African Journal of Biotechnology Vol. 10(16), pp. 3062-3069, 18 April, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.1990
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Genetic diversity of *Jatropha curcas* L. populations in Kenya using RAPD molecular markers: Implication to plantation establishment

Machua J.[1]*, Muturi G[2]., Omondi S. F[3]. and Gicheru J[1].

[1]Biotechnology Laboratory, Kenya Forestry Research Institute, P.O. Box 20412-00200 Nairobi.
[2]Drylands Research, Kenya Forestry Research Institute, P.O. Box 20412-00200 Nairobi.
[3]Tree breeding and improvement Laboratory, Kenya Forestry Research Institute, P.O. Box 20412-00200 Nairobi.

Accepted 12 January, 2011

***Jatropha curcas* L is an economically potential tree species gaining interest globally because of its feasible contribution towards production of commercial biofuel. Little is known however, of its genetic variation patterns within Kenyan accessions for maximum exploitation. Eight populations covering most of its distribution range in Kenya were sampled and leaves of 160 individual trees collected. Analysis of molecular diversity was carried out using random amplified polymorphic DNA (RAPD) markers. Ten random primers generated 251 loci that were scored for diversity. Shannon's diversity index varied from 0.116 (Likoni) to 0.360 (Namanga). Analyses of molecular variance (AMOVA) showed that, more variation (53%; P = 0.01) was partitioned among populations while 47% (P = 0.01) variation was partitioned within populations. Nested analysis of variance showed no variation across regions (0%; P > 0.01). The level of genetic structure and diversity may be explained by the modes of germplasm introductions and the biological traits of *J. curcas*. Based on these results, *Jatropha* accessions are quite variable and seeds collected from various populations are bound to exhibit wide variation. Selection of a *J. curcas* breeding population should encompass a large number of individuals across all accessions in order to capture the wide genetic variation.**

Key words: Genetic diversity, population structure, *Jatropha curcas,* biofuel.

INTRODUCTION

Jatropha curcas L. (tropical physic nut) is a perennial multipurpose shrub or tree belonging to the family Euphorbiaceae (Openshaw, 2000; Kumar and Sharma, 2008). It is distributed naturally in the tropical Americas, from where it has been introduced and become naturalized in many parts of the tropical and subtropical regions of the world (Heller, 1996; Gohil and Pandya, 2008). *J. curcas* grows to between 5 to 8 m height and has large green to pale-green deciduous leaves with several yellowish flowers and 2.5 to 4 cm long bell-shaped green capsules (fruits), which turn yellow when ripe (Little et al., 1974; Morton, 1977). The species is adaptable to a wide range of soils and climates (0 to 500 m above sea level; mean annual temperature ranging from 20 to 28°C with mean annual rainfall of between 100 to 2000 mm or more) and grows well without any special nutrition regime (Patil and Singh, 1991; Beentje, 1994). It can grow well under any unfavorable agro climatic conditions, because of its low moisture demands, fertility requirements and tolerance to high temperatures (Diwaker, 1993; Tiwari et al., 1994).

J. curcas has medicinal values and is commonly grown as hedges to protect gardens and fields from animals (Openshaw, 2000). However, in the recent years, the species has gained tremendous significance as a potential biodiesel plant (Umamaheswari et al., 2010), which is characterized as a safe, healthy environment and renewable resource, alternative to petrol diesel (Ambrosi et al., 2009; Ikbal et al., 2010; Shen et al., 2010). *J. curcas* produces large quantity of oil-seed within 2 to 3 years after planting. The species is highly open pollinated and

*Corresponding author. E-mail: machuaj@yahoo.com. Tel: 254-722810171.

can be propagated from seed or cuttings of stem or branch. It always starts flowering and fruiting 1 year after planting, but economic yield starts after 3 years and continues to produce seeds for a period of 50 years (Aker, 1997). A *Jatropha* seed contains 46 to 58% of oil on kernel weight and 30 to 40% on seed weight (Subramanian et al., 2005). The oil is a rich source of hydrocarbon (27 to 48.5% of seed oil) hence, its potential as a biofuel crop (Umamaheswari et al., 2010).

J. curcas was first introduced into Kenya by Portuguese navigators in the 16th century, but there were no records of the source origin or where it was first planted and additional introductions have been made, but no reliable information is available due to poor record keeping. The species has since been cultivated in Kitui, Thika, Namanga, Kajiado, Malindi, Nyanza, Nakuru, Marakwet, Naivashaand in Meru and is still raising a lot of interest among many farmers (Muok and Kallback, 2008).

The economic importance of *J. curcas* and its ability to grow in a wide range of climate and soil conditions has generated wide interest in understanding the genetic diversity of the species as an initial step towards selection and breeding of superior genotypes (Mohamad et al., 2009). Little work has been done in Kenya so far on the germplasm collection and evaluation of genetic diversity in order to preserve this species. It is therefore, necessary to detect and document the amount of variation existing within and between populations of *J. curcas* in Kenya so as to guide the improvement and conservation efforts.

Morphological markers are routinely used for estimating genetic diversity but are not very successful due to strong influence of the environment (Tanksley, 1983). Presently, rapid use of molecular markers has complemented the classical technology and enabled the characterization of several plant genotypes. These markers are independent of the influence of environment, growth conditions, age and the type of tissue being analyzed (Vainstein and Ben Meir, 1994). In *Jatropha*, isozymes, randomized amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP) and inter simple sequence repeat (ISSR) markers have been used to determine genetic relatedness, interspecific hybridization and genetic diversity in several countries (Sujatha and Prabakaran, 2003; Basha and Sujatha, 2007; Subramanyam et al., 2009). When compared with other molecular markers, RAPD has the advantage of being simple, cheap and faster in obtaining result despite its weakness of low reproducibility (Nejia et al., 2007). The technique has earlier been successfully used in assessing genetic diversity in wider array of agriculture and forest tree crops and produced results similar to other molecular markers (Belaj et al., 2001; Deshwall et al., 2005). RAPD markers have thus been chosen for the current study. The aim of the present study was to assess the level of genetic diversity within and between eight *J. curcas* populations in Kenya to draw consequences for the management and improvement of the species.

MATERIALS AND METHODS

Population sampling

Three populations were identified at the Coastal region (Likoni, Kilifi and Kwale), three in South Rift (Ngurumani, Kajiado and Namanga) and two in Eastern Kenya (Kibwezi and Kitui) (Figure 1). One hundred and sixty individual trees of *J. curcas* were randomly sampled from the eight populations in Kenya representing the species distribution range and areas of high germplasm sourcing (Table 1). Within each population, young and healthy leaf tissues were collected randomly from 11 to 30 adult trees. A minimum distance of 100 m between individual trees was used to avoid the risk of selecting closely related individuals. The leaf tissues were dried on silica gel and stored at -20°C until DNA extraction.

DNA extraction and PCR amplification

Total genomic DNA was isolated from the leaves following a modified sodium dodecyl sulphate (SDS) procedure (Machua et al., 2007) and DNA quantification performed through both Bio-photometer readings and comparison with low DNA mass ladder (Invitrogen) in ethidium bromide-stained 1% agarose gels. A total of 40 arbitrary primers were screened for RAPD amplification. Ten of the RAPD primers were found to show clear and analyzable bands. Polymerase chain reaction (PCR) amplification was carried out in a 25 µl reaction volume containing 1XPCR buffer (10 mM Tris-HCL pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$), 200 µM each of dNTPs, 2 µM of primer, 0.5 units of Taq polymerase (Invitrogen) and 25 ng DNA template.

PCR amplification was performed in a TECHNE TC - 412 Thermal Cycler (UK), with an initial denaturation at 94.5°C for 5 min, followed by 40 cycles. Each cycle consisted of denaturation at 94°C for 45 s, primer annealing at 37°C for 1 min and extension at 72°C for 2 min with a final extension at 72°C for 5 min. PCR products were separated on 1.5% agarose gel in 0.5 × TBE (Tris-Borate EDTA) buffer and stained in ethidium bromide (10 mg/ml). The sizes of the amplified fragments were determined using a 100 bp DNA ladder (Invitrogen) run along the sides of the amplified products. The amplified products were visualized under ultraviolet light and photographed with gel documentation system (Gel LOGIC 200 imaging system-Kodak MI SE).

Data analysis

Unequivocally scorable and consistently reproducible amplified DNA fragments (bands) were scored manually and transformed into binary character matrix (1 = presence, 0 = absence). Percentage of polymorphic loci was calculated for each population. T_0 assess molecular variation, Shannon's diversity index was used. Nei's unbiased genetic distance (*D*) was determined using POPGENE 1.32 software (Yeh et al., 1997). The Nei's genetic distance matrix was used to generate the phylogenetic tree using unweighted pair group arithmetic average method (UPGMA) method in MEGA software (Tamura et al., 2007). Analysis of molecular variance (AMOVA) and principal coordinate analysis (PCA) were performed using GenAlEx 6.4 software (Peakall and Smouse, 2006).

RESULTS

Out of the 40 primers screened to assess the genetic

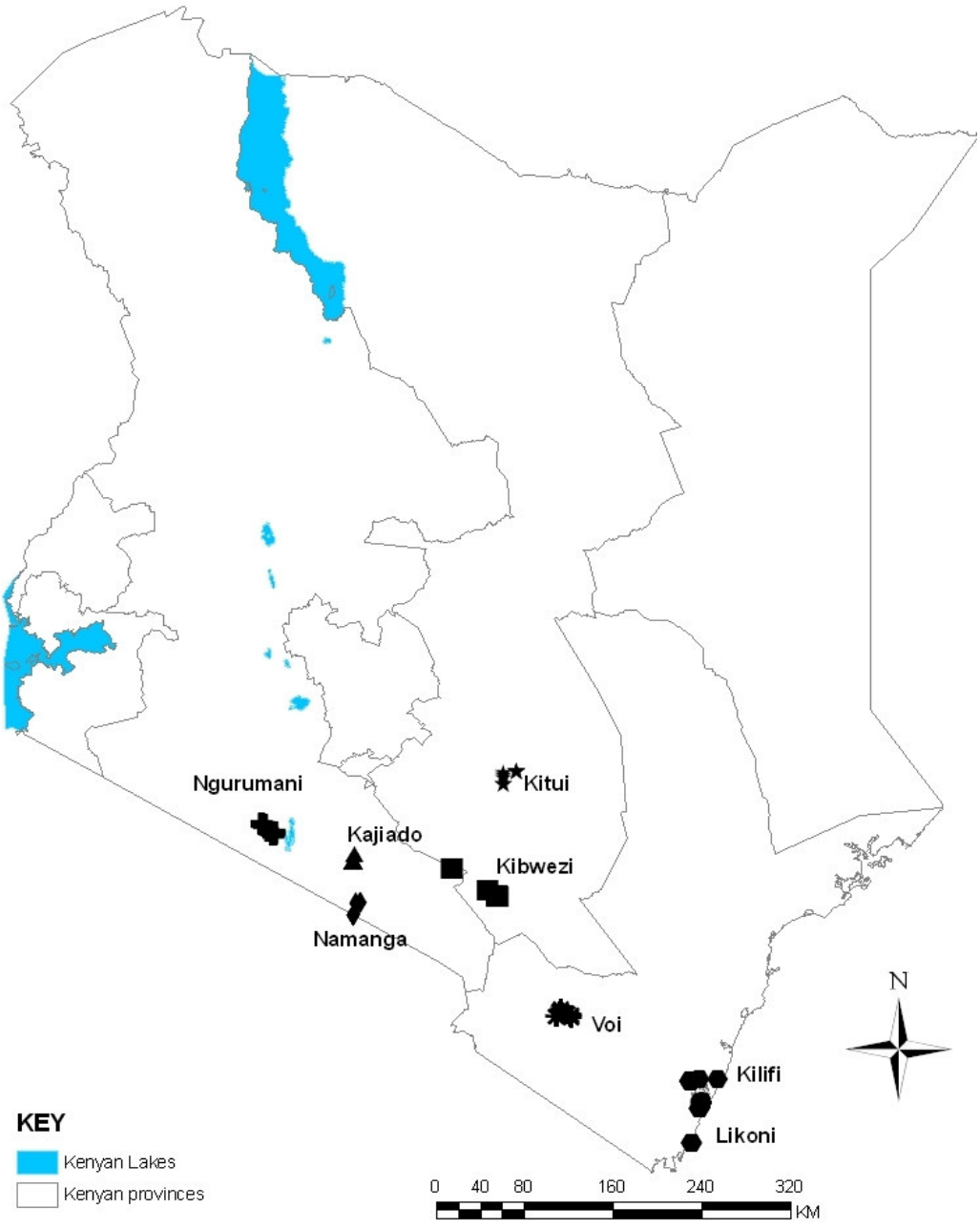

Figure 1. Locations of the *J. curcas* populations studied in Kenya.

diversity among the 8 populations of *J. curcas* in Kenya, only 10 (Table 2) produced unambiguous polymorphic and reproducible fragments while the rest resulted in either no amplification or smeared profiles. The 10 primers yielded 251 polymorphic loci ranging from 150 to 1500 bp in size. The number of amplified fragments per primer ranged from 23 to 28 with an average of 25 bands per primer (Table 2). Moderate to high genetic diversity was observed from each primer with all showing over 60% polymorphism. An example of the molecular profile generated using KFP 10 is as shown in Figure 2.

The level of genetic diversity as shown by Shannon's diversity index (I) was average for most of the populations varying from 0.116 (Likoni) to 0.360 (Namanga). Comparing the variation between the regions, eastern had the highest mean diversity (0.295) followed by southern (0.252) with coastal region having the least index (0.164). Based on the mean percentage polymorphism of the loci within populations, the diversity ranged from 25% (Likoni) to 73.31% (Namanga) with the overall mean of 55.33%. The 10 primers detected 10 unique loci (markers) in 5 populations ranging from 1 (Kajiado and

Table 1. Geographical locations of the *J. curcas* populations in Kenya used in genetic diversity analysis.

Population	Latitude	Longitude	Mean annual rainfall (mm)	Mean annual temperature (°C)
Kitui	1.37087S	36.13110E	500-1050	18-30
Kibwezi	2.39045S	37.96156E	800-1200	20-25
Kajiado	2.09737S	36.79166E	300-1250	15-30
Ngurumani	1.84674S	36.10066E	300-800	23-32
Namanga	2.54621S	36.78860E	300-800	26-33
Kilifi	3.93382S	39.53496E	400-1200	22-34
Voi	3.37835S	38.53742E	200-1200	20-30
Likoni	4.23333S	39.80000E	500-1500	23-32

Table 2. Primers used for study in *J. curcas* populations in Kenya. Tm-melting temperature.

Primer code	Primer sequence	GC content (%)	Tm	No. of amplified bands	No. of polymorphic bands	Polymorphic bands (%)
KFP-1	GGCTCGTACC	70	34	23	18	78.3
KFP-2	CGTCCGTCAG	70	34	24	16	66.7
KFP-3	GTTAGCGGCG	70	34	24	21	87.5
KFP-4	CGGAGAGTAC	60	32	25	18	72.0
KFP-5	CCTGGCGAGC	80	36	28	22	78.6
KFP-6	TCCCGACCTC	70	34	26	24	92.3
KFP-7	CCAGGCGCAA	70	34	26	21	80.8
KFP-8	ACGCGCTGGT	70	34	28	23	82.1
KFP-9	GACTGGAGCT	60	32	23	18	78.3
KFP-10	ACGGTGCGCC	80	36	24	19	79.2

Kibwezi) to 3 (Namanga and Likoni) per population (Table 3).

Analysis of molecular variance (AMOVA) revealed significant variation among the populations (53%; P = 0.01) higher than within population variation (47%; P = 0.01) (Table 4). No variation was partitioned among the regions (P > 0.01), showing no structuring based on geographical regions (Table 4).

The average Nei's standard unbiased genetic distance (*D*) between the populations was 0.236 with the pairwise population matrix (Table 5). The shortest genetic distance was observed between Kibwezi and Kitui (0.142), while the most distant populations (0.342) were Likoni and Ngurumani (Table 5)

The genetic relationships among the 8 populations were summarized using both principal coordinate analysis (PCA) and UPGMA cluster analysis. Results from both analysis showed similar structuring defying the underlying geographic regions (Figure 3 and 4). In both cases 3 groups were identified, whereby Likoni population was isolated from the others. The cluster analysis clumped together Kibwezi, Kitui, Kilifi and Ngurumani in one group and Voi, Kajiado and Namanga in another (Figure 4). In the PCA analysis (Figure 3), the first axis accounted for 31.5% of the total variation and clearly isolated Likoni population from the others.

DISCUSSION

The *Jatropha* plant is a new system and only recently exposed to molecular investigations, mainly due to its increasing popularity as a biodiesel feedstock and valuable co-products (Kohli et al., 2009). This study constitutes the first successful attempt to assess genetic diversity of *J. curcas* using molecular markers in Kenyan provenances when compared with India, where a lot of studies have been done. RAPD technique has been successfully used in variety of taxonomic and genetic diversity studies of *Jatropha* and the present results corroborate this conclusion (Rodriguez et al., 1999). The number of alleles per locus, a potentially more sensitive measure of genetic diversity, cannot be determined from our dominant marker data. Studies show that, dominant markers predictably can underestimate genetic diversity (Wu et al., 1999) and therefore, true diversity of *J. curcas* in the present study might be higher than reported here.

During this study, the polymorphisms detected with the 10 primers were high (Table 4) and considerable levels of genetic diversity could be detected even with some populations showing low variation. These figures (53.33%) were higher than those reported about *J. curcas* in India (40%) using RAPD markers (Basha and Sujatha, 2007). The species has the ability to propagate vegetatively and

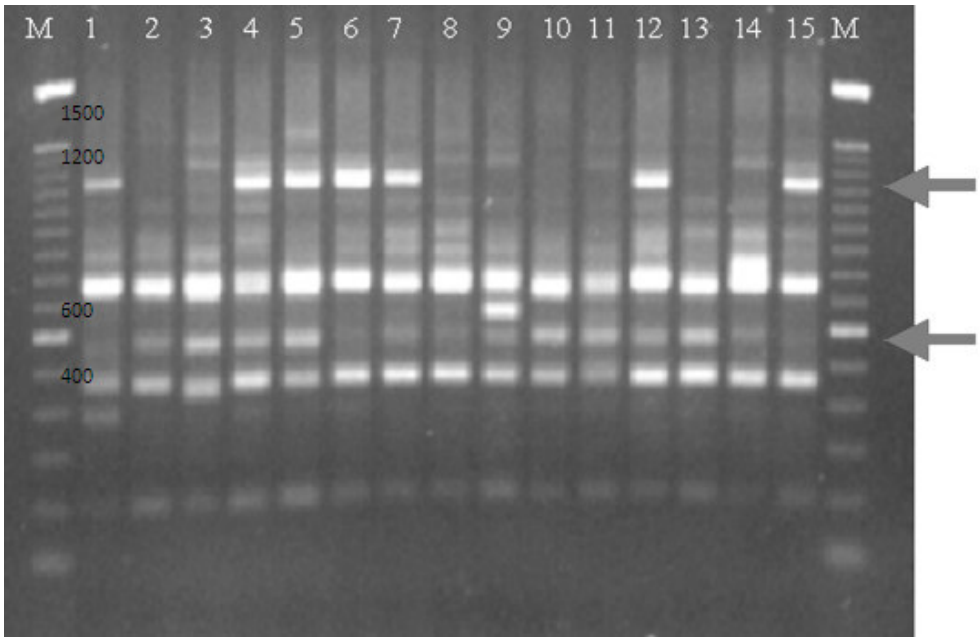

Figure 2. *J. curcas* Kibwezi population RAPD profile generated using primer KFP-10 (The arrows show polymorphic bands scored. M; 100bp ladder by Invitrogen, USA. 1 to 15; *J. curcas* samples).

Table 3. Genetic diversity indices of the eight populations of *J. curcas* populations in Kenya.

Population	N	P	P (%)	PSL	I	H
Voi	20	147	58.17	0	0.266	0.174
Kajiado	11	139	55.38	1	0.170	0.097
Kibwezi	19	182	72.51	1	0.259	0.160
Ngurumani	20	150	53.78	2	0.225	0.144
Namanga	20	190	73.31	3	0.360	0.238
Kilifi	20	107	35.86	0	0.152	0.098
Kitui	20	186	68.53	0	0.331	0.218
Likoni	30	70	25.10	3	0.116	0.076
Mean	20	146	55.33	1.3	0.235	0.151

N, Number of samples; P, number of loci; P (%), percentage polymorphic loci; PSL, population specific loci; H, Nei's gene diversity (Nei, 1973); I, Shannon's information index (Lewontin, 1972).

Table 4. Analysis of molecular variance for 160 individuals of *J. curcas* from eight populations in Kenya.

Source of variation	df	SS	MS	Variance	V (%)	P-value
Among regions	2	938.599	469.300	0.000	0	0.940
Among pops	5	2358.335	471.667	22.982	53	0.010
Within pops	152	3126.028	20.566	20.566	47	0.010
Total	159	6422.963		43.548	100	

DF, degrees of freedom; SS, sum of squares; MS, mean sum of squares; % V, percentage variance.

local farmers normally collect planting materials from neighbors or friends as hedge materials, this leads to the distribution of a narrow gene pool, hence, low genetic diversity within populations. However, within population

Table 5. Pairwise population matrix of Nei's unbiased genetic distance (Nei, 1978) of the 8 populations of *J. curcas* in Kenya.

	Kitui	Kibwezi	Kajiado	Ngurumani	Namanga	Kilifi	Voi	Likoni
Kitui	0.000							
Kibwezi	0.143	0.000						
Kajiado	0.230	0.172	0.000					
Ngurumani	0.165	0.172	0.269	0.000				
Namanga	0.264	0.226	0.204	0.257	0.000			
Kilifi	0.192	0.142	0.179	0.206	0.228	0.000		
Voi	0.276	0.262	0.231	0.339	0.206	0.247	0.000	
Likoni	0.269	0.158	0.287	0.342	0.332	0.270	0.337	0.000

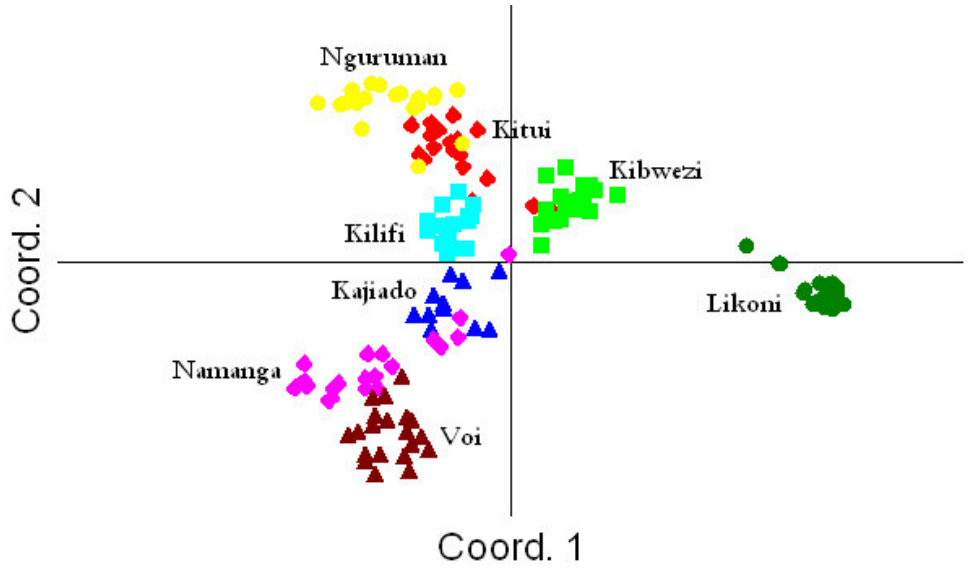

Figure 3. Principal coordinate analysis (PCA) of the 160 individuals of *J. curcas* from 8 populations.

variation (47%) was almost similar to that detected among the populations (53%). This was comparable to results obtained in India (Basha and Sujatha, 2007). The low genetic diversity observed in some populations (Likoni, Kilifi and Kajiado) could be due to very few individual per introduction that have been distributed and clonally propagated. In other cases, higher population differentiation realized might have been brought about by numerous introductions from different sources. It is believed that *J. curcas* germplasm in Kenya came from different countries in Africa and Asia. Some propagules entered the country through Sudan, Ethiopia, Egypt and India (Personal communication). These materials, which might have had different genetic background, were directed to different locations in the country. This led to availability materials with diverse genetic constitution, hence, the population differentiation. In addition, there has not been any improvement programme towards the species and most of the plantings have relied on naturally occurring unadapted populations.

The genetic distances estimated on the basis of 10 primers exhibited a wider range (Table 5) suggesting that *J. curcas* germplasm collection represents genetically diverse populations. This may be attributed to the high level of cross-pollination nature of this species and interaction of materials from different genetic sources (Ikbal et al., 2010). The high diversity revealed by RAPD markers in this study is in agreement with the general belief that out-breeding plant species always exhibit considerable diversity, same as in the species of the present study. Similar result was found by Subramanyam et al. (2009). It is also generally believed that, availability and maintenance of higher genetic diversity within populations are favored by the genetic systems of the species such as gene flow, mating systems, mutations, etc. Therefore, the out crossing nature of *J. curcas* might have promoted higher diversity observed (Ikbal et al., 2010).

Cluster analysis based on the Nei's unbiased genetic

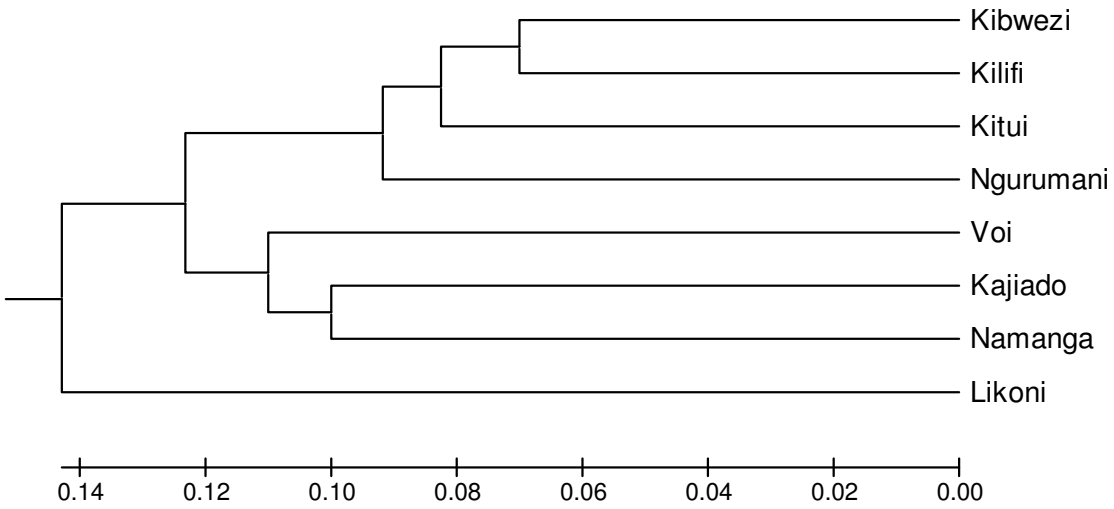

Figure 4. UPGMA Cluster analysis of the 8 populations of *J. curcas* using Nei's (1978) unbiased genetic distance.

distance (D) classified *J. curcas* in Kenya into 3 main groups (Figure 4). There was no clear pattern of grouping based on the geographic location. This may be due to sources of planting materials and their distribution within the country. Since germplasm are collected and distributed by farmers from different sources, there tend to be a mixture of planting materials from different regions hence, leading to no structuring of the regions. This kind of mixed distribution of propagules from different gene pools may also be the source of high levels of genetic variations realized. However, ecological and geographical differentiations are important factors, which influence breeding and sampling strategies (Namkoong, 1986). As has been reported in many studies, variation in genetic diversity and structure within the species is related to its geographic range, mode of reproduction, mating systems, seed dispersal and fecundity (Loveless, 1992). The results reported here might be due to all the stated factors. Similar conclusions were made in India about *J. curcas* using RAPD markers where modest to high genetic diversity was reported. The results of the present study showed that, *J. curcas* germplasm from Kenya constitute a broad genetic base rich for a breeding and improvement program. From the clustering patterns and the genetic relationship obtained, selection for breeding programmes can be done from the different clusters realized to capture in entirety the available gene pool. Due to the high genetic variation observed, random seed collection for plantation establishment are likely to result in varied seed yield hence, affects uniform production anticipated in a plantation.

ACKNOWLEDGEMENTS

The Desert Margin Initiative (DMP) project and the Government of Kenya through Farm Forestry Program of KEFRI supported this study.

REFERENCES

Aker CL (1997). Growth and reproduction of *Jatropha curcas*. Biofuels and Industrial Products from *Jatropha curcas*. In: Gubitz GM, Mittelbach M, Trabi M (eds). Proceedings of 'Jatropha 97'. Dbv-Verlag für die Technische Universität Graz, pp. 2-18.

Ambrosi DG, Purelli M, Galla G, Fabbri A, Barcaccia G (2009). Genetic diversity and reproductive biology of *Jatropha curcas* L. In: Proceedings of the 53rd Italian Society of Agricultural Genetics Annual Congress Torino, Italy-16/19 September, 2009.

Basha SD, Sujatha EM (2007). Inter and intra-population variability of *Jatropha curcas* (L). Characterized by RAPDs and ISSR markers and development of population specific SCAR markers. Euphytica, 156: 375-386.

Beentje HJ (1994). Kenya trees, shrubs and lianas. Kenya, NMK, Nairobi.

Belaj A, Trujilo I, Rosa R, Rallo L, Gimenez MJ (2001). Polymorphism and discrimination capacity of randomly amplified polymorphic markers in an olive germplasm bank. J. Am. Soc. Hort. Sci. 126: 64-71.

Deshwall RPS, Singh R, Malik K, Randhawa GJ (2005). Assessment of genetic diversity and genetic relationships among 29 populations of Azadirachta indica A. Juss. Using RAPD markers. Genet. Resour. Crop Evol. 52: 285-292.

Diwaker VJM (1993). 16th November. Energy from the humble castor. The Indian express.

Gohil RH, Pandya JB (2008). Genetic diversity assessment in physic nut (*Jatropha curcas* L.). Int. J. Plant Prod. 2(4): 321-326.

Heller J (1996). Physic nut-*Jatropha curcas* L. In: Promoting the conservation and use of underutilized and neglected crops. International Plant Genetic Resources Institute, Rome, Italy. http://www.ipgri.cgiar.org/publications/pdf/161.pdf

Ikbal K, Boora S, Dhillon RS (2010). Evaluation of genetic diversity in Jatropha curcas L. using RAPD markers. Indian J. Biotechnol. 9: 50-57.

Kohli A, Raorane M, Popluechai S, Kannan U Syers KJ, O'Donnell AG (2009). *Jatropha curcas* as a novel, non-edible oilseed plant for biodiesel. In: Ferry N, Gatehouse AMR eds. Environmental impact of genetically modified novel crops. Chapter 14. CAB International, London, UK. pp. 294-322.

Kumar A, Sharma S (2008). An evaluation of multipurpose oil seed crop for industrial uses (*Jatropha curcas* L.): A review. Ind. Crop Prod. 28: 1-10.

Lewontin RC (1972). The apportionment of human diversity. Evol. Biol. 6: 381-398.
Little EL, Woodbury RO, Wadsworth FH (1974). Trees of Puerto Rico and the Virgin Islands, vol. 2. Ag. Handbook 449. USDA, Washington, DC.
Loveless MD (1992). Isozyme variation in tropical trees. New For. 6: 67-94.
Machua J, Muturi G, Gicheru J (2007). Genetic diversity within *Brachylaena hullensis* (O. Hoffim) populations in Kenya: Implication for conservation of a wood carving tree. Disc Innovat. 19(1): 32-36.
Mohamad AJ, Janagoudar BS, Biradar DP, Ravikumar RL, Koti RV, Patil SJ (2009). Genetic diversity analysis of elite *Jatropha curcas* (l.) genotypes using randomly amplified polymorphic DNA markers. Karnataka J. Agric. Sci. 22(2): 293-295.
Morton JF (1977). Major medicinal plants. C.C. Thomas Press. Springfield.
Muok B, Källbäck L (2008). Feasibility study of *Jatropha curcas* as a biofuel feedstock in Kenya**.** www.pisces.or.ke/JatrophaFeasibilityStudyFinal.pdf
Namkoong G (1986). Genetics and the forests of the future. Unasylva, 152: 2-18.
Nei M (1973). Analysis of gene diversity in subdivided populations. Genetics, 70: 3321-3323.
Nei M (1978). Estimation of average heterozygozity and genetic distance from a small number of individuals. Genetics, 89: 583-590.
Nejia Z, Ichraf C, Badra B, Mahamoud G, Hassene Z, Adbelwahed G, Ahmed M (2007). Molecular based assessment of genetic diversity within Barbary fig (*Opuntia indica*) in Tunisia. Sci. Hortic. 113: 134-141.
Openshaw K (2000). A review of of *Jatropha curcas*: an oil plant with unfulfilled promise. Biomass Bioenergy, 19: 1-5
Patil V, Singh K (1991). Oil gloom to oil boom-*Jatropha curcas* a promising agro-forestry crop. Shree Offset Press, Nashik.
Peakall R, Smouse PE (2006). GENALEX 6: Genetic analysis in Excel. Population genetic software for teaching and research. Mol. Ecol. Nat. 6: 288-295.
Rodriguez JM, Berke T, Engle L, Nienhuis J (1999). Variation among and within *Capsicum* species revealed by RAPD markers. Theor. Appl. Genet. 99: 147-156.
Shen J, Jia X, Ni H, Sun P, Niu S, Chen X (2010). AFLP analysis of genetic diversity of Jatropha curcas grown in Hainan, China. Trees, 24: 455-462.
Subramanian KA, Singal SK, Saxena M, Singhal S (2005). Utilization of liquid biofuels in automotive diesel engines: an Indian perspective. Biomass Bioenergy, 29: 65-72.
Subramanyam K, Muralidhararao D, Devanna N (2009). Genetic diversity assessment of wild and cultivated varieties of *Jatropha curcas* (L.) in India by RAPD analysis. Afr. J. Biotechnol. 8(9): 1900-1910.
Sujatha M, Prabakaran AJ (2003). New ornamental *Jatropha* hybrids through interspecific hybridization. Genet. Resour. Crop Evol. 50: 75-82.
Tamura K, Dudley J, Nei M, Kumar S (2007). MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol. Biol. Evol. 24: 1596-1599.
Tanksley SD (1983). Molecular markers in plants breeding. Plant Mol. Biol. Rep. 1: 3-8.
Tiwari DN (1994). Brouchur on Jatropha. Dehra Dun: ICFRI.
Umamaheswari D, Paramathma M, Manivannan N (2010). Molecular genetic diversity analysis in seed sources of Jatropha (*Jatropha curcas* L) using ISSR markers. Elect. J. Plant Breed. 1(3): 268-278.
Vainstein A, Ben Meir H (1994). DNA fingerprint analysis of rose. Am. Soc. Hortic. Sci. 119: 1099-1103.
Wu J, Krutovskii KV, Strauss SH (1999). Nuclear DNA diversity population differentiation and phylogenetic relationships in California closed-cone pines based on RAPD and Allozyme markers. Genome, 42: 893-908.
Yeh FC, Yang R, Boyle T (1997). Popgene Version 1.32. Ag/For Molecular Biology and Biotechnology Centre, University of Alberta and Center for International Forestry Research.