Chanpen Boontong 2007: Development of microsatellite Markers in Indian Neem (*Azadirachta indica* A.Juss. var. *indica*) and Thai Neem (*Azadirachta indica* A.Juss. var. *siamensis*). Master of Science (Genetic Engineering), Major Field: Genetic Engineering, Interdisciplinary Graduate Program.
Thesis Advisor: Associate Professor Suree Bhumibhamon, D.F. 91 pages.

The neem tree (*Azadirachta* spp.) has multiple uses throughout its tropical and subtropical distribution with many useful products derived from nearly every part of the plant such as azadirachtin in seeds used as pest managements. Due to its potentials and wide natural distribution in Thailand, neem has been established for plantation and breeding for genetic improvement. Despite its high medicinal and cultural values there exists very few knowledge about the genetics of this species. For the purpose of conserving genetic resources of the species investigation of its genetic diversity and reproductive biology are essential. In order to study the population genetics of this species, highly polymorphic codominant markers like microsatellites are necessary. Therefore, the objective of this study was to develop polymorphic microsatellite markers for neem.

To develop the microsatellite markers in neem, the genomic DNA fragments were hybridized with oligonucleotide $(CT)_{10}$ and the fragments containing microsatellites were separated magnetically by using MagneSphere? . According to the sequence analysis, the results showed that out of 68, 47 (69.1 %) of clones from the genomic libraries contained microsatellite motifs. Out of 47 fragments, 42 fragments contained one motif and other 5 fragments contain two motifs. Out of 52 motifs comprising of 35 (67%) AG/TC, 12 (23%) TG/AC and 5 (10%) TA/AT.

Out of 47 microsatellite fragments, 26 pairs of primer could be designed to amplify DNA containing microsatellite. However, only 8 primer pairs could be used to amplify which were polymorphic DNA products. The average number of alleles per locus, observed heterozygosity and expected heterozygosity at all loci of Indian neem and Thai neem were 6.625, 6.125, 0.4639, 0.7674, 0.4193 and 0.6994, respectively. The cluster analysis by UPGMA showed Indian neem and Thai neem were grouped separately. Test of Hardy-Weinberg equilibrium showed the highest significant departure from the equilibrium in both neem populations at locus NpCT_14. F_{st} values of in Indian neem and Thai neem were 0.2152 and 0.1851, respectively. The implications of these results for differentiation of Indian neem and Thai neem are also discussed.