

Short Research Communication

No Variation at 29 Microsatellites in the Genome of *Jatropha curcas*

Gen Hua Yue ^{1,2}, Loong Chueng Lo ^{1,3}, Fei Sun ¹, Su Ying Cao ¹, Cheng Xin Yi ³, Yan Hong ³ and Wei Bang Sun ⁴

1. Molecular Population Genetics Group, Temasek Life Sciences Laboratory, 1 Research Link, National University of Singapore, 117604 Singapore.
2. Department of Biological Sciences, National University of Singapore, 14 Science Drive 4, 117543 Singapore.
3. JOIL (S) Pte, Singapore, 117604 Singapore.
4. Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, 650201, Yunnan, China.

✉ Corresponding author: Dr. G.H. Yue, Temasek Life Sciences Laboratory, 1 Research Link, National University of Singapore, 117604 Singapore. Tel: +65-68727405 Fax: +65-68727007 Email: genhua@till.org.sg.

© Ivyspring International Publisher. This is an open-access article distributed under the terms of the Creative Commons License (<http://creativecommons.org/licenses/by-nc-nd/3.0/>). Reproduction is permitted for personal, noncommercial use, provided that the article is in whole, unmodified, and properly cited.

Published: 2014.03.01

Abstract

Genetic variation in the genome of a given species is the basis for natural selection and genetic improvement through selective breeding. We applied 29 microsatellites located on 11 linkage groups to study genetic variation in 276 accessions of *J. curcas* collected from nine locations in five countries in South America, Asia and Africa to initiate a breeding program. To our surprise, we did not detect any genetic diversity at all 29 microsatellites loci. All the 276 accessions were homozygous at all loci and shared the same genotype at each locus, suggesting no microsatellite variation in the genome of *Jatropha curcas*. This result is quite unusual, and may have a profound influence on the breeding strategies and genome study of this species.

Key words: *Jatropha*; DNA markers; genetic variation; epigenetics; breeding.

Introduction

Jatropha curcas (Euphorbiaceae) is oil-bearing tree species tree and grows naturally in countries of the equatorial Americas [1]. It has been introduced to other tropical countries in Africa and Southeast Asia (Indian, Indonesia, Malaysia and China) by Portuguese sailors 400 years ago [1]. *J. curcas* is considered a potential bioenergy tree. It does not compete with food crops for fertile land resources [2]. Therefore, it was introduced rapidly in various rural programmes with the expectation of producing biodiesel sustainably and profitably [3,4]. Genetic variation is the basis for natural selection and genetic improvement through selective breeding. Genetic variation can be measured at DNA level [5]. Microsatellites are one of the most preferred co-dominant DNA markers for studying genetic diversity [6] because of their abun-

dance in a given genome, high polymorphism and the ease of genotyping with automatic DNA sequencers. In *J. curcas*, microsatellites have been recently developed [7,8] and used in constructing a linkage map [8], and studying genetic diversity within and among populations [7,9,10]. Results of studies on genetic diversity of *J. curcas* are controversial [11]. Some reported no genetic variation at microsatellite loci [8,10,12], while some reported limited variation [13,14].

To initiate a breeding program for genetic improvement of *J. curcas*, we collected seeds from nine locations in five countries in South America, Asia and Africa since 2006 and 2007 (Table 1). The collected seeds were germinated in Singapore, and 276 trees were planted and managed under the same condition

in our experimental farm in 2007. We observed substantial variations of phenotypic traits, such branch number, tree height and seed yield [10]. We isolated DNA from fresh leaves of each tree using a plant DNA isolation kit (Qiagen, Hilden, Germany). DNA was examined in 1% agarose gels to ensure sufficient quality and quantity for PCR. We selected 29 microsatellites from over 1000 markers developed in our lab [8] because they were able to amplify DNA from *J. curcas* and the related species *J. integerrima*. Among the 29 microsatellites, 21 named with *Jatr-* were developed from a *J. curcas* individual, while the re-

maining 8 named with *Jcuint-* were cloned from a hybrid (*J. curcas* X *J. integerrima*) individual. Sixteen markers were already mapped onto 11 linkage groups of jatropha [8], and the remaining 13 had not been mapped yet (Table 2). We applied the reference families, which were used to construct the first generation linkage map of jatropha [8], to examine the inheritance patterns of alleles at the 29 loci. All microsatellites were genotyped using the DNA sequencer ABI3730xl (Applied Biosystems, CA, USA) as described previously [8].

Table 1. Sampling locations of *Jatropha curcas*, *Jatropha integerrima* and their hybrid (*J. curcas* x *J. integerrima*) on three continents

Population	Species	Location	Country/Continent	Number
Jcu01	<i>J. curcas</i>	Java	Indonesia/Asia	32
Jcu02	<i>J. curcas</i>	Sumatera	Indonesia/Asia	48
Jcu03	<i>J. curcas</i>	Yunnan	China/Asia	48
Jcu04	<i>J. curcas</i>	Sichuan	China/Asia	32
Jcu05	<i>J. curcas</i>	Tamil Nadu	India/Asia	32
Jcu06	<i>J. curcas</i>	Sulawesi	Indonesia/Asia	8
Jcu07	<i>J. curcas</i>	Suriname	Suriname/South America	32
Jcu08	<i>J. curcas</i>	Tanzania	Tanzania/Africa	32
Jcu09	<i>J. curcas</i>	Mixed	Not known	12
Jint01	<i>J. integerrima</i>	Singapore	Singapore/Asia	1
Hybrid	<i>J. cu. x J. int.</i>	Singapore	Singapore/Asia	1

Table 2. Genotypes of 276 *J. curcas* individuals, one *J. integerrima* individual and one hybrid (*J. curcas* x *J. integerrima*) individual in 29 microsatellite loci*

LG	Locus	Genbank Accession no.	<i>J. curcas</i>	<i>J. integerrima</i>	<i>J. curcas</i> x <i>J. integerrima</i>
1	Jcuint091	JF502118	215/215	189/189	189/215
2	Jatr691	EU664836	142/142	173/177	142/173
3	Jatr901	EU664920	159/159	163/169	159/163
3	Jcuint221	JF502150	394/394	373/375	373/394
4	Jcuint034	JF502095	338/338	334/338	334/338
4	Jcuint298	JF502166	229/229	225/227	225/229
5	Jcuint061	JF502107	358/358	340/340	340/358
5	Jcuint192	JF502143	214/214	196/208	196/214
6	Jatr152	EU664742	192/192	188/204	188/192
6	Jatr190	EU664749	95/95	75/75	75/95
7	Jatr220	EU664757	223/223	223/235	223/235
8	Jatr679	EU664757	171/171	186/186	171/186
9	Jcuint079	JF502114	162/162	145/171	145/162
10	Jatr153	EU664743	260/260	242/242	242/260
11	Jatr154	JF502114	462/462	454/454	454/462
11	Jatr229	EU664743	151/151	161/163	151/163
UN	Jatr161	EU664646	250/250	234/246	234/250
UN	Jatr211	EU664755	283/283	259/283	259/283
UN	Jatr219	KC934904	356/356	362/362	356/362
UN	Jcuint240	KC934905	393/393	435/435	393/435
UN	Jatr194	KC934906	126/126	114/114	114/126
UN	Jatr221	EU664758	133/133	146/146	133/146

UN	Jatr030	EU664686	178/178	170/170	170/178
UN	Jatr079	KC934907	195/195	204/206	195/206
UN	Jatr093	KC934908	87/87	89/89	87/89
UN	Jatr095	KC934909	185/185	185/185	185/185
UN	Jatr102	EU664717	390/390	382/394	382/390
UN	Jatr103	KC934910	139/139	135/137	135/139
UN	Jatr105	KC934911	315/315	319/325	315/319

*: All the 276 *J. curcas* trees showed the same genotypes at each of the 29 microsatellite loci. LG: Linkage group where the markers were mapped. UN: Unmapped. Primer sequences of mapped microsatellites can be found in the paper of Wang et al (2011) [8], while the primer sequences of unmapped markers were submitted to GenBank. The numbers (e.g. 215/215) under each species are the sizes of alleles in base pairs (bp) at each locus.

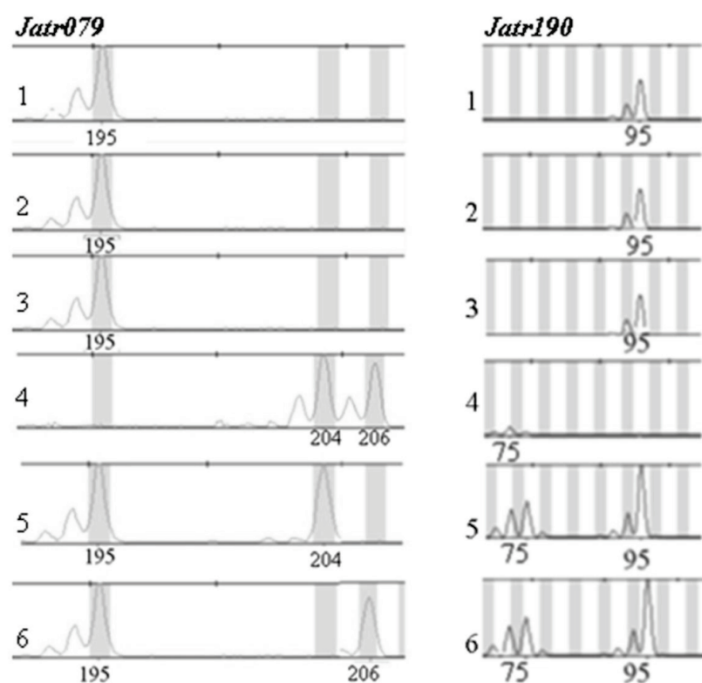


Fig 1. No microsatellite variation in *Jatropha curcas*, but some variation in one *Jatropha integerrima* and two hybrid (*J. curcas* x *J. integerrima*) individuals at microsatellite loci *Jatr079* and *Jatr190*. 1-3: three *Jatropha curcas* individuals, 4: one *J. integerrima* individual and 5-6: two hybrid (*J. curcas* x *J. integerrima*) individuals. The numbers of each peak are the sizes of allele in base pairs (bp).

To further examine microsatellite variation, we blasted the sequences of the 29 selected microsatellites against the draft genome sequence of a *J. curcas* individual from Japan [15]. We also examined the reproduction patterns of *J. curcas* using artificial pollination. We tested whether the female flowers developed into fruits by covering the female flowers with paper bags to prevent pollinations and by pollinating the female flowers with pollens from individuals of *J. curcas* or closely related species *J. integerrima*. If seeds were obtained, we germinated the seeds and genotyped them with the microsatellites to see whether the offspring obtained alleles from the father side.

All the 29 microsatellites amplified specific PCR products in *J. curcas* and *J. integerrima* (Table 2). Surprisingly, all the 276 *J. curcas* individuals were identical and homozygous in all 29 loci, whereas in the individuals from the species *J. integerrima* and the

hybrid, most microsatellites were heterozygous and polymorphic (Table 2 and Figure 1). These results suggest that (A) there is no genetic variation in the 29 microsatellite loci in *J. curcas*, (B) technical limitations prevent detection of genetic variation; or (C) the *J. curcas* accessions are cloned from a single tree recently, or (D) *J. curcas* is haploid or double haploid or (E) *J. curcas* is capable of apomixes. However, we can rule out the possibilities B-E. As mentioned above, we used one (i.e. ABI3730xl sequencer) of the most advanced genotyping platforms for genotyping microsatellites. The markers showed Mendelian inheritance in the two reference families used for constructing the linkage map of jatropha [8], thus excluding the possibility of errors in genotyping. The 276 trees used in this study were collected from five countries on three continents, and some seeds were directly collected from the wild. Therefore, it is im-

possible that they were cloned from single individuals recently. Even if all individuals evolved from a single ancestor, after the first introduction 400 years ago (ca. 400 generations), microsatellites should have shown some degree of variation. This is because microsatellites usually show a higher degree of mutation (10^{-4} /generation) than other regions (10^{-7} /generation) of the genome [6]. Karyotype analysis showed that *J. curcas* was diploid, containing 11 chromosome pairs [16], thus excluding the possibility of it being haploid. When the female flowers were covered with paper bags to prevent pollination, the female flowers aborted, whereas using artificial pollination, the female flowers developed into fruits. Microsatellite genotyping showed that the offspring inherited an allele at each locus from the father side (Table 2 and Figure 1). These data indicate that *J. curcas* can reproduce sexually. Thus, our results suggest that there is no microsatellite variation among the 276 individuals.

Furthermore, in the linkage map of *Jatropha* [8], all 216 mapped microsatellites and 290 SNPs were homozygous in the *J. curcas* parent, while they were

heterozygous in the two hybrid parents. By blasting the sequences of the 22 microsatellites isolated from *J. curcas* against the draft genome sequence of an individual from Japan [17], we did not find any difference in the number of repeat unit (Figure 2), indicating that there is no microsatellite variation between our *J. curcas* individual and the individual from Japan. By blasting the eight microsatellites isolated from the hybrid individual against the *J. curcas* genome sequence, we detected variation of both sequence and repeat length (Figure 2) in 7 of the eight microsatellites. Only in the locus *Jcuint221*, there was no repeat length difference, probably the sequence of *Jcuint221* originated from *J. curcas*. The sequence similarity of the seven microsatellites ranged from 89% for *Jcuint091* to 97% for *Jcuint079*, while the number of repeat unit changed by 1-10 (Figure 2), indicating that there is microsatellite variation between *J. curcas* and *J. integerrima* and within *J. integerrima*. Yi et al. [10] used 12 SSR primer pairs developed by Sudheer et al [7] to genotype 162 *J. curcas* samples from five countries. They did not detect any polymorphism and found

that all the individuals were homozygous, sharing the same genotype in each locus. In a recent study [12] using 28 microsatellites, no microsatellite variation was detected in *J. curcas* accessions collected from many places outside Mexico, strongly supporting that there are no microsatellite variation in the genome of *J. curcas*.

```
Query: 126  ttottgatttttgaatggtgactgaagccttcttggaaatcaaaacaactgctcctgaag 185
          |||
Sbjct: 4541  ttottgatttttgaatggtgactgaagccttcttggaaatcaaaacaactgctcctgaag 4600

Query: 186  tgcatgccaaatgcttcgacgaaatgcataaatggaagacatgtaacagcgagaagaggt 245
          |||
Sbjct: 4601  tgcatgccaaatgcttcgacgaaatgcataaatggaagacatgtaacagcgagaagaggt 4660

Query: 246  aatggagggtctgttttggggctaaaattttctagcagcatcttctaaactcttacaat 305
          |||
Sbjct: 4661  aatggagggtctgttttggggctaaaattttctagcagcatcttctaaactcttacaat 4720

Query: 306  attaaaaacaataaagacaaggaagccatgactttgcttgggttgg 350
          |||
Sbjct: 4721  attaaaaacaataaagacaaggaagccatgactttgcttgggttgg 4765
```

Jatr161

```
Query: 300  gtttatgaccagatgaaacagaaggggttcaagtatacaaaatgatcattgtgggg 359
          |||
Sbjct: 41610 gtttatgaccagatgaaacaga-----atacaaaaatgatcattgtgggg 41656

Query: 360  atggaagagaactcagcagaataaactatattgtctatttcaacagaattataatgtgg 419
          |||
Sbjct: 41657 atggaagagaactcagcagaataaactatattgtctatttcaacagaattataatgtgg 41716

Query: 420  ctttgatctagtattggacatcattcgaattatgacgttttaagttgaagcagatctct 479
          |||
Sbjct: 41717 ctttgatctagtattggacatcattcgaattatgacgttttaagttgaagcagatctct 41774

Query: 480  ctctcttctctctctctctctctctctctctctctctctctctcaacacatca-tgcccatacctatgcttt 538
          |||
Sbjct: 41775 ctctc-----tctctctctctctctctctctctctctctctctctctcaacacatca-tgcccatacctatgcttt 41812

Query: 539  aagtgattatgaccattgcttggtaaaactgggtgttaaaacctatgacgctctaata 598
          |||
Sbjct: 41813 aagtgattatgaccattgcttggtaaaactgggtgttaaaacctatgacgctctaata 41872
```

Jcuint221

Fig 2. Alignment of DNA sequences of two microsatellite markers (*Jatr161* and *Jcuint221*) against the genome sequence of a *J. curcas* individual from Japan. At the locus *Jatr161* isolated from *J. curcas*, there is not repeat and sequence variance, whereas at the locus *Jcuint221* isolated from the hybrid (*J. curcas* x *J. integerrima*), both DNA sequences and the number of repeats are different.

The phenomenon of no microsatellite variation, but high phenotypic variation in *J. curcas* is quite unusual and worthy further studying. Epigenetic variations may play an important role in the phenotypic difference [10]. Examining epigenetic patterns across the whole genome and in many individuals of *J. curcas* using whole genome bisulfite sequencing [18] may shield new insights into how the epigenetic variations contribute to the phenotypic variation. In fact, understanding more about the genetic and epigenetic variations in *J. curcas* and the mechanism underlying phenotypic variations are surely helpful in designing breeding programs to improve the oil yield of *J. curcas* for sustainable and profitable production of biodiesel.

Competing Interests

The authors have declared that no competing interest exists.

References

- Heller J. *Physic nut, Jatropha curcas* L. Rome, Italy: Bioversity International. 1996.
- Fairless D. Biofuel: The little shrub that could - maybe. *Nature* 2007; 449: 652-655.
- Pan BZ, Xu ZF. Benzyladenine treatment significantly increases the seed yield of the biofuel plant *Jatropha curcas*. *J Plant Grow Reg* 2011; 30: 166-174.
- Ariza-Montobbio P, Lele S. *Jatropha* plantations for biodiesel in Tamil Nadu, India Viability, livelihood trade-offs, and latent conflict. *Ecol Econom* 2010; 70: 189-195.
- Weiner MP, Gabriel SB, Stephens JC. *Genetic variation: a laboratory manual*. USA: Cold Spring Harbor Laboratory Press. 2007.
- Goldstein DB, Schlotterer C. *Microsatellites: Evolution and Applications*. Oxford: Oxford University Press. 1999.
- Sudheer P, Rahman H, Mastan SG, Reddy MP. Isolation of novel microsatellites using FIASCO by dual probe enrichment from *Jatropha curcas* L. and study on genetic equilibrium and diversity of Indian population revealed by isolated microsatellites. *Mol Bio Report* 2010; 37: 3785-3793.
- Wang CM, Liu P, Yi CX, Gu KY, Sun F, et al. A first generation microsatellite-and SNP-based linkage map of *Jatropha*. *PloS one* 2011; 6: e23632.
- Sun QB, Li LF, Wu Y, Ge GJ. SSR and AFLP markers reveal low genetic diversity in the biofuel plant in China. *Crop Sci* 2008; 48: 1865-1871.
- Yi CX, Zhang SL, Liu XK, Bui HTN, Hong Y. Does epigenetic polymorphism contribute to phenotypic variances in *Jatropha curcas* L.? *BMC Plant Bio*. 2010; 10: 295.
- Surwenshi A, Kumar V, Shanwad V, Jalageri B. Critical review of diversity in *Jatropha curcas* for crop improvement: A candidate biodiesel crop. *Res J Agric Sci* 2011; 2: 193-198.
- Vischi M, Raranciu S, Baldini M. Evaluation of genetic diversity between toxic and non toxic *Jatropha curcas* L. accessions using a set of simple sequence repeat (SSR) markers. *Afri J Biotechnol* 2013; 12: 265-274.
- Rosado TB, Laviola BC, Faria DA, Pappas MR, Bhering LL, et al. Molecular markers reveal limited genetic diversity in a large germplasm collection of the biofuel crop *Jatropha curcas* L. in Brazil. *Crop Sci* 2010; 50: 2372-2382.
- Zhang ZY, Guo XL, Liu BY, Tang L, Chen F. Genetic diversity and genetic relationship of *Jatropha curcas* between China and Southeast Asian revealed by amplified fragment length polymorphisms. *Afri J Biotechnol* 2011; 10: 2825-2832.
- Sato T, Pal S. A two-step site and mRNA-level model for predicting microRNA targets. *BMC Bioinformatics* 2010; 11: 612.
- Carvalho CR, Clarindo WR, Praca MM, Araujo FS, Carels N. Genome size, base composition and karyotype of *Jatropha curcas* L., an important biofuel plant. *Plant Sci* 2008;174: 613-617.
- Sato S, Hirakawa H, Isobe S, Fukai E, Watanabe A, et al. Sequence analysis of the genome of an oil-bearing tree, *Jatropha curcas* L. *DNA Res* 2011; 18: 65-76.
- Li N, Ye M, Li Y, Yan Z, Butcher LM, et al. Whole genome DNA methylation analysis based on high throughput sequencing technology. *Methods* 2010; 52: 203-212.