



Review

Molecular markers for genetic diversity studies in *Jatropha (Jatropha curcas L.)*

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Abstract: The molecular markers namely random amplified polymorphic DNA, amplified fragment length polymorphism, sequence-characterized amplified region, inter simple sequence repeats, simple sequence repeats and single-nucleotide polymorphism, etc. have been successfully used for genetic diversity studies in *J. curcas*. The assessment of genetic variations among *Jatropha* germplasms using molecular markers show the presence of high genetic diversity for the Central and South American regions and insignificant genetic variation from Asia and Africa. The use of molecular markers for the assessment of phylogenetic relationships among different *Jatropha* species has been restricted only to the well adapted and acclimatized species in India. The significant genetic variability in the genus *Jatropha* for vegetative and floral traits for seed oil content, productivity, toxicity (phorbol esters, curcin), fatty acid profiles, etc. has been studied in the past decade using molecular markers. Genetic enhancement of *Jatropha* through conventional breeding and interspecific gene transfer can be attempted by exploiting the diverse genetic resource form *J. curcas* and their wild species. This review focuses on the importance and use of molecular markers towards studying diversity analysis in *J. curcas* growing at different areas.

Keywords: *Jatropha*; germplasm; molecular markers; genetic diversity; breeding

1. Introduction

Jatropha, commonly called as physic nut, belongs to family Euphorbiaceae and comprises of 172 species of which the most primitive species is *Jatropha curcas* L. ($2n = 22$) [1]. *Jatropha curcas* has been recognized as an important crop for biodiesel production. The *Jatropha* species is perennial shrub and serves as an important wasteland crop facilitating soil conservation by protecting soil against erosion via wind and animals interference. It is widely distributed in Central and South America, Africa, India and South East Asia. The *J. curcas* originated in Central and South America, and distributed to other tropical countries [2]. It is mainly evolved for dryland and adapt well in limited water source. Several characteristics of *J. curcas* such as drought resistance, speedy growth, easy propagation and wide adaptation to soil conditions have led to the wide spread of *J. curcas* [3–5]. It has acclimatized in different agro-climatic regions because of its strong adaptation at morphological and physiological level. *J. curcas* is considered as the best source for restoration of ruined lands and rural development. The *J. curcas* seeds have semi-drying oil (32–35%) and yield biodiesel of European (EN 14214) and American (ASTM D6751) standards [3,6] via trans-esterification. *J. curcas* seed oil used in the production of biodiesel is eco-friendly, biodegradable, non-toxic and renewable to petroleum diesel [7,8]. It can be used as a traditional vegetable oil or trans-esterified to produce biodiesel for use in standard diesel engines. These properties of *J. curcas* have fascinated globally to develop a sustainable alternative feedstock for biodiesel production [9,10]. After oil extraction, the remaining seed cake can be used as organic fertilizer. Seed cake can be also used for direct combustion or charcoal conversion [11]. *J. curcas* has been used for the isolation and production of pharmaceutical and pesticides in China [12]. Anti-viral (against herpesvirus I and II) and antibacterial (*Staphylococcus aureus* and *Monilia albicans*) skin disinfectors are commercialized [13]. Additional products from *J. curcas* are A2-Jetfuel kerosene and polyol biodegradable foam for use in packaging and insulation industry, paint from the bark [14].

DNA (or molecular) markers are used predominantly due to their abundance, plentiful in number and are unaltered by environmental factors and/or the plant developmental stage. Molecular markers develop from different classes of DNA mutations such as rearrangements (insertions or deletions), substitution (point mutations), or errors in replication of tandemly repeated DNA. Molecular markers have several applications in plant breeding such as (i) evaluation and selection of breeding materials for genetic diversity, parental selection, cultivar identity and assessment of cultivar purity, (ii) trait pyramiding, and (iii) backcrossing.

Molecular markers have played an important role in the detection of unique alleles between two or more species which can be applied in the species characterization and improvement. Molecular markers have been abundantly used to evaluate biodiversity, phylogenetic relationships, construction of genetic linkage maps and in tagging and mapping of useful traits. Currently, genetic diversity studies in the genus *Jatropha* are focused on *J. curcas* and few wild species that are commonly distributed in India. Different types of single and multi-locus molecular markers such as random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), sequence-characterized amplified region (SCAR), inter simple sequence repeats (ISSRs), simple sequence repeats (SSRs) and single-nucleotide polymorphism (SNPs), etc. were used for assessment of genetic diversity in the available germplasm. This review mainly focus on the different studies carried out on genetic variability present in the *Jatropha* species other than Central and South-American and African regions.

2. Genetic diversity studies in *J. curcas* using Molecular Markers

2.1. Amplified fragment length polymorphism (AFLP)

The AFLP is trustworthy, high throughput and less expensive. Many researchers from India, China, Brazil and Mexico used AFLP markers for genetic diversity analysis in *J. curcas* accessions and reported the presence of low to high genetic variability. Some researchers reported that germplasms from Mexico and Central America have high genetic diversity by using AFLP markers (Table 1). The different accessions from Mexico and Central America regions with high oil content and other characters are shown link with its productivity. These genetically contrary germplasm may provide critical resources for future genetic improvement of *J. curcas*. Zhang et al. [15] studied the genetic diversity within 240 samples by AFLP from three Asian countries, two African countries and different geographical regions in China. The germplasm of *J. curcas* showed a narrow genetic diversity in China and Southeast Asia with molecular polymorphism of 14.8%. Genotypic and phenotypic evaluation of a total of 182 accessions from Asia (91), Africa (35), South America (9) and Central America (47) was carried out to estimate the genetic variation. A very large genetic variation by DNA-marker in the pool of Central American accessions was observed as compared from other regions. According to Luis et al. [16] the Central American accessions can be considered as the most important source for plant breeding as they have highest phenotypic variation. Further, it is also stated that Mexico and Central America have highest genetic diversity in *J. curcas* than in other parts of the world because the Mexico and Central America (Mesoamerican region) may be a centre of origin and diversity of *J. curcas* [17]. The genetic diversity study consisted of 114 accessions from 15 populations of 4 different species: *J. curcas*, *J. costaricensis*, *J. gossypifolia* and *J. stevensii* collected from Costa Rica were analysed by AFLP. The results showed that the species that obtained the highest average of polymorphic loci was *J. curcas*, followed by *J. gossypifolia*, *J. costaricensis*, and *J. stevensii* [18]. A broad genetic base of 48 *J. curcas* germplasm from six different states in India was reported [19]. Genetic and fatty acid variability in four datasets of accessions and pre-breeding lines of *J. curcas* was analysed. The results revealed that both genetic as well as fatty acid variability was significantly higher in the newly created pre-breeding lines and the level of fatty acid variability in these datasets was different and highly correlated to the level of their genetic variability as revealed by AFLP markers [20].

2.2. Randomly amplified polymorphic DNA (RAPD)

The RAPD technique is simple, rapid, low-cost and can be deployed even in the absence of any prior information related the genome or DNA of the plant. The RAPD technique has been used for assessment of genetic diversity for *J. curcas* (Table 2) and has revealed that low to moderate level of genetic diversity of Indian germplasms. Assessment of genetic diversity among 43 germplasm collected from different regions in India showed moderate level of genetic variation, however, wide genetic variation was observed between the Indian and Mexican genotypes [21]. Hybrid conformity using RAPD markers was carried out for a backcross involving *J. curcas* and *J. integerrima*, thereby, indicating the potential of employing the RAPD analysis for pre-breeding and genetic enhancement of *J. curcas* through interspecific hybridization [22]. A narrow genetic diversity in 192 *J. curcas* accessions of Brazil was revealed by using RAPD markers [23].

Table 1. Genetic Diversity of *J. curcas* by using AFLP Marker.

Sl. No.	No. of accessions studied	Country/Region	Trait	No. of primers used	Reference/s
1	58	China and Malaysia	Genetic Diversity	7	[27]
2	7	India	Genetic Diversity	56	[8]
3	38	India, Nigeria and Thailand	Genetic Diversity	32	[34]
4	48	India	Genetic Diversity	7	[19]
5	5	China, Indonesia, Suriname, Tanzania and India	Agronomic traits	23	[35]
6	28	India	Genetic Diversity	30	[36]
7	38	China and Indonesia.	Genetic diversity	9	[37]
8	134	Chiapas, Mexico: Soconusco, Isthmus, Center, Frailesca and Border	Genetic Diversity	209	[17]
9	88	Mexico	Genetic diversity on floral traits.	6	[38]
10	240	Africa, China and Asia	Genetic Diversity	6	[15]
11	182	Asia, Africa , South America and Central America	Oil yield	20	[16]
12	114 (accessions from 15 populations of 4 different species of <i>J. curcas</i> .)	Costa Rica	Agronomic Traits	3	[18]
13	65 (<i>J. curcas</i> accessions (13), BC1 (28), BC1F2 (12) and single seeds (12). The BC1, BC1F2 and single seed dataset were derived from an interspecific cross between <i>J. curcas</i> and <i>J. integerrima</i>)	India	Fatty acid composition	4	[20]
14	6 populations amounting to a total number of 70 genotypes of <i>Jatropha curcas</i> L. originating from Africa (Senegal, Mali, Burkina Faso and Madagascar)	Africa	Genetic Diversity	2	[39]

Table 2. Genetic Diversity of *J. curcas* by using RAPD Marker.

Sl. No.	No. of accessions studied	Country/Region	Trait	No. of primers used	Reference/s
1	43	India and Mexico	Genetic Diversity	400	[21]
2	18	India	Genetic Diversity	11	[40]
3	12	India	Genetic Diversity	26	[41]
4	13	India	Genetic Diversity	20	[42]
5	7	India	Genetic Diversity	5	[43]
6	34 accessions comprising eight agronomically important <i>Jatropha</i> species	India	Agronomic traits	100	[22]
7	26	India	Genetic Diversity	55	[44]
8	7	India	Genetic Diversity	180	[8]
9	38	India, Nigeria and Thailand	Genetic Diversity	10	[45]
10	19	Mexico, South America, Asia and Africa	Genetic Diversity	10	[5]
11	40	India	Genetic Diversity	50	[46]
12	28	India	Genetic Diversity	52	[35]
13	192	Brazil	Genetic Diversity	96	[23]
14	10	India	Genetic Diversity	43	[47]
15	24	China	Genetic Diversity	5	[48]
16	4	India	Genetic Diversity	13	[49]
17	160	Kenya	Genetic Diversity	10	[4]
18	48	Malaysia	Genetic Diversity	8	[50]
19	29	India	Genetic Diversity	47	[51]
20	24	Indonesia	Genetic Diversity	22	[52]

The genetic diversity and genetic structure analysis of 160 individuals from eight Kenya populations, suggested that the Kenya germplasms have a broad genetic base and are therefore, important and useful for breeding programmes of *Jatropha* [4].

2.3. Inter simple sequence repeats (ISSRs)

The ISSR analysis, is technically simple, it has been used for evaluating the genetic variation and genetic relatedness of the *Jatropha* species and high level of genetic variation is predicted in *Jatropha* by using ISSR markers (Table 3). The genetic variability among 332 *J. curcas* cultivated accessions from Brazil were investigated using ISSR primers revealed high genetic diversity of *J. curcas* at species level [24]. A high level of genetic diversity in 224 *J. curcas* accessions in China was reported based on ISSR molecular profiles [25]. Genetic diversity analysis of *J. curcas* accessions from Mexico revealed a high genetic diversity in germplasm [26]. Characterization of *Jatropha* species occurring in India is using nuclear and organelle specific primers for supporting interspecific gene transfer. The *J. tanjorensis* is considered as a natural hybrid between *J. gossypifolia* and *J. curcas* via ISSR markers [22].

2.4. Simple sequence repeats (SSRs)

The SSRs (microsatellites), with their larger number and immense potential for variation, form an indispensable component of genetic diversity studies. There is a wide scope and utility for these SSR markers towards diversity estimation and marker assisted breeding of *J. curcas* (Table 4). A total of 182 accessions were evaluated at genetic and phenotypic level using SSRs for genetic diversity assessment revealed moderate diversity [16]. Low genetic diversity was recorded among 192 *J. curcas* accessions collected from different geographical regions throughout Brazil using SSR markers [23]. Very low genetic diversity was observed among the 58 accessions of *J. curcas* collected across China as out 17 genomic SSRs, only one was polymorphic [27]. Pamidimarri DVNS et al. [28] analysed 12 microsatellite markers, and found seven polymorphic markers between the toxic and non-toxic varieties at molecular level.

2.5. Expressed sequence tag (EST)-SSRs

The EST-SSRs markers are important to investigate genetic diversity of *J. curcas*. The SSR (including EST-SSRs) have predicted lower genetic diversity of *J. curcas* germplasm as compared to AFLP, RAPD and ISSR makers in several research reports (Table 5). This is SSR markers identify the variation in repeat regions only whereas entire genome is used by AFLP, RAPD and ISSR makers for diversity analysis. Moderate level of genetic diversity was observed among 45 *J. curcas* accessions using EST-SSRs [29]. The genetic relationships determined by EST-SSRs among 25 *J. curcas* accessions were grouped into three main clusters which reveals low genetic diversity among accessions [30]. Out of 432 EST-SSR primer pairs, 269 were polymorphic among the *Jatropha* and *Jatropha*-related species [31].

Table 3. Genetic Diversity of *J. curcas* by using ISSR Marker.

Sl. No.	No. of accessions studied	Country/Region	Trait	No. of primers used	Reference/s
1	43	India and Mexico	Genetic Diversity	100	[21]
2	9	China	Genetic Diversity	10	[53]
3	13	India	Genetic Diversity	25	[42]
4	34 accessions comprising eight agronomically important <i>Jatropha</i> species	India	Agronomic traits	100	[22]
5	11	India	Genetic Diversity	9	[54]
6	19	Mexico, South America, Asia and Africa	Genetic Diversity	6	[5]
7	224	China and Myanmar	Genetic Diversity	100	[25]
8	10	India	Genetic Diversity	12	[55]
9	17	India and Zimbabwe	Genetic Diversity	13	[51]
10	24	China	Genetic Diversity	12	[48]
11	332	Brazil	Genetic Diversity	32	[24]
12	39 accessions with six different <i>Jatropha</i> sps. and the last and most distinct group was <i>Ricinus communis</i> .)	Mexico, China, Vietnam and Thailand	Genetic Diversity	86	[26]
13	16	Malaysia	Genetic Diversity	8	[9]
14	29	India	Genetic Diversity	25	[29]
15	24	Indonesia	Genetic Diversity	9	[52]
16	66	Brazil	Genetic variability	10	[56]
17	10	Brazil	Genetic Diversity	9	[57]
18	24	Mexico	Genetic Diversity	6	[58]

Table 4. Genetic Diversity of *J. curcas* by using SSR Marker.

Sl. No.	No. of accessions studied	Country/Region	Trait	No. of primers used	Reference/s
1	58	China and Malaysia	Genetic Diversity	17	[27]
2	7	India	Genetic Diversity	12	[28]
3	19	Mexico, South America, Asia and Africa	Genetic Diversity	10	[5]
4	192	Brazil	Genetic Diversity	6	[23]
5	41	Brazil	Genetic Diversity	9	[59]
6	29	Mexico, South America and Africa	Toxic trait	40	[60]
7	4	2 toxic (Africa and South America) 2 Nontoxic (Mexico)	Phorbol esters	5	[61]
8	182	Asia , Africa , South America and Central America	Oil yield	29	[16]
9	93	Mexico	Genetic Diversity	10	[62]
10	158	Ecuador	Genetic Diversity	10	[63]

Table 5. Genetic Diversity of *J. curcas* by using EST-SSR Marker.

Sl. No.	No. of accessions studied	Country/Region	Trait	No. of primers used	Reference/s
1	45	Indonesia, China, Grenada and South America	Genetic Diversity	187 EST-SSR and 54 Genomic SSR	[29]
2	25	India	Genetic Diversity	12080 Sequences	[30]
3	2 <i>J. curcas</i> and and four Jatropha-related species.	Thailand	Phorbol esters	432 Sequences	[31]
4	50	Costa Rica	Genetic Diversity	21	[64]

2.6. Single-nucleotide polymorphisms (SNPs)

In recent years, the employment of SNP markers towards assessing population genetic structure in different species have increased. The unavailability of DNA sequence information restricts the use of SNPs for assessing the population genetic structure of the diverse *J. curcas* accessions. As SNPs show the most abundant source of genetic polymorphism, there is an immediate need to develop SNP markers and use them in various studies in *J. curcas* (Table 6). A narrow level of genetic diversity was observed among 148 global collections of *J. curcas* lines using SNPs [32]. The genetic diversity analysis among 273 *J. curcas* accessions using SNP markers revealed the presence of low genetic diversity [33]. Twenty candidate SNPs were identified from *J. curcas* accessions having low and high phorbol esters (PEs), and interestingly, one gene involved PE biosynthesis pathway was also identified [31].

Table 6. Genetic Diversity of *J. curcas* by using SNP Marker.

Sl. No.	No. of accessions studied	Country/Region	Trait	No. of primers used	Reference/s
1	148	India, North America, South America and Africa	Oil yield	103	[32]
2	273	Africa, Asia and America	Genetic Diversity	8	[33]
3	2 <i>J. curcas</i> and four <i>Jatropha</i> -related species.	Thailand	Phorbol esters	20	[31]

3. Conclusions

The review suggests that the analysis of *J. curcas* germplasm via different molecular markers have led to identification of low genetic diversity of accessions in the Asian region and also a close clustering of African and Asian accessions highlighting the existence of a common ancestor. The low genetic base of the African and Asian accessions could be attributed to less introduction from other areas, the prevalence of asexual reproduction and/or occurrence of apomix. According to the [2], *J. curcas* distribution and spread at the tropical belt was *via* the Cape Verde Islands, which was further confirmed by analysing diversity employing various molecular markers. The South American, Mexican and Meso-American regions show the existence of rich allelic diversity with useful and novel genes. These accessions can be an important resource for improving genetic base of *J. curcas*. Although molecular markers suggest variations, however, only limited quantitative genetic variability is predicted [27]. Hence, detailed evaluation of molecular diversity is the need of the hour, to identify divergent material for molecular breeding, construction of linkage maps, diversity analysis and QTL/association mapping for *J. curcas*.

Contributions by authors

All authors have equally contributed towards writing and have read and finalized the MS.

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Conflict of interest

The authors declare there is no conflict of interest.

References

1. Dehgan B, Webster GL (1979) Morphology and infrageneric relationships of the genus *Jatropha* (Euphorbiaceae). *University of California Publications in Botany* 74: 1–73.
2. Heller J, Physic Nut—*Jatropha curcas* L. Promoting the Conservation and use of Underutilized and Neglected Crops. 1. International Plant Genetic Resources Institute, Rome, Italy, 1996. Available from: <http://www.ipgri.cgiar.org/publications/pdf/161.pdf>.
3. Jones N, Miller JH (1991) *Jatropha curcas*—a multipurpose species for problematic sites. Land Resources Series—Asia Technical Department, World Bank 1: 1–12.
4. Machua J, Muturi G, Omondi SF, et al. (2011) Genetic diversity of *Jatropha curcas* L. populations in Kenya using RAPDs molecular markers: Implication to plantation establishment. *Afr J Biotechnol* 10: 3062–3069.
5. Ambrosi DG, Galla G, Purelli M, et al. (2010) DNA Markers and FCSS analyses shed light on the genetic diversity and reproductive strategy of *Jatropha curcas* L.. *Diversity* 2: 810–836.
6. Wood D (2007) Target properties for biofuels in Thailand. In: Keith SJ, Wood D, Pongmanee T, editors, Proceedings of the International Technical Workshop on Feasibility of Non-Edible Oilseed Crops for Biofuel Production, 25–27 May, Chiang Rai, Thailand.
7. Berchmans HJ, Hirata S (2008) Biodiesel production from crude *Jatropha curcas* L. Seed oil with a high content of free fatty acids. *Bioresour Technol* 99: 1716–1721.
8. Pamidimarri DVNS, Pandya N, Reddy MP, et al. (2009) Comparative study of interspecific genetic divergence and phylogenetic analysis of genus *Jatropha* by RAPD and AFLP. *Mol Biol Rep* 36: 901–907.
9. Noor Camellia NA, Thohirah Lee A, Abdullah NAP (2012) Genetic relationships and diversity of *Jatropha curcas* accessions in Malaysia. *Afr J Biotechnol* 11: 3048–3054.
10. Fairless D (2007) Biofuel: the little shrub that could: may be. *Nature* 499: 652–655.
11. Anand KGV, Kubavat D, Trivedi K, et al. (2015) Long-term application of *Jatropha* cake promotes seed yield by enhanced carbon sequestration, microbial biomass and enzymatic activity in soils of semi-arid tropical wastelands. *Eur J Soil Biol* 69: 57–65.
12. Xu Y, Tang L, Wang SH, et al. (2007) The research on comprehensive utilization of *Jatropha curcas*. In: International Workshop on the Development of the JCL Industry. 29–31 October 2007, China; 55.
13. Chen F (2007) Advances in *Jatropha* industry research and development. In: International Workshop on the Development of the JCL Industry. 29–31, Hainan, China; 7–8.

14. Jansen R (2008) *Jatropha* for investors. In: *Jatropha International Congress*, 17–18 December, Singapore; K5.
15. Zhang Z, Guo X, Lui B, et al. (2011) Genetic diversity and genetic relationship of *Jatropha curcas* between China and Southeast Asian revealed by amplified fragment length polymorphisms. *Afr J Biotechnol* 10: 2825–2832.
16. Luis RMO, Andres FTS, Raymond EEJ, et al. (2014) High level of molecular and phenotypic biodiversity in *Jatropha curcas* from Central America compared to Africa, Asia and South America. *BMC Plant Biol* 14: 77.
17. Ovando-Medina I, Sánchez-Gutiérrez A, Adriano-Anaya L, et al. (2011) Genetic Diversity in *Jatropha curcas* Populations in the State of Chiapas, Mexico. *Diversity* 3: 641–659.
18. Roberto A, Elmer GD, Marta VM, et al. (2015) Genetic Diversity Analysis of *Jatropha* Species from Costa Rica Using AFLP Markers. *Am J Plant Sci* 6: 2426–2438.
19. Tatikonda L, Wani PS, Kannan S, et al. (2009) AFLP-based molecular characterization of an elite germplasm collection of *Jatropha curcas* L., biofuel plant. *Plant Sci* 176: 505–513.
20. Pratima S, Md Aminul I, Madan SN, et al. (2015) Analysis of genetic diversity and fatty acid composition in a prebreeding material of *Jatropha*. *J Plant Biochem Biotechnol* 25: 111–116.
21. Basha SD, Sujatha M (2007) Inter and intra-population variability of *Jatropha curcas* L. characterized by RAPD and ISSR markers and development of population-specific SCAR markers. *Euphytica* 156: 375–386.
22. SD Basha, M Sujatha (2009) Genetic analysis of *Jatropha* species and interspecific hybrids of *Jatropha curcas* using nuclear and organelle specific markers. *Euphytica* 168: 197–214.
23. Rosado TB, Laviola BG, Faria DA, et al. (2010) Molecular markers reveal limited genetic diversity in a large germplasm collection of the biofuel crop *Jatropha curcas* L. in Brazil. *Crop Sci* 50: 2372–2382.
24. Grativol C, Lira Medeiros CDF, Hemerly AS, et al. (2011) High efficiency and reliability of inter-simple sequence repeats (ISSR) markers for evaluation of genetic diversity in Brazilian cultivated *Jatropha curcas* L. accessions. *Mol Biol Rep* 38: 4245–4256.
25. Cai Y, Sun D, Wu G, et al. (2010) ISSR based genetic diversity of *Jatropha curcas* germplasm in China. *Biomass Bioenergy* 34: 1739–1750.
26. Tanya P, Taepayoon P, Hadkam Y, et al. (2011) Genetic diversity among *Jatropha* and *Jatropha* related species based on ISSR markers. *Mol Biol Rep* 29: 252–264.
27. Sun QB, Li LF, Li Y, et al. (2008) SSR and AFLP markers reveal low genetic diversity in the biofuel plant *Jatropha curcas* in China. *Crop Sci* 48: 1865–1871.
28. Pamidimarri DVNS, Singh S, Mastan SG, et al. (2009) Molecular characterization and identification of markers for toxic and non-toxic varieties of *Jatropha curcas* L. using RAPD, AFLP and SSR markers. *Mol Biol Rep* 36: 1357–1364.
29. Wen M, Wang H, Xia Z, et al. (2010) Development of EST-SSR and genomic-SSR markers to assess genetic diversity in *Jatropha curcas* L. *BMC Res Notes* 3: 42.
30. Hemant KY, Alok R, Mehar HA, et al. (2011) EST-derived SSR markers in *Jatropha curcas* L.: development, characterization, polymorphism, and transferability across the species/genera. *Tree Genet Genomes* 7: 207–219.
31. Kularb L, Patcharin T, Prakrit S, et al. (2015) De novo Transcriptome Analysis of Apical Meristem of *Jatropha* spp. Using 454 Pyrosequencing Platform, and Identification of SNP and EST-SSR Markers. *Plant Mol Biol Rep* 4: 786–793.

32. Priya G, Asif I, Shrikant M, et al. (2012) Discovery and use of single nucleotide polymorphic (SNP) markers in *Jatropha curcas* L. *Mol Breed* 30: 1325–1335.
33. Daniele T, Eleni G, Ezzeddine S, et al. (2015) Assessment of genetic diversity in different accessions of *Jatropha curcas*. *Ind Crops Prod* 75: 35–39.
34. Pecina-Quintero V, Anaya JL, Zamarripa A, et al. (2011) Molecular characterisation of *Jatropha curcas* L. genetic resources from Chiapas, México through AFLP markers. *Biomass Bioenergy* 35: 1897–1905.
35. Yi C, Zhang S, Liu X, et al. (2010) Does epigenetic polymorphism contribute to phenotypic variances in *Jatropha curcas* L.? *BMC Plant Biol* 10: 259.
36. Pamidimarri DVNS, Mastan SG, Rahman H, et al. (2010) Molecular characterization and genetic diversity analysis of *Jatropha curcas* L. in India using RAPD and AFLP analysis. *Mol Biol Rep* 37: 2249–2257.
37. Shen JL, Jia XN, Ni HQ, et al. (2010) AFLP analysis of genetic diversity of *Jatropha curcas* grown in Hainan, China. *Trees* 24: 455–462.
38. Pecina-Quintero V, Anaya-López JL, Zamarripa-Colmenero A, et al. (2014) Genetic structure of *Jatropha curcas* L. in Mexico and probable center of origin. *Biomass Bioenergy* 60: 147–155.
39. Konan NO, Nacoulima LN, Diouf FH (2018) AFLP Analysis of Genetic Diversity Among Different *Jatropha curcas* L. Genotypes from Africa and Ecuador. *Tropicultura* 36: 11–22.
40. Ranade SA, Srivastava AP, Rana TS, et al. (2008) Easy assessment of diversity in *Jatropha curcas* L. plants using two single-primer amplification reaction (SPAR) methods. *Biomass Bioenergy* 32: 533–40.
41. Ganesh Ram S, Parthiban KT, Kumar RS, et al. (2008) Genetic diversity among *Jatropha* species as revealed by RAPD markers. *Genet Resour Crop Evol* 55: 803–809.
42. Gupta S, Srivastava M, Mishra GP, et al. (2008) Analogy of ISSR and RAPD markers for comparative analysis of genetic diversity among different *Jatropha curcas* genotypes. *Afr J Biotechnol* 7: 30–43.
43. Abdulla JM, Janagoudar BS, Biradar DP, et al. (2009) Genetic diversity analysis of elite *Jatropha curcas* (L.) genotypes using randomly amplified polymorphic DNA markers. *Karnataka J Agric Sci* 22: 293–295.
44. Kumar RV, Tripathi YK, Shukla P, et al. (2009) Genetic diversity and relationships among germplasm of *Jatropha curcas* L. revealed by RAPDs. *Trees* 23: 1075–1079.
45. Popluechai S, Breviario D, Mulpuri S, et al. (2009) Narrow Genetic and Apparent Phenetic Diversity in *Jatropha curcas*: Initial Success with Generating Low Phorbol Ester Interspecific Hybrids. *Nature Proceedings*, 1–44.
46. Ikbal K, Boora S, Dhillon RS (2010) Evaluation of *Jatropha curcas* L. using RAPD markers. *Indian J Biotechnol* 9: 50–57.
47. Subramanyam K, Muralidhara RD, Devanna N, et al. (2010) Evaluation of genetic diversity among *Jatropha curcas* L. by RAPD analysis. *Indian J Biotechnol* 9: 283–288.
48. Chen K, Ren P, Ying C, et al. (2011) Genetic relationships among *Jatropha curcas* L. clones from Panzhihua, China as revealed by RAPD and ISSR. *Afr J Agric Res* 6: 2582–2585.
49. Leela T, Naresh B, Srikanth RM, et al. (2011) Morphological, physico-chemical and micropropagation studies in *Jatropha curcas* L. and RAPD analysis of the regenerants. *Appl Energy* 88: 2071–2079.

50. Rafi MY, Shabanimofrad M, Puteri-Edaroyati MW, et al. (2012) Analysis of the genetic diversity of physic nut, *Jatropha curcas* L. accessions using RAPD markers. *Mol Biol Rep* 39: 6505–6511.
51. Varsha KK, Sumita K, Kothari SL (2012) Characterization of genetic diversity in *Jatropha curcas* L. germplasm using RAPD and ISSR markers. *Indian J Biotechnol* 11: 54–61.
52. Darmawan S, Sri Hartati RR, Asep S, et al. (2017) Genetic diversity of Indonesian physic Nut (*J. curcas*) based on molecular marker. *Agrivita J Agril Sci* 39: 160–171.
53. He W, Guo L, Wang L, et al. (2007) ISSR analysis of genetic diversity of *Jatropha curcas* L. *Chinese J Appl Envir Biol* 13: 466–470.
54. Senthil Kumar R, Parthiban KT, Govinda Rao M (2009) Molecular characterization of *Jatropha* genetic resources through inter simple sequence repeat (ISSR) markers. *Mol Biol Rep* 36: 1951–1956.
55. Mittal N, Dubey AK (2010) A novel set of highly polymorphic chloroplast microsatellite and ISSR for the biofuel crop *Jatropha curcas*. *Eurasia J Biosci* 4: 119–131.
56. Díaz BG, Argollo DM, Franco MC, et al. (2017) High genetic diversity of *Jatropha curcas* assessed by ISSR. *Genet Mol Res* 16: gmr16029683.
57. Ribeiro DO, Silva-Mann R, Alvares-Carvalho SV, et al. (2017) Genetic variability in *Jatropha curcas* L. from diallel crossing. *Genet Mol Res* 16: gmr16029651.
58. Juan USV, Neith P, Guadalupe LP, et al. (2018) Behavior of genetic diversity in F₁ crosses of selected accessions of *J. curcas*. *Ind Crops Prod* 122: 669–674.
59. Bressan EDA, Scotton DC, Ferreira RR, et al. (2012) Development of microsatellite primers of *Jatropha curcas* (Euphorbiaceae) and transferability to congeners. *Am J Bot* 99: 237–239.
60. Massimo V, Steluta R, Mario B (2013) Evaluation of genetic diversity between toxic and non toxic *Jatropha curcas* L. accessions using a set of simple sequence repeat (SSR) markers. *Afr J Biotechnol* 12: 265–274.
61. Massimo V, Steluta R, Claudio F, et al. (2014) Study on genetic control for phorbol esters accumulation in *Jatropha curcas* genotypes. *Plant Cel Biotechnol Mol Bi* 15: 1–10.
62. Salvador-Figueroa M, Magaña-Ramos J, Va'zquez-Ovando JA, et al. (2015) Genetic diversity and structure of *Jatropha curcas* L. in its centre of origin. *Plant Genet Resour C* 13: 9–17.
63. Heriberto M, Javier M, Julio L, et al. (2017) Genetic variability in the collection of physic nut (*Jatropha Curcas* L.) Of the National Institute of Agricultural Research of Ecuador using microsatelite markers. *Revista Tecnica* 17: 18–29.
64. Marcela VM, Eric JF, Eduardo JH, et al. (2017) Molecular characterization and genetic diversity of *Jatropha curcas* L. in Costa Rica. *Peer J* 5: e2931.



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