Malaysian Journal of Science **30** (2): 119-126(2011)

ANTHER AND OVULE DEVELOPMENT OF *Johannesteijsmannia lanceolata* J. DRANSF. (ARECACEAE)

Y.M. Chan[1] and A.L. Lim[2]

[1]Forest Biodiversity Division, Forest Research Institute Malaysia, 52109 Kepong, Selangor, Malaysia, [2]Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia.
*yokemui@frim.gov.my (Corresponding author)
Received on 22nd July 2011, accepted in revised form 23rd July 2011.

ABSTRACT The anther is tetrasporangiate with four wall layers. For the first time in Arecaceae, both Monocotyledonous and Dicotyledonous type of anther wall development are reported for a species. Cytokinesis in microsporocytes is simultaneous, forming tetrahedral (rarely isobilateral) tetrads. The pollen grains are elliptic, monosulcate with a smooth exine and are shed at the two-celled stage. The ovule is anatropous, bitegmic, crassinucellate and the micropyle is formed by both the integuments. Megasporogenesis produces linear or T-shaped (rarely) tetrads. The embryo sac development is of the monosporic, 8-nucleate *Polygonum* type. The antipodals are ephemeral and degenerate soon after formation. Twin archesporia and double megaspore tetrads within an ovule were observed in some sections but multiple mature embryo sacs have not been detected.

ABSTRAK Anter jenis tetrasporangiat dan dinding anter terdiri daripada empat lapisan sel. Untuk pertama kali, kedua-dua jenis perkembangan dinding anter, ia itu jenis Monokotiledon dan Dikotiledon dilaporkan dalam satu spesies.Sitokinesis dalam mikrosporosit adalah serentak membentuk tetrad tetrahedral dan jarang sekali isobilateral. Debunganya eliptik, monosulkat dengan eksin licin disebarkan pada peringkat dua sel. Ovul jenis anatropous, bitegmik, krasinuselat dan mikropil dibentuk daripada kedua-dua integument. Megasporogenesis menghasilkan tetrad linear atau tetrad berbentuk T (jarang). Perkembangan pundi embrio jenis 8-nukleus monosporik *Polygonum*. Antipodal merosot seketika selepas dibentuk. Arkesporia dan tetrad megaspora kembar dalam satu ovul diperhatikan dalam beberapa keratan tetapi pundi embrio matang multiple tidak dapat dikesan.

(**Keywords**: anther, pollen, embryo sac, ovule, *Johannesteijsmannia*)

INTRODUCTION

The genus *Johannesteijsmannia*, comprising of only four species (i.e. *J. altifrons, J. magnifica, J. perakensis* and *J. lanceolata*) are slow growing solitary palms with undivided blades belonging to the family Arecaceae, subfamily Coryphoideae. They are acaulescent (with very short, subterranean stem) except for *J. perakensis* which has a trunk and have broad diamond-shaped leaves (except for *J. lanceolata* which are lanceolate), which make them a very attractive and popular ornamental plant. *Johannesteijsmannia lanceolata* is endemic to Peninsular Malaysia and rare, constantly being threatened by deforestation and ornamental trade [1, 2]. The reproductive biology, floral organogenesis and fruit development of *Johannesteijsmannia* have been described [3, 4] but no account of its embryology has been presented.

Arecaceae is a large family with 183 genera and over 2,000 species [5], but very little embryological work has been done compared to the other angiosperm families. Embryological data which is important in breeding and cultivation practices, and could be a useful tool in plant taxonomy, is very much lacking in Arecaceae. Recent studies show that microsporogenesis is important in evolutionary and systematic studies of palms [6, 7]. In the subfamily Coryphoideae, there are eight tribes with 46 genera [5], and only ten genera have been described for the anther and ovule development i.e. *Borassus, Caryota, Hyphaene, Licuala, Livistona, Phoenix, Pritchardia, Sabal, Trachycarpus* and

Washingtonia [8, 9, 10, 11 and 12]. Here, we investigated the anther and ovule development of *J. lanceolata*, another member of the Coryphoideae.

MATERIALS AND METHODS

Buds and flowers at different stages of development were collected from seven plants cultivated in the Forest Research Institute Malaysia in Selangor, Malaysia. The specimens were fixed in Craf III solution and dehydrated through an ascending tertiary-butyl-alcohol (TBA) series. Serial sections using the paraffin technique [13] were prepared, with modification to the infiltration schedule, i.e. after dehydration, the specimens were suctioned at 25–30 atmospheric pressure at 60 °C for 10 minutes to improve wax infiltration into the specimens. The specimens were sectioned at 6–8 μm with a Reichert rotary microtome and stained in Safranin-O/Fast Green-FCF.

Fresh pollen grains were acetolysed according to Erdtman [14], critically point dried, coated with gold and viewed under a Jeol JSM-6400 scanning electron microscope. Some of the acetolysed pollen grains were stained in Safranin and observed under a light microscope. Additional fresh pollen grains were fixed in 50% alcohol, and stained in 1% Safranin. The acetolysed and untreated pollen grains (N = 54 and 79 respectively) were measured using the Leica QWin Image Processing and Analysis System Version 3.

RESULTS

About 2–3 weeks before anthesis, the young flower buds of c. 1 mm in diameter were undergoing meiosis and tetrad formation. Both the anther and ovule underwent the micro- and mega-sporogenesis simultaneously. When the anther was at the tetrad stage, the ovule was also at the same stage or at the two-nucleate embryo sac stage. One week before anthesis, the ovule was undergoing megagametogenesis, while the microspore tetrads had separated into individual microspores. The tapetum was degenerating and the endothecium had fibrous thickening.

Anther development

Anther wall development. The early stages of the anther wall development could not be fully examined because it was difficult to obtain young inflorescences without damaging the plant, as they were fully sheathed and protected by the leaf sheaths. Nevertheless, in a limited series of sections of several young buds, we observed the primary sporogenous and primary parietal cells in young anthers. The sporogenous cells divided mitotically to form microsporocytes while the primary parietal cells divided and differentiated into an endothecium, one middle layer and a tapetum **(Fig. 1B)**. We observed both Dicotyledonous and Monocotyledonous types of anther wall formation, with the former commonly encountered **(Fig. 1A)**. The endothecium enlarged before meiosis of the microsporocytes and the middle layer was crushed when the anther locule enlarged during microsporogenesis.

The anther was tetrasporangiate, with four wall layers comprising of an epidermis, an endothecium, a middle layer, and a tapetum. The epidermis and the endothecium were persistent, while the middle layer and the tapetum were ephemeral. The secretory or glandular tapetum was inititally uninucleate and became binucleate just before meiosis of the microsporocytes.

Microsporogenesis. At prophase I, the nucleus of the microsporocyte became dense; thick callose was observed at metaphase I enveloping the cell **(Fig. 1C)** which later underwent anaphase and telophase I. Usually cytokinesis did not occur after the telophase stage although two cells were observed to have a cell plate. After meiosis II, microspore tetrads were formed from simultaneous cytokinesis **(Fig. 1D).** The tetrads were tetrahedral, occasionally isobilateral, and they later separated into individual grains **(Fig. 1E).** The middle layer was crushed just before meiosis while the tapetum began to degenerate soon after microsporogenesis, creating empty space in the anther. During microgametogenesis, the nucleus of the individual microspore divided to form the mature two-celled pollen grain.

Pollen morphology. The pollen grain was elliptic, monosulcate with a smooth, foveolate exine **(Fig. 1F)**. The aperture was as long as the polar length of the grain at equatorial view. The ratio (in percentage) of the polar length (P) to the equatorial diameter (E) was 126 and 108, for the acetolysed and untreated pollen grains respectively. The acetolysed grains were bigger than the untreated grains. The pollen grains were small in size (mean length 19.7–23.4 μm); subprolate for the acetolysed grains and prolate spheroidal for the untreated grains, based on Erdtman [15].

Ovule development

The ovule was straight when young and it curved to 90° (hemianatropous) at the megaspore mother cell stage, and further to about 135° at the megaspore tetrad stage and finally became completely inverted

(anatropous), towards the funiculus **(Fig. 2I)**. The micropyle was straight, formed by both the inner and outer integuments. The nucellus was two-layer thick at the megaspore mother cell stage **(Fig. 2A**). Hence, the ovule was anatropous, bitegmic and crassinucellate, with an obturator formed at the funiculus near the micropyle at the mature embryo sac stage.

Megasporogenesis. We saw both single and twin archesporia in the ovules, although the latter was rare **(Fig. 2B)**. The archesporia had large nuclei with dense cytoplasm. When two megaspore mother cells were observed in a single ovule, they divided asynchronously, e.g., one of the megaspore mother cells was at meiosis I, dividing into dyads while the other megaspore mother cell remained undivided; or when one had developed into a two-nucleate embryo sac, the other was undergoing meiosis II **(Fig. 2C).** Twin embryo sacs could be formed, but this was not seen throughout the study. The archesporium divided into a primary parietal cell and a primary sporogenous cell **(Fig. 2D).** The nucellus was one-layer thick and the integument initials were formed simultaneously. The primary parietal cell further divided into two wall layers, while the sporogenous cell functioned as the megaspore mother cell. The integumentary and nucellar cells divided periclinally and anticlinally to keep up with the expansion and development of the megaspore mother cell. The megaspore mother cell divided meiotically, forming a linear megaspore tetrad and T-shaped tetrads occasionally (observed in two out of approximately 12 megaspore tetrads) **(Fig. 2E, F).**

Megagametogenesis. The chalazal megaspore developed into an embryo sac, while the three megaspores at the micropylar end degenerated. The chalazal megaspore divided mitotically into a two-, four- and finally an eight-nucleate embryo sac. The three nuclei at the micropylar end differentiated into the egg apparatus (two synergids and an egg) while the three chalazal nuclei formed the antipodal cells **(Fig. 2G, H).** The remaining two nuclei fused into a secondary nucleus at the centre of the embryo sac. Thus, the embryo sac development conformed to the monosporic *Polygonum* type. The antipodals and synergids were ephemeral and they soon degenerated. As the embryo sac matured, the nucellus degenerated and was resorbed, enlarging the embryo sac **(Fig. 2I).** Tanniniferous materials were deposited into the chalazal cells, inner and outer integuments, and particularly one layer of thickened inner integument, strengthening the structure and protecting the embryo sac.

DISCUSSION

The type of anther wall formation is not known in Arecaceae [16, 17] except in *Chamaedorea elegans* [18] and *Jubaeopsis* [19], which is of the Basic type with two middle layers. In *Johannesteijsmannia lanceolata*, we observed both the Monocotyledonous and Dicotyledonous type of anther wall development, with the latter seemed to be common. It is very rare to find both types of anther wall formation in a single species, and this phenomenon has been reported in the Dicotyledonous family, Caryophyllaceae (*Stellaria media*) [16]. Other families known to have more than one type of anther wall formation among species are Thymelaeaceae, Sterculiaceae, Combretaceae and Euphorbiaceae [16]. From this study, we now know that Arecaceae is a family with several types of anther wall formation i.e., the Basic, Dicotyledonous and Monocotyledonous types. As the type of anther wall formation could be a useful taxonomic characteristic, more such developmental studies should be conducted in the family to assess its diversity.

In Coryphoideae, simultaneous cytokinesis during microsporogenesis is reported in *Hyphaene indica* [10], *Caryota urens* and *C. mitis*, *Borassus*, *Licuala*, *Sabal* and *Trachycarpus* [11, 12] and *Copernicia hospita* [7]. Both simultaneous and successive cytokinesis have been reported in *Serenoa repens*, *Caryota mitis* and *Hyphaene coriacea* [7] and this is also seen in the present study of *Johannesteijsmannia lanceolata*. *Phoenix sylvestris* is the only member reported to have the successive type [20].

Most palm species studied show simultaneous cytokinesis, mainly in Arecoideae i.e. *Jubaeopsis* [19], *Howea*, *Ptychosperma*, *Areca*, *Syagrus* and *Bactris* [12], *Allagoptera*, *Butia*, *Dypsis* and *Veitchia* [7]. *Hydriastele* and *Pinanga* (Arecoideae) have both the simultaneous and successive types [6]. *Nypa fruticans* (Nypoideae) [6], *Pinanga disticha* [16], and Calamoideae [21] are reported to be of the successive type which is usually associated with monocotyledonous plants [6, 16].

The secretory tapetum with binucleate cells observed in the present study is consistent in the Coryphoideae and the tapetum can be one- or two-layered [10, 11]. Tapetal cells in the family have been reported to be uni-, bi- or multi-nucleate [10, 11, 19]. Mature pollen grains are shed at the two-celled stage as in most investigated species of palms [17] but three-celled pollen grains have also

been reported in *Calamus gamblei* and *C. rotang* [21].

Acetolysis changes the size of pollen grains to a certain degree [22] and in this study, we observed that the acetolysed pollen grains were bigger than those not acetolysed. The acetolysis process seemed to have ruptured many of the grains, as they appeared flattened or crushed. Our results corroborate the earlier findings that *J. lanceolata* has smooth, elliptical (25 μm x 18 μm) monosulcate pollen [5], and the size measured in this study, i.e. 23 μm x 19 μm is very close to the size reported.

The family Arecaceae is highly eurypalynous showing a great diversity of palynomorphs. The Coryphoideae has elliptic or asymmetric, monosulcate pollen grains with elliptic apertures. Monosulcate perforate pollen grains are common in the presumed primitive Coryphoideae, and have been reported in *Livistona*, *Sabal* and *Licuala* [23]. Pollen grains with extended sulcate or rarely subcircular or trichotomosulcate apertures are present in *Licuala*, and trichotomosulcate in *Cryosophila* [24, 25] and occasionally in *Pritchardia affinis* [26] and the occurrence of pontoperculate pollen in *Chamaerops humilis* [24, 26]. In other family members, triradiate or trisulcate grains are found in Arecoideae (*Elaeis guineensis*, *Astrocaryum aculeatum*), while bisulcate or bicolpate grains are found in Calamoideae (*Calamus* and *Metroxylon sagu*) and Nypoideae (*Nypa fruticans*) [23], monoporate in the Lepidocaryeae (*Mauritia*), diporate or dicolpate in the Calameae, and annulocolpate in the Nypoideae [27].

The ovule is anatropous, bitegmic and crassinucellate in *J. lanceolata*. Evolutionary trends in the type of ovules from anatropous to hemiana-, or campylo- or ortho-tropous are evident in Coryphoideae with 46 genera, as all the four types of ovules are found in the subfamily [5]. *Johannesteijsmannia lanceolata* has anatropous ovule which is considered the most primitive type within the family. The type of ovule could be characteristic of some genera or groups of genera, whereby anatropy is diagnostic for Calamoideae, *Nypa* and *Phoenix*, while hemianatropy is distinct in Chamaedoreeae. Both heminanatropous and campylotropous ovules are found in Ceroxyloideae and Arecoideae. Hemianatropy, anatropy and orthotropy are reported in Cocoseae [5].

The monosporic *Polygonum* type of embryo sac development is common in palms, although the *Allium* and *Adoxa* types are also reported [17]. In the Coryphoideae, the *Polygonum* type is found in *Pritchardia*, *Licuala*, *Livistona*, *Trachycarpus*, *Washingtonia*, *Sabal*, *Caryota* [11] and *Johannesteijsmannia* (present study) while the *Allium* type is reported in *Hyphaene* and *Borassus* [17].

The antipodals may be free nuclei, multinucleate or forming cells, and are usually ephemeral (including *J. lanceolata*), but persistent in some Arecoideae, i.e. *Areca catechu*, *Howea*, *Nephrosperma* and *Verschaffeltia* [12, 16].

Twin archesporia, as seen in *Johannesteijsmannia lanceolata*, is of very rare occurrence in the family, as it has previously been reported only in *Elaeis* [17]. More embryological data is needed within the family to ascertain this phenomenon and its implications. Most palms form linear megapore tetrad [16], but four types of tetrads (linear, isobilateral, T- and inverted T-shaped) have been reported in *Elaeis guineensis* [28].

In *J. lanceolata* we have observed that linear tetrads are more common than T-shaped tetrads, but the reverse is true in *Jubaeopsis caffra* [29]. *Jubaeopsis caffra* is similar to *Johannesteijsmannia lanceolata* in having bitegmic, crassinucellate ovules with tanniniferous integuments, both linear and T-shaped tetrads, and monosporic *Polygonum* type of embryo sac development with ephemeral antipodals. However, *Johannesteijsmannia lanceolata* does not have a postament as reported in *Jubaeopsis caffra*, and the former has basal placentation while the latter has free-central placentation. Postament formation and embryo-sac haustorium are also observed in *Areca*, *Dypsis*, *Howea* and *Ptychosperma* [12], and may be diagnostic for the tribe Areceae.

The presence of tannin content in floral parts and ovules is very common in palms [12, 30]. *Johannesteijsmannia lanceolata* has abundant of tannins in floral parts probably to protect the buds from herbivory, as the buds are long exposed and unprotected by the peduncular bracts before anthesis. Similar to *J. lanceolata*, the outer integument and chalaza of Arecoideae also accumulate tannin at early stages [12]. In Coryphoideae, *Phoenix* and *Thrinax* have fibrous sheaths, tannin and raphides in floral structures [30], while *Licuala* has raphides, tannin, and sclereids in floral parts [31].

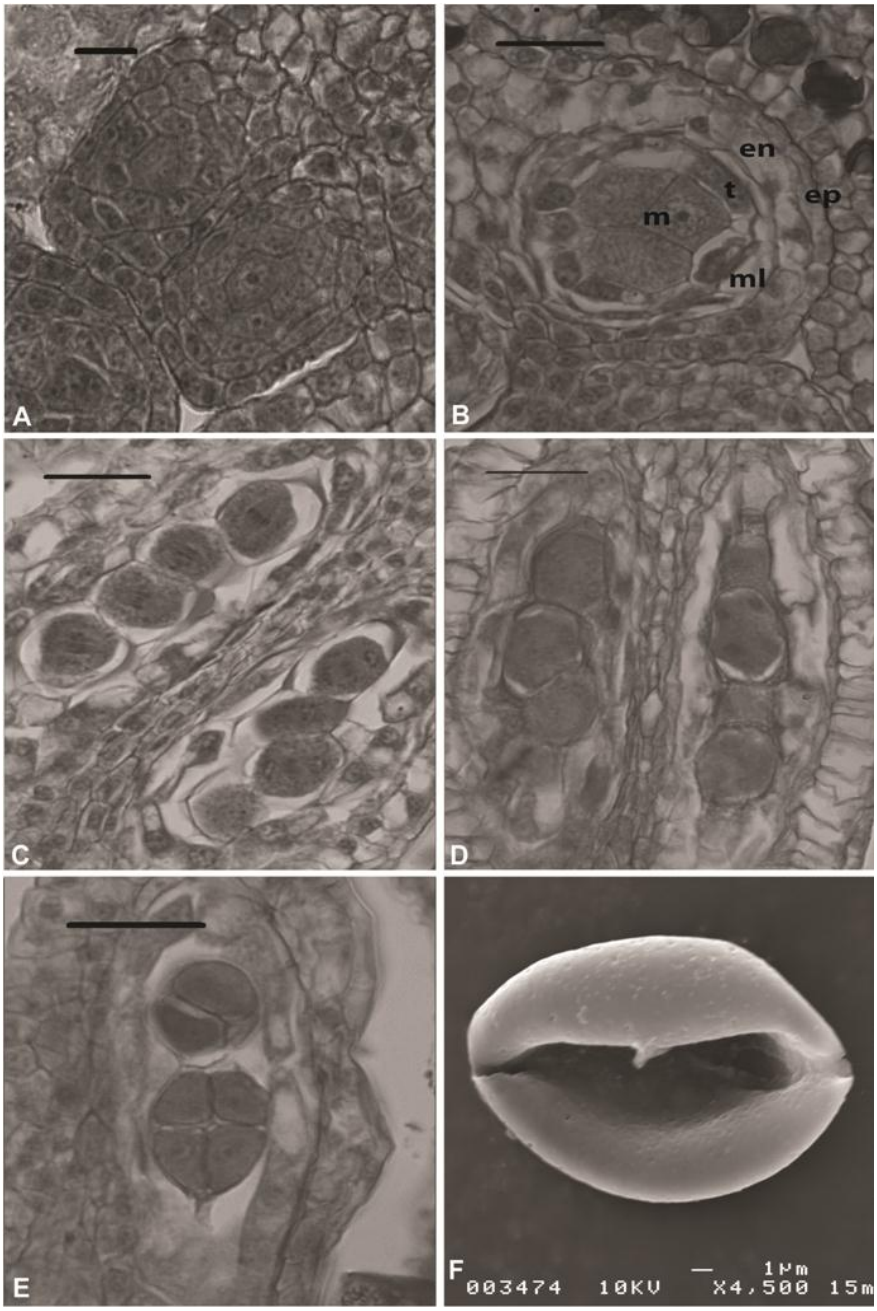

Figure 1. Anther development of *Johannesteijsmannia lanceolata*. (A) Dicotyledonous type of anther wall development (lower right lobe) with the outer parietal layer dividing into a middle layer and an endothecium. (B)–(E) Microsporogenesis. (B) Microsporocytes at late prophase. (C) Metaphase I (upper left) and Anaphase I (bottom right). (D) Tetrads formed by simultaneous cytokinesis. (E) Tetrahedral and isobilateral tetrads. All L.S. except (A) and (B) (T.S). Scale bar = 10 μm (A) and 20 μm (B)–(E), m = microsporocyte, t = tapetum, ml = middle layer, en = endothecium, ep = epidermis. (F) Acetolysed pollen grain at equatorial view.

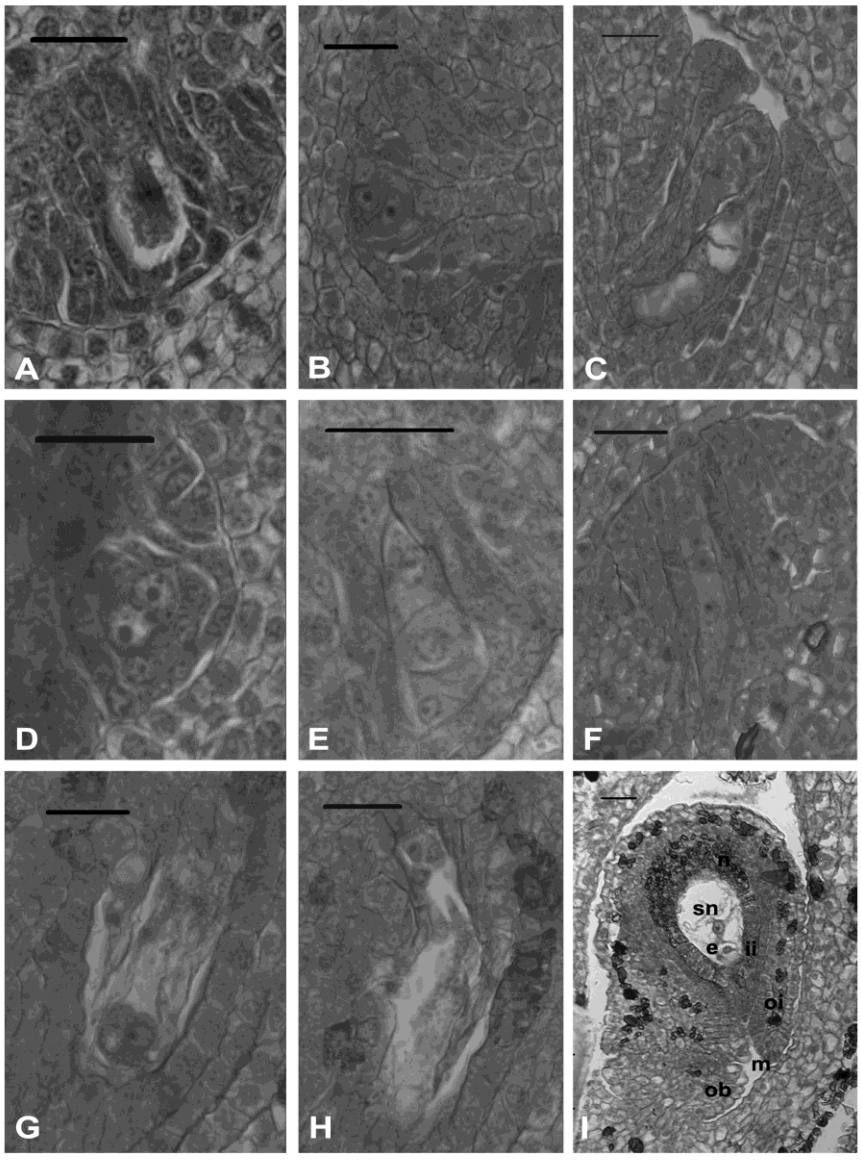

Figure 2. Ovule development of *Johannesteijsmannia lanceolata*. (A)–(I) Megasporogenesis. (A) Meiosis I. (B) Multiple archesporia. (C) Two megaspore mother cells dividing asynchronously during Meiosis I. (D) Archesporia dividing into primary parietal and primary sporogenous cells. (E) T-shaped tetrad. (F) Linear tetrad. (G)–(I) Eight-nucleate embryo sac with an egg and two synergids (G), three antipodals (H) and a secondary nucleus (I). (I) A mature ovule. All L.S. Scale bar = 20μm, n = nucellus, sn = secondary nucleus, e = egg cell, ii = inner integument, oi = outer integument, m = micropyle, ob = obturator

ACKNOWLEDGEMENTS

This work was funded by the University of Malaya's Postgraduate Research Fund [P0124/2006C, F0159/2005B], and was a part of MSc thesis undertaken by the first author. We thank Mdm. Patricia Loh for her laboratory assistance, Dr. Yong Kien Thai for the loan of microscope facilities and Ms. Aslina Baharum for touching up the figures.

REFERENCES

1. Chan Y.M. and Saw L.G. 2009. The uses of *Johannesteijsmannia* by indigenous communities and the current ornamental trade in the genus. *Palms* 53(3): 147–152.

2. Chan Y.M., Chua L.S.L. and Saw L.G. 2011. Way towards the conservation of Malaysian *Johannesteijsmannia* (Palmae). *Gard. Bull. Sing.* (in press).

3. Dransfield J. 1972. The genus *Johannesteijsmannia* H.E. Moore, Jr. *Gard. Bull. Sing*. 26: 63–83.

4. Chan Y.M., Lim A.L. and Saw L.G. 2011. Reproductive biology of the endangered and endemic *Johannesteijsmannia lanceolata* (Arecaceae). *Jour. Trop. F. Sci*. 23(2): 213–221.

5. Dransfield J., Uhl N.W., Asmussen C.B., Baker W.J., Harley M.M. and Lewis C.E. 2008. *Genera Palmarum. Evolution and Classification of the Palms*. Royal Botanic Gardens, Kew.

6. Furness C.A. and Rudall P.J. 1999. Microsporogenesis in Monocotyledons. *Ann. Bot*. 84: 475–499.

7. Sannier J., Nadot S., Forchioni A., Harley M. and Albert B. 2006. Variations in the microsporogenesis of monosulcate palm pollen. *Bot. J. Linn. Soc*. 151: 93–102.

8. Biradar N.V. 1968. Studies on palms: embryology of *Phoenix pusila* Gaertn., *P. acaulis* Buch. and *P. reclinata* Jacq. *Proc. Ind. Acad. Sci*. 67: 165–173.

9. Biradar N.V. and Mahabale T.S. 1968. Studies on palms: embryology of *Phoenix robusta* Hook. *Proc. Ind. Acad. Sci*. 67: 1–8.

10. Mahabale T.S. and Chennaveeraiah M.S. 1957. Studies on *Hyphaene indica* Becc. I. Morphology. *Phytomorphology* 7: 184–194.

11. Rao C.V. 1959. Contributions to the embryology of Palmae. I. Sabaleae. *Proc. Nat. Inst. Sci. India*. 29: 143–169.

12. Rao C.V. 1959. Contributions to the embryology of Palmae. II. Ceroxylinae. *J. Indian Bot. Soc*. 38: 46–75.

13. Johansen D.A. 1940. *Plant Microtechnique*. McGraw-Hill, New York.

14. Erdtman G. 1969. *Handbook of Palynology An Introduction to the Study of Pollen Grains and Spores*. Munksgaard, pp. 213–216.

15. Erdtman G. 1966. *Pollen Morphology and Plant Taxonomy – Angiosperms*. Hafner Publishing Company, New York and London pp. 15–18.

16. Davis G.L. 1966. *Systematic Embryology of the Angiosperms*. John Wiley & Sons, New York.

17. Johri B.M., Ambegaokar K.B. and Srivastava P.S. 1992. *Comparative Embryology of Angiosperms.* Vol. 2. Springer-Verlag, Berlin.

18. González-Cervantes E., Mena A.M., Quero H.J. and Márquez-Guzmán J. 1997. Embryology of *Chamaedorea elegans* (Arecaceae): microsporangium, microsporogenesis and microgametogenesis. *Principes* 41(3): 131–137.

19. Robertson B.L. 1976. Embryology of *Jubaeopsis caffra* Becc.: I. Microsporangium, microsporogenesis and microgametogenesis. *Journal of South African Botany.* 42: 97–108.

20. Mahabale T.S. and Biradar N.V. 1967. Studies on palms: embryology of *Phoenix sylvestris* Roxb. *Proc. Ind. Acad. Sci*. 67(2): 77–96.

21. Krishna Kumar H.N. and Ramaswamy S.N. 2003. Contributions to the study of microsporogenesis in *Calamus* L. (Arecaceae). *Taiwania* 48(3): 180–193.

22. Nair P.K.K. 1985. *Essentials of Palynology*. Today and Tomorrow's Printers and Publishers, New Delhi. pg. 129.

23. Mahabale T.S. 1967. Pollen grains in Palmae. *Rev. Palaeobot. Palynol*. 4: 299–304.

24. Ferguson I.K. and Harley M.M. 1993. The significance of new and recent work on pollen morphology in the Palmae. *Kew Bull*. 48(2): 205–243.

25. Harley M.M. 1990. Occurrence of simple, tectate, monosulcate or trichotomosulate pollen grains within the Palmae. *Rev. Palaeobot. Palynol*. 64: 137–147.

26. Sowunmi M.A. 1972. Pollen morphology of the Palmae and its bearing on taxanomy. *Rev. Palaeobot. Palynol*. 13: 1–80.

27. Sowunmi M.A. 1968. Pollen morphology in The Palmae, with special reference to trends in aperture development. *Rev. Palaeobot. Palynol*. 7: 45–53.

28. Kajale L.B. and Ranade S.G. 1952. Occurrence of four kinds of megaspore tetrads in *Elaeis guineensis* Jacq. *Curr. Sci*. 21: 170.

29. Robertson B.L. 1976. Embryology of *Jubaeopsis caffra* Becc.: 2. Megasporangium, megasporogenesis and megagametogenesis. *Journal of South African Botany* 42: 173–184.

30. Uhl N.W., and Moore H.E. Jr. 1977. Correlations of inflorescence, flower structure, and floral anatomy with pollination in some palms. *Biotropica* 9: 170–190.

31. Barfod A.S., Burholt T. and Borchsenius F. 2003. Contrasting pollination modes in threespecies of *Licuala* (Arecaceae: Coryphoideae). *Telopea* 10(1): 207–223.