

Choladda Sae-Eung 2012: Analysis of DNA Methylation Pattern Involving Sex Determination Mechanism of *Cycas* in Thailand using Methylation-Sensitive Amplification Polymorphism (MSAP) Technique. Master of Science (Genetics), Major Field: Genetics, Department of Genetics.
Thesis Advisor: Assistant Professor Vipa Hongtrakul, Ph.D. 99 pages.

DNA methylation profiles of ten *Cycas* species and one subspecies localized in Thailand were studied using the methylation sensitive amplification polymorphism (MSAP) technique. Eleven MSAP primer combinations were used and 720 MSAP bands were generated. The percentages of DNA methylation estimated from MSAP fingerprints were in the range of 36.95% to 57.44%. MSAP fingerprints were also used for phenogram construction and principal component analysis, based on nucleotide polymorphism and methylation polymorphism. Genetic similarity estimated from nucleotide polymorphism among these cycads ranged from 0.86 to 0.92, whereas genetic similarities of methylation polymorphism estimated from *EcoRI-MspI* and *EcoRI-HpaII* data set ranged from 0.78 to 0.94 and 0.79 to 0.95, respectively. Cluster analysis using nucleotide polymorphism and methylation polymorphism resulted in different groupings for some cycads. The results showed that polymorphism of *Cycas* in Thailand were not only limited in the nucleotide level, but also in the pattern of DNA methylation. It was proposed that the percentage and pattern of DNA methylation as well as nucleotide polymorphism were sources of variation in these cycad species. The total of 720 methylation markers was also used in methylation analysis between sexes. The results showed that percentage of DNA methylation were not statistically significant between male and female cycads. DNA methylation pattern in male and female cycads were compared based on genetic similarity from MSAP bands. The results revealed similarity around 60 to 70% between male and female in all cycads, indicating methylation patterns around 30 to 40% were different between sexes. Nineteen sex-specific MSAP bands were cloned and sequenced. Similarity search of majority of polymorphic bands using NCBI BLAST resulted in unknown sequences, while some sequences did match the known genes, reported to be involved in reproductive development. The present study showed the differences of DNA methylation between male and female *Cycas*. The possible role of DNA methylation in sex determination of *Cycas* was discussed. However, the further intensive study should be applied.

Student's signature

Thesis Advisor's signature