SHORT COMMUNICATION: CYTOLOGICAL ANALYSIS OF OIL PALM POLLEN MOTHER CELLS (PMCs)

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ABSTRACT

This short paper describes the cytological analysis of oil palm pollen mother cells (PMCs) in an effort to identify the pachytene and uninucleate microspore stages for application in other experiments. The chromosomal pachytene stage will be used in the localization of transgenes in transgenic palms while the uninucleate microspore stage will be utilized in microspore culture for haploid production.

Keywords: anthers, pollen mother cells (PMCs), meiosis.

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INTRODUCTION

The oil palm, *Elaeis guineensis* (2n = 32) fruit type *tenera* (DxP), is a major economic crop in Malaysia, and it is reported to yield 3.7 t ha^{-1} yr^{-1} of oil (Kushairi and Rajanaidu, 2000). This perennial monocot, starts to bear fruit as early as three years after field planting and continues to do so, even at 25 years. *Elaeis guineensis* (subfamily Cocoideae) belongs to the family Palmae that contains over 225 genera and 2600 species. The chromosome numbers of 111 genera and approximately 250 species of the Palmae family including *E. guineensis* have been reported (Palomino and Quero, 1992). Maria *et al*. (1995) reported on the mitotic chromosome numbers of *E. guineensis* as 2n = 32 and found that the chromosomes can be divided into three groups on the basis of length. Group I consists only of chromosome pair No. 1 (the longest pair), group II of chromosome Nos. 2-9 (medium length) and group III of pair Nos. 10-16 (short chromosomes).

Meiosis is the unique and essential part of the life cycle of all sexually reproducing organisms in which a diploid cell of the sporophyte gives rise to haploid cells which develop further to the gametophyte (in plants) and gametes (John, 1990; Schwarzacher, 2003). It involves two divisions (Meiosis I and II) that are linked together without any further DNA replication. The first division involves the pairing of homologous chromosomes and their subsequent segregation, and the second resembles mitosis in which the sister chromatids are segregated (Armstrong and Jones, 2003; Schwarzacher, 2003).

However, studies on oil palm meiotic chromosomes are minimal except for that by Hardon and Tan (1969). They reported on the cytology of *E. guineensis* x *E. oleifera* (the other species of *Elaeis*) F_1 hybrids which showed normal chromosome pairing at pachytene except for the occasional unpaired chromosome segments but no inversions, deficiency loops or translocation configurations. Due to the paucity of information on the meiotic and nuclear stages of *E. guineensis* pollen mother cells (PMCs), this study on male meiosis was undertaken mainly to facilitate other experiments such as fluorescence *in situ* hybridization (FISH) of transgenes on transgenic oil palm pachytene chromosomes and also microspore culture to produce haploids and double haploids for breeding purposes. Objectively, this cytological analysis was done to identify the pachytene stage of meiotic chromosomes and the uninucleate microspore stage for use in microspore culture.

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EXPERIMENTAL

Plant Material

Tenera male inflorescences with flower buds containing uninucleate and tetrad stage PMCs were collected from fronds just before anthesis of the subtended inflorescence. The inflorescences can anthesize from Frond 15 to Frond 30. Frond 1 being the youngest fully opened frond. The male flower buds were removed from the spikelets and fixed in 3:1 absolute ethanol:glacial acetic acid overnight at 4°C before storage in 70% ethanol at 4°C. The male flower buds containing uninucleate PMCs were approximately 2 mm in length, 1.5 mm in diameter, soft in texture and whitish in colour, while the ones containing tetrad PMCs were approximately 4 mm in length, 1.5 mm in diameter, hard in texture and yellowish in colour. The anthers were excised from the flower buds and treated using the Feulgen staining method.

Feulgen Staining Method

The procedure was adapted from the method of Greilhuber and Temsch (2001). The anthers were acid hydrolyzed in 5N HCl for 90 min at 20°C. Upon completion, the anthers were rinsed 5 x 2 min in ice-cold distilled water and immersed in Schiff's reagent at 4°C (12-15 hr or overnight). Next, they were rinsed in sulfide water for 3x5 minutes followed by 3x10 min, and finally, by a short rinse in distilled water. At this stage, the anthers can be stored in distilled water at 4°C for a long time. When needed, the PMCs were squeezed out from the cut made in the middle of the anthers into 60% acetic acid mixed with antifade medium on a glass slide. The suspension was covered with a cover slip followed by light tapping and observed under fluorescence microscopy. Images were captured using a ProgResC12 (Jenoptik) camera and saved in TIFF format.

To prepare permanent slides, the PMCs were squeezed into 60% acetic acid, covered with a cover slip, followed by light tapping. The slides were then frozen on dry ice and the cover slips flipped off with a razor blade. They were then dehydrated in an ethanol series of 50%, 75%, 90% and 100% for 5 min each followed by air-drying. A drop of DPX or mountant was then placed on the sample area, covered with a cover slip and stored in a slide box at room temperature.

RESULTS AND DISCUSSION

The following *Figures* illustrate the different meiotic activities in the PMCs and the different nuclear stages produced. The first meiotic process involved pairing of the homologous chromosomes during prophase I (covering leptotene, zygotene and pachytene) after a long interphase. *Figure 1* illustrates the feasibility of using the pachytene chromosome spreads in fluorescence *in situ* hybridization (FISH) for characterization of the transgenes in transgenic palms. At pachytene, the chromosome arms are more extended and less compact compared to the metaphase chromosomes. De Jong *et al*. (1999) showed that the resolution limits achievable on pachytene chromosomes of tomato are about 1.2 Mb in heterochromatin and 120 kb in euchromatin. Compared to metaphase chromosomes, the resolution on the highly condensed metaphase chromosomes is usually restricted to 2 Mbp. *In situ* hybridization of the less condensed pachytene chromosomes is often used without losing the ability to identify the individual chromosomes (Shen *et al*., 1987; Zhong *et al*., 1996).

At metaphase I, the bivalent chromosome pairs align at the equatorial plane with their centromeres attached to the kinetochore microtubules (*Figure 2*). During this stage, the PMCs are in a uninucleate or one nucleus state. At anaphase I, the bivalent chromosome pairs separate to opposite poles and became univalents followed by diakinesis or nuclear division. During this stage, the PMCs are in dyad or two-nuclei state (*Figures 3a* and *3b* arrow). The univalent chromosomes will again align at the equatorial plane in metaphase II (*Figure 3b*-arrow) and the sister chromatids separate to opposite poles to form tetrad or four-nuclei stage PMCs (*Figure 4*). These tetrads give rise to immature pollen, also described as uninucleate microspores, which are used for microspore culture of many plants to produce haploid offsprings. For example, Ma *et al*. (2004) used uninucleate microspores from four winter genotypes of *Secale cereale* to produce haploids.

In conclusion, this study illustrates the importance of basic information on oil palm male meiosis to assist in other applied experiments such as fluorescence *in situ* hybridization of single copy sequence on pachytene chromosomes and also microspore culture.

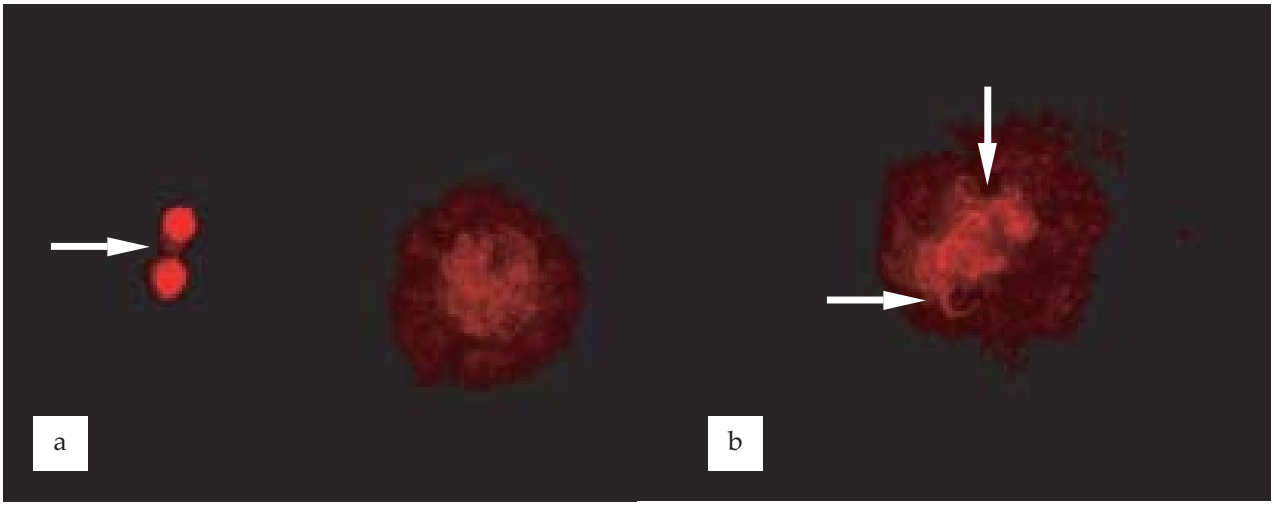

Figure 1. Uninucleate state of PMCs. (a) Two tapetum cells (arrow) with a pachytene chromosome spread, and (b) pachytene spread with chromosome arms extended (arrows).

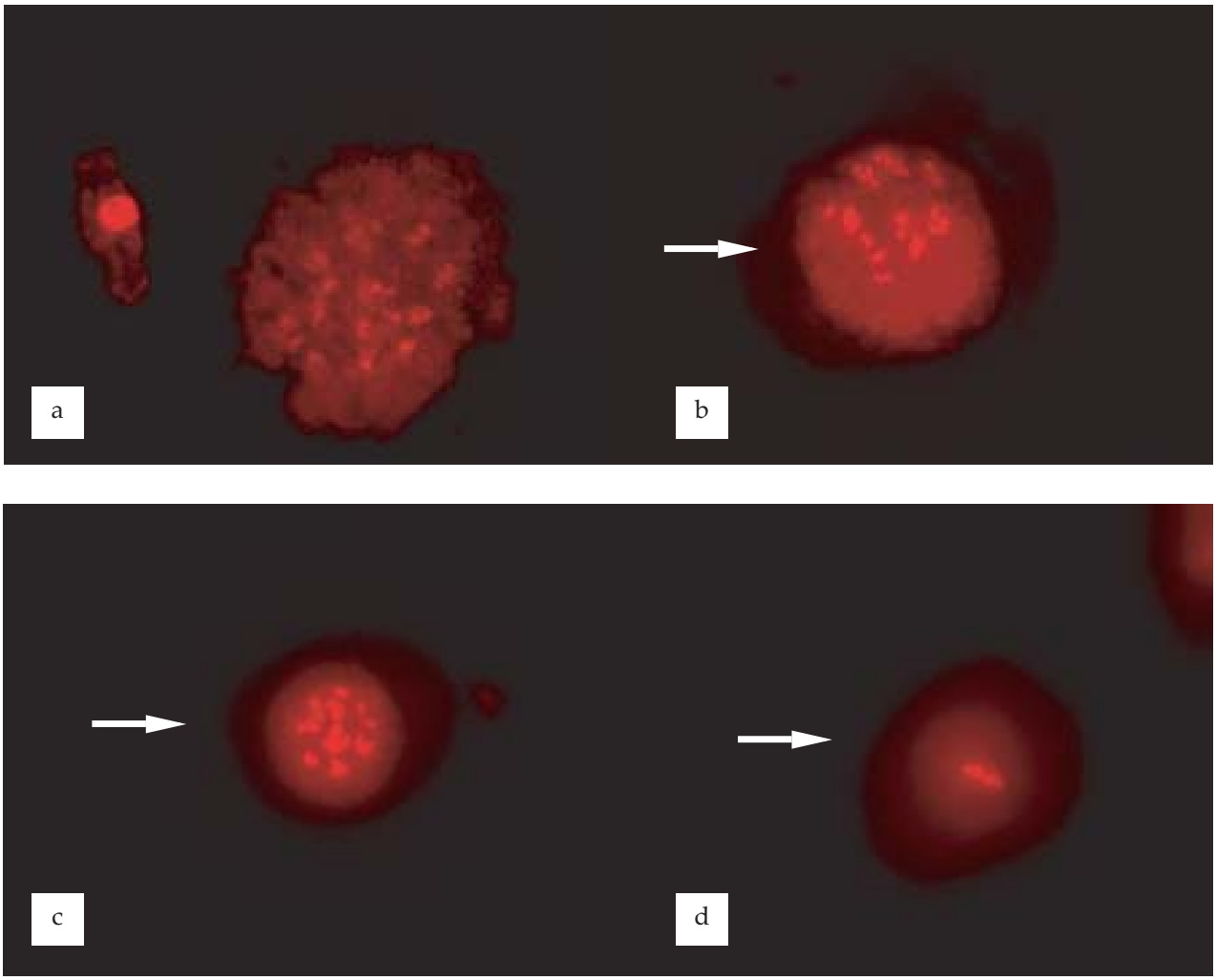

Figure 2. Uninucleate state of PMCs. (a) Bivalents condensing after pachytene and diplotene, (b and c) further condensation of chromosome homologues, and (d) pairing of chromosome homologues at the equatorial plate with centromeres attached to the kinetochore microtubules at metaphase I followed by separation at anaphase I.

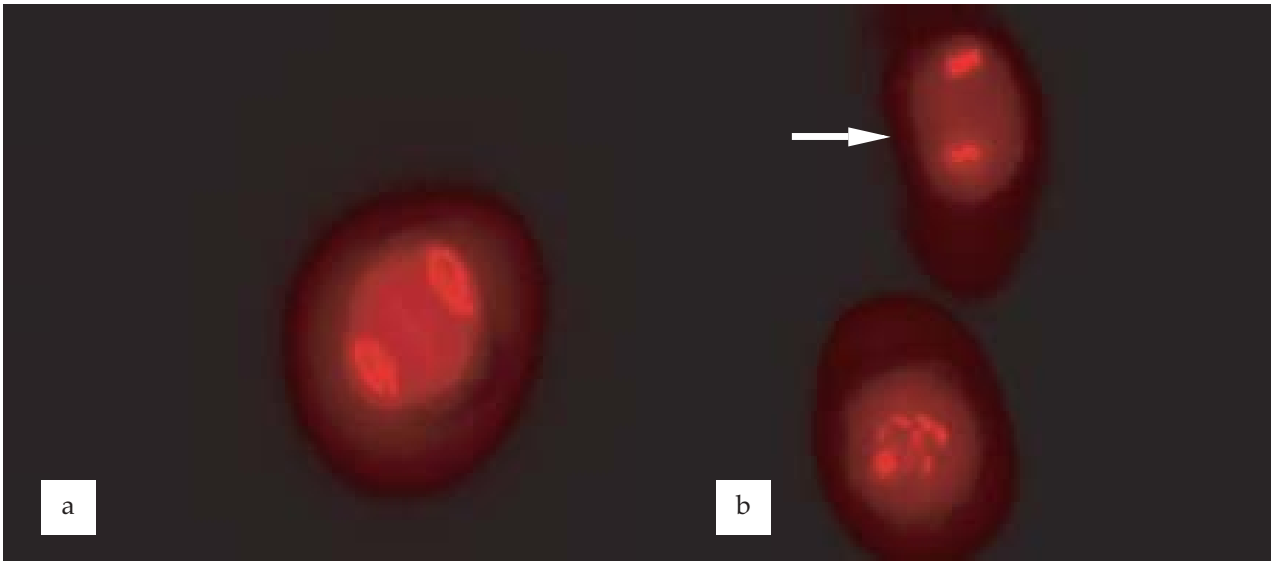

Figure 3. Dyad state of PMCs in a and b (arrow). (a) At anaphase I, the bivalents go through a reductional phase or separate to opposite poles to be univalents, (b) the univalents align at the equatorial plane (arrow) at metaphase II before undergoing anaphase II.

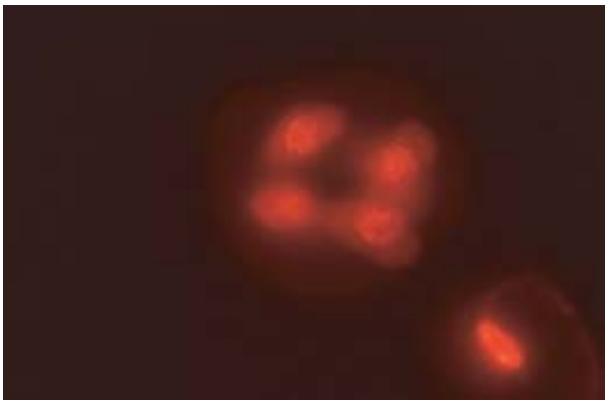

Figure 4. On completion of meiosis I and II, the uninucleate PMCs give rise to tetrad PMCs, or uninucleate microspores, upon release from the sac.

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