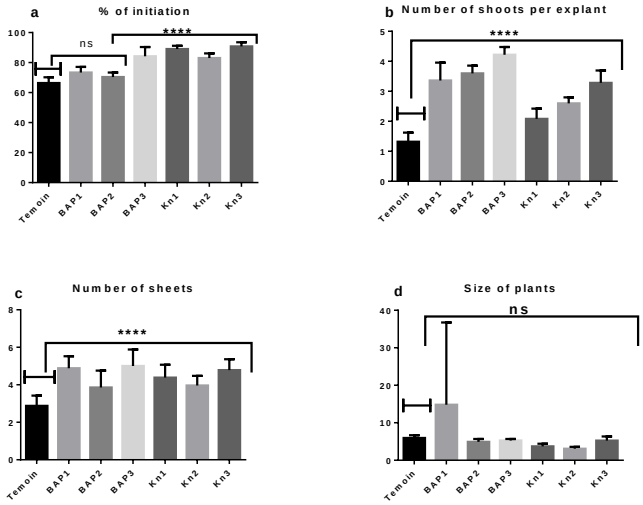


Figure 4. (a) Percentage of initiation (one way ANOVA, F (6, 28) = 32.27, p < 0.0001); (b) Number of shoots per explant (one way ANOVA, F (6, 28) = 37.42, p < 0.0001): (c) Number of sheets (one way ANOVA, F (6, 28) = 5.836, p = 0.0001); (d) Size of plants (one way ANOVA,

F (6, 28) = 1.123, p = 0.3744). Each data point represents an average of three replicates. ns indicates no significance, ****p < .0001. Values are presented as mean ± standard deviation.

3.4 Micropropagation study

Different combinations of Kinetin and BAP were used for in-vitro regeneration of elite J. curcas axillary bud, and the results are shown in figure 3. The highest percentage of initiation was obtained with BAP (0.3mg/l), Kin (0.1-0.3mg/l) with respectively 84.20, 89.00, 83.00, and 90.80 %.

For shoot induction and number of leaves per plant, the best percentage value was observed in BAP (0.3 mg/l) with 4.22 and 5.00 respectively. However, no significant difference was observed regarding the size of plant between different mediums. Different combinations of ANA and IBA were used for rooting induction and the results are shown in table 3. The best value of rooting induction was 45.23±0.85, observed in MS/2 when combined with 0.5mg/l of ANA and IBA.



Figure 5. Micropropagation of best ecotype of jatropha curcas

4 Discussion

The physical properties of different biotypes collected from different regions (Mali and China) of various states were studied for bio-physicochemical characteristics. The highest oil content was observed in Konseguela (M3) and lowest in Teriyabougou (M2), with 28.55ºC, 880mm and 29.75ºC, 730mm as temperature and rainfall respectively for Mali side. High seed oils content has been reported to be suitable for non-edible vegetable oil feedstock in oleochemical industries (Atabani et al. 2013; Gudeta 2016). According to our finding, we can deduce that the environment and climate factors play an important role in the variation of the quantity of oil. Similar results have been observed by Mariana et al. after extracting the Calotropis procera oil. The mean annual temperature of 18-28ºC, an extreme minimum temperature of -4ºC, and a high yearly accumulative temperature are reported as preferable for j.curcas production.

Soil conditions have been reported to play a significant role in causing variations in oil yield. Similar results have been reported by Barbosa et al (Barbosa et al. 2014), where they found that geographical distribution has some role in that variation, after determining the oil content of Calotropis Proceravaried. However, according to Nzikou and colleagues, the variation in oil yield could be due to differences in the growing climate, variety of plants and the harvesting time of the seeds (Nzikou et al. 2009). The best J. curcas seed oils for biodiesel production would be an oil containing a large amount of monounsaturated fatty acids because polyunsaturated fatty acids tend to exhibit poor oxidation stability and cannot be used at low temperature due to the highest pour point (Emil et al. 2010; Pullen and Saeed 2012). The presence of high oil content conferred a special attention to Jatropha seeds as a biodiesel source (Emil et al. 2010). In the present report, the iodine value was determined to evaluate the chemical stability property of substance against oxidation. It has also been reported that the higher iodine value, the higher the number of double bond and hence lesser stability (Knothe 2002a; Unyime Ebong 2019).

The highest value of iodine observed in this study shows that fossil diesel is more stable compared to biodiesel. Similar findings have been reported by Verma et al (Verma and Verma 2015). The saponification value of the extracted oil was determined and found to be in the range of 102 to 101 with a non-significant difference. The high saponification value reported in our study indicated that the oil is normal triglyceride, and it is very useful in the production of soap and shampoo.

The oil from Jatropha seeds was also characterized in terms of fatty acid composition. The total saturated fatty acid content ranged from 25.50 to 29.07%, while the total unsaturated fatty acid content varied from 70.50 to 73.23%. The highest proportion of unsaturated fatty acids was recorded in ecotype C2.
A comparison of ecotypes M3 and C2 reveals interesting differences in the seed quality of Jatropha curcas. M3 exhibited the highest oil content (64.81 ± 0.78%), highlighting its potential for oil production. However, this value was not significantly different from those recorded for several other ecotypes. C2, on the other hand, showed the highest proportion of unsaturated fatty acids (73.23%), indicating a distinct fatty acid profile. These differences may be partly related to the climatic and environmental conditions prevailing in the regions where the ecotypes originated. Overall, M3 was characterized by its higher oil content, whereas C2 stood out for its greater proportion of unsaturated fatty acids. These findings highlight the importance of considering both oil yield and fatty acid composition when selecting Jatropha ecotypes for industrial applications.
Previous studies have reported that the high proportion of unsaturated fatty acids in Jatropha oil makes it suitable for biodiesel production (Verma and Juneja 2014; Verma et al. 2016). However, the oxidative stability of fatty acid methyl esters is one of the most important factors affecting biodiesel quality. Several studies have demonstrated that fatty acid composition directly influences biodiesel properties (Falk and Meyer-Pittroff 2004; Islam et al. 2013; Ramos et al. 2009). In particular, a high proportion of polyunsaturated fatty acids can reduce oxidative stability. The number and position of double bonds, as well as exposure to air, are among the factors influencing the oxidative stability of biodiesel (Knothe 2002b; Silitonga et al. 2013). The plant growth regulators are used in combination or alone at different concentrations (Mukherjee et al. 2011).
Kinetin was found to be a less suitable plant hormone than BAP for the induction of shoot buds in terms of percent explant response; the average number of shoot buds per explant increased with increasing concentration of one of the cytokinins. Furthermore, the wide variations of different explants are dependent on the concentration of NAA and IBA (Verma and Verma 2015) which has also been supported by Mangal and coworkers (Rathore et al. 2015)

5 Conclusion

The high percentage of unsaturated fatty acid (up to 78.83 %) of J. curcas oil, high oil yield and lesser fatty acid content makes the jatropha oil more suitable for biodiesel production. In summary, we report that seeds from China were found to be a best quality with respect to level of oil content, FFA content. A considerable variability exists in the seed oil of jatropha collected from different geographical locations, which offer further scope for selection. The regeneration was found to be promising and useful in stabilizing the nursery industry to raise healthy plants, i.e. rapid clonal propagation of virus-free plants.

6 Acknowledgements

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