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Comparative analyses of amplified fragment length polymorphism (AFLP) and simple sequence repeat (SSR) in genetic diversity of Teak (*Tectona grandis* L.f)

Inza Jesus Fofana[1]*, Souleymane Silue[1], Nafan Diarrassouba[1], Adjoumani A Kadio[2] and Abdourahamane Sangare[3]

[1]Université Péléforo Gon Coulibaly, UFR des Sciences Biologiques, Département de Biologie et Environnement, B. P. 1328 Korhogo, Côte d'Ivoire.
[2]Société de Développement des Forêts (SODEFOR), 01 BP. 3770, Abidjan 01, Côte d'Ivoire.
[3]Conseil Ouest et Centre Africain pour la Recherche et le développement Agricole (CORAF), Programme Biotechnologie et Biosécurité, B. P. 48, Dakar RP, Sénégal.

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ABSTRACT

The performance of different molecular markers in the assessment of population structure was tested using samples of *Tectona grandis* collected in Côte d'Ivoire. A total of 229 individuals belonging to 26 population samples were analysed using 15 simple sequence repeats (SSR) and four amplified fragment length polymorphisms (AFLP). Considering the global qualitative patterns, a correlation between SSR and AFLP in detecting genetic differentiation among samples was found. The percentage of individuals correctly assigned to their population of origin was higher with SSR than with AFLP. The higher power of discrimination and the comparative technical ease of obtaining data from SSR with respect to AFLP suggests the use of SSR for many population genetics studies. The proportion of the total genetic variation resides within provenances with 71.87% and 80.52% according AFLP and SSR markers, respectively. The two molecular markers showed a clear differentiation of the populations introduced in Côte d'Ivoire with an Fst=0.21. Moreover, this study highlighted an important rate of heterozygotes with SSR loci in all the populations thus affirming the mode of allogame reproduction of the species.

INTRODUCTION

Tectona grandis of the family Verbanaceae is one of the most economically important tropical timber tree species. The species is native to the tropical deciduous forests of India, Laos, Thailand, Indonesia and Myanmar (Troup, 1921). Teak, classified as one of the finest and most valuable timber species in the tropics exhibits desirable technical and decorative properties. The timber is suitable for various purposes including house construction, shipbuilding, furniture making, poles, veneer, carvings, etc. The broad product suitability of the timber, its high demands and price on the international market and short rotation have triggered extensive planting programme throughout the tropics (Fofana et al., 2008).

Since 1969, teak was given a top priority for provenance investigations by the FAO (Food and Agriculture Organisation) Panel of Experts on Forest Genetic Recourses (FAO, 1969; Fofana et al., 2008). Due to this, a series of internationally co-ordinated Teak provenance trials were established throughout the tropical regions especially in Asia, Africa and Central

*Corresponding author. E-mail: gomeled@yahoo.fr. Tel: (225) 36 86 50 16.

America (Kjaer and Lauridsen, 1995). Teak constitutes about 75% of the world's high-quality tropical hardwood plantations (FAO, 2001). About 43% of all Teak plantations are located in India, 31% in Indonesia, 7% in Thailand, 6% in Myanmar and 5% in tropical Africa. The provenance trials showed that plantations based on seeds imported from Kerala (India) had better form and grew 30% faster than plantations made with seeds from the landraces (Kjaer et al., 1995). This shows that domestication with planting stock selected from superior tress can increase the benefits. Hence, it is important to initiate and support selection and testing of superior individuals in local breeding activities.

The utility of molecular markers for analysis of the genetic structure and identification of markers linked with important traits are of prime importance in the domestication, improvement and conservation of the species. Nevertheless, there are only few reports (Kartadikara and Prat, 1995a; 1995b) on molecular genetic diversity in Teak. There are also powerful tools for assessing genetic variation within and among populations (Powell et al., 1995).

Among PCR-based markers, microsatellites [simple sequence repeats (SSR)] and amplified fragment length polymorphisms (AFLP) are probably the most informative at the population taxonomic level. SSR are nuclear codominant DNA markers consisting of short (1–6 base pairs) tandem repeats that appear to be highly abundant and dispersed throughout the genome of eukaryotes (Jarne and Lagoda, 1996). Variation in the number of repeat units often produces an abundance of alleles distinguishable by molecular size. AFLP are generated by selective amplification of restriction fragments obtained from the digestion of total genomic DNA (Vos et al., 1995). The resulting markers are dominant (heterozygotes cannot be distinguished from dominant homozygotes) and bi-allelic (for a given size, the fragment is either present or absent). The use of these methods in the assessment of population genetic structure presents both advantages and drawbacks. In contrast with SSR that are generally species-specific loci markers, the AFLP technique does not require any prior knowledge about the genome, and a large number of markers are easily and quickly available. The drawbacks of this method are dominance and non-specificity of the PCR. In addition, since homology of comigrating fragments is rarely tested, there is also the possibility that bands scored as identical in different individuals are homoplastic. In forest organisms, empirical studies describing genetic diversity and population structure obtained from SSR are abundant in literature whereas, in spite of the versatility of the method, the number of genetic surveys using AFLP is limited (Bensch and Akesson, 2005). In addition, comparisons between AFLP and SSR markers' efficiency are still rarely found, since the majority of these studies were carried out to identify cultivars and varieties of crop species involved in breeding programs (Nybom, 2004; Woodhead et al., 2005). In comparison with morphological traits, molecular makers have many advantages. The molecular markers are not subject to environmental change, making them specially informative and superior to traditional methods (Melchinger et al., 1994), including restriction fragment length polymorphism (RFLP) markers (Manoj et al., 2013); randomly amplified polymorphic DNA (RAPD) (Balakrishnan and Vadivel, 2012), AFLP (Lombard et al., 2000), and SSR (Verhaegen et al., 2005). These markers give broad and different ranges of information and substantially differ in terms of practicability and reproducibility.

To evaluate the efficiency of different DNA-based marker systems in resolving genetic structure in Teak provenances, we have examined genetic variation of the samples of the two tests of Teak descents from two sites of the SODEFOR by analysing 15 SSR and 4 AFLP loci. The objective was to compare estimates of population diversity and differentiation obtained using both markers and to compare the usefulness of AFLP and SSR in the assignment of individuals to their population of origin. This kind of comparative analysis should elucidate the relative efficiency of different markers such as SSR and AFLP in resolving gene flow and genetic population structure among samples of *T. grandis.*

The AFLP and SSR are two powerful DNA fingerprinting techniques. A number of polymorphic fragments can be detected in an experiment and there is a higher reproducibility of banding patterns by AFLP. SSR markers have several advantages over other molecular markers for their co-dominant inheritance, large number of alleles per locus, and abundance in genomes. However, there are few reports concerning AFLP and SSR techniques for genetic diversity and relationship among *T grandis* hybrids. Therefore, we have compared the level of information provided by AFLP and SSR markers for estimating genetic relationships among Teak hybrids. This study provides useful information for Teak breeding program.

MATERIALS AND METHODS

Plant material and DNA isolation

Based on the seed collection and distribution made by Danida Forest Seed Centre in 1971–1973 (Keiding et al., 1986), 166 trees were identified as representative of the main natural occurrences of the species in India, Thailand and Laos. The aim of the seed collection was to obtain as much as possible a broad representation of the whole range of distribution, covering the most typical and distinctly different types of environments. The seeds from the natural area which were produced by open pollination

Table 1. Studied provenances.

Sample site	Country	Trials' situation	Provenance name	Number of trees studied	Annual rainfall (mm)
Populations from Africa	Cote d'ivoire	Séguié	Bamoro A29	5	1200
		Séguié	Bamoro A20	5	1200
		Téné	Bouake 3037	7	1200
		Téné	Bouake TB73	6	1200
	Senegal	Séguié	Djibelor	6	1640
		Séguié	Kalounayes	5	1640
	Benin	Séguié	Djigbe	5	1100
		Séguié	Toffo Lama	6	1100
	Cameroun	Séguié	Bambuku7	5	1780
		Téné	Bambuku3067	5	1900
	Togo	Téné	Tove	5	1300
	Tanzanie	Séguié	Mtibwa	10	1160
		Séguié	Kihuhwi	10	1880
		Téné	Bigwa	14	900
		Séguié	Mae Huat	6	1300
Populations from natural area	Thailande	Séguié	Pong Salee	6	1500
		Séguié	Huoi-Na-Soon	7	1350
		Téné	Ban Chm Pui	16	1100
		Téné	Ban Pha Lai	11	1100
	India	Séguié	Nellicutha(15)	22	2700
		Séguié	Nellicutha(16)	18	2700
		Séguié	Virnoli Range	7	2030
		Téné	Masale Valley	9	1300
		Téné	Purunakote	10	1300
	Laos	Téné	Paskse	5	2000
		Téné	Pak Lay	13	1200
	Total			229	

The studied provenances cover a wide area geographically with a range of 110 to 2700 mm of annual rainfall. Teak grow naturally mainly in mixed deciduous forests with a distinct seasonal climate (wet and dry seasons). Trials situation are localised in Côte d'Ivoire.

were sowed and raised in a nursery then planted in two comparative provenance trials in Côte d'Ivoire (Téné and Séguié) by SODEFOR. The provenances trial plots were established in 1970 and 1974 for Séguié and Téné, respectively. For this study, individuals were collected in these sites. It consists of a total of 26 provenances from India, Thailand, Laos (natural area) and landraces from Africa. Leaf samples were taken from all the 'plus-trees' marked on each plot. Another five trees per provenance were collected based on characters such as crooked-ness, buttressing, forking, etc. to represent the maximum variability within each provenance. A total of 229 leaf samples were collected in the populations of 133 from the 3 natural areas and 96 from Africa populations (Table1).

The leaf samples were then washed with clean water dried with clean towels and placed in paper envelopes. Each paper envelope was sealed and then placed in a plastic envelope containing 10 g of silica gel to absorb moisture from the leaf samples as well as preserving the green colour. The plastic envelopes were sealed and

kept in an air-conditioned laboratory maintained at 16°C. The samples were used for molecular analysis at the laboratory of CIRAD in Montpellier (France). Total DNA was extracted from 300 mg of dry leaf material using a Mixed Alkyl Trimethyl Amminium Bromide (MATAB) method derived from Gawel and Janet (1991) and stored at -20°C.

Simple sequence repeat (SSR) analysis

Teak samples were screened for variation at 15 SSR loci. PCR amplifications were carried out as described by Brondani et al. (2002) for loci 1TA6 1TB3 1TF5 1TG2 1TH10 2TB7 2TC3 3TA11 3TB2 3TD9 3TE6 3TF1 4TD12 4TF2 4TH9. Genotyping of individuals was performed by assessing allele size on IR^2 DNA analyzer (LI-COR Inc., USA), using forward primers labelled with IRDye 700 or IRDye 800 as internal standard. Allele sizing was performed using the Saga analysis software Generation 3.2.

Amplified fragment length polymorphism (AFLP) analysis

AFLP marker profiles were generated using procedures described by Vos et al. (1995). Three *Eco* RI/*Mse* I primer combinations was used in the second selective amplification (selective nucleotides AG/CAA, AG/CTA, AG/CAT and TA/CAC) to generate markers ranging from 70 to 400 bp in size. In each case, the *Eco* RI selective primer was labelled with the fluorescent dye. Selective PCR products were separated on IR^2 DNA analyzer (LI-COR Inc., USA) which uses a laser system to detect IR fluorescence chemistry and laser technology for detection of alleles. Electropherograms were subsequently analysed using Quantar pro.5. The intensity of each individual peak was normalised on the basis of the total signal intensity and the peak was considered only if its intensity exceeded a fixed threshold. All the polymorphic peaks were considered in the analysis. To test the repeatability of AFLP procedure, height individuals were completely replicated starting from the same DNA extraction and no differences between the two analyses were observed.

Data analyses

Within-population genetic diversity, SSR measures based on allele frequencies, percentage polymorphic loci, mean number of alleles, estimates of observed and expected heterozygosity, genetic distances and genetic identity were calculated using software GENETIX 4.05 (Belkhir et al., 2001).

The significance of the genetic differentiation between samples was tested by comparison of the observed Fst with a distribution of Fst under the hypothesis of no genetic structure, obtained by means of 1000 random permutations of individuals among groups. Fst was calculated for SSR using Weir and Cockerham's (1984) estimator as computed by the method of Jackknife (Weir, 1990) with GENETIX software. For AFLP, Fst was calculated using Popgene version 1.3.1 (Yeh et al., 1999). This program uses the approach of Lynch and Milligan (1994) to calculate population genetic parameters on the basis of the expected heterozygosity of dominant marker loci. For SSR, the inbreeding coefficient inbreeding coefficients (Fis) (Weir and Cockerham, 1984) was estimated using GENETIX.

In order to test the consistency of SSR and AFLP markers in estimating genetic differentiation parameters, hierarchical analysis of molecular variance (Amova) (Excoffier et al., 1992) were calculated with the program Arlequin, version 2.0 (Schneider et al., 2000), in which significance levels for the populations values were determined after 1000 permutations.

RESULTS

Simple sequence repeat (SSR) markers

Allelic diversity

For the 15 loci microsatellites, 189 different alleles were observed with an average of 12.6 alleles by locus. A total of 67 specific alleles to only one geographic area were found. Among these alleles, 39 were observed only in India area, 4 alleles were specific to Thailand-Laos and 24 alleles were identified in Africa (8 for West Africa and 16 alleles for Tanzania). 100 alleles were observed one to three times in natural area and one to four times in Africa. Among the 189 alleles observed, 59 common alleles were observed in all the populations with an average frequency varying from 2.09% (3TF1-212) to 85% (1TG02-166). Allele 218 of the locus 3TE6 was fixed in 8 populations. Allele 157 of the locus 4TH9 was fixed in 4 provenances, whereas this locus presents heterozygotes deficits in 7 populations. The allele's 3TD9-208, 4TF2-227 and 3TA11-157 were fixed in three populations. In natural area, the analysis indicates that the number of alleles varies from 33 (Pakse) to 119 (Nellicutha16). The number of allele by locus ranged from 3 (1TG2) to 19 (1TA6). The locus 1TG2 presents a deficit of heterozygotes in 11 provenances and for 6 of these populations, the locus was fixed. It was panmictic equilibrium in only one population. Five loci were fixed in Pakse which presents as if it only a deficit of heterozygotes on 9 loci. The loci 4TH9, 3TA11, 3TE6, 4TF2 and 1TG2 were fixed in at least a population. Of the 189 alleles, 177 were present in India with 113 being

Table 2. Descriptive statistics over all SSR loci for each population.

Sample site	Provenance	N	He	Ho	P(0.95)	Ave	Fis
Populations from natural area	Nellicutha15	22	0.708 (0.12)	0.715 (0.141)	100	6.866	0.013ns
	Nellicutha16	18	0.726 (0.124)	0.763 (0.189)	100	7.667	-0.021ns
	Massale	9	0.732 (0.128)	0.785 (0.211)	100	6.533	-0.014ns
	Virnoli	7	0.685 (0.146)	0.733 (0.240)	100	5.200	0.008ns
	Purunakote	10	0.609 (0.23)	0.626 (0.257)	93.33	6.200	0.024ns
	Pong-Salee	6	0.346 (0.277)	0.366 (0.316)	73.33	2.600	0.032ns
	Mae Huat	6	0.350 (0.294)	0.400 (0.349)	66.67	2.600	-0.053ns
	Ban Cham	16	0.354 (0.289)	0.391 (0.334)	60	3.200	-0.073ns
	Ban Pha	11	0.468 (0.247)	0.521 (0.302)	86.67	3.800	-0.065ns
	Huoi-Na	7	0.341 (0.281)	0.323 (0.321)	66.67	2.467	0.128*
	Pak Lai	13	0.304 (0.278)	0.312 (0.316)	66.67	2.733	0.013ns
	Pakse	5	0.249 (0.284)	0.320 (0.391)	53.33	2.067	-0.178ns
Populations from Africa	Djibelor	6	0.594 (0.230)	0.577 (0.307)	93.33	4.200	0.18*
	Kalounayes	5	0.522 (0.208)	0.586 (0.315)	100	3.600	-0.011ns
	Bamoro A29	5	0.613 (0.204)	0.746 (0.266)	100	4.333	-0.109ns
	Bamoro A20	5	0.629 (0.167)	0.666 (0.317)	100	4.133	0.052ns
	Bouaké3037	7	0.606 (0.206)	0.723 (0.272)	100	4.600	-0.118ns
	BouakéTB73	6	0.556 (0.243)	0.666 (0.339)	86.67	3.800	-0.109ns
	Tové	5	0.589 (0.217)	0.640 (0.253)	100	3.933	0.025ns
	Djigbé	5	0.642 (0.178)	0.720 (0.224)	100	4.467	-0.009ns
	Toffo Lama	6	0.569 (0.260)	0.655 (0.336)	93.33	4.333	-0.061ns
	Bambuku7	5	0.440 (0.251)	0.520 (0.309)	86.67	3.000	-0.072ns
	Bambuku3067	5	0.565 (0.226)	0.720 (0.3)	93.33	4.067	-0.168ns
	Kihuwi	10	0.598 (0.181)	0.613 (0.277)	100	5.133	0.028ns
	Mtibwa	10	0.580 (0.218)	0.573 (0.231)	100	5.400	0.064ns
	Bigwa	14	0.578 (0.254)	0.601 (0.321)	86.67	5.933	-0.004ns

N, Number of individuals; He, expected proportion of heterozygotes; Ho, observed proportion of heterozygotes; P (0.95), percentage of polymorphism α=0.5; Ave, average number of alleles per locus; Fis, the inbreeding coefficient; ns, p-value adjusted using sequential Bonferroni procedure not significant; * p-value adjusted using sequential Bonferroni significant < 0.05.

specific, whereas Thailand had 75 and 11 being specific. Of the 94 individuals of the 14 African's populations of Africa, a total of 162 different alleles were observed which is equivalent to an average of 10.8 alleles per locus. The number of alleles by locus ranged from 3 (1TG2) to 16 (1TH10). The microsatellites highlighted a variation of the number of loci in the populations, ranged from 53 (Kalounayes) to 91 (Bigwa). The locus 1TG2 presents a deficit of heterozygotes in 13 populations and it is fixed in 4. The locus 3TE6 is fixed in Djibelor and Mtibwa. The Tanzania's populations (Bigwa, Kihuwi and Mtibwa) are those which present the greatest number of alleles with 91, 78 and 86 alleles respectively.

Genetic diversity

From the natural area, analysis of the 15 loci by applying the criterion of a maximum frequency of 95% for the most common allele showed percentage of polymorphism which was higher than 50% for all the populations with an average percentage of 80.55% (Table 2). It varies from 53.33 (Pakse) to 100% (Nellicutha 15, Nellicutha 16, Masale Vallée and Virnoli). In the natural area, Thailand shows 73.33% of polymorphism and 100% in India. The average observed heterozygosity for the 15 analyzed loci ranged from 0.31 (Pak Lai) to 0.78 (Massale Valley). The average observed heterozygosity was 0.52 and the value of Hardy-Weinberg equilibrium was 0.48 (Table 2). In Huoi-Na-Soon, the expected heterozygosity (0.32) is significantly higher than the observed heterozygosity (0.34). Heterozygotes deficit was observed in this population except for Huoi-Na-Soon were the differences between the observed heterozygosity and the expected hétérozygosity was not different from the other populations, meaning that they are in Hardy-Weinberg equilibrium. The mean observed heterozygosity was 0.72 in India and 0.38 in Thailand, whereas the values of

expected heterozygosity were 0.77 and 0.41 respectively for India and Thailand. Within each area, these values are not significantly different. The number of alleles by locus ranged from 2.06 (Pakse) to 7.66 (Nellicutha16) with a mean of 4.32 by locus. Thailand presents 5.06 alleles by locus while India has 11.8. The allelic richness of India was more than the double of Thailand. The average Wright's inbreeding coefficient (F_{IS} = -0,016^{ns}) indicate a Hardy-Weinberg equilibrium. It ranged from -0.17 (Pakse) to 0.12 (Huoi-Na-Soon). From African provenances, the average percentage of polymorphic locus is 95.7% with a mean range of 86.67% (Bigwa, Bambuku 7 and Bouaké TB) to 100% (Table 2). 8 populations showed the maximum polymorphism average. The polymorphism percentage in African's populations is 100%. The average observed heterozygosity ranged from 0.52 (Bambuku 7) to 0.746 (Bamoro A20) for a mean observed heterozygosity of 0.635. The value of expected heterozygosity was 0.648. These two values are not significantly different. In the l African's populations (unknown origin), only Djibelor showed a mean of expected heterozygosity significantly higher than the mean of observed heterozygosity. A deficit in heterozygotes was thus observed in this population. The average observed heterozygosity and expected heterozygosity were not significantly different from the other populations. These populations were in Hardy-Weinberg equilibrium. The number of alleles by locus ranged from 3.00 (Bambuku7) to 5.93 (Bigwa) with an average of 4.35 by locus. The general average of alleles by locus on the level of the populations is half of what was observed in Africa (10.8). The indices of fixing (Fis) ranged from -0.168 (Bambuku 3067) to 0.118 (Djibelor). For these populations, the average was positive but not significant (0.02). An analysis of molecular variance revealed that 5.5% occurred among populations within regions and 80.52% occurred within populations. The AMOVA analysis showed most variation occurs at the within population. However, a significant amount of variation was also attributed to in between population. The same analysis revealed that 13.98% of the total genetic variance was distributed among regions. The Fst (0.2***) value was positive and highly significant, indicating clearly a differentiation between the geography area of Teak. Nevertheless, the pattern of between and within population genetic variation was very unevenly distributed.

AFLP markers

Allelic diversity

196 different markers were observed during the study of the genetic diversity of the samples with only four couples of starters used. The primers differed in their ability to detect polymorphism within populations. agcaa and atcac markers were presented in 33 alleles forms in the populations. For agcat and agcta loci, they are observed by 75 and 55 markers respectively. The frequencies of 196 markers AFLP in the studied populations showed that agcaa185, agcat221 and atcac132 markers were missing in India. In Thailand's populations, 17 alleles were missing. Among the 196 alleles observed, only atcac344 and atcac229 were not found present in Africa. In the natural area, the four couples of loci used present 196 polymorphic alleles. The presence of the markers varies from 10 to 103 in all of the samples. 92 individuals present at more than 50 alleles. Of the 196 markers, 131 were present at more than 5 individuals. 65 markers are present more than 50 times in the natural area. The population of Pakse showed the weakest rate of polymorphic alleles (88 alleles). The AFLP markers present 193 alleles in India against 179 in Thailand. In African populations (unknown origin), the most polymorphic marker (agcta138) was 82 times present in the samples and agcaa320, the least polymorphic is present only five times. Forty five markers are present more than 50 times. The samples i024 (Bouaké TB73) i024, i031, i032 (Djibelor), i035 (Djigbé), i055, i063, i152 (Kihuwi) and i053, i057, i059, i148, i149 (Mtibwa) have more half (98) markers analyzed.

Genetic diversity

In the natural area, the percentage of polymorphic markers per population is higher than 50% in 11 populations (Table 3). The percentage of polymorphism bands varies from 44.89% (Pakse) with 86.73% (Nellicutha15, Nellicutha16 and Massale Valley). The polymorphic variations are not correlated with the samples number because Masale valley, Nellicutha 15 of Nellicutha 16 observed the same polymorphisms values with different number of sample. The percentage of polymorphic bands is 84.18% in India and 62.24% in Thailand with approximately same samples. From African provenances, the number of polymorphic alleles varies 59 (Bambuku 7) to 176 (Mtibwa). The average rate of polymorphic alleles in Africa is 57.14%; 175 markers (89.79%) were observed in Mtibwa. Bamuku 7 introduces to the low value (30.10%) of polymorphic markers. 9 populations present a percentage of polymorphism higher than 50% of which the maximum is 89.79. The average over all populations of polymorphic bands amplified per primer pair combination varied from 33 (E-AG/M-CAA, E-TA/M-CAC) to 75 (E-AG/M-CAT). The percentage of polymorphic bands for a given primer combination ranges form 16% (EcoRI AG + MseI CAA, EcoRI TA + MseI CAC) to 38% (EcoRI AG + MseI CAT). An analysis of molecular variance revealed that 12.6% of the total genetic variance occurred among group, 15.5% among

Table 3. Descriptive statistics over all AFLP loci for each population.

Sample site	Provenance	N	PS	PM P	PP (100%)
Populations from natural area	Ban Cham Pui	16	196	167	85.20
	Ban Pha Lai	11	196	135	68.87
	Huoi-Na-Soon	7	196	109	55.61
	Mae Huat	6	196	111	56.63
	Pak Lai Main	13	196	135	68.87
	Pakse	5	196	88	44.89
	Pong Salee	6	196	107	54.59
	Purunakote	10	196	135	68.87
	Virnoli	7	196	145	73.97
	Nellicutha15	22	196	170	86.73
	Nellicutha16	18	196	170	86.73
	Masale Valley	9	196	170	86.73
Populations from Africa	Bambuku3067	5	196	80	40.81
	Bambuku 7	5	196	59	30.10
	Bamoro A20	5	196	110	56.12
	Bamoro A29	5	196	99	50.51
	Bigwa	14	196	160	81.63
	Bouaké 3037	7	196	125	63.77
	BouakéTB 73	6	196	120	61.24
	Djibelor	6	196	129	65.81
	Djigbe	5	196	95	48.46
	Kalounayes	5	196	89	45.40
	Kihuwi	10	196	143	72.95
	Mtibwa	10	196	176	89.79
	Toffo Lama	6	196	108	55.10
	Tove	5	196	76	38.77

N, Number of individuals; PS, number of bands selected; PM, polymorphic markers; PP, percentage of polymorphic markers.

populations within groups and 71.9% occurred among individuals within populations.

DISCUSSION

Comparison between AFLP and SSR marker systems

The main aim of this study was to compare the performance of SSRs and AFLPs in the assessment of genetic divergence of *T. grandis*. The estimates of genetic variability obtained using SSRs were higher than those calculated from AFLP data. The large variance of the genetic variability estimates detected across the 15 SSR loci could reflect large differences in mutation rates among loci or rather result from a different exposure of these loci to selective forces acting on the genomic regions or loci to which SSRs are linked. Mutation rate may have a significant effect on the allele variation shown by SSR loci. Depending on the genomic position of the locus under consideration, SSR mutation rates from 10^{-3} to 10^{-4} have been reported in the literature (Estoup et al., 1998). In addition, several authors suggest that AFLP loci are characterized by lower mutation rates compared to SSRs (10^{-4}-10^{-6}/locus/generation; Gaudeul et al., 2004). As a consequence, absolute values of diversity calculated on data from different types of markers, especially from dominant and codominant markers, should not be directly compared. The aforementioned differences in number of alleles and mutation rates might also affect the power of different markers to detect population differentiation. Because indices estimating sample genetic divergence (for example, Fst) are calculated as ratios of among/over within-population genetic variance (Weir and Cockerham, 1984), the degree of differentiation assessed through SSRs is expected to be lower than that calculated using AFLPs (Balloux et al., 2000). On the contrary, we did not find a significant correlation between Fst and estimates of genetic variability, suggesting that the efficiency of SSR loci in detecting genetic divergence is independent from their polymorphism. No significant correlation between

size and frequencies of AFLP fragments was found in this study.

In our study, the polymorphism revealed by the AFLP and SSR markers was higher than 50%, which supported the previous reports. However, the SSR methodology exhibited a higher level of polymorphism (96%) than AFLP approach (62.7%). Although the percentage of polymorphic bands of the AFLP was lower than that of the SSR, but the polymorphic bands detected by each AFLP primer (49) were much higher than SSR (12.6). This could be attributed to the different mechanisms of polymorphisms detection using different marker systems. SSR markers detect multiple alleles at a given locus while AFLP detect multiple loci distributed throughout the genome. On the other hand, it can be explained by this mechanism that replication slippage is thought to occur more frequently than nucleotide mutations and insertion/deletion events, which generate the polymorphisms detectable by AFLP (Powell et al., 1996). This result is in agreement with other studies comparing the level of polymorphism detected with AFLP and SSR markers (Li et al., 2011). In our data set, overall and pairwise Fst estimates obtained with SSRs were higher than those obtained with AFLPs. However, in spite of differences, a consistency of results obtained from both marker types was found.

Genetic diversity

Teak has a considerable allelic richness (188 alleles for 15 loci microsatellites and 196 for 4 loci AFLP), in spite of the fact that number of samples of the 26 analyzed provenances are not very important, the number of alleles is twice higher in the India area than Thailand and four times than Laos. The African populations and north India have a same number of alleles as south India; they thus constitute an important reserve of variability. The average number of alleles by locus microsatellites of *Tectona* is the same order found at the forest trees (3.7 to 16 for *Symphonia globulifera* (Aldrich et al., 1998), 3.4 to 4.2 at *Vittelaria paradoxa* according to Kelly et al. (2004). An average of 12.5 alleles by locus was observed on the totality of the samples.

In the total of Teak population, genetic diversity is rather well organized. An excess of polymorphism is found on the level of the markers. With the 15 loci microsatellites the polymorphisms percentages are very high. However, the number of individuals by population does not seem to affect this result since the population Pong Salee, with six samples, showed 73.33% as polymorphism mean. With a higher number of samples (16 samples), the population of Ban cham Pui presents only 60% of polymorphism. The variations of hetero-zygosity means are not inevitably correlated with those of the locus polymorphic percentage. Thus, if the population of Masale valley has a maximum percentage of loci polymorphic (100%) and has the observed heterozygosity mean most important (0.785), some most polymorphic populations have a low heterozygosity average (Pong-Salee). In this study, the contrary situation does not exist (populations with heterozygosity mean higher and a lower polymorphism). AFLP analysis resulted in 196 polymorphic loci which are similar to figures reported for the tropical tree *Euterpe edulis* (Cardoso et al., 2000). A same proportion of polymorphic loci was observed in *T. grandis* (Nicodemus et al., 2003) using RAPDs and (Madan et al., 2005) using AFLPs. This suggests high levels of genetic variation in heart of Teak.

The diversity analysis made it possible to highlight a level of observed heterozygosity (Ho) relatively high within the 26 populations. This observation is in adequacy with allogamy reproduction which would be that of the species. Indeed the observations showed that Teak is a species preferentially allogam (Hedegart, 1973; Mathew et al., 1987). The Fis averages on the level of the populations are not significant. The average value of Fis is negative (-0,021) and does not significantly translates a Hardy-Weinberg equilibrium on the populations in contrast with the results of Kartadikara and Prat (1995b) out of nine Teak populations. Gene flows thus are sufficiently distributed to ensure genetic mixings. This result confirms the observations on the heterozygotes means in agreement with the reproduction mode of our species. However, the Fis observed was positive and significant in the Huoi-Na-Soon population (0,128*) which supposes a deficit of heterozygotes in this population. This deficit can be explained by the presence of null alleles observed or by a frequency of crossing among individuals in this population.

At Teak, a clear differentiations among areas and populations are observed, which results in high value of Fst (0.21). These high values are compared with those generally observed for other tropical forest species: *Vouacapoua americana* with Fst of 0.08 (Dutech et al., 2004) and *Vitellaria paradoxa* with Fst 0.04 (Sanou et al., 2005). This study showed that most of the genetic diversity observed at Teak comes from an individual variability. Our study indicated that 5.5% of the variation occurred among populations with SSR markers. The partitioning of genetic variation observed in populations of Teak with the AFLP was 71.8% within populations, 12.6% among groups and 15.5% among populations within groups. The value for within population component is in accord with expectations for woody, perennial, predominantly outcrossed species which maintain most variation within populations (Hamrick and Loveless, 1989). Such results are in contrast with Madan et al. (2005) of which the proportion within and among population diversity was 57 and 43% respectively. The amount of within populations' variation is the same as that observed for other woody, predominantly outcrossed,

long lived perennial species using different molecular markers (80%) (Sanou et al., 2005). Our study indicated that 15.5% of the variation occurred among populations, which is lower than the 43% reported by Madan et al. (2005), 22% of variation for 10 Teak populations from India using RAPD markers (Nicodemius et al., 2003) and 21% observed for 16 Teak populations from Thailand (Changtragoon and Szmidt, 2000). From these observations, the discordance between AFLPs and SSRs may be ascribed to the different efficiency of the marker systems in detecting genetic divergence and AFLP analysis suggests that Bambuku 7 and Africa samples of Teak are genetically isolated.

Many authors have published in the past about the genetic diversity of rapeseed cultivars using AFLP and SSR markers (Jin et al., 2006). The results of this work clearly demonstrate that both AFLP and SSR markers can be successfully used for genetic diversity and relationship among *T. grandis*, although only limited numbers of hybrids and primer combinations were analyzed. It is necessary to utilize a larger number of AFLP primer combinations and SSR primer pairs on a wide range of cultivars to distinguish all the hybrids. The SSR technique was the best choice for the evaluation of diversity and assessing the genetic relationships among Teak, AFLP technique was an optimal method for DNA fingerprinting of *T*. *grandis* germplasm. Moreover, the AFLP and SSR techniques could be used in a complementary way to unambiguously distinguish hybrids. For the first stage, the SSR is used to distinguish most hybrids; then, at the second stage, the AFLP is further employed to characterize the most similar ones due to the high polymorphism.

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