SSR GENOTYPING—GENETIC DIVERSITY AND FINGERPRINTING OF TEAK (*TECTONA GRANDIS*) CLONES

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HUANG GH, LIANG KN, ZHOU ZZ & MA HM. 2016. SSR genotyping—genetic diversity and fingerprinting of teak (*Tectona grandis*) clones. A DNA-based fingerprint technique was developed for teak with 15 microsatellite markers, based on 26 widely cultivated teak clones. The same set of markers was used to investigate genetic variations and relationships of the 26 teak clones. Using the fingerprint technique, all teak clones could be unequivocally identified. In terms of genetic diversity among 26 clones, 160 alleles were detected with mean of 10.67 alleles per locus. The observed heterozygosity ranged from 0.3333 to 1.0000, averaging 0.6567. Despite the limited sample, there was substantial genetic variation that could be exploited in teak breeding programme. In addition, the cluster analysis separated teak clones of India origin from Myanmar and essentially confirmed historically known or speculated origin of the clones. Implication on clonal identification and management as well as the protection of legitimate interests of breeders and growers was discussed.

Keywords: Clone discrimination, clone characterisation, microsatellite marker, genetic variation, genetic relationship, clone management

INTRODUCTION

Teak, *Tectona grandis*, is naturally distributed in tropical forests of India, Myanmar, Thailand and Laos. Its high quality and aesthetically appreciated wood have made teak the luxury timber for furniture making, carving and building. Due to its economic importance, the species has been introduced widely in tropical regions since the 19th century, especially into Asia, Africa, Central America and South America (Alcantara & Veasey 2013).

Teak provenances were collected and systematic genetic breeding was carried out with international provenance trials since 1970. Substantial variations in growth (Bendale et al. 2005, Lai et al. 2011) between different provenances as well as individual trees within provenances have been observed. From these trials, teak plus trees were selected on the basis of their superior phenotypes with regard to vigour (height and girth), straightness and cylindrical woody stem, crown compactness, and incidence of pest and disease. The selected plus trees were used as source material for production of planting stock by clonal propagation for large scale plantation or as parents in breeding programmes for development of superb clones. However, teak clone trees possess small number of distinct morphological characters. The recognition of teak clones based on phenotypic characters is often problematic. Due to this problem, the ortets and ramets of plus tree clones might not have been correctly identified and matched for long periods of time. In fact, such mistakes caused by conventional clone management have been revealed after DNA analyses were introduced to the practice of clone management (Li et al. 2011). On the other hand, although genetic variation and relationships are basic information in a breeding programme, they have not become available for these widely cultivated clones. Molecular markers have been proven to be an important way to increase selection efficiency (Liesebach et al. 2010, Roubos et al. 2010).

The markers are highly heritable, stable and exhibit sufficient polymorphism to discriminate very closely related genotypes at any developmental stage without environmental

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interference (Narayanan et al. 2007). In teak, a variety of molecular marker techniques have been applied to genotype identification and genetic diversity analysis, including random amplified polymorphic DNA(Nicodemus et al. 2003, Parthiban et al. 2003), amplified fragment length polymorphism (AFLP) (Shrestha et al. 2005), simple sequence repeat (SSR) (Verhaegen et al. 2005, 2010, Fofana et al. 2008, 2009) and inter simple sequence repeat (ISSR) (Narayanan et al. 2006, Ansari et al. 2012). Of these, the SSR technology was found to be more reliable and adapted to our current objectives, which was in accordance with findings of other species (Rahman & Rajora 2002, Fossati et al. 2005). Microsatellite markers are excellent for genetic characterisation 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). So far, 143 genomic microsatellites (Verhaegen et al. 2005) have been developed for teak, enhancing significantly the capacity of molecular characterisation of teak germplasm. Recently, SSR markers have been used in teak for a variety of purposes, including the evaluation of genetic diversity (Fofana et al. 2008, 2009) and origin certification (Verhaegen et al. 2010). In the present study, we used 15 microsatellite markers to genotype 26 widely cultivated teak clones in China and explore their genetic variations. The objectives were to develop the DNA-based fingerprint of each clone and determine the genetic relationships of these clones.

MATERIALS AND METHODS

Plant materials and DNA extraction

A total of 26 teak clones were collected. Each clone was represented by a ramet conserved in stock pools at the nursery. These clones were propagated by tissue culture. A complete list of accession descriptions and origins is given in Table 1. Among these accessions, 1 to 12 were plus trees originated from India and 13 to 23 were Myanmar sources, 24 was from Nigeria, and 25 and 26 were unknowns.

Genomic DNA of each clone was extracted from 0.02 g dry leaf (dried by silica gel) using DNA plant kit. DNA concentrations were estimated and standardised against known concentrations of 100 bp standard DNA ladder on 1% agarose gel. DNA samples were diluted five times when used for amplification.

SSR genotyping

SSR genotyping analysis was carried out using the method of Li et al. (2011) with modifications because of primer specificity for teak. Primer pairs were synthesised by Generay Biotech Co, Shanghai, China. Primers described in Verhaegen et al. (2005, 2010) were screened in a preliminary test. A total of 13 of 15 primer pairs that produced good amplification and intensity fluorescence signal were employed in this study. CIRAD3TeakB02 (AJ968937) and CIRAD3TeakE06 (AJ968939) primers did not provide any fluorescence signal for most clones under our experimental conditions. This might be due to the fact that insertions or deletions could neither be detected nor excluded due to incomplete sequences or polymerase chain reaction (PCR) production of the two primer pair failure in competitive incorporation with F-dUTP effectively. Somemore primer pairs were designed according to SSR sequences gained from EMBL web site and TgAC12 (AJ511753) as well as TgAC28 (AJ511764) were selected to substitute AJ968937 and AJ968939 in this study considering the same quantity loci would be more comparative with previous study. At the same time, Tm of some primer was optimised to get rid of unwanted fluorescence signal after a preliminary test. The descriptions of 15 markers used in genotyping teak clones are shown in Table 2.

The reaction was amplified running on DNA engine thermal cycler using touchdown conditions: 94 °C for 4 min; 20 cycles of 94 °C for 30 s, Tm +10 °C to Tm °C depending on microsatellite marker (Table 2) for 30 s with a decrease of 0.5 °C per cycle and 72 °C for 1 min; 26 cycles of 94 °C for 30 s, Tm °C for 30 s and 72 °C for 1 min; and a final extension at 72 °C for 10 min.

The PCR products (2 µL) were diluted 2:10 with loading buffer (7.82 µL deionised formamide and 0.16 µL internal standard GeneScan 500LIZ) and then denatured at 95 °C for 5 min followed by rapid cooling on ice.

Table 1 Information of 26 commercial teak clones with code number, name, provenance name and geographical origin

Code	Clone Name	Provenance name	Geographical origin
Clones from India provenances			
1	7013	3070	Sungam, Kerala, India
2	7029	3070	Sungam, Kerala, India
3	715	3071	Stuart Mt., Tamilnadu, India
4	7112	3071	Stuart Mt., Tamilnadu, India
5	7114	3071	Stuart Mt., Tamilnadu, India
6	7122	3071	Stuart Mt., Tamilnadu, India
7	7138	3071	Stuart Mt., Tamilnadu, India
8	7146	3071	Stuart Mt., Tamilnadu, India
9	7137	3071	Stuart Mt., Tamilnadu, India
10	7210	3072	Masale, Valley, Mysore, India
11	7219	3072	Masale, Valley, Mysore, India
12	7412	3074	Virnoli, Mysore, India
Clones from Myanmar provenances			
13	J731	731	Bago Yoma, Myanmar
14	Z408	408	Letpangon, Myanmar
15	7509	7509	Planted provenance, Ledong, Hainan Island, China (Myanmar source)
16	7514	7514	Planted provenance, Ledong, Hainan Island, China (Myanmar source)
17	7531	7531	Planted provenance, Ledong, Hainan Island, China (Myanmar source)
18	7544	7544	Planted provenance, Ledong, Hainan Island, China (Myanmar source)
19	7542	7542	Planted provenance, Ledong, Hainan Island, China (Myanmar source)
20	7549	7549	Planted provenance, Ledong, Hainan Island, China (Myanmar source)
21	7552	7552	Planted provenance, Ledong, Hainan Island, China (Myanmar source)
22	7555	7555	Planted provenance, Ledong, Hainan Island, China (Myanmar source)
23	7559	7559	Planted provenance, Ledong, Hainan Island, China (Myanmar source)
Clone from Nigeria provenance			
24	3078-5	3078	Gambari, Nigeria
Clones from planted provenances of no origin records			
25	8301	8301	Planted provenance, Ledong, Hainan Island, China
26	108	108	Planted provenance, Guangzhou, Guangdong, China

Data analysis

For each locus, IDENTITY 1.0 (Wagner & Sefc 1999) was used to estimate the observed heterozygosity (H_o) (Nei 1973), number of alleles (NA), allele frequencies, probability of identity (PI) (Paetkau et al. 1995) and probability of paternity exclusion (PE) (Weir 1996). Overall PI and PE across all loci were calculated with this software. The number of observed genotypes (NG) and number of unique alleles (NC) were computed empirically according to alleles and were carried out using the method of Li et al. (2011). The effective number of alleles (ENA) was estimated as $1/\Sigma\ p_i^2 = 1/(1 - H_e)$ where p_i = frequency of allele i and H_e = expected heterozygosity (Kimura & Crow 1964). The polymorphism information content (PIC) (Botstein et al. 1980) was calculated with the EXCEL MICROSATELLITE TOOLKIT version 3.1 (Park 2001). The genetic relationship among the clones was determined by calculating Jaccard's coefficients of similarity and a dendrogram was constructed with UPGMA (unweighted pair-

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

Locus name	Accession no. EMBL database	Primer sequence	Repeat motif	Mg^{2+} (mM)	T_m (')
CIRAD1TeakA06	AJ968929	F: 5'-CAAAACAAAACCAATAGCCAGAC-3'	$(GA)_{15}$	2.0	53
		R: 5'-TTTCATCATCATCATCAACATCC-3'			
CIRAD1TeakB03	AJ968930	F: 5'-AACAACCCCTCCTCTCTTCACTA-3'	$(TC)_5$ TG $(TC)_8$ $(AC)_5$ $(N)_{65}$ $(AC)_{14}$	2.0	51
		R: 5'-CACTACCACTCATCATCAACACA-3'			
CIRAD1TeakF05	AJ968931	F: 5'-CTTCTGCAACCCTTTTTCAC-3'	$(GA)_{20}$ GT $(GA)_3$	2.0	53
		R: 5'-AGCCATATCTTCCTTTCTCT-3'			
CIRAD1TeakG02	AJ968932	F: 5'-TTAACGCCAAATCCCAAAG-3'	$(TC)_{10}$	2.0	51
		R: 5'-CACAAAGAGAACCGACGAG-3'			
CIRAD1TeakH10	AJ968933	F: 5'-CGATACCTGCGATGCGAAGC-3'	$(TC)_{16}$	2.0	53
		R: 5'-CGTTGAATACCCGATGGAGA-3'			
CIRAD2TeakB07	AJ968934	F: 5'-GGGTGCTGATGATTTTGAGTT-3'	$(TC)_{14}$	2.0	53
		R: 5'-CTAAGGAGTGAGTGGAGTTTT-3'			
CIRAD2TeakC03	AJ968935	F: 5'-AGGTGGGATGTGGTTAGAAGC-3'	$(GA)_{17}$	2.0	51
		R: 5'-AAATGGTCATCAGTGTCAGAA-3'			
CIRAD3TeakA11	AJ968936	F: 5'-AAACCATGACAGAAACGAATC-3'	$(GA)_{16}$	2.0	53
		R: 5'-TTGGGAATGGGAGGAGAAGT-3'			
CIRAD3TeakDa09	AJ968938	F: 5'-CTCGCTTCTTTCCACATT-3'	$(AC)_{10}$	2.0	51
		R: 5'-ATCATCGCGCATCGTCAA-3'			
CIRAD3TeakF01	AJ968940	F: 5'-GCTCTCCACCAACCTAAACAA-3'	$(TC)_{16}$	2.0	51
		R: 5'-AAAACGTCTCACCTTCTCACT-3'			
CIRAD4TeakDa12	AJ968941	F: 5'-CGCACACCAGTAGCAGTAGCC-3'	$(GA)_4$ $(N)_5$ $(GA)_{11}$ A $(GA)_4$	2.0	51
		R: 5'-GCCGGAAAAAGAAAAACCAAA-3'			
CIRAD4TeakF02	AJ968942	F: 5'-CCGGTAAAAAGGTGTGTCA-3'	$(TC)_4$ $(AC)_3$ $(N)_{16}$	2.0	51
		R: 5'-GAGTGGAAGTGCTAATGGA-3'			
CIRAD4TeakH09	AJ968943	F: 5'-GCAAACCAACCTTACT-3'	$(GA)_{14}$	2.0	53
		R: 5'-CCGTTAGCACTCCATT-3'			
TgAC12	AJ511753	F: 5'-TGGTGCAGTTGCTACAGTTCCTGA-3'	$(AG)_{12}$	1.5	56
		R: 5'-CCCACCACATTACTTCTCACATGCCC-3'			
TgAC28	AJ511764	F: 5'-CCGATGCATGGCATGTTCTACCCA-3'	$(CA)_{11}$	2.0	51
		R: 5'-GGTACCATGATGGGGGACGGC-3'			

group method with arithmetic average) (Sokal & Michener 1958) using software NTSYS-pc 2.02 (Rohlf 1998).

RESULTS

Microsatellite polymorphisms

All the 15 marker loci were polymorphic (Table 3). A total of 160 distinct alleles were generated with an average of 10.67 alleles per locus. The number of alleles per locus ranged from 4 at AJ968938 locus to 17 at AJ511753 locus. Allele size ranged from 110 bp in AJ968934 to 271 bp in AJ968935 and AJ968936. The frequency at which an allele occurred was highly variable, ranging from 1.92 to 62.50% (result not shown). All the SSRs amplified unique alleles that appeared only once in the whole set of clones, ranging from 1 (AJ968931, AJ968933, AJ968938 and AJ968941) to 8 (AJ968934) and reaching a total of 51 (31.875%) (result not shown). In addition, the observed heterozygosity ranged from 0.3333 at AJ968930 to 1.0000 at AJ511753 across all the clones, with a mean value of 0.6567 over all the 15 microsatellite loci.

Genotyping of clones

The parameters reflected relatively consistently the discrimination power of a locus. The average PIC value for the 15 SSR loci was 0.7687 and the PIC value ranged from 0.4855 for AJ968938 to 0.8929 for AJ511753 among the 26 teak clones (Table 3). Most microsatellite loci displayed high PIC values, enabling the identification of all individuals. The PIC results indicated which of the 14 loci could be classified as highly informative (PIC > 0.5). Specifically, 11 of the loci could be classified as useful for genetic mapping (PIC > 0.7).

The two loci AJ511753 and AJ968929 were the most informative as the former had the highest values for ENA (10.081), PIC (0.8929), and PE (0.8019), while the latter had the highest NG (19). In contrast, AJ968938 was the least informative with the lowest PIC (0.4855), NG (5), ENA (2.177) and PE (0.2967) but the highest PI (0.3766). Since low PI indicated high marker efficiency, the best marker according to PI was AJ511753.

Genetic relationship among clones

Genetic distances ranged from 0.033 between clone 7114 (Stuart Mt., Tamilnadu, India) and clone 108 (Guangzhou, China) to 1.000 (Table 4). There were four incidence of genetic distance of 1.000, between clone 7412 (Virnoli, Mysore, India) and four other clones 7544 (Ledong, Hainan Island, China, Myanmar source), clone 7531 (Ledong, Hainan Island, China, Myanmar source), clone 7549 (Ledong, Hainan Island, China, Myanmar source) and clone J731 (Bago Yoma, Myanmar). The fingerprint (result not shown) showed that all 15 primer pairs had the same result when amplified for clones 7114 (Stuart Mt., Tamilnadu, India) and 108 (Guangzhou, China) except for AJ511753.

The UPGMA cluster analysis based on Nei's (1978) unbiased genetic distances showed that three groupings could be recognised from the dendrogram (Figure 1). The first group consisted of clones 1 to 11 (India) as well as clones 24 (Gambari, Nigeria) and 26 (Guangzhou, China). The second group comprised genotypes 13 to 23 (Myanmar) as well as clone 25 (Ledong, Hainan, China). Unexpectedly, clone 12 (Virnoli, Mysore, India) was alone in the third group.

DISCUSSION

This study demonstrated that this 'package' of technologies based on fluorescence-dUTP and ABI 3130xl genetic analyser was effective in identifying teak clones. The 15 microsatellite loci selected were good discrimination markers except for locus AJ968938 which was less informative (PIC < 0.5), as indicated by Roubos et al. (2010). Total PI from the 15 microsatellite markers was 3.248×10^{-16}, corresponding to a cumulative PE of 0.999999892975, which meant that the chance of any clone having been incorrectly characterised as synonymous when it was only 0.000000107025%. Many of the 26 accessions could be uniquely genotyped based on their microsatellite profiles at a single locus without resorting to their multilocus configuration, and all of the clones could be distinguished from one another with three loci (AJ968929, AJ968936 and AJ511753). The technique would allow future verification of clone identity and assessment of correspondence between the reference and

Table 3 Genetic parameters obtained from the 15 microsatellite markers used to evaluate the 26 teak clones

Accession no. EMBL database	ASR (bp)	H_o	H_e	NA	NG	NC	ENA	PI	PE	PIC
AJ968929	174–210	0.7307	0.8727	12	19	2	7.855	0.0530	0.7470	0.8605
AJ968930	227–237	0.3333	0.6180	6	7	2	2.618	0.2771	0.3847	0.5743
AJ968931	233–261	0.4166	0.8472	10	12	1	6.545	0.0723	0.7010	0.8309
AJ968932	135–153	0.9615	0.6937	8	7	4	3.265	0.2263	0.4596	0.6496
AJ968933	205–233	0.6666	0.8758	12	16	1	8.052	0.0479	0.7565	0.8650
AJ968934	110–142	0.8461	0.8010	14	15	8	5.025	0.1003	0.6342	0.7808
AJ968935	245–271	0.5000	0.7795	12	15	6	4.535	0.0971	0.6178	0.7634
AJ968936	249–271	0.6800	0.8608	11	19	3	7.184	0.0648	0.7217	0.8456
AJ968938	178–196	0.4583	0.5407	4	5	1	2.177	0.3766	0.2967	0.4855
AJ968940	180–214	0.6956	0.8648	14	14	5	7.396	0.0522	0.7404	0.8535
AJ968941	111–123	0.7272	0.8016	7	8	1	5.040	0.1224	0.6121	0.7739
AJ968942	183–209	0.7916	0.8871	12	17	2	8.857	0.0439	0.7730	0.8768
AJ968943	128–142	0.3500	0.6612	8	8	5	2.952	0.2540	0.4272	0.6148
AJ511753	212–262	1.0000	0.9008	17	15	7	10.081	0.0334	0.8019	0.8929
AJ511764	198–226	0.6923	0.8742	13	15	3	7.949	0.0494	0.7531	0.8630
Mean/Overall		0.6567	0.7919	10.67	12.80	3.40	5.969	0.1247	0.6285	0.7687
cumulative								3.248×10^{-16}	0.999999	

ASR = allele size range, H_o = observed heterozygosity, H_e = expected heterozygosity, NA = number of alleles, NG = number of observed genotypes, NC = number of unique alleles, ENA = effective number of alleles, PI = probability of identity, PE = paternity exclusion probability, PIC = polymorphism information content

Table 4 Nei's (1978) unbiased measures of genetic identity (above diagonal) and genetic distance (below diagonal) for 26 teak clones

Clone	7013	7029	715	7112	7114	7122	7138	7146	7210	7219	3078-5	108	7412	7509	7514	7531	7544	7549	7552	7555	7559	8301	J731	Z408	7137	7542
7013	**	0.214	0.346	0.321	0.464	0.231	0.385	0.286	0.269	0.308	0.231	0.500	0.115	0.115	0.179	0.115	0.179	0.154	0.115	0.143	0.107	0.214	0.154	0.179	0.875	0.115
7029	0.786	**	0.250	0.300	0.333	0.321	0.250	0.357	0.269	0.346	0.286	0.300	0.071	0.143	0.100	0.143	0.133	0.179	0.143	0.250	0.200	0.100	0.143	0.133	0.375	0.179
715	0.654	0.750	**	0.393	0.393	0.192	0.786	0.385	0.542	0.292	0.308	0.429	0.077	0.192	0.214	0.231	0.179	0.192	0.308	0.269	0.250	0.143	0.214	0.286	0.375	0.269
7112	0.679	0.700	0.607	**	0.400	0.250	0.429	0.179	0.462	0.692	0.286	0.400	0.179	0.179	0.200	0.107	0.167	0.179	0.214	0.214	0.267	0.200	0.107	0.233	0.375	0.143
7114	0.536	0.667	0.607	0.600	**	0.321	0.429	0.357	0.423	0.346	0.321	0.967	0.143	0.179	0.133	0.179	0.233	0.214	0.179	0.250	0.167	0.133	0.250	0.133	0.438	0.214
7122	0.769	0.679	0.808	0.750	0.679	**	0.269	0.269	0.333	0.250	0.154	0.321	0.115	0.115	0.071	0.192	0.179	0.154	0.143	0.192	0.143	0.179	0.231	0.143	0.286	0.214
7138	0.615	0.750	0.214	0.571	0.571	0.731	**	0.423	0.542	0.333	0.269	0.464	0.038	0.154	0.179	0.231	0.179	0.192	0.269	0.231	0.286	0.143	0.250	0.250	0.438	0.231
7146	0.714	0.643	0.615	0.821	0.643	0.731	0.577	**	0.269	0.192	0.269	0.321	0.115	0.154	0.071	0.115	0.071	0.115	0.115	0.179	0.179	0.071	0.154	0.143	0.375	0.077
7210	0.731	0.731	0.458	0.538	0.577	0.667	0.458	0.731	**	0.375	0.333	0.462	0.167	0.167	0.154	0.167	0.115	0.125	0.208	0.154	0.154	0.077	0.167	0.192	0.313	0.333
7219	0.692	0.654	0.708	0.308	0.654	0.750	0.667	0.808	0.625	**	0.292	0.346	0.167	0.125	0.038	0.083	0.077	0.083	0.083	0.154	0.192	0.077	0.083	0.115	0.375	0.167
3078-5	0.769	0.714	0.692	0.714	0.679	0.846	0.731	0.731	0.667	0.708	**	0.321	0.115	0.346	0.107	0.154	0.179	0.231	0.231	0.346	0.214	0.071	0.077	0.107	0.250	0.231
108	0.500	0.700	0.571	0.600	0.033	0.679	0.536	0.679	0.538	0.654	0.679	**	0.143	0.179	0.133	0.179	0.233	0.214	0.179	0.250	0.167	0.133	0.250	0.133	0.438	0.214
7412	0.885	0.929	0.923	0.821	0.857	0.885	0.962	0.885	0.833	0.833	0.885	0.857	**	0.038	0.036	0.000	0.000	0.000	0.038	0.077	0.071	0.036	0.000	0.036	0.071	0.038
7509	0.885	0.857	0.808	0.821	0.821	0.885	0.846	0.846	0.833	0.875	0.654	0.821	0.962	**	0.429	0.423	0.500	0.536	0.385	0.500	0.286	0.250	0.385	0.321	0.214	0.346
7514	0.821	0.900	0.786	0.800	0.867	0.929	0.821	0.929	0.846	0.962	0.893	0.867	0.964	0.571	**	0.393	0.500	0.429	0.607	0.286	0.400	0.533	0.393	0.533	0.063	0.286
7531	0.885	0.857	0.769	0.893	0.821	0.808	0.769	0.885	0.833	0.917	0.846	0.821	1.000	0.577	0.607	**	0.393	0.500	0.385	0.462	0.286	0.286	0.923	0.357	0.125	0.346
7544	0.821	0.867	0.821	0.833	0.767	0.821	0.821	0.929	0.885	0.923	0.821	0.767	1.000	0.500	0.500	0.607	**	0.500	0.357	0.536	0.300	0.367	0.357	0.300	0.188	0.321
7549	0.846	0.821	0.808	0.821	0.786	0.846	0.808	0.885	0.875	0.917	0.769	0.786	1.000	0.464	0.571	0.500	0.500	**	0.423	0.615	0.286	0.357	0.462	0.286	0.143	0.500
7552	0.857	0.857	0.692	0.786	0.821	0.857	0.731	0.885	0.792	0.917	0.769	0.821	0.962	0.615	0.393	0.615	0.643	0.577	**	0.269	0.571	0.286	0.308	0.536	0.071	0.357
7555	0.885	0.750	0.731	0.786	0.750	0.808	0.769	0.821	0.846	0.846	0.654	0.750	0.923	0.500	0.714	0.538	0.464	0.385	0.731	**	0.250	0.250	0.385	0.214	0.188	0.462
7559	0.893	0.800	0.750	0.733	0.833	0.857	0.714	0.821	0.846	0.808	0.786	0.833	0.928	0.714	0.600	0.714	0.700	0.714	0.429	0.750	**	0.267	0.214	0.433	0.125	0.250
8301	0.786	0.900	0.857	0.800	0.867	0.821	0.857	0.929	0.923	0.923	0.929	0.867	0.964	0.750	0.467	0.714	0.633	0.643	0.714	0.750	0.733	**	0.357	0.300	0.125	0.143
J731	0.846	0.857	0.786	0.893	0.750	0.769	0.750	0.846	0.833	0.917	0.923	0.750	1.000	0.615	0.607	0.077	0.643	0.538	0.692	0.615	0.786	0.643	**	0.321	0.188	0.308
Z408	0.821	0.867	0.714	0.767	0.867	0.857	0.750	0.857	0.808	0.884	0.893	0.867	0.964	0.679	0.467	0.643	0.700	0.714	0.464	0.786	0.567	0.700	0.679	**	0.188	0.250
7137	0.125	0.625	0.625	0.625	0.563	0.714	0.563	0.625	0.688	0.625	0.750	0.563	0.929	0.786	0.938	0.875	0.813	0.857	0.929	0.813	0.875	0.875	0.813	0.813	**	0.143
7542	0.885	0.821	0.731	0.857	0.786	0.786	0.769	0.923	0.667	0.833	0.769	0.786	0.962	0.654	0.714	0.654	0.679	0.500	0.643	0.538	0.750	0.857	0.692	0.750	0.857	**

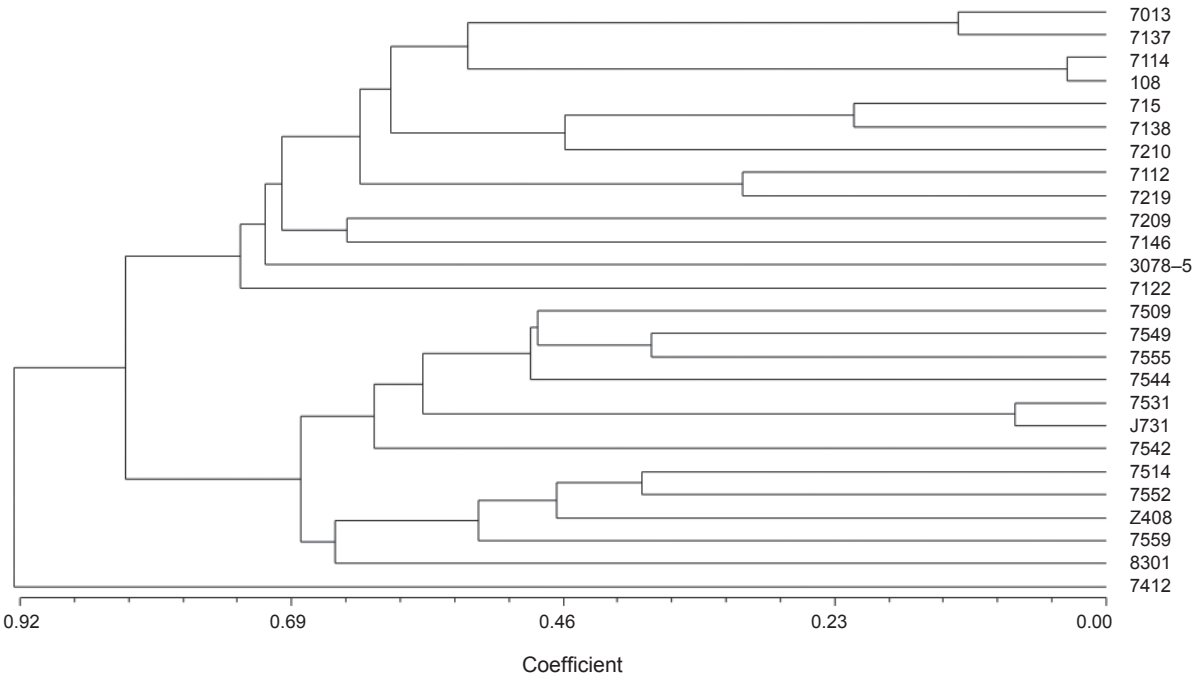

Figure 1 Dendrogram generated by unweighted pair-group method with arithmetic average clustering showing relationship between 26 clones of *Tectona grandis*, based on the Nei's (1978) genetic distances

the declared or suspected identity of clones. It would also play a significant role in registering clone, applying patent, and controlling stocking material in vegetative propagation and commercial exchanges.

This work provided a deep insight into the genetic variation of teak clones. The mean number of 10.67 alleles per locus obtained here was lower than that of 14.7 reported by Verhaegen et al. (2005) for 265 teak individuals from India, Thailand–Laos, Indonesia and Africa, and 13.4 reported by Fofana et al. (2009) for 166 trees from 17 natural provenances both using 15 SSR markers and denatured DNA. The discrepancy in these estimates could be attributed to sample size, diversity within samples and estimation of fragment sizes (Oraguzie et al. 2005). However, this value was higher than that reported by Alcantara and Veasey (2013) who obtained 5 alleles per locus when they analysed teak clones planted in west Brazil, although they used 10 SSR markers and non-denaturing system.

The means of H_o (0.6567) and H_e (0.7919) were higher than those reported elsewhere by SSR markers such as Verhaegen et al. (2005), Fofana et al. (2009) and Verhaegen et al. (2010). The H_e value was also higher than that of Ansari et al. (2012), which was based on 29 Indian teak by ISSR markers (0.40), and greatly higher than that of Sreekanth and Balasundaran (2012) (0.2282) for 31 clones of a seed orchard in south Kerala, India by AFLP markers. Furthermore, our H_e value was higher than the total gene diversity (0.4065) of another tropical forest tree *Casuarina equisetifolia*, as revealed by AFLP markers (Huang et al. 2009). This indicated large genetic variation within the clones in this investigation. In fact, teak is self-incompatible (up to 95%; Suwan & Owens (1997)), with a reproductive system characterised by frequent allogamy. The high level of observed heterozygosity, though with only 26 clones, could predominantly reflect the hybrid nature of the majority of the clones.

The UPGMA dendrogram matched essentially the historically known or speculated origin of the clones. This cluster intuitively separated clones of Indian origin from Myanmar sources. This is probably a reflection of significant divergence of the original seedlots from which these clones were derived. However, a few cases of inconsistency appeared in the dendrogram, i.e. the clones selected from different Indian provenances were not clustered by provenance

or did not group according to their territorial distribution. For example, clone 7013 (Sungam, Kerala, India) and 7029 (Sungam, Kerala, India) originated from the same provenance, 3070 (Sungam, Kerala, India), however, did not cluster closely with one another, suggesting either a common genetic base or frequent natural or anthropogenic gene flow among various teak populations from where the clone selections were made. In other words, there is a possibility that clone 7013 (Sungam, Kerala, India) and 7029 (Sungam, Kerala, India), at least one or two of them, might originate from natural hybridisation of provenance 3070 (Sungam, Kerala, India) and 3071 (Stuart Mt., Tamilnadu, India) crossed in previous generations. The same situations might occur with clone 7210 (Masale, Valley, Mysore, India) and 7219 (Masale, Valley, Mysore, India).

Clone 7412 selected from Indian provenance 3074 (Virnoli, Mysore, India) was clustered separately in a third group that was neither in the Indian nor Myanmar accessions group. This is not surprising. However, as an Indian clone, the genetic identity between 7412 (Virnoli, Mysore, India) and Indian clones were higher than the genetic identity between 7412 (Virnoli, Mysore, India) and Myanmar clones, indicating this clone still kept co-ancestry heredity with Indian clones. In contrast, there were highest genetic distances (1.000) between clone 7412 (Virnoli, Mysore, India) and Myanmar clones 7544, 7531, 7549 (Ledong, Hainan Island, China, Myanmar source), and clone J731 (Bago Yoma, Myanmar). Mislabelled case and sample collection mistake of clone 7412 (Virnoli, Mysore, India) was impossible. The exceptional cluster of 7412 (Virnoli, Mysore, India) may be accounted by its distinctive variation between clone 7412 (Virnoli, Mysore, India) and other clones. This could be proven by DNA fingerprinting (result not shown), whereby 13 of the 15 markers showed special fragments for clone 7412 (Virnoli, Mysore, India). Hence, more attention should be paid to the source material of clone 7412 during teak breeding.

It is interesting to note that clone 3078-5 (Gambari, Nigeria) selected from Nigeria provenance was clustered in the Indian group, indicating that provenance 3078 might be originally introduced from India but the offspring still kept ancestor hereditary of Indian provenance. Similarly, clone 108 (Guangzhou, China) was classified together with Indian clones and 8301 (Ledong, Hainan, China) with Myanmar clones, suggesting that the ancestors of clone 108 might be from India and those of 8301 from Myanmar. It was likely that both clones, 3078-5 (Gambari, Nigeria) and 108 (Guangzhou, China), were derived from provenance 3071 (Stuart Mt., Tamilnadu, India) because they had closer genetic identity with clones from provenance 3071 (Stuart Mt., Tamilnadu, India) and clustered in the same group. Although there were no records of the origins of early planted provenances coded 15 to 23 (Ledong, Hainan, China, Myanmar source) in this study, the genetic relationships between these Myanmar clones had been clearly revealed by similarity matrix and cluster analysis. This information on genetic relatedness of candidate clones would be useful in teak breeding programme in terms of maintaining genetic diversity.

Plus tree selection and clonal test are essential for teak genetic improvement. It normally takes more than a decade before teak clones are available. Field tests demonstrated that these 26 teak accessions performed well in different traits. Myanmar clones were cold resistant and fast growing whereas Indian clones were fast growing and wind resistant for their narrow or compact crown. Indian clone 7029 (Sungam, Kerala, India) is really a super clone selected from provenance 3070 (Sungam, Kerala, India), which combines virtues of Myanmar and Indian clones. The use of SSR markers to identify the most divergent varieties can aid in genetic improvement programme. Clone 7412 (Virnoli, Mysore, India) was shown to be more divergent compared with other Indian materials. Indian materials are divergent with Myanmar materials, so it is possible to use these genotypes in controlled pollination for breeding programmes. At the same time, quality control of planting material is essential for ensuring reliability and future of teak clone forestry. Therefore, the application of DNA fingerprinting lends emphasis not only to our seriousness in developing the best quality planting materials but also the usefulness of such information. The results achieved in this study combined with appropriate clone testing are important for superior clone identification and wise management.

CONCLUSIONS

A total of 26 commercial teak accessions could be accurately and rapidly fingerprinted using microsatellite markers. The microsatellite-based clone genotyping would have significant contributions to the clonal registration and identity control during vegetative propagation, plant stock handling and commercial exchanges. Additionally, the 26 teak accessions contained a wide range of genetic diversity. Their relationships and original information should be valuable to breeding programmes and genetic resource management.

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