Genetic variation and origin of teak (*Tectona grandis* L.f.) native and introduced provenances

By G. H. HUANG[1)], K. N. LIANG[1),*)], Z. Z. ZHOU[1)], J. M. XU[1)] and H. M. MA[1)]

(Received 4th June 2014)

Abstract

A total of 420 individuals belonged to 18 native teak (*Tectona grandis* L.f.) provenances from all four distributed countries and 10 introduced provenances were analyzed to for genetic variation, structure and genetic origin using SSR markers. The unbiased gene diversity for each provenance ranged from 0.4692 to 0.8523 with a mean value 0.6612, showing high variation within teak provenances and variation in India provenances was highest than in other countries' provenances.

AMOVA analysis showed that the majority of variation existed within provenances (84.760%) and also substantial variation among countries (10.586%). As more as possible plus trees from large population should be selected or conserved in order to keep genetic variability for future improvement. Different countries populations should be preserved in the natural habitat or collected for *ex site* conservation with cooperation and region-wise strategies.

A Mantel test revealed significant correlation between genetic distances and geographic distances of teak provenances ($R = 0.7355$, $P < 0.001$). The cluster analyses by UPGMA, PCA and STRUCTURE methods gave very similar results, showing India provenances were firstly differentiated, and Laos provenances clustered with Thailand provenances, then introduced provenances and Myanmar provenances successively joined in the clusters. The introduced provenances no. 19, 20, 22, 23, 25, 27 and 28 appeared to be very closely linked to Laos provenances (especially no. 17) and Thailand provenances (especially no. 5 and 6), while provenances no. 21, 24, 26 may be originated from Myanmar provenance (especially no. 16).

[1)] Research Institute of Tropical Forestry, CAF, Guangzhou 510520, P. R. China.

[*)] Corresponding author: K. N. LIANG.
Tel.: +86-20-87032929, Fax: +86-20-87032929.
E-mail: hghmhx@163.com

Key words: Tectona grandis, genetic variation, genetic structure, genetic relationship, geographic origin.

Introduction

Teak (*Tectona grandis* L.f.) has a large natural distribution area in the tropical forests of India, Myanmar, Thailand and Laos (MOHANAN et al., 1997; KAOSA-ARD, 1981; KADAMBI, 1972), and has been introduced widely in the tropical regions since 19th century, especially in Asia, Africa and Central America due to its valuable timber for furniture making, carving and as an excellent building material around the world (WHITE, 1991).

Teak provenances (including all four native countries provenances and early planted provenances) were collected and systematic genetic breeding was carried out with international provenance trials since 1970. Marked variation in growth (KUANG and ZHENG, 1991; LIANG et al., 2011; LAI et al., 2011; BAGCHI et al., 1989; BEDELL, 1989; BENDALE et al., 2005) and timber characteristics (BHAT and PRIYA, 2004; KJÆR et al., 1999; PRIYA and BHAT, 1998, 1999; VARGHESE et al., 2000) between different provenances, as well as individual trees within provenances, has been observed. Genetic variation of teak provenances at molecular level has been reported by different countries used Isozyme (KJÆR et al., 1996), RAPD (PARTHIBAN et al., 2003), AFLP (SHRESTHA et al., 2005), SSR (FOFANA et al., 2009; VERHAEGEN et al., 2010; MINN et al., 2014) and ISSR (ANSARI et al., 2012) markers, most of the genetic diversity has been found within populations. HANSEN et al. (2015) firstly made a comprehensive study of the genetic resources of teak over its whole natural distribution range using SSR makers, the result supports that teak has its diversity center in India, and Myanmar provenances had higher genetic diversity than Thailand and Laos provenances. However, the field tests in China showed that

DOI:10.1515/sg-2015-0003
edited by Thünen Institute of Forest Genetics

some of Myanmar provenances have superior performance as cold resistive materials.

On the other hand, introduction of teak genetic material has been more than a century in different areas. Many plantations have been established using offspring of earlier local teak trees. Although some of the introduced provenances may exist abundant variation and could be used in the planting programs and breeding programs, the really problem is that there were no records of the origins of the seed which was first introduced to these places. This situation raises a number of problems for researchers because there is no information about genetic variability of the provenances and the genetic relationship between the origins, which is required for the estimation of heritability and genetic gain, and also for genetic resources management. In fact, the above mentioned problem can be solved after DNA analyses were introduced to the practice of

Table 1. – The geographic information of 28 teak provenances sampled for investigation.

Pro. No.	Seedlot No.	Provenance name	Country	Sample size	Latitude	Longitude	Altitude (m)
Native provenances							
1	3070	Sungam, Kerala	India	20	08° 00′ N	77° 20′ E	700
2	3071	Stuart Mt., Tamilnadu	India	20	10° 30′ N	76° 47′ E	640
3	3072	Masale, Valley, Mysore	India	20	11° 55′ N	76° 10′ E	823
4	3074	Virnoli, Mysore	India	20	15° 12′ N	74° 28′ E	488
5	1006	Ban Cham Pui, Lampang	Thailand	11	18° 29′ N	99° 49′ E	520
6	1007	Ban Makut Luang, Tak	Thailand	20	16° 49′ N	98° 36′ E	220
7	1008	Ban Pha Lai, Phrase	Thailand	16	18° 13′ N	99° 59′ E	200
8	1009	Ngao, Lampang	Thailand	12	18° 30′ N	99° 52′ E	350
9	82006	TIC2, Lampang	Thailand	10	18° 40′ N	99° 55′ E	350
10	82271	TIC1, Lampang	Thailand	10	18° 37′ N	99° 50′ E	350
11	1306	Mae Huat, Lampang	Thailand	5	18° 35′ N	99° 55′ E	350
12	1307	TIC SPA, Lampang	Thailand	7	18° 38′ N	99° 45′ E	350
13	1308	Mae Ta SPA, Prae	Thailand	8	18° 00′ N	99° 45′ E	175
14	SO	Letpangon	Myanmar	20	18° 19′ N	95° 50′ E	700
15	BG	Bago Yoma	Myanmar	20	19° 44′ N	96° 06′ E	
16	8601	Mandalay	Myanmar	7	21° 00′ N	95° 30′ E	
17	7004	Lak10/Louangpabang/LPB	Laos	20	19° 05′ N	102° 09′ E	433
18	3054	Pakse South II	Laos	10	15° 07′ N	105° 51′ E	120
Introduced provenances							
19	8014	Yaxian, Hainan	China	20	18° 25′ N	109° 50′ E	
20	7787	Jinghong, Yunan	China	8	22° 02′ N	100° 80′ E	
21	3078	Gambari	Nigeria	19	07° 10′ N	03° 52′ E	122
22	8204	Jianfeng Ledong, Hainan	China	18	18° 42′ N	108° 49′ E	20
23	8001	Baoting, Hainan	China	18	18° 64′ N	109° 70′ E	
24	8003	Longchuan, Yunnan	China	20	24° 33′ N	97° 96′ E	
25	8005	Baoting, Hainan	China	9	18°14′ N	109° 70′ E	
26	8010	Tunchang, Hainan	China	20	19° 36′ N	110° 10′ E	
27	83022	Ledong, Hainan	China	13	18° 73′ N	109° 17′ E	
28	83017	Guangzhou, Guangdong	China	19	23° 06′ N	113° 18′ E	

DOI:10.1515/sg-2015-0003
edited by Thünen Institute of Forest Genetics

genetic resource management. The markers are highly heritable, stable and exhibit sufficient polymorphism to discriminate genetic relationship and origin of different provenances without environmental interference (NARAYANAN et al., 2007). The SSR technology was found to be more reliable and adapted to our current objectives. Microsatellite markers are excellent for genetic characterization of plant material due to co-dominant, multi-allelic, reproducible and highly polymorphic nature as well as abundant distribution within the genome (POWELL et al., 1996; VARSHNEY et al., 2005). In previous reports with teak, the microsatellite teak bank (VERHAEGEN et al., 2005) and a database containing SSR markers for teak in its natural range have been reported (FOFANA et al., 2009), SSR markers have been used in teak for a variety of purposes, including the evaluation of genetic diversity (FOFANA et al., 2009, 2008) and origin identification (VERHAEGEN et al., 2010).

The purpose of this study is to (1) systematic evaluate and compare genetic variation of teak provenances from the whole four native countries with large range of distribution, covering the more typical and distinctly different types of environments, (2) identify the relationships among teak provenances and genetic origin of early planted provenances of potential useful. This work will provide valuable information for further genetic management and breeding program for teak.

Materials and Methods

Plant materials and DNA extraction

Leafs of 256 teak plants belong to 18 native provenances including whole four natural distribution countries were collected from international provenance trials in south of China. And 164 plants from 10 early planted provenances that have been identified as potential useful provenances were collected in this study. The geographic and ecological parameters of sampled provenances are shown in *Table 1*.

Genomic DNA of each plants were extracted from 0.02 g dry leaf (dried by silica gel) using Generay Biotech Co. (Shanghai, China), Ltd DNA plant kit following the manufacturer's instructions. DNA samples were diluted 5 times when used for amplification.

SSR genotyping

SSR genotyping analysis was carried out by the method of LI and GAN (2011) with some modifications because of primers specificity for teak. Primer pairs were synthesized by Generay Biotech Co. (Shanghai, China). Primers described in (VERHAEGEN et al., 2010, 2005) were screened in a preliminary test, 13 of 15 primer pairs gave good amplification with intensity fluorescence signal were employed in this study. Some more primer pairs were designed according to SSR sequences gained from EMBL web site and two were selected to substitute B02 and E06 in this study considering the same quantity loci will be more comparative with previous study. At the same time, T_m values and Mg^{2+} concentration of some primers have been optimized to get rid of other unwanted fluorescence signal after a preliminary test. The descriptions of 15 markers used to genotyping teak clones were shown in *Table 2*.

Data analysis

GenAlEx v6 (PEAKALL and SMOUSE, 2006) was used for data analysis. For each locus, polymorphic information content (*PIC*), observed heterozygosity (H_o) and expected heterozygosity (H_e) were estimated for each teak provenances. Number of alleles (*NA*), observed heterozygosity (H_o) and unbiased gene diversity (H_z, NEI, 1987) across all loci were calculated for each teak provenance. Allelic richness (A_R) for each provenance was calculated using the FSTAT ver. 2.9.3.2 to account for different sample sizes. Differentiation coefficient F-statistics (F_{st}) and gene flow (N_m) were only calculated for each pair of 18 native provenances assuming Hardy-Weinberg equilibrium. The mean *PIC*, *NA*, H_o, H_e, F_{st} over all loci and all provenances was calculated using Excel soft. *NA*, H_o and H_z across all loci were calculated for each four countries (countries) by Excel soft.

The data of 18 native provenances was used to an analysis of molecular variance (AMOVA) using this soft. The genetic variation among countries, among provenances within countries, among individuals within provenances and variation within individual were carried out. N_m, F_{st} and inbreeding coefficient (F_{is}, fixation index) across all loci and 18 native provenances were acquired with AMOVA analysis.

DOI:10.1515/sg-2015-0003
edited by Thünen Institute of Forest Genetics

Table 2. – Microsatellite locus, primer sequences, repeat motif, Mg^{2+} concentration in PCR reaction and annealing temperature used in this study.

Locus name	Accession no. EMBL Database	Primer sequence	Repeat motif	Mg^{2+} (mM)	T_m (°C)
CIRAD1TeakA06	AJ968929	F: 5′-CAAAACAAAACCAATAGCCAGAC-3′ R: 5′-TTTCATCATCATCATCAACATCC-3′	$(GA)_{15}$	2.0	53
CIRAD1TeakB03	AJ968930	F: 5′-AACAACCCCTCCTCTCTTCACTA-3′ R: 5′-CACTACCACTCATCATCAACACA-3′	$(TC)_5TG(TC)_8(AC)_5(N)_{65}(AC)_{14}$	2.0	51
CIRAD1TeakF05	AJ968931	F: 5′-CTTCTGCAACCCTTTTTCAC-3′ R: 5′-AGCCATATCTTCCTTTCTCT-3′	$(GA)_{20}GT(GA)_5$	2.0	53
CIRAD1TeakG02	AJ968932	F: 5′-TTAACGCCAAATCCCAAAG-3′ R: 5′-CACAAAGAGAACCGACGAG-3′	$(TC)_{10}$	2.0	51
CIRAD1TeakH10	AJ968933	F: 5′-CGATACCTGCGATGCGAAGC-3′ R: 5′-CGTTGAATACCCGATGGAGA-3′	$(TC)_{16}$	2.0	53
CIRAD1TeakB07	AJ968934	F: 5′-GGGTGCTGATGATTTTGAGTT-3′ R: 5′-CTAAGGAGTGAGTGGAGTTTT-3′	$(TC)_{14}$	2.0	53
CIRAD1TeakC03	AJ968935	F: 5′-AGGTGGGATGTGGTTAGAAGC-3′ R: 5′-AAATGGTCATCAGTGTCAGAA-3′	$(GA)_{17}$	2.0	51
CIRAD1TeakA11	AJ968936	F: 5′-AAACCATGACAGAAACGAATC-3′ R: 5′-TTGGGAATGGGAGGAGAAGT-3′	$(GA)_{16}$	2.0	53
CIRAD1TeakDa09	AJ968938	F: 5′-CTCGCTTCTTTCCACATT-3′ R: 5′-ATCATCGCGCATCGTCAA-3′	$(AC)_{10}$	2.0	51
CIRAD1TeakF01	AJ968940	F: 5′-GCTCTCCACCAACCTAAACAA-3′ R: 5′-AAAACGTCTCACCTTCTCACT-3′	$(TC)_{16}$	2.0	51
CIRAD1TeakDa12	AJ968941	F: 5′-CGCACACCAGTAGCAGTAGCC-3′ R: 5′-GCCGGAAAAAGAAAAACCAAA-3′	$(GA)_4(N)_5(GA)_{11}A(GA)_4$	2.0	51
CIRAD1TeakF02	AJ968942	F: 5′-CCGGTAAAAAGGTGTGTCA-3′ R: 5′-GAGTGGAAGTGCTAATGGA-3′	$(TC)_4(AC)_3(N)_{16}$	2.0	51
CIRAD1TeakH09	AJ968943	F: 5′-GCAAACCAACCTTACT-3′ R: 5′-CCGTTAGCACTCCATT-3′	$(GA)_{14}$	2.0	53
TgAC12	AJ511753	F: 5′-TGGTGCAGTTGCTACAGTTCCTGA-3′ R: 5′-CCCACCACATTACTTCTCACATGCCC-3′	$(AG)_{12}$	1.5	56
TgAC28	AJ511764	F: 5′-CCGATGCATGGCATGTTCTACCCA-3′ R: 5′-GGTACCATGATGGGGGACGGC-3′	$(CA)_{11}$	2.0	51

The unbiased measures of genetic identity (GID) and genetic distance (GD) among 28 provenances was calculated using NEI's (1978), and the two genetic distance matrixes were used to construct dendrogram using unweighted pair group method analysis (UPGMA) by SHAN in NTSYS software (ROHLF, 1998) and perform principle coordinate analysis (PCA) by GenAlEx v6. In addition, the geographic distance among native teak provenances was generated by their latitude and longitude using GenAlEx v6, and a Mantel test

DOI:10.1515/sg-2015-0003
edited by Thünen Institute of Forest Genetics

for correlation between genetic distances and geographic distances among native provenances was carried out by GenAlEx v6.

Finally, Bayesian cluster analyses were implemented in STRUCTURE ver. 2.3.1 (PRITCHARD et al., 2000) as an alternative approach to describing the genetic structure of provenances. Ten clustering runs were made for each *K* from 1 to 8, each with a burn-in time and run length of 100,000. To infer the true number of clusters (*K*), we used the delta *K* method developed by EVANO et al. (2005) as implemented in STRUCTURE HARVESTER program (EARL and VONHOLDT, 2012). The HARVESTER results were taken as input data using CLUMMP ver. 1.2.2 (JAKOBSSON and ROSENBERG, 2007) and DISTRUCT software (ROSENBERG, 2004) was used for better graphical presentation.

Results

Genetic variation within native and introduced provenances

For each locus, the *PIC* values for every teak provenance were shown in *Table 3*. The microsatellite loci have different detecting capability for teak provenances. The *PIC* value across all SSR loci for each provenance ranged from 0.39 for provenance eight to 0.81 for provenance three, the *PIC* value over all provenances for each of 15 SSR loci ranged from 0.22 for G02 to 0.81 for B07. The average *PIC* value across all SSR loci and all provenances was 0.56. This study detected null alleles at four loci. However, all loci were used for analyses because frequencies of null alleles (from 0.026 to 0.167) lower than 0.20 and could not be significantly influenced for H_E estimation according to MUZZALUPO et al. (2014).

The provenances variation based on 15 Microsatellite markers were shown in *Table 4*. The mean numbers of alleles (*NA*) for each provenance were generated from 3.27 (at provenance 8 and provenance 11) to 11.60 (at provenance 3) with an average of 6.04 alleles per provenance. The unbiased gene diversity (H_z) ranged from 0.4692 (provenance 8) to 0.8523 (provenance 3), with a mean value of 0.6612 over all the 15 microsatellite loci. In addition, the observed heterozygosity (H_o) ranged from 0.5302 at provenance 7 to 0.7451 at provenance 3, with a mean value of 0.6444. At the country level, teak heterozygosity was found clearly higher in India provenances than in Thailand, Myanmar or Laos provenances. Teak heterozygosity of India provenances > Myanmar provenances > Thailand provenances > Laos provenances in this study which consistent with HANSEN et al. (2015). The allelic richness (A_R) ranged from 2.3030 in Thailand provenance no. 8 to 4.3951 in India provenance no. 3 (*Table 4*). Allelic richness was also significantly higher in

Table 3. – The polymorphic information content (*PIC*) values based on 28 teak provenances for each locus.

Locus /Pro.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	Mean
AJ968929	0.87	0.88	0.82	0.87	0.39	0.32	0.46	0.36	0.45	0.44	0.37	0.53	0.32	0.41	0.00	0.53	0.44	0.53	0.52	0.39	0.63	0.69	0.58	0.56	0.54	0.5[illegible]	0.33	0.62	0.48
AJ968930	0.58	0.49	0.80	0.5[illegible]	0.55	0.55	0.33	0.27	0.37	0.50	0.41	0.46	0.45	0.55	0.42	0.69	0.53	0.30	0.72	0.55	0.81	0.64	0.59	0.58	0.57	0.59	0.59	0.54	0.51
AJ968931	0.84	0.77	0.86	0.67	0.42	0.66	0.70	0.09	0.61	0.65	0.55	0.55	0.46	0.65	0.48	0.60	0.40	0.65	0.49	0.24	0.84	0.64	0.66	0.78	0.52	0.80	0.65	0.41	0.56
AJ968932	0.33	0.44	0.4[illegible]	0.40	0.08	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.12	0.21	0.22	0.60	0.00	0.00	0.33	0.40	0.40	0.33	0.21	0.28	0.39	0.74	0.15	0.34	0.22
AJ968933	0.88	0.83	0.90	0.9[illegible]	0.65	0.86	0.72	0.63	0.60	0.74	0.68	0.77	0.85	0.81	0.76	0.84	0.80	0.81	0.84	0.72	0.83	0.79	0.70	0.77	0.67	0.77	0.78	0.89	0.75
AJ968934	0.89	0.84	0.90	0.84	0.91	0.89	0.88	0.75	0.81	0.73	0.80	0.79	0.82	0.83	0.76	0.90	0.78	0.77	0.92	0.86	0.89	0.80	0.87	0.90	0.79	0.9[illegible]	0.79	0.88	0.81
AJ968935	0.85	0.55	0.78	0.68	0.73	0.84	0.79	0.70	0.79	0.81	0.60	0.62	0.70	0.31	0.56	0.79	0.53	0.77	0.75	0.77	0.89	0.76	0.80	0.67	0.74	0.73	0.57	0.79	0.68
AJ968936	0.89	0.89	0.93	0.68	0.47	0.48	0.43	0.16	0.40	0.10	0.27	0.12	0.21	0.45	0.70	0.60	0.14	0.27	0.41	0.57	0.84	0.49	0.33	0.52	0.32	0.54	0.35	0.52	0.44
AJ968938	0.59	0.50	0.80	0.60	0.47	0.56	0.44	0.38	0.29	0.45	0.38	0.38	0.43	0.60	0.47	0.38	0.70	0.58	0.44	0.26	0.39	0.33	0.45	0.22	0.21	0.54	0.43	0.34	0.43
AJ968940	0.86	0.84	0.88	0.89	0.86	0.85	0.75	0.56	0.68	0.50	0.65	0.76	0.67	0.74	0.61	0.62	0.63	0.82	0.68	0.74	0.86	0.80	0.73	0.80	0.69	0.77	0.79	0.78	0.71
AJ968941	0.72	0.68	0.8[illegible]	0.80	0.66	0.66	0.53	0.50	0.72	0.67	0.60	0.72	0.78	0.74	0.59	0.62	0.73	0.68	0.70	0.63	0.76	0.70	0.75	0.48	0.67	0.59	0.66	0.55	0.64
AJ968942	0.91	0.86	0.77	0.80	0.27	0.57	0.36	0.30	0.29	0.53	0.58	0.45	0.40	0.55	0.38	0.67	0.36	0.64	0.70	0.61	0.76	0.69	0.58	0.70	0.70	0.6[illegible]	0.57	0.72	0.55
AJ968943	0.78	0.71	0.77	0.73	0.50	0.65	0.58	0.52	0.57	0.38	0.38	0.58	0.38	0.51	0.55	0.55	0.65	0.58	0.55	0.38	0.44	0.49	0.55	0.71	0.54	0.53	0.57	0.54	0.53
A1511753	0.91	0.85	0.9[illegible]	0.82	0.66	0.62	0.56	0.32	0.35	0.27	0.27	0.38	0.30	0.31	0.63	0.64	0.56	0.49	0.74	0.68	0.87	0.68	0.72	0.82	0.64	0.52	0.78	0.66	0.57
A1511764	0.74	0.50	0.85	0.74	0.77	0.84	0.49	0.37	0.50	0.50	0.47	0.38	0.27	0.54	0.31	0.62	0.52	0.60	0.53	0.66	0.83	0.52	0.42	0.71	0.57	0.74	0.58	0.66	0.54
Mean	0.78	0.71	0.8[illegible]	0.73	0.56	0.63	0.53	0.39	0.49	0.48	0.46	0.50	0.48	0.55	0.50	0.62	0.52	0.57	0.62	0.56	0.74	0.62	0.60	0.63	0.57	0.66	0.57	0.62	0.56

DOI:10.1515/sg-2015-0003
edited by Thünen Institute of Forest Genetics

the India provenances. The H_e, H_o, NA and A_R values over provenances of India region were 0.8056, 0.7114, 10.2 and 4.0606, respectively.

Genetic structure and differentiation among native provenances

Differentiation coefficient F-statistics (F_{st}) and gene flow (N_m) calculated for each pair of native provenances was shown in *Table 5*. The F_{st} values among provenances were ranged from 0 (between provenances 9 and 10) to 0.236 (between provenances 8 and 15).

An analysis of molecular variation (AMOVA) for native provenances showed variance component 84.760% among individuals within provenances, 4.654% among provenances, and remain 10.586% among four countries, both were highly significant ($P < 0.001$). A high gene flow $N_m = 1.458$ ($N_m = [(1/F_{st}) - 1]/4$) and

Table 4. – The diversity parameters of unbiased gene diversity (H_z), observed heterozygosity (H_o), number of alleles (NA) with their standard deviations (SD) and allelic richness (A_R) across all loci for each teak provenance.

Pro. No.	H_z	H_z SD	H_o	H_o SD	NA	NA SD	A_R
1	0.8223	0.0383	0.7206	0.0263	11.47	5.19	4.2075
2	0.7627	0.0387	0.6510	0.0292	8.67	3.50	3.7771
3	0.8523	0.0297	0.7451	0.0260	11.60	4.40	4.3951
4	0.7852	0.0323	0.7287	0.0272	9.07	3.81	3.8628
5	0.6269	0.0599	0.5826	0.0403	5.67	3.22	3.1288
6	0.6730	0.0606	0.6328	0.0283	8.00	3.51	3.3732
7	0.5891	0.0596	0.5302	0.0330	6.00	3.07	2.9208
8	0.4692	0.0631	0.5366	0.0401	3.27	1.67	2.3030
9	0.5766	0.0603	0.6016	0.0421	4.13	2.26	2.7693
10	0.5717	0.0645	0.6722	0.0407	4.07	2.34	2.7433
11	0.5846	0.0589	0.6567	0.0556	3.27	1.49	2.7115
12	0.6100	0.0651	0.6095	0.0506	3.67	2.02	2.8208
13	0.5676	0.0623	0.5675	0.0475	4.13	2.42	2.7912
14	0.6124	0.0491	0.5749	0.0313	5.40	3.20	2.9104
15	0.5608	0.0577	0.5716	0.0307	4.47	1.85	2.6588
16	0.7512	0.0290	0.6444	0.0486	4.87	2.36	3.5097
17	0.5801	0.0627	0.5416	0.0308	4.93	2.46	2.7801
18	0.6453	0.0625	0.6000	0.0408	4.40	2.20	3.1109
19	0.6778	0.0430	0.6628	0.0294	6.80	3.47	3.2618
20	0.6637	0.0503	0.6345	0.0477	4.60	2.13	3.1401
21	0.7824	0.0458	0.6721	0.0304	9.33	3.24	4.0139
22	0.6852	0.0406	0.7028	0.0291	5.93	1.83	3.2389
23	0.6617	0.0474	0.7476	0.0284	5.87	2.72	3.1155
24	0.6870	0.0509	0.6843	0.0282	6.93	3.53	3.3350
25	0.6699	0.0436	0.6946	0.0445	4.20	1.37	3.0900
26	0.7260	0.0295	0.6871	0.0292	6.13	3.58	3.3934
27	0.6408	0.0530	0.6935	0.0360	5.40	2.13	3.0719
28	0.6788	0.0435	0.6976	0.0287	6.87	3.72	3.2494
Average	0.6612	0.0501	0.6444	0.0357	6.04	2.81	3.2030

DOI:10.1515/sg-2015-0003
edited by Thünen Institute of Forest Genetics

Table 5. – The differentiation coefficient F-statistics (F_{st}, below diagonal) and gene flow (N_m, above diagonal) values among 18 native teak provenances based on 15 SSR loci.

Pro. No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	***	20.322	9.030	4.113	1.828	1.676	1.349	1.148	1.517	1.358	1.316	1.494	1.397	1.440	1.030	2.430	1.265	1.394
2	0.012	***	5.959	3.972	1.359	1.309	1.032	0.944	1.240	1.101	0.992	1.192	1.086	1.225	0.923	1.801	1.094	1.143
3	0.027	0.040	***	5.892	2.089	2.048	1.587	1.275	1.728	1.522	1.444	1.705	1.480	1.681	1.341	2.910	1.500	1.734
4	0.057	0.059	0.041	***	1.504	1.419	1.164	1.049	1.189	1.117	1.087	1.338	1.061	1.196	0.927	1.946	1.156	1.227
5	0.120	0.155	0.107	0.143	***	3.530	4.435	4.013	2.940	3.108	3.256	3.403	3.032	1.526	0.950	2.471	3.271	6.137
6	0.130	0.160	0.109	0.150	0.066	***	9.481	2.180	3.016	3.979	2.745	4.773	3.700	2.172	1.453	2.877	1.836	3.832
7	0.156	0.195	0.136	0.177	0.053	0.026	***	4.080	4.302	8.197	5.342	6.417	9.483	1.577	1.087	2.237	2.074	3.851
8	0.179	0.209	0.164	0.192	0.059	0.103	0.058	***	2.764	3.526	4.940	4.263	2.680	1.159	0.809	1.744	2.812	3.117
9	0.142	0.168	0.126	0.174	0.078	0.077	0.055	0.083	***	0.000	4.516	4.186	7.426	1.877	1.198	1.949	2.255	2.647
10	0.155	0.185	0.141	0.183	0.074	0.059	0.030	0.066	0.000	***	5.720	10.429	11.153	1.943	1.091	1.968	2.544	3.704
11	0.160	0.201	0.148	0.187	0.071	0.083	0.045	0.048	0.052	0.042	***	8.897	5.360	1.269	0.824	1.861	2.152	3.619
12	0.143	0.173	0.128	0.157	0.068	0.050	0.038	0.055	0.056	0.023	0.027	***	8.685	1.987	1.106	2.383	2.991	5.490
13	0.152	0.187	0.145	0.191	0.076	0.063	0.026	0.085	0.033	0.022	0.045	0.028	***	1.945	0.969	2.278	1.915	2.606
14	0.148	0.169	0.129	0.173	0.141	0.103	0.137	0.177	0.118	0.114	0.165	0.112	0.114	***	4.507	2.179	1.277	1.444
15	0.195	0.213	0.157	0.212	0.208	0.147	0.187	0.236	0.173	0.186	0.233	0.184	0.205	0.053	***	1.162	0.989	1.057
16	0.093	0.122	0.079	0.114	0.092	0.080	0.101	0.125	0.114	0.113	0.118	0.095	0.099	0.103	0.177	***	1.495	1.916
17	0.165	0.186	0.143	0.178	0.071	0.120	0.108	0.082	0.100	0.089	0.104	0.077	0.115	0.164	0.202	0.143	***	17.354
18	0.152	0.180	0.126	0.169	0.039	0.061	0.061	0.074	0.086	0.063	0.065	0.044	0.088	0.148	0.191	0.115	0.014	***

Table 6. – Nei's (1978) unbiase measures of genetic identity (above diagonal) and genetic distance (below diagonal) for 28 teak provenances.

Pro.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
1	***	0.96	0.88	0.84	0.56	0.55	0.52	0.46	0.53	0.47	0.46	0.45	0.48	0.47	0.44	0.53	0.48	0.49	0.55	0.54	0.66	0.53	0.51	0.57	0.56	0.36	0.52	0.54
2	0.04	***	0.82	0.83	0.44	0.45	0.39	0.31	0.43	0.36	0.34	0.32	0.35	0.37	0.36	0.42	0.39	0.40	0.43	0.42	0.58	0.38	0.40	0.47	0.40	0.27	0.41	0.44
3	0.13	0.20	***	0.87	0.62	0.65	0.60	0.52	0.57	0.52	0.50	0.52	0.53	0.53	0.52	0.60	0.53	0.57	0.59	0.58	0.79	0.57	0.56	0.62	0.58	0.46	0.52	0.58
4	0.17	0.19	0.14	***	0.56	0.57	0.52	0.47	0.50	0.46	0.48	0.50	0.45	0.47	0.44	0.59	0.49	0.52	0.56	0.52	0.72	0.49	0.54	0.56	0.54	0.37	0.51	0.52
5	0.58	0.83	0.47	0.57	***	0.85	0.90	0.89	0.83	0.84	0.87	0.83	0.84	0.64	0.56	0.71	0.88	0.94	0.85	0.85	0.76	0.85	0.80	0.77	0.82	0.63	0.83	0.79
6	0.61	0.80	0.43	0.56	0.17	***	0.96	0.80	0.84	0.89	0.82	0.89	0.87	0.79	0.76	0.78	0.76	0.89	0.85	0.70	0.81	0.86	0.82	0.81	0.80	0.71	0.69	0.73
7	0.66	0.94	0.51	0.66	0.10	0.04	***	0.91	0.90	0.96	0.92	0.95	0.96	0.72	0.68	0.77	0.81	0.91	0.83	0.74	0.78	0.84	0.80	0.79	0.79	0.66	0.76	0.71
8	0.78	1.07	0.66	0.75	0.12	0.22	0.09	***	0.85	0.89	0.93	0.89	0.85	0.59	0.54	0.72	0.87	0.90	0.77	0.77	0.68	0.67	0.69	0.70	0.71	0.53	0.77	0.63
9	0.64	0.83	0.56	0.69	0.19	0.17	0.10	0.17	***	0.99	0.91	0.89	0.95	0.71	0.67	0.70	0.78	0.83	0.74	0.76	0.74	0.81	0.73	0.72	0.71	0.55	0.78	0.73
10	0.75	1.02	0.66	0.77	0.17	0.12	0.04	0.12	0.01	***	0.93	0.94	0.95	0.69	0.64	0.71	0.81	0.89	0.78	0.67	0.70	0.80	0.77	0.72	0.74	0.61	0.78	0.68
11	0.77	1.09	0.69	0.74	0.14	0.20	0.09	0.07	0.10	0.07	***	0.97	0.91	0.65	0.57	0.70	0.81	0.88	0.77	0.75	0.68	0.80	0.73	0.67	0.69	0.56	0.78	0.67
12	0.81	1.13	0.65	0.70	0.18	0.12	0.05	0.12	0.12	0.06	0.03	***	0.91	0.71	0.65	0.72	0.83	0.93	0.79	0.68	0.72	0.78	0.78	0.75	0.74	0.59	0.79	0.66
13	0.72	1.04	0.64	0.79	0.17	0.14	0.04	0.16	0.06	0.05	0.06	0.06	***	0.74	0.64	0.73	0.76	0.84	0.75	0.70	0.71	0.83	0.76	0.71	0.73	0.64	0.76	0.70
14	0.75	1.00	0.63	0.76	0.45	0.24	0.33	0.53	0.34	0.37	0.43	0.34	0.30	***	0.95	0.71	0.57	0.66	0.73	0.63	0.69	0.70	0.72	0.69	0.76	0.61	0.59	0.73
15	0.83	1.03	0.66	0.82	0.59	0.28	0.39	0.62	0.40	0.45	0.57	0.43	0.45	0.05	***	0.63	0.54	0.61	0.69	0.60	0.73	0.67	0.68	0.70	0.71	0.59	0.56	0.67
16	0.64	0.86	0.51	0.53	0.34	0.25	0.26	0.33	0.36	0.35	0.36	0.32	0.31	0.34	0.46	***	0.65	0.72	0.78	0.74	0.71	0.70	0.69	0.78	0.76	0.68	0.64	0.74
17	0.74	0.95	0.63	0.72	0.13	0.28	0.21	0.14	0.24	0.21	0.21	0.19	0.27	0.56	0.62	0.43	***	0.99	0.85	0.82	0.69	0.76	0.82	0.75	0.80	0.55	0.89	0.71
18	0.72	0.93	0.57	0.66	0.06	0.12	0.10	0.11	0.19	0.12	0.13	0.07	0.18	0.42	0.50	0.33	0.01	***	0.86	0.81	0.76	0.82	0.86	0.80	0.81	0.65	0.86	0.74
19	0.60	0.85	0.53	0.57	0.16	0.17	0.19	0.26	0.29	0.25	0.34	0.24	0.28	0.32	0.37	0.25	0.17	0.15	***	0.85	0.82	0.88	0.93	0.91	1.01	0.75	0.85	0.83
20	0.61	0.86	0.55	0.65	0.16	0.35	0.31	0.26	0.28	0.40	0.29	0.39	0.35	0.46	0.51	0.30	0.19	0.22	0.16	***	0.85	0.79	0.74	0.83	0.82	0.60	0.84	0.82
21	0.41	0.54	0.24	0.33	0.27	0.21	0.25	0.38	0.30	0.36	0.39	0.33	0.34	0.37	0.32	0.34	0.37	0.28	0.19	0.16	***	0.80	0.80	0.85	0.82	0.70	0.73	0.76
22	0.64	0.97	0.56	0.71	0.16	0.15	0.17	0.40	0.21	0.22	0.22	0.24	0.19	0.36	0.40	0.35	0.28	0.19	0.13	0.24	0.22	***	0.91	0.82	0.90	0.73	0.78	0.83
23	0.68	0.91	0.57	0.62	0.23	0.20	0.22	0.37	0.31	0.26	0.32	0.24	0.27	0.34	0.38	0.37	0.20	0.15	0.07	0.30	0.22	0.10	***	0.84	0.93	0.74	0.86	0.80
24	0.55	0.75	0.47	0.59	0.27	0.22	0.23	0.35	0.32	0.33	0.40	0.29	0.34	0.37	0.36	0.25	0.29	0.22	0.09	0.18	0.17	0.20	0.18	***	0.89	0.77	0.81	0.88
25	0.59	0.92	0.55	0.61	0.20	0.22	0.24	0.34	0.35	0.29	0.37	0.30	0.31	0.28	0.34	0.28	0.23	0.21	0.00	0.19	0.20	0.11	0.07	0.11	***	0.78	0.83	0.87
26	1.04	1.32	0.78	0.99	0.47	0.35	0.41	0.64	0.59	0.49	0.57	0.53	0.45	0.50	0.53	0.38	0.59	0.43	0.29	0.51	0.36	0.31	0.30	0.26	0.25	***	0.60	0.79
27	0.66	0.89	0.66	0.68	0.19	0.37	0.28	0.26	0.25	0.25	0.25	0.23	0.27	0.53	0.58	0.45	0.12	0.15	0.16	0.17	0.32	0.24	0.16	0.21	0.19	0.51	***	0.86
28	0.61	0.82	0.54	0.66	0.23	0.31	0.35	0.46	0.32	0.38	0.41	0.41	0.35	0.31	0.39	0.30	0.35	0.31	0.18	0.20	0.27	0.19	0.22	0.13	0.14	0.24	0.15	***

DOI:10.1515/sg-2015-0003
edited by Thünen Institute of Forest Genetics

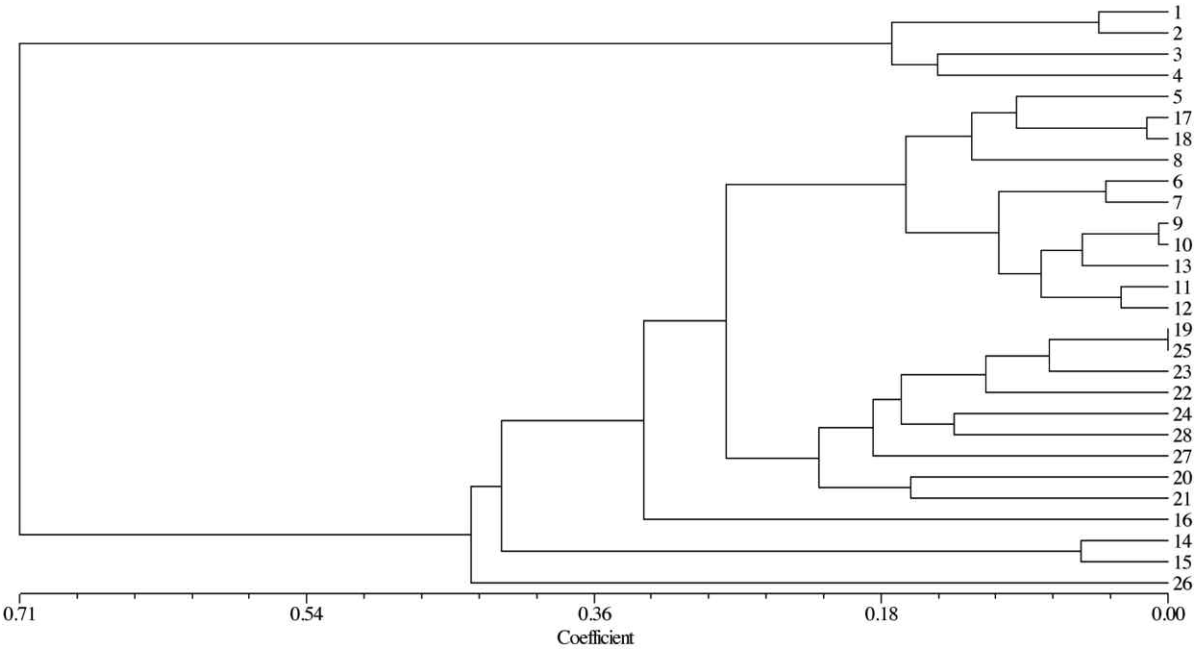

Figure 1. – Dendrogram generated by UPGMA clustering for 28 native and introduced provenances of teak, based on the NEI's (1978) genetic distances.

moderate coefficient of genetic differentiation $F_{st} = 0.146$ were detected among native teak provenances.

Relationships among provenances

Genetic identity and genetic distances for each pair of provenances were calculated and shown in *Table 6*. The genetic distance ranged from 0 (between provenance 19 and provenance 25) to 1.32 (between provenance 2 and provenance 26). The genetic identity ranged from 0.27 (between provenance 2 and provenance 26) to 1.01 (between provenance 19 and provenance 25).

The UPGMA clusters analysis based on NEI's (1978) unbiased genetic distances for all 28 provenances (*Figure 1*) was performed to further show the genetic relationships among provenances. The cluster showing two groupings could be recognized from the dendrogram: the first group consisted of India provenances

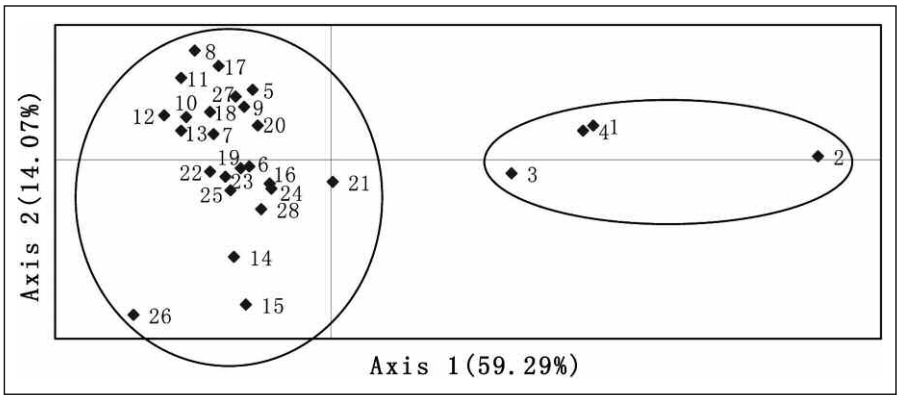

Figure 2. – The principle coordinate analysis (PCA, principal coordinates axis 1 versus axis 2) for 28 native and introduced provenances of teak, based on the NEI's (1978) genetic distances.

DOI:10.1515/sg-2015-0003
edited by Thünen Institute of Forest Genetics

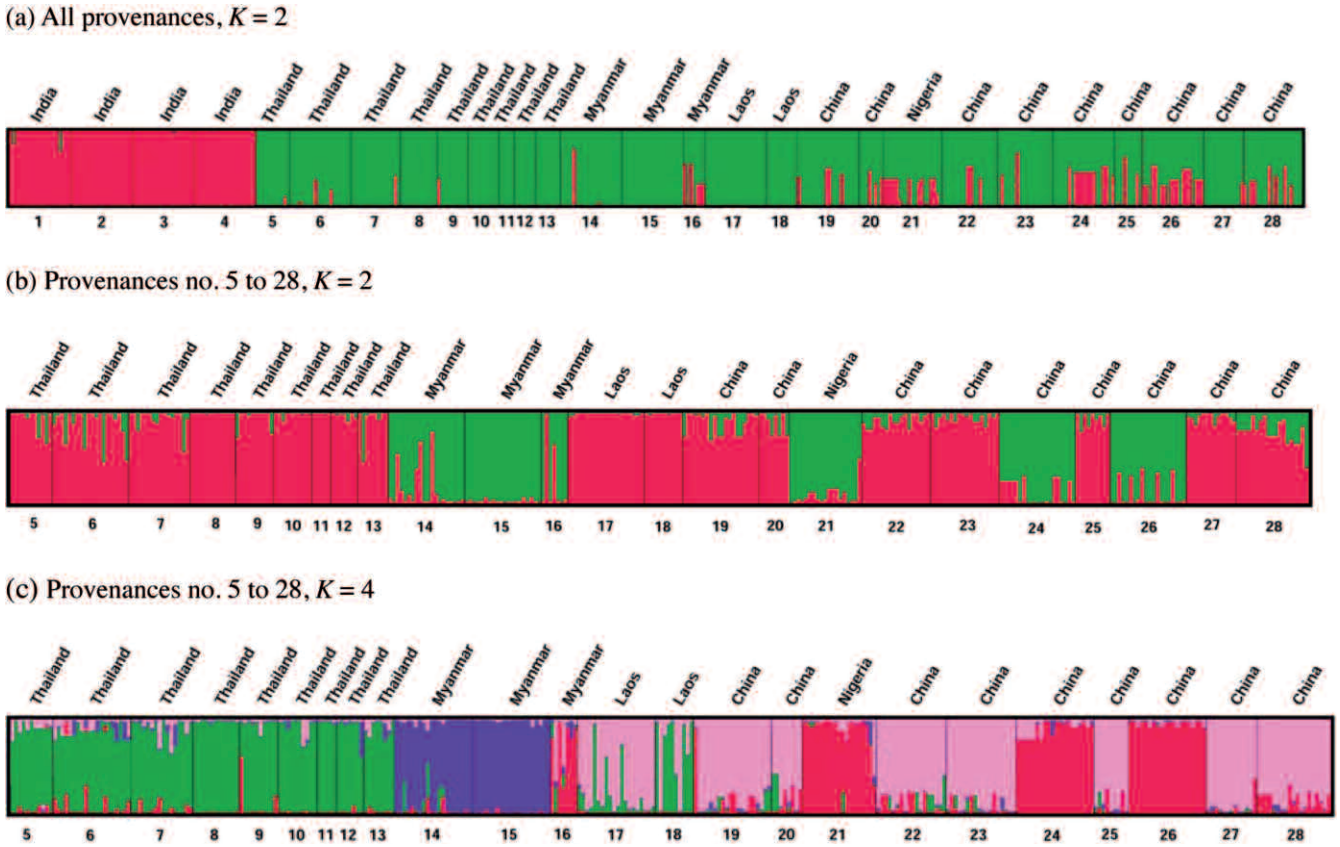

Figure 3. – lllustration of results from STRUCTURE analysis. a: STRUCTURE analysis for all 28 provenances with $K=2$, b: second round of STRUCTURE analysis for provenances no. 5 to 28 with $K=2$, c: second round of STRUCTURE analysis for provenances no. 5 to 28 with $K=4$.

no. 1 to 4, while the second group comprised remain provenances. For subdivision in the second group, Laos provenances firstly clustered with Thailand provenances, then the ten introduced provenances (except no. 26) together clustered with Laos and Thailand provenances, at last Myanmar provenances joined in the second group. A Mantel test with 999 random permutations revealed high correlation between pairwise genetic distances and geographical distances among native provenances (correlation coefficient of $R = 0.7355$, $P < 0.001$). The principle coordinate analysis (PCA, *Figure 2*) further respected and confirmed UPGMA cluster. The two PCA axes explained 73.36% of the overall genetic variability. The first axis and second axis explained 59.29% and 14.07% of genetic variability, respectively.

When analyzing all 28 provenances with STURCTURE, the Evanno method gave a highest estimation $K = 2$ for the number of clusters (*Figure 3a*), the cluster 1 consisted of India provenances no. 1 to 4, while the cluster 2 comprised remain provenances, corresponding to the UPGMA clusters.

The highest delta $K = 2$ (*Figure 3b*) was observed for sub-clusters analysis for provenances no. 5 to 28. Sub-cluster 1 consisting Thailand provenances, Laos provenances and introduced provenances no. 19, 20, 22, 23, 25, 27 and 28. Sub-cluster 2 consisting Myanmar provenance and introduced provenances no. 21, 24 and 26. Thereafter, likelihood and posterior probability increased for $K = 4$ (*Figure 3c*), after which the variance of log-likelihood among runs became plateau when for sub-clusters analysis for provenances no. 5 to 28. Sub-cluster 1 consisting Thailand provenances and Laos provenance no. 18. Sub-cluster 2 consisting Myanmar provenances no. 14 and 15, Sub-cluster 3 consisting Myanmar provenance no. 16 and introduced provenances no. 21, 24 and 26, Sub-cluster 4 consisting Laos provenance no. 17 and remain seven introduced provenances.

DOI:10.1515/sg-2015-0003
edited by Thünen Institute of Forest Genetics

Discussion

This study demonstrates clearly that this "package" of technologies based on fluorescence-dUTP and ABI 3130xl genetic analyzer was very effective to analysis teak resources. The 15 microsatellite loci selected in this study were high power of discrimination markers. Most microsatellite loci displayed high *PIC* values, enabling the high variation detection of teak provenances analyzed. The *PIC* results indicated which of the 11 loci could be classified as highly informative (*PIC* > 0.5). Four loci as less informative marker (*PIC* < 0.5), as indicated by ROUBOS et al. (2010). Therefore, the high *PIC* levels of eleven loci (*PIC* > 0.5) in our analyses suggested that this combination of SSR markers is a reliable tool for variation analysis of teak germplasm resource.

This work provides a deep insight into the genetic variation of teak provenances including all four native countries. Different parameters (*NA*, H_z and H_o) showed a broad genetic variation within provenances sampled and suggesting a bright future in teak improvement. The *NA* (6.04) and H_o (0.6444) were higher than isozyme investigation for nine teak provenances (2.8 and 0.32, respectively) (KERTADIKARA and PRAT, 1995) and higher than SSR investigation for 17 native populations (4.6514 and 0.5124, respectively) (FOFANA et al., 2009). Mean H_z (0.6612) was higher than the highest NEI's genetic diversity (0.40) reported by ANSARI et al. (2012) for 29 India teak populations using ISSR markers. The mean H_z of India, Thailand, Myanmar and Laos provenances were 0.7113, 0.5988, 0.5970 and 0.5708, respectively, suggesting India provenances have highest variation and should be managed as the diversity center of teak. The results were consistent with former reports (SHRESTHA et al., 2005; FOFANA et al., 2009, 2008; NICODEMUS et al., 2003; HANSEN et al., 2015). The H_o (0.597) of three Myanmar provenances in this study was little higher than MINN et al. (2014, 0.564) but lower than HANSEN et al. (2015, 0.700) analyzed Myanmar provenances by SSR. As found by MINN et al. (2014), teak can maintain high genetic diversity in adult provenances or seedling provenances, in undisturbed or disturbed provenances. In fact, teak is found naturally in moist and dry mixed, deciduous forests below 1200 m in elevation and grows on a variety of sites with very different ecological conditions. Genetic variation between stands is therefore possible as a result of adaptation to different environmental conditions. On the other hand, teak is a mainly outcrossing species. The outcrossing rates are high and range between 89% and 95% (KJÆR and SUANGTHO, 1995). It may be hypothesized that high gene diversity and observed heterozygosity in teak provenances are maintained by early exclusion of self material, and by progressive selection against homozygous genotypes during stand life. Consanguineous trees were suppressed and only most heterozygous genotypes attain the reproductive stage. As a result, seedlings from heterozygous seeds dominate the population at maturity, thereby tremendously increasing the intra-population gene diversity. On the other hand, the allelic richness (A_R) of provenances investigated in this study were lower than Myanmar provenances in MINN et al. report (2014). This may be mainly due to different provenances and sample sizes. There were more than 40 individuals in MINN et al. study (2014) and lower than 20 individuals in the present study, and the result of FSTAT software illustrated that A_R is independent of sample size but based on minor sample size of collected provenances in a study.

It is interesting to find out that some planted provenances especial provenance no. 21 was higher in genetic variation than many native provenances. The similar result attained in KJÆR et al. (1996) and FOFANA et al. (2008) reports which analyzing introduced teak provenances. MINN et al. (2014) result also shows that all genetic diversity estimates of teak from Benin ($A = 7.9$, $H_E = 0.643$, $H_O = 0.642$) were higher than those in Myanmar ($A = 6.8$, $H_E = 0.586$, $H_O = 0.564$). Several reasons may be account for this situation. First, this may be duty to different sample size of each provenance in the present study. Second, reflecting the important implications on the introductions into these sites, namely multiple seed sources could have been collected to establish these plantations. Similarly, presumption that other introduced provenances may have been derived from multiple seed sources can be seen from the higher variation (provenances no. 26, 24, 19, 22 and 28) than some native provenances. These new areas with teak plantations have offered teak further variation in order to adapt to local climates and soils with its own distinctive characteristics after hundreds of years and can be used in future plantation.

DOI:10.1515/sg-2015-0003
edited by Thünen Institute of Forest Genetics

AMOVA analysis was similar to the result reported by ANSARI et al. (2012) that most genetic diversity observed in the teak populations, in comparison to genetic diversity among population and among countries. Some other investigations (SHRESTHA et al., 2005; FOFANA et al., 2008; NICODEMUS et al., 2003) reported AMOVA analysis as two levels but all showing the consistent result that most genetic variation within populations. The variation among four countries was lower than HANSEN et al. (2015) result (18.1%), this may be due to only 6 microsatellites were used in HANSEN et al. (2015) study and different provenances were investigated.

A moderate coefficient of genetic differentiation $F_{st} = 0.146$ and high gene flow $N_m = 1.458$ indicated the gene flow among native teak provenances sampled in present study was substantial. The coefficient of genetic differentiation was lower than FOFANA et al. (2009) reported for 17 native populations by SSR markers (0.22) and HANSEN et al. (2015) observed across all four native regions but approximated with ANSARI et al. (2012) analyzed 29 teak populations using ISSR markers (0.1533) and a little higher than MINN et al. (2014) studied for Myanmar populations (0.116). The moderate level of genetic differentiation may be accounted by three reasons, first, might be due to more adjacent provenances investigated in the present work. Second, teak is mainly pollinated by small insects (HEDEGART, 1973), however, the high gene flow $N_m = 1.458$ indicating the gene flow among teak provenances was substantial and close neighbor breeding may occur in natural populations. Finally, N_m value > 1 shows substantial movement of gametes across neighbor populations satisfying the minimum number of migrants per generation needed to avoid differentiation by genetic drift (SLATKIN, 1987).

The UPGMA cluster analysis for all 28 provenances which was confirmed by principle coordinate analysis, indicating a distinct differentiation between the Indian and other three countries' provenances, and less differentiation within second cluster among Myanmar, Thailand and Laos provenances. This result confirmed moderate coefficient of genetic differentiation F_{st}. The Laos provenances together clustered with Thailand provenances and was respected and confirmed by VERHAEGEN et al. (2010).

Although some of the introduced provenances detected in this study existed abundant variation and could be used in the planting and breeding programs, the really problem is that the origins of the seed which was first introduced to these places should be identified. It is necessary to determine the primary origin of the various planted teak populations, i.e. which native countries (India, Myanmar, Thailand or Laos) or which provenances they were initially imported. In general, if native provenances originated from the same hypothetical ancestors and underwent stable evolution, geographically close provenances should show the closest genetic relationships. The dendrogram revealed that provenances from the same country clustered together indicating a clear linkage with the historical and geographical factors. The present result showed there was distinct pattern of genetic distances correlated with geographic distances. A Mantel test revealed high correlation between pair-wise genetic distances and geographical distances among native provenances (correlation coefficient of $R = 0.7355$, $P < 0.001$) suggesting planted provenance origins can be identified by cluster tree and genetic distance matrix if they were from or close with the native provenances sampled in this study. The cluster analysis by UPGMA, PCA and STRUCTURE methods gave very similar results and corresponding with former studies (VERHAEGEN et al., 2010; HANSEN et al., 2015), all showing the India provenances was firstly differentiated from other provenances, Laos provenances clustered with Thailand provenances, then introduced provenances and Myanmar provenances successively joined in the clusters. The ten introduced provenances together with Laos, Thailand and Myanmar provenances clustered in one large group, we can infer that the introduced provenances probably originated from these three native countries other than India. From the UPGMA cluster and genetic distance matrix, we can further know that the introduced provenances have most close relationship with Laos provenances (17 or 18) and Thailand provenances (5 or 6). Similarly, the STRUCTURE analysis suggested introduced provenances may be early from Laos provenance no. 17 and Myanmar provenance no. 16 or their adjacent provenances. On the whole, from the results of different cluster methods, we can infer that the introduced provenances no. 19, 20, 22, 23, 25, 27 and 28 may be originated from Laos prove-

DOI:10.1515/sg-2015-0003
edited by Thünen Institute of Forest Genetics

nances (especially provenance no. 17 or its nearby provenances) and Thailand provenances (especially provenances no. 5 or 6 or their nearby provenances), introduced provenances no. 21, 24, 26 may be from Myanmar provenance (especially provenance no. 16 or its nearby provenances). The result (multiple provenances origin) confirmed that multiple seed sources could have been collected to establish these plantations. Actually, teak genetic resources have been dramatically altered and introduced or exchanged in the past 50–100 years because of uncontrolled mixing of germplasm (SHRESTHA et al., 2005). Furthermore, teak has been planted in China for more than 190 years, some early adjacent plantations probably have cross-pollinated with each other and seeds from these plantations should have been collected and used for other planting programs. These cause the difficulty for origin identification. Sampling of provenances in the native range must be much denser than presently undertaken with chloroplast DNA pattern are likely to yield better results over the identities of the originating native provenances.

In summary, the knowledge on genetic diversity and provenances structure of teak obtained from the present study will provide valuable information for further genetic management and breeding program for teak. The SSR markers revealed that the majority of variation existed within provenances and also substantial variation among countries, emphasizing more attention should be paid to the two aspects when considering conservation measures for teak. As more as possible plants or their seeds from different large populations should be collected *ex site* or conserved *in site* in order to keep maximum genetic diversity and to capture genetic variability of traits of economic interests, which may be utilized for future improvement of timber productivity and quality. At the same time, different countries populations should be preserved *in situ* plots for maintenance of broad genetic base in the natural habitat or collected for *ex site* conservation with cooperation among these countries as substantial variation among four countries was detected. Another important finding in this study is that some of the early introduced provenances were detected exist abundant variation and could be used in future planting programs and breeding programs especially when their origins were recorded or identified. This study will be a reference of genetic origin test of global introduced provenances in many teak-growing countries outside the natural distribution area.

Acknowledgements

This work was financially supported by the National Natural Science Foundation of China (No. 31200501) and the Fundamental Research Funds for the Central Non-profit Research Institution of RITF-CAF (No. RITFYWZX2011-06), also supported by the national '12th five-year forestry programme' (No. 2012BAD01B0504). The authors would like to thank S. M. GAN for kind help and good advices in this study. We also thank anonymous reviewer for valuable comments.

References

ANSARI, S. A., C. NARAYANAN, S. A. WALI, R. KUMAR, N. SHUKLA and S. R. KUMAR (2012): ISSR markers for analysis of molecular diversity and genetic structure of Indian teak (*Tectona grandis* L.f.) populations. Annals of Forest Research **55**(1): 11–23.

BAGCHI, S. K., V. P. SHARMA and P. K. GUPTA (1989): Developmental instability in leaves of *Tectona grandis*. Silvae Genetica **38**: 1–6.

BEDELL, P. E. (1989): Preliminary observations on variability of teak in India. Indian Forest **115**: 72–81.

BENDALE, V. W., R. Y. NAIK, J. L. MEHTA, S. G. BHAVE and U. B. PETHE (2005): Variability studies in teak. J. Ecobiol. **17**: 29–34.

BHAT, K. M. and P. B. PRIYA (2004): Influence of provenance variation on wood properties of teak from the Western Ghat region in India. Int. Assoc. Wood Anat. J. **25**: 273–282.

EARL, D. A. and B. M. VONHOLDT (2012): STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv Genet Resour **4**: 359–361.

EVANNO, G., S. REGNAUT and J. GOUDET (2005): Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol **14**: 2611–2620.

FOFANA, I. J., D. OFORI, M. POITEL and D. VERHAEGEN (2009): Diversity and genetic structure of teak (*Tectona grandis* L.f) in its natural range using DNA microsatellite markers. New forests **37**: 175–195.

FOFANA, I. J., Y. J. LIDAH, N. DIARRASSOUBA, S. P. A. N'GUETTA, A. SANGARE and D. VERHAEGEN (2008):

DOI:10.1515/sg-2015-0003
edited by Thünen Institute of Forest Genetics

Genetic structure and conservation of Teak (*Tectona grandis*) plantations in Côte d'Ivoire, revealed by site specific recombinase (SSR). Tropical Conservation Science **1**(3): 279–292.

HANSEN, O. K., S. CHANGTRAGOON, B. PONOY, E. D. KJÆR, Y. MINN, R. FINKELDEY, K. B. NIELSEN and L. GRAUDAL (2015): Genetic resources of teak (*Tectona grandis* Linn. f.) – strong genetic structure among natural populations. Tree Genetics & Genomes **11**: 802.

HEDEGART, T. (1973): Pollination of Teak (*Tectona grandis* L.). Silvae Genetica **22**: 124–128.

JAKOBSSON, M. and N. A. ROSENBERG (2007): CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. Bioinformatics **23**: 1801–1806.

KADAMBI, K. (1972): Silviculture and management of teak. School of Forestry, Austin State University, Nacogdoches, Texas.

KAOSA-ARD, A. (1981): Teak (*Tectona grandis*): Its natural distribution and related factors. Nat. Hist. Bull. Siam Soc. **29**: 55–74.

KERTADIKARA, A. W. S. and D. PRAT (1995): Genetic structure and mating system in teak (*Tectona grandis* L.f.) provenances. Silvae Genetica **44**: 104–110.

KJÆR, E. D., H. R. SIEGISMUND and V. SUANGTHO (1996): A multivariate study on genetic variation in teak (*Tectona grandis* L.f.). Silvae Genetica **45**: 361–368.

KJÆR, E. D., S. KAJORNSRICHON and E. B. LAURIDSEN (1999): Heartwood, calcium and silica content in five provenances of teak (*Tectona grandis* L.). Silvae Genetica **48**: 1–3.

KJÆR, E. D. and V. SUANGTHO (1995): Outcrossing rate of teak (*Tectona grandis*). Silvae Genetica **44**: 175–177.

KUANG, B. C. and S. Z. ZHENG (1991): Genetic improvement of teak (*Tectona grandis*) in China. Forest Research **4**(2): 139–146.

LAI, M., LIANG, K. N., G. H. HUANG, M. P. LING, Z. Z. ZHOU and H. M. MA (2011): Genetic variation and comprehensive evaluation in growth and wood relevant properties of different provenances of *Tectona grandis*. Forest Research **24**(2): 234–238.

LI, F., S. GAN, Z. ZHANG, Q. WENG, D. XIANG and M. LI (2011): Microsatellite-based Genotyping of the Commercial *Eucalyptus* Clones Cultivated in China. Silvae Genetica **60**(5): 216–223.

LIANG, K. N., M. LAI, G. H. HUANG, M. P. LING, Z. Z. ZHOU and H. M. MA (2011): Growth and adaptability of ten provenances of *Tectona grandis* at 27-year-old. Journal of Central South University of Forestry and Technology **31**(4): 8–12.

MINN, Y., K. PRINZ AND R. FINKELDEY (2014): Genetic variation of teak (*Tectona grandis* Linn. f.) in Myanmar revealed by microsatellites. Tree Genetics and Genome **10**: 1435–1449.

MOHANAN, C., J. K. SHARMA and E. J. M. FLORENCE (1997): Nursery diseases of teak in India, pp. 107–112. *In:* Proceeding of the International Teak Symposium, edited by S. C. BASHA, Kerala Forest Research Institute, Kerala.

MUZZALUPO, I., G. G. VENDRAMIN and A. CHIAPPETTA (2014): Genetic biodiversity of Italian olives (*Olea europaea*) germplasm analyzed by SSR markers. The Scientific World Journal 2014: 296590.

NARAYANAN, C., A. K. MANDAL and S. A. ANSARI (2007): Molecular markers and their applications in forest trees, pp. 31–56. *In:* Biotechnology Current Perspectives and Potential Applications, edited by P. C. TRIVEDI, Aavishkar Publishers & Distributors, Jaipur.

NEI, M. (1987): Molecular evolutionary genetics. Columbia University Press, New York.

NEI, M. (1978): Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics **89**: 583–590.

NICODEMUS, A., B. NAGARAJAN, C. NARAYANAN, M. VARGHESE and K. SUBRAMANIAN (2003): RAPD Variation in Indian Teak Populations and its Implications for Breeding and Conservation, pp. 321–330. *In:* Quallity timer products of teak from sustainable forest management, edited by K. M. BHAT, K. K. N. NAIR, K. V. BHAT, E. M. MURALIDHARAN and J. K. SHARMA, Kerala Forest Research Institute and International Tropical Timber Organization Publication, Kerala.

PARTHIBAN, K. T., C. SURENDRAN, M. PARAMATHMA and K. SASIKUMAR (2003): Molecular Characterization of Teak Seed Source Using RAPD's, PP. 331–337. *In:* Quallity timer products of teak from sustainable forest management, edited by K. M. BHAT, K. K. N. NAIR, K. V. BHAT, E. M. MURALIDHARAN and J. K. SHARMA, Kerala Forest Research Institute and International Tropical Timber Organization Publication, Kerala.

PEAKALL, R. and P. E. SMOUSE (2006): Genalex 6: genetic analysis in Excel, Population genetic software for teaching and research. Mol. Ecol. Notes **6**: 288–295.

POWELL, W., G. C. MACHRAY and J. PROVAN (1996): Polymorphisms revealed by simple sequence repeats. Trend. Plant Sci. **1**: 215–222.

PRITCHARD, J. K., M. STEPHENS and P. DONNELLY (2000): Inference of population structure using multilocus genotype data. Genetics **155**: 945–959.

PRIYA, P. B. and K. M. BHAT (1999): Influence of rainfall, irrigation and age on the growth periodicity and wood structure in teak (*Tectona grandis*). Int. Assoc. Wood Anat. **20**: 181–192.

PRIYA, P. B. and K. M. BHAT (1998): False ring formation in teak (*Tectona grandis* L.f.) and the influence of environmental factors. For. Ecol. Manag. **108**: 215–222.

ROHLF, F. J. (1998): NTSYS-pc: numerical taxonomy and multivariate analysis system, version 2.02. Exeter Software, Setauket, New York.

ROSENBERG, N. A. (2004): DISTRUCT: a program for the graphical display of population structure. Mol Ecol Notes **4**: 137–138.

DOI:10.1515/sg-2015-0003
edited by Thünen Institute of Forest Genetics

ROUBOS, K., M. MOUSTAKAS and F. A. ARAVANOPOULOS (2010): Molecular identification of Greek olive (*Olea europaea*) cultivars based on microsatellite loci. Genetics and Molecular Research **9**(3): 1865–1876.

SHRESTHA, M. K., H. VOLKAERT and D. V. D. STRAETEN (2005): Assessment of genetic diversity in *Tectona grandis* using amplified fragment length Polymorphism markers. Canadian Journal of Forest Research **35**(4): 1017.

SLATKIN, M. (1987): Gene flow and the geographic structure of natural populations, Science **236**: 787–792.

VARGHESE, M., A. NICODEMUS, P. K. RAMTEKE, G. ANBAZHAGI, S. S. R. BENNET and K. SUBRAMANIAN (2000): Variation in growth and wood traits among nine populations of teak in Peninsular India. Silvae Genetica **49**: 201–205.

VARSHNEY, R. K., A. GRANER and M. E. SORRELLS (2005): Genic microsatellite markers in plants: features and applications. Trends Biotechnol. **23**: 48–55.

VERHAEGEN, D., D. OFORI, I. FOFANA, M. POITEL and A. VAILLANT (2005): Development and characterization of microsatellite markers in *Tectona grandis* (Linn. f). Molecular Ecology Notes **5**(4): 945–947.

VERHAEGEN, D., I. J. FOFANA, Z. A. LOGOSSA and D. OFORI (2010): What is the genetic origin of teak (*Tectona grandis* L.) introduced in Africa and Indonesia? Tree genetics and genomes **6**: 717–733.

WHITE, K. J. (1991): Teak: Some aspects of research and development. RAPA publication, Bangkok.

Investigation of gas exchange and biometric parameters in isogenic lines of poplar differing in ploidy

By L. ATANET ALÍA[1)], D. LÜTTSCHWAGER[2)] and D. EWALD[3),*)]

(Received 6th August 2015)

Abstract

Three poplar clones of section *Populus* (Brauna 11 [*Populus tremula*], L447 [*Populus canescens*] and Esch 5 [*Populus tremula* x *Populus tremuloides*]) were used to analyse the effects of ploidy levels on primary productivity and water use efficiency. The clones were established in tissue culture (2N) and lines with different ploidy levels (2N/4N and 4N) were generated via colchicine treatment. Light response curves were modelled based on gas exchange measurements carried out three times during the growing season on the 1st fully developed leaf under controlled conditions. The plants were harvested in September to analyse biometric parameters. The photosynthetic capacity was greatest in May, decreased throughout the season and increased slightly again in September. The decrease in Brauna 11 and Esch 5 varied from 20–50% compared with values in May and it was not as pronounced in L 447. Photosynthesis and intrinsic water use efficiency differed between clones, but not among the single isogenic lines within each

[1)] LUCÍA ATANET ALÍA, Leibniz-Center for Agricultural Landscape Research (ZALF). Institute of Landscape Biogeochemistry, Eberswalder Straße 84, D-15374 Müncheberg, Germany. Phone: +34659295403, Fax: +493343282343. E-Mail: luciaatanet@hotmail.com

[2)] DIETMAR LÜTTSCHWAGER, Leibniz-Center for Agricultural Landscape Research (ZALF). Institute of Landscape Biogeochemistry, Eberswalder Straße 84, D-15374 Müncheberg, Germany. Phone: +493343282108, Fax: +493343282343. E-Mail: dluettschwager@zalf.de

[3)] Thünen-Institute of Forest Genetics, Eberswalder Chaussee 3a, D-15377 Waldsieversdorf, Germany.

[*)] Communicating author: DIETRICH EWALD. Phone: +4933433157170, Fax: +4933433157199. E-Mail: dietrich.ewald@ti.bund.de

DOI:10.1515/sg-2015-0004
edited by Thünen Institute of Forest Genetics