Botanical Studies (2008) 49: 139-146.

Embryology of *Phalaenopsis amabilis* var. *formosa*: embryo development

Yung-I LEE[1, 4], Edward C. YEUNG[2], Nean LEE[3], and Mei-Chu CHUNG[1,*]

[1]*Institute of Plant and Microbial Biology, Academia Sinica, 11529, Taipei, Taiwan, ROC*
[2]*Department of Biological Sciences, University of Calgary Calgary, Alberta T2N 1N4, Canada*
[3]*Department of Horticulture, National Taiwan University, No 1, Sec. 4, Roosevelt Rd., 106, Taipei, Taiwan, ROC*

(Received November 30, 2006; Accepted October 17, 2007)

ABSTRACT. *Phalaenopsis amabilis* var. *formosa* is an endemic epiphytic orchid variety native to Taitung and Lanyu of Taiwan. A ⊥-shaped, four-celled embryo is produced by two successive cell divisions of a zygote. Soon after, two of the four cells toward the micropyle enlarge and divide two more times resulting in the formation of eight tubular suspensor cells. The suspensor cells are highly vacuolated; the bottom tier of suspensor cells elongates towards the micropyle, and the upper tier elongates towards the chalazal end of the seed. During the early stages of embryo development, lipid droplets appear in the elongating suspensor cells and disappear soon afterwards, indicating the suspensor functions in nutrient uptake and as a temporary food storage site for the developing embryo. In the mature seed, a differentiated apical zone containing the relatively small cells can be seen in the embryo proper. Protein and lipid bodies are the main storage products in the embryo proper cells. The results of Nile red staining indicate that a cuticular layer is present only on the surface walls of the embryo proper, but is absent from the suspensor cell wall Cuticular material is also present in the outermost layer of the seed coat and persists through seed maturation.

Keywords: Embryo; Orchid; *Phalaenopsis amabilis* var. *formosa*; Suspensor.

INTRODUCTION

The genus *Phalaenopsis* (Orchidaceae) comprises about 63 species that have produced numerous attractive hybrids and cultivars (Christenson, 2001). Of these, *Phalaenopsis amabilis* var. *formosa* is an endemic epiphytic orchid variety native to Taiwan's Taitung and Lanyu Island (lat. 22° N, long. 121°3' E) (Lin, 1988). During the past few decades, *P. amabilis* var. *formosa* has been used extensively in breeding for *Phalaenopsis* hybrids, and now it is one of the most important species for the floriculture industries in Taiwan.

Orchid seeds are tiny, and most contain a globular-shaped embryo and lack a well-defined endosperm (Arditti, 1992; Yam et al., 2002). Additionally, the orchid embryos have a diversified suspensor morphology. This led Swamy (1949) to propose a classification scheme for orchid embryo development based in part on the form and the pattern of suspensor development. According to Swamy (1949), the suspensor of *Phalaenopsis* contains eight filamentous cells which elongate toward the chalazal end of the seed, surrounding the embryo proper. Although the general pattern of embryo developmental is known, detailed structural information is not available.

The objectives of this study were to document the anatomical events in the embryo development of *P. amabilis* var. *formosa* from fertilization to seed maturity, and to detail the formation of the suspensor. The information presented here will provide valuable background information for both propagation and future molecular biology studies on the embryogenesis of *Phalaenopsis* species.

MATERIALS AND METHODS

Plant Materials

Plants of *Phalaenopsis amabilis* var. *formosa* were grown in greenhouses at National Taiwan University in Taipei, Taiwan. To ensure a good fruit set and seed quantity, flowers were hand pollinated. Developing fruits were harvested at regular intervals after pollination. Approximately 50 developing fruits were gathered for this study.

Light Microscopy

Transverse sections, approximately 2 mm thick of developing and mature fruits were fixed in 2.5% glutaraldehyde and 1.6% paraformaldehyde buffered with 0.05 M phosphate buffer, pH 6.8, for 24 h at 4°C. After fixation,

[4]Present address: Botany Department, National Museum of Natural Science, Taichung, Taiwan, ROC.
*Corresponding author: E-mail: bomchung@gate.sinica.edu.tw; Tel: 886-2-27892701; Fax: 886-2-27827954.

the sections were dehydrated in methyl cellosolve (BDH Chemicals) for 24 h, followed by two changes of 100% ethanol for 24 h each at 4°C. The samples were infiltrated gradually (3:1, 1:1, and 1:3, 100% ethanol: Historesin, 24 h each) with Historesin (Leica Canada, Markham, Ontario), followed by two changes of pure Historesin. The tissues were then embedded according to Yeung (1999). Longitudinal serial sections, 3 μm thick, were obtained using Ralph knives on a Reichert-Jung 2040 Autocut rotary microtome. Sections were stained with the periodic acid-Schiff's (PAS) reaction for total insoluble carbohydrates and counter-stained with either 0.05% (w/v) toluidine blue O (TBO) in benzoate buffer for general histology or 1% (w/v) amido black 10B in 7% acetic acid for protein (Yeung, 1984). The sections were viewed, and the images were captured digitally using a CCD camera (Cool Snap fx, Photometrics, Tucson, AZ) attached to a microscope (Axioplan, Carl Zeiss AG, Germany).

For detecting the storage lipid, some tissues were post-fixed in 1% OsO_4 in the same buffer for 4 h at room temperature and then rinsed in three 15-min changes of buffer. Following fixation, the materials were dehydrated in an acetone series and embedded in Spurr's resin (Electron Microscope Sciences, Washington, PA). Sections, 1 μm thick, were obtained by using a diamond knife on an Ultracut E ultramicrotome, and were stained with 0.1% (w/v) alkaline TBO in benzoate buffer for 1 min at 60°C on a hot plate. The grayish color indicated the presence of unsaturated lipid (Yeung, 1990).

The presence of a cuticle was detected by using Nile red as detailed in Yeung et al. (1996). The sections were stained with 1 μg ml^{-1} of Nile red (Sigma Chemical Co., St. Louis, Mo.) for 10 min, briefly washed in distilled water, and mounted in water containing 0.1% n-propyl gallate (Sigma Chemical Co., St. Louis, Mo.), an antifading compound. The fluorescence pattern was examined using an epifluorescence microscope (Imager A1, Carl Zeiss AG) equipped with the Zeiss filter set 15 (546/12 nm excitation filter and 590 emission barrier filter).

RESULTS

The main structural changes occurring within the ovary of *Phalaenopsis amabilis* var. *formosa* from 60 days after pollination (DAP) until seed maturity at 150 DAP are summarized in Table 1. At 60 DAP, most of the ovules had been fertilized and embryo development had just commenced. During the early stages of embryo development (60-90 DAP), the seeds are white and moist. As the seeds approach maturity (120 DAP), they are beginning to turn yellowish white and desiccate. At maturity (150 DAP), they are yellowish brown and become dry and detached from placentas.

After fertilization, the zygote had an ovoid shape (Figure 1). The nucleus which had a prominent nucleolus was located toward the chalazal end. Judging from the location of the mitotic apparatus, the first division of the zygote was unequal (Figure 2). This division produced a smaller terminal cell and a larger basal cell (Figure 3). The terminal cell eventually gave rise to the embryo proper while the basal cell formed the suspensor and also contributed cells to the embryo proper. The endosperm nuclei and the chalazal nuclei formed a complex and appeared as a dis-

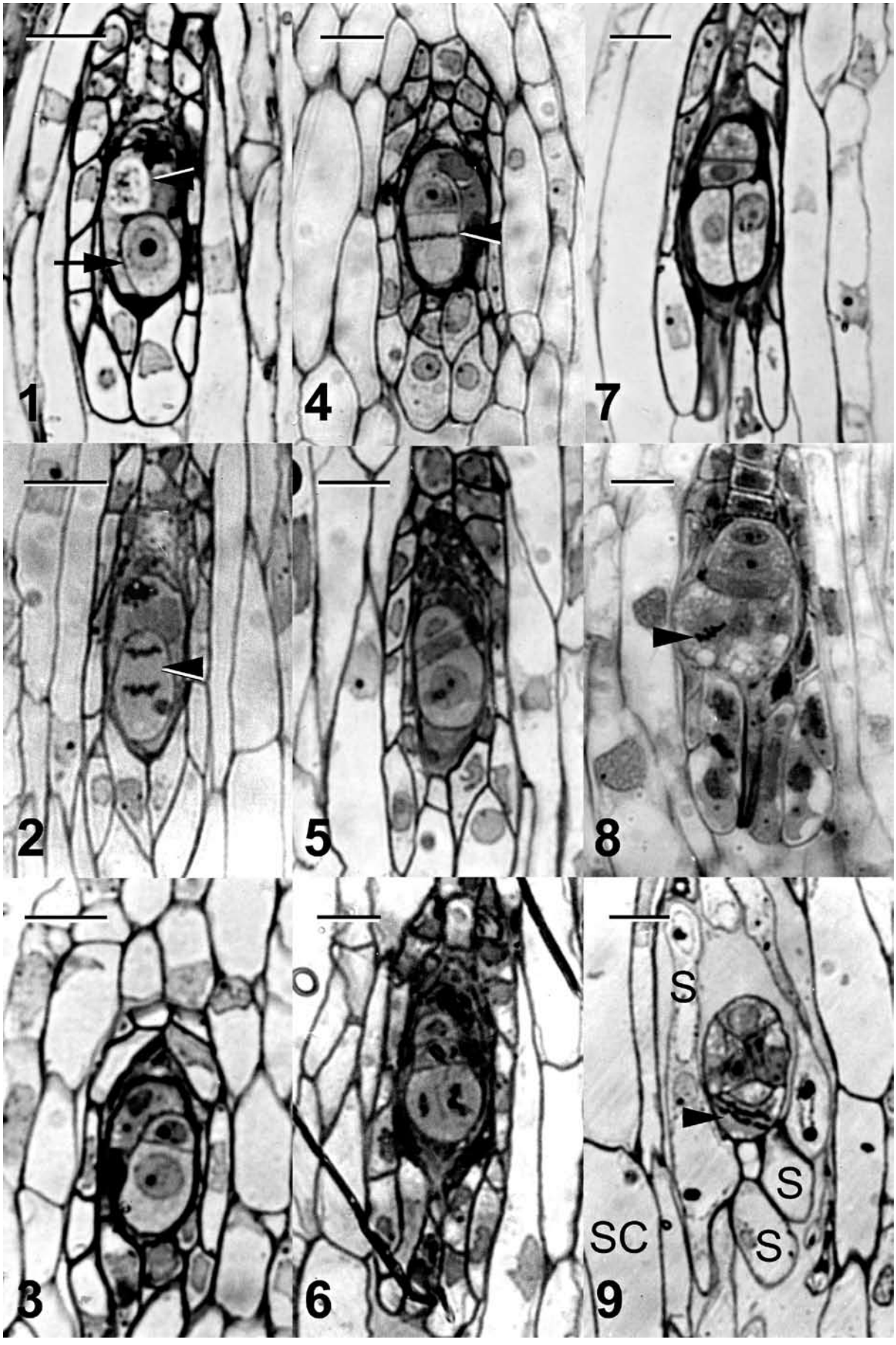
Table 1. Major developmental events occurring in developing fruits of *Phalaenopsis amabilis* var. *formosa* after fertilization.

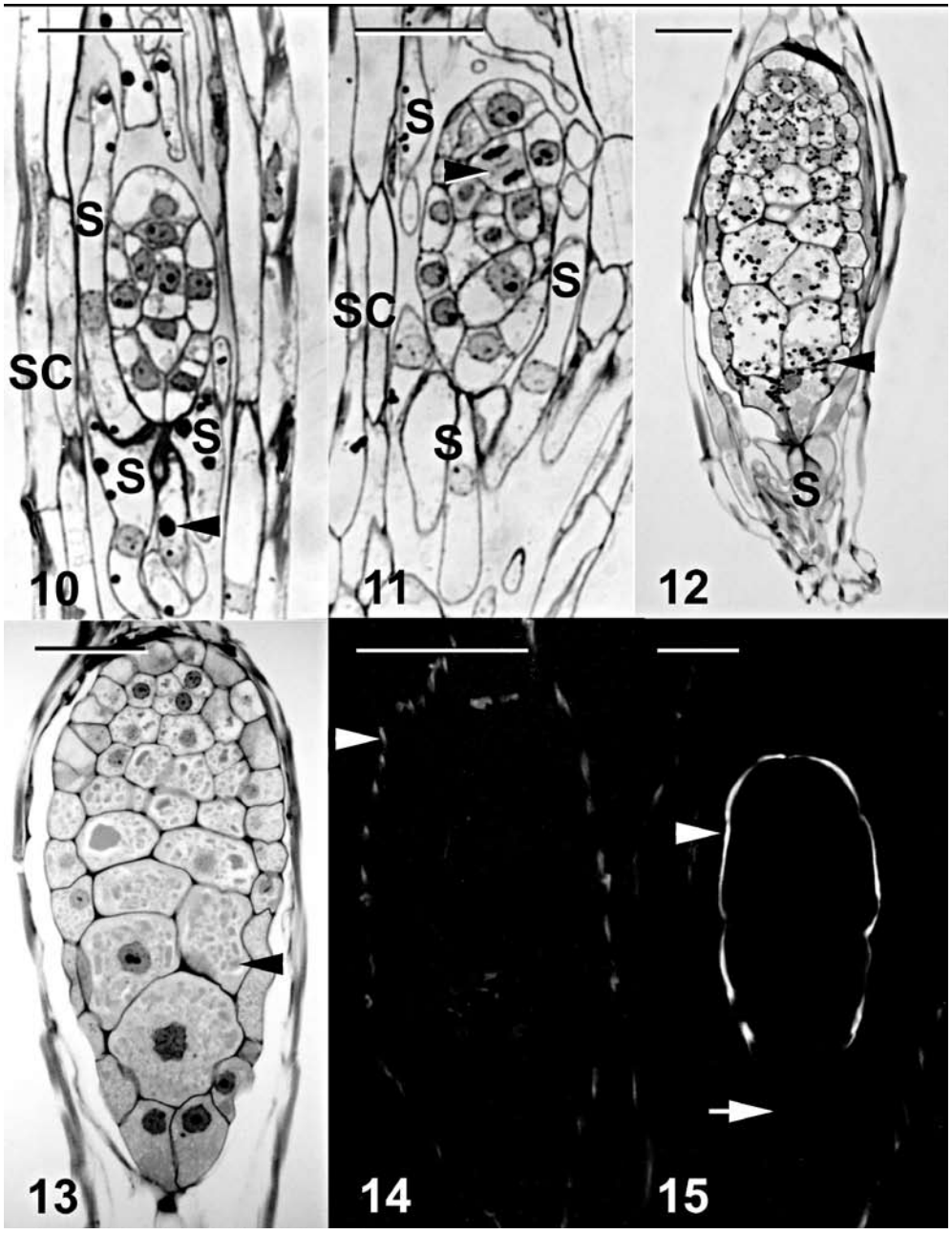
DAP*	Developmental Stage	Seed color
60	Zygote, and 2 to 4 celled embryo	White
70	Proembryo	White
80	Early globular to globular embryo	White
90	Globular embryo	White
120	Late globular embryo, and the suspensor starts to degenerate	A mixture of yellowish white and brown seeds
150	Dry, mature seed	Yellowish brown

*DAP = days after pollination.

→

Figures 1-9. (1) Light micrograph of a zygote (arrow) after fertilization at 60 DAP. The zygote has a dense cytoplasm and a prominent nucleus located at the chalazal end of the cell. Endosperm fails to develop in this species. The polar-chalazal complex (arrowhead) includes the degenerating chalazal nuclei and the polar nuclei. Scale bar = 20 μm; (2) Light micrograph showing the first cell division of the zygote. The mitotic apparatus (arrowhead) is located toward the chalazal end of the cell. Scale bar = 20 μm; (3) The first cell division of the zygote results in the formation of a smaller terminal cell and a larger basal cell. Scale bar = 20 μm; (4) Light micrograph showing a further cell division within the two-celled embryo. The basal cell divides first (arrowhead) and results in two cells of different sizes. Scale bar = 20 μm; (5) Light micrograph showing a three-celled embryo resulting from the transverse division of the basal cell. The cell towards the micropylar end is larger with a prominent nucleus. This section was stained with PAS-TBO. Scale bar = 20 μm; (6) An anticlinal division occurring in the basal cell of a three-celled embryo. Scale bar = 20 μm; (7) Light micrograph showing a four-celled embryo stage. The two cells toward the chalazal end remain small and have a dense cytoplasm while the other two cells toward the micropylar end continue to enlarge. Scale bar = 20 μm; (8) Additional cell divisions (arrowhead) occurring in the cells toward the micropylar end at 70 DAP. The daughter cells subsequently differentiate into suspensor cells. Vacuoles also become more prominent in the cytoplasm of these dividing cells. Scale bar = 20 μm; (9) The cells of the embryo proper divides obliquely (arrowhead), which signals the formation of the globular shaped embryo. At this stage, the suspensor cells (S) enlarge and elongate rapidly within the endosperm cavity towards both the micropylar and chalazal ends of the seed cavity. SC = seed coat. Scale bar = 20 μm. The sections from figures 1-9, excluding figure 8, were stained with PAS-TBO, and that from figure 8 was stained with PAS- amido black 10B.


1
4
7
2
5
8
3
6
9
S
S
S
SC



Figures 10-15. (10) Light micrograph showing a longitudinal section through an early globular embryo at 80 DAP. The suspensor cells (S) are well developed and surround the embryonic mass. In this Spurr's resin section, it is worth noting that the suspensor cells contained several lipid droplets (arrowhead) after TBO staining. SC = seed coat. Scale bar = 40 μm; (11) At 90 DAP, the cells at the chalazal region of the embryo proper divide (arrowhead) more frequently while those at the micropylar region begin to enlarge. A distinct protoderm layer can also be found at this stage. The suspensor cells (S) are highly vacuolated and continue to expand toward the chalazal and micropylar ends. SC = seed coat. Scale bar = 40 μm; (12) At 120 DAP, cell division has ceased within the embryo proper. The embryo proper consists of two cell types: smaller cells in the chalazal region and larger cells in the micropylar end near the suspensor. Starch grains (arrowhead) are abundant and tend to congregate around the nucleus. The suspensor cells (S) are undergoing the dehydration and the compression in the late stage of embryo development. Scale bar = 40 μm; (13) Light micrograph showing a longitudinal section through a mature seed at 150 DAP. Numerous tiny protein bodies (arrowhead) can be found within the embryo proper. Although lipid could not be preserved in this preparation, the spaces between the protein bodies are supposed to be the storage lipid bodies. At this stage, the suspensor cells have degenerated, and the embryo is enveloped by the shriveled seed coat. Scale bar = 40 μm. The sections of figures 10 and 11 are Suprr's-embedded specimens that have been postfixed with osmium tetroxide, and the sections are stained with alkaline TBO. The sections of figures 12 and 13 are stained with PAS-amido black 10B; (14) Nile red staining fluorescence micrograph of an orchid seed at the stage similar to Figure 9. A weak fluorescence indicates a positive staining in the seed coat (arrowhead). Only a trace of fluorescence is noted at the surface of the embryo proper. Scale bar = 40 μm; (15) Nile red staining fluorescence micrograph of an orchid seed at the stage similar to Figure 11. As the embryo matures, the surface wall of the embryo proper fluoresces brightly (arrowhead). Note that the fluorescence of the suspensor wall (arrow) is absent during development. Scale bar = 40 μm.

tinct structure within the endosperm cavity (Figures 1 and 3). In this species, the endosperm failed to develop. Soon after, the developing embryo occupied the endosperm cavity, and the endosperm nuclei and the chalazal nuclei were eventually absorbed by the embryo.

Suspensor development

A transverse division occurred first in the basal cell giving rise to a three-celled embryo (Figure 4). The micropylar end cell of the 3-celled embryo enlarged more than the two chalazal end cells (Figure 5). It then divided once anticlinally (Figure 6) resulting in a ⊥-shaped, 4-celled embryo. Subsequently, two of the four cells toward the micropyle enlarged further through the process of vacuolation while the remaining two cells located at the terminus remained small with a dense cytoplasm (Figure 7). The two larger cells at the base continued to divide (Figure 8) and resulted in the formation of eight filamentous suspensor cells. The suspensor cells elongated and expanded rapidly by the process of vacuolation (Figure 9 and 16). The four cells at the very base grew towards the micropyle. The other four cells grew towards the chalazal end of the seed, surrounding the embryo proper (Figure 10). It is worth noting that the suspensor cells contained several dark blue bodies after osmium post-fixation and TBO staining from the proembryo to the early globular stage (Figures 9 and 10). From the TEM observation (unpublished data), these dark blue bodies are highly osmiophilic, indicating they are most likely lipid droplets. As the globular embryo formed, these droplets vanished from the suspensor cells (Figure 11). Eventually, the suspensor cells extended beyond the micropyle opening of the inner integument and grew into the lumen enclosed by the outer integument (Figure 11). However, the suspensor never extended beyond the micropyle of the outer integument (Figure 12). During embryo development, the suspensor cells were always tightly appressed against the seed coat. As the embryo matured, the suspensor cells began to collapse as they became dehydrated (Figure 13).

Embryo proper development

In the four-celled embryo (Figure 7), the terminal cytoplasmic cells eventually gave rise to the embryo proper. Oblique cell divisions were readily observed during the early stages of embryo proper formation, resulting in the formation of a spheroidic embryo proper (Figure 9). At 90 DAP, a distinct protoderm layer of the embryo proper had formed (Figure 11). At the same time, additional cell divisions occurred in the inner cell tiers. It should be noted that the cells toward the chalazal end divided more frequently than the cells toward the micropylar end. At maturity, an ellipsoidal embryo consisted of different cell sizes: the cells toward the chalazal end are smaller than those toward the micropylar end (Figure 12).

Storage products

During the early stages of embryo development, the cytoplasm reacted strongly with amido black 10B, a protein stain. However, no distinct protein body-like structure is observed within the cytoplasm of the embryo proper. Starch grains are not detected in the proembryo stage. After the cells had ceased to divide (120 DAP), starch grains began to accumulate first within the embryo proper (Figure 12) and they tended to congregate around the nucleus of the cells. At the same time, small protein bodies began to appear and accumulate within the cytoplasm of the embryo proper. As the seed approached maturity (150 DAP), the large vacuoles had broken down and the starch grains had vanished (Figure 13). Concomitant with the disappearance of the large vacuoles, lipid bodies began to accumulate within the cytoplasm. In the cytoplasm of suspensor cells, no storage products were found. At the time of fruit dehiscence, protein and lipid bodies were the major storage products within the embryo proper (Figure 13).

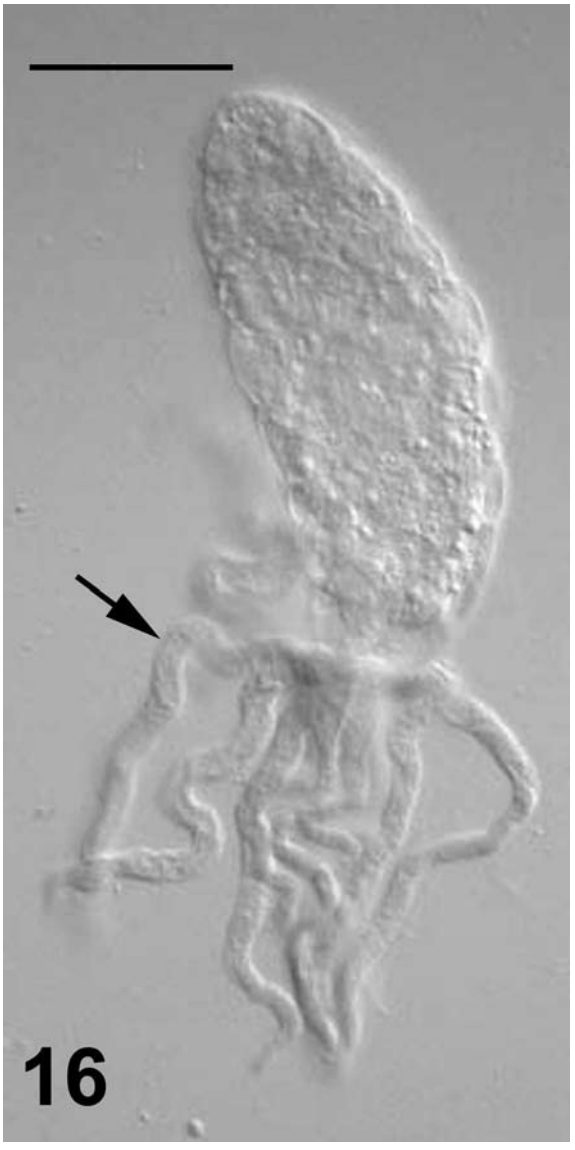


Figure 16. The Nomarski image showing a developing embryo at the stage similar to Figure 11. It is clear that the suspensor (arrow) contains eight filamentous cells. Scale bar = 40 μm.

Testa

The mature seed coat was only composed of the outer integument and was about two-three cells thick. The cells of the inner integument gradually degenerated during the early stages of embryo proper formation (Figures 9 and 10), and their cell content was presumably absorbed by the embryo. At the proembryo stage (Figure 9), the radial walls of the outermost layer of the seed coat were stained greenish blue with the TBO stain, indicating the presence of a secondary wall. The autofluorescence of the seed coat indicated that lignin was also present in the secondary wall. In addition, the secondary walls reacted positively to the Nile red stain (Figures 14 and 15). At maturity, the cells of the seed coat became dehydrated and compressed into a thin layer (Figure 13).

The pattern of Nile red staining indicates that a cuticular substance was absent over the walls of the suspensor cells through their development and maturation (Figures 14 and 15). The cell wall also gave a purple color when stained with toluidine blue O, indicating the absence of phenolic compounds in the wall.

DISCUSSION

One of the unique characteristics of orchid embryos is the diverse morphology of their suspensors. Based on the cell division pattern of the developing embryo and the suspensor morphology, orchid embryos have been classified into five types (Swamy, 1949). *Phalaenopsis* and some genera of the Vandoids group in the orchid family belonged to the type IV group (Swamy, 1949; Poddubnaya-Arnoldi, 1967). In this group, a set of eight suspensor cells is formed following three divisions by the suspensor initial cell. At the 3-celled embryo stage, the larger basal cell of the 3-celled embryo divides vertically, resulting in the formation of a ⊥-shaped four-celled embryo (Figure 6). After two further divisions of the two basal cells, eight suspensor cells are formed, and they elongated rapidly towards the micropylar and chalazal end of the developing embryo proper. In the Nun orchid, cytoskeletal elements, i.e. microtubules and actin filaments play an important role in the morphogenesis of the suspensor cell (Ye et al., 1997). One would predict that the cytoskeletal elements may also be involved in the growth and elongation of the suspensor cells in *Phalaenopsis amabilis*.

The suspensor, a short-lived embryonic organ is essential to embryo development in flowering plants (Yeung and Meinke, 1993). Since symplastic connections are absent between the developing embryo and the maternal tissues, at the very least, the suspensor can serve as the nutrient conduit between these two compartments. In *Cymbidium sinense* (Yeung et al., 1996) as well as in this present study, the tubular suspensor cells are highly vacuolated, indicating that they can store water and other dissolved substances for embryo development. In addition, the cuticular substance is absent in the suspensor wall. This would facilitate nutrient transport and uptake from the maternal tissues through its walls as no apoplastic barrier is present. In *Paphiopedilum delenatii*, we clearly demonstrated that its suspensor takes on transfer cell morphology as wall ingrowths are present (Lee et al., 2006). In developing seeds, the main function of transfer cells is the uptake of solutes such as sugars and amino acids for storage product synthesis (Thompson et al., 2001). In this study, although wall ingrowths are not detected at the light microscope level, lipid droplets appear in the elongating suspensor cells at the globular stage and disappear soon afterwards, indicating the suspensor can function in nutrient uptake and as a temporary site of food storage for the developing embryo. The transient accumulation of storage products, e.g. starch grains in the suspensor cells, has been observed during embryo development in a number of orchid species (Yeung and Law, 1992; Lee et al., 2007). In the Nun orchid *Phaius tankervillae* the positive staining of protein can be seen within the vacuolar sap of the enlarged suspensor cell (Yeung and Lee, unpublished results). All this evidence indicates that the suspensor can play a nutritive role supporting the development of the embryo proper. However, it is important to note that in orchids such as *Spiranthes australis* (Clements, 1999), which lack a structurally defined suspensor, further studies concerning nutrient acquisition by the developing embryo proper are needed.

In mature orchid embryos, a defined tissue pattern similar to that in other flowering plants cannot be found. Usually, only a protoderm is present and, depending on the species, a gradient of small to large cells can be seen with the smaller cells located at the future shoot pole (Andronova, 2006). This may be an indication of structural differentiation within the embryo. It is interesting to note that in difficult-to-germinate species such as *Calypso bulbosa*, there is no marked differentiation within the embryo proper (Yeung and Law, 1992). The embryo proper has cells of similar sizes. In contrast, the easy-to-germinate species *Epidendrum ibaguense* has a well-differentiated apical zone in the embryo proper. In the present study, a marked gradient of cell size also exists in the embryo proper of the mature seeds of *P. amabilis* var. *formosa*. The seeds of this species readily germinate upon *in vitro* culture and have an average germination percentage above 90%. The absence of distinct differentiation within the embryo proper may further prolong the germination process as additional events might be needed leading to the differentiation of a shoot pole.

During embryo development, cuticular materials can accumulate in different layers of the seed coat as reported in *Cymbidium* (Yeung et al., 1996), *Cypripedium* (Lee et al., 2005), *Calanthe* (Lee et al., 2007), and *Paphiopedilum* (Lee et al., 2006). The differences in accumulations of cuticular materials may influence asymbiotic seed germination. In those difficult-to-germinate species, the accumulation of cuticular materials usually formed a uniform layer enveloping the embryo proper. In our previous reports (Lee et al., 2005; Lee et al., 2007), both the outer

and inner layers of the seed coat fluoresce strongly in *Cypripedium formosanum* after Nile red staining, while one layer of the outer walls of the seed coat fluoresces brightly in *Calanthe tricarinata*. In these two terrestrial species, the accumulation of cuticular substances in the testa appears to be the main reason for the low germination percentage observed. The tightly fitted cuticular coating could form a physical barrier restricting embryo growth. On the other hand, for the easy-to-germinate species, such as *P. amabilis* var. *formosa*, the cuticular materials form a discontinuous layer covering the embryo proper. This may enable the embryo to access water and nutrients from the environment and involve fewer physical constraints on embryo growth and development.

In conclusion, the key anatomical events in the embryo development of *P. amabilis* var. *formosa* from fertilization to seed maturity are detailed in this report. The major developmental events will provide valuable data for further molecular studies on embryo development of *Phalaenopsis*.

Ackonwledgements. This work was supported by grants from the Council of Agriculture of Taiwan to Nean Lee of the Academia Sinica, Taiwan and to Mei-Chu Chung, and by grants from the Natural Sciences and Engineering Research Council of Canada to Edward C. Yeung.

LITERATURE CITED

Andronova, E.V. 2006. Embryogenesis in Orchidaceae. *In* T.B. Batygina (ed.), Embryology of Flowering Plants vol. 2: seed. Science Pub., New Hampshire, pp. 355-359.

Arditti, J. 1992. Fundamentals of Orchid Biology. John Wiley and Sons, Inc., New York.

Clements, M.A. 1999. Embryology. *In* A.M. Pridgeon, P.J. Cribb, M.W. Chase, and F.N. Rasmussen (eds.), Genera Orchidacearum vol. 1: General Introduction, Apostasioideae, Cypripedioideae. Oxford University Press, Oxford, pp. 38-58.

Christenson, E.A. 2001. Phalaenopsis: A monograph. Timber Press, Oregon.

Lee, Y.I., N. Lee, E.C. Yeung, and M.C. Chung. 2005. Embryo development of *Cypripedium formosanum* in relation to seed germination *in vitro*. J. Am. Soc. Hortic. Sci. **130:** 747-753.

Lee, Y.I., E.C. Yeung, N. Lee, and M.C. Chung. 2006. Embryo development in the lady's slipper orchid, *Paphiopedilum delenatii* with emphases on the ultrastructure of the suspensor. Ann. Bot. **98:** 1311-1319.

Lee, Y.I., E.C. Yeung, N. Lee, C.F. Lur, and M.C. Chung. 2007. Changes in endogenous abscisic acid levels and asymbiotic seed germination of a terrestrial orchid, *Calanthe tricarinata* Lindl. J. Am. Soc. Hortic. Sci. **132:** 246-252.

Lin, T.P. 1988. Native Species of Taiwan vol. 2. Southern Materials Center, Inc., Taipei, Republic of China.

Poddubnaya-Arnoldi, V.A. 1967. Comparative embryology of the Orchidaceae. Phytomorphology **17:** 312-320.

Thompson, R.D., G. Hueros, H.A. Becker, and M. Maitz. 2001. Development and functions of seed transfer cells. Plant Sci. **160:** 775-783.

Swamy, B.G.L. 1949. Embryological studies in the Orchidaceae. II. Embryology. Am. Midl. Nat. **41:** 202-232.

Yam, T.W., E.C. Yeung, X.L. Ye, S.Y. Zee, and J. Arditti. 2002. Orchid embryos. *In* T. Kull and J. Arditti (eds.), Orchid Biology: Reviews and Persectives, VIII. Kluwer Academic Publisher, Dordrecht. pp. 287-385.

Ye, X.L., S.Y. Zee, and E.C. Yeung. 1997. Suspensor development in the nun orchid, *Phaius tankervilliae*. Intl. J. Plant Sci. **158:** 704-712.

Yeung, E.C. 1984. Histological and histochemical staining procedures. *In* I.K. Vasil (ed.), Cell Culture and Somatic Cell Genetics of Plants. Academic Press, Orlando. pp. 689-697.

Yeung, E.C. 1990. A simple procedure to visualize osmicated storage lipids in semithin epoxy sections of plant tissue. Stain Technol. **65:** 45-47.

Yeung, E.C. 1999. The use of histology in the study of plant tissue culture systems - some practical comments. In Vitro Cell. Dev. Biol.-Plant **35:** 137-143.

Yeung, E.C. and S.K. Law. 1992. Embryology of *Calypso bulbosa*. II. Embryo development. Can. J. Bot. **70:** 461-468.

Yeung, E.C. and D.W. Meink. 1993. Embryogenesis in angiosperms: development of the suspensor. Plant Cell **5:** 1371-1381.

Yeung, E.C., S.Y. Zee, and X.L. Ye. 1996. Embryology of *Cymbidium sinense*: Embryo development. Ann. Bot. **78:** 105-110.

台灣蝴蝶蘭的胚發育

李勇毅[1, 4] Edward C. YEUNG[2] 李 咔[3] 鍾美珠[1]

[1]中央研究院 植物暨微生物學研究所

[2]Department of Biological Sciences, University of Calgary, Calgary, Alberta T2N 1N4, Canada

[3]國立台灣大學 園藝學系

[4]目前地址：國立自然科學博物館 植物學組

台灣蝴蝶蘭 (*Phalaenopsis amabilis* var. *formosa*) 為原生於台東和蘭嶼的著生類蘭花。在受精完成後，接合子連續進行兩次細胞分裂產生一個⊥形的四細胞胚。緊接著，此四細胞胚中接近珠孔端的兩個細胞擴增體積，並且進行兩次細胞分裂，形成八個管狀的胚柄細胞。這些管狀的胚柄細胞呈現高度的液胞化，向下擴展延伸至近珠孔端，向上則到達合點端。在胚發育早期，在胚柄細胞內可觀察到脂質體形成，在後續的發育階段則消失。因此胚柄在胚胎發育過程中擔任養分吸收的角色，並提供暫時的存放空間。當種子成熟時，可觀察到胚體頂部有一群分化程度較高的細胞。台灣蝴蝶蘭的胚體細胞內主要的儲藏物質為蛋白質體與脂質體。由尼羅紅 (Nile red) 染色所散發的螢光顯示:角質層只累積於胚體的表層細胞壁中，在胚柄的細胞壁中並無累積。在種子成熟時，角質亦累積於種皮的最外層細胞壁。

關鍵詞：胚；蘭花；台灣蝴蝶蘭；胚柄。