BIOLOGIA PLANTARUM 55 (2): 387-390, 2011

BRIEF COMMUNICATION

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

C. PHUMICHAI[1]*, T. PHUMICHAI[2], N. KONGSIRI[1], A. WONGKAEW[1], P. SRIPICHIT[1] and R. KAVEETA[1]

Department of Agronomy, Faculty of Agriculture, Kasetsart University, Bangkok 10900, Thailand[1]
Rubber Research Institute of Thailand, Department of Agriculture, Bangkok 10900, Thailand[2]

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 amoonium 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 *Tru*9I 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 cm^3 reaction mixture. The ligated DNA (50 ng) was amplified by polymerase chain reaction (PCR) in a 0.05 cm^3 mixture containing 1× PCR buffer, 10 mM dNTPs, 1.5 mM $MgCl_2$, 10 µM *Tru*9I primer (5'-GATGAGTCCTGA

Received 14 November 2009, *accepted* 8 June 2010.
Abbreviations: AFLP - amplified fragment length polymorphism; ISSR - inter simple sequence repeat; PIC - polymorphic information content; RAPD - randomly amplified polymorphic DNA; SSR - simple sequence repeats.
Acknowledgments: The authors gratefully acknowledge Drs. Kazuyoshi Hosaka and Paul G. Johnson for useful comments and improvement of the manuscript and Anna Saimaneerat, Panie Tongpumnuk and Isara Sooksathan for providing *J. curcas.* This research was financially supported by the Thai Research Fund and the Thai Commission on Higher Education.
* Author for correspondence; fax: (+66) 2579 8580, e-mail: agrcpp@ku.ac.th

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 electropo-ration. 190 clones were grown overnight in 2 cm^3 of liquid medium containing ampicilin (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'-CGACTCACTATAGGGAGAGC GGC-3' and 5'-AAGAACATCGATTTTCCATGGCAG-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 cm^3 reaction volumes containing approximately 10 ng templates DNA, 50 mM KCl, 20 mM Tris-HCl buffer (pH 8.0), 1.5 mM $MgCl_2$, 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 cm^3 of loading dye (95 % formamide, 0.25 % bromophenol blue and 0.25 % xylene cyanol) and denatured. 0.002 cm^3 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 corresponsed 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	AACAATCCCCATTCCTCCTC
JCT17[abc]	AB512289	$(GA)_{6}$…$(GA)_{11}(GT)_{21}$	60	TCTCTCATTGTTGCGCTGTC	TAACAAGTCCTCCCCCTCCT
JCT27[ab]	AB512290	$(CT)_{17}$	60	GCCATTAGAATGGACGGCTA	TGCGTGAAGCTTTGATTTGA
JCT31[ab]	AB512291	$(TC)_{18}$	59	TGGAAAACGAATGAGGCTCT	GGACACTCTGGAAAGGAACG
JCT34[ab]	AB512292	$(GT)_{16}$…$(TA)_{9}$	57	TGACTCAATAAATGTGGACTGG	GGTGCATTCCCAGAAAAAGT
JCT37[ab]	AB512294	$(AG)_{20}$	54	ATTCGACAATCTACGGGATA	CACCTTATACGTCTCTCTCTCTC
JCT53[ab]	AB512297	$(GA)_{19}$	59	AAAGCAATCAACCCAAGAGG	TCTCTCTCTCTCTCTCTCTCTTCTC
JCT59[abc]	AB512295	$(CT)_{10}(CA)_{14}$	60	GGTGACTCCTGAATGCTTGG	TACCCTGAAACTCCCAGGAA
JCT68[abc]	AB512293	$(AG)_{11}$…$(GA)_{7}$…$(A)_{11}(GAAA)_{4}$	60	AGCGATAATCGGCCTACCTT	CAACGTCACTGCCTCCTACC
JCT81[abc]	AB512296	$(CT)_{18}$	54	CCATTTAGAACCACAACCAT	GATGTCCAATAAGCCTGAAT
JCT1[c]	AB525641	$(GC)_{4}(AC)_{5}(AG)_{8}$	54	CAGCAGAAGAGTAAAGGA	GCTTATGGTGTATTGCAA
JCT3[c]	AB525642	$(AG)_{12}$	54	ATCTGCCATCAACCGTA	AACGCGTCACTAAGAGA
JCT5[bc]	AB525643	$(GA)_{7}$…$(GA)_{4}$…$(GA)_{8}$	55	CATGCTAACGATAGAGGA	TTTTCAGCCACTACCTCA
JCT7[c]	AB525644	$(AG)_{11}$	54	CGAAGTGAATGCACAACACA	TGCTATTCAAATGGAACAAGTGA
JCT8	AB525645	$(GT)_{4}$…$(GT)_{4}$	54	ATGTGTATGCTTTGTGCA	GACGAACGCCTAATCGA
JCT10[c]	AB525646	$(TGT)_{4}$…	54	ATATCGAACCATGAACGA	AGCCGTTTATCATTACGA
JCT12[c]	AB525647	$(CT)_{4}$…$(TC)_{5}$	58	TCATAGCCGCAGATCACA	GAGATCGGACGTGGTGA
JCT13[c]	AB525648	$(GA)_{4}$…$(AG)_{4}$…$(GA)_{7}(GT)_{15}$	56	GAACCCTGATAGTGAGGA	GCCGATAGACCATAGACA
JCT14	AB525649	$(AT)_{8}$…$(GT)_{31}$	54	TGCTCCAACTTGAGGAGTGTT	TGTGAATGGGAAACAAGAGTG
JCT18[c]	AB525650	$(GA)_{4}$…$(GA)_{4}$	58	GGAGGAATCAATGAAAGGACA	TGCTTGTTGAACCCTGTGAA
JCT19[c]	AB525651	$(A)_{32}$	60	CATAGGAGCAGGGGTAAACAA	GCCTAGGCCCTGCTAGAGAC
JCT23[c]	AB525652	$(GA)_{17}$	58	ACCAGACCCAGGAAAAGGAC	TGTTGCTGTTGCGATTTCTC
JCT28[c]	AB525654	$(GA)_{15}$	56	CGCAGCCATCTTGAAGGTTA	CAAAATTTCAAGCCATGCTC
JCT31[c]	AB525655	$(TC)_{18}$	58	TGGAAAACGAATGAGGCTCT	GGACACTCTGGAAAGGAACG
JCT32	AB525656	$(AG)_{9}$	56	GCAAATATGTATTACTGGAAAGAAAAA	GAAATGTTTGGCTTTGGATCA
JCT34[c]	AB525657	$(GT)_{16}$…$(TA)_{9}$	54	TGACTCAATAAATGTGGACTGG	GGTGCATTCCCAGAAAAAGT
JCT36[c]	AB525667	$(CT)_{18}$	54	TTCTGATTTGCCCTTTATGT	GAAAATCGCAGAAAAGAAGA
JCT45	AB525668	$(AG)_{8}$	56	AGTCGATTGGTACCCTTTCT	AGACGCTTCTTTTTCCTCTT
JCT47[c]	AB525669	$(CT)_{4}TT(CT)_{12}$	56	GAAGCCTTACTCCCATTTTC	GAAGGCTATGGCAATATGAT
JCT50[c]	AB525670	$(TC)_{5}$…$(TC)_{3}$…$(TC)_{7}$…$(TATC)_{7}$	54	TCCCAAGTCATGATTCAATA	AAGGCCGTTAGAATCTCATT
JCT51[c]	AB525671	$(GA)_{14}$	56	CATGGAATGCATTTGTGTGA	CCTTGACCTTCCTTCCAACA
JCT60[c]	AB525672	$(CT)_{12}$	58	TTGGACAGGCTTTTGTTGTG	GACAGTCAATATTAGGTACTTCAG
JCT74[c]	AB525673	$(TC)_{9}$	58	CGCTTACGAGAAAGAAAATCCA	GGTCAGCTCAGCTCATCTCC
JCT76	AB525674	$(AG)_{12}$	56	ATTTGTTTGCTGTGTGACTG	TCTCAGCTCTCATTTCAGGT
JCT80	AB525675	$(GA)_{17}$	58	TCTCCATCCTGGAGTTTCTA	TGCTGATAAACCACAGATAAAC
JCT86[bc]	AB525676	$(GA)_{17}$	56	TATTTCCTCTTCCTGCACAT	GTTTGGCTAAAAAGGTGATG
JCT89[bc]	AB525677	$(CT)_{16}$	54	GCCGATAAACCACAGATAAA	GAAAAATAAAGCCAGCAAGA
JCT92[bc]	AB525678	$(AG)_{10}AT(AG)_{5}$	56	CACCGCTCATCTATGATTCT	CCAGTGCCAATTTTCTACAT
JCT103[bc]	AB525679	$(AG)_{17}$	54	CAACGACTCTTTGAAGAAAAA	GCCGATAAACCACAGATAAA
JCT106	AB525680	$(TC)_{9}$	56	TAATGCTCTCTTCCCTAAGC	TTCCAGGTTTACACACCTTT
JCT124[c]	AB525681	$(CAA)_{5}$…$(GGA)_{3}$…$(GAGT)_{5}$	58	GTCACCTCGATCACCAAC	GATCTGCGAAAGAGAGAGAA
JCT131	AB525682	$(TC)_{7}CC(TC)_{5}$…$(T)_{39}$	58	CTGAACAGTCCTTTTCATCG	AAGCCCTTATAAAGTTCAAGC
JCT135[c]	AB525683	$(CT)_{8}(CA)_{13}$	58	GGAAAACAGTCCTTCACTTG	GACTAAGGGCAACACTGAAC
JCT136	AB525684	$(AG)_{8}(TG)_{14}$	58	ATGAACCCTGATAGTGAGGA	GCCGATAGACCATAGACAAA
JCT141[c]	AB525685	$(GA)_{12}$	54	TGTTAGTCAATGCAAGAAGGT	TTTGAAACAAGTTTCCTGCT
JGAA1[b]	AB525658	$(GAA)_{9}$	56	AAAGGTCACAGTGTTTCAAAG	TTCTTTCTCAACTTCCTCCA
JGAA28	AB525659	$(CTT)_{8}$	56	TCTTTCTCAACTTCCTCCAA	GGTCACAATGGTTCAAAGTT
JGAA31	AB525660	$(TCT)_{5}$…$(CTT)_{13}$	56	TCTTTCTCAACTTCCTCCAA	GAGGGTGAAGAAGAAAACAA
JGAA35[bc]	AB525661	$(CTT)_{4}$…$(CTT)_{4}$…$(CTT)_{4}$	55	TTCTCCTGCTCCTTCTTCTA	CAAAGGAAAAGCGAAGTTAG
JGAA47	AB525662	$(GAA)_{5}$	56	AAAAGGGGAAAGGAAAATTA	CTTTCTCTATGGCACTTTCC
JGAA53	AB525663	$(AAG)_{5}$…$(GAA)_{4}$	54	GAAAAAGAAGTTGCTGAGGA	CTCCAATTTTCCTTTTCCTT
JGAA55[c]	AB525664	$(GAT)_{11}$…$(GAA)_{5}$	54	TAATGGTATCCGGTATGTGG	CCTTTCCAAATCAACATCAT
JGAA56[b]	AB525665	$(CTT)_{4}$…$(CTT)_{4}$…$(CTT)_{3}$…$(CTT)_{4}$	56	TCAGCAGCACTCTCTTTTTCC	AAGAAAAGAAGGAGGAAGCA
JGAA57	AB525666	$(GAA)_{10}$	54	CGGAGATCGAAGGAGAGGTA	AATCGAACCAAAATGGGCTA

References

Akkak, A., Scariot, V., Torello Marinoni, D., Boccacci, P., Beltramo, C., Botta, R.: Development and evaluation of microsatellite markers in *Phoenix dactylifera* L. and their transferability to other *Phoenix* species. - Biol. Plant. **53**: 164-166, 2009.

Akritidis, P., Mylona, P.V., Tsaftaris, A.S., Polidoros, A.N.: Genetic diversity assessment in greek *Medicago truncatula* genotypes using microsatellite markers. - Biol. Plant. **53**: 343-346, 2009.

Basha, S.D., Sujatha, M.: Genetic analysis of *Jatropha* species and interspecifichybrids of *Jatropha curcas* using nuclear and organelle specific markers. - Euphytica **168**: 197-214, 2009.

Benbouza, H., Jacquemin, J.M., Baudoin, J.P., Mergeai, G.: Optimization of a reliable, fast, cheap and sensitive silver staining method to detect SSR markers in polyacrylamide gels. - Biotechnol. Agron. Soc. Environ. **10**: 77-81, 2006.

Connell, J.P., Sujata, P., Iqbal, M.J., Huizinga, T., Ready, A,S.: A high through-put procedure for capturing microsatellite from complex plant genomes. - Plant. mol. Biol. Rep. **16**: 341-349, 1998.

Doyle, J.J., Doyle, J.L.: Isolation of plant DNA from fresh tissue. - Focus **12**: 13-15, 1990.

Ghosh, A., Chaudhary, D,R., Reddy, M,P., Rao, S,N., Chikara, J., Pandya, J.B.: Prospects for *Jatropha* methyl ester (biodiesel) in India. - Int. J. environ. Stud. **64**: 659-674, 2007.

Hu, J.B., Zhou, X.Y., Li, J.W.: Development of novel chloroplast microsatellite markers for *Cucumis* from sequence database. - Biol. Plant. **53**: 793-796, 2009.

Liu, K., Muse, S,V.: PowerMarker: an integrated analysis environment for genetic marker analysis. - Bioinformatics **21**: 2128-2129, 2005.

Sudheer, P.D.V.N., Nirali, P., Reddy, M.P., Radhakrishnan, T.: Comparative study of interspecific genetic divergence and phylogenic analysis of genus *Jatropha* by RAPD and AFLP. - Mol. Biol. Rep. **36**: 901-907, 2009a.

Sudheer, P.D.V.N., Singh, S., Mastan, S.G., Patel, J., Reddy, M.P.: 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, 2009b.