

BRIEF COMMUNICATION

Isolation of 55 microsatellite markers for *Jatropha curcas* and its closely related species

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Abstract

Jatropha curcas L. (physic nut) is native to Central America and now naturalized widely in many tropical and subtropical areas. Microsatellite markers were isolated and characterized. Eleven out of 55 markers showed polymorphisms, and the allelic variation was investigated using 26 accessions of *J. curcas* collected from several provinces in Thailand. Each marker showed 2 to 5 alleles and the average polymorphic information content (PIC) was 0.49. Thirty four markers (62 %) were also successfully amplified in *J. integerrima*, *J. gossypifolia* and *J. podagrica*.

Additional key words: genetic diversity, microsatellite-enriched genomic library, physic nut.

Jatropha curcas L., or physic nut, is a tree or shrub belonging to the family *Euphorbiaceae*. *J. curcas* is native to Central America, and has become grown in many tropical and subtropical areas, including India, Africa, North America and South East Asia. Because of its high oil content in seeds, *J. curcas* is a potential biodiesel source (Ghosh *et al.* 2007). Understanding of the genetic relationships in *Jatropha* species is important for efficient management, conservation, characterization and utilization (Akkak *et al.* 2009, Akritidis *et al.* 2009, Hu *et al.* 2009). Basha and Sujatha (2009) assessed the genetic relationship among *Jatropha* species from India using RAPD and ISSR markers. Sudheer *et al.* (2009a) developed 12 micro-satellite, or simple sequence repeat (SSR) markers useful for Indian *J. curcas*, and using these markers and RAPD and AFLP markers as well, discriminated non-toxic from toxic cultivars of *J. curcas*. However, Sudheer *et al.* (2009b) found low levels of genetic variation based on surveys of RAPD and AFLP markers in inter- and intraspecific diversity among *Jatropha* species. In this study, we isolated 55 new microsatellite markers for *Jatropha* and the variability

was evaluated using 18 accessions of *J. curcas* collected from several provinces in Thailand and eight Mexican accessions (Table 1) and one accession each of related three species *J. integerrima*, *J. gossypifolia* and *J. podagrica*. All the accessions were obtained from the germplasm collection of the Inseechandrasatitya Institute for Crop Research and Development, Kasetsart University, Thailand.

Genomic DNA was isolated from fresh young leaves using the modified cetyltrimethyl ammonium bromide (CTAB) method (Doyle and Doyle 1990). An SSR-enriched genomic library was constructed by a modified protocol based on the methods of Connell *et al.* (1998). Genomic DNA (500 ng) was digested with *Tru9I* restriction enzyme (*Promega*, Madison, WI, USA), and compatible adapters (5'-GACGATGAGTCCTGAG-3'/5'-TACTCAGGACTCAT-3') ligated to the fragment end using T4 DNA ligase (*Promega*) in a 0.01 cm³ reaction mixture. The ligated DNA (50 ng) was amplified by polymerase chain reaction (PCR) in a 0.05 cm³ mixture containing 1× PCR buffer, 10 mM dNTPs, 1.5 mM MgCl₂, 10 μM *Tru9I* primer (5'-GATGAGTCCTGA

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Abbreviations: AFLP - amplified fragment length polymorphism; ISSR - inter simple sequence repeat; PIC - polymorphic information content; RAPD - randomly amplified polymorphic DNA; SSR - simple sequence repeats.

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Table 1. List of the 26 *Jatropha* accessions included in this study and the province where the accession was collected. ^a- Mexican accessions were obtained from the Inseechandrasatitya Institute for Crop Research and Development, Kasetsart University, Thailand.

Accession number	Province	Accession number	Province
KUBP249	Saraburi	AP136	Suphanburi
A05	Nakhonprathom	AP145	Phrae
DOA1	Chachoengsao	AP147	Phrae
Mex1 ^a	Nakhon Ratchasima	KUBP233	Kanchanaburi
Mex9 ^a	Nakhon Ratchasima	KUBP165	Nong Khai
Mex10 ^a	Nakhon Ratchasima	KUBP244	Lop Buri
Mex11 ^a	Nakhon Ratchasima	KUBP256	Tak
Mex12 ^a	Nakhon Ratchasima	KUBP266	Mae Hong Son
Mex13 ^a	Nakhon Ratchasima	KUBP285	Lumphun
Mex4 ^a	Nakhon Ratchasima	RKPS2	Nakhonprathom
Mex15 ^a	Nakhon Ratchasima	RKPS9	Nakhonprathom
MYA1	Nakhonprathom	RKPS18	Nakhonprathom
AP2	Phitsanulok	RKPS21	Nakhonprathom

GTAA-3'), and 1 unit of *Taq* DNA polymerase (*Fermentas*, Ontario, Canada). The profile of thermal cycling was: 20 cycles of 94 °C for 30 s, 56 °C for 1 min and 72 °C for 1 min. The amplified fragments were selected by hybridization to biotinylated oligonucleotides (GA)₁₅, (CTT)₁₅ and captured with streptavidin-conjugated magnetic beads (*Dyna beads M-280*, *Dynal*, Oslo, Norway). SSR-enriched DNA fragments were cloned using *CloneJET*[™] PCR cloning kit (*Fermentas*) and transformed into the competent *Escherichia coli* strain DH10B (*Invitrogen*, Carlsbad, USA) using electroporation. 190 clones were grown overnight in 2 cm³ of liquid medium containing ampicillin (100 µg dm⁻³). Plasmid DNA was extracted using a high-speed plasmid mini kit (*Geneaid*, Taipei, Taiwan). Each plasmid DNA was PCR-amplified using 0.2 µM each of pJET1.2 sequencing primer (5'-CGACTCACTATAGGAGAGC GGC-3' and 5'-AAGAACATCGATTTCCATGGCAG-3') under the following PCR conditions: 95 °C for 3 min followed by 25 cycles of 30 s at 94 °C, 30 s at 60 °C and 1.0 min at 72 °C and a final 72 °C extension for 5 min. 132 amplified PCR products showed single-banding patterns by electrophoresis, which were sequenced by First BASE Laboratories (*Malaysia*). 56 actually contained microsatellite sequences, for which primer pairs were designed using the program *Primer3* (<http://frodo.wi.mit.edu/>). PCR reactions were prepared in 0.01 cm³ reaction volumes containing approximately 10 ng templates DNA, 50 mM KCl, 20 mM Tris-HCl buffer (pH 8.0), 1.5 mM MgCl₂, 0.2 µM of each primer, 0.4 mM of each dNTP, and 0.5 unit of *Taq* DNA polymerase (*Fermentas*). The reaction mixture was subjected to PCR amplification in a *T1 Thermocycler* (*Biometra*, Göttingen, Germany) using a PCR program, 2 min at 94 °C, followed by 35 cycles of 94 °C for 30 s,

54 - 60 °C annealing temperature for 30 s, and 72 °C for 1.0 min, followed by 5 min at 72 °C. After amplification, PCR reactions were mixed with 0.02 cm³ of loading dye (95 % formamide, 0.25 % bromophenol blue and 0.25 % xylene cyanol) and denatured. 0.002 cm³ of the sample were separated by electrophoresis in 6 % denaturing polyacrylamide gels (*Sequi-Gen1 GT* nucleic acid electrophoresis cell, *Bio-Rad*, Hercules, USA) at 40 W constant powers for 2 h and visualized by silver staining as described by Benbouza *et al.* (2006). The band sizes were compared using a 10 bp DNA ladder (*Invitrogen*).

Initially, 55 primer pairs were assessed by a small sample set of 4 accessions of *J. curcas*, which corresponded to RKPS2, A05, Mex1 and MYA1 and one accession each of *J. integerrima*, *J. gossypifolia* and *J. podagrica*. Of these, 19 produced polymorphic bands among the four accessions, while the others showed no polymorphism or no amplification (JCT8, JCT14 and JCT131) in *J. curcas*. 34 markers (62 %) were successfully amplified in all four *Jatropha* species. Only 11 primer pairs were further evaluated with 26 accessions of *J. curcas*. *PowerMarker* version 3.25 software (Liu and Muse 2005) was used to measure the variability at each locus in terms of number of alleles and polymorphism informative content (PIC). The 11 polymorphic loci produced a total of 36 alleles, ranging from 2 to 5 alleles per locus with an average of 3.27. The average PIC was 0.49 (Table 2).

Therefore, at least 19 microsatellite markers are useful for investigating intraspecific variation in *J. curcas*. Among them 34 SSRs, that are successfully amplified in the 4 *Jatropha* species, are not polymorphic. They will be useful in large germplasm collection, conservation genetic studies, and breeding programs.

Table 2. Characterization of 55 microsatellite markers used for genotyping in *Jatropha*. Primer pairs were evaluated with 26 accessions of *J. curcas*^a, produced polymorphic bands of small sample set of 4 accessions of *J. curcas*^b, successfully amplified in all four *Jatropha* species^c. Ta - annealing temperature.

Locus	Accession number	Repeat motif	Ta [°C]	Forward primers	Reverse primers
JCT15 ^{abc}	AB512287	(A) ₂₂ ...(CT) ₁₀	60	AATTCTCTTTCCGCGATCCT	CGTAGACCTTCCAACAGCAA
JCT16 ^{abc}	AB512288	(GT) ₁₁	60	GCCTCCAGCATCTTTCAATC	AACAATCCCCATTCTCTCTC
JCT17 ^{abc}	AB512289	(GA) ₆ ...(GA) ₁₁ (GT) ₂₁	60	TCTCTCATTTGTTGCGCTGTC	TAACAAGTCTCTCCCCTCTCT
JCT27 ^{ab}	AB512290	(CT) ₁₇	60	GCCATTAGAAATGGACGGCTA	TGCGTGAAGCTTTGATTGTA
JCT31 ^{ab}	AB512291	(TC) ₁₈	59	TGGAAAACGAATGAGGCTCT	GGACACTCTGGAAGGAACG
JCT34 ^{ab}	AB512292	(AG) ₁₆ ...(TA) ₉	57	TGACTCAATAAATGTGGACTGG	GGTGCATCCCGAGAAAAGT
JCT37 ^{ab}	AB512294	(AG) ₂₀	54	ATTCGACAATCTACGGGATA	CACCTTATACGTCTCTCTCTCTC
JCT53 ^{ab}	AB512297	(GA) ₁₉	59	AAAGCAATCAACCCAAGAGG	TCTCTCTCTCTCTCTCTCTCTCTCT
JCT59 ^{abc}	AB512295	(CT) ₁₀ (CA) ₁₄	60	GGTGACTCCTGAATGCTTGG	TACCTGAAACTCCCAGGAA
JCT68 ^{abc}	AB512293	(AG) ₁₁ ...(GA) ₇ ...(A) ₁₁ (GAAA) ₄	60	AGCGATAATCGGCCTACCTT	CAACGCTCACTGCTCTACCT
JCT81 ^{abc}	AB512296	(CT) ₁₈	54	CCATTTAGAACCAACCAT	GATGTCCAATAAGCCTGAAT
JCT1 ^c	AB525641	(GC) ₄ (AC) ₅ (AG) ₈	54	CAGCAGAAGAGTAAAGGA	GCTTATGGTGTATTGCAA
JCT3 ^c	AB525642	(AG) ₁₂	54	ATCTGCCATCAACCGTA	AACGCGTCACTAAGAGA
JCT3 ^{bc}	AB525643	(GA) ₇ ...(GA) ₄ ...(GA) ₈	55	CATGTAACGATAGAGGA	TTTTACGCCAGCTGCTCA
JCT7 ^c	AB525644	(AG) ₁₁	54	CGAAGTGAATGCACAACACA	TGCTATTCAAATGGAACAAGTGA
JCT8	AB525645	(GT) ₄ ...(GT) ₄	54	ATGTGTATGCTTTGTGCA	GACGAACGCCTAATCGA
JCT10 ^c	AB525646	(TGT) ₄ ...	54	ATATCGAACCATGAACGA	AGCCGTTTATCATTTACGA
JCT12 ^c	AB525647	(CT) ₄ ...(TC) ₅	58	TCATAGCCGAGATACACA	GAGATCGGACGTGGTGA
JCT13 ^c	AB525648	(GA) ₄ ...(AG) ₄ ...(GA) ₇ (GT) ₁₅	56	GAACCCGTGATAGTGAGGA	GCCGATAGACCATAGACA
JCT14	AB525649	(AT) ₈ ...(GT) ₃₁	54	TGCTCCAACCTTGAGGAGTGT	TGTGAATGGGAAACAAGAGTG
JCT18 ^c	AB525650	(GA) ₄ ...(GA) ₄	58	GGAGGAATCAATGAAAAGGACA	TGCTTGTGAACCCCTGTGAA
JCT19 ^c	AB525651	(A) ₃₂	60	CATAGGAGCAGGGGTAAACAA	GCCTAGGCCCTGCTAGAGAC
JCT23 ^c	AB525652	(GA) ₁₇	58	ACCGAGCCAGGAAAAGGAC	TGTTGCTGTTGCGATTCTCTC
JCT28 ^c	AB525654	(GA) ₁₅	56	CGCAGCCATCTTGAAGGTTA	CAAAATTTCAAGCCATGCTC
JCT31 ^c	AB525655	(TC) ₁₈	58	TGGAAAACGAATGAGGCTCT	GGACACTCTGGAAGGAACG
JCT32	AB525656	(AG) ₉	56	GCAAATATGTATTACTGAAAAGAAAAA	GAAATGTTTGGCTTTGGATCA
JCT34 ^c	AB525657	(GT) ₁₆ ...(TA) ₉	54	TGACTCAATAAATGTGGACTGG	GGTGATCTCCCGAGAAAAGT
JCT36 ^c	AB525667	(CT) ₁₈	54	TTCTGATTTGCCCTTTATGT	GAAAATCGCAGAAAAGAAGA
JCT45	AB525668	(AG) ₈	56	AGTCGATTGGTACCCCTTCT	AGACGCTTCTTTTCTCTCT
JCT47 ^c	AB525669	(CT) ₄ TT(CT) ₁₂	56	GAAGCCTTACTCCCATTTC	GAAGGCTATGGCAATATGAT
JCT50 ^c	AB525670	(TC) ₅ ...(TC) ₃ ...(TC) ₇ ...(TATC) ₇	54	TCCCAAGTCATGATTCAATA	AAGGCCGTTAGAATCTCAT
JCT51 ^c	AB525671	(GA) ₁₄	56	CATGGAATGCATTTGTGTGA	CCTTGACCTTCCTTCCAACA
JCT60 ^c	AB525672	(CT) ₁₂	58	TGGACAGGCTTTTGTGTG	GACAGTCAATATTAGGTACTTCAG
JCT74 ^c	AB525673	(TC) ₉	58	CGCTTACGAGAAAGAAAATCCA	GGTCAGCTCAGCTCATCTCC
JCT76	AB525674	(AG) ₁₂	56	ATTGTGTTGTGTGTGACTG	GCTCATTCTATTTCAGGT
JCT80	AB525675	(GA) ₁₇	58	TCTCCATCCTGGAGTTTCTA	TGCTGATAAAACACAGATAAAC
JCT86 ^{bc}	AB525676	(GA) ₁₇	56	TATTTCTCTTCTGTCACAT	GTTTGGCTAAAAAGGTGATG
JCT89 ^{bc}	AB525677	(CT) ₁₆	54	GCCGATAAACACAGATAAA	GAAAAATAAAGCCAGCAAGA
JCT92 ^{bc}	AB525678	(AG) ₁₀ AT(AG) ₅	56	CACCGCTCATCTATGATTCT	CCAGTGCCAATTTTCTACAT
JCT103 ^{bc}	AB525679	(AG) ₁₇	54	CAACGACTCTTTGAAGAAAAA	GCCGATAAACACAGATAAA
JCT106	AB525680	(TC) ₉	56	TAATGCTCTCTCCCTAAGC	TTCCAGGTTTACACACCTTT
JCT124 ^c	AB525681	(CAA) ₅ ...(GGA) ₃ ...(GAGT) ₅	58	GTCACCTCGATCACCAAC	GATCTGCGAAAAGAGAGAGAA
JCT131	AB525682	(TC) ₇ CC(TC) ₅ ...(T) ₃₉	58	CTGAACAGTCCTTTTCATCG	AAGCCCTTATAAAGTTCAAGC
JCT135 ^c	AB525683	(CT) ₈ (CA) ₁₃	58	GGAAAACAGTCCTTCACCTTG	GACTAAGGGCAACACTGAAC
JCT136	AB525684	(AG) ₈ (TG) ₁₄	58	ATGAACCCGTGATAGTGAGGA	GCCGATAGACCATAGACAAA
JCT141 ^c	AB525685	(GA) ₁₂	54	TGTTAGTCAATGCAAGAAGGT	TTTGAAACAAGTTTCTCTGCT
JGAA1 ^b	AB525658	(GAA) ₉	56	AAAGGTCACAGTGTTCCTAAAG	TTCTTTCTCAACTTCTCTCCA
JGAA28	AB525659	(CTT) ₈	56	TCTTTCTCAACTTCTCTCAA	GGTCACAATGGTTCAAAGTT
JGAA31	AB525660	(TCT) ₅ ...(CTT) ₁₃	56	TCTTTCTCAACTTCTCTCAA	GAGGGTGAAGAAGAAAACAA
JGAA35 ^{bc}	AB525661	(CTT) ₄ ...(CTT) ₄ ...(CTT) ₄	55	TCTCTCTGCTCTCTCTCTCTA	CAAAGGAAAAGCGAAGTTAG
JGAA47	AB525662	(GAA) ₅	56	AAAAGGGGAAAGGAAAATTA	CTTTCTCTATGGCACTTTCC
JGAA53	AB525663	(AAG) ₅ ...(GAA) ₄	54	GAAAAAGAAGTTGCTGAGGA	CTCCAATTTCTCTTTCTCTT
JGAA55 ^c	AB525664	(GAT) ₁₁ ...(GAA) ₅	54	TAATGGTATCCGGTATGTGG	CCTTTCCAAATCAACATCAT
JGAA56 ^b	AB525665	(CTT) ₄ ...(CTT) ₄ ...(CTT) ₃ ...(CTT) ₄	56	TCAGCAGCACTCTCTTTTCC	AAGAAAAGAAGGAGGAAGCA
JGAA57	AB525666	(GAA) ₁₀	54	CGGAGATCGAAGGAGAGGTA	AATCGAACCAAAATGGGCTA

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