LEGITIMACY ASSESSMENT OF POLLEN WITH SIMPLE SEQUENCE REPEATS MARKERS

YEN-YEN KWAN*; EZDIAWATI YAHAYA*; NUR ADIBAH ISHAK** and LING-JIUN LIAO**

ABSTRACT

It is essential that only good quality planting materials are used in oil palm breeding and commercial seed production. Best parents are usually selected from the best families for commercial production. Stringent quality measures of seed had already been employed to produce high quality planting materials. However, pollination with illegitimate pollen still arise in oil palm breeding programme and compromise quality of planting materials. To date, legitimacy of pollen is traced through data recording. Therefore, illegitimate pollen due to errors occur during pollen processing may have been overlooked. Concurring genotyping profiles generated by panel of Simple Sequence Repeats (SSR) markers in leaf and pollen grains of the same palm, facilitate assessment of pollen legitimacy. Utilisation of this assessment may exclude utilisation of illegitimate pollen in oil palm breeding programme and improve the quality of breeding procedure.

Keywords: oil palm, pollen, SSR markers.

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INTRODUCTION

Parentage information of families and individuals are very important in breeding programme as oil palm breeders usually select the best individual plants within the best families (Corley, 2005) based on progeny testing results. Progeny testing results of unknown illegitimate individuals in the families is not useful for selection process as the true parental is unknown and is therefore, cannot be exploited for commercial production and breeding improvement.

Illegitimacy and contamination did occur sporadically in the past as reflected by Pol-820, which is the ancestor of AVROS materials (Corley, 2005). Typical detection of illegitimacy or contamination is through fruit typing which exhibits occurrences of unexpected fruit form or anomaly segregation ratios. Subsequent detection accuracy has improved tremendously with genetic markers, where discrimination between individuals can be

performed based on variation in the genomic loci. Among the available genetic markers, microsatellite markers are the most widely used due to its straightforward PCR procedure and also providing informative alleles per locus.

Controlled pollination is susceptible to various sources of error, which could contribute to illegitimacy or contamination of a cross. Errors may occur in pollen collection or storage, labelling of pollinated bunches on the palm, seed storage, nursery or during field planting of the trials (Corley, 2005). Despite the implementation of quality control measures of seed production process (Chin, 1995) to produce high quality planting materials, utilisation of illegitimate pollen still lingers and detection remains a challenge. Here, we report that panel of Simple Sequence Repeats (SSR) markers is capable to assess pollen legitimacy and propose to be employed as QC tool in seed production process.

MATERIALS AND METHODS

Plant Materials

Pollen and leaf samples were collected from six designated palms at Pusat Penyelidikan Pertanian

* Felda Global Ventures Research & Development Sdn Bhd, FGV Innovation Centre, PT 23417 Lengkok Technology, 71760 Bandar Enstek, Negeri Sembilan, Malaysia. E-mail: speedwell.yen@gmail.com

** Felda Global Venture Research & Development Sdn Bhd, Pusat Penyelidikan Pertanian Tun Razak, 26400 Bandar Jengka, Pahang, Malaysia.

Tun Razak, Felda Global Venture Research & Development Sdn Bhd (FGV), at Jerantut, Pahang, Malaysia. GMH-43, AB1-15, FJ2-7 and GNR-4 were *pisifera* of Yangambi origin. On the other hand, DF-64 and FJ6-3 were Ulu Remis Deli *dura* and introgressed NPM x Yangambi *tenera*, respectively.

Extraction of Genomic DNA

Genomic DNA was extracted from pollen and leaf as previously described by Seng and Faridah (2006). The concentration of the extracted genomic DNA for each sample was determined using Nanodrop ND-1000 spectrophotometer (Thermo Scientific, USA). The integrity of the extracted genomic DNA was evaluated by resolving on a 1% agarose gel stained with RedSafe™ (iNtRON Biotechnology Inc., Korea) and visualised with transilluminator (UVItc Limited, UK).

Microsatellite Analysis

Eight primers were selected from FGV's Microsatellite Legitimacy Panel Version 2.0 (Siti Hawa *et al.*, 2015). These primers were selected based on SSR loci isolated by the French Centre de Coopération en Recherche Agronomique pour le Développement (CIRAD) and Malaysian Palm Oil Board (MPOB) (Table 1). The forward primers were added with M13 sequence 5'-GGA AAC AGC TAT GAC CAT-3' (Oetting *et al.*, 1995), permitting concurrent fluorescence labelling of polymerase chain reaction (PCR) products by a third primer (M13) with an incorporated multiple fluorescent dye label (6-FAM™, VIC®, NED™ and PET®) together with the reverse primer (Boutin-Ganache *et al.*, 2001).

PCR amplification was performed in a 10 µl reaction mixture containing 100 ng genomic DNA, 1x MyTaq™ HS mix, 0.42 µl of primer mix (10 µM of M13-tailed forward primer, 10 µM reverse primer and 1 µM of fluorescent labelled-M13 primer).

TABLE 1. PRIMERS USED IN SIMPLE SEQUENCE REPEATS (SSR) ANALYSIS AND ALLELE SIZE OF EACH PRIMER

Primer name	Repeat motif	Allele size
mEgCIR0886	(GA) ₉	150 – 190
mEgCIR0894	(GA) ₁₈	170 – 230
mEgCIR2427	(GA) ₁₄	120 – 180
mEgCIR3358	(GA) ₁₅	200 – 260
mEgCIR3400	(GA) ₁₆	120 – 180
mEgCIR3428	(GA) ₁₅	160 – 200
mEgCIR3663	(GA) ₂₁	200 – 260
mEgCIR3785	(GA) ₂₁	250 – 300

PCR amplifications were performed in Mastercycler pro S (Eppendorf, Germany) using the following programme; pre-denaturation at 95°C for 1 min, followed by 35 cycles of 95°C for 15 s, 52°C-57°C for 15 s and 72°C for 10 s. PCR amplicons were analysed along a size standard, (GeneScan™ 600 LIZ® Size Standard) using ABI3500XL Capillary Electrophoresis Genetic Analyser (Applied Biosystems, USA). The alleles were sized and genotyped using GeneMapper version 5.0 software (Applied Biosystems, USA).

RESULTS AND DISCUSSION

Isolation of Genomic DNA from Pollen

Genomic DNA was successfully extracted from the pollen collected from GMH-43, ABI-15, FJ2-17, GNR-4, FJ6-3 and DF-64 palms. Intact genomic DNA with no visible signs of DNA degradation were observed in the agarose gel (Figure 1). Purity of the extracted genomic DNA from pollen ranged from 1.74 to 1.86, comparable to those extracted from leaf samples (Table 2). Utilising 0.1 g of pollen as starting material is sufficient to generate good quality of genomic DNA with the yields ranging between 38 – 51 µg for subsequent SSR markers analysis.

Figure 1. Genomic DNA extracted from pollen samples. Lane 1, Lambda DNA; 2, GMH-43; 3, ABI-15; 4, FJ2-7; 5, GNR-4; 6, FJ6-3 and 7, DF-64.

Figure 2. Electropherogram for Simple Sequence Repeats (SSR) marker analysis with mEgCIR0886 (blue), mEgCIR0894 (green), mEgCIR2427 (black) and mEgCIR3358 (red) on pollen (A, C, E, G, I, K) and leaf (B, D, F, H, J, L). A and B, AB1-15; C and D, DF-64; E and F, FJ2-17; G and H, FJ6-3; I and J, GMH-43; K and L, GNR-4. The allele size of the amplicons is labelled in panel A, C, E, G, I and K.

TABLE 2. QUANTITY AND QUALITY OF THE EXTRACTED GENOMIC DNA

Sample ID	Source	DNA yield (ng μl^{-1})	DNA purity (A_{260}/A_{280})
GMH-43	Leaf	443	1.79
GMH-43	Pollen	346	1.85
ABI-15	Leaf	336	1.80
ABI-15	Pollen	291	1.83
FJ2-17	Leaf	535	1.83
FJ2-17	Pollen	254	1.80
GNR-4	Leaf	501	1.82
GNR-4	Pollen	255	1.85
FJ6-3	Leaf	333	1.83
FJ6-3	Pollen	307	1.86
DF-64	Leaf	407	1.78
DF-64	Pollen	284	1.74

Concurring Microsatellite Profiles in Leaf and Pollen Grains

Microsatellite profiles derived from leaf exhibited one or two alleles per SSR marker indicating diploidy nature of oil palm. On the other hand, pollen grains are haploid and therefore should exhibit 1:1 segregation ratio in loci with two alleles in mother palm. Gómez *et al.* (2001) reported *in vitro*-induced haploid embryos from anther cultures of *Quercus suber* L. exhibiting parental tree segregation. Only one allele per locus was amplified in each embryo of the tested *Q. petraea* microsatellite loci. Furthermore, Mendelian segregation of 1:1, is statistically confirmed by squared test in one case.

As mix of pollen grains harvested from a male inflorescence were used for genomic DNA

Figure 3. Electropherogram for Simple Sequence Repeats (SSR) marker analysis with mEgCIR0886 on pollen (A, C, E, G, I, K) and leaf (B, D, F, H, J, L). A and B, ABI-15; C and D, DF-64; E and F, FJ2-17; G and H, FJ6-3; I and J, GMH-43; K and L, GNR-4.

extraction, microsatellite profile generated by pollen DNA concurred with leaf profile of the same palm. No differences in microsatellite profiles were observed between leaf and pollen DNA of the same palm (*Figures 2 and 3*) in eight tested loci. Agreement of microsatellite profiles in leaf and pollen of the same palm indicating this molecular marker data comparison could be exploited as legitimacy tool for the harvested pollen.

Quality Control on Breeding Procedure

It is essential that crosses have to be made correctly to exclude illegitimacy and contamination in breeding programmes. Breeding glitches could occur at various stages, from pollen collection to field planting, compromising the quality of the breeding crosses made.

Utilisation of microsatellite markers in fidelity (Bakoume *et al.*, 2011) and legitimacy testing (Hama-Ali *et al.*, 2014; Siti Hawa *et al.*, 2015) have been reported in oil palm with leaf genomic DNA. These testing hitherto conducted at the earliest in the nursery, after controlled pollination. However, oversight could also occur at the earlier stages, at pollen collection or storage.

Our results demonstrated concurrence profiles generated by SSR genotyping on pollen and leaf of the same palm, indicating legitimacy assessment of the harvested pollen can be determined before proceeding with cross pollination activity. Consolidating legitimacy assessment of pollen into standard operating procedure of breeding crosses could help to exclude the usage of incorrect pollen for pollination process and subsequently refine the quality control measures in seed production.

CONCLUSION

Concurring microsatellite profiles generated in leaf and pollen grains of the same palm demonstrated that it is viable to determine pollen status with panel of SSR markers. Integrating pollen legitimacy assessment into current standard operating procedure of seed processing will help to improve the quality of planting materials.

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