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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 guinensis Jacq.*) was constructed from a cross between two heterozygous 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.

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## Introduction

The species *Elaeis guineensis* Jacquin is an allogamous aborescent monocot of the *Arecaceae* family (tribe: *Cocoineae*) (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 m $M$ Tris-HCl (pH 8), 100 m $M$ KCl, 0.05% (w/v) gelatin and 2.0 m $M$ $MgCl_2$] containing 0.08 $\mu M$ of the M13-labeled primer, 0.1 $\mu M$ of the other primer, 160 $\mu M$ dNTP, 1 U *Taq* DNA polymerase (Life Technologies, Gaithersburg, Md.) and 0.06 $\mu M$ 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 *Eco*RI/*Mse*I or *Taq*I/*Hin*dIII 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% denaturating 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 *Eco*RI/*Mse*I or *Taq*I/*Hin*dIII 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 (*Cococus 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 ($17\pm 4$ repeats), nine $(GT)_n$ SSRs ($12\pm 7$ repeats) and nine $(CCG)_n$ SSRs ($6\pm 2$ repeats). Compound microsatellites usually involved two different dinucleotide motifs, which accumulated 23 repeats on average (± 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 |
|---|---|
| *mEgCIR0037* | AJ578497 |
| *mEgCIR0055* | AJ578498 |
| *mEgCIR0059* | AJ578499 |
| *mEgCIR0067* | AJ271636 |
| *mEgCIR0074* | AJ578500 |
| *mEgCIR0146* | AJ578501 |
| *mEgCIR0163* | AJ578502 |
| *mEgCIR0173* | AJ578503 |
| *mEgCIR0177* | AJ578504 |
| *mEgCIR0192* | AJ578505 |
| *mEgCIR0195* | AJ578506 |
| *mEgCIR0219* | AJ271637 |
| *mEgCIR0230* | AJ271639 |
| *mEgCIR0243* | AJ578507 |
| *mEgCIR0246* | AJ578508 |
| *mEgCIR0254* | AJ271640 |
| *mEgCIR0257* | AJ578509 |
| *mEgCIR0268* | AJ578510 |
| *mEgCIR0280* | AJ578511 |
| *mEgCIR0328* | AJ578512 |
| *mEgCIR0332* | AJ271933 |
| *mEgCIR0353* | AJ271935 |
| *mEgCIR0359* | AJ578514 |
| *mEgCIR0366* | AJ578515 |
| *mEgCIR0369* | AJ578516 |
| *mEgCIR0380* | AJ578517 |
| *mEgCIR0399* | AJ578518 |
| *mEgCIR0408* | AJ578519 |
| *mEgCIR0409* | AJ578520 |
| *mEgCIR0425* | AJ578521 |
| *mEgCIR0433* | AJ578522 |
| *mEgCIR0439* | AJ578523 |
| *mEgCIR0445* | AJ578527 |
| *mEgCIR0446* | AJ578524 |
| *mEgCIR0465* | AJ271940 |
| *mEgCIR0521* | AJ578525 |
| *mEgCIR0551* | AJ578526 |
| *mEgCIR0555* | AJ578528 |
| *mEgCIR0580* | AJ578529 |
| *mEgCIR0588* | AJ578530 |
| *mEgCIR0772* | AJ578531 |
| *mEgCIR0773* | AJ578532 |
| *mEgCIR0774* | AJ578533 |
| *mEgCIR0775* | AJ578534 |
| *mEgCIR0778* | AJ578535 |
| *mEgCIR0779* | AJ578536 |
| *mEgCIR0781* | AJ578537 |
| *mEgCIR0782* | AJ578538 |
| *mEgCIR0783* | AJ578539 |
| *mEgCIR0785* | AJ578540 |
| *mEgCIR0786* | AJ578541 |
| *mEgCIR0787* | AJ578542 |
| *mEgCIR0788* | AJ578543 |
| *mEgCIR0790* | AJ578544 |
| *mEgCIR0793* | AJ578545 |
| *mEgCIR0795* | AJ578546 |
| *mEgCIR0800* | AJ578547 |
| *mEgCIR0801* | AJ578548 |
| *mEgCIR0802* | AJ578549 |
| *mEgCIR0803* | AJ578550 |
| *mEgCIR0804* | AJ578551 |
| *mEgCIR0825* | AJ578552 |
| *mEgCIR0827* | AJ578553 |
| *mEgCIR0832* | AJ578554 |
| *mEgCIR0836* | AJ578555 |
| *mEgCIR0840* | AJ578556 |

**Table 1** (Contd.)

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

**Table 1** (Contd.)

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

**Table 1** (Contd.)

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

[a]The *mEgCIR0905* SSR primer revealed one duplicated locus—*mEgCIR0905a* and *mEgCIR0905b*

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

[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 | 42 |
| | 2 | – | – | – |
| | 3 | 86 | 18 | 103 |
| Bridge loci of type: | 5 | 2 | 2 | 1 |
| | 6 | 2 | 2 | 2 |
| | 7 | 1 | 1 | 1 |
| | 8 | 35 | 38 | 36 |
| | 9 | 73 | 76 | 70 |
| AFLP: | | | | |
| Parent specific loci of type: | 1 | 450 | 296 | 665 |
| Bridge loci of type: | 2 | 27 | 22 | 23 |
| Locus *Sh*: | | | | |
| Parent-specific loci of type: | 1 | 1 | 0 | 1 |
| 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 |

[a]According to Hulbert et al. (1988)
[b]Made 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, *mEgCIR3275*. 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 (Rouppe 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 unu-

**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

| Position | Marker |
|---|---|
| 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-CAT257 |
| **35** | **mEgCIR3788** |
| 41 | E-gAg/M-TTA255 |
| 43 | E-gAA/M-TgA180 |
| 45 | E-ACT/M-CTT117 |
| 47 | E-gAA/M-TCA131 |
| **49** | E-ACC/M-CAA70; **mEgCIR3819**; E-gAA/M-TAC126 |
| 50 | E-AAT/M-CAg97 |
| 51 | E-AAT/M-CAC>330-1 |
| 52 | E-AgC/M-CAT>330-4 * |
| **55** | **mEgCIR0399** ¤; **mEgCIR3297** ¤ |
| **57** | E-AgA/M-CAT182; **mEgCIR3316** ¤ |
| 62 | E-Agg/M-CAg268 |
| **63** | **mEgCIR0380** |
| 65 | E-AgC/M-CAg165; E-gAA/M-TAA341 |
| **65** | **mEgCIR3428** |
| 71 | H-Agg/H-CTA93 |
| 73 | E-AAT/M-CAA>330-5; E-ACA/M-CTA78 |
| **74** | **mEgCIR0268** |
| **75** | **mEgCIR3813** |
| 77 | E-AAC/M-CAT>330-5 |
| 80 | E-AgA/M-CAC282; 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

| Position | Marker |
|---|---|
| 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-Agg/M-CTA260 |
| 31 | E-AAg/M-CAA181 |
| 38 | E-AAC/M-CTA235 |
| 41 | E-Agg/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-Agg/H-CAT250 |
| 71 | E-Agg/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** |
| 90 | E-AAT/M-CAg>330-4; 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

| Position | Marker |
|---|---|
| 0 | H-Agg/H-CTA252 |
| 4 | E-Agg/M-CTC275 |
| 7 | E-AgC/M-CAC>330-2 |
| 9 | E-AAg/M-CAT155 |
| **10** | **mEgCIR3698** |
| 11 | E-AAC/M-CTT62 |
| **12** | **mEgCIR2518** ¤; 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

| Position | Marker |
|---|---|
| 0 | E-AAA/M-CAC>330-2 |
| **11** | **E-Agg/M-CAA132** |
| **16** | **Sh** |
| **28** | **E-ACT/M-CTA>330-1** |
| 35 | E-ACA/M-CAg273 |
| **39** | **mEgCIR3275** |
| 43 | E-ACC/M-CAT113 |
| 47 | E-gAC/M-Tgg360 |
| 51 | E-AgC/M-CTA95 |
| 54 | E-AAA/M-CAT188 |
| 58 | E-AAA/M-CTg282 |
| 64 | E-Agg/M-CAg>330-1 |
| **65** | **mEgCIR3413/3194**; **mEgCIR3716** |
| 66 | E-AgA/M-CTg231; 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 |
| **78** | **mEgCIR3526**; E-gAC/M-TgT145 |
| **79** | **mEgCIR1917** |
| **80** | **mEgCIR0786** |
| **81** | **mCnCIR213** ¤ |
| 82 | E-AAC/M-CTT270 |
| **83** | **mEgCIR3439** ¤ |
| 84 | E-Agg/M-CAA229 |
| 85 | E-ACT/M-CAA95 |
| 86 | E-ACA/M-AAgb2 |
| 87 | E-gAC/M-TTg158 |
| 88 | H-ACA/H-CTT148 |
| 89 | E-gAA/M-TAA186 |
| 90 | E-AAg/M-CTT>330-2; E-AAA/M-CTg202 |
| 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 |
| **124** | E-AAC/M-CTg207; **mEgCIR2423** |
| 125 | E-AgA/M-CAA91 |
| **126** | **mEgCIR1716** ¤ |
| 128 | E-AAC/M-CAT196 |
| 130 | E-AAT/M-CTC125 |
| 131 | E-AAC/M-CAT115 |
| **132** | **mEgCIR3693** |
| 133 | E-AAT/M-CAA>330-7 |
| 135 | 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-CTg103 |
| 154 | E-ACA/M-CTC95 |
| 155 | E-AAA/M-CTC171 |
| **156** | **mEgCIR2595** |
| **157** | **mCnCIRC03'** |
| 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

0 E-gAC/M-TTA260
2 E-ACA/M-AAgb4
4 E-AgA/M-CAA199
5 mEgCIR3691
E-Agg/M-CAA>330-2
6 E-AgA/M-CAC192
8 E-ACA/M-CAg>330-1
10 E-gAg/M-TgT51
11 E-AAT/M-CAA280
14 E-AgC/M-CAT140
20 mEgCIR3828
23 E-AAT/M-CTC195
27 E-AAg/M-CAg116
32 E-AAA/M-CTT156
35 E-AAg/M-CTC330
38 mEgCIR3902 ¤
43 E-ACA/M-CTC278
46 mEgCIR3574
51 mEgCIR2813 ¤
59 mEgCIR3427

LG6

0 E-gAA/M-TCT93
7 E-AAC/M-CAT204
10 E-AAT/M-CAg200
11 E-AAT/M-CTA327
17 E-Agg/M-CAA285
18 E-gAg/M-TTT148
20 E-gAg/M-TgT74
24 E-gAC/M-TTT80
30 E-Agg/M-CTT226
32 E-AAA/M-CTC138
33 E-gAC/M-Tgg180
35 E-AgA/M-CAA114
36 mEgCIR3281
38 mEgCIR3358
39 E-AgA/M-CTT320
43 E-AAg/M-CTA108
46 E-AAA/M-CTg213
47 mEgCIR3747
48 E-gAg/M-TTg415
49 E-AAT/M-CAT111
E-AAg/M-CAA130
50 E-AgA/M-CTg>330-1
51 E-AAT/M-CAA255
E-AAC/M-CAg316
E-AgA/M-CAA>330-1
52 E-ACC/M-CTg>330-7
53 mCnCIR105
54 E-AAC/M-CTg>330-2
55 mCnCIR069
56 E-gAC/M-TTT250
57 E-gAA/M-TAg55
58 E-AAT/M-CTC241
59 E-AAA/M-CAg265
60 E-gAg/M-TTg145
61 E-AAA/M-CTg145
64 E-AgC/M-CAT290
69 mEgCIR3643
71 mEgCIR3543
74 E-AAg/M-CTg>330-2
75 mEgCIR0804
77 E-ACA/M-CTA>330-1
81 mEgCIR0195
83 E-ACT/M-CAT>330-2
84 E-AAA/M-CTT310
85 mEgCIR3869 ¤
88 E-AgA/M-CTg310
90 E-ACT/M-CAA182
93 H-ACg/H-CTA190
95 mEgCIR0219
99 E-AAC/M-CAC>330-1
100 E-Agg/M-CAA310
102 E-AAC/M-CAC>330-2
104 E-AAT/M-CAC183
107 E-ACA/M-CTT194
E-AAC/M-CTA198
108 E-AgA/M-CTg166
109 mEgCIR3383
111 mEgCIR0580
113 E-AgA/M-CTg>330-4
117 E-AgA/M-CTT107
120 E-AAA/M-CTT209
124 H-AAg/H-CAg202
127 mEgCIR0783 ¤

LG7

0 E-ACg/M-CTC160
7 E-gAg/M-TTA550
10 mEgCIR3590
11 E-Agg/M-CAA177
13 mEgCIR0894
14 mEgCIR3365 ¤
16 mEgCIR3307
19 E-AgA/M-CAA122
20 mEgCIR0905b
22 E-AAT/M-CTA155
23 H-AAC/H-CAA275
28 mEgCIR3389
31 mCnCIR146
32 E-AAC/M-CTg>330-1
33 E-AAA/M-CAT272
34 mCnCIR139 ¤
37 mCnCIR236 ¤
39 E-ACT/M-CAC>330-3
41 E-AgC/M-CAT83
42 E-ACT/M-CTT315 *
43 mEgCIR3567/0055 ¤*
44 E-AgC/M-Ctg320
45 E-ACT/M-CTC105
50 E-AgA/M-CAA>330-3
52 mEgCIR3300
E-ACA/M-CTA280
E-ACA/M-AAgb6
53 H-AAC/H-CAA273
E-AgA/M-CTA228
54 E-Agg/M-CTg227
56 E-Agg/M-CAT>330-1
57 mEgCIR2387
58 E-Agg/M-CAT>330-2
60 E-AAC/M-CAA98
66 mEgCIR2029
67 E-AAT/M-CAC203
76 mEgCIR2600

LG8

0 E-gAA/M-TCT50
6 E-AAT/M-CAT113
10 E-AAC/M-CAT>330-3
15 E-Agg/M-CTg>330-1
18 mEgCIR3728
28 E-AgA/M-CAA184
33 mEgCIR3563
37 mEgCIR0359
41 E-ACT/M-CTA278
44 mEgCIR0555
45 E-gAA/M-TAA82
47 E-AAA/M-CTC>330-3
E-ACC/M-CAC186
mEgCIR0910
49 mEgCIR3622
51 mCnCIR192
52 E-AAT/M-CAT105
53 E-gAC/M-Tgg105
54 mEgCIR0439 ¤
55 mEgCIR0886/0246
56 E-AgA/M-CTT293
57 E-ACC/M-CTC>330-1
59 mEgCIR3711/3808
60 mEgCIR0778
63 E-AAT/M-CAC255
66 E-ACC/M-CAT120
68 mEgCIR2291 ¤
73 E-ACA/M-CAg330
75 mEgCIR3376
E-ACC/M-CTg>330-1
77 E-ACT/M-CAT>330-4
79 mEgCIR0774
82 mEgCIR0163
84 E-AAT/M-CTg>330-1
85 E-ACA/M-CAA242
89 E-gAC/M-TTA61
93 mCnCIR109 *
100 mEgCIR3328
102 mEgCIR0775 *
103 mEgCIR0836 * *
104 mEgCIR3111 *
112 E-AAC/M-CAA>330-2
114 E-Agg/M-CAA266
115 mEgCIR3890 ¤
119 E-gAA/M-TCg183
120 E-gAA/M-TCg201
121 mEgCIR3732 *
124 E-gAA/M-TgA345
127 mEgCIR2440 * *
133 mCnCIRG09 ¤
142 E-Agg/M-CTg115
143 E-AAT/M-CAA88
145 E-ACA/M-CTg151
146 mEgCIR3363/1740
E-gAA/M-TAA421
148 mEgCIR2887
150 mEgCIR1996
151 E-AAT/M-CTg>330-5
152 E-AgA/M-CAC115
153 E-AAC/M-CTT274
156 E-ACA/M-CTC117 *
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

| Position | Marker |
|---|---|
| 0 | E-gAA/M-TCg60 |
| 11 | mEgCIR2224 |
| 14 | H-AAg/H-CAg100 |
| 21 | mEgCIR3787 |
| 22 | mEgCIR3592/3886 |
| 22 | mEgCIR3271 ¤ |
| 23 | H-AAg/H-CAg152 |
| 27 | E-AAg/M-CAC278 |
| 28 | E-ACC/M-CAg267 |
| 36 | E-ACC/M-CTT295 |
| 40 | E-ACT/M-CAT237 |
| 58 | E-AAg/M-CAA191 * |
| 62 | mEgCIR3878 |
| 63 | E-ACg/M-CAT152 * |
| 65 | E-ACC/M-CAA163 |
| 66 | mEgCIR3305 |
| 68 | mCnCIRE11 |
| 69 | E-ACg/M-CTg330 |
| 71 | mEgCIR0844 |
| 72 | mEgCIR2188 |
| 73 | mEgCIR3384 |
| 74 | E-AAT/M-CAA273 |
| 75 | mEgCIR2332/3684 |
| 75 | E-AAC/M-CAg>330-1 |
| 75 | E-AAT/M-CAg>330-2 |
| 76 | mEgCIR3663 |
| 76 | E-AAT/M-CTA140 |
| 77 | E-ACC/M-CAA229 |
| 77 | E-Agg/M-CTg192 |
| 77 | E-AAT/M-CAT>330-4 |
| 78 | E-Agg/M-CTg86 |
| 79 | E-AAT/M-CTA184 |
| 79 | E-Agg/M-CTT211 |
| 81 | E-AAA/M-CTg254 |
| 83 | E-AAT/M-CAA320 |
| 86 | E-Agg/M-CTA100 |
| 91 | E-ACT/M-CTA97 |
| 93 | mEgCIR0803 |
| 100 | E-AgC/M-CAg>330-2 |
| 104 | E-gAA/M-TCC80 |
| 105 | mEgCIR3296 ¤ |
| 107 | E-ACC/M-CAC76 |

LG10

| Position | Marker |
|---|---|
| 0 | E-gAA/M-TAg80 |
| 2 | mCnCIRG06 |
| 8 | E-AgC/M-CAA300 |
| 11 | mCnCIRE10 |
| 13 | E-ACT/M-CTg120 |
| 16 | E-AgA/M-CAA>330-2 |
| 17 | mEgCIR0825 |
| 18 | E-ACg/M-CTT134 |
| 20 | E-ACA/M-CTC>330-2 |
| 21 | E-AgA/M-CAC>330-3 |
| 24 | mEgCIR3826 |
| 26 | E-ACC/M-CTT163 |
| 27 | mEgCIR0243 |
| 29 | E-AgA/M-CTT106 |
| 30 | mEgCIR0788 |
| 31 | E-AAC/M-CAC>330-3 |
| 31 | E-AgA/M-CAC168 |
| 32 | E-AgA/M-CAC179 |
| 34 | E-AAC/M-CAT288 |
| 35 | mEgCIR3213/2492 |
| 36 | E-gAA/M-TCC431 |
| 41 | mEgCIR0840 ¤ |
| 43 | mEgCIR2628 |
| 44 | mEgCIR3519 |
| 48 | E-ATA/M-ACC149 |
| 53 | E-AgA/M-CTA109 |
| 54 | E-ACA/M-CTT122 |
| 58 | E-ACT/M-CTA252 * |
| 59 | E-gAC/M-TTA500-2 |
| 60 | mEgCIR0146 |
| 61 | E-AgA/M-CAC>330-2 |
| 61 | E-AgA/M-CTC184 |
| 61 | E-AgA/M-CAC>330-1 |
| 62 | E-AAT/M-CAT79 |
| 63 | E-AAC/M-CTg>330-3 |
| 65 | mEgCIR0551/2020 |
| 66 | E-gAA/M-TgC102 |
| 67 | E-gAg/M-TTg250 |
| 72 | mEgCIR0433 |
| 75 | E-AAC/M-CAA180 |
| 79 | mEgCIR3321 ¤ |
| 82 | E-gAA/M-TCC53 |
| 85 | mEgCIR0366 |
| 91 | E-AAT/M-CTg175 |
| 96 | mEgCIR0445/0446 |
| 101 | mEgCIR3696 |
| 104 | mEgCIR3785 |

LG11

| Position | Marker |
|---|---|
| 0 | E-AAT/M-CTT280 |
| 6 | E-ACT/M-CTT220 |
| 7 | E-AAC/M-CAT>330-1 |
| 8 | E-ACA/M-CTA105 |
| 12 | E-AAg/M-CAC232 |
| 13 | mEgCIR3400 |
| 14 | mEgCIR3722 * |
| 16 | mEgCIR3362/3587 |
| 17 | mEgCIR0878 |
| 18 | mEgCIR2110 |
| 21 | E-AgA/M-CAA81 |
| 22 | E-gAC/M-TTA185 |
| 23 | E-AgA/M-CAT>330-3 |
| 24 | mEgCIR3653 |
| 25 | E-AAg/M-CAA96 |
| 25 | E-AAg/M-CAT320 |
| 26 | E-gAA/M-TCC269 |
| 27 | mEgCIR0192 |
| 30 | E-ACT/M-CTC130 |
| 31 | E-gAA/M-TgA105 |
| 32 | E-ACA/M-CTg294 |
| 32 | E-AAT/M-CTA>330-2 |
| 34 | E-Agg/M-CAA>330-6 |
| 35 | E-AgC/M-CTC>330-4 |
| 36 | mEgCIR3766 |
| 38 | E-AgA/M-CTA>330-2 |
| 39 | E-Agg/M-CTg245 |
| 42 | E-Agg/M-CTC98 |
| 45 | E-AgA/M-CAT260 |
| 52 | mEgCIR1977 |
| 54 | E-ACA/M-ACgb1 |
| 57 | E-gAA/M-TCA230 |
| 59 | mEgCIR3382 |
| 63 | E-AgA/M-CTC122 |
| 68 | H-AAg/H-CTA278 |

LG12

| Position | Marker |
|---|---|
| 0 | mEgCIR0067 |
| 3 | mEgCIR2417 |
| 8 | E-gAC/M-TTA108 |
| 9 | E-AgA/M-CAT141 |
| 12 | E-ACA/M-CAC82 |
| 16 | mEgCIR3672mCnCIRB05 |
| 17 | E-AAA/M-CAC229 |
| 20 | E-AAg/M-CAC132 |
| 22 | E-gAg/M-TTT105 |
| 23 | mEgCIR2414 |
| 24 | E-gAA/M-TgC90 |
| 29 | E-AAg/M-CTC170 |
| 31 | E-AgC/M-CTT170 |
| 32 | E-ACC/M-CTA>330-2 |
| 33 | E-gAA/M-TCC91 |
| 34 | E-ACg/M-CAA212 |
| 36 | E-AgA/M-CAg184 |
| 38 | E-ACT/M-CTT98 |
| 39 | mEgCIR2525 ¤ |
| 40 | E-AAg/M-CAT142 |
| 41 | E-ACA/M-CTC285 |
| 42 | E-AgA/M-CAT>330-2 |
| 43 | E-AAC/M-CAg182 |
| 45 | mEgCIR2422 |
| 46 | mEgCIR2621 |
| 47 | E-AgC/M-CTA>330-1 |
| 48 | E-AAA/M-CTC260 |
| 49 | E-AAg/M-CAA267 |
| 50 | E-AAg/M-CTA195 * |
| 50 | E-AgA/M-CTC>330-2 |
| 51 | mEgCIR0827 |
| 51 | E-gAg/M-TgT345 |
| 52 | mEgCIR2893 |
| 53 | E-gAg/M-TTg62 |
| 53 | mEgCIR1773 |
| 54 | E-gAA/M-TAC84 |
| 54 | E-gAA/M-TCg241 |
| 54 | E-AAT/M-CTA225 |
| 56 | E-gAg/M-TTA72 * |
| 57 | E-AgC/M-Ctg322 |
| 58 | E-AAC/M-CAT87 |
| 60 | E-gAg/M-TTA140 |
| 61 | mEgCIR0906 |
| 65 | E-gAA/M-TAg180 |
| 66 | E-ACA/M-CAT280 |
| 68 | E-AgA/M-CAC260 |
| 69 | E-AAT/M-CAC197 |
| 73 | E-AAC/M-CTg219 |
| 77 | mEgCIR0465 * |
| 82 | E-ACA/M-CAA115 |
| 83 | E-ACA/M-CAg214 |
| 84 | E-ACA/M-CAT166 |
| 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 detection 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

**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-Agg/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−(0.047×0.116)] reliability. Subject to their validation, these markers open up prospects for efficient management of the experimental areas by structuring 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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