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Original Article

Genetic diversity of teak populations in native regions and plantations in Myanmar detected by microsatellite markers

THWE-THWE-WIN*1, Atsushi WATANABE*2, Tomonori HIRAO*3, Keiya ISODA*4, Wataru ISHIZUKA*5, Susumu GOTO*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; resistance to fungi, termites and weathering; and a beautiful grain and color (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 from natural forests and plantations. Previous population genetic studies have used various DNA markers such as allozymes (KERTADIKARA and PRAT, 1995; KJAER and SEIGISMUND, 1996), sequence characterized amplified regions (SCAR) (ISODA *et al*., 2000), random amplified polymorphic DNA (RAPD) (WATANABE *et al*., 2004), amplified fragment length polymorphisms (AFLP) (SHRESTHA *et al*., 2005), inter simple sequence repeats (ISSR) (NARAYANAN *et al*., 2007), and simple sequence repeats (SSR) (FOFANA *et al*., 2008, 2009; 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).

*1 The University Forest, Graduate School of Agriculture and Life Sciences, The University of Tokyo
*2 Forest Sciences, Agro-environmental Sciences, Faculty of Agriculture, Kyushu University
*3 Forest Tree Breeding Center, Forestry and Forest Research Institute, Independent Administrative Institution
*4 Kansai Regional Breeding Office, Forest Tree Breeding Center, Forestry and Forest Product Research Institute
*5 Local Independent Administrative Agency Hokkaido Research Organization Forestry Research Institute

Large genetic variation was observed in natural teak provenances at isozyme variation (KERTADIKARA and PRAT, 1995; KJAER and SEIGISMUND, 1996). FOFANA *et al.* (2009) used SSRs for investigating the genetic diversity within 17 natural populations of Indian, Thailand and Laotian teak from a provenance trial. They 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). Thus, geographic variation pattern of teak was clearly detected in the previous studies and genetic diversity of teak seems to decline to eastward direction. However, genetic diversity of teak from its indigenous four countries has not been studied yet using same number of identical markers. In general, larger populations show higher genetic diversity than smaller populations (HAMRICK *et al.*, 1992, LAMMI *et al.*, 1999). Myanmar's teak populations are therefore predicted to have a higher level of genetic diversity than those in other native countries because Myanmar contains nearly 60% of the total cover of natural teak forests (GYI and TINT, 1998; KYAW, 2003).

Teak plantations have been established in Myanmar since 1700 (SAW, 2003) to reduce the impact on natural teak forests and to meet the high demand for teak. Teak plantations cover ca. 390,000 ha in Myanmar representing 9% of teak plantations worldwide (KOLLERT and CHERUBINI, 2012). If the level of genetic diversity in these plantations is similar to that in natural populations, plantations established in Myanmar would be candidates for ex situ gene conservation. Traditionally, seeds were collected by local farmers from different stands and mixed before being used to establish seedlings for plantations. Parentage analyses in natural forests revealed that most offspring or seeds come from only a few adults trees (ALDRICH and HAMRICK, 1988). If the area for collecting seed is limited, the genetic diversity of plantations may be low due to the founder effect. However, details of seed collection procedures and the genetic diversity of plantations are largely unknown in Myanmar.

In Myanmar, teak plantations with superior phenotypes are transformed into seed production areas from which candidate plus trees are selected. Seed production areas are interim approaches that can be established easily for producing quality seeds in a short time period until genetically improved seeds are produced through genetic improvement programs. SIVAKUMA *et al.* (2011) stated that culling phenotypically inferior trees in seed production areas enhances the quality of seeds and seedlings of Acacia auriculiformis. If plantations are considered seed production areas, the maintenance of their genetic diversity becomes more important (FINKELDEY and HATTEMER, 2007). Thus, genetic diversity of teak plantation in Myanmar was investigated whether they can be used as seed sources of future plantations.

Two working hypotheses were considered in this study. First, genetic diversity of Myanmar teak is the highest among populations of teak in its four native countries because Myanmar has the largest area of natural teak forest. Second, genetic diversity in plantations is lower than in natural populations due to the founder effect since seeds for the plantations come from a limited number of mother trees and there is insufficient mixing of seeds. We first surveyed the genetic

diversity and genetic differentiation among four natural populations in Myanmar teak based on the same microsatellite markers that have been used in previous studies (FOFANA *et al*., 2009; VERHAEGEN *et al*., 2010). Then, to fill in the gap of genetic information of teak in native regions, we compared the genetic diversity of Myanmar teak with teak from other native countries. In addition, the genetic diversity of teak from six plantations in Myanmar was examined to determine the role of teak plantations in conservation. We discuss the genetic status of Myanmar teak compared with other native populations and the role of Myanmar teak in a global conservation strategy. Finally, we compared the genetic diversity of Myanmar teak in plantations with that in natural populations, and we discuss the role of teak plantations in ex situ conservation and the designation of seed production areas.

Materials and Methods

Sampling design and DNA extraction

A total of 316 leaf samples from 10 populations including four natural populations and six plantations were used to investigate the genetic diversity of natural and planted populations of teak in Myanmar (Fig. 1, Table 1). Thirty fresh leaf samples were collected from each of two plantations, Pyay and Pyinmana. The other 256 samples were collected from a provenance trial established at Pyinmana, Myanmar in 2007 representing four natural and four planted populations with seed sources from Bago, Phyu, Oktwin and Kanbalu for the natural populations, and from Taungoo, Paukkhaung, Nattalin and Thabeikkyin for the plantations. From this provenance trial, we collected leaves from 32 individuals per population and fresh leaves were collected and dried overnight at 80˚C and stored in silica gel at room temperature.

Total DNA was extracted from the 316 samples 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 1. Detailed information of teak populations investigated in this study

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
5	Taungoo	Plantation (70 yr)	32	19° 4'N	96°33'E	126	Provenance trial
6	Paukkhaung	Plantation (93 yr)	32	19° 7'N	95°46'E	293	Provenance trial
7	Nattalin	Plantation (32 yr)	32	18°29'N	95°54'E	288	Provenance trial
8	Thabeikkyin	Plantation (84 yr)	32	22°51'N	96° 7'E	437	Provenance trial
9	Pyay		30	19°59'N	95°59'E	36	Plantation (154 yr)
10	Pyinmana		30	18°47'N	95°13'E	193	Plantation (45 yr)

Samples of eight populations were collected from a 3-year-old provenance trial and two populations (Pyay and Pyinmana) were collected directly from plantations. Numbers in parenthesis indicate the age of plantations from which seeds were collected for provenance trail and leaves for this study.

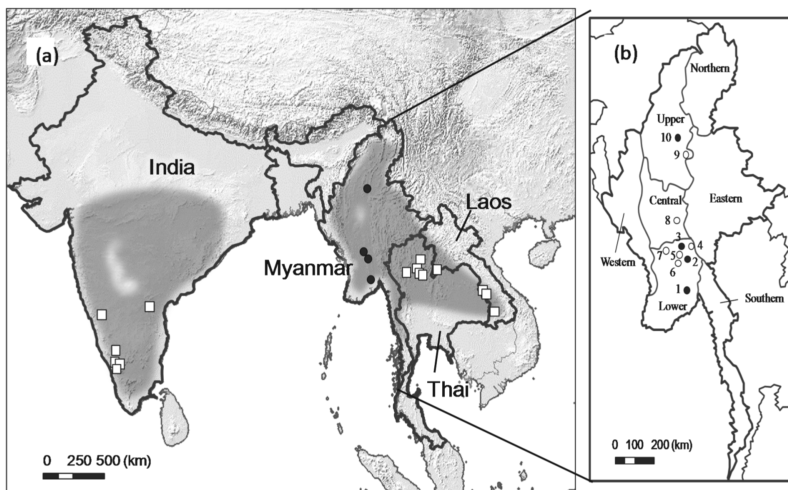

Fig. 1. Maps of (a) the distribution of teak in India, Myanmar, Laos and Thailand and (b) the locations of the ten sampled populations of teak in Myanmar. In (a), open squares indicate the locations of the teak populations from a previous study (FOFANA *et al.*, 2009) and closed circles represent Myanmar teak populations. In (b), closed and open circles refer to natural and planted populations, respectively. The shaded area shows the natural distribution of teak in its native regions.

Molecular and statistical analyses

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; Table 2). To compare the genetic diversity of Myanmar teak with other teak from its native regions, we must use the same number of markers. Therefore, 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'-CTGTGCCTTCTAGTTGCCAGCGCAAGAGCTGAAAGCAAC C-3' and 5'-GGCCGTTAGC ACTCCATTTA-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 (Table 2), 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'-ATGCTAGTTATTGCTCAGCGG-3'), reverse complement of BGH-R primer (VIC-5'-CTGTGCCTTCTAGTTGCCAGC-3'), reverse complement of pCold-R primer (NED-5'-TTGGGTGCAATGAGAATGCG-3') and pCold TF-F1 primer (PET-5'-CCACTTTCAACGAGCTGATG-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™ 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).

The following genetic diversity parameters for each locus over the four natural populations: 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}) (hereafter) 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).

The genetic diversity parameters such as *A*, *R*, H_E and fixation index, F_{IS} for each of ten populations including four natural populations and six plantations from Myanmar were estimated across 14 loci. Those ten populations were separated into two groups; natural populations and planted populations. At the natural population and plantation group levels, we measured the genetic diversity in terms of *R*, H_E, and fixation index; F_{IS} and F_{ST}. FSTAT ver. 2.9.3 software (GOUDET, 2001) was used to calculate the above mentioned genetic diversity parameters, the significance of the fixation index for each locus and population, and the significance of the differences of each parameter between the natural and planted population groups. 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. Lositan software (ANTAO *et al.*, 2008) was run at the 95% confidence level to detect outlier loci (i.e., loci that behave differently from the rest of genome) in the evaluation of the neutral genetic diversity of teak. Mega 5 (TAMURA *et al.*, 2011) was used for constructing UPGMA tree based on the Nei' s standard genetic distance (NEI *et al.*, 1983) for clustering analysis.

Table 2. Genetic diversity of four natural populations of Myanmar teak estimated by 15 simple sequence repeat (SSR) loci

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

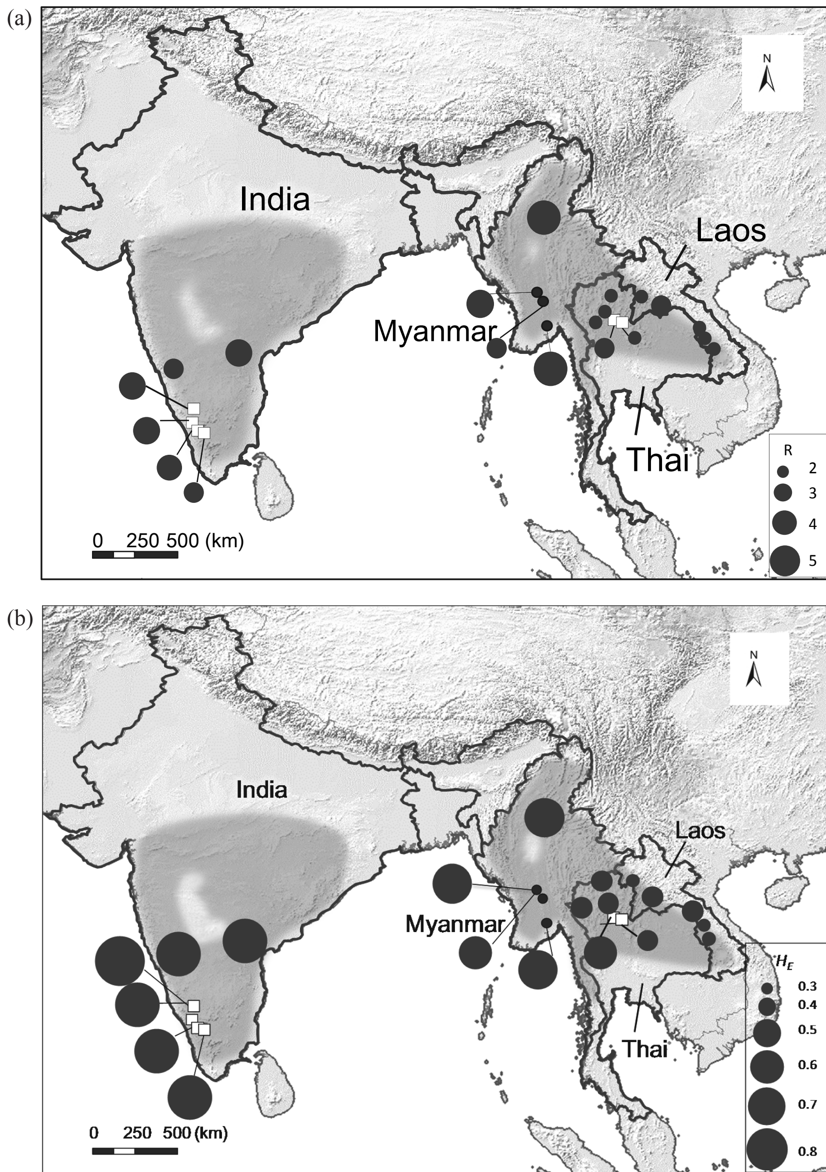

Fig. 2. Distribution of genetic diversity parameters of teak for (a) allelic richness and (b) expected heterozygosity. The diameter of the circles is proportional to the level of allelic richness or expected heterozygosity and numbers indicate values. Parameters for Myanmar were calculated in this study and those for Indian, Thai and Laotian populations are from FOFANA *et al*. (2009).

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). Out of 15 microsatellite loci, one locus (CIRAD1TeakB03) was detected as an outlier using positive selection in a stepwise mutation model and an infinite mutation model. This locus was excluded from the dataset of 10 populations of Myanmar teak for the evaluation of neutral genetic variation in teak.

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 3). Allelic richness of Myanmar teak was significantly higher than that of Indian, Thai and Laotian teak (Fig. 2a). 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. 2b).

Results of the genetic structure of the 10 populations of Myanmar teak using 14 microsatellite loci indicated similar levels of high genetic diversity in the Kanbalu natural population and in the Thabeikkyin plantation; however, the former population had the highest number of alleles and allelic richness (Table 4). The Phyu natural population had the lowest genetic diversity based on all parameters. High genetic diversity and significant F_{IS} values at the 5% significant level were detected in the Bago, Kanbalu, Paukkhaung and Thabeikkyin populations. As a group, natural populations had slightly lower genetic diversity (allelic richness R = 6.79, genetic diversity H_E = 0.599, fixation index F_{ST} = 0.074, F_{IS} = 0.084) than plantations (R = 7.03, H_E = 0.613, F_{IS} = 0.096, F_{ST} = 0.096). The permutation tests showed no significance differences for allelic richness, expected heterozygosity, genetic differentiation among populations and inbreeding coefficient values. The UPGMA tree depicted the genetic divergence of four populations (two natural populations: Oktwin and Phyu, and two plantations: Taungoo and Thabeikkyin) that were different from the other populations (Fig. 3).

Table 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. The weighted average values of H_E and R of populations from each country were 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 of each country was calculated. p: probabilities in R and H_E using 3,000 permutations. F_{ST}: genetic differentiation among populations within country.

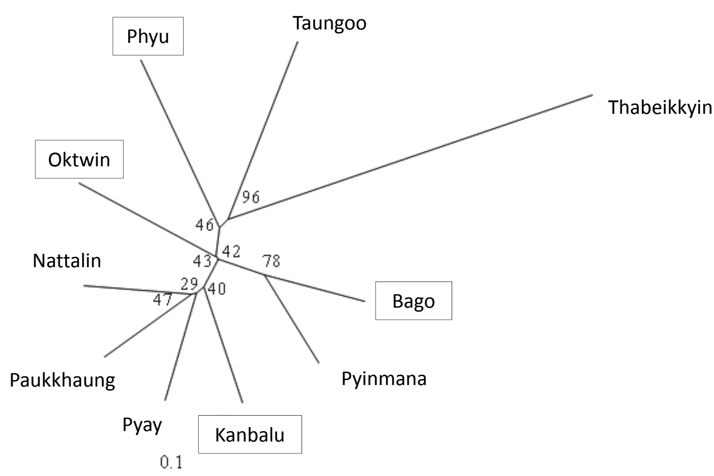

Fig. 3. UPGMA dendogram demonstrating the genetic relationships among four natural populations and six plantations of Myanmar teak. Branch length indicates the Nei's standard genetic distance. The numbers show the bootstrap values. Populations in the boxes represent natural populations.

Table 4. Genetic diversity parameters of each teak population in Myanmar based on 14 simple sequence repeat (SSR) loci

No.	Population	*N*	*A*	*R*	H_E	F_{IS}
	Natural populations					
1	Bago	32	8.07	7.51	0.632	0.109*
2	Phyu	32	5.36	5.09	0.537	0.010
3	Oktwin	32	6.57	6.19	0.582	0.044
4	Kanbalu	32	9.14	8.38	0.643	0.159*
	Planted populations					
5	Taungoo	32	6.71	6.27	0.592	0.052
6	Paukkhaung	32	8.79	8.14	0.635	0.116*
7	Nattalin	32	7.57	7.17	0.609	0.041
8	Pyay	32	6.29	6.09	0.614	-0.005
9	Pyinmana	30	7.71	7.40	0.584	0.137*
10	Thabeikkyin	30	7.57	7.14	0.645	0.225*

N: number of individuals, *A*: number of alleles, *R*: allelic richness, H_E: expected heterozygosity, F_{IS}: fixation index, *p* values were generated using 1 000 permutations.

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 (LAMII *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 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.

Our finding of similar levels of genetic diversity of natural populations compared to plantations is not consistent with our hypothesis. Similar results have been detected in many forest tree species including Pinus strobes (HAMELIN *et al.*, 1995), Cedrus atlantica (RENAU-MORATA *et al.*, 2005), Pinus brutia (ICGEN *et al.*, 2006) and Brazilian pine (STEFENON *et al.*, 2008). The

admixture effect increases genetic diversity (COMPAS *et al*., 2000). Thus, collecting and mixing seed from various sources before producing seedlings might lead to a population admixture and explain the similar level of genetic diversity of plantations. However, individual-based PCA analysis showed not much variation of genetic components between natural populations and plantations of teak in Myanmar (data not shown). The Wahlund principle tells us that the inbreeding coefficient due to barriers is always positive since the coefficient is defined with respect to the variance of gene frequency (YASUDA, 1967). Moreover, differences in allele frequencies among subpopulations can lead to deviation from the Hardy-Weinberg equilibrium in a population (HARTL and CLARK, 2007). Thus, mixing seeds from subpopulations might explain the significant F_{IS} values of the Bago, Kanbalu, Paukkhuang, Pyinmana and Thabeikkyin populations. Although the traditional seed collection method in Myanmar seems to be useful for maintaining the genetic resource of teak, the seed collection strategy would change to commercial strategies in the near future if mass seedling production becomes common in Myanmar. Therefore, genetic and climate information would be needed to formulate guidelines for seed transfer in Myanmar.

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. To sustain the genetic resources of teak, the Kanbalu natural population with high genetic diversity and the highest allelic richness should be prioritized for in situ conservation. Our study revealed that plantations in Myanmar have high genetic diversity and similar genetic composition as natural populations. These plantations, especially the Paukkhaung and Thabeikkyin populations with their high estimates of genetic diversity and genetic divergence, would play an important role for ex situ conservation to complement in situ conservation of teak. Furthermore, plantations with high genetic diversity and superior phenotypic trees might be candidates for seed production areas. For conservation of genetic resources of teak, plantations from Myanmar are important as well as natural populations.

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Summary

Teak (*Tectona grandis* L.f) is naturally distributed in four Asian countries; India, Myanmar, Thailand and Laos. Its genetic diversity in all of these regions other than Myanmar has previously been investigated using various molecular markers. In this study, four natural populations in Myanmar were studied using 15 microsatellite loci to compare the genetic diversity of teak from Myanmar with that of teak in its other native countries. In addition, we investigated six planted populations in Myanmar to determine whether teak plantations were genetically different from natural populations of teak. Allelic richness, a genetic diversity parameter, was significantly higher for populations in Myanmar (R = 4.91) compared to populations in India, Thailand and Laos (R = 4.20, 2.68 and 2.14, respectively). The expected heterozygosity of Myanmar teak (H_E = 0.609) was significantly lower than that of Indian teak (H_E = 0.748), but significantly higher than that of Thai and Laos teak (H_E = 0.450 and 0.356, respectively). A moderate level of genetic differentiation (F_{ST} = 0.079) between populations was observed in Myanmar teak. The preservation of natural teak populations in Myanmar will therefore be essential for global gene conservation due to their high genetic diversity and moderate genetic divergence. The genetic diversity parameters of planted populations were not significantly different from those of natural populations. In addition, a UPGMA dendrogram indicated that planted populations were not different from natural populations. We discuss the role of Myanmar teak as a genetic resource in its native regions and the roles of plantations in Myanmar in conservation and as seed production areas.

Keywords: Gene conservation, Native regions, Myanmar, *Tectona grandis*, Seed production areas

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マイクロサテライトマーカーで明らかにされた自生地域における チーク天然集団とミャンマーにおける人工林の遺伝的多様性

トウェ・トウェ・ウィン *1・渡辺敦史 *2・平尾知士 *3・磯田圭哉 *4・

石塚航 *5・後藤晋 *1

*1 東京大学大学院農学生命科学研究科附属演習林教育研究センター
*2 九州大学農学研究院森林環境科学
*3 独立行政法人　森林総合研究所材木育種センター
*4 独立行政法人　森林総合研究所材木育種センター関西育種場
*5 地方独立行政法人　北海道立総合研究機構林業試験場

要　旨

チークはインド，ミャンマー，タイ，ラオスのアジア 4 ヶ国に天然分布する。ミャンマー以外では，さまざまな分子マーカーを用いた既往研究がある。本研究では，3 ヶ国のチークの遺伝的多様性と比較するため，ミャンマーの 4 天然集団について 15 のマイクロサテライトマーカーを用いて遺伝的多様性を調べた。さらに，ミャンマーの 6 つの人工林を対象に，それらが天然集団と遺伝的に違うのかどうかを調べた。ミャンマーのアレリックリッチネスは 4.91 で，4.20 のインド，2.68 のタイ，2.14 のラオスと比べて有意に高かった。ヘテロ接合度の期待値では，ミャンマー（0.609）はインド（0.748）よりも有意に低く，タイ（0.450）とラオス（0.356）よりも有意に高かった。また，集団間の遺伝的分化度は 0.079 で中程度であった。ミャンマーの天然集団が高い遺伝的な多様性と中程度の遺伝的分化度を示したことから，世界的にもその保全が重要だと考えられた。人工林の遺伝的多様性は天然集団と有意な違いはなく，UPGMA 法によるクラスター解析でも両者に違いはなかった。本研究では，天然分布域の遺伝子資源としてのミャンマーのチークの役割とミャンマーにおける遺伝子保全や種子生産林としての人工林の役割を論じた。

キーワード：遺伝子保全，自生地域，ミャンマー，チーク，種子生産林