Research Article

Genetic structure and conservation of Teak (*Tectona grandis*) plantations in Côte d'Ivoire, revealed by site specific recombinase (SSR)

Inza J. Fofana[1*] Yehili J.Lidah[1] Nafan Diarrassouba[2] Simon P. A. N'guetta[1] Abdourahamane Sangare[2] and Daniel Verhaegen[3]

[1]Laboratoire de génétique à l'UFR Biosciences Université de Cocody-Abidjan 22 BP. 582 Abidjan 22, Côte-d'Ivoire. *Email: gomeled@yahoo.fr.
[2]Laboratoire Central de Biotechnologie du CNRA 01 BP. 1740 Abidjan 01, Côte d'Ivoire.
[3]CIRAD Biological System Department Research Unit 39: "Genetic Diversity and Breeding of Forest Tree Species" Campus International de Baillarguet TA A-39/C 34398 Montpellier Cedex 5, France.

Abstract
Teak (*Tectona grandis* L.f; Verbenaceae) is a diploid species (2n=36). It is native to the tropical deciduous forests of India, Myanmar, Thailand and Laos. Snuffed for its aesthetic physical properties and its qualities, teak is becoming increasingly important in the forest plantation development in Côte d'Ivoire. To preserve the genetic resources of this species and ensure the supply of genetically superior quality germplasm for improvement and plantations, a core collection of superior genotypes with large genetic diversity is a prerequisite. This paper reports the use of site-specific recombinase (SSR) technology using microsatellite DNA markers to investigate the level of genetic variability, distribution of genetic variation and genetic relatedness in *Tectona grandis* grown in Côte d'Ivoire. The proportion of the total genetic variation resides within provenances (80.52 %) with 5.5 % of the variation occurring among populations of one region and 13.98 % among regions. The SSR markers showed a clear differentiation of the populations introduced in Côte d'Ivoire with an Fst = 0.21. The populations coming from the natural area were characterized by three clusters corresponding to South India, North India and Thailand. The study on the origin of African teak was close to North of India. However, Bambuku 7 population was an exception, as it seemed to have some affinity with Thailand populations. The use of SSR markers for conservation of teak forest diversity is discussed.

Key words: *Tectona grandis*, Teak wood, tree genetic diversity, tree genetic structure conservation, West Africa, Côte d'Ivoire.

Résumé
Le teck (*Tectona grandis L.f*) est une espèce feuillue de la famille des Verbenacées avec 2n = 36 chromosomes. Il pousse naturellement dans toute la péninsule indienne, au Myanmar, en Thaïlande et au Laos. Prisé pour ses propriétés physiques et ses qualités esthétiques, le teck est la première essence de reboisement en Côte d'Ivoire. Pour conserver les ressources génétiques de cette espèce et assurer un approvisionnement de la qualité génétique du genoplasme, une collection des génotypes supérieurs avec une grande diversité génétique est une condition préalable. Cet article montre la technologie utilisée par les marqueurs microsatellites de l'ADN pour la caractérisation de la variabilité génétique, la distribution de la variation génétique et les liaisons génétiques du *Tectona grandis* en Côte d'Ivoire. La proportion de la variation génétique totale réside à l'intérieur des provenances (80.52%) avec 5.5% de la variation qui se produit entre les populations d'une région et 13.98% entre les régions. Les marqueurs microsatellites ont montré une nette différenciation des populations introduites en Côte d'Ivoire avec un Fst = 0.21. Les populations provenant des aires naturelles ont été caractérisées par trois groupes correspondant à l'Inde du sud, à l'Inde du nord et à la Thaïlande. La recherche des origines des tecks africains a montré que ces derniers sont probablement originaires de l'Inde du nord. Cependant, la population Bambuku 7 a fait une exception, car elle semble avoir une affinité avec les populations de la Thaïlande. L'utilisation des marqueurs microsatellites pour la conservation de la diversité forestière du teck est discutée.

Mots clés: *Tectona grandis*, bois de Teck, diversité génétique de l'arbre, conservation de la structure génétique de l'arbre, Afrique de l'Ouest, Côte d'Ivoire.

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Introduction

Teak, *Tectona grandis* (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, and Myanmar [1]. Teak is mainly an outcrossing species. The outcrossing rates are high and range between 89% and 95% [2]. It has a long history as a plantation species due to its valuable timber, and has been introduced successfully as an exotic species to many parts of the tropical world [3]. Today it is considered to be one of the most promising plantation species in the tropics [4]. The genetic origin of the stands found outside the natural distribution area is largely unknown, as historical records mostly are inadequate. However, in the case of introduction to Côte d'Ivoire, some knowledge is available. Teak was introduced to Côte d'Ivoire from Togo in 1926 [5].

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, and carvings. 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. Société de Développement des Forêts (SODEFOR) in Côte d'Ivoire administers approximately 60.000 ha of teak plantations. Today, these trials have reached an age of almost 30 years, representing a unique source of information for teak growers in the region. (Aimé Kadio, pers. com).

The populations develop heritable adaptations to local environmental factors in order to survive in different ecological conditions. In 1969, Teak was given a top priority for provenance investigations by the FAO (Food and Agriculture Organization) Panel of Experts on Forest Genetic Recourses [6]. Due to this, a series of internationally co-ordinated Teak provenance trials were established throughout the tropical regions especially in Asia, Africa and Central America [7, 8]. These involved provenances from India, Laos, Thailand and Indonesia and landraces from Africa and Latin America.

The provenance trials have shown that plantations based on seeds imported from Kerala, India had better form and also grows 30 % faster than plantations made with seeds from the landraces [8]. This shows that domestication with planting stock selected from superior trees can increase the benefits. Hence, it is important to initiate and support selection and testing of superior individuals in local breeding programmes.

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 [9] on molecular genetic diversity in teak. Microsatellites are short tandem repeats of di-, tri- or tetra- nucleotides which are produced by mutations, uneven crossing over or DNA slippage [10]. They are abundant in eukaryotic genomes [11] and have been good sources of genetic markers in many plants such as *Vitellaria paradoxa* [12] and *Punis sylvestris* [13] due to their hypervariability in nature. They have been used in estimating gene flow, mating parameters and paternity coefficients [14]. *T*wo types of simple sequence (poly (dA).poly (dT) or poly (dG-dT).poly (dC-dA)) have been shown to be repetitive and interspersed in many eukaryotic genomes [15]. In humans, (TG)n repeats have been found in several sequenced regions [16].

Microsatellites are also powerful tools for assessing genetic variation within and among populations. The objectives of this study were to measure genetic variability within and among teak populations in Côte d'Ivoire, with the aim of seeking the probable origins of the African populations.

Methods

Plant material and DNA isolation

Individuals were collected throughout the forest of Téné and Séguié managed by SODEFOR (Société de Développement des Forêts) society. The provenance trials were established in 1970 and 1974 at Séguié and Téné, respectively. A total of 26 provenances from India, Thailand, Laos and African landraces were compared in complete randomized repetitions.

Leaf samples were taken from the "best or plus-trees" (trees with a good phenotype) on each plot and from five unselected trees per provenance in order to represent the maximum variability within each provenance. A total of 229 trees were collected in the 26 populations, 133 trees were coming from the natural area and 96 trees from the african populations. The number of trees studied and the main characteristics of the populations are given in Table 1

After collection, the leaf samples were then washed with clean water, dried with clean towels and placed in paper envelopes. Each paper envelope containing a leaf sample was sealed and then placed in a plastic envelope containing 10 g of silicagel to absorb moisture from the leaf samples. The plastic envelopes were sealed and kept in an air-conditioned laboratory maintained at 16 °C. Total DNA was extracted from 300 mg of dry leaf material using a Mixed Alkyl Trimethyl Amminium Bromide (MATAB) method and stored at -20 °C.

Screening and selection of SSR primers

Thirty six SSR primers were obtained from genomic bank of Montpellier Cirad-forest. Polymorphic tests were carried out on 8 samples. The purpose of these tests was to select the most informative loci for this study. Each 25 µl reaction mixture contained 15 ng DNA, two mM $MgCl_2$, 200 µM dNTPs, 0.2 µM each of forward and reverse primers, one unit of Taq polymerase and 1x enzyme buffer according to [17] method. A drop of oil was put on the surface of PCR mix in each tube to prevent evaporation. The surface of PCR mix in each well was overlaid with a drop of ultra-pure mineral oil and then sealed with polythene thermowell sealer. DNA was subjected to amplification in a robotic thermocycler under the following conditions: initial denaturation temperature of 94 °C for 4 minutes, followed by 36 cycles of 94 °C for 30 sec, annealing temperatures of 47 °C, 51 °C depending of the primer for 30 sec and 72 °C elongation for 1 min. Final elongation phase of 72 °C for 5 min.

The PCR amplified products were electrophoresed on 1 % horizontal agarose gel stained with ethidium bromide to check for positive amplification. The DNA fragments were visualised under UV transilluminator and photographed using a polaroid camera. Primers that produced one or two clearly defined bands per accession were selected for electrophoresed on Licor™ according to the protocol described in following paragraph. The PCR revelation on sequencer was printed and the polymorphic loci selection was done. This screening made it possible to select 15 loci for this study [18]. The purpose of this test was also to determine the Series of PCR products dilutions before the samples migration.

Polymorphism

The PCR mix was amplified under the following conditions, using the robotic Stratagene Robocycler with the heated lid on. An initial denaturation of 94 °C for 4 min then followed by 35 cycles of 94 °C for 30 sec, 48 °C for 45 sec and 72 °C for 45 sec. A final elongation phase of 72 °C for 5 min was done. The PCR products were stored at 4 °C until analysis. The amplification strategy used a 19 base pair extension on the 5' end of the reverse primer. The reverse PCR primers were probed with a 19 base extension at its 5' tail end with the sequence 5'-CACGACGTTGTAAAACGAC-3. This sequence is identical to an IR labelled universal M13 forward sequencing primer, which is included in the PCR reaction. The tailed primer generates a complementary sequence which is subsequently utilised for priming in the amplification reaction thereby generating IR labelled PCR products. The samples were electrophoresed on IR^2 DNA analyzer (LI-COR Inc., USA) using a laser system to detect IR fluorescence chemistry and laser technology for detection of alleles. It is fitted with two laser systems that permits the detection of alleles marked with both IRDye 700 and IRDye 800 at the same time. Thus, a multiplex of four PCR primers was loaded in one well. The samples were first mixed in a microplate and then 0.8 µl of the mix loaded on the gel. Series of dilutions of the PCR products ranging from four to 30 times were evaluated for best resolution. Thirty times dilution were found to be the best that permits easy and accurate scoring of allele size. When the band detection was made on the IR DNA sequencer, one or two clearly distinct alleles were produced. The IR based technique allows multiplexing of four PCR products in a single well for the detection of alleles. This system combines IR fluorescence chemistry and laser technology, thus eliminating the need for gel handling required with silver staining and flour detection systems. The on-line detection does not require post-electrophoretic

DNA staining as required by silver staining and allows immediate visualisation of the images and alleles. By loading a 64-lane gel with a multiplex of four PCR products, two man days' work can be accomplished in two hours. DNA samples from 224 trees from the 26 provenances trial were amplified using the 15 primers and were genotyped using Saga Generation 3.2.

Data analysis

Measures of genetic diversity 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 [19]. The structure among individual provenances was estimated using Fst based on the method of Jackknife. This fixing index [20] is calculated based on the formulae of [21]. Hierarchical analyses of molecular variance (Amova) [22] were calculated using the program Arlequin, version 2.0 [23], in which significance levels for the populations values were determined after 1000 permutations. The relationships between provenances were portrayed graphically using Darwin 5.0 software [24] after the computation of genetic matrix [25] using UPGMA algorithm.

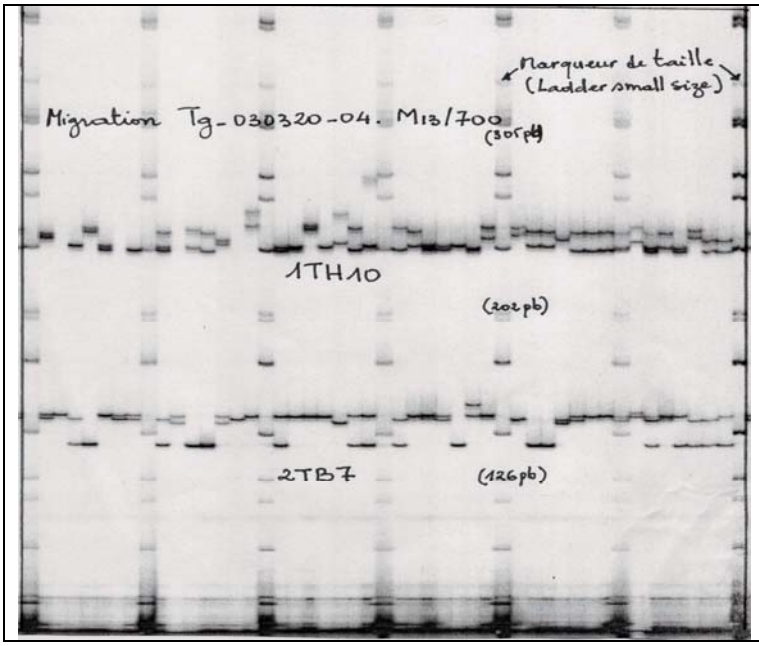

Fig.1. Example of segregation of bands in the samples. In this figure two loci are multiplexed (1TH10 and 2TB7). The alleles of the locus 1TH10 ranged from 305 to 202 pb and the locus 2TB7, the pairs of bases ranged from 198 to 126. The ladder small size is deposited after all seven samples. The individuals are diploid, the presence of two alleles shows that this sample is heterozygote for this locus and one allele represents a homozygote individual.

Results

Allelic diversity

For the 15 loci microsatellites, 189 different alleles were observed, with an average of 12.6 alleles per locus. An example of allelic richness is presented in Fig. 1.

A total of 67 specific alleles to only one geographic area were found. Among these alleles, 39 were observed only in South India. Four alleles were specific to Thailand-Laos and 24 alleles were identified in Africa including 8 for West Africa and 16 alleles for Tanzania. No specific allele was found in North of India. One hundred 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) with 85 % (1TG02-166).

All the populations present heterozygotes deficit on at least a locus except for the locus 1TF5. Allele 218 of the locus 3TE6 is fixed in seven populations (Ban Cham Pui, Huoi-Na-Soon, Mae Huat, Pal Lai, Pakse, Pong Salee and Purunakote). Allele 157 of the locus 4TH9 is fixed in four provenances (Pakse, Mae Huat, Huoi-Na-Soon and Ban Cam Pui) whereas this locus presents heterozygotes deficits in seven populations (Pakse, Mae Huat, Huoi-Na-Soon, Ban Cam Pui, Pong Salee, Pak Lai and Ban Pha Lai). The allele's 3TD9-208, 4TF2-227 and 3TA11-157 are fixed in three different populations each.

Table 1. *Tectona grandis* provenances used in this study

Trials situation	Provenance name	N	Latitude	Longitude	Annual rainfall (mm)
COTE D'IVOIRE					
Séguié	Bamoro A29**	5	7°48	5°5 W	1200
Séguié	Bamoro A20**	5	7°48	5°5 W	1200
Téné	Bouake 3037**	7	8°	5° W	1200
Téné	Bouake TB73**	6	8°	5° W	1200
SENEGAL					
Séguié	Djibelor**	6	12°35	16°6 W	1640
Séguié	Kalounayes**	5	12°45	16°5 W	1640
BENIN					
Séguié	Djigbe**	5	6°25	2°2 E	110
Séguié	Toffo Lama**	6	6°25	2°07 E	110
CAMEROON					
Séguié	Bambuku7**	5	4°15	9°15 E	1780
Téné	Bambuku3067**	5	4°	9° E	1900
TOGO					
Téné	Tove**	5	7°	0° E	1300
Séguié	Mtibwa**	10	6°	37°39 E	1160
Séguié	Kihuhwi**	10	5°12	38°39 E	1880
Téné	Bigwa**	14	7°	39° E	900
THAILAND					
Séguié	Mae Huat*	6	18°6	99° E	1300
Séguié	Pong Salee*	6	19°8	100°1 E	1500
Séguié	Huoi-Na-Soon*	7	18°7	100°8 E	1350
Téné	Ban Chm Pui*	16	18°	100° E	1100
Téné	Ban Pha Lai*	11	18°	100° E	1100
INDIA					
Séguié	Nellicutha(15)*	22	11°17	76°14 E	2700
Séguié	Nellicutha(16)*	18	11°17	76°14 E	2700
Séguié	Virnoli Range*	7	15°	74° E	2030
Téné	Masale Valley*	9	12°	76° E	1300
Téné	Purunakote*	10	20°	84° E	1300
LAOS					
Téné	Paskse*	5	15°	105° E	2000
Téné	Pak Lay*	13	18°	101°E	1200
TOTAL		229			

The studied provenances cover a wide area geographically with a range of 110 to 2700 mm of annual rainfall. Teak grows naturally mainly in mixed deciduous forests with a distinct seasonal climate (wet and dry seasons). Trials situation are localised in Côte d'Ivoire, *: populations from natural area, **populations from Africa, N: Number of studied trees, E: East and W: West.

In natural population, the analysis indicates that the number of alleles varies from 33 in Pakse to 119 in Nellicutha16. The number of allele per locus ranged from three (1TG2) to 19 (1TA6). The locus 1TG2 presents a deficit of heterozygotes in 11 provenances and for Ban Cham Pui, Ban Pha Lai, Huoi-Na-Soon, Pong-Salee and Pakse, the locus is fixed. Five loci are fixed in Pakse which presents a heterozygote deficit on nine loci. The loci 4TH9, 3TA11, 3TE6, 4TF2 and 1TG2 are fixed in at least a population. Of the 189 alleles, 177 are from Indian origin (113 private) and 75 from Thailand origin (11 private).

Of the 94 individuals of the 14 populations of Africa, a total of 162 different alleles were observed. This is equivalent to an average of 10.8 alleles per locus. The number of alleles per locus ranged from three (1TG2) to 16 (1TH10). The number of microsatellite alleles ranged from 53 (Kalounayes) to 91 (Bigwa). The locus 1TG2 presents a deficit of heterozygotes in 13 populations and it is fixed in bambuku3067, Bamoro A20, Bouaké TB73 and Djigbé. The locus 3TE6 is fixed in Djibelor and Mtibwa. The Tanzania's populations (Bigwa, Kihuwi, Mtibwa) are those which present the greatest number of alleles with 91, 78 and 86 alleles, respectively.

Genetic diversity In Teak from outside Africa

After analysis of the 15 loci and using the criterion of a maximum frequency of 95 % for the most common allele, the percentage of polymorphism is 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 showed 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 not statistically significant (0.52) while the expected value under Hardy-Weinberg was 0.48 (Table 2). In Huoi-Na-Soon, the expected heterozygosity (0.34) was significantly higher than observed heterozygosity (0.32). Except for Huoi-Na-Soon, the rest of the populations were 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 were not significantly different. The number of alleles per locus ranged from 2.06 (Pakse) to 7.66 (Nellicutha16) with a mean of 4.32 per locus. Thailand presents 5.06 alleles per locus while India has 11.8. In India, the allelic Richness was more of the double than that of Thailand.

Genetic diversity in Teak from African provenance.

The average percentage of polymorphic locus is 95.7% with a mean ranged of 86.67 % (Bigwa, Bambuku 7 and Bouaké TB) to 100 % (Table 3). Eight populations showed 100 % polymorphism average. The polymorphism percentage in African's populations is 100 %. The heterozygosity observed, ranged from 0.52 (Bambuku 7) to 0.746 (Bamoro A20) with a mean of 0.635. This value is not significantly different from expected heterozygosity which is 0.648. With African's populations (unknown origin), only Djibelor shows a mean of expected heterozygosity (0.594) significantly higher than the mean of observed heterozygosity (0.577). A deficit in heterozygotes is thus observed in this population. The average observed heterozygosity and expected heterozygosity are not significantly different from the other populations because these populations are in Hardy-Weinberg equilibrium. The number of alleles per locus ranged from 3.00 (Bambuku7) to 5.93 (Bigwa) with an average of 4.35 per locus. The general mean of alleles by locus on the level of the populations is half of what was observed in Africa (10.8).

Table 2. Descriptive statistics over all loci for each population of natural area.

Provenances	N	He	Ho	P(0.95)	Ave
Nellicutha15	22	0.708 (0.12)	0.715 (0.141)	100.000	6.866
Nellicutha16	18	0.726 (0.124)	0.763 (0.189)	100.000	7.667
Massale	9	0.732 (0.128)	0.785 (0.211)	100.000	6.533
Virnoli	7	0.685 (0.146)	0.733 (0.240)	100.000	5.200
Purunakote	10	0.609 (0.23)	0.626 (0.257)	93.330	6.200
India	66	0.772(0.121)	0.724(0.141)	100.000	11.800
Pong-Salee	6	0.346 (0.277)	0.366 (0.316)	73.330	2.600
Mae Huat	6	0.350 (0.294)	0.400 (0.349)	66.670	2.600
Ban Cham	16	0.354 (0.289)	0.391 (0.334)	60.000	3.200
Ban Pha	11	0.468 (0.247)	0.521 (0.302)	86.670	3.800
Huoi-Na	7	0.341 (0.281)	0.323 (0.321)*	66.670	2.670
Pak Lai	13	0.304 (0.278)	0.312 (0.316)	66.670	2.733
Pakse	5	0.249 (0.284)	0.320 (0.391)	53.330	2.067
Thailand	64	0.414 (0.290)	0.384 (0.277)	73.330	5.060
Ave G		0.489	0.521	80.556	4.328

NB. 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, Ave G: General average, * p value significant < 0.05.

Molecular variance.

An analysis of molecular variance revealed that 5.5 % of variation occurred among populations within regions (AMOVA: df = 23, $p < 0.001$) and 80.52 % occurred within populations (AMOVA: df = 422, $p < 0.001$). Cluster analysis also showed that most variation occurs at the intra-population level. However, a significant amount of variation was also attributed to the variation between populations. This analysis revealed also that 13.98 % of the total genetic variation was distributed among regions (AMOVA: df = 2, $p < 0.001$). The Fst (0.2***) value is positive and highly significant thus indicating clearly a differentiation between the geographical area of teak.

Origin of African teak.

The phylogenetic tree (Fig. 2) showed the formation of three groups and the robustness of nodes varied from 552 to 1000. The group obtained with south India was clearly separated from the other clusters. Bambuku 7 and Thailand populations were clustered together and were separated from the others Africa-Purunakote populations. Africa-Purunakote cluster is subdivided in two sub groups. Mtibwa, Bigwa (Tanzania) and Bambuku 3067 (Cameroun) are attached to Purunakote (north India) population and the other African populations form under homogeneous cluster clearly distinct. No African population is close to the south Indian genotypes.

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

Provenances	N	He	Ho	P(0.95)	Ave
Djibelor	6	0.594 (0.230)	0.577 (0.307)*	93.330	4.200
Kalounayes	5	0.522 (0.208)	0.586 (0.315)	100.000	3.600
Bamoro A29	5	0.613 (0.204)	0.746 (0.266)	100.000	4.333
Bamoro A20	5	0.629 (0.167)	0.666 (0.317)	100.000	4.133
Bouaké3037	7	0.606 (0.206)	0.723 (0.272)	100.000	4.600
BouakéTB73	6	0.556 (0.243)	0.666 (0.339)	86.670	3.800
Tové	5	0.589 (0.217)	0.640 (0.253)	100.000	3.933
Djigbé	5	0.642 (0.178)	0.720 (0.224)	100.000	4.467
Toffo Lama	6	0.569 (0.260)	0.655 (0.336)	93.330	4.333
Bambuku7	5	0.440 (0.251)	0.520 (0.309)	86.670	3.000
Bambuku3067	5	0.565 (0.226)	0.720 (0.300)	93.330	4.067
Kihuwi	10	0.598 (0.181)	0.613 (0.277)	100.000	5.133
Mtibwa	10	0.580 (0.218)	0.573 (0.231)	100.000	5.400
Bigwa	14	0.578 (0.254)	0.601 (0.321)	86.670	5.933
Ave G	94	0.648 (0.212)	0.635 (0.231)	100.000	10.800

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, Ave G: General average, * p value significant < 0.05

Discussion

Molecular diversity.

Teak has a considerable allelic richness (188 alleles for 15 loci microsatellites), in spite of the fact that number of samples of the 26 analyzed provenances are not important. The average number of alleles per microsatellite locus of *Tectona* is similar to those found in other tree species (3.7 to 16 for *Symphonia globulifera* [26], 3.4 to 4.2 for *Vittelaria paradoxa* according to [27]. An average of 12.5 alleles per locus was observed on the whole samples. A study undertaken on various tropical woody species [28] reveals that in the whole population, the average number of polymorphic alleles per locus is 2.6. This mean is lower compared to that observed with the teak (4.3). This difference can be explained by the highly individual variability of the teak. With 15 loci microsatellites, the percentages of polymorphism are very high. However, the number of individuals per population does not seem to affect this result since the population of Pong-Salee, with six samples, shows 73.33 % as mean polymorphism. With a higher number of samples (16 samples), the population of Ban cham Pui presents only 60 % of polymorphism. The variations of mean heterozygosity are not inevitably correlated with those of the locus polymorphic percentage. Thus, if the population of Masale valley has a maximum percentage of polymorphic loci (100 %) and has the most important observed heterozygosity mean (0.785), some polymorphic populations have a low average heterozygosity (Pong-Salee).

In this study, the populations with higher mean heterozygosity were also polymorph. The observations showed that the teak is preferentially allogamous species [29, 30]. Self-fertilization of the tree led to the fruit with low germination [31, 29]. On the mode of

reproduction of six maternal descents in free fecundation coming from India confirms these observations. Its results reveal that more than 90 % of the individuals are resulting from allofecondation. Gene flows thus are sufficiently distributed to ensure genetic mixings.

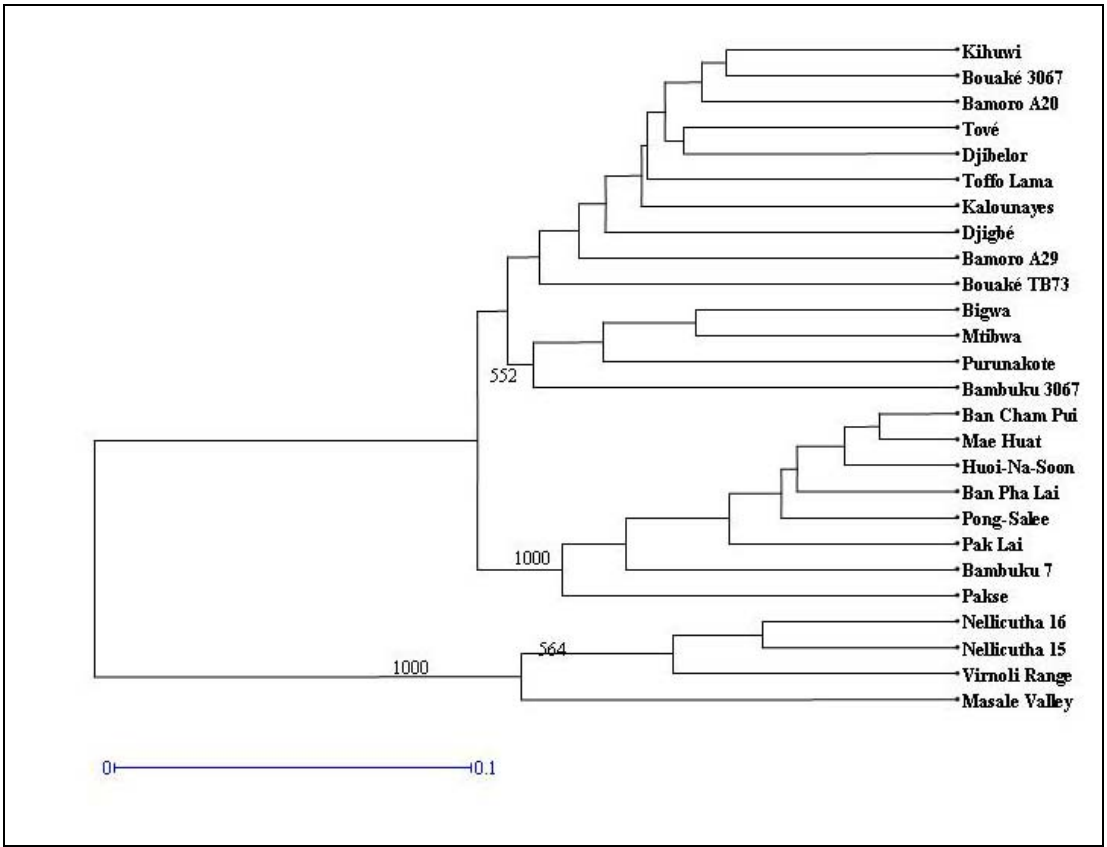

Fig. 2. UPGMA cluster analysis showing the relationships among 26 provenances of teak on the basis of Nei 1983 matrix value with SSR markers. Bootstraps values higher than 500 (test out of 1000 bootstraps) are represented.

For teak, a clear differentiation among areas and populations has been 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 [32] and *Vitellaria paradoxa* 0.04 [12]. Differences in genetic diversity measured as average expected heterozygosity was found in this study. The main differences were found between the populations from Thailand and the populations from India. With an average of 0.414, Thailand populations had a low expected heterozygosity. Similar low diversity of Thailand populations compared to populations from India was found by [33]. Also, the general level of diversity in their study was much higher. The origin of the apparently relatively lower diversity of the Thailand populations compared to the Indian populations found in the present study is not known. The Indian and Thailand parts of the natural distribution area are geographically separated and probably have been so for thousands of years [34].

This study showed that most of the genetic diversity observed in the teak populations comes from an individual variability which is specific to all the forest species. This study indicated that 5.5 % of the variation occurred among populations. That variation is lower than the 43 % reported by [35] using AFLP markers, 22 % of variation for 10 teak populations from India using RAPD markers [36] and 21 % observed for 16 teak populations from Thailand [37] With phylogenic trees, three geographical areas were observed: south India, north India and Thailand. These three areas could be regarded as the “diversity centers” of teak which is in contrast with [38]. This author indicated that India,

Thailand and Indonesia are the three “centers of diversity” of the teak. The assumption of this study is more reasonable because the teak is an exotic species in Indonesia. Indeed, a molecular analysis of diversity carried out on the teak populations introduced in Ghana does not differentiate Indonesian teak from African populations (Daniel Ofori, per.com). [33] found no clear separation between three provenances: from southern India, central India and Thailand. Other literature showed incomplete groupings of provenances without extension to the whole teak natural area. Isozyme markers have distinguished three southern Indian provenances (Sakrebail, Virnoli, Thithimathy) and two Thai provenances (Tam Bah Thai, Mae Huat) [9], or one southern Indian provenance (Sadiuaval) and two Thai provenances (Ban Cham Pui, Mae Huat) [39]. AFLP markers do not distinguish the Thai and Indian populations, but comparison between Indian populations suggests that the northwestern Allapally plain population is distinct from the two southern Indian populations [35].

Origin of African teak.

This study showed a regional structuring of the teak introduced in Côte d’Ivoire. The bibliography showed that the first introductions of teak in Côte d’Ivoire were from Togo. This assertion was confirmed by the low values of genetic distance found between the populations of Côte d’Ivoire and Tové. Exceptions to Bambuku7 which clustered with Thailand, all of african populations were clustered with north India. Mtibwa, Bigwa (Tanzania) and Bambuku 3067 (Cameroun) populations were attached to Purunakote population and the other african provenances gather between them to form under homogeneous group. This second sub group can be regarded as an isolated genetic group. It would be difficult to trace the origin of this sub-group from this study. Even though the individuals contain some alleles from the African populations other north Indian populations were also not studied.

African teak would probably come from only one origin: North India, but the seeds were probably collected on several trees because individual genetic diversity is very strong. However the tendency of the populations to gather in under geographical area could justify the existence of local races generated by the lack of genetic mixing and often because of a disjoined geographical area. African populations are closer to Purunakote (India of north) more than the other diversity centers of teak. The fact that south India’s populations are very distinct from African shows these two areas are geographically isolated for a long time. The results are in contrast with [40] on the bringing together of the African populations to Thailand.

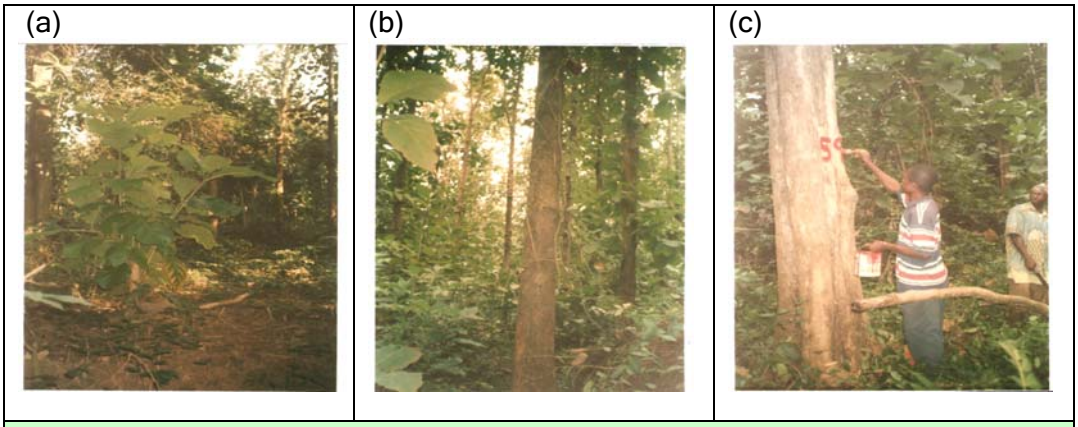

Fig: 3. (a) Trial provenance 1970 at Séguié, (b) Trial provenance 1974 at Téné,
(c) Marking of the trees selected for the molecular analysis (Photos by Inza Fofana).

Implications for Conservation.

During the last five decades, the demand for teak has increased several folds, resulting in extraction of trees from old plantations and from natural forest. Extraction of best teak from forest has resulted in the loss of good genotypes [41]. Genetic conservation of teak is urgent because most of the natural teak forests have been gradually converted into teak monoculture. Indeed in Côte d'Ivoire teak plantations are not associated with other species (Fig 3). The gene diversity has been reduced with each round of teak plantations as seeds are collected from selected trees of the existing plots. Transformation of the natural forests into plantation caused numerous problems including site deterioration due to repeated fires, heavy grazing and water erosion, poor quality of planting stock raised from genetically inferior seeds, and attack by teak defoliators, *Hyblea puera* [42]. In the absence of gene conservation plots and protected forests, the choice is limited to identifying appropriate plantations for long-term conservation. In India the biodiversity of teak conservation stands may be estimated within each genecological zone taking into consideration the extent of population differentiation within each zone [41]. A conservation plan for teak in Thailand has been developed with the objective to protect this precious genetic resource for future use. The conservation plan is based on so-called genecological zonation where variation in ecological conditions within the distribution area is investigated and uniform zones are established based on available data. A network of conservation stands based on this zonation is recommended rather than a few populations [43].

Molecular genetic studies, carried out on many forest tree species around the world, are contributing to a better understanding of patterns of variation and supporting the development of improved management practices, and monitoring species turnover in time and in space. Studies of intraspecific variation can contribute to the development of conservation strategies, by identifying appropriate units for conservation [44]. Integrating new tools, such as modeling simulations with molecular research will improve our knowledge of landscape patterns of genetic diversity within species distribution, and help develop resource management plans [45].

The genetic variability of teak remains at an acceptable level, but the risks are mainly due to overexploitation, anthropological pressure, fire, loss of the most valuable trees through international and national demand, and the conversion of natural populations.

The molecular data in particular the microsatellite markers can be of great use in defining the best the methods of genetic conservation and to insure tracking of future evolution of variability. They can also be very effective to combat illegal logging or to certify wood provenance. On the other hand, the molecular data cannot be used regardless of the other markers. Various sources of information as phenotypic and ecological data will be necessary to protect and manage the genetic variability of teak.

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