

Yatavee Rattanamanee 2010: Development of Microsatellite Markers for *Jatropha curcas* L..
Master of Science (Genetics), Major Field: Genetics, Department of Genetics. Thesis Advisor:
Assistant Professor Vipa Hongtrakul, Ph.D. 120 pages.

This research is to develop microsatellite DNA markers for classification and varietal specific identification in physic nut (*Jatropha curcas* L.) from the enrich library using biotin-oligonucleotide probes of B-(GA)₁₅, B-(CA)₁₅, B-(ACC)₁₀ and B-(CCT)₁₀. One hundred and eight positive clones were selected by dot blot hybridization and 97 clones were sent for sequencing. A total of 58 clones (59.79 %) were found to contain microsatellite sequences. Most abundant iterated sequences were dinucleotide repeat of (GA)_n (32.76 %) and trinucleotide repeat of (GGA)_n (20.70 %). Thirty eight primer pairs were designed and were used to evaluate genetic diversity among 125 physic nut samples collected from Thailand and foreign countries. Only five polymorphic markers were obtained and only two alleles per marker were observed. Polymorphic Information Contents (PICs) ranged from 0.15-0.22 with an average PIC score of 0.21. Cluster analysis using computer program NTSYSpc-2.20k was performed and could classify all 125 samples into 2 groups, group I consisted of toxic samples from Thailand and foreign countries and group II was composed of 2 subgroups containing non toxic samples from Mexico. Genetic similarity ranged from 0.72-1.00. Genetic relationship among 67 samples was also estimated using 8 ISSR primers. A total of 96 bands or markers were generated with 16 polymorphic markers observed. PICs ranged from 0.00-0.36 with an average PIC score of 0.06. Cluster analysis could separate all 67 physic nut samples into 2 groups of toxic and non toxic samples. Genetic similarity ranged from 0.91-1.00. AS-PCR (Allele Specific PCR) markers were developed specific for 6 genes in fatty acid synthesis pathway, i.e. *KASIII* (*beta-ketoacyl-ACP synthase III*), *KASII* (*beta-ketoacyl-ACP synthase II*), *SAD* (*stearoyl-ACP desaturase*), *FatA* (*acyl-ACP thioesterase*), *FAD3* (ω -3 fatty acid desaturase) and *FAD6* (ω -6 fatty acid desaturase) based on plant gene sequences available in GenBank. All gene specific markers were used to produce DNA fingerprints of 125 physic nut samples using DFLP (DNA Fragment Length Polymorphism), SSCP (Single Strand Conformational Polymorphism) techniques. The results showed no polymorphism among 125 physic nut samples, suggesting very low genetic variation in this plant. However, the specific markers developed were found to be polymorphic among plants in the genus *Jatropha* (*Jatropha gossypifolia*, *Jatropha integerrima* and *Jatropha multifida*) and *Ricinus communis* which was also in the family Euphorbiaceae. These gene specific markers could be used for hybridity test from both interspecific and intergeneric hybridizations and for the improvement project of physic nut in the future.

Student's signature

Thesis Advisor's signature