Send Orders for Reprints to reprints@benthamscience.ae

The Open Renewable Energy Journal, 2015, *8*, 1-6 1

Open Access

Utility of Markers for Determination of Genetic Diversity in *Jatropha*: A Review

R. M. Jingura and R. Kamusoko*

Chinhoyi University of Technology, Private Bag 7724, Chinhoyi, Zimbabwe

Abstract: *Jatropha* is largely a semi-wild plant under domestication. There is wide variation in morphological and agronomic traits of *Jatropha*. There are several endeavours to improve the genetic quality of *Jatropha*. Various seed yield ranges have been reported for *Jatropha*, for example, 0.4 – 12 t ha^{-1}. Proper identification and characterisation of the plant's germplasm is central in genetic improvement of the plant. This paper evaluates the utility of markers for determination of genetic diversity in *Jatropha*. Several marker techniques are available for genetic characterisation of *Jatropha*. These include morphological and DNA-based markers. DNA-based markers such as RAPD, AFLP, RFLP, SSR and ISSR have been applied in evaluation of genetic diversity in *Jatropha*. Each of these techniques has its own advantages and limitations that determine its applicability in plant genetic diversity studies. This paper recommends application of a combination of markers as a reliable approach for determination of intra-specific genetic diversity in *Jatropha*.

Keywords: Accessions, DNA-based markers, germplasm, isozymes, morphological markers.

INTRODUCTION

Jatropha has emerged in recent years as an energy plant suitable for production of biofuels. The popularity of *Jatropha* is derived from the numerous potential benefits that can be derived from its cultivation. These range from the multiple uses of its oil, ability to reclaim degraded lands [1] to promoting rural entrepreneurship. *Jatropha* is an oil tree that grows well under a wide range of climates and physiographic conditions [2, 3]. It is largely a semi-wild plant with much work pending or in vogue in attempts to make it a successful energy crop [4]. Traits of economic importance in *Jatropha* are seed yield, oil content and oil quality. These are the main targets for genetic improvement of the plant. A few provenance trials that have been conducted in Africa have found genotype by environment interaction in these traits [5]. Wide variation has been reported in morphological and agronomic traits of *Jatropha*. The variation can be between and within varieties under the same growth conditions [4]. For example, various seed yield ranges have been reported for *Jatropha*. The range is as wide as 0.4 – 12 t ha^{-1} [6]. Empirical data also show too much variability in seed yield among individual trees. Annual seed yield variation among 19 trees of 0 – 850 g dry seed per tree was reported [7].

What is worth noting is that seed yields reported in literature are accompanied by little or no information on genetic provenances. This information would be useful for sagacious interpretation of the variation. There is consensus in literature that the greatest prospect for improving the productivity of *Jatropha* lies in genetic improvement [7]. The starting point is genetic characterisation of different provenances as a process towards selection and breeding of superior genotypes. Genetic characterisation enables the identification of key features of the available genetic resources. Breeding programmes can be developed using this knowledge. In terms of methodology for genetic characterisation, various markers have been used to detect genetic diversity in different plant species. These markers include morphological, protein-based, and DNA-based markers. Morphological and protein-based markers have a long history in genetic characterisation of plants. Since the 1990s, DNA-based markers have become common tools in genetic evaluation due to the advent of polymerase chain reaction (PCR).

Genetic marker techniques include random amplified polymorphic DNA (RAPD) [8], amplified fragment length polymorphism (AFLP) [9], restriction fragment length polymorphism (RFLP) [10], simple sequence repeat (SSRs) or microsatellites [11] and inter simple sequence repeat (ISSR) [12]. This paper provides a review of use of these genetic markers in evaluation of biodiversity of *Jatropha* and their possible applications in genetic improvement of *Jatropha*.

DESCRIPTION AND COMPARISON OF DIFFERENT GENETIC MARKER TECHNIQUES

There are three major types of markers that can be used to determine genetic diversity in plant species. These are morphological, protein-based and DNA-based markers. The typology of DNA-based markers is shown in Table **1**.

*Address correspondence to this author at the Chinhoyi University of Technology, Private Bag 7724, Chinhoyi, Zimbabwe; Tel: +263 67 29464;
E-mail: rkamusoko@cut.ac.zw

1876-3871/15 2015 Bentham Open

Table 1. Markers for determining genetic diversity.

Type of Marker	Year Introduced	Reference
DNA-based		
Restriction fragment length polymorphism (RFLP)	1980	[10]
Random amplified polymorphic DNA (RAPD)	1990	[8]
Amplified fragment length polymorphism (AFLP)	1993	[9]
Simple sequence repeats (SSR) or microsatellites	1989	[11]
Inter simple sequence repeat (ISSR)	1994	[12]

Table 2. Recent experiences in genetic characterisation of *Jatropha*.

Type of Marker	Work Done on Jatropha	Genetic Variation	% Polymorphism	Reference
Morphological	-	-	-	-
Isozymes	Isozyme polymorphism to detect genetic diversity between 55 Indian accessions	Low	26.67	[13]
RAPD	Genetic characterisation of *J. curcas* clones from China	Low	34	[14]
	Evaluation of genetic diversity in eco-geographical populations of India	High	93.90	[15]
	Analysis of molecular diversity in 160 accessions from 8 populations of Kenya	Moderate to high	>60	[3]
ISSR	Genetic characterisation of *J. curcas* clones in China	Low	34	[14]
	Genetic relationship among different accessions of *J. curcas*	High	61.53	[16]
	Assessment of the extent of genetic diversity between 16 *J. curcas* accessions in Malaysia	Low	40	[17]
SSR	Genetic diversity of 192 *J. curcas* germplasm collected in Brazil	Very low	0.26	[18]
	Genetic relationship among different accessions of *J. curcas*		33.33	[16]
AFLP	Genetic diversity and relationship of *J. curcas* in China and Asian countries	Narrow	14.78	[19]
	Genetic diversity in 5 *J. curcas* populations from Chiapas in Mexico	High	81.18	[20]

EXPERIENCES WITH DIFFERENT MARKERS

Several studies have been carried out with different markers in genetic characterisation of *Jatropha*. Some of the work has produced useful information. A summary of experiences in genetic characterisation of *Jatropha* is shown in Table **2**.

Morphological Markers

Morphological markers are routinely used for estimating genetic diversity of plants since they are cheap and fast. Morphological differences arise due to selection and/or genetic drift, and phenotypic variation [21]. The commonly used traits for morphological characterisation include phenotypic variability of plant organs such as flowers, leaves and stems. An example of morphological characterisation of *Jatropha* is work done in India on 24 accessions collected from different zones [22]. This work reported moderate genetic diversity and that variation was higher for phenotype than genotype between the accessions. Significant differences ($P<0.05$) in seed size, 100-seed weight and oil content between the accessions were reported [22].

Another study in India, [23] found moderate variation in plant height, stem girth, branches per plant and seed weight

among 34 accessions. Earlier work in Thailand reported no intra-specific morphological variations among 40 *Jatropha* lines from different locations [24].

Morphological markers are influenced by environmental conditions. Thus, observations may not represent true genetic differences or similarities [15]. As stated earlier, a few provenance trials reported in Africa found genotype by environment interaction for *Jatropha* [5]. The imperative then is to go beyond morphological characterisation in order to establish true genetic variation in different provenances of *Jatropha*.

Protein-based Markers

Protein profiles (isozymes) were the first molecular markers to be used in genetic characterisation and are still used today. The relatedness of the genus *Jatropha* and *Ricinus* were determined using isozymes [25]. Work done in India by [13] showed that only three out of fifteen enzyme systems (formate dehydrogenase, peroxidase, malate dehydrogenase) were useful in polymorphic studies of 55 *Jatropha* accessions. Very low heterozygosity was revealed by dendrogram, narrowing the scope for exploitation of hybrid vigour. As a result, [13] concluded that initiating breeding programmes for *Jatropha* in India may not lead to change in both quality and quantity of economic traits.

Electrophoretic mobilities of bands (R_f) shown by peroxidase enzyme system from leaf samples of six *Jatropha* species *viz*., *J. curcas*, *J. gossypifolia* Jacq, *J. integerrima* var. Rosea, *J. multifida* L., J. *podagrica* Hook, *J.tanjorensis* and an F_1 hybrid between female *J. curcas* and male *J. Integerrima* were studied [26]. Two bands with mobilities of 0.376 and 0.476 were revealed by peroxidase isozymes of *J. tanjorensis*. Band mobilities of 0.376 and 0.476 were found in *J. gossypifolia* and *J. curcas,* respectively. An artificial hybrid between *J. curcas* and *J. integerrima* produced three bands of mobilities 0.376, 0476 and 0.471 in all the progenitors.

Comparison of isozymes (peroxidase, esterase and glutamate oxaloacetate transaminase) of 15 accessions of *Jatropha* from four States of Brazil showed differences in electrophoretic profiles of accessions for the different enzymatic systems [27]. However, extensive divergence was not found except in one genotype which presented only 55% of similarity with the rest of the biological materials tested [27]. Isozyme analysis is simple, fast and cheaper than DNA-based methods. However, the limited number of loci available for study limits their usefulness.

DNA-based Markers

DNA-based molecular markers are increasingly becoming important tools in plant genetic diversity analysis due to their sensitivity and specificity. The first advancement of DNA-based markers came with the introduction of restriction fragment length polymorphism (RFLP). Since then, the advent of polymerase chain reaction (PCR) has provided new marker systems for diagnosis of genetic diversity to improve plant breeding programs. These are simple and quick techniques such as randomly amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), inter simple sequence repeat (ISSR), and simple sequence repeat (SSR) or microsatellites [28].

Use of DNA-based markers has precedence in genetic characterisation of *Jatropha*. In India and China a wide variety of molecular marker systems have been used to assess intra-specific genetic diversity within species of *Jatropha* involving accessions from different agro-climatic zones [28]. Molecular characterisation to distinguish between toxic and non-toxic species of *Jatropha* has also been attempted using PCR based techniques particularly RAPD and AFLP techniques.

Restriction Fragment Length Polymorphism (RFLP)

Restriction Fragment Length Polymorphism markers were first used by [29] to differentiate three synthetic North American alfalfa (*Medicago sativa*) varieties. Since that time, RFLP markers have been used in other plant species. RFLP markers were used for identification of quantitative trait loci (QTL) in soybean (*Glycine max* L. Merr.) for seed protein and oil content [30]. They were also used for identification of alfalfa ecotypes [31].

Genetic diversity of several other plants such as rubber and cassava has also been established using RFLP markers [32]. The relationship between the Korean and Japanese Tea plants (*Camellia sinencis*) was also determined by RFLP markers [33]. It is plausible to state that RFLP markers can help in assessing molecular diversity of *Jatropha* germplasm and provide information that can be used in breeding programs [34, 35]. However, there is yet no empirical evidence on *Jatropha* diversity-related applications. The RFLP markers need high quality DNA. Therefore they are expensive, slow, and laborious and cannot be mechanised or scaled up.

Randomly Amplified Polymorphic DNA (RAPD)

Most of the work that has been done on determination of genetic diversity of *Jatropha* has used RAPD markers. For example, Chen *et al*. [14] used RAPD primers to determine genetic relationships among *J. curcas* clones in China. Their results showed that five RAPD primers generated reproducible amplification of 43 polymorphic bands out of 126 bands scored, accounting for low polymorphism of 34% across the clones. In Kenya, [3] studied molecular diversity of eight *Jatropha* populations and reported moderate to high genetic diversity with all showing over 60% polymorphism. The work reported by [15] showed high genetic variability and 93.90% polymorphism on evaluation of a population of *J. curcas* L. from eco-geographical populations of India.

There are other examples of the application of the RAPD technique. Seventy-nine percent polymorphism was reported in seven genotypes of *Jatropha* in India [36]. Twenty RAPD primers were used to analyse phylogenetic relationships of 13 *Jatropha* genotypes in India and reported a polymorphism range of 40 to 100% [37]. Genetic similarities studied between 24 accessions of *Jatropha* in Brazil using the RAPD technique indicated existence of high genetic divergence

[38]. However, RAPD technique has the problem of low reliability and reproducibility [4].

Amplified Fragment Length Polymorphism (AFLP)

The AFLP technique is a combination of RFLP and PCR technologies based on selective PCR amplification of restriction fragments from a total digest of genomic DNA [9]. In Southeast Asian countries and China, AFLP markers were successfully used to survey genetic diversity of *Jatropha*. Examples include work done by [19] where 14.78% polymorphism was reported [19], and [39] generated 246 fragments, of which 72 (29.3%) were polymorphic among 38 populations of *J. curcas* in China. The results showed low genetic diversity and lack of variation among these populations.

In India, [40] studied genetic diversity of 48 accessions of *Jatropha* using AFLP markers and found 88% polymorphism which provided high discriminative power for classification of germplasm accessions into different clusters. High genetic variability of 81.18% polymorphism was also observed by [20] who studied genetic diversity of five *J. curcas* populations in Mexico using AFLP markers. It is worth noting now that work on use of AFLPs to analyse genetic diversity in populations of *Jatropha* is increasing.

The AFLP is a highly efficient molecular marker that is stable and repeatable and allows the simultaneous analysis of large numbers of marker loci throughout the genome [19]. However, AFLP requires more DNA (300–1000 mg per reaction) and is more technically demanding and time consuming in the laboratory than RAPD [41].

Simple Sequence Repeats (SSR)

Simple sequence repeats (SSRs) or microsatellites often present high levels of inter- and intra-specific polymorphism, particularly when the tandem repeats number is at least ten [42]. Microsatellite markers are commonly used for quantitative trait mapping [16].

Microsatellites have been used to determine genetic diversity of *Jatropha.* For example, [43] studied genetic relationships of 58 accessions of *Jatropha* in China using simple sequence repeat (SSR). Only one out of 17 microsatellite markers was polymorphic with two alleles [43]. In another study, use of 18 highly polymorphic chloroplast microsatellite markers (ccSSRs) yielded only six primers (33.33%) that resulted in good amplification and polymorphism [16]. In these two cases genetic variation was very low among accessions. New microsatellite markers were also applied by [44] to classify non-toxic and toxic *J. curcas* accessions from different countries. Eight out of 25 markers that were able to amplify bands were polymorphic indicating usefulness in assessing diversity of *Jatropha* and identifying toxic and non-toxic *Jatropha*.

Compared to multi-locus markers such as RAPD and AFLP, microsatellites have advantages such as locus specificity, high reproducibility, co-dominance nature, and substantial size polymorphism [27]. The technique is simple (consisting of two, three or four nucleotides), quick and can be repeated many times. Therefore, they remain the most stringent markers in detection of variability. The problem of these markers is the development of correctly functioning primers which is often a tedious and costly process [45].

Inter Simple Sequence Repeat (ISSR)

There are few reports in literature of the ISSR technique with *Jatropha*. The ISSRs have high capacity to reveal polymorphism as compared to other arbitrary primers such as RAPD [36]. A novel set of polymorphic ISSR markers to mark the genetic relationship among different accessions of *Jatropha* were identified in India [31]. Molecular polymorphism was 35.5% with 100 ISSR markers indicating modest levels of genetic variation within Indian germplasm [31].

In a study in Brazil, ISSR markers were selected to evaluate their potential in accession of *Jatropha* [46]. Only five primers were found that resulted in acceptable levels of polymorphism and robustness of bands. Recently, [17] analysed the extent of genetic diversity among 16 accessions of *J. curcas* in Malaysia using ISSR markers and reported 40% polymorphism.

The ISSRs are inexpensive to develop, simple, precise, require small amounts of DNA for PCR amplifications. Therefore, they are the least technically demanding and offer a fast method for providing information from a large number of loci especially in species where studies have not previously been undertaken. The problem of lack of reproducibility limits their application in studies on genetic diversity of *Jatropha* [16].

UTILITY OF MARKERS FOR APPLICATION PURPOSES

The information provided in the preceding sections provides brief descriptions of the various markers that can be used for genetic characterisation of *Jatropha*. These techniques can be considered to be applicable where the appropriate technical environment is in vogue. However, use of such novel techniques is not axiomatic in all environments. It is worthwhile to provide an analysis of the applicability of these techniques. This will provide information useful in planning technical approaches to genetic characterisation of *Jatropha*. The inadequacies of morphological markers are well documented. They cannot be a reliable measure of genetic differences. On the other hand, isozymes though a good option, have limitations in the number of loci available.

DNA-based markers are at the forefront of technological advancement. What is important is to develop appropriate criteria that can be used to compare applicability and efficacy of the different DNA-based marker techniques. In so doing, the critical variables that will influence choice of technique need to be identified. The choice of markers depends on low assay cost, affordable hardware, throughput, convenience and ease of assay development and automation [47, 48]. Major limitations for technological adoption in developing countries include cost and sophistication of requisite infrastructure. The criteria used by [49] to compare different marker techniques are adopted in this paper. The nine

Table 3. Comparison of different genetic marker techniques[a].

Feature	RFLP	RAPD[b]	AFLP	SSR
Development cost	Medium	Low	Low	High
Running cost	High	Low	Medium	Low
Samples/day	Low	High	High	High
Level of skill required	Low	Low	Medium	Low-medium
Automation	Difficult	Yes	Yes	Yes
Radioactivity necessary	Yes-no	No	Yes-no	Yes-no
Reliability	High	Low-medium	High	High
Dominant or codominant	Codominant	Dominant	Dominant (codominant)	Codominant
Polymorphism	medium	medium	high	high

[a][49]
[b]ISSR as a variant

factors used by [49] in comparison of different marker techniques are shown in Table **3**. Table **3** provides useful information that can be used to determine the most appropriate techniques to use in genetic characterisation of *Jatropha*.

RECOMMENDATIONS

It is not the objective of this paper to prescribe a 'best-fit' option but provide an array of useful information to guide work on genetic characterisation of *Jatropha*. The RAPD is the simplest and fastest method among the DNA-based techniques and can be applied in early phases of genetic characterisation programs. The AFLP, RFLP, SSR and ISSR methods all have varying advantages and disadvantages. A multi-method approach which seeks to optimise the merits of each of these methods is recommended.

CONCLUSION

Markers are useful tools for measuring genetic diversity of *Jatropha*. Morphological markers and isozymes have limited use in determination of genetic diversity and cannot adequately measure true genetic differences on their own. DNA-based molecular markers provide a more efficient and powerful tool to study inter- and intra-specific genetic differences in *Jatropha*. Use of such techniques for germplasm characterisation will facilitate the conservation and utilisation of *Jatropha* genetic resources, permitting the identification of unique genotypes or sources of genetically diverse genotypes. Application of more than one method (a combination of markers) in a reliable manner on widely collected germplasm of *Jatropha* is highly recommended for determination of intra-specific genetic diversity of *Jatropha*.

CONFLICT OF INTEREST

The authors declare that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

Declared none.

REFERENCES

[1] Francis, G.; Edinger, R.; Becker, K. A concept for simultaneous wasteland reclamation, fuel production and socio-economic development in degraded areas in India: Need, potential and perspectives of *Jatropha* plantations. *Nat. Resour. Forum*., **2005**, *29*, 12-24.

[2] Jingura, R.M.; Matengaifa, R.; Musademba, D.; Musiyiwa, K. Characterisation of land types and agro-ecological conditions for production of *Jatropha* as a feedstock for biofuels in Zimbabwe. *Biomass. Bioenergy*, **2011**, *35*(5), 2080-2086.

[3] Machua, J.; Muturi, G.; Omondi, S.F.; Gicheru, J. Genetic diversity of *Jatropha curcas* L. populations in Kenya using RAPDS molecular markers: Implication to plantation establishment. *Afr. J. Biotechnol*., **2011**, *10*(16), 3062-3069.

[4] Yan, H. *Genetic Markers for Jatropha Biodiversity Evaluation and Breeding*. International Consultation on Pro-poor *Jatropha* development, Rome, Italy, **2008**

[5] Heller, J. Physic nut. *Jatropha curcas L.* Promoting the conservation and use of underutilised and neglected crops. 1. Institute of Plant Genetics and Crop and Plant Research, Gatersleben/ International Plant Genetics Resource Institute, Rome, Italy, **1996**.

[6] Openshaw, K. A review of *Jatropha curcas*: An oil plant of unfulfilled promise. *Biomass Bioenergy*, **2000**, *19*(1), 1-5.

[7] Henning, R.K. Identification, selection and multiplication of high yielding *Jatropha curcas* L. plants and economic key points for viable *Jatropha* oil production costs, Paper presented to: International Consultation on Pro-poor *Jatropha* Development 10-11 April 2008, Rome, IFAD, **2008**.

[8] Williams, J.G.K.; Kubelik, A.R.; Livak, K.J.; Rafalski, J.A.; Tingey, S.V. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucl. Acids. Res*., **1990**, *18*, 6531- 6535.

[9] Vos, P.; Hogers, R.; Bleeker, M.; Reijans, M.; van de Lee, T.; Hornes, M.; Frijters, A.; Pot, J.; Peleman, J.; Kuiper, M.; Zabeau, M. AFLP: A new technique for DNA fingerprinting. *Nucl. Acids. Res.,* **1995**, *23*(21), 4407-4414.

[10] Botstein, D.; White, R.L., Skolnick, M.; Davis, R.W. Construction of a genetic linkage map in man using restriction fragment length polymorphism. *Am. J. Hum. Genet*., **1980**, *32*(3), 314-331.

[11] Tautz, D. Hypervariability of simple sequences as a general source of polymorphic DNA markers. *Nucleic Acids. Res*., **1989,** *17*(16), 6463-6471.

[12] Zietkiewicz E, Rafalski A, Labuda D. Genome fingerprinting by simple sequence repeats (SSR) –anchored PCR amplification. *Genomics*, **1994**, *20*(2), 176-183.

[13] Warrier, R.R.; Priyadharshini, P.; Senthil, V.S.; Devika, N.B.; Savitha, C.; Anandalakshmi, R.; Nicodemus, A.; Gurudev, S.B. Isozyme polymorphism to detect genetic diversity of *Jatropha curcas* (L.) in India. *Ann. Trop. Res*., **2010**, *32*(1), 92-111.

[14] Chen, K.; Ren, P.; Ying, C.; Jiang, Q.; Jia, X. Genetic relationships among of *Jatropha curcas* L. clones from Panzhihua, China as revealed by RAPD and ISSR. *Afr. J. Agric. Res*., **2011**, *6*(11), 2582-2585.

[15] Ikbal, K.; Boora, S.; Dhillon, R.S. Evaluation of *Jatropha curacs* L. using RAPD markers. *Ind. J. Biotechnol*., **2010**, *9*, 50-57.

[16] Mittal, N.; Dubey, A.K. A novel set of highly polymorphic chloroplast microsatellite and ISSR for the biofuel crop *Jatropha curcas. EurAsia. J. BioSci*., **2010**, *4*, 119-131.

[17] Camellia, N.A.; Thohirah Lee, A.; Abdullah, N.A.P. Genetic relationship and diversity of *Jatropha curcas* accessions in Malaysia. *Afr. J. Biotechnol*., **2012**, *11*(13), 3048-3054.

[18] Rosado, T.B.; Laviola, B.G.; Faria, D.A.; Pappas, M.R.; Bhering.; L.L.; Quirino, B.; Grattapaglia, D. Diversity in a Large Germplasm Collection of the Biofuel Crop *Jatropha curcas* L. in Brazil. *Crop. Sci*., **2010**, *50*, 2372-2382.

[19] Zhang, Z.; Guo, X.; Lui, B.; Tang, L.; Chen, F. Genetic diversity and genetic relationship of *Jatropha curcas* between China and Southeast Asian revealed by amplified fragment length polymorphisms. *Afr .J. Biotechnol*., **2011**, *10*(15), 2825-2832.

[20] Ovando-Medina, I., Sánchez-Gutiérrez, A.; Adriano-Anaya, L.; Espinosa-García, F.; Núñez-Farfán, J.; Salvador-Figueroa, M. Genetic Diversity in *Jatropha curcas* Populations in the State of Chiapas, Mexico. *Diversity*, **2011**, *3*, 641-659.

[21] Serebryanaya, A.; Shipunov, A. Morphological variation of plants on the uprising islands of northern Russia. *Ann. Bot. Fennici*., **2009**, *46*, 81-89.

[22] Kaushik, N.; Kumar, K.; Kumar, S.; Kaushik, N.; Roy, S. Genetic variability and divergence studies in seed traits and oil content of Jatropha (*Jatropha curcas* L.) accessions. *Biomass Bioenergy*, **2007**, *31*, 497-502.

[23] Saikia, S.P.; Bhau, B.S.; Rabha, A.; Dutta, S.P.; Choudhari, S.P.; Chetia, M.; Mishra, B.P.; Kanjilal, P.B. Study of Accession source variation in morpho-physiological parameters and growth performance of *Jatropha curcas* Linn. *Curr Sci*., **2009**, *96*, 1631-1636.

[24] Sakaguchi, S.; Somabhi, M. Exploitation of promising crops of Northeast Thailand. Agricultural Development Research Center. **1987**; p. 61.

[25] Sathaiah, V., Reddy, T.P. Seed protein file of castor (*Ricinus communis* L.) and some *Jatropha* species. *Genet. Agric*., **1985**, *39*, 35-43.

[26] Prabakaran, A.J.; Sujatha, M. *Jatropha tanjorensis* Ellis & Saroja, a natural interspecific hybrid occurring in Tamil Nadu, India. *Genet. Res. Crop. Evol*., **1999**, *46*(1), 213-218.

[27] Bomfim-Gois, I.B.; Silva, R.; Boari, A.J.; Santos-Oliveira, A.; Fraga, A.C. Caracterização isoenzimática de accesos de pinhão-manso (Jatropha curcas L.). In: Proceedings of the 2nd Brazilian Congress of Mamona (Ricinus communis) held at Sergipe, Brazil, EMBRAPA, **2006.**

[28] Singh, P.; Singh, S.; Mishra, S.P.; Bhatia, S.K. Molecular Characterisation of Genetic Diversity of *Jatropha curcas* L. *Genes. Genomes. Genomics*, **2010**, *4*(1), 1-8.

[29] Brummer, E.C.; Kochert, G.; Bouto, N.J.H. RFLP variation in diploid and tetraploid alfalfa. *Theor. Appl. Gen*., **1991**, *83*, 89-96.

[30] Diers, B.W.; Keim, P.; Fehr, W.R.; Shoemaker, R.C. RFLP analysis of soybean seed protein and oil content. *Theor. Appl. Genet*., **1992**, *83*, 608-612.

[31] Pupilli, F.; Labombarda, P.; Scotti, C.; Arcion, S. RFLP analysis allows for the identification of alfalfa ecotypes. *Plant. Breed*., **2000**, *119*(3), 271-276.

[32] Basha, S.D.; Sujatha, M. Inter and intra-population variability of *Jatropha curcas* (L.) characterised by RAPD and ISSR markers and development of population-specific SCAR markers. *Euphytica*, **2007**, *156*, 375-386.

[33] Matsumoto, S.; Kiriiiw.; Y.; Yamaguchi, S. The Korean Tea Plant (*Camellia sinensis*): RFLP Analysis of Genetic Diversity and Relationship to Japanese Tea. *Breed. Sci*., **2004**, *54*(3), 231-237.

[34] Mohan, M., Nair, S., Bhagwat, A., Krishna, T.G., Yano, M., Bhatia, C.R.; Sasaki, T. Genome, mapping, molecular markers and marker-assisted selection in crop plants. *Mol. Breed.,* **1997**, *3*, 87-103.

[35] Kumar, S.L. DNA marker in plant improvement: an overview. *Biotechnol. Adv*., **1999**, *17*, 143-182.

[36] Jubera, M.A.; Janagoudar, B.S.; Biradar, D.P.; Ravikumar, R.L.; Koti, R.V.; Patil, S.J. Genetic diversity analysis of elite *Jatropha curcas* (l.) genotypes using randomly amplified polymorphic DNA markers. *Karnataka. J. Agric. Sci*., **2009**, *22* (2), 293-295.

[37] Gupta, S.; Srivastava, M.; Mishra, G.P.; Naik, P.K.; Chauran, R.S.; Tiwari, S.K.; Kumar, M.; Singh, R. Analogy of ISSR and RAPD markers for comparative analysis of Genetic Diversity among different Jatropha genotypes. *Afr. J. Biotechnol*., **2008**, *7*(23), 4230-4243

[38] Oliveira, A.S.; Silva-Maan, R.; Santos, M.F.; Gois, I.B.; Carvalho, S.V.; Borai, A.J.; Fraga, A.C.; Castro-Neto, P. Prospecção e caracterização de acessos de *Jatropha* sp. In: Proceedings of the 2nd Brazilian Congress of Mamona (*Ricinus communis*) held at Sergipe, Brazil, EMBRAPA, **2006**.

[39] Shen, J.; Jia, X.; Ni, H.; Sun, P.; Niu, S.; Chen, X. AFLP analysis of genetic diversity of *Jatropha curcas* grown in Hainan, China. *Trees-Struct. Funct*., **2010**, *24*(3), 455-462.

[40] Tatikonda, L.; Wani, S.P.; Kannan, S.; Beerelli, N.; Sreedevi, T.K.; Hoisington, D.A.; Devi, P.; Varshney, R.K. AFLP-based molecular characterization of an elite germplasm collection of *Jatropha curcas* L.: A biofuel plant. *Plant. Sci*., **2009**, *176*, 505-513.

[41] Karp, A.; Kresovich, S.; Bhat, K.V.; Ayad, W. G.; Hodgkin, T. Molecular Tools in Plant Genetic Resources Conservation: A Guide to the Technologies IPGRI Technical Bulletin No. 2; International Plant Genetic Resources Institute; Rome, Italy, **1997**.

[42] Queller, D.C.; Strassman, J.E.; Hughes, C.R. Microsatellites and Kinship. *Trends. Ecol. Evol*., **1993**, *8*(8), 285-288.

[43] Sun, Q.B.; Li, L.F.; Li ,Y.; Wu, G.J.; Ge, X.J. SSR and AFLP markers reveal low genetic diversity in the biofuel plant *Jatropha curcas* in China. *Crop. Sci*., **2008**, 48, 1865-1871.

[44] Tanya, P.; Dachapak, S.; Tar, M.M.; Srinives, P. New microsatellite markers classifying nontoxic and toxic *Jatropha curcas. J. Genet*., **2011**, *90*(3), 76-78.

[45] Jarne, P.; Lagoda, P.J.L. Microsatellites, from molecules to populations and back. *Trends. Ecol. Evol.*,**1996**, 11, 424-429.

[46] Hartmann-Neto, I.; Alencar, J.; Diniz, F.; Souza, V.; Araujo, E.; Lima, P. Seleção de primers para uso de ISSR na analise de diversidade genética em 28 pinhão-manso (*Jatropha curcas* L.). In: Proceedings of the 2nd Brazilian Congress of Mamona (*Ricinus communis*) held at Sergipe, Brazil, EMBRAPA, **2006**.

[47] Rafalski, J.A.; Tingey, S.V.Genetic diagnostics in plant breeding: RAPDs, microsatellites and machines. *Trends. Genet*., **1993**, *9*(8), 275-280.

[48] Rafalski, J.A. Novel genetic mapping tools in plants: SNPs and LD-based approaches. *Plant Sci*., **2002b**, *162*, 329-333.

[49] Caetano-Anoless, G.; Gresshoff, P.M. *DNA markers: Protocols, Applications and Overviews*, Willey & Sons: New York, **1997**.

Received: March 12, 2014 Revised: October 15, 2014 Accepted: November 20, 2014

© Jingura and Kamusoko; Licensee *Bentham Open*.

This is an open access article licensed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/3.0/) which permits unrestricted, non-commercial use, distribution and reproduction in any medium, provided the work is properly cited.