AFLP Analysis of Genetic Diversity Among Different *Jatropha curcas* L. Genotypes from Africa and Ecuador

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Summary

Six populations amounting to a total number of seventy genotypes of Jatropha curcas *L. originating from Africa (Senegal, Mali, Burkina Faso and Madagascar) and Ecuador were investigated for genetic diversity using two AFLP primer combinations. The results revealed a high genetic diversity in the populations studied. The population with greatest genetic diversity was Madagascar (He= 0.2638 and I= 0.4066) and the least diverse was Senegal-Tamba (He= 0.1962 and I= 0.3079). AMOVA (analysis of molecular variance) detected the highest proportion of variation within populations (81% of the total molecular variation). This may be attributed to the high level of allogamy observed in this species. The Nei's standard unbiased genetic distance (D) between the populations ranged from 0.010 (Senegal-Tamba and Burkina Faso) to 0.131 (Mali and Ecuador); the average was 0.063. Analysis of the genetic relationships among the 6 populations using both neighbor-joining cluster analysis and principal component analysis (PCoA) showed five clusters with globally, groupings of i) most of Burkina Faso and Senegal-Tamba genotypes, ii) most of Mali and Senegal-Diobass genotypes, iii) most of Madagascar and Ecuador genotypes, and iv) some mixings of genotypes with different origins. Considering the distance existing between the different origins there are prospects to develop F1 hybrids. The greatest heterosis might be expected from crossing involving genotypes of cluster I and cluster V which group the more distant genotypes. Such crossing schemes might produce greater success in the production of genetic variability and might maximize the exploitation of heterosis and segregation.*

Résumé

Analyse par les marqueurs AFLP de la diversité génétique de différents génotypes de *Jatropha curcas* provenant d'Afrique et d'Equateur

Soixante-dix génotypes de Jatropha curcas *L. originaire d'Afrique (Sénégal, Mali, Burkina Faso et Madagascar) et d'Equateur ont été analysés pour leur diversité génétique en utilisant deux combinaisons de marqueurs AFLP. Les résultats ont révélé une importante diversité génétique dans les populations étudiées. La population avec la plus grande diversité génétique était celle de Madagascar (He= 0.2638 and I= 0.4066) et la moins diverse celle du Senegal-Tamba (He= 0.1962 and I= 0.3079). L'AMOVA (analysis of molecular variance) a détecté la plus grande variation (81% de la variation moléculaire totale) dans les populations contre 19% entre populations. Cela pourrait être dû au haut niveau d'allogamie observé chez cette espèce. La distance génétiquestandard non biaisée de Nei allait de 0.010 (Senegal-Tamba et Burkina Faso) à 0.131 (Mali et Ecuador); la moyenne était de 0,063. L'analyse des relations génétiques entre les 6 populations en utilisant la méthode du* neighbor-joining cluster analysis *et l'analyse des composantes principales (PCoA) a montré 5 clusters avec globalement les regroupements de i) la plupart des génotypes Burkina Faso et du Sénégal-Tamba, ii) la plupart des génotypes du Sénégal-Diobass et du Mali, iii) la plupart des génotypes d'Equateur et de Madagascar, et iv) quelques mélanges par endroit. En considérant les distances génétiques existant entre les différentes origines, il y a des perspectives de développement d'hybrides F1. Le croisement des génotypes des clusters I et V qui sont les plus éloignés pourrait permettre l'obtention d'hybrides exhibant les meilleurs effets d'hétérosis.*

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Introduction

Jatropha curcas L. (tropical physic nut) is a perennial small tree of 5 to 8 m height belonging to the Euphorbiaceae family (12, 21, 27). The species is native to tropical America, from where it has been distributed widely in tropical and subtropical regions of the world (9, 14, 27). The genus *Jatropha* is morphologically diverse with more than 200 species composed mainly of diploids (2n= 2x= 22), although triploid and tetraploid chromosome numbers have rarely been reported (7, 14). *J. curcas* is drought resistant and grows well under a wide range of climatic and soil conditions (2, 11, 14, 33). It is a monoic plant with unisexual flowers, producing male and female flowers in the same inflorescence (5, 17). It usually starts flowering and fruiting 1 year after planting, and continues to produce seeds for a period of 50 years (14). *J. curcas* reproduction is essentially via amphimixis, mainly by outcrossing (enthomophylous pollination), even if the species is self-compatible and hence selfing is also possible (1). Only a small proportion of seeds are set through apomixis. The plant can also be propagated clonally by anthropic management via cuttings of stem or branch. *J. curcas* is commonly cultivated because the plant has medicinal values and it fits easily into agricultural system in the form of hedges, windbreak, and erosion barrier or as a source of firewood (6, 17). In the recent years, the species has gained tremendous significance for its potential as a feedstock in the lipid, biopharmaceutical, cosmetic and biopesticide industries, and especially for the use of its seed oil as a commercially viable alternative source of fuel (6, 31). Actually, *J. curcas* is a source of oil-rich seeds (46 to 58% of oil on kernel weight and 30 to 40% on seed weight) not edible as it contains toxic compounds and antinutritional factors. Jatropha oil is considered as an environment friendly renewable source of energy (1, 7). Therefore, genetic improvement of such an economic important plant is of prime importance in order to alter its current wild or semi-domesticated status to a cultivated crop with varieties having desirable characters like a high seed yield and oil content, resistance to pests and adaptability to different agro-climatic conditions (18). Understanding the genetic diversity of the species is an initial step towards selection and breeding of superior genotypes (14). Among the different breeding strategies that can be followed, hybrid breeding is one of the most promising (19). For optimal exploitation of heterosis, it is important to identify genetically distant germplasm pools (28). In the present study, we investigated genetic diversity of *J. curcas* genotypes coming from Africa and Ecuador, using AFLP markers in order to identify possible parents to be included in a hybrid breeding program.

Materials and methods

Plant material

Dried leaves of six populations of of *J. curcas* genotypes originated from Senegal (Tamba: 5 genotypes; Diobass: 12 genotypes), Mali (11 genotypes), Burkina Faso (12 genotypes), Madagascar (14 genotypes) and Ecuador (16 genotypes) were used in this study (Table 1).

DNA isolation

Total genomic DNA was isolated from 20 mg of dried leaves following a slightly modified mixed alkyltrimethylammonium bromide (MATAB) method as described by Lacape *et al.* (13). Briefly, leaves were ground in 2 ml safe-lock microtubes (Eppendorf, Belgium) with stainless steel beads using a Qiagen TissueLyser mixer-mill (Qiagen, Belgium) and dissolved in 800 µL of MATAB buffer (0.1 M Tris HCl, pH 8.0, 1.5 M NaCl, 20 mM EDTA, 2% MATAB, 1% polyethylene glycol 6000, 0.5% sodium sulphite) at 72 °C. The samples were incubated for 1 hour at 72 °C and cooled for 5 minutes at room temperature. Then, 960 µL of chloroform-isoamyl alcohol (24:1) was added to each sample. All samples were homogenized by inversion for 5 minutes, before centrifugation at 6200 g for 20 minutes at room temperature. The supernatant (800 µL) was collected and the DNA was precipitated with 640 µL of isopropanol by gentle shaking. After a centrifugation at 6200 g for 20 minutes, the pellet was washed with 600 µL of 70% ethanol through a centrifugation at 6200 g for 10 minutes, dried at 60 °C for 10 minutes and dissolved in 500 µL of TE buffer (10 mM Tris HCl, pH 8; 0,1 mM EDTA). RNA was removed by RNAse treatment at 37 °C for 1 h. DNA quality and concentration were evaluated by electrophoresis in SYBR Safe (Invitrogen, Belgium) stained 1% agarose gels through comparison with low DNA mass ladder.

AFLP Analysis

Two AFLP primer combinations (E-ACT/M-CAT and E-AAC/M-CTT) selected on the basis of their good profile in a preliminary study (not presented here) were used. AFLP was carried out using the “AFLP Analysis System I/AFLP starter primer kit” (Invitrogen, Belgium) following the protocol proposed by Invitrogen. Briefly, genomic DNA (250 ng) was double digested with EcoR I and Mse I restriction endonucleases. The digested DNA fragments were ligated to EcoR I and Mse I adaptors with T4 DNA ligase to generate template DNA for amplification by PCR. Two consecutive PCR were performed: a pre-selective and selective PCR. In the pre-selective reaction, DNA was amplified using an AFLP pre-amp primer pair complementary to the adaptors and each having one selective nucleotide. Pre-selective PCR amplification was used as template for the selective amplification using AFLP primers, each containing three selective nucleotides.

Table 1
Genotypes, country and region of origin of the 75 Jatropha curcas used in the study.

N°	Collection Id.	Country	Region
1	T A3 (9/18)	Senegal (Tamba)	West Africa
2	T A4 (13/24)	Senegal (Tamba)	West Africa
3	T A4 (3/8)	Senegal (Tamba)	West Africa
4	T A4 (13/35)	Senegal (Tamba)	West Africa
5	T A4 (4/37)	Senegal (Tamba)	West Africa
6	D A3 (6/32)	Senegal (Diobass)	West Africa
7	D A3 (14/21)	Senegal (Diobass)	West Africa
8	D A4 (12/16)	Senegal (Diobass)	West Africa
9	D A4 (13/15)	Senegal (Diobass)	West Africa
10	D A4 (10/16)	Senegal (Diobass)	West Africa
11	D A4 (10/24)	Senegal (Diobass)	West Africa
12	D A4 (10/23)	Senegal (Diobass)	West Africa
13	D A4 (9/16)	Senegal (Diobass)	West Africa
14	D A4 (13/12)	Senegal (Diobass)	West Africa
15	D A5 (3/18)	Senegal (Diobass)	West Africa
16	D A5 (10/12)	Senegal (Diobass)	West Africa
17	D A5 (7/14)	Senegal (Diobass)	West Africa
18	B A5 (1/21)	Burkina Faso	West Africa
19	B A5 (5/7)	Burkina Faso	West Africa
20	B A5 (4/13)	Burkina Faso	West Africa
21	B A5 (9/36)	Burkina Faso	West Africa
22	B A5 (1/26)	Burkina Faso	West Africa
23	B A6 (1/26)	Burkina Faso	West Africa
24	B A6 (1/7)	Burkina Faso	West Africa
25	B A6 (1/9)	Burkina Faso	West Africa
26	B A6 (7/22)	Burkina Faso	West Africa
27	B B3 (6/30)	Burkina Faso	West Africa
28	B B3 (7/29)	Burkina Faso	West Africa
29	B B3 (7/31)	Burkina Faso	West Africa
30	M A3 (13/43)	Mali	West Africa
31	M A4 (12/24)	Mali	West Africa
32	M A5 (2/28)	Mali	West Africa
33	M A5 (6/32)	Mali	West Africa
34	M A5 (2/29)	Mali	West Africa
35	M B3 (5/6)	Mali	West Africa

36	M B3 (8/15)	Mali	West Africa
37	M B3 (10/56)	Mali	West Africa
38	M B3 (8/12)	Mali	West Africa
39	M B3 (9/13)	Mali	West Africa
40	M B4 (6/18)	Mali	West Africa
41	MAD 1	Madagascar	Madagascar
42	MAD 2	Madagascar	Madagascar
43	MAD 3	Madagascar	Madagascar
44	MAD 4	Madagascar	Madagascar
45	MAD 5	Madagascar	Madagascar
46	MAD 6	Madagascar	Madagascar
47	MAD 8	Madagascar	Madagascar
48	MAD 9	Madagascar	Madagascar
49	MAD 10	Madagascar	Madagascar
50	MAD 11	Madagascar	Madagascar
51	MAD 13	Madagascar	Madagascar
52	MAD 14	Madagascar	Madagascar
53	MAD 15	Madagascar	Madagascar
54	MAD 16	Madagascar	Madagascar
55	EQ 1	Ecuador	America
56	EQ 2	Ecuador	America
57	EQ 3	Ecuador	America
58	EQ 4	Ecuador	America
59	EQ 5	Ecuador	America
60	EQ 6	Ecuador	America
61	EQ 7	Ecuador	America
62	EQ 8	Ecuador	America
63	EQ 9	Ecuador	America
64	EQ 10	Ecuador	America
65	EQ 11	Ecuador	America
66	EQ 12	Ecuador	America
67	EQ 13	Ecuador	America
68	EQ 14	Ecuador	America
69	EQ 15	Ecuador	America
70	EQ 16	Ecuador	America

Amplification products were separated on 6.0% denaturing polyacrylamide gel and visualized by silver stain according to the following protocol: fixing the gel for 3 minutes in a fixing solution (10% ethanol, 0.5% acetic acid, in water), staining for 5 minutes in a staining solution (0.2% silver nitrate, in water), rinsing in de-ionized water for 3 seconds, developing in a cold (4–10 °C) developer solution (0.15% sodium hydroxide, 0.2% formaldehyde, in water) until the DNA bands became visible. The gel was rinsed for 1 min in the fixing solution, air-dried and DNA bands were analyzed and photo-documented using a Nikon digital camera (Nikon, Belgium).

Data collection and statistical analysis

The scoring of bands was done as present [1] or absent [0] for AFLP marker loci and data were entered in a binary data matrix as discrete variables. Only unambiguous polymorphic bands were scored. POPGENE software (version 1.32) was used to calculate observed number of alleles (*na*), effective number of alleles (*ne*), Nei's genetic diversity (*h*) and Shannon's information index (*I*).

Within species diversity (*Hs*), total genetic diversity (*Ht*), mean coefficient of gene differentiation (*Gst*) and estimate of gene flow (*Nm*) were also calculated by POPGENE software. Dissimilarity coefficients between the genotypes, in a pairwise comparison, were computed using Jaccard's coefficient of dissimilarity to estimate relationships between the genotypes studied. The resulting dissimilarity matrix was subjected to cluster analysis by the neighbor-joining method and a dendrogram, showing the distance-based interrelationship among the genotypes, was generated using FreeTree software (23). Robustness of the clustering pattern was evaluated by bootstrapping analysis using 1000 repetitions, with FreeTree software. TreeView drawing program:
(http://taxonomy.zoology.gla.ac.uk/rod/treeview.html)
was then used to draw the dendrogram. Analysis of Molecular Variance (AMOVA) was carried out with GenALEx 6.5 software (24) to examine total genetic variation among and within accessions; in addition, Principal Component Analysis (PCoA) was performed in order to more effectively view the patterns of genetic distance. GenALEx software was also used to calculate Nei's unbiased genetic distance among the different populations. Nei's unbiased genetic distance is an accurate estimate of the number of gene differences per locus when populations are small (20).

Results

The primer combinations produced a total of 54 polymorphic bands, with 29 bands for E-ACT/M-CAT combination and 25 bands for E-AAC/M-CTT combination. The percentage of polymorphic bands in the populations varied from 66.67% (Senegal-Tamba) to 90.74% (Ecuador and Madagascar), with an average of 82.10% (Table 2).
Analysis of genetic variability across the populations gave data consigned in table 2. For all the six populations analyzed, the effective number of alleles (Ne) was between 1.3082 and 1.4376 with an average of 1.372 (0.019); the Nei's genetic diversity (He) ranged from 0.1962 to 0.2638 with an average of 0.229 (0.010); the Shannon's diversity index (*I*) ranged from 0.3079 to 0.4066, the average was 0.358 (0.013). These results indicate a significant genetic diversity in the populations studied. The population with greatest genetic diversity was Madagascar. The least diverse was Senegal-Tamba, suggesting a few introduced plants and their vegetative propagation activity in this region.
Ht value was 0.2771 while Hs value was 0.2293 (+/- 0.0217). Mean coefficient of gene differentiation (*Gst*) was 0.1723 (0.0131), indicating that 82.77% of the genetic diversity resided within the population. Estimate of gene flow in the population was found as 2.4013.

Analysis of Molecular Variance (AMOVA) partitioned the overall AFLP variations into variance among populations and within population. The highest proportion of variation was found within populations with 81% of the total molecular variation (Figure 1). The Nei's standard unbiased genetic distance (*D*) between the populations was between 0.010 and 0.131with the pairwise population matrix (Table 3); the average was 0.063. The shortest genetic distance was observed between Senegal-Tamba and Burkina Faso, while the most distant populations were Mali and Ecuador (Table 3). The dendrogram plotted basing on Nei's genetic distance between the six populations grouped them in 3 groups: Senegal-Tamba and Burkina Faso, Madagascar and Ecuador, and Senegal-Diobass and Mali (Figure 2).
A neighbor-joining method based dendrogram (Figure 3) was obtained from the binary data deduced from the DNA profiles of the samples analyzed. The Jaccard's dissimilarity coefficients used, ranged from 0.1 (low genomic dissimilarity) to 1 (full genomic dissimilarity) with a mean of 0.723. The lowest genetic dissimilarity coefficient [0.1] was observed between 6-Senegal-*Di* and 7-Senegal-*Di*, while the highest value [1] was measured between several plants but mainly between 27-Bur Faso and several plants of Madagascar and Ecuador.
The dendrogram divided the 70 *Jatropha* genotypes into 5 major clusters (I, II, III, IV and V, Figure 3). Cluster I contained 21 genotypes from Burkina Faso (9 genotypes), Ecuador (1 genotype), Madagascar (3 genotypes), Senegal-Diobass (4 genotypes) and almost all the Senegal-Tamba genotypes (4 genotypes). Cluster II had 21 genotypes coming essentially from Mali (10 genotypes) and Senegal-Diobass (8 genotypes). Cluster III contained 5 genotypes (2 from Madagascar, 2 from Ecuador and 1 from Senegal-Tamba). Cluster IV involved 21 genotypes originated essentially from Madagascar (7 genotypes) and Ecuador (13 genotypes). Cluster V had 2 genotypes, coming from Madagascar. In this study, globally, genotypes coming from the same region tended to cluster together indicating a relation between eco-geographical distribution of genotypes and genetic diversity. The PCoA plots (Figure 4) obtained, showed clustering similar to that of the dendrogram. The first three axes explained 45.46% (19%, 13.68% and 12.78%) of the total variation.

Table 2
Genetic variability across the six populations of *J. curcas* investigated, using AFLP primers.

	Pop size	Observed no. of alleles (Na)	Effective no. of alleles (Ne)	Nei's gene diversity (He)	Shannon's Information index (I)	% of polymorphism
Pop1 (Senegal-Tamba)	5	1.6667 (0.4758)	1.3082 (0.3132)	0.1962 (0.1709)	0.3079 (0.2477)	66,67
Pop1 (Senegal-Diobass)	12	1.8889 (0.3172)	1.4314 (0.3544)	0.2599 (0.1734)	0.4014 (0.2298)	88,89
Pop2(Burkina)	12	1.7778 (0.4196)	1.3268 (0.3338)	0.2030 (0.1775)	0.3196 (0.2461)	77,78
Pop3 (Mali)	11	1.7778 (0.4196)	1.4024 (0.3746)	0.2372 (0.1920)	0.3616 (0.2632)	0,7778
Pop5 (Madagascar)	14	1.9074 (0.2926)	1.4376 (0.3484)	0.2638 (0.1732)	0.4066 (0.2296)	90,74
Pop6 (Ecuador)	16	1.9074 (0.2926)	1.3269 (0.2864)	0.2157 (0.1476)	0.3505 (0.1989)	90,74
Mean		1.654 (0.042)	1.372 (0.019)	0.229 (0.010)	0.358 (0.013)	82.10% (3.96%)

The values in the brackets are standard deviation.

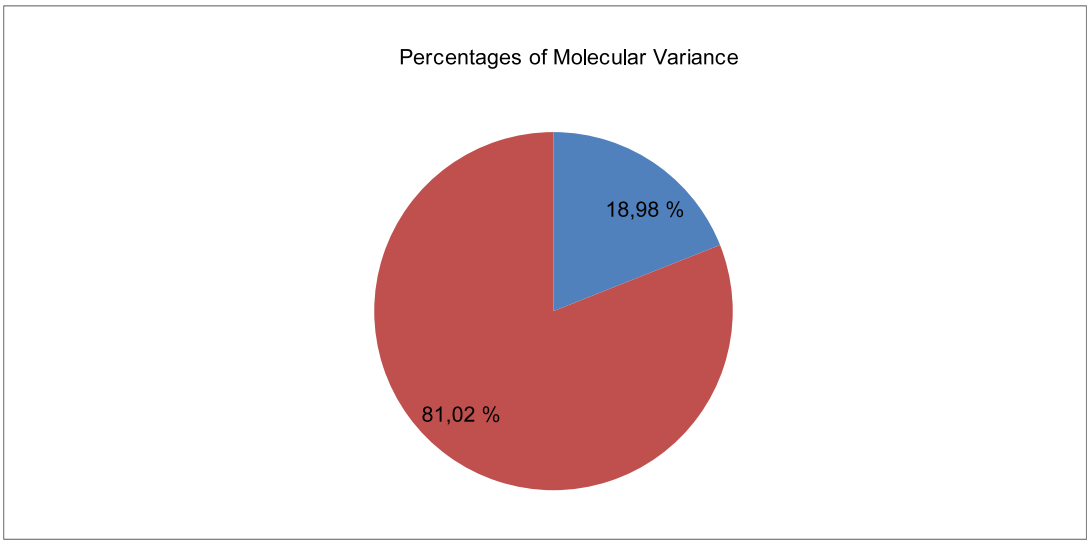

Figure 1: Result of analysis of molecular variance (AMOVA) showing the percentage of variation among and within populations for the six populations of *J. curcas*.

Table 3
Pairwise population matrix of Nei unbiased genetic distance calculated with AFLP data obtained in the six populations of *J. curcas* studied.

	Senegal-Ta	Senegal-Di	Burkina Faso	Mali	Madagascar	Ecuador
Senegal Ta	0					
Senegal Di	0,057	0				
Burkina Faso	0,01	0,046	0			
Mali	0,125	0,041	0,096	0		
Madagascar	0,031	0,069	0,041	0,112	0	
Ecuador	0,029	0,086	0,052	0,131	0,013	0

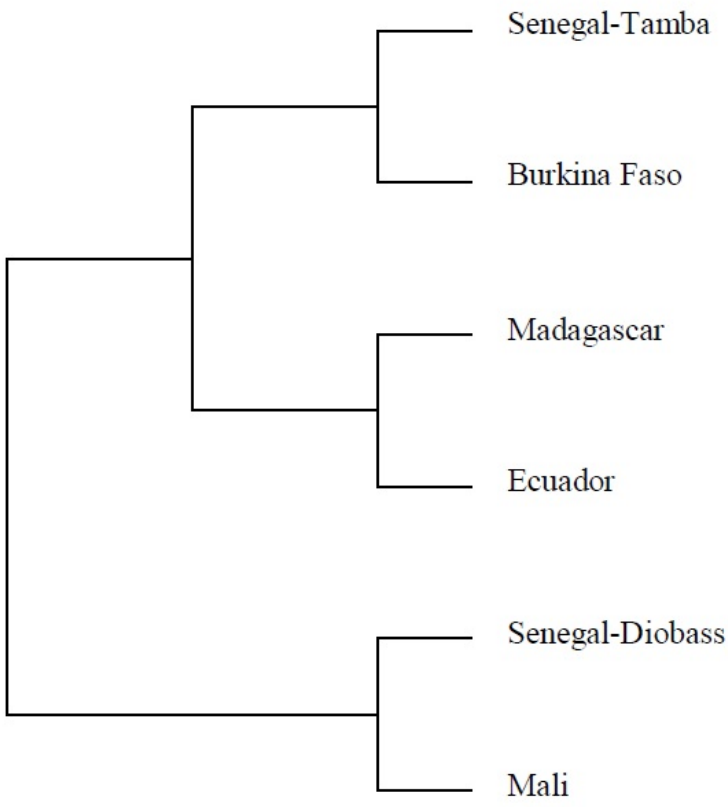

Figure 2: Dendrogram based Nei's genetic distance between the six *Jatropha* populations studied.

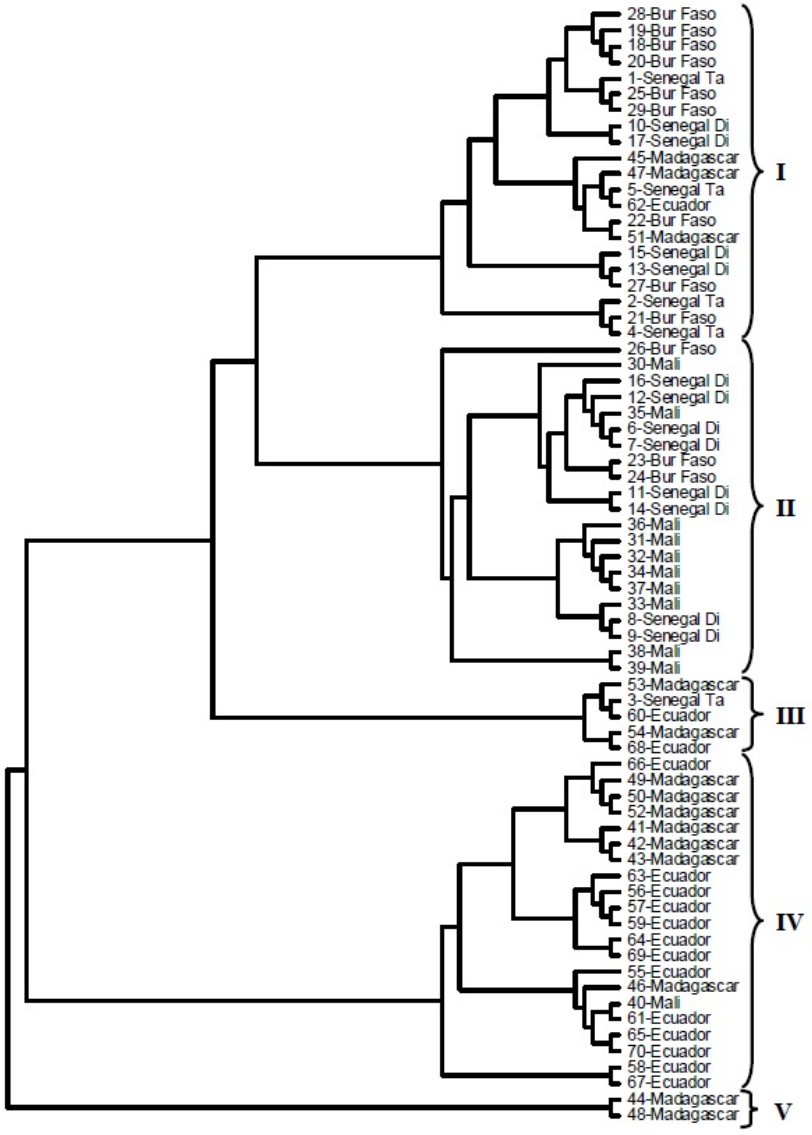

Figure 3: Dendrogram generated using AFLP data and neighbor-joining method, showing relationships between *J. curcas* genotypes investigated.

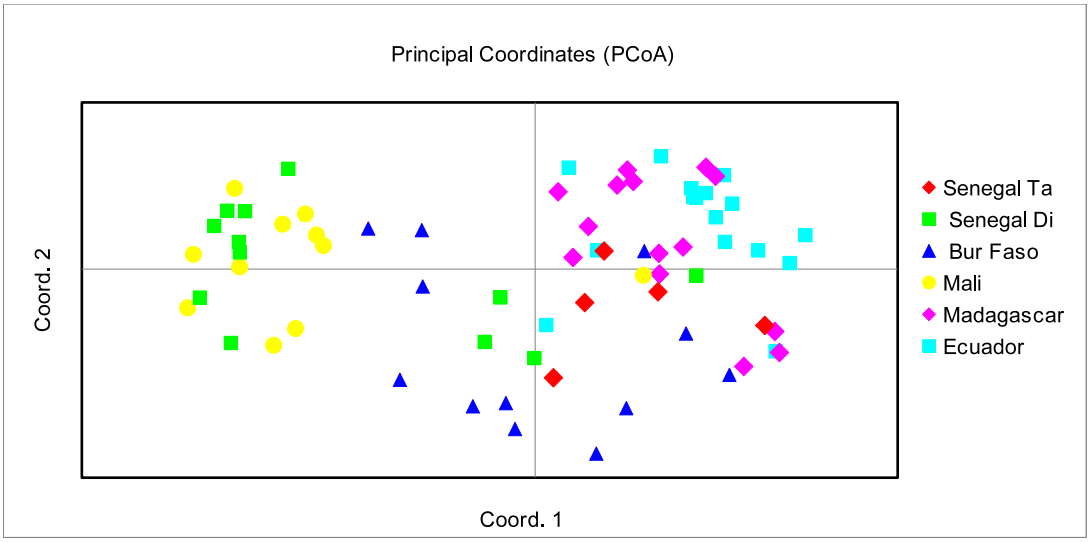

Figure 4: Two-dimensional plot of principal component analysis (PCoA) of the seventy *Jatropha* genotypes investigated using AFLP analysis.

Discussion

In the present study, the percentages of polymorphic loci found in the populations were important (66.67% to 90.74% with an average of 82.10) suggesting high level of genetic variation among the genotypes studied. Such levels of polymorphisms have already been reported by several authors working on *J. curcas* accessions. In Asia, Gupta *et al.* (8), working on 13 accessions from India, detected 84.26% and 76.54% polymorphism with RAPD and ISSR markers, respectively. Likewise, Ram *et al.* (26) and Ikbal *et al.* (10) reported, respectively, 80.2% and 93.90% polymorphism across accessions from India, using RAPD markers. With ISSR markers across 224 accessions from China, Cai *et al.* (4) found a polymorphism of 75%. Using the same marker technique, Mavuso *et al.* (16) obtained 85,19% polymorphism in 78 accession in Taiwan.

In America, Medina *et al.* (17), with AFLP markers, reported 71.7% to 92.1% polymorphism in 5 populations of 134 *J. curcas* genotypes from Mexico. Avendaño *et al.* (2) detected 80.24% polymorphism across 28 genotypes from Costa Rica using AFLP markers. For his part, Pioto *et al.* (25) found, with AFLP markers, polymorphisms ranged from 70.65 to 89.76% across 5 populations of *J curcas* from Brazil. Concerning *J. curcas* from Africa, Ouattara *et al.* (22), using 33 SSR markers, accross populations of Senegal, Mali and Burkina Faso, obtained almost no polymorphism. But, Maghuly *et al.* (15) with three AFLP combinations and five ISSRs across 907 *J. curcas* accessions from Africa (Cape Verde, Guinea Bissau, Burkina Faso, Ethiopia, Kenya, Mali, Tanzania) America and Asia found nearly the same polymorphism for the 3 continents with 31.4% for America, 31.2% for Asia and 33.1% for Africa suggesting that levels of polymorphisms found in Asia and America are about the same among *J. curcas* from Africa.

The results of the study of Machua *et al.* (14) are in line with this hypothesis; using RAPD markers in eight *J. curcas* populations from Kenya, they detected polymorphisms ranging from 25% to 73.31% with an overall mean of 55.33%.

The conflicting results obtained regarding the genetic diversity of *J. curcas* in Africa may be related to sampling size or to the use of limited number of markers. Both factors could result in overestimating or underestimating the diversity indices (32). SSR-based studies have generally detected less diversity in Jatropha compared with those found with dominant markers like AFLP, RAPD or ISSR (6, 32). This is probably because AFLP, RAPD, ISSR makers make possible the detection of variations across the entire genome, whereas SSR markers are confined to the repeat regions of the DNA (32). AFLP is a rich information marker system due to its ability to generate a large number of polymorphic/informative loci simultaneously in a single lane with a single-primer combination, and this marker system does not require any prior DNA sequence information before utilization contrary to SSR (2).

Parameters of genetic diversity (polymorphism rates, effective number of alleles (*Ne*), Shannon diversity index (*I*), genetic diversity of Nei (*He*) obtained in the present study revealed a high genetic variance in the populations studied. AMOVA detected that the highest proportion of variation was found within populations (81% of the total molecular variation). This result is in accordance with several works reporting that the genetic variation was found mainly within *J. curcas* populations (2, 8, 10, 15, 17, 30). The high level of within population variation is frequently observed in allogamous species (9, 14, 29). The allogamous status of Jatropha and the dominance and efficiency of cross-fertilization in this species as reported by Santos *et al.* (14, 29), support our finding. The Nei's

standard unbiased genetic distance (*D*) between the populations was low (0.063 on average), confirming the low genetic variation between them. The dendrogram plotted basing on the pairwise population matrix of *Nei* unbiased genetic distance grouped the six populations in 3 groups: Senegal-Tamba and Burkina Faso, Madagascar and Ecuador and Senegal-Diobass and Mali. The existence of 3 groups rather than one group means that there is a certain differentiation among the 6 populations. A measure of this differentiation gave a *Gst* value of 0.1723. Since *Gst* values between 0.00 and 0.05, 0.05 and 0.15, 0.15 and 0.25, and >0.25 are known to reveal respectively low, moderate, high and very high differentiation among populations (3), the *Gst* value found in the present work indicates a rather high population differentiation.

For a better comprehension of this differentiation, the genetic relationships among the 6 populations were examined through both neighbor-joining cluster analysis and principal component analysis (PCA). Results from both analysis showed similar structuring. In both cases the analysis grouped together i) most of Ecuador and Madagascar genotypes, ii) most of Senegal-Diobass and Mali genotypes, and iii) Most of Burkina Faso and Senegal-Tamba genotypes with a part of Madagascar, Senegal-Diobass and Ecuador genotypes. These results show that, the genotypes of *J. curcas* used in this study were globally correlated with their geographic origins, although some mixings occurred. Madagascar and Ecuador genotypes, despite the great geographic distance between them, clustered together revealing that the phylogenetic characteristics of *J. curcas* from South America, close to the Central America region where *J. curcas* originated, and from Madagascar, where the species was spread after the discovery of America, were very similar. The existence of Senegal, Burkina Faso, Madagascar and Ecuador genotypes in a same group suggests that all these populations had a common background. This finding supports the hypothesis of a common ancestor and confirms the observation of Heller (9), who showed the distribution and spread of *J. curcas*. The study of the genetic relationships among the 6 populations showed also that most of the genotypes of Senegal-Tamba and Burkina Faso grouped together, while most of the Senegal-Diobass and Mali genotypes formed a cluster.

This observation indicates a differentiation between population from Diobass and Tamba which are two regions of the same country, Senegal. Moreover, the separate clustering of *J. curcas* populations from the same West African region, on one the hand, and the separation of these West African genotypes from some of Madagascar and Ecuador, on the other hand, suggests the existence of a differentiation between some germplasms from these regions.

This result supports the great variations in important phenotypic, physiological and biological traits such as plant height, seed size, water use efficiency and seed oil content previously reported in *J. curcas* germplasm (30, 32). But, our result is opposite to previous works that reported no or minor differentiation in African *J.curcas* populations (22, 32). The level of genetic diversity and differentiation of *J. curcas* is partly attributed to the mode of its introduction in many countries, prior to anthropogenic management, selective environmental pressure, or possible cross hybridization (30). Its interpretation requires an understanding of the history of *J. curcas* introduction in the different parts of the tropical regions of the world as an exotic species. According to Heller (9), *J. curcas* is native to Central and South America, from where it spread to other tropical countries. It has been transported via the Cape Verde and Guinea Bissau to Africa by Portuguese traders before 1810 approximately (9, 14, 30). If this is correct, the time in which it was introduced into Africa represents a very short time to evolve new alleles. This time was not long enough to give rise to a genetic variation; so the level of genetic variation present in Africa is determined largely by the material originally exported from America, and possibly also from reciprocal trade movements between these continents (14, 27).

Consequently, the relatively high population differentiation found in the present work might be explained by the introduction in different parts of Africa and Madagascar of germplasms with particular genetic backgrounds corresponding probably to different geographic origins.

Among the different breeding strategies (clone breeding, line breeding, hybrid breeding, and population breeding) available in plant breeding, hybrid breeding of *Jatropha* constitutes one of the most promising (19). It can allow an optimum exploitation of heterosis, if genetically distant germplasm pools are identified and involved in the breeding program (19, 28). Determination of genetic relationships among species is critical for the management of genetic resources and success of hybridization. The success of *J. curcas* genetic improvement program requires germplasm collection that has broad genetic base. The result in this study indicates the existence of a certain diversity that can be used by breeders. There are prospects to develop F1 hybrids considering the distance existing between the different origins. The grouping of germplasm in divergent pools is advantageous to maximize the expected heterosis (28). The choice of the parents can be based on the complementarily characteristics existing in elite genetic stocks that will be selected in the different populations. This work will be followed by assessing the combining ability of these elite materials.

Regarding the dendrogram generated, the greatest heterosis might be expected from crossing schemes involving genotypes of cluster I and the more distant genotypes (genotypes of cluster IV and V). Such crossings might permit to benefit from heterotic increase due to differences in allele frequencies (19), allowing for greater success in the production of genetic variability and thus might maximize the exploitation of heterosis and segregation.

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