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

Dissertation for
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Chapter 1

Introduction

The rate of deforestation significantly increased across tropical Asia and the highest level of deforestation was experienced in Southeast Asia during 1990s (Wright 2005, Miettinen et al., 2011). Economically important forest tree species (e.g., *Tectona grandis, Shorea siamensis* and *Pterocarpus macrocarpus*) are naturally distributed in this region. For teak, only four countries from Southeast Asia, India, Myanmar, Thailand and Laos are native regions (Fig 1.1). Now that the teak plantation has been widely established beyond its indigenous countries, planting materials from various sources have been used. Both conservation and breeding programs for teak are therefore urgently required to save natural genetic resources and to provide the genetically improved materials. 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). It is also essential in evaluating potential for genetic gain through breeding programs. Geographic variation and genetic structure are necessary to develop strategy for effective and efficient conservation and breeding programs for target species.

1.1 Species characteristics of 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 from dry forests shows poor flowering, fruiting and seed fillings (Dabral and Amin, 1975). Natural regeneration is almost absent or very poor due to various factors such as overwood removal and control of understory. Moreover, low seed production and poor germination of teak account for scant natural regeneration in teak forests. Therefore, the natural teak forests have suffered from inadequate natural regeneration.

The recorded latitudinal and longitudinal limits of natural range for teak 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 amount of natural teak forest is ca 27.9 million ha and out of them 8.9 million ha in India (Tewari 1992), 2.5 million ha in Thailand (Kaosa-ard 1991), and 0.016 million ha in Laos (Anon 1992). The rest of total area approximately is 16.5 million ha in Myanmar (Pengduoang 1991).

Teak has been regarded as one of the world's most important tropical tree species due to precious timber qualities (Pandey and Brown, 2000; Kaosa-ard 2003). Four beautiful colors of teak timber are golden yellow, light brown, dark brown and black stripe (Fig 1.2). Furthermore, 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 make increasing its demand and endangered species (Gyi and Tint, 1998; Kaosa-ard 1998). Thus, it is recognized

worldwide as the most important wood for multipurpose particularly for ship building and furniture.

Natural populations of teak in its native countries nearly to be depleted 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 countries in the late 1980s except Myanmar (Pandey and Brown, 2000). Nevertheless, teak is now a threatened and endemic species so conservation effort is urgently needed to safeguard the genetic resources of teak from degraded natural teak forests.

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 exist within 25˚30'N and 10˚00' N latitude and widely distributed (Fig 1.3). In Myanmar, teak grows throughout the Shan state except the area where the altitude is more than 1000 m 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 grows on the west bank of the Ayeyarwady into the foothills of Rhakhine 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 grows in three major forest types; the semi-evergreen forests, mixed deciduous forests and deciduous dipterocarp forests (Fig 1.3). The composition of teak in natural forests varies with forest types, from 4-12% in semi-evergreen forests,

deciduous dipterocarp, mixed deciduous forests. Teak is usually found as scattered individuals or in small groups with little or no regeneration present in the semi-evergreen forest (Kermode, 1964). The mixed deciduous forest includes moist upper mixed deciduous forests, dry upper mixed deciduous forests, and lower mixed deciduous forests. In the lower mixed deciduous forest, teak may be found gregariously or in patches with a large girth and height and fluted trees. In the moist upper mixed deciduous forests, teak are cleaner and straight boles. In the deciduous dipterocarp forests including Indaing forest in Myanmar, teak grows small size with poor quality.

1.3 Genetic information of native teak

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. 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 studies of population genetics have used various genetic markers such as allozyme (Kertadikara and Prat, 1995, Kjaer and Seigismund, 1996), amplified fragment length polymorphism (AFLP) (Shrestha et al., 2005, Fofana et al., 2013), inter simple sequence repeat (ISSR) (Narayanan et al., 2007), and simple sequence repeats (SSR) markers (Fofana et al., 2008, 2009; 2013; Minn et al., 2014).

The southern Indian populations possessed the highest genetic diversity, followed by the northern Indian, Thailand and Laotian populations using SSR markers Fofana et al. (2009, 2013). Similar results were obtained by using AFLP markers (Shrestha et al*.,* 2005; Sreekanth et al., 2012). Higher genetic divergence of Indian teak was reported on isozyme variation (Kertadikara and Prat, 1995; Kjaer and Seigismund, 1996). Recently, significant genetic differentiation was detected between regions within Myanmar (Minn et al*.,* 2014). However, comparison of genetic diversity of teak in its native areas; India, Myanmar, Thai and Laos, has not been conducted yet. Furthermore, overall geographic variation and genetic structure of teak within Myanmar is still unclear.

Chloroplast markers are promising tools to understand geographic variation and genetic structure, because chloroplast genomes are haploid and maternally inherited in angiosperms. Especially, chloroplast single nucleotide polymorphism (cpSNP) markers are promising to understand phylogeographic pattern (Petit et al., 2005). Nevertheless, no chloroplast markers have been developed for teak.

1.4 Teak plantation in Myanmar

To conserve the natural resources and supply the demands in a market, teak plantation was initiated in 1700 in Myanmar. During the past 20 years, teak production from natural forests has decreased. Then, the interests for plantations have developed (Pandey and Brown, 2000). Over the years, most of the natural teak forests have been gradually converted into teak monoculture plantations. Until 2007, the Forestry Department took the responsibility for establishing teak plantations using native seeds in Myanmar. Since 2007, private sectors were allowed to establish

the large-scale of teak plantations in deforestation areas. Seeds from various regions were used for establishment of teak plantation without considering geographic structure. For currently established plantations by private companies, not only native seeds but also exotic teak from other introduced countries; Indonesia, China and Costa Rica, have been used without information of their genetic background. Therefore, genetic structure and genetic composition of these commercial plantations should be checked whether they are significantly different from native teak populations in Myanmar.

1.4 Problem statements

Within two decades natural teak forest area in Myanmar was drastically diminished. Decreasing the area of natural teak forest in Myanmar might result in decreasing genetic diversity, disrupting gene flow and genetically isolating tree populations. Despite that knowledge of the genetic variation of extant populations covering natural distribution is essential for the conservation of genetic resources (Neale and Kremer, 2011). However, genetic information is very limited for Myanmar teak among its native regions. Without genetic information of Myanmar teak, it is difficult to discover the genetic center of natural teak. Commercial plantation of teak may be established by using seeds from various sources of Myanmar teak and from exotic teak. There will be a risk of genetic disturbance to natural genetic resources. However, there is no clear instruction for seed transfer to plantation sites. Thus, the information of geographic variation and genetic structure of teak is needed for balancing conservation and breeding programs.

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 based on nuclear and chloroplast DNA markers
3. To designate seed and planting zones within Myanmar to fulfil conservation and breeding purposes
4. To clarify genetic component of commercial plantations established by private sectors to prevent genetic disturbance

1.6 Composition of the study

To complete the objectives of this study, first, the level of genetic diversity of Myanmar teak was investigated using nuclear SSR markers and compared with that of other native teak in chapter 2. After developing cpSNP markers for teak in chapter 3, phylogeographic variation and genetic structure of Myanmar teak were investigated using newly developed cpSNP and nrSSR markers in chapter 4. Genetic components of exotic teak planted in Myanmar were elucidated by using nrSSR makers in chapter 5. In chapter 6, findings from this study were finally discussed to retain the natural genetic resources of Myanmar teak through balancing conservation and breeding activities and to evaluate the potential for risk of genetic disturbance by commercial plantation established by private sectors.

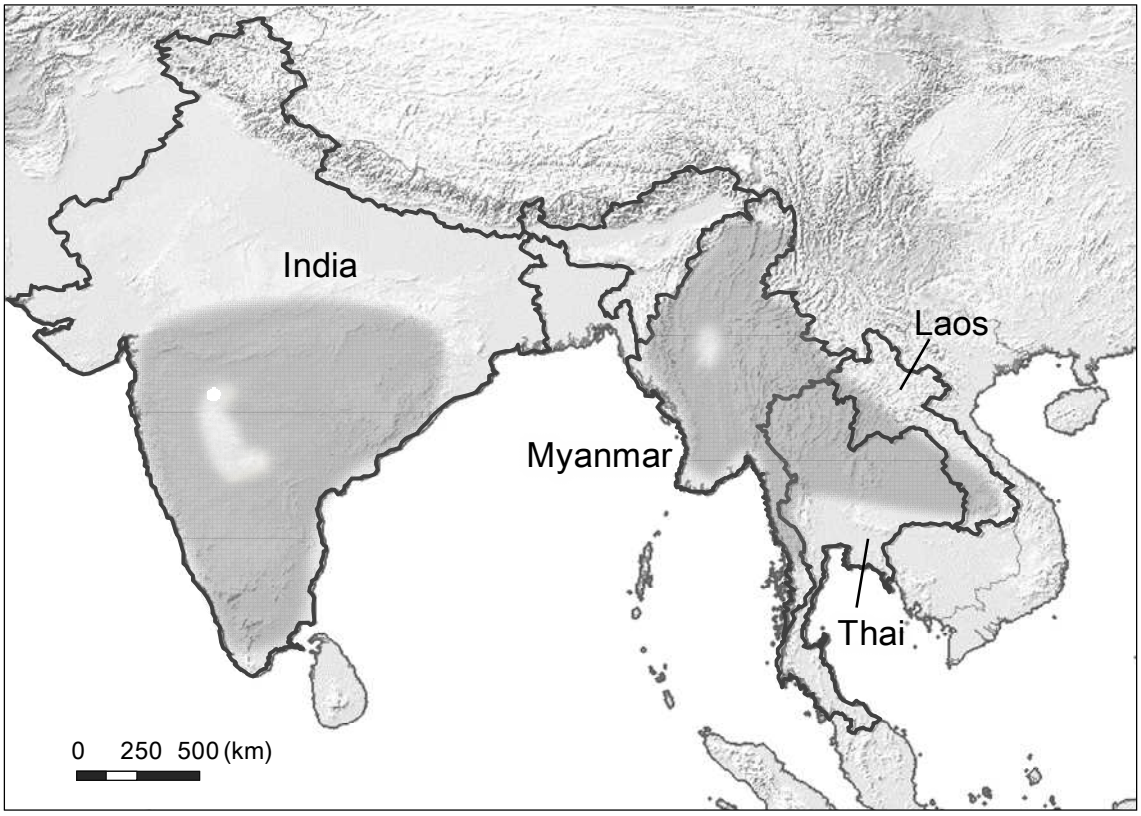

Figure 1.1 Natural distribution of teak in its native regions, India, Myanmar, Laos and Thailand.

The shaded areas show natural distribution of teak in each respective country **(**Kyi and Tint, 1998).

Figure 1.2 Color variation of teak

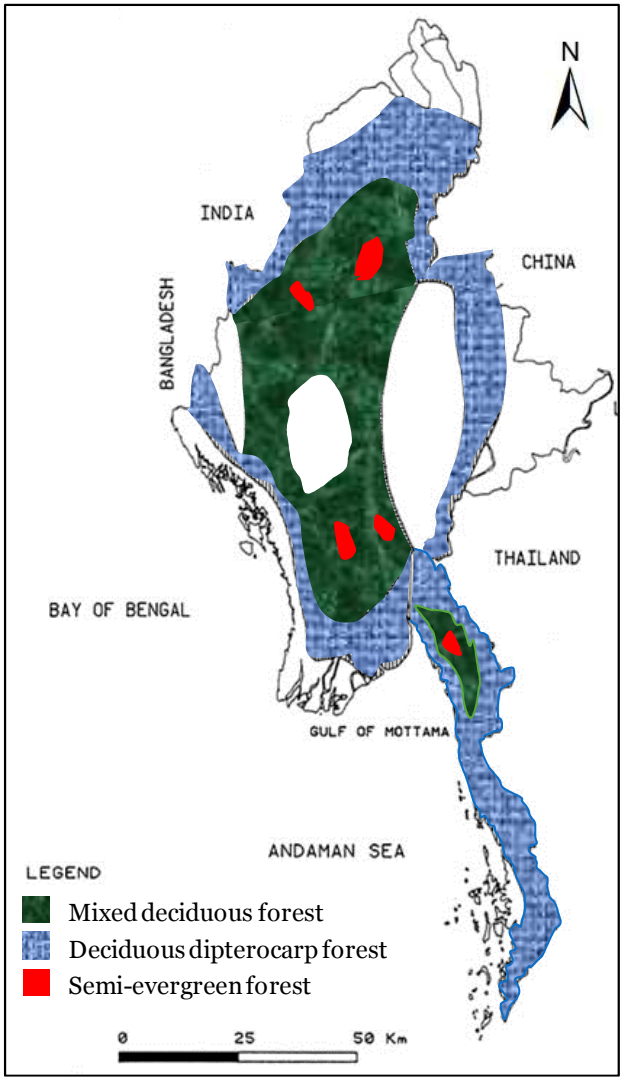

Figure1.3 Natural teak bearing forests in Myanmar (Source: FD-Myanmar).

Chapter 2

Genetic diversity of teak in its native region

2.1 Introduction

Natural distribution of teak is limited to a discontinuous range in South and Southeast Asia from the Indian subcontinent to Myanmar, Thailand and Laos (Khanduri et al., 2008). Since teak provides premium timber with a number of very desirable properties such as high durability, strength and workability (Pandey and Brown, 2000; Kaosa-ard 2003), its demand increases in the timber market. Natural teak forests have decreased because of the supply of high demand. Rapid decreasing of limited natural teak forests alarm the urgent needed of conservation effort. 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). SSR markers are arguably the most informative of these marker types due to

their hyper-polymorphic nature and co-dominance (Powell et al., 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). Recently, significant geographic variation pattern of Myanmar teak was detected between different regions (Minn et al., 2014). However, comparison of genetic diversity of teak in its native areas has never been conducted yet. Therefore, this study was conducted to figure out level of 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 (Table 2.1; Fig 2.1). Those samples were collected from a provenance trial established at Pyinmana, Myanmar in 2007 and the collected samples represented natural populations 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 modified CTAB 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).

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 those of populations from India, Thailand and Laos (Fofana et al., 2009). The locus CIRAD4TeakH09 was modified 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'-CTGTGCCTTCTAGTTGCC AGCGCAAGAGCTGAAAGCAACC-3' and 5'-GGCCGTTAGCACTCCATTTA-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, 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 were used. The florescent universal tail primers, T7 terminator primer (FAM-5'-ATGCTAGTTATTGCTCAGCGG-3'), reverse complement of BGH-R primer (VIC-5'-CTGTGCCTTCTAGTT GCCAGC-3'), reverse complement of pCold-R primer (NED-5'- TTGGGTG CAATGAGAATGCG-3') and pCold TF-F1 primer (PET-5'-CCACTTTCAACGAG CTGATG-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 each teak microsatellite markers used in this study. 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 followed 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 to ensure complete amplification, the products were stored at 4˚C. Then, 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 LIZ size standard (Applied Biosystems). After denaturing the mixed products at 95˚C for 5 min, they were determined 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 with FSTAT ver. 2.9.3. 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 test with 3 000 permutations.

2.3 Results

The number of alleles at each locus over 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).

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 (Table 2.3; Fig 2.2). However, the expected heterozygosity of Myanmar teak was significantly lower than that of Indian teak, but significantly higher than that of Thai and Laotian teak (Table 2.3; Fig. 2.3).

2.4 Discussion

Myanmar teak exhibited the high level of genetic diversity in term of allelic richness and the expected heterozygosity among four native countries (Fig. 2.2, Fig 2.3). Genetic diversity of large and continuous populations tends to be higher than small and scattered populations (Hamrick et al., 1992). In contrast, genetic diversity is expected to be lower in small isolated populations, as a consequence of bottlenecks, founder effects, and inbreeding (Lammi et al., 1999). Myanmar possesses the largest area of teak natural distribution among four native countries, India, Myanmar, Thai, and Laos (Gyi and Tint, 1998). Thus, genetic diversity of Myanmar teak was hypothesized to be highest among native countries, which is mostly supported by this

study. Myanmar teak harbored significant higher level of genetic diversity than that of Thai and Laotian teak although its genetic diversity was significant lower value of H_E than that of India teak. Allelic richness of Myanmar teak is the highest among four countries (Table 2.3; Fig. 2.2). Furthermore, the expected heterozygosity of Myanmar teak was significantly higher than that of Thai and Laos teak (Table 2.3; Fig 2.3). Small population size of Thai and Laos teak might have accounted for lower genetic diversity of Thai and Laos teak among native regions. Hamrick et al., (1992) suggested that the current genetic diversity should be explained by not only species distribution patterns and life history but also demographic history of the target species. Previous studies revealed that genetic diversity of India teak is apparently higher than that of Thai and Laos teak (Shrestha et al., 2005; Fofana et al., 2009). Populations that possessed high genetic diversity and allelic richness might be genetic origin such as refugia during Last Glacial Maximum (Petit et al., 2003). Therefore, this discrepancy might be historical movement of teak. Eastward migration of teak was supposed by recent phylogeography study (Hasen et al., 2015). The genetic relationship between Myanmar and Indian teak might be future prospective for understanding phylogeographic structure of teak.

The large level of genetic differentiation and continuous populations tends to be smaller than that of small and isolated populations (Hamrick et al., 1992). The F_{ST} value of Myanmar teak is moderate among natural teak (Table 2.3), despite of largest natural areas of teak distribution. Minn et al. (2014) indicated that teak populations in Myanmar are genetically differentiated. They indicated that large environmental variations affect genetic differentiation in Myanmar teak. In contrast, the F_{ST} value is highest in Laos teak (Table 2.3). Laos teak populations might be

experienced by severe fragmentation. Genetic drift would affect the level of genetic differentiation in Laotian teak.

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. 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). 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 of conservation and producing teak planting materials.

2.5 Conclusion

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 purpose. Furthermore, Myanmar teak with high genetic diversity parameters and the best timber quality should be concentrated for breeding purpose.

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

No.	Population	N	Latitude	Longitude	Altitude (m)
1	Bago	32	18° 7'N	96° 4'E	134
2	Phyu	32	18°28'N	96°20'E	399
3	Oktwin	32	18°55'N	96° 1'E	245
4	Kanbalu	32	23°30'N	95°52'E	274
	Total	128			

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

Locus Name	*N*	*A*	*R*	*Ho*	H_E	F_{ST}	F_{IS}	*P-value*
CIRAD1TeakA06	127	10	6.89	0.614	0.650	0.079	0.059	0.186 (NS)
CIRAD1TeakB03	127	15	10.21	0.788	0.755	0.128	-0.042	0.864 (NS)
CIRAD1TeakF05	128	12	8.07	0.391	0.572	0.056	0.276	0.001 (*)
CIRAD1TeakG02	127	7	3.94	0.173	0.211	0.095	0.030	0.040 (*)
CIRAD1TeakH10	128	20	14.14	0.820	0.851	0.047	0.229	0.192 (NS)
CIRAD2TeakB07	128	18	8.83	0.477	0.574	0.090	0.125	0.001 (*)
CIRAD2TeakC03	116	14	10.08	0.827	0.799	0.086	-0.037	0.826 (NS)
CIRAD3TeakA11	128	14	9.41	0.664	0.758	0.036	0.039	0.002 (*)
CIRAD3TeakB02	128	20	12.91	0.695	0.730	0.093	0.292	0.141 (NS)
CIRAD3TeakDa09	126	8	5.59	0.313	0.375	0.093	0.109	0.012 (*)
CIRAD3TeakE06	127	12	8.64	0.487	0.693	0.062	0.311	0.001 (*)
CIRAD3TeakF01	128	13	9.35	0.641	0.722	0.074	-0.087	0.009 (*)
CIRAD4TeakDa12	128	7	4.03	0.367	0.338	0.055	0.009	0.910 (NS)
CIRAD4TeakF02	128	9	6.55	0.547	0.564	0.111	0.162	0.329 (NS)
CIRAD4TeakH09	127	12	7.50	0.660	0.546	0.084	-0.203	0.999 (NS)
Mean	127	13	8.408	0.564	0.609	0.079	0.085	

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, F_{IS}: inbreeding coefficient, *P* values for the HWE test, (NS) means non-significant, (*) Significance threshold at 5 %.

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

Country	No. of populations	N	R	H_E	F_{ST}	Reference
Myanmar	4	128 (32)	4.91	0.609	0.079	This study
South India	6	71 (7 - 22)	4.20 (0.030)	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)

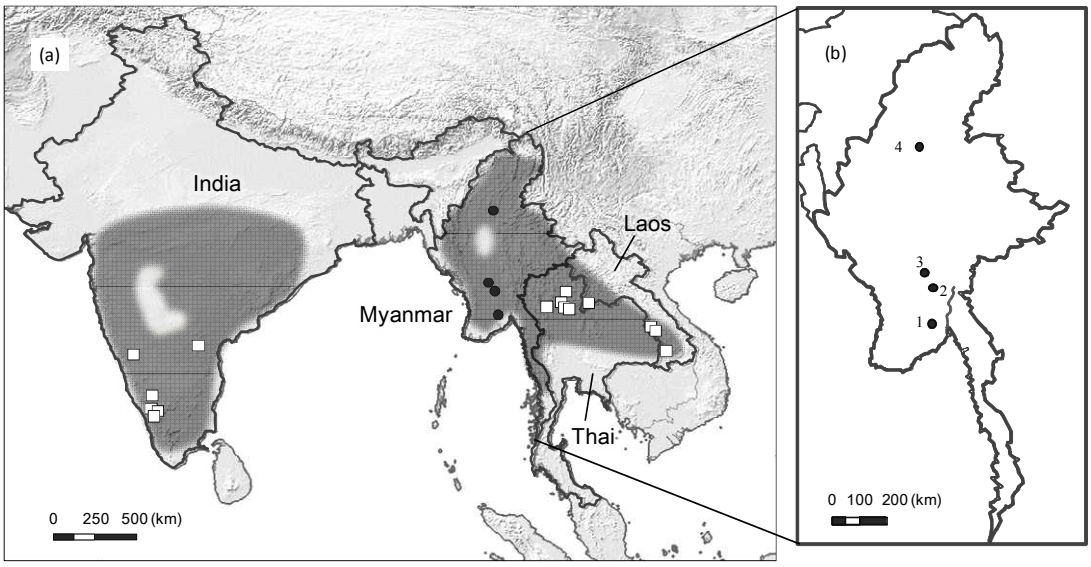

Figure 2.1 (a) Maps of the distribution of teak in India, Myanmar, Laos and Thailand and (b) the locations of the four sampled populations of teak in Myanmar. In (a), open squares indicate the locations of the teak populations from a previous study (Fofana et al., 2009) and closed circles represent Myanmar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

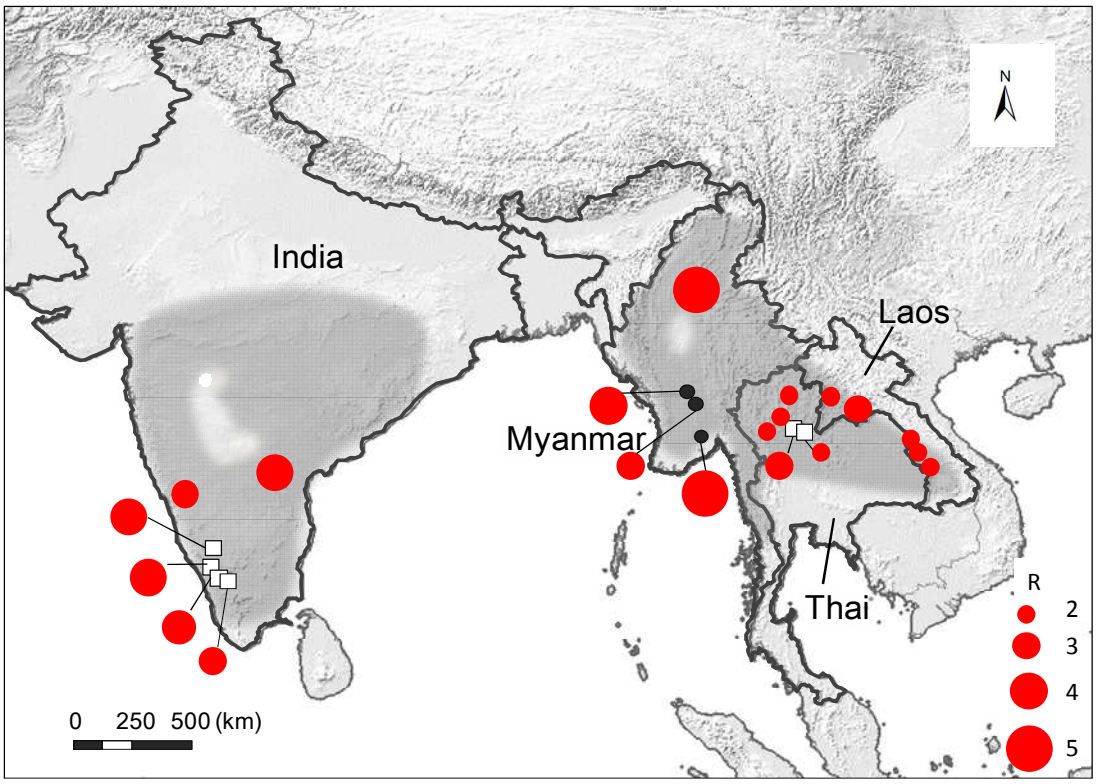

Fig. 2.2 Genetic diversity parameter, allelic richness (*R*) for teak from native regions.

The diameter of the circles is proportionate to the level of allelic richness (*R*) and numbers indicate the values of allelic richness which was calculated for Myanmar teak in this study and those for Indian, Thai and Laotian populations are obtained from Fofana et al. (2009).

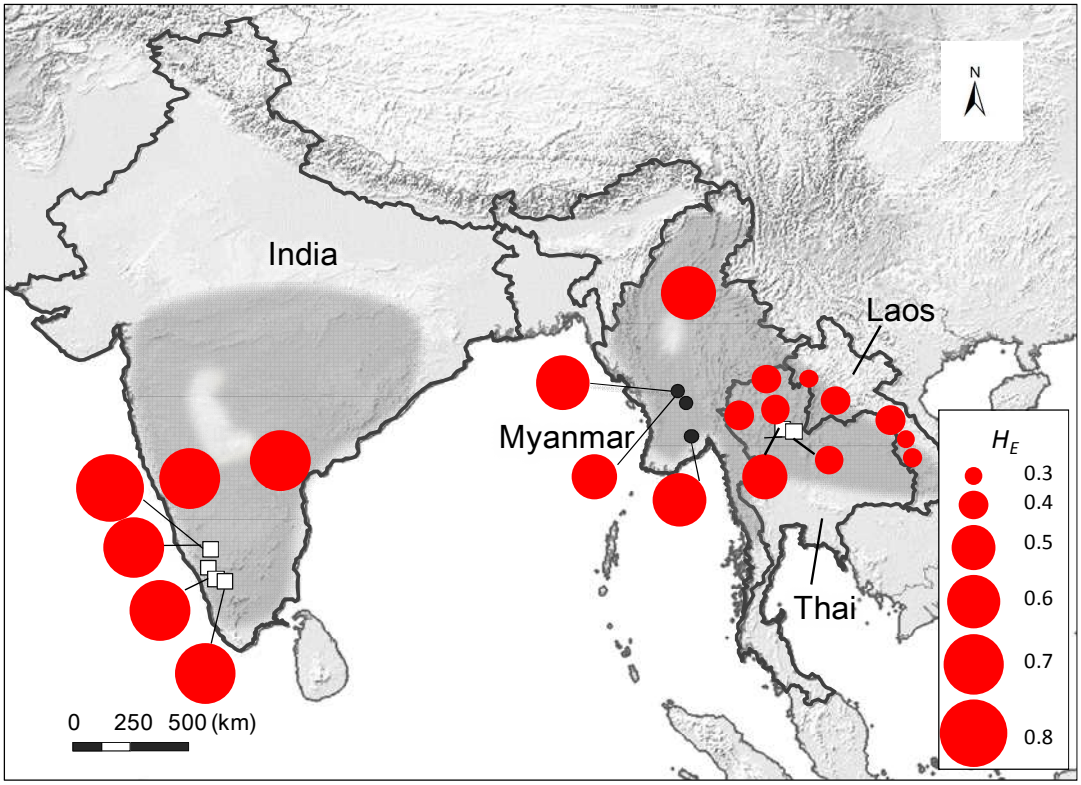

Fig 2.3 Genetic diversity parameter, the expected heterozygosity (H_E) of teak from native regions.

The diameter of the circles is proportionate to the level of the expected heterozygosity (H_E). The expected heterozygosity for Myanmar teak was from this study and for Indian, Thai and Laotian populations was obtained from Fofana et al. (2009).

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. Even though approximately 60% of the total natural forest area occurs in Myanmar few genetic studies of Myanmar teak has been conducted, no comparison between Myanmar teak and from its indigenous countries has been reported. Natural teak forest in Myanmar drastically diminished due to over logging, illegal cutting and transforming landuse systems. Furthermore, large scale of teak plantations was established in Myanmar using planting materials from other countries. Therefore conservation and breeding programs of Myanmar teak is urgently needed to retain the natural genetic resources of teak and supplying the genetically improved planting materials in the world. Knowledge of the genetic variation of extant populations over the entire range of their distribution is therefore essential for both conservation of genetic resources and breeding purpose. 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. Allelic richness of Myanmar teak is highest among native countries. Myanmar teak has significantly higher than that of Thai and Laos teak, but significantly lower than that of India teak. Thus, Myanmar teak plays an important role for genetic resource management in the world.

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. For the development of cpSNP markers for teak, a total of 43,734 bp were sequenced for eight individuals using 58 walking primers in Chapter 3. As a result, three SNPs were detected and then three cpSNP markers of teak were developed based on findings of three SNPs.

Geographic variation and genetic structure of Myanmar teak were 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 phylogeographic structure of Myanmar teak. In contrast, 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 and tree breeding.

Teak plantation in Myanmar has been started using local seeds since 1700 to replenish the degraded natural forests. In 2007 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 exotic teak from

Indonesia, China and Costa Rica without information on their genetic background. To prevent genetic disturbance for Myanmar natural teak, genetic component of recently established teak plantation by private sectors were investigated using three cpSNP markers and 10 nrSSR markers. Then, genetic diversity and component of exotic were compared with that of natural teak and old teak plantation. Exotic teak showed low genetic diversity and significant level of genetically differentiated from Myanmar teak, especially China and Indonesian teak. Higher genetic diversity and less genetic differentiation among populations of recently established teak plantation supported the assumption of various seeds sources used for private plantations. Furthermore, mixture of all haplotypes and genetic clusters indicated that various seeds source of private teak plantations in Myanmar.

Finally, 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. Exotic teak introduced to Myanmar for planting purpose should be restricted. Seeds from exotic teak should be avoided for the establishment of teak plantation in Myanmar because those seeds may be products of outbreeding between exotic and Myanmar teak with high genetic divergence. Instead of using the exotic teak, Myanmar teak should be focused on producing the planting materials through breeding programs. Retaining natural genetic resources of Myanmar are useful for supplying the high demand of teak with good timber qualities. Thus, genetic

information of Myanmar teak observed in this study may take a part of role for the conservation of natural genetic resource and breeding program of teak.

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