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A HISTOLOGICAL STUDY OF OIL PALM (*Elaeis guineensis*) ENDOSPERM DURING SEED DEVELOPMENT

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ABSTRACT

Information on histological features of oil palm is scarce, especially on seed development. This study aims to examine the cell structure and anatomy of developing oil palm seed. The seeds were analysed by histochemical technique and light microscopy. At early developmental stages, a vacuole fluid-filled endosperm was formed. As the seed developed further, the enlargement of the endosperm size was observed accompanied by cellularisation. Cells were formed from the periphery towards the centre of the endosperm. Accumulation of storage reserves within the cells started at week 10 after anthesis. Polysaccharides were stored in the form of thickened walls whilst lipid and protein were stored in the cytoplasm. At late developmental stages, the endosperm cavity was fully cellularised and storage reserves accumulated within the entire cell. A small cylindrical embryo was seen embedded within the massive endosperm tissue. The endosperm functions as a nutrient reservoir for the embryo. This histological study of developing oil palm seeds provides information on the nature and anatomical changes in endosperm tissues as well as shedding light on the growing points of seed development.

Keywords: morphology, embryo, zygotic embryogenesis, *tenera* palms, light microscopy.

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INTRODUCTION

The genus *Elaeis* which belongs to the palm family Arecaceae, is an important member of the monocot group under the order Arecales. Arecaceae, the sole family in the Arecales order, a monophyletic group comprising of 183 genera and over 2500 species is divided into five subfamilies: Calamoideae, Nypoideae, Coryphoideae, Ceroxyloideae and Arecoideae (Dransfield *et al.*, 2008). Oil palm belongs to the subfamily Arecoideae in the tribe Cocoseae and the subtribe Elaeidinae (Adam *et al.*, 2011). According to Dransfield *et al.* (2005), Arecoideae is the largest subfamily of Arecaceae and has been classified into 112 genera. Within the genus *Elaeis*, two species of oil palm are distinguished as economically important: *Elaeis guineensis* (African origin) and *Elaeis oleifera* (American origin).

The African oil palm (*Elaeis guineensis* Jacq.) is the commercial planting material in Malaysia and Indonesia. It is a single-stemmed palm with a single natural vegetative localised at the centre of the leaf crown known as meristem (Adam *et al.*, 2011). Oil

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palm is a pleonanthic species, whereby a single shoot in which the inflorescences appear in the axils of vegetative leaves and continue to be produced as the palm continues its vegetative extension (Tomlinson, 1990; Adam *et al.*, 2011). As a monoecious palm, male and female inflorescences are produced successively on the same plant but at different time points.

A fertilised female inflorescence may develop and give rise to a fruit bunch at about 22 – 26 weeks later (Ngando-Ebongue *et al.*, 2012). Oil palm fruit is a drupe, varying in shape from nearly spherical to ovoid or elongated and bulging at the top (Corley and Tinker, 2003). The pericarp of each fruit comprises three layers, namely outer exocarp or skin, mesocarp or pulp and endocarp or shell. In general, exocarp is included with mesocarp while endocarp together with kernel forms the seed. The kernel consists of a grayish-white endosperm which is surrounded by a dark brown testa covered with a fibrous network (Latiff, 2000). A small embryo is embedded in the endosperm.

The seeds of oil palm are endospermous, ellipsoid and their lengths range between 1 and 1.6 cm at mature phase. Study on biochemical characterisation of oil palm seeds has shown that moisture content of mature seed is about 30%. Lipid is the major storage reserve which accounted for 55% of total dry weight followed by carbohydrate and protein; 18% and 17% respectively (Kok *et al.*, 2013).

Histological studies on most seeds have been neglected especially for mature endosperm structure (Bhatnagar and Sawhney, 1981). In oil palm, detailed histochemical investigations on developing seeds are still lacking. This is probably due to the hard and thick-walled characteristics of mature endosperm tissues which are difficult to fix and embed (DeMason, 1986). Despite that, the aim of this study is to provide a histological description on oil palm seed development.

MATERIALS AND METHODS

Plant Material

Oil palm fruits (*Elaeis guineensis* Jacq.) were collected from the Malaysian Palm Oil Board-Universiti Kebangsaan Malaysia (MPOB-UKM) Research Station located in Bangi, Selangor, Malaysia. All palms were of *tenera* variety, products from a cross of *dura* (thick shell palm) and *pisifera* (shell-less palm). The palms underwent an open pollinated regime. To study oil palm seed development, inflorescences were tagged randomly based on their appropriate physiological state at anthesis (fully opened florets with the moist surface of their stigma exposed). Developing fruits were harvested at defined stages: two weeks after anthesis (WAA), five WAA, eight WAA, 10 WAA, 13 WAA and 16 WAA.

Histochemistry and Light Microscopy

Kernels were removed from the fruit and dissected longitudinally or transversely. Dissected kernels were fixed in fixation buffer 25% (v/v) glutaraldehyde and 10% (v/v) paraformaldehyde in 0.2 M phosphate buffer, pH 7.2 containing 1% (w/v) caffeine. Specimens were then dehydrated through a graded ethanol series: 30% (v/v) for 30 min, 50% (v/v) for 45 min, 70% (v/v) for 45 min, 80% (v/v) for 60 min, 90% (v/v) for 60 min, 95% (v/v) for 60 min, and twice in absolute ethanol for 60 min each. After that, specimens were bathed in butanol solution three times for a minimum of 24 hr for each bath and embedded in Technovit resin (Technovit 7100 Embedding Kit, Heraeus Kulzer, Germany) according to the method of Buffard-Morel *et al.* (1992). Blocks were sectioned at 5 μm thickness using a microtome (Leica RM2165, USA). Sections were stained with Periodic Acid Schiff (PAS) for carbohydrates and counterstained with naphthol blue-black for proteins (Feder and O'Brien, 1968). Observations were made with a Nikon Eclipse TS100 microscope and images were captured by a colour PAXcam ARC camera. The overlapping images were automatically stitched together using the PAX-it software (MIS Inc., USA) into a mosaic image.

RESULTS AND DISCUSSION

Based on literature search and to our best knowledge, no structural studies on developing endosperm tissues of *Elaeis guineensis* have been carried out previously. The structural features of a seed have been reported only in a few palm species (Meier, 1958; DeMason and Thomson, 1981; Meier and Reid, 1982; Alang *et al.*, 1988; Panza *et al.*, 2004).

Oil palm fruits are drupaceous and usually one-seeded (*Figures 1* and 2). At the beginning, oil palm endosperm was in liquid form (*Figure 1A-I*). The endosperm was initially observed at two WAA and its pericarp was in fawn-white colour (*Figure 1A*). Early stage of endosperm was in liquid form and housed in a cavity that is small in size, with a diameter of 2 to 3 mm (*Figure 1D*). The early endosperm formation was observed as fluid-filled cavity and enclosed by cell boundaries that stained with naphthol blue black, which is an undifferentiated seed coat. Meanwhile, brown deposits were observed in the pre-endocarp (*Figure 1G*). The early endosperm in liquid form suggests that initial cell division in this tissue was not followed by cell wall formation.

Pericarps of five WAA oil palm fruit changed colour to deep violet at apex and pale greenish at the

base (*Figure 1B*). Endosperm at this stage was still in liquid form and the cavity that held the endosperm was larger (*Figure 1E*). Cell boundaries enclosed the endosperm became thinner and brown deposits were densely distributed along the well-developed endocarp (*Figure 1H*).

At eight WAA stage, endosperm increased in size and translucent jelly-like endosperm tissue was observed closely adhering to the wall of seed coat (*Figure 1C*). Histological analysis revealed that the endocarp was more solid and filled densely with brown deposits (*Figure 1F*). In addition, cellularisation was observed in the thin layer of semi-solid endosperm (*Figure 1I*). A similar phenomenon had been observed in coconut endosperm too (Abraham and Mathew, 1963). All cells develop from existing cells by cell division (Virchow, 1860; Fahn, 1982). Cell division is a process whereby one cell divides into two daughter cells. As endosperm develops further, new cells are formed from the peripheral region (underneath endocarp) and continue to grow towards the centre of cavity. Endosperm cellularisation in cereals is also formed from the peripheral cells extending towards the central cavity (Olsen, 2004). Newly formed cells within oil palm endosperm were vacuolated and cell walls were not well-established yet (*Figure 1I*). The vacuoles play an essential role in regulating flow of water and solutes in the cell, *i.e.* in osmoregulation and for storage (Fahn, 1982).

Iljin (1957) reviewed that plant cells which tolerate desiccation must survive the mechanical stresses associated with volume reduction. This may be achieved by reduction of the volume of fluid-filled vacuoles by shrinkage, breaking up of one or a few large vacuoles into many considerably smaller ones, or replacing it with insoluble reserve material (Panza *et al.*, 2004). Our study showed that oil palm endosperms also go through these developmental phenomena where a large vacuole of liquid endosperm divides into many cells, and each cell is filled with storage reserves as endosperm develops further. Hence, oil palm endosperms attain the ability as a food storage reserve for germination and to tolerate desiccation.

A greyish-white endosperm was observed once cellularisation had occurred (*Figures 2A-O*). At 10 WAA, endosperm was in semi-gelatinous form (*Figure 2A*) and the endosperm cells were formed from peripheral towards centre of endosperm cavity and the innermost endosperm cells were stained intensely with the PAS stain (*Figure 2D*). The cells within the endosperm differed in shape and contents depending on their position. Those peripheral cells

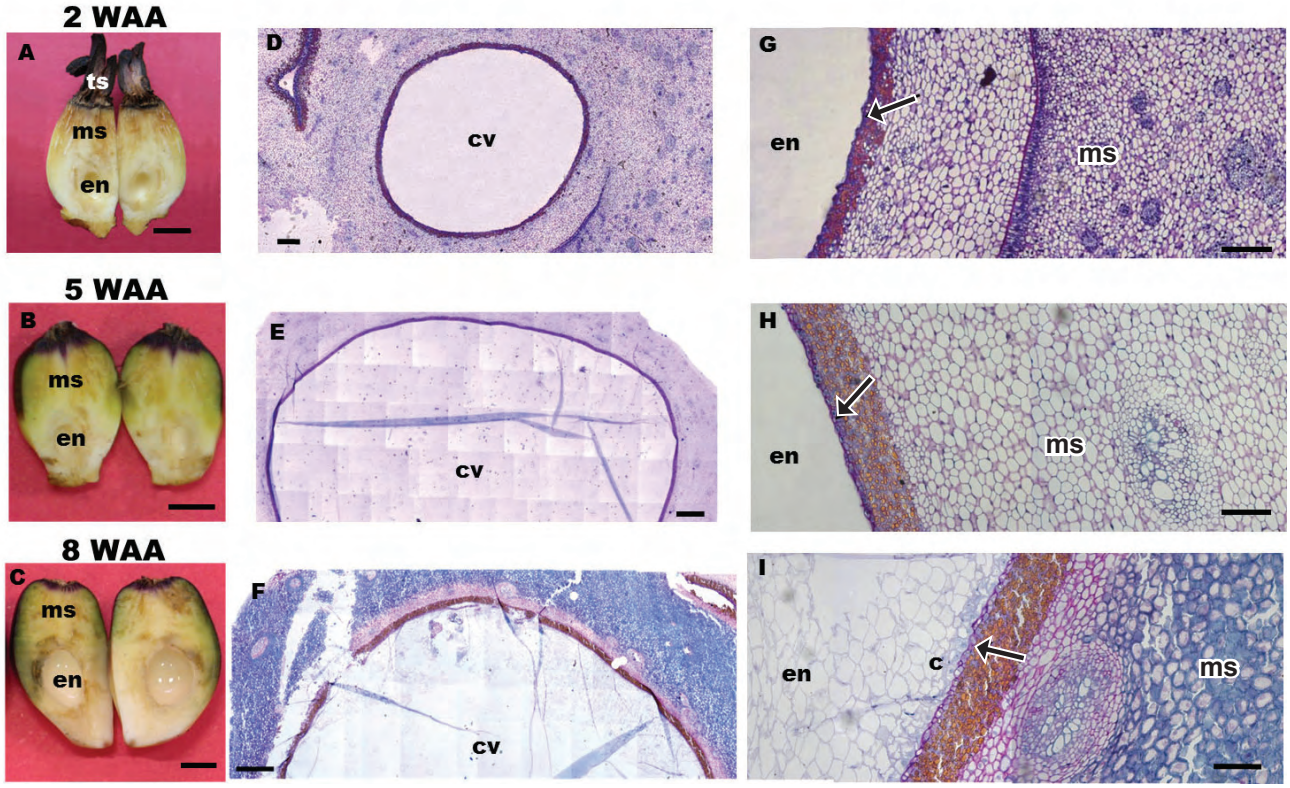

Figure 1. Macroscopic and microscopic views of developing oil palm fruits. A-C, photographs of median longitudinal section of oil palm fruits at stage of two weeks after anthesis (WAA) (A), five WAA (B) and eight WAA (C). Scale bar = 5 mm. D-I, Cross-sections of early seed development in oil palm visualised by light microscope. Sections stained with Periodic Acid Schiff (PAS) (carbohydrate stain pink) and napthol blue black (protein stain blue). D-F, Endosperm of two WAA (D), five WAA (E) and eight WAA (F) formed as fluid-fill cavity. Scale bar = 100 µm. G-I, Higher magnification of a portion of the developing seed; two WAA (G), five WAA (H) and eight WAA (I). Arrows indicate seed coat. Scale bar = 50 µm. C: cell; cv: cavity; en: endosperm; ms: mesocarp; ts: trifid stigma.

beneath the seed coat were vacuolated and round in shape (*Figure 2G*). The peripheral thin-walled cells reacted positively with the PAS stain, indicating the presence of carbohydrates. Cytoplasm of peripheral cells did not react with any stains. In the middle of endosperm, the interior cells were rectangular in shape and cell walls were stained intensely with the PAS stain (*Figure 2J*). The cells were thick-walled except for the area of two adjacent cells. Cytoplasm of interior endosperm cells stored protein which stained with naphthol blue black. New cells found near the centre of cavity were not deep-seated thus these cells were ruptured during dissecting (*Figure 2M*). The endosperm cell walls at week 10 were observed to have irregular thickenings and narrow constrictions. According to Rudall (2007), pits often remain as thin areas of the wall even after secondary wall has been formed. Thus, thin areas of two adjacent cells found in oil palm endosperm might be pits. This observation is in accordance to Vaughan (1970) findings, who reported that palm kernel is marked with pitted nature. The presence of pits is important to connect two adjacent cells for cell-cell communication (Fahn, 1982).

At 13 and 16 WAA stages, the pericarps were in black reddish colour at the top while yellow at the base (*Figures 2B* and *2C*). In both stages, the endosperm was in solid form with an embedded embryo at apex region with approximately 3 mm in length (*Figures 2E* and *2F*). The endosperm tissue in both stages were surrounded by a dark and thin seed coat (*Figures 2H* and *2I*), where cells were small quadrangular in shape with dense tannoid contents (Hussey, 1958). Overall, endosperm cells of both stages reacted positively to PAS and napthol blue black. The endosperm cell walls were mostly stained with the PAS stain implying the cell walls are the site of polysaccharide storage. This could possibly cause the tissue to become more rigid. Hence, an intact section of the whole kernel was hard to obtain.

At 13 WAA, endosperm cells at peripheral area were longer and narrower than those cells in the centre (*Figure 2H*). The peripheral cells stored few proteins and were thin-walled. While, the interior cells had thicker wall except in areas of potentially primary pit fields and contained more storage proteins (*Figure 2K*). At 16 WAA, both outermost and inner endosperm cells were thick-walled and

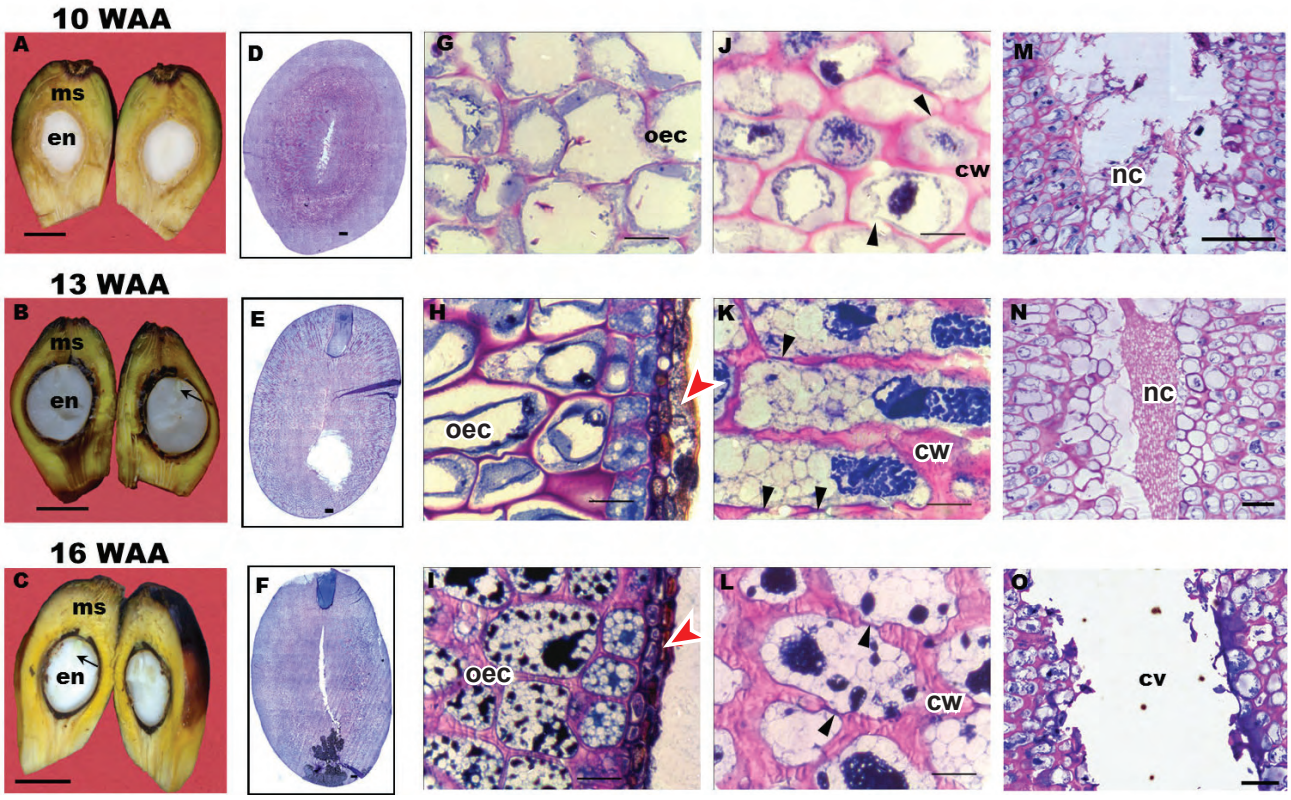

Figure 2. Macroscopic and microscopic views of developing oil palm fruits. A-C, photographs of median longitudinal section of oil palm fruits at stage of 10 weeks after anthesis (WAA) (A), 13 WAA (B) and 16 WAA (C). Arrow indicates embryo. Scale bar = 1 cm. D-O, Longitudinal sections of developing oil palm seed visualised by light microscope. Sections stained with Periodic Acid Schiff (PAS) (carbohydrate stain pink) and napthol blue black (protein stain blue). D-F, Overviews of semi-gelatinous, 10 WAA (D) and solid endosperms, 13 WAA (E) and 16 WAA (F). Incomplete section of whole endosperm tissue and presence of air bubbles due to its hard and thick-walled characters resulting in poor fixation and infiltration. Scale bar = 500 µm. G-O, Higher magnification of different portion of the developing endosperm. G-I, Outermost endosperm layers of 10 WAA (G), 13 WAA (H) and 16 WAA (I). Red arrows indicate seed coat. J-L, Inner endosperm layers of 10 WAA (J), 13 WAA (K) and 16 WAA (L). Arrowheads indicate primary pit fields. Scale bar = 5 µm. M-O, Central endosperm layers of 10 WAA (M), 13 WAA (N) and 16 WAA (O). Scale bar = 100 µm. Cv: cavity; cw: cell wall; en: endosperm; ms: mesocarp; nc: new cells; oec; outermost endosperm cells.

cytoplasm was rich in protein and lipid (*Figures 2I* and *2L*). Primary pit fields were observed in the inner endosperm cells of 16 WAA. Formation of new cells was observed in the centre of 13 WAA endosperm tissue (*Figure 2N*). The new cells were thin-walled and vacuolated. These young cells in the growing region were all relatively small in size. Meanwhile, a fissure found in the centre of 16 WAA endosperm and the cells were rich in storage reserves (*Figure 2O*). Yampolsky (1922) reported that oil palm endosperm has two cavities; one in which the outline form of the embedded embryo and the other in the centre of kernel in longitudinal section which is seen as a fissure running up and down. These two cavities actually are connected by a tiny canal which facilitates substances passage from the embryo to the endosperm (Yampolsky, 1922).

A membrane intensely stained with the PAS stain demarcated the embryo from the endosperm tissue (*Figure 3*). Both endosperm and embryo cells at 13 WAA were vacuolated and with thin walls which stained weakly with PAS (*Figure 3A*). Meanwhile, the 16 WAA endosperm cells adjacent to the embryo stained intensively with PAS and naphthol blue black (*Figure 3B*). The 16 WAA embryo cell walls stained lightly with PAS meanwhile the cytoplasm stained intensely with naphthol blue black (*Figure 3B*). Cytoplasm and cell wall of both endosperm and embryo tissues of 16 WAA were rich in storage reserves.

At 13 and 16 WAA stages, endosperm development comprises of the filling of reserves into the endosperm cells. In oil palm, endosperm consists of living cells storing carbohydrate in the form of thickened cell walls, and lipid and protein in the cytoplasm. At 16 WAA stage, embryo cells are matured and in cylindrical form: these cells consist of thin cell wall and protein present in cytoplasm. Alang *et al.* (1988) reported that oil palm endosperm stored polysaccharide *e.g.* galactomannan in the cell walls but absent in embryo. Hence, the endosperm and embryo are heterogeneous tissues. Endosperm cell walls that constitute polysaccharides are the main characteristics of members of the palm family (Meier and Reid, 1982; Boesewinkel and Bouman, 1984; DeMason, 1986; Panza *et al.*, 2004). It is obvious that the major function of the endosperm in *Elaeis* is as an organ for accumulation of storage reserves. The endosperm functions as an embryonic annex that supports embryo development and germination (Olsen, 2001).

CONCLUSION

The histochemical characteristics show the physical change, orientation of cell growth and reserve materials deposition in the developing oil palm endosperm. These observed characteristics will be useful in taxonomy studies of this genus. This study also provides the fundamental cellular knowledge on endosperm organisation in the maturing seed of oil palm which will be useful for future molecular, biochemical and physiological analyses.

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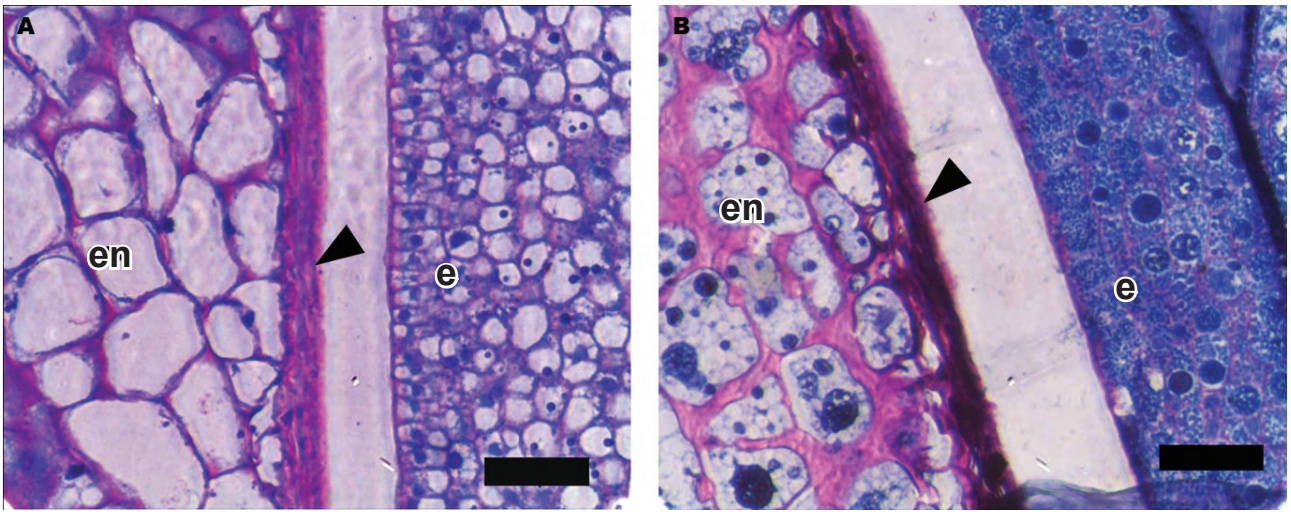

Figure 3. Longitudinal sections of developing oil palm endosperm cells next to embryo at 13 weeks after anthesis (WAA) (A) and 16 WAA (B). Sections stained with Periodic Acid Schiff (PAS) (carbohydrates stain purplish pink) and naphthol blue black (proteins stain blue). Endosperm and embryo cells at week 13 are vacuolated and have thin cell walls which stained lightly with PAS. Endosperm and embryo cells at week 16 contain protein and lipid bodies; thick cell wall which stained intensively with PAS. Arrowheads indicate PAS-positive membrance which demarcated embryo from endosperm. Scale bar = 10 µm. E: embryo; en: endosperm.

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