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Using Monomorphic Microsatellite Markers in Oil Palm (*Elaeisguineensis* Jacq.).

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Research Article

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ABSTRACT

Molecular markers in oil palm characterization and breeding began two decades ago. Microsatellite markers are a system that is commonly used in oil palm research since its development. Monomorphic SSR markers have been eliminated from all evolutionary and population genetics studies by researchers because of their lack of genetic variability. The goals of this study were to review polymorphic DNA microsatellite marker system also known as simple sequence repeats(SSR) in oil palm research since its development and to employa monomorphic SSR marker for detection of illegitimacy in oil palm breeding programs. Ten monomorphic SSR markers and two half-sib families were used in this study. Illegitimate offspring IDs 97 and 180 were found by four monomorphic locimEgCIR0425, mEgCIR3477, mEgCIR3769, and mEgCIR3902 in Family-1and Family-2. In addition, five loci (mEgCIR3574, mEgCIR3607, mEgCIR3672, mEgCIR3785 and mEgCIR3807) detect one illegitimate offspring ID 180.This study showed that monomorphic SSR markers are suitable for the detection of illegitimate offsprings in oil palm breeding programs.

INTRODUCTION

The oil palm is a perennial oil crop, diploid with 16 pairs of chromosomes,classified into two species, *Elaeisguineensis* and *Elaeisoleifera* which are also known as African and American oil palms based on their native lands respectively. The American species is attractive because it possesses a number of good characteristics such as slow increase in height and resistance to diseases [1]. Therefore, *E. oleifera*is used for hybridization with *E. guineensis*to transfer its useful characters to commercial materials. The *E. guineensis* is a source of commercial oil palm materials, the most important oil palm type in producing oil is *tenera,* the result of *dura*× *pisifera*(D×P) crosses. The oil palm belongs to the Arecaceae (Palmae) family which is the second most economically important monocot family after Poaceae (Gramineae).The latest classification by Dransfieldet *al*. [2]for the palm family, Arecaceae are 187 catalogued genera including *Elaeis.*

The oil palm has gained many different names like “Cash crop” or “Golden crop” because of its important contributions to the Malaysian economy over the last 50 years. In 2011, Malaysian and Indonesian palm oil productions reached 18,912 and 21,449 million tons, respectively; while the world total was 47,704 million tons [3]. This economic growth was the result of research and development (R&D) by the Department of Agriculture and passed through many organizations such as the Palm Oil Research Institute of Malaysia (PORIM) until the establishment of the Malaysian Palm Oil Board (MPOB). Now, R&D has expanded into several areas such as food, nutrition, oleo chemical products, genomics, biotechnology and biofuel. Oil palm R&D is well organized for the breeding programs which put increasing yields as the first priority, followed by oil quality, and disease tolerance of palm trees. Breeding research and expansion

of plantation areas have led to increases in yield and production. The majority of these increases have come from changes in the genetic materials as a result of breeding work. The changeover from the old planting material *dura* to the *tenera* (D × P) hybrid seed was the first step which led to a 30% increase of yield, the second step caused the increase in the productivity per unit area and [4] reported that the next step in yield improvement would be clonal planting material. Also, biotechnology in general and molecular

Table 1: Summary of studies conducted on the oil palm using genomic and EST-SSR markers.

No	Type of SSR	Objective	No of SSRs	Other marker systems	Reference
1	Genomic SSR	genetic diversity	21	-	[9]
2	Genomic SSR	genetic diversity	12	-	[14]
3	Genomic SSR	genetic diversity	14	-	[15]
4	Genomic SSR	genetic diversity	8	-	[16]
5	Genomic SSR	genetic diversity	20	-	[17]
6	EST-SSR	genetic diversity	10	-	[18]
7	EST-SSR	genetic diversity	9	-	[19]
8	EST-SSR	genetic diversity	15	-	[20]
9	EST-SSR	genetic diversity	9	-	[21]
10	EST-SSR	genetic diversity	10	-	[22]
11	Genomic SSR	QTL	371	-	[23]
12	Genomic SSR	QTL	255	AFLP	[8]
13	Genomic SSR/ EST-SSR	QTL	22	AFLP/ cDNA–RFLP	[24]
14	Genomic SSR	QTL	278	AFLP	[25]
15	Genomic SSR	QTL	331	AFLP/ PCR–RFLP	[26]
16	Genomic SSR/ EST-SSR	QTL	161	AFLP/ PCR–RFLP	[11]
17	Genomic SSR	hybrid verification	9	RAPD	[12]
18	Genomic SSR/ EST-SSR	Association mapping	58	-	[13]
19	Genomic SSR	Illegitimacy test and sibship assignments	30	-	[13]

markers in particular plays a big role in oil palm genetic studies. Molecular markers have been exploited in oil palm research over the last twenty years in areas like DNA fingerprinting using restriction fragment length polymorphism (RFLP) [5], genetic diversity by implementing both randomly amplified polymorphic DNA (RAPD)[6] and amplified fragment length polymorphism (AFLP) [7], and QTL mapping using microsatellites [8]. Each DNA marker type has its pros and cons, but the single locus DNA microsatellite marker system also known as simple sequence repeats (SSR) is the best used DNA marker in oil palm research because of its good features such as polymorphic information content, reproducibility, co-dominance, widespread distribution in genomes and abundance. Microsatellites are common in oil palm, and it can be used for all kinds of genetic studies (Table 1). Billotte*et al.* [9] reported the first successful development of SSRs from enriched DNA libraries in the oil palm, with high percentage of positive clones. Since the development of these markers, they have been used commonly in oil palm research. Table 2.2 shows the use of SSRs markers in numerous research studies which have been conducted in the oil palm. The purpose of this review is to show the significance of and to update the research work which used SSRs markers in the oil palm. The source of SSRs is from either enriched DNA libraries or EST-SSR. Nineteen studies that used SSR markers were found. Genomic SSR was used in eleven studies, EST-SSR was used in five studies, and only three studies used a combination of them. Genomic SSRs are commonly employed in both genetic diversity and QTL mapping research. EST-SSRs are mostly utilized in genetic diversity work thus showing that they are helpful in detecting genetic variations among germ plasms. Also, Tranbarger*et al.*[10] suggested that EST-SSRs derived from cDNA can be used in functional diversity studies. The number of SSRs in the genetic diversity studies ranged from 9 to 21. For QTL mapping, the number of SSRs used ranged from 22 to 371, but in all QTL studies, a combination of SSRs with other marker systems had been

used. In the study of Ting *et al.*[11]the final number of SSRs markers in the maps was less than the number of markers in other work on QTLs, but they demonstrated that their parental maps were not saturated because of the large genome size of the oil palm. Also in another study conducted for hybrid verification, they used 9 SSRs [12]. Recently, SSRs had been employed in association study as a screening tool to determine oil palm resistance against *Ganoderma* in a breeding program and for illegitimacy testing and sibship assignments in oil palm half-sib families [13].

The above mentioned studies used polymorphic SSRs in their oil palm research. However, in this study we have used monomorphic SSRs for detecting illegitimate palms in the oil palm breeding programs.

MATERIALS AND METHODS

Plant materials and DNA extraction

Two half-sib families were selected from previous work [13] of the control cross *D* × *P* (*dura* ×*pisifera*). Each family had 50 offsprings , FP1/10 and FP1/28 were sibs and the male parents (*pisifera*) and the female parents of the Dura fruit type were FD6 and FD1/224 respectively for the Family-2 (FD 6 × FP1/10) and Family-4 (FD 1/224 × FP1/28) respectively.

Leaf samples from seedlings and parents were used to extract genomic DNA using the modified cetyl trimethyl ammonium bromide (CTAB) method [27]. DNA quality was checked by gel electrophoresis in a 1% agarose gel, and the NanoDrop H ND-1000 spectrophotometer (Nano Drop Technologies Inc) was used to measure the concentration and check the quantity of the extracted DNA.

Microsatellite genotyping

A total of nine monomorphic microsatellite loci were tested on all offsprings. Table 2 provides information on these loci. PCR amplification for offsprings were carried out in 15 µL volumes containing 50 ng DNA, 1×PCR buffer (-$MgCl_2$), 0.2 mMd NTP mix, 0.2 U *Taq* polymerase (Invitrogen, Brazil), 2.0 mM $MgCl_2$, 0.2 ug/µL BSA, 0.3 mM M13-tailed forward primer, 0.3 mM untailed reverse primer, and 005 mM IRD-labeled M13 primer (IR700 or IR800). PCR was performed on a My Cycler Thermal Cycler (Bio Rad) with initial denaturation for 3 min at 95 ºC, 35 cycles denaturation for 30 s at 95 ºC, annealing for 1 min at 52 ºC, extension for 2 min at 72 ºC, and final extension for 15 min at 72 ºC. All forward primers were tailed with an M13 5'-GGA AAC AGC TAT GAC CAT- 3', which allowed the simultaneous amplification of PCR products by the IRD labeled-M13 primer [26].

Microsatellite genotyping was conducted using LI-COR 4300 DNA Analysis System (Li-COR BioScience, Lincoln, NE, USA). Labelled PCR products IR700 or IR800 were mixed and then separated in 6.5% KB Plus acrylamide gel matrix (LI-COR Inc.). The allele size was detected and scored using the SAGA Generation 2 software (LI-COR Inc.) with three standards of size 50-350 bp in each gel (LI-COR Inc.).

Table 2: Microsatellite, primer sequences, annealing temperature (TA) and expected amplicon size (AS).

No.	Microsatellite ID	Forward Primer	Reverse Primer	TA(ºC)	AS (bp)
1.	mEgCIR0425	AGCAAGAGCAAGAGCAGAACT	CTTGGGGGCTTCGCTATC	58	232
2.	mEgCIR3477	CCTTCAAGCAAAGATACC	GGCACCAAACACAGTAA	52	232
3.	mEgCIR3574	AGAGACCCTATTTGCTTGAT	GACAAAGAGCTTGTCACAC	52	207
4.	mEgCIR3607	ATTGCAGAGATGATGAGAAG	GAGATGCTGACAATGGTAGA	52	188
5.	mEgCIR3672	AAAGCCATTCCAGACTAC	CTCATAGCCTTTGTTGTGT	52	159
6.	mEgCIR3722	GGCAGGTTAGGTTAGATGAT	GAGTTGAAAGAAAAGGAGTG	52	268
7.	mEgCIR3769	TCTCTCCAACTAGAAGTTGTAGAC	CATGGGCCAGTAGGAAGTAG	52	122
8.	mEgCIR3785	AAGCAATATAGGTTCAGTTC	TCATTTTCTAATTCCAAACAAG	52	284
9.	mEgCIR3807	CCTATTCCTTACCTTTCTGT	ATACATCCCCTCCCCTCTCT	52	116
10.	mEgCIR3902	ACAATAACCTGAGACAACAAGAAAC	TCATTTTCTAATTCCAAACAAG	52	284

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RESULTS AND DISCUSSION

Manual analysis of the LI-COR gels was conducted for 10 monomorphic loci (Table2) in two half-sib families during genotyping.In the previous work of [13] they detect two illegitimate palms IDs 97 and 180 in Family-2 and Family-4 respectively using polymorphic SSRs markers. Figure 1 shows the gel images ofthe monomorphic locus mEgCIR0425 for Family-2 and Family-4. For example, at this locus all offsprings in Family-2 had only one allele, whileoffspring ID 97 had two alleles.

In Family-4 all offsprings had two alleles butoffspring ID 180 hadtwo different alleles. Similar results were found at locus mEgCIR3477, mEgCIR3769, and mEgCIR3902 in Family-1and Family-2. These results indicated that offspring IDs 97 and 180 in Family-2 and Family-4 respectively had different alleles from the other offsprings. Therefore, these offspring were illegitimate and did not belong to any of the families typed. The results of the present study are in agreement with those of [13], who reported that offspring IDs 97 and 180 in Family-2 and Family-4were illegitimate based on polymorphic SSR markers and computational analysis.

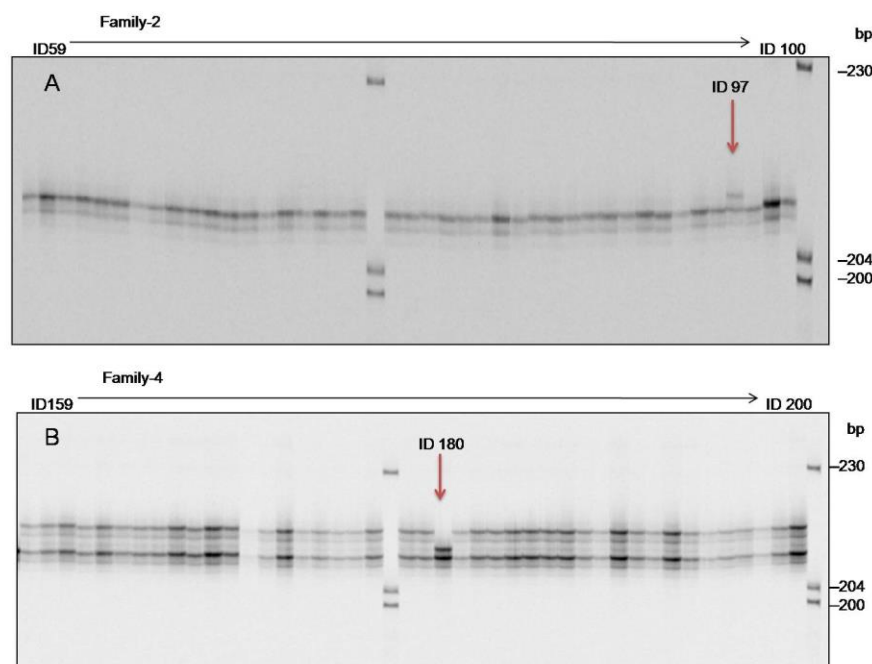

Figure 1: LI-COR gel images for locus mEgCIR0425 showing detection of illegitimate offspring ID 97 (A) in Family-2, and of illegitimate offspring ID 180 (B) in Family-4.

In addition, fivemonomorphic loci (mEgCIR3574, mEgCIR3607, mEgCIR3672, mEgCIR3785 and mEgCIR3807) just detected one illegitimate offspring ID 180 and they did not detected illegitimate offspring ID 97. Based on [13] study'soffspring ID 97 was due to pollen contamination during the control cross. In contrast, admixed (illegitimate) offspring ID 180 might have been caused by errors during the nursery stage. This error could be due to mislabeling at the nursery stage. However, monomorphic locus mEgCIR3722did not detect illegitimate offsprings ID 97 and ID 180. This study showed that monomorphic SSR markers are useful for illegitimacy testing in oil palm breeding programs.

Also, pervious work by [28] reported monomorphic SSR markers as being useful for genetic analysis and [29] reported of converting monomorphic microsatellite markers to polymorphic markers. The conversion of monomorphic microsatellite markers to polymorphic markers were also investigated successfully [30] in cotton (*Gossypium*L.).

CONCLUSION

In conclusion, this important finding satisfied the goals of this study. Due to the successful detections of illegitimate palms within a breeding program by monomorphic SSR markers, this study showed that monomorphic molecular markers are also suitable for illegitimacy testing in the oil palm.

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