

Jirapa Dathong 2012: Development of Molecular Markers for Hybrid Detection in Waterlily (*Nymphae* spp.) and Lotus (*Nelumbo* spp.). Master of Science (Genetics), Major Field: Genetics, Department of Genetics. Thesis Advisor: Assistant Professor Vipa Hongtrakul, Ph.D. 113 pages.

Twelve gene specific markers were developed based on plant gene sequences available in database and used for DNA fingerprinting of 69 lotus (*Nelumbo* spp.) samples collected from Thailand and foreign countries. All 12 specific markers developed exhibited monomorphism when detected in 1% agarose gel (DFLP: DNA Fragment Length Polymorphism technique), but when analysis of the PCR product in non-denaturing polyacrylamide gel (SSCP: Single Strand Conformational Polymorphism technique) resulted in two polymorphic loci, *fruitful* (*ful*) and *AGAMOUS-like* (*AG*). Polymorphic Information Contents (PICs) of these two loci were 0.611 and 0.548, respectively. Cluster analysis using NTSYSpc-2.20k based on DNA variation at the 2 loci (*ful* and *AG*) was performed and could classify all 69 samples into 3 groups. Group I and II consisted of lotus samples from Thailand and foreign countries, while group III was composed of only lotus samples from People Republic of China. Genetic similarity among lotus samples ranged from 0.328-0.748. Twenty six lotus samples out of 31 samples from the intraspecific hybridization were confirmed to be the hybrids using SSCP *ful* gene specific marker. Seven intersubgeneric waterlily (*Nymphaea* spp.) samples were verified by PCR-RFLP (PCR-Restriction Fragment Length Polymorphism) technique. The PCR products of *rRNA* ITS region were cut by *AluI*, *MseI* and *RsaI* restriction enzymes. All 7 samples were confirmed to be hybrids using SSCP ITS specific marker. In addition, 5 gene specific markers, *Apetala 3* (*AP3*), *AGAMOUS-like* protein (*AGL*), *Leafy* (*LFY*), *Pistillata* (*PI*) and *Sepallata1* (*SEP1*), were developed based on waterlily genome. Only *LFY* and *PI* gene specific markers were polymorphic based on DFLP technique. PIC scores of these two loci were 0.444 and 0.152, respectively. Using SSCP technique with 12 waterlily samples, *AP3*, *LFY*, *PI*, *SEP1* and ITS of *rRNA* gene specific markers were found to be polymorphic. PIC scores of *AP3*, *LFY*, *PI*, *SEP1* and ITS of *rRNA* specific markers were 0.219, 0.607, 0.607, 0.468 and 0.290, respectively. DNA fingerprints of 12 waterlilies based on 5 polymorphic markers were analyzed using NTSYSpc-2.20k and could classify all samples into 2 groups. Group I consisted of subgenus of *Nymphaea* and their hybrids and group II consisted of subgenus of *Brachyceras*. Genetic similarity was ranged from 0.333-0.933. Markers specific to subgenus *Nymphaea* and subgenus *Brachyceras* (hybrid parent) were developed by sequencing of AFLP (Amplified Fragment Length Polymorphism) subgenus specific DNA bands. All gene specific and subgenus specific markers developed could be used to verify all 7 intersubgeneric hybrids and could be used for hybrid detection in waterlily breeding program in the future.

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Thesis Advisor's signature