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TITLE: Population Genetic Analysis of *Xylia xylocarpa* (Roxb.) W. Theob. var. *kerrii* I. C. Nielsen in Thailand

NAME: Mr. Tanat Wattanakulpakin

THIS THESIS HAS BEEN ACCEPTED BY

THESIS ADVISOR

(Associate Professor Siriluck Iamtham, Ph.D.)

THESIS CO-ADVISOR

(Mr. Hugo Volkaert, Ph.D.)

GRADUATE COMMITTEE CHAIRMAN

(Miss Laksana Kantama, Ph.D.)

APPROVED BY THE GRADUATE SCHOOL ON _____

DEAN

(Associate Professor Gunjana Theeragool, D.Agr.)

THESIS

POPULATION GENETIC ANALYSIS OF *Xylia xylocarpa* (Roxb.) W. Theob. var. *kerrii* I. C. Nielsen IN THAILAND

TANAT WATTANAKULPAKIN

A Thesis Submitted in Partial Fulfillment of
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Tanat Wattanakulpakin 2014: Population Genetic Analysis of *Xylia xylocarpa* (Roxb.) W. Theob. var. *kerrii* I. C. Nielsen in Thailand. Master of Science (Economic Botany), Major Field: Economic Botany, Division of Science. Thesis Advisor: Associate Professor Siriluck Iamtham, Ph.D. 130 pages.

A study of the genetic diversity in populations of *Xylia xylocarpa* (Roxb.) W. Theob. var. *kerrii* I. C. Nielsen in Thailand was performed using molecular markers to reveal differentiation within and between populations and the geographical distribution of alleles. Samples were collected from individual trees in 14 National Parks and 2 community forests. DNA was isolated from leaves and 8 single locus gene markers were used to identify different alleles using PCR – SSCP combined with sequencing. For easier detection and differentiation of some alleles, 15 SNPs were converted into CAPS assays and 5 SNPs were converted to AS-PCR assays. From 8 to 34 alleles were identified for the different loci though the effective number of alleles ranged between 1.1 and 7.6. Several loci showed deviation from Hardy-Weinberg equilibrium with the CAT2 locus having the most significant deviations. Genetic distances calculated according to Nei (G_{st}) and Jost (D_{est}) indicated the largest genetic distances between Pang Sida National Park and Mae Ngao National Park. Direct sequencing of PCR amplified fragments revealed several alleles in addition to those distinguished by SSCP. The alleles found among *Xylia xylocarpa* differed from one another due to one or just a few substitution polymorphisms. Unexpectedly, more non-synonymous than synonymous substitutions were found in the exons of the genes. STRUCTURE revealed very weak support for population differentiation in two or maybe three subgroups. Only the most western populations (LamKhlongNgu, Erawan, MaeNgao, ThaTaFang) seemed to be slightly separated from the others in STRUCTURE and in principal component analysis. Different pollination syndromes and seed dispersal mechanisms may explain this difference in population structure between *Xylia* and teak, where clear geographically-linked population genetic structure can be observed.

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Student's signature Thesis Advisor's signature

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LIST OF ABBREVIATIONS

ASPE	=	allele-specific primer extension
bp	=	base pair
˚C	=	degrees (Celsius)
cm	=	centimeter
DH	=	direct hybridization
DNA	=	deoxyribonucleic acid
dNTP	=	deoxynucleotide triphosphate
F	=	forward (PCR primer)
g	=	gram
hr	=	hour
Hz	=	Hertz
IPTG	=	Isopropyl β-D-1-thiogalactopyranoside
LB	=	lysogeny broth, Luria-Bertani broth
µl	=	microliter
µg	=	microgram
m	=	meter
mg	=	milligram
min	=	minutes
NA	=	not available
mm	=	millimeter
mM	=	millimolar
ng	=	nanogram
OL	=	oligonucleotide ligation
PCR	=	polymerase chain reaction
pmole	=	picomole
R	=	reverse (PCR primer)
RFD	=	Royal Forestry Department
rpm	=	rotation per minute
SBE	=	single-base extension
sec	=	Second

LIST OF ABBREVIATIONS (Continued)

SSCP	=	Single strand conformation polymorphism
t	=	ton
T_A	=	annealing temperature
TBE	=	Tris/Borate/EDTA
V	=	Volt
W	=	Watt
X-gal	=	5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside

POPULATION GENETIC ANALYSIS OF *Xylia xylocarpa* (Roxb.) W. Theob. var. *kerrii* I. C. Nielsen IN THAILAND

INTRODUCTION

Forest cover in Thailand has decreased dramatically in recent times. According to an official estimate, current forests cover is 26 % of the country, down from 53 % in 1961 (Bhumibhamon, 1986). In addition, large areas of the remaining forest have been partly degraded. Forest degradation has been blamed for occasional severe flash floods in mountainous areas in the south and north of the country. Concern about the decrease in forest cover has lead to the issuance of a complete logging ban in 1989. More recently, the awareness about the threat of global warming has heightened the interest in preservation of the remaining forests and in replanting trees. To benefit from the potential of CO_2 sequestration through reforestation, it would be useful to have knowledge about the diversity of forest tree populations, their adaptation to the local environment and their responses to changing climate conditions.

Xylia xylocarpa (Roxb.) W. Theob. var. kerrii Nielsen**,** vernacular name 'Daeng' (แดง) or 'Maidaeng' (ไม้แดง), is a long living, medium to large size tree of the monsoon deciduous forests in Southeast Asia. It occurs mixed with teak (*Tectona grandis*), *Pterocarpus*, *Afzelia*, *Lagerstroemia* and other species. The species belongs to the family Fabaceae (Leguminosae) – Mimosoidaea. In Thailand it can be found in the North, Central and Northeastern parts of the country (RFD, 1993).

The reddish brown wood is hard and durable and makes excellent material for all kind of construction, boats, railway sleepers, furniture, turnery, and household implements. *Xylia* can be established in plantations, though at present it is rarely planted. However, forest managers in Malaysia have begun to take an interest in growing *X. xylocarpa* because it has no problem with diseases and insects. It is hoped that in Thailand people should turn to take an interest in its afforestation.

Molecular genetic analysis techniques are now widely used to study genetic diversity in many organisms. This work aimed to develop specific nuclear DNA markers and use them for studying the genetic diversity of *X. xylocarpa* within and between populations in its natural range in Thailand. Tissue samples were collected from trees in various locations. Polymorphism between *X. xylocarpa* populations was studied by PCR-SSCP technique and DNA sequencing. Bayesian analysis of the population genetic data was applied to detect population structure within *X. xylocarpa* from Thailand.

The molecular marker and population genetic structure information that has been obtained from this study may be used as an efficient tool for conservation of genetic resources of *X. xylocarpa* and in breeding programs.

OBJECTIVES

1. To develop nuclear DNA markers for *Xylia xylocarpa*

2. To study the genetic diversity and population structure of *X. xylocarpa* in Thailand

Literature Review

Botanical description

The genus *Xylia* (haploid chromosome number = 12) belongs to the family Fabaceae (Leguminosae), subfamily Mimosoideae and contains about 12 species mostly from Africa and Madagascar. Only one species occurs in Southeast Asia: *X. xylocarpa*. This species may be divided into two morphological varieties: *Xylia xylocarpa* var. *xylocarpa* from India and Myanmar with almost glabrous leaflets and anthers with glands, and *Xylia xylocarpa* (Roxb.) W. Theob. var. *kerrii* I. C. Nielsen from Myanmar, Thailand and Indo-China with hairy leaflets and anthers without glands (Ba, 1998). Vernacular names include "daeng" (แดง), "maidaeng" (ไม้แดง) (Thailand), "pyinkado" (Burma), "so-kra:ch" (Cambodia), "deng" (Laos), "c[aw]mxe" in Vietnam, and "irul" in Kerala, India (Ba, 1998).

X. xylocarpa is a medium to large deciduous tree, in dry areas usually up to about 20- 25 m tall; old trees on moist and fertile sites occasionally reach up to 40 m. The bole is often straight and cylindrical albeit sometimes with buttress development and somewhat fluted. The bark is flaky, grey to light yellow-brown or pinkish, peeling off into irregular fragments. Leaves are spirally arranged, bipinnately compound with 1 pair of pinnae, each with 3-6 pairs of opposite oval leaflets, largest terminal pair of leaflets up to 10-15 cm long and 5-6 cm wide; basal leaflets much smaller (Figure l). Each leaflet has a basic gland (Ba, 1998; Schmidt, 2004). The flowers are small, pale yellow, in dense spherical heads, 1.5-2 cm, solitary or in very short, unbranched clusters in axils of fallen leaves. Head stalks 3.5-5 cm, individual flowers without stalks. 5 petals, 3.5-4.5 mm, slightly fused at base, hairy outside. 10-12 free stamens, 5-12 mm, much longer than petals, 5 stamens longer than others, anthers without glands (Gardner *et al.,* 2000). The flowers are hermaphroditic or male. Main flowering is normally before or just after leaf flushing.

The fruit is a flat, dehiscent, compressed, woody pod, 10-15 cm long and 5 - 6 cm wide, containing 5-10 seeds. The pod is light brown with prominent cross nervation outside and glossy red-brown inside; curling up when dry (Schmidt, 2004).

The seed is often round or ellipsoid, compressed, glabrous, light to dark brown, 7.5 - 9 mm wide, 12 - 14 mm long, and 1.5 - 2 mm thick. The pleurogram (a U-shaped or elliptic fracture line surrounding the areole on the side of the seed in Mimosoideae or *Cassia*) is inconspicuous and same shape as the seed (Schmidt, 2004). Seed weight is 245- 260 grams per 1000 seed, or about 4000 seeds per kg. The seedling germinates epigeal with emergent cotyledons and an elongated hypocotyl. The first pair of leaves is opposite, with 1(-2) pairs of leaflets, some 3-foliolate, while the subsequent leaves are arranged spirally (Ba, 1998).

Reproductive success is variable from site to site and year to year. In poor seed years, pods may develop without or with very few developed seed (Schmidt, 2004).

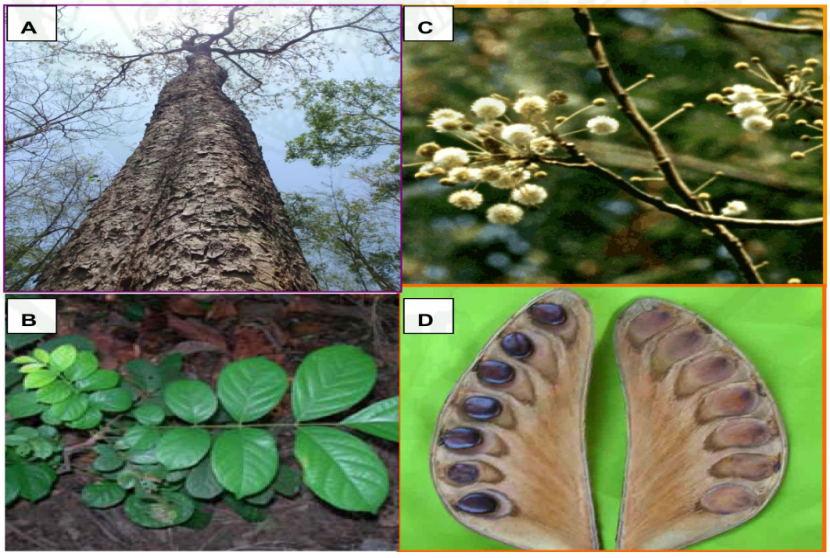

Figure l *X. xylocarpa* is a medium to large deciduous tree (A). Leaves are bipinnately compound (B). Flowers are small, pale yellow, clustered in dense spherical heads (C). Woody pods with round or ellipsoid seed (D).

Ecology

X. xylocarpa occurs in dry evergreen forest, mixed deciduous forest and dry deciduous dipterocarp forest, on well-drained, sandy and rocky soils, up to 850 m altitude (Ba, 1998; Gardner et al., 2000). It occurs often together with teak (*Tectona grandis* L. f.), *Lagerstroemia*, *Pterocarpus macrocarpus*, *Dalbergia* spp, *Afzelia xylocarpa, Dipterocarpus alatus, Dipterocarpus obtusifolius*, and *Tetrameles nudiflora*.

Saelim (1997) studied differences in response of *X. xylocarpa* seedlings to high temperature stress. Different seed sources grown under different growth temperatures showed similar photosynthesis and thermotolerance of seedlings acclimated to high temperature was correlated with changes in protein expression. Fresh seed needs no pretreatment but dried, stored seeds need pre-treatment for imbibition and germination to take place. Pretreatment can be done by a quick dip in 70-80 (-95)°C warm water and left to cool in the water, or manual scarification (clipping, filing, burning) (Schmidt, 2004).

Flowering occurs right before or during leafing out at the beginning of the hot season (from second half of February through March). At that time the flowers are heavily visited by bees, stingless bees and other insects.

Seed collection and germination

Fruits can be harvested when they have turned dry and yellow and the first fruits have started to open. Seeds should have lost their greenish immature colour. Fruits may be harvested directly from the tree before dehiscence, as the seeds are lost when the pods open. Harvesting implies climbing or use of long handled tools. The peduncle breaks relatively easily when the pods are about mature. Where the ground can be cleaned reasonably well from vegetation, the seeds can be collected on nets or tarpaulins under the trees or mounted as funnels. However, for large trees seeds may

be dispersed quite far away from the trunk, so there is likely to be a large loss by that method (Schmidt, 2004).

Harvested fruits are dried in the sun to dehisce. Often a relatively strong drying is required. The pedicel attachment is not strong, so the seeds are released when the pods open. The pods are removed manually. Seed can be cleaned by screening and blowing; it is easy to achieve a high purity (Schmidt, 2004).

The seed is orthodox and poses little problems when stored dry (< 8-10% moisture content) and cool in airtight containers. Reduction of moisture content to about 3% is possible by additional sun-drying. Seeds can be stored for > 3 years at ambient temperature although storage at ambient temperature may cause insect problems (Schmidt, 2004).

Best sowing medium is sandy loam. The seeds will start germination after 3-4 days and germination is complete with most seedlings of a seed-lot with unfolded first pair of leaflets after 5-8 days (Schmidt, 2004). The use of coconut husk as the covering media resulted in the highest germination percentage (Sawantarat, 2003). The germination rate of the seed can vary between different seed collections (Wangeiad, 2003), but it is unclear whether these differences are due to seed quality or genetic differences between sources.

Uses of *Xylia xylocarpa*

The wood is hard and durable, moderately resistant to termites and makes excellent material for all kind of construction, boats, railway sleepers, furniture, turnery, and household implements. Though it is difficult to work and plane and has a serious blunting effect on tools, a good finish can be obtained. Green stock, however, is easier to saw. The wood properties of nine-year-old *X. xylocarpa* planted at the Luasong Forestry Centre, Tawau, Sabah ($117^{o}23'$E and $4^{o}36'$N) were almost comparable to a number of more mature local popular heavy hardwood species. The location of the plantation is approximately 132 m above sea level, and the mean

annual rainfall is more than 2000 mm. The seedlings were line-planted at an initial spacing of 3 m x 3 m (Josue, 2004).

Charcoal made from branches, has a calorific content of 7,384 calories/gram (RFD, 1993).

Large supplies of timber of *X. xylocarpa* are available, particularly in Burma. At the beginning of the 1980s the annual production of logs in Burma was about 20,000 t (Ba, 1998). Thailand imported about 1,252 cubic meter of *X. xylocarpa* timber worth 5,274,000 baht in 1979 (RFD, 1993).

Bark and fruits are used in traditional medicine (Ba, 1998). In a study of ethanolic extracts from 20 species of Mimosoideae, *Xylia xylocarpa* pericarp, seed, leaves and bark extract showed the highest ability to scavenge free radicals with half maximal inhibitory concentration (IC_{50}) less than 0.1 μg/ml (Ramli *et al.,* 2008). Moreover, the stem, leaves, branch and bark of *Xylia xylocarpa* also displayed high α-glucosidase inhibitory activity (Tunsaringkarn *et al.,* 2008) which indicates that this species should be further studied for the treatment diabetes mellitus (type II) as the glucosidase inhibitor is of interest for lowering of blood glucose level**.**

Mature *Xylia xylocarpa* seeds contained 29.5% crude protein, 14.78% crude fat, 8.02% crude fibre, 5.11% ash and 42.6% crude carbohydrates (Siddhuraju *et al.,* 1995). The seeds appeared to be a good source of potassium, magnesium, phosphorus and iron. The major components of the total seed proteins were globulins and albumins; the latter constituting 32% of the total extractable proteins. The seed lipids contained a high proportion of unsaturated fatty acids with linoleic acid (51.3%) as the predominant fatty acid. The total seed proteins were rich in most of the essential amino acids and they were deficient only in cysteine and methionine (Siddhuraju *et al.,* 1995).

X. xylocarpa, like any tree species, are sinks of CO_2. CO_2 and other greenhouse gases absorb and emit radiation within the thermal infrared range. More

recently, the awareness about the threat of global warming has heightened the interest in preservation of the remaining forests and in replanting trees. To benefit from the potential of CO_2 sequestration through reforestation, it would be useful to have knowledge about the diversity of forest tree populations, their adaptation to the local environment, and their responses to changing climate conditions. The estimated biomass (carbon) allocation was 7.8% in the leaves, 22.2% in the branches, 52.2% in the bole and 17.8% in the roots. Aye *et al.,* (2011) discovered that the biomass of the trees in a *X. xylocarpa* plantation (80.4 tons ha^{-1}) was higher than that in a *Pterocarpus macrocarpus* plantation (77.2 tons ha^{-1}). Likewise, the mean annual increment of the carbon content in the *X. xylocarpa* plantation was estimated at about 2.7 tons ha^{-1} while the *P. macrocarpus* plantation accounted for 2.5 tons ha^{-1}. This study suggested that it is very important in the management of plantations to focus not only on the planted trees but also on the undergrowth vegetation, litter layer and soil layer, which play a significant role in the stand-level carbon content.

Silviculture of *Xylia xylocarpa*

X. xylocarpa is a native tree of local economic importance. At present, the Royal Forest Department has established only a few *Xylia* plantations and there is not much interest from the private sector because the return on the investment is considered low when compared to others tree species that are faster growing, teak in particular. However in Malaysia foresters have begun to take an interest in growing *X. xylocarpa* because it has no problem with diseases and insects. The private sector in Thailand should take an interest in afforestation with this species as the wood is valuable (RFD, 1993).

Molecular Markers

DNA (deoxyribonucleic acid) is organized in pairs of chromosomes, each inherited from one of the parents in diploid organisms. Genes are scattered along chromosomes, separated by long, mainly repetitive, DNA sequences. Genes consist of coding sequences (exons) separated by introns and surrounded by regulatory DNA

sequences. Phenotypic diversity among organisms is a result of variations in DNA sequences and of environmental effects. DNA variations are mutations resulting from substitution of single nucleotides (single nucleotide polymorphisms – SNPs), insertion or deletion of DNA fragments of various lengths, or duplication or inversion of DNA fragments. DNA variations are classified as "neutral" when they cause no change in metabolic or phenotypic traits, and hence are not subjected to positive, negative, or balancing selection; otherwise, they are referred to as "functional selective". Mutations in key nucleotides of a coding sequence may change the amino acid composition of a protein and lead to new functional variants. Such variants may have an increased or decreased metabolic efficiency compared to the original "wild type", may lose their functionality completely, or alternatiuely gain a novel function. Mutations in regulatory regions may affect levels and patterns of gene expression; for example, turning genes on/off or under/over-expressing proteins in specific tissues at different development or physiological stages (FAO, 2007)

Genetic polymorphism is defined as the simultaneous occurrence of two or more discontinuous variants (alleles or genotypes) of a trait in the same population. Genetic markers are gene or DNA sequences with a known location on a chromosome that can be used to identify individuals or species. DNA markers are useful in phylogenetic analysis and can be applied for marker assisted selection, paternity testing, and food traceability. Although DNA sequencing is a straightforward approach for identifying variations at a locus, it is expensive and laborious. A wide variety of techniques have, therefore, been developed for obtaining DNA sequence polymorphism information.

DNA marker technologies

Restriction fragment length polymorphisms (RFLP) are variations in the DNA sequence that can be detected by breaking the DNA into fragments with restriction enzymes, separating the fragments by gel electrophoresis, and detection of individual fragments hybridizing to particular DNA probes. RFLP analysis can identify DNA sequence substitution differences when they occur within a restriction enzyme

recognition site and can identify insertion/deletion mutations when they occur between conserved recognition sites. Analysis of RFLP variation was an important tool in genome mapping, localization of genetic disease genes, determination of risk for a disease, genetic fingerprinting, and paternity testing *(Saiki* et al., 1985)**.**

The term DNA-fingerprinting was introduced by Jeffrey *et al*. (1985) to describe barcode-like DNA fragment patterns generated by multilocus probes after electrophoretic separation of genomic DNA restriction enzyme fragments. Composed of short sequences that are repeated in tandem, the multilocus probes reveal regions are that are hypervariable, primarily due to the variation in the number of repeat units at each locus. Polymorphisms for minisatellite loci are detected by cutting genomic DNA with a 4-cutter restriction enzyme, separating resulting fragments by agarose gel electrophoresis, Southern blotting to nylon, and hybridizing to repeat sequence probes to identify fragment length differences that arise from variation in repeat number. The emerging patterns make up a unique feature of the individual and are currently considered to be the ultimate tool for biological individualization. More recently, the term DNA fingerprinting/profiling is also used to describe the combined use of several single locus detection systems and is being used as versatile tools for investigating various aspects of plant genomes. These include characterization of genetic variability, genome fingerprinting, genome mapping, gene localization, analysis of genome evolution, population genetics, taxonomy, plant breeding, and diagnostics.

Random Amplification of Polymorphic DNA (RAPD) is a type of PCR reaction, but the segments of DNA that are amplified are not predetermined. The PCR is performed using genomic DNA template and a single arbitrary, short primer (8-12 nucleotides), expecting that some fragments will amplify. By resolving the resulting patterns, a semi-unique profile can be gleaned from a RAPD reaction (Williams *et al.,* 1990).

Amplified fragment length polymorphism is a PCR-based method used in genetic research and DNA fingerprinting. It has many advantages when compared to

other marker technologies including RAPD, RFLP, or microsatellites. AFLP not only has higher reproducibility, resolution, and sensitivity at the whole genome level compared to other techniques (Mueller and Wolfenbarger, 1999), but it also has the capability to amplify between 50 and 100 fragments at one time. In addition, no prior sequence information is needed for amplification (Meudt and Clarke 2007). As a result, AFLP has been applied to the study of taxa including bacteria, fungi, and plants, where much is still unknown about the genomic makeup of various organisms. However AFLP are very not well defined as the type of mutation is unknown.

Microsatellites, or Simple Sequence Repeats (SSRs), are polymorphic loci present in nuclear and organellar DNA that consist of repeating units of 1-6 base pairs in length (Condit and Hubbell, 1991). They are typically neutral, multi-allelic and are used as molecular markers which have wide-ranging applications in the field of genetics, including kinship and population studies. Most microsatellite loci are located in genomic regions of unknown function which are generally highly polymorphic. Null alleles, where some sequence variants do not amplify in PCR, are a major concern for microsatellite scoring and could lead to erroneous population genetic analysis (Chapuis and Estoup, 2006). SSRs have a very high resolution at the individual locus.

Single strand conformation polymorphism (SSCP), is a powerful and rapid technique for DNA fragment analysis particularly for detection of point (Orita *et al.,* 1989). SSCP can identify heterozygosity of DNA fragments of the same length and in many instances can even detect mutations of a single nucleotide base as the mobility of the single-stranded DNA conformation changes depending on the exact DNA sequence. To overcome problems of re-annealing and complex banding patterns, an improved technique called asymmetric-PCR SSCP was developed (Ainsworth *et al.,* 1991), wherein the denaturation step was eliminated and a large-sized sample could be loaded for gel electrophoresis, making it a potential tool for high throughput DNA polymorphism. It was found useful in the detection of heritable human diseases. In plants, however, it is not well developed although its application in discriminating progenies can be exploited, once suitable primers are designed for agronomically

important traits (Fukuoka *et al.,* 1994). Such consensus degenerate primers may be convenient if the same gene is to be amplified from different organisms as the genes themselves are probably similar but not identical. The other use for degenerate primers is when primer design is based on protein sequence. As several different codons can code for one amino acid, it is often difficult to deduce which codon is used in a particular case. Use of degenerate primers can greatly reduce the specificity of the PCR amplification (Gibson and Muse, 2004).

Kuhn *et al.,* (2008) correlated the estimation of genetic diversity and genetic distance in a population or germplasm collection when measured by 13 highly polymorphic microsatellite markers or 20 SSCP markers. A significant correlation in pairwise genetic distances of 82 individuals in an international cacao germplasm collection was observed. Both sets of markers could distinguish each individual in the population. These data provide strong support for the use of SSCP markers in the genotyping of plant species where development of microsatellites would be difficult or expensive. SSCP markers are PCR-based and scored by electrophoretic mobility but, because they are based on SNPs rather than length differences, occur more frequently and are easier to develop than microsatellites.

The genetic analysis of known single nucleotide polymorphisms (SNPs) can be achieved by various means, including restriction fragment length polymorphism analysis (RFLP, Botstein *et al.,* 1980) and the related PCR-based version of this technique, cleaved amplified polymorphic sequence (CAPS) analysis (Konieczny and Ausubel, 1993), allele-specific PCR, oligonucleotide ligation, or microarrays.

CAPS polymorphisms are differences in restriction fragment lengths caused by SNPs or INDELs that create or abolish restriction endonuclease recognition sites in PCR amplicons produced by locus-specific oligonucleotide primers. In the derived cleaved amplified polymorphic sequences (dCAPS) assay a mismatch in one of the PCR primers is used to create a restriction endonuclease (RE)-sensitive polymorphism based on the target mutation. This technique is useful for genotyping known mutations and genetic mapping. Similar to the CAPS technique, this method is

simple, relatively inexpensive, and uses the ubiquitous technologies of PCR, restriction digestion and standard agarose gel electrophoresis (Neff *et al.,* 1998).

Allele-specific PCR is a variation of the polymerase chain reaction which is used as a diagnostic or cloning technique, to identify or utilize single-nucleotide polymorphisms (SNPs) (single base differences in DNA). AS-PCR requires the sequence of the target DNA fragment, including differences between the alleles. It uses a primer whose 3' ends encompasses the SNP in addition to a primer some distance away. PCR amplification under stringent conditions is much less efficient in the presence of a mismatch between template and primer, so successful amplification with a SNP-specific primer signals presence of the specific SNP in a sequence (Saiki *et al.,* 1986)

Other methods to genotype SNPs include single-base extension (SBE), allele-specific primer extension (ASPE), oligonucleotide ligation (OL), and direct hybridization (DH). Lee *et al,* (2004) compared the accuracy, efficiency, and cost of single-base extension (SBE), allele-specific primer extension (ASPE), oligonucleotide ligation (OL), and direct hybridization (DH). All four assay methods used the same Luminex 100 flow cytometer platform. Fifty-eight F2-derived soybean [*Glycine max* (L.) Merr.] lines from a cross between inbred lines G99-G725 and N00-3350 were genotyped at four SNPs. On the basis of cost and labor, ASPE is more cost-effective and simpler than SBE, and would therefore be a good method for genetic mapping and diversity studies which require a large number of markers and a high level of multiplexing. DH appears to be the most economical assay for marker-assisted selection, though optimization for DH would be required for some SNP markers.

Osman *et al.,* (2003) used DOP primers (a variant of RAPD) to amplify DNA fragments in *Eurycoma longifolia* and detected SNPs by direct sequencing of resulting PCR products. They found that the occurrence of these SNPs reflects the geographic origins of individual plants and can distinguish different natural populations. Thus they demonstrated the rapid development of molecular genetic markers in a species for which little or no genomic sequence information is available.

Genetic diversity

Genetic diversity refers to the variation at the level of heritable characters (polymorphism) and provides a mechanism for populations to adapt to their ever changing environment. The more variation, the higher the chance that at least some of the individuals will have an allelic variant that is suited for the new environment, and will produce offspring with the variant and will in turn reproduce and continue the population into subsequent generations. When a new allele appears in a population, it has the potential to change the genetic make-up of successive generations. Harmful mutations will likely not persist because the affected individual will either not survive, or will have limited reproductive success. However, some mutations may be passed on to successive generations because an organism with that allele is better equipped to survive in its environment, that is, it has a selective advantage. Those individuals that produce a greater number of offspring that survive are said to be more fit. Other mutations may have no effect on phenotype, and may persist simply by chance (genetic drift). It is the selective advantage that drives evolution, albeit momentarily, in one direction or another (Russell, 2003).

Population genetic Analysis

Several parameters which indicate genetic diversity within a population and between populations can be estimated.

The number of alleles per locus: the count of the number of alleles at each locus in each sample and in the overall population. To obtain the average number of alleles per locus, the total number of alleles is divided by the number of loci.

Determination of allele frequencies: the allele frequencies in each sample and overall average can be estimated. The overall allele frequencies can be presented either by weighted by sample size or non-weighted frequencies.

Allelic richness (estimate allelic richness per locus and sample (*Rs*))

Allelic richness is the mean number of alleles per locus, and includes monomorphic loci (Lowe *et al.,* 2006). El Mousadik and Petit (1996) proposed to estimate the number of alleles expected in a sample of specified size using rarefaction. The principle is to estimate the expected number of alleles in a subsample of 2n gene, given that 2N genes have been sampled. n is fixed as the smallest number of individuals carrying a locus in a sample. Allelelic richness is then a calculated as:

$$R_s = \sum_{i=1}^{n_{ij}} \left(1 - \frac{\binom{2N - N_i}{2n}}{\binom{2N}{2n}} \right)$$

Where

Ni = the number of alleles of types i among the 2N genes.

n = number of individuals in smallest sampled population

N = the number of samples across all populations

The effective number of alleles (ENA) equals the number of equally frequent alleles it would take to achieve a given level of gene diversity. It allows one to compare populations where the number and distribution of alleles differ drastically. effective number of alleles is then a calculated as:

$$ENA = 1 / (\Sigma p_i^2)$$

Where

Σp_i^2 = the sum of the squared population allele frequencies.

Heterozygosity

Heterozygosity is a measure of heterozygote frequencies per locus. It refers to the fraction of loci within an individual that are heterozygous. It is normally used to refer to the population as a whole. Typically, the observed (*Ho*) and expected (*He*)

heterozygosities are compared, defined as follows for diploid individuals in a population:

Observed heterozygosity

$$H_0 = \frac{\sum_{i=1}^{n} (1 \text{ if } a_{i1} \neq a_{i2})}{n}$$

Where

n = the number of individuals in the population

a_{i1} and a_{i2} = the alleles of individual *i* at the target locus

Expected heterozygosity

$$H_e = 1 - \sum_{i=1}^{m} (f_i)^2$$

Where

m = the number of alleles at the target locus

fi = the allele frequency of the *ith* allele at the target locus

The Hardy–Weinberg principle states that allele and genotype frequencies in a population will remain constant from generation to generation in the absence of evolutionary influences. These influences include non-random mating, mutation, selection, genetic drift, gene flow and meiotic drive. Because one or more of these influences are typically present in real populations, the Hardy–Weinberg principle

describes an ideal condition against which the effects of these influences can be analyzed. In the simplest case of a single locus with two alleles denoted A and B with frequencies freq(A) = p and freq(B) = q, the expected genotype frequencies are freq(AA) = p^2 for the AA homozygotes, freq(BB) = q^2 for the BB homozygotes, and freq(AB) = 2pq for the heterozygotes. The genotype proportions p^2, 2pq, and q^2 are called the Hardy-Weinberg proportions. [Note that $(p + q) = (p + q)^2 = p^2 + 2pq + q^2 = 1$].

If the union of gametes to produce the next generation is random, it can be shown that the new freq(B') = $q^2 + pq = (q)(q + p) = q$. That is, allele frequencies are constant between generations. This principle was named after G. H. Hardy and Wilhelm Weinberg, who first demonstrated it mathematically.

Deviations from HW equilibrium can be calculated and tested for significance using Genepop (Raymond and Rousset, 1995). GENEPOP uses an exact test of significance instead of the Chi square test.

Analysis of Molecular Variance (AMOVA) is a method of estimating population differentiation directly from molecular data and testing hypotheses about such differentiation. A variety of molecular data – molecular marker data (for example, RFLP or AFLP), direct sequence data, or phylogenetic trees based on such molecular data – may be analyzed using this method (Excoffier, *et al.* 1992).

The program STRUCTURE uses multi-locus genotype data to investigate population structure. Its uses include inferring the presence of distinct populations, assigning individuals to populations, studying hybrid zones, identifying migrants and admixed individuals, and estimating population allele frequencies in situations where many individuals are migrants or admixed. It can be applied to most of the commonly-used genetic markers, including microsatellites, RFLPs and SNPs.

The Mantel test is a statistical test of the correlation between two matrices. The matrices must be of the same rank, in most applications they are matrices of

interrelations between the same vectors of objects. The test is commonly used in ecology, where the data are usually estimates of the "distance" between objects such as species of organisms. For example, one matrix might contain estimates of the genetic distances (i.e., the amount of difference between two different genomes) between all possible pairs of taxa in the study, while the other might contain estimates of the geographical distance between the locations of each taxon. If there are n objects, and the matrix is symmetrical (so the distance from object a to object b is the same as the distance from b to a) such a matrix contains n(n − 1)/2 distances. Because distances are not independent of each other – since changing the "position" of one object would change n − 1 of these distances (the distance from that object to each of the others) – we can't assess the relationship between the two matrices by simply evaluating the correlation coefficient between the two sets of distances and testing its statistical significance. The Mantel test deals with this problem.

Null hypothesis statement

The null hypothesis for this research is that:

1 there is no population genetic structure in *Xylia xylocarpa* in Thailand,

2 the population as a whole and each of the individually sampled populations are in Hardy-Weinberg equilibrium.

MATERIALS AND METHODS

Materials

Plant materials

X. xylocarpa vegetative tissues (40-60 mg of leaves or flower), were collected from trees growing in their natural habitat in 14 national parks (permission number: ทส 0907.1/4792; ทส 0907/15941), and 2 community forest (Table 1). The number of trees sampled in each population ranged from 11 to 44 for a total of 539 trees. The location of each tree was recorded by GPS device (Figure 2-17, Appendix Table 1).

Table 1 List of collecting sites.

Population No.	Geographic region	Sample size (trees)
Xx-01	LamKhlongNgu National Park, Kanchanaburi. West	41
Xx-02	Erawan National Park, Kanchanaburi. West	36
Xx-05	Vichienburi Community Forest, *Phetchabun.* Central	23
Xx-06	PangSida National Park, Sakaew. Northeast	41
Xx-07	PhuJongNaYoi National Park, *Ubon Ratchathani*. Northeast	30
Xx-08	PhuPhaLek National Park, Sakonnakhon. Northeast	37
Xx-09	KhlongTron National Park, *Uttaradit*. North	44
Xx-10	ThamPhaTai National Park, Lampang. North	41
Xx-11	PhaDaeng National Park, Chiangmai. North	37
Xx-12	DoiJong National Park, Lampang. North	41
Xx-13	KhlongWangJao National Park, *Kamphaeng* Phet. Northwest	36
Xx-14	MaeNgao National Park, *Maehongson*. Northwest	36
Xx-15	ThaTaFang community forest area, MaeHongSon. Northwest	11
Xx-16	MaePing National Park, Lampang. North	28
Xx-17	KaengJedKwae National Park, Phitsanulok. North	25
Xx-18	WiangKosai National Park, *Phrae*. North	32
total		539
Individual trees	Various locations	10
Grand total		549

Girth at breast height was measured for each sampled tree and any notes about tree morphology (aspect of the bark etc) were recorded. In addition, 10 individual trees were sampled at various locations across Thailand. Tissues were dried and taken to the laboratory for further processing.

Figure 2 Location of the 14 national parks and 2 community forests where *Xylia* trees were sampled**.** (1=LamKhlongNgu National Park; 2=Erawan National Park; 5=Vichienburi Community Forest; 6=PangSida National Park; 7=PhuJongNaYoi National Park; 8=PhuPhaLek National Park; 9=KhlongTron National Park; 10=ThamPhaTai National Park; 11=PhaDaeng National Park; 12=DoiJong National Park; 13=KhlongWangJao National Park; 14=MaeNgao National Park; 15 = ThaTa Fang area; 16=MaePing National Park; 17=KaengJedkwae National Park; 18=WiangKosai National Park)

Source: Google Earth 6.2

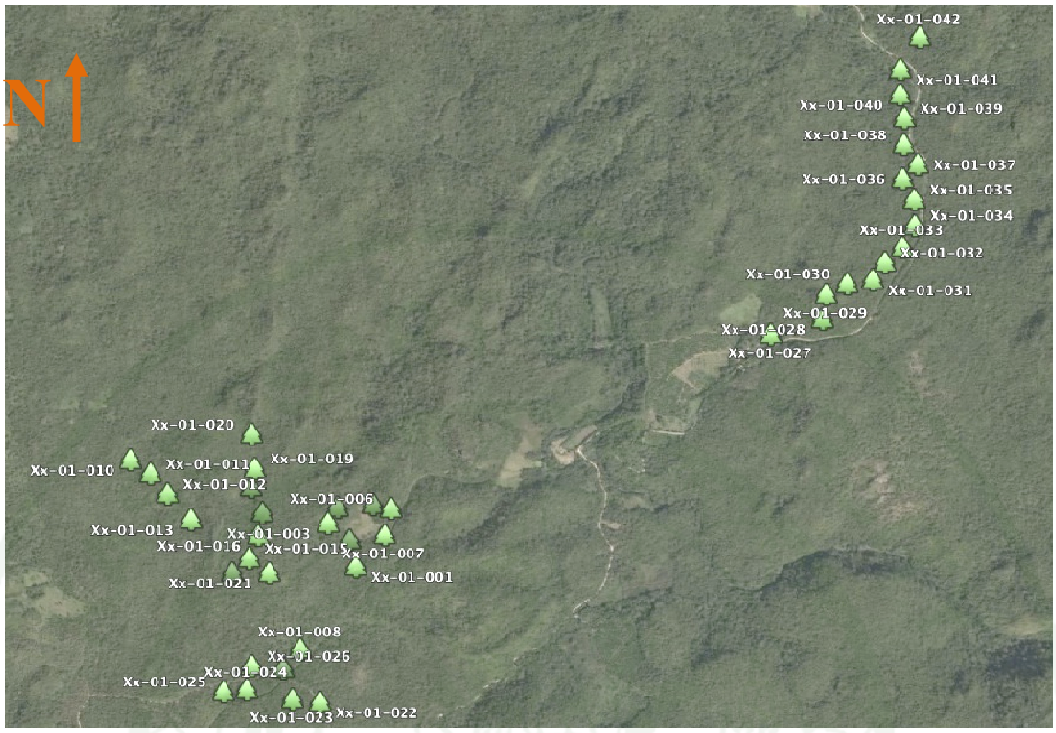

Figure 3 *Xylia* samples collected in LamKhlongNgu National Park (Xx-01).

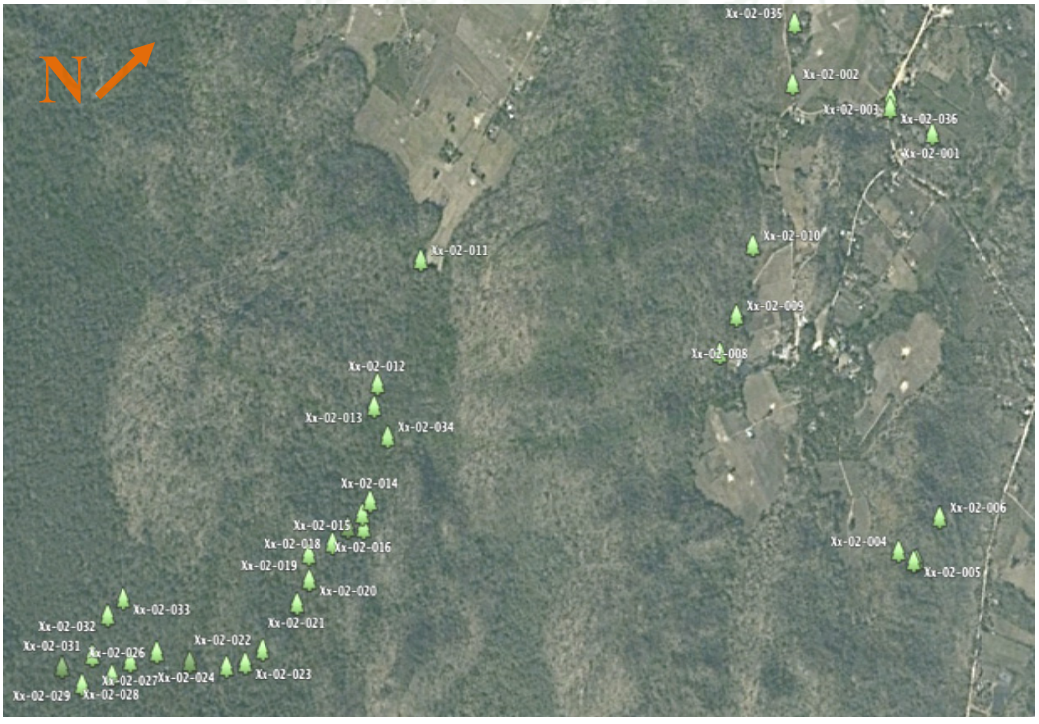

Figure 4 *Xylia* samples collected in Erawan National Park (Xx-02).

Figure 5 *Xylia* samples collected in Vichienburi (Xx-05).

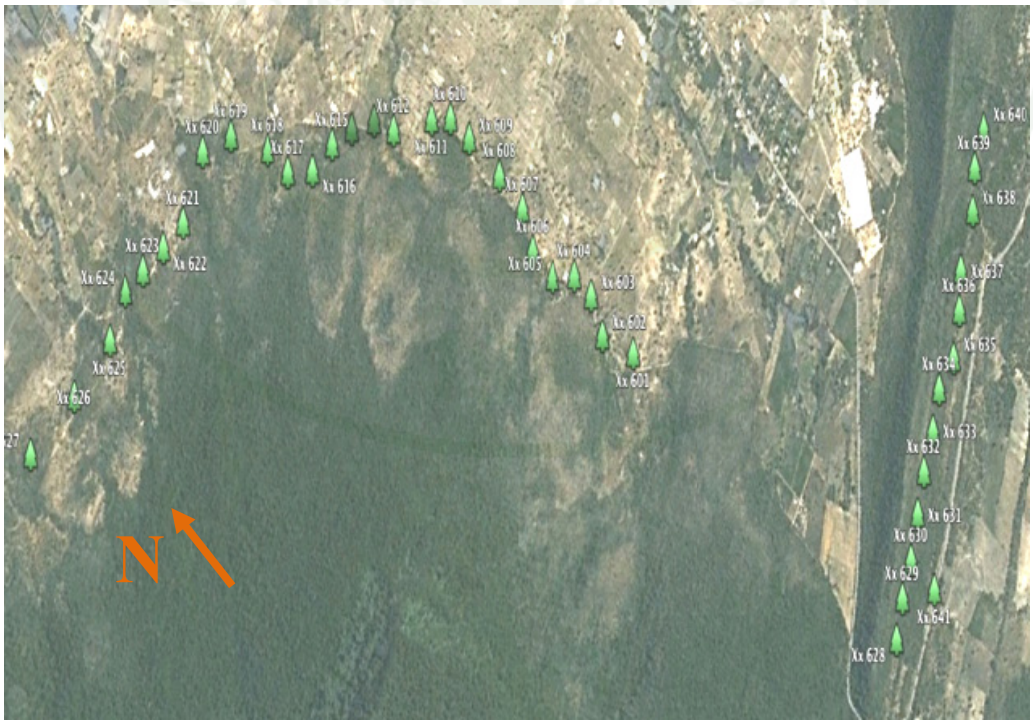

Figure 6 *Xylia* samples collected in PangSida National Park (Xx-06).

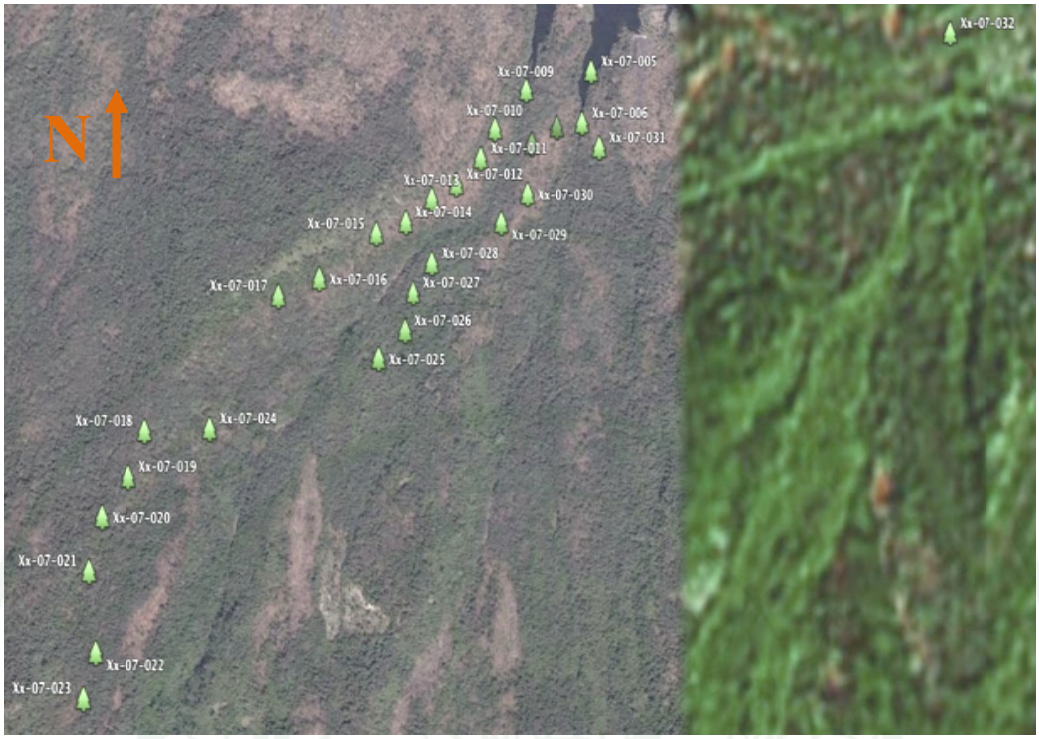

Figure 7 *Xylia* samples collected in PhuJongNaYoi National Park (Xx-07).

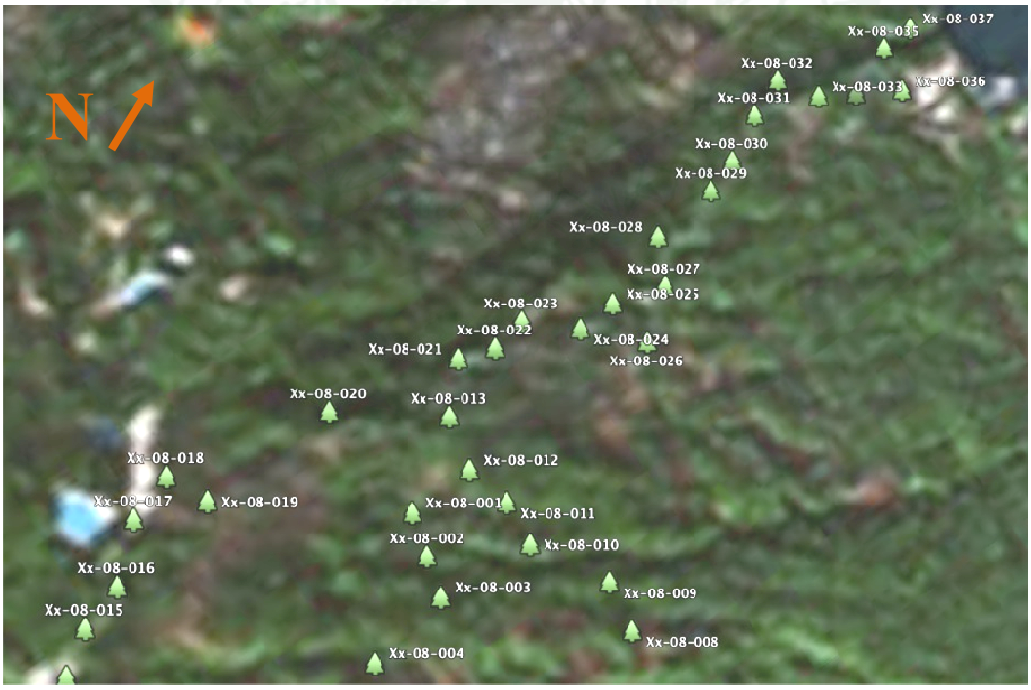

Figure 8 *Xylia* samples collected in PhuPhaLek National Park (Xx-08).

Figure 9 *Xylia* samples collected in KhlongTron National Park (Xx-09).

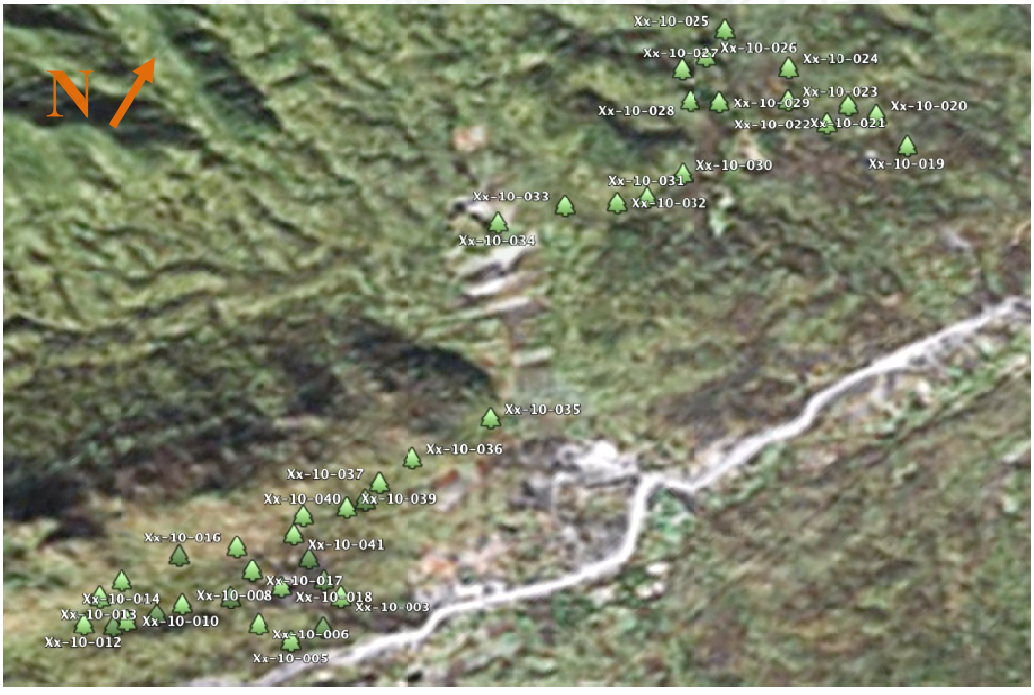

Figure 10 *Xylia* samples collected in ThamPhaTai National Park (Xx-10).

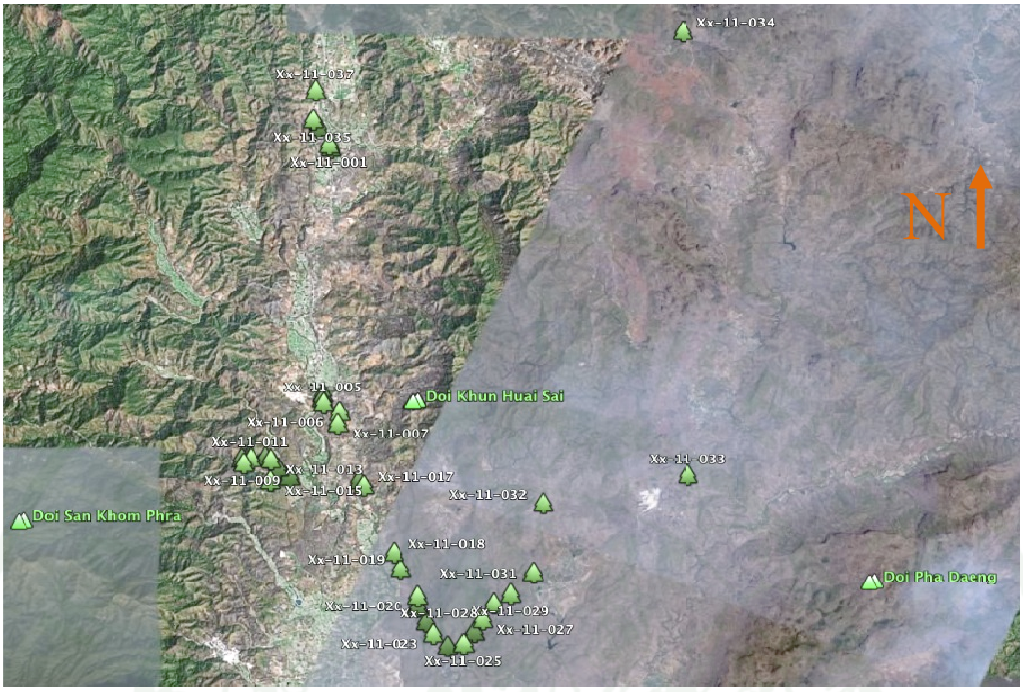

Figure 11 *Xylia* samples collected in PhaaDaeng National Park (Xx-11).

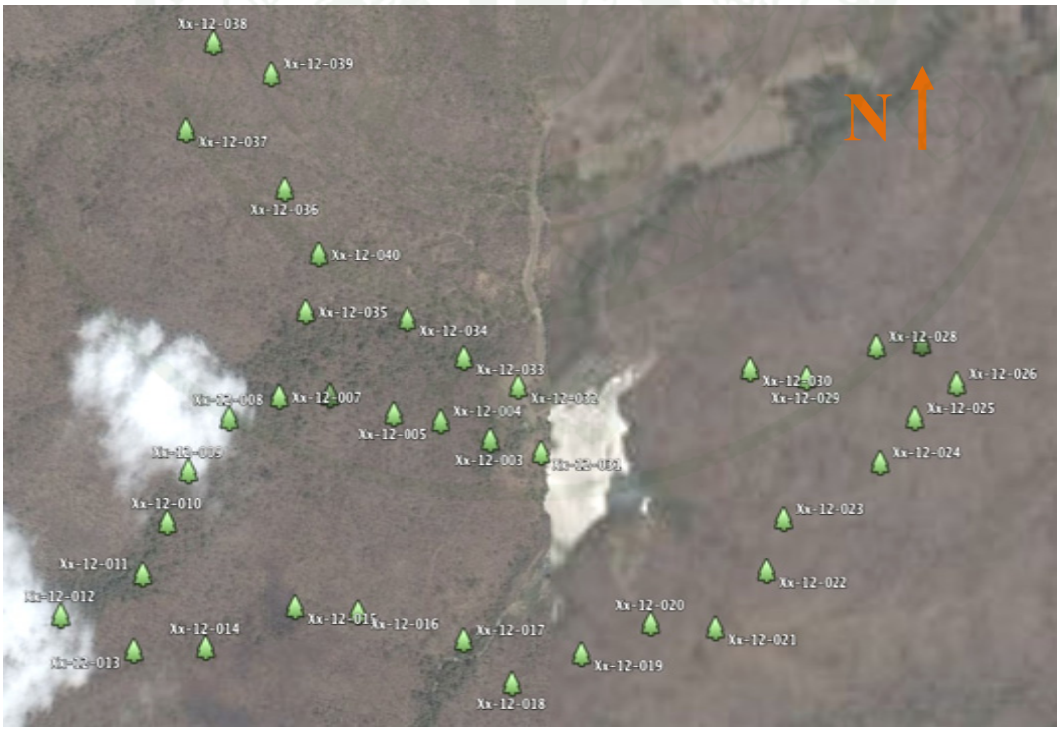

Figure 12 *Xylia* samples collected in DoiJong National Park (Xx-12).

Figure 13 *Xylia* samples collected in KhlongWangJao National Park (Xx-13).

Figure 14 *Xylia* samples collected in MaeNgao National Park (Xx-14).

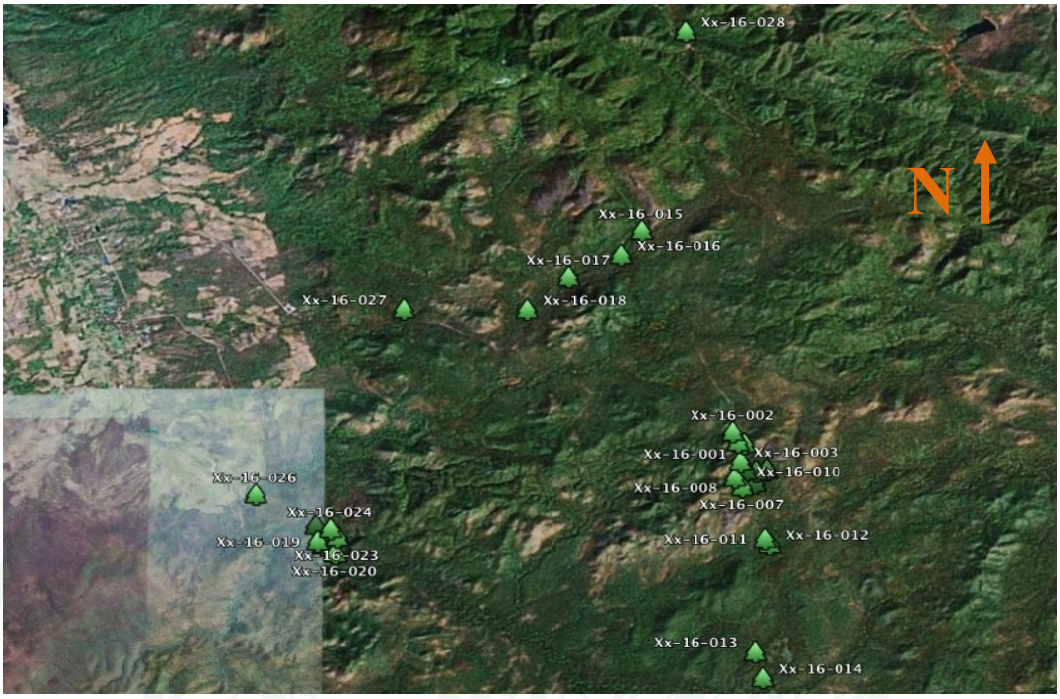

Figure 15 *Xylia* samples collected in MaePing National Park (Xx-16).

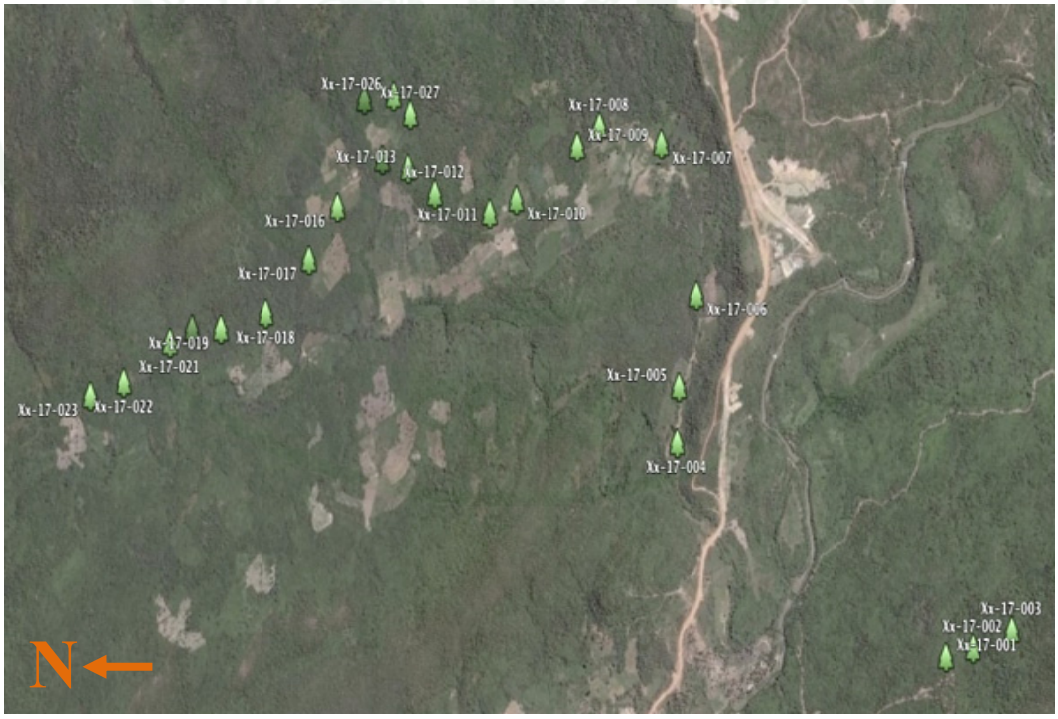

Figure 16 *Xylia* samples collected in KaengJedKhwae National Park (Xx-17).

Figure 17 *Xylia* samples collected in ViengKosai National Park (Xx-18).

Methods

DNA extraction

A leaf area corresponding to about 60 mg fresh weight was put in a tube with a stainless steel ball and crushed to a very fine powder by Tissuelyser (Retsch®) for 3 min at 30 Hz. Genomic DNA was extracted from the powdered tissue using the Genomic DNA Mini Kit (Plant) (Geneaid, Taiwan), following the manufacturer's protocol. The DNA concentration of the samples was estimated on agarose gel by comparing to a standard of known concentration (Lambda DNA Cat# SD0011, Fermentas, UAB, Inc), stained with ethidium bromide and photographed with GeneGenius Bioimaging system using GeneSnap version 4.1.

Development of gene based markers

Because no DNA sequences of genes in *X. xylocarpa* were available in public databases, primers for PCR amplification of specific genes were designed to match conserved codons among genes sequenced in a multitude of other plant species. These primers contained one or more positions where mixtures of nucleotides were incorporated during synthesis (degenerate PCR primers, Table 2). The genes chosen to develop consensus primer are low-copy-number genes families mostly coding for metabolic enzymes i.e. catalase (*CAT*), isopentenyl diphosphate isomerase (*IPI*), aspartate aminotrantransferase (*AAT, GOT*), sucrose synthase (*SUS*), isocitrate dehydrogenase (*IDH*), leucine aminopeptidase (*LAP*), glyceraldehyde-3-phosphate dehygrogenase (*GAPC*), isocitrate lyase (*ICL*), abscissic acid insensitive 3 (*ABI3*), cystein proteinase inhibitor (*CPI*), and auxin response factor 6&8 (*ARF6, ARF8*).

Table 2 Nucleotide sequence of the degenerate primers used for PCR amplification of specific gene families.

Primer	Sequence	bp	Degeneracy
IDH (F)	AAGAGTGAAGGAGGNTAYGTNTGG	24	8
IDH (R)	GCATCAATGAAYTCYTCNGTRTT	23	32
IPI (F)	ACAAACACTTGYTGYAGYCAYCC	23	16
IPI (R)	CCTTTCTCNACRTGRTCCCACCA	23	16
LAP (F)	GTTGCAGCTTGTGARAAYATGAT	23	4
LAP (R)	CCTGATTTCATTGAYTCCCARTA	23	4
SUS (F)	GGTTATCCTGATACYGGNGGNCA	23	32
SUS (R)	ACAAGGTTTCCATCACTRTARTTNCC	26	16
CAT (F)	GGTTTCTTTGARGTYCANCAYGA	23	32
CAT (R)	TGATGAGCACAYTTTGGNGCRTT	23	16
AATcy (F)	CATGCTTGTGCTCATAAYCCNACYGG	26	16
AATcy (R)	AAAGTGAACATTCCDATYTGYTT	23	12

Table 2 (Continued)

Primer	Sequence	bp	Degeneracy
ABI3 (F)	AAGGTGTTGAAGCARAGYGAYGT	23	8
ABI3 (R)	ATCACTATGAARTCTCCYTCYTG	23	8
CPI (F)	GCCGTCGATGARCAYAAYAARAA	23	16
CPI (R)	TGTGGNGTCATCCARAACCA	20	8
ICL (F)	TCTCCATCTTTCAACTGGGAY	21	2
ICL (R)	TTGAATTGCTCTTCTGTNCANCCT	24	4
GAPC (F)	ACTCAGAAGACTGTTGAYGGNCC	23	8
GAPC (R)	TAACCCCATTCRTTRTCRTACCA	23	8
ARF6/8 (F)	AGGTTGGTTGGGATGARTCANC	22	8
ARF6/8 (R)	GTCAACRAATACAAGYTGCCA	21	4

D=(A/C/T) N=(A/G/C/T) R=(A/G) Y=(C/T)

DNA fragments were PCR amplified from two or three samples using the consensus primer pairs in a total reaction mixture of 25 µl, containing 200 µM dNTPs (Fermentas), 5 pmoles of each primer, 1x PCR buffer (RBC) with $MgCl_2$ adjusted to 2 mM, 0.3 units of *Taq* DNA polymerase (Fermentas), and 5 ng of genomic DNA template. Amplification was carried out at 94^{o}C for 3 min, followed by 40 cycles of 45 sec at 94^{o}C, 45 sec at the appropriate annealing temperature (46-51^{o}C), 1 min 30 sec at 72^{o}C, and a final extension at 72^{o}C for 5 min. PCR products were separated on agarose gel.

PCR reactions showing reproducible fragment bands were selected and purified. The purified fragments were then ligated into pGEM-T® plasmid vector (Promega, USA) and transformed into competent cell (*Escherichia coli* 'DH10B') by electroporation using MicroPulser™ (Bio-Rad, USA). The cells were spread onto LB (Luria-Bertani) medium agar plates containing 100 µg/ml of antibiotic (ampicilin), 100 µl IPTG (100 mM), and 20 µl X-gal (50 mg/ml). The bacteria were allowed to

grow overnight at 37^{o}C. Blue-white colony selection was used to identify transformants. Individual colonies were picked and checked for the presence of an insert by PCR amplification using the degenerate primer pairs. The remainder of the same single colony was grown overnight in an incubator shaker at 37^{o}C, 200 rpm in 5 ml of LB medium broth with 100 µg/ml of ampicilin. Plasmids were extracted using High-Speed Plasmid Mini Kit (Geneaid, Taiwan) following the manufacturer's instruction, and then sent for sequencing at 1stBase, Malaysia**.**

Sequence analysis and design of specific primers

The obtained sequences were compared with those from other plant species to check that the targeted gene had been obtained. Specific primer pairs for the amplification of unique DNA fragments from *X. xylocarpa* were then designed based on the obtained sequences from each gene family. Intron-exon boundaries (GT-AG) were determined in *X. xylocarpa* sequences by comparison with cDNA sequences from other species. Specific primers for each locus were designed in such a way that different loci in each gene family could be amplified separately from the *X. xylocarpa* genome (Table 3).

Single-Strand Conformation Polymorphism (SSCP)

PCR amplification conditions were optimized for each of the loci of interest. PCR fragments were single bands of 450-890 bp. Non-denaturing polyacrylamide gels (6-8 % poly-acrylamide gels with ratio 99:1 Acrylamide/Bis-acrylamide) were prepared in 0.6x TBE buffer. Alternatively, a commercial solution, (SequaGel® MD, National diagnostics, USA.) was used. PCR product (1.5 µl) was mixed with 4 volumes of loading dye (95% (v/v) formamide, 0.025% bromophenol blue, 0.025% xylene cyanol and 10 mM NaOH). The double stranded DNA was denatured by heating at 95^{o}C for 10 min and cooled on ice to stabilize single strands. Aliquots (2.5 µl) were loaded on the gel. The gels were run at a constant power of 10-20 Watt for 12-24 hr depending on the size of PCR product in a refrigerator (4-8^{o}C) with 1xTBE

as running buffer. The DNA fragments were revealed by silver staining (Bassam *et al.,* 1991).

Table 3 Nucleotide sequence of the specific primers for each locus with annealing temperature and fragment size.

Specific primers	T_A	size (bp)	Sequence 5′-3′
Isocitrate dehydrogenase1			
Xx-IDH-F	56	744	AGTGATTTCTTAGCCCAAGGTG
Xx-IDH1-R			AAGTCCAACAATCTTGCATTTCC
Isopentenyl diphosphate isomerase1			
Xx-IPI1-F	55	449	CTCATTGAAGAGCAAGCCCTCG
Xx-IPI1-R			AACAGCTCCTTCAATTGATCACG
Sucrose synthase1			
Xx-SUS1-F2	56	515	AGACTGACTACCCTGCACGGTTCA
Xx-SUS1-R2			GCACCTTTAGTGTCTGCTATATAG
Sucrose synthase2			
Xx-SUS2-F2	56	495	AGACTCGCTCAATTTCACCCTGCC
Xx-SUS2-R2			GCTCCCTTTGTGTCAGCAATGCA
Leucine aminopeptidase1			
Xx-LAP1-F	57	884	ATGCGGCCTGGAGATATTGTCAC
Xx-LAP1-R			GTGTTGTTAACCTATACAAGAGG
Catalase1			
Xx-CAT1-F	57	880	CCTGTTATTGTCCGCTTCTCTAC
Xx-CAT1-R			AATGTTTCTATTCAAGACCAGGCG
Catalase2			
Xx-CAT2-F	57	847	CCTGTCATTGTCCGTTTCTCGAC
Xx-CAT2-R			AATGTTCTTATTCAAGACCAAACG
Aspartate aminotransferase (cytoplasmic isoform)			
Xx-AATcy-F2	54	489/493	ATCCTCAAGGACCGGTAATCAG
Xx-AATcy-R2			TTGCCTTCAACTCAACAGTCCA

Haplotype network construction

Different alleles were identified on SSCP gels and PCR amplification products of corresponding samples were directly sequenced. Additionally sequences were obtained from fragments that did not give clear SSCP genotyping results. The haplotypes deduced from the sequence data were aligned and SNPs were detected. A haplotype network was constructed using the program TCS 1.21 (Clement *et al.,* 2000). Each insertion or deletion (indel) was reduced to single mutation event. For the analysis, indels (gaps) were considered as a fifth character state.

Development of *Xylia* allele specific assays

Based on sequence data, 5 of the SNPs were developed into allele-specific PCR amplification assays (AS-PCR) using the program BatchPrimer3 website (http://probes.pw.usda.gov/batchprimer3/). For the allele-specific PCR assays an extra mismatching nucleotide was incorporated at the -3 or -4 position in addition to the allele specific 3′ terminal nucleotide. The annealing temperature for PCR was optimized for each SNP assay.

Allele-specific DNA fragments were amplified by PCR in two separate reactions each with one allele specific primer and a common primer. Details of the primers are presented in Table 4. The PCR reaction mixtures contained 10 ng of plant genomic DNA, 1.7 mM of Mg^{+2}, 10 pmoles of F and R primers, 200 µM of dNTPs, 10x reaction buffer with $(NH_4)_2SO_4$, 0.38 unit of *Taq* DNA polymerase (Fermentas UAB, Lithuania), in a total volume of 10 µl. The PCR thermocycling reaction was performed on a Biometra® T1 Thermocycler using the following steps: the first cycle consisted of denaturation of DNA template at 94°C for 3 min., followed by 40 cycles at 94°C for 45 sec., primer annealing for 45 sec., primer extension 72°C for 45 sec. and finally incubation at 72°C for 5 min. The annealing temperature for PCR was optimized for each primer assay (details of annealing temperature in Table 4). The reaction products were checked by 1.5% agarose gel electrophoresis.

CAPS assays were designed to differentiate 10 SNPs occurring in 5 loci: IDH1 (3 SNPs), CAT1 (1 SNP), CAT2 (3 SNPs), LAP1 (1 SNP) and IPI1 (2 SNPs). The dCAPS Finder 2.0 website (http:helix.wust.edu/dcaps/dcaps.html) was used to determine appropriate restriction enzyme assays. The CAPS-PCR reaction mixtures and condition were the same as those used for the AS-PCR protocol. The primer sequences are given in Table 4.

The amplified PCR product was digested with *Dde*I (New England Biolabs, USA) for IDH1 2 SNP. A reaction mixture containing 4 µl of PCR product 1.2 µl of restriction enzyme buffer 10xNEBuffer 3 (1X NEBuffer 3 contained 50 mM Tris-HCl, 100 mM NaCl, 10 mM $MgCl_2$, 1 mM Dithiothreitol, pH 7.9 at 25°C), 1 U of restriction enzyme *Dde*I, in a total volume of 12 µl, was incubated at 37°C 2 hr. and separated on 2% SeaKem agarose (Cambrex, USA). The other restriction enzyme with IDH1 (1 SNP) PCR product was *Bcc*I (New England Biolabs, USA). A reaction mixture containing 4 µl of PCR product 1.2 µl of 10xNEBuffer 1 (1XNEBuffer 1 contained 10 mM Bis-Tris-Propane-HCl, 10 mM $MgCl_2$, 1 mM Dithiothreitol, pH 7.0 at 25°C), 1.2 µl of 10xBSA, 1 U of *Bcc*I, in total volume of 12 µl, was incubated at 37°C 2 hr. and separated on 3% SeaKem agarose

The CAT1 PCR fragment (1 SNP) was digested with *Alu*I (New England Biolabs, USA). A reaction mixture containing 4 µl of PCR product 1.2 µl of 10xNEBuffer 4 (1xNEBuffer 4 contained 50mM potassium acetate, 20 mM Tris-acetate, 10 mM magnesium acetate, 1 mM dithiothreitol, pH 7.9 at 25°C), 1 U of *Alu*I, in a total volume of 12 µl, was incubated at 37°C 2 hr. and separated on 3% SeaKem agarose.

The CAT2 PCR fragment (1 SNP) was digested with *Nla*III (New England Biolabs, USA) A reaction mixture containing 4 µl of PCR product 1.2 µl of 10xNEBuffer 4, 1.2 µl of 10xBSA, 1 U of *Nla*III, in total volume of 12 µl, incubated at 37°C 2 hr. and separated on 3% SeaKem agarose.

The amplified PCR product was digested with *Taa*I (Fermentas UAB, Lithuania) for LAP1 (1 SNP) and CAT2 (1 SNP). A reaction mixture containing 5 µl of PCR product 1.2 µl of 10x Fastdigest® Buffer, 1 U of *Taa*I, in total volume of 12 µl, incubated at 65°C 10 min. and separated on 3% SeaKem agarose. The other restriction enzyme with CAT2 (1 locus) PCR product was *Tas*I (Fermentas UAB, Lithuania) set reaction mixture containing 4 µl of PCR product 1.2 µl of 10x Buffer B (1x Buffer B contained 10 mM Tris-HCL pH 7.5 at 37°C, 10 mM $MgCl_2$ and 0.1 mg/ml BSA), 1.5 U of *Tas*I, in total volume of 12 µl, incubated at 65 °C 2 hr. and separated on 3% SeaKem agarose.

The IPI1 PCR fragment (1 SNP) was digested with *Vsp*I (Fermentas UAB, Lithuania) A reaction mixture containing 6 µl of PCR product 1.2 µl of 10x Tango Buffer, 1 U of *Vsp*I, in total volume of 12 µl, incubated at 37°C 2 hr. and separated on 3% SeaKem agarose. The amplified PCR product was digested with *Taq*$^{\alpha}$I (New England Biolabs, USA) for IPI1 (1 SNP). A reaction mixture containing 6 µl of PCR product 1.2 µl of 10x *Taq*$^{\alpha}$I Buffer, 1 U of *Taq*$^{\alpha}$I, in total volume of 12 µl, incubated at 65°C 2 hr. and separated on 3% SeaKem agarose.

Table 4 PCR primer pairs and reaction conditions for SNP detection in the AS-PCR and CAPS assays.

Primer	T_A	Size (bp)	SEQ 5′-3′	Assay Type	Position	Restriction enzyme
Xx-LAP1-F	60	265	ATGCGGCCTGGAGATATTGTCAC	CAPS	225	*Taa*I (CAN^GT)
Xx-LAP1-Tsp-R			TATTCAACCGGTTcTGCAGCAG			Fermentas/ Fastdigest®
Xx-IDH1-CAPS-F1	56	217	AGTGATTTCGTAGCCCAAGGT	CAPS	46	*Dde*I (C^TNAG)
Xx-IDH1-CAPS-R1			AATCCTGCAAAGGGATAGGGA			New England Biolabs
Xx-IDH1-CAPS-F2	54	400	ATGCATGGGTTGGAAAATGT	CAPS	204	*Dde*I (C^TNAG)
Xx-IDH1-CAPS-R2			GACCAAGCAAAAATGGATGC			New England Biolabs
Xx-IDH1-CAPS-F2	54	400	ATGCATGGGTTGGAAAATGT	CAPS	353	*Bcc*I (GGTAGN^)
Xx-IDH1-CAPS-R2			GACCAAGCAAAAATGGATGC			New England Biolabs
Xx-IDH1-508-F	57	212	AGCTTGAGATGCTTCATTTGGT	AS	508	-
Xx-IDH1-508-R-A			GAAGCCTAAAACAACAAAAGTA			
Xx-IDH1-508-R-T			GAAGCCTAAAACAACAAAAGTT			
Xx-IDH1-558-F-A	58	210	TCCCCATTTGCTCCCAAAGAA	AS	558	-
Xx-IDH1-558-F-C	53		TCCCCATTTGCTCCCAAAGAC			
Xx-IDH1-RL			TTTGGTCCATGAACAAGAAGCG			

Table 4 (Continued)

Primer	T_A	Size (bp)	SEQ 5′-3′	Assay Type	Position	Restriction enzyme
Xx-IDH-F	58	273	AGTGATTTCTTAGCCCAAGGTG	AS	253	-
Xx-IDH1-253-R-T			CAAAGAAAAGGAAGAATGCCA			
Xx-IDH1-253-R-C			CAAAGAAAAGGAAGAATGCCG			
Xx-CAT1-709-F-C	53	270	CAGTCATGCAACTCAAGACATC	AS	709	-
Xx-CAT1-709-F-T			CAGTCATGCAACTCAAGACATT			
Xx-CAT1-709-R			ATCATCTGAGTAGTATATTCCAGG			
Xx-CAT1-F	54	233	CCTGTTATTGTCCGCTTCTCTAC	CAPS	131	*Alu*I (AG^CT) New England Biolabs
Xx-CAT1-AluI-R			CAATTAGGCTTGTTTCCCTAAG			
Xx-CAT2-Tas-F	52	358	AAGTTCCCCATTTTGACTAAGG	CAPS	457	*Tas*I (^AATT) Fermentas
Xx-CAT2-Tas-R			ATTTTACATAGTGTGCTTTCCC			
Xx-CAT2-Tsp-F	62	251	ATCCAGGAGAACTGGAGGATCC	CAPS	577	*Taa*I (CAN^GT) Fermentas/ Fastdigest®
Xx-CAT2-Tsp-R			CTGTGGTTGGATCCTCCAACCC			
Xx-CAT2-Nla-F	58	256	GGGTGTCCCACAAGATTACAGG	CAPS	637	*Nla*III (CATG^) New England Biolabs
Xx-CAT2-Nla-R			ATGGTCAGGATCCATTGTCTGG			

Table 4 (Continued)

Primer	T_A	Size (bp)	SEQ 5′-3′	Assay Type	Position	Restriction enzyme
Xx-CAT2-610-F	53	526	ATCTTCTGGATTCTGGTCAGTG	AS	610	-
Xx-CAT2-610-R-G			GCCTCCTCCTCCAATAGAAAC			
Xx-CAT2-610-R-A			GCCTCCTCCTCCAATAGAAAT			
Xx-IPI1-F	56	449	CTCATTGAAGAGCAAGCCCTCG	CAPS	392	*Vsp*I (AT^TAAT) Fermentas
Xx-IPI1-R			AACAGCTCCTTCAATTGATCACG			
Xx-IPI1-F	56	449	CTCATTGAAGAGCAAGCCCTCG	CAPS	414	*Taq*$^{\alpha}$I (T^CGA) New England Biolabs
Xx-IPI1-R			AACAGCTCCTTCAATTGATCACG			

Population Genetic Analysis

Genetic diversity parameters within and between populations were calculated using GenAlEx 6.4 excluding the 10 individual trees that had been sampled. The programme GENEPOP (version 1.2) (Raymond and Rousset 1995) at the http://genepop.curtin.edu.au/ website was used to test for deviations from HW equilibrium. Jost's measure of genetic differentiation among populations, D, (Jost, 2008) was calculated using the programme SMOGD version 1.2.5 website (http://www.ngcrawford.com/django/jost/) (Crawford, 2010). Based on the calculated matrices of pairwise genetic distances neighbor-joining phylogenies were calculated using the programme NEIGHBOR from the PHYLIP package version 3.69 (Felsenstein, 2009). The programme FigTree version 1.4.0 (Rambaut, 2009) was used to display the population-based phylogenetic trees. AMOVA analysis was done using GenAlEx 6.4. For the Mantel test for correlation between linear genetic and geographical distance was performed in GenAlEx 6.4. A principal component analysis of the genotype data was performed using the programme DARwin version 5 (Perrier *et al.,* 2003) to analyse phylogenetic relationships among all genotyped *Xylia* individuals. DARwin version 5 calculates a genetic distance based on the number of shared alleles among diploid organisms. This genetic distance information was also used to construct a phylogenetic tree of all individual samples. Population genetic structure was analysed using the programme STRUCTURE version 2.3.3 (Pritchard *et al*., 2007). STRUCTURE tests different number of hypothetical subpopulations (K) to find the highest likelihood. The STRUCTURE programme was run once for each number of subpopulation (K) values, ranging from 1 to 5, using the admixture and correlated allele frequency, admixture and independent allele frequency, no admixture and correlated allele frequency, no admixture and independent allele frequency model with 100,000 steps for burn-in and 500,000 steps of the MCMC sampling after burn-in. The final population subgroups were determined based on Ln P(D) and Var[Ln P(D)] for the model choice criterion to refer as true number of population (K). The number of populations considered by STRUCTURE is not the same as the number of populations sampled.

RESULTS AND DISCUSSION

Results

Analysis of Specific nuclear DNA markers

Consensus PCR primers complementary to conserved regions of some metabolic enzyme coding genes could amplify one or a small number of fragments in *Xylia*. Twenty clones containing inserts were isolated and sent for sequencing. The obtained results indicated that twelve of these fragment inserts corresponded to the targeted genes. These consensus primers can be used to specifically amplify fragments in several plant species and can be applied to study evolution, population genetics, and mating patterns.

Based on the obtained sequences, specific primer pairs were designed for 6 enzyme systems encoded by 8 loci: *SUS* (2 loci), *AAT* (1 locus), *IDH* (1 locus), *LAP* (1 locus), *CAT* (2 loci) and *IPI* (1 locus). Table 3 lists the primer sequences and annealing temperature for each targeted fragment. Multiple alleles were revealed at each of these loci by PCR-SSCP resulting in a multi-locus genotype for each diploid tree.

The Xx-IDH1 (Isocitrate dehydrogenase1) primers successfully amplified a fragment from all *X. xylocarpa* populations. On agarose gel, a single band of approximately 500 bp was visible. Different alleles could not be distinguished (Figure 18). However, using SSCP, several alleles could be tentatively identified in all populations.

Figure 18 PCR products were amplified from *X. xylocarpa* samples from Khlong Tron National Park using Xx-IDH1 primers and compared with a size standard (λ/*Hind*III+*Eco*RI) in lane M. The size of the PCR product is approximately 500 bp.

Figure 19 shows some of the different alleles from *X. xylocarpa* in SSCP. Several of the SSCP banding patterns were difficult to interpret. Therefore, PCR fragments of several individuals were sent for direct sequencing which revealed a number of additional haplotypes. Allele-specific assays were then designed to detect some of these variants (see below) in the *Xylia* populations.

Figure 19 SSCP polyacrylamide gel of the Xx-IDH1 marker amplified from individual *Xylia xylocarpa* trees. Different alleles are assigned. Allele designations were deduced from both allele specific and SSCP assays.

The Xx-IPI1 (Isopentenyl diphosphate isomerase1) primers successfully amplified a single fragment from all *X. xylocarpa* populations. On agarose gel, a single band of approximately 450 bp was visible. Different alleles could not be distinguished (Figure 20). However, using SSCP, 4 alleles could be identified. Figure 21 shows different alleles from *X. xylocarpa* in SSCP.

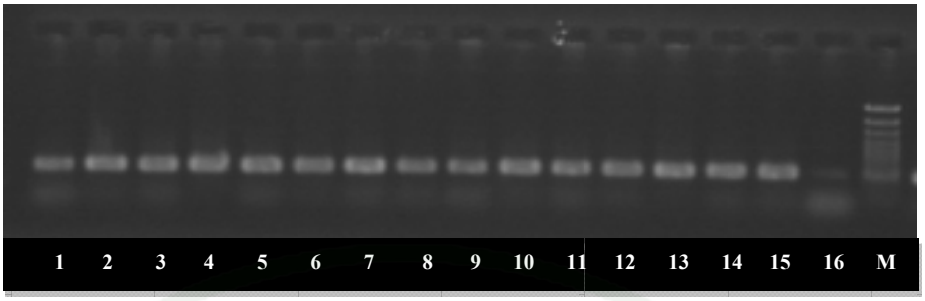

Figure 20 PCR products were amplified from *X. xylocarpa* samples from Khlong Tron National Park using Xx-IPI1 primer and compared with a size standard (λ/*Hind*III+*Eco*RI) in lane M. The size of the PCR product is approximately 450 bp.

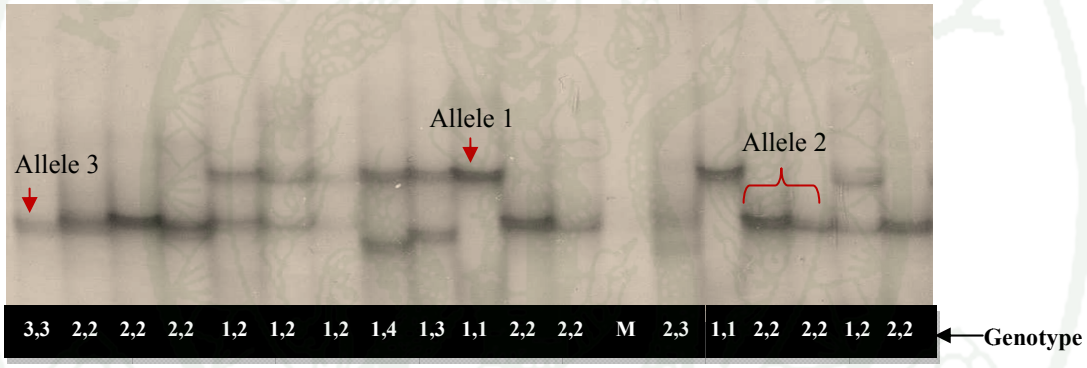

Figure 21 SSCP polyacrylamide gel of the Xx-IPI1 marker amplified from individual *Xylia xylocarpa* trees. Different alleles are assigned.

Some of the SSCP banding patterns were difficult to interpret. Therefore, PCR fragments of several individuals were sent for direct sequencing which revealed additional haplotypes. Allele-specific assays were designed to detect some of these variants (see below).

The Xx-SUS1 (Sucrose synthase1) and Xx-SUS2 (Sucrose synthase2) primers successfully amplified a fragment from all *X. xylocarpa* populations. On agarose gel, a single band of approximate 500 and 600 bp, respectively, was visible. Different alleles could not be distinguished (Figure 22). However, using SSCP, 4 and 6 alleles

could be identified in all populations for Xx-SUS1 and Xx-SUS2, respectively. Figure 23 shows some of the different alleles from *X. xylocarpa* in SSCP.

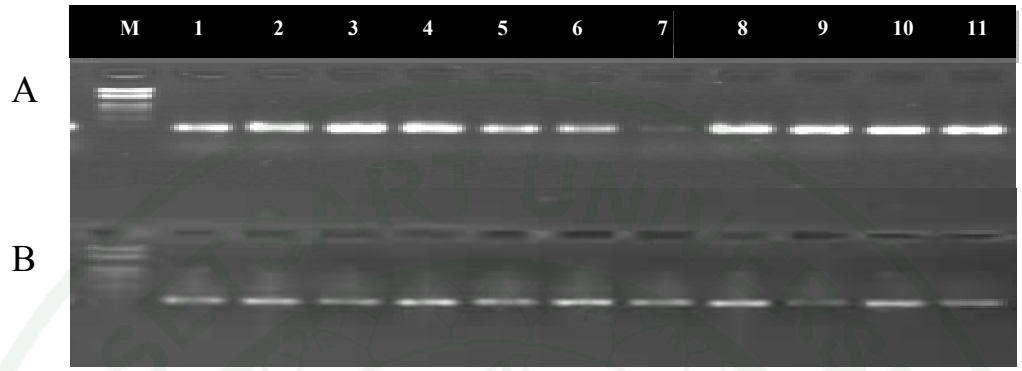

Figure 22 PCR products were amplified from *X. xylocarpa* samples from Khlong Wang Jao National Park (1), Mae Ngao National Park (2-6), Kaeng Jed Kwae National Park (7), and Wiang Kosai National Park (8-11) using the Xx-SUS1 (A) and Xx-SUS2 (B) primers and compared with a size standard (λ/*Hind*III+*Eco*RI) in lane M. The size of the PCR product is approximately 500 and 600 bp.

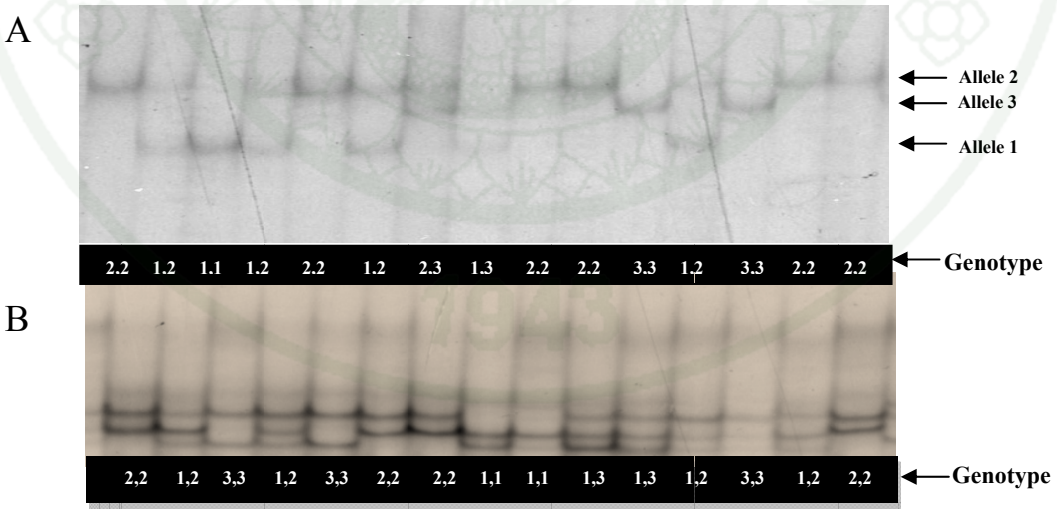

Figure 23 An example of SSCP polyacrylamide gel of the Xx-SUS1 (A) and Xx-SUS2 (B) marker amplified from individual *Xylia xylocarpa* trees. Different alleles are assigned.

The Xx-LAP1 (Leucine Aminopeptidase1) primers successfully amplified a single fragment from all *X. xylocarpa* populations. On agarose gel, a single band of approximately 800 bp was visible. Different alleles could not be distinguished (Figure 24). However, using SSCP conditions, 7 alleles could be identified in all populations. Figure 25 shows different alleles from *X. xylocarpa* in SSCP.

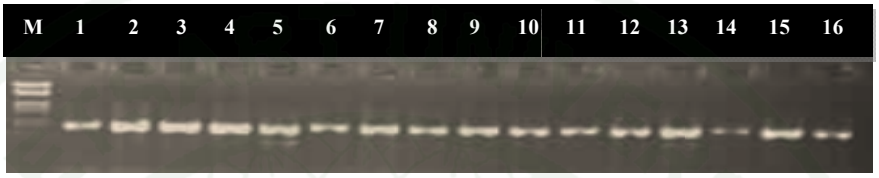

Figure 24 PCR products were amplified from *X. xylocarpa* samples from Wiang Kosai National Park using Xx-LAP1 primers and compared with a size standard (λ/*Hind*III+*Eco*RI) in lane M. The size of the fragments is approximately 500 bp.

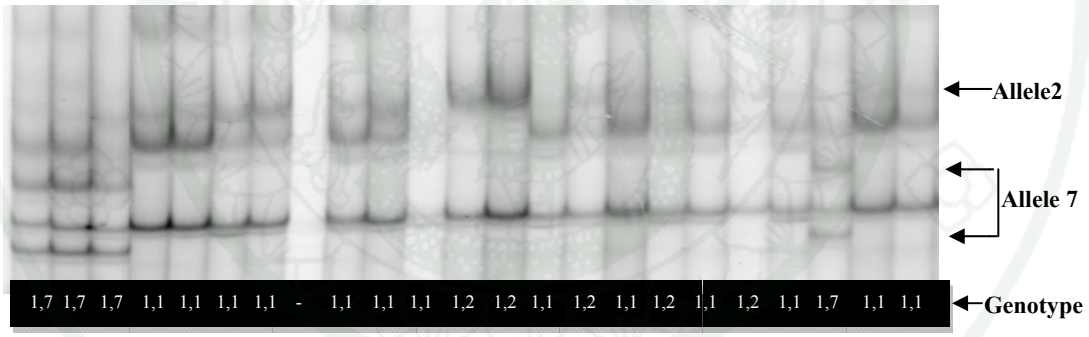

Figure 25 SSCP polyacrylamide gel of the Xx-LAP1 marker amplified from individual *Xylia xylocarpa* trees. Different alleles are assigned.

The Xx-CAT1 (Catalase1) and Xx-CAT2 (Catalase2) primers successfully amplified a fragment from all *X. xylocarpa* populations. On agarose gel, a single band of approximately 880 and 850 bp, respectively, was visible. Different alleles could not be distinguished (Figure 26). However, using SSCP, 6 alleles could be identified in each of Xx-CAT1 and Xx-CAT2. Figure 27 shows different alleles from *X. xylocarpa* in SSCP.

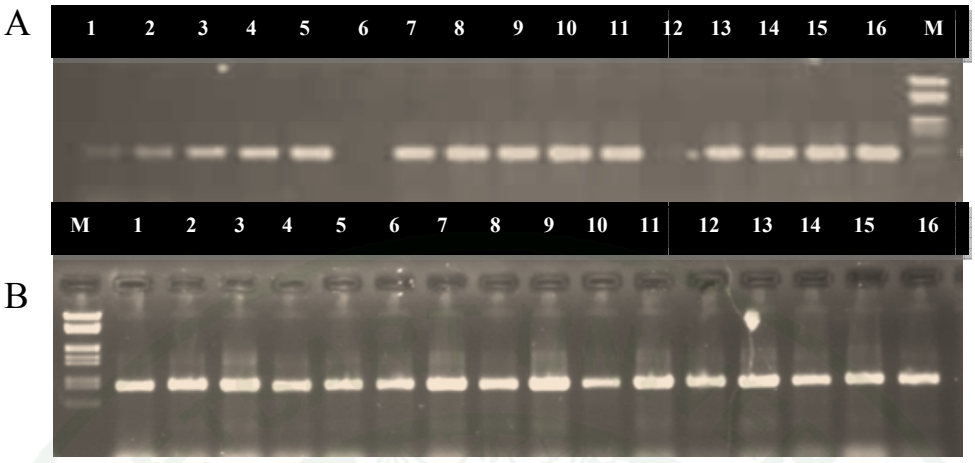

Figure 26 PCR products were amplified from *X. xylocarpa* trees using the Xx-CAT1 (A) and Xx-CAT2 (B) primers and compared with a size standard (λ/*Hind*III+*Eco*RI) in lane M. The size of the PCR product is approximately 880 and 850 bp.

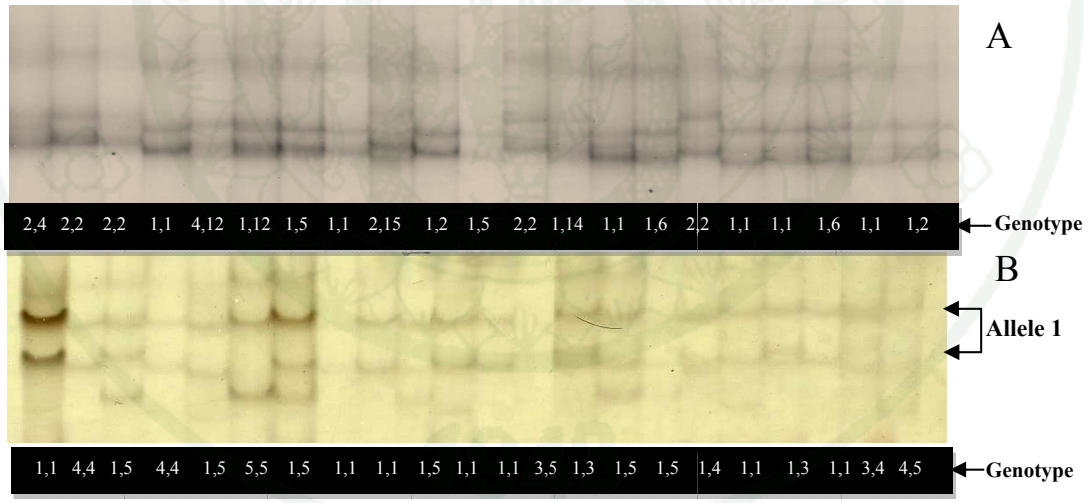

Figure 27 A and B are SSCP polyacrylamide gel of the Xx-CAT1 and Xx-CAT2 marker amplified from individual *Xylia xylocarpa* trees. Different alleles are assigned.

The Xx-AATcy (Aspartate AminoTransferase, cytoplasmic isoform) primers successfully amplified a fragment from all *X. xylocarpa* populations. On agarose gel, a single band of approximately 500 bp was visible. Different alleles could not be

distinguished (Figure 28). However, using SSCP, 3 alleles could be identified in all populations. Figure 29 shows the different alleles from *X. xylocarpa* in SSCP.

Figure 28 PCR products were amplified from *X. xylocarpa* samples from Mae Ngao National Park (1-7), Wiang Kosai National Park (8-15) using Xx-AATcy primers and compared with a size standard (λ/*Hind* III+*Eco*RI) in lane M. The size of the PCR product is slightly less than 500 bp.

Figure 29 SSCP polyacrylamide gel of the Xx-AATcy marker amplified from individual *Xylia xylocarpa* trees. Different alleles are assigned.

DNA sequence analysis

At each of the studied loci, one to three accessions from each presumed allele were selected for sequencing directly from PCR amplified fragments. Additionally, in some cases PCR products were cloned into a plasmid vector and subsequently sequenced. The individual samples that were sent for direct sequencing or cloning of alleles are listed in Appendix 2. The haplotypes were deduced from the combined information of direct sequencing and cloned fragments (Table 5). Many additional alleles were discovered by sequencing, most of them though low-frequency alleles. For all singleton haplotypes at the IDH1 locus and for most of them at other loci, the

sequencing was repeated once to confirm the presence of the variant. From the haplotype sequence data, allele-specific PCR and CAPS assays could be developed to detect particular alleles among the sampled *Xylia* trees. The combination of AS-PCR and CAPS screening greatly speeds up the allele determination of a large number of individuals for phylogeographic study: the SSCP combined with AS-PCR and CAPS assays provides information on the allele frequencies and population genetic parameters, and the sequencing of selected alleles allows the phylogenetic interpretation.

For all loci combined, 43 nucleotide substitution polymorphisms were noted in exons and 104 in introns with an additional 13 insertion / deletion mutations (Table 6). Haplotype networks were constructed for each locus (Figure 30-37).

Table 5 List of the different haplotypes differentiated by SSCP and detected through sequencing.

No	Locus	Enzyme	SSCP	Haplotypes
1	Xx-IDH1	Isocitrate Dehydrogenase	1, 2, 3/4	35
2	Xx-IPI1	Isopentenyl diphosphate isomerase	1, 2/3, 4,5	14
3	Xx-SUS1	Sucrose synthase	1, 2, 3	12
4	Xx-SUS2	Sucrose synthase	1, 2, 3	11
5	Xx-LAP1	Leucine Aminopeptidase	1, 2, 7	21
6	Xx-CAT1	Catalase	1, 2	22
7	Xx-CAT2	Catalase	1, 2/4, 5	10
8	Xx-AATcy1	Aspartate aminotransferase (cytoplasmic isoform)	1, 2, 3	21

Table 6 Nucleotide substitutions and indel polymorphisms in exons and introns for each of the studied loci.

Locus	Intron		Exon
	Substitution	Insertion/Deletion	Substitution (Synonymous, Non- synonymous)
Xx-IDH1	11	4	5 (0,5)
Xx-IPI1	11	0	1 (0,1)
Xx-SUS1	6	1	3 (1,2)
Xx-SUS2	5	0	16 (4,12)
Xx-LAP1	42	4	0
Xx-CAT1	13	0	5 (3,2)
Xx-CAT2	4	2	0
Xx-AATcy1	12	2	8 (4,4)
Total	104	13	44 (13,31)

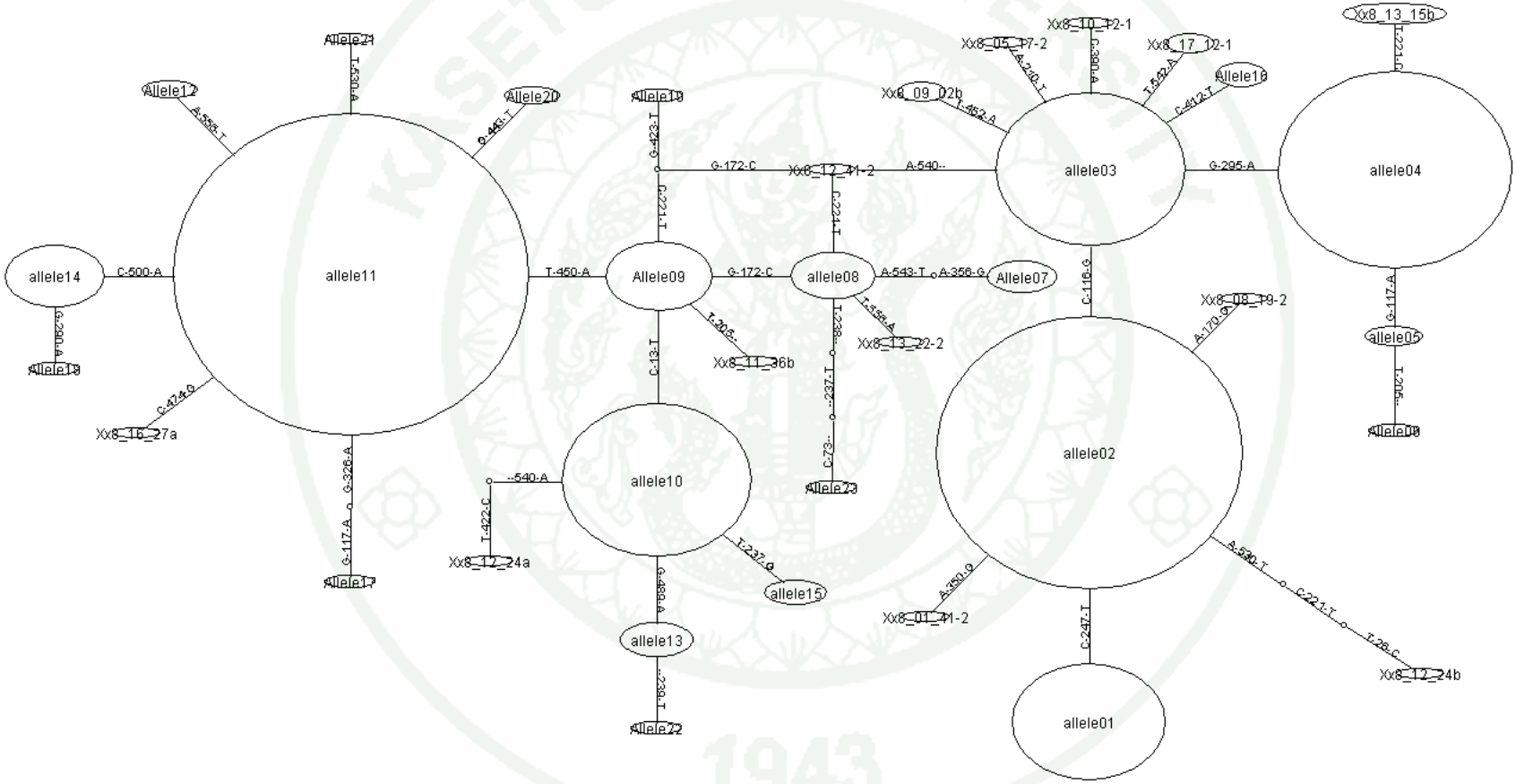

Figure 30 *Xylia xylocarpa* haplotypes at the Xx-IDH1 locus. The haplotype network was constructed using the program TCS 1.21 (Clement *et al*., 2000). 35 alleles were identified from sequencing data. The size of the circles is proportional to the frequency in the total set of sequences for this locus.

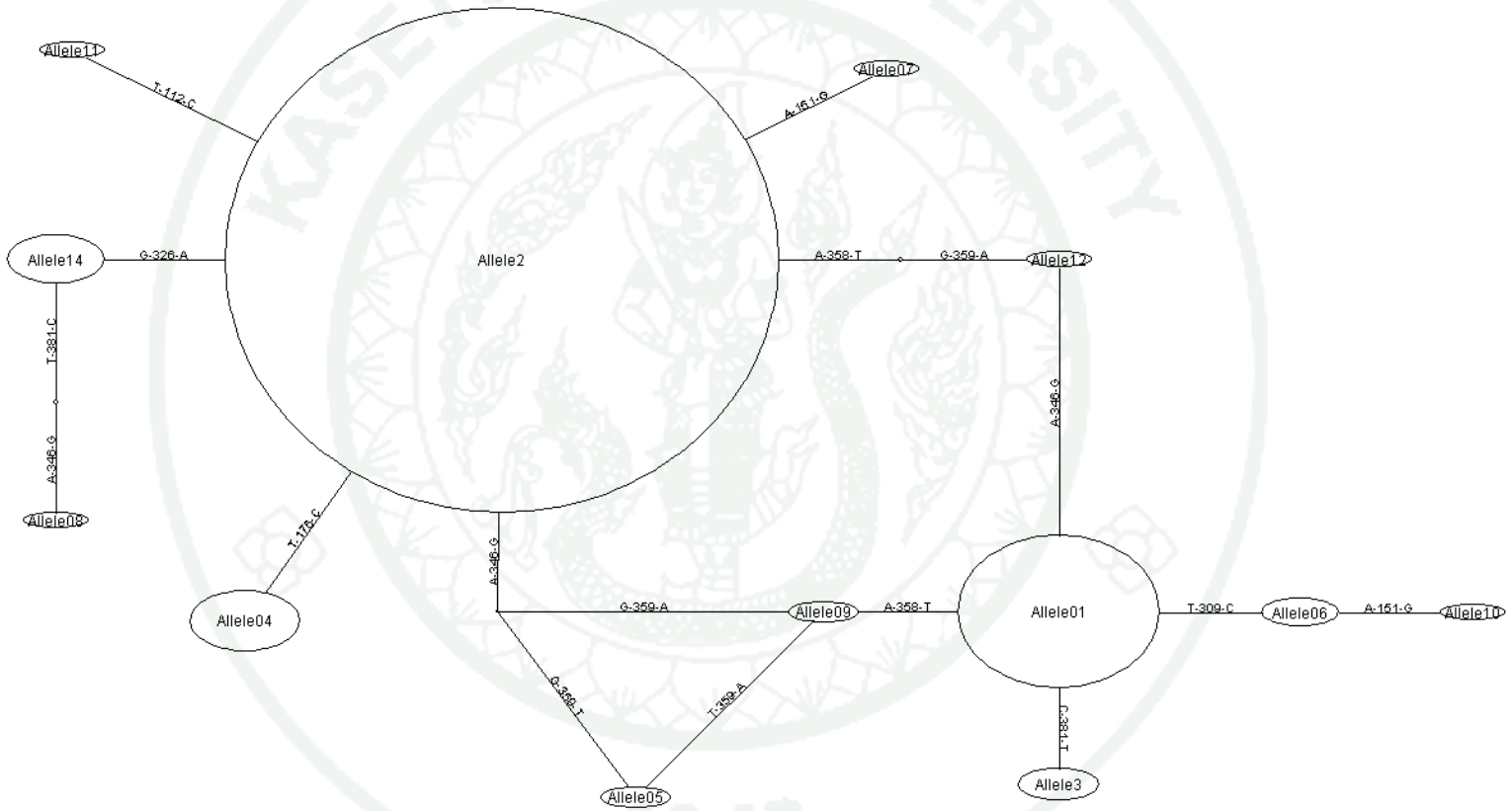

Figure 31 *Xylia xylocarpa* haplotypes at the Xx-IPI1 locus. The haplotype network was constructed using the program TCS 1.21 (Clement *et al*., 2000). 14 alleles were identified from sequencing data. The size of the circles is proportional to the frequency in the total set of sequences for this locus.

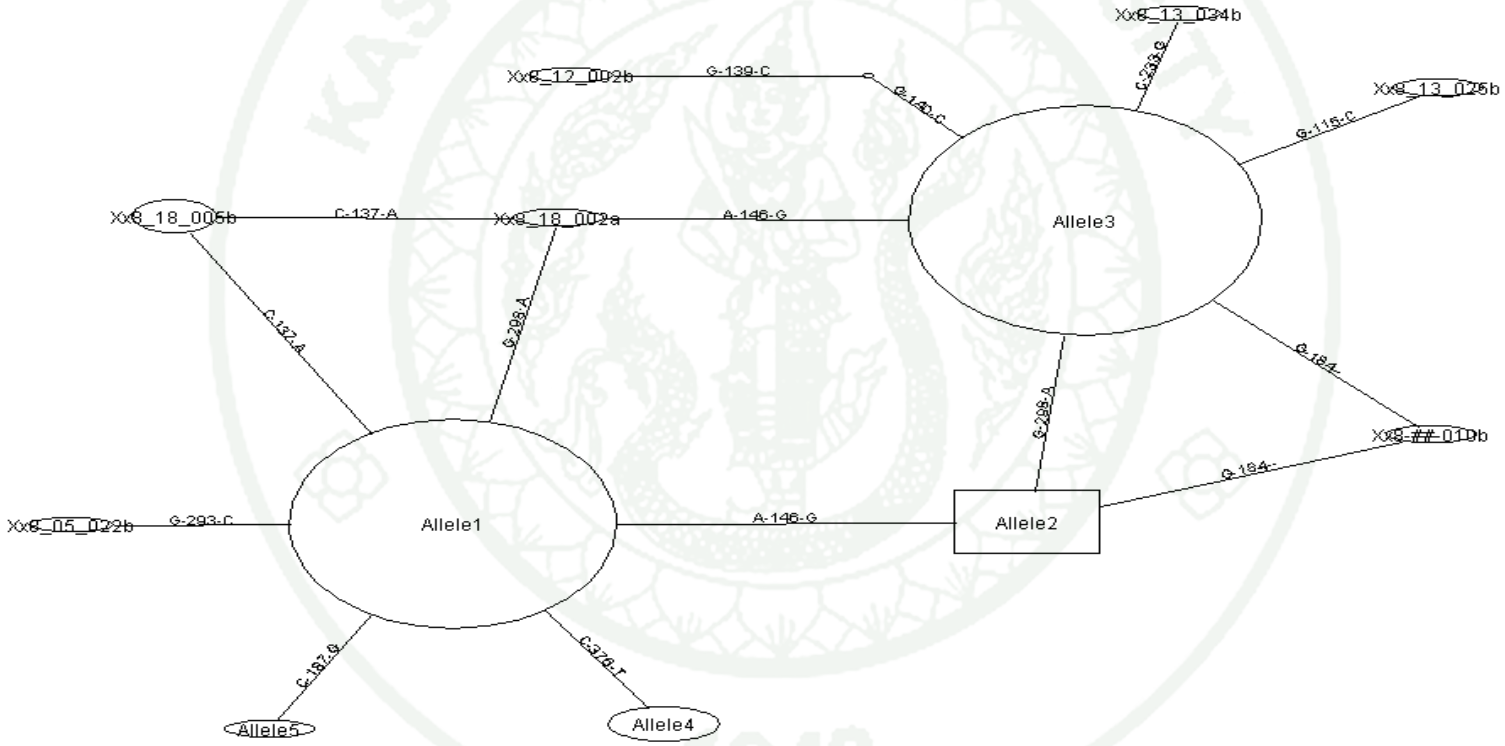

Figure 32 *Xylia xylocarpa* of Xx-SUS1 haplotypes. The haplotype network was constructed using the program TCS 1.21 (Clement *et al*., 2000). 12 alleles were identified from sequencing data. The size of the circles is proportional to the frequency in the total set of sequences for this locus.

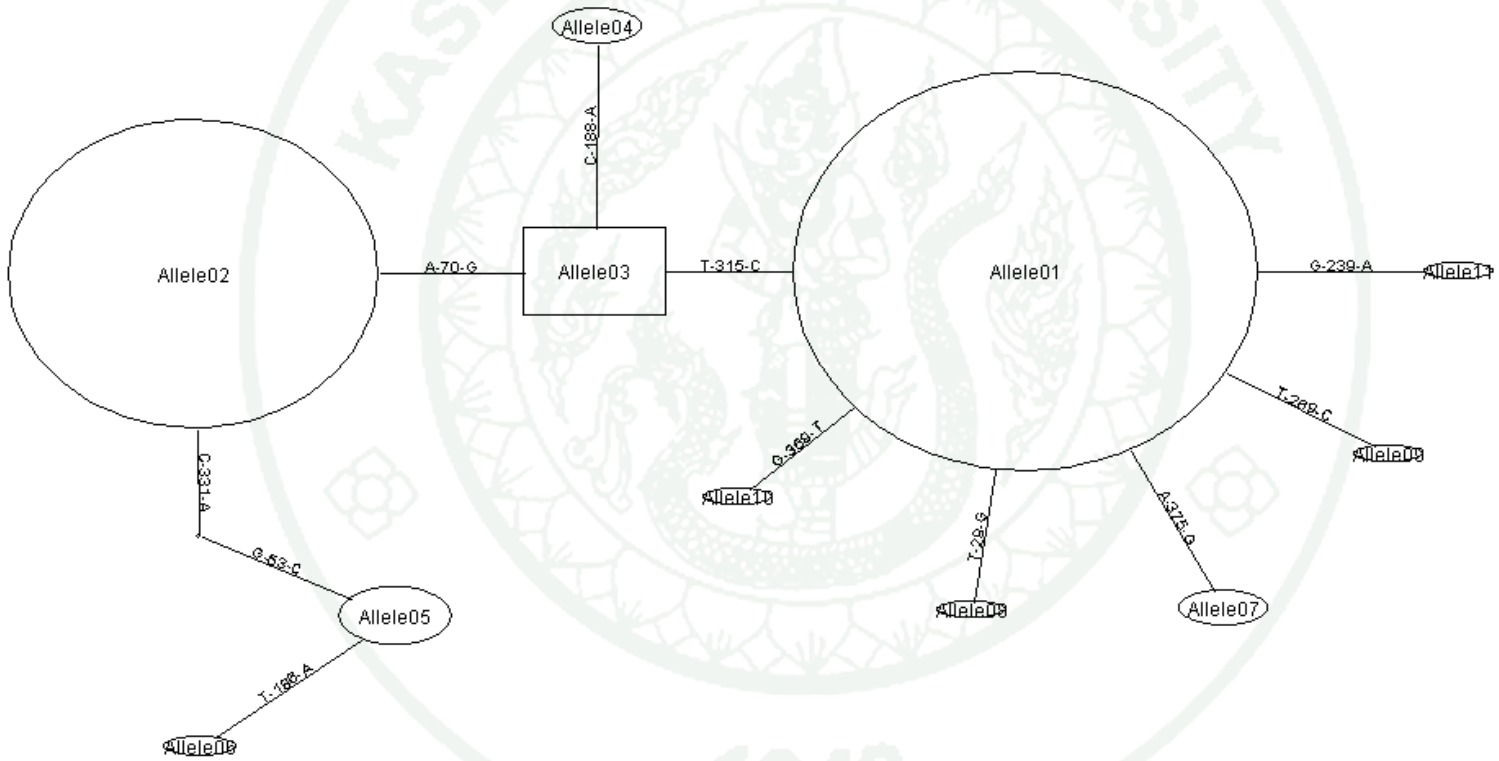

Figure 33 *Xylia xylocarpa* of Xx-SUS2 haplotypes. The haplotype network was constructed using the program TCS 1.21 (Clement *et al*., 2000). 11 alleles were identified from sequencing data. The size of the circles is proportional to the frequency in the total set of sequences for this locus.

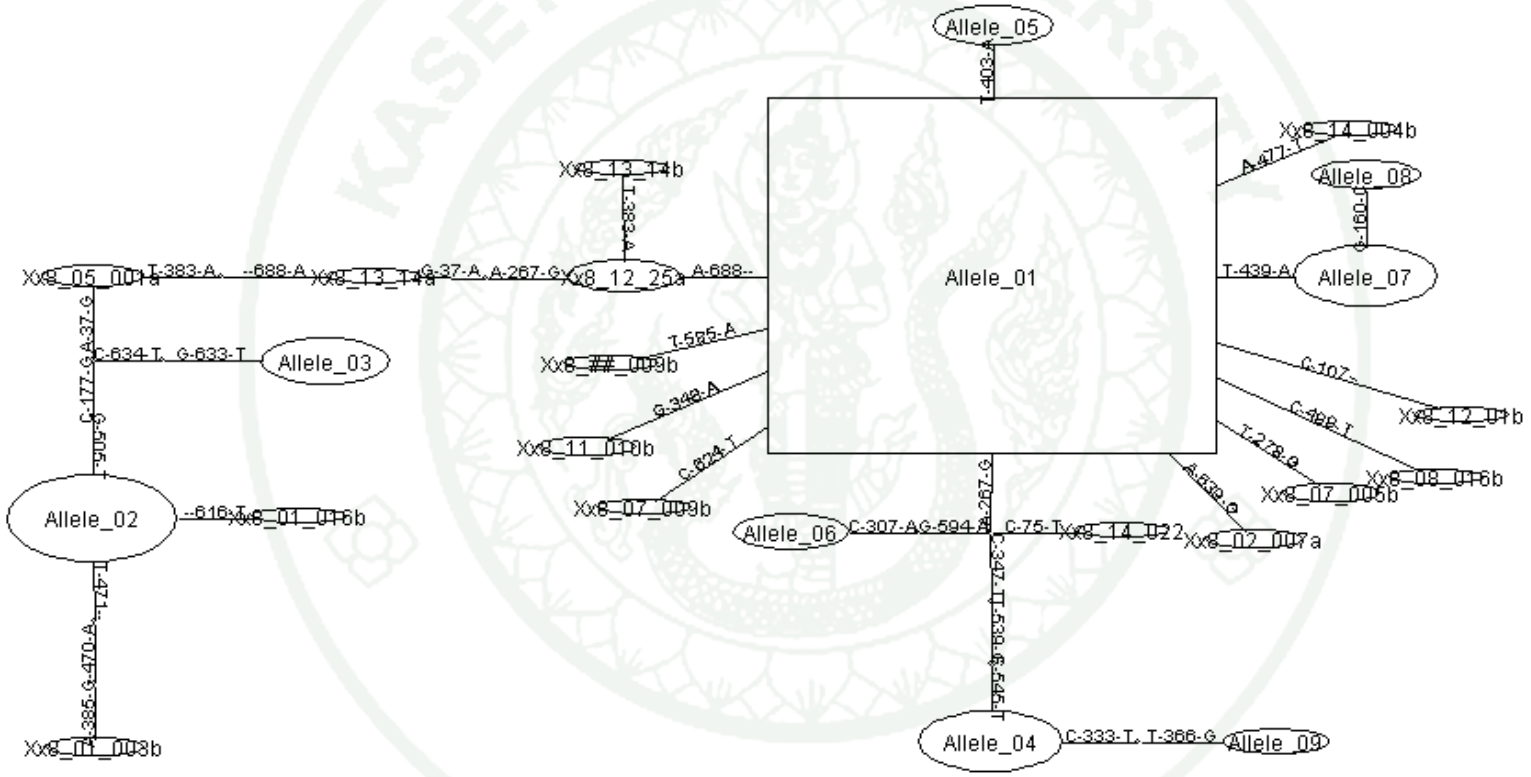

Figure 34 *Xylia xylocarpa* of Xx-LAP1 haplotypes. The haplotype network was constructed using the program TCS 1.21 (Clement *et al*., 2000). 24 alleles were identified from sequencing data. The size of the circles is proportional to the frequency in the total set of sequences for this locus.

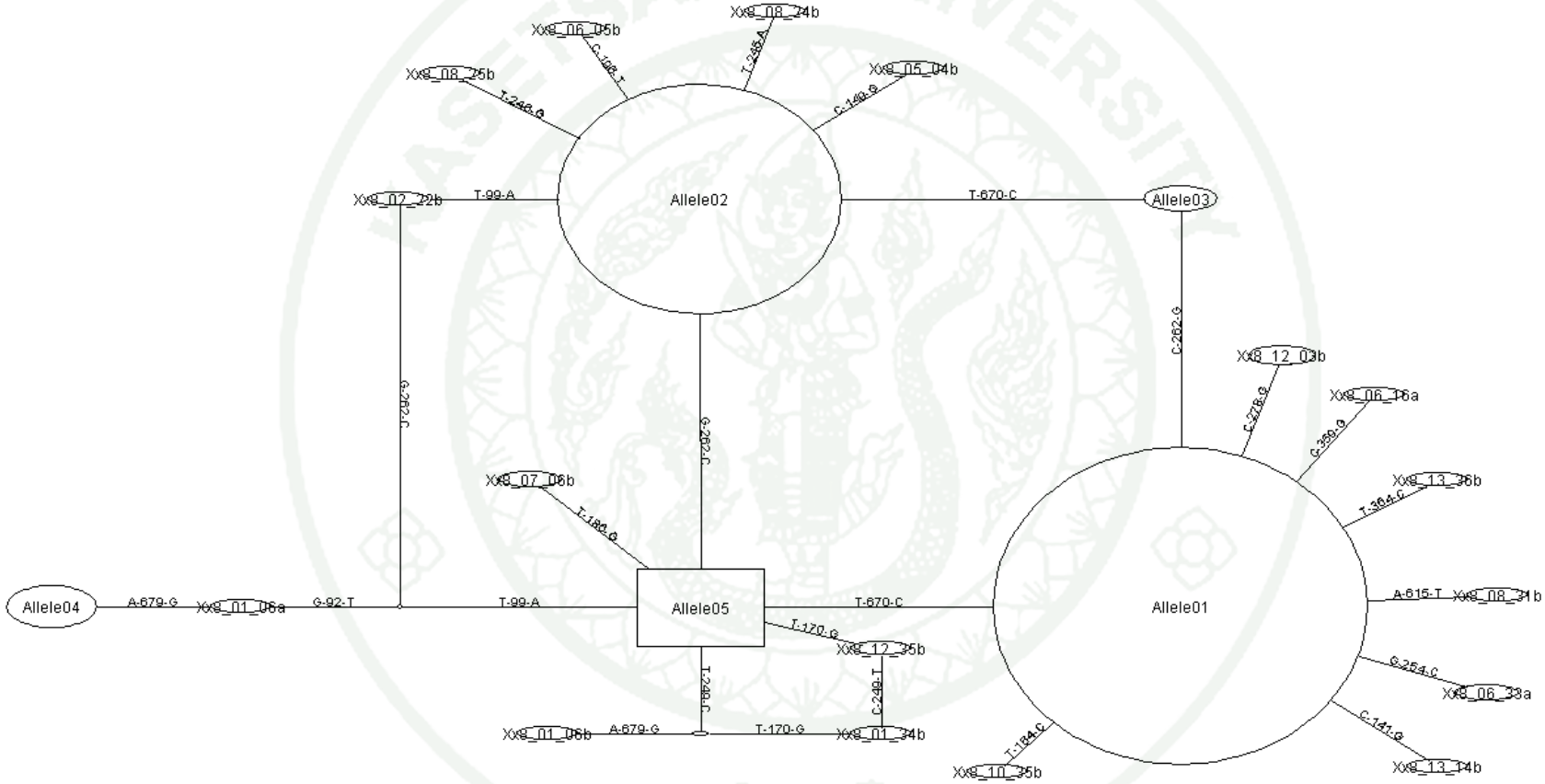

Figure 35 *Xylia xylocarpa* of Xx-CAT1 haplotypes. The haplotype network was constructed using the program TCS 1.21 (Clement *et al*., 2000). 22 alleles were identified from sequencing data. The size of the circles is proportional to the frequency in the total set of sequences for this locus.

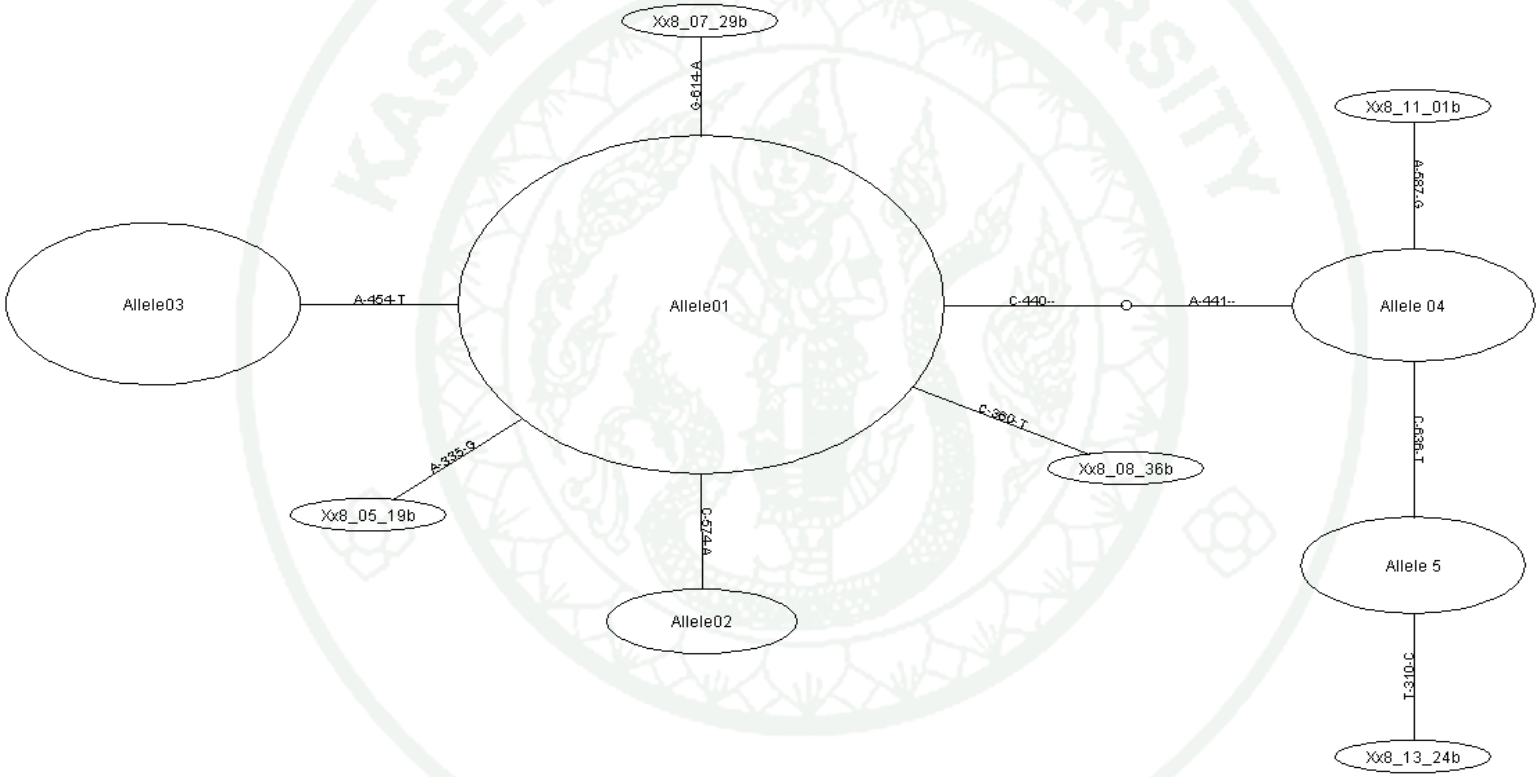

Figure 36 *Xylia xylocarpa* of Xx-CAT2 haplotypes. The haplotype network was constructed using the program TCS 1.21 (Clement *et al*., 2000). 10 alleles were identified from sequencing data. The size of the circles is proportional to the frequency in the total set of sequences for this locus.

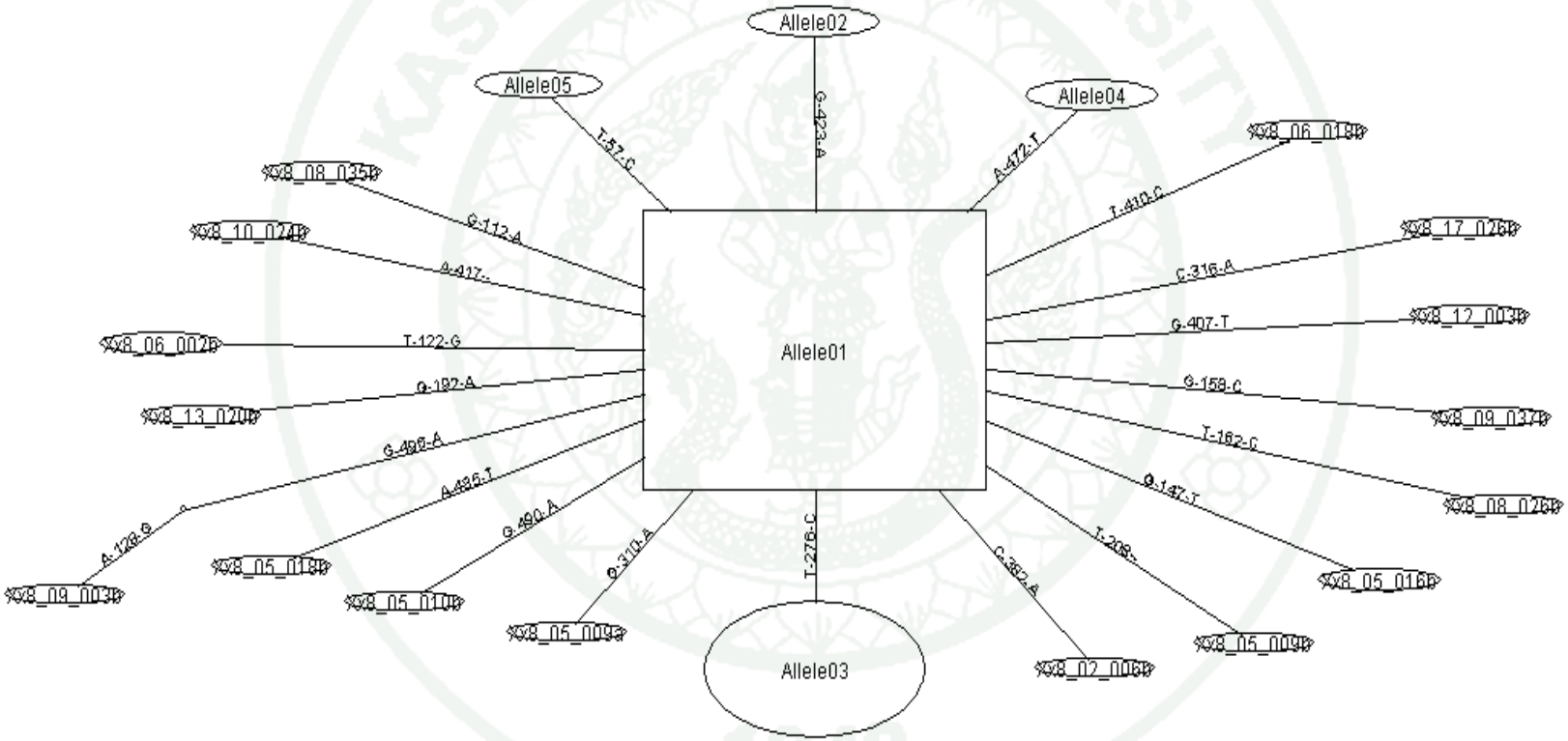

Figure 37 *Xylia xylocarpa* of Xx-AATcy1 haplotypes. The haplotype network was constructed using the program TCS 1.21 (Clement *et al*., 2000). 21 alleles were identified from sequencing data. The size of the circles is proportional to the frequency in the total set of sequences for this locus.

Development of *Xylia* allele specific assays

Sequencing indicated that the alleles found among *Xylia xylocarpa* differed due to one or a few substitution polymorphisms, though also a few insertion / deletions were detected. Because some alleles were difficult to distinguish on SSCP gels, allele specific assays were designed using allele-specific PCR or CAPS approach. Assays were designed for a total of 15 SNPs from 5 loci: IDH1 (6), LAP (1), CAT1 (2), CAT2 (4) and IPI1 (2). Ten SNPs were detected by digestion of a PCR product with restriction enzymes (CAPS). Five nucleotide polymorphisms were converted into AS-PCR tests incorporating an extra mismatching nucleotide at the -3 position (3 SNPs), three for the IDH1 locus and one each for the CAT1 and CAT2 loci. The allele-specific polymerase chain reaction (PCR) assay could distinguish the different alleles at Xx-IDH1-508, Xx-IDH1-253 and Xx-IDH1-558 (Figure 38-40).

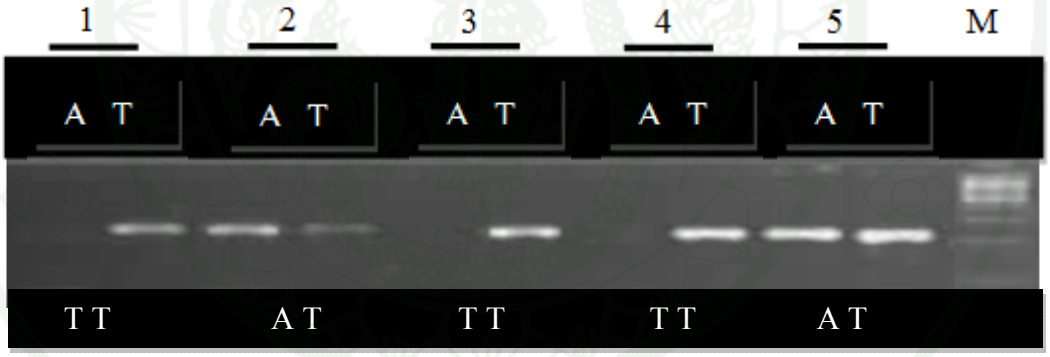

Figure 38 The allele-specific polymerase chain reaction (PCR) assay for detecting marker Xx-IDH1-508. The IDH1 fragment is polymorphic at position 508 with either A or T. The PCR amplified fragment is 212 bp long. The T and A alleles were amplified in separate PCR reactions. The deduced genotypes for the individuals are indicated.

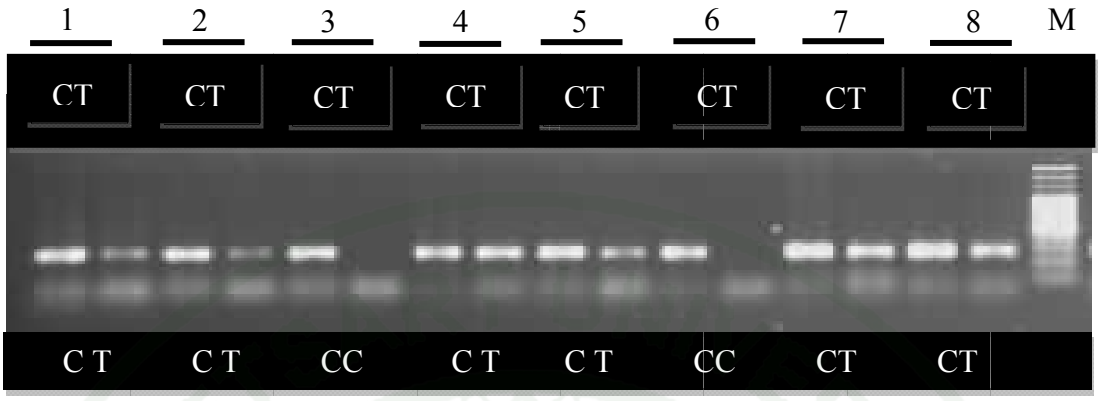

Figure 39 The allele-specific polymerase chain reaction (PCR) assay for detecting marker Xx-IDH1-253. The IDH1 fragment is polymorphic at position 253 with either C or T. The PCR amplified fragment is 273 bp long. The C and T alleles were amplified in separate PCR reactions. The deduced genotypes for the individuals are indicated.

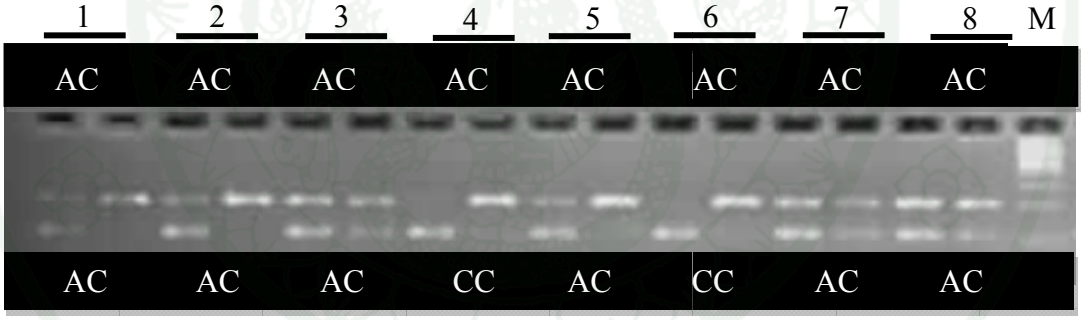

Figure 40 The allele-specific polymerase chain reaction (PCR) assay for detecting marker Xx-IDH1-558. The IDH1 fragment is polymorphic at position 558 with either A or C. The target fragment is 210 bp long. The A and C alleles were amplified in separate PCR reactions. The deduced genotypes for the individuals are indicated.

A total of ten SNPs in IDH1 (3), CAT1 (1), CAT2 (3), LAP1 (1) and IPI1 (2) were converted into a CAPS assay using the dCAPS Finder 2.0 website. The CAPS-PCR reaction mixtures and condition were the same as those used for the AS-PCR protocol. The restriction enzyme with IDH1 (1 locus) PCR product was *Bcc*I (New

England Biolabs, USA). Incubated at 37°C 2 hr. and separated on 3% SeaKem agarose (Figure 41).

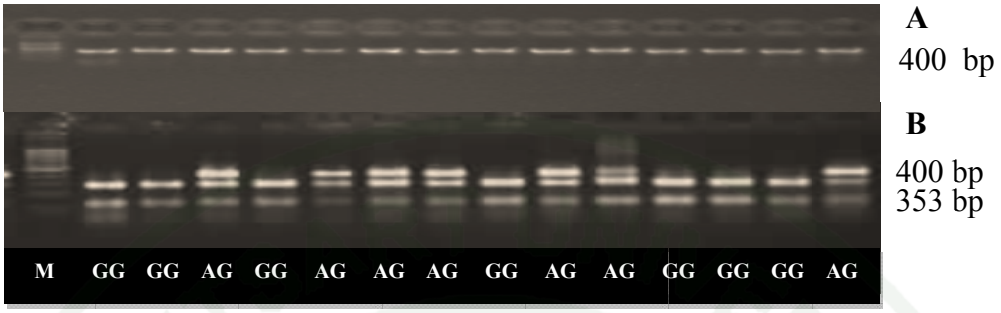

Figure 41 The CAPS marker Xx-IDH1 *Bcc*I. The IDH1 fragment is polymorphic at position 353 with either A or G. The PCR amplified fragment is 400 bp long. The G allele can be cut by the *Bcc*I restriction enzyme (GGTAGN^) A PCR fragment before digestion, B after *Bcc*I digestion.

The amplified PCR product was digested with *Dde*I for IDH1 2 loci. Detect marker Xx-IDH1-CAPS-F1 + Xx-IDH1-CAPS-R1 (Figure 42) and Xx-IDH1-CAPS-F2 + Xx-IDH1-CAPS-R2 (Figure 43) incubated at 37°C 2 hr. and separated on 2% agarose gel.

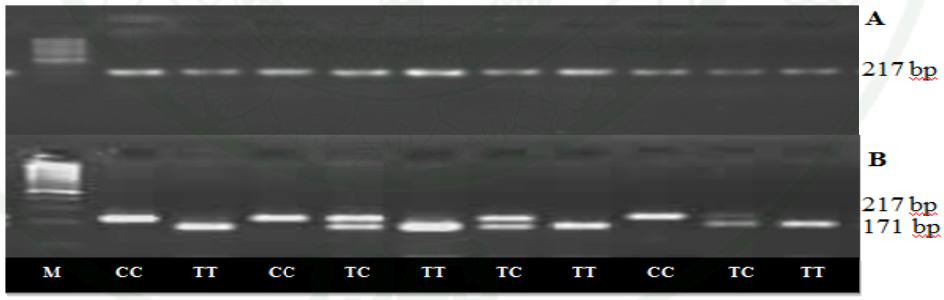

Figure 42 The CAPS marker Xx-IDH1 *Dde*I. The IDH1 fragment is polymorphic at position 46 with either T or C. The PCR amplified fragment is 217 bp long. The T allele can be cut by the *Dde*I restriction enzyme (C^TNAG) which results in a fragment of 171 and 46 bp (not visible on gel). A PCR fragment before digestion, B after *Dde*I digestion.

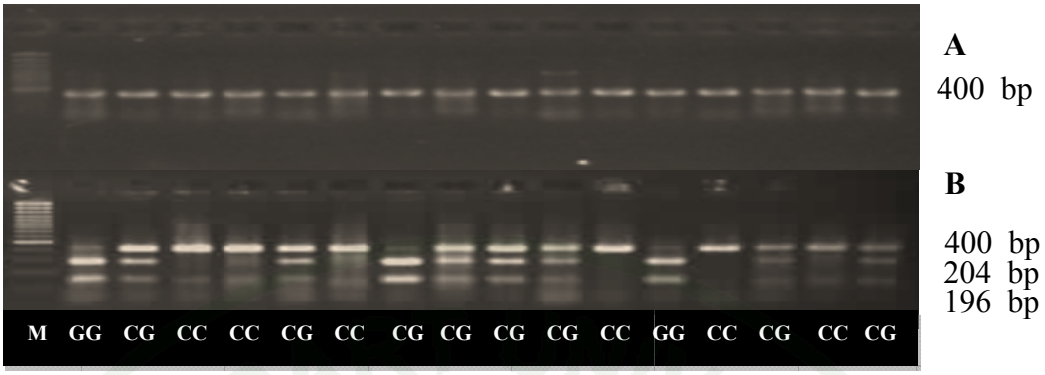

Figure 43 The CAPS marker Xx-IDH1 *Dde*I. The IDH1 fragment is polymorphic at position 204 with either A or G. The PCR amplified fragment is 400 bp long. The G allele can be cut by the *Dde*I restriction enzyme (C^TNAG) A PCR fragment before digestion, B after *Dde*I digestion.

The amplified PCR product was digested with *Alu*l for CAT1 1 locus. Detect marker Xx-CAT1-F + Xx-CAT1-AluI-R (Figure 44). One amplified PCR product was digested with *Nla*III for CAT2 1 locus. Detect marker Xx-CAT2-Nla-F + Xx-CAT2-Nla-R (Figure 45) incubated at 37^{o}C 2 hr. Two PCR product digested with *Taa*I for LAP1 (1 locus) Xx-LAP1-F + Xx-LAP1-Tsp-R and CAT2 (1 locus) Xx-CAT2-Tsp-F + Xx-CAT2-Tsp-R**.** The other restriction enzyme with CAT2 (1 locus) PCR product was *Tas*I. Detect marker Xx-CAT2-Tas-F + Xx-CAT2-Tas-R (Figure 46)**.**

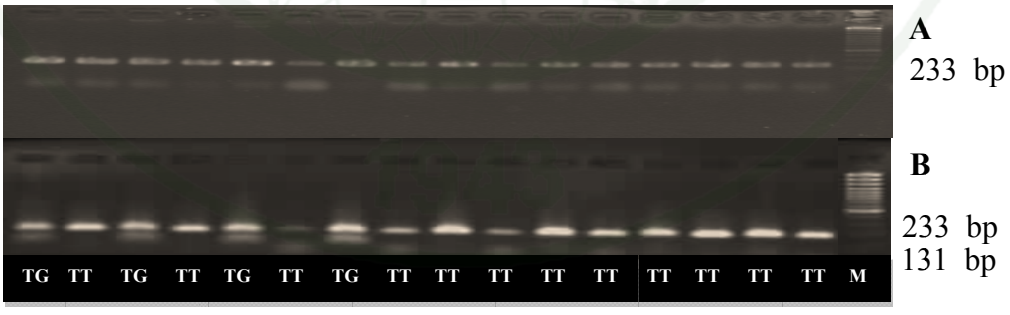

Figure 44 The CAPS marker Xx-CAT1 *Alu*l. The CAT1 fragment is polymorphic at position 131 with either T or G. The PCR amplified fragment is 233 bp long. The G allele can be cut by the *Alu*l restriction enzyme (AG^CT) which results in a fragment of 131 and 102 bp (not visible on gel). A PCR fragment before digestion, B after *Alu*l digestion.

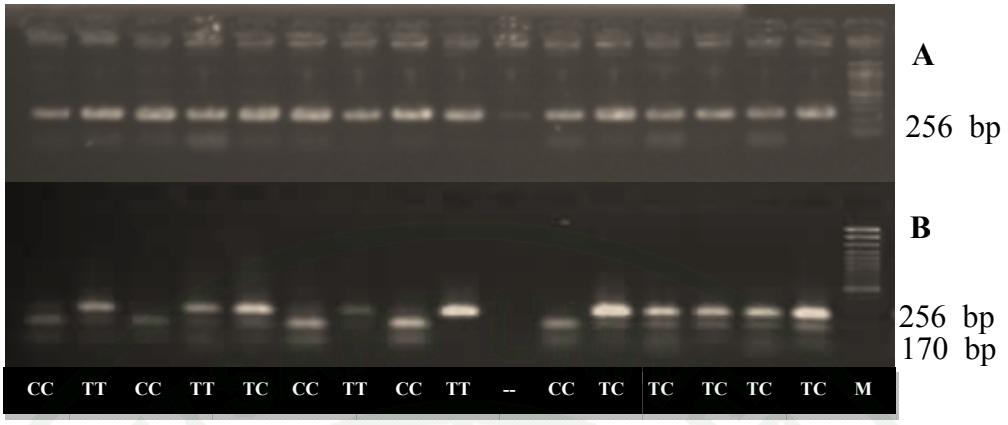

Figure 45 The CAPS marker Xx-CAT2 *Nla*III. The CAT2 fragment is polymorphic at position 637 with either T or C. The PCR amplified fragment is 256 bp long. The C allele can be cut by the *Nla*III restriction enzyme (CATG^) which results in a fragment of 170 and 86 bp (not visible on gel). A PCR fragment before digestion, B after *Nla*III digestion.

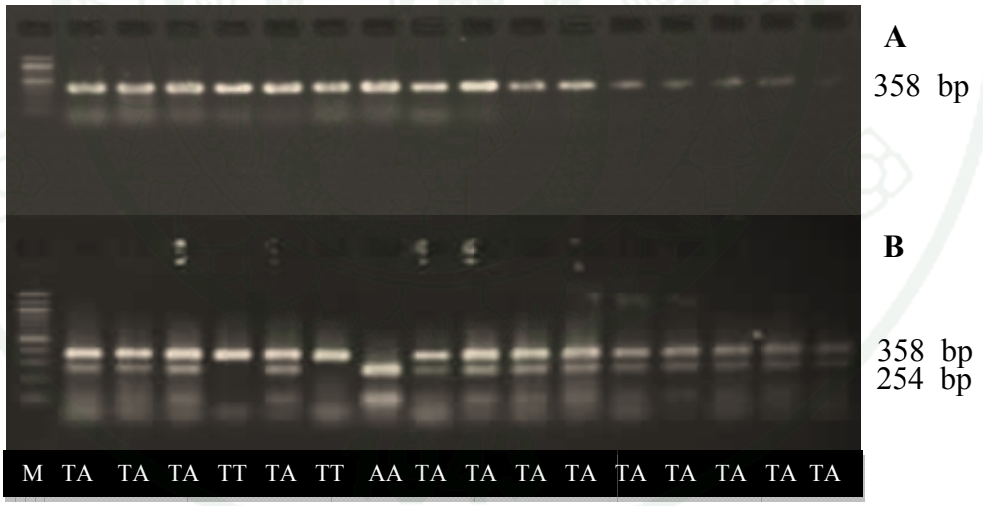

Figure 46 The CAPS marker Xx-CAT2 *Tas*I. The CAT2 fragment is polymorphic at position 457 with either T or A. The PCR amplified fragment is 358 bp long. The A allele can be cut by the *Tas*I restriction enzyme (^AATT) which results in a fragment of 254 and 104 bp (not visible on gel). A PCR fragment before digestion, B after *Tas*I digestion.

The IPI1 locus was amplified with the regular PCR primers and the obtained fragment was digested with *Vsp*I (37°C 2 hr) (Figure 47) and separately with *Taq*$^{\alpha}$I (65°C 2 hr) (Figure 48).

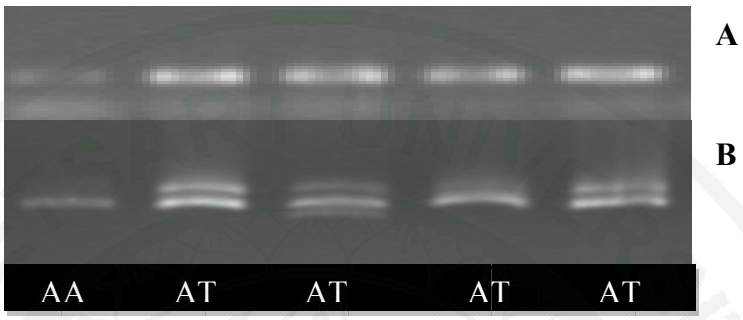

Figure 47 The CAPS marker Xx-IPI1. The IPI1 fragment is polymorphic at position 392 with either A or T. The PCR amplified fragment is 449 bp long. The A allele can be cut by the *Vsp*I restriction enzyme (TA^ATTA) which results in a fragment of 392 and 449 bp. A PCR fragment before digestion, B after *Vsp*I digestion.

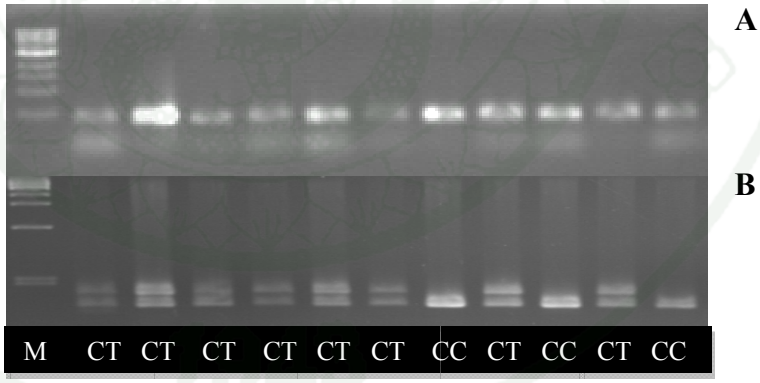

Figure 48 The CAPS marker Xx-IPI1. The IPI1 fragment is polymorphic at position 414 with either C or T. The PCR amplified fragment is 449 bp long. The C allele can be cut by the *Taq*$^{\alpha}$I restriction enzyme (T^CGA) which results in a fragment of 414 and 449 bp. A PCR fragment before digestion, B after *Taq*$^{\alpha}$I digestion.

Structure of the Leucine Aminopeptidase1 gene.

The fragment amplified from the LAP1 locus was 884 bp long and consisted of the 3' end of the 6th exon and the complete 6th intron. One polymorphism in the intron was detected with a CAPS assay (Figure 49) using a specially designed reverse primer for the PCR reaction. The PCR product was digested with *Taa*I at position 225 with either C or G. The C allele can be cut by the *Taa*I restriction enzyme (CAN^GT).

Structure of the Catalase genes.

DNA fragments were amplified from two loci in the genomic DNA coding for catalase genes. The fragments were 880 and 847 bp long, respectively, and contained the 3' end of the third exon and most of the 4th, plus the intervening intron. One SNP in the CAT1 locus was detected with an allele-specific test and another SNP was converted to a CAPS marker. Similarly in the CAT2 locus, three SNPs were converted into CAPS assays and one was detected by AS-PCR. To detect marker Xx-CAT1-F + Xx-CAT1-AluI-R and Xx-CAT1-709 (Figure 50) of Catalase1 gene, one PCR product digested with *Alu*l, position 131 with either T or G. The G allele can be cut by the *Alu*l restriction enzyme (AG^CT), One AS-PCR fragment is polymorphic at position 709 with either T or C. The PCR amplified fragment is 270 bp long. The T allele can be amplified Xx-CAT1-709-F-T + Xx-CAT1-709-R, The C allele can be amplified by Xx-CAT1-709-F-C + Xx-CAT1-709-R.

CAT2 marker Xx-CAT2-Tas-F + Xx-CAT2-Tas-R, Xx-CAT2-Tsp-F + Xx-CAT2-Tsp-R, Xx-CAT2-Nla-F + Xx-CAT2-Nla-R and Xx-CAT2-610 (Figure 51) of Catalase2 gene, One PCR product digested with *Tas*I, position 457 with either T or A. The A allele can be cut by the *Tas*I restriction enzyme (^AATT), One PCR product digested with *Taa*I, position 577 with either C or A. The A allele can be cut by the *Taa*I restriction enzyme (CAN^GT) and PCR product digested with *Nla*III, position 637 with either C or T. The C allele can be cut by the *Nla*III restriction enzyme (CATG^). AS-PCR fragment is polymorphic at position 610 with either G or A. The PCR amplified fragment is 526 bp long. The G allele can be amplified Xx-CAT2-610-

F + Xx-CAT2-610-R-G, The A allele can be amplified by Xx-CAT2-610-F + Xx-CAT2-610-R-A

Structure of Isocitrate dehydrogenase1 gene.

A DNA fragment was amplified from the *Xylia* genome coding for an isocitrate dehydrogenase gene. The fragment was 744 bp long and consisted of the 3' end of the 11 th exon to the 14th, including the three intervening introns. Six SNPs in the IDH1 locus for allele specific PCR 6 loci were genomic DNA to detect marker Xx-IDH1-CAPS-F1 + Xx-IDH1-CAPS-R1, Xx-IDH1-CAPS-F2 + Xx-IDH1-CAPS-R2 (2 loci), Xx-IDH1-508, Xx-IDH1-558 and Xx-IDH1-253 (Figure 52) of Isocitrate dehydrogenase1 gene, Two PCR product digested with *Dde*I position 46, 204 with either T or C for position 46, G or C for position 204. T (position 46), G (position 204) alleles can be cut by the *Dde*I restriction enzyme (C^TNAG) and One PCR product digested with *Bcc*I, position 353 with either G or A. The G allele can be cut by the *Bcc*I restriction enzyme (GGTAGN^). Three AS-PCR fragment are polymorphic at position 253, 508 and 558 with either T or C (position 253), T or A (position 508) and A or C (position 558). PCR amplified fragment is 273 bp (position 253), 212 bp (position 508) and 210 bp (position 558) long. The position 253 T allele can be amplified Xx-IDH-F + Xx-IDH1-253-R-T, The C allele can be amplified by Xx-IDH-F + Xx-IDH1-253-R-C. The position 508 T allele can be amplified Xx-IDH1-508-F + Xx-IDH1-508-R-T, The A allele can be amplified by Xx-IDH1-508-F + Xx-IDH1-508-R-A. The position 558 A allele can be amplified Xx-IDH1-558-F-A + Xx-IDH1-RL, The C allele can be amplified by Xx-IDH1-558-F-C + Xx-IDH1-RL.

Structure of Isopentenyl diphosphate isomerase1 gene.

A DNA fragment was amplified from the *Xylia* genome coding for an isopentenyl diphosphate isomerase gene. The fragment was 449 bp long and consisted of most of the first exon to the 5' end of the third, including the two short intervening introns. Two SNPs were detected by restriction enzyme digestion of the full IPI1 fragment (Figure 53). A restriction enzyme digestion with *Vsp*I detected a SNP at

position 392 with either A or T. The A allele can be cut by the type II restriction enzyme (TA^ATTA). Another SNP at position 414 was detected after digestion with *Taq*$^{\alpha}$I, with either C or T. The C allele can be cut by the *Taq*$^{\alpha}$I restriction enzyme (T^CGA).

Figure 49 Structure of the Leucine Aminopeptidase1 locus showing exon – intron structure and SNP location (CAPS, triangle).

Figure 50 Structure of the Catalase1 locus showing exon – intron structure and SNP location (CAPS, triangle; AS-PCR, diamond).

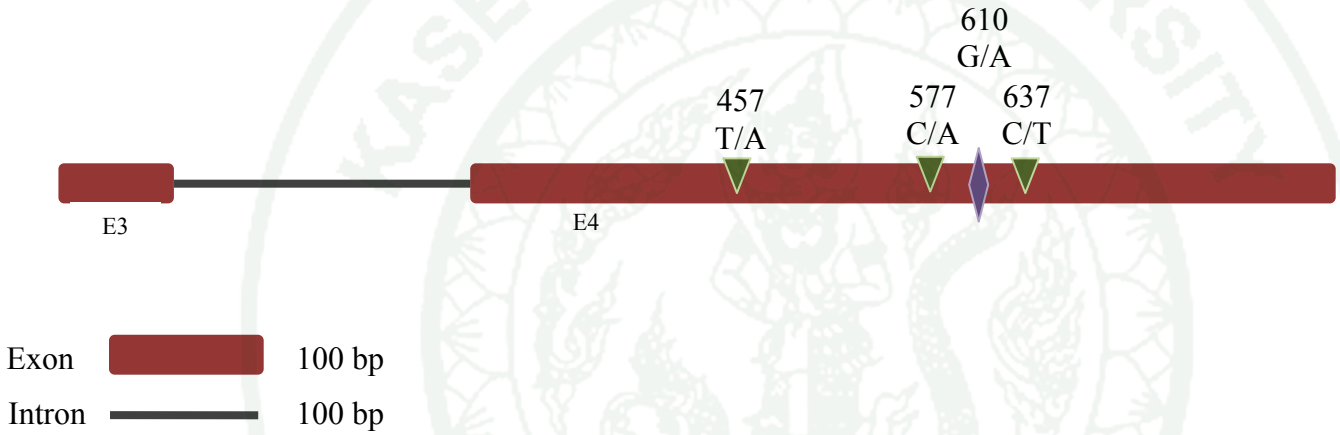

Figure 51 Structure of the Catalase2 locus showing exon – intron structure and SNP location (CAPS, triangle; AS-PCR, diamond).

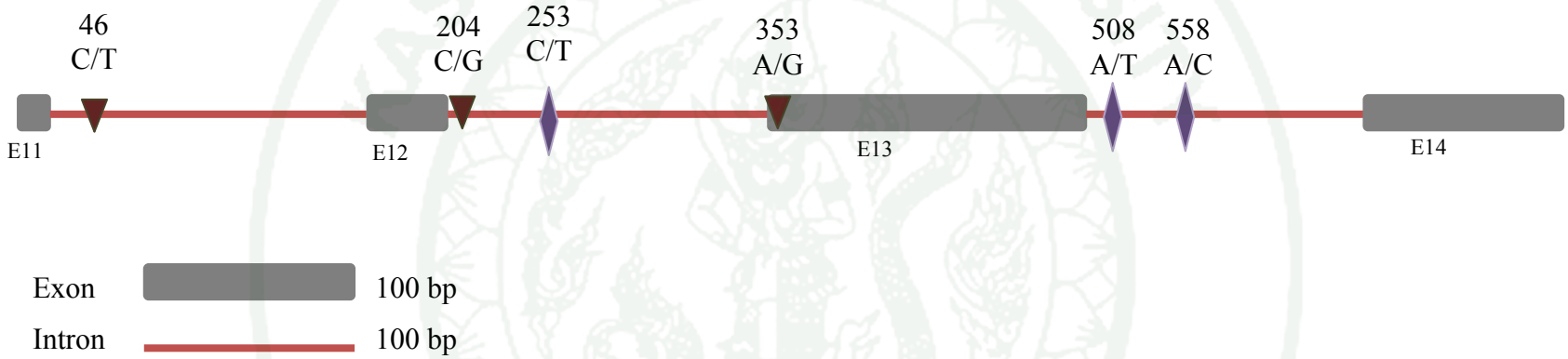

Figure 52 Structure of the Isocitrate dehydrogenase1 locus showing exon – intron structure and SNP location (CAPS, triangle; AS-PCR, diamond).

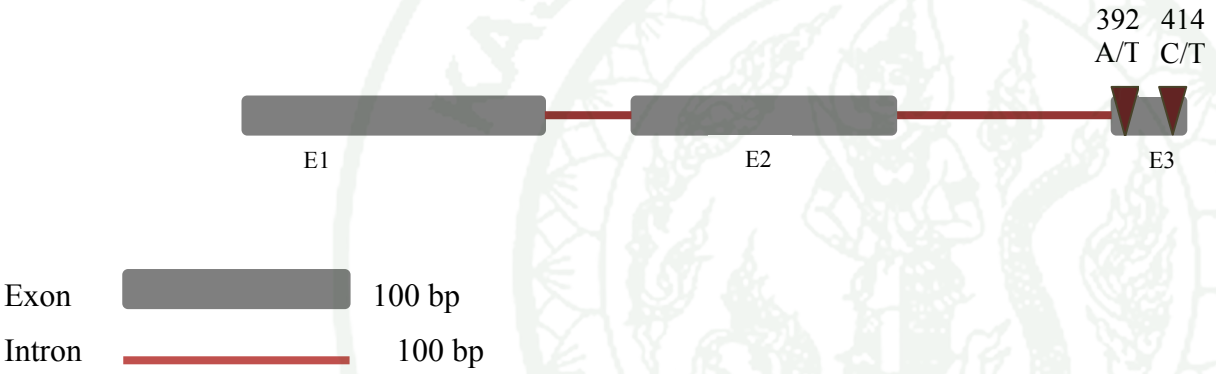

Figure 53 Structure of the Isopentenyl diphosphate isomerase1 locus showing exon – intron structure and SNP location (CAPS, triangle.).

Genetic parameters.

Summary population genetic parameters of *Xylia* at 8 loci (SUS1, SUS2, AATcy1, CAT1, CAT2, IDH1 LAP1 and IPI1) were calculated using GENALEX 6.4 using options appropriate for codominant markers. The number of alleles, heterozygosity (H_o and H_e), F-statistics and polymorphism per locus were obtained for each population (Table 7) and for the combined sample. The effective number of alleles which is the number of equally frequent alleles it would take to achieve a given level of genetic diversity, ranged from 1.1 (AATcy) to 7.6 (IDH1) but was much lower than the actual number of alleles for each locus where 20 alleles were observed for AATcy and 34 for the IDH1 locus. This is due to the presence of a large number of rare alleles, most of which were revealed by direct sequencing of PCR products. The observed heterozygosity ranged from 0.091 at the AATcy locus to 0.819 at the IDH1 locus with a mean of 0.460 for the 8 loci. CAT1, CAT2 and IPI1 had higher inbreeding coefficient F_{IS} = 0.223, 0.204 and 0.155, respectively. SUS1 and IDH1 had lowest F_{IS}. Diversity was also estimated using the Shannon diversity index. The value of the effective number of migrants (Nm) was higher than 1 for all loci, with a mean of 4.291, indicating a high level of geneflow among the populations. Deviations from Hardy-Weinberg Equilibrium per population were calculated using the GENEPOP web-based service. The SUS1 locus was the only marker that was in HWE in all populations and the CAT1 locus had the largest number (9) of populations that were not in HWE (Table 8).

Genetic distances and AMOVA.

Pairwise population genetic distances were calculated according to Nei's unbiased genetic distance (G_{st}) in GENALEX 6.4 and the Jost D_{est} was calculated with SMOGD. Both measures showed a similar pattern with most of the largest genetic distances between the ThaTaFang area and MaeNgao National Park on one hand and Pangsida and PhuJongNaYoi National Parks on the other (Table 9). A neighbour-joining phenetic tree calculated based on these distances shows the relationships among the 16 populations clearly (Figure 54). Overall though, the genetic distances

were quite small as even the geographically most distant populations (LamKhlongNgu, Erawan, MaeNgao, ThaTaFang from western Thailand vs. PangSida, PhuJongNaYoi, PhuphaLek from northeastern Thailand.) shared several genotypes. A Mantel test showed a low correlation between the genetic and geographical distances (Rxy = 0.041) which was however significant (P = 0.02).

An analysis of molecular variance (AMOVA) for the 538 *Xylia* trees from 16 populations was done in GENALEX 6.4. Results of the AMOVA indicate that for all loci the total F_{st} = 0.093, F_{is} = 0.078 and F_{it} = 0.163. The AMOVA derived F statistics per locus and for all loci combined with their probabilities are presented in Table 10. Most of the total variation was distributed within individuals (84%) with the remaining among population (9%), and among individuals within populations (7%). The variation for all sources was significant (*P* = 0.01) (Figure 55).

STRUCTURE, DARwin5.

The programme DARwin5 was used for a principal component clustering analysis and neighbor joining tree calculation. DARwin5 calculates a genetic distance among individuals based on the number of shared alleles in a diploid organism. Neither the principal component analysis of all *Xylia* genotypes nor the unweighted neighbor-joining tree revealed a clustering of the 548 individuals, including the 10 individual trees sampled from different locations in Thailand (Figure 56). The trees from western Thailand (LamKhlongNgu, Erawan, MaeNgao National Parks and ThaTaFang area) have been indicated in green. The program STRUCTURE tries to cluster individuals based on their genotypes by optimizing likelihoods in a Bayesian analysis using Markov Chain. The analysis is repeated for several possible values of K, the assumed number of (ancestral) populations. Based on the calculated likelihood (Ln P(D)) and its variance the true number of populations (K) can be deduced. The STRUCTURE analysis of all 548 trees lends weak support for a division of all *X. xylocarpa* trees into two groups when admixture is allowed. When using the "No admixture" option, the highest likelihood was obtained for K=3 (correlated allele frequencies) or K = 4 (allele frequencies independent) (Table 11). When assuming

two populations (K=2), the trees from western Thailand (Lam Khlong Ngu, Erawan, MaeNgao and ThaTaFang, indicated in red) separate from the other populations. When assuming three populations (K=3) no clear signal for further population differentiation was obtained (Figure 57).

Table 7 Summary of population genetic parameters per locus for all *Xylia xylocarpa* samples.

Locus	Sample Size	No. Alleles	No. Effective Alleles	Observed Heterozygosity	Unbiased Expected Heterozygosity	Fixation Index	Information Index	Effective migrants
	N	Na	Ne	Ho	UHe	F	I	Nm
SUS2	548	11	2.267	0.513	0.559	0.082	1.026	4.864
AATcy	548	20	1.116	0.091	0.104	0.123	0.319	4.885
SUS1	547	8	2.422	0.559	0.588	0.047	1.005	4.254
CAT1	548	15	2.666	0.485	0.625	0.223	1.223	7.399
CAT2	547	8	2.119	0.420	0.529	0.204	1.106	2.748
LAP1	547	15	1.448	0.289	0.310	0.067	0.801	4.498
IPI1	547	14	2.470	0.503	0.596	0.155	1.284	1.744
IDH1	548	34	7.601	0.819	0.869	0.057	2.317	3.936
Mean	547.5	15.625	2.764	0.460	0.522	0.120	1.135	4.291

* = P<0.05, ** = P<0.01, *** = P<0.001.

Table 8 Summary of tests for Hardy-Weinberg Equilibrium per population and locus.

Location	Locus							
	SUS2	AATcy	SUS1	CAT1	CAT2	LAP1	IPI1	IDH1
LamKhlongNgu	0.1978	0.4655	0.1436	0.0055**	0.1721	0.0003***	0.0108*	0.0031**
Erawan	0.2419	0.1332	0.5018	0.3225	0.0801	0.3939	0.0050**	0.0535
Vichienburi	1.0000	0.0663	0.7602	0.1222	1.0000	0.0697	0.0023**	0.0062**
Pangsida	0.1540	1.0000	1.0000	0.2313	0.6514	0.3454	0.0209*	0.0206*
PhuJongNaYoi	0.0461*	NA	0.7263	0.0016**	0.0001***	0.2798	0.1397	0.5347
PhuphaLek	0.9231	1.0000	0.5266	0.0000***	0.0567	0.0026**	0.2194	0.3080
KhlongTron	0.2615	0.1787	0.6818	0.0079**	0.0954	0.0372*	0.0000***	0.0282*
ThamPhaThai	0.2649	1.0000	0.4762	0.1598	0.0778	0.0183*	0.4452	0.6732
PhaDaeng	0.0918	NA	0.4676	0.2442	0.0039**	0.5604	0.1020	0.0377*
DoiJong	0.9052	1.0000	0.1068	0.5018	0.0006***	1.0000	0.8002	0.1792
KlongWangJao	0.5401	0.0194*	0.2616	0.1219	0.9041	1.0000	0.6059	0.1388
MaeNgao	0.0016**	1.0000	0.5801	0.0000***	0.0064**	0.1160	0.0291*	0.4857
ThaTaFang	0.1090	0.2756	0.1475	0.0044**	NA	1.0000	0.5003	0.3811
MaePing	1.0000	NA	1.0000	0.0006***	0.4932	1.0000	0.7502	0.7847
KaengJedKhwae	0.0851	1.0000	0.7154	0.0567	0.2004	1.0000	0.1537	0.1292
ViengKosai	0.3527	1.0000	0.0693	0.0000***	0.0002***	1.0000	0.7692	0.1628
All	0.0126*	0.5249	0.5894	0.0000***	0.0001***	0.0018**	0.0000***	0.0001***

* = P<0.05, ** = P<0.01, *** = P<0.001. NA = not available

Table 9 Matrix of pairwise population genetic distances. The Nei unbiased genetic distance (G_{st}) is given below the diagonal, the Jost D_{est} measure is given above the diagonal.

1	2	5	6	7	8	9	10	11	12	13	14	16	17	18	15	
--	0.005	0.037	0.085	0.055	0.047	0.040	0.038	0.020	0.060	0.038	0.018	0.028	0.076	0.041	0.013	LamKhlongNgu
0.008	--	0.013	0.059	0.040	0.024	0.028	0.017	0.012	0.047	0.012	0.017	0.016	0.066	0.050	0.021	Erawan
0.104	0.047	--	0.022	0.019	0.000	0.015	0.004	0.013	0.022	0.009	0.060	0.040	0.037	0.021	0.066	Vichienburi
0.168	0.094	0.035	--	0.028	0.012	0.024	0.040	0.049	0.031	0.023	0.094	0.044	0.028	0.023	0.110	Pangsida
0.125	0.072	0.045	0.047	--	0.010	0.023	0.009	0.017	0.015	0.032	0.069	0.015	0.025	0.013	0.086	PhuJongNaYoi
0.102	0.042	0.001	0.018	0.023	--	0.011	0.000	0.008	0.012	0.008	0.055	0.024	0.027	0.019	0.062	PhuphaLek
0.050	0.039	0.050	0.072	0.057	0.045	--	0.018	0.005	0.003	0.003	0.039	0.005	0.009	0.008	0.056	KhlongTron
0.075	0.029	0.011	0.050	0.025	0.004	0.036	--	0.004	0.012	0.013	0.039	0.014	0.021	0.016	0.049	ThamPhaTai
0.042	0.020	0.030	0.059	0.045	0.020	0.013	0.013	--	0.002	0.001	0.035	0.002	0.010	0.002	0.057	PhaDaeng
0.078	0.052	0.040	0.045	0.039	0.022	0.007	0.020	0.010	--	0.002	0.069	0.002	0.001	0.002	0.096	DoiJong
0.073	0.041	0.025	0.031	0.052	0.020	0.010	0.022	0.009	0.005	--	0.051	0.001	0.010	0.001	0.078	KlongWangJao
0.057	0.044	0.125	0.169	0.152	0.114	0.074	0.078	0.071	0.103	0.097	--	0.052	0.089	0.077	-0.002	MaeNgao
0.058	0.044	0.050	0.064	0.043	0.037	0.012	0.033	0.012	0.005	0.008	0.106	--	0.007	0.000	0.075	MaePing
0.106	0.087	0.063	0.040	0.043	0.041	0.015	0.043	0.028	0.006	0.018	0.122	0.019	--	0.001	0.110	KaengJedKhwae
0.092	0.069	0.039	0.034	0.034	0.022	0.028	0.029	0.013	0.007	0.008	0.132	0.004	0.006	--	0.105	ViengKosai
0.046	0.046	0.148	0.232	0.177	0.140	0.096	0.103	0.102	0.134	0.145	0.000	0.138	0.164	0.177	--	ThaTaFang

1.Lam Khlong Ngu National Park, 2.Erawan National Park, 5.Vichienburi community forest, 6.Pang Sida National Park, 7.Phu Jong Na Yoi National Park, 8.Phu Pha Lek National Park, 9.Khlong Tron National Park, 10.Tham Pha Tai National Park, 11.Pha Daeng National Park, 12.Doi Jong National Park, 13.Khlong Wang Jao National Park, 14.Mae Ngao National Park, 16.Mae Ping National Park, 17. Kaeng JedKhwae National Park, 18.Vieng Kosai National Park and 15.ThaTaFang area.

Table 10 Analysis of molecular variance of 538 *Xylia* trees from 16 populations. (A) Overall AMOVA table and (B) the derived F-statistics per locus and for all loci combined with their probabilities.

A **Source**	**df**	**SS**	**MS**	**Est. Var.**
Among Pops	15	132.933	8.862	0.205
Among Indiv	522	1125.971	2.157	0.156
Within Indiv	538	993.000	1.846	1.846
Total	1075	2251.904		2.206

B	**SUS2**	**AATcy**	**SUS1**	**CAT1**	**CAT2**	**LAP**	**IPI1**	**IDH1**	**Total**
Fst	0.034 (0.010)	0.047 (0.010)	0.076 (0.010)	0.030 (0.010)	0.116 (0.010)	0.070 (0.010)	0.201 (0.010)	0.084 (0.010)	0.093 (0.010)
Fis	0.052 (0.090)	0.104 (0.010)	0.009 (0.400)	0.209 (0.010)	0.165 (0.010)	0.040 (0.050)	0.060 (0.020)	0.015 (0.130)	0.078 (0.010)
Fit	0.084 (0.030)	0.146 (0.010)	0.084 (0.040)	0.232 (0.010)	0.262 (0.010)	0.107 (0.010)	0.249 (0.010)	0.097 (0.010)	0.163 (0.010)

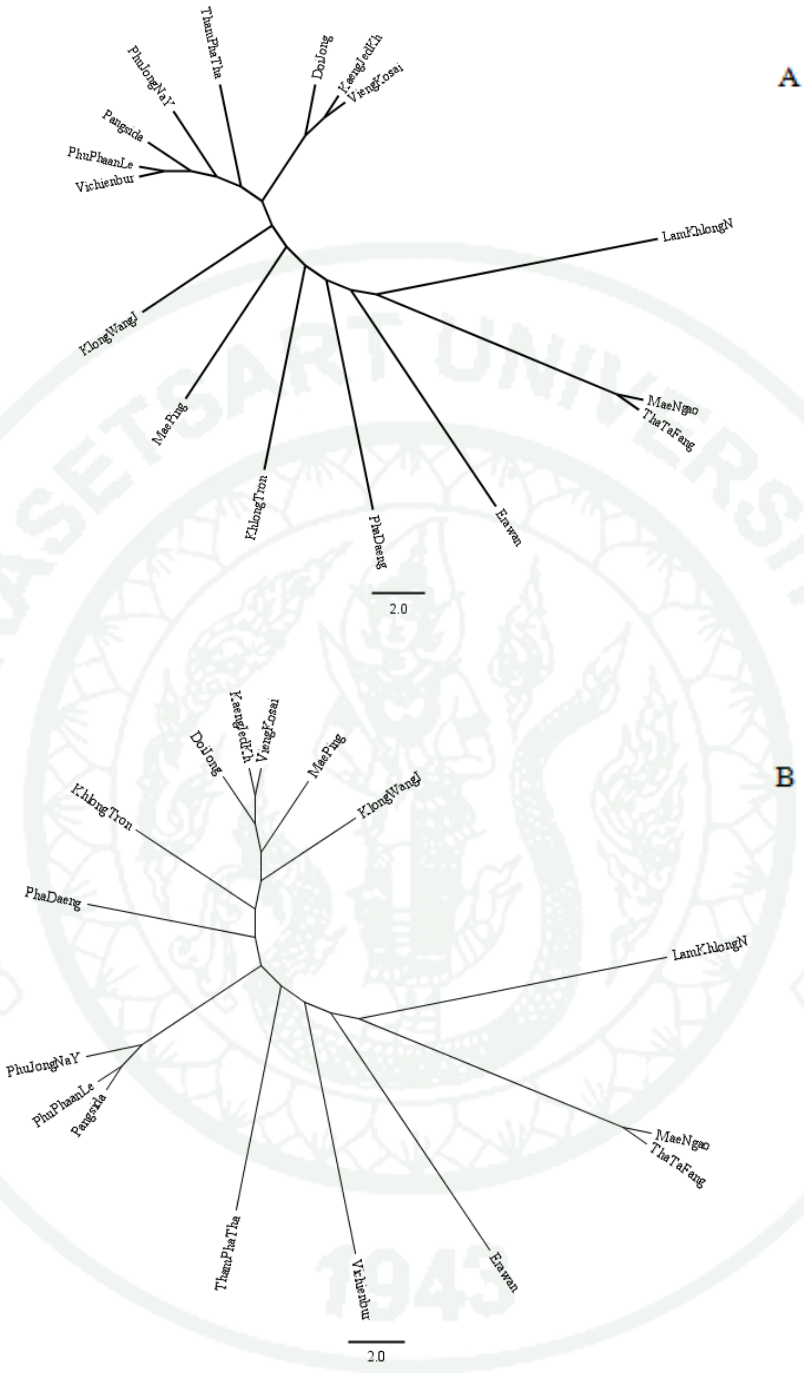

Figure 54 Neighbour-joining tree based on pairwise distances between all pairs of *Xylia* populations. The tree was annotated with the program FigTree. (A) Nei Unbiased Genetic Distance (G_{st}). (B) Jost D_{est} Distance.

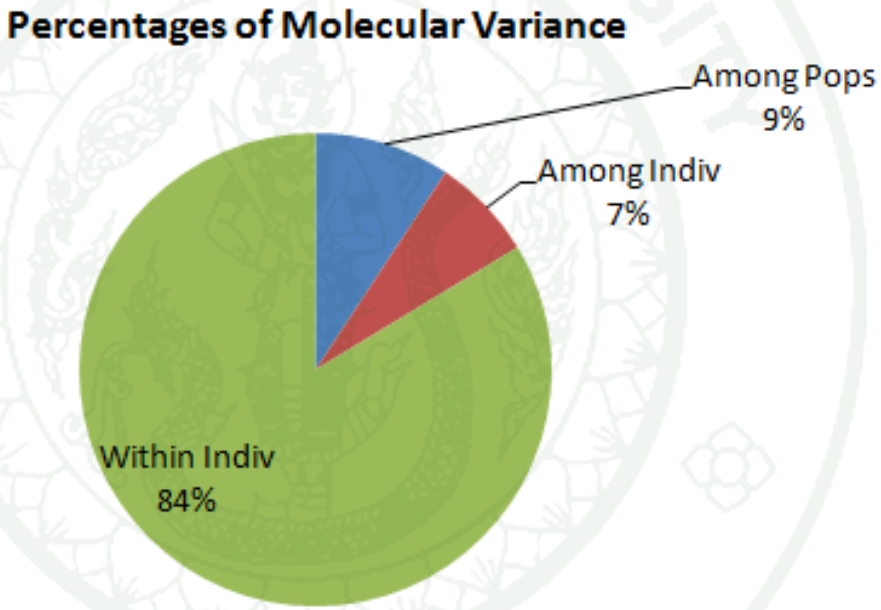

Figure 55 Distribution of genetic diversity based on F statistics from AMOVA. The variation for all sources was significant ($P = 0.01$).

Table 11 Parameter settings and likelihoods as calculated by the program STRUCTURE for K = 1 to K = 5 with 100,000 steps for burn-in and 500,000 steps of the MCMC sampling after burn-in.

Admixture, Correlated allele frequencies	K	Ln P(D)	Var[LnP(D)]	Alpha [a]	Fst_1	Fst_2	Fst_3	Fst_4	Fst_5
	1	-10032.2	57.7	-	0.1055	-	-	-	-
	2	-10040.9	642.5	1.3382	0.2836	0.0975	-	-	-
	3	-10147.9	999.3	1.5009	0.4836	0.3501	0.0003	-	-
	4	-10176.1	1279.4	1.1426	0.4411	0.5697	0.0003	0.3036	-
	5	-10332.8	1868.9	0.8219	0.3895	0.5891	0.353	0.4822	0.0002
Admixture, independent allele frequencies	K	Ln P(D)	Var[LnP(D)]	Alpha	Fst_1	Fst_2	Fst_3	Fst_4	Fst_5
	1	-10039.3	64.6	-	-	-	-	-	-
	2	-10006.9	509.6	0.5138	-	-	-	-	-
	3	-10089.9	976.3	0.2728	-	-	-	-	-
	4	-10050.6	1153.5	0.1558	-	-	-	-	-
	5	-10176.7	1487.4	0.1012	-	-	-	-	-

Table 11 (Continued)

No Admixture, Correlated allele frequencies	K	Ln P(D)	Var[LnP(D)]	Alpha	Fst_1	Fst_2	Fst_3	Fst_4	Fst_5
	1	-10032.3	57.8	-	0.1061	-	-	-	-
	2	-9820	303.4	-	0.1015	0.0702	-	-	-
	3	-9753.2	583.6	-	0.0551	0.076	0.0787	-	-
	4	-10668.4	2725	-	0.0517	0.0354	0.0846	0.0773	-
	5	-10781.2	3092	-	0.0758	0.0151	0.0576	0.0705	0.0618
No Admixture, independent allele frequencies	K	Ln P(D)	Var[LnP(D)]	Alpha	Fst_1	Fst_2	Fst_3	Fst_4	Fst_5
	1	-10039.4	66.7	-	-	-	-	-	-
	2	-9923.1	318.6	-	-	-	-	-	-
	3	-9945.8	541.9	-	-	-	-	-	-
	4	-9899.3	887.1	-	-	-	-	-	-
	5	-9908.6	904.8	-	-	-	-	-	-

[a] Alpha is the measure of admixture (thus not calculated for settings assuming no admixture) and Fst are the parameters of allele correlation.

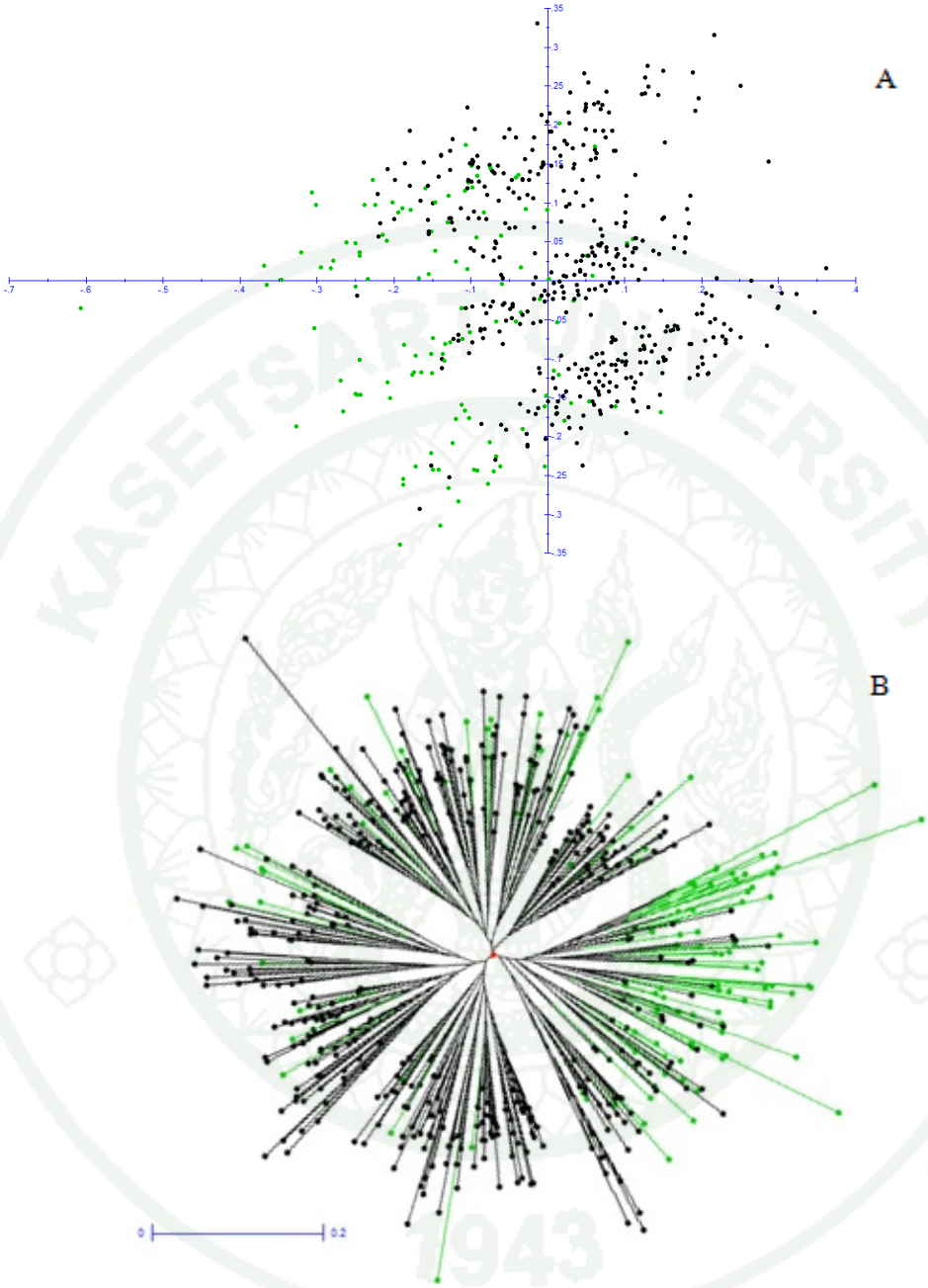

Figure 56 DARwin5 analysis (A) Principal component analysis of all 548 *Xylia* genotypes and (B) unweighted neighbor-joining tree representation of the same data. The trees from western Thailand (LamKhlongNgu, Erawan, MaeNgao and ThaTaFang) have been indicated in green.

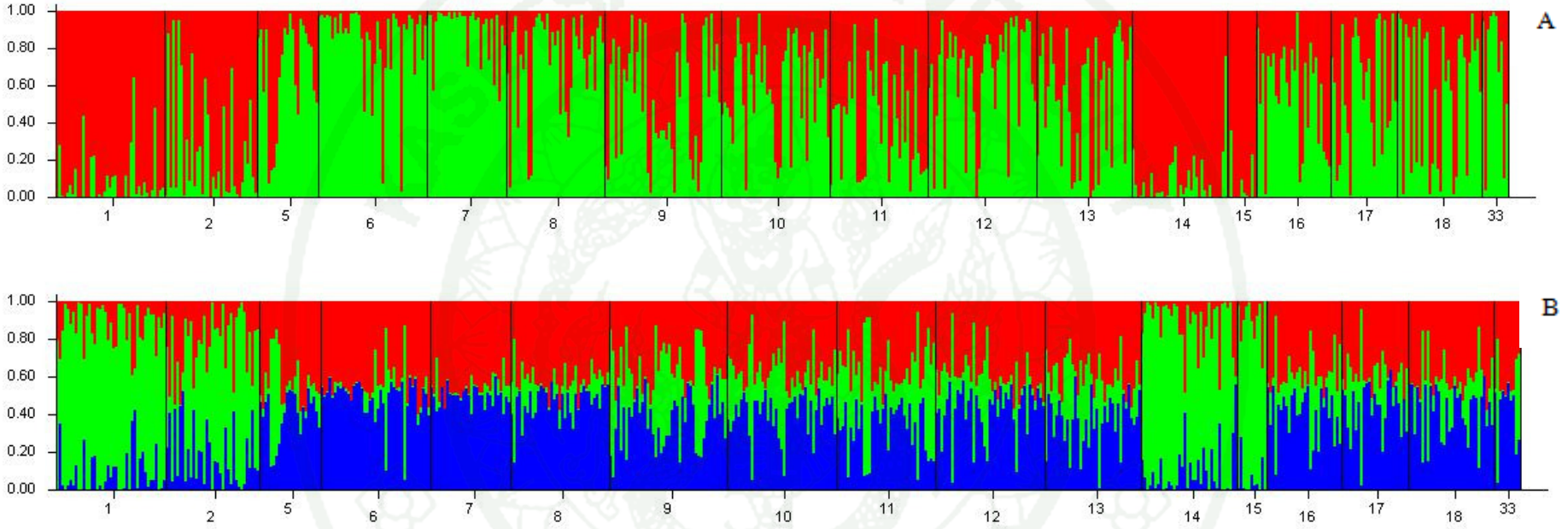

Figure 57 Bayesian clustering analysis results for K = 2 (A) and K = 3 (B) with admixture and correlated allele frequencies.

1.LamKhlongNgu National Park, 2.Erawan National Park, 5.Vichienburi community forest, 6.PangSida National Park, 7.PhuJongNaYoi National Park, 8.PhuPhaLek National Park, 9.KhlongTron National Park, 10.ThamPhaThai National Park, 11.PhaDaeng National Park, 12.DoiJong National Park, 13.KhlongWangJao National Park, 14.MaeNgao National Park, 15.ThaTaFang area, 16.MaePing National Park, 17. KaengJedKhwae National Park, 18.WiengKosai National Park.

Discussion

Analysis of Specific nuclear DNA markers

Based on the nucleotide sequences obtained from PCR fragments cloned after amplification of DNA fragments using consensus primers targeting low-copy nuclear genes in *X. xylocarpa*, specific primer sets were designed in such a way that each locus could be amplified separately in each gene family (Table 2). The sequences of these specific primers consisted of about 20 nucleotides, without any degenerate base positions (Table 3). Because each amplification product was designed to include at least one intron, and introns are known to be more polymorphic than coding exons (Graur and Li, 2000), a high level of polymorphism could be anticipated. This approach has also been called exon-primed intron-crossing PCR (EPIC-PCR) (Palumbi and Baker, 1994). Single amplification products can then easily be screened for variation by a number of methods.

Development of *Xylia* allele specific assays.

On agarose gel the PCR products were visible as single bands and different alleles could not be distinguished. Various techniques for detection of genetic variants at the molecular level can be selected and optimized based on costs, available equipment and the number of SNPs to be tested. In this study, three simple assays, PCR-SSCP, CAPS and AS-PCR that are inexpensive, reliable and sensitive for mutation detection were applied for developing the gene based markers of *Xylia*. SSCP polyacrylamide gel electrophoresis was chosen because of its high sensitivity to detect single nucleotide polymorphisms even in sequences of identical length (Orita *et al.,*1989). The SSCP analysis of amplified DNA fragments revealed a large diversity of amplification products, many of which could be tentatively assigned to alleles (Thakaew, 2011). PCR products were then sequenced from those trees with different identifiable alleles and from other samples where the SSCP patterns were ambiguous. The haplotypes deduced from the sequencing analysis indicated the presence of more

alleles than could be detected by SSCP (Table 5). To reveal those alleles in the natural populations, specific SNPs were converted into highly specific assays.

Allele specific assays were designed using allele-specific PCR or CAPS approach. Primers were designed for a total of 15 SNPs. Five SNPs were developed into allele-specific PCR assays. For three assays an extra mismatching nucleotide at the -3 position in addition to the allele specific 3′ terminal nucleotide (Saiki *et al.,* 1986) was incorporated into the PCR primer. Two other assays had two specific nucleotides at the 3′ end and thus no additional mismatch was introduced. The annealing temperature for PCR was optimized for each assay. In the AS-PCR assay, an identical fragment is amplified in separate reaction with either of two allele-specific primers in combination with a common third primer. The amplification products are then revealed by agarose gel electrophoresis and scored as presence or absence for each of the targeted alleles.

Ten SNPs in IDH1 (3), CAT1 (1), CAT2 (3), LAP1 (1) and IPI1 (2) were converted into CAPS assays using the dCAPS Finder 2.0 website (http:helix.wust.edu/dcaps/dcaps.html). The CAPS assays combined PCR with restriction enzyme digestion and simple agarose gel electrophoresis to detect the resulting restriction site polymorphisms. This cost-effective technique has been widely used for assaying SNPs in many crop species such as barley (Kota *et al.,* 2008), rice (Komori and Nitta, 2005) and banana (Toprasi, 2012).

The combination of SSCP with AS-PCR and CAPS screening, greatly speeds the analysis of a large number of individuals for phylogeographic study: the SSCP assay provides information on the most common alleles while the specific CAPS and AS-PCR assays can reveal the presence of particular variants that may be difficult to differentiate otherwise. The characterization of the alleles present in each tree yields estimates of population genetic parameters, and the sequencing of each allele allows a phylogenetic interpretation.

Population genetic Analysis

Population genetic parameters were calculated with GenAlEx version 6.4. The GenAlEx programme has been widely used for genetic analysis in many plants species such as *Justicia adhatoda* (Syed *et al.,*2011), an endangered tropical island tree *Medusagyne oppositifolia* (Finger *et al.,*2011), *Astragalus ampullarioides* (Breinholt *et al*., 2009) and oak (Homolka *et al*., 2012).

For the different loci, between 8 and 34 different alleles were observed in *X. xylocarpa*. However, many alleles occurred in very low frequency and thus the effective number of alleles, which is the number of equally frequent alleles it would take to achieve a given level of gene diversity, was only between 1.1 and 7.6. The effective number of alleles for IDH1 (7.601) was almost threefold higher than that for the second highest, CAT1 (2.666). The observed level of heterozygosity was moderately high for 7 loci (ranging from 0.289 to 0.819) but low for the AATcy locus. CAT1, CAT2 and IPI1 has higher measure of population differentiation with fixation index F = 0.223, 0.204 and 0.155, respectively, while SUS1 and IDH1 had lowest F. It is noteworthy that among the nucleotide polymorphisms identified in the amino acid-coding exons, more were non-synonymous, while one would expect polymorphisms mostly as synonymous substitutions in the third position of codons.

Deviations from Hardy-Weinberg Equilibrium per locus and per population were estimated by GENEPOP (Raymond and Rousset, 1995). CAT1 has the highest number (9) of *significant* deviations from HWE, while none were observed for the SUS1 marker.

Pair-wise population genetic distances were calculated using the Nei unbiased genetic distance (G_{st}) in GENALEX 6.4 and using the Jost D_{est} measure in the programme SMOGD. The largest genetic distances were observed between the ThaTaFang area and MaeNgao National Park on one side and the PangSida and PhuJongNaYoi National Park on the other. These populations are also geographically distant, from the Thai-Myanmar border in the West to the Thai-Cambodian border in

the East. It has to be remarked that only 11 trees were collected in the ThaTaFang area. Although the largest genetic distances were thus found between populations that are geographically very distant, a Mantel test to detect correlations between geographical and genetic distances showed only a very low, though significant, correlation (0.041).

A principal component analysis in DARwin5 was not able to reveal identifiable clusters among the total set of 548 *Xylia* trees. There was a slight trend for the trees from Western Thailand (ThaTaFang area, MaeNgao NP, LamKhlongNgu NP, and Erawan NP) to cluster in one area of the plot, but several trees from other origins in Thailand were still intermingled with them.

Also the Bayesian clustering algorithm implemented in STRUCTURE revealed gave only very weak support for population differentiation in two, or maybe three, groups.

The limited clustering of genetic diversity along geographically separated population structure within Thailand might indicate a high level of geneflow over long distances. The estimated number of effective migrants (Nm) averaged for all loci was 4.291, and as high as 7.399 for the CAT1 locus. Probably the pollination syndromes and seed dispersal mechanisms can explain this. *Xylia* flowers are small, pale yellow, in dense spherical heads. The flowers are hermaphroditic or male. The main flowering time is normally before or just after leaf flushing. At the time of flowering, the flowers are heavily visited by various insects, among them social and solitary bees and stingless bees. However, the foraging distances travelled by these insects is unknown. Insect pollination and wind-dispersed seeds are thought to promote relatively long distance gene dispersal (Dick *et al.,* 2003; Lander *et al.,* 2010). The seed dispersal in *Xylia* is probably rather limited. When pods dry they tend to open suddenly and the seed can be flung away for some distance. However, the seed does not have any adaptation for further dispersal through the air or once on the ground.

The large number of low frequency alleles, and the large proportion of non-synonymous nucleotide substitutions in the exons, combined with limited geographical structure, might indicate that these low frequency alleles play a role in *Xylia* adaptation and evolution. The conservation of such low frequency alleles would necessitate setting up a large number of conservation areas in different regions. The limited spatial genetic structure would indicate that the exact location of conservation areas may not be as important.

CONCLUSION AND RECOMMENDATION

A study of the genetic diversity in populations of *Xylia xylocarpa* (Roxb.) W. Theob. var. kerrii (Craib & Hutch.) I. C. Nielsen in Thailand was performed using specific primer pairs designed for 8 nuclear gene encoding loci: SUS (2 loci), AAT (1 locus), IDH (1 locus), LAP (1 locus), CAT (2 loci) and IPI (1 locus) to reveal differentiation within and between populations and the geographical distribution of alleles. Samples were collected from individual trees in 14 national parks, 2 community forests and a small number of isolated individual trees. Specific primers amplified a unique fragment at each locus and showed a high level of polymorphism when analysed by sequencing. SSCP assay was able to distinguish most of the alleles at each locus, but for some loci additional allele-specific assays were done to reveal diversity detected by sequencing but not by SSCP. The sequence analysis indicated that the majority of the variation was found in the introns. Therefore DNA based markers developed from low copy nuclear genes have considerable potential as DNA markers for use in population genetic studies in *Xylia xylocarpa* and other plant species.

Primers were designed for a total of 15 SNPs from 5 loci IDH (6), LAP (1), CAT1 (2), CAT2 (4) and IPI1 (2). Five SNPs were developed into AS-PCR tests incorporating an extra mismatching nucleotide at the -3 position and ten SNPs were converted into a CAPS assay. This approach quite useful at identifying broadly applicable primers targeted to low copy-number nuclear genes. Wider application of this approach should be effective at greatly increasing the amount of genetic information available for a diversity of plant nuclear genomes.

Population genetic analysis of *X. xylocarpa* populations with 8 gene-linked loci (SUS1, SUS2, AATcy1, CAT1, CAT2, IDH1 LAP1 and IPI1) using GENALEX 6.4 revealed codominant and binary genetic distance, heterozygosity, F-statistics and polymorphism by population for codominant data CAT1, CAT2 and IPI1 has higher measure of population differentiation due to genetic structure F_{ST} = 0.223, 0.204 and 0.155 respectively. SUS1 and IDH1 had low F_{ST}. Effective number of alleles for

IDH1 (7.601) and CAT1 (2.666) has higher measure to shows the number of equally frequent alleles it would take to achieve a given level of gene diversity. The effective number of alleles was between 1.1 (AATcy) and 7.6 (IDH1). Diversity within and between populations and deviations from HW equilibrium were estimated using GENEPOP.

Matrix of pairwise population genetic distances with either the Nei unbiased genetic distance (G_{st}) or the Jost D_{est} measure shows same measure between Pang Sida National Park in the East of Thailand with Mae Ngao National Park in the West has the most genetic distance of *X. xylocarpa*. Geographical origin of the populations as even the geographically most distant populations (LamKhlongNgu, Erawan, MaeNgao, ThaTaFang from western Thailand vs PangSida, PhuJongNaYoi, PhuPhaanLek from northeastern Thailand.) share related genotypes. We used GENALEX 6 to compute genetic distance and analysis of molecular variance (AMOVA) haploid of 538 *Xylia* trees from 16 geographical populations. Results of AMOVA for total total Fst = 0.093, Fis = 0.078 and Fit = 0.163. The AMOVA derived F statistics per locus and for all loci combined with their probabilities. Percentages of molecular variance to show analysis in the distribution of genetic differentiation based on F statistics. AMOVA revealed variation Among Population 9%, among Individuals 7% and within Individuals 84% of the total. The variation for both sources was significant ($P = 0.01$).

The programme DARwin5 phylogenetic tree also could be differentiated population trees in identifiable clusters. Principal component analysis of all *Xylia* genotypes and unweighted neighbor-joining tree representation of the same data. The trees from western Thailand (Lam Khlong Ngu, Erawan, MaeNgao and ThaTaFang) have been indicated in green. Using the program STRUCTURE revealed based on Ln P(D) and Var[LnP(D)], for the model choice criterion to refer as true number of populations (K), which was evident when the results from different model of subpopulations were compared. Population structure analysis indicated that the 548 *X. xylocarpa* trees could be clustered into be 2 groups with admixture and correlated allele frequency model or 3 to 4 groups when no admixture and correlated or independent allele frequency models. The result of STRUCTURE based on 8 loci for

K = 2 indicates that the trees from western Thailand (Lam Khlong Ngu, Erawan, MaeNgao and ThaTaFang) form a distinct cluster from populations from northeastern Thailand (PangSida, PhuJongNaYoi, and PhuPhaLek) in green. K =3 yielded no clear signal for further population differentiation.

A large number of alleles were found for each locus, especially through sequencing. The weak support for population structure does not allow us to delimit seed zones concerning *Xylia* breeding or conservation. However, the large number of low frequency alleles that we found would indicate that sizeable populations at various locations need to be protected for the effective conservation of the genetic diversity in *Xylia xylocarpa*. This is in contrast to teak where more clear patterns of geographical structure can be detected.

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APPENDIX

Appendix Table 1 Location of each tree as recorded by GPS device.

Lam Khlong Ngu

Tree identifier	Latitude N			Longitude E			Elevation (m)	Tree GBH (cm)	Sample
Xx8-01-001	14°	53'	1.9"N	98°	49'	30.4"	558	36	leaf
Xx8-01-002	14°	53'	6.5"N	98°	49'	29.5"	569	104	leaf
Xx8-01-003	14°	53'	9.5"N	98°	49'	26.3"	600	135	leaf
Xx8-01-004	14°	53'	12.2"N	98°	49'	27.7"	586	52	leaf
Xx8-01-005	14°	53'	12.7"N	98°	49'	33.1"	594	138	leaf
Xx8-01-006	14°	53'	12."N	98°	49'	35.9"	599	189	leaf
Xx8-01-007	14°	53'	7.4"N	98°	49'	34.9"	593	64	leaf
Xx8-01-008	14°	52'	47.6"N	98°	49'	21.9"	575	43	leaf
Xx8-01-009	14°	52'	44.3"N	98°	49'	19.3"	577	79	leaf
Xx8-01-010	14°	53'	20.4"N	98°	48'	56."	578	121	leaf
Xx8-01-011	14°	53'	18."N	98°	48'	59.1"	580	114	leaf
Xx8-01-012	14°	53'	14.5"N	98°	49'	1.7"	581	diam>60	flower
Xx8-01-013	14°	53'	10.1"N	98°	49'	5.3"	580	84	leaf
Xx8-01-014	14°	53'	.9"N	98°	49'	11.5"	583	92	leaf
Xx8-01-015	14°	53'	3.4"N	98°	49'	14.1"	579	142	leaf
Xx8-01-016	14°	53'	7.3"N	98°	49'	15.5"	584	76	leaf
Xx8-01-017	14°	53'	11.2"N	98°	49'	16.1"	571	134	leaf
Xx8-01-018	14°	53'	15.6"N	98°	49'	14.4"	555	55	leaf
Xx8-01-019	14°	53'	18.9"N	98°	49'	15."	566	55+36	leaf
Xx8-01-020	14°	53'	24.8"N	98°	49'	14.6"	559	89	leaf
Xx8-01-021	14°	53'	.9"N	98°	49'	17.2"	574	95	leaf
Xx8-01-022	14°	52'	38.6"N	98°	49'	25."	577	136	leaf
Xx8-01-023	14°	52'	39.1"N	98°	49'	20.8"	575	76	leaf
Xx8-01-024	14°	52'	40.8"N	98°	49'	13.8"	577	67	leaf
Xx8-01-025	14°	52'	40.4"N	98°	49'	10.2"	577	55	leaf
Xx8-01-026	14°	52'	44.8"N	98°	49'	14.5"	585	55	leaf
Xx8-01-027	14°	53'	42."N	98°	50'	34.4"	577	?	leaf
Xx8-01-028	14°	53'	44.6"N	98°	50'	42.1"	609	133	leaf
Xx8-01-029	14°	53'	48.8"N	98°	50'	42.6"	618	108	leaf
Xx8-01-030	14°	53'	50.6"N	98°	50'	45.8"	623	154	leaf
Xx8-01-031	14°	53'	51.3"N	98°	50'	49.7"	625	81	leaf
Xx8-01-032	14°	53'	54.3"N	98°	50'	51.6"	610	125	leaf
Xx8-01-033	14°	53'	56.9"N	98°	50'	54.3"	609	58	leaf
Xx8-01-034	14°	54'	.7"N	98°	50'	56.2"	607	126	leaf

Appendix Table 1 (Continued)

Tree identifier	Latitude N			Longitude E			Elevation (m)	GBH(cm)	Sample
Xx8-01-035	14°	54'	5.2"N	98°	50'	56.2"	598	118	leaf
Xx8-01-036	14°	54'	8.8"N	98°	50'	54.5"	585	157	leaf
Xx8-01-037	14°	54'	11.3"N	98°	50'	56.8"	592	117	leaf
Xx8-01-038	14°	54'	14.7"N	98°	50'	54.7"	586	161	leaf
Xx8-01-039	14°	54'	19.2"N	98°	50'	54.7"	588	154	leaf
Xx8-01-040	14°	54'	23.2"N	98°	50'	54."	592	174	leaf
Xx8-01-041	14°	54'	27.5"N	98°	50'	53.8"	594	137	leaf
Xx8-01-042	14°	54'	32.9"N	98°	50'	56.8"	608	67	leaf

Erawan

Tree identifier	Latitude N			Longitude E			Elevation (m)	GBH(cm)	Sample
Xx8-02-001	14°	24'	23.5"N	99°	4'	40.8"	561	373	leaf
Xx8-02-002	14°	24'	7.5"N	99°	4'	31.1"	590	198	leaf
Xx8-02-003	14°	24'	19.3"N	99°	4'	35.9"	577	260	leaf
Xx8-02-004	14°	24'	8.7"N	99°	5'	20.4"	615	120	leaf
Xx8-02-005	14°	24'	10.3"N	99°	5'	21.9"	593	152	leaf
Xx8-02-006	14°	24'	14.6"N	99°	5'	18.5"	640	113	leaf
Xx8-02-007	14°	24'	10.7"N	99°	5'	21.9"	592	120	leaf
Xx8-02-008	14°	23'	51.7"N	99°	4'	54.9"	556	126	leaf
Xx8-02-009	14°	23'	54.7"N	99°	4'	51.8"	575	69	leaf
Xx8-02-010	14°	23'	58.5"N	99°	4'	45.5"	588	177	leaf
Xx8-02-011	14°	23'	17.1"N	99°	4'	35.5"	597	116	leaf
Xx8-02-012	14°	23'	8.5"N	99°	4'	46.1"	605	225	leaf
Xx8-02-013	14°	23'	7.5"N	99°	4'	48.2"	608	191	leaf
Xx8-02-014	14°	23'	4.6"N	99°	4'	57.3"	618	47	leaf
Xx8-02-015	14°	23'	3.3"N	99°	4'	58.3"	623	47	leaf
Xx8-02-016	14°	23'	3.1"N	99°	4'	59.7"	630	67+150	leaf
Xx8-02-017	14°	23'	1.2"N	99°	4'	59.1"	631	96	leaf
Xx8-02-018	14°	22'	58.9"N	99°	5'	.1"	640	42+150	leaf
Xx8-02-019	14°	22'	55.7"N	99°	5'	.4"	652	155	leaf
Xx8-02-020	14°	22'	55.1"N	99°	5'	2.9"	648	159	leaf
Xx8-02-021	14°	22'	53."N	99°	5'	4.8"	640	164+69	leaf

Appendix Table 1 (Continued)

Tree identifier	Latitude N			Longitude E			Elevation (m)	GBH(cm)	Sample
Xx8-02-022	14°	22'	47.5"N	99°	5'	8.1"	638	84	leaf
Xx8-02-023	14°	22'	45."N	99°	5'	8.8"	641	91	leaf
Xx8-02-024	14°	22'	42.6"N	99°	5'	8.5"	647	172	leaf
Xx8-02-025	14°	22'	38.2"N	99°	5'	6.8"	664	131	leaf
Xx8-02-026	14°	22'	34.4"N	99°	5'	4.7"	661	100	leaf
Xx8-02-027	14°	22'	30.9"N	99°	5'	4.7"	660	52	leaf
Xx8-02-028	14°	22'	28.2"N	99°	5'	5.4"	658	176	leaf
Xx8-02-029	14°	22'	24.3"N	99°	5'	5.3"	659	119	leaf
Xx8-02-030	14°	22'	22.3"N	99°	5'	2.9"	656	114	leaf
Xx8-02-031	14°	22'	26.3"N	99°	5'	2.8"	658	97	leaf
Xx8-02-032	14°	22'	29.2"N	99°	4'	59.4"	654	64	leaf
Xx8-02-033	14°	22'	31.6"N	99°	4'	58.3"	659	91	leaf
Xx8-02-034	14°	23'	8.4"N	99°	4'	51.6"	625	57+66	leaf
Xx8-02-035	14°	24'	9.3"N	99°	4'	25.2"	587	81	leaf
Xx8-02-036	14°	24'	19."N	99°	4'	36.8"	580	137	leaf

Vichienburi

Tree identifier	Latitude N			Longitude E			Elevation (m)	GBH(cm)	Sample
Xx8-05-001	15°	38'	57.4"N	101°	1'	39.4"	188	3	leaf
Xx8-05-002	15°	38'	59.8"N	101°	1'	35.4"	217	48	leaf
Xx8-05-003	15°	39'	6.1"N	101°	1'	29.7"	223	-	leaf
Xx8-05-004	15°	39'	9.6"N	101°	1'	27.7"	235	54	leaf
Xx8-05-005	15°	39'	12.4"N	101°	1'	25.7"	252	54	leaf
Xx8-05-006	15°	39'	15.4"N	101°	1'	23.4"	263	54	leaf
Xx8-05-007	15°	39'	20."N	101°	1'	22.7"	248	38	leaf
Xx8-05-008	15°	39'	23.2"N	101°	1'	20.4"	269	42	leaf
Xx8-05-009	15°	39'	26.6"N	101°	1'	19.4"	292	23	leaf
Xx8-05-010	15°	39'	22.4"N	101°	1'	17.1"	333	17	leaf
Xx8-05-011	15°	39'	18.6"N	101°	1'	18.6"	338	49	leaf
Xx8-05-012	15°	39'	15."N	101°	1'	20.7"	333	53	leaf
Xx8-05-013	15°	39'	11.7"N	101°	1'	22.1"	331	14	leaf
Xx8-05-014	15°	39'	54.3"N	101°	1'	40.6"	206	3	leaf

Appendix Table 1 (Continued)

Tree identifier	Latitude N			Longitude E			Elevation (m)	GBH(cm)	Sample
Xx8-05-015	15°	38'	46.2"N	101°	1'	47.5"	200	35	leaf
Xx8-05-016	15°	38'	43.1"N	101°	1'	47.8"	192	39	leaf
Xx8-05-017	15°	38'	37.2"N	101°	1'	50.4"	178	19	leaf
Xx8-05-018	15°	38'	33.8"N	101°	1'	51.9"	178	14	leaf
Xx8-05-019	15°	38'	32.4"N	101°	1'	55.6"	153	3	leaf
Xx8-05-020	15°	38'	37."N	101°	1'	54.1"	148	58	leaf
Xx8-05-021	15°	38'	41.7"N	101°	1'	51.5"	152	44	leaf
Xx8-05-022	15°	38'	49.1"N	101°	1'	46.6"	146	55	leaf
Xx8-05-023	15°	38'	52.5"N	101°	1'	48.1"	129	20	leaf

Pang Sida

Tree identifier	Latitude N			Longitude E			Elevation (m)	GBH(cm)	Sample
Xx8-06-001	14°	00	34.00"N	102°	34'	46.50"E	129	33+50	leaf
Xx8-06-002	14°	00	39.50"N	102°	34'	45.40"E	132	48	leaf
Xx8-06-003	14°	00	42.90"N	102°	34'	47.80"E	130	47	leaf
Xx8-06-004	14°	00	46.40"N	102°	34'	48.10"E	127	73	leaf
Xx8-06-005	14°	00	49.90"N	102°	34'	46.10"E	129	48	leaf
Xx8-06-006	14°	00	54.10"N	102°	34'	46.60"E	128	41	leaf
Xx8-06-007	14°	00	57.60"N	102°	34'	49.30"E	126	99	leaf
Xx8-06-008	14°	01	02.60"N	102°	34'	50.20"E	124	38	leaf
Xx8-06-009	14°	01	09.00"N	102°	34'	50.80"E	123	103	leaf
Xx8-06-010	14°	01	12.80"N	102°	34'	50.90"E	124	83	leaf
Xx8-06-011	14°	01	15.70"N	102°	34'	49.20"E	125	36	leaf
Xx8-06-012	14°	01	21.20"N	102°	34'	45.00"E	125	39	leaf
Xx8-06-013	14°	01	24.70"N	102°	34'	44.20"E	125	40	leaf
Xx8-06-014	14°	01	27.80"N	102°	34'	41.60"E	124	81	leaf
Xx8-06-015	14°	01	30.20"N	102°	34'	38.70"E	125	39	leaf
Xx8-06-016	14°	01	32.10"N	102°	34'	34.80"E	128	56	leaf
Xx8-06-017	14°	01	35.90"N	102°	34'	32.60"E	126	29	leaf
Xx8-06-018	14°	01	40.20"N	102°	34'	32.70"E	125	47	leaf
Xx8-06-019	14°	01	46.60"N	102°	34'	30.70"E	126	113	leaf
Xx8-06-020	14°	01	50.30"N	102°	34'	27.00"E	128	48+47	leaf

Appendix Table 1 (Continued)

Tree identifier	Latitude N			Longitude E			Elevation (m)	GBH(cm)	Sample
Xx8-06-021	14°	01	50.20"N	102°	34'	19.40"E	128	28	leaf
Xx8-06-022	14°	01	52.20"N	102°	34'	15.60"E	129	54	leaf
Xx8-06-023	14°	01	54.40"N	102°	34'	11.90"E	130	50	leaf
Xx8-06-024	14°	01	56.20"N	102°	34'	08.70"E	128	53	leaf
Xx8-06-025	14°	01	56.60"N	102°	34'	03.20"E	128	38	leaf
Xx8-06-026	14°	01	59.60"N	102°	33'	55.50"E	131	65	leaf
Xx8-06-027	14°	02	03.90"N	102°	33'	47.00"E	138	53	leaf
Xx8-06-028	13°	59	40.70"N	102°	34'	44.60"E	171	50	leaf
Xx8-06-029	13°	59	41.50"N	102°	34'	48.50"E	171	48	leaf
Xx8-06-030	13°	59	41.80"N	102°	34'	52.50"E	168	42	leaf
Xx8-06-031	13°	59	42.60"N	102°	34'	57.00"E	167	65+64+25	leaf
Xx8-06-032	13°	59	43.40"N	102°	35'	01.10"E	169	48	leaf
Xx8-06-033	13°	59	43.70"N	102°	35'	05.30"E	170	63	leaf
Xx8-06-034	13°	59	44.50"N	102°	35'	09.30"E	171	53	leaf
Xx8-06-035	13°	59	43.60"N	102°	35'	13.20"E	167	41	leaf
Xx8-06-036	13°	59	44.60"N	102°	35'	17.80"E	167	42	leaf
Xx8-06-037	13°	59	46.10"N	102°	35'	21.30"E	169	47	leaf
Xx8-06-038	13°	59	46.70"N	102°	35'	27.40"E	166	53	leaf
Xx8-06-039	13°	59	48.20"N	102°	35'	31.20"E	169	58+24	leaf
Xx8-06-040	13°	59	48.40"N	102°	35'	35.50"E	167	68	leaf
Xx8-06-041	13°	59	36.90"N	102°	34'	52.00"E	147	98	leaf

Phu Jong Na Yoi

Tree identifier	Latitude N			Longitude E			Elevation (m)	GBH(cm)	Sample
Xx8-07-001	14°	26'	3.5"N	105°	15'	27.3"	269	151	leaf
Xx8-07-002	14°	26'	13.5"N	105°	14'	33.5"	211	100	leaf
Xx8-07-003	14°	23'	17.4"N	105°	12'	31.8"	196	192	leaf
Xx8-07-004	14°	23'	21.3"N	105°	12'	32.3"	199	126	leaf
Xx8-07-005	14°	23'	39."N	105°	8'	42.9"	188	180	leaf
Xx8-07-006	14°	23'	33.9"N	105°	8'	41.6"	195	212	leaf
Xx8-07-007	14°	23'	33.5"N	105°	8'	38."	203	107	leaf
Xx8-07-008	14°	23'	31.8"N	105°	8'	34.4"	211	70	leaf

Appendix Table 1 (Continued)

Tree identifier	Latitude N			Longitude E			Elevation (m)	GBH(cm)	Sample
Xx8-07-009	14°	23'	37.1"N	105°	8'	33.6"	202	69	leaf
Xx8-07-010	14°	23'	33.2"N	105°	8'	29.2"	214	110+106	leaf
Xx8-07-011	14°	23'	30.2"N	105°	8'	27.1"	227	175	leaf
Xx8-07-012	14°	23'	27.7"N	105°	8'	23.8"	241	187	leaf
Xx8-07-013	14°	23'	26.2"N	105°	8'	20.4"	255	92	leaf
Xx8-07-014	14°	23'	23.9"N	105°	8'	16.7"	250	74	leaf
Xx8-07-015	14°	23'	22.7"N	105°	8'	12.7"	263	69	leaf
Xx8-07-016	14°	23'	18.3"N	105°	6'	5."	292	135	leaf
Xx8-07-017	14°	23'	16.6"N	105°	7'	59.5"	309	62	leaf
Xx8-07-018	14°	23'	3.8"N	105°	7'	41.4"	346	151	leaf
Xx8-07-019	14°	23'	59.5"N	105°	7'	39.3"	356	69	leaf
Xx8-07-020	14°	22'	55.8"N	105°	7'	36."	374	333	leaf
Xx8-07-021	14°	22'	50.8"N	105°	7'	34.5"	387	97	leaf
Xx8-07-022	14°	22'	43.3"N	105°	7'	35.6"	401	87	leaf
Xx8-07-023	14°	22'	39.1"N	105°	7'	34.1"	413	57	leaf
Xx8-07-024	14°	23'	4."N	105°	7'	49.9"	327	160	leaf
Xx8-07-025	14°	23'	10.7"N	105°	8'	13.1"	130	100	leaf
Xx8-07-026	14°	23'	13.4"N	105°	8'	16.7"	283	132	leaf
Xx8-07-027	14°	23'	17.1"N	105°	8'	17.8"	263	182	leaf
Xx8-07-028	14°	23'	20."N	105°	8'	20.4"	244	52	leaf
Xx8-07-029	14°	23'	24."N	105°	8'	30.1"	232	108	leaf
Xx8-07-030	14°	23'	26.9"N	105°	8'	33.8"	215	132	leaf
Xx8-07-031	14°	23'	31.4"N	105°	8'	44."	218	163	leaf
Xx8-07-032	14°	23'	42.7"N	105°	9'	34."	205	44	leaf

Phu Pha Lek

Tree identifier	Latitude N			Longitude E			Elevation (m)	GBH(cm)	Sample
Xx8-08-001	17°	15'	54.7"N	103°	27'	36.8"	466	97	leaf
Xx8-08-002	17°	15'	53.7"N	103°	27'	40.8"	473	91	leaf
Xx8-08-003	17°	15'	52.7"N	103°	27'	44.5"	483	80	leaf
Xx8-08-004	17°	15'	45."N	103°	27'	46."	504	65	leaf
Xx8-08-005	17°	15'	31.9"N	103°	27'	55.4"	534	64	leaf

Appendix Table 1 (Continued)

Phu Pha Lek

Tree identifier	Latitude N			Longitude E			Elevation (m)	GBH(cm)	Sample
Xx8-08-006	17°	15'	35.2"N	103°	27'	55.8"	510	103	leaf
Xx8-08-007	17°	15'	38.4"N	103°	27'	58.8"	497	71	leaf
Xx8-08-008	17°	16'	4.2"N	103°	27'	57."	413	71	leaf
Xx8-08-009	17°	16'	5."N	103°	27'	52.1"	420	71	leaf
Xx8-08-010	17°	16'	1.2"N	103°	27'	45."	442	67+22+48	leaf
Xx8-08-011	17°	16'	1.7"N	103°	27'	40.8"	450	115	leaf
Xx8-08-012	17°	16'	.7"N	103°	27'	36.5"	464	57	leaf
Xx8-08-013	17°	16'	1.9"N	103°	27'	31.5"	471	124	leaf
Xx8-08-014	17°	15'	24.1"N	103°	27'	31.9"	603	78	leaf
Xx8-08-015	17°	15'	27.7"N	103°	27'	29.3"	592	54	leaf
Xx8-08-016	17°	15'	31.6"N	103°	27'	27.8"	581	106	leaf
Xx8-08-017	17°	15'	35.7"N	103°	27'	23.7"	561	190	leaf
Xx8-08-018	17°	15'	39.8"N	103°	27'	22.1"	553	59	leaf
Xx8-08-019	17°	15'	41.4"N	103°	27'	25.9"	547	59	leaf
Xx8-08-020	17°	15'	53.8"N	103°	27'	25.2"	516	111	leaf
Xx8-08-021	17°	16'	5.3"N	103°	27'	27.6"	474	78	leaf
Xx8-08-022	17°	16'	8.4"N	103°	27'	28.6"	462	46+43	leaf
Xx8-08-023	17°	16'	11.6"N	103°	27'	27.9"	449	100	leaf
Xx8-08-024	17°	16'	15.4"N	103°	27'	31.4"	433	65	leaf
Xx8-08-025	17°	16'	19."N	103°	27'	31.1"	418	44+38	leaf
Xx8-08-026	17°	16'	19.6"N	103°	27'	35.8"	406	97	leaf
Xx8-08-027	17°	16'	23.7"N	103°	27'	32.4"	390	72	leaf
Xx8-08-028	17°	16'	25.7"N	103°	27'	28.2"	376	71+46	leaf
Xx8-08-029	17°	16'	32.1"N	103°	27'	27.4"	358	79	leaf
Xx8-08-030	17°	16'	35.2"N	103°	27'	26.2"	350	102	leaf
Xx8-08-031	17°	16'	39.3"N	103°	27'	23.7"	336	43	leaf
Xx8-08-032	17°	16'	42.9"N	103°	27'	22.1"	321	70	leaf
Xx8-08-033	17°	16'	45.1"N	103°	27'	25.5"	312	44	leaf
Xx8-08-034	17°	16'	48.2"N	103°	27'	27.3"	300	63	leaf
Xx8-08-035	17°	16'	52.9"N	103°	27'	25.1"	281	97	leaf
Xx8-08-036	17°	16'	52.1"N	103°	27'	29.4"	269	48	leaf
Xx8-08-037	17°	16'	56.1"N	103°	27'	34.8"	253	63	leaf

Appendix Table 1 (Continued)

Khlong Tron

Tree identifier	Latitude N			Longitude E			Elevation (m)	GBH(cm)	Sample
Xx8-09-001	17°	35'	53.3"N	100°	32'	49.1"	231	89	leaf
Xx8-09-002	17°	36'	.7"N	100°	32'	42.2"	185	48	leaf
Xx8-09-003	17°	35'	38.4"N	100°	33'	8."	202	42	leaf
Xx8-09-004	17°	35'	42."N	100°	33'	11."	210	53	leaf
Xx8-09-005	17°	35'	44.4"N	100°	33'	14.2"	205	42	leaf
Xx8-09-006	17°	35'	46.5"N	100°	33'	17."	209	46	leaf
Xx8-09-007	17°	35'	51.3"N	100°	33'	20.1"	204	44	leaf
Xx8-09-008	17°	35'	49.1"N	100°	33'	23.1"	209	45	leaf
Xx8-09-009	17°	35'	52."N	100°	33'	27.1"	208	38	leaf
Xx8-09-010	17°	35'	52."N	100°	33'	27.2"	208	39	leaf
Xx8-09-011	17°	35'	55.6"N	100°	33'	31."	208	61	leaf
Xx8-09-012	17°	35'	54.3"N	100°	33'	34.4"	209	69	leaf
Xx8-09-013	17°	35'	56.1"N	100°	33'	39.6"	210	70	leaf
Xx8-09-014	17°	35'	58.6"N	100°	33'	42.5"	214	54	leaf
Xx8-09-015	17°	36'	."N	100°	33'	43.7"	214	64+80	leaf
Xx8-09-016	17°	36'	3.2"N	100°	33'	45.5"	216	55	leaf
Xx8-09-017	17°	36'	6.4"N	100°	33'	47.2"	216	50	leaf
Xx8-09-018	17°	36'	10.4"N	100°	32'	47."	216	42+33+30	leaf
Xx8-09-019	17°	36'	4.9"N	100°	33'	51.4"	217	49	leaf
Xx8-09-020	17°	36'	8.1"N	100°	33'	54."	217	42+29	leaf
Xx8-09-021	17°	36'	12.3"N	100°	33'	56.7"	221	50	leaf
Xx8-09-022	17°	36'	11.2"N	100°	33'	59.8"	220	47+47+54	leaf
Xx8-09-023	17°	36'	12.2"N	100°	34'	5.2"	220	53	leaf
Xx8-09-024	17°	36'	15.3"N	100°	34'	8.9"	225	135	leaf
Xx8-09-025	17°	36'	6.5"N	100°	33'	58.7"	218	129	leaf
Xx8-09-026	17°	36'	3.6"N	100°	34'	2.6"	226	46	leaf
Xx8-09-027	17°	35'	58.2"N	100°	34'	7.9"	239	75	leaf
Xx8-09-028	17°	36'	2.5"N	100°	34'	14."	242	157	leaf
Xx8-09-029	17°	36'	7.8"N	100°	34'	14."	242	74	leaf
Xx8-09-030	17°	36'	14.3"N	100°	34'	17.6"	244	90	leaf
Xx8-09-031	17°	35'	57.4"N	100°	34'	3."	207	53	leaf
Xx8-09-032	17°	35'	49.9"N	100°	34'	6.2"	201	78	leaf
Xx8-09-033	17°	35'	51.4"N	100°	34'	.8"	200	58	leaf
Xx8-09-034	17°	35'	57.8"N	100°	33'	57.2"	198	71	leaf

Appendix Table 1 (Continued)

Tree identifier	Latitude N			Longitude E			Elevation (m)	GBH(cm)	Sample
Xx8-09-035	17°	36'	.8"N	100°	32'	43.2"	208	65	leaf
Xx8-09-036	17°	36'	37."N	100°	33'	1.8"	199	67	leaf
Xx8-09-037	17°	36'	41.8"N	100°	32'	59.9"	204	49	leaf
Xx8-09-038	17°	36'	47.7"N	100°	32'	56.8"	202	56	leaf
Xx8-09-039	17°	36'	49.2"N	100°	33'	6."	229	46	leaf
Xx8-09-040	17°	36'	53.9"N	100°	33'	18.3"	219	115	leaf
Xx8-09-041	17°	37'	6.4"N	100°	33'	18."	216	71	leaf
Xx8-09-042	17°	39'	23.5"N	100°	34'	8.8"	181	239	leaf
Xx8-09-043	17°	35'	36.8"N	100°	32'	12.1"	200	62	leaf
Xx8-09-044	17°	35'	37.8"N	100°	31'	52.1"	170	54	leaf

Tham Pha Thai

Tree identifier	Latitude N			Longitude E			Elevation (m)	GBH(cm)	Sample
Xx8-10-001	18°	36'	17.8"N	99°	53'	55.4"	330	368	leaf
Xx8-10-002	18°	36'	16.7"N	99°	53'	59.8"	331	79	leaf
Xx8-10-003	18°	36'	16.5"N	99°	54'	3.7"	330	103	leaf
Xx8-10-004	18°	36'	10.9"N	99°	54'	5.6"	339	201	leaf
Xx8-10-005	18°	36'	6."N	99°	54'	3.6"	347	78	leaf
Xx8-10-006	18°	36'	4.6"N	99°	53'	57.9"	335	118	leaf
Xx8-10-007	18°	36'	4.9"N	99°	53'	51.4"	340	202	leaf
Xx8-10-008	18°	35'	59.1"N	99°	53'	46.8"	359	103	leaf
Xx8-10-009	18°	35'	55.3"N	99°	53'	45.2"	364	164	leaf
Xx8-10-010	18°	35'	51.7"N	99°	53'	42.7"	362	171	leaf
Xx8-10-011	18°	35'	49.5"N	99°	53'	41.9"	370	129	leaf
Xx8-10-012	18°	35'	46.8"N	99°	53'	38.4"	366	167	leaf
Xx8-10-013	18°	35'	51.9"N	99°	53'	36.8"	376	279	leaf
Xx8-10-014	18°	35'	56.2"N	99°	53'	37.3"	376	120	leaf
Xx8-10-015	18°	36'	4.6"N	99°	53'	40.5"	372	133	leaf
Xx8-10-016	18°	36'	12."N	99°	53'	45.8"	368	134	leaf
Xx8-10-017	18°	36'	10.5"N	99°	53'	50.4"	353	205	leaf
Xx8-10-018	18°	36'	11.6"N	99°	53'	55.8"	360	95	leaf
Xx8-10-019	18°	38'	10.1"N	99°	54'	11.6"	348	94	leaf

Appendix Table 1 (Continued)

Tree identifier	Latitude N			Longitude E			Elevation (m)	GBH(cm)	Sample
Xx8-10-019	18°	38'	10.1"N	99°	54'	11.6"	348	94	leaf
Xx8-10-020	18°	38'	10.5"N	99°	54'	4.3"	350	135	leaf
Xx8-10-021	18°	38'	8.7"N	99°	53'	59.9"	349	85	leaf
Xx8-10-022	18°	38'	4.2"N	99°	53'	59.7"	346	205	leaf
Xx8-10-023	18°	38'	2.9"N	99°	53'	52.5"	342	169	leaf
Xx8-10-024	18°	38'	6.8"N	99°	53'	48.5"	343	196	leaf
Xx8-10-025	18°	38'	4.6"N	99°	53'	36.7"	347	201+152	leaf
Xx8-10-026	18°	37'	59.5"N	99°	53'	37.9"	349	197	leaf
Xx8-10-027	18°	37'	55.2"N	99°	53'	36.7"	371	192	leaf
Xx8-10-028	18°	37'	52.4"N	99°	53'	41.5"	360	65	leaf
Xx8-10-029	18°	37'	55.5"N	99°	53'	45."	349	125	leaf
Xx8-10-030	18°	37'	43."N	99°	53'	49.7"	342	51	leaf
Xx8-10-031	18°	37'	36.4"N	99°	53'	48.6"	344	103	leaf
Xx8-10-032	18°	37'	32.6"N	99°	53'	45.9"	348	104	leaf
Xx8-10-033	18°	37'	26.9"N	99°	53'	40.4"	338	104	leaf
Xx8-10-034	18°	37'	17.8"N	99°	53'	35."	343	97	leaf
Xx8-10-035	18°	36'	53.7"N	99°	53'	58.4"	357	104	leaf
Xx8-10-036	18°	36'	40.7"N	99°	53'	54.6"	357	130	leaf
Xx8-10-037	18°	36'	34.2"N	99°	53'	53.9"	354	140	leaf
Xx8-10-038	18°	36'	30.7"N	99°	53'	54.4"	346	203	leaf
Xx8-10-039	18°	36'	27.9"N	99°	53'	53.3"	350	199	leaf
Xx8-10-040	18°	36'	22.6"N	99°	53'	49.4"	355	151	leaf
Xx8-10-041	18°	36'	19.2"N	99°	53'	50.7"	347	166	leaf

Pha Daeng

Tree identifier	Latitude N			Longitude E			Elevation (m)	GBH(cm)	Sample
Xx8-11-001	19°	36'	7.5"N	98°	57'	38.4"	500	77	leaf
Xx8-11-002	19°	36'	4."N	98°	57'	39.9"	494	62	leaf
Xx8-11-003	19°	31'	53.7"N	98°	57'	32.4"	456	76	leaf
Xx8-11-004	19°	31'	48.6"N	98°	57'	31.4"	462	50	leaf
Xx8-11-005	19°	31'	44.8"N	98°	57'	33.2"	455	71	leaf
Xx8-11-006	19°	31'	35.4"N	98°	57'	47.9"	461	224	leaf

Appendix Table 1 (Continued)

Tree identifier	Latitude N			Longitude E			Elevation (m)	GBH(cm)	Sample
Xx8-11-007	19°	31'	23.4"N	98°	57'	46.3"	479	94	leaf
Xx8-11-008	19°	30'	29.1"N	98°	57'	1.4"	482	83	leaf
Xx8-11-009	19°	30'	43.3"N	98°	56'	18.5"	478	147	leaf
Xx8-11-010	19°	30'	48.2"N	98°	56'	16.6"	493	106	leaf
Xx8-11-011	19°	30'	48.2"N	98°	56'	25.5"	472	95	leaf
Xx8-11-012	19°	30'	49.4"N	98°	56'	39.1"	463	81+63	leaf
Xx8-11-013	19°	30'	47."N	98°	56'	44.4"	463	40	leaf
Xx8-11-014	19°	30'	38.4"N	98°	56'	51."	469	103	leaf
Xx8-11-015	19°	30'	25.5"N	98°	56'	43.9"	500	53	leaf
Xx8-11-016	19°	30'	25.4"N	98°	58'	5.4"	448	77	leaf
Xx8-11-017	19°	30'	20.3"N	98°	58'	9.9"	450	102	leaf
Xx8-11-018	19°	29'	11.7"N	98°	58'	38.7"	441	195	leaf
Xx8-11-019	19°	28'	55.6"N	98°	58'	44.6"	440	76	leaf
Xx8-11-020	19°	28'	27.5"N	98°	59'	1."	433	84+103	leaf
Xx8-11-021	19°	28'	16.7"N	98°	59'	1.6"	437	90	leaf
Xx8-11-022	19°	28'	.2"N	98°	59'	9.1"	431	109	leaf
Xx8-11-023	19°	27'	49.1"N	98°	59'	15.9"	437	95	leaf
Xx8-11-024	19°	27'	36.3"N	98°	59'	29.3"	441	177	leaf
Xx8-11-025	19°	27'	38.7"N	98°	59'	44.2"	435	120	leaf
Xx8-11-026	19°	27'	50.9"N	98°	59'	53.6"	438	112	leaf
Xx8-11-027	19°	28'	3.6"N	99°	'	1.4"	438	97	leaf
Xx8-11-028	19°	28'	20.3"N	99°	'	12."	442	104	leaf
Xx8-11-029	19°	28'	29.9"N	99°	'	28."	459	216	leaf
Xx8-11-030	19°	28'	44.3"N	99°	'	34.8"	477	201	leaf
Xx8-11-031	19°	28'	51.6"N	99°	'	48.7"	503	137	leaf
Xx8-11-032	19°	30'	2.7"N	99°	'	58.5"	537	223	leaf
Xx8-11-033	19°	30'	31.6"N	99°	3'	13.3"	540	260	leaf
Xx8-11-034	19°	38'	.2"N	99°	3'	8.3"	755	238	leaf
Xx8-11-035	19°	36'	32.9"N	98°	57'	23.6"	529	123	leaf
Xx8-11-036	19°	36'	25.1"N	98°	57'	28.1"	524	113	leaf
Xx8-11-037	19°	37'	2.7"N	98°	57'	26."	511	138	leaf

Appendix Table 1 (Continued)

Doi Jong

Tree identifier	Latitude N			Longitude E			Elevation (m)	GBH(cm)	Sample
Xx8-12-001	17°	55'	22.6"N	99°	16'	28.1"	265	95	leaf
Xx8-12-002	17°	55'	37.4"N	99°	15'	49.4"	295	54	leaf
Xx8-12-003	17°	55'	.4"N	99°	14'	56.8"	330	111	leaf
Xx8-12-004	17°	55'	1.7"N	99°	14'	52.7"	322	141	leaf
Xx8-12-005	17°	55'	2.2"N	99°	14'	48.8"	329	101	leaf
Xx8-12-006	17°	55'	3.5"N	99°	14'	43.6"	336	236	leaf
Xx8-12-007	17°	55'	3.4"N	99°	14'	39.5"	341	92	leaf
Xx8-12-008	17°	55'	1.9"N	99°	14'	35.1"	348	127	leaf
Xx8-12-009	17°	54'	58.3"N	99°	14'	32.2"	355	119	leaf
Xx8-12-010	17°	54'	54.8"N	99°	14'	30.6"	366	96	leaf
Xx8-12-011	17°	54'	51.3"N	99°	14'	28.7"	372	137	leaf
Xx8-12-012	17°	54'	48.6"N	99°	14'	23.2"	389	148	leaf
Xx8-12-013	17°	54'	46.1"N	99°	14'	28."	372	70	leaf
Xx8-12-014	17°	54'	46.2"N	99°	14'	33.6"	361	146	leaf
Xx8-12-015	17°	54'	48.9"N	99°	14'	40.8"	346	137	leaf
Xx8-12-016	17°	54'	48.5"N	99°	14'	45.9"	343	80	leaf
Xx8-12-017	17°	54'	46.5"N	99°	14'	54.6"	330	146	leaf
Xx8-12-018	17°	54'	43.5"N	99°	14'	58.6"	331	90	leaf
Xx8-12-019	17°	54'	45.5"N	99°	15'	4.4"	335	65	leaf
Xx8-12-020	17°	54'	47.6"N	99°	15'	10.1"	343	132	leaf
Xx8-12-021	17°	54'	47.3"N	99°	15'	15.5"	347	63	leaf
Xx8-12-022	17°	54'	51.3"N	99°	15'	19.7"	345	85+69	leaf
Xx8-12-023	17°	54'	54.9"N	99°	15'	21.1"	341	55	leaf
Xx8-12-024	17°	54'	58.8"N	99°	15'	29.2"	332	54	leaf
Xx8-12-025	17°	55'	1.9"N	99°	15'	32.1"	335	103	leaf
Xx8-12-026	17°	55'	4.3"N	99°	15'	35.6"	329	65	leaf
Xx8-12-027	17°	55'	7.1"N	99°	15'	32.7"	320	108	leaf
Xx8-12-028	17°	55'	6.9"N	99°	15'	28.9"	305	60	leaf
Xx8-12-029	17°	55'	4.7"N	99°	15'	23."	319	142	leaf
Xx8-12-030	17°	55'	5.3"N	99°	15'	18.3"	326	63	leaf
Xx8-12-031	17°	54'	59.5"N	99°	15'	1."	312	110	leaf
Xx8-12-032	17°	55'	4.1"N	99°	14'	59.1"	316	117	leaf
Xx8-12-033	17°	55'	6.1"N	99°	14'	54.6"	314	104	leaf
Xx8-12-034	17°	55'	8.8"N	99°	14'	49.9"	320	124	leaf
Xx8-12-035	17°	55'	9.3"N	99°	14'	41.6"	332	57	leaf

Appendix Table 1 (Continued)

Khlong Wang Jao

Tree identifier	Latitude N			Longitude E			Elevation (m)	GBH(cm)	Sample
Xx8-12-036	17°	55'	17.8"N	99°	14'	39.8"	333	130	leaf
Xx8-12-037	17°	55'	21.8"N	99°	14'	31.8"	338	71	leaf
Xx8-12-038	17°	55'	27.8"N	99°	14'	34.1"	353	72	leaf
Xx8-12-039	17°	55'	25.7"N	99°	14'	38.8"	347	73	leaf
Xx8-12-040	17°	55'	13.3"N	99°	14'	42.6"	331	68	leaf
Xx8-12-041	17°	55'	34.8"N	99°	15'	28."	312	80	leaf
Xx8-13-001	16°	30'	17.1"N	99°	10'	6.7"	216	242	leaf
Xx8-13-002	16°	30'	22.5"N	99°	10'	2.9"	231	232	leaf
Xx8-13-003	16°	30'	27.3"N	99°	10'	7.5"	230	232+62	leaf
Xx8-13-004	16°	30'	31.1"N	99°	10'	10.6"	233	53	leaf
Xx8-13-005	16°	30'	32."N	99°	10'	14.4"	221	166	leaf
Xx8-13-006	16°	30'	33.9"N	99°	10'	21.7"	219	146	leaf
Xx8-13-007	16°	30'	18."N	99°	10'	17.8"	241	147	leaf
Xx8-13-008	16°	30'	12."N	99°	10'	20.3"	250	151	leaf
Xx8-13-009	16°	30'	7.1"N	99°	10'	23.5"	264	55	leaf
Xx8-13-010	16°	30'	.4"N	99°	10'	25.7"	266	85	leaf
Xx8-13-011	16°	29'	56.1"N	99°	10'	28.1"	269	57	leaf
Xx8-13-012	16°	29'	48.3"N	99°	10'	31.2"	278	75	leaf
Xx8-13-013	16°	29'	42."N	99°	10'	31.5"	288	70	leaf
Xx8-13-014	16°	29'	39.1"N	99°	10'	27.2"	288	77	leaf
Xx8-13-015	16°	29'	38.4"N	99°	10'	21.3"	268	192	leaf
Xx8-13-016	16°	29'	40.1"N	99°	10'	17.2"	253	268	leaf
Xx8-13-017	16°	29'	45."N	99°	10'	14.7"	269	215	leaf
Xx8-13-018	16°	29'	46.8"N	99°	10'	11.1"	283	269	leaf
Xx8-13-019	16°	29'	53.5"N	99°	10'	6.6"	287	188	leaf
Xx8-13-020	16°	29'	50.8"N	99°	10'	2."	295	105	leaf
Xx8-13-021	16°	29'	49.7"N	99°	9'	57.4"	320	90	leaf
Xx8-13-022	16°	29'	40.5"N	99°	9'	59.9"	339	119	leaf
Xx8-13-023	16°	29'	35.5"N	99°	10'	.5"	368	170	leaf
Xx8-13-024	16°	29'	30.6"N	99°	10'	.8"	378	132	leaf
Xx8-13-025	16°	29'	27.1"N	99°	9'	57.9"	363	225	leaf
Xx8-13-026	16°	29'	26.1"N	99°	9'	52.6"	368	118	Leaf
Xx8-13-027	16°	29'	33.3"N	99°	9'	53.7"	361	79	leaf

Appendix Table 1 (Continued)

Tree identifier	Latitude N			Longitude E			Elevation (m)	GBH(cm)	Sample
Xx8-13-028	16°	29'	41.3"N	99°	9'	53."	368	122	leaf
Xx8-13-029	16°	29'	47.6"N	99°	9'	50.8"	414	158	leaf
Xx8-13-030	16°	29'	53.2"N	99°	9'	53.5"	359	131	leaf
Xx8-13-031	16°	29'	59.4"N	99°	9'	53.5"	326	43	leaf
Xx8-13-032	16°	30'	.5"N	99°	9'	58.3"	296	122	leaf
Xx8-13-033	16°	30'	4."N	99°	10'	1.7"	270	50	leaf
Xx8-13-034	16°	30'	9.5"N	99°	9'	58.6"	248	61	leaf
Xx8-13-035	16°	30'	11.3"N	99°	9'	42."	285	337	leaf
Xx8-13-036	16°	29'	41.2"N	99°	9'	12.7"	356	198	leaf
Xx8-13-037	16°	29'	45.1"N	99°	9'	8.6"	355	216	leaf

Mae Ngao

Tree identifier	Latitude N			Longitude E			Elevation (m)	GBH(cm)	Sample
Xx8-14-001	17°	50	44.90"N	97°	58'	35.90"E	150	277	leaf
Xx8-14-002	17°	50	39.50"N	97°	58'	39.50"E	151	277	leaf
Xx8-14-003	17°	50	35.50"N	97°	58'	43.00"E	156	263	leaf
Xx8-14-004	17°	50	32.30"N	97°	58'	46.40"E	172	139	leaf
Xx8-14-005	17°	50	26.30"N	97°	58'	52.90"E	159	163	leaf
Xx8-14-006	17°	50	20.80"N	97°	58'	55.70"E	156	227	leaf
Xx8-14-007	17°	50	14.90"N	97°	58'	57.00"E	174	149	leaf
Xx8-14-008	17°	50	06.90"N	97°	59'	00.10"E	181	180	leaf
Xx8-14-009	17°	50	00.50"N	97°	58'	59.50"E	188	218	leaf
Xx8-14-010	17°	49	55.80"N	97°	58'	56.60"E	192	80	leaf
Xx8-14-011	17°	49	40.20"N	97°	59'	03.00"E	204	127	leaf
Xx8-14-012	17°	49	36.60"N	97°	59'	03.10"E	197	266	leaf
Xx8-14-013	17°	49	37.30"N	97°	59'	08.40"E	215	114	leaf
Xx8-14-014	17°	49	28.30"N	97°	59'	05.90"E	182	251	leaf
Xx8-14-015	17°	49	13.60"N	97°	59'	07.80"E	181	189	leaf
Xx8-14-016	17°	48	55.80"N	97°	58'	55.30"E	190	287	leaf
Xx8-14-017	17°	48	48.20"N	97°	59'	00.20"E	195	109	leaf
Xx8-14-018	17°	48	44.90"N	97°	59'	06.80"E	181	153	leaf
Xx8-14-019	17°	48	41.80"N	97°	59'	19.20"E	198	133	leaf

Appendix Table 1 (Continued)

Tree identifier	Latitude N			Longitude E			Elevation (m)	GBH(cm)	Sample
Xx8-14-020	17°	48	41.20"N	97°	59'	25.20"E	212	129	leaf
Xx8-14-021	17°	52	03.10"N	97°	57'	56.30"E	217	141	leaf
Xx8-14-022	17°	52	06.70"N	97°	58'	05.00"E	204	155	leaf
Xx8-14-023	17°	52	03.60"N	97°	58'	12.10"E	174	204	leaf
Xx8-14-024	17°	52	01.70"N	97°	58'	17.20"E	176	136	leaf
Xx8-14-025	17°	51	57.10"N	97°	58'	22.40"E	169	329	leaf
Xx8-14-026	17°	51	49.50"N	97°	58'	25.70"E	162	204	leaf
Xx8-14-027	17°	51	45.80"N	97°	58'	26.00"E	160	223	leaf
Xx8-14-028	17°	51	41.20"N	97°	58'	22.50"E	160	114	leaf
Xx8-14-029	17°	51	37.20"N	97°	58'	18.90"E	158	150	leaf
Xx8-14-030	17°	51	33.00"N	97°	58'	20.20"E	164	123+116+134+170	leaf
Xx8-14-031	17°	51	28.90"N	97°	58'	20.20"E	168	162	leaf
Xx8-14-032	17°	51	23.70"N	97°	58'	12.90"E	170	190	leaf
Xx8-14-033	17°	51	11.40"N	97°	58'	15.50"E	157	184	leaf
Xx8-14-034	17°	50	59.50"N	97°	58'	25.50"E	167	268	leaf
Xx8-14-035	17°	50	58.10"N	97°	58'	25.10"E	176	267	leaf
Xx8-14-036	17°	50	49.60"N	97°	58'	33.80"E	154	196+153	leaf

ThaTaFang

Tree identifier	Latitude N			Longitude E			Elevation (m)	GBH(cm)	Sample
Xx8-15-001	18°	04'	14.40"N	97°	41'	27.20"E	148	86	leaf
Xx8-15-002	18°	04'	09.40"N	97°	41'	29.70"E	135	272	leaf
Xx8-15-003	18°	04'	02.90"N	97°	41'	18.70"E	156	255	leaf
Xx8-15-004	18°	04'	09.20"N	97°	41'	17.00"E	149	163	leaf
Xx8-15-005	18°	04'	07.60"N	97°	41'	14.00"E	166	205	leaf
Xx8-15-006	18°	04'	07.80"N	97°	41'	05.40"E	125	113	leaf
Xx8-##-001	18°	00'	28.80"N	97°	50'	10.90"E	379	126	leaflet
Xx8-##-002	18°	00'	46.80"N	97°	50'	40.20"E	478	169	leaflet
Xx8-##-003	18°	01'	15.00"N	97°	51'	08.20"E	578	114	leaflet
Xx8-##-004	18°	01'	41.30"N	97°	52'	34.50"E	321	225	leaflet
Xx8-##-005	18°	01'	59.60"N	97°	53'	03.90"E	266	145	leaflet

Appendix Table 1 (Continued)

Mae Ping

Tree identifier	Latitude N			Longitude E			Elevation (m)	GBH(cm)	Sample
Xx8-16-001	17°	36'	05.50"N	98°	52'	42.60"E	673	82	leaf
Xx8-16-002	17°	36'	12.60"N	98°	52'	36.60"E	679	155	leaf
Xx8-16-003	17°	36'	06.40"N	98°	52'	39.30"E	675	94	leaf
Xx8-16-004	17°	35'	49.00"N	98°	52'	48.70"E	661	177	leaf
Xx8-16-005	17°	35'	49.90"N	98°	52'	54.10"E	652	151	leaf
Xx8-16-006	17°	35'	41.90"N	98°	52'	48.10"E	653	199	leaf
Xx8-16-007	17°	35'	39.70"N	98°	52'	41.50"E	667	159	leaf
Xx8-16-008	17°	35'	45.30"N	98°	52'	37.70"E	676	78	leaf
Xx8-16-009	17°	35'	49.50"N	98°	52'	43.40"E	660	110	leaf
Xx8-16-010	17°	35'	54.80"N	98°	52'	40.60"E	663	145	leaf
Xx8-16-011	17°	35'	08.40"N	98°	52'	53.20"E	610	106	leaf
Xx8-16-012	17°	35'	05.20"N	98°	52'	55.60"E	594	151	leaf
Xx8-16-013	17°	34'	00.50"N	98°	52'	48.20"E	577	255	leaf
Xx8-16-014	17°	33'	44.80"N	98°	52'	52.10"E	550	265	leaf
Xx8-16-015	17°	38'	13.50"N	98°	51'	49.20"E	670	145	leaf
Xx8-16-016	17°	37'	58.80"N	98°	51'	38.60"E	650	156	leaf
Xx8-16-017	17°	37'	45.70"N	98°	51'	11.50"E	637	74	leaf
Xx8-16-018	17°	37'	26.10"N	98°	50'	49.70"E	612	199	leaf
Xx8-16-019	17°	35'	06.80"N	98°	49'	01.30"E	367	127	leaf
Xx8-16-020	17°	34'	59.70"N	98°	49'	10.40"E	463	105	leaf
Xx8-16-021	17°	35'	04.60"N	98°	49'	04.30"E	429	75	leaf
Xx8-16-022	17°	35'	08.10"N	98°	49'	07.30"E	411	117	leaf
Xx8-16-023	17°	35'	09.30"N	98°	49'	11.50"E	408	223	leaf
Xx8-16-024	17°	35'	14.30"N	98°	49'	08.00"E	400	189	leaf
Xx8-16-025	17°	35'	16.30"N	98°	49'	00.40"E	382	184	leaf
Xx8-16-026	17°	35'	35.10"N	98°	48'	29.00"E	333	230	leaf
Xx8-16-027	17°	37'	26.30"N	98°	49'	45.90"E	550	216	leaf
Xx8-16-028	17°	40'	13.30"N	98°	52'	11.90"E	589	128	leaf

Appendix Table 1 (Continued)

Kaeng Jed-khwae

Tree identifier	Latitude N			Longitude E			Elevation (m)	GBH(cm)	Sample
Xx8-17-001	17°	10'	32.70"N	100°	23'	53.10"E	179	80	leaf
Xx8-17-002	17°	10'	26.30"N	100°	23'	54.60"E	146	82	leaf
Xx8-17-003	17°	10'	17.10"N	100°	23'	57.60"E	157	80	leaf
Xx8-17-004	17°	11'	37.10"N	100°	24'	26.40"E	94	85	leaf
Xx8-17-005	17°	11'	36.80"N	100°	24'	35.40"E	89	86	leaf
Xx8-17-006	17°	11'	32.90"N	100°	24'	50.20"E	93	80	leaf
Xx8-17-007	17°	11'	41.60"N	100°	25'	14.40"E	90	87	leaf
Xx8-17-008	17°	11'	56.30"N	100°	25'	16.90"E	96	82	leaf
Xx8-17-009	17°	12'	01.50"N	100°	25'	13.60"E	104	85	leaf
Xx8-17-010	17°	12'	16.00"N	100°	25'	04.80"E	153	82	leaf
Xx8-17-011	17°	12'	22.30"N	100°	25'	02.50"E	168	83	leaf
Xx8-17-012	17°	12'	35.30"N	100°	25'	05.40"E	170	82	leaf
Xx8-17-013	17°	12'	41.70"N	100°	25'	09.40"E	165	88	leaf
Xx8-17-014	17°	12'	47.90"N	100°	25'	10.70"E	162	82	leaf
Xx8-17-016	17°	12'	58.40"N	100°	25'	02.90"E	182	81	leaf
Xx8-17-017	17°	13'	05.00"N	100°	24'	54.30"E	165	82	leaf
Xx8-17-018	17°	13'	15.10"N	100°	24'	45.50"E	150	85	leaf
Xx8-17-019	17°	13'	25.90"N	100°	24'	42.80"E	152	83	leaf
Xx8-17-020	17°	13'	32.70"N	100°	24'	42.70"E	154	82	leaf
Xx8-17-021	17°	13'	37.90"N	100°	24'	40.40"E	153	83	leaf
Xx8-17-022	17°	13'	48.70"N	100°	24'	33.80"E	164	82	leaf
Xx8-17-023	17°	13'	56.70"N	100°	24'	31.40"E	162	81	leaf
Xx8-17-025	17°	12'	52.30"N	100°	25'	20.30"E	159	82	leaf
Xx8-17-026	17°	12'	45.40"N	100°	25'	21.00"E	157	82	leaf
Xx8-17-027	17°	12'	41.30"N	100°	25'	18.10"E	153	81	leaf

Appendix Table 1 (Continued)

Wieng Kosai

Tree identifier	Latitude N			Longitude E			Elevation (m)	GBH(cm)	Sample
Xx8-18-001	17°	42'	58.70"N	99°	18'	30.10"E	304	39	leaf
Xx8-18-002	17°	43'	02.80"N	99°	18'	23.70"E	310	124	leaf
Xx8-18-003	17°	43'	17.90"N	99°	18'	13.80"E	324	47	leaf
Xx8-18-004	17°	43'	21.50"N	99°	18'	12.80"E	328	70	leaf
Xx8-18-005	17°	43'	27.80"N	99°	18'	10.40"E	334	67	leaf
Xx8-18-006	17°	43'	33.40"N	99°	18'	10.70"E	338	59	leaf
Xx8-18-007	17°	43'	38.50"N	99°	18'	10.50"E	347	54	leaf
Xx8-18-008	17°	43'	32.20"N	99°	18'	13.90"E	354	54	leaf
Xx8-18-009	17°	43'	04.90"N	99°	18'	18.20"E	328	70	leaf
Xx8-18-010	17°	42'	47.40"N	99°	18'	46.10"E	310	226	leaf
Xx8-18-011	17°	42'	23.80"N	99°	19'	03.60"E	301	206	leaf
Xx8-18-012	17°	42'	09.40"N	99°	19'	02.30"E	299	47	leaf
Xx8-18-013	17°	41'	48.70"N	99°	19'	02.20"E	279	80	leaf
Xx8-18-014	17°	42'	05.80"N	99°	20'	07.90"E	282	166	leaf
Xx8-18-015	17°	42'	03.10"N	99°	20'	15.60"E	275	117	leaf
Xx8-18-016	17°	42'	02.50"N	99°	20'	20.90"E	296	193	leaf
Xx8-18-017	17°	42'	02.30"N	99°	20'	27.30"E	295	63	leaf
Xx8-18-018	17°	41'	59.00"N	99°	20'	31.90"E	284	87	leaf
Xx8-18-019	17°	41'	59.60"N	99°	20'	35.60"E	289	116	leaf
Xx8-18-020	17°	42'	01.60"N	99°	20'	40.10"E	293	76	leaf
Xx8-18-021	17°	42'	05.40"N	99°	20'	42.50"E	297	71	leaf
Xx8-18-022	17°	42'	05.50"N	99°	20'	47.00"E	292	135	leaf
Xx8-18-023	17°	42'	07.70"N	99°	20'	50.30"E	274	172	leaf
Xx8-18-024	17°	42'	11.70"N	99°	20'	57.10"E	281	92	leaf
Xx8-18-025	17°	42'	15.60"N	99°	20'	56.90"E	273	78	leaf
Xx8-18-026	17°	42'	21.00"N	99°	20'	59.00"E	265	112	leaf
Xx8-18-027	17°	42'	23.20"N	99°	20'	55.80"E	269	60	leaf
Xx8-18-028	17°	42'	28.90"N	99°	20'	51.90"E	271	84	leaf
Xx8-18-029	17°	42'	31.70"N	99°	20'	54.90"E	304	68	leaf
Xx8-18-030	17°	42'	28.20"N	99°	20'	58.60"E	303	71	leaf
Xx8-18-031	17°	42'	26.80"N	99°	20'	59.00"E	306	187	leaf
Xx8-18-031	17°	42'	26.80"N	99°	20'	59.00"E	306	187	leaf
Xx8-18-032	17°	42'	17.00"N	99°	21'	00.40"E	292	-	leaf

Appendix Table 1 (Continued)

Individual trees

Tree identifier	Latitude N			Longitude E			Elevation (m)	GBH(cm)	Sample
Xx8-##-006	19°	36'	53.75"N	100°	00'	05.38"E	418	422	leaflet
Xx8-##-007	14°	22'	57.70"N	98°	52'	27.40"E	149	314	leaflet
Xx8-##-008	14°	22'	44.50"N	98°	52'	40.70"E	86	197	leaflet
Xx8-##-009	16°	04'	36.60"N	103°	31'	31.20"E	293	56	leaflet
Xx8-##-010	17°	58’	07.9"N	99°	35’	31"E	254	88	leaflet
Xx8-##-011	14°	00'	18.70"N	100°	00'	02.40"E	12m	160	leaf
Xx8-##-012	18°	56'	01.40"N	99°	02'	45.20"E	357m	134	leaf
Xx8-##-013	18°	56'	05.20"N	99°	02'	40.70"E	380m	167	leaf
Xx8-##-014	14°	42'	17.10"N	98°	35'	08.60"E	256m	201	leaf
Xx8-##-015	14°	42'	41.30"N	98°	39'	03.60"E	217m	219	leaf

Appendix Table 2 Individual accessions used for direct sequencing and cloning of alleles.

Locus	Accession
Xx-IDH1	Xx8-##-001, Xx8-##-002, Xx8-##-003, Xx8-##-004, Xx8-##-005, Xx8-##-006, Xx8-##-007, Xx8-##-008, Xx8-##-012, Xx8-##-013, Xx8-##-015, Xx8-01-001, Xx8-01-002, Xx8-01-003, Xx8-01-004, Xx8-01-005, Xx8-01-006, Xx8-01-009, Xx8-01-013, Xx8-01-014, Xx8-01-018, Xx8-01-019, Xx8-01-020, Xx8-01-021, Xx8-01-022, Xx8-01-023, Xx8-01-024, Xx8-01-025, Xx8-01-027, Xx8-01-032, Xx8-01-033, Xx8-01-034, Xx8-01-037, Xx8-01-040, Xx8-01-041, Xx8-02-002, Xx8-02-003, Xx8-02-006, Xx8-02-007, Xx8-02-008, Xx8-02-009, Xx8-02-011, Xx8-02-012, Xx8-02-013, Xx8-02-017, Xx8-02-018, Xx8-02-019, Xx8-02-020, Xx8-02-021, Xx8-02-022, Xx8-02-023, Xx8-02-024, Xx8-02-028, Xx8-02-031, Xx8-02-033, Xx8-02-035, Xx8-05-001, Xx8-05-005, Xx8-05-013, Xx8-05-014, Xx8-05-015, Xx8-05-016, Xx8-05-017, Xx8-05-018, Xx8-05-019, Xx8-05-021, Xx8-05-023, Xx8-06-002, Xx8-06-004, Xx8-06-005, Xx8-06-008, Xx8-06-009, Xx8-06-010, Xx8-06-012, Xx8-06-014, Xx8-06-015, Xx8-06-016, Xx8-06-017, Xx8-06-018, Xx8-06-019, Xx8-06-021, Xx8-06-024, Xx8-06-029, Xx8-06-030, Xx8-06-035, Xx8-06-036, Xx8-06-039, Xx8-06-040, Xx8-06-041, Xx8-07-003, Xx8-07-006, Xx8-07-007, Xx8-07-009, Xx8-07-010, Xx8-07-011, Xx8-07-015, Xx8-07-016, Xx8-07-018, Xx8-07-021, Xx8-07-022, Xx8-07-023, Xx8-07-024, Xx8-07-027, Xx8-07-029, Xx8-07-031, Xx8-07-032, Xx8-08-001, Xx8-08-003, Xx8-08-004, Xx8-08-006, Xx8-08-007, Xx8-08-008, Xx8-08-010, Xx8-08-011, Xx8-08-012, Xx8-08-013, Xx8-08-014, Xx8-08-015, Xx8-08-016, Xx8-08-019, Xx8-08-020, Xx8-08-022, Xx8-08-023, Xx8-08-024, Xx8-08-026, Xx8-08-030, Xx8-08-034, Xx8-08-036, Xx8-08-037, Xx8-09-001, Xx8-09-002, Xx8-09-003, Xx8-09-004, Xx8-09-006, Xx8-09-008,

Appendix Table 2 (Continued)

Locus	Accession
	Xx8-09-010, Xx8-09-011, Xx8-09-012, Xx8-09-013, Xx8-09-014, Xx8-09-016, Xx8-09-018, Xx8-09-019, Xx8-09-020, Xx8-09-022, Xx8-09-024, Xx8-09-026, Xx8-09-028, Xx8-09-030, Xx8-09-034, Xx8-09-036, Xx8-09-039, Xx8-09-040, Xx8-09-041, Xx8-09-042, Xx8-09-043, Xx8-09-044, Xx8-10-001, Xx8-10-002, Xx8-10-003, Xx8-10-004, Xx8-10-007, Xx8-10-009, Xx8-10-010, Xx8-10-012, Xx8-10-014, Xx8-10-015, Xx8-10-018, Xx8-10-023, Xx8-10-027, Xx8-10-028, Xx8-10-029, Xx8-10-030, Xx8-10-032, Xx8-10-034, Xx8-10-035, Xx8-10-036, Xx8-10-038, Xx8-10-039, Xx8-10-040, Xx8-10-041, Xx8-11-001, Xx8-11-004, Xx8-11-005, Xx8-11-007, Xx8-11-008, Xx8-11-009, Xx8-11-011, Xx8-11-014, Xx8-11-018, Xx8-11-019, Xx8-11-022, Xx8-11-028, Xx8-11-029, Xx8-11-030, Xx8-11-031, Xx8-11-032, Xx8-11-033, Xx8-11-036, Xx8-11-037, Xx8-12-001, Xx8-12-002, Xx8-12-003, Xx8-12-004, Xx8-12-006, Xx8-12-009, Xx8-12-010, Xx8-12-011, Xx8-12-013, Xx8-12-014, Xx8-12-015, Xx8-12-016, Xx8-12-017, Xx8-12-020, Xx8-12-021, Xx8-12-024, Xx8-12-027, Xx8-12-028, Xx8-12-031, Xx8-12-032, Xx8-12-034, Xx8-12-036, Xx8-12-037, Xx8-12-039, Xx8-12-041, Xx8-13-001, Xx8-13-002, Xx8-13-004, Xx8-13-006, Xx8-13-007, Xx8-13-008, Xx8-13-013, Xx8-13-014, Xx8-13-015, Xx8-13-018, Xx8-13-020, Xx8-13-022, Xx8-13-023, Xx8-13-025, Xx8-13-026, Xx8-13-027, Xx8-13-028, Xx8-13-029, Xx8-13-030, Xx8-13-031, Xx8-13-035, Xx8-14-001, Xx8-14-002, Xx8-14-004, Xx8-14-005, Xx8-14-006, Xx8-14-008, Xx8-14-011, Xx8-14-012, Xx8-14-013, Xx8-14-015, Xx8-14-016, Xx8-14-017, Xx8-14-018, Xx8-14-019, Xx8-14-023, Xx8-14-024, Xx8-14-027, Xx8-14-031, Xx8-14-035, Xx8-15-002, Xx8-15-003, Xx8-15-005, Xx8-16-001, Xx8-16-002,

Appendix Table 2 (Continued)

Locus	Accession
	Xx8-16-004, Xx8-16-005, Xx8-16-010, Xx8-16-017, Xx8-16-020, Xx8-16-021, Xx8-16-024, Xx8-16-025, Xx8-16-026, Xx8-16-027, Xx8-16-028, Xx8-17-001, Xx8-17-006, Xx8-17-009, Xx8-17-012, Xx8-17-013, Xx8-17-014, Xx8-17-016, Xx8-17-017, Xx8-17-018, Xx8-17-019, Xx8-17-020, Xx8-17-021, Xx8-17-022, Xx8-17-023, Xx8-17-026, Xx8-18-001, Xx8-18-005, Xx8-18-006, Xx8-18-008, Xx8-18-011, Xx8-18-014, Xx8-18-017, Xx8-18-019, Xx8-18-022, Xx8-18-023, Xx8-18-026, Xx8-18-027, Xx8-18-028, Xx8-18-030, Xx8-18-032, Xx8-01-020, Xx8-13-015, Xx8-17-012, Xx8-02-001, Xx8-02-036, Xx8-06-020, Xx8-10-017, Xx8-11-034, Xx8-12-030, Xx8-14-010, Xx8-17-004, Xx8-18-024, Xx8-##-004, Xx8-09-002, Xx8-01-008, Xx8-02-010, Xx8-05-002, Xx8-06-026, Xx8-10-021, Xx8-11-035, Xx8-12-033, Xx8-14-021, Xx8-17-005, Xx8-18-025, Xx8-##-007, Xx8-10-012, Xx8-01-011, Xx8-02-014, Xx8-05-004, Xx8-06-031, Xx8-10-025, Xx8-12-007, Xx8-13-010, Xx8-14-022, Xx8-17-007, Xx8-18-029, Xx8-15-003, Xx8-08-019, Xx8-01-012, Xx8-02-016, Xx8-05-007, Xx8-07-025, Xx8-11-002, Xx8-12-008, Xx8-13-011, Xx8-14-029, Xx8-17-008, Xx8-##-009, Xx8-16-020, Xx8-13-022, Xx8-01-015, Xx8-02-027, Xx8-05-011, Xx8-08-028, Xx8-11-006, Xx8-12-018, Xx8-13-019, Xx8-14-030, Xx8-18-003, Xx8-##-010, Xx8-##-001, Xx8-12-041, Xx8-01-028, Xx8-02-030, Xx8-05-020, Xx8-08-031, Xx8-11-015, Xx8-12-019, Xx8-13-032, Xx8-16-008, Xx8-18-004, Xx8-##-011, Xx8-12-031, Xx8-05-017, Xx8-01-035, Xx8-02-032, Xx8-06-006, Xx8-09-032, Xx8-11-016, Xx8-12-022, Xx8-13-033, Xx8-16-015, Xx8-18-013, Xx8-##-014, Xx8-16-027, Xx8-01-041, Xx8-01-039, Xx8-02-034, Xx8-06-011, Xx8-09-035, Xx8-11-027, Xx8-12-026, Xx8-14-009, Xx8-16-023, Xx8-18-021

Appendix Table 2 (Continued)

Locus	Accession
	405 accessions for direct sequencing and 4 clones.
Xx-IPI1	Xx8-01-001, Xx8-01-004, Xx8-01-007, Xx8-01-018, Xx8-02-007, Xx8-02-009, Xx8-02-013, Xx8-02-027, Xx8-06-002, Xx8-06-008, Xx8-06-021, Xx8-06-029, Xx8-06-034, Xx8-06-036, Xx8-07-007, Xx8-07-012, Xx8-07-013, Xx8-07-015, Xx8-07-021, Xx8-07-028, Xx8-07-029, Xx8-07-030, Xx8-07-031, Xx8-07-032, Xx8-08-001, Xx8-08-002, Xx8-08-003, Xx8-08-004, Xx8-08-005, Xx8-08-006, Xx8-08-007, Xx8-08-008, Xx8-08-009, Xx8-08-010, Xx8-08-011, Xx8-08-012, Xx8-08-013, Xx8-08-017, Xx8-08-018, Xx8-08-019, Xx8-08-022, Xx8-08-023, Xx8-08-028, Xx8-08-029, Xx8-09-028, Xx8-09-030, Xx8-09-032, Xx8-09-036, Xx8-10-027, Xx8-10-035, Xx8-11-012, Xx8-11-019, Xx8-11-022, Xx8-11-023, Xx8-11-024, Xx8-12-005, Xx8-12-011, Xx8-12-016, Xx8-12-023, Xx8-12-030, Xx8-12-032, Xx8-12-038, Xx8-12-040, Xx8-13-001, Xx8-13-002, Xx8-14-013, Xx8-14-016, Xx8-14-017, Xx8-14-023, Xx8-14-031, Xx8-16-002, Xx8-16-022, Xx8-16-023, Xx8-16-024, Xx8-17-006, Xx8-17-021, Xx8-17-023, Xx8-18-004, Xx8-18-005, Xx8-18-020, Xx8-18-025, Xx8-##-006, Xx8-##-012, Xx8-##-014, Xx8-##-015, Xx8-01-023, Xx8-05-023, Xx8-05-016, Xx8-02-014, Xx8-09-029, Xx8-01-015, Xx8-09-002, Xx8-##-001, Xx8-##-002, Xx8-##-003, Xx8-01-012, Xx8-01-038, Xx8-05-002, Xx8-05-011, Xx8-06-023, Xx8-06-038, Xx8-07-011, Xx8-09-041, Xx8-10-029, Xx8-11-031, Xx8-13-012, Xx8-16-017, Xx8-01-017, Xx8-01-040, Xx8-05-003, Xx8-05-012, Xx8-06-025, Xx8-07-002, Xx8-07-014, Xx8-10-006, Xx8-10-030, Xx8-17-016, Xx8-17-019, Xx8-16-018, Xx8-01-025, Xx8-02-001, Xx8-05-004, Xx8-05-013, Xx8-06-027, Xx8-07-003, Xx8-07-016, Xx8-10-015, Xx8-10-032, Xx8-12-010, Xx8-13-032, Xx8-17-025, Xx8-01-028, Xx8-02-004, Xx8-05-005, Xx8-05-018,

Appendix Table 2 (Continued)

Locus	Accession
	Xx8-06-030, Xx8-07-004, Xx8-07-017, Xx8-10-017, Xx8-10-034, Xx8-12-021, Xx8-14-007, Xx8-16-021, Xx8-01-032, Xx8-02-006, Xx8-05-006, Xx8-05-019, Xx8-17-003, Xx8-07-005, Xx8-09-027, Xx8-10-018, Xx8-10-036, Xx8-12-027, Xx8-14-026, Xx8-16-026, Xx8-01-033, Xx8-02-016, Xx8-05-008, Xx8-05-020, Xx8-06-033, Xx8-07-006, Xx8-09-031, Xx8-10-024, Xx8-10-037, Xx8-12-039, Xx8-15-001, Xx8-16-027, Xx8-01-035, Xx8-02-035, Xx8-05-009, Xx8-05-022, Xx8-06-035, Xx8-07-009, Xx8-09-038, Xx8-10-025, Xx8-10-040, Xx8-13-007, Xx8-16-010, Xx8-17-002, Xx8-01-037, Xx8-02-036, Xx8-05-010, Xx8-06-022, Xx8-06-037, Xx8-07-010, Xx8-17-010, Xx8-10-026, Xx8-11-021, Xx8-17-017, Xx8-17-020 190 accessions direct sequencing.
Xx-SUS1	Xx8-01-012, Xx8-01-013, Xx8-01-022, Xx8-01-026, Xx8-01-027, Xx8-01-029, Xx8-01-030, Xx8-01-031, Xx8-01-035, Xx8-01-037, Xx8-02-024, Xx8-05-002, Xx8-05-004, Xx8-05-010, Xx8-05-012, Xx8-05-017, Xx8-05-020, Xx8-05-022, Xx8-05-023, Xx8-07-003, Xx8-07-007, Xx8-07-009, Xx8-07-010, Xx8-07-018, Xx8-07-021, Xx8-07-023, Xx8-07-029, Xx8-08-001, Xx8-08-002, Xx8-08-006, Xx8-09-002, Xx8-09-012, Xx8-09-014, Xx8-09-020, Xx8-09-028, Xx8-09-034, Xx8-09-039, Xx8-09-041, Xx8-09-044, Xx8-10-005, Xx8-10-010, Xx8-10-019, Xx8-10-027, Xx8-10-039, Xx8-11-005, Xx8-11-006, Xx8-11-017, Xx8-12-005, Xx8-12-021, Xx8-12-030, Xx8-12-035, Xx8-12-039, Xx8-13-011, Xx8-##-004, Xx8-##-006, Xx8-##-010, Xx8-##-012, Xx8-##-013, 56 accessions direct sequencing.
Xx-SUS2	Xx8-01-012, Xx8-01-024, Xx8-01-026, Xx8-01-038, Xx8-01-040, Xx8-02-002, Xx8-02-004, Xx8-02-006, Xx8-02-015, Xx8-02-019, Xx8-02-021, Xx8-02-022, Xx8-02-023, Xx8-02-028, Xx8-02-030,

Appendix Table 2 (Continued)

Locus	Accession
	Xx8-02-033, Xx8-02-034, Xx8-05-009, Xx8-05-010, Xx8-05-014, Xx8-05-018, Xx8-06-001, Xx8-06-002, Xx8-06-003, Xx8-06-004, Xx8-06-005, Xx8-06-006, Xx8-06-007, Xx8-06-008, Xx8-06-009, Xx8-06-010, Xx8-06-011, Xx8-06-012, Xx8-06-013, Xx8-06-014, Xx8-06-015, Xx8-06-016, Xx8-06-017, Xx8-06-018, Xx8-06-019, Xx8-06-020, Xx8-06-021, Xx8-06-022, Xx8-06-023, Xx8-06-024, Xx8-06-025, Xx8-06-026, Xx8-06-027, Xx8-06-028, Xx8-06-029, Xx8-06-030, Xx8-06-031, Xx8-06-032, Xx8-06-033, Xx8-06-034, Xx8-06-035 Xx8-06-036, Xx8-06-037, Xx8-06-038, Xx8-06-039, Xx8-06-040, Xx8-06-041, Xx8-07-003, Xx8-07-009, Xx8-07-030, Xx8-08-001, Xx8-08-002, Xx8-08-006, Xx8-08-026, Xx8-09-007, Xx8-09-017, Xx8-10-012, Xx8-10-018, Xx8-10-022, Xx8-10-024, Xx8-10-027, Xx8-10-039, Xx8-11-001, Xx8-11-011, Xx8-11-012, Xx8-11-018, Xx8-11-019, Xx8-11-020, Xx8-11-021, Xx8-11-023, Xx8-11-024, Xx8-11-026, Xx8-11-027, Xx8-11-030, Xx8-12-002, Xx8-12-005, Xx8-12-009, Xx8-12-011, Xx8-12-014, Xx8-12-015, Xx8-12-020, Xx8-12-021, Xx8-12-023, Xx8-12-027, Xx8-12-030, Xx8-12-034, Xx8-12-035, Xx8-12-036, Xx8-12-038, Xx8-12-039, Xx8-12-040, Xx8-13-001, Xx8-13-002, Xx8-13-003, Xx8-13-007, Xx8-13-008, Xx8-13-011, Xx8-13-022, Xx8-13-024, Xx8-13-034, Xx8-13-036, Xx8-##-001, Xx8-##-002, Xx8-##-003, Xx8-##-004, Xx8-##-005, Xx8-##-009, Xx8-##-011, Xx8-##-012, Xx8-##-013, 124 accessions direct sequencing.
Xx-LAP1	Xx8-01-003, Xx8-01-004, Xx8-01-005, Xx8-01-013, Xx8-01-016, Xx8-01-017, Xx8-01-019, Xx8-02-002, Xx8-02-003, Xx8-02-007, Xx8-02-011, Xx8-02-012, Xx8-02-013, Xx8-02-018, Xx8-02-019, Xx8-02-021, Xx8-02-022, Xx8-02-023, Xx8-02-036, Xx8-05-001, Xx8-05-004, Xx8-06-018, Xx8-06-021, Xx8-06-022, Xx8-06-025,

Appendix Table 2 (Continued)

Locus	Accession
	Xx8-06-026, Xx8-06-029, Xx8-07-003, Xx8-07-005, Xx8-07-007, Xx8-07-009, Xx8-07-011, Xx8-07-014, Xx8-07-017, Xx8-07-020, Xx8-07-023, Xx8-07-026, Xx8-08-001, Xx8-08-010, Xx8-08-015, Xx8-08-016, Xx8-08-020, Xx8-08-027,Xx8-08-028, Xx8-09-001, Xx8-09-009, Xx8-09-012, Xx8-09-019, Xx8-09-021, Xx8-09-023, Xx8-09-025, Xx8-09-028, Xx8-09-029, Xx8-09-036, Xx8-09-037, Xx8-09-038, Xx8-09-042, Xx8-10-004, Xx8-10-007, Xx8-10-012, Xx8-10-013, Xx8-10-023, Xx8-10-031, Xx8-10-034, Xx8-10-037, Xx8-11-009, Xx8-11-010, Xx8-11-018, Xx8-11-019, Xx8-11-020, Xx8-11-032, Xx8-12-001, Xx8-12-011, Xx8-12-012, Xx8-12-013, Xx8-12-014, Xx8-12-016, Xx8-12-017,Xx8-12-022, Xx8-12-025, Xx8-12-030, Xx8-12-031, Xx8-12-036, Xx8-12-037, Xx8-12-040, Xx8-13-001, Xx8-13-002, Xx8-13-006, Xx8-13-008, Xx8-13-014, Xx8-13-015, Xx8-13-016, Xx8-13-023, Xx8-13-026, Xx8-13-028, Xx8-13-030, Xx8-13-031, Xx8-##-007, Xx8-##-009, Xx8-##-013 99 accessions direct sequencing.
Xx-CAT1	Xx8-12-021, Xx8-11-032, Xx8-11-024, Xx8-08-019, Xx8-01-002, Xx8-01-003, Xx8-01-004, Xx8-01-006, Xx8-01-015, Xx8-01-016, Xx8-01-026, Xx8-01-029, Xx8-01-032, Xx8-01-033, Xx8-01-034, Xx8-01-040, Xx8-02-001, Xx8-02-004, Xx8-02-006, Xx8-02-007, Xx8-02-008, Xx8-02-009, Xx8-02-011, Xx8-02-019, Xx8-02-022, Xx8-02-035, Xx8-05-003, Xx8-05-004, Xx8-05-023, Xx8-06-004, Xx8-06-005, Xx8-06-016, Xx8-06-021, Xx8-06-029, Xx8-06-031, Xx8-06-033, Xx8-06-034, Xx8-06-035, Xx8-06-036, Xx8-06-037, Xx8-06-039, Xx8-06-040, Xx8-07-003, Xx8-07-006, Xx8-07-015, Xx8-07-016, Xx8-07-020, Xx8-07-031, Xx8-07-032, Xx8-08-002, Xx8-08-012, Xx8-08-016, Xx8-08-020, Xx8-08-024, Xx8-08-025, Xx8-08-031, Xx8-08-037, Xx8-09-001, Xx8-09-005, Xx8-09-006,

Appendix Table 2 (Continued)

Locus	Accession
	Xx8-09-018, Xx8-09-019, Xx8-09-021, Xx8-09-028, Xx8-09-033, Xx8-09-036, Xx8-09-039, Xx8-09-042, Xx8-10-013, Xx8-10-015, Xx8-10-016, Xx8-10-017, Xx8-10-032, Xx8-10-033, Xx8-10-035, Xx8-10-037, Xx8-11-001, Xx8-11-002, Xx8-11-006, Xx8-11-009, Xx8-11-012, Xx8-11-014, Xx8-11-018, Xx8-12-003, Xx8-12-007, Xx8-12-009, Xx8-12-014, Xx8-12-019, Xx8-12-027, Xx8-12-029, Xx8-12-030, Xx8-12-034, Xx8-12-035, Xx8-12-041, Xx8-13-004, Xx8-13-008, Xx8-13-012, Xx8-13-014, Xx8-13-016, Xx8-13-018, Xx8-13-019, Xx8-13-023, Xx8-13-028, Xx8-13-034, Xx8-13-036, Xx8-12-021, Xx8-11-032, 104 accessions direct sequencing.
Xx-CAT2	Xx8-01-002, Xx8-01-003, Xx8-01-006, Xx8-01-020, Xx8-01-021, Xx8-01-033, Xx8-01-039, Xx8-02-021, Xx8-02-024, Xx8-02-026, Xx8-02-027, Xx8-02-034, Xx8-05-004, Xx8-05-005, Xx8-05-006, Xx8-05-009, Xx8-05-010, Xx8-05-012, Xx8-05-016, Xx8-05-018, Xx8-05-019, Xx8-06-003, Xx8-06-007, Xx8-06-013, Xx8-06-015, Xx8-06-018, Xx8-06-021, Xx8-06-022, Xx8-06-027, Xx8-06-028, Xx8-06-029, Xx8-06-031, Xx8-06-032, Xx8-06-040, Xx8-07-006, Xx8-07-009, Xx8-07-015, Xx8-07-020, Xx8-07-021, Xx8-07-027, Xx8-07-028, Xx8-07-029, Xx8-08-002, Xx8-08-010, Xx8-08-013, Xx8-08-014, Xx8-08-017, Xx8-08-020, Xx8-08-023, Xx8-08-024, Xx8-08-026, Xx8-08-027, Xx8-08-030, Xx8-08-036, Xx8-08-037, Xx8-09-008, Xx8-09-015, Xx8-09-023, Xx8-09-025, Xx8-09-026, Xx8-09-027, Xx8-09-030, Xx8-09-036, Xx8-09-037, Xx8-09-038, Xx8-09-041, Xx8-10-001, Xx8-10-005, Xx8-10-007, Xx8-10-009, Xx8-10-010, Xx8-10-015, Xx8-10-016, Xx8-10-019, Xx8-10-029, Xx8-10-031, Xx8-10-035, Xx8-10-038, Xx8-11-001, Xx8-11-004, Xx8-11-006, Xx8-11-010, Xx8-11-014, Xx8-11-018, Xx8-11-019,

Appendix Table 2 (Continued)

Locus	Accession
	Xx8-11-020, Xx8-11-022, Xx8-11-023, Xx8-11-024, Xx8-11-032, Xx8-11-033, Xx8-12-002, Xx8-12-007, Xx8-12-009, Xx8-12-013, Xx8-12-014, Xx8-12-015, Xx8-12-016, Xx8-12-017, Xx8-12-018, Xx8-12-019, Xx8-12-026, Xx8-12-027, Xx8-12-030, Xx8-12-038, Xx8-13-001, Xx8-13-002, Xx8-13-003, Xx8-13-004, Xx8-13-006, Xx8-13-007, Xx8-13-008, Xx8-13-013, Xx8-13-015, Xx8-13-016, Xx8-13-019, Xx8-13-021, Xx8-13-023, Xx8-13-024, Xx8-13-025, Xx8-13-028, Xx8-13-030, Xx8-13-032, Xx8-13-035, Xx8-13-036, Xx8-13-037, Xx8-14-002, Xx8-14-008, Xx8-14-013, Xx8-14-015, Xx8-16-003, Xx8-16-010, Xx8-16-011, Xx8-16-021, Xx8-16-022, Xx8-16-024, Xx8-17-004, Xx8-17-006, Xx8-17-007, Xx8-17-010, Xx8-17-011, Xx8-17-018, Xx8-17-019, Xx8-17-020, Xx8-17-021, Xx8-17-026, Xx8-17-027, Xx8-18-003, Xx8-18-005, Xx8-18-007, Xx8-18-010, Xx8-18-011, Xx8-18-013, Xx8-18-014, Xx8-18-017, Xx8-18-018, Xx8-18-020, Xx8-18-022, Xx8-##-002, Xx8-##-007, Xx8-##-008, Xx8-##-009, Xx8-##-012, Xx8-##-015 164 accessions direct sequencing.
Xx-AATcy1	Xx8-01-009, Xx8-01-010, Xx8-01-012, Xx8-01-015, Xx8-01-018, Xx8-01-026, Xx8-02-006, Xx8-02-019, Xx8-02-026, Xx8-02-029, Xx8-02-030, Xx8-05-009, Xx8-05-010, Xx8-05-016, Xx8-05-018, Xx8-07-030, Xx8-08-026, Xx8-09-003, Xx8-09-010, Xx8-09-011, Xx8-09-013, Xx8-09-016, Xx8-09-037, Xx8-10-022, Xx8-10-024, Xx8-12-003, Xx8-12-007, Xx8-13-003, Xx8-13-004, Xx8-13-011, Xx8-13-020, Xx8-13-030, Xx8-##-005, Xx8-##-012, Xx8-##-013, Xx8-06-023, Xx8-06-027, Xx8-06-034,
38 clones	38 accessions direct sequencing.

CURRICULUM VITAE

NAME **:** Mr. Tanat Wattanakulpakin

BIRTH DATE **:** January 19, 1986

BIRTH PLACE : Kamphaeng Phet

EDUCATION **:**

YEAR	**INSTITUTION**	**DEGREE/DIPOMA**
2007	KMITL	B. Sc (Agronomy)