博士論文

# Geographic variation and genetic structure of teak (*Tectona grandis*) in Myanmar revealed by cpSNP and nrSSR markers

（葉緑体 SNP と核 SSR マーカーで明らかにされたミャンマーにおけるチーク（*Tectona grandis*）の地理変異と遺伝構造）

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Contents

## Acknowledgement

I am very first indebted to my parents, U San Thein and Daw Win Sein for giving me a human life, looking after till now and guiding how to survive in life. I'd like to gratitude Dr. Kurunobu, Dr. Nagame for favoring me to start my study at The University of Tokyo. I deep in thank Dr. Yuji Ide for the kind supports to my studies. My special thanks also go to Dr. Susumu Goto and his family, my supervisor for valuable advice and supports for my master and doctorate studies. Deepest thanks convey to Dr. Atsushi Watanabe from Kyushu University, his wife and Dr. Tomonori Hirao from Forest Tree Breeding Center for their great contribution and supports to do the experiments at FTBC, and care when I stayed in Hitachi and encouragement whenever I faced the difficulties. My sincere thanks go to Dr. Wataru Ishizuka for lending his hands for both studying and my personal affairs since the beginning of my stay in Japan. It is grateful for Dr. Makoto Takahashi, Dr. Hiroshi Iwata and Dr. Sakaguchi, for teaching me concerned with data analysis. I'd like to thank the MEXT from Japan for the financial assistance for studying and living cost in Japan for six years. My heartfelt thanks go to Takayuki Sato and his wife and Akira Sakai and his family for their care a family member during my stay in Japan. Dr. C. Lian and K. Ushiyama are acknowledged for their advices and reviewing my dissertation.

Authorized persons from The Republic of Myanmar, particularly from the Ministry of Environmental Conservation and Forestry are specially thanked for giving the permission to study in abroad. I'd like to convey my deepest thanks to the Staff from Forest Tree Breeding Center from Japan for helping me during my study and from the Ministry of Forestry for collecting samples. I deeply appreciate Ma Khin Moe Kyi, my best friend and Ma Soe Soe New, lovely junior for favoring me

without vexation whenever I make a request. I also thank to Myanmar friends for trying together to fix up with new environment and Japanese friends for giving a hand to face the difficulties in your land.

My special heartfelt thanks go to my brothers' families, Ko Zaw Naing Oo and Ma Khin Nyein Chan, Ko Win Thein Oo and Ma Toe Toe Lwin, sisters, Ma Nu Nu Win and Ma Swe Swe Win and relatives, U Aung Moe and Ma Htay Htay Myint, and my lovely nieces Dr. May Phyu Moe, Aye Chan Moe and May Myo Myat Khin, nephews, Ent Zin Ko and Ye Wint Ko for your caring our beloved mother, being patient to me and physical and mental supports to see my dream in real. Your kindness refreshes and enhances me when I am away from you all. At last but not least, heartfelt thanks go to my beloved Ko Zaw Zaw Toe, my fiancé, for your encouragement, kindness and special care. Everyone who favors and contributes to my study is deeply appreciated though I left to express the individual's name.

# Chapter 1

# Introduction

Deforestation rates significantly increased across tropical Asia and the highest level of deforestation experienced in Southeast Asia during 1990s (Wright, 2005, Miettinen et al. 2011). Four countries of teak native regions, Thai and Laos included in Southeast Asia (Fig 1.1). Due to the increasing demand, teak plantations were widely established in the world including its indigenous countries and stands third position of planted species. Thus, both conservation and breeding programs are urgently required to save the natural genetic resources of teak and to provide the genetically improved materials for teak plantations. Information on genetic variation is important to maintain the natural population as evolutionarily viable units which are adaptable to changing environments in the long term (Zuo et al. 2010). Genetic information particularly phylogeographic variation and genetic structure are indispensible for effectively and efficiently implementing the conservation and breeding programs for target species through formulating the useful strategies.

## 1.1 Teak

The study species, teak (*Tectona grandis*) is one of the most economically important tropical deciduous timber species. It belongs to family Lamiaceae and genus *Tectona*. Teak requires a high light intensity for its growth and development and it has been classified as a pioneer species. Teak is angiosperm, diploid, monoecious and allogamous species (Gill, et al., 1983; Mathew, et al., 1987; Kertadikara and Prat, 1994). It is primarily an outcrossing and insect pollinated species, but self-pollination is possible. No self-pollinated flowers develop into

mature fruits, although many of the fruits develop to different sizes before they abort (Tangmitchroen and Owens, 1997).

Teak (*Tectona grandis* L.f) has been regarded as one of the world's most precious tropical tree species due to precious timber qualities (Pandey and Brown, 2000; Kaosa-ard, 2003) and increasing demand in the world market. It is recognized worldwide as the most important wood for multipurpose particularly for ship building and furniture. The unique qualities of teak such as durability, ease of seasoning without splitting and cracking, workability, beautiful color and grain, and resistance to termite, fungus and weathering, etc. make increasing its demand and endangered species (Gyi and Tint, 1998; Kaosa-ard, 1998). Timber color of teak varies with four types, golden yellow, light brown, dark brown and black stripe (Fig 1.2).

The recorded latitudinal limits of its natural range are between 9˚ to 26˚ N latitude and 73˚ to 104˚ E longitude (White, 1991). Its natural distribution is limited to a discontinuous range in South and Southeast Asia from the Indian subcontinent to Myanmar, Thailand and Laos (Khanduri, et al., 2008). The total amounts of teak bearing forest is ca 27.9 million ha and out of them 8,900,000 ha in India (Tewari, 1992), 2,500,000 ha in Thailand (Kaosa-ard, 1991), and 16,000 ha in Laos (Anon, 1992). The rest of area about 16,517, 700 ha are in Myanmar (Pengduoang, 1991).

Natural populations of teak in its native countries are nearly to be depleted through over-exploitation, illegal cutting and other factors such as the transformation of land-use systems. Teak logging from the natural forest has been banned in its native regions in the late 1980s except Myanmar (Pandey and Brown, 2000). Logging from natural forests in India, Thailand and Laos has been banned since the

late 1980s. Teak is now a threatened species so conservation effort is urgently needed to safeguard the genetic resources of teak from degraded natural teak forests. Teak plantations were widely planted not only in native regions but also Asia, Africa and Central America to supply the high demand of teak. Therefore, conservation and breeding program should be balanced to retain the irreplaceable natural genetic resource of teak and to produce the genetically qualified planting materials for the commercial plantations. Genetic diversity and genetic variation are key components of the stability of forest resources (Rajora, et al., 2000). It is therefore important to evaluate the genetic diversity and genetic divergence of natural populations in native countries to facilitate conservation efforts aimed at maintaining species' genetic resources.

## 1.2 Teak in Myanmar

Myanmar is geographically situated between latitudes 09º 32' to 28º31' N and longitudes 92º10' to 101º 11' E. In Myanmar, natural teak forests occurs within 25˚30'N and 10˚00' N latitude and widely distributed from the sea level to 1000 m elevation (Fig 1.3). Today, beautiful natural teak stands can be seen only in Myanmar where is home to the best quality of teak. Myanmar has the largest area of natural teak forests and its timber quality is declared as the best in the market. In Myanmar, teak grows throughout the Shan state except over limited elevation and extends beyond the frontier into Thailand and Laos on the east. In the northwest it does not extend beyond the western watershed of the Ayeyarwady and Chindwin rivers; in the southwest it occurs on the west bank of the Ayeyarwady into the foothills of Rhakhin Yomas in decreasing abundance to approximately 18˚ N latitude. It does not occur

abundantly in the dry zone of central Myanmar, or in the tidal regions of the delta area.

The forests in Myanmar are classified into six major forest types. Teak naturally occurs in major three types of forests; the semi-evergreen forests, mixed deciduous forests (Moist upper mixed deciduous forests, dry upper mixed deciduous forests, and lower mixed deciduous forests) and deciduous dipterocarp or Indaing forests (Fig 3). The composition of teak in natural forests varies with forest types from 4-12%. Teak is usually found as scattered individuals or in small groups with little or no regeneration present in the semi-evergreen forest (Kermode, 1964). In the lower mixed deciduous forest, teak may be found gregariously or in patches with a large girth and height and fluted trees while teak with cleaner and straight boles in the moist upper mixed deciduous forest. Small size and poor quality of teak grows in Indaing forest in Myanmar.

### 1.3 Genetic information of native teak

Genetic studies on teak populations in its native countries of India, Thailand and Laos have been conducted using plant materials derived from international provenance trials established in the early 1970s (Keiding et al., 1986, Kjaer et al., 1995) and natural populations. Previous population genetic studies have used various DNA markers such as allozymes (Kertadikara and Prat, 1995, Kjaer and Seigismund, 1996), amplified fragment length polymorphisms (AFLP) (Shrestha, et al., 2005, Fofana et al., 2013), inter simple sequence repeats (ISSR) (Narayanan, et al., 2007), and simple sequence repeats (SSR) (Fofana, et al., 2008, 2009; 2013; Minn, et al., 2014). SSRs are arguably the most informative of these marker types due to their

hyper-polymorphic nature and co-dominance (Powell, et al. 1996).

Molecular markers of teak showed the genetic variation of teak. AFLP markers showed large genetic variation in natural teak provenances within India and also natural populations from native regions; India, Thai and Laos teak (Shrestha, et al., 2005; Sreekanth, et al. 2012) and higher genetic divergence of Indian teak at isozyme variation (Kertadikara and Prat, 1995; Kjaer and Seigismund, 1996). Fofana, et al. (2009, 2013) found that the southern Indian populations possessed the highest genetic diversity, followed by the northern Indian, Thailand and Laotian teak populations. Similar results were obtained using AFLP markers (Shrestha, et al., 2005). Significant geographic variation pattern of Myanmar teak was recently detected among southern and northern populations (Minn, et al., 2014). However, comparison of genetic diversity of teak in its native areas; India, Myanmar, Thai and Laos, has not investigated yet.

## 1.4 Problem statements

Economically and ecologically important tree species, teak have suffered from particularly severe pressure because of selective logging of phenotypically superior trees. Diminishing the area of natural teak forest in native regions might be resulted in decreased in genetic diversity, disrupted gene flow and genetically isolated tree populations. Thus, conservation and breeding programs are urgently needed for teak.

Although knowledge of the genetic variation of extant populations over the entire range of natural distribution is essential for the conservation of genetic resources (Neale and Kremer, 2011), genetic information is very limited for

Myanmar teak among its native regions. Without genetic information of Myanmar teak, it is impossible to discover the genetic center of the teak in the world. Tropical forests were decreased due to various reasons including the Myanmar forests. Within two decades natural teak forest area in Myanmar was drastically diminished, but genetic information resulted from very few studies of teak is insufficient to apply to conservation or breeding purposes. Large scale area of teak plantation was widely established using seeds from various sources of Myanmar teak and also from alien teak without knowing their genetic background that poses a risk of genetic disturbance to natural genetic resources. There was no clear instruction for seed transfer to plantation sites. For commercial teak plantation, breeding program is important to produce the genetically improved planting materials, while focusing on the conservation of natural genetic resource of teak. Thus, geographic variation and genetic structure of teak needed to understand for balancing conservation and breeding processes.

## 1.5 Objectives

1. To evaluate the genetic diversity of Myanmar teak comparing with other native teak
2. To reveal phylogeographic variation and genetic structure of Myanmar teak
3. To formulate seed transfer guidelines and designate zones for conservation and breeding
4. To elucidate genetic component of alien teak planted in Myanmar by private companies

5. To retain the natural genetic resources of Myanmar teak and prevent from genetic disturbance of alien teak

6. To provide the genetic information of Myanmar teak for conservation

To complete the objectives of this study, first, level of genetic diversity of Myanmar teak among native teak was investigated using nuclear SSR markers by the comparison with that of other native teak in chapter 2. After developing cpSNP marker for teak in chapter 3, phylogeographic variation and genetic structure of Myanmar were investigated using newly developed cpSNP and nrSSR markers in chapter 4. Genetic components of alien teak planted in Myanmar were elucidated using nrSSR makers in chapter 5. In chapter 6, the findings from this study were finally discussed to retain the natural genetic resources of Myanmar teak through balancing conservation and breeding activities and to alarm the genetic disturbance of alien teak which was genetically different from Myanmar teak.

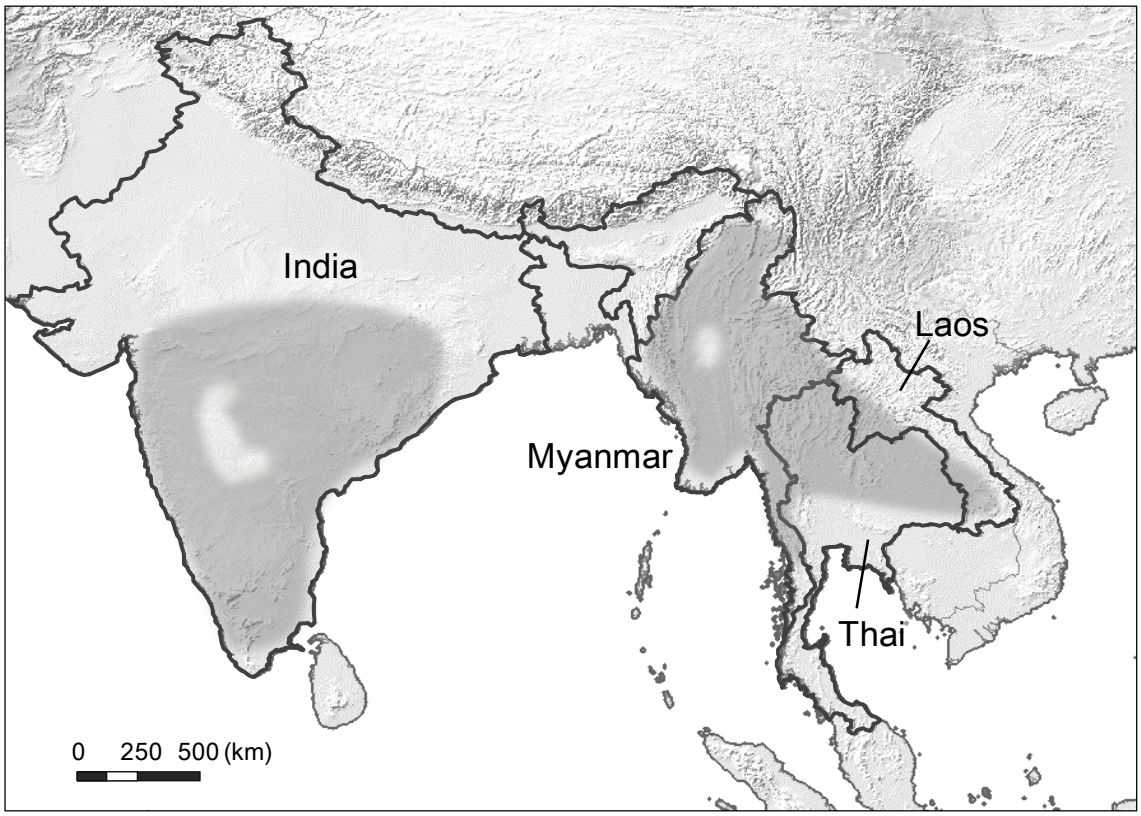


**Figure 1.1 Natural distribution of teak in its native regions, India, Myanmar, Laos and Thailand.** The shaded areas show natural distribution of teak in each respective country.

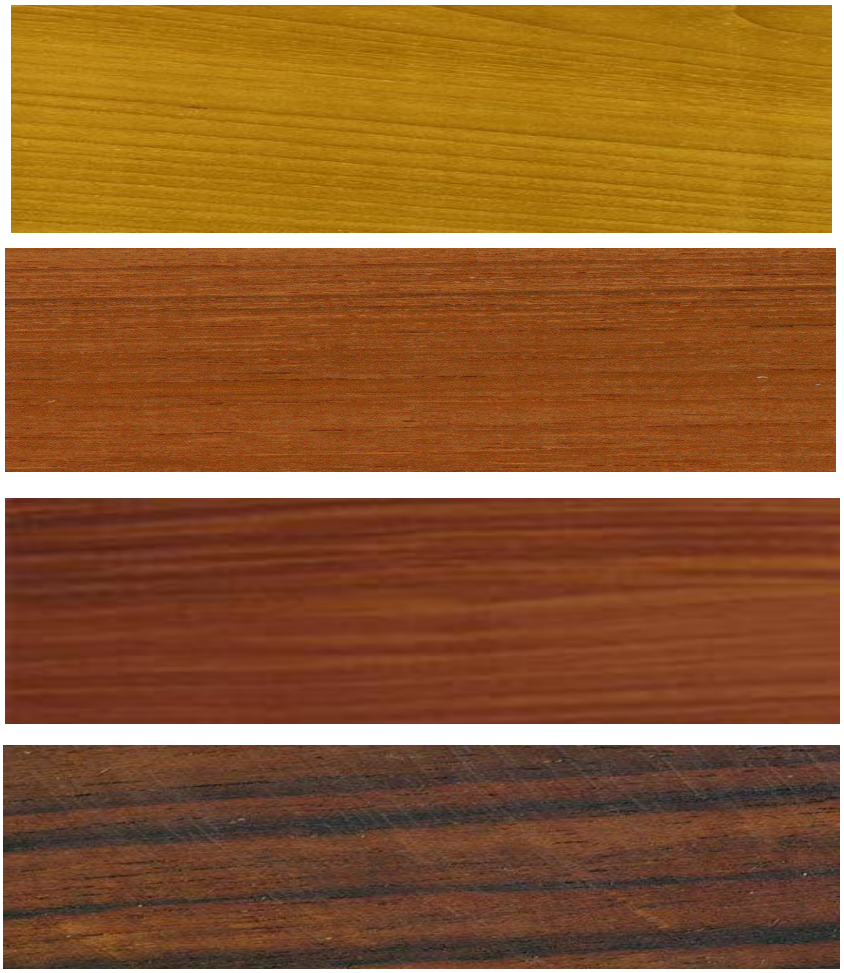

**Figure 1.3 Color variation of teak**

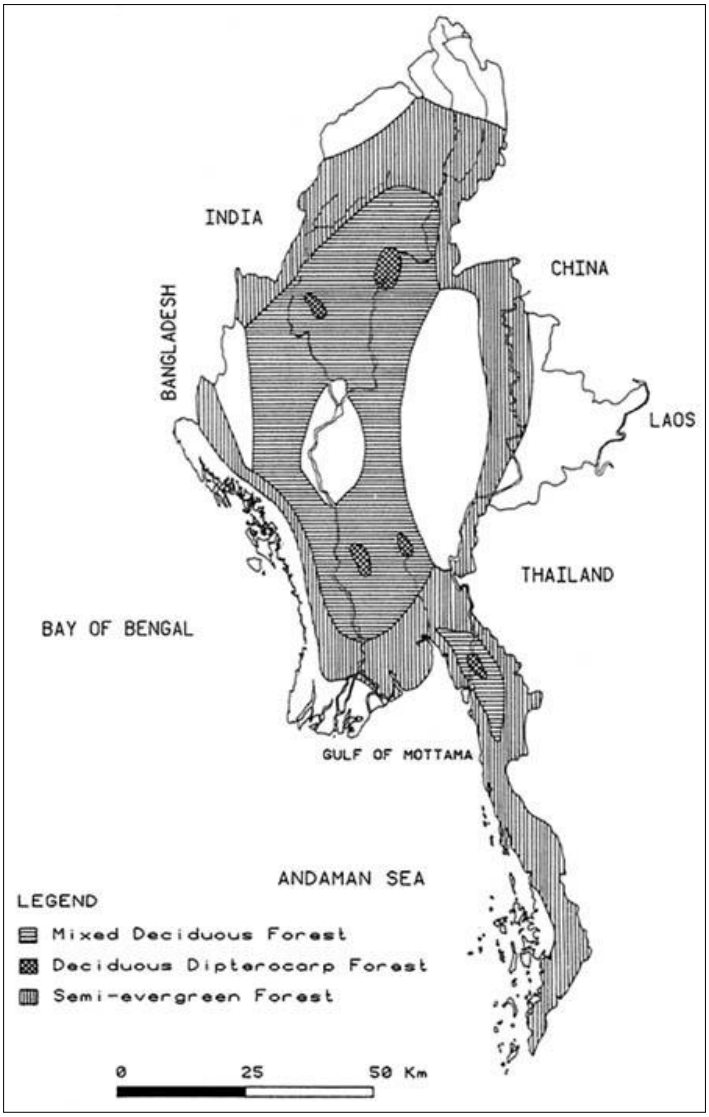


**Figure 1.2 Natural teak bearing forests in Myanmar (FD-Myanmar)**

# Chapter 2

# Genetic diversity of teak in its native region

## 2.1 Introduction

Teak (*Tectona grandis* L.f) has been regarded as one of the world's most precious tropical tree species because it provides premium timber with a number of very desirable properties including high durability, strength and workability (Pandey and Brown, 2000; Kaosa-ard, 2003). Its natural distribution is limited to a discontinuous range in South and Southeast Asia from the Indian subcontinent to Myanmar, Thailand and Laos (Khanduri, et al., 2008). Natural populations of teak in its native countries have decreased through over-exploitation, illegal cutting and other factors such as the transformation of land-use systems, so that logging from natural forests was banned in the late 1980s in India, Thailand and Laos but not in Myanmar (Pandey and Brown, 2000). Teak is now a threatened species and conservation effort is urgently needed to safeguard the genetic resources of teak from degraded natural teak forests. Genetic diversity and genetic variation are key component of the stability of forest resources (Rajora, et al., 2000). It is therefore important to evaluate the genetic diversity and genetic divergence of natural populations in native countries to facilitate conservation efforts aimed at maintaining species' genetic resources.

Genetic studies on teak populations in its native countries of India, Thailand and Laos have been conducted using plant materials derived from international provenance trials established in the early 1970s (Keiding, et al., 1986, Kjaer, et al.,

1995) and natural populations. Previous population genetic studies have used various DNA markers such as allozymes (Kertadikara and Prat, 1995, Kjaer and Seigismund, 1996), amplified fragment length polymorphisms (AFLP) (Shrestha, et al., 2005, Fofana et al., 2013), inter simple sequence repeats (ISSR) (Narayanan, et al., 2007), and simple sequence repeats (SSR) (Fofana, et al., 2008, 2009; 2013; Minn, et al., 2014). SSRs are arguably the most informative of these marker types due to their hyper-polymorphic nature and co-dominance (Powell, 1996) and therefore useful for elucidating the spatial structure of genetic diversity and the demographic patterns of variation which have resulted from migration (Neale and Ingvarsson, 2008) and drift as well as through evolutionary history.

In the previous studies, large genetic variation was observed in natural teak provenances and higher genetic divergence of Indian teak at isozyme variation (Kertadikara and Prat, 1995; Kjaer and Seigismund, 1996). Fofana, et al. (2009, 2013) found that the southern Indian populations possessed the highest genetic diversity, followed by the northern Indian, Thailand and Laotian teak populations. Similar results were obtained using AFLP markers (Shrestha, et al., 2005). Significant geographic variation pattern of Myanmar teak was recently detected among southern and northern populations (Minn, et al., 2014). However, comparison of genetic diversity of teak in its native areas has not investigated yet. Therefore, this study was conducted to figure out the genetic diversity of Myanmar teak by comparing that of Indian, Thailand, and Laotian teak.

## 2.2 Materials and Methods

### 2.2.1 Sampling design and DNA extraction

A total of 128 leaf samples from four natural populations were used to investigate the genetic diversity of Myanmar teak (Fig 2.1, Table 2.1). Those samples were collected from a provenance trial established at Pyinmana, Myanmar in 2007 and the collected samples represented natural populations with seed sources from Bago, Phyu, Oktwin and Kanbalu. From this provenance trial, fresh leaves were collected and dried overnight at 80 °C and stored in silica gel at room temperature.

Total DNA was extracted following the method of Shiraishi and Watanabe (1995). Approximately 100 mg of leave sample was frozen in liquid nitrogen and ground in a homogenizer. Each homogenized sample was mixed with 1 ml of CTAB (hexadecetyltrimethylammonium bromide) buffer (100 mM Tris-HCl, pH 9.0, 20 mM EDTA, 2% CTAB), with 0.1% beta-mercaptoethanol added immediately prior to use. The mixture was incubated at 65 °C for 1 hr and centrifuged for 10 min at 12 000 xg; 600 µl of the supernatant was then transferred to a 1.5 ml microcentrifuge tube. The supernatant was mixed twice with phenol/chloroform/isoamyl alcohol (25:24:1) and centrifuged for 10 min at 12 000 xg. DNA was precipitated from the aqueous phase by adding 0.1 volume of 3 M sodium acetate and 2.5 volumes of ethanol. The precipitate was washed twice with 70% ethanol and dissolved in water. Extracted DNA was further purified using the DNeasy Plant Mini kit (Qiagen).

**Table 2.1 Geographic and climatic information of four natural population of teak in Myanmar**

| No. | Population Name | Seed source of provenance trial | N | Latitude | Longitude | Altitude (meter) | Sampling Site |
|---|---|---|---|---|---|---|---|
| 1 | Bago | Natural | 32 | 18° 7'N | 96° 4'E | 134 | Provenance trial |
| 2 | Phyu | Natural | 32 | 18°28'N | 96°20'E | 399 | Provenance trial |
| 3 | Oktwin | Natural | 32 | 18°55'N | 96° 1'E | 245 | Provenance trial |
| 4 | Kanbalu | Natural | 32 | 23°30'N | 95°52'E | 274 | Provenance trial |

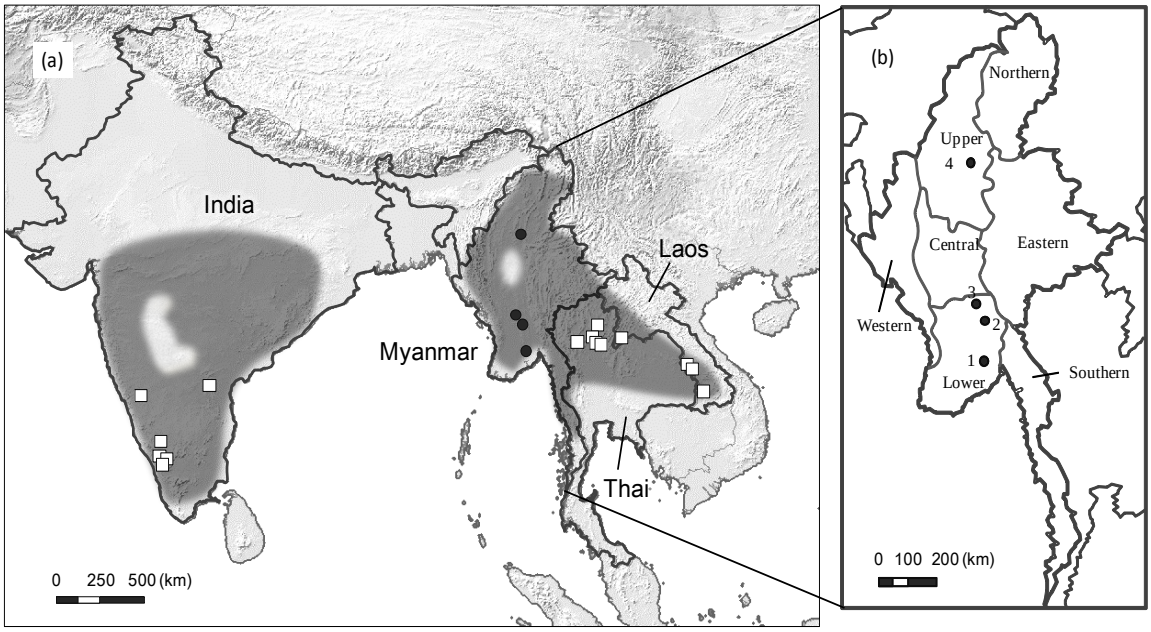


**Figure 2.1 (a) Maps of the distribution of teak in India, Myanmar, Laos and Thailand and (b) the locations of the ten sampled populations of teak in Myanmar.** In (a), open squares indicate the locations of the teak populations from a previous study (Fofana, et al., 2009) and closed circles represent Myanmar teak populations. In (b) the shaded area shows the natural distribution of teak in its native regions.

2.2.2 Molecular genotyping

Fifteen microsatellite markers (Verhaegen, et al., 2005) were used to compare the genetic diversity of natural populations of teak from Myanmar with that of teak from India, Thailand and Laos (Fofana, et al., 2009). To compare the genetic diversity of Myanmar teak with other teak from its native regions, we must use the same number of markers. We modified the locus CIRAD4TeakH09 based on the sequence obtained from Genbank as it could not depict the clear amplification of peaks. The modified forward and reverse primer sequences of CIRAD4TeakH09 are 5'-CTGTGCCTTCTAGTTGCCAGCGCAAGAGCTGAAAGCAACC-3' and 5'-GG CCGTTAGCACTCCATTTA -3'. The microsatellite genotyping was conducted with four fluorescent dyes detected using multiple-tailed primers to allow simultaneous genotyping of four different microsatellite loci (Missiaggia and Grattapaglia, 2006). For PCR, we used the QIAGEN multiplex PCR kit with 2xQIAGEN multiplex PCR master mix (final concentration, 1x), a 0.25 µM concentration of each set of primer, 2.5 µL of distilled water, and 2 µL of DNA for a total volume of 10 µL. The florescent universal tail primers, T7 terminator primer (FAM-5'-ATGCTAGTTA TTGCTCAGCGG-3'), reverse complement of BGH-R primer (VIC-5'-CTGTGCCT TCTAGTTGCCAGC-3'), reverse complement of pCold-R primer (NED-5'-TTGGGTGCAATGAGAATGCG-3') and pCold TF-F1 primer (PET-5'-C CACTTTCAACGAGCTGATG-3') were developed (Hirao et al., unpublished) based on the TAKARA universal primers (TAKARA Shuzo, Japan). These oligo tails were added to the 5' end of forward primers of developed teak microsatellite markers to complement the sequences of different loci in the PCR reaction. PCR amplifications

were carried out in a PTC-200 thermocycler (MJ Research) using the multiplex-touchdown-PCR protocol (QIAGEN Multiplex PCR kit, QIAGEN): denaturing at 94˚C for 15 min, an initial 10 cycles of denaturing at 94˚C for 30 s, annealing at 55°C for 90 s with a decrease of 0.5˚C per cycle, and an extension at 72˚C for 1 min with the annealing temperature of the remaining 20 cycles set at 50˚C for 90 s. After a final extension at 72˚C for 10 min was used to ensure complete amplification, the products were stored at 4˚C. A 1 μL aliquot of the PCR product was mixed with 11.7 μL of Hi-Di$^{TM}$ formamide (Applied Biosystems) including 0.3 μL of Genescan-500 size standard (Applied Biosystems). After denaturing the mixed products at 95˚C for 5 min, they were examined using electrophoresis on an ABI 3130xl Genetic Analyzer (Applied Biosystems, USA) and their fragment lengths were assayed using GeneMapper software (Applied Biosystems).

2.2.3 Statistical analysis

The following genetic diversity parameters for each locus over the four natural populations of Myanmar: the number of alleles (*A*), allelic richness (*R*), observed heterozygosity ($H_O$), expected heterozygosity ($H_E$), and fixation indices; genetic differentiation among populations ($F_{ST}$) and inbreeding coefficient ($F_{IS}$) were computed. To compare the genetic diversity of Myanmar teak with other teak from its native regions, the genetic diversity parameters; *R*, $H_E$ and $F_{ST}$ were measured for each natural population across 15 loci. Samples of each natural population were randomly excluded to reduce to the minimum sample size of population from Fofana et al., 2009 for the calculation of allelic richness due to rarefaction method (Leberg,

2002). Weighted average values of $H_E$ and $R$ of populations from each country were used for the comparison of genetic diversity of teak from each native country as accurate as possible and calculated as following. The sample of each population was divided by total sample size of each country and multiplied by $H_E$ or $R$ values of correspondent population. Then average $H_E$ or $R$ of all populations from each country was calculated. We tested the significance of the differences in the $R$ and $H_E$ between Myanmar teak and Indian, Thailand and Laos teak populations using permutation tests with 3 000 permutations.

## 2.3 Results

The number of alleles at each locus from the four natural populations varied from 7 (CIRAD4TeakDa12) to 20 (CIRAD3TeakB02 and CIRAD1TeakH10) with an average of 13. The mean allelic richness was 8.41 and ranged from 3.94 (CIRAD1TeakG02) to 14.14 (CIRAD1TeakH10). Average expected heterozygosity was 0.611 with a range from 0.177 (CIRAD1TeakG02) to 0.851 (CIRAD1TeakH10). Seven of fifteen loci showed significant $F_{IS}$ values with minimum and maximum $F_{IS}$ values observed at CIRAD4TeakH09 (-0.203) and CIRAD3TeakE06 (0.311), respectively, (Table 2.2).

**Table 2.2 Genetic information of 15 SSR markers across four natural populations of Myanmar teak**

| Locus Name | *N* | *A* | *R* | *Ho* | $H_O$ | $H_E$ | $F_{ST}$ | *P-value* |
|---|---|---|---|---|---|---|---|---|
| CIRAD1TeakA06 | 127 | 10 | 6.89 | 0.614 | 0.614 | 0.650 | 0.079 | 0.186 (NS) |
| CIRAD1TeakB03 | 127 | 15 | 10.21 | 0.788 | 0.788 | 0.755 | 0.128 | 0.864 (NS) |
| CIRAD1TeakF05 | 128 | 12 | 8.07 | 0.391 | 0.391 | 0.572 | 0.056 | 0.001 (*) |
| CIRAD1TeakG02 | 127 | 7 | 3.94 | 0.173 | 0.173 | 0.211 | 0.095 | 0.040 (*) |
| CIRAD1TeakH10 | 128 | 20 | 14.14 | 0.820 | 0.820 | 0.851 | 0.047 | 0.192 (NS) |
| CIRAD2TeakB07 | 128 | 18 | 8.83 | 0.477 | 0.477 | 0.574 | 0.090 | 0.001 (*) |
| CIRAD2TeakC03 | 116 | 14 | 10.08 | 0.827 | 0.827 | 0.799 | 0.086 | 0.826 (NS) |
| CIRAD3TeakA11 | 128 | 14 | 9.41 | 0.664 | 0.664 | 0.758 | 0.036 | 0.002 (*) |
| CIRAD3TeakB02 | 128 | 20 | 12.91 | 0.695 | 0.695 | 0.730 | 0.093 | 0.141 (NS) |
| CIRAD3TeakDa09 | 126 | 8 | 5.59 | 0.313 | 0.313 | 0.375 | 0.093 | 0.012 (*) |
| CIRAD3TeakE06 | 127 | 12 | 8.64 | 0.487 | 0.487 | 0.693 | 0.062 | 0.001 (*) |
| CIRAD3TeakF01 | 128 | 13 | 9.35 | 0.641 | 0.641 | 0.722 | 0.074 | 0.009 (*) |
| CIRAD4TeakDa12 | 128 | 7 | 4.03 | 0.367 | 0.367 | 0.338 | 0.055 | 0.910 (NS) |
| CIRAD4TeakF02 | 128 | 9 | 6.55 | 0.547 | 0.547 | 0.564 | 0.111 | 0.329 (NS) |
| CIRAD4TeakH09 | 127 | 12 | 7.50 | 0.660 | 0.660 | 0.546 | 0.084 | 0.999 (NS) |
| Mean | 127 | 13 | 8.408 | 0.564 | 0.564 | 0.609 | 0.079 | |

*N*: number of samples, *A*: mean number of alleles, *R*: allelic richness, $H_O$: the observed heterozygosity, $H_E$: the expected heterozygosity, $F_{ST}$: genetic differentiation among populations, *P* values for the HWE test, (NS) means non-significant, (*) Significance threshold at 5 % and (**) Significance threshold at 1 %.

Genetic diversity parameters calculated from 15 loci for Myanmar natural teak were $R$ = 4.91, $H_E$ = 0.609, and $F_{ST}$ = 0.079. The weighted average values of the expected heterozygosity and allelic richness of six natural populations from India, five from Thailand and five from Laotian teak obtained from Fonfana, et al., (2009) were calculated and compared with Myanmar teak (Table 2.3). Allelic richness of Myanmar teak was significantly higher than that of Indian, Thai and Laotian teak (Fig 2.2). However, expected heterozygosity of Myanmar teak was significantly lower than that of Indian teak, but significantly higher than that of Thai and Laotian teak (Fig. 2.3).

**Table 2.3 Statistical comparison of genetic diversity estimates between Myanmar teak and Indian, Thai and Laotian teak**

| Country | No. of populations | *N* | *R* (*p*- value) | $H_E$ (*p*- value) | $F_{ST}$ | Reference |
|---|---|---|---|---|---|---|
| Myanmar | 4 | 128 (32) | 4.91 | 0.609 | 0.079 | This study |
| South India | 6 | 71 (7 - 22) | 4.20 (0.03) | 0.748 (0.004) | 0.030 | Fofana et al. 2009 |
| North Thai | 5 | 46 (5 - 13) | 2.68 (0.003) | 0.450 (0.016) | 0.120 | Fofana et al. 2009 |
| Laos | 5 | 39 (5 - 13) | 2.14 (0.002) | 0.356 (0.002) | 0.050 | Fofana et al. 2009 |

*N*: number of samples (numbers in parenthesis indicate the range among different populations), *R*: weighted average of allelic richness, $H_E$: weighted average of expected heterozygosity. *p*: probabilities in *R* and $H_E$ using 3,000 permutations. $F_{ST}$: genetic differentiation among populations. (*) Significance threshold at 5 % and (**) Significance threshold at 1 %.

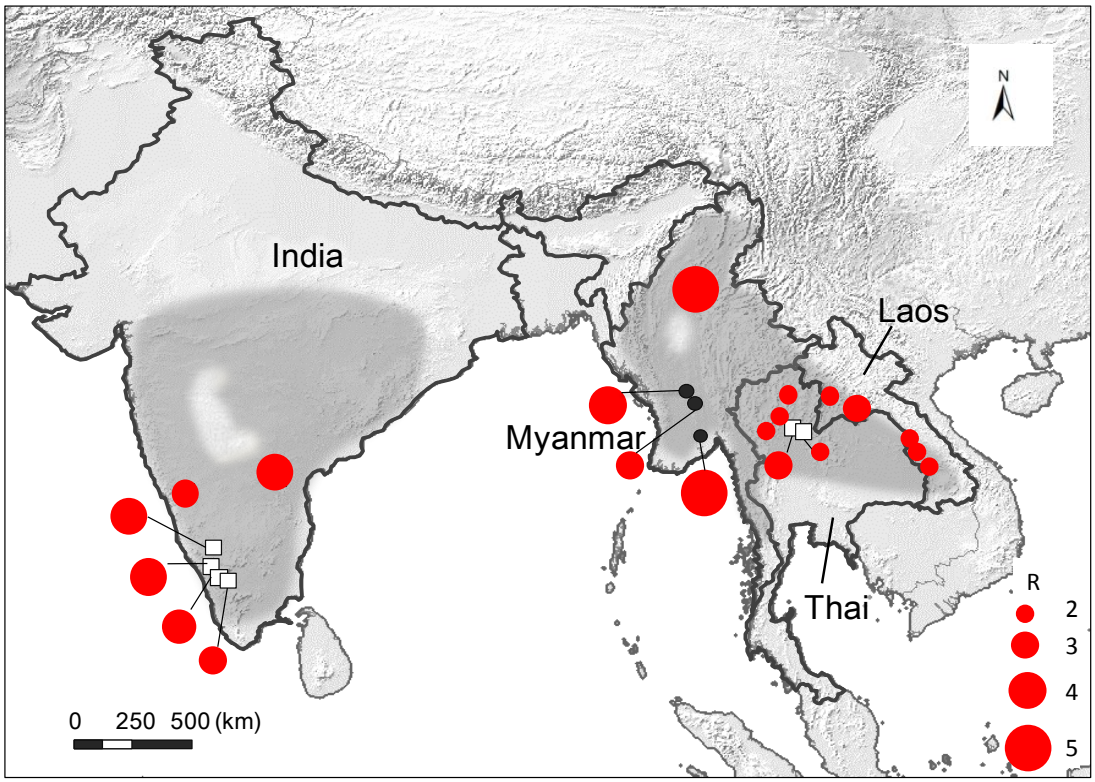


**Fig. 2.2 Distribution of genetic diversity parameters of teak for allelic richness.**

The diameter of the circles is proportionate to the level of allelic richness or expected heterozygosity and numbers indicate values. Parameters for Myanmar were calculated in this study and those for Indian, Thai and Laotian populations are from Fofana et al. (2009).

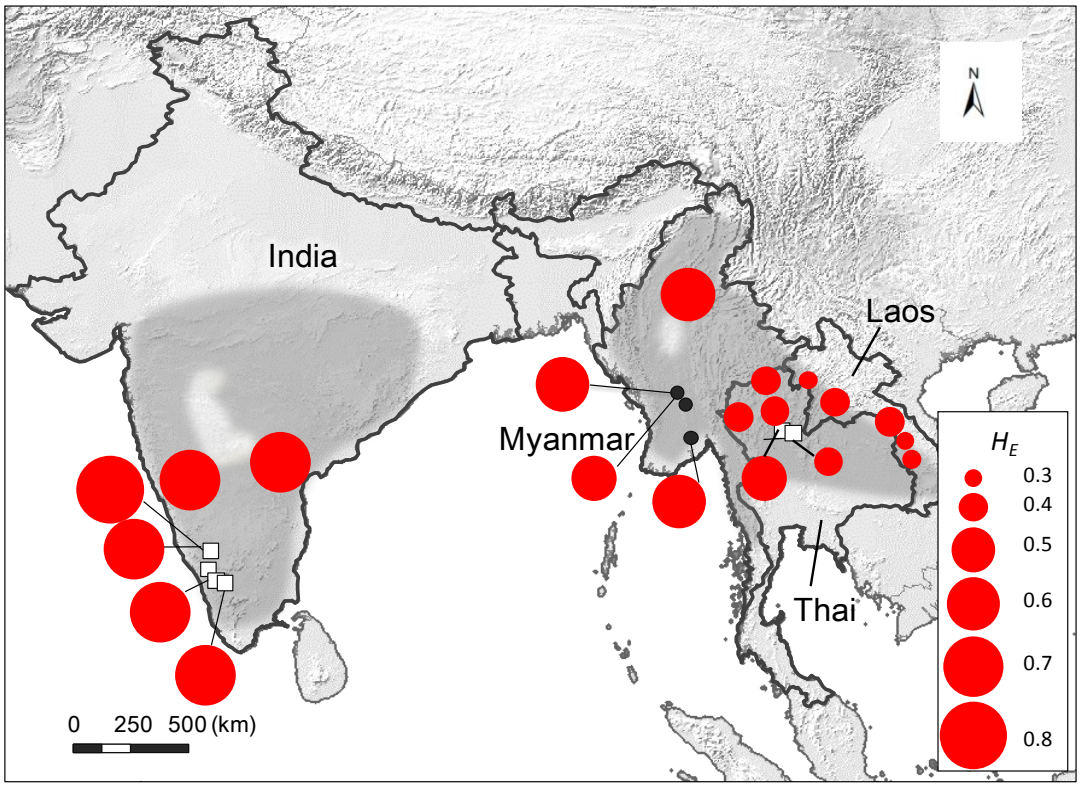


**Fig 2.3 Distribution of genetic diversity parameters of teak for expected heterozygosity.**

## 2.4 Discussion

Our results of high allelic richness and the expected heterozygosity of teak in Myanmar compared to other countries (except for the expected heterozygosity in India) does not support our hypothesis that Myanmar teak has the highest genetic diversity among the four native countries. However, genetic diversity of Myanmar teak is significantly higher than that of Thailand and Laotian teak. Genetic diversity is expected to be lower in small isolated populations, such as Thailand and Laos, as a consequence of bottlenecks, founder effects, and inbreeding (Lammi, et al., 1999). Finding in this study is consistent with the summarizing of the previous studies that genetic diversity of teak is decreasing with the eastward direction; from south India, north India, Myanmar, Thai and Laotian teak. However, natural teak forests cover a much larger area in Myanmar which therefore has higher genetic diversity and a moderate level of genetic differentiation compared to those in other teak native regions (Table 2.3). Both population divergence and diversity are important for conservation because they contribute to total species diversity (Petit, et al., 1998). Thus, Myanmar teak populations with high genetic diversity and moderate genetic differentiation among populations would be an important global genetic resource.

## 2.5 Conclusion

For conservation, more attention should be given to genetic diversity, allelic richness and genetic divergence (Petit, et al., 1998, Steven, 2004; Shrestha, et al., 2005). We found that teak populations from Myanmar possessed high genetic diversity, the highest allelic richness and moderate genetic divergence compared to

other native countries. Genetic resources of Myanmar teak should therefore be a priority for *in situ* conservation programs. However, Myanmar and Indian teak might be future prospective for understanding geographic patterns in the genetic structure of teak.

# Chapter 3

## Development of chloroplast single nucleotide polymorphism (cpSNP) markers of teak

This chapter is going to publish in soon.

# Chapter 4

## Geographic variation pattern of Myanmar teak revealed by newly developed cpSNP and nrSSR markers

This chapter is under the process of submission.

# Chapter 5

## Comparison of genetic composition between alien and native teak in Myanmar using simple sequence repeat (SSR) markers

Chapter 5 is also in preparation for publication.

# Chapter 6

## General Discussion

Among four native countries of teak, the highest genetic diversity was observed in India teak. This finding was congruent with Hasen, et al., 2015. At the age of the inversion of Myanmar central Basin, ca 10 million years ago, the opening of the Andaman Sea affected sharply bending northeastward the India relative to Myanmar motion (Bertrand and Rangin, 2003). The tectonic movement and the level of genetic diversity of teak from native regions indicated that teak might have migrated into eastward direction from India to Myanmar. Anyhow, natural teak forest in India was nearly to be depleted since the late 1980s. In the long term, reduction in population size may decrease the genetic variation (Ledig, 1992) that is important for adaptation to environmental changes. Moreover, genetic erosion has occurred in natural teak in India due to uncontrolled logging and unrestricted movement of planting materials (Ansari, et al., 2012). Myanmar teak with high genetic diversity is therefore important as natural gene resources of teak in the world. Moreover, Myanmar has the largest area of natural teak forests with the best timber quality. Thus, Myanmar teak should be concentrated not only for conservation but also for the production of genetically improved materials through breeding and tree improvement program.

Knowledge of variation patterns in intraspecific chloroplast DNA (cpDNA) is useful to examine the numerous aspects of evolutionary genetics including migration patterns and rates, drift, and population structure (Golden and Bain, 2000). Among three regions, tranK-rps16 was described as the best choice for molecular studies because of its phylogenetically informative character (Powell et al. 1996).

The region psbK-psbI has the highest discrimination power and is useful for species identification (Zuo et al. 2010). The regions developed cpSNP markers, psbI-psbK, trnK-rps16 and rpl16 regions The developed three cpSNP markers are applicable to population and phylogeographic study of teak. Anyhow, the better resolution of cpSNP markers of teak should be increased to see the clear picture of geographic structure. Furthermore, those chloroplast SNP markers might cross amplify in related species; *Tectona hamilitonia and Tectona philipino.*

It was unclear for weak geographic variation of Myanmar teak for cpSNP markers. Generation time is one of the biological factors influence on rates of nucleotide sequence evolution (Wu and Li, 1985). A prolonged or severe demographic bottleneck in recent times might have resulted in low haplotype diversity and nucleotide diversity (Avise, 2000). The widespread distribution of common haplotype indicated ancient population bottleneck (Liu, et al., 2012). Myanmar teak possessed low haplotype diversity and widely distribution of common haplotype (H1). Thus, severe bottleneck after glaciations or rapid expansion of founder populations or long life span of teak or human interference to natural populations might account for weak cpDNA variation of Myanmar teak. Only the genetic data is insufficient to distinguish between natural dispersal and migration (Gong, et al., 2008). Fossil data and genetic data are required to confirm the human propagation, natural dispersal and migration of teak.

Clear genetic structure of Myanmar teak for nrSSR markers showed limited pollen flow of teak. Both economical and ecological traits are largely varied for different provenances (Keiding, et al., 1986, Kjaer, et al., 1999, Monteuuis, et al., 2011). Three major zones are proposed to designate based on genetic structure of teak revealed by

nrSSR markers. In addition to the knowledge of genetic structure, natural climatic and physiographic divisions should be considered for designating the boundaries of seed zones (Ledig, 1992). The boundary should therefore be made between upper and lower regions due to the difference in climatic conditions. However, provenance test should be conducted to designate profoundly the seed zones, breeding zones or plantation zones. High genetic diversity within populations of Myanmar teak indicated its importance for conservation and breeding purposes. The populations with high level of genetic diversity have the adaptability the capacity of rapid adaptive changes (Lefevre, et al., 2004). Thus, HMB, TDG, POL and KTA should be conserved not only for retaining the natural genetic resources of Myanmar teak but also for breeding programs to use as raw populations.

Teak is an important source of tropical timber and planted not only in its native region but also outside of its natural distribution. Teak plantation stands on the third position of world plantation to supply the demand of timber market because of decreasing the capacity of natural teak bearing forest. Expanding the ranges of species of economic value may lead to genetic divergence and mixing divergent populations will contaminate local gene pools and homogenizing species structure (Ledig, 1992). Gene flow of foreign genes into natural populations, exotic or genetically modified plants by hybridization and introgression can cause genetic pollution (Linacre and Ades, 2003). Secondary evolution can occur through the hybridization between the indigenous species and related exotic populations (Lefevre, et al., 2004). Furthermore, non-local tree cross with native populations may increase the genetic diversity of next generation but with negative consequences for local adaptation (Ledig, 1992). Thus, to retain the natural genetic resource of Myanmar

teak and also to supply the demand, the best way is balancing conservation and breeding program of teak. Timber quality of Myanmar teak is famous for the best timber quality in the world. Conserved populations with high genetic diversity and genetic divergence can be use as breeding population that satisfied major economic needs. Therefore, instead of introducing the alien teak for plantations, producing the genetically superior quality of planting materials should be concentrated by implementing the breeding program for Myanmar teak.

## Summary

The tropical deciduous and semi ever-green tree species, teak, is one of the most economically important tree species. It naturally occurs in India, Myanmar, Thai and Laos. Genetic information of teak from its native regions has been investigated using molecular markers and they showed south India teak has the highest genetic diversity followed by teak from North India, Thai and Laos. About 60% of the total natural forest area occurs in Myanmar that is the largest area of natural teak forest. Few study for genetic diversity of Myanmar teak has been conducted but no comparison between Myanmar teak and from its indigenous countries has been reported. The same markers used in the previous study were applied for evaluating the level of genetic diversity of Myanmar teak to compare with that of teak from other native countries in chapter 2. As the results, Myanmar teak has significantly lower genetic diversity than that of India teak, but significantly higher than that of Thai and Laos teak.

Natural teak forest in Myanmar drastically diminished due to over logging, illegal cutting and transforming landuse systems, therefore conservation of Myanmar teak is urgently needed to retain the natural genetic resources of teak in the world. Furthermore, teak plantation was widely established at about 43 countries including its native countries, Myanmar. Knowledge of the genetic variation of extant populations over the entire range of their distribution is therefore essential for the conservation of genetic resources. Microsatellite markers (nrSSRs), which are highly polymorphic, are useful for elucidating the spatial genetic structure and the demographic patterns of variation which have resulted from migration and drift as well as through evolutionary history. Chloroplast markers are also useful for

phylogeographic studies and gene conservation, because chloroplast genomes, which are haploid, are maternally inherited in angiosperms and hence transmitted by seeds. Nevertheless, no chloroplast markers for teak have been developed yet. Thus, cpSNP markers for teak have been developed to determine phylogeographic structure of Myanmar teak. After sequencing about one third of complete genome of teak about 43,734 bp, three cpSNP markers of teak were developed to study the geographic variation of teak in Myanmar.

Geographically genetic structure of Myanmar teak was examined using total 480 individuals of 20 natural populations from five regions representing almost natural teak forests in Myanmar and two types of molecular markers; three newly developed cpSNP markers and 10 nrSSR markers in chapter 4. The combined studied of cpSNP and nrSSR markers suggested there are at least four genetic resources of Myanmar teak. Randomized distribution of four haplotypes showed by cpSNP markers did not depicted clear geographic structure of Myanmar teak. On the other hand, four genetic clusters of 20 natural populations depicted by nrSSR markers suggested clear geographic genetic structure of Myanmar teak. The putative genetic boundaries of 20 populations suggested at least three zones such as planting or seed zones can be designated based on combined cpSNP and nrSSR data. Of 20 populations, four populations with their high contribution to total genetic diversity were found to be prioritized for conservation.

Teak plantation in Myanmar has been started using local seeds since 1700 to replenish the degraded natural forests. A couple of years ago, private sectors were allowed to establish teak plantation at deforested area or some were around natural teak forests. No seed guideline of teak is formulated in Myanmar. Therefore, seeds

from wherever available were used for teak plantation without considering their genetic component. Moreover, teak plantation established by private companies used alien teak from Indonesia, China and Costa Rica without information on genetic background. To prevent genetic disturbance for Myanmar natural teak, genetic component of recently established teak plantation by private sectors were investigated using 10 nrSSR markers and compared with that of natural teak and old teak plantation. Higher genetic diversity and less genetic differentiation among populations of recently established teak plantation supported the assumption of various seeds sources used for those plantations. Alien teak showed low genetic diversity and significant level of genetically differentiated from Myanmar teak especially Indonesian teak.

At last, gene conservation and afforestation strategy for Myanmar teak were discussed based on findings obtained in this study. Among four native countries of teak, Myanmar with the largest natural teak forests and high genetic diversity may be genetic core of teak in the world. The current four genetic resources of Myanmar teak should be retained not to be deteriorated by genetic erosion by designating the planting zones or seeds zone based on geographic genetic structure of Myanmar teak. Alien teak introduced to Myanmar for planting purpose should be restricted. Seeds from alien teak should be avoided for the establishment of next teak plantation in Myanmar because those seeds may be products of outbreeding between alien teak and Myanmar teak with high genetic divergence. Instead of using the alien teak, genetically improved planting materials, Myanmar teak should be focused on producing the planting materials through breeding and tree improvement programs. In doing so, retaining natural genetic resources of Myanmar and supplying the high

demand of teak can be implemented. Genetic information of Myanmar teak observed in this study may take a part of role for the conservation of natural genetic resource of teak.

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