中国特有河口异叶苣苔（苦苣苔科）胚胎学研究[*]

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摘要：对河口异叶苣苔的胚胎学观察旨在为该属的系统学研究提供参考。该种的花药药壁由表皮、药室内壁、中层和绒毡层4层细胞组成。2～3－核细胞在绒毡层频繁出现。胚珠属倒生，单珠被和薄珠心型。胚囊发育属蓼型。该种胚囊发育中的双大孢子母细胞现象，分别为并列和前后排列型。前者发育至双并列四分体，后者发育到呈棱形的4个大孢子。胚乳的发育属细胞型，并在合点端和珠孔端分别具有吸器。珠孔吸器发育早期为单核、2－细胞，后期为两核、2－细胞或单核、4－细胞，有时为多细胞，并在发育过程中向外伸长形成外珠孔。合点吸器为两核。由于合点吸器和珠孔吸器的活动，位于珠被最外层细胞和珠被绒毡层之间的2～3层细胞逐渐解体和被吸收。胚的发生和发育属柳叶菜型。在胚的发育过程中，胚乳几乎被吸收耗尽，仅剩下一层胚乳细胞紧贴内种皮。成熟种子的种皮由珠被最外层细胞和珠被绒毡层发育而来。本文对河口异叶苣苔的胚胎发育过程同苦苣苔科其它类群进行了广泛的比较和讨论。

关键词：小孢子；胚囊；双大孢子母细胞；胚；胚乳；河口异叶苣苔；苦苣苔科

中图分类号：Q 944.4　**文献标识码**：A　**文章编号**：0253－2700(2001)01－0072－09

Embryology of *Whytockia hekouensis* (Gesneriaceae)[*]

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Abstract: The embryology of *Whytockia hekouensis* Y. Z. Wang, as a representative of the genus *Whytockia*, was investigated in order to provide information on the systematics of the genus. The anther wall comprises four layers—epidermis, endothecium, middle layer and tapetum. 2—3－nucleate cells are frequently observed in the tapetum. The anatropous, unitegminal and tenuinucellar ovules, and the embryo sac of the Polygonum type conform to those of the most members of the Gesneriaceae. Twin megaspore mother cells occasionally occur during megasporogenesis. The development of the endosperm is of cellular type. The micropylar haustorium of *Whytockia* is 2－celled uninucleate in the early development and 2－celled binucleate or 4－celled uninucleate, and sometimes multi－celled in the late development, while the chalazal haustori-

um is binucleate. The extra – micropylar structure has been observed during the development of the micropylar haustorium. The development of the onagrad type embryo consumes up the endosperm except one layer that remains in the mature seed. The coat of a mature seed consists of two layers which develop respectively from the outer epidermal cells and the endothelial cells of the integument. The embryology of *W. hekouensis* is compared and discussed with that of others in the Gesneriaceae.

Key words: Pollen; Embryo sac; Twin megaspore mother cell; Embryo; Endosperm; *Whytockia hekouensis*; Gesneriaceae

Despite a large size of the Gesneriaceae, there is a paucity of work on the embryology of this family. Embryological study has become of crucial importance in plant systematics today. The comparative embryological data can help profoundly in the resolution of difficulties in determining homologies of mature reproductive structures, and further provide independent evaluations of relationships hypothesized via use of other characters (Stuessy, 1990). The genus *Whytockia* W. W. Smith endemic to China has been considered as the most primitive representative of the relict group – the tribe Epithemateae in the Gesneriaceae. Nevertheless, no embryological information of the genus has been reported up to now.

The goal of the present work is mainly to provide data in a wide range on the developmental process of embryology in *Whytockia*. This investigation includes the microsporogenesis, the megasporogenesis and the development of the endosperm and embryo, and the structure of the seed. The developmental process of embryology in *Whytockia* will be compared and discussed with that of others in the Gesneriaceae, which would contribute to the systematics of the genus.

Materials and Methods

The materials of *W. hekouensis* Y. Z. Wang were collected from Hekou county of Yunnan. Flowers and fruits at different developmental stages were fixed in formalin – acetic acid – alcohol (FAA). The materials were dehydrated in ethyl alcohol – xylol series and imbedded in paraffin wax following customary methods. Serial sections of 6 – 8 (m thick were stained either with iron – haematoxylin or safranin – fast green.

Results

W. hekouensis is a small perennial herb. Leaves are opposite with one large leaf and one small leaf. Simple and pair – flowered cymes emerge from the axils of large leaves in the upper parts of stems. No bract subtends inflorescences and flowers. Calyxes are five – lobed and corolla bilabiate with a three – lobed lower lip longer than the two – lobed upper one. The androecium consists of four fertile stamens and one staminode. Ovaries are bilocular with axile placentation. In each locule, the placentae are divergent bearing numerous anatropous ovules.

1 Microsporangium and microsporogenesis

The anther contains four microsporangia (Plate I: 1). Prior to maturity the anther wall comprises

four layers – – epidermis, endothecium, middle layer and tapetum (Plate I: 2). Tapetal cells are uninucleate at first but later become binucleate or trinucleate (Plate I: 2, 3, 5). The primary sporogenous tissue within the anther wall differentiates into the microspore mother cells (Plate I: 2, 3). Meiotic divisions in the microspore mother cells are accompanied by simultaneous cytokinesis, and form four microspores arranged in a tetrahedral manner (Plate I: 4). The middle layer degenerates early during maturity of the microsporangium (Plate I: 5, 6). At maturity of the microsporangium, the tapetum becomes degenerated (Plate I: 6), while the endothecium acquires prominant fibrous thickenings (Plate I: 6, 7). Each microspore enlarges and divides to form a large vegetative cell and a small generative cell (Plate I: 7, 8). The pollen grains are shed at the two – celled stage and possess a thin intine and a comparatively thick smooth exine with three germ pores (Plate I: 8).

2 Megasporangium and female gametophyte

Each ovular primordium develops into a unitegmic, anatropous, and tenuinucellate ovule. Usually a single hypodermal archesporial cell differentiates at the same time when the integument is initiated in the young ovule (Plate I: 9). Afterwards, it grows and functions directly as the megaspore mother cell (Plate I: 10) and undergoes a meiotic division to form a linear tetrad of megaspores (Plate I: 11 – 13). The mitotic divisions of two cells in the dyad occur almost at the same time. The three megaspores near the micropyllar part degenerate soon and the nucleus in the chalazal megaspore develops (Plate I: 14). The nucleus undergoes three divisions to form an eight – nucleate embryo sac of polygonum type (Plate I: 15 – 20). The plate of the first division is almost paralleling or 30°angle to the minor axis of the sac (Plate I: 15, 16). The two nucleus from the first division are located in the chalazal part (Plate I: 17). They undergo the second division respectively to form four nuclei in which two nuclei are located in the micropylar part and another two in the chalazal end (Plate I: 18). The mature embryo sac usually comprises a dilated micropylar portion and a comparatively narrow of the chalazal part, and the endothelium is restricted to the chalazal region of the embryo sac (Plate I: 19, 20). The egg apparatus consisting of two synergids and one egg cell is located in the micropylar end (Plate I: 20). The two polar nuclei move to the center of the embryo sac and fuse to form the secondary nucleus (Plate I: 19, 20). And the three antipodal nuclei in the chalazal end are ephemeral (Plate I: 19, 20).

Occasionally, twin megaspore mother cells occur during the megasporogenesis. The twin megaspore mother cells are in a parallel or pre – post arrangement (Plate I: 21, 22). Four megaspores in diamond arangement developed from the twin megaspore mother cells have been observed (Plate I: 23). However, twin mature embryo sacs have not been found in the present investigation.

3 Endosperm

The primary endosperm nucleus divides prior to the zygote. A transverse wall separates the embryo sac into a micropylar chamber and a small chalazal chamber, and the next division is longitudinal and occurs only in the micropylar chamber (Plate II: 24). In the micropylar chamber, the further transverse division separates the micropylar haustorium from the endosperm proper (Plate II: 25). The endosperm proper undergoes a vertical division at first and then a transverse division, and each division

follows the formation of the cell wall to form a 4 – celled initial endosperm (Plate II: 25). The following divisions are also accompanied by the formation of cell walls. The development of the endosperm in *W. hekouensis* thus belongs to the cellular type (Plate II: 38 – 39, 41 – 42).

4 Haustoria

The single nucleus in the chalazal chamber divides without wall formation to form two nuclei (Plate II: 24). The binucleate chalazal chamber functions directly as a chalazal haustorium (Plate II: 26). The activity of the chalazal haustorium results in the disorganization of the integumentary cells lying between the endothelium and the outer epidermis (Plate II: 25, 42).

During early stages, when the chalazal haustorium is very active, the micropylar haustorium remains deeply stained (Plate II: 25). The micropylar haustorium gradually becomes active when the fertilized egg develops up to an octant or 16 – cell proembryo (Plate II: 31). The micropylar haustorium is 2 – celled and uninucleate in the early development (Plate II: 31 – 33). And later, the 2 – celled uninucleate micropylar haustorium gradually divides to become 2 – celled binucleate (Plate II: 34). Several 4 – celled and multi – celled micropylar haustoria have been observed, but the cell walls seem incomplete (Plate II: 35 – 37, 40). The cells of the micropylar haustorium are elongated considerably, and extend and grow out of the micropyle to form an extra – micropylar structure (Plate II: 33, 35). At the late developmental stage of endosperm, the micropylar haustorium gradually brings about the disgeneration of the surrounding cells of the integument in the micropyllar portion (Plate II: 41, 42), and remains prominent for a long time in the seed (Plate II: 44).

5 Embryo and seed coat

The fertilized egg elongates considerably (Plate II: 24), and its first division takes place only after the endosperm has undergone its initial development. The first division of the fertilized egg is transverse resulting in a terminal cell ca and a basal cell cb (Plate II: 25). The former then is divided by a vertical wall to form two juxtaposed cells (Plate II: 28) which undergo a similar division at the right angle to the first bringing about the formation of the quadrant q (Plate II: 29). The basal cell undergoes a transverse division producing two cells c_i and m (Plate II: 27). Next, the cell c_i undergoes a transverse division forming two cells n and n' (Plate II: 28, 29). The quadrant then gives rise to an octant by a transverse division (Plate II: 30). The two tiers of the octant are represented by l and l' (Plate II: 30). Up to 16 – cell and 32 – cell proembryo, the dermatogen is differentiated from a group of inner cells by the periclinal division of the periphery cells (Plate II: 31). Meanwhile, the cell m divides transversely to form d and f (Plate II: 30). The former functions as the hypophysis and undergoes a vertical division to form d' and d'', while the latter contributes to the suspensor (Plate II: 31). Afterwards, the embryo gradually becomes a spherical shape (Plate II: 32, 37). With the continuous development of the proembryo, further periclinal divisions in the inner group of cells gradually form the periblem and plerome (Plate II: 38, 39). At the same time, n and n' undergo further transverse division to give rise to a uniseriate row of cells as the major part of suspensor (Plate II: 38, 39, 41). With the regional development, the top of the embryo becomes flattened and gradually gives rise to the cotyledons and stem tip (Plate II: 41 – 43). The hypocotyledonary portion is below them. The suspen-

sor degenerates in later stages. The development of the embryo thus follows the capsella type. During the development of embryo, the endosperm is almost consumed (Plate II: 44), and leaves only one layer pressing close to the endothelium in the mature seed (Plate II: 40). The embryo occupies almost the whole region within the seed coat (Plate II: 45).

During the development of the endosperm and embryo, the cells lying between the endothelium and the outer epidermis of the integument become crushed as a result of the activities of the chalazal and micropylar haustorium. At a later stage, the outer tangential walls of the epidermal cells of integument are thickened lamellately to become the outer coat of the mature seed (Plate II: 42, 44, 45). Meanwhile, the cells of the endothelium remain and exhibit a thickening both along the inner and outer tangential walls (Plate II: 41, 42, 44). In a mature fruit, the endothelium persists as the inner coat of seed (Plate II: 45).

Discussion

The four layer structures of anther walls in *W. hekouensis* follow the same pattern as other members investigated in the Gesneriaceae. The tapetal cells are 2 - or 3 - nucleate in *Rhynchoglossum notoniana* (Thathachar, 1942). The 2 - 5 - nucleate cells were observed respectively in the tapetum in *Didymocarpus tomentosa* and *Platystemma violoides*, while 2 - nucleate cells only in Epithema carnosum (Thathachar, 1942; Adatia *et al*, 1971). The 2 - 3 - nucleate tapetum of *W. hekouensis* is similar to that of *R. notoniana*.

The anatropous, unitegminal and tenuinucellar ovules, and the embryo sac of Polygonum type in *W. hekouensis* conform to those of the most members of the Gesneriaceae. Nevertheless, twin megaspore mother cells in a parallel arrangement are only found in *W. hekouensis* and *R. notoniana* (Arekal, 1961). The twin megaspore mother cells in a pre - post arrangement and the four megaspores in a diamond arrangement in *W. hekouensis* are first reported in the Gesneriaceae. However, no twin embryo sac in an ovule has been found in the present investigation.

In the development of the cellular type of endosperm in the Gesneriaceae, the primary endosperm nucleus usually divides to give rise to micropylar and chalazal chambers in which the micropylar haustorium and chalazal haustorium respectively develops. The presence of a 2 - celled uninucleate micropylar haustorium is common to a large majority of the taxa investigated up to date (Johri *et al*, 1992). 4 - celled uninucleate micropylar haustorium is only reported in *Ramondia and Haberlea* (Glišič, 1928; Arekal, 1961). In *Alloplectus sanguineus*, the two cells of the micropylar haustorium is uninucleate at early time but later becomes binucleate (Arekal, 1961). In the present study, the 2 - celled uninucleate micropylar haustorium in *W. hekouensis* divides to give rise to 2 - celled binucleate or 4 - celled one in the late development of the endosperm. The multi - celled micropylar haustorium occasionally occurs in *W. hekouensis*. The great diversity of micropylar haustrium in *W. hekouensis* is first reported in the Gesneriaceae.

The extra - micropylar embryo - sac was observed in *Rhynchoglossum zeylanica* (*Klugia zeylanica*) (Schnarf, 1921), *Monophyllaea horsfieldii* (Oehlkers, 1923) and Roettlera sp. (Glišič, 1934).

The embryo sac of *W*. *hekouensis* presents no tendencies for an extra – micropylar development, however, haustorial cells develop beyond the micropyle to form an extra – micropylar structure. This phenomenon was only reported in *Didymocarpus tomentosa* (Thathachar, 1942).

The uninucleate chalazal haustorium has been observed in the most investigated taxa in this family. However, several taxa, like *D*. *tomentosa* (Thathachar, 1942), are characteristic of binucleate chalazal haustorium. Occasionally, the chalazal haustorium is 2 – celled in *Haberlea* or 4 – celled in *Prohaberlea* (Adatia, 1971; Johri *et al*, 1992). The binucleate chalazal haustorium in *W*. *hekouensis* is similar to that of *D*. *tomentosa* reported to date in the Gesneriaceae.

As the most investigated taxa in the subfamily Cyrtandroideae, the embryogeny of *Whytockia* conforms to the onagrad type. The development of the embryo in *Whytockia* consumes up almost all the endosperm, but one layer of endosperm remains in the mature seed. In *R*. *notoniana*, all the integumentary cells within the epidermis including the endothelial cells become disorganized as a result of the activity of the chalazal haustorium. Thus, the coat of the mature seed consists of only one – layer cells of the outer epidermis of integument (Arekal, 1961). However in *W*. *hekouensis*, the cells of endothelium are elongated and thickened during maturity of seeds, and persist in the mature seed as the inner coat. The coat of the mature seed in *W*. *hekouensis* comprises two layers that are developed respectively from the outer epidermal cells and endothelial cells of the integument. This situation of the seed in *Whytockia* differs from that of others investigated up to date in the Gesneriaceae.
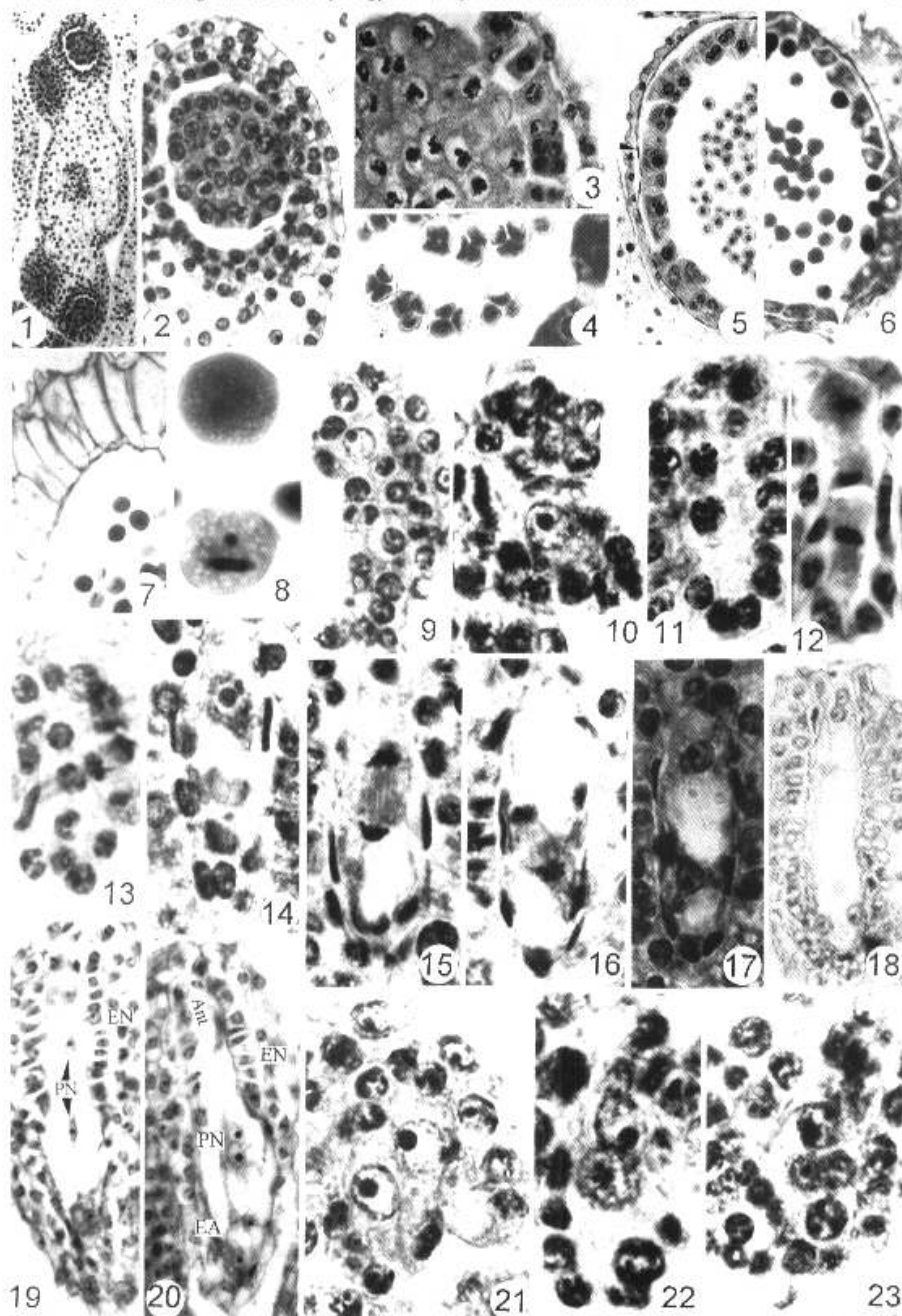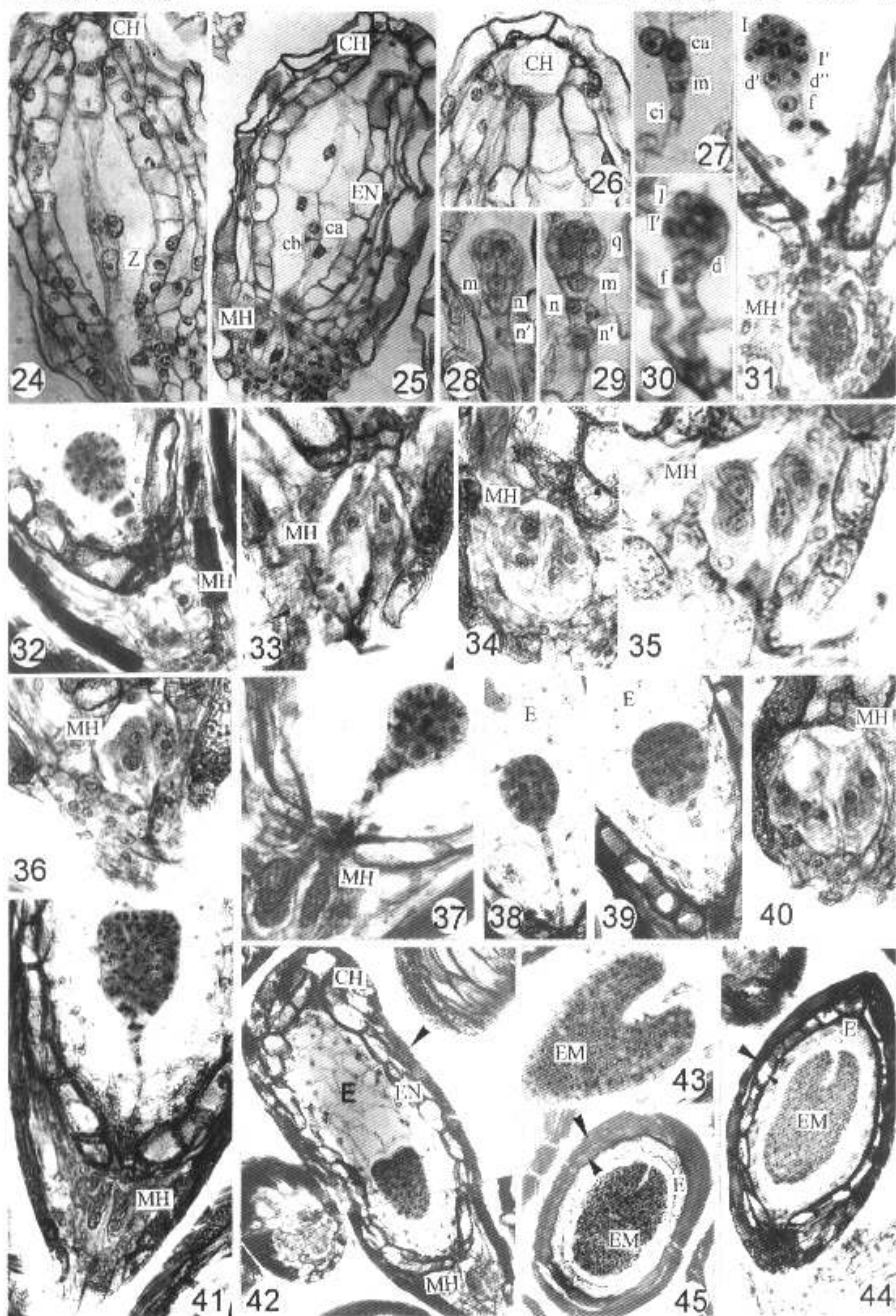
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Explanation of Plates

Plate I Microsporanqium, microsporogenesis, megasporangium and megasporogenesis of *Whytockia hekouensis*. 1–8. Microsporanqium and microsporogenesis. 1. Showing young anther ($\times 100$). 2. Anther wall comprising an epidermis, an endothecium, a middle layer and tapetum, and the primary sporogenous tissue ($\times 400$). 3. Microspore mother cells, showing binucleate tapetum ($\times 400$). 4. Microspore tetrads ($\times 400$). 5. Microspore, showing trinucleate tapetum and degeneration of the middle layer (arrowhead, $\times 300$). 6. Pollen grains and degeneration of tapetal cells ($\times 300$). 7. Pollen grains, showing fibrous thickening of the endothecium ($\times 300$). 8. Mature pollen grains with two nuclei and three germ pores ($\times 1000$). 9–23. Megasporangium and megasporogenesis. 9. Differentiation of a single hypodermal archesporial cell and initiation of the integument ($\times 300$). 10. Megaspore mother cell ($\times 400$). 11. Dyad ($\times 400$). 12. Division of dyad cells ($\times 400$). 13. Linear tetrad of megaspores ($\times 300$). 14. Functional megaspore ($\times 400$). 15–16. First division of the megaspore ($\times 400$), showing the division parallel (15) and $30°$ angel (16) to the minor axis of the sac (15). 17. Two-nucleate embryo sac ($\times 200$). 18. Four-nucleate embryo sac ($\times 150$). 19. Eight-nucleate and organized embryo sac, showing two polar nuclei (PN) near the center of the embryo sac and the endothelium in the chalazal part ($\times 200$). 20. Mature embryo sac, showing two polar nuclei fusing in the center of embryo sac, degenerated antipodal nuclei (Ant) and egg apparatus (EA) ($\times 200$). 21. Two paralleling megaspore mother cells (arrowhead, $\times 400$). 22. Two megaspore mother cells in pre-post arrangement (arrowhead, $\times 400$). 23. Four megaspores in diamond arrangement developed from the double megaspore mother cells ($\times 400$).

Plate II Development of endosperm, embryo and maturation of seed in *W. hekouensis*. 24. A transverse wall separating micropylar chamber from a small chalazal chamber (CH) containing two nuclei, and the zygote (Z) having been left from the micropylar part and elongated, and longitudinal division of endosperm nucleus ($\times 200$). 25. A terminal cell (ca) and a basal cell (cb) of the pre-embryo and 4-celled initial endosperm, chalazal and micropylar haustorium and elongation of the endothelial cells (EN) ($\times 200$). 26. Binucleate chalazal haustorium (CH) ($\times 400$). 27. Terminal cell and two cells ci and m produced from the division of the basal cell ($\times 300$). 28. Juxtaposed cells ($\times 400$). 29. The quadrant ($\times 400$). 30. The octant ($\times 400$). 31. Cells d', d'' and f, and 2-celled uninucleate micropylar haustorium (MH) ($\times 400$). 32. Spherical pre-embryo and 2-celled uninucleate micropylar haustorium ($\times 400$). 33. 2-celled uninucleate micropylar haustorium ($\times 320$). 34. 2-celled binucleate micropylar haustorium ($\times 320$). 35. 4-celled micropylar haustorium ($\times 480$). 36. 4-celled micropylar haustorium ($\times 320$). 37. Spherical pre-embryo and 4-celled micropylar haustorium ($\times 400$). 38. Uniseriate cells of the long suspensor, the regional development of pre-embryo and the cellular type of the endosperm (E) ($\times 300$). 39. Pre-embryo with the top gradually becoming flattened and the cellular type of the endosperm ($\times 400$). 40. Multi-celled micropylar haustorium ($\times 320$). 41. Uniseriate cells of the long suspensor, the regional development of pre-embryo and thickening both along the inner and outer tangential walls of endothelial cells ($\times 400$). 42. Appearance of the two cotyledons and lamellated thickening of the outer wall of the epidermal cells (arrowhead) and the cellular type of the endosperm ($\times 100$). 43. The two cotyledons of the young embryo (EM) ($\times 300$). 44. Late developmental stage of seed, showing the thickened endothelial cells and the outer wall of the epidermal cells (arrowhead) ($\times 150$). 45. Mature seed, showing the outer coat and inner coat (arrowhead) and one layer endosperm pressing close to the inner coat (E) ($\times 150$).