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# Variability in seed traits, oil content and genetic diversity in local and exotic accessions of *Jatropha curcas* L. in Senegal

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**Variability in seed traits, oil content and genetic diversity of *Jatropha curcas* L. according to rainfall gradient in Senegal are hereby reported and discussed. Seed oil variability ranged from 58.61% in Sudanian zone to 46.94% in Sahelian zone. Seed oil content and seed thickness were correlated to rainfall with a correlation coefficient of 0.62 and 0.48 respectively. However, seed length, breadth and 20 seed-weight were not correlated to rainfall. The random amplification of polymorphic DNA (RAPD) primers used to assess genetic variation showed a clear polymorphism. The mean polymorphism rate was 42.68%. A low variability was observed in the accessions. The genetic diversity was not correlated to geographic position. On the basis of coefficient similarity values, the accessions were genetically diverse. Cluster analysis based on similarity values classified *Jatropha curcas* L. accessions into three major clusters of which cluster I was the largest group. The lowest genetic distance (0.029) was recorded between Karang and Kaffrine accessions whereas the highest genetic distance (0.274) was observed between Bignona and Karang accessions. Accession from Bignona recorded the highest intra-population variation. Special attention must be accorded to accessions with high oil content, seed weight and high intra-population variation for future selection programs.**

**Key words:** *Jatropha curcas L*., seed size, oil content, genetic diversity, Senegal.

## INTRODUCTION

In the last years, the tendency in increasing petroleum products price was observed. The increment of fuel demand especially from countries like China and India, the word geopolitical features, especially in Middle Eastern region, and weather related supply shocks have fuelled the continual rise in crude oil prices (Asif and Muneer, 2007). This situation would lead to a risk of perturbation of fuel supply in the future. To prevent a risk of lack of fuel, the government of Senegal has developed biofuel options through cultivation of wild species. In this context, cultivation of *Jatropha curcas* L. (*J. curcas*) has gained special attention like in others countries from West African Monetary Economic Union (UEMOA). *J. curcas* is a small tree or large shrub that has been domesticated in a widespread manner in Africa and Asia mainly due to its ability to grow in a number of climatic zones in tropical and subtropical regions of the world particularly in marginal lands (Rao et al., 2008).

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It belongs to the Euphorbiaceae family; it is a native of Latin America and has been introduced in Africa and Asia through Portuguese traders (Jubera et al., 2008). The plant is drought resistant, can grow on marginal lands and produce till 50 years (Heller, 1996). It has multi-purpose uses. The uses includes as anti erosive fence, its oil is used in soap manufacturing and many parts of the plant are used in traditional medicine. In addition to the above, *J. curcas* is being promoted for its seed oil (35 %) which can easily be converted to biofuel (Azam et al., 2005). *J. curcas* has gained popularity all over the world in comparison to other tree-borne oil seed crops because of its better adaptation to a wide range of environmental conditions, low cost of seeds, high oil content, small gestation period and smaller plant size that makes the seed collection easier (Sujatha, 2006).

In spite of its enormous potential, the introduction of *J. curcas* in crop system raises many concerns and questions related to its productivity, the impacts of its cultivation on soil properties, the impacts of climate change on its production, its germplasm management, potential competition with food for arable land, etc.

Until now, this crop has not been fully domesticated. In Senegal, information on germplasm of *J. curcas* is not well documented. The success of commercial cultivation of *J. curcas* is much dependent on the use of high yielding genotypes. Therefore, genetic improvement and development of high-yielding genotypes of this crop is vital and key to biodiesel production. The improvement work should start with assessment of the local genotypes. This is because local genotypes are best adapted to local climate and soil conditions as revealed in case of *Populus davidiana* (Zhang et al., 2005).

In Senegal, variability in local germplasm of *J. curcas* has been reported in germination behavior (Ouattara et al., 2011) and seedling growth inoculated with arbuscular mycorhizae (Leye et al., 2009). These studies indicate the existence of potential genetic variability in *J. curcas* germplasm in Senegal. The present study aimed at exploring variability of seed size, oil content and genetic diversity in *J. curcas* accessions in Senegal and three exotic accessions.

## MATERIALS AND METHODS

*J. curcas* accessions were collected from Sahelian, sudano-sahelian and sudanian zones, three agro-ecologic zones of Senegal for their rainfall (Cisse et al., 2003). A total of 30 accessions were collected; 10 accessions from each zone (Table 1). From each of the accessions, fruits were collected from at least ten trees. Accessions were about 30 km apart from each other in order to avoid narrowing down of the genetic base due to relatedness or inbreeding. *J. curcas* is not native to Senegal, so fruits were collected from old plantations which are generally live fences.

Fruits were air dried under similar conditions of temperature and humidity until constant weight. Then, seeds from capsule were extracted through manual threshing and kept at room temperature, about 25°C, in the laboratory.

Seeds from three exotic accessions (India, Mozambique and Tanzania) were also assessed in order to compare them with the local accessions.

### Observations and measures

#### *Seed traits*

To assess seeds weight, three replicates of 20 undamaged seeds of each of the 33 local and exotic accessions were chosen randomly and weighted using an electronic scale. For each replicate, the seeds sizes (Length, breadth and thickness) of each seed were measured using an electronic vernier caliper

#### *Oil content*

Oil content was determined by soxhlet method (Pant et al., 2006) using three replicate of 20 seeds for each seed lot including all the local and exotic accessions. Seed kernels were dried in oven at 65°C for 48 h. Kernels were ground and powders were used for oil extracting. Seed oil content was determined using the following formula:

% oil = (oil weight/powder weight) *100

### Molecular analysis

#### *DNA extraction*

DNA was extracted from young fresh leaves of 20 plants of each accession following the standard CTAB method with minor modifications (Benbouza et al., 2006). 100 mg of fresh leaves were ground to fine powder, then homogenized in 750 µl of extraction buffer [2% (p/v) CTAB, 20 mM EDTA, 2% (p/v) PVP, 2 M NaCl, 100 mM Tris-HCl pH 8.0 and 5% (v/v) β-mercaptoethanol] and incubated at 65°C for 1 h.

The supernatant was extracted with chloroform isoamylalcohol (24:1 v/v). The DNA was precipitated with isopropanol and washed with a washing buffer (76% ethanol and 10 mM ammonium acetate). The pelleted DNA was air dried at room temperature and dissolved in 300 µl of 1X TE. The solution was treated with RNase A (100 µg/ml), incubated at 37°C for 30 min before storing at 4°C overnight. The following day, DNA concentration was determined electrophoretically on 1% agarose gel using known amount λ DNA as standard. DNA was diluted in 1X TE to a concentration of 5 ng/µl for use in PCR analysis.

**Table 1.** Seed source origin characteristics of *J. curcas,* (* indicates accessions used in molecular characterization).

| Accession | Code | Zone | Rainfall (mm) (Cisse et al., 2003) |
|---|---|---|---|
| Tivaouane2* | P3 | Sahelian | 250-500 |
| Notto | P38 | Sahelian | 250-500 |
| Tivaouane1* | P6 | Sahelian | 250-500 |
| Notto2 | P13 | Sahelian | 250-500 |
| Notto3 | P15 | Sahelian | 250-500 |
| Notto4 | P14 | Sahelian | 250-500 |
| Ndialite | P12 | Sahelian | 250-500 |
| Keur ndiogou Ndiaye | P11 | Sahelian | 250-500 |
| Bambey | P9 | Sahelian | 250-500 |
| Tawaye | P8 | Sahelian | 250-500 |
| Bignona* | P35 | Sudanian | 900-1100 |
| Sindian* | P34 | Sudanian | 900-1100 |
| Kilou | P32 | Sudanian | 900-1100 |
| Tendouck | P1 | Sudanian | 900-1100 |
| Bourofaye | P89 | Sudanian | 900-1100 |
| Kilou2 | P2 | Sudanian | 900-1100 |
| Kagnobon | P5 | Sudanian | 900-1100 |
| Vater | P33 | Sudanian | 900-1100 |
| Tendouck2 | P17 | Sudanian | 900-1100 |
| Kagnobon2 | P90 | Sudanian | 900-1100 |
| Ndoffane | P48 | Sudano-sahelian | 500-900 |
| Toubacouta | P52 | Sudano-sahelian | 500-900 |
| Samba gueye | P50 | Sudano-sahelian | 500-900 |
| Latmingue | P69 | Sudano-sahelian | 500-900 |
| Latmingue2 | P83 | Sudano-sahelian | 500-900 |
| Ndary | P77 | Sudano-sahelian | 500-900 |
| Banfadjiré | P85 | Sudano-sahelian | 500-900 |
| Kaffrine* | P95 | Sudano-sahelian | 500-900 |
| Pane abdlay Diop | P94 | Sudano-sahelian | 500-900 |
| Karang* | P51 | Sudano-sahelian | 500-900 |
| Mozambique* | - | Exotic | - |
| Inde* | - | Exotic | - |
| Tanzanie* | - | Exotic | - |

***RAPD analysis***

50 RAPD primers were tested in a first analysis and five of them, producing clear amplification profiles, were chosen to investigate the genetic variability. A total of six accessions, two accessions from each agro-ecologic zone (20 individuals per accession), were used for molecular analysis. The PCR amplification reaction (12.5 ml) consisted of 10 ng of DNA, 1X PCR Buffer (10 mM Tris-HCl pH 8.8, 50 mM KCl, 1.5 mM $MgCl_2$ ); 3.5 mM $MgCl_2$; 0.2 mM of each of the four dNTPs; 0.15 mM of RAPD primers and 0.4 U of Taq DNA polymerase (SBS Gentech).

PCR amplifications were performed in a ‘’PRIMUS 96 plus’’ thermal cycler with a initial denaturation at 94°C for 3 min followed by 45 cycles at 94°C for 45 s, 36°C for 30 s and 72°C for 2 min with a final extension at 72°C for 7 min. The PCR products were separated on 1.8% agarose gel in 1X TBE buffer by electrophoresis at 100 V for 3 h and visualized after staining with ethidium bromide for 45 mn.

**Table 2.** Results of ANOVA of seed traits

| Seed trait | DF | S.C | S.M | F | Probability |
|---|---|---|---|---|---|
| Thickness (mm) | 32 | 7.04833 | 0.22026 | 8.68 | 0.00 |
| Breadth (mm) | 32 | 4.17142 | 0.13036 | 6.63 | 0.00 |
| Length (mm) | 32 | 31.9721 | 0.99913 | 13.6 | 0.00 |
| Oil content (%) | 32 | 1906.46 | 59.5768 | 64.0 | 0.00 |
| 20 seeds weight (g) | 32 | 334.126 | 10.4414 | 21.9 | 0.00 |

**Table 3**. Correlation coefficients between rainfall and seed traits.

| Seed trait | Coefficient of correlation (Pearson) |
|---|---|
| Length (mm) | $0.299^{ns}$ |
| Breadth (mm) | $0.253^{ns}$ |
| Thickness (mm) | 0.476** |
| 20 Seed-weight (g) | 0.429* |
| Oil content (%) | 0.616** |

Ns, not significant.

#### Statistical analysis

Seed traits and oil content data were subjected to analysis of variance and Significant difference test using Statistix 8.1 software. Linear correlation coefficients were calculated among the studied traits. Data of seed traits were suggested for factorial analysis.

For each RAPD primer, the presence or absence of bands in each accession was visually scored and set in a binary matrix. The number of polymorphic and monomorphic fragments for each primer pair was scored and the monomorphic markers were excluded from the analysis. Genetic diversity was estimated by computing the percentage of polymorphic loci, and mean number of allele per locus. The binary matrix was read by Dissimilarity Analysis and Representation (DARwin5) software package with Jaccard's similarity coefficients and genetic distances for all pairwise comparisons between accessions were estimated with GenAlex software. Dendrogram was constructed based on pooled marker data using neighbor joining method. Genetic distance matrix was subjected to factorial analysis. Variability within accession and among accession was estimated using molecular analysis of variance.

## RESULTS

### Seed weight and oil content

There were significant ($P<0.05$) differences in seed weight and oil content of the accessions (Table 2). Among the local accessions, Kagnobon from Sudanian zone recorded the highest 20 seeds weight (15.09 g) and the lowest was recorded in Bambey (8.33 g) from Sahelian zone. The highest 20 seeds weight among the exotic accessions was recorded in accession from India (12.85 g) and the lowest in accession from Tanzania (7.93 g).

Seed oil content varied from 58.61 to 36.40%. The top ranking accession was Kagnobon from Sudanian zone followed by Ngodiba (50.41%) and Banfadjiré (50.21%) both from Sudano-Sahelian zone. Low oil content was recorded in accessions from Sahelian zone with Ndialite (44.93%) Tawaye (44.67%) and Notto (44.66%). Seed oil content and seed thickness were positively correlated to rainfall (Table 3).

The factorial analysis based on seed traits grouped the 33 accessions in two main clusters (Figure 1). The two axes of factorial analysis explained 64 and 15% variation among accessions. Almost all the accessions from Senegal were clustered together with accessions from India. While accessions from Mozambique and Tanzania were grouped together with P12 and P9 all from Sahelian zone.

### Genetic variability and relationship among *J. curcas* accessions

The five tested primers were polymorphic and produced amplification products with all the nine accessions (six local and three exotic accessions). Figure 2 illustrates DNA fingerprint using OPB-10. The number of bands amplified per primers varied between 3 (OPB-01) and 8 (OPB-07) with an average of 5.6 bands per primer (Table 4).

A total of 28 bands were amplified of which 14 were polymorphic resulting in a polymorphism frequency of 50% and an average of 2.8 polymorphic bands per primer. The extent of polymorphism per primer ranged from 25 (OPB-03) to 71.43% (OPB-10). Accession from Bignona recorded the highest intra-population variation whereas the lowest was recorded in Tivaouane1 accession (Table 5). Genetic distances using Jaccard's coeffi-

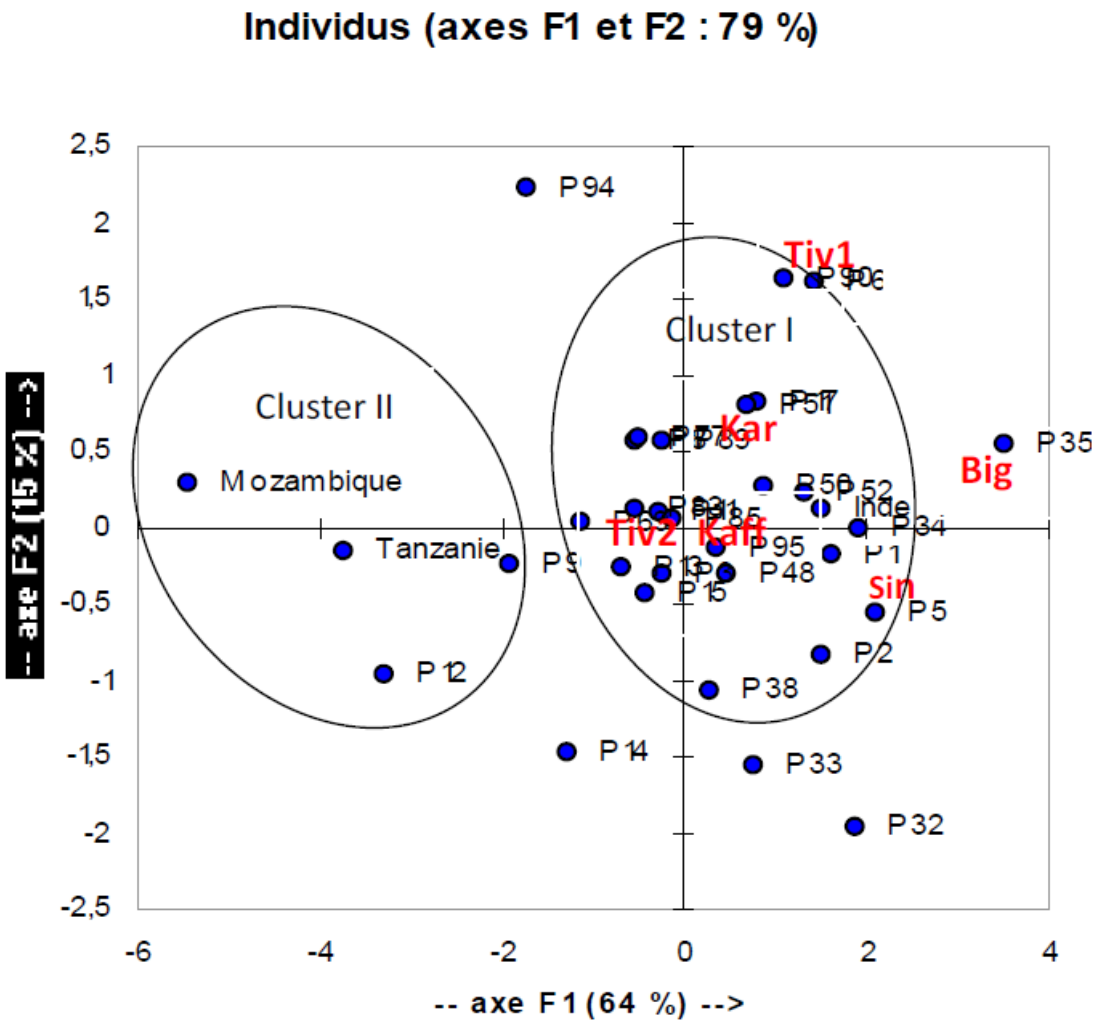


**Figure 1.** Two-dimensional scaling of 33 accessions of *J. curcas* by Factorial analysis based on pooled data of seed traits. Kar, karang; Tiv 1, Tivaoune 1; Big, Bignona; Sind, Sindian; Tiv 2, Tivaouane 2; Kaff, Kaffrine (P14, P33, P32…, are accessions code).

cient ranged from 0.029 between Karang and Kaffrine accessions to 0.274 between Bignona and Tivaouane2 accessions.

Clustering of accessions based on dendrogram using ‘’neighbor joining’’ method separated accessions out to three major clusters (Figure 3). Cluster I comprised accessions from Bignona, Tivaouane1 and Sindian. Cluster II included accessions from Kaffrine, Karang and Tivaouane2. Cluster III regrouped individuals from kaffrine and Karang accessions (8 individuals of Kaffrine and 2 of Karang accessions).

In comparison to factorial analysis representation, minor modifications have been observed. The six local accessions used in molecular analysis are grouped together in factorial analysis scaling based on seed traits except Bignona accession. Tivaouane2 and Bignona accessions seem to be the most distant in the factorial analysis representation based on seed traits. This con-

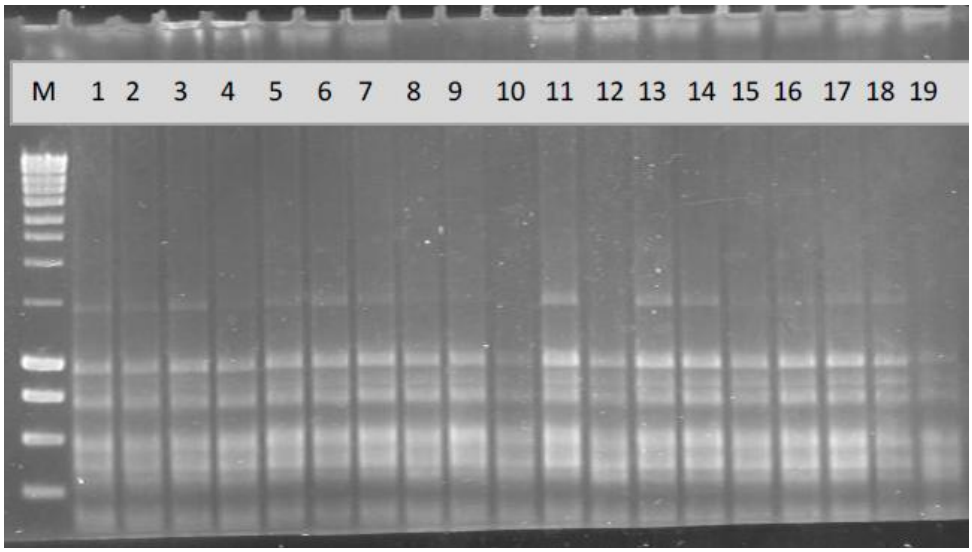


**Figure 2**. DNA fingerprint using RAPD primer: OPD-10 (sample 2-20; M, DNA Marker).

**Table 4.** Amplified bands and polymorphism generated in *J. curcas* genotypes using 5 RAPD primers.

| Primer | Total band | Polymorphic loci | Polymorphism (%) |
|---|---|---|---|
| OPB-01 | 3 | 2 | 66.67 |
| OPB-03 | 4 | 1 | 25 |
| OPB-05 | 6 | 3 | 50 |
| OPB-07 | 8 | 3 | 37.5 |
| OPB-10 | 7 | 5 | 71.43 |
| Total | 28 | 14 | |
| Mean | 5.6 | 2.8 | 50.12 |

firms the highest genetic distance observed between these two accessions (Table 6).

A few differences in clustering were observed between ‘’neighbor joining’’ clustering and Factorial analysis based on genetic distances matrix. Two individuals of Bignona accession formed distinct cluster in factorial analysis while all individuals of this accession grouped together with ‘’neighbor joining’’ method. Cluster formed with individuals of Kaffrine and karang (Cluster III) with’’ neighbor joining’’ method was not observed with factorial analysis (Figure 4).

Molecular variance analysis (AMOVA) indicated 70% variability within accessions whereas variability among accessions was 30% (Figure 5).

## DISCUSSION

Significant difference ($p < 0.05$) was recorded for seed length, breadth, thickness, 20 seed-weight and oil content. However, breadth values varied closely from 11.42 to 10.34 mm compared to other seed sizes. In general, seed size increased from Sahelian zone where rainfall was low compared with Sudanian zone (wet zone). For example, 20 seeds weight varied from 8.33 g in Sahelian zone to 15.09 g in Sudanian zone. Variability in seed traits

**Table 5.** polymorphism rate detected in the different accessions used.

| Accession | Polymorphism (%) |
|---|---|
| Karang | 46.43 |
| Tivaouane 1 | 28.57 |
| Bignona | 53.57 |
| Sindian | 39.29 |
| Tivaouane 2 | 42.86 |
| Kaffrine | 46.43 |
| Mean | 42.86 |

might be explained by the differences in climatic conditions and variability in soil nature of seed origins (Kaushik et al., 2007). Similar results have been reported in a number of species. Kaushik et al. (2007) and Ginwal et al. (2005) reported similar variations in *J. curcas* seeds. Similar findings have also been reported in *Azadirachta indica* (Jindal et al., 1999) and *Acacia nilotica* (Kundu et al., 1997).

Material (seed) from those accessions having high seed weight and oil content might gain attention for further breeding programs. Indeed, seed germination and seedling growth are improved through seed size in case of *Hardwickia binnata* (Ponnammal et al., 1993) and *J. curcas* (Kaushik et al., 2001). Larger and heavier seeds have been found to produce better quality of seedling in *Pinus brutia* (Aslan, 1975). The interest of seed weight in delimiting and understanding the geographical variation has been reported because of the least plasticity of this character (Harper et al., 1970).

Seed oil content varied from 58.61 to 36.40% with highest oil content in Bignona accession. These results are consistent with those found by others authors worldwide. Oil content in India accessions ranked from 46.22 to 58.12% (Ginwal et al., 2004). Pant et al. (2006) studying the influence of the altitude on seed oil content of *J. curcas* in India reported variability in oil content from 42.34 to 45%.

However, Ginwal et al. (2004) reported highest oil content (58.12%) in Chhindwara accession with less rainfall and moderately high temperature. In the present study, Bignona location belonging to wet region in Senegal recorded the highest oil content. Better soil depth and soil fertility could explain these results. Indeed Srivastava et al. (1999) reported that soil conditions play significant role in causing variation in oil yield. The role of Phosphorus in increasing oil yield in the case of castor beans was reported by De Geus (1973) in East Africa.

Significant and positive correlations are recorded between seed traits. Seed weight and oil content are correlated. Similar results have been reported in *J. curcas* (Kaushik et al., 2007) and *A. indica* (Kundu et al., 1997; Jindal et al., 1999). However, no correlation was found between seed length and seed breadth as reported by Kaushik et al. (2007). On the basis of the present findings, seed oil content that is correlated to all the seed traits might be used as important traits in early selection of seed sources.

Factorial analysis representation based on seed traits clustered most of the local accessions with accession from Indian while accessions from Tanzania and Mozambique were clustered in another group. In 2007, the government of Senegal distributed *J. curcas* seeds from India to the farmers in order to promote the cultivation of *J. curcas* (ISRA, 2009). This might explain the grouping of India accession with the local accessions. Indeed *J. curcas* has cross-pollination (Qing *et al.,* 2007) and seeds collected during our prospection might be the result of cross-pollination between local and introduced accessions. In the present study, RAPD primers, despite being dominant markers, allowed the analysis of intra and inter accessions variability. Only clear and unambiguous bands were scored to reduce these risks. The RAPD primers (5) revealed 42.86% of polymorphism with 2.8 polymorphic bands per primer. These results indicate low genetic diversity in accessions from Senegal. These results were in consistence with the studies of Basha et al. (2009) where 42% of polymorphism was found in *J. curcas* accessions from India. Intra accession variability was highest in Bignona accessions compared to Tivaouane I which recorded the lowest intra accession variability.

Genetic distance between local accessions varied from 0.272 to 0.029. Similar results was found by Sujatha et al. (2006) who reported narrow genetic diversity among eight *J. curcas* accessions from India.

Analysis of molecular variance showed 30% of variability among accessions. These results are consistent to those of Basha and Sujatha (2007) who reported low molecular diversity in germsplasm from India.

Geographic range, mode of reproduction, mating system, seed dispersal and fecundity are usually related to variation in genetic diversity (Loveless, 1992). However, despite large distribution of *J. curcas* in different eco-geographical zones of Senegal, low genetic diversity was observed. The main reasons are probably low genetic

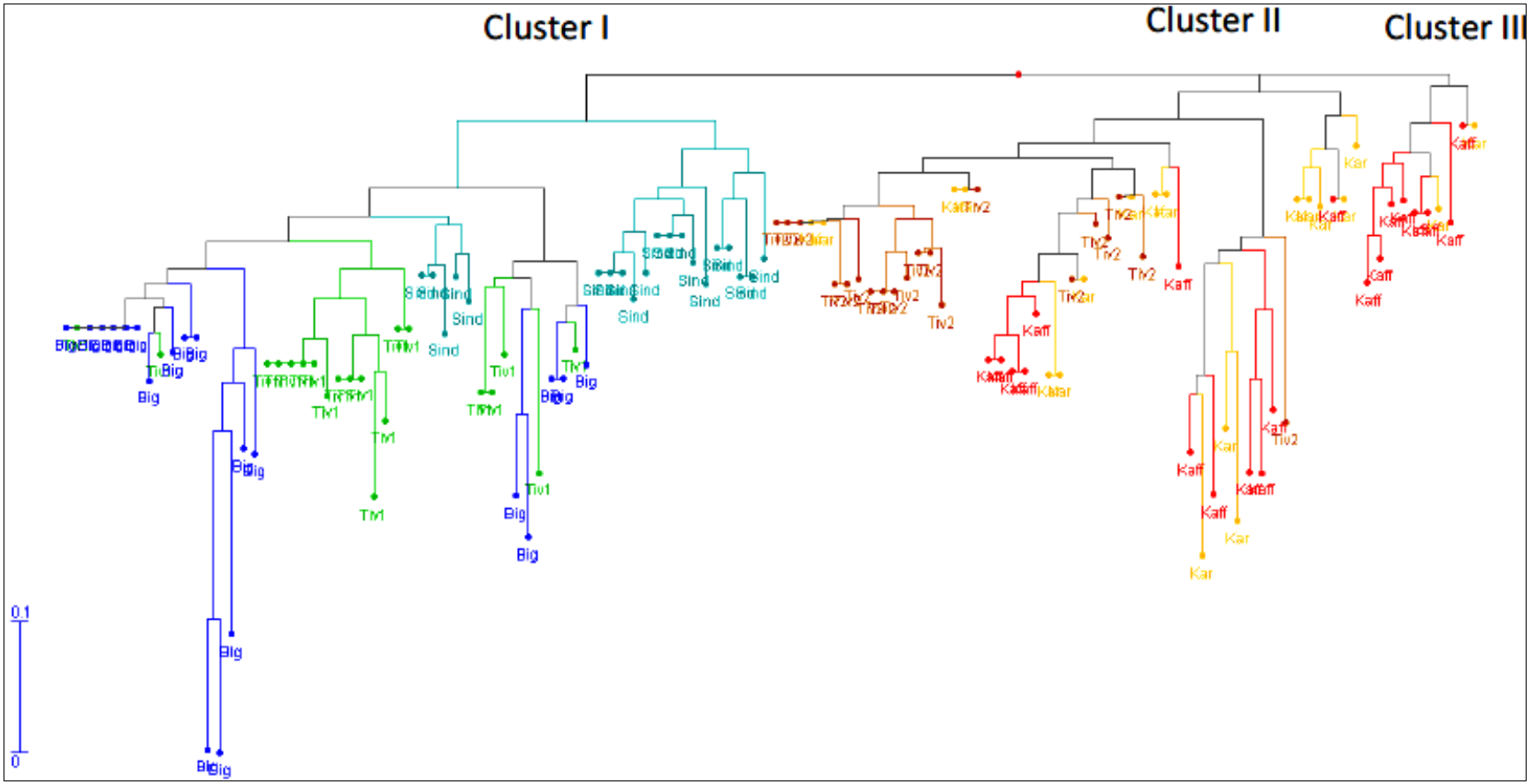


**Figure 3.** dendrogram showing relationship between individuals of six accessions from Senegal. Kar, karang; Tiv 1, Tivaoune 1; Big, Bignona; Sind, Sindian; Tiv 2, Tivaouane 2; Kaff, Kaffrine).

**Table 6**. Genetic distance between accessions using RAPD markers.

| | Kar | Tiv I | Big | Sind | Tiv II | Kaff |
|---|---|---|---|---|---|---|
| Kar | 0.000 | | | | | |
| Tiv 1 | 0.221 | 0.000 | | | | |
| Big | 0.225 | 0.038 | 0.000 | | | |
| Sind | 0.137 | 0.099 | 0.109 | 0.000 | | |
| Tiv 2 | 0.051 | 0.260 | 0.274 | 0.178 | 0.000 | |
| Kaff | 0.029 | 0.205 | 0.235 | 0.136 | 0.099 | 0.000 |

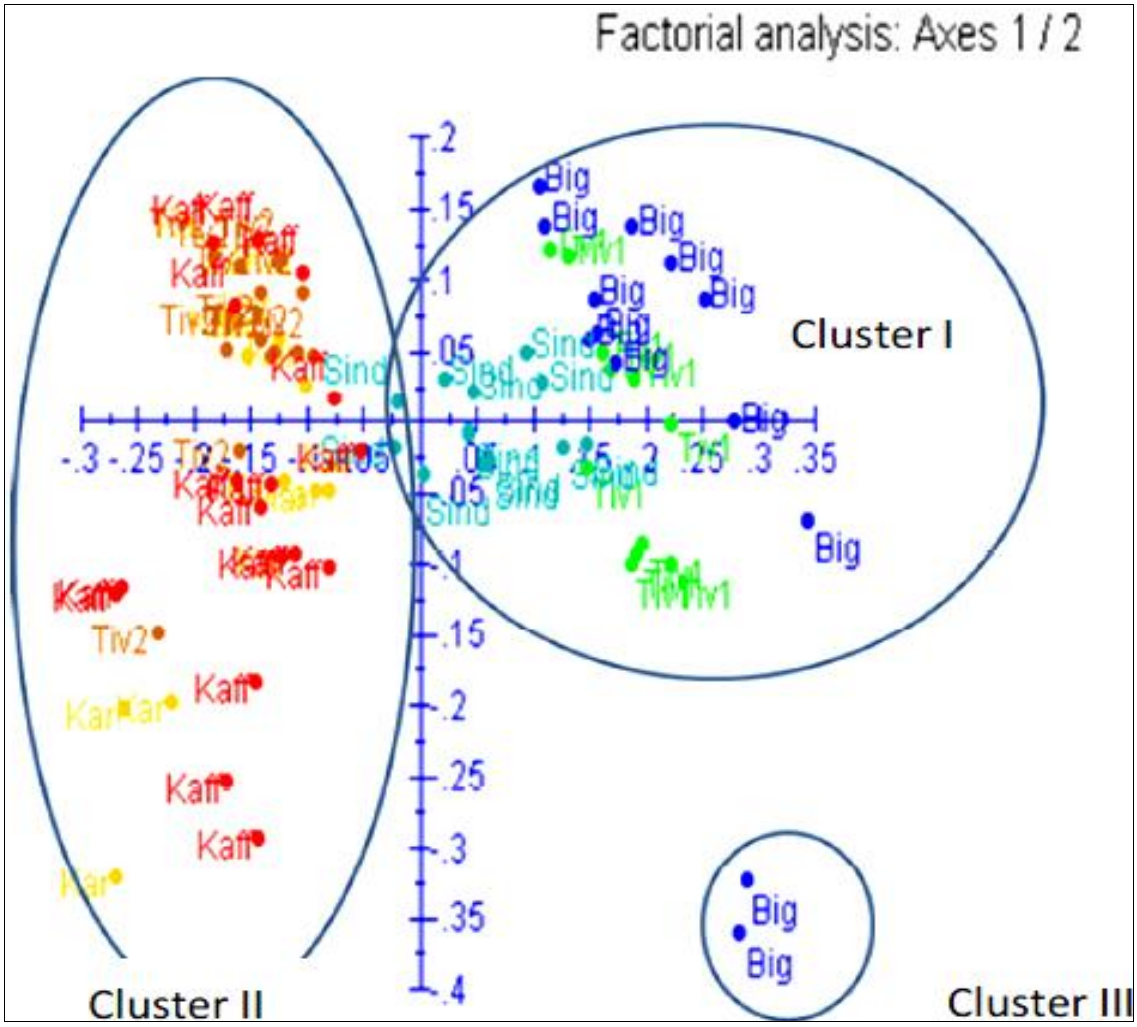


**Figure 4**. factorial analysis scaling of 6 accessions of *J. curcas* using Jaccard's similarity coefficients based on pooled data of RAPD primers Kar, karang; Tiv 1, Tivaoune 1; Big, Bignona; Sind, Sindian; Tiv 2, Tivaouane 2; Kaff, Kaffrine.

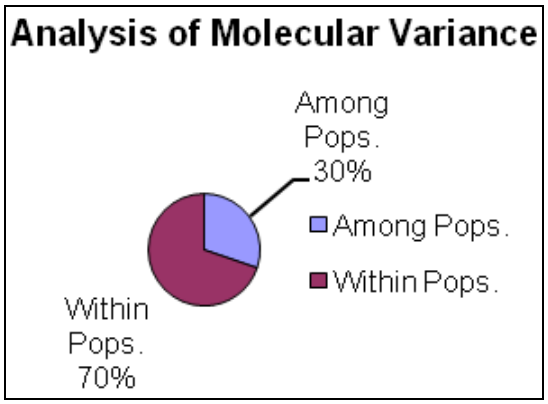


**Figure 5**. Molecular variance among and within the six accessions of *J. curcas* from Senega.l

base of the introduced materials and propagation by cuttings as highlighted by several elders encountered during the collection.

The results of the present study show that *J. curcas* germplasm within Senegal exhibits high phenotypic variability and low genetic base. The cluster pattern proved that geographical diversity need not necessarily be related to genetic diversity.

From the clustering pattern and genetic relationship obtained using RAPD markers, breeders can identify the diverse genotypes from different clusters and employ them in their future breeding program. Accession from Kagnobon which exhibited high values of seeds traits (seeds weight, oil content) and accession from Bignona which showed high intra-accession genetic variability deserve special attention in future breeding programme.

We can conclude from the present study that high variability in oil content with highest value (58.61%) was observed in Sudanian, wet zone, and the lowest in Sahelian zone (45.94%). Rainfall was significantly and positively correlated to seed thickness and oil content. However, similar correlation was not found with seed length, breadth and seed weight.

Individuals from the same accession were closely grouped although low genetic diversity was found between accessions. Kaffrine and Karang were found to be closed and Tivaouane 2 and Bignona to be most distant. Though accessions were geographically distant, close genetic base was observed. In future studies co-dominant and more polymorphic markers and large sample will be useful in analysis of genetic extent of *J. curcas* in Sénégal.

The results of the present study will be valuable for seed zone delineations, strategies for conservation of genetic variation, prospects of improvement and assessment of the potential of locally adapted seed source. It is reported that larger and heavier seeds produced better quality of seedling. This relationship can be exploited for screening of genotypes to have an early indication on their oil yield and growth performance.

In the present study, geographical diversity did not represent genetic diversity because individuals from different accessions were grouped together.

Local accessions are closely linked to accession from India. Future research is needed to understand the relationships between both Senegalese and Indian *J. curcas* germplasm.

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