

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO			
RAD:	12-124171- -7	FECHA:	2015-02-17 09:26:19
DEP:	2020 DIRECCION DE NUEVAS CREACIONES	EVE:	378 FASE NACIONAL INCLUIDO CAPITULO I
TRA:	11 PCT-PATENTE DE INVENCION CAPITULOS I Y II	FOLIOS:	8
ACT:	519 PRESENTACION		

DIRECCIÓN DE NUEVAS CREACIONES

2020
Bogotá, D.C.,

Abogado(a)/Señor(a)
ALICIA LLOREDA RICAURTE

Asunto: Radicación: 12-124171- -7
 Trámite: 11
 Evento: 378
 Actuación: 519
 Oficio: 1725
 Folios: 8

Patente de Invención	X			Patente de Modelo de Utilidad	
Vía Nacional					
Vía PCT (Fase Nacional)	X	Capítulo I	X	Capítulo II	
No. Solicitud Internacional	PCT/MY2011/000003				
Fecha de Presentación Internacional	07/01/2011				

Como resultado del examen de fondo realizado, con fundamento en el Artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se comunica:

1. Documentos estudiados

Descripción:	Radicado No 12-124171-00000-0000	24/Julio/2012
Reivindicaciones:	Radicado No 12-124171-00000-0000	24/Julio/2012
	Radicado No 12-124171-00003-0000	25/Junio/2013
	Radicado No 12-124171-00006-0000	15/Agosto/2014
Dibujos:	Radicado No 12-124171-00000-0000	24/Julio/2012
Prioridad:	PI2010000118	11/Enero/2010

2. Análisis de la Prioridad

Se acepta la reivindicación de prioridad porque ésta solicitud fue presentada dentro del plazo establecido (Art. 9 D 486) y su contenido técnico corresponde con el de la prioridad reivindicada

3. Contrato de acceso (Art. 26 D 486 literal (h))

Debido a que la invención se refiere a la palma *Elaeis oleifera* especie que proviene de Colombia, se requiere al solicitante anexar el contrato de acceso a recursos genéticos o productos derivados, expedido por el Ministerio de Ambiente, Vivienda y Desarrollo Territorial, o indicar el estado de trámite del mismo.
Se requiere al solicitante hacer las aclaraciones necesarias.

4. Certificado de depósito (Art. 29 D 486)

Debido a que la solicitud se refiere a material biológico, es necesario presentar el Certificado de Depósito para el germoplasma utilizado en el método

5. Objeto de la invención

De acuerdo con lo establecido en el numeral 1.2.2.2 del Título X de la Circular Única para efectos del examen de patentabilidad, se tendrá en cuenta la tasa pagada por el solicitante al momento de comenzar el examen para las reivindicaciones adicionales; esto es que cuando la solicitud contenga más de diez (10) reivindicaciones y no se haya pagado la tasa complementaria, sólo se estudiarán las cubiertas por la tasa pagada. En la presente solicitud se estudiarán las reivindicaciones desde la 1 hasta la 13 de los folios 5 a 7 Radicado No 12-124171-00006-0000.

Título de la solicitud: "Identificación de marcadores moleculares asociados con composición de ácido graso en plantas"

El problema planteado en esta solicitud consiste en la necesidad de incrementar los niveles de ácidos grasos insaturados en palmas.

Para resolver este problema técnico la solicitud en estudio proporciona un método para identificar marcadores genéticos.

6. De la solicitud de patente

Reivindicaciones (Art 30 D 486, Art 22 PCT)

- 1- El capítulo reivindicatorio contiene 3 reivindicaciones independientes de método (reiv. 1, 8 y 10), lo que corresponde a una falta de unidad de invención de la solicitud.
- 2- Las reivindicaciones 1, 8 y 10 mencionan un método pero éste no se define mediante características como alguna etapa ó alguna condición específica en que debiera realizarse, como lo es las etapas para detectar un marcador genético. Se sugiere, entonces, definir la invención por sus características esenciales indicando la secuencia recombinante exacta del marcador genético.
- 3- Las reivindicaciones 1, 8 y 10 mencionan secuencias nucleótidas que no tienen sustento en el capítulo descriptivo por cuanto no hay divulgación para cada una de las secuencias en formato ST25, adicionalmente el porcentaje de homología para definir las o la mención a los nucleótidos contiguos de la secuencia no permite tener la certeza de la secuencia nucleótida recombinante específica. Se sugiere definir cada una de las secuencias a partir de la secuencia de nucleótidos que contiene e indicar el número de la misma (SEQ ID NO).
- 4- Las reivindicaciones 1, 8 y 10 mencionan un método de selección de la planta, pero éste no se debe definir en términos del metabolito de interés, sino por la secuencia del polinucleótido introducida que permite mejorar la eficiencia en la producción de dicho metabolito. De igual manera la referencia a que la presencia del marcador genético indicará un FAC saturado alto o la ausencia indicará un FAC insaturado alto, es un resultado a alcanzar y no definen al método, puesto que la reivindicación incluiría no sólo la solución propuesta por el solicitante, sino todas las alternativas presentes o futuras que lleguen a ese resultado. Se sugiere reemplazarlo por las características esenciales, que contribuyen a la obtención de ese efecto, definiendo por una secuencia lógica de etapas.
- 5- Las reivindicaciones 3 -7, 12 y 13 mencionan el nivel de FAC saturado, que es un resultado a alcanzar y no definen al método, puesto que la reivindicación incluiría no sólo la solución propuesta por el solicitante, sino todas las alternativas presentes o futuras que lleguen a ese resultado. Además el método de selección de la planta, no se debe definir en términos del metabolito de interés, sino por la secuencia del polinucleótido introducida que permite mejorar la eficiencia en la producción de dicho metabolito. Se sugiere reemplazar la referencia al metabolito por las características esenciales, que contribuyen a la obtención de ese efecto.

6- Se recuerda a la solicitante que cualquiera de las secuencias mencionadas deben ser caracterizadas por su secuencia completa, la cual además debe ser adjuntada en un listado aparte con su respectivo SEQ ID NO, y que no se aceptan definiciones indicando porcentajes de homología o identidad, puesto que esta definición no permite establecer la secuencia nucleótida exacta a la que se refiere.

Descripción (Art 28 D 486)

La descripción no divulga la invención de manera suficientemente completa para que la persona del oficio normalmente versada en la materia pueda ejecutarla ó reproducirla, como lo es en el caso de la tabla A Pág. 12 en la que solamente se indica el número de la secuencia y la designación por referencia al clon en el que se encuentra y no están las listas de las secuencias nucleótidas que deben presentarse en forma separada de la descripción, en formato ST25 bajo el título “Listado de secuencias” y deben estar anexadas en formato digital.

7. Unidad de invención (Art 25 D 486)

No hay unidad de invención entre las tres reivindicaciones independientes porque se hizo un estudio a posteriori Con los documentos D2 y D3 que anticipan el concepto común que corresponde a la cantidad de ácido graso que produce la planta, pero éste no puede ser concepto común porque es un resultado que se pretende alcanzar con la modificación genética, además como son 3 secuencias diferentes las causantes del efecto entonces se tienen 3 métodos que no pueden coexistir bajo la misma solicitud de patente de invención, por cuanto no hay relación estructural entre las mismas.

Por lo tanto, esta invención presenta 3 diferentes conceptos técnicos comunes inventivos así:

- 1° Método para seleccionar una planta con base en el contenido de ácido graso (FAC) que comprende detectar un marcador genético que corresponde a una secuencia nucleótida que tiene al menos el 90% de identidad para al menos 20 nucleótidos contiguos de la secuencia SEQ ID NO 3
- 2° Método para seleccionar una planta con base en el contenido de ácido graso (FAC) que comprende detectar un marcador genético que corresponde a una secuencia nucleótida que tiene al menos el 90% de identidad para al menos 20 nucleótidos contiguos de la secuencia SEQ ID NO 56.
- 3° Método para seleccionar una planta con base en el contenido de ácido graso (FAC) que comprende detectar un marcador genético que corresponde a una secuencia nucleótida que tiene al menos el 90% de identidad para al menos 20 nucleótidos contiguos de la secuencia SEQ ID NO 65.

Se sugiere presentar las solicitudes divisionales que considere necesarias, o limitar la solicitud inicial a una sola invención, eliminando de ella las otras invenciones.

8. Determinación del Estado de la Técnica

El estado de la técnica se determinó con base en la, fecha de presentación de la solicitud de prioridad: enero 11 de 2010, y se encontraron los siguientes documentos que anticipan el objeto de esta solicitud:

Ítem	Documento	Título	Fecha de Publicación*
D2	NRC. N. Billote, E. Barcelos (Pág. 413-425)	“Development, characterisation, and across-taxa utility of oil palm (Elaeis guineensis Jacq.) microsatellite markers”	22/Mayo/2001
D3	Theor Appl Genet (Pág. 754-765)	“Microsatellite-based high density linkage map in oil palm (Elaeis guineensis Jacq)”	26/Enero/2005

El documento D2¹ enseña la aplicación de marcadores de microsatélites en la detección de 21 loci en plantas de palma *E. guineensis* y *E. oleífera* (resumen).

El El documento D3² enseña la construcción de microsatélites para identificar linajes de alta densidad entre palmas de aceite heterocigotos (Pág. 754)

9. Examen de Patentabilidad (Art 14 D486)

Nivel inventivo (Art18 D486)

La reivindicación 1 consiste en “Un método para seleccionar una planta con base en el contenido de ácido graso (FAC) del aceite aislado de dicha planta, dicho método comprende...”

Comparación de las características técnicas esenciales, con las del estado de la técnica:

Características esenciales	D2	D3
Método para seleccionar una planta con base en el contenido de ácido graso (FAC) del aceite aislado de dicha planta que comprende:	Método para detectar microsatélites en palma de aceite que comprende:	
Detectar la presencia del marcador genético en la planta que corresponde a una secuencia de nucleótidos que tiene al menos el 90% de identidad para al menos 20 nucleótidos contiguos de la SEQ ID NO 3 o una secuencia complementaria a esta.	Detectar micro satélites en palmas <i>Elaeis guineensis</i> , <i>Elaeis oleifera</i> (Pág. 415-416)	Detección de microsatélites en palma de aceite (Pág. 756)
Detectar la ausencia del marcador genético en la planta que corresponde a una secuencia de nucleótidos que tiene al menos el 90% de identidad para al menos 20 nucleótidos contiguos de la SEQ ID NO 3 o una secuencia complementaria a esta.	-	

El documento D2 se considera el estado de la técnica cercano a la invención definida en la reivindicación 1 porque divulga un método de detección de microsatélites en palmas de aceite (Pág. 415-416).

El documento D3 divulga la detección de microsatélites en palma de aceite (Pág. 756).

La diferencia consiste en que la solicitud divulga una secuencia de nucleótidos que tiene al menos el 90% de identidad para al menos 20 nucleótidos contiguos de la SEQ ID NO 3.

No se evidencia un efecto técnico asociado a un método para detectar la presencia o ausencia de un marcador genético porque no se conoce la secuencia del marcador detectado.

Por lo tanto, el problema técnico objetivo que pretende resolver esta invención se puede formular así: cómo proporcionar un método de detección de marcador genético alternativo.

Sin embargo, la utilización de microsatélites en la detección de rasgos como contenido de aceite en palmas del género *Elaeis*, ya está divulgada en el documento D3 que se refiere a la utilidad de técnicas de microsatélites en procedimiento de identificación de característica deseadas en palmas (Pág. 755).

11<http://libgen.asia/644dbd2e11093db7693d18edf951a921/billotte2001.pdf>
22http://www.corsat.agr.ku.ac.th/doc/oilpalm/SSR_oilpalm_2005.pdf

En consecuencia, la persona del oficio normalmente versada en la materia, incluiría técnicas de detección de marcadores genéticos del documento D3 en el procedimiento divulgado en el documento D2 para llegar así al objeto de la reivindicación 1 de la solicitud. Por lo cual se considera obvia.

En conclusión, la reivindicación 1 cumple el requisito de novedad (Art. 16), pero no cumple con el requisito de nivel inventivo (Art. 18).

Las reivindicaciones dependientes 2-7 no mencionan alguna característica que haga inventivo el método para seleccionar una planta de la reivindicación 1, por lo cual se considera que tampoco tienen nivel inventivo.

La reivindicación 8 consiste en “Un método para selecciona runa planta con base en el contenido de ácido graso (FAC) del aceite aislado de dicha planta, dicho método comprende...”

Comparación de las características técnicas esenciales, con las del estado de la técnica:

Características esenciales	D2	D3
Método para seleccionar una planta con base en el contenido de ácido graso (FAC) del aceite aislado de dicha planta que comprende:	Método para detectar microsatélites en palma de aceite que comprende:	
Detectar la presencia del marcador genético en la planta que corresponde a una secuencia de nucleótidos que tiene al menos el 90% de identidad para al menos 20 nucleótidos contiguos de la SEQ ID NO 56 o una secuencia complementaria a esta.	Detectar micro satélites en palmas <i>Elaeis guineensis</i> , <i>Elaeis oleifera</i> (Pág. 415-416)	Detección de microsatélites en palma de aceite (Pág. 756)
Detectar la ausencia del marcador genético en la planta que corresponde a una secuencia de nucleótidos que tiene al menos el 90% de identidad para al menos 20 nucleótidos contiguos de la SEQ ID NO 56 o una secuencia complementaria a ésta.	-	

El documento D2 se considera el estado de la técnica cercano a la invención definida en la reivindicación 8 porque divulga un método de detección de microsatélites en palmas de aceite (Pág. 415-416).

El documento D3 divulga la detección de microsatélites en palma de aceite (Pág. 756).

La diferencia consiste en que la solicitud divulga una secuencia de nucleótidos que tiene al menos el 90% de identidad para al menos 20 nucleótidos contiguos de la SEQ ID NO 3.

No se evidencia un efecto técnico asociado a un método para detectar la presencia o ausencia de un marcador genético porque no se conoce la secuencia del marcador detectado.

Por lo tanto, el problema técnico objetivo que pretende resolver esta invención se puede formular así: cómo proporcionar un método de detección de marcador genético alternativo.

Sin embargo, la utilización de microsatélites en la detección de rasgos como contenido de aceite en palmas del género *Elaeis*, ya está divulgada en el documento D3 que se refiere a la utilidad de técnicas de microsatélites en procedimiento de identificación de característica

deseadas en palmas (Pág. 755).

En consecuencia, la persona del oficio normalmente versada en la materia, incluiría técnicas de detección de marcadores genéticos del documento D3 en el procedimiento divulgado en el documento D2 para llegar así al objeto de la reivindicación 8 de la solicitud. Por lo cual se considera obvia.

En conclusión, la reivindicación 8 cumple el requisito de novedad (Art. 16), pero no cumple con el requisito de nivel inventivo (Art. 18).

La reivindicación dependiente 9 no menciona alguna característica que haga inventivo el método para seleccionar una planta de la reivindicación 8, por lo cual se considera que tampoco tiene nivel inventivo.

La reivindicación 10 consiste en *“un método para selecciona runa planta con base en el contenido de ácido graso (FAC) del aceite aislado de dicha planta, dicho método comprende...”*

Comparación de las características técnicas esenciales, con las del estado de la técnica:

Características esenciales	D2	D3
Método para seleccionar una planta con base en el contenido de ácido graso (FAC) del aceite aislado de dicha planta que comprende:	Método para detectar microsatélites en palma de aceite que comprende:	
Detectar la presencia del marcador genético en la planta que corresponde a una secuencia de nucleótidos que tiene al menos el 90% de identidad para al menos 20 nucleótidos contiguos de la SEQ ID NO 65 o una secuencia complementaria a esta.	Detectar micro satélites en palmas <i>Elaeis guineensis</i> , <i>Elaeis oleifera</i> (Pág. 415-416)	Detección de microsatélites en palma de aceite (Pág. 756)
Detectar la ausencia del marcador genético en la planta que corresponde a una secuencia de nucleótidos que tiene al menos el 90% de identidad para al menos 20 nucleótidos contiguos de la SEQ ID NO 65 o una secuencia complementaria a ésta.	-	

El documento D2 se considera el estado de la técnica cercano a la invención definida en la reivindicación 10 porque divulga un método de detección de micro satélites en palmas de aceite (Pág. 415-416).

El documento D3 divulga la detección de microsatélites en palma de aceite (Pág. 756).

La diferencia consiste en que la solicitud divulga una secuencia de nucleótidos que tiene al menos el 90% de identidad para al menos 20 nucleótidos contiguos de la SEQ ID NO 3.

No se evidencia un efecto técnico asociado a un método para detectar la presencia o ausencia de un marcador genético porque no se conoce la secuencia del marcador detectado.

Por lo tanto, el problema técnico objetivo que pretende resolver esta invención se puede formular así: cómo proporcionar un método de detección de marcador genético alternativo.

Sin embargo, la utilización de microsatélites en la detección de rasgos como contenido de aceite en palmas del género *Elaeis*, ya está divulgada en el documento D3 que se refiere a

la utilidad de técnicas de microsátélites en procedimiento de identificación de característica deseadas en palmas (Pág. 755).

En consecuencia, la persona del oficio normalmente versada en la materia, incluiría técnicas de detección de marcadores genéticos del documento D3 en el procedimiento divulgado en el documento D2 para llegar así al objeto de la reivindicación 10 de la solicitud. Por lo cual se considera obvia.

En conclusión, la reivindicación 10 cumple el requisito de novedad (Art. 16), pero no cumple con el requisito de nivel inventivo (Art. 18).

Las reivindicaciones dependientes 11-13 no mencionan alguna característica que haga inventivo el método para seleccionar una planta de la reivindicación 10, por lo cual se considera que tampoco tiene nivel inventivo.

10. Conclusión

Los requisitos de Patentabilidad de la presente solicitud están afectados así:

Ítem	Documento	Reivindicaciones afectadas	Requisito que afecta
D2	NRC. N. Billote, E. Barcelos (Pág. 413-425)	1-13	Nivel inventivo
D3	Theor Appl Genet (Pág. 754-765)	1-13	Nivel inventivo

En cumplimiento del artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de sesenta (60) días contados a partir del día siguiente de la fecha de notificación de la presente comunicación. En caso de no dar respuesta al presente requerimiento, o si a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente.

Se invita al solicitante se sirva indicar, si así lo considera, su renuncia al término faltante de sesenta (60) días para dar respuesta a este requerimiento y presentarla por escrito.

Finalmente, se le recuerda al solicitante que si como respuesta a este requerimiento llegara a modificar el capítulo reivindicatorio y éste contenga más de diez (10) reivindicaciones, no pagará la tasa de modificación voluntaria pero sí la correspondiente al pago por reivindicación adicional.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única No. 10 de 2001.



JOSÉ LUIS SALAZAR LÓPEZ
Director de Nuevas Creaciones (c)

El anterior requerimiento fue notificado por fijación en lista, de fecha 2015-02-24

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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
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Microsatellite-based high density linkage map in oil palm (*Elaeis guineensis* Jacq.)

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Abstract A microsatellite-based high-density linkage map for oil palm (*Elaeis guineensis* Jacq.) was constructed from a cross between two heterozygous

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parents, a *tenera* palm from the La Mé population (LM2T) and a *dura* palm from the Deli population (DA10D). A set of 390 simple sequence repeat (SSR) markers was developed in oil palm from microsatellite-enriched libraries and evaluated for polymorphism along with 21 coconut SSRs. A dense and genome-wide microsatellite framework as well as saturating amplified fragments length polymorphisms (AFLPs) allowed the construction of a linkage map consisting of 255 microsatellites, 688 AFLPs and the locus of the *Sh* gene, which controls the presence or absence of a shell in the oil palm fruit. An AFLP marker *E-Agg/M-CAA132* was mapped at 4.7 cM from the *Sh* locus. The 944 genetic markers were distributed on 16 linkage groups (LGs) and covered 1,743 cM. Our linkage map is the first in oil palm to have 16 independent linkage groups corresponding to the plant's 16 homologous chromosome pairs. It is also the only high-density linkage map with as many microsatellite markers in an *Arecaceae* species and represents an important step towards quantitative trait loci analysis and physical mapping in the *E. guineensis* species.

Introduction

The species *Elaeis guineensis* Jacquin is an allogamous aborescent monocot of the *Arecaceae* family (tribe: *Cocoinae*) (Hartley 1988). Its diploid genome comprises 16 pairs of homologous chromosomes ($2n=32$), and its physical size has been estimated to be 3.79 pg/2C by flow cytometry (Rival et al. 1997). The high oil content in the mesocarp and kernel of oil palm fruits has led man to exploit this tropical perennial plant industrially since the 1920s. Three varietal types of oil palm exist in the natural state. These have been classified on the basis of the presence or absence of a shell in the fruit, which is

governed by a major gene called *Sh*, for shell (Beirnaert and Vanderweyen 1941): *dura*, the homozygous genotype (*Sh*+/*Sh*+), which produces fruits with a thick shell; *pisifera* (*Sh*-/*Sh*-), which is without a shell in its rare fruits; *tenera* (*Sh*+/*Sh*-), which is a hybrid of these two variety types and produces fruits with an intermediate shell.

The *tenera* varieties, which are naturally more productive in oil, are derived from *dura*×*pisifera* crosses and *dura*×*tenera* crosses and are usually improved through reciprocal recurrent selection (Meunier and Gascon 1972). Molecular markers have made it possible to enrich the genome through the phenotypic selection of important traits by means of indirect selection based on markers tightly linked to the genes of interest (Gallais 1996). This necessarily requires prior genetic mapping of the species (Mohan et al. 1997)—i.e., a map in which the markers are located at a density such that any locus of the genome is strongly linked to at least one of them (saturated map). The establishment of a linkage map in oil palm was the purpose of the present investigation, with the principle goal being the identification of the chromosomal portion(s) bearing the genes responsible for a quantitative trait (quantitative trait locus, QTL) by relating the molecular polymorphism of the markers with the phenotypic variation of that trait (Charcosset 1996).

The restriction fragment length polymorphism (RFLP) genetic mapping carried out by Mayes et al. (1997) and the QTL detection enabled by it (Rance et al. 2001) amounted to the first step towards such a rational use of molecular markers in oil palm. Two complementary studies combined linkage mapping and bulked segregant analysis (BSA), a methodology published by Michelmore et al. (1991), and these were successful in identifying randomly amplified polymorphic DNA (RAPD) markers (Moretzsohn et al. 2000) or amplified fragment length polymorphism (AFLP) markers (Billotte et al. 2001a, b) linked to the *Sh* gene. Particular attention is being paid to the use of molecular techniques that have been tested on other plants and to the possible future transfer of results from this study to small research units at overseas experimental oil palm stations. Application of the microsatellite technique has revealed, by PCR, the length of hypervariable, ubiquitous simple sequence repeat (SSR) loci that are well distributed in eukaryote genomes. Locus-specific, co-dominant and highly polymorphic marker DNA fragments showing Mendelian heredity have been produced (Delseny et al. 1983). The AFLP technique developed by Vos et al. (1995), which is also PCR-based, generates a large number of reproducible dominant markers useful for saturating a map when its density is insufficient (Ritter et al. 2002).

The main components of the study reported here are: (1) the development and testing of PCR primers for the amplification of a large number of SSRs; (2) the mapping of these SSRs on a *tenera*×*dura* cross to establish a chromosomal cover as dense as possible; (3) the filling of

the gaps with AFLP markers and establishment of a dense genetic linkage map for oil palm.

Materials and methods

Plant material

The progeny consisted of 116 full-sibs derived from a cross between two heterozygous *Elaeis guineensis* Jacquin parents from the CNRA oil palm breeding program (La Mé, Ivory Coast): LM2T, a *tenera* palm belonging to the La Mé African population, and DA10D, a *dura* palm selected from a Deli population introduced into Indonesia in the 19th century. The *dura* or *tenera* variety phenotype of each palm was determined on the basis of its fruit morphology. Total genomic DNA was extracted from freeze-dried leaf samples of each individual and of the parents according to the DNA isolation protocol of Risterucci et al. (2000).

E. guineensis SSR primer pairs

(GA)_n, (GT)_n or (CCG)_n microsatellite-enriched libraries were constructed following a hybridization-based capture methodology (Billotte et al. 1999) or the membrane-bound oligonucleotide-enrichment technique of Edwards et al. (1996). DNA fragmentation prior to the SSR-enrichment procedure was performed either by sonication or by endonuclease restriction with *Pst*I or *Rsa*I based on the hypothesis put forward by Chen et al. (1997) whereby physically sheared and enzyme-digested DNA produces independent sources of SSRs. The SSR-containing DNA fragments were sequenced by the Genoscope centre (Evry, France) following the protocol of Artiguenave et al. (2000). PCR primer pairs for microsatellite amplification were designed from 444 SSR sequences using OLIGO 4.06 primer analysis software (National Biosciences, Plymouth, Minn.) and the parameters described by Billotte et al. (2001b).

Microsatellite and AFLP analyses

SSRs were genotyped as described by Roy et al. (1996) using an automated infrared fluorescence technology of a Li-Cor IR2 sequencer (Lincoln, Neb.). For any one given locus, one of the SSR primers was designed with a 5'-end M13 extension, 5'-CACGACGTTGTAAAACGAC-3' (Steffens et al. 1993). The PCR amplification was performed in a MJ Research PTC-100 thermocycler (Waltham, Mass.) on 25 ng of DNA in a 10-μl final volume of buffer [10 mM Tris-HCl (pH 8), 100 mM KCl, 0.05% (w/v) gelatin and 2.0 mM MgCl₂] containing 0.08 μM of the M13-labeled primer, 0.1 μM of the other primer, 160 μM dNTP, 1 U *Taq* DNA polymerase (Life Technologies, Gaithersburg, Md.) and 0.06 μM of M13 primer-fluorescent dye IR700 or IR800

(Biolegio, The Netherlands). The PCR program consisted of an initial denaturation at 95°C for 1 min; 35 cycles of 94°C for 30 s, 52°C for 60 s and 72°C for 120 s; a final elongation step at 72°C for 8 min. IR700- or IR800-labeled PCR products were diluted tenfold and fourfold, respectively, subjected to electrophoresis on a 6.5% polyacrylamide gel and then sized by the IR fluorescence scanning system of the sequencer. AFLP analyses (Vos et al. 1995) were performed with 126 *EcoRI/MseI* or *TaqI/HindIII* AFLP primer combinations. The E-Agg/M-CAA AFLP primer combination, which generated a 132-bp marker (*E-Agg/M-CAA132*) of the *Sh* locus (Billotte et al. 2001a), was one of these combinations. Final selective amplifications were performed under radioactive conditions. PCR products were separated on a 5% denaturing polyacrylamide gel as described by Risterucci et al. (2000). The gels were dried for 30 min at 80°C then exposed to a Kodak XLS X-ray film (Rochester, N.Y.) for 96 h. Microsatellite loci were named mEgCIR when revealed by oil palm SSR primers and mCnCIR when revealed by coconut SSR primers. AFLP markers were denoted by the *EcoRI/MseI* or *TaqI/HindIII* primer combination and by the size of the amplified band. The genotype configurations of SSRs or AFLPs segregating in the mapping population, as well as of the *Sh* locus, were identified and coded according to the nomenclature of Ritter et al. (1990) for a cross between two heterozygous parents. Chi-square tests for segregation distortion were carried out for all locus situations, comparing the observed ratio with the expected one for each specific locus configuration (1:1, 3:1, 1:1:1:1 or 1:2:1). Chi-square analyses were performed at thresholds of $P=0.05$ and $P=0.01$. Skewed AFLP markers at $P \leq 0.01$ were excluded as we considered that such AFLPs could be due to identical or very close electrophoretic mobilities of several non-homologous fragments that do not allow adequate scoring (Vos et al. 1995). AFLP or SSR loci with 100% similarity were discarded to simplify the computation of locus order.

Linkage mapping analyses

Our cross between heterozygous parents was considered to be a double pseudo-test cross (Grattapaglia and Sederoff 1994). Most probable linkage phases between markers and linkage mapping analyses were performed using JOINMAP ver. 3.0 (van Ooijen and Voorrips 2001). The Kosambi mapping function was used to convert recombination frequencies into map distances (Kosambi 1944). A microsatellite map and subsequently a complete linkage map were constructed for each parent, at LOD 4.5 for LM2T and at LOD 4.2 for DA10D. JOINMAP proceeds in three successive rounds of ordering. For the first two rounds, the constraints applied were LOD 3.0, Rec carthagene software (Schiex and Gaspin 1997), which simultaneously generates and estimates the reliability of several maximum likelihood

multipoint maps. An integrated linkage map of the cross was constructed using fully informative loci heterozygous for both parents, which enables homologous linkage groups to be merged (Ritter et al. 1990). The genome size (G) of each parental linkage group was estimated from complete parent linkage data by the method of Hulbert et al. (1988), as follows: $G = N(N-1) X/K$ at an LOD threshold of T , where N is the number of markers mapped on the linkage group, X is the average distance between two markers for which the expected value of the LOD score is T and K is the observed number of pairs of markers having an LOD score equal to or greater than T .

Results

E. guineensis SSR sequences, polymorphism and segregation of SSRs and AFLPs

A set of 369 functional SSR markers was developed in oil palm. These markers and 21 previously developed SSRs (Billotte et al. 2001b) were screened for polymorphism between LM2T and DA10D. A total of 256 SSRs was selected for mapping. A list of these loci, along with the corresponding EMBL accessions numbers, is given in Table 1. The detailed SSR information including primer sequences is available under the web page <http://tropgenedb.cirad.fr/oilpalm/publications.html>. In addition, 21 coconut (*Cocos nucifera* L.) SSR markers (Lebrun, unpublished), which were functional on oil palm DNA and polymorphic between the parents, were used. All SSR primer pairs amplified a single locus, except for one, *mEgCIR905*, which was revealed to be a duplicated locus in the genome. Microsatellite sequences could be classified into three major classes of SSRs as defined by Weber (1990), with perfect (220), compound (26) or interrupted (10) microsatellite stretches. Perfect microsatellites included 203 (GA)_n SSRs (17 ± 4 repeats), nine (GT)_n SSRs (12 ± 7 repeats) and nine (CCG)_n SSRs (6 ± 2 repeats). Compound microsatellites usually involved two different dinucleotide motifs, which accumulated 23 repeats on average ($\pm$ seven; $n=26$). No significant difference in repeat number was observed between the coconut and oil palm SSRs (data not shown). The SSR allele patterns showed seven of the nine genotype configurations defined by Ritter et al. (1990), with one to four alleles segregating in the cross between heterozygous parents (Fig. 1). These SSR configurations were case nos. 1, 3, 5, 6, 7, 8 and 9. About 18% (46) of the SSR primer pairs revealed loci belonging to genotype configuration no. 1—i.e., the locus was not amplified in one of the parents, and in the other parent only one allele was amplified, the other being null. The *Sh* locus and its *E-Agg/M-CAA132* AFLP marker belonged to configuration no. 1 with a single detectable allele segregating from LM2T. Fully informative loci represented 43% of the scored SSR patterns, against 10% for AFLPs. In total, 1,397 marker

Table 1 List of 256 *Elaeis guineensis* SSR loci along with their EMBL accession numbers

Locus name	EMBL accession number
<i>mEgCIR0037</i>	AJ578497
<i>mEgCIR0055</i>	AJ578498
<i>mEgCIR0059</i>	AJ578499
<i>mEgCIR0067</i>	AJ271636
<i>mEgCIR0074</i>	AJ578500
<i>mEgCIR0146</i>	AJ578501
<i>mEgCIR0163</i>	AJ578502
<i>mEgCIR0173</i>	AJ578503
<i>mEgCIR0177</i>	AJ578504
<i>mEgCIR0192</i>	AJ578505
<i>mEgCIR0195</i>	AJ578506
<i>mEgCIR0219</i>	AJ271637
<i>mEgCIR0230</i>	AJ271639
<i>mEgCIR0243</i>	AJ578507
<i>mEgCIR0246</i>	AJ578508
<i>mEgCIR0254</i>	AJ271640
<i>mEgCIR0257</i>	AJ578509
<i>mEgCIR0268</i>	AJ578510
<i>mEgCIR0280</i>	AJ578511
<i>mEgCIR0328</i>	AJ578512
<i>mEgCIR0332</i>	AJ271933
<i>mEgCIR0353</i>	AJ271935
<i>mEgCIR0359</i>	AJ578514
<i>mEgCIR0366</i>	AJ578515
<i>mEgCIR0369</i>	AJ578516
<i>mEgCIR0380</i>	AJ578517
<i>mEgCIR0399</i>	AJ578518
<i>mEgCIR0408</i>	AJ578519
<i>mEgCIR0409</i>	AJ578520
<i>mEgCIR0425</i>	AJ578521
<i>mEgCIR0433</i>	AJ578522
<i>mEgCIR0439</i>	AJ578523
<i>mEgCIR0445</i>	AJ578527
<i>mEgCIR0446</i>	AJ578524
<i>mEgCIR0465</i>	AJ271940
<i>mEgCIR0521</i>	AJ578525
<i>mEgCIR0551</i>	AJ578526
<i>mEgCIR0555</i>	AJ578528
<i>mEgCIR0580</i>	AJ578529
<i>mEgCIR0588</i>	AJ578530
<i>mEgCIR0772</i>	AJ578531
<i>mEgCIR0773</i>	AJ578532
<i>mEgCIR0774</i>	AJ578533
<i>mEgCIR0775</i>	AJ578534
<i>mEgCIR0778</i>	AJ578535
<i>mEgCIR0779</i>	AJ578536
<i>mEgCIR0781</i>	AJ578537
<i>mEgCIR0782</i>	AJ578538
<i>mEgCIR0783</i>	AJ578539
<i>mEgCIR0785</i>	AJ578540
<i>mEgCIR0786</i>	AJ578541
<i>mEgCIR0787</i>	AJ578542
<i>mEgCIR0788</i>	AJ578543
<i>mEgCIR0790</i>	AJ578544
<i>mEgCIR0793</i>	AJ578545
<i>mEgCIR0795</i>	AJ578546
<i>mEgCIR0800</i>	AJ578547
<i>mEgCIR0801</i>	AJ578548
<i>mEgCIR0802</i>	AJ578549
<i>mEgCIR0803</i>	AJ578550
<i>mEgCIR0804</i>	AJ578551
<i>mEgCIR0825</i>	AJ578552
<i>mEgCIR0827</i>	AJ578553
<i>mEgCIR0832</i>	AJ578554
<i>mEgCIR0836</i>	AJ578555
<i>mEgCIR0840</i>	AJ578556

Table 1 (Contd.)

Locus name	EMBL accession number
<i>mEgCIR0844</i>	AJ578557
<i>mEgCIR0874</i>	AJ578558
<i>mEgCIR0878</i>	AJ578559
<i>mEgCIR0882</i>	AJ578560
<i>mEgCIR0886</i>	AJ578561
<i>mEgCIR0894</i>	AJ578562
<i>mEgCIR0905^a</i>	AJ578563
<i>mEgCIR0906</i>	AJ578564
<i>mEgCIR0910</i>	AJ578565
<i>mEgCIR0912</i>	AJ578566
<i>mEgCIR1492</i>	AJ578567
<i>mEgCIR1713</i>	AJ578568
<i>mEgCIR1716</i>	AJ578569
<i>mEgCIR1729</i>	AJ578570
<i>mEgCIR1730</i>	AJ578571
<i>mEgCIR1740</i>	AJ578572
<i>mEgCIR1753</i>	AJ578573
<i>mEgCIR1773</i>	AJ578574
<i>mEgCIR1917</i>	AJ578575
<i>mEgCIR1977</i>	AJ578576
<i>mEgCIR1996</i>	AJ578577
<i>mEgCIR2020</i>	AJ578578
<i>mEgCIR2029</i>	AJ578579
<i>mEgCIR2110</i>	AJ578580
<i>mEgCIR2144</i>	AJ578581
<i>mEgCIR2149</i>	AJ578582
<i>mEgCIR2188</i>	AJ578583
<i>mEgCIR2212</i>	AJ578584
<i>mEgCIR2215</i>	AJ578585
<i>mEgCIR2224</i>	AJ578586
<i>mEgCIR2291</i>	AJ578587
<i>mEgCIR2320</i>	AJ578588
<i>mEgCIR2332</i>	AJ578589
<i>mEgCIR2347</i>	AJ578590
<i>mEgCIR2380</i>	AJ578591
<i>mEgCIR2387</i>	AJ578592
<i>mEgCIR2407</i>	AJ578593
<i>mEgCIR2409</i>	AJ578594
<i>mEgCIR2414</i>	AJ578595
<i>mEgCIR2417</i>	AJ578596
<i>mEgCIR2422</i>	AJ578597
<i>mEgCIR2423</i>	AJ578598
<i>mEgCIR2427</i>	AJ578599
<i>mEgCIR2433</i>	AJ578600
<i>mEgCIR2436</i>	AJ578601
<i>mEgCIR2440</i>	AJ578602
<i>mEgCIR2450</i>	AJ578603
<i>mEgCIR2492</i>	AJ578604
<i>mEgCIR2518</i>	AJ578605
<i>mEgCIR2525</i>	AJ578606
<i>mEgCIR2569</i>	AJ578607
<i>mEgCIR2575</i>	AJ578608
<i>mEgCIR2577</i>	AJ578609
<i>mEgCIR2590</i>	AJ578610
<i>mEgCIR2595</i>	AJ578611
<i>mEgCIR2600</i>	AJ578612
<i>mEgCIR2621</i>	AJ578613
<i>mEgCIR2628</i>	AJ578614
<i>mEgCIR2659</i>	AJ578615
<i>mEgCIR2670</i>	AJ578616
<i>mEgCIR2763</i>	AJ578617
<i>mEgCIR2813</i>	AJ578618
<i>mEgCIR2860</i>	AJ578619
<i>mEgCIR2887</i>	AJ578620
<i>mEgCIR2893</i>	AJ578621
<i>mEgCIR3040</i>	AJ578622
<i>mEgCIR3111</i>	AJ578623

Table 1 (Contd.)

Locus name	EMBL accession number
<i>mEgCIR3160</i>	AJ578624
<i>mEgCIR3194</i>	AJ578625
<i>mEgCIR3213</i>	AJ578626
<i>mEgCIR3232</i>	AJ578627
<i>mEgCIR3260</i>	AJ578628
<i>mEgCIR3271</i>	AJ578629
<i>mEgCIR3275</i>	AJ578630
<i>mEgCIR3281</i>	AJ578631
<i>mEgCIR3282</i>	AJ578632
<i>mEgCIR3286</i>	AJ578633
<i>mEgCIR3292</i>	AJ578634
<i>mEgCIR3293</i>	AJ578635
<i>mEgCIR3295</i>	AJ578636
<i>mEgCIR3296</i>	AJ578637
<i>mEgCIR3297</i>	AJ578638
<i>mEgCIR3298</i>	AJ578639
<i>mEgCIR3300</i>	AJ578640
<i>mEgCIR3301</i>	AJ578641
<i>mEgCIR3305</i>	AJ578642
<i>mEgCIR3307</i>	AJ578643
<i>mEgCIR3310</i>	AJ578644
<i>mEgCIR3311</i>	AJ578645
<i>mEgCIR3316</i>	AJ578646
<i>mEgCIR3321</i>	AJ578647
<i>mEgCIR3328</i>	AJ578648
<i>mEgCIR3346</i>	AJ578649
<i>mEgCIR3350</i>	AJ578650
<i>mEgCIR3358</i>	AJ578651
<i>mEgCIR3362</i>	AJ578652
<i>mEgCIR3363</i>	AJ578653
<i>mEgCIR3365</i>	AJ578654
<i>mEgCIR3376</i>	AJ578669
<i>mEgCIR3382</i>	AJ578655
<i>mEgCIR3383</i>	AJ578656
<i>mEgCIR3384</i>	AJ578657
<i>mEgCIR3387</i>	AJ578658
<i>mEgCIR3389</i>	AJ578659
<i>mEgCIR3392</i>	AJ578660
<i>mEgCIR3399</i>	AJ578661
<i>mEgCIR3400</i>	AJ578662
<i>mEgCIR3402</i>	AJ578663
<i>mEgCIR3407</i>	AJ578664
<i>mEgCIR3413</i>	AJ578665
<i>mEgCIR3427</i>	AJ578666
<i>mEgCIR3428</i>	AJ578667
<i>mEgCIR3433</i>	AJ578668
<i>mEgCIR3439</i>	AJ578670
<i>mEgCIR3477</i>	AJ578671
<i>mEgCIR3519</i>	AJ578672
<i>mEgCIR3526</i>	AJ578673
<i>mEgCIR3533</i>	AJ578674
<i>mEgCIR3534</i>	AJ578675
<i>mEgCIR3535</i>	AJ578676
<i>mEgCIR3538</i>	AJ578677
<i>mEgCIR3543</i>	AJ578678
<i>mEgCIR3544</i>	AJ578679
<i>mEgCIR3546</i>	AJ578680
<i>mEgCIR3555</i>	AJ578681
<i>mEgCIR3557</i>	AJ578682
<i>mEgCIR3563</i>	AJ578683
<i>mEgCIR3567</i>	AJ578684
<i>mEgCIR3569</i>	AJ578685
<i>mEgCIR3574</i>	AJ578686

Table 1 (Contd.)

Locus name	EMBL accession number
<i>mEgCIR3587</i>	AJ578687
<i>mEgCIR3590</i>	AJ578688
<i>mEgCIR3592</i>	AJ578689
<i>mEgCIR3593</i>	AJ578690
<i>mEgCIR3607</i>	AJ578691
<i>mEgCIR3622</i>	AJ578692
<i>mEgCIR3633</i>	AJ578693
<i>mEgCIR3639</i>	AJ578694
<i>mEgCIR3641</i>	AJ578695
<i>mEgCIR3643</i>	AJ578696
<i>mEgCIR3649</i>	AJ578697
<i>mEgCIR3653</i>	AJ578698
<i>mEgCIR3655</i>	AJ578699
<i>mEgCIR3663</i>	AJ578700
<i>mEgCIR3668</i>	AJ578701
<i>mEgCIR3672</i>	AJ578702
<i>mEgCIR3683</i>	AJ578703
<i>mEgCIR3684</i>	AJ578704
<i>mEgCIR3691</i>	AJ578705
<i>mEgCIR3693</i>	AJ578706
<i>mEgCIR3696</i>	AJ578707
<i>mEgCIR3698</i>	AJ578708
<i>mEgCIR3705</i>	AJ578709
<i>mEgCIR3711</i>	AJ578710
<i>mEgCIR3716</i>	AJ578711
<i>mEgCIR3718</i>	AJ578712
<i>mEgCIR3722</i>	AJ578713
<i>mEgCIR3727</i>	AJ578714
<i>mEgCIR3728</i>	AJ578715
<i>mEgCIR3732</i>	AJ578716
<i>mEgCIR3737</i>	AJ578717
<i>mEgCIR3739</i>	AJ578746
<i>mEgCIR3745</i>	AJ578718
<i>mEgCIR3747</i>	AJ578719
<i>mEgCIR3750</i>	AJ578720
<i>mEgCIR3755</i>	AJ578721
<i>mEgCIR3766</i>	AJ578722
<i>mEgCIR3769</i>	AJ578723
<i>mEgCIR3775</i>	AJ578724
<i>mEgCIR3782</i>	AJ578725
<i>mEgCIR3785</i>	AJ578726
<i>mEgCIR3787</i>	AJ578727
<i>mEgCIR3788</i>	AJ578728
<i>mEgCIR3792</i>	AJ578729
<i>mEgCIR3803</i>	AJ578730
<i>mEgCIR3807</i>	AJ578731
<i>mEgCIR3808</i>	AJ578732
<i>mEgCIR3809</i>	AJ578733
<i>mEgCIR3813</i>	AJ578734
<i>mEgCIR3819</i>	AJ578735
<i>mEgCIR3825</i>	AJ578736
<i>mEgCIR3826</i>	AJ578737
<i>mEgCIR3828</i>	AJ578738
<i>mEgCIR3847</i>	AJ578739
<i>mEgCIR3850</i>	AJ578740
<i>mEgCIR3869</i>	AJ578741
<i>mEgCIR3878</i>	AJ578742
<i>mEgCIR3886</i>	AJ578743
<i>mEgCIR3890</i>	AJ578744
<i>mEgCIR3902</i>	AJ578745

^aThe *mEgCIR0905* SSR primer revealed one duplicated locus—*mEgCIR0905a* and *mEgCIR0905b*

Genotype configuration by Ritter <i>et al.</i> (1990) as figured by Lespinasse (1999)						Number of segregating marker loci in the cross					
Code n°	Segregating marker alleles	Parent genotypes		Progeny phenotype classes		LM2T parent			DA10D parent		Total on the cross
		P1	P2	Allelic pattern	Segregation ratio	SSR	AFLP	Gene <i>Sh</i>	SSR	AFLP	
1	1 allele	$\frac{A_1}{A_0}$	$\frac{A_0}{A_0}$	$\rightarrow$	1 : 1	29	642	1	17	370	1059
2		$\frac{A_1}{A_0}$	$\frac{A_1}{A_0}$	$\rightarrow$	3 : 1	not scored	106		not scored	106	106
3	2 alleles	$\frac{A_1}{A_2}$	$\frac{A_0}{A_0}$	$\rightarrow$	1 : 1	91 ^b			21		112 ^b
4		$\frac{A_1}{A_2}$	$\frac{A_1}{A_0}$	$\rightarrow$	1 : 2 : 1	0			0		0
5		$\frac{A_1}{A_2}$	$\frac{A_1}{A_2}$	$\rightarrow$	1 : 2 : 1	3			3		3
6		$\frac{A_1}{A_0}$	$\frac{A_2}{A_0}$	$\rightarrow$	1 : 1 : 1 : 1	2			2		2
7	3 alleles	$\frac{A_1}{A_2}$	$\frac{A_3}{A_0}$	$\rightarrow$	1 : 1 : 1 : 1	1			1		1
8		$\frac{A_1}{A_2}$	$\frac{A_1}{A_3}$	$\rightarrow$	1 : 1 : 1 : 1	38			38		38
9	4 alleles	$\frac{A_1}{A_2}$	$\frac{A_3}{A_4}$	$\rightarrow$	1 : 1 : 1 : 1	76 ^a			76 ^a		76 ^a
Total number of segregating marker loci						240	748	1	158	476	1397
						989			634		

^a: including a locus *mEgCIR905a* revealed by the SSR primers *mEgCIR905*. ^b: including a duplicated locus *mEgCIR905b* revealed by the *mEgCIR905* primers.

Fig. 1 Genotype configuration and distribution of segregating marker loci in the LM2T×DA10D cross between heterozygous parents of *Elaeis guineensis* Jacq

loci were scored in our cross, with 989 and 684 marker loci in LM2T and DA10D, respectively. Thirty-five AFLPs with 100% similarity were detected and discarded. Skewed segregation was detected at $P=0.05$ for 16 (6%) of the 278 SSRs. Ninety-one skewed AFLPs (8%) were detected at $P=0.05$.

Genetic linkage map construction

An integrated SSR map of the cross was produced with 282 marker loci (258 SSRs, 23 AFLPs and the *Sh* locus) distributed on 16 linkage groups (data not shown). This map spanned 1,415 cM with an average marker density of 5 cM and a linkage group length varying between 51 cM and 140 cM. In a second step, 1,289 marker loci (278 SSRs, 1,010 AFLPs and the *Sh* locus) were used to construct the complete linkage maps of the parents, LM2T and DA10D (Table 2). The LM2T map was 1,597 cM long with an average marker density of 2.3 cM, while the DA10D map was 1,528 cM long with an average marker density of 3.2 cM. The genome sizes estimated by the method of Hulbert *et al.* (1988) was 1,562 cM for LM2T and 1,685 cM for DA10D. The colinearity of fully informative loci between the parental genomes was good, apart from a few local inversions of closely linked markers (<5 cM) which were probably due to statistical inaccuracy linked to the limited number of individuals studied. The two-point recombination rates between fully informative loci showed good overall homogeneity

from one parent to the next, except for LG 6 and a half-portion of LG 11 (its size in DA10D was double that in LM2T) as well as a few local heterogeneities detected by chi-square tests between linked markers. The homologous linkage groups of each parental map were combined using 133 fully informative loci (110 SSRs, 23 AFLPs). Fully informative SSR loci all segregated in a 1:1:1:1 ratio. Those SSR loci showing a 1:2:1 ratio were not used to combine parent maps due to the impossibility in determining their linkage phases. Linkage phases could be estimated for only 23 of the 106 fully informative AFLP loci showing a 3:1 segregation ratio. These 23 AFLP loci were those loci successfully used to combine the parental maps, generally with a likelihood support for order of 3.0. In a final step, we produced an integrated linkage map of the cross using 944 markers (255 SSRs, 688 AFLPs, allele *Sh*—) distributed on 16 linkages groups spanning 1,743 cM of the genome (Fig. 2). This map had an average of one marker every 1.8 cM. The lengths of the linkage groups varied between 59 cM and 192 cM. SSRs were rather well distributed along the genome, except for some SSR gaps on LGs 2, 9 and 14. A core map of high confidence was defined using a subset of 115 SSR loci chosen from among SSR loci that could be mapped using a likelihood support for order of 3.0 and distributed every 10–20 cM on average along the genome (Fig. 2). AFLPs filled gaps between SSRs and extended the high-density map size by 23% relative to the integrated SSR map. The determination of 77 cM terminal segment on LG 4 bearing the *Sh* locus was

Table 2 Segregating loci and a description of the parental and integrated linkage maps

	LM2T map	DA10D map	Integrated map
Initial number of segregating loci	989	634	1,397
Segregating loci excluded from linkage analyses:			
AFLP identical loci	27	8	35
Skewed AFLP loci (1%)	53	26	73
Number of mapped loci			
SSR:			
Parent-specific loci of type:	1	25	17
	2	—	—
	3	86	18
Bridge loci of type:	5	2	2
	6	2	2
	7	1	1
	8	35	38
	9	73	76
AFLP:			
Parent specific loci of type:	1	450	296
Bridge loci of type:	2	27	22
Locus <i>Sh</i> :			
Parent-specific loci of type:	1	1	0
Total:	702	472	944
Linkage analysis LOD threshold	4.5	4.2	—
Number of linkage groups	16	16 ^b	16
Unlinked markers	71	58	—
Unmapped markers	136	70	93
Map length (cM)	1,597	1,528	1,743
Estimated genome size ^a (cM)	1,562	1,685	—
Linkage group average length (cM)	100	96	109
Marker density	2.3	3.2	1.8

^aAccording to Hulbert et al. (1988)

^bMade from 23 initial linkage sub-groups, referring to bridge loci and LM2T map information

based on markers segregating in the LM2T parent, except for one SSR fully informative locus, *mEg-CIR3275*. A relatively low marker density was also observed for that particular segment, with one marker every 3.1 cM instead of the 1.2 cM that was found for the rest of the linkage group. No marker was located closer to the *Sh* locus than *E-Agg/M-CAA132* mapped at 4.7 cM. When the whole map was considered, the DA10D-inherited markers were absent in some segments on six linkage groups (nos. 1, 3, 4, 6, 8 and 12), while LM2T-inherited markers were relatively well distributed along the genome. Skewed AFLPs (at $P=0.05$) were quite diffuse along the genome. Skewed SSRs ($P=0.01$ or 0.05) were mapped on seven different linkage groups, but one-half of these skewed SSRs belonged to LG 8 and were heterozygous in both parents, while most of the other loci of this linkage group were only heterozygous in the LM2T parent.

Discussion

Construction strategy and description of the linkage maps

In our investigation, we adopted the strategy of Grattapaglia and Sederoff (1994) in order to break down the segregation information on each parent considered to be a pseudo-testcross, for which first a microsatellite map then a high density map was constructed. The high informativeness of numerous fully informative SSRs was decisive in the comparative identification, construction and integration of parental homologous linkage groups, all the more so because less informative comigrating AFLPs (3:1 segregation ratio), despite their theoretical ability to align genetic maps (Roupe van der Voort et al. 1997), could not be used in our investigation due to the impossibility of identifying the linkage phases for about 80% of them. The underlying causes of some local recombination-frequency differences between parents remain unclear. We did not find significant clusters of mapped SSRs, and some of the large gaps observed between these loci seemed to be due to the homozygosity of the genome studied or to the non-uniform distribution of recombination events (Castiglioni et al. 1999). The observed clusters of AFLPs might correspond to reduced recombination frequencies, such as those found in centromeric regions (Keim et al. 1997). A limited proportion of SSRs or AFLPs revealed a segregation distortion. Similar skewed markers found in cocoa or rubber tree (Lespinasse et al. 2000; Risterucci et al. 2000) were attributed to linkage with closely positioned genes subject to direct selection or displaying lethal alleles, particularly when they were located in a common region of the genome, like LG 8. The equivalent total length of the parental maps reflects a similar degree of saturation of the parental genomes. Our estimations of genome size were similar and comparable to the genome size of a *tenera* parent estimated at 1,604 cM by Moretzsohn et al. (2000) on a partial RAPD genetic map. This means our synthetic map is rather well saturated despite a few remaining gaps. The total length of the synthetic map is 1,743 cM for $2n=16$ and 944 markers. For comparison purposes, coconut, which is taxonomically close, also with 16 pairs of homologous chromosomes, displays a double quantity of nuclear DNA, with 3.6 pg per haploid cell (Röser et al. 1997). This probably partly explains why our map is shorter than the SSR-AFLP map for coconut, which is 1,971 cM for 227 markers (Lebrun et al. 2001). The mean and variation in the lengths of the linkage groups tallies with that found in the plant species, but the larger size of LG 4 was nonetheless un-

Fig. 2 Oil palm linkage map based on the cross LM2T×DA10D (Kosambi distance). The map encompasses 944 markers (255 SSRs, 688 AFLPs, locus *Sh*). *mEgCIR*; *E. guineensis* SSR marker, *mCnCIR*; *Cocos nucifera* SSR marker. SSR markers belonging to a core map of high consistency (LOD support: 3.0) are indicated by

marker names that have been shifted to the left. Single asterisk; Skewed marker at $P=0.05$, double asterisk; skewed marker at $P=0.01$, spiked circle enclosed in a square; SSR dominant marker (genotype configuration no. 1 with null alleles)

LG1

0 E-AAg/M-CTA110
 5 E-gAA/M-TCC141
 8 E-ACA/M-CTT151
 9 E-AAT/M-CTg96
 14 E-ACA/M-CAT231
 17 **mEgCIR1713**
 20 **mEgCIR0257**
 25 E-AAT/M-CAT95
 32 E-AgA/M-CTA257
 35 **mEgCIR3788**
 41 E-gAg/M-TTA255
 43 E-gAA/M-TgA180
 45 E-ACT/M-CTT117
 47 E-gAA/M-TCA131
 E-ACC/M-CAA70
 49 **mEgCIR3819**
 E-gAA/M-TAC126
 E-AAT/M-CAG97
 51 E-AAT/M-CAC>330-1
 52 E-AgC/M-CAT>330-4*
 55 **mEgCIR0399** □
mEgCIR3297 □
 E-AgA/M-CAT182
 57 **mEgCIR3316** □
 E-AgA/M-CAG268
 63 **mEgCIR0380**
 E-AgC/M-CAG165
 E-gAA/M-TAA341
 65 **mEgCIR3428**
 H-AgA/H-CTA93
 71 E-AAT/M-CAA>330-5
 73 E-ACA/M-CTA78
 74 **mEgCIR0268**
 75 **mEgCIR3813**
 E-AAC/M-CAT>330-5
 77 E-AgA/M-CAC282
 80 E-AgA/M-CTg110
 81 **mEgCIR3809**
 82 **mEgCIR0874**
 83 E-gAA/M-TgA194
 86 **mEgCIR3782** □
 E-AgA/M-CTg>330-6
 87 **mEgCIR2763**
 90 **mEgCIR3847**
 96 E-AgA/M-CTg305
 97 E-gAg/M-TgT60
 98 E-ACA/M-CTA257
 99 E-AAT/M-CAC70
 110 **mCnCIR067** □
 112 **mEgCIR0332** □
 113 **mEgCIR0802**

LG2

0 E-AgA/M-CAT85
 1 E-AAC/M-CTg99
 12 **mEgCIR3792**
 16 **mEgCIR2149**
 23 **mEgCIR0800**
 24 E-gAA/M-TAA76
 25 **mEgCIR3282** *
 26 **mEgCIR0793** * □
 29 E-AgA/M-CTA260
 31 E-AAg/M-CAA181
 38 E-AAC/M-CTA235
 41 E-AgA/M-CTT138
 43 E-ACT/M-CAC>330-1
 47 E-gAC/M-TTT312
 55 E-AAg/M-CAG245
 62 E-AAC/M-CAA187
 64 **mEgCIR0408** □
 66 E-ACT/M-CAT242
 67 H-AgA/H-CTA250
 71 E-AgA/M-CAA260
 73 E-AAC/M-CAA322
 75 E-ACg/M-AAT69
 76 E-AAT/M-CAC83
 77 E-ACA/M-CAG177
 81 E-gAg/M-TgT174
 82 E-AAC/M-CAT158
 83 H-ACg/H-CTA152
 84 **mEgCIR3649**
 85 E-ACC/M-CTT>330-1
 86 **mEgCIR2575**
 87 E-AAA/M-CTC229
 88 E-ACA/M-CTg>330-1
 89 E-ACA/M-CAA>330-1*
 90 **mEgCIR2215**
 E-AAT/M-CAG>330-4
 90 E-AAT/M-CTg>330-2
 E-gAA/M-TCT259
 91 E-AAT/M-CAC286
 E-ACC/M-CTg>330-3
 92 E-AAT/M-CAT>330-1
 93 H-ACA/H-CTT270
 94 E-AAT/M-CAG118
 96 E-AAA/M-CTC225
 E-ACA/M-CAA75
 97 E-AAT/M-CTC>330-2
 99 E-AAT/M-CAG208
 100 E-AAT/M-CTT>330-1
 102 E-ACA/M-CTT159
 103 **mEgCIR3683**
 106 E-gAg/M-TTg45
 107 E-AAT/M-CTC176
 108 E-gAA/M-TCA175
 109 E-ACA/M-CAA119
 112 E-AAT/M-CAG113
 117 E-ACC/M-CAT245
 122 E-ACA/M-CAG99
 128 E-AAA/M-CTg197

LG3

0 H-Ag g/H-CTA252
 4 E-AgA/M-CTC275
 7 E-AgC/M-CAC>330-2
 9 E-AAg/M-CAT155
 10 **mEgCIR3698**
 11 E-AAC/M-CTT62
mEgCIR2518 □
 12 E-AgA/M-CAT>330-5
 E-AAT/M-CAT>330-3
 13 **mEgCIR0882**
 14 **mEgCIR0369**
 15 E-gAA/M-TAg119
 16 E-AAC/M-CAT>330-2
 17 E-gAg/M-TgT245
 22 **mCnCIRH06**
 25 E-gAC/M-TgT60
 27 E-gAg/M-TTT330
 31 **mEgCIR0425**
 34 **mEgCIR0173**
 35 E-AAg/M-CTA290
 36 **mEgCIR0912** □
 38 **mEgCIR3544**
 46 **mEgCIR3301**
 54 **mEgCIR2347**
 62 **mEgCIR3260**
 73 E-AAA/M-CTg117

LG4

0 E-AAA/M-CAC>330-2
 11 **E-Agg/M-CAA132**
Sh
 16 **E-ACT/M-CTA>330-1**
 28 E-ACA/M-CAG273
 35 **mEgCIR3275**
 39 E-ACC/M-CAT113
 43 E-gAC/M-Tgg360
 47 E-AgC/M-CTA95
 51 E-AAA/M-CAT188
 54 E-AAA/M-C Tg282
 58 E-Agg/M-CAG>330-1
 64 **mEgCIR3413/3194**
 65 **mEgCIR3716**
 E-AgA/M-C Tg231
 66 E-AAT/M-CAA>330-3
 67 **mEgCIR3286**
 69 E-AAA/M-CAC132
 70 E-ACC/M-CTT92
 75 E-AgA/M-CAT153
 77 E-AgC/M-CAT295
mEgCIR3526
 E-gAC/M-TgT145
 78 **mEgCIR1917**
 79 **mEgCIR0786**
 80 **mCnCIR213** □
 81 E-AAC/M-CTT270
 82 **mEgCIR3439** □
 83 E-Agg/M-CAA229
 84 E-ACT/M-CAA95
 85 E-ACA/M-AAg b2
 86 E-gAC/M-TTg158
 87 H-ACA/H-CTT148
 88 E-gAA/M-TAA186
 89 E-AAg/M-CTT>330-2
 90 E-AAA/M-C Tg202
 91 E-ACC/M-CTA>330-3
 92 H-ACg/H-CTA185
 94 E-AAC/M-CAA>330-3
 95 E-gAA/M-TAT127
 103 E-AAT/M-CTg258
 107 E-ACg/M-CTT189
 108 **mEgCIR3232**
 109 E-gAA/M-TAT131
 112 E-gAA/M-TCg62
 114 E-AAT/M-CTg259
 117 **mEgCIR3535**
mEgCIR3310
 118 E-gAA/M-TgA91
 120 E-AAC/M-CAC143
 121 E-ACg/M-CAA155
 122 E-gAA/M-TCT150
 123 E-AAC/M-CTg204
 E-AAC/M-CTg207
 124 **mEgCIR2423**
 E-AgA/M-CAA91
 125 **mEgCIR1716** □
 128 E-AAC/M-CAT196
 130 E-AAT/M-CTC125
 131 E-AAC/M-CAT115
 132 **mEgCIR3693**
 E-AAT/M-CAA>330-7
 133 E-AgA/M-CAA186
 139 E-ACA/M-CAT219
 141 E-ACA/M-CTT179
 144 **mEgCIR3533**
 147 **mEgCIR1753/3769**
 148 **mEgCIR0074**
 149 **mCnCIR246**
 150 E-gAg/M-TgT185
 151 E-gAC/M-Tgg400
 152 E-AAA/M-C Tg103
 154 E-ACA/M-CTC95
 155 E-AAA/M-C TC171
 156 **mEgCIR2595**
 157 **mCnCIR03'**
 158 E-ACA/M-CAG215
 162 **mEgCIR3705**
 163 E-AAg/M-CAG165
 164 E-ACT/M-CAA255
 168 E-ACC/M-CAA214
 171 E-gAA/M-Tgg150
 172 **mEgCIR3040** □
 173 E-AAg/M-CAA278
 174 **mEgCIR3477**
 175 E-gAA/M-TCC108
 177 E-AAT/M-CTg85
 179 **mEgCIR0059**
 180 **mEgCIR3775**
 185 E-ACA/M-CAA190
 E-AAT/M-CTT158
 186 E-AAC/M-CTT94
 189 E-Agg/M-CAG238
 192 **mEgCIR3557**

LG5	LG6	LG7	LG8
0 E-gAC/M-TTA260	0 E-gAA/M-TCT93	0 E-ACg/M-CTC160	0 E-gAA/M-TCT50
2 E-ACA/M-AAgb4	7 E-AAC/M-CAT204	7 E-gAg/M-TTA550	6 E-AAT/M-CAT113
4 E-AgA/M-CAA199	10 E-AAT/M-CAG200	10 mEgCIR3590	10 E-AAC/M-CAT>330-3
5 mEgCIR3691	11 E-AAT/M-CTA327	11 E-Agg/M-CAA177	15 E-Agg/M-CTg>330-1
6 E-Agg/M-CAA>330-2	17 E-Agg/M-CAA285	13 mEgCIR0894	18 mEgCIR3728
8 E-ACA/M-CAG>330-1	18 E-gAg/M-TTT148	14 mEgCIR3365	28 E-AgA/M-CAA184
10 E-gAg/M-TgT51	20 E-gAg/M-TgT74	16 mEgCIR3307	33 mEgCIR3563
11 E-AAT/M-CAA280	24 E-gAC/M-TTT80	19 E-AgA/M-CAA122	37 mEgCIR0359
14 E-AgC/M-CAT140	30 E-Agg/M-CTT226	20 mEgCIR0905b	41 E-ACT/M-CTA278
	32 E-AAA/M-CTC138	22 E-AAT/M-CTA155	44 mEgCIR0555
	33 E-gAC/M-Tgg180	23 H-AAC/H-CAA275	45 E-gAA/M-TAA82
	35 E-AgA/M-CAA114	28 mEgCIR3389	47 E-AAA/M-CTC>330-3
20 mEgCIR3828	36 mEgCIR3281	31 mCnCIR146	49 E-ACC/M-CAA186
23 E-AAT/M-CTC195	38 mEgCIR3358	32 E-AAC/M-C Tg>330-1	51 mEgCIR0910
	39 E-AgA/M-CTT320	33 E-AAA/M-CAT272	52 mEgCIR3622
27 E-AAg/M-CAG116	43 E-AAg/M-CTA108	34 mCnCIR139	53 mCnCIR192
	46 E-AAA/M-CTg213	37 mCnCIR236	52 E-AAT/M-CAT105
	47 mEgCIR3747	39 E-ACT/M-CAC>330-3	53 E-gAC/M-Tgg105
32 E-AAA/M-CTT156	48 E-gAg/M-TTg415	41 E-AgC/M-CAT83	54 mEgCIR0439
35 E-AAg/M-CTC330	49 E-AAT/M-CAA111	42 E-ACT/M-CTT315	55 mEgCIR0886/0246
38 mEgCIR3902	50 E-AAg/M-CAA130	43 mEgCIR3567/0055	56 E-AgA/M-CTT293
	51 E-AgA/M-CTg>330-1	44 E-AgC/M-CTg320	57 E-ACC/M-CTC>330-1
	52 E-AAC/M-CAg316	45 E-ACT/M-CTC105	59 mEgCIR3711/3808
43 E-ACA/M-CTC278	53 E-AgA/M-CAA>330-1	50 E-AgA/M-CAA>330-3	60 mEgCIR0778
46 mEgCIR3574	54 E-ACC/M-CTg>330-7	52 E-AgC/M-CTA280	63 E-AAT/M-CAC255
	55 mCnCIR105	53 E-AgA/M-AAgb6	66 E-ACC/M-CAT120
51 mEgCIR2813	56 E-AAC/M-CTg>330-2	54 H-AAC/H-CAA273	68 mEgCIR2291
	57 mCnCIR069	55 E-AgA/M-CTA228	73 E-ACA/M-CAg330
	58 E-gAC/M-TTT250	56 E-Agg/M-CTg227	75 mEgCIR3376
	59 E-AAg/M-TAg55	57 E-Agg/M-CAT>330-1	77 E-ACC/M-CTg>330-1
	60 E-AAT/M-CTC241	58 mEgCIR2387	79 E-ACT/M-CAT>330-4
	61 E-AAA/M-CAg265	59 E-Agg/M-CAT>330-2	82 mEgCIR0774
59 mEgCIR3427	62 E-gAg/M-TTg145	60 E-AAC/M-CAA98	84 mEgCIR0163
	63 E-AAA/M-CTg145	66 mEgCIR2029	85 E-AAT/M-CTg>330-1
	64 E-AgC/M-CAg290	67 E-AAT/M-CAC203	89 E-ACA/M-CAA242
	69 mEgCIR3643	76 mEgCIR2600	89 E-gAC/M-TTA61
	71 mEgCIR3543		93 mCnCIR109
	74 E-AAg/M-CTg>330-2		100 mEgCIR3328
	75 mEgCIR0804		102 mEgCIR0775
	77 E-ACA/M-CTA>330-1		103 mEgCIR0836
	81 mEgCIR0195		104 mEgCIR3111
	83 E-ACT/M-CAT>330-2		112 E-AAC/M-CAA>330-2
	84 E-AAA/M-CTT310		114 E-Agg/M-CAA266
	85 mEgCIR3869		115 mEgCIR3890
	88 E-AgA/M-CTg310		119 E-gAA/M-TCg183
	90 E-ACT/M-CAA182		120 E-gAA/M-TCg201
	93 H-AgC/H-CTA190		121 mEgCIR3732
	95 mEgCIR0219		124 E-gAA/M-TgA345
	99 E-AAC/M-CAC>330-1		127 mEgCIR2440
	100 E-Agg/M-CAA310		133 mCnCIRG09
	102 E-AAC/M-CAC>330-2		142 E-Agg/M-CTg115
	104 E-AAT/M-CAC183		143 E-AAT/M-CAA88
	107 E-ACA/M-CTT194		145 E-ACA/M-CTg151
	108 E-AAC/M-CTA198		146 mEgCIR3363/1740
	109 E-AgA/M-CTg166		148 E-gAA/M-TAA421
	111 mEgCIR3383		148 mEgCIR2887
	111 mEgCIR0580		150 mEgCIR1996
	113 E-AgA/M-CTg>330-4		151 E-AAT/M-CTg>330-5
	117 E-AgA/M-CTT107		152 E-AgA/M-CAC115
	120 E-AAA/M-CTT209		153 E-AAC/M-CTT274
	124 H-AAg/H-CAg202		156 E-ACA/M-CTC117
	127 mEgCIR0783		160 E-AAC/M-CTC320
			168 E-AAg/M-CAC98

Fig. 2 (Contd.)

sual. This was due to the long terminal portion bearing the *Sh* locus and a few markers that were almost all specific to the *tenera* parent, LM2T. The most probable terminal mapping of those markers was verified on LM2T using different algorithms of CARTHAGENE. The low density of the surrounding markers might correspond to a region highly homozygous and subject to higher recombination frequencies.

Quality of the high-density map

Despite the multipoint analyses that were carried out with both JOINMAP and CARTHAGENE, the resolution of our map is far below the average marker density of 1.8, with only 116 individuals, which could mean only 232 meioses

when only the fully informative markers are considered. Saturating AFLPs alone constitute a large collection of polymorphic fragments that are generally useless for future mapping efforts, while SSR loci constitute more valuable markers that breeders would invest time and effort into for the purposes of comparative mapping and QTL validation among different pedigrees. In the present investigation, our development of a core SSR map of high confidence is, in fact, the most important result. We saw that a rather high proportion of SSR loci revealed null alleles, with a single allele amplified in one of the two map parents. This configuration is not rare with application of the SSR technique, and it generates less informative dominant markers. Such cases, probably due to insertion-deletion events, in primer sites have already been observed with *E. guineensis* SSR markers (Billotte

LG9	LG10	LG11	LG12
0 E-gAA/M-TCg60	0 E-gAA/M-Tag80	0 E-AAT/M-CTT280	0 mEgCIR0067
11 mEgCIR22 24	2 mCnCIRG06	6 E-ACT/M-CTT220	3 mEgCIR2417
14 H-AAg/H-CAG100	8 E-AgC/M-CAA30 0	7 E-AAC/M-CAT>330-1	8 E-gAC/M-TTA108
21 mEgCIR37 87	11 mCnCIRE10	8 E-ACA/M-CTA105	9 E-AgA/M-CAT 141
22 mEgCIR35 92/38 86	13 E-ACT/M-CTg 120	12 E-AAg/M-CAC232	12 E-ACA/M-CAC82
23 mEgCIR3271	16 E-AgA/M-CAA>330-2	13 mEgCIR3400	16 mEgCIR3672mCnCIRB05
27 H-AAg/H-CAG152	17 mEgCIR0825	14 mEgCIR3722 *	17 E-AAA/M-CAC229
27 E-AAg/M-CAC278	18 E-AcG/M-CTT134	16 mEgCIR3362/3587	20 E-AgA/M-CAC132
28 E-ACC/M-CAg267	20 E-ACA/M-CTC>330-2	17 mEgCIR0878	22 E-gAg/M-TT105
36 E-ACC/M-CTT295	21 E-AgA/M-CA>330-3	18 mEgCIR2110	23 mEgCIR2414
40 E-ACT/M-CA T237	24 mEgCIR3826	21 E-AgA/M-CAA81	24 E-gAA/M-TgC90
58 E-AAg/M-CAA191 *	26 E-ACC/M-CTT 163	22 E-gAC/M-TTA185	29 E-AgA/M-CTC170
62 mEgCIR38 78	27 mEgCIR0243	23 E-AgA/M-CA T>330-3	31 E-AgC/M-CTT 170
63 E-AcG/M-CAT1 52 *	29 E-AgA/M-CTT 106	24 mEgCIR3653	32 E-ACC/M-CTA>330-2
65 E-ACC/M-CAA1 63	30 mEgCIR0788	25 E-AAg/M-CAA96	33 E-gAA/M-TCC91
66 mEgCIR33 05	31 E-AAC/M-CAC>330-3	26 E-AAg/M-CAT320	34 E-AcG/M-CAA212
68 mCnCIRE11	32 E-AgA/M-CA C16 8	26 E-gAA/M-TCC269	36 E-AgA/M-CAg 184
69 E-AcG/M-CTg330	34 E-AAC/M-CA T28 8	27 mEgCIR0192	38 E-ACT/M-CTT98
71 mEgCIR08 44	35 mEgCIR3213/2492	30 E-ACT/M-CTC130	39 mEgCIR2525
72 mEgCIR21 88	36 E-gAA/M-TCC431	31 E-gAA/M-TgA105	40 E-AgA/M-CAT 142
73 mEgCIR33 84	41 mEgCIR0840	32 E-AcA/M-CTg294	41 E-ACA/M-CTC285
74 E-AAT/M-CAA273	43 mEgCIR2628	32 E-AAT/M-CTA>330-2	42 E-AgA/M-CAT>330-2
75 mEgCIR23 32/36 84	44 mEgCIR3519	34 E-AgA/M-CA A>330-6	43 E-AAC/M-CAg182
75 E-AAC/M-CAg>330-1	48 E-ATA/M-ACC149	35 E-AgC/M-CTC>330-4	45 mEgCIR2422
75 E-AAT/M-CAg>330-2	53 E-AgA/M-CTA 109	36 mEgCIR3766	46 mEgCIR2621
76 mEgCIR36 63	54 E-ACA/M-CTT122	38 E-AgA/M-CTA>330-2	47 E-AgC/M-CTA>330-1
76 E-AAT/M-CTA140	58 E-ACT/M-CTA252 *	39 E-AgA/M-CTg245	48 E-AAA/M-CTC260
77 E-ACC/M-CAA229	59 E-gAC/M-TTA500-2	42 E-AgA/M-CTC98	49 E-AgA/M-CAA267
77 E-AgA/M-CTg192	60 mEgCIR0146	45 E-AgA/M-CAT260	50 E-AgA/M-CTC>330-2
78 E-AAT/M-CA T>330-4	61 E-AgA/M-CA C>330-2	52 mEgCIR1977	51 mEgCIR0827
78 E-AgA/M-CTg 86	62 E-AgA/M-CTC184	54 E-ACA/M-ACgb1	51 E-gAg/M-TgT345
79 E-AAT/M-CTA184	63 E-AAT/M-CA T7 9	57 E-gAA/M-TCA230	52 mEgCIR2893
81 E-AgA/M-CTg254	63 E-AAC/M-CTg>330-3	59 mEgCIR3382	53 E-gAg/M-TT g62
83 E-AAT/M-CAA320	65 mEgCIR0551/2020	63 E-AgA/M-CTC122	53 mEgCIR1773
86 E-AgA/M-CTA100	66 E-gAA/M-TgC10 2	68 H-AAg/H-CTA278	54 E-gAA/M-TAC84
91 E-ACT/M-CTA97	67 E-gAg/M-TTg250		54 E-gAA/M-TCg241
93 mEgCIR08 03	72 mEgCIR0433		56 E-AAT/M-CTA225
100 E-AgC/M-CAg>330-2	75 E-AAC/M-CAA 180		56 E-gAg/M-TTA 72
104 E-gAA/M-TCC80	79 mEgCIR3321		57 E-AgC/M-CTg322
105 mEgCIR32 96	82 E-gAA/M-TCC53		58 E-AAC/M-CA T87
107 E-ACC/M-CAC76	85 mEgCIR0366		60 E-gAg/M-TTA 140
	91 E-AAT/M-CTg175		61 mEgCIR0906
	96 mEgCIR0445/0446		65 E-gAA/M-Tag180
	101 mEgCIR3696		66 E-ACA/M-CA T280
	104 mEgCIR3785		68 E-AgA/M-CA C260
			69 E-AAT/M-CA C197
			73 E-AAC/M-CTg219
			77 mEgCIR0465 *
			82 E-ACA/M-CAA 115
			83 E-ACA/M-CAg214
			84 E-ACA/M-CA T166
			92 mEgCIR3825
			95 mEgCIR3538
			97 E-AAA/M-CTC92
			100 E-AAT/M-CTA136
			102 E-ACC/M-CTg>330-2
			107 mEgCIR0790
			110 mEgCIR3311
			124 E-ACC/M-CAT188

Fig. 2 (Contd.)

et al. 2001b). An awareness of the existence of null alleles of these loci is very important as their presence probably means that corresponding SSR markers are not easily transferable from one genotype to another—which is why these markers were definitively not chosen for the core set of markers for the reference map. Despite this disadvantage, these loci as well as all other SSR loci, including transferable coconut SSRs, could be of potential interest to any research team working on *E. guineensis* genetic mapping and QTL analysis projects.

Potential applications for molecular breeding

The fact that the LM2T×DA10D cross could be incorporated into on-going genetic improvement schemes made it a material of choice for establishing a genetic linkage map that will very quickly be used for the detec-

tion of QTL of agronomic interest in view of marker-assisted selection. The numerous SSRs produced for this study virtually permits the use of any parent involved in genetic improvement programs. It will be possible to identify fully informative loci with co-segregating alleles in different selected parents, whose genetic maps will be able to be combined or aligned more cheaply to compare their results and those derived from QTL analyses on other populations. Multi-parental genetic mapping recommended by Muranty et al. (1996) is a potential field of application. Our genetic linkage map could also be an interface between genetic and physical mapping in oil palm by increasing the population size and the density of markers in areas carrying QTLs. As such, these markers could be used to identify and contig bacterial artificial chromosome (BAC) clones within the framework of cloning and tagging genes (Tanksley et al. 1995). Our synthetic linkage map of oil palm is also the first to have

LG13

0 E-Ag/M-CAA1 15
 8 E-Ag/M-CTA278
 20 **mEgCIR3555**
 23 E-AAT/M-CTC281
 25 E-Ag/M-CTA155
 26 E-Ag/M-CTC123 *
 33 **mEgCIR2569**
 38 E-Ag/M-CTA98
 42 E-Ag/M-CTA>330-1
 46 E-AA/M-CAC209
 52 E-Ag/M-CTA125
 54 **mEgCIR0832**
 59 **mCnCIR038**
 64 **mEgCIR2212** □
 65 E-ACT/M-CTg85
 70 E-gAC/M-TTC225
 71 E-gAg/M-TTT68
 74 E-ACA/M-CAA203 *
 77 H-Ag/H-CTA>330-1
 78 E-ACA/M-CTg>330-2
 79 E-ACA/M-CAA85
 81 E-gAA/M-TCg430
 82 E-gAA/M-TAC340
 85 E-gAA/M-TCC155
 86 **mEgCIR3399**
 88 E-gAC/M-Tgg99
 90 E-gAA/M-TAg84
 91 E-gAg/M-CAA89
 92 E-AAT/M-CAA206
 93 E-AAT/M-CTT>330-2
 94 E-AAT/M-CTC>330-4
 96 E-Ag/M-CAg235
 97 E-gAA/M-TCA100
 99 E-gAA/M-TAA161
 100 E-AAT/M-CTg139
 110 E-ACC/M-CAT130
 111 E-gAA/M-TAC91

LG14

0 E-AAC/M-CAA240
 5 E-ACC/M-CTA>330-1
 7 E-Ag/M-CTT147
 9 **mEgCIR3569**
 16 **mEgCIR2427**
 17 E-AAA/M-CTA>330-3
 19 E-AA/M-CAA130
 21 **mEgCIR0779/3350**
 23 E-AAC/M-CTT>330-2
 25 E-Ag/M-CAA87
 27 **mCnCIR124**
 40 **mEgCIR3546**
 53 E-Ag/M-CAC150
 54 E-Ag/M-CAA240
 55 E-AA/M-CAC245
 56 E-AA/M-CTC153
 57 E-AA/M-CAA183
 59 E-Ag/M-CTg>330-3
 64 E-AAA/M-CAC228
 66 **mEgCIR3607**
 70 E-Ag/M-CTT131
 72 **mEgCIR0772**
 74 E-AA/M-CTA171
 75 E-gAg/M-TTT150
 78 E-CT/M-CAg132
 81 E-gAg/M-TgT213
 81 **mEgCIR0588**
 84 E-AAC/M-CTA>330-3
 85 E-Ag/M-CAA111
 87 E-Ag/M-CTg84
 91 **mEgCIR3633**
 98 E-AA/M-CTA220
 100 E-Ag/M-CTT220
 110 E-gAg/M-TTT225

LG15

0 E-gAA/M-TCC81
 5 E-AAT/M-CTA>330-4
 8 **mEgCIR3433**
 9 **mEgCIR3402**
 12 E-gAA/M-TAT175
 16 **mEgCIR0787/1729** □
 17 E-gAA/M-TgA122
 17 **mEgCIR0328** □
 18 **mEgCIR0230**
 19 E-Ag/M-AAT70
 20 **mEgCIR2380**
 21 **mEgCIR0773**
 24 **mEgCIR3295**
 25 E-AAT/M-CTg149
 27 E-Ag/M-CTT>330-2
 29 **mEgCIR0521/2590**
 34 **mEgCIR2860** □
 35 E-gAC/M-TgT230
 36 **mEgCIR2144**
 37 E-Ag/M-CAA255
 38 E-gAA/M-TAA124
 39 **mEgCIR3718**
 41 E-Ag/M-CTC165
 44 E-AAA/M-CTT>330-1
 46 E-gAA/M-TCA151
 47 E-gAC/M-TTA112
 48 **mEgCIR3655**
 49 **mEgCIR3534**
 50 E-gAC/M-TTA164
 53 **mEgCIR3737**
 55 E-Ag/M-CTC>330-1
 58 **mCnCIR09**
 60 E-AAA/M-CTA99
 61 E-Ag/M-CTg>330-5
 62 E-gAg/M-TTg179
 66 E-Ag/M-CTg227
 67 **mEgCIR3727**
 68 **mEgCIR3593**
 69 **mEgCIR2670/2320** □
 70 **mEgCIR0409**
 72 **mEgCIR0177**
 73 E-AAA/M-CAT329
 75 E-Ag/M-CTg233
 75 E-AAC/M-CAC176
 79 E-gAC/M-TTT207
 80 E-AAT/M-CAT216
 80 **mEgCIR3807**
 81 E-ACT/M-CTA265
 82 E-Ag/M-CTC>330-2
 84 E-gAA/M-TAA251
 85 E-AAA/M-CAC>330-1
 87 **mEgCIR1492** □
 88 E-ACA/M-CAC329
 88 E-Ag/M-CAC251
 89 **mEgCIR0781** □
 89 **mEgCIR0037**
 90 E-ACC/M-CTg>330-8
 91 E-ACA/M-CAT135
 92 E-gAA/M-TAA500
 93 **mEgCIR3850**
 94 E-ACC/M-CTg>330-6
 96 E-AAC/M-CTg220
 101 E-Ag/M-CTC>330-4
 102 E-AAA/M-CAC165 *
 108 E-AAT/M-CAA220
 114 E-ACA/M-AAg1

LG16

0 E-ACC/M-CAT300 *
 2 E-ACT/M-CAA187 *
 4 E-ACC/M-CAg308
 5 E-AAT/M-CAA>330-9
 6 E-ACC/M-CAA171
 7 E-gAA/M-TAA248
 8 E-gAA/M-TgA110
 9 E-gAA/M-TgC325
 10 E-AAA/M-CTA>330-5
 11 E-gAg/M-TTA173
 11 E-ACA/M-CTT99
 13 E-AAT/M-CTC172
 16 E-AAT/M-CAT152
 16 E-gAg/M-TgT80
 17 E-Ag/M-CTg>330-2
 18 **mEgCIR3745**
 20 **mEgCIR3639**
 22 E-gAA/M-TTC209
 24 E-ACA/M-CAA187
 25 E-ACA/M-CTA>330-3
 26 **mEgCIR0353**
 27 **mEgCIR3298**
 28 E-gAg/M-TTA220
 29 **mEgCIR2436**
 30 E-AAC/M-CTg107
 32 E-Ag/M-CAT175
 33 E-ACC/M-CTg96
 34 E-ACA/M-CAA243
 35 E-AAA/M-CAg>330-2
 41 E-ACC/M-CTg97
 45 **mEgCIR3750**
 53 E-Ag/M-CTA83
 54 E-gAA/M-TgC178
 57 **mEgCIR0782**
 69 **mEgCIR0905a**

Fig. 2 (Contd.)

16 independent linkage groups corresponding to the 16 homologous chromosome pairs of the plant. It is also the only map with as many SSR markers in an *Arecaceae* species. In that sense, our map provides reference information for future molecular work on oil palm and its relatives. To date, the *E-Ag/M-CAA132* AFLP marker is the only one to have been mapped relatively close to the *Sh* locus. An AFLP marker (*E-ACT/M-CTA>330-1*) flanks the *Sh* gene at 11.6 cM on its other side. The combined use of the two AFLPs flanking the *Sh* locus enables prediction of the variety type directly from the nursery stage, with around 99.5% [$1 - (0.047 \times 0.116)$] reliability. Subject to their validation, these markers open up prospects for efficient management of the experimental areas by struc-

turing genetic trials according to the variety type of selected descents. Likewise, it will be possible to detect *dura* or *pisifera* genotypes separately in seed gardens. This type of selection has already been applied to S_1 selfed progenies of LM2T. However, the true adult phenotype of the parents will still have to be checked before vast quantities of seeds can be harvested from them. Given that recombination affects the association between alleles of a given locus and those of its close anonymous markers, only one intragenic marker of the *Sh* gene will enable 100% reliable molecular prediction of the varietal type.

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