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RESEARCH ARTICLE

Study on Seed Morphogenesis of Orobanchaceae in Taiwan

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ABSTRACT: Seed morphogenesis of Orobanchaceae was not completely investigated previously. Here, we observed seed development of Orobanchaceous species in Taiwan using light and scanning electron microscopies. Results indicated that seeds of *Aeginetia indica*, *Boschniakia himalaica*, and *Orobanche caerulescens* all consisted of embryo, endosperm and testa. Ontogeny of the embryo in *A. indica* was Solanad type, while in both *B. himalaica* and *O. caerulescens* was Onagrad type. The mature embryos of the three species lacked embryonic organs, and their endosperm development was the cellular type and, at maturity, appeared as several cell layers of storage tissue. Ontogeny of the testa was all non-multiplicative, with the residues of the outermost cell layer and reticulately-thickened secondary walls of its cells at maturity. Mature seeds of *A. indica* and *O. caerulescens* were ovate whereas those of *B. himalaica* were oblate. As for *Christisonia hookeri*, due to lack of samples, only the cellular-typed endosperm was determined. The comparative development of Orobanchaceous seeds was discussed.

KEY WORDS: Embryo, endosperm, Orobanchaceae, seed development, testa.

INTRODUCTION

Orobanchaceae is a dicot family, which consists of annual and perennial plants distributing from tropical to subarctic regions, predominately in temperate regions (Kuijt, 1969). In Taiwan, there are four species in Orobanchaceae, including *Aeginetia indica* L., *Boschniakia himalaica* Hooker & Thomson, *Christisonia hookeri* C. B. Clarke & Hooker, and *Orobanche caerulescens* Stephan & Willd. (Yang and Lu, 1998). Members of this family are achlorophyllous, consisting of a fleshy body with spike or raceme inflorescences. They are all root-parasitic plants and obtain water and nutrients from hosts via their invading haustoria. To set up the parasitism, chemical signals from host roots are essential for the seeds of these parasitic plants to germinate and attach themselves to the host roots, then further form the haustoria to establish a nutrient bridge with their hosts (Parker and Riches, 1993; Westwood *et al.*, 2010; Xie *et al.*, 2010). Several species in Orobanchaceae have been reported to cause yield losses of host crops, e.g. *A. indica* and *Christisonia wightii* on sugar-canes in Asia, and some of the genus *Orobahche* on different crops mainly in the Mediterranean Basin (Riches and Parker, 1995; Parker, 2009). Thus studies on controlling these parasitic weeds have also been intensively conducted (Sauerborn *et al.*, 2007; Fernandez-Aparicio *et al.*, 2008).

A typical dicot embryogenesis can be defined as a series of stages, including (1) zygote stage: the egg cell is fertilized, (2) 2-celled proembryo stage: zygote's first division into two cells, the earliest embryonic stage, (3) tetrad proembryo stage: cells of the 2-celled proembryo divide into four cells, (4) quadrant proembryo stage: the tetrad proembryo forms a quadrant structure at its end, (5) octant proembryo stage: cells of the quadrant proembryo divide to form an octant one, (6) globular proembryo stage: cells of the octant divide to form a globular-shaped proembryo with differentiation of the protoderm, (7) heart embryo stage: the initial of cotyledons appears, transforming the embryo into a heart shape, (8) torpedo embryo stage: elongation of the cotyledons and hypocotyl-radicle axis, (9) mature embryo stage: differentiation of embryonic organs completes.

Orobanchaceous plants usually produce numerous tiny seeds. The seeds consist of an embryo, endosperm and testa. Studies on *A. indica* (Juliano, 1935; Tiagi, 1952b), *B. tuberose*, *B. himalaica* (Tiagi, 1963), *Cistanche tinctoria* (Kadry, 1955), *Cistanche tubulosa* (Tiagi, 1952a), *O. aegyptiaca*, and *O. cernua* (Tiagi, 1951) all agree that the common morphological features of the seeds include (1) the mature embryo deficient in organs such as radicle, hypocotyls and cotyledons, (2) the cellular-type of development for the endosperm, and (3) reticulate thickenings in the walls of the outermost-layered cells of the testa. In some other species, e.g. *B. hookeri* (Olsen and Olsen, 1980), *Christisonia subacaulis* (Raju and Chamaiah, 1972), *O. lucorum* (Tiagi and Sankhla, 1963), and *O. uniflora* (Cassera, 1935), even though only portions of the seed structures were described, they share similar features as

above. Moreover, development of embryo in *Cistanche tinctoria* is the Caryophyllad-type, i.e. the apical cell of the 2-celled proembryo divides transversely and its later divisions constitute the embryo proper (Johansen, 1950; Kadry, 1955), while it is the Onagrad type in *O. aegyptiaca* and *O. cernua* (Johansen, 1950; Tiagi, 1951), which is similar to the Caryophyllad type but the apical cell first divides longitudinally. Thus far there has been more information on the development of embryo and endosperm but less on the testa development, and it is still not enough to tell us a complete story about the seed development of Orobanchaceous plants.

In the present study, we collected the four Orobanchaceous species in Taiwan to observe the seed development. Besides the embryo and the endosperm, we also described the complete structure and formation of the testa. A complete description of seed morphogenesis of a certain Orobanchaceous species was shown here.

MATERIALS AND METHODS

Plant materials

Plants with mature ovaries and fruits at different developmental stages were collected at field sites in Taiwan as shown in Table 1, including the Orobanchaceous species *Aeginetia indica* L., *Boschniakia himalaica* Hooker & Thomson, *Christisonia hookeri* C. B. Clarke & Hooker and *Orobanche caerulescens* Stephan & Willd., but only one collection for *C. hookeri* due to its rarity. The inflorescence samples at different fruiting stages were fixed with FPA (formalin : propionic acid : alcohol = 1 : 1 : 1 [v]) and then preserved in 70% alcohol. Some mature fruits were directly air-dried under 55°C. The preserved ovaries and fruits were dissected for the ovules and seeds to be processed for further light and scanning electron microscopies.

Light microscopy (LM)

The dissected samples were dehydrated with alcohol-acetone series to pure acetone, infiltrated with acetone-Spurr's resin (Electron Microscopy Sciences, USA) series and finally embedded in pure resin. Embedded samples were sectioned 1-2 μm in thickness using Leica RM2145 rotary microtone; then the sections were stained with 0.5% toluidine blue O (Clark, 1981) and examined under Olympus BX60 light microscope.

Scanning electron microscopy (SEM)

For the external morphology of seed development, immature seeds were dehydrated with alcohol-acetone series to pure acetone and substituted with liquid CO_2 for critical point drying (critical point drier, Ladd Model 28000, USA). The mature seeds were air-dried and used directly for SEM examination. All samples were mounted on stubs, coated with gold or platinum (for gold coating, the setting was 0.05 torr vacuum, 20 mA current, and coating for 90 s [JBS E150, Canada]; platinum coating, 0.05 torr, 15 mA, 180 s [Hitachi E-102, Japan]), and observed under field-emission scanning electron microscope at 15 kV (Hitachi S-4200, Japan).

For the pattern of cell wall thickening of testa, samples were dehydrated by alcohol-tert-butanol series to pure tert-butanol, infiltrated with liquid wax and finally embedded in pure wax. The embedded samples were sectioned using America Optical 820 rotary microtone, among which *A. indica* and *O. caerulescens* were 30 μm, and *B. himalaica* 50 μm in thickness. Sections were adhered to cover slides with Haupt's adhesive, and were de-waxed in xylene and air-dried, then followed by the same coating procedure and SEM examination as the above.

RESULTS

(i) Structure of the ovule

Aeginetia indica L.: The ovule was composed of an embryo sac and a single integument full with starch grains (Fig. 1A). The embryo sac consisted of seven cells, including one egg cell and two synergids at the micropylar end, three antipodal cells at the chalazal end, and a central cell in the middle. During the seed development, one of the synergids became degraded when the fertilized egg cell (zygote) began to develop but the other remained intact till the seed matured; whereas for the antipodal cells, all broke down before the globular proembryo stage.

Boschniakia himalaica Hooker & Thomson: The structure of the ovule as shown in Fig. 1B was similar to *A. indica* in their single integument, cell arrangement of the embryo sac and fate of the synergids and the antipodals. The difference was that the integument consisted of more cell layers without starch grain.

Christisonia hookeri C. B. Clarke & Hooker: As shown in Fig. 1C, the structure of the ovule was very similar to *A. indica*, with the same cell arrangement of the embryo sac and the single integument. However, the integument contained much less starch grains than *A. indica*.

Orobanche caerulescens Stephan & Willd.: Fig. 1D showed the structure of the ovule. Compared to *A. indica*, it possessed similar structure for both the

Table 1. Sampling information of seed morphogenesis of Orobanchaceae in this study.

Species	Site	Date	Inflorescence/fruit*
Aeginetia indica	Ren-ai Township, Nantou County	2000/9 – 2000/11	67/178
	Dahu Township, Miaoli County	2000/9; 2001/8	22/88
	Shihtan Township, Miaoli County	2000/10	9/31
Boschniakia himalaica	Heping Township, Taichung County	1999/4; 2000/3 – 2000/6; 2001/4 – 2001/6	84/189
	Ren-ai Township, Nantou County	1998/7; 2001/6 – 2001/8	19/63
Cristisonia hookeri	Heping Township, Taichung County	2001/8	25/35
Orobanche caerulescens	Gongliao Township, Taipei County	1999/4; 2000/4; 2001/4	22/109
	Dajia Township, Taichung County	2000/5; 2001/4	11/47
	Sinyi Township, Nantou County	1998/8; 2000/8; 2001/5 – 2001/6	35/168

*The number of sampled inflorescences and fruit used in our observations.

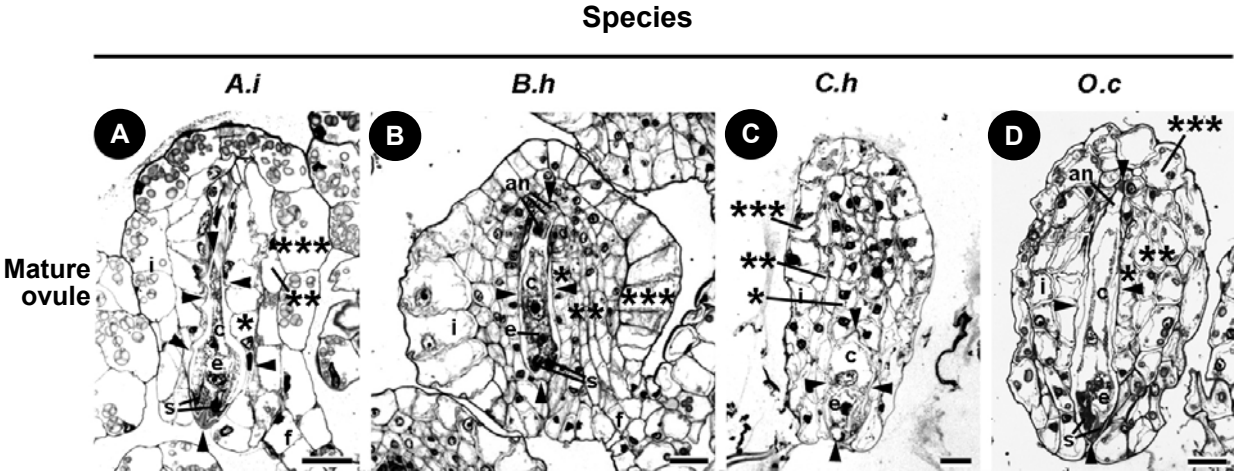

Fig. 1. LM micrographs of the structure of the mature ovule. All figures in our results were oriented corresponding to the chalazal end at the top and micropylar end at the bottom. Species labels: *A.i*, *Aeginetia indica*; *B.h*, *Boschniakia himalaica*, *C.h*, *Christisonia hookeri*; *O.c*, *Orobanche caerulescens*. Abbreviations: an, antipodal; c, central cell; e, egg cell; f, funicle; i, integument; s, synergid; *, inner layer of integument; ** middle layers of integument; *, outer layer of integument. The area pointed by arrowheads was the embryo sac. A: Ovule of *A. indica*. The particles in cells of the outer layer of integument were starch grains. The antipodals were not shown in this section. B: Ovule of *B. himalaica*. C: Ovule of *C. hookeri*. Since this section was cut obliquely, only part of the embryo sac was shown. D: Ovule of *O. caerulescens*. A few starch grains were present in cells of the outer layer of integument. Only one antipodal was shown. Bars= 30 μm.**

integument and embryo sac and similar fate for the synergids and the antipodals. Unlike *A. indica*, less starch grains were present.

(ii) Development of the embryo

To correlate the developmental events of the seed, we followed the description of a typical dicot embryo development. The development of the embryos of three species was summarized in Fig. 2 and described as below, except for *C. hookeri* due to lack of samples.

Aeginetia indica L.: The developmental process of the embryo is shown in Figs. 3A-D. After fertilization, the zygote elongated along the chalaza-micropyle axis and then divided transversely into two cells. The upper one (close to the chalaza) was the apical cell, and the lower one (close to the micropyle) was the basal cell, both constituting the 2-celled proembryo (Fig. 3A). The following transverse divisions of both cells rendered the linear tetrad proembryo (Fig. 3B). The upper three cells underwent further divisions into the 6-celled embryo proper, whereas the lower one stayed undivided and directly differentiated into the suspensor. The suspensor degraded before the next cell division of embryo proper. Following the degeneration of the suspensor, the 6-celled embryo proper continued to divide to form the globular proembryo (Fig. 3C). Finally at maturity of the seed, the embryo appeared as an oval-shaped cell

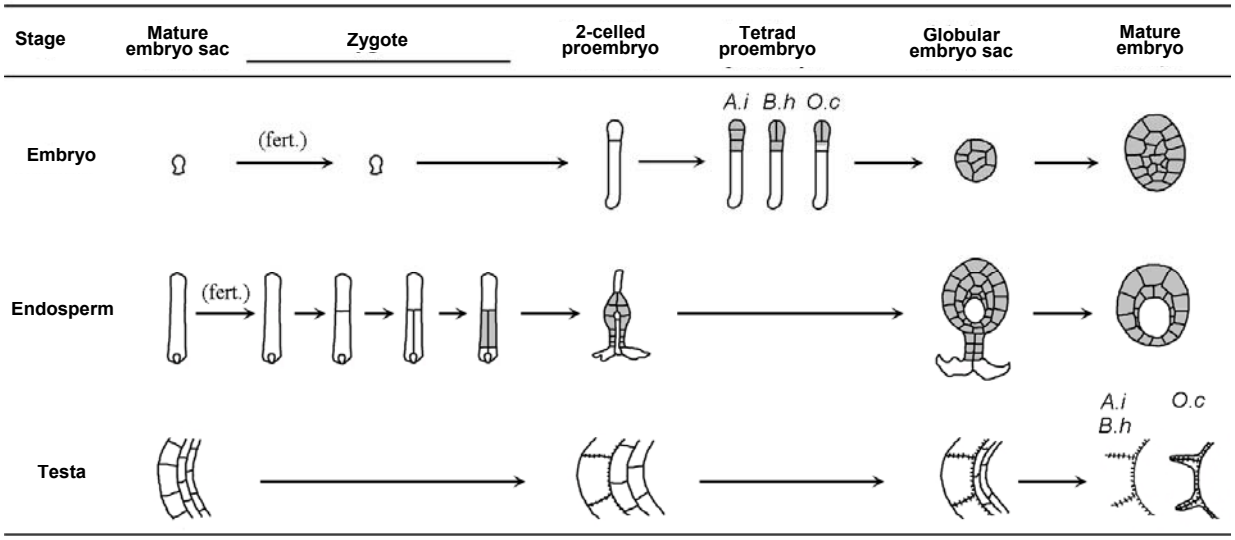

Fig. 2. Comparison of the development of the seed components of Orobanchaceae in Taiwan. Abbreviations: *A.i*, *Aeginetia indica*; *B.h*, *Boschniakia himalaica*; fert., fertilization; *O.c*, *Orobanche caerulescens*. The grey area marks the cell lineages of embryo proper and endosperm proper. Drawings of testa indicate the wall thickening and changes of cell layers. All species possessed similar organizations, except the differences indicated for each species.

cluster without any organs (Fig. 3D). Based on Johansen's classification of the embryo morphogenesis (1950), since the apical cell first divided transversely, and one of the two cells by the first division of the basal cell also constituted the embryo proper, it was the Solanad type in *A. indica*.

Boschniakia himalaica Hooker & Thomson: The developmental process of the embryo is shown in Figs. 3E-H. The 2-celled proembryo consisted of the apical and basal cell (Fig. 3E). Subsequently, the apical cell divided longitudinally, and the basal cell transversely, to form a T-shape tetrad proembryo, in which the upper three cells served as the embryo proper whereas the other one as the suspensor (Fig. 3F). Among the embryo proper cells, the upper two divided to form the quadrant, whereas the third cell divided into two progeny cells, resulting in a 6-celled embryo proper. Meanwhile, the suspensor degraded. After the degeneration of the suspensor, the quadrant divided to form the octant. The subsequent cell divisions of the embryo proper rendered the proembryo to be globular (Fig. 3G). At maturity, the embryo appeared as a nearly round cell cluster with wave-shaped outline and without any organs (Fig. 3H). We ruled out the possibility of poor fixation to cause this wave-shaped outline because first, the samples we observed all shared similar phenomenon, and second, at the globular stage the proembryo appeared slightly wave-shaped outline, implying it would start to transform the shape from round to wavy as a result of maturation. Since the apical cell first divided longitudinally, and one of the two cells by the first division of the basal cell also constituted the embryo proper, the embryo morphogenesis of *B. himalaica* was the Onagrad type (Johansen, 1950).

Orobanche caerulescens Stephan & Willd.: The developmental process of the embryo as shown in Figs. 3I-L was similar to *B. himalaica* and classified to the Onagrad type (Johasen, 1950). However the difference was that in the tetrad proembryo, after the upper cell (one of the two derived from the basal cell) divided transversely into two cells, only the progeny adjacent to the quadrant joined the constituent of the embryo proper. Meanwhile, the other progeny, together with the cells near the chalazal end, served as the multicellular suspensor. No organ was identified in the mature embryo (Fig. 3L).

(iii) Development of the endosperm.

The development of the endosperms of four species was summarized in Fig. 2 and described as below.

Aeginetia indica L.: The developmental process of the endosperm began earlier than the zygote, and the entire process was classified as cellular type, i.e. each nuclear division followed by a cytokinesis (Johri, 1984), as shown in Figs. 4A-F. After fertilization, the central cell divided into two cells, occupying the micropylar and the chalazal chambers, respectively (Fig. 4A). The cell at the micropylar chamber then divided into two cells (Fig. 4B). These two cells further divided to give rise to a 4-celled state surrounding the zygote, with two

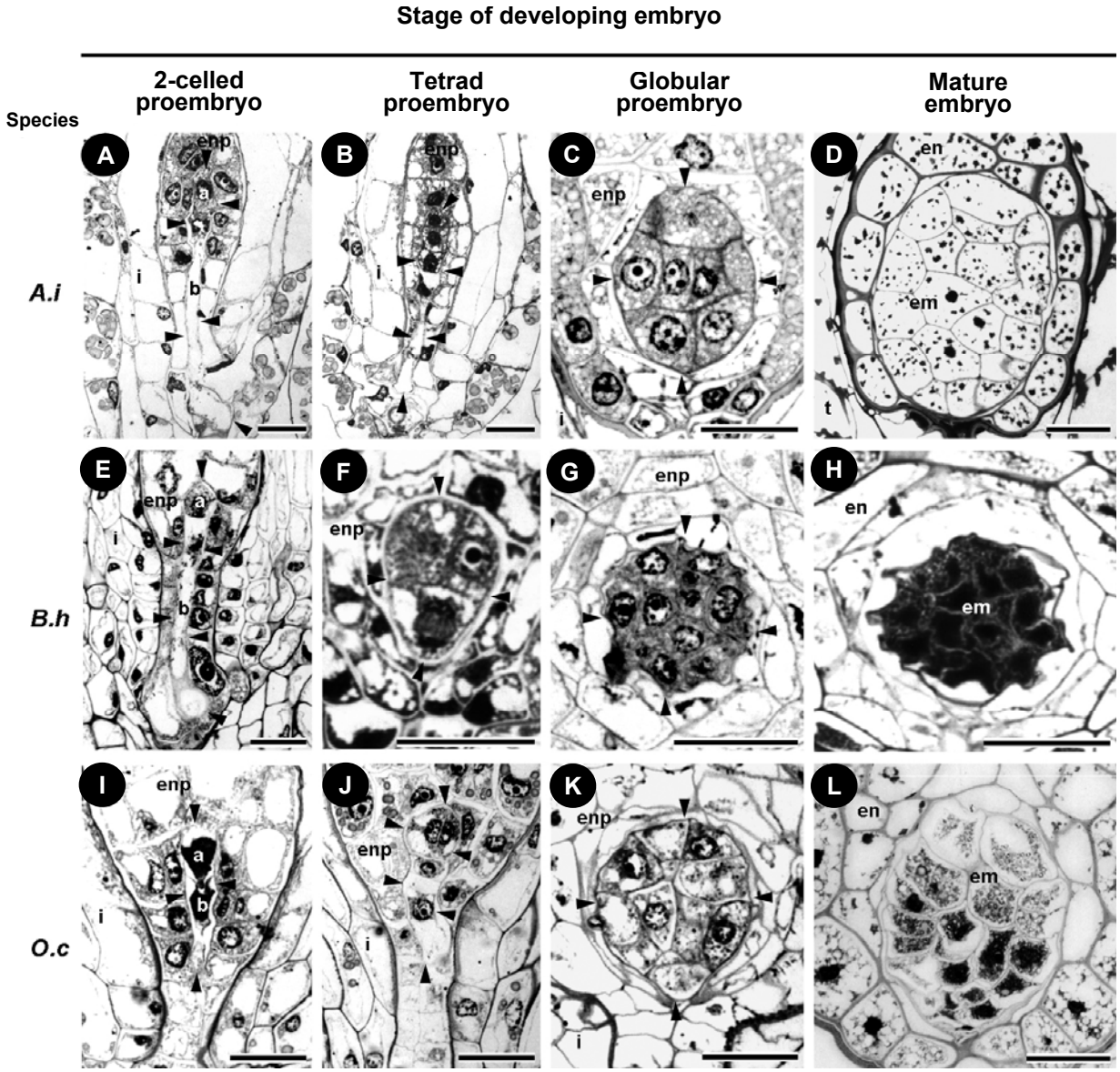

Fig. 3. LM micrographs of the embryo development. Abbreviations: a, apical cell; b, basal cell; em, mature embryo; en, mature endosperm; enp, endosperm proper; i, integument; t, testa. The area pointed by arrowheads was the proembryo. The figures are tabulated to compare the developing embryos of the three species. Among which the basal cell in Fig. 3I was partially shown; the tetrad was linear in Fig. 3B and T-shape in Figs. 3F & J. In Fig. 3F only the three embryo proper cells were shown, while the lowest cell was partially masked by endosperm in Fig. 3J. Bars= 30 μm.

cells comprising the "inner tier" of endosperm, and the other two locating at micropylar end (Fig. 4C). As to the chalazal chamber, it remained unicellular (Figs. 4B & C). At the 2-celled proembryo stage, the cell at the chalazal chamber became a chalazal haustorium and later degraded after the tetrad proembryo stage. On the other hand, at the micropylar chamber the two cells close to the micropylar end became amoeboid micropylar haustoria and intruded into the surrounding integument tissue through intercellular spaces (Fig. 4D). The micropylar haustoria remained intact until the seed became matured, and then degraded.

The cells of the "inner tier" of the 4-celled state served as the endosperm proper (the endospermal structure besides the chalazal and micropylar haustoria) and divided several times to form single layer of endosperm cells enclosing the 2-celled proembryo (Fig. 4D). At the globular proembryo stage, the endosperm cells initially surrounding the apical cell of the 2-celled proembryo proliferated and differentiated into a storage

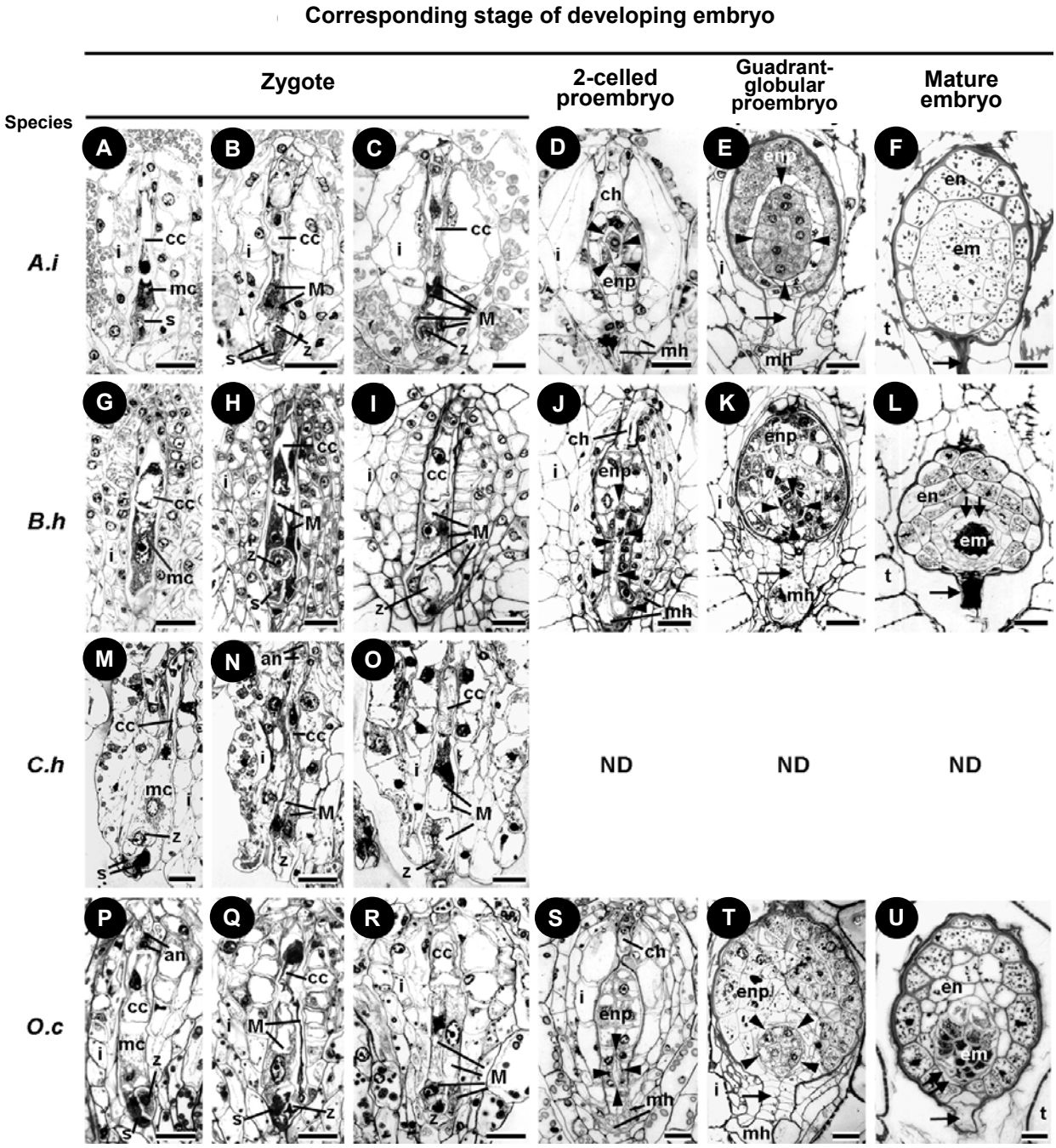

Fig. 4. LM micrographs of the endosperm development. Abbreviations: an, antipodal; cc, cell at chalazal chamber; ch, chalazal haustorium; em, mature embryo; en, mature endosperm; enp, endosperm proper; i, integument; M, progeny cells at micropylar chamber resulted from the 2nd and 3rd divisions of the developing endosperm; mc, cell at micropylar chamber resulted from the 1st division of the fertilized central cell; mh, micropylar haustorium; s, synergid; t, testa; z, zygote. Single-arrow, isthmus; double-arrow, degraded endosperm cell near the embryo. The area pointed by arrowheads was the proembryo. The figures are tabulated by the corresponding stages of the developing embryos for four species to compare the development of endosperm. However, for *C. hookeri*, due to the lack of samples, only early development was shown here. A, G, M & P: The 1st division of the fertilized central cell formed the cells at the micropylar and chalazal chamber. B, H, N & Q: The 2nd division for the developing endosperm occurred at the micropylar chamber, forming two progeny cells. C, I, O & R: At the micropylar chamber, the 3rd division occurred into four progeny cells. F, L & U: The mature endosperm. Bars= 30 μm. ND, no data.

tissue with starch grains, whereas the endosperm cells surrounding the basal cell differentiated into the isthmus and connected with the micropylar haustoria (Figs. 4E). At first, the isthmus covered the suspensor of the proembryo. Following the degradation of the suspensor, the isthmus cells enlarged, causing the whole proembryo to be enclosed by the endosperm (Figs. 4E). During the entire process of development, the storage tissue of endosperm was filled with starch grains. As the embryo continued to grow, the cell layers of the endosperm adjacent to the proembryo gradually decomposed. When the embryo matured, the remaining endosperm was only one-cell-layered with sparse starch grains. Meanwhile, there was a significant thickening on the outer periclinal walls of the endosperm; the isthmus disintegrated (Fig. 4F).

Boschniakia himalaica Hooker & Thomson: The developmental process as shown in Figs. 4G-L was very similar to *A. indica.* However, there was more storage tissue during the endosperm development in *B. himalaica*.

Christisonia hookeri C. B. Clarke & Hooker: Due to its rarity and limitation of sampling in Taiwan, only the early stages of endosperm development were described here. As shown in Figs. 4M-O, the early endosperm development was identical to *A. indica*.

Orobanche caerulescens Stephan & Willd.: The developmental process as shown in Figs. 4P-U was similar to *A. indica.* The difference was that there was more storage tissue during endosperm development in *O. caerulescens*.

(iv) Development of the testa.

The development of the testa of three species was summarized in Fig. 2 and described as below, except for *C. hookeri* due to lack of samples. For convenience, we arbitrarily defined some terms for the integument: the outermost cell layer as the "outer layer", the innermost cell layer the "inner layer", and those in-between the "middle layers". Further for the outer layer, the cell wall facing outside as the "outer periclinal wall", the cell wall abutting against the middle layers the "inner periclinal wall", and the cell wall connecting these two periclinal walls the "anticlinal wall".

Aeginetia indica L.: The development of the testa as shown in Figs. 5A-D was the non-multiplicative type, i.e. the integument cells do not divide (Corner, 1976). During the testa development, all cells of the integument enlarged at first, among which cells in both inner and outer layers were the most significant ones, especially those near the chalazal end. At the 2-celled proembryo stage, the enlargement of integument cells ceased, and the middle layers and the inner layer were gradually crushed by the developing endosperm at later stages (Figs. 5A & C). When the seed matured, almost all cells of both middle layers and inner layer disappeared, but a few cells remained at the micropylar end and appeared dead without cell contents (Fig. 5D). As to the changes of the cells in the outer layer, the anticlinal and inner periclinal walls started to thicken at the 2-celled proembryo stage till the globular proembryo stage (Figs. 5A, B & C). On the other hand, the starch grains in the cells disappeared (Fig. 5C) When the seed matured, the outer layer was the only layer of the testa (Fig. 5D), in which the cells were empty, with their outer periclinal walls torn off, showing the interior reticulate thickenings of cell walls (Fig. 6D).

Boschniakia himalaica Hooker & Thomson: The cells of the outer layer, especially those near the transverse axis (perpendicular to chalaza-micropyle axis), were much larger than cells of the other layers of the integument (Fig. 1B). In addition, there were 2-3 layers of cells in the middle layers.

As shown in Figs. 5E-H, the development was the non-multiplicative type (Corner, 1976). During the development, all cells of integument enlarged at first. The cells of the outer layer underwent a more significant expansion as they were closer the transverse axis, which changed the shape of the seed from spherical to oblate. Meanwhile, the cells of the inner layer also expanded as the same fashion as the outer layer, resulting in transversely flattened cells. The integument cells kept enlarging until the 2-celled proembryo stage (Fig. 5E). Later the inner layer and the middle layers were crushed by the growing endosperm and degraded finally (Figs. 5G & H). Similar to *A. indica*, the wall-thickening duration of the outer layer was from the 2-celled proembryo stage (Figs. 5E & F) to the globular proembryo stage. Testa of the mature seed constituted only the outer layer, where the cells became empty (Fig. 5H). The outer periclinal walls were torn off, showing the interior reticulate thickenings of cell walls (Fig. 6H).

Orobanche caerulescens Stephan & Willd.: It presented the similar integumental structure (Fig. 1D) and developmental process (Figs. 5I-L) to *A. indica.* The outer layer was the only layer remained, with reticulately thickened interior cell walls (Fig. 6L, inset). However, the difference was that most of its outer periclinal walls remained parenchymatous and complete (Fig. 5L).

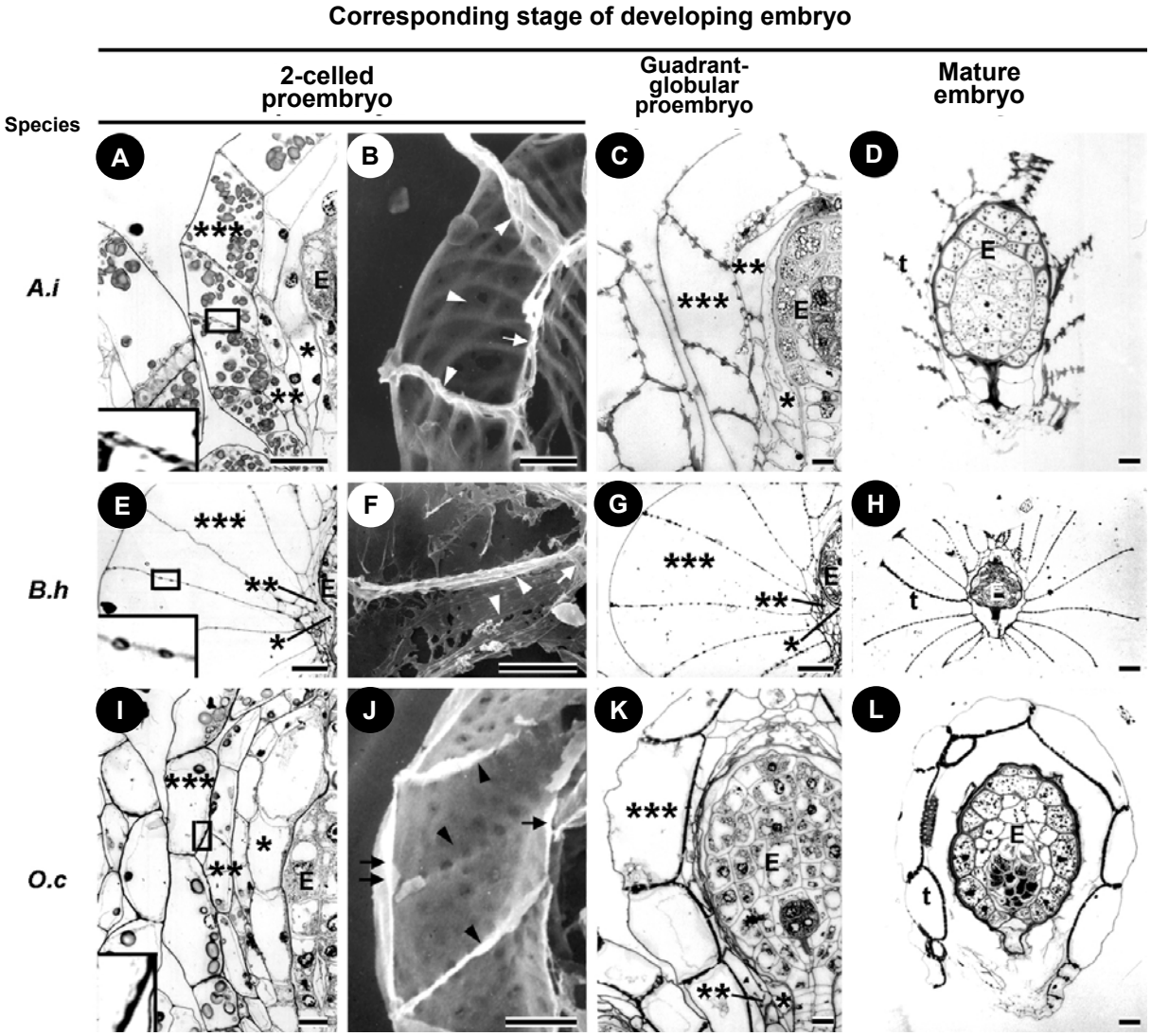

Fig. 5. The testa development. Figs. 5B, F & J are SEM micrographs whereas the others are LM micrographs. Abbreviations: E, embryo and endosperm; t, testa. *, inner layer of integument; ** middle layers of integument; *, outer layer of integument. Single-arrow, inner periclinal wall of the outer-layer cell; double-arrow, outer periclinal wall of the outer-layer cell; arrowhead, anticlinal wall of the outer-layer cell. The figures are tabulated by the corresponding stages of the developing seeds for three species to compare the development of testa. A, E & I: The insets are magnified views of wall thickenings in the bracket regions. The particles in the outer-layer cells shown in Figs. 5A & I were starch grains. B, F & J: The wall thickening of the individual outer-layer cell. The outer periclinal walls in Figs. 5B & F were lost due to sample processing. C: At globular proembryo stage. G: At quadrant proembryo stage. K: At octant proembryo stage. D, H & L: The mature testa. Bars= 15 μm in A-D; 90 μm in E-H; 20 μm in I-L.**

(v) External morphological changes during seed development.

Aeginetia indica L.: The ovule was anatropous and ovate (Fig. 6A). Before fertilization the length of longitudinal (chalaza-micropyle) and transverse axes of the ovule were 141 ± 17 μm and 97 ± 12 μm, respectively, and both axes increased to 1.6-1.8 folds at the 2-celled proembryo stage (Figs. 6A & B). The size of the seed did not become fixed until the globular proembryo stage. Meanwhile, the cells of the outer layer enlarged, stretching the outer periclinal walls and the edges of thickened anticlinal walls were clearly visible as opposed to the thin outer periclinal walls (Fig. 6C). Finally, the outer periclinal walls were torn apart, showing reticulate thickenings of the remaining walls, which were the interior surfaces of cells of the outer layer (Fig. 6D). Size of a mature seed was 318 ± 32 μm longitudinally and 211 ± 26 μm transversely.

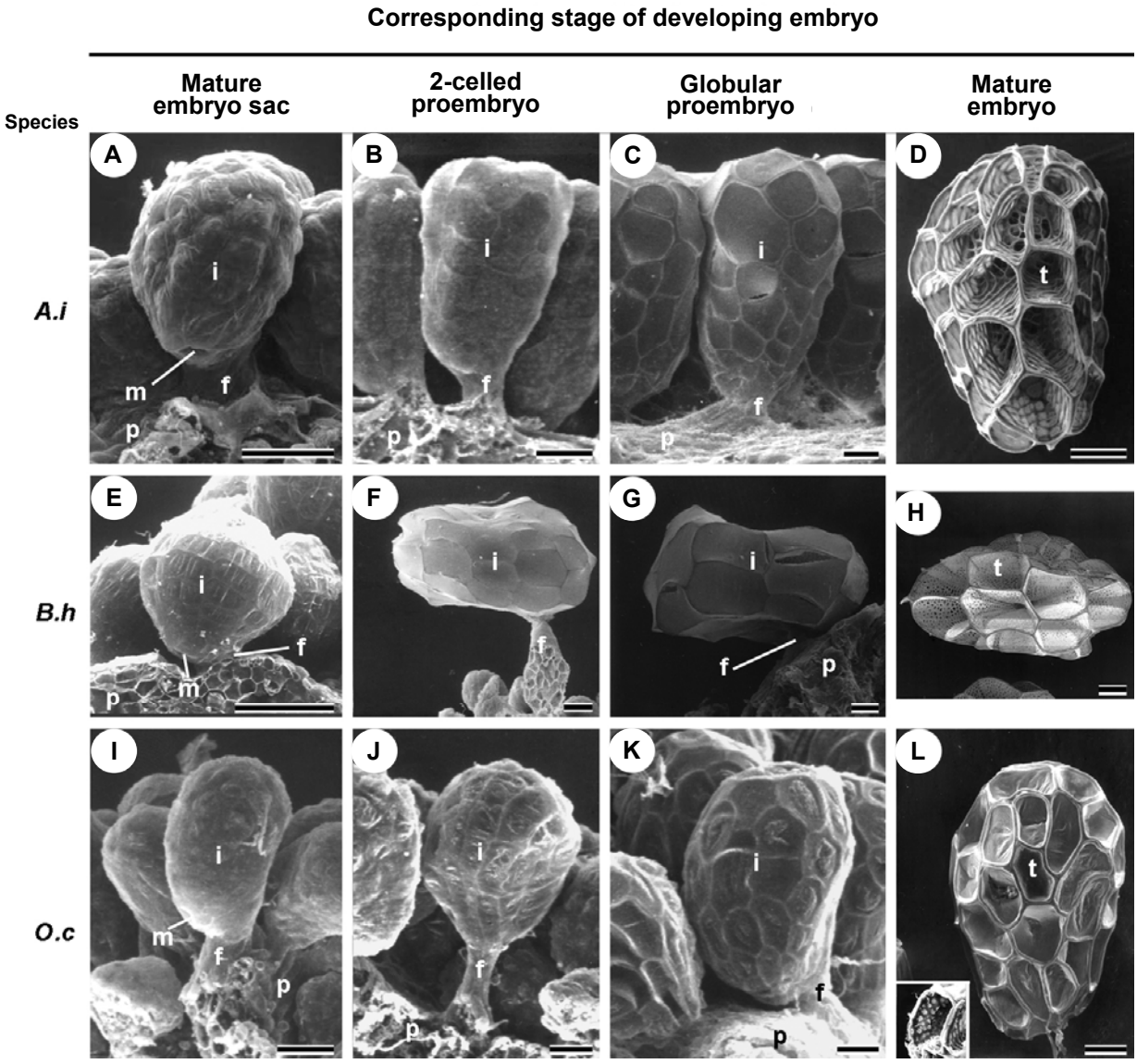

Fig. 6. SEM micrographs of external morphogenesis of seeds. Abbreviations: f, funicle; I, integument; m, micropyle; p, placenta; t, testa. The figures are tabulated by the corresponding stages of the developing embryos for three species to compare the external morphology of seeds. The surface of the integument appeared with reticulate ridges, which delineated one cell of the outer layer. D & H: The outer periclinal walls of the testa have torn away, showing the reticulately thickened inner walls of each cell. L: The outer periclinal cell walls of the testa sank inwards, making the exterior reticulate. The inset showed the interior thickened walls when the outer periclinal wall torn off. Bars= 60 μm in A-D & I-L; 120 μm in E-H.

Boschniakia himalaica Hooker & Thomson: The ovule was anatropous and nearly spherical with 214 ± 21 μm longitudinally (along the chalaza-micropyle axis) and 226 ± 21 μm transversely (Fig. 6E). When the seed grew to the 2-celled proembryo stage, its size got a 2.3-fold increase longitudinally and 3.3-fold increase transversely such that the seed turned to be oblate (Fig. 6F). The size of the seed stopped increasing at the globular proembryo stage. Due to the enlargement of cells of the outer layer to stretch the outer periclinal walls, the edges of thickened anticlinal walls were clearly visible as opposed to the outer periclinal walls (Fig. 6G). Finally, the outer periclinal walls were torn apart, showing reticulate thickenings of the interior walls (Fig. 6H). Size of a mature seed was 446 ± 70 μm longitudinally and 753 ± 25 μm transversely.

Orobanche caerulescens Stephan & Willd.: The ovule was anatropous and oval (Fig. 6I). Before fertilization the lengths of the ovule were 190 ± 23 μm

longitudinally (chalaza-micropyle) and 123 ± 15 μm transversely; and, after fertilization, both lengths increased to 1.7-1.9 folds at the 2-celled proembryo stage (Figs. 6I & J). The size of the seed kept increasing until the globular proembryo stage. At globular proembryo stage, the cells of outer layer enlarged, making the edge of the outer periclinal walls stretched to be flattened whereas the middle region of the walls appeared wrinkled. Meanwhile, as opposed to the outer periclinal walls, the edges of thickened anticlinal walls were clearly visible (Fig. 6K). Finally, when the fruit (capsule) broke, mature seeds were air-dried causing the outer periclinal walls to shrink inwards, resulting in concaved cells (Fig. 6L). Most mature seeds had the outer periclinal walls; only a few cases the outer periclinal walls torn apart, showing the reticulate interior walls (Fig. 6L, inset). Size of a mature seed was 392 ± 52 μm longitudinally and 241 ± 24 μm transversely.

DISCUSSION

We provide a complete portrait of seed morphogenesis for the Orobanchaceae in Taiwan and summary in Fig. 2. Although due to sample limitation, the examinations of *C. hookeri* were incomplete, its ovule organization and developmental type of endosperm were determined. Seeds of *A. indica*, *B. himalaica*, and *O. caerulescens* exhibited highly similar developmental stages in their embryo, endosperm, and testa. This means that the constituent counterparts of the seeds in the three species were formed in the same growth pattern. Structurally, they also shared many characters but some significant differences existed.

Embryological traits, on the whole, are much more constant at the family or genus than at the taxa of higher levels (Palser, 1975). In a more detailed analysis for Myrtales, the embryogenic type appears consistent in the section level (Tobe, 1989). Here, we identified two embryogenic types in Orobanchaceae, including Solanad type in *A. indica* and Onagrad type in both *B. himalaica* and *O. caerulescens*. The third type, the Caryophyllad type, was found in *C. tinctoria* (Kadry, 1955). As the same genus with *O. caerulescens*, *O. aegyptiaca* and *O. cernua* were also classified to be Onagrad type (Tiagi, 1951). We proposed that the consistency of embryogenic type for Orobanchaceae was at the genus level.

Our results as well as the other previous researches (Cassera, 1935; Kadry, 1955; Tiagi, 1951, 1952a, 1952b, 1963; Tiagi and Sankhla, 1963; Raju and Chamaiah, 1972) indicated that cellular type of endosperm development is conserved in Orobanchaceae and this is consistent with Palse (1975) and Tobe's (1989) statement. On the other hand, an argument about the endosperm structure of *A. indica* was raised previously. Juliano (1935) stated that the micropylar haustorium is poorly developed but Tiagi (1952b) pointed out that it is well developed and aggressive. Our results of *A. indica* from the three sampling sites were all identical to what Tiagi (1952b) described. It is possible that there might be diversities of the micropylar haustorium occurring in some *A. indica* populations.

Testa ontogeny of *A. indica*, *B. himalaica* and *O. caerulescens* was non-multiplicative, with the residues of the outer layer and reticulately-thickened secondary walls of its cells. We could not conclude whether this ontogenic process is prevalent in Orobanchaceae due to inefficient samples. Nonetheless, the seed shapes and testa features make these seeds distinguishable from each other. In *B. himalaica*, its oblate seed shape is different from the ovate shape of *A. indica* and *O. caerulescens*. The latter two species could be distinguished by the presence or absence of outer periclinal walls of the outer-layer cells, i.e. present in *O. caerulescens*, but absent in *A. indica*. The differences in cell shape and wall thickening appearance also aid in telling the seeds of these three species apart.

Mature embryos of Orobanchaceae are deficient in organs. Reduction of embryonic organs seems to be prevalent in parasitic plants (Kuijt, 1969). By comparing the Scrophulariaceae and Orobanchaceae, which are closely related and comprise autotrophs, hemiparasites and holoparasites in total, Teryokhin and Nikiticheva (1982) found that hemiparasitic members display intermediate embryo anatomy between those of autotrophs and holoparasites. They stated that the more dependent on hosts, the more reduced the embryonic organs could be. Phylogeny based on *rps2* and *matK* indicated that all parasites in these two families form a monophyletic group (dePamphilis *et al*., 1997; Young *et al*., 1999). It seems that the evolution of reduced embryonic organs of Orobanchaceae correlates to the parasitic behavior.

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臺灣產列當科植物之種子形態發生研究

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摘要：列當科種子之形態形成於先前未有完整的研究。於此，我們以光學與掃描式電子顯微鏡對臺灣產列當科植物進行種子發育之解析。結果顯示野菰、丁座草與列當之種子皆由胚、胚乳與種皮組成。於胚的層面，野菰之發育屬於茄型，而丁座草與列當屬於柳葉菜型；成熟的胚皆未分化。關於胚乳，所有種類之發育皆屬於細胞型；成熟時則由數層儲藏組織之細胞所構成。在種皮方面，所有種類之發育皆為非複分裂型；最終只剩最外層細胞，並具有網狀加厚之次生壁。野菰與列當之成熟種子呈現卵圓形，而丁座草則為扁圓形。至於假野菰則因材料不足，僅判定其胚乳發育屬於細胞型。列當科之種子發育比較於此討論之。

關鍵詞：胚、胚乳、列當科、種子發育、種皮。