SHORT COMMUNICATION

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CORRELATION OF MICROSPORE NUCLEAR DEVELOPMENT WITH MALE INFLORESCENCE MORPHOLOGY IN *Elaeis oleifera, Elaeis guineensis* AND THE OxG HYBRID

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ABSTRACT

Microspore nuclear stages of the Elaeis *species and their OxG hybrid were examined and correlated with the respective male inflorescence morphology. Isolated pollen mother cells (PMC) and microspores obtained from squashed male flower buds were stained with blue fluorescent 4',6-diamidino-2-phenylindole (DAPI) dye and observed with the Zeiss Axioplan epifluorescent microscope to determine their nuclear stages. The male inflorescence from both the* Elaeis *species and the (OxG) hybrid with a perfectly closed spathe, having whitish inflorescences and friable individual spikelets indicated that PMC activities were on-going within the anthers of male flower buds. In* E. guineensis*, the whitish, pale yellow or light green spikelets with flexible texture obtained from a perfectly closed spathe appeared to be morphological markers for uninucleate microspores. For* E. oleifera *inflorescences, the colour varies from pale beige to umber brown with flexible spikelets, while the spathe varied from being perfectly closed to slightly open. The observed morphology indicated the presence of uninucleate microspores in* E. oleifera*. For the OxG hybrid morphological markers resembled that of* E. oleifera*. By staining a plethora of male flower buds from various oil palm male inflorescences, the meiosis and mitosis phases were also profiled.*

Keywords: microspore, nuclear development, male inflorescence.

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INTRODUCTION

Generating functional embryos via microspore culture for haploid production requires a suitable cytological stage of its explants. The cytological stage of interest is unique for each species. Small-grained cereals and self-pollinating shrubs such as wheat, *Triticum aestivum L.* (Hu and Kasha, 1997), *Brassica napus* (Chuong and Beversdorf, 1985), maize (Pescitelli *et al*., 1990), and *Nicotiana tabacum* (Sunderland and Wicks, 1971) have undergone successful anther or microspore culture to produce haploid plantlets (Snape, 1989).

Cytological analysis is a crucial step for generating functional embryos from microspore through *in vitro* culture techniques. Each variety within a species exhibits unique nuclear activity for a successful switch from the gametophytic pathway to the sporophytic pathway. However, the most common requirement for successful

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haploid production via microspore culture is to have the samples at the late uninucleate stage where the chromosomes are active in a single nucleus (Cordewener *et al.,* 1998). Therefore, a process to identify or predict the microspore nuclear activity in oil palm male inflorescence will be very useful in efforts at generating haploids through microspore culture.

The genus *Elaeis* (Arecaceae) has two species, the economically valuable *E. guineensis* and a South American species with unique traits, *E. oleifera*. Hybridisation has resulted with an OxG hybrid. Harvesting the oil palm male flower inflorescence for microspore culture samples is a very tedious task. It is a labour intensive and tedious process to cut down the exterior fronds in order to obtain the inflorescence of interest. The oil palm male flower buds have to be stained to confirm the cytological stages, and more often than not, the stages of interest are not obtained.

While exploring microspore cytological stages of both *Elaeis* species and their OxG hybrid, the correlation between microspore development and male inflorescence morphology was observed. This knowledge is useful and will make it easier for researchers to select the inflorescence that meets their requirement for sampling. Therefore, this article describes the microspore nuclear activities at the various morphological stages of the male inflorescence. This information would be useful as it would help to avoid unnecessary inflorescence and frond pruning.

MATERIALS AND METHODS

Plant Material

A total of 59 oil palm male inflorescences were sampled from *E. guineensis*, *E. oleifera* and their hybrids. The samples were harvested every two weeks where possible, from the research stations at MPOB Kluang, MPOB Hulu Paka, MPOB Keratong and MPOB Universiti Kebangsaan Malaysia, Malaysia. The male inflorescences were enclosed within their spathe and kept in a cold room of 4°C to 6°C in complete darkness overnight, if necessary, before processing.

Cytological Screening Method of Male Flower Buds within Male Inflorescence

The morphology of the male inflorescence was observed and the different zones for cytological screenings are as indicated in *Figure 1*. Initially, closed male inflorescence was numbered vertically,

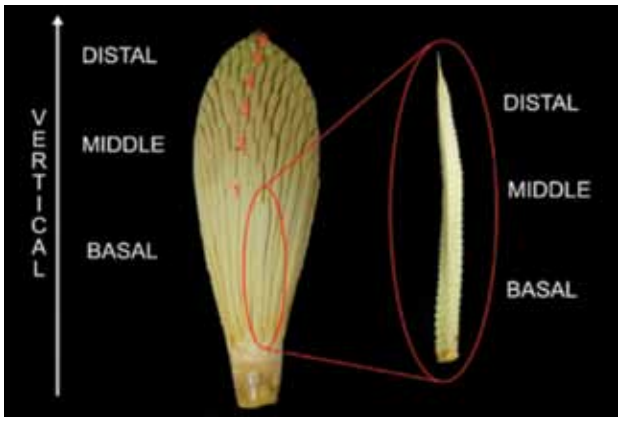

Figure 1. Oil palm male inflorescence and cytological screening of male flower buds obtained from the spikelets.

followed by the cytological screening of male flower buds obtained arbitrarily from the basal, middle and distal regions of each numbered spikelet. The buds were isolated using a pair of tweezers and placed on a precleaned microscope glass slide. The 11 μl of 1.25 μg m^{-1} DAPI (4',6-diamidino-2-phenylindole) was dropped on the bud and squashed. The remaining husk was removed using the same pair of tweezers to evenly spread the microspore sample before applying a cover slip. Microscopical observations were performed using the ultraviolet light of a Zeiss Axioplan epifluorescent microscope to analyse the cytological stages.

RESULTS AND DISCUSSIONS

Morphology of Male Inflorescence Bearing Anthers Containing Pollen Mother Cells (PMC)

Observation of male inflorescence bearing anthers containing pollen mother cells (PMC) was based on regular and consistent sampling activity, where the oil palm male inflorescence spathe aperture, colour of the spikelets and the individual spikelet texture also served as useful morphological markers. The three morphological criteria vary slightly yet distinctively between the two *Elaeis* species and their hybrid as shown in *Figures 2*, *3* and *4*.

The PMC were borne within the anthers of inflorescence enclosed with a perfectly closed spathe of whitish and greenish colour with the tip of spathe turning brown (*Figure 2a*). Their spirally arranged spikelets were whitish in colour and sometimes for *E. guineensis* they were pale yellow (*Figures 2b, 2c* and *2d*). These finger-like spikelets were friable for *E. guineensis* (*Figure 2e)* but for *E. oleifera* they are also tightly clumped (*Figure 2f*) which made it more laborious to isolate. At an early phase of microspore

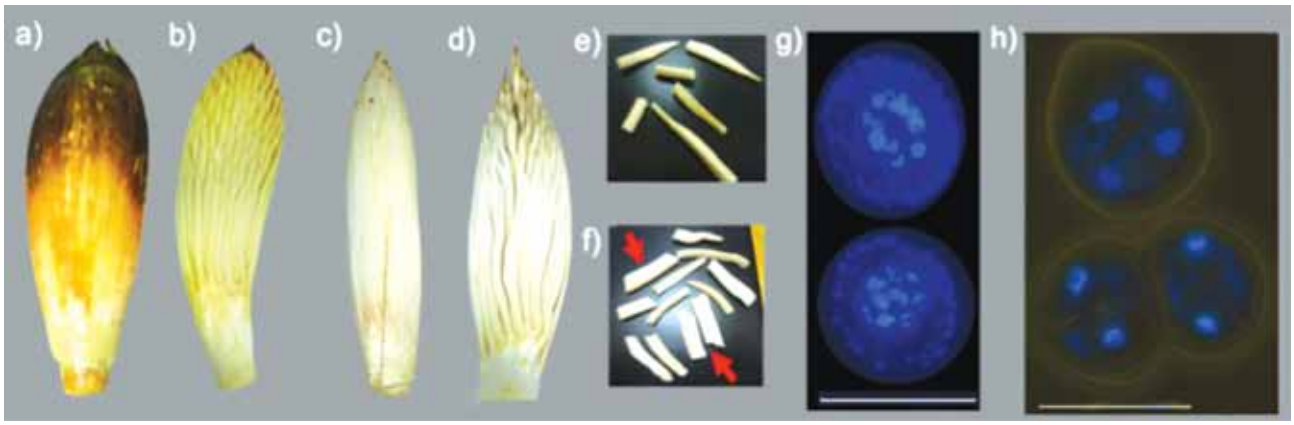

Figure 2. Oil palm male inflorescences morphology bearing pollen mother cell (PMC) (a) perfectly closed spathe; (b) E. guineensis*; (c)* E. oleifera*; (d) OxG hybrid; (e)* E. guineensis *friable spikelets and (f)* E. oleifera *friable and clumpy spikelets (arrows). PMC nuclear activity at (g) Meiosis I and (h) Meiosis II (bar=30µm).*

production, meiotic activities were held within PMC (*Figure 2g*) until the tetrad stage (*Figure 2h*) of meiosis II (Madon *et al*., 2005).

Morphology of Male Inflorescence Bearing Anthers Containing Uni- and Binucleate Microspores

Young immature pollen released from PMC are known as uninucleate microspore comprising of G_1 stage till anaphase of its first mitotic division. Uninucleate microspores have been used for microspore culture in many plant species. Male inflorescences enclosed within a brownish spathe with slight rupture at the tip (*Figure 3a*) contain the unicellular microspores. *E. guineensis* spikelets were whitish or pale yellow with slight greenish colour at the tip of the male inflorescence (*Figures 3b* and *3c*), *E. oleifera* clumpy spikelets were pale brown to umber brown (*Figures 3d* and *3e*) and OxG hybrid spikelets were light beige to beige (*Figures 3f* and *3g*). Oil palm uninucleate microspore spikelets were flexible with various colour tones according to their species (*Figures 3h* and *3i*). The unicellular microspores of *E. guineensis* and OxG hybrids are shown in *Figures 3j* and *3k* respectively.

Uninucleate microspores progress through mitosis and become binucleate microspores. Male inflorescence harbouring binucleate microspores were marked morphologically as having wide spathe aperture (*Figure 4a*), while for *E. guineensis*, slight aperture indicated binucleate microspore stage. For *E. guineensis,* the spikelets were green and dirty yellow (*Figure 4b*) while for *E. oleifera* and OxG hybrid their flexible spikelets were usually dark brown (*Figures 4c, 4d* and *4eii*). The binucleate microspores (*Figure 4f*), contain small dense nucleus and big diffuse nucleus, which are generative and vegetative nuclei respectively (Fan *et al*., 1988; Gervais *et al*., 2000).

Despite the criteria described above, the oil palm microspore nuclear activity is dynamic within each spikelet of an inflorescence. Although the general morphology of the inflorescence that contains the desired cytological stage is obvious, the different

Figure 3. Oil palm male inflorescences morphology bearing uninucleate microspores (a) slight spathe aperture; (b-c) E. guineensis*; (d-e)* E. oleifera*; (f-g) OxG hybrid; (h)* E. guineensis *flexible spikelet; (i)* E. oleifera *flexible spikelet; (j)* E. guineensis *uninucleate microspore and (k) OxG hybrid uninucleate microspore (bar = 30 µm).*

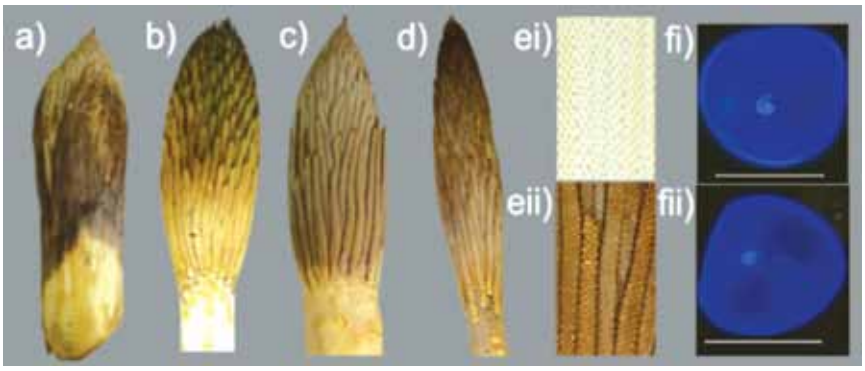

Figure 4. Oil palm male inflorescences morphology bearing binucleate microspores (a) wide spathe aperture; (b) E. guineensis*; (c)* E. oleifera*; (d) OxG hybrid; (e)* E. oleifera *spikelets, young and smooth (top) hence old and coarse (bottom) (f)* E. oleifera *binucleate microspore (bar = 30µm).*

parts of the inflorescence may still show variation. This is obvious in *Figure 3b* where the distal region of the male inflorescence was greenish indicating that microspores had entered the binucleate stage while at the whitish and pale yellow basal region, the microspores were mostly in the uninucleate stage and suitable for culture.

Meiotic Activities of PMC and Mitotic Activities of Oil Palm Microspore

The formation of pollens starts from the meiotic activities of the nucleus within the PMC. During Meiosis I, PMC nucleus divides into two (dyad) and in Meiosis II the nuclei will further divide to produce four nuclei (tetrad). *Figure 5A* shows the progressive stages of Meiosis I, while in *Figure 2h,* the upper PMC shows the tetrad stage. The PMC walls then degrade to release the immature pollens or microspores. At this stage, the uninucleate microspores would go through a post mitotic activity to give rise to two nuclei (binucleate stage) as shown in *Figure 5B,* while *Figure 6* shows a microspore with n=16 chromosomes at a prometaphase stage. The microspore would then continue to mature and finally become pollen.

CONCLUSION

The ability to correlate the morphological markers of the male inflorescences with the PMC or microspores nuclear activity will greatly assist in the sampling of suitable inflorescences. This will minimise unnecessary inflorescence pruning. In addition, the morphological markers of spathe aperture and spikelets colour and texture will make it easier for researchers to identify the stage of interest for microspore culture experiments.

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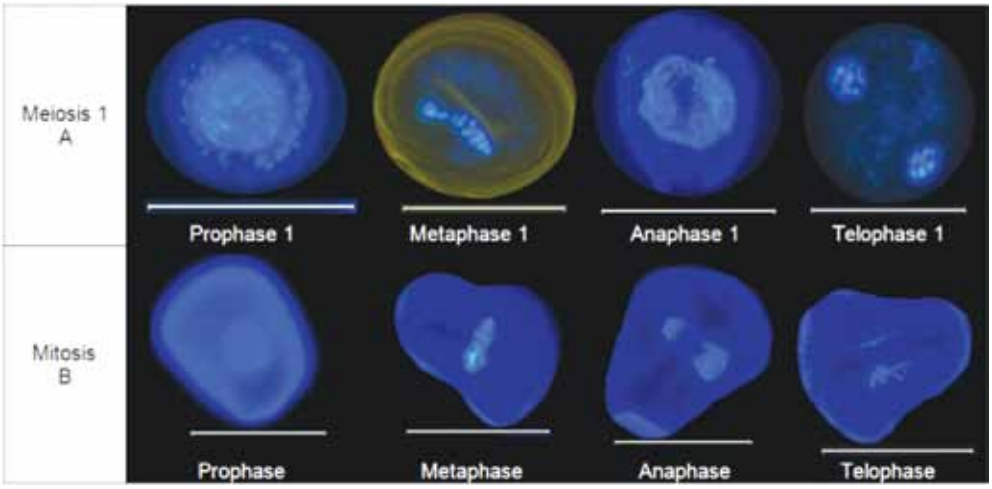

Figure 5. Nuclear activities of OxG hybrid (A) pollen mother cell (PMC) at Meiosis I and (B) post mitotic activities in microspores to produce vegetative and generative nuclei (bar = 30 µm).

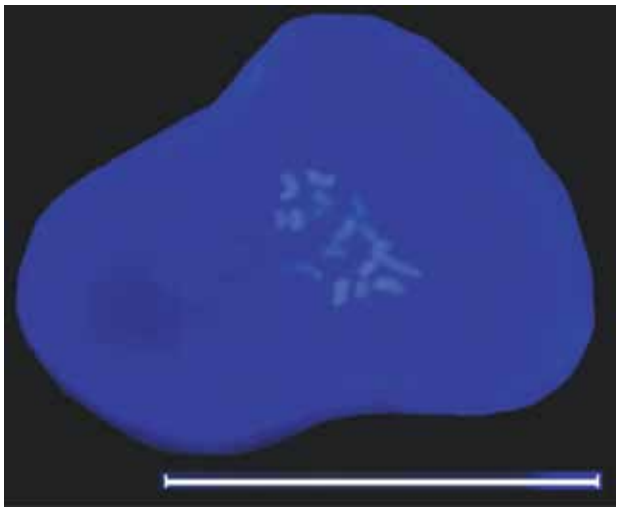

Figure 6. A uninucleate OxG hybrid microspore at prometaphase stage with n=16 chromosomes (bar=30 µm).

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